

Dual appearance of xanthophores, and ontogenetic changes in other pigment cells during early development of Japanese flounder *Paralichthys olivaceus*

Mitsuo Nakamura · Tadahisa Seikai · Masato Aritaki ·
Reiji Masuda · Masaru Tanaka · Masatomo Tagawa

Received: 11 October 2009 / Accepted: 15 December 2009 / Published online: 4 February 2010
© The Japanese Society of Fisheries Science 2010

Abstract Flatfishes display a left–right asymmetry that is unique in the animal kingdom. In order to clarify the mechanisms of the asymmetrical development of pigment cells, changes in pigment cell densities were examined in Japanese flounder *Paralichthys olivaceus*. During development from symmetrical larvae to asymmetrical juveniles, pigment cell densities were monitored on the skin on

both the left side (ocular side in juvenile; eventually has two eyes) and the right side (blind side in juvenile; eventually has no eyes). A symmetrical and constant decrease was observed in leucophores and larval type melanophores. A mostly symmetrical (slightly delayed on the blind side) and constant increase in iridophores from metamorphosis was observed. Adult-type melanophores appeared and then increased only after metamorphosis on the ocular side. However, the pattern of xanthophores was complicated: they first existed symmetrically and decreased symmetrically until metamorphosis, and they later increased only on the ocular side. The dual appearance of the xanthophores, as well as the differences between their depths and sizes on the ocular and blind sides, may suggest the presence of two types of xanthophores—just as melanophores are well known to exhibit two types. The ontogenetic study of pigment cells described here is likely to help to elucidate the process of abnormal pigmentation in flatfishes.

M. Nakamura · M. Tanaka · M. Tagawa (✉)
Division of Applied BioSciences, Graduate School of
Agriculture, Kyoto University, Kitashirakawa, Sakyo, Kyoto
606-8502, Japan
e-mail: tagawa@kais.kyoto-u.ac.jp

T. Seikai
Faculty of Marine Biosciences, Fukui Prefectural University,
Obama, Fukui 917-0003, Japan

M. Aritaki
Miyako Station, National Center for Stock Enhancement,
Fisheries Research Agency, Miyako, Iwate 027-0097, Japan

Present Address:
M. Aritaki
Coastal Fisheries and Aquaculture Division, Seikai National
Fisheries Research Institute, Fisheries Research Agency,
Taira, Nagasaki, Nagasaki 851-2231, Japan

R. Masuda
Maizuru Fisheries Research Station, Field Science Education
and Research Center, Kyoto University, Nagahama, Maizuru,
Kyoto 625-0086, Japan

M. Tanaka · M. Tagawa
Field Science Education and Research Center, Kyoto University,
Kitashirakawa, Sakyo, Kyoto 606-8502, Japan

Present Address:
M. Tanaka
Borneo Marine Research Institute, Universiti Malaysia Sabah,
88999 Kota Kinabalu, Sabah, Malaysia

Keywords Japanese flounder · Pigment cell ·
Metamorphosis · Asymmetrical development ·
Xanthophore · Larval and adult type · Ontogeny ·
Flatfish

Introduction

The adult Japanese flounder *Paralichthys olivaceus* displays a unique asymmetry in body form, and one of its distinctive characteristics is skin coloration. Skin coloration is different between the ocular side and the blind side. In the early stages of development, like other fish, larval skin coloration is symmetric. However, after metamorphosis, which includes its dramatic eye migration, juveniles develop a dark brown to olive coloration only on the ocular side.

Ectothermic vertebrates, such as fishes or amphibians, have diverse skin colorations. It is known that the skin coloration of Japanese flounder is based on the density and distribution of four pigment cell types: dark melanophores, yellow to orange xanthophores, white leucophores, and reflective iridophores for silvery coloration [1].

Malpigmentation is common in hatchery-reared individuals of both right-eye and left-eye flatfishes, including Japanese flounder [2]. Malpigmentation seems to result from a disruption of the development of pigment cells at metamorphosis, and may take the form of pseudoalbinism on the ocular side or hypermelanosis on the blind side. The design of a rational solution to the problem of defective pigmentation will require knowledge of normal skin color formation [2].

In Japanese flounder, melanophores play the most important role in skin color formation. Melanophores are present as two types: the large-sized larval type and the small-sized adult type. In flounder with normal pigmentation, larval-type melanophores exist on both sides during the larval stage, while adult-type melanophores only appear with a high density on the ocular side after metamorphosis [3, 4].

Xanthophores, leucophores and iridophores also play important roles in skin color formation. Among all of these, xanthophores are indispensable for generating the unique olive coloration of Japanese flounder, since they express a yellowish color and therefore provide dark olive coloration on the ocular side together with the black color provided by melanophores. However, until now, these pigment cells have not been examined and reported in detail. The aim of the present study was to clarify the behaviors of three types of pigment cells during metamorphosis in Japanese flounder, which result in an asymmetric differentiation of skin coloration.

