Biology of the Chromatophores of the Ice Goby, Leucopsarion petersii

Authors: Goda, Makoto, and Fujii, Ryozo

Source: Zoological Science, 13(6) : 783-793

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.783

ZOOLOGICAL SCIENCE **13**: 783–793 (1996)

Biology of the Chromatophores of the Ice Goby, *Leucopsarion petersii*

Makoto Goda and Ryozo Fujii*

Department of Biomolecular Science, Faculty of Science, Toho University, Miyama, Funabashi, Chiba 274, Japan

ABSTRACT—Limited numbers of melanophores and xanthophores exist in the skin and also inside the body of the ice goby, *Leucopsarion petersii*. These chromatophores show fundamentally identical fine structural features to those described in many other fishes examined hitherto. When the ice goby is adapted to a dark or white background, chromatosomes in the cells disperse or aggregate, respectively. Interestingly, the melanophores existing inside the body, namely, those in the peritoneum and those close to the vertebrae, are likewise responsive. Studies on chromatophores in the excised tissue pieces show that they are responsive to various agents known to affect chromatophores. Although the population of the chromatophores in the ice goby is much smaller than that of many other fish, these cells may bear functional significance in the strategy for survival.

INTRODUCTION

Larvae or young fish are very commonly pellucid. In Japan, the very transparent young sardines are inclusively called "shirasu" which means "white youngs". Other representative instances include leptocephalus larvae of fish belonging to various orders, such as Anguilliformes, Elopiformes, Notacanthiformes, and Gonorynchiformes. During their migration upstream a river, for example, larvae of the eel (e.g., *Anguilla* spp., Anguillidae) take advantage of their transparency to avoid being eaten by predators.

Even among adult forms of teleosts, there exist some transparent ones. We can enumerate, for example, the transparent glass catfish *Kryptopterus bicirrhis* (Siluridae, Siluriformes), the African glass catfish *Physailia pellucida* (Schilbeidae, Siluriformes) and the icefish *Salangichthys microdon* (Salangidae, Salmoniformes). In many such cases, their common names and/or scientific names exquisitely signify their optical characteristics. It may be noted that their bodies are compressed, although not so much as the leptocephalus larvae mentioned above.

The ice gobies, *Leucopsarion petersii* (Gobiidae, Perciformes) are also famous for their bodily transparency. Like other gobiid fish, their trunks are not so compressed as many other transparent fish. It is also known that this one-year fish shows neotenic development in that it retains a larval structural plan even in its adult life. Because of the difficulty in obtaining live specimens for study, we still have very little information about the biology of the species, and practically no information is available on their pigmentary system.

On the other hand, we now have a considerably large amount of information about the chromatophores as well as the coloration of fish, to which readers can refer to current review articles, such as those from our laboratory (Fujii, 1993a, b; Fujii and Oshima, 1986, 1994). Even in these articles, however, the implications of the paucity of chromatophores or of the bodily transparency in fish has never been dealt with. In order to provide some clues for understanding such problems, we have now examined the pigmentary system of this interesting species of fish, and have further tried to discuss the situation in due consideration of the ethological significance of the existing bodily transparency.

MATERIALS AND METHODS

Materials

Adult forms of the ice goby, *Leucopsarion petersii*, were employed. As one may imagine from the common name of the species, the macroscopical appearance of the body is very transparent. Among gourmets in Japan, these fish are well known for their deliciousness of fresh, raw material. Usually in the vicinity of their habitat, therefore, they are served alive, immediately after they are caught in the river when they go upstream for breeding in springtime. Therefore, live specimens for experiments can be obtained from fishermen or from those who manage fish food stalls along the rivers in which the species go upstream during the season.

In the present study, we used fish caught by fishermen in a river in Maizuru city, Kyoto Prefecture, in April. They were kept for a few days in a freshwater aquarium in the facility of the Fisheries Research Station of Kyoto University, Maizuru. Some of them were used there for preliminary examinations. Others were transferred to our laboratory in Funabashi, Chiba, where they were used for further detailed studies, while being maintained in a freshwater aquarium. During that season, we could easily identify the sex of the individuals, because females held visible numbers of eggs. In most cases, we examined female fish, although there is practically no difference in the physiological

* To whom correspondence should be addressed.

properties of the chromatic system between the sexes.

Preliminary examination of chromatophores

The distribution of chromatophores was initially examined with the naked eye or through a binocular dissecting microscope (SMZ-10, Nikon, Tokyo). Photographic recordings were made with a 35-mm camera (OM-4, Olympus, Tokyo) with a close-up lens (Zuiko Automacro, 50 mm, Olympus). For a more precise investigation of the morphological features of the chromatophores, we employed an ordinary transmission light microscope (Optiphot, Nikon) along with a photomicrographic apparatus (UFX, Nikon).

Observations of the melanophores were occasionally made on specimens fixed with an ice-cold fixative (1 part 37% formalin, 9 parts physiological saline). The physiological saline solution employed was for teleosts, and had the following composition (in mM): NaCl, 125.3; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.8; D-(+)-glucose, 5.6; Tris-HCl buffer, 5.0 (pH 7.3). After fixation, the specimens were dehydrated through a graded ethanol series, cleared in xylol and mounted for examination.

Fine structure of chromatophores

To examine the structural details of chromatophores and of the tissues surrounding them, conventional transmission electron microscopy was employed. The procedures used were essentially the same as those described elsewhere (Goda *et al.*, 1994). Ultrathin sections were viewed with a JEM-1210 electron microscope (JEOL, Tokyo).

Background adaptation experiments

To examine whether the integumentary chromatophores were actually functioning in chromatic adaptation to the backgrounds, observations were made by placing the fish in a small plastic aquarium ($20 \times 13 \times 12$(H) cm), the outside of which was painted either with black or white acrylic paint.

Fish adapted to either of the backgrounds for more than 3 hr were quickly thrown into the ice-cold formalin fixative and the fixed specimens were processed as described above. Each specimen was transferred into a small Petri dish with xylol, in which the specimen could be held in various positions so that we could observe integumentary chromatophores vertical to the optical axis of a close-up lens of a camera or of a low power objective lens (5 ×) of the microscope.

Physiological experiments in vitro

During preliminary observations, we recognized that in many parts of the body, the chromatophores were not appropriate for precise and quantitative assessment of their motile responses. However, the chromatophores existing along the dorsal median line of the trunk were found to be rather good objects for study, because their dendritic processes extended almost parallel to the plane of the skin.

The xanthophores of this species were rather faintly colored. Thus, it was by no means easy to measure their responses. The quantitative recordings of the motile responses were therefore performed mainly on the melanophores, although the responses of xanthophores in the same microscopic field could frequently be observed for comparison.

In some experiments, skin pieces were stimulated in a field of sine-wave alternating current generated by a CR oscillator (AG-203, Kenwood, Tokyo). It is known that such as electrical field stimulates sympathetic fibers to liberate neurotransmitters (Fujii and Novales, 1968). The stimulating waves were monitored by a storage oscilloscope (5111A, Tektronix, Beaverton, OR).

In order to confirm that melanophores to be examined would show normal motile responsiveness, a K^+-rich saline solution, in which the concentration of K^+ ions was raised to 50.0 mM, was commonly applied to the skin piece prior to the examination of the effects of various agents. In this solution, the concentration of Na^+ was compensatorily decreased to 78.0 mM in order to keep its osmolarity the same as that of normal saline. We adopted this type of chemical stimulation for testing cellular responsiveness because the induced aggregation of melanosomes was quickly reversible, and the aftereffects of the stimulation were minimal. Such a sudden increase in the concentration of K^+ ions is known to give rise to the liberation of the neurotransmitter from presynaptic elements of nerves that control melanophores, thus acting as a sympathetic stimulus (Fujii, 1959). The positive responsiveness to the heightened K^+ concentration indicated that the cell was normally innervated, in addition to the normal responsiveness of the cell itself.

