Hyperplastic development and hypertrophic growth of muscle fasciculi in *Macrobrachium rosenbergii* (De Man 1879)

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**ABSTRACT**

Muscle growth dynamics was analyzed in the giant freshwater prawn, *Macrobrachium rosenbergii*, using 30 full-sib families, 10 each of three stocks collected from different parts of India. The objectives of this study were to understand the muscle growth pattern in *M. rosenbergii* and to identify stocks with high performance. The diameters of individual fasciculi were measured and total fasciculi numbers per mm² at different hapa-ages (i.e., 60, 90, 120, 150 and 180 days) were calculated. The average muscle fasciculi diameter ranged from 13.50 ± 2.5 to 23.96 ± 2.2 μm at different hapa-ages. Significant variations were observed between the stocks at all hapa-ages except 150 and 180 days and between tanks at certain hapa-ages. The average muscle fasciculi numbers per mm² varied with hapa-age from 11.16 ± 2.6 to 23.96 ± 4.3; significant variations were observed in the fasciculi numbers per mm² between stocks and sires at certain hapa-ages. By using non-parametric smoothing technique, it was found that the muscle growth in *M. rosenbergii* is mainly due to hypertrophy but the recruitment of the muscle fasciculi do takes place at around 120 days of hapa-age. The findings of this study appear to have enormous potential in genetic improvement programmes.

Keywords: Fasciculi, Hyperplasia; Hypertrophy, *Macrobrachium rosenbergii*; Muscle fibre, Skeletal muscle
edible portion of fish can be directly affected by muscle cellularity and muscle growth dynamics. Since there is a potential to manipulate muscle cellularity through aquaculture practices and selective breeding programs, the ultimate goal of such applied work would be to produce fast-growing animals with maximal muscle mass.

Currently, there is lack of literature describing skeletal muscle growth in *M. rosenbergii*. Therefore, in this experiment, muscle fasciculi diameter and numbers at different ages was studied to identify the mechanism of muscle growth in *M. rosenbergii* and understand the environmental conditions that result in optimal growth and body weight at specific ages which could be used to improve *M. rosenbergii* breeding programmes.

**Materials and methods**

**Stocks**

*M. rosenbergii* brooders were collected from a natural reservoir in Dapchery of Thane district of Maharashtra, the Narmada River in Baruch district of Gujarat and the Mahanadi River in Cuttack district of Orissa. The collected brooders were placed in six circular Fibreglass Reinforced Plastic (FRP) tanks (220 cm in diameter and 150 cm deep) separately with respect to stock and sex for acclimatization, prior to breeding.

**Breeding and harvesting of full-sibs**

A total of 45 pairs of mature brooders, 15 pairs from each stock were separately kept for mating in uniformly sized FRP tanks at CIFE centre, Balabhadrapuram, Andhra Pradesh. Of the 15 pairs, 10 males and 10 females showing better maturity conditions were paired and each pair was kept in isolation until the mating stage. Larvae were grown to the PL15 stage and then transferred to nurseries in Balabhadrapuram, for full-sib family studies on juvenile prawns. Standard aquaculture practices for feeding, water quality parameters, light and stocking density were followed throughout the experimental period as described by New (2002).

**Histology of skeletal muscle tissue**

Muscle samples from five randomly selected individuals belonging to each full-sib family were collected monthly, from 60 days to 180 days hapa-age. The animals were de-shelled, muscle tissue of sixth abdominal segment was dissected and about 0.2 g of tissue was fixed in 10% neutral buffered formalin (NBF).

**Tissue processing and staining**

The fixed tissues were washed in running water for 15 h and then dehydrated gradually by immersing in different concentrations of ethanol. After dehydration, the tissue samples were dipped in ethanol: propanol (50:50) mixture, followed by propanol, propanol: acetone (50:50) mixture, and finally, in acetone. Each dip lasted for 10 min. The dehydrated tissue was impregnated in paraffin (Qualigens Fine Chemicals, Mumbai, India) at 55 ºC for 45 min. and then transferred to fresh paraffin for another 45 min. This whole procedure was repeated thrice. The tissues were embedded in paraffin blocks and then sectioned into 5 μm thick sections using a microtome and mounted onto slides. After drying, the slides were immersed in xylene, this step was repeated twice to remove the wax completely. The deparaffinized tissue sections were rehydrated and stained with haematoxylin and counter stained with eosin. The sections were mounted using DPX.

**Estimation of fasciculi number**

The stained skeletal muscle sections were observed under a microscope fitted with a 5 mm² ocular micrometer, and the number of fasciculi per mm² was counted as follows. Five fields per slide were randomly chosen and the total fasciculi in each corner square (area = 1 mm²) and the central square of the ocular micrometer were counted. The average of the five measurements was estimated to arrive at an accurate fasciculi number for each slide. A total of three slides were analyzed for each animal.