We clarified the process of pigmentation development by observing changes in the appearance of pigment cells, and found that the xanthophores presented a dual appearance. The two populations of xanthophores showed different cell sizes and depths in the skin. Potential similarities of the two possible populations of xanthophores to larval-type and adult-type melanophores are discussed.

Materials and methods

Fish

Japanese flounder eggs were provided by Miyazu Station, National Center for Stock Enhancement, Fisheries Research Agency, and the Niigata Prefectural Fisheries and Marine Research Institute. The eggs were transported to the Maizuru Fisheries Research Station, Kyoto

University, and maintained in a 500-L transparent plastic tank. The water temperature was adjusted to 18–22°C after hatching.

Larvae were fed with the rotifer *Brachionus plicatilis* [2–30 days after hatching (DAH)] and *Artemia* sp. nauplii (15–40 DAH). The rotifers and *Artemia* nauplii were enriched for 6–10 h with DHA oil (BioDHA Marine Growth; Nissin Marine Tech Co., Ltd). Larvae were then fed with an artificial diet (Otohime, Marubeni Nisshin Feed, Tokyo, Japan) (21 DAH).

Following Minami [5], the flounder larvae and juveniles were classified into the following developmental stages: D stage [6.1 mm total length (TL) in this study]; E stage (8.5 mm TL); F stage (9.8 mm TL); G stage (11.2 mm TL); H stage (12.5 mm TL); and I stage (13–20 mm TL). In this study, the I stage was further subdivided into an I-1 stage (13 mm TL) and an I-2 stage (17–20 mm TL). Seven larger juveniles (50–80 mm TL), which were provided by Miyako Station, National Center for Stock Enhancement, Fisheries Research Agency, were also used in this study.

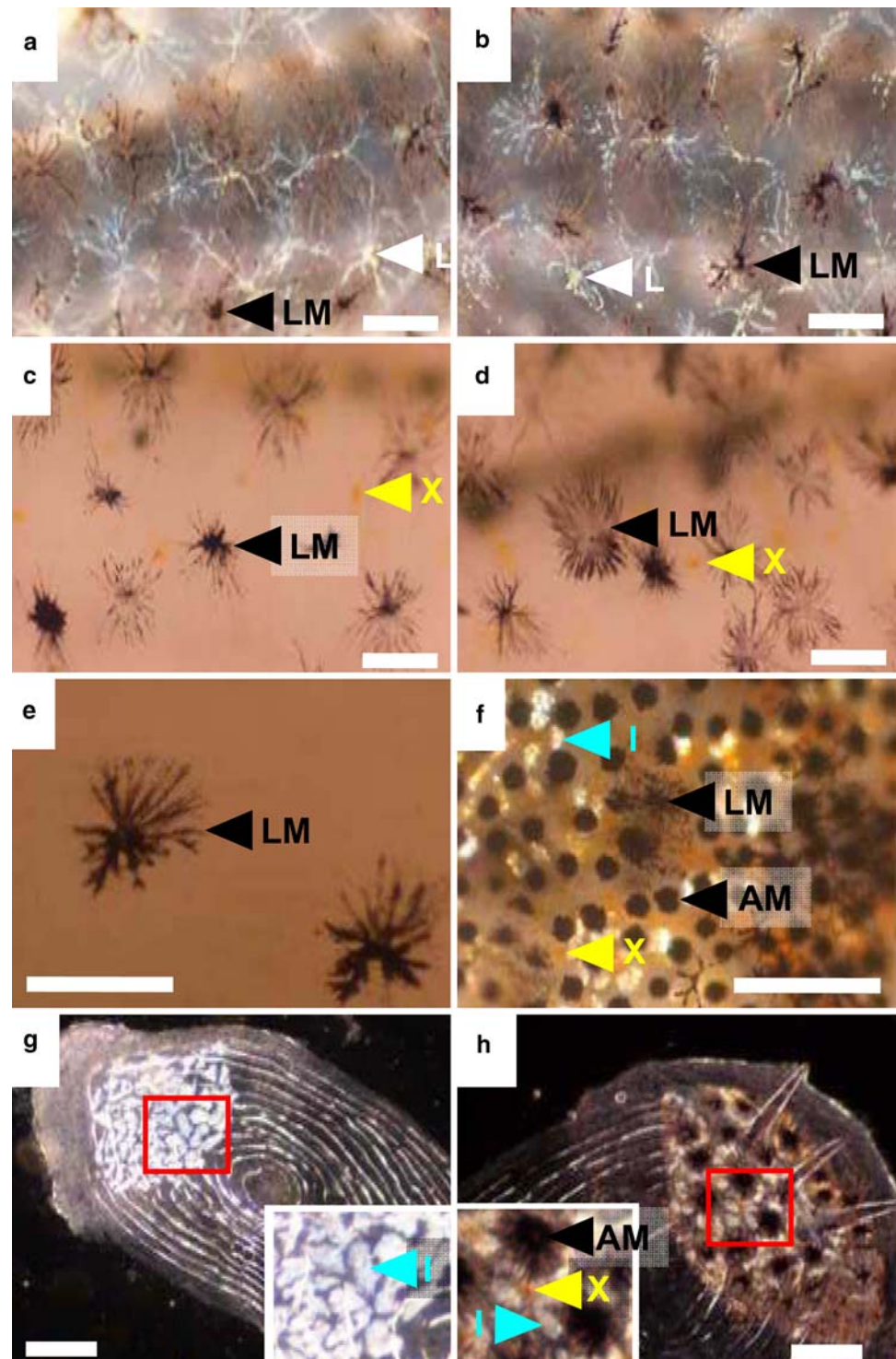
Identification of pigment cell types and density measurement

