稚魚期から若魚期におけるクロマグロ体側筋の発達

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Lateral Muscle Development of the Pacific Bluefin Tuna, *Thunnus thynnus orientalis*, from Juvenile to Young Adult Stage under Culture Condition

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Abstract: The volume of lateral muscle, cross-sectional area of red and white fibers, and the number of fibers were examined for artificially hatched bluefin tuna, *Thunnus thynnus orientalis*, from juvenile to young adult stage within the size range of 19.5-163.0 mm body length (BL). The red and white muscle volumes increased exponentially with BL. At a size larger than 80.0 mm BL, both the volume increases were significantly accelerated. The proportion of red muscle volume in the total lateral muscle volume slightly increased with BL. The cross-sectional area and the total number of red and white fibers at the point of maximum body height increased in the BL range examined. The small red fibers (<a 100 μm^2 in size) of a cross-sectional area gradually disappeared with the growth of BL. In contrast, there existed the white small fibers (200-300 μm^2 in size) at all body sizes. Increases of both fiber numbers were approximately accelerated at sizes larger than 85 mm BL. The size of 80-85 mm BL, at which the phase change of muscle development occurred, corresponded to the transitional stage from juvenile to young adult.

Key words: *Thunnus thynnus orientalis*; ontogenetic development; lateral muscle

The seedstock production of the Pacific bluefin tuna has developed remarkably in recent years in Japan owing to the improvement of rearing techniques[1,2]. In the near future, the aquaculture of the Pacific bluefin tuna without wild-caught seedlings will be accomplished. However, there still exist obstacles to aquaculture using artificially hatched seedlings, for example, large losses during the first 10 days after hatching[1], cannibalism at the juvenile stage[1], loss by collisions with the net wall at the young adult stage[1,3], and difficulties in handling*3,4 and in transportation of live fish[1,4]. Some of these problems are caused by the characteristic behavior of the Pacific bluefin tuna.

Collisions are frequently observed when the fish reach the body length (BL) of 80 to 160 mm. The Pacific bluefin tuna from the juvenile stage are very susceptible to noise and vibration, and easily panicked into colliding with the tank wall[3]. There appears to be a causal relationship between the development of swimming ability and the sensory system, and collisions. The lateral muscles of fish directly provide the swimming power[5] and in the tuna are roughly divided into red and white muscle the same as in other vertebrates[6]. Red and white muscles consist of red and white fibers, respectively[5]. Slow-twitch and aerobic red fibers chiefly work for sustainable swimming, while fast-twitch and anaerobic white fibers chiefly work to give bursts of speed[7]. Therefore, the development of

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these muscles is closely related to the development of behavior, and such information is useful for the preventing collisions of bluefin tuna. In this study, we examined the change of muscle volume of the Pacific bluefin tuna from juvenile to young adult. In addition, changes of the cross-sectional area and number were examined for both red and white muscle fibers.

Materials and Methods

Fish

Artificial hatched the Pacific bluefin tuna was obtained as following method: approximately 130 broodstock, which were wild-caught and raised for 9-11 years in captivity at the Ohshima Experimental Station of the Fisheries Laboratory of Kinki University, spawned naturally from June to August in 1996 and 1998. Eggs were transported to the laboratory of the Fish Nursery Center of Kinki University in Shirahama about 60 km away and transferred to a 100 m^3 concrete tank. The feeding scheme for larvae, juveniles, and young adult fish was as follows: rotifers, *Brachionus rotundiformis*, from 2 days after hatching, *Artemia* nauplii from 10 days, live striped beakperch larvae, *Oplegnathus fasciatus*, from 10 days, minced sand lance, *Ammodytes personatus*, from 20 days (Fig. 1). During the experiment, water temperature ranged from 24.8 to 27.4°C and from 24.8 to 27.5°C in 1996 and 1998, respectively. Currency of rearing tank was 0-1, 3-5, and 5-8 cm/s from 0-10, 11-30, and 31-70 days after hatching, respectively. Rearing density was approximately 12000 ind./m^3 in 40 m^3 rearing tank on 0 day after hatching. Fish decreased in number for various reasons[1], and estimated the densities were 9000, 750, and 200 ind./m^3 at 2, 14, and 18 days after hatching. At 32 days after hatching, the fish were transferred two larger rearing tanks of 80 m^3 volume. The average densities at 32, 40, 60, and 70 days after hatching were about 11.6, 7.5, 1.5, and 0.8 ind./m^3, respectively.

