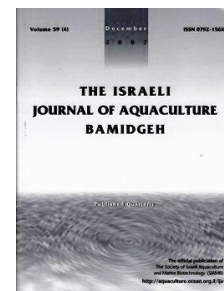




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## Effect of Background Color of Tanks on Growth and Survival of Juvenile Yellow Seahorse, *Hippocampus kuda* (Bleeker 1852), in the Pelagic Phase

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### Abstract

As with many species of seahorses, wild populations of the yellow seahorse, *Hippocampus kuda*, are subject to uncontrolled exploitation. Seahorse aquaculture is one way to meet the growing demand for seahorses while promoting the recovery of wild stocks. Attempts to culture seahorses on a mass scale have met with limited success due to heavy juvenile mortality, particularly during the pelagic phase. This communication reports the results of an experiment conducted to see if background tank color affects the growth and survival of juvenile *H. kuda*. Juveniles were reared from 0 to 21 days after birth (DAB) in one of six tank colors: green, blue, red, or black (dark backgrounds), yellow or transparent (light backgrounds). Growth and survival were significantly higher in the dark backgrounds than in the light ( $p < 0.05$ ). At 21 DAB, the maximum juvenile growth ( $34.58 \pm 1.15$  mm,  $55.43 \pm 6.71$  mg) and survival (70%) were obtained in the red, blue, and green backgrounds. The order of growth increment and percent survival at DAB 21 was red > green > blue > black > yellow > transparent and blue > green > red > black > yellow > transparent, respectively. Ingestion of the air-bubble accounted for 65% of the total mortality in all backgrounds and occurred more often in light backgrounds. Juveniles reared in blue and red backgrounds reached a settlement phase on 17 DAB; those raised in the transparent tanks reached this stage at 21 DAB. In conclusion, a carefully chosen background can optimize growth and survival during the pelagic phase of juvenile *H. kuda*.

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## Introduction

The current demand for seahorses appears to far exceed the supply, with at least 25 million seahorses (>70 tons dry weight) traded globally (Project Seahorse, 2006; Salin and Mohankumran, 2006). High exploitation rates in India, Indonesia, Philippines, Thailand, and Vietnam resulted in a decline of 25-75% of the wild population of some seahorse species in just five years (Vincent, 1996). At least 77 nations and territories are involved in the seahorse trade (Koldewey and Martin-Smith, 2010). India alone was responsible for about 30% of the global seahorse trade until 2001 (Salin and Mohankumaran, 2006).

The yellow seahorse, *Hippocampus kuda* (Bleeker 1852), is one of 46 extant species native to the Indo-Pacific region (Kuitert, 2009) and heavily traded for traditional Chinese medicine (Lourie et al., 1999). It has a fairly smooth appearance and often exhibits a pale yellow color, traits that are preferred by overseas markets of the live aquarium and souvenir trades (Lourie et al., 1999). A great part of the demand for seahorses and other syngnathids for the traditional medicine market is met by wild caught animals (Foster and Vincent, 2004). Seahorse aquaculture can integrate both conservation and sustainable development goals by reducing pressure on wild stocks as well as providing an alternative subsistence livelihood to fisher-folk in the source countries.

Optimization of environmental conditions and feeding regimes during rearing of juveniles is critical for successful captive breeding programs. The rearing of ornamental marine fishes is often problematic because of their bipartite life style which involves both pelagic and settlement behavior (Victor, 1991). The initial period of 0-15 days after birth (DAB), when juveniles switch from a pelagic to settlement phase, is very critical in seahorse husbandry (Woods, 2000). Attempts to culture seahorses on a mass scale have met with limited success as a result of severely high mortality due to inadequate nutrition and disease. In captivity, new-born seahorses exhibit pelagic behavior and require 24-h illumination as they are visual feeders. Inadequate light can result in wall tapering behavior around tank corners, leading to starvation and death (Blaxter, 1980; Murugan et al., 2009).

