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Effects of substrate color, light intensity and temperature on survival and skin color change of juvenile seahorses, *Hippocampus erectus* Perry, 1810

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ABSTRACT

Seahorses are popular ornamental fish for marine aquaria, with more colorful individuals commanding higher prices. We investigated the effects of substrate color, light intensity and water temperature on the survival and skin color change of the juvenile seahorse, *Hippocampus erectus*. During the 6-week study, the juveniles cultured with substrates of mixed colors (green and orange, green and red) had higher survival and color change rates than those with a single color substrate (green, orange, red, or black, $F_{5,\ 18}=2.764$, $F_{5,\ 18}=56.225$, $P<0.05$). Color change rate of the juveniles increased with increasing light intensity (500, 1000, 1500, 2000, 2500 and 3000 lx) with juveniles cultured at 500 lx had the highest survival rate ($85.35\pm5.18\%$). The wet weight, standard body length and age of the juveniles that began to change their skin color ranged from 0.054 ± 0.007 g, 3.12 ± 0.31 cm and 17.26 ± 2.23 days post partum (green and orange substrate mixture) to 0.082 ± 0.013 g, 3.68 ± 0.59 cm and 25.66 ± 6.21 days post partum (black) among the substrate color treatments; and from 0.043 ± 0.009 g, 3.09 ± 0.62 cm, and 14.57 ± 2.35 days post partum (3000 lx) to 0.067 ± 0.008 g, 3.87 ± 0.49 cm, and 18.41 ± 3.73 days post partum (500 lx) among the light intensity treatments. Survival rate and color change rate of the juveniles cultured at three temperatures (23 °C, 26 °C, and 29 °C) differed significantly after 5 weeks ($F_{2,\ 9}=11.053$, $P<0.05$). The juveniles cultured at the temperature of 29 °C had the highest color change rate of $80.43\pm4.27\%$. These results demonstrate that appropriate mixed color substrate, light intensity and temperature can improve the survival and skin color change rates of the juvenile *H. erectus*, thus affecting their market value.

1. Introduction

Ornamental fish production is an important component of the aquaculture industry (Wilson and Vincent, 1998; Hargrove, 1998; Woods, 2003a; Palma et al., 2008). In the United States, ornamental fish production is the fourth largest sector in aquaculture (Tlusty, 2002). Seahorses are popular fish for marine aquaria because of their unique appearance and unusual reproduction (Vincent and Sadler, 1995; Masonjones and Lewis, 2000). However, the natural populations of seahorses have sharply declined in recent years because of the destruction of estuarine and reef habitats (Lourie et al., 1999), and overfishing to satisfy the strong demand for seahorses from the Chinese traditional medicine (Vincent, 1996; Lourie et al., 1999; Lin et al., 2006, 2007a). Aquaculture is an alternative to the capture of wild seahorses, and may reduce the impacts of exploitation on natural populations (Vincent, 1996; Forteath, 1997; Hilomen-Garcia, 1999; Job et al., 2002; Woods, 2000, 2003a,b).

Low survival of juvenile seahorses and low reproductive efficiency of the parent seahorses have restricted seahorse culture for many years (Scarratt, 1995; Wilson and Vincent, 1998; Wong and Benzie, 2003; Woods, 2003a,b; Lin et al., 2006, 2007a). Extensive research on the survival of the different seahorse species has been carried out (Correa et al., 1989; Payne and Rippingate, 2000; Job et al., 2002; Woods, 2000, 2003a,b; Wong and Benzie, 2003; Lin et al., 2007a,b, 2008a,b). Woods (2000, 2003a) and Martinez-Cardenas and Purser (2007) have also studied the effects of tank color and light intensity on the growth and survival of juvenile seahorses. Environmental factors such as light intensity, background color, salinity and temperature could affect the skin color of the pot-bellied seahorse *Hippocampus abdominalis* (Wardley, 2001). In general, the yellow seahorses have higher market value in the aquarium trade, and these seahorses can maintain their colorful skin for life if the environmental conditions are appropriate (Foster et al., 2003).

The lined seahorse, *H. erectus* Perry, 1810, which has been included in the International Union for the Conservation of Nature (IUCN,

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2008) and listed on the Appendix II of Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) (CITES, 2008), is a highly valued species in both medicinal and aquarium trades, and the yellow or orange individuals are more popular in the aquarium trade (Correa et al., 1989; Foster et al., 2003). It is mainly found from Nova Scotia along the western Atlantic coast, through the Gulf of Mexico and Caribbean to Venezuela, in shallow inshore areas to depths of over 70 m (Lourie et al., 1999; Fritzche and Vincent 2002; Foster and Vincent, 2004). To date, research works on *H. erectus* have shown that this species has a high potential for commercial production (Scarratt, 1995; Lin et al., 2008a, 2009a,b). The present study aimed to investigate the effects of substrate color, light intensity and temperature on the survival and skin color change of juvenile *H. erectus* in order to improve culture efficacy and market value.

2. Materials and methods

2.1. Juvenile seahorse

The study was conducted at the Vero Beach Marine Laboratory, Vero Beach, Florida, USA. Juveniles sampled from 12 broods of F_3 generation male *H. erectus*, each with 320–480 individuals, were used in this study. Following birth, all the juveniles were temporarily cultured in re-circulating holding tanks (50×25×30 cm, 27 L). Seawater was pumped directly from the Atlantic Ocean and treated with sand filtration and water flow rate was maintained at 0.2–0.4 L/min. Temperature, salinity, dissolved oxygen, light intensity and photoperiod in the holding tanks were maintained at (mean ± S.D.) 28 ± 0.5 °C, 35 ± 1.0‰, 6.5 ± 0.5 mg/L, 1500 lx, and 16 h L (0700–2300 h): 8 h D (2300–0700 h), respectively. Tanks were aerated gently so as not to form excessive air bubbles and turbulence. Plastic plants (tree-shape, 21 cm in height and 7 cm in width) of different colors were used as substrate and holdfasts for the juvenile seahorses.