The volume of lateral muscle was determined for 117 specimens from a BL of 19.5 mm (24 days after hatching, juvenile stage) to 163.0 mm (63 days after hatching, young adult stage) reared in 1996 and 1998. The cross-sectional area of muscle fiber was determined for 50 specimens from 19.5 mm to 163.0 mm in BL reared in 1998. The fiber number was determined for 15 specimens from 19.5 to 163.0 mm in BL using the same sample as for the cross-sectional area of muscle fiber. These specimens were caught by scooping with a soft mesh net or fishing in the rearing tank, and immediately fixed in 10% neutralized formalin solution.

Lateral muscle volume

The lateral muscle volume was measured by the sectional area method[8]. The body of the fish from the posterior of the pectoral fin to the posterior of the caudal keel was cut into 8 to 13 portions at even intervals. Digital images of the transverse sections were obtained with an image scanner (Nikon co. inc., AX-1200) aided by a computer (Apple co. inc., Power Macintosh 7600). These portions were embedded in paraffin, cut at 6 μm in thickness with a microtome around the horizontal septum, and stained by Mayer's hematoxylin and eosin. Red and white

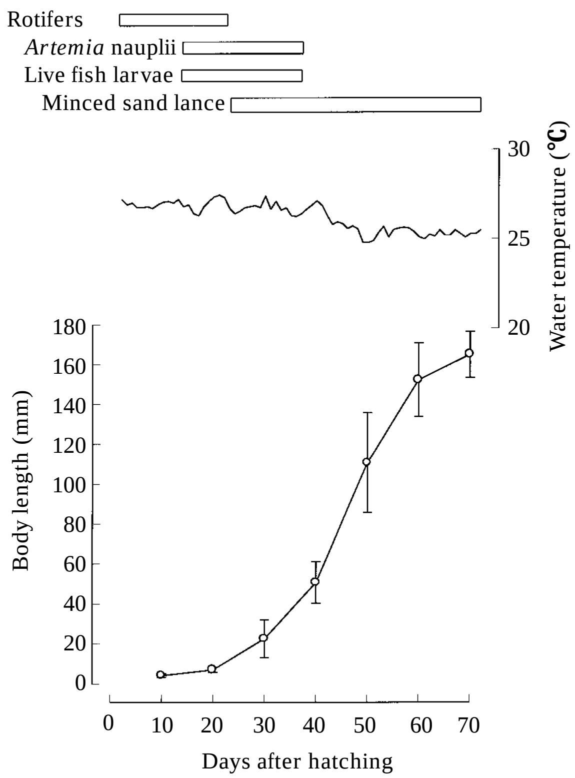

Fig. 1. Growth, rearing temperature, and feeding schedule of artificially hatched the Pacific bluefin tuna. Open circles indicate the mean of body length at 10-day intervals. Vertical bars show standard deviations.

muscles were distinguished by muscle colors referring to tissue sections, which indicated the difference of distribution pattern of myofibrils within fibers[5,8]. The areas of red and white muscle were determined at each transverse section using the public domain NIH Image program (The U.S. National Institutes of Health, version 1.60). Each area of red and white muscle was integrated to estimate the whole volume of fish body.

Results were expressed by allometric equation showing the relationship between morphometric measurements (y) and body length (x). Slopes of simple linear regression lines of the allometric equation ($\log y = \log a + b \cdot \log x$, where a and b are constants) were obtained by the least square method. Inflection points of these lines were determined subjectively at the most likely positions, however the statistical significance ($p < 0.01$) of the difference of regression line slopes was checked before and after inflection points referring t distribution[9].

Cross-sectional areas of red and white muscle fibers

The cross-sectional areas of red and white fibers were determined at the point of maximum body height as follows. Transverse sections of the superficial red muscle and the white muscle epaxial portion were embedded in paraffin, cut at 4-6 μm in thickness, and stained with hematoxylin and eosin. Digital pictures were taken with a CCD camera (Sony co. inc., MODEL DXC-108) attached to the microscope, and area measurements of muscle fibers were done by using the NIH Image program. The area measurements were made for 100 fibers both red and white for each section. Results of the area measurements were expressed by histogram at representative stages in relation to the morphology and behavior at 19.5, 47.0, 75.5, and 163.0 mm BL.

Total numbers of red and white muscle fibers

The total numbers of red and white muscle fibers were determined at the point of maximum body height. The total fiber number was estimated by counting the fiber number in a 100×100 μm transverse sectional area and measuring the total muscle transverse sectional area.

