Bull. Nansei Reg. Fish. Res. Lab. (10) 1～16 (1977)

Some Morphological Observations on Larvae and Juveniles of the Kurodai, *Mylio macrocephalus* (Sparidae : TELEOSTEI) Reared in the Laboratory

Osamu FUKUHARA

In recent years, *Mylio macrocephalus* (Basilewsky) is receiving increasing consideration among aquaculturists as a suitable species for profitable cultivation. Although considerable efforts have been undertaken to improve culture techniques for various marine fish species, including kurodai, the knowledge of the development of morphological characters and their ecological significance are still not adequately known for many species. Thus, environmental requirements for optimum development, suitable methods for and optimum size at liberation of fry into natural habitats as well as the biological importance of pre-liberation protection of seedlings in enclosures or embayments are not yet fully understood. They may provide an indispensable basis for a sound development of a farming-fisheries industry for various marine fishes. Experimental studies are needed in order to obtain these informations for both the laboratory and the natural habitat.

Morphological characteristics during early development under almost natural conditions are not known in detail for the kurodai. Embryonic stages and pre-larvae have been described briefly by SENO (1912)¹⁾ and KISHINOUYE (1915)²⁾. Young larvae and juveniles from plankton catches are considered by UCHIDA *et al.* (1958)³⁾ and MITO (1963)⁴⁾.

This paper describes the development of the functional morphology of kurodai, especially that of fins, scales and transverse stripes, in relation to behaviour of the larvae and juveniles. A detailed knowledge of morphological characteristics developed during successive stages may be considered as important for an effective utilization and observation of hatchery reared seedlings planted in natural habitats.

Material and Methods

Fertilized eggs were collected on 21 May 1976 at Hiroshima Prefectural Fisheries Experimental Station on Kurahashi Island. These eggs had been spawned by parental fish kept in a floating net cage on the 20 th of May at ambient temperature of about 18.0 °C. Eggs were transferred to the Nansei Regional Fisheries Research Laboratory, Ohno-cho. Transportation took about 2 hours.

Received : April 12, 1977. Contribution No. 65 from the Nansei Regional Fisheries Research Laboratory.

Eggs were stocked into incubation tanks immediately upon arrival. Running sea water (ambient temperature; filtered through a sandfilter) was provided throughout the incubation time. Immediately after hatching, yolk sac larvae were stocked into two translucent circular tanks of 500 *l* capacity each (diameter: 105 cm; water depth: 70 cm; stocking density: 12 larvae per liter). The rearing tanks were placed in the laboratory near the window facing south. Plastic blinds were used to eliminate direct sunlight during day time. No artificial illumination was provided. Sediments and dead larvae which had accumulated at the bottom were removed daily by a siphon or a pipet. Each rearing tank was gently aerated by an airstone. Tank water was partly exchanged (about 1/5 of the total volume) every three or four days during the first 30 days of the experiment. Running water was employed throughout the remaining experimental period of 70 days. The upward trend in ambient water temperature during late spring was also observed in the rearing tanks, ranging from 18.8 to 26.3 °C during the experimental period.

From day 3 to day 25 after hatching, the larvae were fed on rotifer, *Brachionus plicatilis*. From day 15 to day 35 the copepod *Tigriopus japonicus* was employed as an additional food organism. Food organisms had been cultured in separate tanks. They were added to the rearing tanks in regular intervals in order to maintain an average density of 5 to 10 individuals per liter. Minced meat of mussels and small fishes was offered as food to post-larvae and juveniles after day 30.

In intervals of 5 to 10 days, 15 to 20 specimens were removed from rearing tank in order to measure growth and to observe the development of meristic characters, such as fins, scales and transverse stripes of melanophores. All observations on external features of the fish were carried out on the left side of the specimens sampled. Detailed methods of observations were similar to those described previously in a paper on the madai, *Chrysophrys major* (FUKUHARA 1976 a, b)[5) 6)]

Specimens of 20 mm or less in standard length were preserved in 5 % buffered formalin solution, and those larger than 20 mm in standard length were kept in 10 % buffered formalin solution. The transverse bands of pigment were observed without staining fish, whereas observations on segmentation, branching of fins and scale formation were made after staining with Alizarin. Measurements were done under an profile projector (Nikon, model V-16) in connection with an electric digital micrometer (Nikon, model RQ-321S). For direct observations on larvae and juvenile specimens a binocular microscope had been employed.

Results

Appearance of eggs

Egg diameter varied from 0.96 to 1.12 mm at ambient salinity of about 32 ‰. At that salinity the perivitelline space appeared to be very narrow. The eggs observed during this experiment contained only one oil globule. There is only little information available on the relationship between incubation temperature and incubation time. In our experiments hatching occurred in 3 to 2 days after fertilization at temperatures of 18.0 to 20.6 °C, respectively.

General remarks on larval morphology

The average size of newly hatched larvae was 2.47 mm in standard length. The mouth opened when the larvae reached an average standard length of about 3.0 mm, 4 to 5 days after hatching at ambient temperatures of 18.0 to 20.6 °C. Yolk absorption was completed within 6 days after hatching, but active feeding started one to two days earlier.

Compared to the larval development of other breams, no remarkable differences were observed during the two weeks following hatching. The form and extend of the primordial fin-fold changed strikingly at the transitional phase between larva and juvenile. This stage occurred between day 25 and 28 after hatching.

Observation on the formation of fins

Fin rays were enumerated for each of the fins after staining the fish with Alizarin. Development of segmentation and branching of fin rays were plotted against standard length of fish (Fig. 1). Segmentation of the unpaired fins was completed when the larvae reached a standard length of about 9 mm. The caudal fin showed a larger number of rays than the other unpaired fins, yet its segmentation as well as the branching of rays occurred considerably earlier. The size difference in standard length of fish between the first appearance of segmented rays and the completion of segmentation was 3.0 mm in the case of the unpaired fins. This size difference of larvae was equivalent to a five day rearing period in this experiment.