**Measurement of fasciculi size**

The diameter of the fasciculi was measured to the nearest μm using a stage micrometer and an ocular micrometer. The diameters of 5 randomly selected fasciculi were measured per slide and the average was estimated. A total of three slides were analyzed for each animal. The conversion of cross-sectional area to equivalent diameter values was made according to Weatherly et al. (1985) assuming that individual fasciculi cross-sections are circular.

**Statistical analysis**

Muscle fasciculi numbers and diameters were tested for normal distribution by employing PROC UNIVARIATE of SAS system (SAS, 2000). Wherever the data were found to deviate from a normal distribution, appropriate transformations (logarithmic or square root) were carried out to fit the data to a normal distribution and means were reported after reconversion of the values. Outliers were eliminated from the analysis to avoid biases in the estimation of parameters.

A kernel density estimation (or KDE) procedure was performed to smooth the normal distribution obtained using the PROC UNIVARIATE of SAS system described above. After applying the PROC KDE system (SAS, 2000) to the various data points, the weighted kernel density estimate was determined as described by Silverman (1986).
An application of the probability density function toward evaluating muscle dynamics has been reported by Johnston et al. (1999). The significant source of factors influencing muscle fasciculi number and diameter at various hapa-ages was estimated by adopting the following model and employing PROC GLM procedure of SAS (SAS, 2000):

$$Y_{ijklm} = \mu + \text{Stock}_i + \text{Tank}_j + \text{Slide}_k + \text{Sire}_{l(i)} + e_{ijklm}$$

Where, $Y_{ijklm}$ is the $m$th individual born to $l$th sire belonging to $i$th stock reared in $j$th tank and measured in $k$th slide; $\mu$ = overall mean; $\text{Stock}_i$ = fixed effect of $i$th stock and $\sum \text{Stock}_i = 0, i = 1, 2, 3$; $\text{Tank}_j$ = fixed effect of $j$th tank and $\sum \text{Tank}_j = 0, j = 1, 2, 3, ..., 10$; $\text{Slide}_k$ = random effect of slide within an animal and $\sum \text{Slide}_k = 0, k = 1, 2, 3, ..., 10$; $\text{Sire}_{l(i)}$ = random effect of $m$th sire and $\sum \text{Sire}_{l(i)} = 0$, and $l = 1, 2, 3, ..., 10$; $e_{ijklm}$ = random error associated with $Y_{ijklm}$. Random errors were assumed to be independently and normally distributed with mean zero and variance one $e\sim[0, 1]$.

Results

Muscle fasciculi diameter and hapa-age

For all three stocks of *M. rosenbergii* analyzed in this study, the average muscle fasciculi diameter increased with hapa-age (Table 1). At 60, 90, 120, 150 and 180 days hapa-age, the average fasciculi diameter was $13.50 \pm 2.50$, $18.39 \pm 3.30$, $17.22 \pm 3.3$, $20.90 \pm 2.3$, and $23.96 \pm 2.2 \mu m$, respectively, with an average muscle fasciculi diameter value of $17.53 \pm 0.14 \mu m$. When the fasciculi diameters at different hapa-ages were plotted on a smooth kernel function, the distributions were multimodal (Fig. 1). Intriguingly, the distribution patterns for all age groups were significantly different from one another. ANOVA results show that stock had significant effect on average muscle fasciculi diameter at 60, 90 and 120 days hapa-age; however the effect was not significant at 150 and 180 days hapa-age. Furthermore, the effect of tank on mean muscle fasciculi diameter was highly significant at 60, and 120 days hapa-age, but not at 90, 150 and 180 days hapa-age. Alternately, the average diameter of muscle fasciculi between different slides within an animal was statistically non-variant at all hapa-age groups. The sire was found to have significant effect on muscle fasciculi diameter at all hapa-ages except 180 days hapa-age.

Muscle fasciculi numbers per mm² and hapa-age

The average muscle fasciculi numbers decreased with age for all three stocks of *M. rosenbergii* (Table 2). For example, the average numbers per mm² at 60, 90, 120, 150 and 180 days hapa-age were $23.96 \pm 4.30$, $12.21 \pm 2.00$, $14.27 \pm 3.90$, $11.27 \pm 2.80$ and $11.17 \pm 2.60$, respectively. The fasciculi numbers at different hapa-ages, plotted on a smooth kernel function (Fig. 2), resulted in a distribution, which was unimodal except that the values at 60 days hapa-age exhibited a wider peak as compared to the other age groups. Differences between the age groups for muscle fasciculi number distributions were not as evident as the differences for fasciculi diameter distributions (Fig. 1 and 2).