Results

Morphological and behavioral change

On day 22 after hatching (14.2 mm BL) fish attained the adult complement of fin rays (juvenile stage). On day 30 after hatching (56.0 mm BL) fish swam continuously all day and actively fed the fish meal of sand lance. On day 40 after hatching (80.5 mm BL) fish had each eight incompletely separated, fan-shaped dorsal and ventral finlets (young stage). At this stage, they were very sensitive against the sound and flash light, and the loss caused by the trauma of collisions seriously increased. On day 61 after hatching (163.0 mm BL) fish swam spreading their pectoral fin when they swam at the cruising speed. The loss by collisions remarkably decreased.

Lateral muscle volume

Red and white lateral muscle volumes both increased exponentially with BL (Fig. 2). Both regression lines in allometries of these muscle volumes had one inflection point at 80.0 mm BL. At a size larger than 80 mm BL, the volume increase was accelerated for both kinds of muscles.

The proportion of red muscle volume against total lateral muscle volume slightly increased from 6 to 9% in the range of 19.5-163.0 mm BL (Fig. 3).

The cross-sectional area of muscle fibers

The cross-sectional areas of red and white fibers at the portion of maximum body height increased exponentially in relation to BL in the range from 19.5 to 163.0 mm BL (Fig.4).

In red fibers, smaller fibers of 100 μm^2 remarkably decreased at the size of 163.0 mm BL (Fig. 5). In white fibers, there were fine fibers of 200-300 μm^2 cross-sectional area in spite of the body size.

Total number of muscle fibers

The total number of red and white fibers in the portion of maximum body height increased with growth (Fig. 6). Increases of both fiber

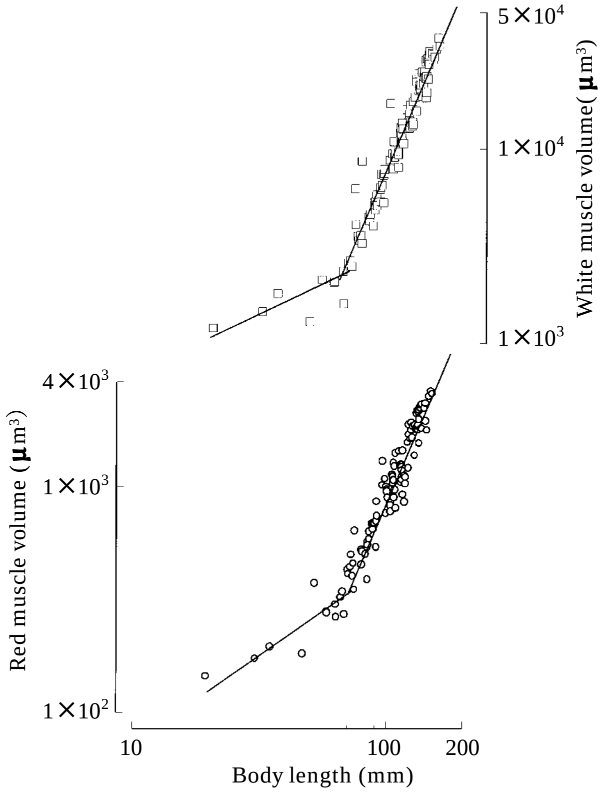

Fig. 2. Allometries of red and white lateral muscle volume of artificially hatched the Pacific bluefin tuna. Figures by regression lines indicate the slopes of lines. Open circles, red muscle; Open squares, white muscle.

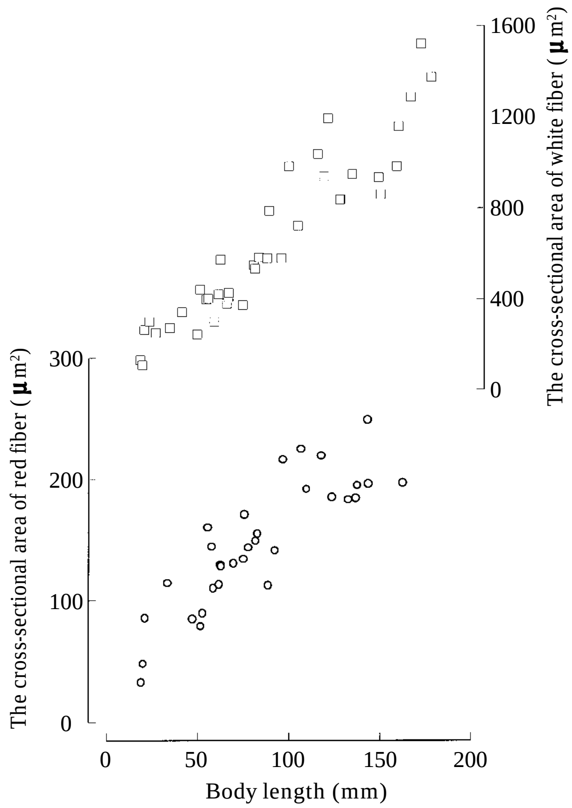

Fig. 4. Ontogenetic changes of the Pacific bluefin tuna red and white fiber cross-sectional area at the body portion of maximum body height.