Branching of rays in unpaired fins began shortly after the larvae had attained a standard length of about 10 mm when segmentation was almost completed. Anal and caudal fin branching was completed at about 20 mm standard length. No further increase in the number of branching of the dorsal fin was observed, when the larvae attained a size of about 30 mm. In general, branching of rays in unpaired fins required a considerable longer time than segmentation of rays. Similar trends had been observed in the development of soft rays of the madai (*Chrysophrys major*) reared under laboratory conditions (FUKUHARA 1976 a)5).

In general, 9 soft rays observed in the specimens smaller than 18.0 mm in

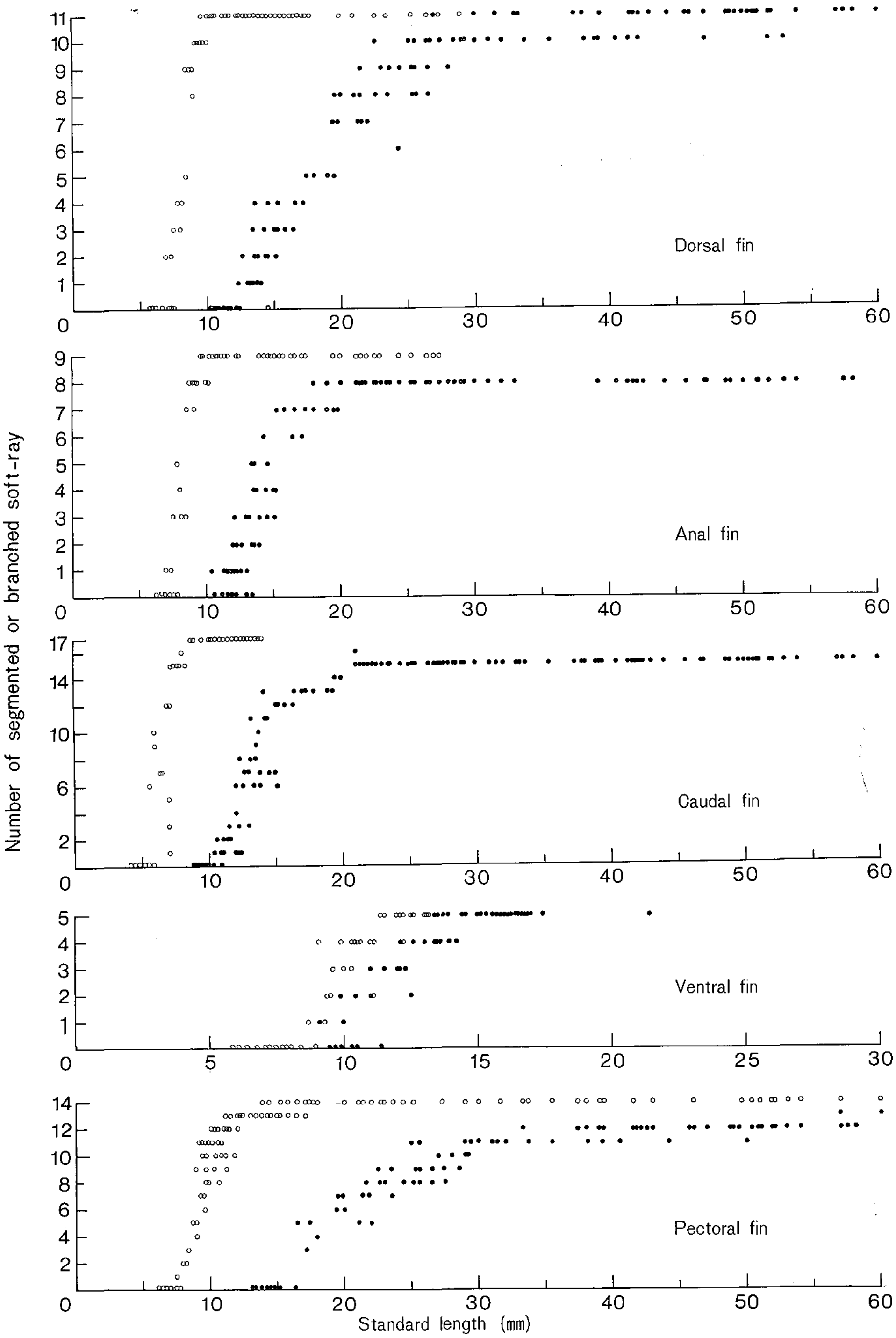


Fig. 1. Segmentation (open circles) and branching (closed circles) of fin ray in laboratory-reared specimen of kurodai, *Mylio macrocephalus*.

standard length. In specimens of 18.0 to 27.5 mm in standard length either 9 or 8 soft rays were observed, whereas in specimens larger than 28.0 mm standard length only 8 soft rays had been counted in the anal fin.

The segmentation and branching of the soft rays in the pectoral fin levelled off to a constant number when the fish reached about 30 mm in standard length. Development of ventral fins was peculiar in that the branching occurred almost concurrently with segmentation within a narrow range of fish size (9-12 mm standard length), whereas in the other fins branching occurred distinctly later than segmentation (Fig. 1). Thus, the development of the ventral fin was completed considerably earlier than that of the others.