Recording of responses of a single melanophore

The physiological and pharmacological methods employed were fundamentally the same as those described in a previous report (Fujii and Miyashita, 1975). The responses of single melanophores were measured by means of the transmission photometric technique described in detail elsewhere (Oshima and Fujii, 1984), but using an electronic processing system that had been partially remodeled for easier and more stable operation (Fujii *et al.*, 1994).

At the end of each series of measurements, a sufficiently strong solution of norepinephrine hydrochloride (NE, racemic modification; Sankyo, Tokyo) made up in the physiological saline was applied for a few min to bring about the full aggregation of melanosomes for reference. Usually, NE at the concentration of 2.5 μM was employed for this purpose, the concentration of NE being expressed in terms of the active L-(-)-isomer, namely, half the concentration of its synthetic racemic modification. In all cases, the magnitude of melanosome aggregation is expressed as a percentage of the maximal response observed during the course of measurements, with the fully dispersed state taken as zero.

Chemicals and drugs used

In addition to norepinephrine, melatonin (Sigma Chemical, St. Louis, MO), adenosine (Kohjin, Tokyo), α-melanophore stimulating hormone (MSH; Sigma Chemical), melanin-concentrating hormone (MCH; Peninsula Lab., Belmont, CA) and acetylcholine (chloride salt, Daiichi Seiyaku, Tokyo) were employed as the common agents known to induce motile responses of the chromatophores. Pharmacological drugs employed included an α-adrenergic agent, phentolamine mesylate (Ciba-Geigy, Basel), and a β-adrenolytic agent, propranolol hydrochloride (Sumitomo Pharmaceuticals, Osaka). Stock solutions of these agents were diluted with physiological saline immediately before use.

All physiological and pharmacological measurements were made at room temperature (20-25°C).

RESULTS

Preliminary observations

Although the species is known for its highly transparent body, the presence of limited numbers of melanophores and xanthophores could be seen (Fig. 1). The distribution of these chromatophores was examined through a binocular dissecting microscope or an ordinary transmission light microscope (Fig. 2). Most chromatophores were generally large compared with those of many other species of fish, sometimes having tip-to-tip diameters of more than 1 mm (Fig. 2D). Many of the melanophores could be identified even with the naked eye (Fig. 1). The xanthophores of this species were rather faintly colored, and were found to exist in the skin. By contrast, the melanophores were distributed within rather restricted portions of the body. There were rather dense populations of melanophores in the skin covering the upper and lower jaws (Fig. 2A), and the opercula (Fig. 2B), and also around the base

and fin-rays of the caudal fin (Fig. 2F). A longitudinal dark stripe running along the dorsal median line was found to be composed of rows of melanophores in the skin (Fig. 2C). Histological examination showed that these melanophores were dermal.

On the ventral median line, too, we could recognize the presence of melanophores forming a long stripe (Fig. 3A, B, D, E). Histologically, however, we found that these melanophores were not in the integument, but were inside the body, namely, on the median plane in the peritoneum. Somewhat anterior to the middle of the trunk, there was an air bladder just under the vertebral column (Figs. 1 and 3C, F). Just posterior to it, a small mass of visceral organs was found enclosed in the peritoneum with a population of melanophores.

To our astonishment, there were melanophores inside the body, notwithstanding the fact that they were clearly visible from the outside. In the female individuals, two rows of very large melanophores were found to exist on the anterior lateroventral portion of the trunk just covering the ovary (Figs. 1A and 2D). Histological examination showed that these

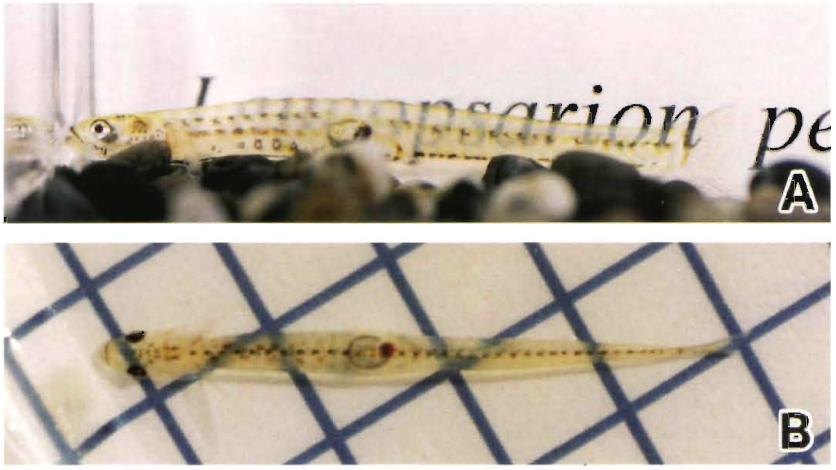

Fig. 1. Photographs of live female specimens of the ice goby, *Leucopsarion petersii*. A: A fish on the bottom of the aquarium, viewed from the side. The genus name printed on a sheet of paper posted outside the back glass can clearly be seen through the body. B: A fish viewed from above. The bottom of the tray was ruled into 10 mm dark-blue squares. A clear, oval structure visible a little anterior to the middle of the trunk is the air bladder. Body lengths of both specimens were about 50 mm.

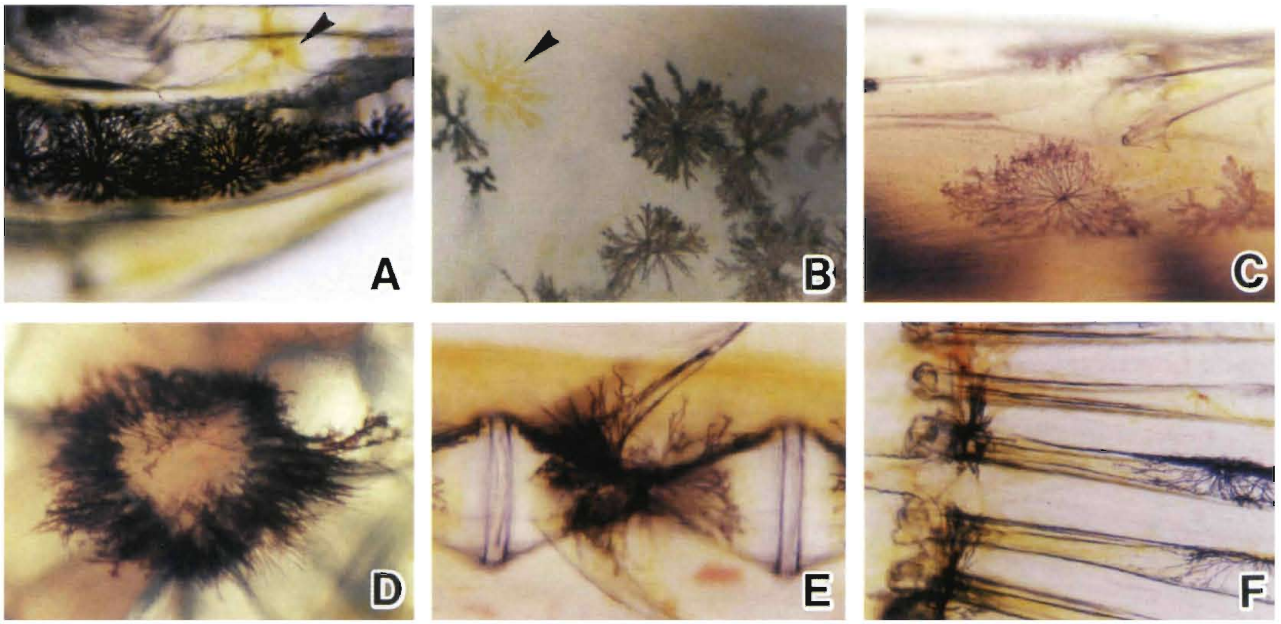

Fig. 2. Low power photomicrographs showing the melanophores in various tissues of the ice goby *in situ*. A: Dermal melanophores on the upper jaw. The arrow-head indicates a xanthophore. B: Melanophores on the skin covering the operculum. The arrow-head indicates a xanthophore. C: A dermal melanophore on the dorsal median line of the trunk. D: A large melanophore in the peritoneum enclosing the ovary. E: A large melanophore attached to the vertebra, its cell body being just on the dorsal surface of the latter. F: Small dermal melanophores around the base (left part) of the caudal fin, and those present adjacent to the fin-rays (right part). × 36.

