Fish Physiol Biochem
https://doi.org/10.1007/s10695-024-01300-1

RESEARCH

Neurotransmitter norepinephrine regulates chromatosomes aggregation and the formation of blotches in coral trout *Plectropomus leopardus*

Nannan Zhao · Ke Jiang · Xiaoyu Ge · Jing Huang · Caiming Wu · Shi Xi Chen

Received: 13 October 2023 / Accepted: 10 January 2024

Abstract Color changes and pattern formations can represent strategies of the utmost importance for the survival of individuals or of species. Previous studies have associated capture with the formation of blotches (areas with light color) of coral trout, but the regulatory mechanisms link the two are lacking. Here, we report that capture induced blotches formation within 4–5 seconds. The blotches disappeared after anesthesia dispersed the pigment cells and reappeared after electrical stimulation. Subsequently, combining immunofluorescence, transmission electron microscopy and chemical sympathectomy, we found blotches formation results from activation of catecholaminergic neurons below the pigment layer. Finally, the in vitro incubation and intraperitoneal injection of norepinephrine (NE) induced aggregation of chromatosomes and lightening of body color, respectively, suggesting that NE, a neurotransmitter released by catecholaminergic nerves, mediates blotches formation. Our results demonstrate that acute stress response-induced neuronal activity can drive rapid changes in body color, which enriches our knowledge of physiological adaptations in coral reef fish.

Keywords Blotches · Coral trout · Chromatosomes aggregation · Catecholaminergic neurons · Norepinephrine

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10695-024-01300-1.

N. Zhao · K. Jiang · X. Ge · J. Huang · S. X. Chen (✉)
State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen 361102, People's Republic of China
e-mail: chenshixi@xmu.edu.cn

C. Wu
State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen 361102, People's Republic of China

S. X. Chen
State-Province Joint Engineering Laboratory of Marine Bioproducts and Technology, Xiamen University, Xiamen 361102, People's Republic of China

Introduction

The spectacular variations in animal's beautiful coloration and conspicuous patterns have attracted a great deal of attention (Goda and Kuriyama 2021; Swierk 2022). For the animals themselves, these color changes and pattern formations can represent strategies that are critical to the survival of an individual or species (Bandaranayake 2006; Goda and Kuriyama 2021). Color change is the ability of an organism to modify its coloration in response to specific stimuli (Figon and Casas 2018). Several biological functions have been proposed to explain it, including thermoregulation, ultraviolet (UV) protection, crypsis,

Published online: 31 January 2024

inter- and intraspecific communication (Figon and Casas 2018; Rudh and Qvarnström 2013). Also, some cryptic coloration and patterns is useful for avoiding attacks by predatory animals (Fujii and Oshima 1994). To date, many studies have been devoted to shedding light on these color characteristics. It is known that, when excited, some animals become lighter in color while others darken. These conditions are called "excitement pallor" and "excitement darkening", respectively (Fujii and Oshima 1994). *Plectropomus leopardus* (Lacépède, 1802), commonly known as the coral trout (Qu et al. 2012), usually exhibits red (dark-, normal- and light-red) or brown (dark-, normal-and light-brown) color patterns (Shimose and Kanaiwa 2022). Studies have reported that when under disturbances, such as water flow stimulation and light change, blotches (areas with light color) appeared on the fishes. Besides, when they are captured, coral trout developed an acute stress response and blotches formed on their skin. Further observations of the location of pigment granules within chromatophores under capture-induced stress and non-stress conditions attributed the formation of blotches to the aggregation of chromatosomes (Zhou et al. 2021). How to link capture to chromatosomes aggregation remains a key question in studies of the mechanisms underlying the diversity of body color variation in grouper species. Specifically, the pathway by which capture cause chromatosomes to aggregate in coral trout are yet to be explored.

In fish, changes in color pattern caused by the motile activities of chromatosomes, aggregation or dispersion, are called “physiological color changes” (Fujii 2000; Ligon and McCartney 2016). The motile activities are regulated by the endocrine and/or nervous system, and the changes are rather rapid and involved in faster chromatic adaptations (Goda and Kuriyama 2021). The coordinating systems for the motile activities of chromatosomes found in fish show rich diversity (Goda and Kuriyama 2021). Primitive fishes are most susceptible to the influence of the hormones, α-melanophore-stimulating hormone (α-MSH) (Yamaguchi et al. 2022), melanophore-concentrating hormone (MCH) (Enami 1955; Nagai et al. 1986), and melatonin (MT) because their physiological color change is mostly controlled by the endocrine system (Fujii 2000). In teleosts, a neural mechanism is also involved. The peripheral chromatic nerves regulating chromatosomes motility belong to the sympathetic division of the autonomic nervous system (Fujii and Oshima 1986). Previous researcher have observed dense networks of nerve fibers around the chromatophores (Ballowitz 1893; Yamada, et al. 1984). Employing radiolabeling method, Kumazawa and Fujii actually showed that norepinephrine (NE) is released from nervous elements in response to nervous stimuli (Kumazawa and Ryozo 1984). Examples of in vitro incubations with NE can be collected from numerous species, e.g., incubation of zebrafish *Danio rerio* melanophores with 5 μM NE resulted in complete aggregation of melanosomes within 5 min (Nguyen et al. 2006); 3 min after application of 5×10^{-6} μM NE, the melanophores and xanthophores on isolated dorsal-fins piece of scalycheek damselfish *Pomacentrus lepidogenys* were completely aggregated (Kasukawa and Oshima 1987). Although the response of pigment cell to NE injection has been observed in winter flounder *Pseudopleuronectes americanus* (Burton 1985), the in vivo effects of NE have not been as well studied. Also, whether NE is released from neuron close to chromatophores remains unclear in coral trout.

The variable and easy-to-observe body color patterns of the coral trout provides a good model for studying the physiological changes of body color. In the present study, using anesthesia followed by electrical stimulation, we determined that the formation of the blotches is regulated by the nervous system. Further immunofluorescence labeling with tyrosine hydroxylase (TH, the catecholaminergic neurons marker (Jones and Beaudet 1987)), transmission electron microscopy and chemical sympathectomy revealed that the catecholaminergic neurons control the formation of blotches. Finally, in vitro incubation and in vivo injection experiments elucidated that the catecholamine neurotransmitters NE mediated the chromatosomes aggregation and body color lightening.

Material and methods

Experimental fish

The coral trout were purchased from Xiao Deng Fisheries Technology Co., LTD and Xia Shang Aquatic Market (Xiamen, Fujian province, China), with body lengths of 30—35 cm and weights of 400—450 g.

After transfer to our laboratory, the coral trout were housed in indoor plastic tanks (upper diameter 50.5 cm, height 54.5 cm, lower diameter 35.5 cm) with seawater at salinity 28 and temperature 25 °C for about one week before use. During this temporary culture period, fish were hand-fed twice daily with a commercial feed (grouper compound feed; TianMa Technology Group Co., Ltd., Fujian).

Capture, anesthesia and electrical stimulation treatments

We pre-installed a video camera (SONY DSC-HX300, Japan) outside the tank to record the changes in body coloration of coral trout. After recording the body color in the unstressed state, the coral trout were captured with a fishing net and transferred to another transparent tank (30 cm × 16.5 cm × 20 cm, length × width × height). Recordings stopped immediately after the blotches appeared on the fish's body. We selected photographs of the unstressed condition and the capture-induced blotches. After the fish were captured, visible blotches appeared on the dorsal, flank, and ventral skin, so we extracted the Red, Green, and Blue (RGB) values and color code of the 3 skin regions using the online tool Color Picker. Three randomly selected locations in each region with intervals greater than 1 cm were measured, and the results are shown as their average values. We then transformed the RGB model to an HSL model that is closer to the human-eye perception (Sonka et al. 2013), where colors are defined by three parameters: hue, saturation, and brightness (Urban et al. 2013). Hue values range from 0° to 360°, each representing a distinct color. Saturation is measured as a percentage from 0% (white) to 100% (fully saturated color). Brightness is measured as percentage from 0% (black) to 100% (fully bright color) (Cubukcu and Kahraman 2008). The experiments were repeated three times on three different fish (n = 3 fish).