High juvenile mortality, particularly during the pelagic phase, has been attributed to inadequate or inefficient feeding responses. Mortalities can possibly be minimized by improved feeding efficiency (Koldewey, 2005; Thangaraj and Lipton, 2008; Garcia et al., 2010) and manipulation of the background (tank) color. Different colored backgrounds induce a variety of responses in relation to food intake, growth, and survival in fishes (Moriya and Miyashita, 1987; Papoutsoglou et al., 2000; Tamazouzt et al., 2000). Some species prefer dark tank walls (Naas et al., 1996) that promote a suitable contrast between the prey and background (Browman and Marcotte, 1987). However, others prefer light backgrounds (Papoutsoglou et al., 2000; Tamazouzt et al., 2000).

This experiment was conducted to test how tank color affects growth and survival during the pelagic phase of juvenile *H. kuda*.

## Materials and Methods

**Fish and facilities.** Juveniles came from captive bred seahorses at the Aquaculture Laboratory of the National Institute of Oceanography in Goa, India. Newly-born juveniles ( $8 \pm 0.9$  mm,  $1.9 \pm 0.45$  mg) from a single brood were used. Flat-bottomed transparent circular plastic tubs were painted red, green, blue, black (dark backgrounds), or yellow (light background) and served as rearing tanks. Oil-based enamel paints were applied on the outside surface of the tanks to prevent the leaching of paint into the rearing sea water. Transparent (unpainted) tanks served as the control (light background). Tanks were filled with 5 l filtered ( $5 \mu\text{m}$ ) UV-treated sea water. They were illuminated 24-h from above with a single 100 W Philips bulb ( $\sim 700$  lx) and gently aerated with air stones embedded in PVC pipes (60 mm diameter) wrapped with serrated muslin cloth ( $20 \mu\text{m}$  mesh) to prevent the formation of aeration-induced currents and excessive air bubbles. Eighteen tanks provided for six treatments in triplicate. The layout of the colored tanks was randomized. Juveniles were stocked and maintained at 2 fish/l.

*Water quality and feeding.* The sea water used for rearing the juveniles was treated by rapid sand filtration, bio-filtration, and ultraviolet radiation. Physico-chemical parameters of the water were within the optimum levels recommended for *H. kuda* culture:  $28.5 \pm 0.5^\circ\text{C}$ , salinity  $31 \pm 1.5$  ppt, dissolved oxygen  $6.1 \pm 0.6$  mg/l, pH  $7.6 \pm 0.3$ ,  $\text{NO}_2\text{-N} < 0.02$  mg/l, and  $\text{NH}_3/\text{NH}_4$  0 mg/l. Mixed zooplankton were collected from the nearby bay-estuarine ecosystem using plankton nets of 60, 120, and 200  $\mu\text{m}$  mesh to feed 0-7 DAB, 8-14 DAB, and 15-21 DAB juveniles, respectively. After collection, the zooplankton were allowed to settle in a large glass bowl to remove detritus. The zooplankton, dominated by nauplii, copepodites, and adult copepods, were fed to juveniles twice a day (10:00 and 21:00) at a density of 6-7 cells/ml after carefully siphoning out feces and uneaten prey organisms (prey mortality was due to unsuccessful prey attacks) from the tank bottom. Fifty percent of the rearing water was exchanged twice a day. Our previous feeding experiments confirmed that growth and survival are better when *H. kuda* juveniles are fed copepods as prey organisms. Before each feeding, the prey density in the rearing tank was estimated and adjusted to ensure that the density never fell below 6-7 cells/ml.