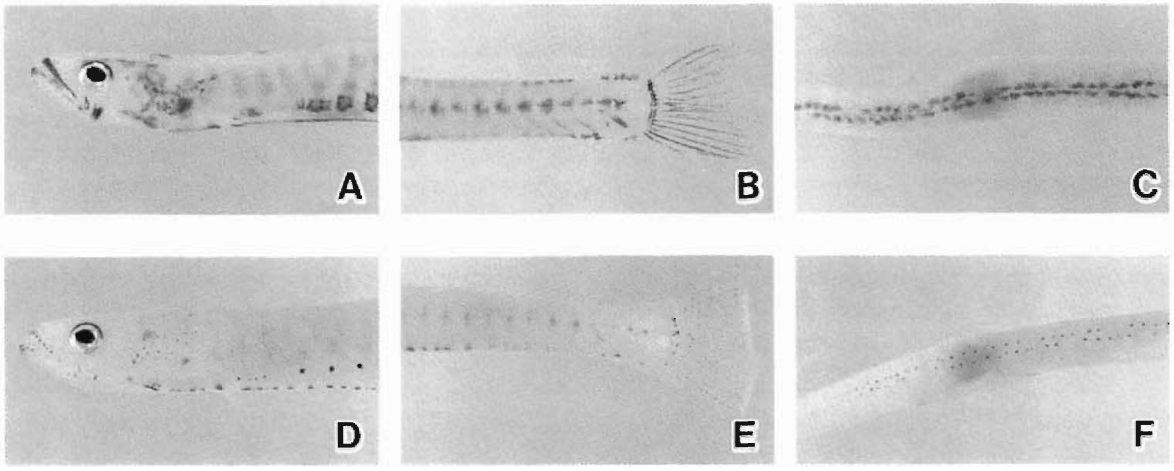

Fig. 3. Close-up photographs of parts of the bodies of two female fish adapted for 3 hr to the black (A-C) or to white (D-F) backgrounds, showing the state of melanophores under those respective conditions. Fixed and cleared specimens. A and D: Anterior parts of the body viewed from the side. B and E: Posterior parts viewed from the side. C and F: Middle parts of the body viewed from the top; the dark, oval images around the center are the silhouettes of the air bladder. Note that in the black-adapted fish, melanosomes in the melanophores in all parts of the body are dispersed, while those in the cells of the white-adapted one are aggregated (cf. Fig. 2). × 3.0 – 3.5.

melanophores were not in the integument, but in the peritoneum enclosing the ovary. In the males, no such melanophores were found in the corresponding portion of the trunk. An earlier report indicated that even in the females these melanophores were not visible when they were premature in the sea (Matsui, 1986), and therefore, these rows of dark spots are probably a kind of nuptial coloration expressed in mature females.

When the fish are viewed from the lateral side, a row of dark spots was observed running all through the dorsolateral region of the trunk (Fig. 1A). One may take it as corresponding to the longitudinal dark band on either side of the trunk rather commonly observed in many species of fish. It was found, however, that there were no melanophores in the corresponding portions of the integument. Instead, the melanophores responsible for the darkness were present deep within the body (Fig. 1B). Being present about the median plane of the body, these large melanophores were closely associated with the vertebral column. Putting their cell bodies on the dorsal dent of each vertebra (Fig. 2E), a large melanophore could be seen with extended dendritic processes three-dimensionally. The extremities of some dendritic processes of these cells were frequently found to reach near the surface of the skin.

During the first stage of our macroscopic examination, we had indeed thought that such melanophores in the peritoneum or close to the vertebrae were integumental, because the descriptions of the species in illustrated encyclopedia of fish had not indicated their locations. However, our study has revealed that they were not in the skin. The transparency of the trunk of this small species of fish allowed us to see them very clearly from the outside.

Fine structural observations

As a representative of typical integumentary melanophores, the dermal ones existing along the dorsal median line of the trunk were first examined for their fine structural features. Similar to many other teleosteans described previously (e.g., gluttonous goby; Fujii, 1968), these melanophores were found just under the subepidermal collagenous lamella (Fig. 4). Including round or somewhat ellipsoidal melanosomes, the fine structural details inside the melanophore were also fundamentally analogous to those of melanophores of many other teleostean species studied earlier (Fujii, 1966, 1993a; Obika, 1976).

The fine structure of the melanophores in the tissues inside the body were then examined. Showing the tissues around the peritoneum enclosing the ovary, Fig. 5 illustrates the presence as well as the structural details of the typical melanophore. Again, the architectural organization of the cell was quite similar to that of the integumental melanophores examined previously in many species of teleosteans.

The fine structure of melanophores found in association with the vertebrae was then studied. In the electron micrograph shown in Fig. 6, tissues near the dorsal part of a vertebra are seen. Cross sections of some dendritic processes of a melanophore are visible, which contained numbers of melanosomes.

Background adaptation experiments

In order to see whether ice gobies can chromatically adapt to the hues of the background, we placed the fish in a black or a white background. After adapting the fish for more than three hours to the black or the white background, melanosomes in the melanophores were dispersed or aggregated into the perikarya. Such changes could be recognized even with the naked eye. Some fish treated in this manner were fixed in an ice-cold fixative, and processed for detailed examination of the state of melanophores. The melanophores from the fish adapted to the dark background can be seen in Fig. 3A-C, while those from the fish adapted to the white background with their pigment aggregated are shown in panels D-F of the