Developmental changes in the primordial fin-fold were similar to those of the madai, the entire fin-fold was replaced by the respective unpaired fins at the transitional phase from post-larva to juvenile when the larva attained 8-10 mm in standard length. At the same time, the hind margin of the caudal fin changed rapidly from a rounded shape to a truncated form. This feature can be used for rough estimates on developmental stages (Table 1). The pectoral fins also changed their shape from a round to a triangular form. The initially

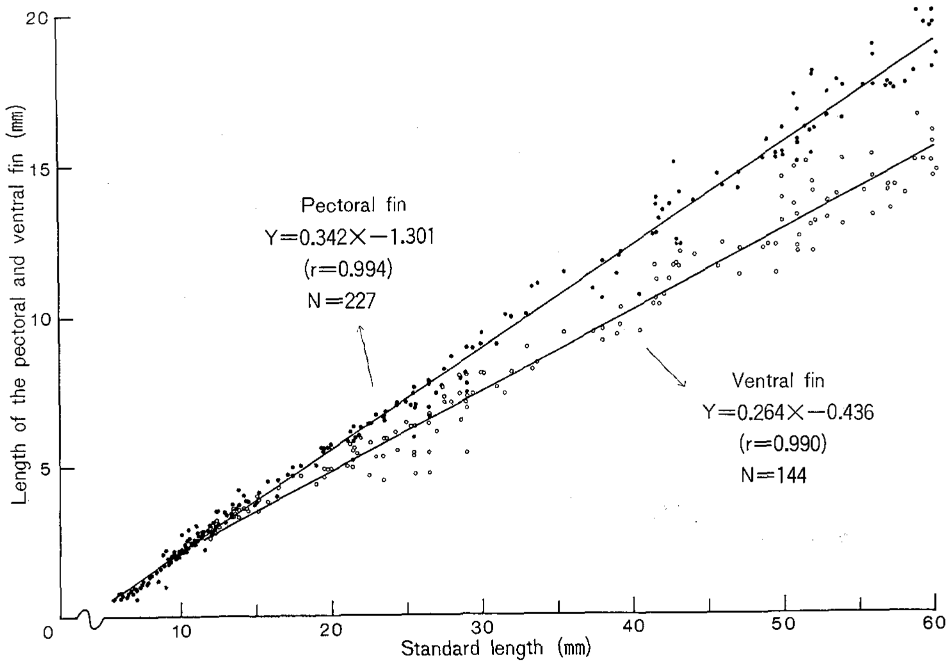


Fig. 2. *Mylio macrocephalus.* Length of pectoral (closed circles) and ventral fin (open circles) in relation to standard length. N= Number of observations; r=coefficient of correlation. Method employed to measure fin length see Fukuhara (1976 a).

triangular ventral fins appeared to be falcated.

Standard length of fish appeared to be positively correlated with pectoral and ventral fin length ($r=0.994$ and 0.990, respectively). Growth rates of both fins are similar up to a fish size of 15 mm standard length. In larger larvae, however, the growth rate of the ventral fin increases over that of the pectoral fin (Fig. 2).

Observations on scale formation

Squamation in kurodai may be divided into 7 stages (A-G). This is shown semidiagrammatically in Figure 3. Each stage was assigned by the two basic criteria: the number of rows of scales and the extend of body coverage of squamation. The characters of each stage was defined as follows:

Stage A, A few scales appear in the posterior portion of the trunk along the lateral line. No teeth are visible on the exposed portion of the scale.

Stage B, There are 4 scale rows and 3 teeth on each scale. Scale formation extends to each direction on the trunk, anteriorly reaching the posterior end of operculum, and posteriorly, the central portion of the peduncle.

Stage C, There are 6 to 7 scale rows. the squamation covers anteriorly a part of the operculum, and posteriorly the base of the caudal fin. The squamated area tapers toward the caudal.

Stage D, There are 9 to 10 scale rows. Squamated area is horizontally almost identical with stage C, but extends more dorso-ventrally. About one third of the lateral area of body on each side is covered with scales.

Stage E, There are 11 to 12 scale rows. Squamation reaches posteriorly to the hind end of the trunk. Two-thirds of the lateral area are squamated.

Stage F, Squamation is almost completed, leaving only the base of the dorsal and anal fin and abdominal portion uncovered.

Stage G, Fully squamated.

Figure 4 shows the distribution of these developmental stages in relation to standard length. The largest larva without scales was about 11.0 mm in standard length, whereas the first appearance of squamation was observed in specimens as small as 10.2 mm. In all specimens larger than 16 mm in standard length the squamation was always completed.

Measurements on scale size were done on 3 to 4 scales taken near the pectoral fin on the left side of the specimens. The mean length of scales are plotted against standard length in Figure 5. A straight line relationship is