Then, anesthetic MS222 (CAS: 886–86-2; E10521, Sigma-Aldrich, USA) was dissolved in seawater of the tank with a final concentration of 100 mg/L. Images were taken before and after the anesthesia. A JL-A electronic stimulator (Shanghai, China) was used to perform the electrical stimulation (Figure S1A). A unidirectional square pulse of constant pulse width (0.6 ms) with no interphase gap, 40 Hz in stimulation frequencies and 50 V in strength were applied for 0.25 s. The front end of the electrode was placed on the skin of the anaesthetized fish (Figure S1B). The stimulation ranges from the position of the electrode to the surrounding radiation of about 2.5 cm, similar to a circle (Figure S1C-D). Images were taken using a camera (SONY DSC-HX300, Japan) before and after the electrical stimulation. Meanwhile, HSL values were acquired (n = 3) during this process using the online tools Color Picker. The video recording was shot in the studio in the same shooting mode. The experiments were repeated three times on three different fish.

Immunofluorescence

Skin with blotches (1 cm * 1 cm) were dissected carefully from the dorsal side with a sterilized scalpel and washed three times in phosphate buffer saline (PBS, Solarbio, Beijing, China). After that, they were fixed in 4% paraformaldehyde (PFA, CAS: 30525–89-4; Sigma-Aldrich, USA) for 12 h at 4℃, immersed in 30% sucrose overnight at 4℃, and then embedded in OCT (Sakura Finetek, Torrance, CA, USA). 40-μm sections were prepared by a cryostat (Leica CM 1950, Leica Biosystems) and used for immunofluorescent staining. First, slides were blocked (10% goat serum and 0.2% Triton X-100 in PBS) for 1 h at room temperature, incubated with primary antibody (TH, mouse, Millipore Cat# MAB318, LOT: 3782107, dilution: 1:100) or blocking solution (control) overnight at 4℃, then incubated with secondary antibody (Goat anti-mouse IgG H&L, Abcam ab150113, 1:250) for 2 h at room temperature. Images were acquired using a Leica DM2500 LED optical microscope (Leica, Wetzlar, Germany) equipped with a Leica DFC7000 T camera (Leica Application Suite X, Leica Microsystems GmbH; Wetzlar, Germany). The experiment was repeated three times on three different fish. In each immunofluorescence experiment, we dissected 3 locations of skin from a fish, sectioned them separately, and finally showed the fluorescence results of one of the pieces that could represent the whole.

Transmission electron microscopy (TEM)

Skin with blotches was isolated from one coral trout, which was later sacrificed. Skin samples were washed three times in PBS and then fixed in 2.5% glutaric

dialdehyde in 0.2 M phosphate buffer at 4 °C overnight. After being post-fixed in 2% osmium tetraoxide in 0.2 M phosphate buffer for 2 h at 4 °C, the skin samples were washed three times in distilled water, dehydrated in a graded series of acetone solutions (50, 70, 90, and 100%) for 15 min at 4 °C and twice each step, soaked in acetone: resin mixture (3:1) twice for 30 min, and immediately embedded in a Spurr resin (SPI, West Chester, USA). Ultrathin (65 nm) sections were cut vertically to the plane of the skin using an ultramicrotome with a diamond knife, and then mounted on coated copper grids. At last, the sections were stained with uranyl acetate and lead citrate, and observed under an electron microscope (Philips CM100, Eindhoven, Netherlands).

Intraperitoneal injection of 6-OHDA

For chemical sympathetic nerve ablation, 6-hydroxydopamine (6-OHDA), the catecholaminergic neurotoxin (Glinka et al. 1997; Kostrzewa and Jacobowitz 1974), was applied. 6-OHDA can ablate the sympathetic nerve efficiently but spared the sensory nerves and other cell types (Shwartz et al. 2020) and is known to promote a specific and reversible degeneration of the nerve terminals (Junqueira and Salles 1978). In reasonable amounts, 6-OHDA will destroy catecholaminergic neurons with a high degree of selectivity (Kostrzewa and Jacobowitz 1974). In this study, 6-OHDA solution was prepared freshly by dissolving oxidopamine hydrobromide powder (CAS: 636–00-0; T12352L, TargetMol, USA) in 0.1% ascorbic acid in 0.9% sterile NaCl. 120 mg/kg (body weight) of 6-OHDA was injected intraperitoneally for two consecutive days with an interval of 24 h. Control fish were injected with equivalent volume of vehicle (0.1% ascorbic acid in 0.9% sterile NaCl). For intraperitoneal injections, a sterile disposable injection needle was carefully inserted into the midline between the pelvic fins. The needles were pointed towards the head and closer to the pelvic girdle than to the anus. The appropriate amount of 6-OHDA or vehicle was injected when the needle was felt to penetrate deep into the body wall, after which the needle was removed. The fish was gently returned to the tank after the injection. The injection experiment was repeated three times with six fish. The color patterns of the skin in two groups were photographed before injection and 48 h after the first injection.

Effects of NE on scales chromatophores in vitro and body color pattern in vivo

Norepinephrine (NE) (CAS: 51–41-2; N814761, Macklin, Shanghai, China) was dissolved into 10 mL PBS to obtain a solution at a concentration of 10 mM and part of the solution was diluted with PBS to a concentration of 10 μM. Then, 10 μM NE were applied to the excised scales and skin, and the PBS was used as control. The morphology changes of chromatophores on scales were observed and photographed using Leica DM2500 LED optical microscope (Leica, Wetzlar, Germany) equipped with a Leica DFC7000 T camera (Leica Application Suite X, Leica Microsystems GmbH; Wetzlar, Germany). The body color pattern change on skin were photographed using a camera (SONY DSC-HX300, Japan) and HSL values were recorded. 1 mL NE (10 mM) and PBS (Control) were intrathoracic injected into the body of coral trout, respectively. For intrathoracic injections, a sterile disposable injection needle was inserted into the scaly depression at the base of the fish's pectoral fins and pierced towards the front of the fish at an angle of 45–60 degrees to the body axis to a depth of approximately 1 cm. The fish was gently returned to the tank after the injection. The changes of body color patterns of coral trout were observed and photographed using a camera (SONY DSC-HX300, Japan). HSL changes during this process were also analyzed. Both in vitro incubation and in vivo injections were carried out three times. Each experiment was carried out with one fish each time. For each fish we randomly chose 3 locations to measure and averaged them to make $n = 1$.

Data analysis

The Software SPSS 19.0 (SPSS Incorporation) was used for the statistical evaluation. SL values under different situations (non-stress vs. capture; before vs. after electrical stimulation; before vs. after adding NE) were compared by two-tailed Student's *t*-test ($p < 0.05$). The results are displayed as changes in the latter situations relative to the former situations. The NE injection data were checked for homogeneity of variances using a Levene's test and then analyzed by

one-way analysis of variance (ANOVA) followed by a Tukey's test ($p < 0.05$). The results are displayed as changes after NE injection relative to pre-injection.

Results

The formation of blotches is controlled by the nervous system

To identify the factors that regulate the formation of blotches, we first photographed and recorded the process of this body color change. When being captured, blotches formed rapidly on the skin of coral trout, especially dorsal, flank and caudal skin, within 4–5 seconds (Fig. 1A, B). The hue of the corresponding skin changed from near red to orange (dorsal: 1°-3° to 12°-13°; flank: 0°-5° to 7°-13°; ventral: 0°-3° to 9°-12°). Brightness has relatively increased, accompanied by a slight decrease in saturation (Fig. 1C). Anesthesia then effectively dispersed the chromatosomes, causing existing blotches to disappear and the skin to become redder or darker (Fig. 2A, B). We therefore hypothesized that the formation of blotches might be regulated by neural mechanisms. Subsequent electrical stimulation caused the re-formation of the blotches-like pattern on the skin (Fig. 2C, D, dotted box areas) and corresponded to a change in hue (2–3° to 15–19°) as well as relatively higher skin lightness and lower saturation (Fig. 2E), which indicated that the blotches are indeed under the control of the nervous system.