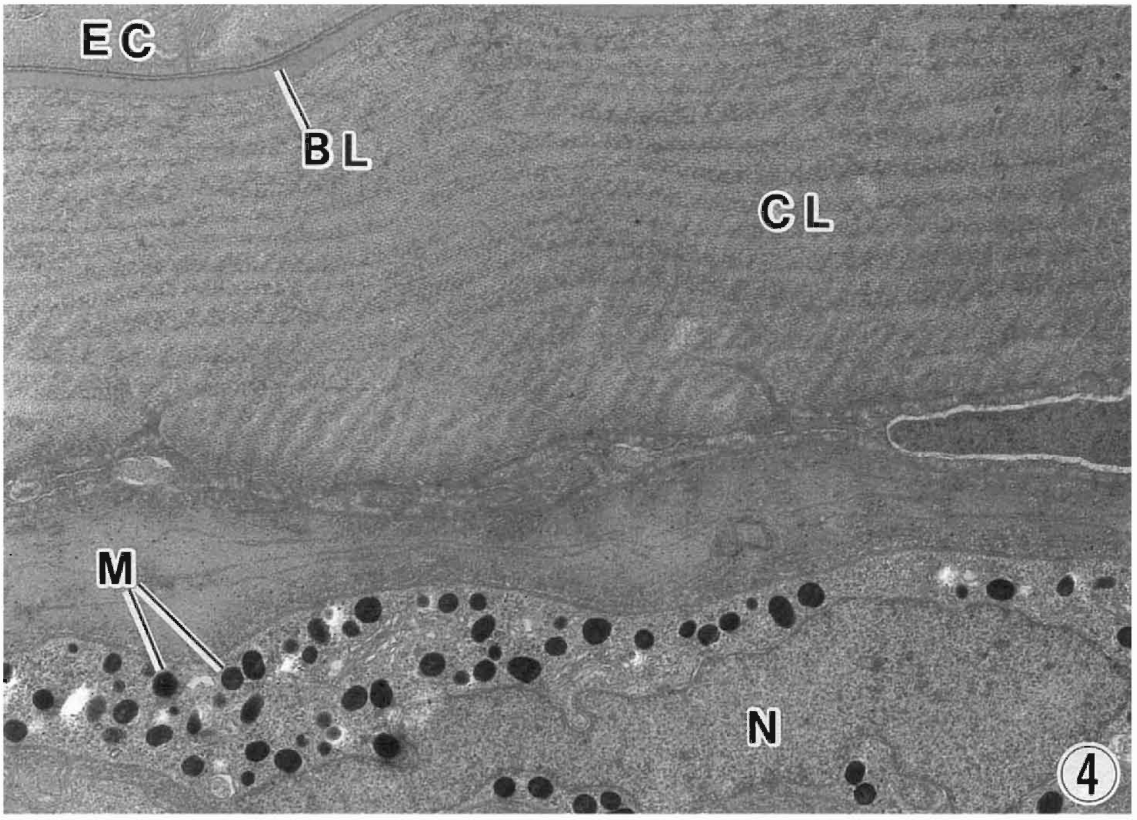

Fig. 4. Electron micrograph of part of the integument of the dorsal median line of the trunk. BL, basal lamina; CL, subepidermal collagenous lamella; EC, epidermal basal cell; M, melanosomes in the melanophore; N, nucleus. × 8,900.

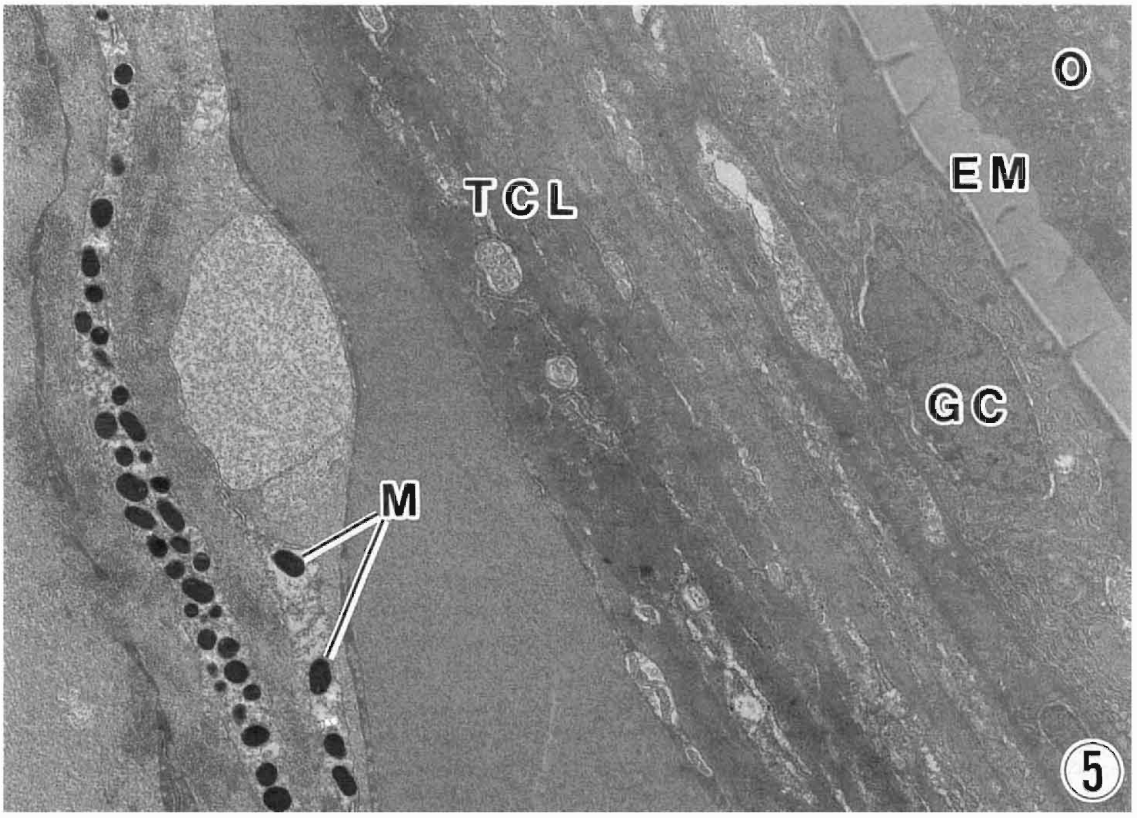

Fig. 5. Electron micrograph of the tissues near the ovary and peritoneum, showing the presence of the melanophore in the peritoneum enclosing the ovary. EM, egg membrane; GC, granulosa cell; M, melanosomes; O, part of ovum; TCL, theca cell layer. × 8,500.

