INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 2, p. 430-438, 2019

RESEARCH PAPER OPEN ACCESS

Effect of different light intensity in the growth and survival of *Hippocampus kuda* (yellow seahorse) under laboratory conditions

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Key words: *Hippocampus kuda,* Conservation, Light, Seahorse.

http://dx.doi.org/10.12692/ijb/14.2.430-438 Article published on February 27, 2019

Abstract

Seahorses are charismatic fishes that are a focus for marine conservation efforts. Among many species of seahorses, the yellow seahorse, *Hippocampus kuda*, are subject to growing market demand. The study aims to identify the suitable light intensity for seahorses under small-scale laboratory set-up, to come up with recommendations for effective seahorse rearing. This investigation examined the effect of different light intensities (500 lx, 1000 lx, 1500 lx) on the growth and survival of male and female seahorses *H. kuda*. Results show an insignificant difference in the average weight and standard length of *H. kuda* as a measure of growth between the different light intensities with p=0.723 for the weight and p= 0.602 for the standard length respectively. However, it was observed that darker environment showed poor prey contrast and therefore increased the efforts in feed intake. For the comparison between male and female *H. kuda,* there is a significant difference in the growth between male and female *H. kuda* in terms of the average length across different light intensities with p= 0.001 wherein, male species of *H. kuda* exhibited a greater rate of growth in terms of weight and length compared to the female. Moreover, males have aggressive behaviors such as tail wrestling and snapping of snout. Results demonstrated that the presence of light can improve the growth rate of male *H. kuda* species.

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Introduction

Over 15 million seahorses are being traded annually in Southeast Asia alone (Garcia and Hilomen-Garcia, 2009). The International Union for the Conservation of Nature (IUCN) included *Hippocampus kuda* in its list of vulnerable species due heavy exploitation for aquarium trading as well as traditional Chinese medicine and patent medical preparations (Lin *et al.*, 2006; Garcia and Hilomen-Garcia, 2009; Celino *et al.*, 2012). In response, in the mid-1990s there was a sudden raise of awareness in countries with large and valuable trade in seahorses and the related concerns regarding the exploitation of wild seahorses. In general, yellow seahorses have higher market value in aquarium trading and *Hippocampus kuda* is one of the 32 existing native species of seahorses in Indo-Pacific waters and is one of the most traded seahorses in the Philippines because of its smooth appearance and pale yellow complexion that are preferred by overseas markets of both traditional Chinese medicine and patent medicinal preparations (Celino *et al.*, 2012). Thus, providing an alternate source of seahorses, the Department of Agriculture in the Philippines embarked on testing seahorse aquaculture with juvenile grow-out in floating cages, but failed due to high juvenile mortality. Despite that, seahorse aquaculture in the Philippines continued to be developed by the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) (Foster *et al.*, 2003; Woods, 2007). Accordingly, it was reported that high mortality of seahorses, particularly during the pelagic phase, has been attributed to inadequate or inefficient feeding responses. Mortalities can possibly be minimized by improved feeding efficiency and manipulation of the light intensity (Koldewey, 2005; Thangaraj and Lipton, 2008; Garcia *et al.*, 2009). Generally, seahorses are visual predators, and as they are not fast swimmers they implement a sit and wait attack strategy (Lovett, 1969; Wong and Benzie, 2003; Martinez-Cardenas and Purser, 2007). Herewith, stressed the role of light intensity on growth and survival of seahorses hence, this study.

Due to the low survival of juvenile seahorses and low Reproductive efficiency of the parent seahorses, cultures have been restricted for many years (Wong and Benzie, 2003; Lin *et al.*, 2009).

Although researches on different seahorse species has been done (Lin *et al.*, 2009) such as the study on the effects of tank color and light intensity on the growth and survival of juvenile seahorses (Martinez-Cardenas and Purser, 2007); the study on the effects of temperature, food, stocking density and light on the growth of *H. whitei* (Wong and Benzie, 2003); tolerance of seahorse *H. kuda* juveniles to various salinities (Hilomen-Garcia *et al.,* 2003); and feeding selectivity of *H. kuda* juveniles (Celino *et al.,* 2012), records on the effect of light intensity to *H. kuda* species are still lacking.

