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Effect of broodstock origin, background and substrate color on skin coloration of three-spotted seahorses *Hippocampus trimaculatus* Leach, 1814

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ABSTRACT

Seahorse performing attractive skin color under sensitive environmental conditions has been of favor for aquarists and aquaculturists. This study investigated the skin coloration and fading process, and the effects of broodstock, background and substrate color on the skin coloration of the three-spotted seahorse *Hippocampus trimaculatus* Leach, 1814. The formation of skin coloration was in the sequence of abdomen, tail, back and head, respectively. Seahorse color changed from black to yellow within 26.41 ± 6.28 s in response to sudden tactile stimuli, and then faded to the original black color within 259 ± 147.5 s. Skin coloration negatively correlated with seahorse body size: the smaller the seahorse, the easier seahorse changed skin color. Compared among six background color treatments (white, red, brown, yellow, blue and clear, respectively), the skin coloration rate (% changed from black to yellow) was the highest in the blue background (86.4 ± 11.6%, $P<0.001$). Seahorses derived from three broodstock origin (A, B and C) had different skin coloration rates of 44.2, 41.6 and 17.1%, respectively ($P=0.012$). When cultured with four different substrate colors (yellow, green, red and the mixture, respectively) for 5 days, 77.8% of the seahorses with the yellow substrate changed their skin color to yellow ($P=0.007$), and all seahorses with the red became yellow or yellow-black skin. As a novelty, this is the first report of the instantaneous skin color change in seahorses, and this study will be of interest for the behavior study and ornamental aquaculture of *H. trimaculatus*.

1. Introduction

Seahorses have been listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Convention on International Trade in Endangered Species of Wild Flora and Fauna, 2008). Traditionally, seahorses are popular in Chinese medicine, and there has been growing interest for marine aquaria because of their unique appearance and unusual reproduction (e.g. Lin et al., 2009a; Lourie et al., 1999; Vincent, 1996). As an important component of the aquaculture industry, ornamental fish production is the fourth largest sector in aquaculture in the United States, and seahorses are one of the most sought-after fish type (Tlusty, 2002; Wilson and Vincent, 1998). As a consequence, over-exploitation of seahorse stocks has led to a decline, even depletion in many tropical source regions (Lourie et al., 1999). Fortunately, seahorse aquaculture may help partially alleviate the fishing pressure on the wild seahorse stocks (Koldewey and Martin-Smith, 2010; Lin et al., 2006, 2007, 2008, 2009a, b; Woods, 2000, 2003a, b).

Environmental factors such as temperature, salinity, light intensity, background color of the tanks and substrates can influence fish stress response (Barcellos et al., 2009; Barton, 2002; Marshall and Messenger, 1996). Effects of environmental substrate on fish have been shown to modulate several physiological and behavioral responses, such as feeding, growth, reproduction, sex competition, aggression, stress response and body coloration (Banan et al., 2011; Imanpoor and Abdollahi, 2011). Body adaptive coloration often differs significantly because of the physiological or morphological mechanism of skin tissues in fish (Bagnara and Hadley, 1973; Logan et al., 2006). Culture vessel background features, including the color, size and patterns, can influence the body coloration and patterns in some cephalopods (Chiao and Hanlon, 2001; Kelman et al., 2008), and even the behavior and physiology in response to the stress (Strand et al., 2007). In the Syngnathidae, reports show that the fish generally change their skin color during the mating season, and that the coloration is an integral part of courtship and mating (Foster and Vincent, 2004; Vincent, 1994).

Seahorses mainly inhabit the sea grasses, mangroves and coral reefs in the shallow sea, and most seahorses display black, gray and yellow coloration, with few displaying red skin color on the skin (Lourie et al., 1999; Vincent, 1996). Knowledge of the behavior features of adaptive coloration in seahorses is relatively scarce. In the previous study, we have demonstrated that the juvenile seahorses *Hippocampus erectus* could change and maintain their skin color when their body size attain certain threshold values (length/weight) with appropriate mixed color substrate, and the light intensity and

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temperature could improve the skin color change rates of the juveniles (Lin et al., 2009c).

The three-spotted seahorse *Hippocampus trimaculatus* Leach, 1814 is widely distributed throughout the Indian Ocean and northwest of Pacific Ocean and the skin color is mainly shallow white in adult and black in the young (Foster and Vincent, 2004; Lourie et al., 1999; Sheng et al., 2006, 2007). The purpose of the present study was to investigate the skin coloration response of the seahorses (e.g. the process of skin color change and fading) while being stimulated, and the effects of broodstock origin, background and substrate color on skin coloration of *H. trimaculatus*.

2. Materials and methods

2.1. Experimental fish

Seahorses used in this study were cultured in Zhanjiang Seahorse Center of South China Sea Institute of Oceanology, Chinese Academy of Sciences (SCSIO-CAS) (latitude 110.04°E, longitude 20.54°N).

