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The Blue Coloration of the Common Surgeonfish, *Paracanthurus hepatus*—II. Color Revelation and Color Changes

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ABSTRACT—Measurements of spectral reflectance from the sky-blue portion of skin from the common surgeonfish, *Paracanthurus hepatus*, showed a relatively steep peak at around 490 nm. We consider that a multilayer thin-film interference phenomenon of the non-ideal type, which occurs in stacks of very thin light-reflecting platelets in iridophores of that region, is primarily responsible for the revelation of that hue. The structural organization of the iridophore closely resembles that of bluish damselfish species, although one difference is the presence of iridophores in a monolayer in the damselfish compared to the double layer of iridophores in the uppermost part of the dermis of surgeonfish. If compared with the vivid cobalt blue tone of the damselfish, the purity of the blue hue of the surgeonfish is rather low. This may be ascribable mainly to the double layer of iridophores in the latter since incident lightrays are complicatedly reflected and scattered in the strata. The dark-blue hue of the characteristic scissors-shaped pattern on the trunk of surgeonfish is mainly due to the dense population of melanophores, because iridophores are only present there in a scattered fashion. Photographic and spectral reflectance studies *in vivo*, as well as photomicrographic, photoelectric, and spectrometric examinations of the state of chromatophores in skin specimens *in vitro*, indicate that both melanophores and iridophores are motile. Physiological analyses disclosed that melanophores are under the control of the sympathetic nervous and the endocrine systems, while iridophores are regulated mainly by nerves. The body color of surgeonfish shows circadian changes, and becomes paler at night; this effect may be mediated by the pineal hormone, melatonin, which aggregates pigment in melanophores.

INTRODUCTION

Many people are attracted by tropical fish which display beautiful colors and patterns. Nowadays, hobbyists appreciate not only freshwater species but also marine ones in their own aquariums. Among such fishes, those which exhibit brilliantly bluish tints are especially enchanting, and are frequently cared for as “living gems”.

Until recently, mechanisms by which the blue hues of such fish are generated were unknown, because blue pigments have not been found in their skin. Lately, studies have been performed on bluish colorations of coral-reef damselfish, including the blue damselfish, *Chrysiptera cyanea* (Oshima *et al*., 1985; Kasukawa *et al*., 1987), and the blue-green damselfish, *Chromis viridis* (Fujii *et al*., 1989), and also on the blue hue of the longitudinal stripe of tetra fishes, including the neon tetra, *Paracheirodon innesi* (Clothier and Lythgoe, 1987; Nagaishi *et al*., 1990). Results of these studies indicate that iridophores in the dermis are primarily responsible for such hues, and that the multilayer thin-film interference phenomena which occur in stacks of light-reflecting platelets within those iridophores are responsible for these phenomena (Fujii, 1993a).

In the dermis of the blue damselfish, small, round or somewhat ellipsoidal iridophores are found densely arranged in a single layer like a bricked pavement. Each cell contains a nucleus located in its apical part, from where a number of piles of very thin light-reflecting platelets radiate into the cytoplasm. Fine structural observations have revealed that reflecting platelets are not more than 5 nm thick (Oshima *et al*., 1985; Fujii *et al*., 1989). It was concluded that the multilayered thin-film interference phenomenon of very far from the ideal status is primarily responsible for the generation of such fluorescence-like blue hues.

Recently, we have found that iridophores very similar to those of damselfish are present in the dermis of the common surgeonfish, *Paracanthurus hepatus* (Acanthuridae, Acanthuroidei, Perciformes). The surgeonfish is also a popular aquarium fish for its beautiful two-tone bluish colors, which are brighter and deeper, and seen over greater parts of the trunk (Goda *et al*., 1994). Although they are described as blue, the hues are rather different from the fluorescence-like blue

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displayed by many damselfish. Having lower purity, the brighter, whitish blue may appropriately be described as "sky blue" or "cerulean blue", and the deeper hue as "midnight blue". Morphological studies have shown that the skin of surgeonfish also contains iridophores which are similar to those of damselfish, but which occur in a double layer. Iridophores of this type were first shown to occur in species of fish that belongs to families other than Pomacentridae (Percoidei, Perciformes). Working on that species of fish, we have tried in this study to analyze the mechanism by which their characteristic blue hue is produced, and to further examine the characteristics of the regulatory system for motile activities of melanophores and iridophores.

MATERIALS AND METHODS

Materials

The material used was the common surgeonfish, or the regal tang, *Paracanthurus hepatus*, which belongs to the family Acanthuridae (suborder Acanthuroidei, order Perciformes) and was used in an earlier paper in this series (Goda *et al*., 1994). Young adult specimens having body lengths between 40 and 60 mm were purchased from local dealers in Tokyo or in the Chiba Prefecture, and were maintained in a seawater aquarium in our facility until used for study.

Observations *in vivo*

Colorations and chromatic responses of live fish were examined by the naked eye, and were recorded photographically with a 35-mm camera (OM-4, Olympus, Tokyo) with a closeup lens (Zuiko Automacro, 50 mm, Olympus). Color negative films (Agfacolor, SXG 100, ISO 100, Agfa-Gevaert, Leverkusen) were used. Evaluation of hues was made by comparing photographic prints from the same strip of film, printed at the same time. Films and prints were processed by a reliable local photographic laboratory.

On some occasions, the light-reflective properties of parts of the integument were studied by means of spectral reflectance measurements. For this purpose, a commercial spectrophotometric system equipped with a 1024-channel photodiode-array (MCPD-100, Otsuka Electronics, Osaka) was employed (Kasukawa *et al*., 1987; Fujii *et al*., 1989). The spectral reflectance of the skin of individual hovering fish was measured directly on the surface of the trunk around the posterior abdominal part to the base of the caudal fin. The aperture of the quartz fiber-optics was immersed in the sea water, and appropriately positioned very close to the surface of the skin. The area to be measured for reflectance was about 2 mm^2, and the spectral reflectance was measured at every 1 nm, from 400 to 700 nm. The sampling time to scan the whole range of the spectrum was set to 500 msec. The data were recorded on floppy disks in a personal computer. The spectra were graphically displayed on a CRT monitor, compiled for representation, and printed out on an X-Y plotter (MC-920, Otsuka Electronics).

Circadian changes of coloration were examined initially in an aquarium with natural sea water, in a room where aquaria were illuminated with light of an approximate 12L-12D regime at ca. 500 lx and 0.1 lx, respectively. When color changes due to the effect of melatonin were examined, the sea water containing the hormone at a final concentration of 1 μM was used. Such a method of applying the hormone was justifiable, because melatonin is known to affect the state of chromatophores by invading the body rather easily, owing to its higher lipid solubility (Reed, 1968; Nishi and Fujii, 1992; Hayashi *et al*., 1993). The color of fish was characterized with photographic and spectrophotometric methods, as described above.