In the observations of pigment cells, 5–10 larvae and juveniles with normal pigmentation were selected at each developmental stage. At the end of rearing, more than 95% of the juveniles were judged to be normal. Live larvae were examined under a dissecting light microscope after being anesthetized in MS-222 (100 ppm in seawater). Although anesthesia may alter the pigment cell conditions, observing the blind side was impossible without it. The specimen was primarily illuminated by transmitted light and dark field light, with epi-illumination performed with a fiber-optic lamp. Types of pigment cells were classified by coloration, and larval- or adult-type melanophores were classified by large or small size, respectively (Fig. 1).

The density of pigment cells for larvae and juveniles was examined by calculating the number of cells in a constant area of skin. The area neighboring the lateral line near the root of the caudal fin was examined, because this area does not have a relatively high possibility of expressing pseudoalbinism [6]. Five areas per individual were examined, and mean values and standard errors were calculated. Statistical analysis was carried out using the Mann–Whitney *U* test in Excel Toukei 2000 (SSRI, Shakai Jyohou Service, Tokyo, Japan).

The skins of the larger juveniles were too thick to examine. For these larger juveniles, skin was detached from the body, together with a scale underneath, and examined. About 20 scales were examined per individual.

Fig. 1 Photomicrographs showing the various pigment cells in the skin of Japanese flounder *Paralichthys olivaceus* at each developmental stage. **a, b** D-stage 6.5 mm TL. **c, d** G-stage 12.3 mm TL. **e, f** I-2 stage 18.9 mm TL. **g, h** Larger juvenile 74.3 mm TL. **a, c, e, g** Blind side. **b, d, f, h** Ocular side. **LM**, Larval-type melanophore; **AM**, adult-type melanophore; **X**, xanthophore; **L**, leucophore; **I**, iridophore. Scale bars: 100 μ m in **a–f**, and 50 μ m in **g, h**, respectively



Examination of pigment cell size and depth

Since the yellow color of the xanthophores disappears upon histological processing, the depths of the melanophores and xanthophores were examined in live fish. The vertical distance from the skin surface to the pigment cell was measured using a scale attached to the fine adjustment knob

of the microscope. The tip of a glass capillary was placed in contact with the skin surface using a micromanipulator in the field of view of the microscope containing a target pigment cell. When the coarse adjustment knob was fixed, the difference in the scale division indicated the distance moved, as controlled using the fine adjustment knob. The minimum scale value was 1 μ m, which was sufficiently

small compared to the depths of the pigment cells in this study. Therefore, the depth of the pigment cell is determined by the difference between scale divisions observed when focusing on the target cell and when focusing on the tip of the glass capillary. About 50 cells per individual were examined on the ocular side and 10–20 cells per individual on the blind side. Three size classes were studied: a 12 mm size class (11.2–13.3 mm TL); a 25 mm size class (24.5–25.3 mm TL), and a 50 mm size class (50.1–53.4 mm TL), with 3 or 4 fish examined per size group.

Skin and scale samples were photographed with a digital camera on the dissecting microscope. The long and short diameters of the target pigment cell were measured. The product of multiplying the long diameter by the short diameter was used to describe cell size. Three size groups were examined: D-stage (about 8 mm TL); I-stage (about 25 mm TL); larger juveniles (50–80 mm TL), with 5–7 fish examined per size group.

Results

Changes in pigment cell density

The density of larval type melanophores was 136 cells/mm² on the ocular side and 141 cells/mm² on the blind side at the D-stage (before the onset of metamorphosis) with no significant differences observed between sides until the I-2 stage (Fig. 2a). The density of larval-type melanophores decreased to about 40 cells/mm² (I-2 stage) consistently on both sides. No significant differences in density were observed between the ocular and blind side at each developmental stage.