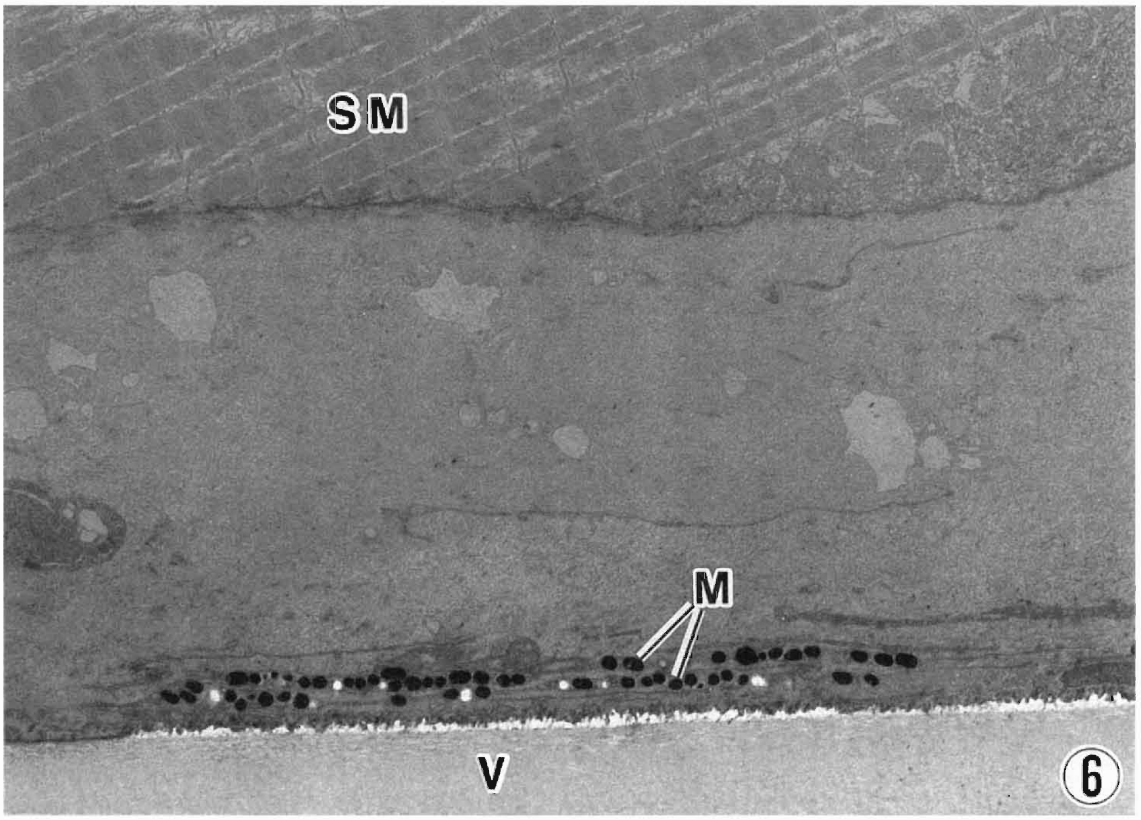

Fig. 6. Electron micrograph of the tissues near the upper part of a vertebra, showing the existence of the melanophore in close proximity of the vertebra. M, melanosomes; SM, part of striated muscle fiber; V, vertebra. × 5,500.

same figure. Since carotenoid pigment(s) in xanthophores are eluted under these conditions, we could not examine the state of these brightly colored cells in fixed specimens. However, observing live specimens, we could confirm that the xanthophores also responded similarly to the melanophores.

Cell physiological experiments

The effects of electrical nervous stimulation and of some chemical substances that are known to affect teleostean chromatophores were also examined. Due to the difficulty of applying the photoelectrical method to xanthophores, we have presented only the motile response of a single melanophore. However, we could examine through the binocular eye-pieces the state of xanthophores while we were measuring the responses of the melanophore. We were thus able to ascertain that both melanophores and xanthophores responded similarly, although the responses of the xanthophores were a little slower and smaller than those of the melanophores. Sometimes, photomicrographic examinations of the responses of chromatophores were made, and the same conclusion was obtained.

Skin pieces excised from various parts of the body were tested. Among them, those excised from the median line of the dorsal skin were most frequently used because the responses of the chromatophores were clearly recognizable. In this type of preparation, the layer of chromatophores was lined with a thin sheet of inclinator muscles for dorsal fin-rays (Fig. 7). In response to electrical stimulation, these skeletal muscle fibers contracted, and the resultant vibratory movement of the skin specimen frequently interfered with the accurate measurement of the cellular response. This was especially true during the initial stage of the measurement, before the fatigue of the musculature. Therefore, when we wanted to record the state of chromatophores photomicrographically, we transiently turned the stimulative pulses off. In Fig. 7, the melanosome aggregation response of melanophores to K^+-rich saline is illustrated.

The responses of larger melanophores visible for nuptial coloration over the ovary in the peritoneum were examined. Since these cells were covered with peritoneal epithelium, it took more time for their responses either to electrical or to chemical stimuli than when the cells were in the excised skin. It was found however, that the responsiveness was fundamentally the same as those in the skin. Other types of excised tissues tested included the skin pieces covering the opercula, the upper and lower jaws, the basal part of the caudal fin and the vertebra with the surrounding connective tissue in which large melanophores were associated.

As the first example of the recording, Fig. 8 exhibits the response to electrical stimulation of a single melanophore in a piece of skin isolated from the dorsal median line. A rapid aggregation of the melanosomes was clearly seen. Phentolamine, an α-adrenergic blocker, effectively interfered with the action of the A.C. stimulation. After a thorough washing

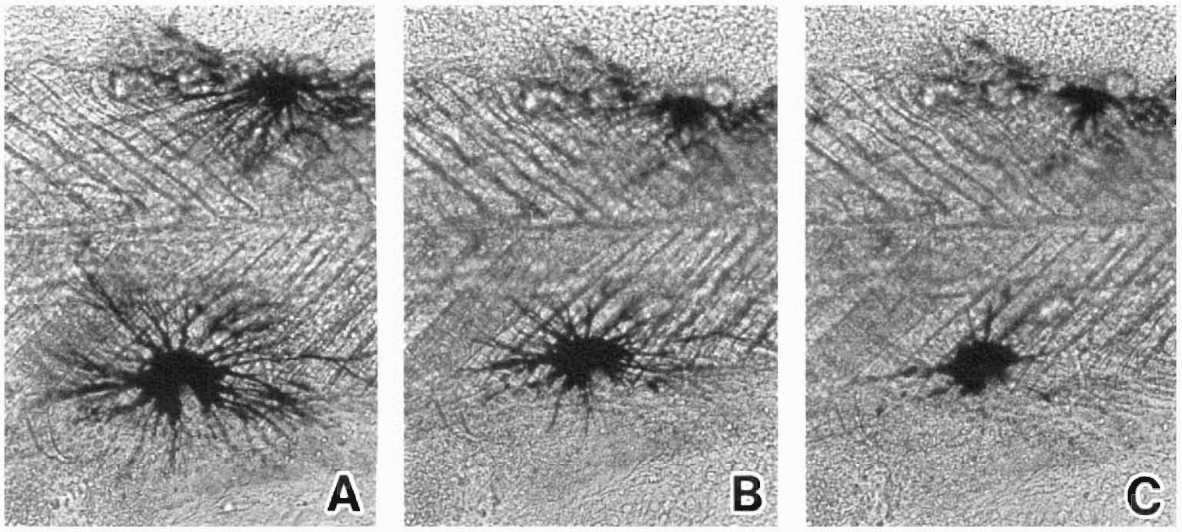

Fig. 7. Serial photomicrographs of a skin specimen excised from the dorsal median line of the trunk, showing the motile responsiveness of melanophores. Rostral side, leftward. Feather-like pattern recognizable around the center shows the ranks of striated, inclinator muscle fibers for dorsal fin, and the rachis line just above the center corresponds to the dorsal medial line. A: Equilibrated in physiological saline; melanosomes in the melanophores are totally dispersed. B: 2 min after the application of 50 mM - K^+-saline; melanosomes have begun to aggregate. C: 4 min after the application of K^+-saline; melanosomes are almost completely aggregated in the perikarya. × 100.

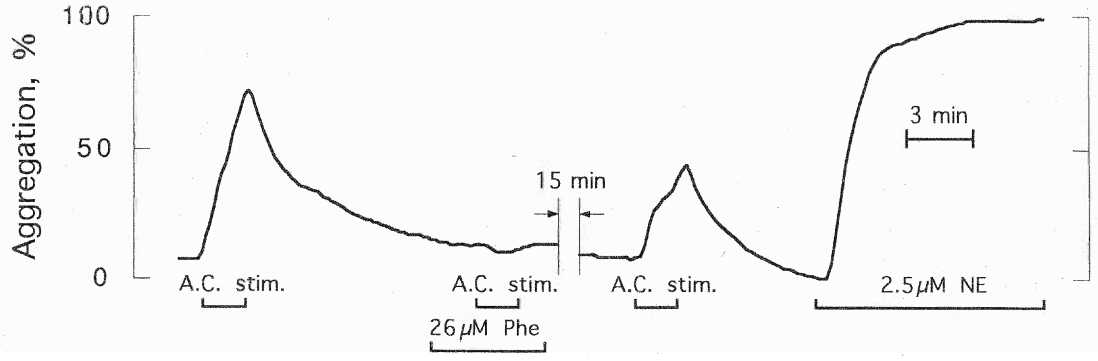

Fig. 8. Typical photoelectric recording of the responses of a single melanophore in the dorsal skin of a female ice goby. Effects of the field stimulation by alternating current (A.C.; sine wave, 10 Hz, 0.7 V/mm), and the influence of an alpha adrenergic blocker, phentolamine (Phe) on the action of the field stimulation were shown. A.C. stimulation induced rapid aggregation of melanosomes, but under the effect of the blocker, it failed to do so. After removing the blocker, the effect of A.C. was restored. Finally, the maximal pigment aggregation was induced by applying 2.5 μM norepinephrine (NE).

with normal saline to remove the blocker, A.C. stimulation regained its effectiveness. At the end of the recording, sufficient norepinephrine was applied to induce the maximal aggregation of melanosomes in the cell. The effectiveness of norepinephrine may indicate that the chromatophores are under the control of the sympathetic nervous system.