Observations of melanophores

In order to investigate possible circadian changes of coloration and the effect of melatonin, individual fish were treated appropriately and were physically fixed by quickly placing in physiological saline at 80°C for 1 min. The physiological saline employed had the following composition (in mM): NaCl 128, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.8, D-(+)-glucose 5.6, Tris-HCl buffer 5.0 (pH 7.2). The fish were then chemically postfixed for about 1 hr in a solution made up of 37% formalin and physiological saline at a ratio of 1 : 9. Pieces of skin excised from various parts of the trunk were mounted in glycerol. Using an industrial light microscope (Optiphot XT-BD, Nikon, Tokyo), these whole mount specimens were observed and photographed on the same color negative films for the reasons described above. Employment of CF-BD plan objective lenses enabled us to observe chromatophores either by transmitted light illumination, by dark-field epi-illumination, or sometimes by both types of illumination.

Histological observations

Histological observations were made on semi-ultrathin sections of fixed skin by transmission light microscopy. In the daytime or at night, they were fixed physically as described above. Pieces of skin (3 mm square) from parts of the trunk were trimmed out, and postfixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hr at room temperature. After rinsing with 0.1 M phosphate buffer (pH 7.2), specimens were dehydrated in a graded ethanol series, and embedded in epoxy resin (Quetol 812; Nisshin EM, Tokyo). One-μm sections were cut vertically to the plane of the skin on a Porter-Blum MT-1 ultramicrotome (Ivan Sorvall, Newtown, CT) with a glass knife. After extending and adhering them to a glass slide, the sections were stained with 1% toluidine blue, and mounted for microscopic observation.

Motile responses of chromatophores

The responses of chromatophores in skin specimens excised from the sky-blue portion of the trunk were studied. The responses of melanophores were examined by ordinary transmission microscopy, while those of iridophores were assessed by the dark-field epi-illumination optics. Changes in the states of chromatophores were followed and recorded photomicrographically by comparing prints obtained from the same strip of color negative film as noted above. To follow changes in the spectral reflectance of the skin due to motile activities of iridophores, the spectrophotometric system (MCPD-100) was again employed, and the spectral reflectance was measured at intervals of 15 sec.

As for several dendritic chromatophores, the responses of melanophores may rather easily be expressed using the terms "aggregation" or "dispersion" of pigmentary organelles within the cells. On the other hand, a consensus about describing motile responses of iridophores has not yet been reached. In order to describe such sequences more clearly, we have recently proposed special terms, namely, the "LR response" and the "SR response" (Oshima *et al*., 1989; cf., also Fujii, 1993a). Being abbreviations for Longer-wavelength light-Reflecting response, the former is recommended to describe changes in the state of the cell when the spectral peak moves towards the longer wavelengths. The latter term indicates the reverse process, and is an abbreviation for Shorter-wavelength light-Reflecting response. These terms are conveniently employed in this communication as well.

The motile responses of melanophores were measured by a photoelectric method which is fundamentally identical to that described before (Oshima and Fujii, 1984), but with electronic portions partly modified for improved stability and easier operation (Fujii *et al*., 1993). To eliminate the possible influence of motile activities of iridophores, a red filter (R-61, Toshiba Glass, Tokyo), which eliminates lightrays shorter than 610 nm in wavelength, was placed between the light source and the condenser.

Chemicals used

The drugs used in pharmacological experiments included norepinephrine hydrochloride (Sankyo, Tokyo), acetylcholine chloride (Sankyo), phentolamine mesylate (Ciba-Geigy, Basel, Switzerland), melanin-concentrating hormone (MCH; Peninsula Lab., Belmont, CA, USA), melatonin (Sigma Chemical, St. Louis, MO, USA), α-melanophore stimulating hormone (α-MSH; Sigma). Stock solutions of these drugs were diluted with physiological saline immediately before use. Since the norepinephrine employed was racemic, its concentration is expressed in terms of its active L-(-)-isomer, namely, half the concentration calculated from the weight content. Physiological experiments were carried out at a room temperature between 19 and 24°C.

Mathematical treatments and graphics

Theoretical considerations of optical events were based primarily upon the exposition by Huxley (1968) on multilayer thin-film interference phenomena. The computer program that enabled us to deal with complex functions, and to display results as graphics in color, was that used in foregoing papers (Fujii *et al*., 1991; Fujii, 1993a). The spectral curves thus displayed on a color CRT monitor were printed on a laser printer in monochrome, and appropriately labeled for exhibition.

RESULTS

Changes of coloration *in vivo*

When we set about this series of studies, we did not know whether this species of fish could change their integumentary coloration. Careful observations of live fish, however, indicated that the darkness of the skin changed in response to various environmental conditions. Figure 1 illustrates such changes displayed by one individual of fish. Compared with the state seen in simple sea water (Fig. 1A), the sky-blue portions and the dark-blue scissors' pattern became paler to some degree after equilibration with 1 μM melatonin for 2 hr (Fig. 1B). During the night, the dark scissors' pattern became remarkably paler, and the sky-blue portion became somewhat paler, changing to a purplish hue (Fig. 1C).

Figure 2 shows actual measurements by MCPD-100 of spectral reflectance from the sky-blue portion of a live fish. In an aquarium with simple sea water, there was a peak of spectral reflectance around 490 nm in the daytime (Curve A), but when the measurement was done at night, the peak was found around 450 nm, being shifted towards the shorter wavelength region (Curve B). Similar displacement of the spectral peak has consistently been observed, although the actual shifts were always limited within this region. By covering the aquarium with a shading box, the fish could be placed in the darkness even in the daytime, and within 1 hr of doing that, the peak of spectral reflectance from the sky-blue portion of the fish shifted toward the shorter wavelength as when the measurement was done at night.

Microscopic observations of whole-mount skin

Skin preparations excised from the fish after various treatments were physically fixed, and observed through an ordinary transmission light microscope. Panels A-C of Fig. 3 are photomicrographs of specimens from the sky-blue part, while those labeled as D-F are from the dark-blue scissors pattern. Epidermal melanophores were not recognized in any of specimens examined, as already described by us (Goda *et al*., 1994). That the melanophores were partially obscured must be due in part to the presence of an overlying layer of iridophores through which the melanophores had to be seen, and to the relatively thick skin of this species.

Iridophores were rather clearly visible as much smaller, round or polygonal contours with diameters of about 10 μm, that were distributed in packets throughout the preparation, shown in Fig. 3A to C, where the sky-blue skin of the trunk is shown. Our recent electron microscopic observations indicated that iridophores constituted a double layer in sky-blue portions of the skin, whereas in dark-blue areas, by contrast, iridophores were found only distributed in scattered patterns (Goda *et al*., 1994). It is clearly shown here that, in both sky-blue and dark-blue portions, blanched skin pieces contained melanophores with aggregated pigment (Fig. 3B, C, E, F).

Histological observations

One-μm sections of resin-embedded specimens from the sky-blue portion were cut perpendicular to the plane of the skin, and stained with toluidine blue. In order to observe the melanophores in which melanosomes were dispersed, skin specimens were excised and fixed from the fish sacrificed during the daytime, whereas those with aggregated melanosomes were observed in skin pieces fixed during the night. Melanophores containing very dark melanosomes were recognizable as very dark particles in the usual transmission microscope, and melanophores with dispersed melanosomes are seen in Fig. 4A. Melanosomes were found even in dendritic processes extending among iridophores. By contrast, in the specimen fixed at night, melanosomes were totally aggregated in the perikarya (Fig. 4B). Iridophores could be seen in both panels as lightly stained double strata, though their nuclei were intensely stained (Fig. 4A, B).