*Juvenile growth and survival.* Height and weight of three randomly selected juveniles were measured 7, 14, and 21 DAB. Height was measured to the nearest 0.5 mm from the tip of the coronet to the tip of the uncurled tail by placing the fish on a submerged plastic-covered 1-mm scaled sheet (Lourie et al., 1999). Wet weight of individual juveniles was measured on an analytical balance to the nearest 0.01 mg (Job et al., 2002). Mortality in each tank was recorded daily. Juveniles showing symptoms of horizontal floating or circular movement in a single place (whirlpool) on the water surface possibly as a result of air-bubble ingestion (creating a buoyant air bubble in the juvenile's stomach) were immediately siphoned out. These were recorded as dead since our previous observations confirmed that such juveniles cannot be revived and perturb healthy juveniles. Dead juveniles were replaced with identifiable tagged juveniles of the same batch and size to maintain the original stocking density and, thereby, prevent an increased growth rate in the remaining juveniles that would enjoy greater access to feed because of less competition from a lower number of tank-mates. The newly-introduced juveniles, however, were not included in the final analyses.

*Statistical analysis.* One way analysis of variance (ANOVA) was used to assess differences in growth between treatments and Tukey's multiple comparison test for post-hoc analysis when differences were statistically significant. Kruskal-Wallis test statistic was used to find differences among survival rates. Significant differences were tested at 5.0% level of significance and are presented as  $p < 0.05$ . All results were processed and analyzed with the Graph Pad Prism 5 statistical package.

## Results

*Growth.* There were no statistical differences in growth between replicate tanks of the same color. Juveniles reared in dark backgrounds grew longer than those reared in the light (yellow and transparent) backgrounds (Table 1). Juveniles grown in the red background consistently attained a greater length in all treatments and on all sampling days. On all three sampling days, the order was similar in terms of wet weight to that of length but, at 14 DAB, post hoc analysis yielded three groups.

*Survival.* There were no statistical differences in survival between replicate tanks of a single color. Kruskal-Wallis statistic indicated significant variability between background colors. At 7 DAB, the highest and lowest mortalities were obtained in the transparent (36.66%) and blue (13.33%) backgrounds, respectively (Fig. 1). At 14 DAB, the highest mortality was in the yellow background and the highest survival (86.67%) was in the green background. No mortalities occurred in any color between 15 and 21 DAB. At 21 DAB, the cumulative survival in juveniles was highest in the green and blue (70%) treatments, followed by the red (66.67%) and black (53.33%). The highest cumulative mortalities were in the transparent (63.33%) and yellow (56.67%) backgrounds.

*Cause of mortality.* The causes of the mortality were either ingestion of an air bubble and/or starvation. The major cause was ingestion of an air bubble, which accounted for

Table 1. Growth of *Hippocampus kuda* juveniles reared in tanks with different background colors (means±sd, n = 9).

Background color	Days after birth		
	7	14	21
<b>Length</b>			
Red	17.58±1.20 <sup>a</sup>	26.42±1.16 <sup>d</sup>	34.58±1.15 <sup>h</sup>
Green	16.83±0.86 <sup>ab</sup>	25.33±1.16 <sup>de</sup>	33.58±1.15 <sup>hi</sup>
Blue	16.75±0.82 <sup>ab</sup>	25.08±1.06 <sup>de</sup>	33±0.94 <sup>hi</sup>
Black	16.25±0.52 <sup>abc</sup>	24.16±1.12 <sup>efg</sup>	31.91±0.58 <sup>i</sup>
Yellow	15.33±0.60 <sup>bc</sup>	22.50±1.00 <sup>f</sup>	29.33±1.12 <sup>j</sup>
Transparent	15.17±0.80 <sup>c</sup>	22.08±1.24 <sup>fg</sup>	28.75±1.50 <sup>j</sup>
<b>Weight</b>			
Red	16.17±1.73 <sup>a</sup>	32.01±2.01 <sup>c</sup>	55.43±6.71 <sup>g</sup>
Green	15.87±2.00 <sup>a</sup>	30.38±1.61 <sup>cd</sup>	52.30±3.63 <sup>g</sup>
Blue	15.6±1.70 <sup>a</sup>	28.71±1.59 <sup>cd</sup>	49.93±3.05 <sup>gh</sup>
Black	14.13±1.51 <sup>ab</sup>	27.46±2.81 <sup>de</sup>	45.7±1.82 <sup>hi</sup>
Yellow	13.37±1.61 <sup>ab</sup>	24.65±2.36 <sup>ed</sup>	39.53±2.07 <sup>ij</sup>
Transparent	12.21±1.54 <sup>b</sup>	23.83±3.16 <sup>f</sup>	38.53±2.03 <sup>j</sup>

Values in a column with different superscripts significantly differ ( $p < 0.05$ ).