Next, the responses of a melanophore to an increase in the concentration of K^+ ions in the bathing medium were examined. When the melanophores at the dorsal median line were stimulated by K^+-rich saline, the same interference from undesired vibration of the skin preparation frequently occurred. This was apparently due to the effect of the K^+-induced contraction of the skeletal muscle cells as described above. Such disturbances were not encountered when we employed specimens from other parts of the body. Notwithstanding these disturbances, the aggregation of melanosomes in response to the increased K^+ concentration could be clearly detected (Fig. 9). Since the chromatosome-aggregating effect of the increased K^+ concentration was counteracted by phentolamine (data not shown), the effect is apparently mediated by the release of sympathetic neurotransmitter as widely known among teleostean fishes (Fujii, 1959; Fujii and Oshima, 1986, 1994). In the presence of phentolamine, the melanosome-aggregating action of norepinephrine was blocked. After thorough removal of the blocker, the amine regained its action, and could be employed for inducing the maximal level of melanosome aggregation.

Melatonin, a pineal hormone (Figs. 10 and 11), and melanin-concentrating hormone (MCH; data not shown) also elicited the aggregation of melanosomes. Acetylcholine, which

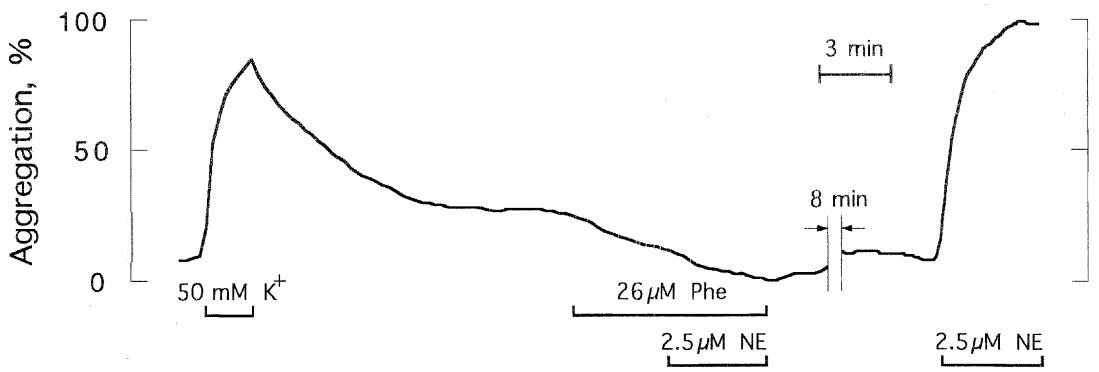

Fig. 9. Responses of a single melanophore in the dorsal skin of a female ice goby, showing the effects of an increased K^+ concentration (K^+: 50.0 mM) in the bathing saline, and of 2.5 μM norepinephrine (NE). The effect of phentolamine (Phe) on the action of NE was also tested. Under the influence of the blocker, NE did not aggregate melanosomes, whereas it acted quite normally after thorough removal of the blocker.

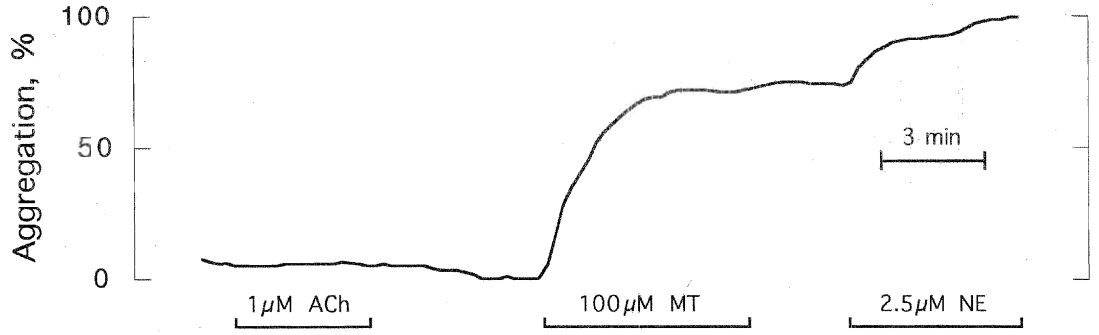

Fig. 10. Responses of a single melanophore in the dorsal skin of a female ice goby, showing the effects of acetylcholine (ACh) and melatonin (MT). ACh failed to aggregate melanosomes, while MT effectively aggregated them. Finally, the maximal level of pigment aggregation was attained by applying 2.5 μM solution of norepinephrine (NE).

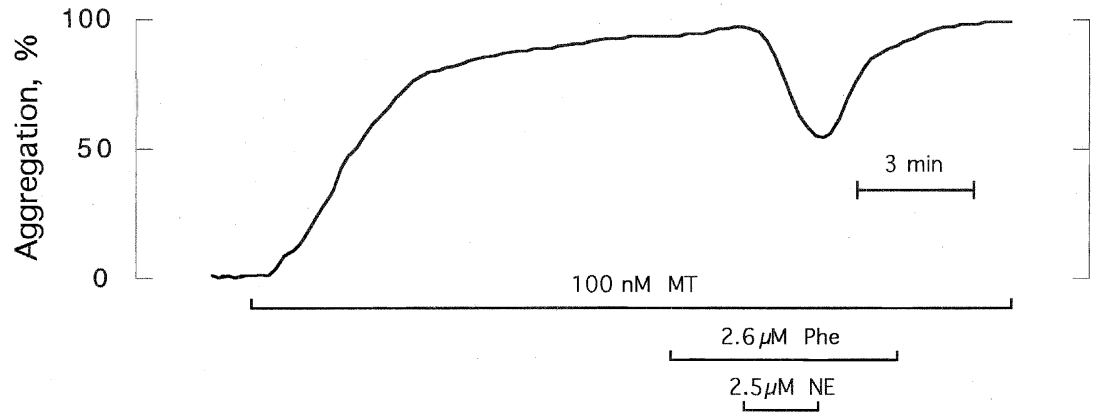

Fig. 11. Responses of a single melanophore in the dorsal skin of a female ice goby, showing melanosome-dispersing effect of norepinephrine (NE) under the influence of phentolamine (Phe). First, 100 nM melatonin (MT) was applied to stimulate the aggregation of pigment in the melanophore, and then, 2.6 μM Phe was added. Two min afterwards, 2.5 μM NE was applied in addition to MT and Phe, and a rapid dispersion of pigment took place.

has been shown to aggregate melanosomes in the melanophores of some fishes (Fujii, 1993a; Fujii and Oshima, 1986, 1994) did not have any effect in this system (Fig. 10).