Control of cellular motility

Motile responses of chromatophores in an excised piece of skin from the sky-blue portion were first examined photomicrographically (Fig. 5). When a skin piece was equilibrated in physiological saline, melanosomes in melanophores were widely dispersed in the cytoplasm (Fig. 5A). Under incident illumination, on the other hand, iridophores appeared dark blue with shades of violet (Fig. 5E). When the irrigating medium was changed to K^+-rich saline, melanosomes in melanophores aggregated (Fig. 5B-D), and concurrently, the color reflected from the iridophores changed. The dark-blue color tinged with violet (Fig. 5E) changed gradually to sky blue (F, G) and then greenish blue (H). The sky blue color corresponds to the normal hue of the trunk of the fish during the day.

By means of spectrophotometry using MCPD-100, changes in the spectral reflectance pattern were studied on excised skin pieces, and a typical series of measurements is displayed in Fig. 6. When the specimen was equilibrated in physiological saline, the spectral peak occurred around 455 nm (Curve A). When the medium was changed to K^+-rich saline, the peak rapidly shifted within 90 sec towards a longer

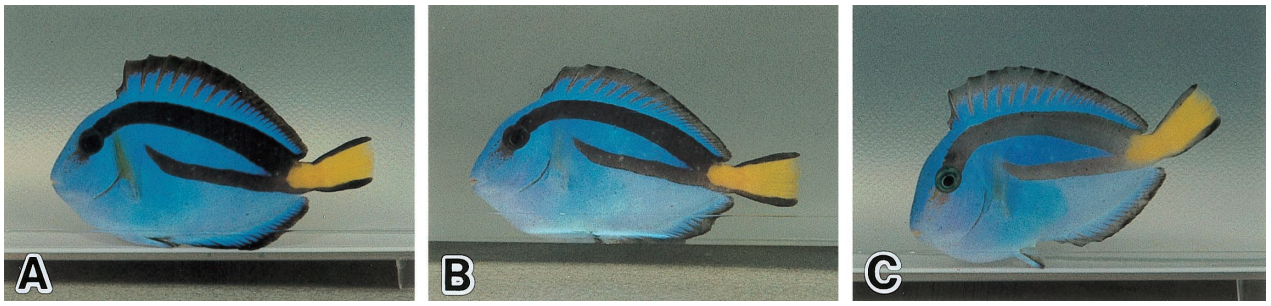

Fig. 1. Photographs of a single young common surgeonfish, *Paracanthurus hepatus*, showing its coloration under three different conditions. The body length of this particular individual was about 60 mm. (**A**) In the aquarium with simple sea water. (**B**) Two hr after adding melatonin to a final concentration of about 1 μM; the sky-blue portion became paler, and the dark-blue scissors pattern blanched slightly. (**C**) Photographed at night (1 a.m.); the sky-blue and the dark-blue portions became paler, and the hue of the sky-blue portion became purplish. Photographs **A** and **B** were taken during the daytime.

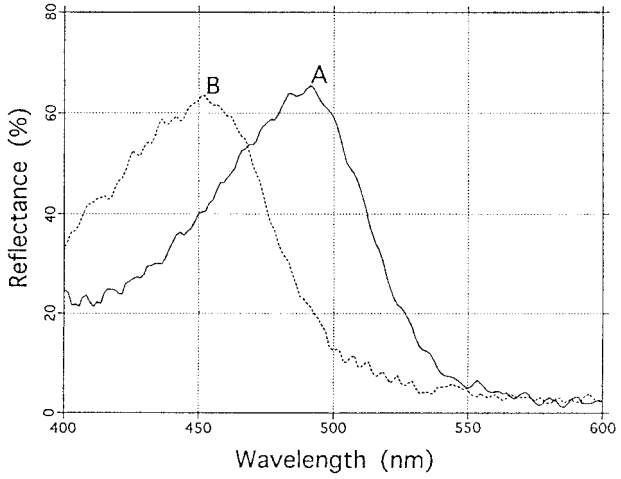

Fig. 2. Typical spectral reflectance curves obtained from the sky-blue portion of an individual fish *in vivo*, while it was hovering in simple sea water. (**A**) Measured during the day. (**B**) Measured at night (1 a.m.).

wavelength about 490 nm, namely, the cells exhibited the LR response (Curves B -D).

The time course of motile responses of iridophores was followed more precisely, and the translocation of the spectral reflectance peak was measured at intervals of 15 sec. Figure 7A shows the LR response of iridophores to K^+-rich saline and then to norepinephrine (NE). An α-adrenolytic agent, phentolamine, effectively blocked the action of NE, while acetylcholine was without effects (data not shown). The pineal hormone, melatonin, was unable to induce an LR response (Fig. 7B) or an SR response (Fig. 7C). Likewise, melanin-concentrating hormone (MCH) and melanophore-stimulating hormone (MSH) were also ineffective in arousing motile responses of iridophores (data not shown).

The regulatory system for motile activities of melanophores in the sky-blue portion of the trunk was also studied. Panels A through C of Fig. 8 show representative photoelectric recordings selected among those for analyzing the sys-

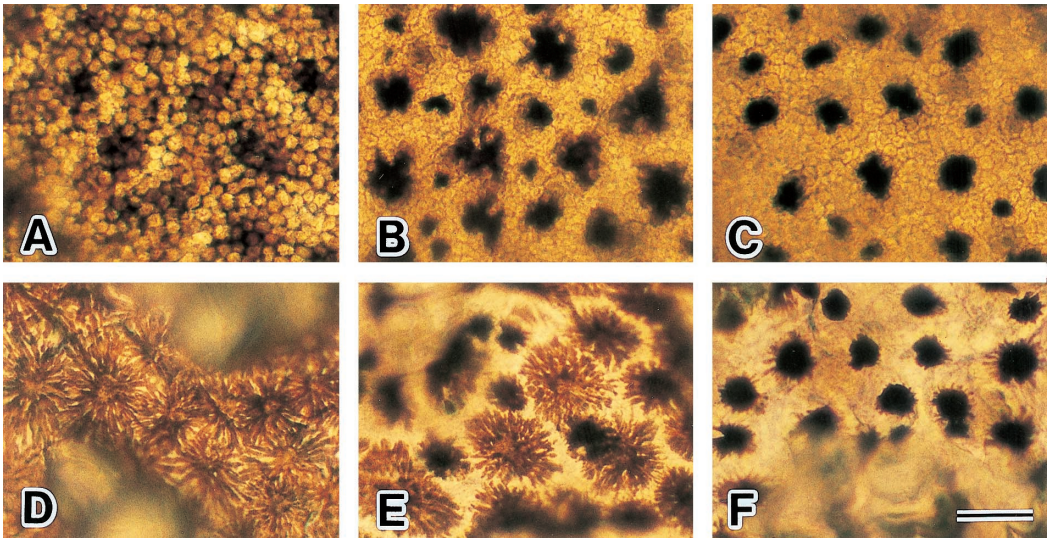

Fig. 3. Photomicrographs of dermal chromatophores in skin specimens from the sky-blue (**A**-**C**) and dark-blue (**D**-**F**) portions of the trunk. Fish adapted to conditions as described below were physically fixed, skin pieces were then isolated, and processed for microscopic observations using ordinary transmission optics. In panels **A**-**C**, iridophores are recognizable as round bodies about 10 nm in diameter, distributed in packets throughout the sky-blue part. (**A**, **D**) Specimens from a fish adapted to simple sea water. (**B**, **E**) Specimens from a fish equilibrated for 2 hr in sea water containing 1 μM melatonin. (**C**, **F**) Specimens from a fish at night (1 a.m.). Scale bar: 50 μm.