The catecholaminergic neurons beneath the pigment layer control the formation of blotches

The sympathetic nervous system was previously reported to be responsible for the flight-or-fight response to stressful stimuli (Kindermann et al. 2014), which is brought about by the release of catecholamines (Maeno and Iga 1992). To determine whether the appearance of blotches are under the control of

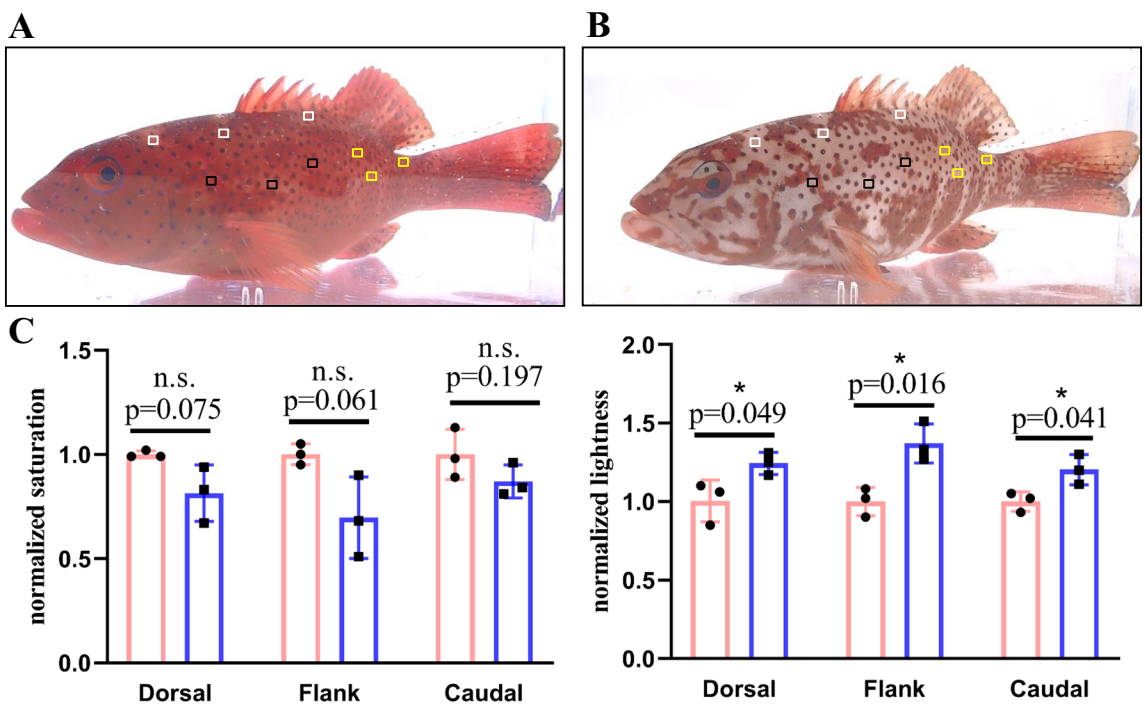

Fig. 1 Body color pattern of the coral trout *Plectropomus leopardus* under different physiological states. **A** In the non-stress condition, the coral trout showed a uniform body color pattern. **B** Blotches formed on the skin 4–5 seconds after being captured. **C** The normalized saturation and lightness values of three skin regions under two conditions. Dorsal (white box in A &B); Flank (black box in A &B); Caudal (yellow box in A &B). n = 3 biological replicates for each condition, two-tailed independent samples *t*-test. All bar graphs are mean ± S.D. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant

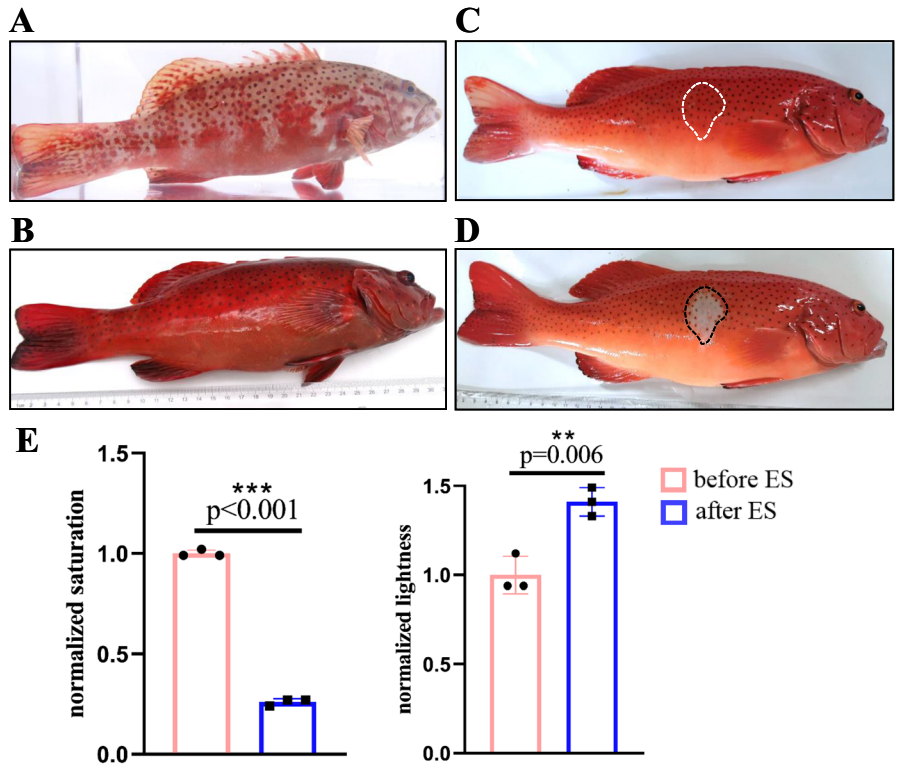

Fig. 2 Changes in blotches of coral trout under different treatments. **A** Blotches formed on the skin after being captured. **B** After MS222 anesthesia, blotches disappeared. **C**, **D** After stimulated with electric current (white dotted box area), blotches-like pattern formed on the skin (black dotted box area). **E** The normalized saturation and lightness values of skin before and after electrical stimulation (ES). n = 3 biological replicates for each condition, two-tailed independent samples *t*-test. All bar graphs are mean ± S.D. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant

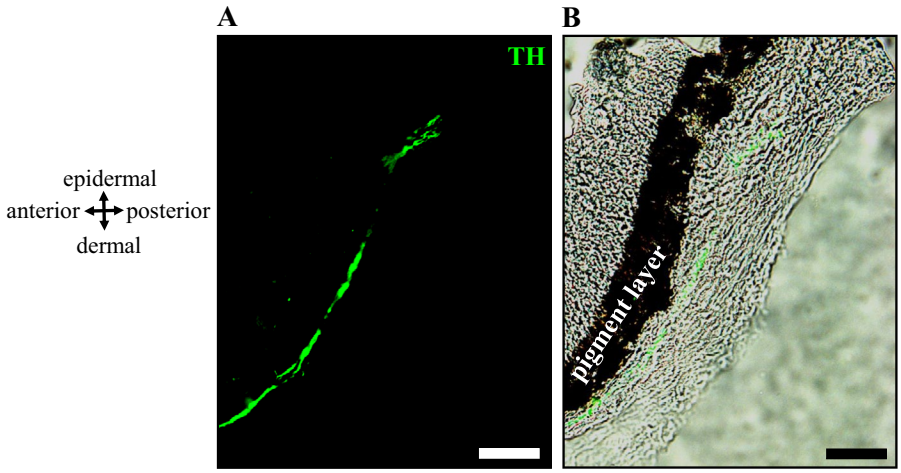

Fig. 3 Immunofluorescence showed catecholaminergic neurons located below the pigment layer. **A** Immunofluorescence with anti-tyrosine hydroxylase (TH) antibody labeling catecholaminergic neurons in skin of the blotches region. **B** Merge image of (**A**) and bright field show the location of TH and pigment layer. Scale bars, 50 μm (**A**, **B**)

catecholaminergic neurons, we performed immunofluorescence. We observed that TH-positive (+) neurons (Fig. 3A) were located under the pigment layer (Fig. 3B), indicating that these neurons are catecholaminergic. In addition, underneath the pigment cells in the dermis (Fig. 4A), TEM results showed neurons containing multiple myelinated axons with compact structure (Fig. 4B).