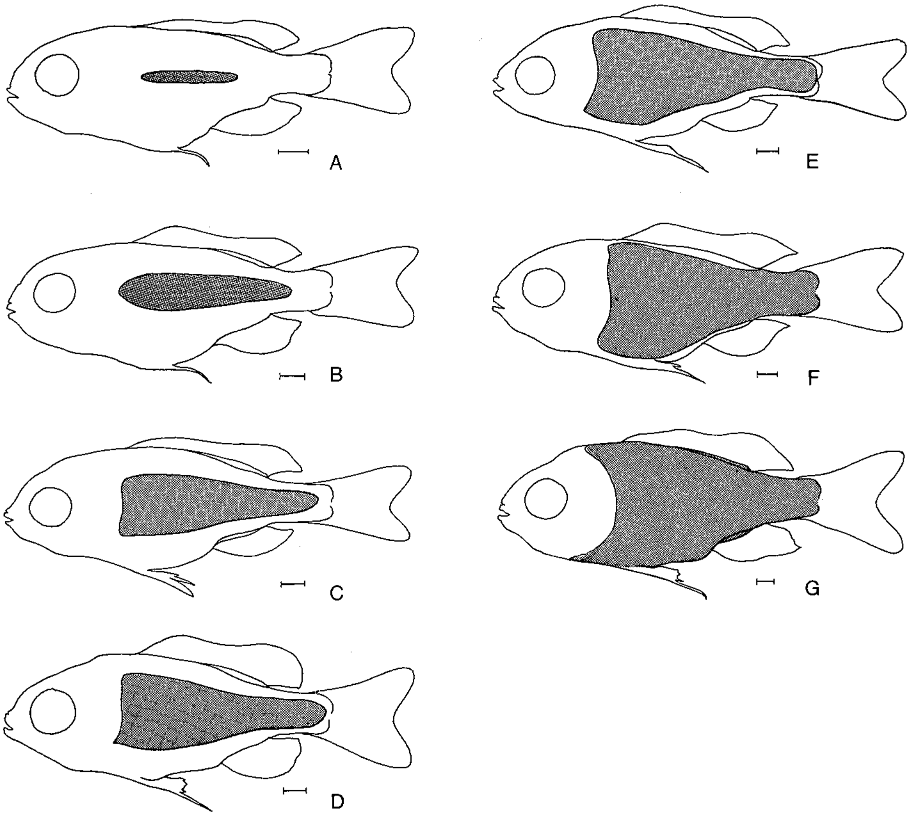


Fig. 3. Semidiagrammatic drawings of young kurodai, *Mylio macrocephalus*, at different stages of squamation, assigned by number of rows and extent of coverage of squamation. Scales denote 1 mm.

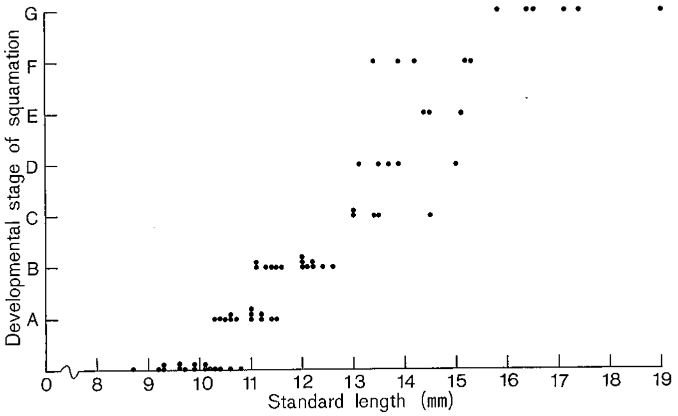


Fig. 4. *Mylio macrocephalus*. Relation between developmental stage of squamation and standard length; illustrations for A-G see Fig. 3.

obvious and may be expressed by the equation R=0.0296 SL−0.280 (r=0.990), where R and SL represent the scale length and standard length, respectively. The concentric lines drawn in the insert of Figure 5 indicate the developmental changes of the scale to final shape of a typical ctenoid scale. The scale assumed characteristics of a ctenoid when it reaches 0.5 mm in length. This scale length corresponds to a standard length of about 25 mm in juvenile fish. The number and size of teeth, grooves and ridges increased as the juvenile grew.

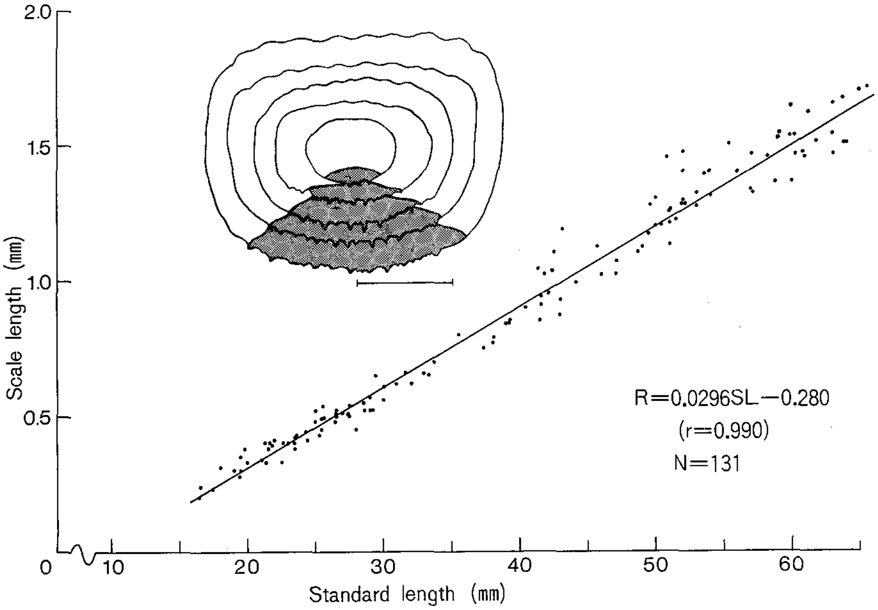


Fig. 5. *Mylio macrocephalus*. Relatioship between scale length (R) and standard length (SL). Insert: The concentric line drawings indicate developmental changes to the final rim of a typical ctenoid scale. The exposed portions are speckled. Scale denote 0.5 mm.

Observations on black stripe formation

A maximum of 7 to 8 transverse stripes are formed on each side of the body. They are most clearly to be distinguished in the late juvenile stage. Their sequence from first appearance to full development is shown semidiagrammatically in Figure 6 and photographically in Plate 1. The first stripe appeared dorsally at the anterior end of the trunk, and the formation proceeded posteriorly. Each stage shown in Figure 6 may be defined as follows:

Stage A, Melanophores are concentrated in the anterior base of the dorsal fin forming a transverse stripe, which tapers ventrally.

Stage B, The second stripe appears between the 4 th and 7 th spine of the dorsal fin, and extends dorso-ventrally further than the first.

Stage C, The third stripe appears at the anterior position of the caudal region between the 1st and 3 rd soft ray of the dorsal fin. This stripe covers the whole depth of the trunk extending posteriorly along the base of the anal fin.

Stage D, The fourth stripe appears between the first and second, and is narrower than the other stripes already present.