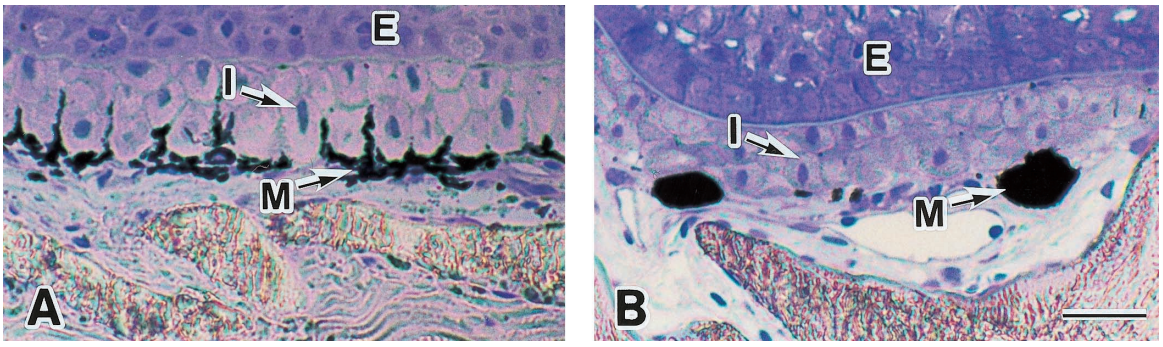

Fig. 4. Photomicrographs of 1-μm sections cut vertically to the surface of the skin from the sky-blue part of the trunk. E, epidermis; I, iridophore; M, melanophore. (**A**) Specimens fixed during the daytime; melanosomes in melanophores were dispersed even into dendritic processes extending into spaces among the iridophores. (**B**) Specimen fixed at night; melanosomes were totally aggregated in the perikarya of melanophores. Scale bar: 20 μm.

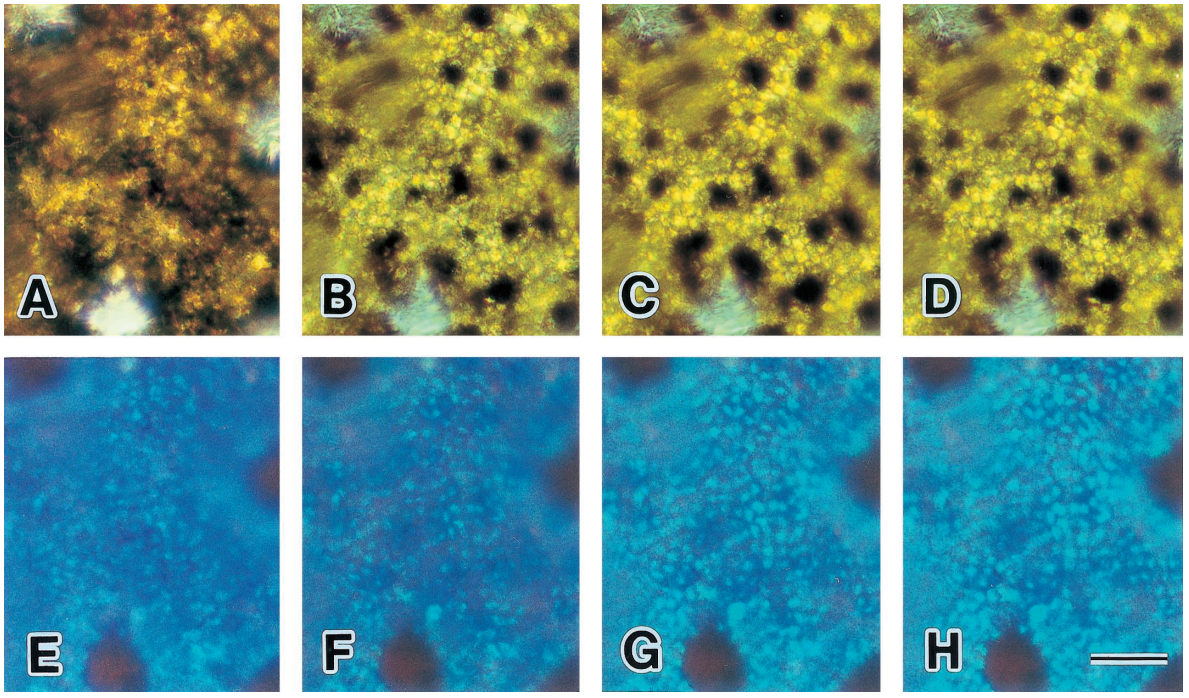

Fig. 5. Photomicrographs showing motile responses of iridophores and melanophores in the dermis of skin excised from the sky-blue part of the trunk. (**A**-**D**) Viewed by ordinary transmission optics for examining responses of melanophores. (**E**-**H**) Viewed by dark-field epi-illumination optics for examining responses of iridophores. (**A**, **E**) Equilibrated in physiological saline. (**B**, **F**) 30 sec after the application of 50-mM K^+ saline. (**C**, **G**) 60 sec after the application of the K^+ saline. (**D**, **H**) 90 sec after the application of the K^+ saline. Scale bar: 50 μm.

tem. An elevation of K^+ ions in the perfusing medium gave rise to the rapid aggregation of melanosomes (A). While ACh did not influence the state of melanophores (A), NE aroused melanosome aggregation very effectively (A-C). The action of NE on melanophores was effectively blocked by phentolamine (A). After thorough removal of the blocker, NE regained its action (A). Thus, NE could be employed for inducing the maximal level of melanosome aggregation. The pineal hormone, melatonin (B), and MCH (C) also elicited aggregation of melanosomes. Under the influence of MCH at a low strength (100 pM), the action of MSH on melanophores was tested, and as illustrated in Fig. 8C, a remarkable dispersion of melanosomes took place.