To further test if activation of catecholaminergic neurons is responsible for the appearance of blotches, we ablated them with 6-hydroxydopamine (6-OHDA) (Fig. 5A). Chemical sympathectomy by 6-OHDA promoted an evident and almost immediate paling of the coral trout and six hours later, they gradually became much darker than the controls (data not shown). Two days after the first injection, 6-OHDA

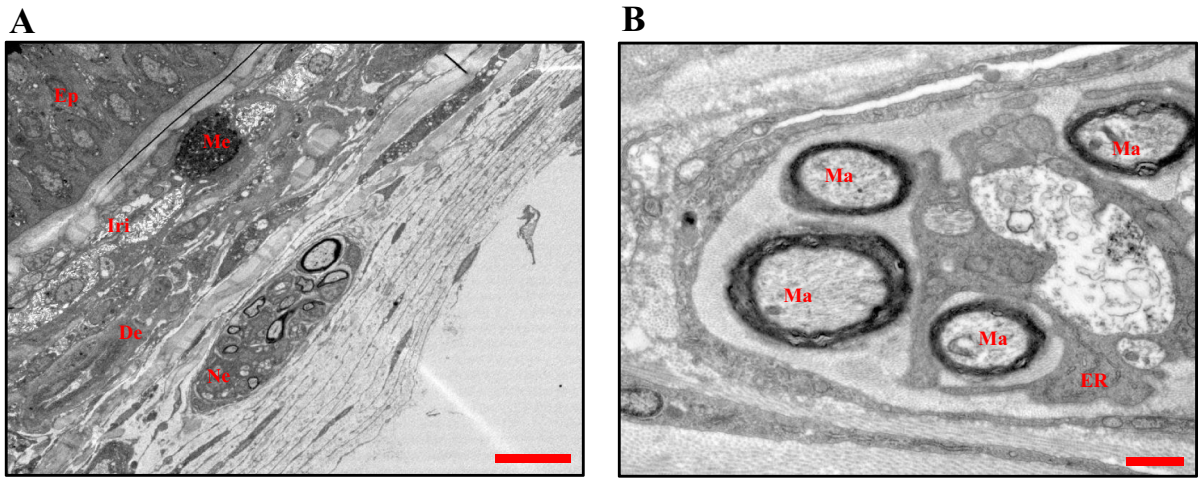

Fig. 4 TEM showed the structure of neuron. **A** The neuron in the dermis. Ep, epidermis; De, dermis; Me, melanophores; Iri, iridophores; Ne, neuron. **B** close-up of (**A**). Ma, myelinated axons; ER, endoplasmic reticulum. Scale bars, 10 μm (**A**); 1 μm (**B**)

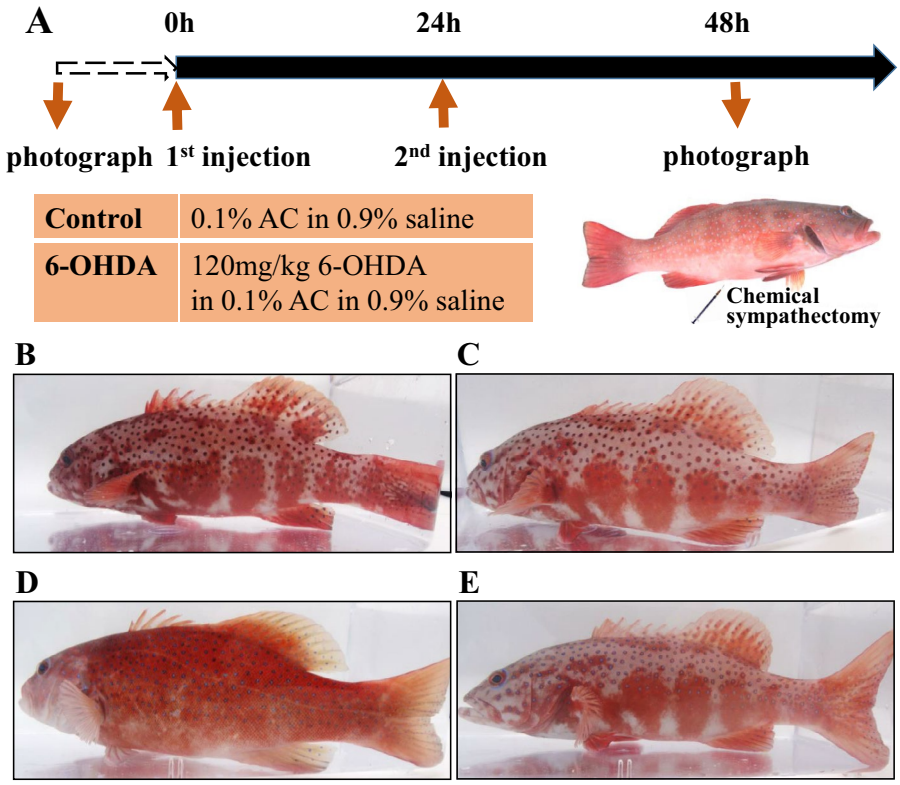

Fig. 5 The effect of 6-OHDA on color pattern change of coral trout. **A** Schematic of experimental design for 6-OHDA injection and observation. Fish in 6-OHDA (**B**) and control (**C**) groups formed blotches after being captured. The coral trout injected with 6-OHDA no longer produce blotches (**D**), while those in control group were unaffected (**E**)

blocked their capacity to show blotches when they are captured (Fig. 5B, D), and blotches still formed in the control group (Fig. 5C, E). This suggested that catecholaminergic nerves indeed control the appearance of capture-induced blotches.

The catecholaminergic neurotransmitter NE mediated the pigment aggregation and body color lightening

NE is an important catecholaminergic neurotransmitter that has been reviewed in several articles and is

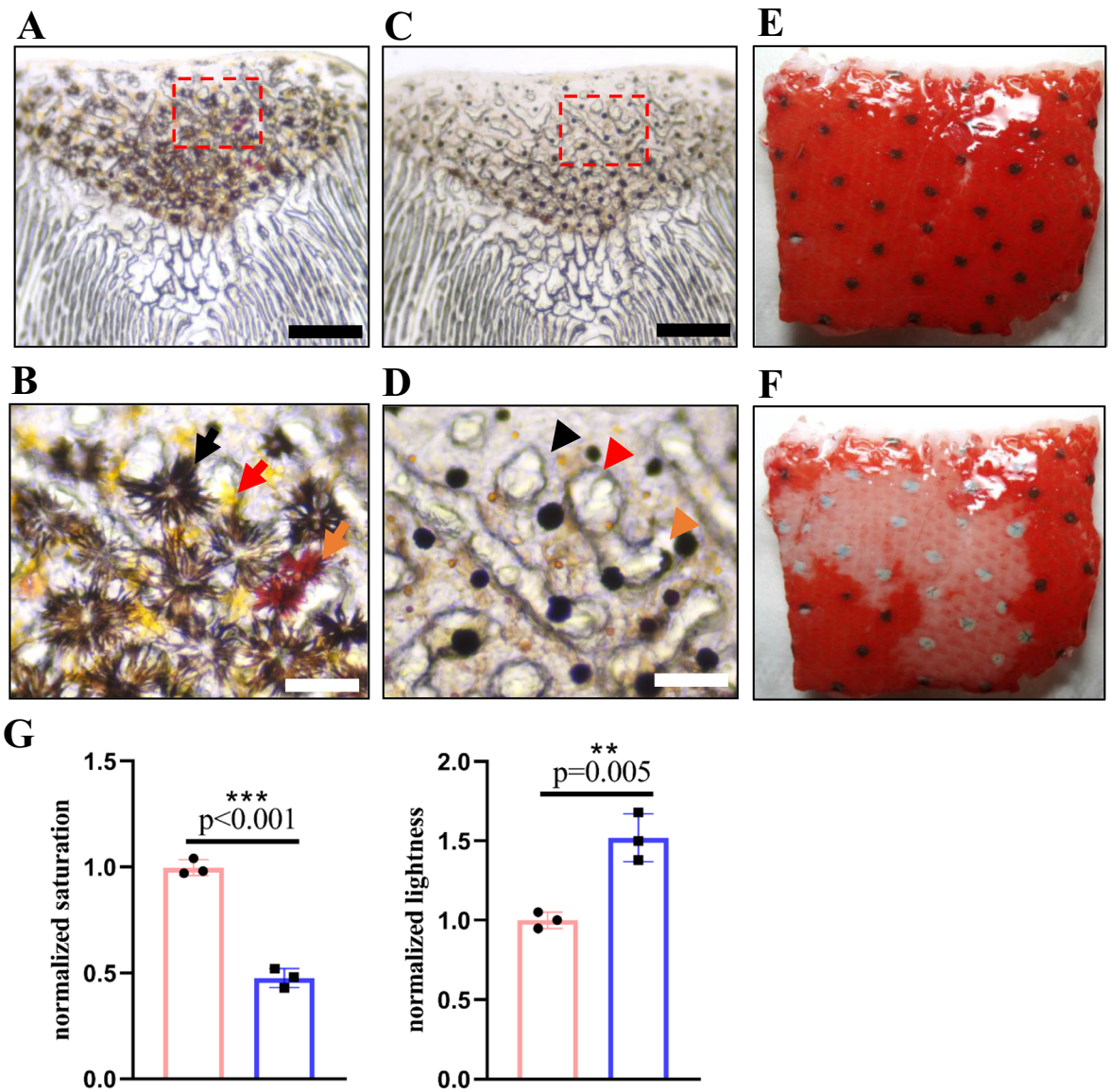

Fig. 6 The effects of NE on chromatosomes in scales and skin. **A** Before NE treatment, the chromasomes were dispersed. **B** close-up of boxed region in (**A**). **C** After NE treatment, the chromasomes were aggregated. **D** close-up of boxed region in (**C**). Black arrow and arrowhead, melanophores; red arrow and arrowhead, xanthophores; Orange arrow and arrowhead, erythrophores. Scale bars, 200 µm (**A**, **C**); 50 µm (**B**, **D**). **E** The uniform color pattern before adding NE. **F** The blotches-like color pattern after adding NE. **G** The normalized saturation and lightness values of skin tissue before and after adding NE. n = 3 biological replicates for each condition, two-tailed independent samples *t*-test. All bar graphs are mean ± S.D. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant

responsible for signal transmission and leads to pigment aggregation in most fish (Fujii 2000; Fujii and Oshima 1994). We therefore assessed the effect of NE on the location of pigment granules within chromatophores in vitro and color pattern in vivo. After NE treatment, the pigment granules inside the chromatophores on the scales (Fig. 6A, B) become aggregated, taking on a compact circular shape (Fig. 6C, D). Skin treated with NE showed a blotches-like color pattern (Fig. 6E, F), along with a change in degrees of hue

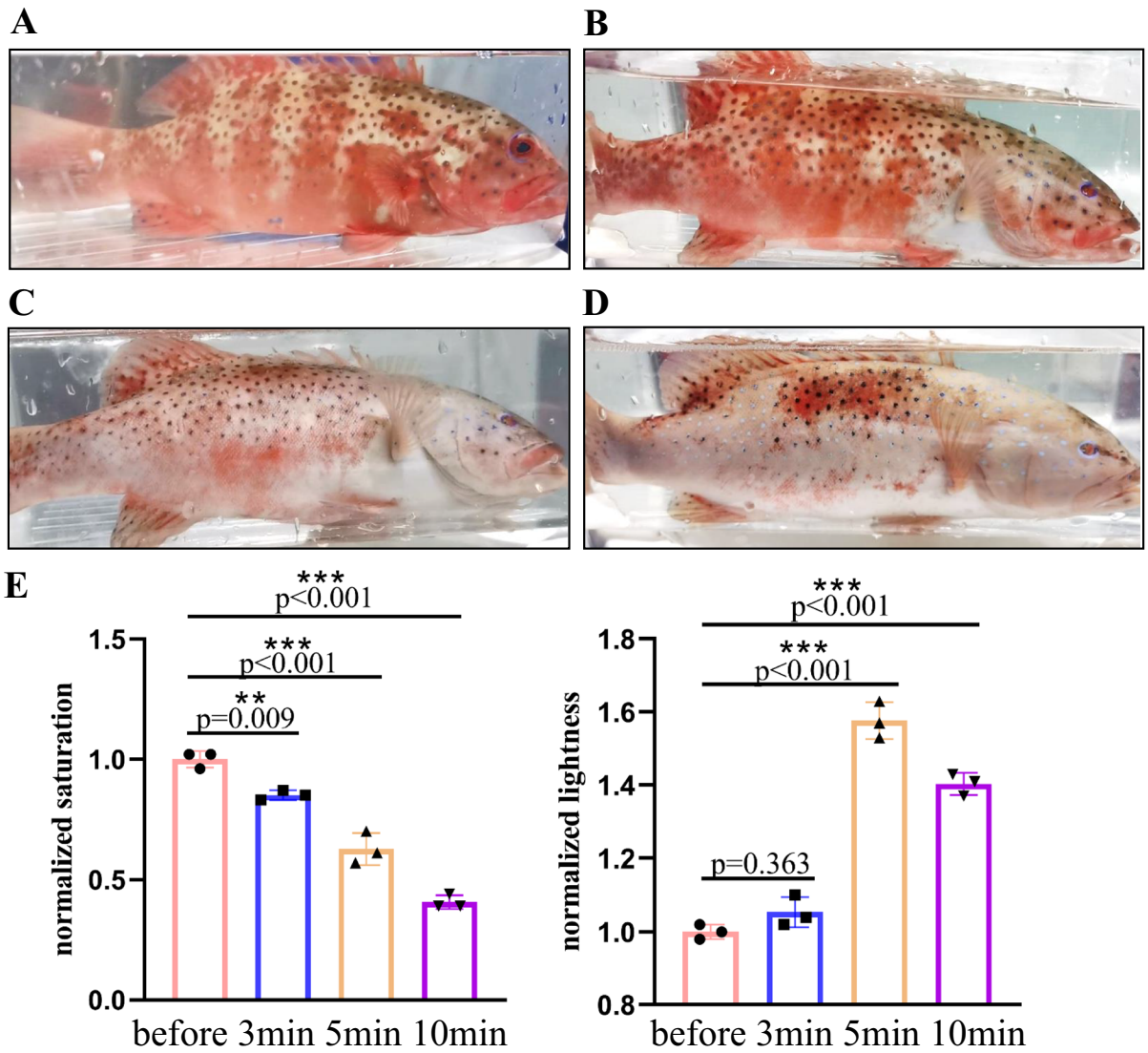

Fig. 7 The effects of NE on body color patterns of coral trout. **A** Before injection, the blotches formed in the skin. **B** 3 min after injection of 10 mM NE. **C** 5 min after injection of 10 mM NE. **D** 10 min after injection of 10 mM NE. **E** The normalized saturation and lightness values of skin before and after NE injection. n = 3 biological replicates for each condition, one-way ANOVA with Tukey's multiple comparisons. All bar graphs are mean ± S.D. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s.: not significant

(4°-6° to 9°-11°), and a relative increase and decrease in lightness and saturation, respectively (Fig. 6G), which is consistent with changes during blotches formation. Next, we injected NE intraperitoneally into the coral trout (Fig. 7A). Three minutes later, the skin around the pectoral fin first turned white (Fig. 7B), followed by a gradual whitening of the posterior skin (Fig. 7C), with only a small portion remaining red after 10 min (Fig. 7D). During this process, the degrees of skin hue is gradually increased (9–11° → 13–14° → 15–16° → 20–22°). Saturation gradually decreases and brightness increases (Fig. 7E). Collectively, these data suggest that the coral trout are sensitive to catecholamine neurotransmitter NE, which mediated the chromatosomes aggregation and body color lightening.

Discussion

Phenotypic plasticity allows species to survive under a wider range of conditions and to respond more quickly to environmental changes (Agrawal 2001). A notable example of phenotypic plasticity in animals is the capacity for color change (Sköld et al. 2016). Studies on color variations in groupers can be found scattered in the literatures (Choi, et al. 2020; CL 1961; Colin 1992; Deloach 1993; Heemstra and Randall 1993; Longley 1917; Sadovy and Eklund 1999; Townsend 1909; 1929; Watson et al. 2014). As previously observed in the coral trout, after being captured, body color lightened rapidly and blotches formed on their skin (Zhou et al. 2021). In this study, we further reveal the mechanism of this chromatic change. We demonstrate that capture leads to the activation of catecholaminergic neurons, where the neurotransmitter NE induces chromatosomes aggregation and blotches formation.

Lightening of skin coloration are usually achieved through rapid chromatosomes aggregation (Aspengren, et al. 2003b; Kindermann and Hero 2016; Ryan et al. 2002), as is the case with coral trout (Zhou et al. 2021). Measurements of chromaticity parameters, the HSL values, provide an objective way to quantify coloration (Yasir and Qin 2009). In this article, we therefore further quantify the skin changes during blotches formation as a change in hue (from near-red toward orange), a relative increase in brightness and a decrease in saturation. Then, the disappearance of blotches and the reddening and darkening of body color after the application of MS222 mean that it promoted the dispersion of chromatosomes. This has been demonstrated in black striped pipefish *Syngnathus abaste* (Cunha et al. 2017) and leech *Placobdella parasitic* (Smith 1942), where anesthetics performed the same function. Second, this result implies that the formation or disappearance of blotches is regulated by the nervous system, since MS222 has been shown to have the ability to block sensory and motor nerves (Ramlochansingh et al. 2014). Following this reasoning, further electrical stimulation allowed chromatosomes aggregation, blotches-like pattern reformation and consistent HSL values changes that clearly supported this idea. As described in previous reviews: the motor response of chromatosomes due to electrical stimulation is a manifestation of the sympathetic control of pigment cells (Fujii 2000).