Moreover, light is extremely variable in nature. It has been reported that, light intensity plays a major role in most species to the point that too intense light can be stressful or even lethal. Receptivity and the developmental status of fish to light profoundly changes according to the species (Boeuf and Bird, 1999). *H. kuda* is one of the most traded seahorse species in the Philippines. However, limited production remains the major problem that should be overcome to improve efficacy and commercial viability of seahorse aquaculture (Celino *et al.*, 2012). The study aimed to determine the effects of different light intensity on the survival and growth of *H. kuda* in order to provide a suitable light intensity for seahorse rearing and serve as a baseline data for future researches.

Materials and methods

Sampling area, specimens and identification

Seahorse specimens were readily available in the laboratory at Premier Research Institute of Science and Technology (PRISM) and donated as live bycatch samples from fishermen. Hence, such opportunity was taken advantage to study the effect of different light intensities on the growth and survival of seahorse, *Hippocampus kuda*. The seahorses were reported to come from Tubod, Lanao del Norte, Philippines. Identification of samples was done through illustrated keys, Guide to the identification of Seahorses (Lourie *et al*., 2004) and consultation of experts. At the start of the study, all seahorses were sexed and initial weights and lengths were obtained, and subtracted to succeeding measurements. The study was conducted for four (4) weeks under laboratory conditions at the Premier Research Institute of Science and Technology (PRISM).

Experimental set-up

For this study, the four treatments were the following: 500lx, 1000lx, 1500lx, (measured on the water surface from an overhead LED light source) and the control set-up (no artificial light source overhead, utilized available natural light only), each with four replicates, and was run for a total of four (4) weeks to test the effect of light intensity on the growth and survival of seahorses. Transparent cylindrical container (23H x 15 W cm) was used, each with 4 liters of seawater, containing a pair of male and female seahorse. A 9W LED lamp was used as the light source in the 500 lux set-up, 12W LED lamp in the 1000 lux set-up, and 15W LED lamp in the 1500 lux set-up.

The lights were kept ON during the entire duration of the experiment. Furthermore, a digital lux meter (FUYI®LX-1010B) was used to ascertain the different light intensities.

Filtered seawater was used in all experimental set-ups and was changed once a week. Moderate aeration was provided using an aerator. Conditions such as salinity were maintained at 30 to 35 ppt and all other parameters were constant to all set-ups with the exception of light intensity. Temperature in each set-up was monitored closely and was maintained at $25^{\circ}C$ to $27^{\circ}C$. The seahorses were fed by *Artemia* once a day. Stones were used as the substrate and holdfasts for the seahorses.

Hatching and enrichment of Artemia cyst

For the decapsulation of *Artemia* cysts, the cysts were soaked for an hour in aerated water, aeration supply should be at the bottom, with an overhead lamp. Household bleach was added after and was soaked for another 10 minutes. Cheesecloth was used to filter and rinsed the *Artemia* until the smell of the bleach was gone. Lastly, the rinsed *Artemia* cysts were soaked in aerated seawater for 20 – 24 hours to hatch.

Collection of data

The wet weight, length and survival rate were observed and recorded for four weeks. Standard length (= head length + trunk length + tail length) (Lourie, 2004) (Fig. 1.), was measured using the imageJ image tool. Filtered seawater was used and debris that settled at the bottom were siphoned out without disturbing the animals. Survival rate (%) was calculated using a formula:

$$\text{Survival Rate (\%)} = \frac{\text{Final Number of Surviving Seahorse}}{\text{Initial Number of Seahorse}} \text{X } 100$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the differences between the different light intensities based on standard length and wet weight. All the variables were tested for normality and homogeneity. ANOVA yield insignificant so, post hoc test was not performed. To test the hypothesis for significant difference in the growth of *H. kuda* between the male and female species across different light intensities, Independent Samples t test was utilized. The software Statistical Package for the Social Science (IBM SPSS Statistics 20) was used to carry out all of the analyses.

Results and discussion

Growth of H. kuda in terms of weight and length

The growth and survival of seahorse, *Hippocampus kuda* was studied across different light intensities. According to Martinez-Cardenas and Purser, 2007, teleost fish are naturally visual feeders and conditions such as light intensity may influence this process.

Table 1 shows the mean and standard deviation between male and female *H. kuda* for each set-up in terms of their wet weight.