DISCUSSION

The dorsal to lateral surfaces of oceanic migratory fish normally display bluish hues, and it has been shown that coexistence of melanophores and iridophores is necessary for revealing such hues (Denton and Nicol, 1966; Land, 1972). The iridophores referred to here have been thought to be immotile cells, and each of them contains a stack or stacks of reflecting platelets about 70–100 nm in thickness. It is also known that the silvery glitter in the ventral skin of these fish is due to the lack of melanophores overlying the iridophores. Some fish, such as cutlassfish or hairtail (*Trichiurus lepturus*) show such extremely high reflectance over almost entire body

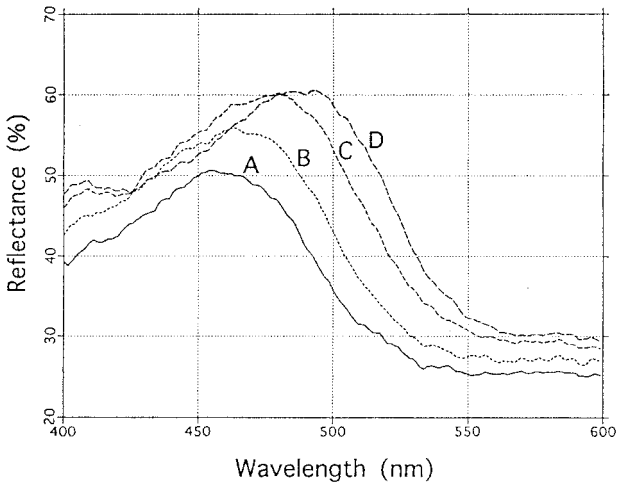

Fig. 6. Typical spectral reflectance curves obtained from a skin piece excised from the sky-blue part of the trunk. A: Equilibrated in physiological saline. B: 30 sec after the application of 50-mM K^+ saline. C: 60 sec after the application of K^+ saline. D: 90 sec after the application of K^+ saline.

surface. Such an incredibly high reflectivity of the skin over the range of visual light is due to the multilayered thin-film interference phenomenon of the ideal type, or the so-called "constructive" interference (Huxley, 1968; Land, 1972; Fujii, 1993a).

In addition, we can now enjoy deep, but vivid, fluorescence-like bluish skin hues exhibited by several species of coral-reef fish and sometimes by tropical freshwater ones, which can be reared in home aquariums. In studies of bluish damselfish, we recently reported that iridophores of a special type were aligned with melanophores and were distributed in packets in a single layer just under the epidermis (Oshima *et al.*, 1985; Kasukawa *et al.*, 1987; Fujii *et al.*, 1989). In the first paper in this series of investigations, we reported that the structural features of iridophores of the common surgeonfish were very similar to those reported for the damselfish (Goda *et al.*, 1994). It should be stressed that the light-reflecting stack of platelets were again extremely thin, being comparable with the cell membrane. The conclusion reached was that those

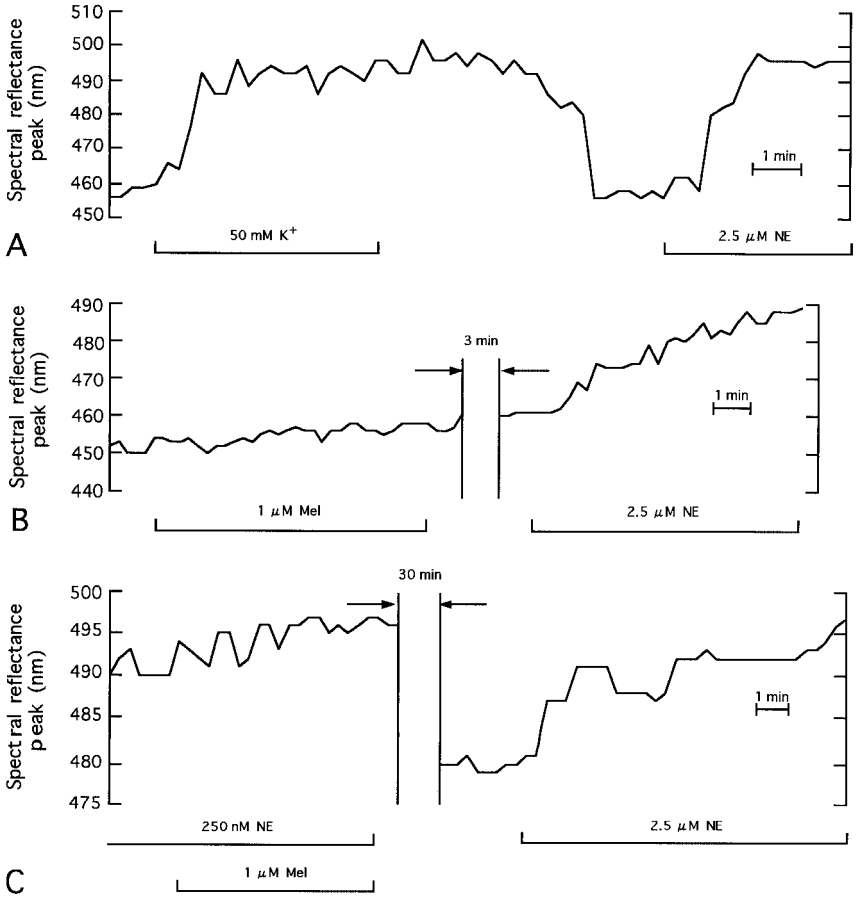

Fig. 7. Typical recording of motile responses of iridophores in the sky-blue portion of an isolated skin piece. Translocation of the position of the spectral peak reflected from the skin was followed by plotting the position of spectral peaks measured by MCPD-100 at intervals of 15 sec. Ordinate: Position of the spectral reflectance peak. Abscissa: time. (**A**) LR responses to an elevated K^+ concentration and to norepinephrine (NE). (**B**) A recording showing that melatonin was unable to induce an LR response. (**C**) A recording showing that melatonin was unable to induce an SR response.