Acute stress is known to cause transient and beneficial "fight-or-flight" responses essential for survival (Zhang 2020). Here, we demonstrate that acute stress can also cause body color pattern changes. The changes may be achieved through activation of the catecholaminergic nervous system, an idea supported first by the observation of TH + cells below the pigment layer of the dermis. And then, the chemical sympathectomy experiment showed that fish injected with 6-OHDA suffered disruption or perhaps degeneration of catecholaminergic nervous connections to chromatophores, as coral trout lost the ability to produce blotches after being captured. The result of this experiment lend strong support to the notion that capture-induced blotches formation is indeed controlled by catecholaminergic neurons. Previous study have also reported that after injection of 6-OHDA, several other fish species lost the capacity to change color according to background (Junqueira and Salles 1978a; Ryan et al. 2002). However, additional assays to the ultrastructure of catecholaminergic neuron after 6-OHDA application is necessary to explore deeper structural mechanisms.

Having established that blotches formation is stimulated by sympathetic nerves, the phenomenon following chemical sympathectomy guided us to further localize to catecholaminergic neurons. Thus, the role of catecholaminergic neurotransmitters has undoubtedly become the next focus of inquiry. Evidence accumulated over the past 30 years suggests that NE is the major neurotransmitter of the sympathetic nervous system (Nardocci et al. 2014). It has been widely demonstrated to play a role in inducing pigment granules aggregation in melanophores from many species of fish, including cuckoo wrasse *Labrus ossifagus* (Andersson et al. 1984; Mårtensson and Andersson 2000), tilapia *Sarotherodon niloticus* (Kumazawa and Ryozo 1984a), flatfish *Pleuronectes americanus* (Burton and Mayo 1995), blue damselfish *Chyrsiptera cyanea* (Kasukawa et al. 1985), blue-green damselfish *Chromis viridis* (Oshima et al. 1989), medaka *Oryzias latipes* (Masazumi et al. 1985; Uchida-oka and Sugimoto 2001), ballan wrasse *Labrus Bergylta* (Svensson et al. 1989), Atlantic Cod *Gadus morhua* (Aspengren et al. 2003a; Nilsson et al. 1996; Sköld et al. 2002), two-spotted gobies *Gobiusculus flavescens* (Sköld

et al. 2008), Siamese fighting fish *Betta splendens* (Amiri and Shaheen 2012), peppered catfish *Corydoras paleatus* (Oshima et al. 2001), Nile tilapia *Oreochromis niloticus* (Oshima et al. 2001), bitterling *Acheilognathus lanceolatus* (Fujishige et al. 2000), winter flounder *Pleuronectes americanus* (Burton and Vokey 2000), White-Spotted Rabbitfish *Siganus canaliculatus* (Amiri 2009), sand goby *Pomatoschistus minutes* (Sköld et al. 2015), spotted snakehead *Channa punctata* (Biswas et al. 2014), koi *Taisho-Sanshoku* (Shinohara et al. 2022) and zebrafish *Danio rerio* (Nguyen et al. 2006). As research progressed, evidence on other pigment cell types has also been reported. For example, in male three-spined stickleback *Gasterosteus aculeatus*, NE had aggregating effects on both melanophore and erythrophore pigments resulting in blue eyes and a pale jaw. In Scalycheek Damsel fish, *Pomacentrus lepidogenys*, xanthophores responded to NE by pigment aggregation. Under the light microscope, we observed three kinds of light-absorbing pigmentary chromatophores in the skin of coral trout, namely melanophores, xanthophores and erythrophores (Fig. 6). After NE incubation of isolated scales, chromatosomes within three kinds of chromatophores all aggregated, suggesting that the treatment with NE showed a general pattern for all pigmentary chromatophore types in coral trout. And as expected, intraperitoneal injection of NE lightens the skin from the injection site to the posterior skin and ultimately the whole body. Altogether, these results strongly indicate that NE is a regulator of the blotches formation in coral trout. Although additional hormones or neurotransmitters may also participate in blotches formation regulation, our both in vitro and in vivo results suggest that NE plays an important role in this process.

One of the extended questions is: after being captured, why do some parts of the skin become lighter while others remain darker? Based on previous reports, we hypothesize that this is due to differential activation of specific areas in the brain regions, most likely the mesencephalon. Previous studies have given us some hints: in the study of bluegill *Lepomis tnacrochirus*, vertical banding was evoked by electrical stimulation in the diencephalon (Demski 1973). Combined electrical stimulation and serially section of brains, Bauer further confirmed the involvement of the teleostean diencephalon in color change and implicates the preoptic area and thalamic-hypothalamic transition zone as the specific diencephalic areas involved in the banding response of green sunfish *Lepomis cyanellus* and bluegill (Bauer and Demski 1980). It is unfortunate that the brain of coral trout is too hard to allow us to make a breakthrough in dissection and localization of electrical stimulation. But anyway this provides a direction for our future work.

The ability to change the color, conformation and brightness of body patterns can have significant ecological and evolutionary implications. It act as a deception mechanism to impair recognition in cases of mimicry or disruptive coloration, or can be used to blend in with background habitats to prevent detection by potential predators or prey (Hanlon et al. 2009; Sköld et al. 2016 Stevens and Merilaita 2011). In the latter scenario, the tropical flounder *Bothus ocellatus* is almost cephalopod-like in its ability to match the substratum on which it settles (Ramachandran et al. 1996). In fact, many coral reef-fish species possess this ability to an extent. For example, species that settle for sleep in coral and reef structures at night also change color, almost invariably becoming pale and blotchy. This color change presumably aid in camouflage through disruption (Marshall et al. 2019). Although animal camouflage is a widespread tactic, dynamic camouflage is relatively uncommon and has been rarely studied in marine teleosts under natural conditions (Watson et al. 2014). There is a small amount of evidence from Nassau groupers *Epinephelus striatus* that they are capable of extreme color changes from uniform to "banded" or "barred" patterns in seconds (rather than minutes), which may depend on the fish's "mood", surroundings and activity (Archer et al. 2012; Heemstra and Randall 1993; Nemtzov et al. 1993). The ethological significance of the rapid blotches formation in coral trout remains obscure. We speculate that capture may have put the coral trout into a stressful "mood" of being pursued by predators, and that the rapid formation of blotches may have been an attempt to escape predators by matching the background of the coral reefs they inhabited. Further behavioral investigations will clarify these issues.

Conclusion

In summary, we demonstrate that the formation of blotches in coral trout in response to capture is controlled by catecholaminergic neurons, a process that can be mediated by the neurotransmitter NE. These results provide valuable evidence for elucidating the regulatory mechanism of color change and pattern formation in coral trout.

Acknowledgements We thank technician Xinming Chen of Xiao Deng Fisheries Technology Co., LTD for his help in obtaining and transporting experimental fish. We appreciate Chenchen Shi (PhD candidate) of the College of Ocean and Earth sciences, Xiamen University for her assistance in collecting samples for ultrastructure observation.

Authors' contributions Study conception and design: Nannan Zhao, Shi Xi Chen; material preparation, data collection and analysis: Nannan Zhao, Ke Jiang, Xiaoyu Ge, Jing Huang; methodology: Nannan Zhao, Caiming Wu; data curation: Nannan Zhao; writing — original draft: Nannan Zhao; writing — review and editing: Shi Xi Chen; Resources: Shi Xi Chen; project administration: Shi Xi Chen. All authors read and approved the final manuscript.

Funding This work was supported by the Key Project of Agriculture in Fujian Province of China (No. 2021N0001, 2021NZ033016).

Data Availability Data will be made available on request.

Declarations

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval The Laboratory Animal Management and Ethics Committee of Xiamen University gave its approval to all animal experiments, which were planned to reduce pain and the number of animals utilized.

Competing interests The authors declare no competing interests.