Table 1. Mean and standard deviation between male and female *H. kuda* for each set-up in terms of their wet weight (g).

Light Intensity	Male		Female	
	Mean	SD	Mean	SD
Control	8.326	0.545	3.540	0.393
500 Lux	6.306	0.423	6.960	0.590
1000 Lux	7.711	0.331	3.123	0.106
1500 Lux	7.250	0.537	6.198	0.449

It was found out that male *H. kuda* species were heavier than female *H. kuda* in all set-ups except the 500 lux treatment. Table 2 shows the mean and standard deviation between male and female *H. kuda* for each set-up in terms of their standard length where, the male *H. kuda* species were longer than female *H. kuda* in all set-ups. Table 3 showed the ANOVA result for the test of significant difference in the growth of *H. kuda* in terms of weight. The results showed a p – value = 0.723 thus, there is an insignificant difference in the average weight of *H. kuda* as a measure of growth comparing the different light intensities.

Table 2. Mean and standard deviation between male and female *H. kuda* for each set-up in terms of their standard length (cm).

Light Intensity	Male		Female	
	Mean	SD	Mean	SD
Control	15.926	1.663	11.830	0.829
500 Lux	14.729	0.770	12.320	2.967
1000 Lux	16.271	1.235	11.234	0.526
1500 Lux	15.608	1.086	14.450	0.825

This was supported by the findings of Wong and Benzie (2003) wherein, *H. whitei* from Australia shows no significant difference on any of the variables tested. Moreover, Table 4 showed the ANOVA test for significant difference in the growth of *H. kuda* in terms of length. Results yield a p – value = 0.602 hence, there is an insignificant difference in the average length of *H. kuda* as a measure of growth comparing the different light intensities. This was in contrast to the findings by Lin *et al.,* 2009, wherein the growth of sub-adult *H. erectus* Perry in the 1500 lx treatment had the highest incremental standard length. However, findings are justifiable since, *H. kuda* is highly variable in nature considering that it is a complex species.

Table 3. One-way analysis of variance (ANOVA) for growth of *H. kuda*, in terms of weight (g) at different light intensities

	Sum of sqrs	df	Mean square	F	p
Between groups:	4.415	3	1.472	0.445	0.723
Within groups:	105.935	32	3.310		
Total:	127.302	28			

*p<0.05 is significant.

In view of the results, supporting studies showed, that light intensity often influences prey capture, survival and stress response in many fish species (Barton, 2002), and high light intensity may cause stress and mortality in fish (Boeuf and Bird, 1999). Depending on the species, light intensity has different effects on growth. In the recent study conducted, 500, 1000, and 1500 lux showed no effect in the growth of *H. kuda* species. As stated by Benfield and Minello, 1996, light intensity only affects feeding of his experimental fish as illumination increased from near darkness to very low levels. Other data suggest that for visual predators, reductions in light intensity may influence reactive distance and reduce predation rates only at very low levels of illumination.

Table 4. One-way analysis of variance (ANOVA) for growth of *H. kuda*, in terms of length (cm) at different light intensities.

	Sum of sqrs	df	Mean square	F	p
Between groups:	8.950	3	2.938	0.629	0.602
Within groups:	151.746	32	4.742		
Total:	160.696	35			

*p<0.05 is significant.

According to James and Heck, 1994, predation rates were not statistically different among low and high light intensities, but were significantly reduced in complete darkness. Thereby, darker environment may show a poor prey contrast and therefore increased the efforts in feed intake for some fish species (Lin *et al.,* 2009). However, light intensity measured at the water surface does not exactly correspond with light intensity perceived by a fish beneath the surface as light is quickly absorbed in water. Consequently, the light levels measured at the water surface serve only as an index of light level and do not represent the real threshold value for drifting (Reichard *et al.,* 2002). Indeed, the aforementioned light intensities have uniform effects to the growth of *H. kuda* species.

Table 5. Test for significant difference in the growth between male and female *H. kuda* in terms of weight (g) across different light intensities.

	Groups	
	Male	Female
Mean	7.398	4.934
SD	0.869	1.673
Mean difference	2.464	
t– value	5.707*	
Degrees of freedom	34	
p– value	0.001	
Decision	Reject H_o	
Interpretation	Significant difference	

*Difference is significant at the 0.05 level (2-tailed).