Adult-type melanophores only appeared on the ocular side after the completion of metamorphosis (Fig. 2b). The density of adult-type melanophores increased rapidly and reached 650 cells/mm² on the ocular side at the I-2 stage. In contrast, on the blind side, adult-type melanophores did not appear at all. These densities at the I-2 stage were equivalent to those for juveniles.

The xanthophore density was 341 cells/mm² on the ocular side and 320 cells/mm² on the blind side at the D-stage (Fig. 2c). Xanthophore density decreased to about 90 cells/mm² consistently on both sides from the D stage to the G stage. No significant differences were observed between the densities on the ocular and blind sides at each developmental stage. After metamorphosis, xanthophore density increased rapidly on the ocular side and reached 400 cells/mm² at the I-2 stage, and 700 cells/mm² in larger juveniles. In contrast, on the blind side, the density of xanthophores continuously decreased, and no xanthophores were observed in larger juveniles.

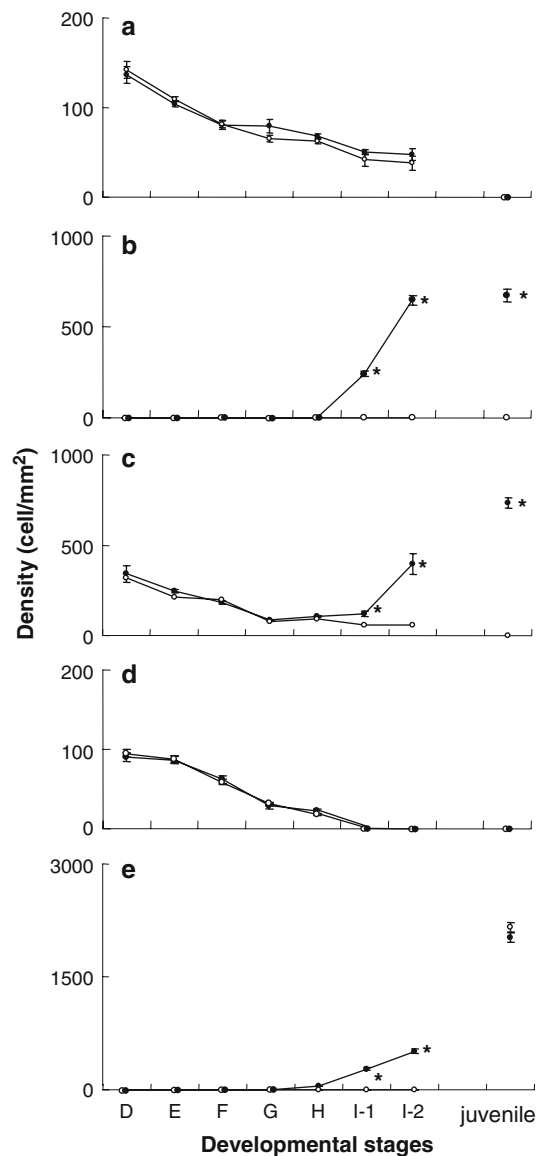


Fig. 2 Developmental changes in pigment cell densities in the skin of Japanese flounder *Paralichthys olivaceus*. **a** Larval-type melanophore; **b** adult-type melanophore; **c** xanthophore; **d** leucophore; **e** iridophore. Closed circles and open circles represent the densities on the ocular side and the blind side, respectively. Symbols and vertical bars represent mean \pm SE. * $p < 0.05$, significant difference between the sides by the Mann–Whitney U test

The leucophore density was 90 cells/mm² on the ocular side and 95 cells/mm² on the blind side at the D-stage (Fig. 2d). No significant differences in density between the ocular and blind sides were observed. Leucophore density decreased to about 20 cells/mm² consistently on both sides from the D stage to the H stage, and there were no significant differences at each developmental stage. After the I-1 stage, the presence of leucophores was not confirmed on either side.

The presence of iridophores was not confirmed on either the ocular side or blind side until the G stage (Fig. 2e). On

the ocular side only, iridophores appeared and increased rapidly at the H stage. On the blind side, iridophores did not appear up to the start of the I-2 stage. For larger juveniles (50–80 mm TL), iridophore density on the ocular side was 1,979 cells/mm², and on the blind side it was 2,166 cells/mm². There was no significant difference between the ocular and blind sides.