References

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Amiri M (2009) Postsynaptic alpha 2-adrenoceptors mediate melanosome aggregation in melanophores of the white-spotted rabbitfish (Siganus canaliculatus). Pak J Biol Sci 12:1

Amiri M, Shaheen HM (2012) Chromatophores and color revelation in the blue variant of the Siamese fighting fish (Betta splendens). Micron 43:159–169

Andersson R, Karlsson J, Grundström N (1984) Adrenergic nerves and the alpha2-adrenoceptor system regulating melanosome aggregation within fish melanophores. Acta Physiol Scand 121(2):173–179. https://doi.org/10.1111/j.1748-1716.1984.tb07444.x

Archer SK, Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, Mccoy CM, Johnson BC (2012) Patterns of color phase indicate spawn timing at a Nassau grouper Epinephelus striatus spawning aggregation. Current Zoology 58:73–83

Aspengren S, Sköld HN, Quiroga G, Mårtensson L, Wallin M (2003a) Noradrenaline-and melatonin-mediated regulation of pigment aggregation in fish melanophores. Pigment Cell Res 16:59–64

Aspengren S, Sköld HN, Quiroga G, Mårtensson L, Wallin M (2003b) Noradrenaline-and melatonin-mediated regulation of pigment aggregation in fish melanophores. Pigment Cell Res 16(1):59–64. https://doi.org/10.1034/j.1600-0749.2003.00003.x

Ballowitz E (1893) Die Nervenendigungen der Pigmentzellen, ein Beitrag zur Kenntnis des Zusammenhanges der Endverzweigungen der Nerven mit dem Protoplasma der Zellen. Zeit. Wiss. Zool. 56:673–706

Bandaranayake WM (2006) The nature and role of pigments of marine invertebrates. Nat Prod Rep 23:223–255

Bauer DH, Demski LS (1980) Vertical banding evoked by electrical stimulation of the brain in anaesthetized green sunfish, Lepomis cyanellus, and bluegills, Lepomis macrochirus. J Exp Biol 84:149–160

Biswas SP, Jadhao AG, Palande NV (2014) Role of catecholamines and nitric oxide on pigment displacement of the chromatophores of freshwater snakehead teleost fish, Channa punctatus. Fish Physiol Biochem 40:457–467

Burton D (1985) Differential in vivo sensitivity of melanophores and xanthophores to catecholamines in winter flounder (Pseudopleuronectes americanus Walbaum) integumentary patterns. J Exp Biol 114:649–659

Burton D, Mayo D (1995) Adrenoceptors in cryptic patterning of a flatfish, Pleuronectes americanus. Proc R Soc London. Ser B: Biol Sci 261:181–186

Burton D, Vokey J (2000) α1-and α2-adrenoceptor mediation in melanosome aggregation in cryptic patterning of Pleuronectes americanus. Comp Biochem Physiol a: Mol Integr Physiol 125:359–365

Choi S-H, Kim B-H, Lee C-H, Lee Y-D (2020) Response of body color change rearing under different light intensity conditions in farmed red spotted grouper, Epinephelus akaara. Fish Aquat Sci 23:1–9

CL S (1961) Synopsis of biological data on groupers (*Epinephelus* and allied genera) of the Western North Atlantic. FAO Fish Biol. Synopses 23:61

Colin PL (1992) Reproduction of the Nassau grouper, Epinephelus striatus (Pisces: Serranidae) and its relationship to environmental conditions. Environ Biol Fishes 34:357–377

Cubukcu E, Kahraman I (2008) Hue, saturation, lightness, and building exterior preference: An empirical study in Turkey comparing architects' and nonarchitects' evaluative and cognitive judgments. Color Res Appl: Endorsed Inter-Soc Color Council, The Colour Group (Great Britain) Canadian Soc Color, Color Sci Assoc Japan, Dutch Soc Study

of Color, Swedish Colour Centre Foundation, Colour Soc Australia, Centre Français De La Couleur 33:395–405
Cunha MA, Berglund A, Monteiro NM (2017) Female ornaments signal own and offspring quality in a sex-role-reversed fish with extreme male parental care. Marine Ecology 38(5):e12461. https://doi.org/10.1111/maec.12461
Deloach N (1993) Reef coral identification: Florida. Jacksonville, FL. New World Publications Inc, Caribbean, Bahamas
Demski L (1969) Behavioral effects of electrical stimulation of the brain in free-swimming bluegills (*Lepomis macrochirus*). University of Rochester, New York, USA, Ph.D. dissertation
Enami M (1955) Melanophore-contracting hormone (MCH) of possible hypothalamic origin in the catfish. Parasilurus Sci 121(3132):36–37. https://doi.org/10.1126/science.1121.3132.1136
Figon F, Casas J (2018) Morphological and physiological colour changes in the animal kingdom. eLS: 1–11. https://doi.org/10.1002/9780470015902.a9780470028065
Fujii R (2000) The regulation of motile activity in fish chromatophores. Pigment Cell Res 13(5):300–319. https://doi.org/10.1034/j.1600-0749.2000.130502.x
Fujii R, Oshima N (1986) Control of chromatophore movements in teleost fishes. Zoolog Sci 3(1):13–47
Fujii R, Oshima N (1994) Factors influencing motile activities of fish chromatophores. In: Arpigny JL, Coyette J, Davail S, Feller G, Fonzé E, Foulkes EC, Frère J-M, Fujii R, Génicot S, Gerday C, Joris B, Lamotte-Brasseur J, Maina JN, Narinx E, Nguyen-Disteche M, Oshima N, Viarengo A, Zekhnini Z (eds) Advances in Comparative and Environmental Physiology. Springer, Berlin, Heidelberg, pp 1–54. https://doi.org/10.1007/978-3-642-78598-6_1
Fujishige A, Moriwake T, Ono A, Ishii Y, Tsuchiya T (2000) Control of melanosome movement in intact and cultured melanophores in the bitterling, Acheilognathus lanceolatus. Comp Biochem Physiol A: Mol Integr Physiol 127:167–175
Glinka Y, Gassen M, Youdim M (1997) Mechanism of 6-hydroxydopamine neurotoxicity. in: Riederer P, Calne DB, Horowski R, Mizuno Y, Poewe W, Youdim MBH (Eds), Advances in Research on Neurodegeneration. J Neural Transm. Supplementa. Springer. Berlin, German, pp. 55-66. https://doi.org/10.1007/978-3-7091-6842-4_7
Goda M, Kuriyama T (2021) Physiological and Morphological Color Changes in Teleosts and in Reptiles. In: Hashimoto H, Goda M, Futahashi R, Kelsh R, Akiyama T (eds) Pigments, Pigment Cells and Pigment Patterns. Springer, Singapore, pp 387–423. https://doi.org/10.1007/978-981-16-1490-3_13
Hanlon R, Chiao C-C, Mäthger L, Barbosa A, Buresch K, Chubb C (2009) Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. Phil Trans R Soc B: Biol Sci 364:429–437
Heemstra PC, Randall JE (1993) FAO species catalogue Vol. 16. Groupers of the world (family: Serranidae, Subfamily: Epinephelinae). An annotated and illustrated catalogue of the grouper, rocked, hind, coral grouper and lyretail species known to date. FAO Fisheries synopsis: FAO, Rome, p. 382
Jones BE, Beaudet A (1987) Distribution of acetylcholine and catecholamine neurons in the cat brainstem: a choline acetyltransferase and tyrosine hydroxylase immunohistochemical study. J Comp Neurol 261(1):15–32. https://doi.org/10.1002/cne.902610103
Junqueira L, Salles L (1978) Effects of 6-hydroxydopamine and 5-hydroxydopamine onlthe teleost melanophores innervation. J Fish Biol 13(4):415–419. https://doi.org/10.1111/j.1095-8649.1978.tb03449.x
Kasukawa H, Oshima N (1987) Divisionistic generation of skin hue and the change of shade in the scalycheek damselfish, Pomacentrus lepidogenys. Pigment Cell Res 1:152–157
Kasukawa H, Sugimoto M, Oshima N, Fujii R (1985) Control of chromatophore movements in dermal chromatic units of blue damselfish–I. The melanophore. Comparative biochemistry and physiology. C, Comp Pharmacol Toxicol 81:253–257
Kindermann C, Hero J-M (2016) Pigment cell distribution in a rapid colour changing amphibian (*Litoria wilcoxii*). Zoomorphology 135(2):197–203. https://doi.org/10.1007/s00435-016-0303-1
Kindermann C, Narayan EJ, Hero J-M (2014) The neuro-hormonal control of rapid dynamic skin colour change in an amphibian during amplexus. PLoS One 9(12):e114120. https://doi.org/10.1371/journal.pone.0114120
Kostrzewa RM, Jacobowitz DM (1974) Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 26(3):199–288
Kumazawa T, Ryozo F (1984) Concurrent releases of norepinephrine and purines by potassium from adrenergic melanosome-aggregating nerve in tilapia. Comp Biochem Physiol Part C: Comp Pharmacol 78(2):263–266. https://doi.org/10.1016/0742-8413(84)90080-X
Ligon RA, McCartney KL (2016) Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. Curr Zool 62(3):237–252. https://doi.org/10.1093/cz/zow051
Longley W (1917) Studies upon the biological significance of animal coloration. I. The colors and color changes of West Indian reef-fishes. J Exp Zool 23:533–601
Mårtensson LG, Andersson RG (2000) Is Ca2+ the second messenger in the response to melatonin in cuckoo wrasse melanophores? Life Sci 66:1003–1010
Maeno N, Iga T (1992) Adrenergic mechanisms associated with the movement of platelets in iridophores from the freshwater goby, *Odontobutis obscura*. Comp Biochem Physiol C, Comp Pharmacol Toxicol 102(2):233–237. https://doi.org/10.1016/0742-8413(92)90106-h
Marshall NJ, Cortesi F, de Busserolles F, Siebeck UE, Cheney KL (2019) Colours and colour vision in reef fishes: Past, present and future research directions. J Fish Biol 95:5–38
Masazumi S, Noriko O, Ryozo F (1985) Mechanisms controlling motile responses of amelanotic melanophores in the medaka, Oryzias latipes. Zoolog Sci 2:317–322
Nagai M, Oshima N, Fujii R (1986) A comparative study of melanin-concentrating hormone (MCH) action on teleost