Moreover, males were naturally stronger and active than females. Table 5 shows the test for significant difference in the growth between male and female *H. kuda* in term of weight across different light intensities. Table 5 shows the p – value = 0.001. Since p – value is much smaller than the level of significance $\alpha = 0.05$, the null hypothesis is rejected inferring that there is a significant difference in the growth between male and female *H. kuda* in terms of the average weight across different light intensities. The result further shows that the male species of *H. kuda* exhibited a greater rate of growth in terms of weight compared to the female species as confirmed in the mean difference of 2.464.

Table 6. Test for significant difference in the growth between male and female *H. kuda* in terms of length (cm) across different light intensities.

	Groups	
	Male	Female
Mean	15.634	12.629
SD	1.273	1.819
Mean difference	3.005	
t– value	5.824*	
Degrees of freedom	34	
p– value	0.001	
Decision	Reject H_o	
Interpretation	Significant difference	

*Difference is significant at the 0.05 level (2-tailed).

Table 6 shows the test for significant difference in the growth between male and female *H. kuda* in terms of length across different light intensities. Table 6 depicts a p – value = 0.001, inferring that there is a significant difference in the growth between male and female *H. kuda* in terms of the average length across different light intensities. The result further shows that the male species of *H. kuda* exhibited a greater rate of growth in terms of length compared to the female species as confirmed in the mean difference of 3.005. This is in contrast to the findings by Woods (2002) wherein the growth of *H. abdominalis* had no significant differences between male and female *H. abdominalis* species.

In addition, male seahorses compete more intensely than females (Vincent, 1994). It was revealed in this study that male *H. kuda* species shows greater rate in growth than female *H. kuda* species. According to Vincent *et al*., 1992, all male syngnathids carry and care for developing embryos that may influence the competition. Activity levels were significantly higher in male competition than in female competition in terms of behaviors common to both sexes and only males exhibited behaviors employed uniquely in aggression (Vincent, 1994). Males exhibit higher levels of behaviour patterns common to both sexes when in the company of another male than do females when another female is present, and the two most overtly aggressive behaviours in courtship (tail wrestling and snapping with the snout) were confined to males, whereas females had no sex-specific competitive behaviours.

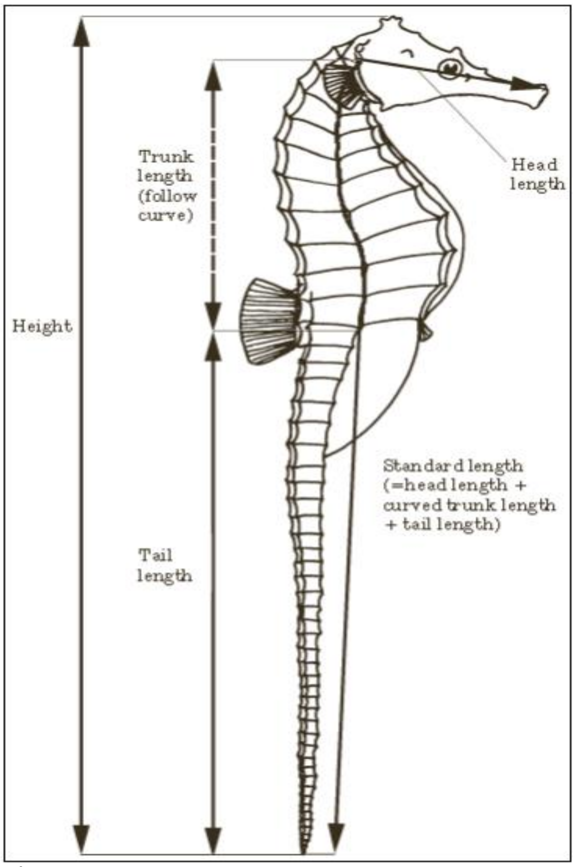

Fig. 1. Standard length (SL) as measured on a seahorse (source: S. A. Lourie, 2004).

The more intense male competition for mates may at least partially derive from the strongly male-biased operational sex ratio amongst unpaired seahorses (Vincent *et al*., 1992). Female competition could have serious consequences despite its lower intensity.