Distribution of pigment cell depth in the skin structure

The depth of larval type melanophores immediately after metamorphosis was about 4–5 μm (mean values) on both the ocular and the blind sides (Table 1). As the flounders grew larger, the distribution of larval type melanophores grew deeper. For the 50 mm size class, the depth of larval-type melanophores reached 40 μm on the ocular side and 26 μm on the blind side. The depth of adult-type melanophores was constant at about 4–5 μm (mean values) for the size class of 12–50 mm, a similar depth to the larval-type melanophores in the 12 mm size class.

The depth of xanthophores in juveniles of the 12 mm size class was about 1–10 μm (mean 4.6 ± 0.4 μm, Fig. 3a) on the ocular side. The depth reached 7.8 ± 0.5 μm for juveniles of the 25 mm size class, and a similar depth was maintained in the 50 mm size class. On the blind side, the depth of xanthophores in juveniles of the 12 mm size class was about 1–10 μm (mean 4.5 ± 0.3 μm). As the flounder grew larger, the depth of xanthophores increased (to about 6–35 μm) with mean values of 17.0 ± 1.3 and 23.3 ± 0.7 μm for 25 mm TL and 50 mm TL, respectively.

The distribution of pigment cell sizes

The size of larval-type melanophores was approximately 8,300 μm² on the ocular side and 11,600 μm² on the blind side at the I stage (Table 2). Adult-type melanophores appeared only on the ocular side, and they were smaller: about 130 μm².

At the D stage, differences in xanthophore size were not observed between the ocular and blind sides, with 80% of the cells being <200 μm² in size (Fig. 4a). Mean areas were 140 ± 17 and 120 ± 20 μm² for the ocular side and

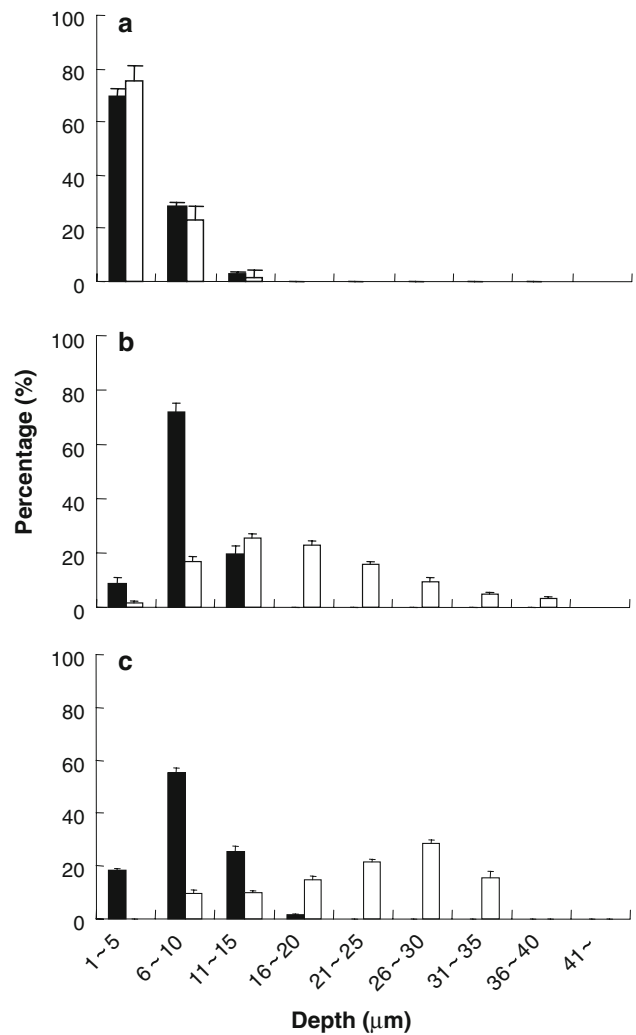


Fig. 3 Developmental changes in the depth distribution of xanthophores. Closed bars and open bars represent mean + SE of the depth on the ocular side and the blind side, respectively. **a** 12 mm size class; **b** 25 mm size class; **c** 50 mm size class

blind side, respectively. At the I stage, on the blind side, 80% of the cells had an area of <200 μm² (Fig. 4b). On the ocular side, the majority of cells had areas of 200–300 μm² with a mean area of 330 ± 29 μm², and cell areas of >400 μm² were also observed (Fig. 4b). The xanthophores on the ocular side were significantly (*p* < 0.05) larger than those on the blind side based on a comparison of mean

Table 1 Developmental changes in the depths of larval-type melanophores and adult-type melanophores

Size class (mm TL)	Larval-type melanophores		Adult-type melanophores	
	Ocular side (μm)	Blind side (μm)	Ocular side (μm)	Blind side (μm)
12	4.5 ± 0.4 (4) ^a	4.5 ± 0.4 (4)	4.5 ± 0.3 (3)	–
25	22.2 ± 0.9 (3)	14.5 ± 0.8 (3)	4.5 ± 0.2 (4)	–
50	40.3 ± 1.9 (6)	26.0 ± 0.9 (6)	4.3 ± 0.1 (6)	–