Stage E, The fifth stripe appears between the 8 th and 9 th spine of the dorsal fin. This is the last one formed on the trunk, and is as wide as the fourth.

Stage F, The sixth stripe appears at posterior end of the caudal peduncle. This is the second stripe in the caudal region and is continuous anteriorly with the third stripe along the ventral margin.

Stage G, The seventh (the third one in the caudal region) appears between

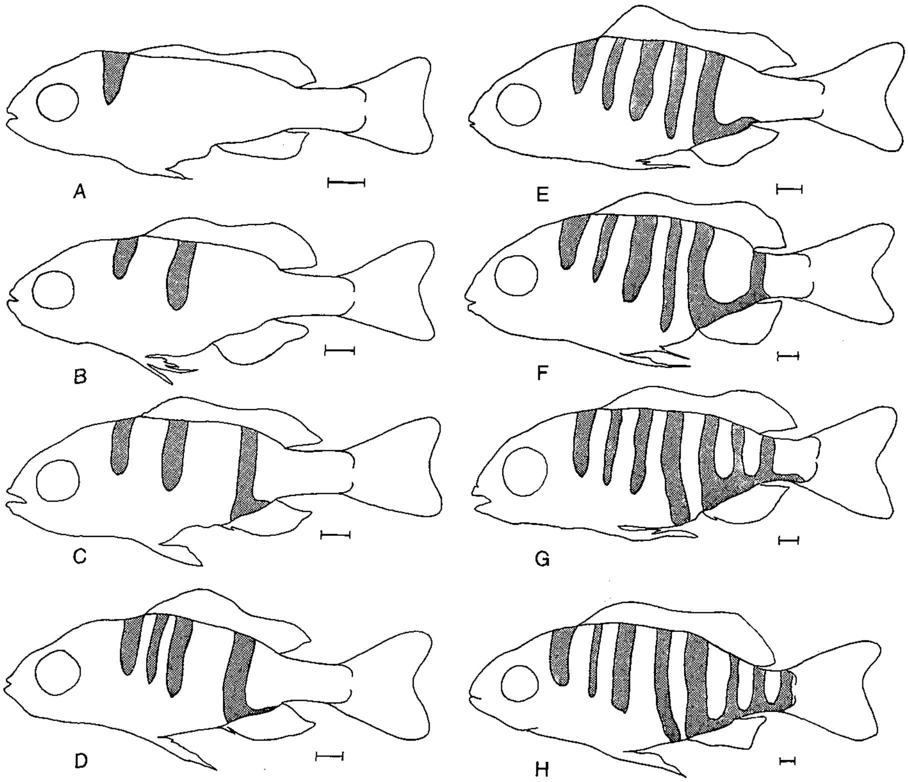


Fig. 6. *Mylio macrocephalus.* Semidiagrammatic drawings showing the developmental sequence of black stripes in kurodai reared in the laboratory. Each stage is defined according to the number of transverse stripes. Scales denote 1 mm.

the third one and the sixth stripe.

Stage H, The eight, stripe appears at the posterior end of the caudal region. All the stripes in the caudal region are connected along the ventral margin.

Figure 7 depicts the relation between stripe formation and standard length. The largest specimen without any stripe development was 11.0 mm and the smallest one on which first stripes occurred was about 9.0 mm in standard length. The development of stripes was completed in all the specimens examined when the larvae attained 25 mm in standard length.

In some specimens the number of stripes were not obvious owing to the development of melanophores between stripes. There were a few variations along the edges of the black stripes, In several case, the second stripe branched toward either the dorsal and ventral end, and in other case the fourth stripe branched toward its dorsal end.

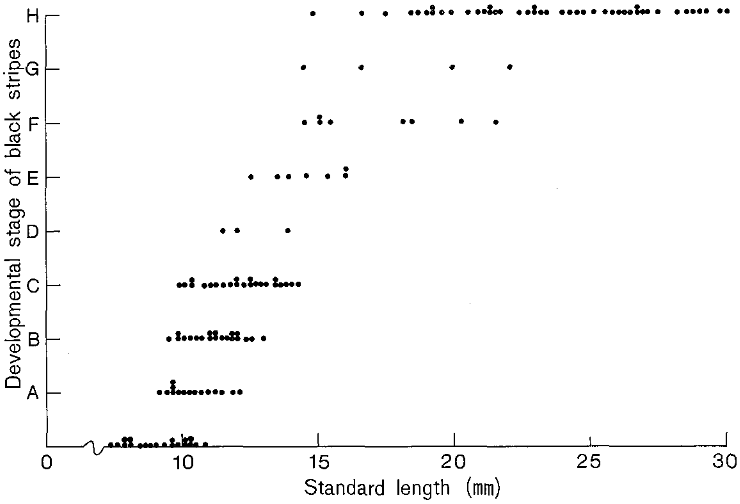


Fig. 7. Development of black stripes plotted against standard length. A – H identical to description of Fig. 6.

Growth and behaviour of reared larvae

Growth in standard length of reared larvae is shown in Figure 8. Number of measured larvae was less than 15 during the first 20 days of the experiment, therefore the standard error was not inserted into the figure.

The size of newly hatched larvae ranged from 2.20 to 2.35 mm in standard length. Due to their buoyancy they are concentrated near the surface and are at the mercy of water movement due to aeration. Active swimming was observed towards the end of yolk absorption, when the larvae attained about 4.0 mm in standard length. At this stage the larvae assumed flexed postures of the body axis in S-shaped form before striking on any food organism, as has been observed in many other fish species (ROSENTHAL 1968,[7] BLAXTER and STAINES 1971[8]).