However, females had no unique aggressive behaviors such as snapping and wrestling (Vincent, 1994).

Survival rate

The survival rate of *H. kuda* seahorses in 1500 lx and control set-up was the highest at 100% and 500 lx and1000 lx both had a survival rate of 50% (Fig. 1). It has similar results with Lin *et al.,* 2010, *Hippocampus erectus* had a high survival rate of 86.75 ± 4.74% under 1500 lx and was low under 500 lx at 43.3±7.8% after a 4-week study.

A possible explanation for this phenomenon is the fact that seahorses, as well as their prey, *Artemia*, were active in high light environment (Barton, 2002). Natural light from the sunlight yield also favorable.

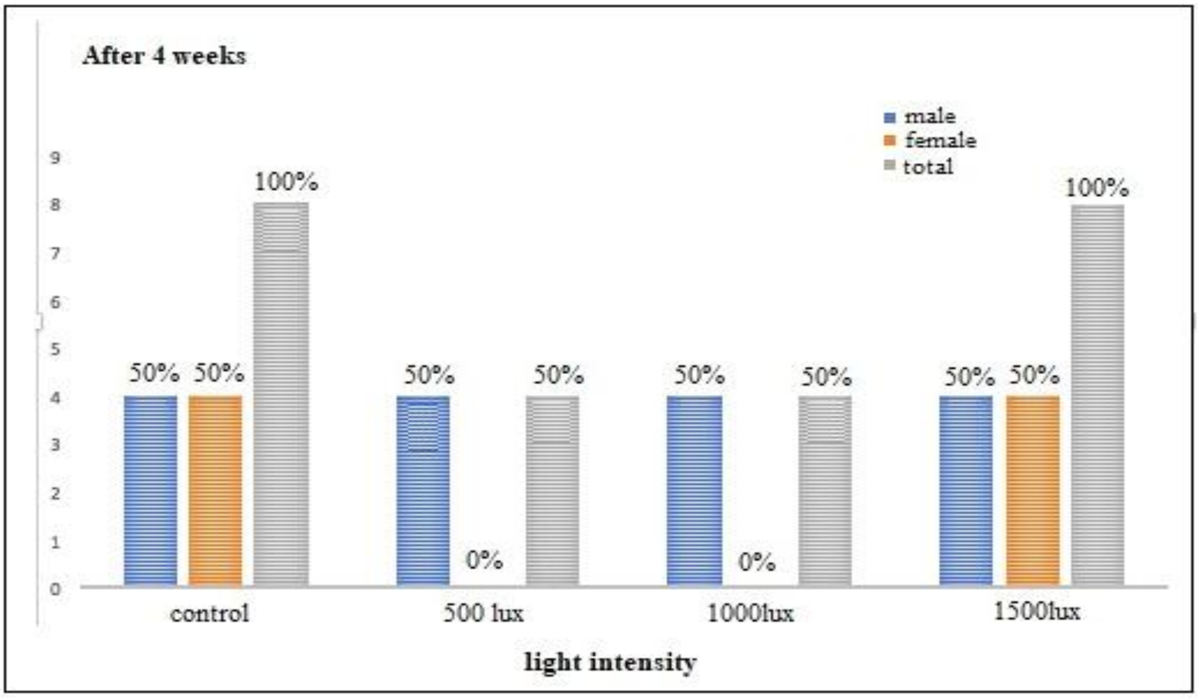

Fig. 2. Survival rate of male and female individuals of *H. kuda* across different light intensity.

Conclusion

Results show an insignificant difference in the average weight and standard length of *H. kuda* as a measure of growth comparing different light intensities. However, for the comparison between male and female *H. kuda* the result show significant difference in growth, in terms of the average length across different light intensities. Herewith, the male species of *H. kuda* exhibited a greater rate of growth in terms of weight and length compared to the female species as confirmed in the mean difference of 2.464 and 3.005 respectively.

Although the initial wet weight and standard length of the samples vary and was observed for a short period of time results demonstrated that light intensity can improve the growth rate of male *H. kuda* species. This study served as a baseline data for future researches.

Acknowledgment

The researchers would like to express their heartfelt gratitude to their families and friends who become a considerable source of inspiration and determination.

This research was done under the Premier Research Institute of Science and Mathematics (PRISM), MSU-IIT.

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