^a Numbers in parentheses indicate numbers of individuals

Table 2 Cell sizes of larval-type melanophores, adult-type melanophores, and iridophores

Pigment cells	Developmental stage	Cell size ($\times 10^2 \mu\text{m}^2$)	
		Ocular side	Blind side
Larval-type melanophores	I stage	83.4 ± 7.4 (7) ^a	115.8 ± 8.4 (7)
Adult-type melanophores	I stage	1.3 ± 0.2 (7)	–
Iridophores	Larger juveniles	2.2 ± 0.1 (5)	2.6 ± 0.1 (5)

^a Numbers in parentheses indicate numbers of individuals

values, although the cell size distributions largely overlapped.

Iridophores existed on both sides in larger juveniles. Differences in iridophore size were not observed between the ocular side and the blind side (Table 2). Most cells had areas of 200–400 μm^2 on both sides, with mean values of 218 and 256 μm^2 for the ocular side and blind side, respectively.

Discussion

Melanophores

The findings concerning melanophores that were obtained in this study were similar to those reported by Seikai [1] and Nakamura (A. Nakamura, unpublished data). Large-sized larval-type melanophores that appeared from the early larval stage onwards existed symmetrically on both sides of the body. Small-sized adult-type melanophores that appeared from the early juvenile stage onwards existed only on the ocular side.

With regard to the depth of their locations, both larval-type and adult-type melanophores were found to be

concentrated near the skin surface in the juvenile stage immediately after metamorphosis (TL, approximately 12 mm), and only the larval-type melanophores changed their locations to deeper depths (Table 1). This relocation could be explained by the thickening of the skin layers. Using electron microscopy, Haga et al. [7] showed that: (1) in the juvenile stage (i.e., 53–56 DAH), the thickness of subepidermal collagenous lamellae reached 15–20 μm , and; (2) melanophores were located at two depths—under the basement membrane and under the subepidermal collagenous lamellae. The results of the present study showed that the shallower layer (5 μm) of melanophores were of the adult type and the deeper layer (26 μm on the blind side and 40 μm on the ocular side) were of the larval type. Immediately after metamorphosis, the two layers of melanophores were located near the skin surface, since the epidermis and dermis consisted of only a few layers [4]. During development, melanophores of the larval type gradually relocate from the skin surface and cover a distance equivalent to the thickness of the subepidermal collagenous lamellae.

Xanthophores

Before metamorphosis, a continuous decrease in the density of xanthophores was observed on both the ocular and the blind side; there was no significant difference between the decreases in density on the two sides, which was similar to that seen in the case of larval-type melanophores. However, after metamorphosis, xanthophore density only increased rapidly on the ocular side, indicating the presence of a secondary xanthophore population that appears at the I-2 stage. This observation is consistent with that reported by Nakamura (A. Nakamura, unpublished data), who studied the Japanese flounder. The pattern of a gradual decrease and a rapid increase in the xanthophore density on the ocular side closely resembles that of the larval-type melanophore before metamorphosis and that of the adult-type melanophore after metamorphosis. We expected the presence of two populations of xanthophores—an early-appearing population and a late-appearing population—similar to the two types of melanophores. Thus, we examined whether qualitative differences were present between the two populations of xanthophores; by analogy

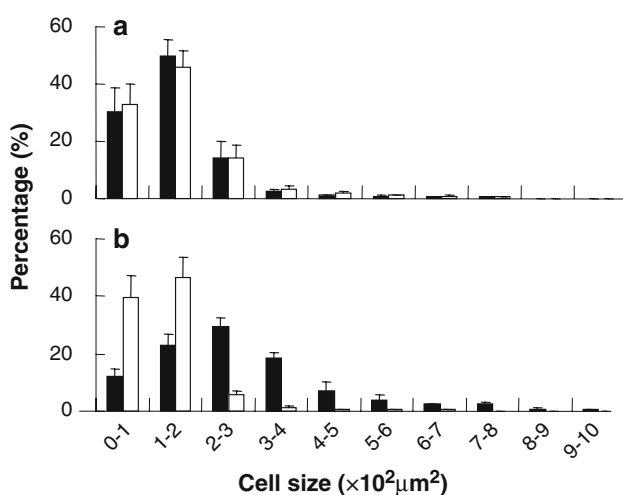


Fig. 4 Developmental changes in the cell size distribution of xanthophores. *Closed bars* and *open bars* represent mean + SE of the cell sizes on the ocular side and the blind sides, respectively. **a** D stage; **b** I stage

with the larval-type and adult-type melanophore distribution, we assumed that the early-appearing population of xanthophores was symmetrically distributed on both sides of the body and that the late-appearing population was specific to the ocular side.