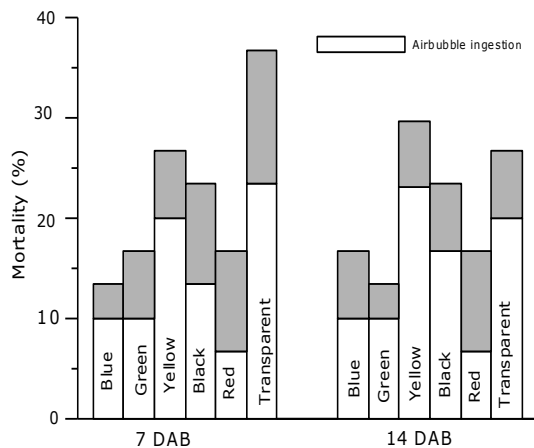


Fig. 1. Mortality of *Hippocampus kuda* juveniles reared in tanks with different background colors 7 and 14 days after birth (DAB).

(Woods, 2000). Building on the design of Woods (2000), prey ingestion, growth, and survival were compared in similar-aged *H. abdominalis* juveniles (3, 7, 42 DAB) under similar environmental conditions in eight tank colors (clear, white, yellow, orange, red, blue, green, black) in short-term and long-term trials (Martinez-Cardenas and Purser, 2007). Results showed, however, that tank color may not be critical in *H. abdominalis* juvenile culture (Martinez-Cardenas and Purser, 2007). The contrasting results might be attributed to different levels of *Artemia* enrichment (Woods, 2007).

In the present experiment, the best growth (height and weight) was obtained in juveniles reared in dark backgrounds with copepods as the prey organisms. The vast differences between our results and those reported by Woods (2000) and Martinez-Cardenas and Purser (2007) suggest that response to color may be species-specific. Further, light intensity can enhance prey detection and capture, and manipulation of prey characteristics such as color can influence feeding success (Dendrinis et al., 1984). The significantly higher growth in the dark backgrounds might have been due to increased visibility. Copepod prey are translucent and may have easily merged into the light colored backgrounds. The incidence of clear copepods was higher in the gut of Atlantic

an average 65% of the total mortality in all backgrounds but was more predominant in the transparent and yellow backgrounds.

## Discussion

Attempts to culture seahorses on a mass scale have met with limited success due to inadequate nutrition and disease resulting in heavy juvenile mortality (Thangaraj and Lipton, 2008; Garcia et al., 2010). Therefore, to successfully rear seahorses, it is necessary to determine the optimum prey density, feed type, system design, and conditions. The initial developmental period (0-21 DAB) in seahorse husbandry, when juveniles switch from a pelagic to a settlement phase, is very critical as mortalities are heavy during this period. In addition to prey density, light orientation, intensity, and wavelength, tank hydro-dynamics, and the color and orientation of prey items, the background color of a tank affects the ability of fish larvae to detect, capture, and ingest food items which, in turn, affect feed efficiency and survival (Duray et al., 1996; Martinez-Cardenas and Purser, 2007; McLean et al., 2008).