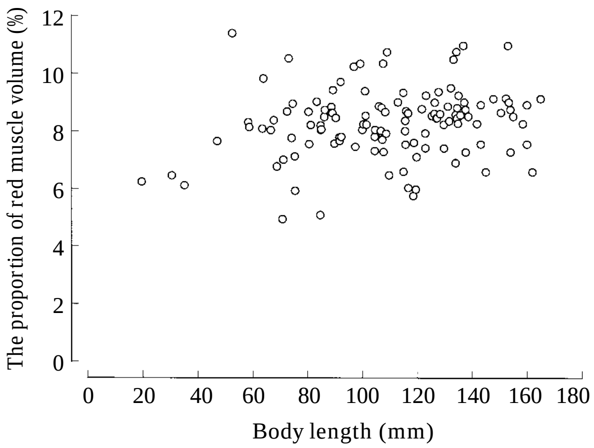

Fig. 3. Proportional change of the red muscle volume against the total lateral muscle volume of artificially hatched the Pacific bluefin tuna.

number were approximately accelerated at sizes larger than 85 mm BL.

Discussion

The existence of an inflection in the allometric growth of muscle volume was reported for other fishes. Inflections occurred at around the onset of feeding for red sea bream *Pagrus major*[10,11], and at the transitional stage from larva to juvenile for Pacific herring *Clupea pallasi*[12]. In the Pacific bluefin tuna, the body size of 80 mm BL, at which an inflection occurred in the allometric volume growth, corresponded to the ending of the juvenile stage[*5].

Fish generally have an area ratio of red muscle to total lateral muscle smaller than 10%[13]. The higher ratio of red muscle in the trunk musculature corresponds to the improved swimming ability[14]. Thus, the high ratio indicates the active life of the Pacific bluefin tuna from an early developmental stage.

For the Pacific bluefin tuna, the development of red fibers was achieved both by the increase of fiber number and the enlarging of fiber cross-sectional area. The cross-sectional area of red fibers enlarged with growth and small fibers disappeared gradually (Fig. 5-A), and the total

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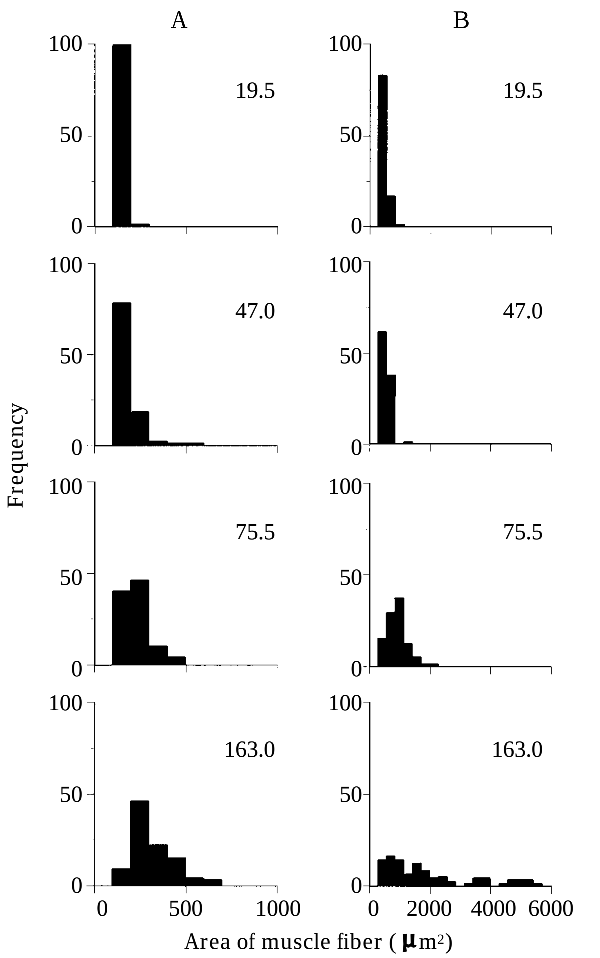

Fig. 5. Frequency distribution of cross-sectional area of muscle fibers at the maximum body height of artificially hatched the Pacific bluefin tuna. Figures in each graph indicate body lengths in mm. A: red fibers. B: white fibers.