In the I-2 stage juveniles, xanthophore density was 395 cells/mm² on the ocular side and 60 cells/mm² on the blind side (Fig. 2). All of the xanthophores on the blind side can be assumed to be from the early-appearing population basis on their early appearance and symmetrical distribution. Because 335 cells/mm² (i.e., 395 – 60) of the xanthophores (approximately 85% of all of the xanthophores) were from the late-appearing population and specific to the ocular side, the characteristics of the xanthophores in the late-appearing population can be represented by those of the major population of xanthophores on the ocular side.

In 25-mm TL (Fig. 3b) and 50-mm TL (Fig. 3c) juveniles, xanthophores on the ocular side and blind side were present at different depths. The depths at which the xanthophores on the ocular side (late-appearing population) occurred were similar to those of the adult-type melanophores (Table 1). In larger juveniles [4, 7], vertical colocalization of melanophores and xanthophores was also observed just under the basement membrane on the ocular side. On the other hand, the depths at which xanthophores on the blind side (early-appearing population) were present were similar to those of the larval-type melanophores (Table 1). Ontogenic changes in the depths of the xanthophores on the blind side were probably due to skin thickening, as discussed for larval-type melanophores, and/or migration of early-appearing xanthophores, as observed in salamander larvae [8]. With regard to the depth and time of appearance, melanophores and xanthophores may have common features; the early-appearing population is located deep in the skin, whereas the late-appearing population is found closer to the surface of the skin.

Our results suggested that xanthophores of the early- and late-appearing population have different cell sizes. At the D stage (before the appearance of the late-appearing xanthophores), no significant difference was observed in cell size between xanthophores on the ocular and blind sides (Fig. 4a). After the appearance of the late-appearing population on the ocular side at the I stage, the cell sizes of the xanthophores on the ocular side were found to be larger than those on the blind side (Fig. 4b). This suggested that the late-appearing population of xanthophores has larger cell sizes. This relative difference in the sizes of early- and late-appearing xanthophores is much smaller than, and contrasts with, that observed for melanophores, in which the cell size of the larval type is much larger. However, we cannot rule out the possibility that the difference in the cell size between the two xanthophore populations is due to

different sensitivities of the cells to MS-222 and stress, and/or simply due to wider variation in late-appearing xanthophores.

On the ocular side, we were unable to detect a cell population that appeared at a similar depth to the larval-type xanthophore (Fig. 3). This was because, during depth measurements, the presence of the larval-type xanthophores was possibly masked by adult-type xanthophores, which were present near the skin surface and interfered with the measurement. Although further studies are needed for confirmation, the dual appearance of the xanthophores, as well as the possible differences in depth and size between the early- and late-appearing populations, may suggest the presence of two types of xanthophores—just as it is well known that there are two types of melanophores.

Leucophores and iridophores

Although Seikai [1] first detected leucophores in the Japanese flounder, this study is the first to elucidate the developmental profile of this cell. We found that leucophore density was symmetrical on both sides of the body and constantly decreased during the larval stages. On the basis of the similarity in the developmental profiles of leucophores, the larval-type melanophores, and the xanthophores of the early-appearing population, it has been speculated that leucophores are pigment cells that are specific to the larval stage and distributed symmetrically.

Iridophores first appeared on the ocular side during metamorphic climax, which was followed by a delayed appearance on the blind side some time before the large juvenile stage. In the larger juveniles, the density of iridophores was similar on the ocular and blind sides. Our observation above was consistent with the results of the study by Nakamura (A. Nakamura, unpublished data) on the Japanese flounder. In the larger juveniles, there was no significant difference in the cell sizes of iridophores between the ocular and the blind sides. Although the time of appearance was slightly different between iridophores on the ocular and blind sides, the presence of only one type of iridophore—the adult type—is suggested.

The difference in the time of appearance could be due to differences in skin development on the ocular and blind sides. In the Japanese flounder, cycloid scales (without spines) appear on both sides just after metamorphosis, and spines subsequently appear only in the scales of the ocular side, resulting in the development of ctenoid scales [9, 10]. This process of scale formation is completed on the ocular side before the blind side in larger juveniles with a TL of approximately 50 mm [9]. Based on this information, it is possible that the different times at which the iridophores appeared on the ocular and blind sides simply reflects the differences in skin development of the two sides.