Metamorphosis occurred when the larvae reached a length of about 9.0 to 10.0 mm. Average growth increment during the rearing period for 100 days was 0.74 mm per day. The growth rate differs between the larva and the juvenile, attaining values of 0.25 mm per day in the former and 0.95 mm per day in the latter. Thus the growth rate during the juvenile stage, especially after attaining 30 mm in standard length was considerably larger than that during the larval stage.

Another striking changes in the behaviour was observed among the 4-week old larvae, in which about one-third of the total survivors stayed at the bottom of the rearing tank and a few other specimens swam near the bottom. The remaining larvae swam in the middle layer of the tank. Metamorphosed

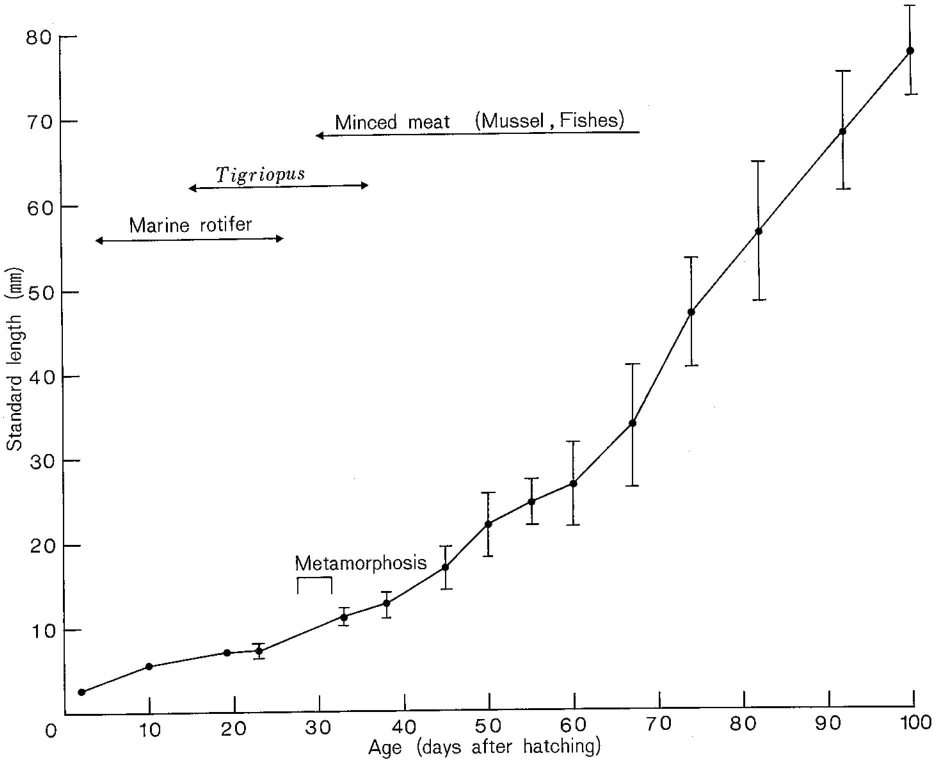


Fig. 8. Growth in length of larvae of kurodai, *Mylio macrocephalus*, reared under laboratory conditions. Vertical bars indicate standard errors. The feeding scheme is also shown. Points are the means of about 20 measurements.

juveniles reacted very sensitive to various stimuli such as noise and light. Aggressive and territorial behaviour was often observed among the survivors at this stage as has been described in madai (YAMAGISHI 1969[9])). No further striking changes in behaviour were observed during the rearing to fish up to 80 and 85 mm in standard length (100 days after hatching).

Discussion

The sequence of development in external characters of kurodai observed in this experiment summarized in Table 1. The horizontal bars indicate the range in length between the largest size of the larvae with undeveloped features and the smallest size of the larvae with fully developed features.

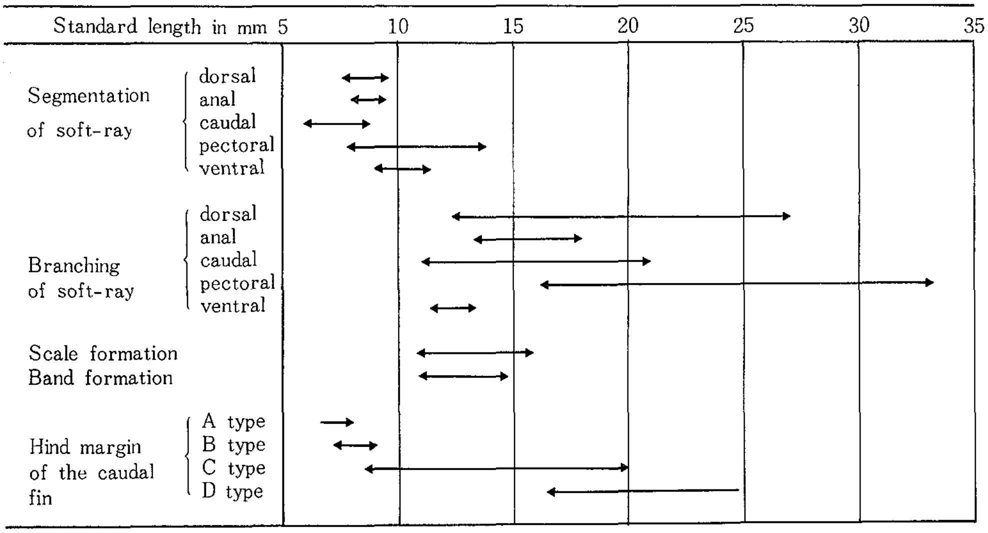
Segmentation in the unpaired fins tended to develop earlier than those in the paired fins. In the caudal fin the rays are segmented earlier than in other unpaired fins. The difference in timing of fin development seems to reflect their role in changing locomotion patterns and development of swimming behaviour. Unpaired fins are the primary organs of balancing and proplusion and the caudal fin plays a leading role in locomotion. As outlined by GOSLINE (1973)[10], the efficiency of the fins is provided by the flexibility and elasticity of the segmented rays. Therefore, it is reasonable to assume that the mode of behaviour of the early juvenile in which fin rays are segmented is fundamentally different from that of the larvae in which movement is effected by undulations of the tail only.