Substances known to disperse chromatosomes in chromatophores, including melanophores, were also tested for their effects. In order to detect such effects, the chromatosomes had to be aggregated beforehand. In the recording illustrated in Fig. 11, melatonin at a relatively low strength (100

nM) was used. Under the influence of the α-adrenergic blocking agent phentolamine, the effect of norepinephrine was tested. A remarkable dispersion of melanosomes was recorded, indicating that a β-adrenergic mechanism is operating in the melanophores of this species similar to many other fishes (Miyashita and Fujii, 1975; Fujii, 1993a; Fujii and Oshima, 1986, 1994). Actually, the pigment-dispersing response to norepinephrine could easily be inhibited by the action of propranolol, a β-adrenolytic agent (data not shown).

Finally, the action of α-melanophore stimulating hormone (MSH) on melanophores of the ice goby was tested, as illustrated in Fig. 12. In this recording, the melanosome aggregation was induced by the action of norepinephrine. During the perfusion with normal saline, when the melanosomes were gradually dispersing, MSH was applied and a rapid dispersion of melanosomes was evident. Although the recording is not presented, adenosine showed the same effect.

DISCUSSION

Spectral ranges of light perception among animals are not so different from one another. Namely, they can sense what is called "visual light", and cannot perceive the rays outside the range, although some smaller animals, such as bees, butterflies and small fish, have been shown to be able to sense near ultraviolet rays (Davson, 1990; Fernald, 1993). Thus, we may rather safely conceive that an organism that looks transparent to us must also be recognized as transparent by other animals. To the predators of small fishes such as the ice goby, namely, the larger fish and possibly birds, the transparent objects may also be seen as transparent. Thus, being transparent should be an excellent strategy for survival, and predators will have considerable difficulty in finding such objects. Incidentally, recent ecological studies on the same species of goby showed that premature individuals in the seawater are mostly preyed upon by larger species of fish, including the horse mackerel, *Trachurus japonicus*, and the common blackish goby, *Acanthogobius flavimanus* (Dotsu and Uchida, 1979), while the mature ones in the river are caught by the eel, *Anguilla japonica*, the chestnut goby, *Chaenogobius castaneus*, the dusky tripletooth goby, *Tridentiger obscurus obscurus*, and the flathead, *Platycephalus indicus* (Matsui, 1986). At the present time, we have no information as to whether some species of birds prey on the ice goby.

Being transparent is the privilege of small animals, especially aquatic ones. If we assume that the opacity of the unit thickness of the body is equal, the opacity increases in proportion to the thickness of the animal. When the animal is terrestrial, the light reflectivity at the surface of the body may not be less than 2.4×10^{-2}, a value based on the Fresnel's equation:

$$R = (n_b - n_a)^2/(n_b + n_a)^2,$$

where R is the reflectivity, namely the proportion of incident energy reflected, and n_b and n_a are defined as the refractive indices of materials comprising the two phases. Usually, n_b is used for representing the higher one for convenience. At the interface between air and the surface of the body, we have adopted 1.37 and 1.00 for n_b and n_a. The value of 1.37 was adopted as representative of the value for cytoplasm obtained using precise measurements on sea urchin eggs (Hiramoto *et al.*, 1979). Being normally keratinized, squamous or cuticulized, the coverings of terrestrial animals may have refractive indices higher than 1.37, and thus, the light reflectivity at the body surface may be somewhat higher than the value given above.

When the animal is in the water, we can adopt 1.33 for n_a, and the reflectivity is calculated to be 2.2×10^{-4}, being strikingly smaller than that obtained for animals in the air. In this way, the reflectivity at the body surface of an aquatic animal is practically negligible. Thus, the coloration of the animal should be ascribable to the absorption, scattering or reflectance of light occurring from the very surface of the body. In the ice goby, neither iridophores nor leucophores are present which are known to take part in reflecting or scattering incident lightrays, respectively, in many other animals. Thus, the absorption of light should be almost exclusively responsible for the opacity, or the coloration exhibited by this species of fish. Studies of the light-absorbing chromatophores must

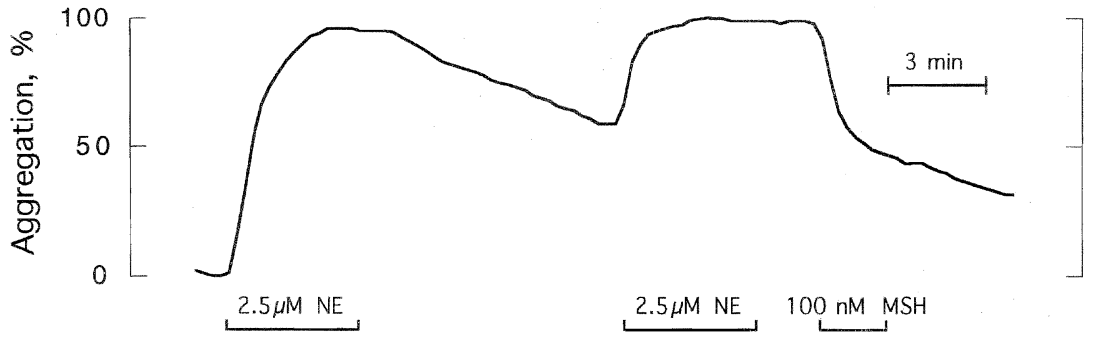

Fig. 12. Responses of a single melanophore in the dorsal skin of a female ice goby, showing the effects of α-melanophore stimulating hormone (MSH). First, 2.5 μM norepinephrine (NE) was applied for 4 min. During subsequent washing with normal saline, only a gradual dispersion of melanosomes was observed. NE at the same concentration was then again applied for 4 min. After washing the specimen with the normal saline for 2 min, 100 nM MSH was applied, and a rapid dispersion of melanosomes took place.

therefore be of utmost importance for understanding chromatic phenomena in this species.

As explained above, transparency seems to be quite favorable for small fishes. For them, however, there are also some difficulties that should be overcome or must remain as unavoidable. The largest weakness is the presence of lateral eyes. In order to increase the quality of vision, the retinas should accompany strata for light absorption (the pigment layer), and frequently for light reflection (the tapetum). In the present study, we have found that the lateral eyes were indeed the most conspicuous figures, especially when the fish are adapted to white background. As in all light-sensing creatures, the eyes cannot be transparent even in this very transparent animal, although a predator might mistake the eyes as grains of sand rolling over the bottom of the river.

Visceral organs are also inevitable apparatuses for animals. However, the ice goby seems to have evolved so as to include them in a space as small as possible. A similar design for housing viscera has been adopted by many pellucid fishes, and we could enumerate a number of such instances, which one may find in an illustrated encyclopedia of fish. During the final stage of their lives in the river, furthermore, both male and female ice gobies seem to take no food (Matsui, 1986). Actually, a large degree of regression has been reported of digestive organs among specimens caught in the river (Tamura and Honma, 1971).

The flowing red blood may also be conspicuous. Observing live specimens through a dissecting microscope, however, we judged that the relative volume of the blood may not be so large as to be easily challenged.

Being aware of the fact that the teleostean air bladder is filled with gas, we can easily imagine the optical situation at the inner surface of the epithelium to be quite analogous to that occurring at the body surface of the terrestrial animals as discussed above. Taking a fairly large value, the reflection of light there can thus be estimated to be no less than 2×10^{-2}. Actually, the air bladder of this species was very conspicuously recognizable through the transparent body (Fig. 1). Such a structure must also be easily recognizable by the predators of the species. It might be mistaken as a small bubble of air in the water, although we are rather afraid that sporadic movement of the possessor may easily be followed by the predators.