There may be an age-dependent effect of tank color (white, black, clear) on the attack rate and capture success in different stages of *H. abdominalis* juveniles (Woods, 2000). At 7 DAB, the attack rate and capture success were highest by juveniles in clear jars, followed by juveniles in black and white jars; at 30 DAB, there were no significant differences in attack rate between jars but capture success was higher in clear and white jars than in black

salmon alevins grown in a red aquarium than in guts of those grown in blue or green aquaria (Browman and Marcotte, 1987), perhaps because of the higher dispersion and scattering of light that make prey appear bright in contrast to a dark background (Naas et al., 1996). The poor ability of juveniles to detect and capture prey in light colored backgrounds may have affected feeding efficiency, resulting in poor growth.

The significantly higher growth of *H. kuda* juveniles reared in dark backgrounds is not surprising considering the natural habitat of this species. *Hippocampus kuda* typically inhabit shallow coastal waters and estuaries and are often found attached to thick seaweed vegetation where light penetration is relatively low. Tanks with black backgrounds provide a light regime that best represents natural conditions for rearing marine fish larvae (Naas et al., 1996). While first-feeding Atlantic salmon alevins possess innate color preferences that can be altered by a single associative learning event (Clarke and Sutterlin, 1985), the presence of such innate color preferences has not been reported in seahorses. A short-term color-conditioning test to determine the preference of *H. abdominalis* juveniles for a specific background color failed to find any conditioning to the color of the tank in which the juveniles were cultured (Martinez-Cardenas and Purser, 2007).

Ingestion of an air-bubble accounted for 65% of the total mortality in all tank colors. Juveniles in the light colored tanks had significantly higher incidences of air-bubble ingestion than juveniles in dark colored tanks. Air bubble ingestion during the pelagic phase in juvenile seahorses occurs due to miss-strike. Seahorse juveniles can engulf atmospheric air if they miss a strike while preying on copepods, leading to formation of an air bubble in the digestive tract. Young juveniles cannot expel ingested air bubbles. They lose their balance and are unable to capture prey, leading to starvation and ultimately death. The highest mortality due to air bubble ingestion occurred in transparent and yellow tanks. Therefore, tank color should be optimized according to prey organism, light conditions, and seahorse species.

The results of the current study, like those of previous studies, are inconclusive from a growth perspective. Nevertheless, they should help to optimize rearing conditions for pelagic phase rearing of the yellow seahorse, *H. kuda*.

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### References

- Blaxter J.H.S.**, 1980. Vision and feeding of fishes. pp. 32-56. In: J.E. Bardach, J.J. Magnuson, R.C. May, J.M. Reinhart (eds.). *Fish Behaviour and its Use in the Capture and Culture of Fishes*. ICLARM Conf. Proc., ICLARM, Manila, Philippines.
- Browman H.I. and B.M. Marcotte**, 1987. Effects of prey color and background color on feeding by Atlantic salmon alevins. *Prog. Fish-Cult.*, 49:141-143.
- Clarke L.A. and A.M. Sutterlin**, 1985. Associative learning, short-term memory, and color preference during first feeding by juvenile Atlantic salmon. *Can. J. Zool.*, 63:9-14.
- Dendrinis P., Dewan S. and J.P. Thorpe**, 1984. Improvement in the feeding efficiency of larval, post larval and juvenile Dover sole (*Solea solea* L.) by the use of staining to improve the visibility of *Artemia* used as food. *Aquaculture*, 38:137-144.
- Duray M.N., Estudillo C.B. and L.G. Alpasan**, 1996. The effect of background color and rotifer density on rotifer intake, growth and survival of the grouper (*Epinephelus suillus*) larvae. *Aquaculture*, 146:217-224.
- Foster S.J. and A.C.J. Vincent**, 2004. Life history and ecology of seahorses: implications for conservation and management. *J. Fish Biol.*, 65:1-61.
- Garcia L.M.B., Hilomen-Garcia G.V. and R.L.M, Calibara**, 2010. Culturing seahorse (*Hippocampus barbouri*) in illuminated cages with supplementary *Acetes* feeding. *Isr. J. Aquacult. - Bamidgeh*, 62:122-129.