2.2. Effect of substrate color on skin color change

Six substrate treatments (green (G), black (B), red (R), orange (O), green (50%) and red (50%) (GR) mixture, and green (50%) and orange (50%) (GO) mixture, respectively), each with 3 replicate tanks, were used to evaluate the effect of substrate color on the color change of the juvenile seahorse (1 day post partum (dpp)) for 6 weeks. In each experimental transparent glass tank (50×25×30 cm, 27 L), four plastic plants were used as the substrate for the juvenile seahorse and two sides of the glass tank were also covered by the corresponding color paper to the plastic plants. Each tank had 50 juveniles haphazardly sampled from the re-circulating holding tanks. Culture conditions in the experimental tanks were the same as in the re-circulating holding tanks and the water flow rate in each experimental tank was kept at 0.3–0.4 L/min. During the first 15 dpp, the juveniles were fed four times (0730 h, 1200 h, 1700 h and 2100 h) a day to satiation with newly hatched *Artemia* nauplii (0.04 ± 0.012 cm in body length) at an approximate density of 4–7 *Artemia*/mL. From 16 dpp to the end of the experiment (42 dpp), the juveniles were fed with *Artemia* (0.53 ± 0.184 cm in body length) enriched with micro-algae *Chlorella* spp. Faeces and uneaten food were siphoned out of each experimental tank at 2230 h daily.

During the experiment, the black juveniles that began to change their skin color to yellow or brown were collected to measure their wet weights and standard body lengths, and their ages (dpp) were also recorded. Then the mean wet weight, standard body length and the age of the juveniles in each treatment were calculated. At the end of the experiment, the numbers of total juveniles and of the juveniles in yellow or brown color at each tank were counted and recorded.

2.3. Effect of light intensity on the skin color change

Six treatments (500, 1000, 1500, 2000, 2500 and 3000 lx, respectively, on the water surface from the overhead fluorescent light source), each with 3 replicates, were run for 5 weeks to test the effect of light intensity on the color change of 1 dpp juveniles. Each re-circulating tank (50×25×30 cm, 27 L) contained 50 juveniles, and plastic plants in mixed colors (2 orange; 1 red and 1 green) were used as substrate and holdfasts for the juveniles. Water flow rate was kept at 0.2–0.4 L/min, and culture conditions (except for the light intensity) in the experimental tanks were the same as in the re-circulating holding tanks. The feeding regime for the juveniles was the same as that in Experiment 2.2 and faeces and uneaten food were siphoned out daily. In each tank, the wet weight and standard body length of the juveniles that began to change their skin color were measured, and their age (dpp) was also recorded.

2.4. Effect of temperature on skin color change

Three treatments (23, 26 and 29 °C), each with three replicates, were run to test the effect of temperature on color change of the juveniles during the 5-week study. The juveniles were cultured under 28 ± 0.5 °C and acclimated to each experimental temperature between the ages of 8 and 10 dpp. Heaters were used to control the water temperature. Each re-circulating tank (50×25×30 cm, 27 L) contained 50 juveniles. Plastic plants of mixed colors (2 orange, 1 red and 1 green) were used as the substrate and holdfasts for the juveniles. Water flow rate was kept at 0.2–0.4 L/min and culture conditions (except for the temperature) in the experimental tanks were the same as in the re-circulating holding tanks. During the first 5 days of the experiment (from 10 to 15 dpp), the juveniles were fed four times (0730 h, 1200 h, 1700 h and 2100 h) a day to satiation with newly hatched *Artemia* nauplii at an approximate density of 4–7 *Artemia*/mL. From 16 dpp to the end of the experiment (45 dpp), the juveniles were fed with *Artemia* (0.53 ± 0.184 cm in body length) enriched with micro-algae *Chlorella* spp. Faeces and uneaten food were siphoned out at 2230 h daily. At the end of the experiment, the number of total juveniles and of those that were yellow or brown color in each tank were counted and recorded.

2.5. Data analysis

Statistical analyses were run using the software SPSS 11.5 (Statistical Program for Social Sciences 11.5). One-way analysis of variance (ANOVA) was used to evaluate the differences in survival rate and color change rate of juvenile seahorses among the treatments. The relationship between light intensity and the color change rate of juvenile seahorses was subjected to a regression analysis. If ANOVA effects were significant, comparisons among the different means were made using post hoc least significant differences (LSD).

3. Results

3.1. Effect of substrate color on survival and skin color change

3.1.1. Survival and color change rates

The survival rate of the juveniles was significantly different among the different substrate treatments after 6 weeks ($F_{5,18} = 2.764, P < 0.05$) (Fig. 1). The juveniles with the substrate of green (50%) and orange (50%) mixture (GO) had the highest survival rate of 83.32 ± 4.19%. The survival rates of the juveniles with green substrate (G) (76.42 ± 4.08%) and green (50%) and red (50%) substrate mixture (GR) (78.14 ± 3.75%) were also high. The juveniles cultured with red substrate (R) had the lowest survival rate of 69.52 ± 7.15%.

The skin color change rate of the juveniles also differed significantly after the 6-week study ($F_{5,18} = 56.225$, $P < 0.05$) (Fig. 1).