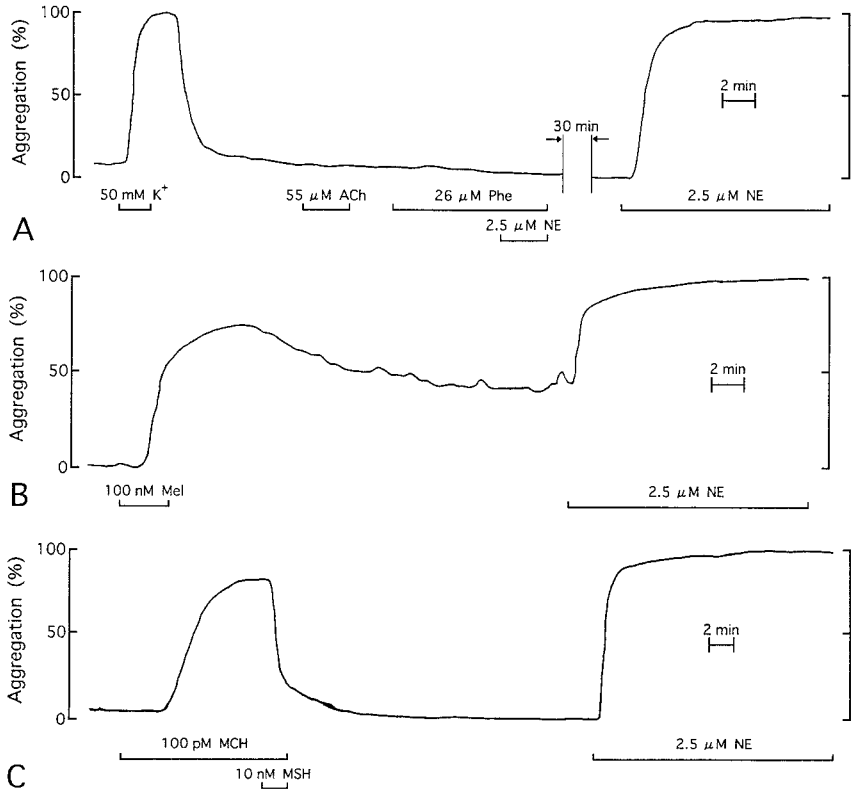

Fig. 8. Typical photoelectric recordings of responses of individual melanophores in the sky-blue portion of the surgeonfish. (**A**) A recording showing effects of K^+-rich saline, acetylcholine (ACh) and norepinephrine (NE). K^+-rich saline effectively aggregated melanosomes, while ACh failed to do so. Under the influence of an α-adrenergic blocking agent, phentolamine (Phe), NE lost its action. The melanosome-aggregating action of NE was regained after thorough washing of the blocker. (**B**) A recording showing the effect of melatonin (Mel); a gradual melanosome aggregation was elicited in response to 100 nM melatonin. (**C**) A recording showing the effect of MCH and MSH. Pigment aggregation was first induced by 100 pM MCH, and then, 10 nM MSH was applied in the presence of MCH. Rapid dispersion of pigment due to the action of MSH took place.

iridophores were primarily responsible for the generation of the characteristic bluish hue through the multilayer thin-film interference phenomenon of the non-ideal type, and that melanophores lining the sheet of iridophores may function to increase the purity of the blue color by shielding stray lightrays.

We have also described the presence in the dermis of surgeonfish of an organized, special combination of iridophores and melanophores which resembles that found in bluish damselfish (Goda *et al*., 1994). Being backed with a monolayer of melanophores, however, the iridophores existed in a double layer, and this current paper is concerned with optical and physiological analyses about the functions of such units of chromatophores.

As mentioned above, light-reflecting platelets in iridophores of bluish damselfish are extremely thin, being not more than 5 nm (Oshima *et al*., 1985; Kasukawa *et al*., 1987; Fujii *et al*., 1989; Fujii, 1993a). Such a situation should favor conditions required for the production of the characteristic fluorescence-like cobalt blue coloration of these species. The thickness of platelets in iridophores of surgeonfish was about 8 nm, i.e. a little larger (Goda *et al*., 1994). Assuming that they were uniformly 8 nm thick, therefore, we have made mathematical analyses based on the exposition by Huxley (1968). Measurements made in this study on light reflectance from the skin indicated that the spectral peak (λ_{max}) occured around 490 nm, although the position moved to a certain degree towards longer or shorter wavelengths in response to various environmental and stimulatory cues (Figs. 2 and 6). In this analysis, therefore, the position of the spectral peak was set at 490 nm.

Figure 9 shows a graphical representation of simulated curves for possible spectral reflectances from a stack of platelets, within the range of wavelengths between 400 and 550 nm. The refractive indices of guanine platelets and the cytoplasm were assumed to be 1.83 and 1.37, respectively (cf. Fujii, 1993a). A dull convex curve like a hill is a reference case, in which the interference system is assumed to be of the ideal type. To satisfy these conditions, the thickness of platelets (d_b) was calculated to be 66.94 nm, while the distance between adjacent platelets (d_a) was 89.42 nm. Both the height and the width of the spectral peak increases as the number of platelets in a pile increases (Land, 1972; Fujii, 1993a). In this figure, the case where the number of platelets in the stack is 4 is shown, but one should understand that, in an ideal system, such high reflectance can be achieved by a pile with such a small number of platelets.

Four other curves with smaller harmonics on both sides

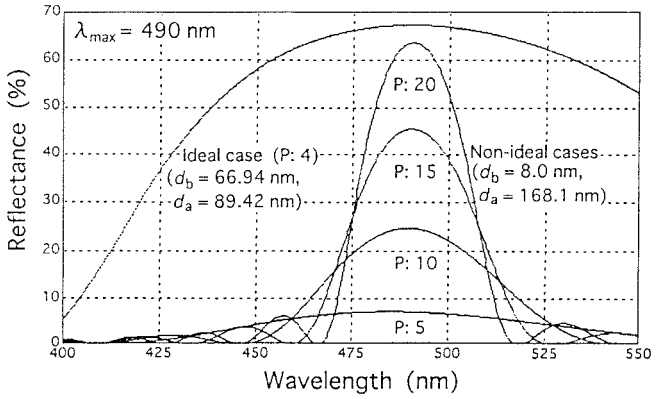

Fig. 9. Simulated spectral reflectance curves based on the mathematical treatment for the multilayered thin-film interference phenomenon of the non-ideal type, when the principal spectral peak (λ_{max}) was set at 490 nm, and the thickness of all platelets constituting a pile (d_b) was assumed to be 8 nm. Under such conditions, the distance between adjacent platelets (d_a) was calculated to be 168.1 nm. Curves generated when the number of platelets in the pile (P) was 5, 10, 15, and 20 are represented. It is clearly seen that, as the number of platelets increases, the height of the peak increases, whereas its width decreases to some extent. The broad peak around the top of the figure indicates the reference case where the interference system is of the ideal type. The thickness of platelets (d_b) and the distance between platelets (d_a) in this case were calculated as indicated in the upper left corner of the figure. Note that in the ideal system, such a high reflectance can be realized even when the number of platelets was only 4, at the expense of the purity of the color.

exhibit other possible cases, when the thickness of platelets was assumed to be constantly 8 nm. Of course, the actual system is far from the ideal type. From the bottom to the top, the curves represent cases in which the numbers of platelets are 5, 10, 15 and 20, respectively. We recognize that as the number of platelets increases, the height of the peaks becomes larger, but in contrast to the case of ideal interference, the width of the peak becomes somewhat smaller (Fujii *et al*., 1989; Fujii, 1993a), namely, the peaks become steeper. Based primarily on fine-structural observations of iridophores of surgeonfish (Goda *et al*., 1994), we presume that no less than 10 platelets are involved with reflection, when lightrays invade parallel to the axis of the pile. Incidentally, we have already presented the results of a similar simulation on the possible optical phenomena which occur in iridophores of bluish damselfish, where the thickness of platelets (d_b) and the position of the spectral peak (λ_{max}) were assumed to be 5 and 470 nm, respectively (Fujii, 1993a).

Although not as steep as damselfish (Kasukawa *et al*., 1987; Fujii *et al*., 1989; Fujii, 1993a), spectral reflectance peaks actually measured in the surgeonfish tegument were still fairly sharp (Figs. 2 and 6). The coloration of brighter portions of the surgeonfish can actually be expressed as “sky blue” or “cerulean blue”. In this connection, we have adequately described the bluish hue of damselfish as “cobalt blue” (Kasukawa *et al*., 1987; Fujii, 1993a). In any case, the results of our analyses on iridophores of surgeonfish favor the view that the existing system in cells can be treated as a thin-film interference phenomenon of the non-ideal type.

