Cone Photoreceptor Arrangement at Metamorphosis in the Marbled Sole

Pleuronectes yokohamae

Rena Shibata1,*, Yasuyuki Uto2,3 and Yoshifumi Miyama2

Abstract: Retinal structure in marbled sole was examined microscopically in their early developmental stage to estimate a linkage of changes of visual ability with the caudal fin loss. Photoreceptor cells in the pre-metamorphosis stage were comprised of single cones exclusively. During metamorphosis, adjacent single cones formed double cones, while a regular square mosaic pattern composed of four double cones surrounding one single cone appeared in post-metamorphosis stage. These morphological changes were assumed to improve the visual ability. As one of possible causes for caudal fin loss, biting other individuals is considered to increase by the help of the improved visual sense.

Key words: Pleuronectes yokohamae; Cone mosaic; Metamorphosis

The marbled sole Pleuronectes yokohamae, commonly found in the coastal waters around Japan, is a commercially high-valued species, and the seed production is the second highest next to Japanese flounder Paralichthys olivaceus in Japan (Seikai 1985). However, the seed production of marbled sole often encounters loss of the caudal fin due to biting (Sakamoto et al. 2006; Sugimoto et al. 2007), which impairs swimming performance and decreases the survival rate due to infections by bacteria (Sugimoto et al. 2007). Aggressive behavior in flatfish has been reported also winter flounder (Fairchild and Howell 2001) and Japanese flounder (Sakakura and Tsukamoto 2002). To improve seed production technology, prevention of fin loss is necessary, and yet the causes of increase of biting are unknown. Adjustment of the volume of feed, ration level and rearing density have been tried to prevent the loss of the caudal fin in this species since the late 1990s (Inoue et al. 1998; Sugimoto et al. 2007), but in vain. These results may imply a possible involvement of a sensory improvement to recognize conspecific individuals, since it has been reported that the visual abilities were improved during the early developmental stage in flatfish species (Kawamura and Ishida 1985; Evans and Fernald 1993; Kvenseth et al. 1996).

Migration of the eyes of flatfish larvae and their morphological change occurs during metamorphosis dramatically (Minami 1984a). That is, it was found that changes in the retinal structure occur during metamorphosis in sole Solea solea (Sandy and Blaxter 1980), Japanese flounder (Kawamura and Ishida 1985), winter flounder Pseudopleuronectes americanus (Evans and Fernald 1993) and Atlantic halibut Hippoglossus hippoglossus (Kvenseth et al. 1996), while no report for marbled sole. Synchronously, they commonly shift their habitats from the pelagic to the benthic where the light intensities are largely decreased (Minami 1984b). Food items of marbled sole change from planktons to benthic organisms (Minami 1981).

Completion of settlement of juvenile marbled sole when they show biting behavior under the artificial seed production occurs soon after the eye migration accomplished (Kondou and Sugino 1994), and we also observed the loss of the caudal fin (unpublished data). To examine the relevance of visual sense with behavioral changes such as biting would offer good suggestions to find effective measures to prevent the caudal fin loss. Thus, this study aimed to examine the retinal structure of marbled sole during the early developmental stage to know a possible linkage of changes of visual ability with the loss of the caudal fin due to biting in marbled sole.

Total number of 1,300,000 newly hatched larvae obtained through artificial fertilization were reared in twenty-four 20 kl tanks with flow-through system at the Seed Production Research Laboratory, Futttsu Sea Farming Section, Chiba Prefectural Fisheries Research Center. Larvae were kept at water temperature of 14°C.
using a thermostat. Thirteen larvae of 32–34 days post hatch [E-J stages, according to Minami (1981)] were randomly scooped from the rearing tank and used as experimental animals. Because the classification of individuals into the stages shown by Minami (1981) was not easy due to lack of clear differences of the external morphology during the transitional period at metamorphosis, the developmental stage was distinguished by their lens diameter using the method of Hoke et al. (2006) and thus the individuals supposed to be classified into G-I stages were picked up. Larvae and juveniles were fixed in Bouin’s fluid, dehydrated through an ethanol series, embedded in paraffin, and then transverse sections with 2–3 μm thickness were made for the hematoxylin-eosin staining, which were microscopically observed thereafter. Thus the histological examination of retinal structures were carried out before and after metamorphosis of which the developmental stage was synchronized with the settlement period as short as only few days (Minami 1984b).