When the mixed ANOVA model was applied to analyze effects of stock on muscle fasciculi numbers, it was observed that the stock had a significant effect on average fasciculi numbers at 60, 90, 120 and 150 days hapa-age; however, its effect was not significant at

<table>
<thead>
<tr>
<th>Fasciculi diameter (µm)</th>
<th>Hapa-age</th>
<th>60 days</th>
<th>90 days</th>
<th>120 days</th>
<th>150 days</th>
<th>180 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>450</td>
<td>450</td>
<td>180</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>$13.50 \pm 2.50$</td>
<td>$18.39 \pm 3.30$</td>
<td>$17.22 \pm 3.30$</td>
<td>$20.90 \pm 2.30$</td>
<td>$23.96 \pm 2.20$</td>
</tr>
<tr>
<td>Gujarat</td>
<td></td>
<td>$11.11 \pm 2.75$</td>
<td>$18.64 \pm 3.70$</td>
<td>$15.37 \pm 3.10$</td>
<td>$20.86 \pm 2.10$</td>
<td>$23.98 \pm 2.90$</td>
</tr>
<tr>
<td>Maharashtra</td>
<td></td>
<td>$14.90 \pm 3.20$</td>
<td>$19.28 \pm 3.60$</td>
<td>$19.49 \pm 5.90$</td>
<td>$20.00 \pm 2.88$</td>
<td>$24.25 \pm 3.20$</td>
</tr>
<tr>
<td>Orissa</td>
<td></td>
<td>$11.51 \pm 2.20$</td>
<td>$17.26 \pm 3.40$</td>
<td>$21.53 \pm 6.10$</td>
<td>$20.85 \pm 2.07$</td>
<td>$23.01 \pm 3.20$</td>
</tr>
</tbody>
</table>

Means bearing same superscript within a group do not differ significantly from each other ($p<0.01$).
seems to be the first attempt to characterize muscle growth in *M. rosenbergii*. In the current study, we found that at 60 day hapa-age, the average fasciculi diameter was 13.50 ± 2.5 μm and the overall average fasciculi number per mm² was 24. However, the Maharashtra stock started with a higher average diameter as compared to the Gujarat and Orissa stocks, but with a lower average fasciculi number (Table 1 and 2). Given that the Maharashtra stock was isolated from a still water source, *i.e.*, a natural reservoir, as compared to the other two riverine stocks it is possible that the Maharashtra stock might have genetically adapted to possess a fasciculi size and number optimally suited to its natural environment. In fact, ANOVA showed that differences amongst stocks become non-significant at later hapa-ages. This is probably because the animals acclimatize to their environments as age increases and, therefore, show uniformity in growth. During the study it was also observed that the effects of different sires on muscle fasciculi diameter and numbers were significant up to 150 days hapa-age but not at 180 days hapa-age. Since our study only included full-sib families, it becomes important to study half-sib families to fully illustrate the sire effect. Furthermore, the tank environment might have significantly affected muscle fasciculi characteristics up to 150 days hapa-age. Notably, the tanks can be equated to environmental conditions encountered by *M. rosenbergii* and are inclusive of factors such as nutrition, feeding regime and husbandry practices. Our data, therefore, indicate that there might be specific contributors to muscle growth and further studies are required to identify these factors. One point to be noted is that, at later ages, our sample size decreased due to large scale mortality which occurred in many hapas and hence, these experiments need to be repeated to obtain more reliable data.

In addition, we observed that as fasciculi diameter increased with age, the average fasciculi number per mm² decreased, however at around 120 day hapa-age, the average fasciculi number increased again. This pattern seems to be the first attempt to characterize muscle growth in *M. rosenbergii*.
suggests that the muscle growth in *Macrobrachium rosenbergii* is mainly due to hypertrophy but at around 120 days of age hyperplasia also occurs. Previous studies in various fish species have also reported similar correlation between the number of fasciculi per mm² and fasciculi diameter as the fish age (Weatherley *et al*., 1980; Weatherley and Gill, 1985; Weatherley *et al*., 1988; Hall *et al*., 1990; Kaumans *et al*., 1993; Henckel *et al*., 1997; Johnston *et al*., 1999; Bugeon *et al*., 2003). This pattern is suggestive of growth by hyperplasia in the initial stages, as indicated by an increase in fasciculi numbers, followed by hypertrophy at later stages, as indicated by an increase in fasciculi diameters.

Overall, the present study highlights the importance of muscle fibre dynamics in *Macrobrachium* growth and accentuates a need for further extensive research. Given that the patterns of muscle fiber recruitment and hypertrophy vary between *Macrobrachium* populations, as in the three different stocks used in this study, this opens up the possibility of using selective breeding programs to specifically manipulate muscle structure and consequently, overall growth and body weight. Such programs can be accelerated by choosing starter populations that exhibit a growth profile naturally enhanced for highly desired qualities.

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**References**