- Job S.D., Do H.H., Meeuwig J.J. and H.J. Hall,** 2002. Culturing the oceanic seahorse, *Hippocampus kuda*. *Aquaculture*, 214:333-341.
- Koldewey H.,** 2005. Syngnathid husbandry in public aquariums. [http://seahorse.fisheries.ubc.ca/Documents/Books/Syngnathid\\_Husbandry\\_Manual2005.pdf](http://seahorse.fisheries.ubc.ca/Documents/Books/Syngnathid_Husbandry_Manual2005.pdf). Retrieved 18 October 2010.
- Koldewey H.J. and K.M. Martin-Smith,** 2010. A global review of seahorse aquaculture. *Aquaculture*, 302:131-152.
- Kuiter R.H.,** 2009. *Seahorses and their Relatives*. Seaford, Aquatic Photographics, Victoria, Australia. 334 pp.
- Lourie S.A., Vincent A.C.J. and H.J. Hall,** 1999. *Seahorses: an Identification Guide to the World's Species and their Conservation*. Project Seahorse, London, UK. 214 pp.
- Martinez-Cardenas L. and G.J. Purser,** 2007. Effect of tank color on *Artemia* ingestion, growth and survival in cultured early juvenile pot-bellied seahorses (*Hippocampus abdominalis*). *Aquaculture*, 264:92-100.
- McLean E., Cotter P., Thain C. and N. King,** 2008. Tank color impacts performance of cultured fish. *Ribarstvo*, 66:43-54.
- Moriya T. and Y. Miyashita,** 1987. Body color and the preference for background color of the Siamese fighting fish, *Betta splendens*. *Zool. Sci.*, 4:881-888.
- Murugan A., Dhanya S., Sreepada R.A., Rajagopal S. and T. Balasubramanian,** 2009. Breeding and mass-scale rearing of three spotted seahorse, *Hippocampus trimaculatus* Leach under captive conditions. *Aquaculture*, 290:87-96.
- Naas K., Huse I. and J. Iglesias,** 1996. Illumination in first feeding tanks for marine fish larvae. *Aquacult. Eng.*, 15:291-300.
- Papoutsoglou S.E., Mylonakis G., Miliou H., Karakatsouli N.P. and S. Chadio,** 2000. Effects of background color on growth performances and physiological responses of scaled carp (*Cyprinus carpio* L.) reared in a closed circulated system. *Aquacult. Eng.*, 22:309-318.
- Project Seahorse,** 2006. *Annual Report*. Univ. British Columbia. 20 pp.
- Salin K.R. and N.C. Mohankumaran,** 2006. Resources and biodiversity of seahorses and the need for their conservation in India. *Aquacult. Asia*, 10:3-8.
- Tamazouzt L., Chatain B. and P. Fontaine,** 2000. Tank wall color and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). *Aquaculture*, 182:85-90.
- Thangaraj M. and A.P. Lipton,** 2008. Survival and growth of captive reared juvenile seahorse (*Hippocampus kuda*) fed live feeds and fishmeal. *Isr. J. Aquacult. - Bamidgeh*, 60:185-189.
- Victor B.C.,** 1991. Settlement strategies and biogeography of reef fishes. pp. 231-260. In: P.F. Sale (ed.). *The Ecology of Fishes on Coral Reefs*. Academic Press, San Diego, CA.
- Vincent A.C.J.,** 1996. *The International Trade in Seahorses*. TRAFFIC Int. 163 pp.
- Woods C.M.C.,** 2000. Improving initial survival in cultured seahorses, *Hippocampus abdominalis* Leeson, 1827 (Teleostei: Syngnathidae). *Aquaculture*, 190:377-388.
- Woods C.M.C.,** 2007. *Aquaculture of the Big-Bellied Seahorse Hippocampus abdominalis, Lesson 1827 (Teleostei: Syngnathidae)*. Ph.D. Thesis, Victoria Univ. Wellington, New Zealand. 251 pp.