All photoreceptor cells observed in the retina of pre-metamorphosis stage were comprised of single cones (Fig. 1A1, 1A2). There are two developmental stages of retina in the transitional period at metamorphosis, depending on the lens diameters: T-I (≤150 μm) and T-II (>150 μm), respectively. Total length of T-I did not differ from that of T-II. The T-I larvae had double cones in the retina peripherally, while single cones centrally (Fig. 1B). In T-II larvae, double cones increase due to fusion of single cones throughout the whole retina (Fig. 1C). Thereafter, the cone cells were aligned into a square mosaic having a pattern of four double cones surrounding one single cone in the post-metamorphosis stage (Fig. 1D, 1E).

The microscopical observations clearly indicated that the retinal structure of marbled sole largely changed throughout metamorphosis. Mosaic formation of visual cell has been reported in other flatfish species as well. Sandy and Blaxter (1980) confirmed the fusion of adjacent single cones into double cones using thymidine labeled with [3H] in sole. Hoke et al. (2006) demonstrated cone mosaic transformation near the peripheral margins of the retina in winter flounder using the same [3H] labeled thymidine technique.

Such structural changes of visual cells are considered to be accompanied with functional changes of the visual sense along with behavioral changes. Evans and Fernald (1993) reported the close relationships of visual function with habitat preference and behavioural performances in the winter flounder which shows morphological changes in the retina during the metamorphosis. The larvae prior to metamorphosis are photopositive, intermittently swimming toward the surface and then passively sinking (Sullivan 1915). After metamorphosis, they become photonegative while still diurnal feeders (Olla et al. 1969). Kawamura and Ishida (1985) also confirmed in Japanese flounder morphological development of the retina and increased the visual sensitivity through metamorphosis, where the relevance to change in visual behaviour and habitat were shown. These linkages with behavioural characteristics would be ascribed to the mosaic arrangement of single and double cones predominant in acute vision (Engström 1963; Kawamura and Tamura 1973; Kawamura et al. 1981).

Pre-metamorphosis marbled sole larvae exclusively feed on diatoms and bivalvia veligers (Minami 1981), that can be easily captured due to their passive movement in the water column (Yamamoto et al. 2005), while

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**Fig. 1.** Photomicrographs showing the chronological change of the retina of marbled sole in early stage. (A1) Transverse section of retina of a pre-metamorphosis larva. Arrows indicate the single cone-like photoreceptor type cells. sc, single cone. (A2) Tangential section of the retina shows only one type of visual cells, i.e. single cone-like photoreceptor type cells. (B) Tangential section of T-I stage larval retina. Retinal transformation has begun in the periphery of the retina and these cone-like cells coupled and formed double cones, but remained as single cones in the center. dc, double cone. (C) Tangential section of T-II stage larval retina. Number of double cones which fused as single cones have increased. Although some cells look being fused into triple cones, it is not easy to clearly prove the existence due to low resolution of the picture. (D) Tangential section of the post-metamorphosis juvenile retina. Regular square mosaic pattern can be seen. (E) The figure shows the expansion of the post-metamorphosis juvenile retina. Mosaic pattern of cones with four double cones surrounding one single cone. N, nasal; T, temporal. Scale, 10 μm.
those of post-metamorphosis feed on polychaetes and copepods (Minami 1985), which are not passive swimmers. These shifts of food organisms are assumed to show the change of feeding characteristics from omnivorous to carnivorous, and may be ascribed to the improvement of visual sense as well as development of swimming ability after metamorphosis. The most regular mosaics have mainly been found in those animals feeding on fast-moving prey (Lyall 1957). The cone mosaic formation would enable the fish to increase visual perception in low light (Engström 1963) owing to the heightened visual acuity (Wagner 1978). Parts or all of these functions, as well as the increasing absolute size of the eye itself, would increase the visual ability (Kvenseth et al. 1996). In marbled sole larvae, the retinal cone mosaic was completed after metamorphosis. This fact infers that the post-metamorphosis stage enable fish to possess a better visibility including conspecific recognition in comparison to the pre-metamorphosis stage. Thus, the present study showed a possible linkage of the morphological changes in visual cells with the loss of the caudal fin in marbled sole.

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References