Branching of the rays developed later and required longer periods than segmentation, with the exception of the ventral fin in which both processes occur almost concurrently within less duration than in the other fins. Short period is required in ray development for the ventral fin and this seems to be due simple to the small number of rays. No information is available about the functional role of branching of fin rays.

According to UCHIDA *et al.* (1958)[3], segmentation of the caudal fin in the wild specimens appeared at 9.8 mm in total length, which is quite a similar size of the specimens reared in this experiment. Unfortunately, no descriptions are available on the developmental sequence of other fins and no data are reported about the branching in the wild larvae.

The smallest larva in which squamation started was about 10 mm in standard length (Fig. 4) and the largest larva with undeveloped features was 11.0 mm in standard length. The smallest larva with fully developed scales measured 16.0 mm in standard length. All juveniles larger than 16.0 mm had attained full squamation. It is generally accepted that the functional role of the scale is to protect the body surface from various external stimuli (KAWAMOTO 1956,[11]

Table 1. Length ranges of which respective meristic characters occurred in kurodai, *Mylio macrocephalus* during experimental rearing. Arrows depict to the left the largest size at which undeveloped condition were observed. The right end of the each arrow indicates the smallest size at which the fully developed condition was observed.



MATSUBARA *et al.* 1965[12]). The juveniles with full squamation can reasonably be assumed to have a better ability to adapt to a wider range of environmental conditions than before. In the wild specimens two or three scale rows were observed at the length of 14.1 mm and the completion of squamation at 18.0 mm in total length. These size are very well comparable to those of the larvae reared in this experiment.

The black stripe began to develop at nearly the same time as in the scale, but was completed later than the latter. Specimens as small as about 9.0 mm in standard length had some of the stripes. The stripe formation seemed to be completed in all the larvae larger than about 25 mm, although large variations between individuals had been observed. The main role of the stripes are generally believed to be in concealment and camouflage (LAGLER *et al.* 1962[13]), MATSUBARA *et al.* 1965[12]), therefore, the stripe formation seems largely to be related to habitat preferences of the larvae, as is the case in scale formation.

The marginal shape of the caudal fin showed a characteristic change until it assumed the final furcated shape. The development may be divided into 3 stages, though the duration of each stage varied greatly from one larvae to another. Major changes, however, were concentrated in most of the specimens at or near the stage of about 8.0 mm in standard length. Since the shape of hind margin of the caudal fin was a useful index to estimate the stage of fin development as reported in the previous paper (FUKUHARA 1976a[5]), it may also be useful to predict the behaviour changes. Thus, the larvae with rounded tail swam undulated fashion in the upper layer of the rearing water. As the hind margin approached the final form, the juvenile swam in the lower layer with increased swimming speed and developed diverse behaviour patterns such as aggression and cannibalism. Comparable changes in swimming behaviour from undulating movements with high amplitude of the tail beat to those with low amplitude but increased net progress were described by ROSENTHAL (1968)[7] for herring larvae during the transitional stage between 18 and 25 mm in total length. At this stage fins were well developed. They played an important role in prey capturing manoeuvres. Therefore, it will be justified to conclude that the morphological changes are closely linked with the development of swimming and feeding behaviour.

Morphologically, development of the reared specimen was almost comparable to those of the wild fish described by UCHIDA *et. al.*, however, comparison of behaviour between the artificially reared specimen and the wild one needs further studies.

Acknowledgment

The author wishes to express his thanks to Dr. Sakaguchi and Dr. Kurata of the Nansei Regional Fisheries Research laboratory and Dr. Harald Rosenthal of the Biologische Anstalt Helgoland for their valuable advices and kindness in critical reading the manuscript. I thanks Mr. Fushimi of Hiroshima prefectural fisheries experimental station and Mr. Umezawa of the laboratory for their help in obtaining the experimental materials and statistical analysis of the collected data.

Summary

1. Morphometric development of fins, scales and black stripes during postembryonic stages are described and figured in detail.

2. Data are obtained from specimens reared in the laboratory (continuous water flow, ambient temperatures between 18.8 to 26.3 °C) from eggs to juvenile fish.

3. The sequence of development of each character is shown in Table 1.

4. Striking changes were observed within the size range from about 9 mm to 11 mm in standard length.

5. Development of unpaired fins was completed when the fish reached a

standard length of about 30 mm.

6. Scale development started at 10.2 mm standard length and full squamation of juveniles was attained at a size of 16.0 mm.

7. Occurrence and number of black stripes are described in relation to larval growth. A total of 8 stripes can be counted in juveniles of 25 mm standard length.

8. Branching of rays of unpaired fins as well as the formation of the final shape of the caudal fin margin rapidly assumed the characters of adult fish.

9. It is suggested from observations made during this rearing experiment, that the development of behaviour in larvae and juveniles is closely linked to the development of morphological characters such as fins, scales and transeverse bands.

References

1) SENO, H., 1912: Kurodai no hatsusei. Zoological Magazine 24 (282), 195-197.

2) KISHINOUYE, K., 1915: Tai rui no hatsusei oyobi seicho. Suisan Gakai Hou. 1 (3), 185-199.

3) UCHIDA, K., S. IMAI, S. MITO, S. FUJITA, M. UENO, Y. SHOJIMA, T. SENTA, M. TAHUKU and Y. DOTU, 1958: Studies on the eggs, larvae and juveniles of Japanese fish. Kyushu Univ. Fac. Agric. Fish. Dep. Second Lab. Fish. Biol. Ser. 1., 89 pp+86 pls.