In addition to the thickness of platelets, there are other differences in the structural organization of skins of bluish damselfish and surgeonfish. First, the epidermis of surgeonfish is considerably thicker than that of many other smaller species, including damselfish. We know that the epidermis is usually fairly transparent to lightrays, but the scattering of light within the tissue inevitably increases in proportion to its thickness. Therefore, thicker skin may result in a lower purity of color, or a lower “chroma” in terms of the Munsell color notation system. Actually, such blue hues with higher purity, such as those of bluish damselfish or of surgeonfish, can not be found among larger fish, where the epidermis is naturally thicker.

We would like to point out, however, that the double layered pattern of iridophores in this species may be more deeply concerned with the dullness of the observed spectral peak, because lightrays reflected from iridophores in the bottom layer should be scattered in a complicated manner by iridophores in the top layer. If iridophores were in a single layer as in blue damselfish, the system for reflecting light would be simpler, and a purer blue color would be generated.

In addition, the general construction of the pigment cell layer in surgeonfish also resembles that described in blue mutants of a frog, *Rhacophorus schlegelii* (Nishioka and Ueda, 1985). The blue hue of the *Rhacophorus* frog is due to a genetic mutation which results in the lack of xanthophores. In normal adult forms of some anuran amphibians and of reptiles, xanthophores, iridophores and melanophores are arranged in layers from top to bottom, forming the so-called “dermal chromatophore unit” (Bagnara *et al*., 1968; Taylor and Hadley, 1970; Nishioka and Ueda, 1985). In iridophores of those amphibians and reptiles, light-reflecting platelets are smaller, but thicker, and are more randomly oriented. In contrast, in iridophores of the common surgeonfish or of the bluish damselfish, light-reflecting platelets are strikingly well-organized in the cytoplasm, and light-rays are reflected by the multilayered interference phenomenon (Kasukawa *et al*., 1987; Fujii *et al*., 1991; Fujii, 1993a). In iridophores of amphibians or reptiles, lightrays are considered to be reflected by Rayleigh scattering or the so-called “Tyndall phenomenon” (Bagnara and Hadley, 1973). By contrast, the arrangement of very thin platelets in iridophores of the surgeonfish is quite similar to that of the damselfish. Therefore, it is natural to suppose that identical optical phenomenon as disclosed in damselfish is responsible for the generation of bluish colors in surgeonfish as well.

A remarkable compression of the trunk is also a characteristic feature of the surgeonfish. In the posterior abdominal region, namely, from just posterior to the base of the caudal fin, for example, lightrays can traverse the body fairly easily. When pigmentary organelles in melanophores aggregated, skin transparency increases, and lightrays which penetrate from the other side of the trunk mingle with the rays simply reflected from iridophores, resulting in a decrease in the purity of coloration, or the chroma in the Munsell system. In other

words, that portion of the skin becomes more whitish. On the other hand, the part of the fish containing viscera is enclosed within the heavily pigmented peritoneum and does not allow the light to penetrate. Thus, the color is entirely due to chromatophores in the skin in front of the observer. Thus, the bluish tone largely remains, even when melanosomes in melanophores which line the strata of iridophores are fully aggregated. It can therefore be suggested that, in smaller fish like the bluish damselfish, or in very compressed fish like the surgeonfish, the dispersion of light-absorbing organelles in melanophores may function to enhance the vividness of the blue coloration characteristic to these beautiful fish.

Using isolated skin specimens of the common surgeonfish, we have confirmed that an elevation of K^+ ions in the perfusing medium induces the aggregation of pigment in melanophores and the LR response of motile iridophores (cf. Materials and Methods section). Norepinephrine had the same effect on both types of chromatophores. These effects were effectively antagonized by an α-adrenergic blocking agent, phentolamine, but acetylcholine had no effect on the state of these chromatophores. Thus, melanophores and iridophores are under the control of the sympathetic division of the autonomic nervous system mediated by α-adrenoceptors. Concerning the hormonal regulation of chromatophores, we have found that MCH, melatonin and MSH exert strong influences on the state of melanophores. These 3 factors are widely known biogenic pigment-motor principles, but none of them influenced motile iridophores, indicating that iridophores are primarily controlled by neural means. The sum of these observations favor the view that the regulatory system for chromatophores in common surgeonfish is quite similar to that of the bluish damselfish (Fujii, 1993a).

Our present observations indicate that, during the nighttime or in the dark, the sky-blue part of the surgeonfish becomes rather whitish with a shade of violet (Fig. 1), and measurement of spectral reflectance from the skin indicated that, under such conditions, the peak shifted towards the shorter wavelengths (Fig. 2). In other words, the SR response of iridophores (cf. Materials and Methods section) may have taken place. At this moment, however, no active intrinsic signals have been found that can induce the SR response in these cells.

We know that the sympathetic system is generally active during the day, while it becomes rather quiescent at night. As a coral-reef dweller, the surgeonfish is a diurnal animal, and thus, the above-mentioned principle should apply. We also know that when an isolated skin specimen of teleosts is equilibrated in physiological saline, melanosomes in melanophores disperse. Upon electrical stimulation of the controlling sympathetic postganglionic fibers, the aggregation of melanosomes takes place. This may indicate the lack of spontaneous firing of sympathetic fibers in excised specimens, as previously discussed (Fujii, 1993a; Fujii and Oshima, 1994). In excised skin pieces of the surgeonfish, melanosomes were also found to disperse in physiological saline. Under the same conditions, iridophores reflect lightrays of shorter wavelengths, i.e. those cells are at the terminal state of the SR response. In the daytime, chromatophores *in vivo* are governed by nervous discharges at a moderate rate, such as 5 Hz (cf. Fujii and Miyashita, 1975; Fujii, 1993a), although the frequency varies in accordance with various environmental and intrinsic conditions. During the night or in the dark, the frequency descreases, and iridophores reflect rays of shorter wavelengths. Namely, the observed shift of the spectral reflectance towards shorter wavelengths is due to the SR response of iridophores which results from a decrease in the frequency of sympathetic discharges.

Being expressed in the common term of "blue", however, the hue of the tegument varied within a rather limited range (Fig. 1), and the actually measured shift of the spectrum was also limited (Figs. 2 and 6). Notwithstanding the fact that the range is limited, we should consider here the cellular mechanism by which LR and SR responses are induced. Remembering the cases of bluish damselfish (Oshima *et al*., 1985; Kasukawa *et al*., 1987; Fujii *et al*., 1989), we naturally assume that those responses are due to simultaneous changes in the distance between light-reflecting platelets constituting the piles within iridophores. Namely, the increase or decrease in distance is the cause of the translocation of the spectral peak towards longer or shorter wavelengths.

In addition to such changes in hue, changes in the darkness of the color, or in the "value" in the Munsell terminology, were also observed in response to the environmental luminosity in the surgeonfish (Fig. 1). It may be said that those changes are more remarkable than those of the hue. The aggregation of melanosomes in melanophores is naturally considered to be the principal cause of the paler night coloration, as confirmed microscopically on fixed skin specimens (Fig. 3C and F; Fig. 4B). In the day-night changes of skin coloration, therefore, melanophores seem to have the larger influence, leaving the involvement of iridophores rather limited.