As is well known, the air bladder of almost all species of fish cannot be seen from outside the body. It is also known that some benthonic fish lack air bladders. Why should the ice goby possess such disadvantageous air bladders? Before they go upstream, ice gobies live in seawater which has specific gravity of 1.02-1.03, being considerably higher than that of the fresh river water. In order to swim upstream in fresh water, therefore, their own specific gravity must be lowered. It is by no means easy for them to lower their specific gravity within a short period of time and thus the air bladders serve an important role in this regard.

In this way, the weak points against the invisibility we have just discussed are unfortunately actualized only when the body of the animal is transparent. It may also be said that the risks of being found have been considerably lowered by the realized transparency of the other, greater part of the body. Looking into the aquarium, we frequently lost sight of the fish. Actually, the eye balls and the air bladders looked as if they were not living things, even though we could rather easily find them; although this impression was by humans, it may also be shared by predators.

When adapted to a white background, chromatosomes either in melanophores and in xanthophores aggregate, and the fish becomes even more transparent (Fig. 3A-C). When adapted to a darker background, by contrast, the chromatosomes become dispersed, and the body darkens to some extent (Fig. 3D-F), indicating that the chromatophores are indeed participating in the chromatic adaptation of the fish.

These results have further shown that several factors known to control the motile activities of chromatophores were effective in aggregating or in dispersing chromatosomes either in melanophores or in xanthophores. Namely, the chromatophores of this very transparent fish are also controlled by the sympathetic nervous system and the endocrine system as in many other teleostean species. We also consider that, although not so large in the extent, the changes of color thus regulated may be advantageous for survival, in addition to the intrinsically existing transparency. Along this line, some ethological experiments are being planned, in which, using larger species of fish as the predators, we like to know as to whether the transparency as well as the color changes of the present material would actually function to lower the rate of being preyed.

Interestingly, the melanophores existing in the peritoneum and those close to the vertebral column were found to show motile responsiveness quite similar to those of the integument. Namely, those non-integumentary melanophores also participated in the active color changes of the possessor animal. Indeed, such a phenomenon can only be realized when the animals are highly transparent, and, so far as we are aware, analogous phenomena have not been described previously.

Believing that those cells were immotile, some earlier researchers have described the melanin-containing cells inside the body as "melanocytes" (cf. Parker, 1948; Bagnara and Hadley, 1973). Among poikilotherm vertebrates, in addition, there are many integumentary melanophores that are immotile. In accordance with the current and widely accepted concept, therefore, the melanophores may be defined as the melanin-producing chromatophores of poikilotherms, irrespective of their location and the motile responsiveness (Fujii, 1993a). Namely, the darkly pigmented cells inside the body with which we have dealt here may also be categorized as melanophores.

Excepting those present in the caudal fin, the chromatophores of this species were very large, and resemble those of the larva of many fishes. Presumably, such a feature of chromatophores is concerned with the neotenic properties of the species. However, further detailed observations on the development of the pigment cell system in this species will be necessary to clarify these interesting issues.

Among the larger melanophores of this species, those in the peritoneum covering the ovaries were prominent, some exceeding 1 mm in diameter (Fig. 2D). Forming two rows of dark spots in either side of the trunk, they are distributed separately from one another. The melanophores on the vertebrae were also very large. Putting its cell body on the dorsal surface of the vertebra, each of them also exists separately from those on the anterior or posterior vertebrae (Fig. 2E). In these cases, a single melanophore constitutes an independent dark spot. Generally, a colored spot on the skin of teleostean adults consists of many chromatophores (cf. Fujii, 1993b). In this species, dark spots with single melanophores are present, which may function as visual signals during ethological encounters among individuals. As touched upon before, the large melanophores around the ovary are considered to display nuptial coloration, in that the dotted lines appearing on the sides of the females may function as the crucial signal to males for initiating mating behavior.

ACKNOWLEDGMENTS

This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. The authors also thank Dr. T. Seikai of the Fisheries Research Station, Faculty of Agriculture, Kyoto University, for the supply of ice gobies, and for making facilities available for carrying out the research during the initial stage of work. Many thanks are also due to Drs. N. Oshima and M. Sugimoto of this laboratory for their interest and critical comments.

REFERENCES

Bagnara JT, Hadley ME (1973) Chromatophores and Color Change. Prentice-Hall, Englewood-Cliffs, NJ

Davson H (1990) Physiology of the Eye. 5th ed, MacMillan, London

Dotsu Y, Uchida T (1979) Outline of life history of gobiid fishes exemplified by the ice-goby. Aquabiology 3: 9–16

Fernald RD (1993) Vision. In "The Physiology of Fishes" Ed by DH Evans, CRC Press, Boca Raton, pp 161–189

Fujii R (1959) Mechanism of ionic action in the melanophore system of fish—I. Melanophore-concentrating action of potassium and some other ions. Annot Zool Japon 32: 47–59

Fujii R (1966) A functional interpretation of the fine structure in the melanophore of the guppy, *Lebistes reticulatus*. Annot Zool Japon 39: 185–192

Fujii R (1968) Fine structure of the collagenous lamella underlying the epidermis of goby, *Chasmichthys gulosus*. Annot Zool Japon 41: 95–106

Fujii R (1993a) Cytophysiology of fish chromatophores. Int Rev Cytol 143: 191–255

Fujii R (1993b) Coloration and Chromatophores. In "The Physiology of Fishes" Ed by DH Evans, CRC Press, Boca Raton, pp 535–562

Fujii R, Miyashita Y (1975) Receptor mechanisms in fish chromatophores—I. Alpha nature of adrenoceptors mediating melanosome aggregation in guppy melanophores. Comp Biochem Physiol 51C: 171–178

Fujii R, Novales RR (1968) Tetrodotoxin: effects on fish and frog melanophores. Science 160: 1123–1124

Fujii R, Oshima N (1986) Control of chromatophore movements in teleost fishes. Zool Sci 3: 13–47

Fujii R, Oshima N (1994) Factors influencing motile activities of fish chromatophores. In "Advances in Comparative and Environmental Physiology Vol 20" Ed by R Gilles, Springer-Verlag, Berlin, pp 1–54

Fujii R, Tanaka Y, Hayashi H (1994) Endothelin-1 causes aggregation of pigment in teleostean melanophores. Zool Sci 10: 763–772

Goda M, Toyohara J, Visconti MA, Oshima N, Fujii R (1994) The blue coloration of the common surgeonfish, *Paracanthurus hepatus*—I. Morphological features of chromatophores. Zool Sci 11: 527–535

Hiramoto Y, Shimoda S, Shoji Y (1979) Refractive index of the protoplasm in sea urchin eggs. Dev Growth Differ 21: 141–153

Matsui S (1986) Studies on the ecology and the propagation of the ice goby, *Leucopsarion petersi* Hilgendorf. Sci Bull Fac Agr Kyushu Univ 40: 135–174

Miyashita Y, Fujii R (1975) Receptor mechanisms in fish chromatophores—II. Evidence for beta adrenoceptors mediating melanosome dispersion in guppy melanophores. Comp Biochem Physiol 51C: 179–187

Obika M (1976) An analysis of the mechanism of pigment migration in fish chromatophores. In "Pigment Cell Vol 3" Ed by V Riley, Karger, Basel, pp 254–265

Oshima N, Fujii R (1984) A precision photoelectric method for recording chromatophore responses *in vitro*. Zool Sci 1: 545–552

Parker GH (1948) Animal Colour Changes and Their Neurohumours. Cambridge Univ Press, Cambridge

Tamura E, Honma Y (1971) Histological changes in the organs and tissues of the gobiid fishes throughout the life span—IV. Digestive organs of the ice-goby. Bull Jpn Soc Sci Fish 37: 831–839

(Received July 1, 1996 / Accepted August 7, 1996)