4) MITO, S., 1963: Pelagic fish eggs from Japanese water-III. Percina. Japan J. Ichthyol. 11 (1-2), 39-64.

5) FUKUHAKA, O., 1976 a: Morphological studies of larvae of red sea bream-I. Formation of fins. Bull. Nansei Reg. Fish. Res. Lab. 9, 1-11.

6) FUKUHARA, O., 1976 b: Morphological studies of larvae of red sea bream-II. Early development of squamation. Bull. Nansei Reg. Fish. Res. Lab. 9, 12-18.

7) ROSENTHAL, H., 1968: Untersuchungen über das Beutefangverhalten bei Larven des Herings Clupea harengus. Marine Biology. 3 (3), 208-221. (In German with English summary)

8) BLAXTER, J. H. S. and M. STAINES, 1971: Food searching potential in marine fish larvae. 467-485. In D. J. Crisp (ed.). Fourth European marine biology smposium. Cambridge University Press.

9) YAMAGISHI, H. 1969: Postembryonal growth and its variability of the three marine fishes with special reference to the mechanism of growth variation in fishes. Researches on Population Ecology., 11 (1), 14-33.

10) GOSLINE, W. A., 1973: Functional morphology and classification of teleostean fishes. vii+208 pp., The University Press of Hawaii.

11) KAWAMOTO, N., 1956: Fish Physiology. First edition, iii+319 pp., Ishizaki shoten, Tokyo.

12) MATSUBARA, K., A. OCHIAI and T. IWAI, 1965: Ichthyology Part 1. 342 pp., Kouseisha Kouseikaku, Tokyo.

13) LAGLER, K. F., J. E. BARDACH and R. R. MILLER, 1962: Ichthyology. xiii+545 pp., Jhon

Wiley and Sons Inc. New York.

クロダイ稚仔魚の二，三の形態について

福 原 修

クロダイ幼期の各発育段階における形態的特性を知るため，室内で人工飼育した標本を用いて鰭，鱗，斑紋などの器官の発達経過を観察した。計測形質と器官発達の点からみると体長範囲9～11㎜の期間を境として形態的に大きな変化がみられ，次の発育段階に達することが明らかとなった。この時期には，観察した諸器官がたがいに関連しながら発達し，行動にも変化がみられることから器官の発達と行動様式が密接に関連していることがうかがわれた。

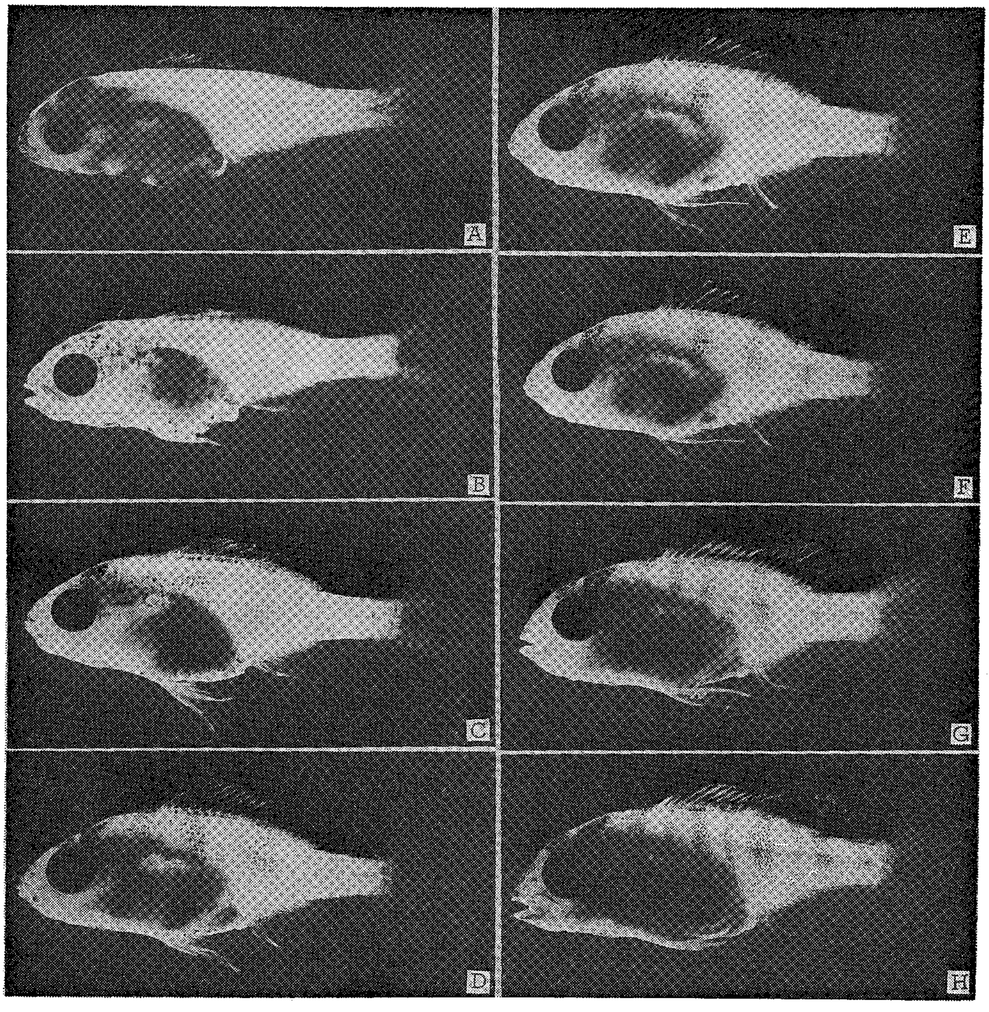


Plate 1. Photographs showing the black stripe formation of the reared kurodai, *Mylio macrocephalus*, under laboratory conditions.