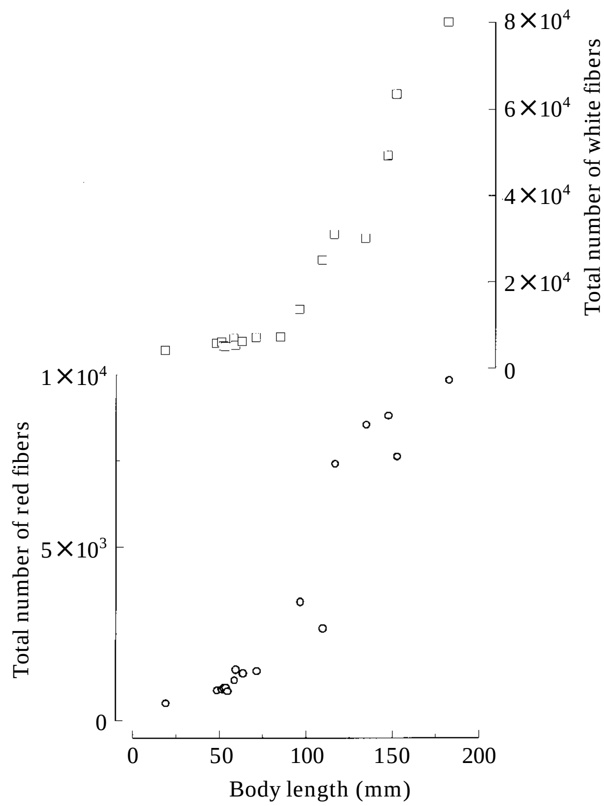

Fig. 6. The total number of red and white fibers of artificially hatched the Pacific bluefin tuna. Open circles, red muscle fibers; Open squares, white muscle fibers.

number of red fibers increased with growth (Fig. 6). White fibers developed, increasing in number and in cross-sectional area, however small fibers did not disappear (Fig. 5-B). The increase of total number of both fibers was accelerated after 85 mm BL (Fig. 6).

The mechanism of an increase in fiber number of the muscle has not yet been made clear. In northern anchovy, *Engraulis mordax*, fiber branching brings an increase in the number of red fibers[15]. In white fibers, the increase in number was accomplished by addition from satellite cells[16] or fiber splitting[17]. For the Pacific bluefin tuna, fiber branching would lead to the increase in the number of red fibers because the total number of red fibers increased and small fibers gradually disappeared with development. In white fibers, the small fibers (200-300 μm^2 in size) did not disappear and various sized fibers were distributed. So, fiber splitting or addition from satellite cell would lead to the increase in the number of white fibers.

There were inflection points at around 80-85 mm BL in the increase both of the lateral muscle volume and of the number of white fibers. This body size corresponded to the transitional stage from juvenile to young adult. After this stage, the cruising speed of the Pacific bluefin tuna in the rearing tank increased*6 and the distance to the nearest neighbor (ODNN)[18] decreased*6. In, addition, the caudal fin shape, which indicates the swimming performance[19] was transformed to the form more adaptive to high cruising speed[1]. Therefore, the swimming performance of the Pacific bluefin tuna was abruptly improved at around this stage. Such correspondence between behavioral and physiological changes and lateral muscle development is observed in other fish[11,12].

*6 N. Hattori, unpublished data.

The difficulty with rearing the Pacific bluefin tuna seedstock is designing a procedure adapted to the behavioral, morphological, and physiological changes that accompany growth. Further investigation of these changes is needed to solve the problems of seedstock production.

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稚魚期から若魚期におけるクロマグロ体側筋の発達

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人工孵化クロマグロの筋体積，筋繊維断面積および筋繊維数を稚魚期から若魚期，体長19.5から163.0 mm の範囲で調べた。血合筋，普通筋ともに体積は体長の成長にともない指数関数的に増加した。血合筋および普通筋の筋体積の相対成長式には，ともに体長80.0 mm で 1 つの屈曲点が存在し，体長80.0 mm 以降で筋体積は有意に高い割合で増加した。全筋体積に対する血合筋体積の割合は 6 から 9%へ緩やかに，また体高最大部での赤色および白色筋繊維断面積とそれらの筋繊維数は体長の成長に伴い増加した。赤色筋繊維では100 μm^2以下の細い筋繊維は体長の成長に伴い徐々に消滅したが，白色筋繊維ではいずれの魚体サイズにおいても200～300 μm^2の筋繊維が残存した。赤色筋繊維の総数は成長に伴い増加し，特に体長85 mm 以降からは高い割合での増加傾向を示した。体長80～85 mm は筋肉の発達に大きな変化が見られるサイズであるとともに，稚魚から若魚への移行期に相当した。