The aggregation of dark organelles in melanophores naturally increases the transparency of the skin. When the environmental luminosity is low, the pineal hormone, melatonin, acts as follows: We know that in many vertebrate forms, melatonin levels increase remarkably at night. Our study shows that in the dark, surgeonfish become whitish and partly transparent, and that in the seawater containing melatonin, very similar changes in coloration are inducible. Melatonin also induces the aggregation of pigment in melanophores *in vitro*. By losing the characteristic sky-bluish hue of the trunk at night, these fish may escape attack by nocturnal predators. Exhibiting sober hues or becoming transparent in the dark may thus increase their rate of survival. We have recently reported such cases of fish becoming undemonstrative during the night in two tetra fishes including the neon tetra *Paracheirodon innesi* and the cardinal tetra *P. axelrodi* (Hayashi *et al*., 1993), and in the medaka *Oryzias latipes* (Hoizumi and Fujii, 1996).

In addition to color changes due to environmental luminosity, changes in response to certain environmental and ethological cues have also been recognized, although our observations in this regard are still in a very primitive stage. In these

cases too, changes in the darkness, or the "value" in the Munsell terminology, are naturally thought to depend on motile activities of melanophores. The hue component in those changes was also detected, although the range was again narrow within the region of blue. Motile iridophores should be responsible for such changes. At present, we do not know whether such delicate changes have some certain physiological or ethological significance or not, but presumably, they might have some significance in social encounters among conspecifics. By contrast, the characteristic scissors-like dark pattern over the sky-blue background color, along with the bright yellow color of the tail fin, may function as social signals for mutual identification among species in their habitat (cf. Fujii, 1993b). Further investigations should clarify these interesting ethological issues.

As a member of the family Acanthuridae, suborder Acanthuroidei, the common surgeonfish belongs to a considerably different group of fish from the damselfish which belong to Pomacentridae, suborder Percoidei. Since they belong to different suborders, they are phylogenetically considerably remote from each other, although both of them belong to the very large order Perciformes. As stated above, iridophores we have found in the common surgeonfish are very similar to those of the damselfish, and it is remarkable that such very similar iridophores exist in fish of different suborders. At this moment, we have no information as to whether iridophores in either group of fish have a common origin or whether they have evolved independently in each group in more recent evolutionary time. We also have no information about the presence of such iridophores among groups of fish that stand phylogenetically between Acanthuridae and Pomacentridae, although we presume that such cells may exist at least in some closely related acanthurid species. Further surveys on other families and orders of teleosts will be necessary to resolve this issue. Such data may provide some clues as to the origin of motile iridophores of the damselfish type among teleostean fishes.

In the Introduction, we noted that chromatophores containing blue pigment have not yet been described in vertebrates. Very recently however, we found novel chromatophores containing blue-colored pigmentary organelles in two coral-reef dwellers that belong to the family Callionymidae, and we named the cells "cyanophores" and the organelles "cyanosomes" (Goda and Fujii, 1995). Cyanosomes aggregate or disperse within cells, resulting in changes in the bluish shades of these species. The mechanism by which the bluish coloration is generated is distinct from that in iridophores of damselfish or of surgeonfish. Finding so many varieties of chromatic systems, we are really struck with admiration by the wonderful diversity existing in the world of poikilotherms. If we recall the long phylogenetic history of teleostean fishes over 200 million years, we may also be able to recognize recent circumstances as a natural consequence of the accumulation of innumerable mutational changes which are advantageous to their strategy for survival. The magical architectural and physiological devices for the revelation of colors and their changes have certainly been developed by animals over generations during their struggle for better adaptation to their environment.

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REFERENCES

Bagnara JT, Taylor JD, Hadley ME (1968) The dermal chromatophore unit. J Cell Biol 38: 67–79

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

Clothier J, Lythgoe JN (1987) Light-induced colour changes by the iridophores of the neon tetra, *Paracheirodon innesi*. J Cell Sci 88: 663–668

Denton EJ, Nicol JAC (1966) A survey of reflectivity in silvery teleosts. J Mar Biol Assoc UK 46: 685–722

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, FL, 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, Kasukawa H, Miyaji K, Oshima N (1989) Mechanism of skin coloration and its changes in the blue-green damselfish, *Chromis viridis*. Zool Sci 6: 477–486

Fujii R, Hayashi H, Toyohara J, Nishi H (1991) Analysis of the reflection of light from motile iridophores of the dark sleeper, *Odontobutis obscura obscura*. Zool Sci 8: 461–470

Fujii R, Tanaka Y, Hayashi H (1993) Endothelin-1 causes aggregation of pigment in teleostean melanophores. Zool Sci 10: 763–772

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

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

Goda M, Fujii R (1995) Blue chromatophores found in two species of callionymid fish. Zool Sci 12: 811–813

Hayashi H, Sugimoto M, Oshima N, Fujii R (1993) Circadian motile response of erythrophopres in the red abdominal skin of tetra fishes and its possible significance in chromatic adaptation. Pigment Cell Res 6: 29–36

Hoizumi T, Fujii R (1996) Becoming transparent of small fishes in dark and its ethological significance. Pigment Cell Res 8: 330

Huxley AF (1968) A theoretical treatment of the reflexion of light by multilayer structures. J Exp Biol 48: 227–245

Kasukawa H, Oshima N, Fujii R (1987) Mechanism of light reflection in blue damselfish motile iridophores. Zool Sci 4: 243–257

Land MF (1972) The physics and biology of animal reflectors. Progr Biophys Mol Biol 24: 75–106

Nagaishi H, Oshima N, Fujii R (1990) Light-reflecting properties of the iridophores of the neon tetra, *Paracheirodon innesi*. Comp

Biochem Physiol 95A: 337–341
Nishi H, Fujii R (1992) Novel receptors for melatonin that mediate pigment dispersion are present in some melanophores of the pencil fish (*Nannostomus*). Comp Biochem Physiol 103: 363–368
Nishioka M, Ueda H (1985) Electron-microscopic observation on the dermal chromatophores of normal frogs and three kinds of color variants in *Rhacophorus schlegelii*. Sci Rep Lab Amphibian Biol Hiroshima Univ 7: 123–155
Oshima N, Fujii R (1984) A precision photoelectric method for recording chromatophore responses *in vitro*. Zool Sci 1: 545–552
Oshima N, Sato M, Kumazawa T, Okeda N, Kasukawa H, Fujii R (1985) Motile iridophores play the leading role in damselfish coloration. In "Pigment Cell 1985: Biological, Molecular and Clinical Aspects of Pigmentation" Ed by JT Bagnara, SN Klaus, E Paul, M Schartl, Univ Tokyo Press, Tokyo, pp 241–246
Oshima N, Kasukawa H, Fujii R (1989) Control of chromatophore movements in the blue-green damselfish, *Chromis viridis*. Comp Biochem Physiol 93C: 239–245
Reed BL (1968) The control of circadian pigment changes in the pencil fish: a proposed role for melatonin. Life Sci Part II 7:961–973
Taylor JD, Hadley ME (1970) Chromatophores and color change in the lizard, *Anolis carolinensis*. Z Zellforsch 104: 282–294

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