Profiles of the three types of pigment cells present during the early ontogeny of the Japanese flounder

In the present study, using Japanese flounder, we clarified the developmental profiles of all of the types of pigment cells (two types of melanophores, two populations of xanthophores, leucophores, and iridophores) for the first time in flatfishes during metamorphosis. We tried to classify these pigment cells into three groups. The first type of cell is the symmetrical larval type (larval-type melanophores, early-appearing population of xanthophores, and leucophores); these cells show maximum density during the larval stages and a constant decrease during and after metamorphosis. The second type is the asymmetrical adult type (adult-type melanophores and late-appearing population of xanthophores); such cells are characterized by their asymmetrical appearance (i.e., they only occur on the ocular side after metamorphosis). The third type was the symmetrical adult type (iridophores), and cells of this type are chiefly characterized by their symmetrical appearance on both sides after metamorphosis.

Abnormal pigmentation is an important issue in hatchery-reared flatfishes, including Japanese flounder. The abovementioned characteristics of pigment cells may help in the development of useful concepts that explain abnormal pigmentation. Pseudoalbino fish have white areas (that lack asymmetrical adult-type pigment cells) on the ocular side. This white area consists of iridophores and cycloid scales (A. Nakamura, unpublished data). This suggests that this process of skin formation on the ocular side is similar to that observed on the blind side in normal fish, since the ocular and blind sides of normal fish are covered with ctenoid and cycloid scales, respectively [9]. In contrast, ambicolored fish exhibit a colored area (where asymmetrical adult-type pigment cells are observed) on the blind side. This colored area consists of ctenoid scales (A. Nakamura, unpublished data), which suggests that the process of skin formation on the blind side is similar to that observed on the ocular side in normal fish. There is a small possibility of the involvement of pigment cells of the symmetrical larval and symmetrical adult types in these malformations. In order to clarify the mechanisms underlying malpigmentation, there is a need to focus on the common process(es) found in the development of pigment cells of the asymmetrical adult type, which are not common in symmetrical types. Since ctenoid scales and pigment cells of the asymmetrical adult type always form in the same areas, the concept of “field” may be required in the future to completely understand the process of

asymmetrical skin development and its underlying mechanisms in the Japanese flounder. In other words, we should attempt to find the factor that alters the “atmosphere of an area,” which in turn induces ocular side characteristics in all of the components (scale and pigment cells, for example) in the area simultaneously, rather than searching for the individual factors that induce ocular side characteristics in each component.

Acknowledgments We are grateful to the staff at Miyazu Station, National Center for Stock Enhancement, Fisheries Research Agency, and those at the Niigata Prefectural Fisheries and Marine Research Institute, for kindly supplying the Japanese flounder eggs. We are also grateful to the staff and the students at the Field Science Education and Research Center, Kyoto University, for their assistance in rearing the fish and conducting experiments. This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, and from the Ministry of Agriculture, Forestry and Fisheries of Japan to M. T.

References

1. Seikai T (1997) Mechanism of abnormal pigmentation. In: Minami T, Tanaka M (eds) *Biology and Stock Enhancement of Japanese Flounder*. Koseisha Koseikaku, Tokyo, pp 63–73 (in Japanese)
2. Bolker JA, Hill CR (2000) Pigmentation development in hatchery-reared flatfishes. *J Fish Biol* 56:1029–1052
3. Seikai T, Matsumoto J, Shimozaki M, Oikawa A, Akiyama T (1987) An association of melanophores appearing at metamorphosis as vehicles of asymmetric skin color formation with pigment anomalies developed under hatchery conditions in the Japanese flounder, *Paralichthys olivaceus*. *Pigment Cell Res* 1:143–151
4. Seikai T (1992) Process of pigment cell differentiation in skin on the left and right sides of the Japanese flounder, *Paralichthys olivaceus*. *Jpn J Ichthyol* 39:85–92
5. Minami T (1982) The early life history of a flounder *Paralichthys olivaceus*. *Bull Jpn Soc Sci Fish* 48:1581–1588 (in Japanese)
6. Seikai T (1985) Influence of feeding periods of Brazilian *Artemia* during larval development of hatchery-reared flounder *Paralichthys olivaceus* on the appearance of albinism. *Bull Jpn Soc Sci Fish* 51:521–527
7. Haga Y, Nataami K, Takeuchi T (2005) Process of true ambicoloration in larval and juvenile Japanese flounder *Paralichthys olivaceus*: an ultrastructural study. *Nippon Suisan Gakkaishi* 71:782–790 (in Japanese)
8. Pederzoli A, Gambarelli A, Restani C (2003) Xanthophore migration from the dermis to the epidermis and dermal remodeling during *Salamandra salamandra salamandra* (L.) larval development. *Pigment Cell Res* 16:50–58
9. Seikai T (1980) Early development of squamation in relation to color anomalies in hatchery-reared flounder, *Paralichthys olivaceus*. *Jpn J Ichthyol* 27:249–255
10. Kikuchi SK, Makino N (1990) Characteristics of the progression of squamation and the formation of ctenii in the Japanese flounder, *Paralichthys olivaceus*. *J Exp Zool* 254:177–185