Six weeks old juvenile seahorses (*H. trimaculatus*, F_1 generation) (0.24 ± 0.18 g, 51.36 ± 10.02 mm) used in the study were temporarily cultured in outdoor re-circulating gray concrete ponds (6.0 × 3.5 × 1.2 m) with the stocking density of 15 seahorses per cubic meter and bamboo branches, plastic plants and corallites as the holdfasts for the seahorses. Seawater was pumped directly from the South China Sea and treated with the double sand filtration, and water flow rate of the re-circulating ponds was 1000 L/day. During the experiment, temperature, salinity and pH were maintained at 25 ± 4.0 °C, 33 ± 1.0‰ and 7.8 ± 0.3, respectively. Seawater quality parameters were as follows: total ammonia nitrogen (TAN) < 0.3 mg/L, nitrite < 0.2 mg/L, nitrate < 4 mg/L and dissolved oxygen > 65%. Black nylon mesh was used to shade the outdoor ponds to keep the light intensity level below 3400 lx (close to the natural light for the habitat of this species). Seahorses were fed one to three times a day depending on availability with copepods, adult *Artemia*, *Mysis* spp. and *Acete* spp. Feces were siphoned out of the tanks daily and seawater was aerated gently.

2.2. Skin coloration response

Skin coloration of the experimental seahorses was defined as changing from black to yellow and the method used in this study is similar to our former study on the skin coloration of *H. erectus* (Lin et al., 2009c). Before the experiment, most juvenile seahorses were observed to change their body color from black to yellow during the transportation among the culture ponds. Therefore, in order to compare skin coloration in each treatment, seahorses were divided into three groups as follows: seahorses either not changing color (B), or changed from black to yellow (Y) or to a mixture of yellow and black (YB: mixture of both colors throughout the body).

Two hundred and forty 6-week old seahorses (0.24 ± 0.18 g, 51.36 ± 10.02 mm) were haphazardly selected from the outdoor ponds, and held by hand for 5 s to simulate threatening stimuli and then transferred into one of 12 recirculating tanks (60 × 40 × 40 cm, water flowing rate of 3 L/h). Among the 12 tanks, 4 treatments (white, blue, yellow and red, respectively), each with three replicates, were performed to investigate the effects of background color on the color change of the "startled" seahorses. Sixty seahorses were settled as three replicates (each had 20 seahorses) for a treatment. The light intensity on the water surface was 2400 ± 100 lx, and no substrate and diet were provided for the seahorses in the tanks during the experiment. A video camera was used on the top of each tank to record the formation of the skin coloration (changing rate and fading time of the abdomen, tail, back and head of the seahorses) when transferring the seahorses to the experimental tanks. Through the recorded video, we obtained a measure of skin coloration and fading time of each seahorse.

After the above measurement for skin coloration, in order to analyze the correlation between the skin coloration and the body sizes of the seahorses, 60 seahorses (including: 20 Y, 20 YB and 20 B, respectively) were haphazardly selected from the 240 seahorses in the experimental tanks. The wet weights (mean ± S.D.) of seahorses were measured after blotted dry on the filter paper (Job et al., 2002) and the body heights (mean ± S.D.) (the sum of the length from the tip of the tail to the mid-point of the cleithral ring (Lourie et al., 1999) were measured).

2.3. Effects of broodstock origin and background color on skin coloration

The combined background color (white, red, brown, yellow, blue and clear) and broodstock (stocks A, B and C) study on the skin coloration of seahorses were performed as a 3 × 6 factorial design with each of the two factors (broodstock and background) and three replicates.

Three hundred and twenty four (108 × 3) 6 weeks old seahorses (0.24 ± 0.18 g, 51.36 ± 10.02 mm) derived from three wild paired parent seahorses (parent pairs A: white-gray in skin; parent pairs B and C: dark-gray in skin) were placed into the re-circulating tanks (60 × 40 × 40 cm, water flowing rate of 3 L/h) at a density of 6 seahorses per tank. Plastic plants with corresponding color to each background color were set up as the substrate and holdfasts for the fish. Seahorses were fed twice a day with copepods and 8 day-old *Artemia* (0.57 ± 0.15 cm) in excess. The temperature, salinity, dissolved oxygen (DO), light intensity and photoperiod in the tanks were as follows: 26 ± 1.0 °C, 33 ± 1.0‰, 6.5 ± 0.5 mg/L, 2000 lx (on water surface), and 16 L: 8 D, respectively. The tanks were aerated gently to prevent the excessive air bubbles and turbulence. After 5 days of culture, the seahorses in each treatment were counted according to their body colors (Y, YB and B, respectively).

2.4. Effects of substrate color

According to the results of the experiment in Section 2.3, juvenile seahorses from the stock A were used to conduct this experiment. Four substrate color treatments (yellow, green, red and the mixture (yellow: green: red = 1:1:1) respectively), each with three replicate tanks (60 × 40 × 40 cm, water flowing rate of 3 L/h), were conducted to evaluate the effects of substrate color on the skin coloration of seahorses during 5 days. In each tank, three plastic plants with the corresponding experimental colors were set up as the substrate for the seahorses, and all the experimental tanks were shaded with the black paint around the tank walls. In this experiment, 18 seahorses (6 Y, 6 YB and 6 B, respectively) in each treatment were selected from stock A. To avoid social effects among the seahorses, 18 seahorses were selected after their skin color was stable when they were cultured in the same tanks. The culture husbandry protocol was the same as that in the above experiment. The skin coloration of seahorses in each treatment was recorded daily at 1:00 p.m. for 5 days.

2.5. Statistical analysis

Statistical analyses were conducted using the software SPSS 19.0 (Statistical Program for Social Sciences 19.0) and Sigma PLOT 10.0 (Version 10.0, Systat Software, Inc. 2002). One-way analysis of variance (ANOVA) was used to assess the differences of the skin coloration rates of the seahorses among the treatments with a confidence level of 0.05. If ANOVA effects were significant, comparisons between the different treatment means were made using post hoc least significant differences (LSD).