melanophores. Biol Bull 171(2):360–370. https://doi.org/10.2307/1541678
Nardocci G, Navarro C, Cortés PP, Imarai M, Montoya M, Valenzuela B, Jara P, Acuña-Castillo C, Fernández R (2014) Neuroendocrine mechanisms for immune system regulation during stress in fish. Fish Shellfish Immunol 40(2):531–538. https://doi.org/10.1016/j.fsi.2014.08.001
Nemtzov SC, Kajiura SM, Lompart CA (1993) Diel color phase changes in the coney, Epinephelus fulvus (Teleostei, Serranidae). Copeia 1993:883–885
Nguyen N, Sugimoto M, Zhu Y (2006) Production and purification of recombinant somatolactin β and its effects on melanosome aggregation in zebrafish. Gen Comp Endocrinol 145:182–187
Nilsson H, Rutberg M, Wallin M (1996) Localization of kinesin and cytoplasmic dynein in cultured melanophores from Atlantic cod, Gadus morhua. Cell Motil Cytoskelet 33:183–196
Oshima N, Kasukawa H, Fujii R (1989) Control of chromatophore movements in the blue-green damselfish, Chromis viridis. Comp Biochem Physiol Part C: Comp Pharmacol 93:239–245
Oshima N, Nakamaru N, Araki S, Sugimoto M (2001) Comparative analyses of the pigment-aggregating and-dispersing actions of MCH on fish chromatophores. Comp Biochem Physiol C: Toxicol Pharmacol 129:75–84
Qu M, Ding S, Xu X, Shen M, You Y, Su Y (2012) Ontogenetic development of the digestive system and growth in coral trout (*Plectropomus leopardus*). Aquaculture 334–337:132–141. https://doi.org/10.1016/j.aquaculture.2011.12.046
Ramachandran V, Tyler C, Gregory R, Rogers-Ramachandran D, Duensing S, Pillsbury C, Ramachandran C (1996) Rapid adaptive camouflage in tropical flounders. Nature 379:815–818
Ramlochansingh C, Branoner F, Chagnaud BP, Straka H (2014) Efficacy of tricaine methanesulfonate (MS-222) as an anesthetic agent for blocking sensory-motor responses in *Xenopus laevis* tadpoles. PloS One 9(7):e101606. https://doi.org/10.1371/journal.pone.0101606
Rudh A, Qvarnström A (2013) Adaptive colouration in amphibians, Seminars in cell & developmental biology. Elsevier, pp 553–561
Ryan RW, Post JI, Solc M, Hodson PV, Ross GM (2002) Catecholaminergic neuronal degeneration in rainbow trout assessed by skin color change: a model system for identification of environmental risk factors. Neurotoxicology 23(4–5):545–551. https://doi.org/10.1016/S0161-813X(02)00063-3
Sadovy Y, Eklund A-M (1999) Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). US Department Commer. National Oceanic and Atmospheric Administration Technical Report. National Marine Fisheries Service 146, and FAO Fisheries Synopsis 157, Seattle, Washington, p 65
Shimose T, Kanaiwa M (2022) Influence of the body color and size on the market value of wild captured coralgroupers (Serranidae, *Plectropomus*): Implications for fisheries management. Fish Res 248. https://doi.org/10.1016/j.fishres.2021.106223
Shinohara Y, Kasagi S, Amiya N, Hoshino Y, Ishii R, Hyodo N, Yamaguchi H, Sato S, Amano M, Takahashi A (2022) Taisho-Sanshoku koi have hardly faded skin and show attenuated melanophore sensitivity to adrenaline and melanin-concentrating hormone. Front Endocrinol 13:994060
Shwartz Y, Gonzalez-Celeiro M, Chen C-L, Pasolli HA, Sheu S-H, Fan SM-Y, Shamsi F, Assaad S, Lin ET-Y, Zhang B (2020) Cell types promoting goosebumps form a niche to regulate hair follicle stem cells. Cell 182(578–593):e519
Sköld HN, Norström E, Wallin M (2002) Regulatory control of both microtubule-and actin-dependent fish melanosome movement. Pigment Cell Res 15:357–366
Sköld HN, Yngsell D, Mubashishir M, Wallin M (2015) Hormonal regulation of colour change in eyes of a cryptic fish. Biology Open 4:206–211
Sköld HN, Aspengren S, Cheney KL, Wallin M (2016) Fish chromatophores—from molecular motors to animal behavior. Int Rev Cell Mol Biol 321:171–219
Sköld HN, Amundsen T, Svensson PA, Mayer I, Bjelvenmark J, Forsgren E (2008) Hormonal regulation of female nuptial coloration in a fish. Horm Behav 54:549–556
Smith RI (1942) Nervous control of chromatophores in the leech *Placobdella parasitica*. Physiol Zool 15(4):410–417. https://doi.org/10.2307/30151654
Sonka M, Hlavac V, Boyle R (2013) Image processing, analysis and machine vision. Springer
Stevens M, Merilaita S (2011) Animal camouflage: mechanisms and function. Cambridge University Press
Svensson S, Karlsson J, Grundström N (1989) Melanophores in isolated scales of Labrus berggylta (ascanius): innervation and alpha2-adrenoceptor-mediated pigment aggregation. Comp Biochem Physiol Part C: Comp Pharmacol 93:247–251
Swierk L (2022) Color change, encyclopedia of animal cognition and behavior. Springer, pp 1522–1525
Townsend CH (1909) Observations on instantaneous changes in color among tropical fishes. Kalkhoff Company
Townsend CH (1929) Records of changes in color among fishes. Zoologica 9:321–378
Uchida-oka N, Sugimoto M (2001) Norepinephrine induces apoptosis in skin melanophores by attenuating cAMP-PKA Signals Via α2-Adrenoceptors in the Medaka, Oryzias Latipes. Pigment Cell Res 14:356–361
Urban J, Štys D, Sergejevová M, Masojídek J (2013) Expertomica Fishgui: comparison of fish skin colour. J Appl Ichthyol 29:172–180
Watson A, Siemann L, Hanlon R (2014) Dynamic camouflage by Nassau groupers Epinephelus striatus on a Caribbean coral reef. J Fish Biol 85:1634–1649
Yamada K, Miyata S, Katayama H (1984) Autoradiographic demonstration of adrenergic innervation to scale melanophores of a teleost fish, Oryzias latipes. J Exp Zool 229:73–80
Yamaguchi D, Kasagi S, Shimizu D, Maeda T, Takahashi A, Mizusawa K (2022) A low body-color regulating ability of spotted halibut *Verasper variegatus*: Evaluation of the roles of melanin-concentrating hormone and proopiomelanocortin systems. Fish Sci 88(3):411–418. https://doi.org/10.1007/s12562-022-01600-6

Yasir I, Qin JG (2009) Effect of light intensity on color performance of false clownfish, Amphiprion ocellaris Cuvier. J World Aquac Soc 40:337–350

Zhang B (2020) Hyperactivation of sympathetic nerves drives melanocyte stem cell depletion. Nature 577:676–681. https://doi.org/10.1038/s41586-020-1935-3

Zhou K, Xin Wen, Cheng Deng, Shuailong Chen, Xingzhu Qi, Jian L (2021) Analysis of pigment cells difference in body color variation of *Plectropomus leopardus*. Acta Hydrobiologica Sinica 45(5):1164–1170. https://doi.org/10.7541/2021.2020.1291