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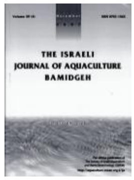

Culturing Seahorse (*Hippocampus barbouri*) in Illuminated Cages with Supplementary *Acetes* Feeding

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Abstract

Juvenile *Hippocampus barbouri* were grown in illuminated cages with or without supplemental daytime feeding of thawed *Acetes* (a planktonic marine crustacean), or in non-illuminated cages with *Acetes* feeding, as a supplement to light-attracted zooplankton prey. After ten weeks, seahorses in illuminated cages fed *Acetes* had the highest mean body weight (2.24 g) and length (8.20 cm), but these did not significantly differ from seahorses in unfed illuminated cages (1.88 g; 7.25 cm), which did not significantly differ from those in fed non-illuminated cages (0.88 g; 6.32 cm). In all treatments, the mean instantaneous growth rate in body weight declined progressively throughout the test but the instantaneous growth rate in stretched length did not vary. Mean survival (76-100%) of seahorses in fed non-illuminated cages and in unfed illuminated cages did not vary significantly over the test period. The mean survival of seahorses in fed illuminated cages was lowest (54%), but did not significantly differ from the other treatments. Juvenile *H. barbouri* grown in illuminated cages had better growth than those in non-illuminated cages, but survival was reduced when seahorses in illuminated cages were fed *Acetes*.

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Introduction

Seahorses are threatened worldwide due to demands of the traditional Chinese medicine market in Asian countries and the marine aquarium trade in Europe, North America, and Japan (Foster and Vincent, 2004). The continuing exploitation of wild stocks of seahorses has drastically reduced their populations worldwide, prompting the inclusion of all seahorse and pipefish species in Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and trade regulations (Foster and Vincent, 2004). To alleviate the increasing intensity of wild seahorse fishing, alternative means of seahorse production are necessary. Seahorse farming has shown potential. Indeed, studies demonstrate that seahorses may be reared in small enclosures in hatcheries when fed live enriched brine shrimp (*Artemia*), thawed mysid shrimp, or copepods (Job et al., 2002; Wong and Benzie, 2003; Woods, 2003; Woods and Valentino, 2003; Sheng et al., 2006; Thangaraj and Lipton, 2008).

Syngnathids are ambush predators that feed during daytime hours on a variety of mobile prey consisting mostly of planktonic crustaceans such as mysid shrimps, amphipods, copepods, or any tiny larvae that fits into their elongated snouts (Kendrick and Hyndes, 2005). Seahorses thrive on these types of zooplankton prey (Woods, 2002; Kitsos et al., 2008) because they are rich in highly unsaturated fatty acids (HUFA), which are essential for the growth and development of juvenile fish (Watanabe et al., 1983). Studies demonstrate that syngnathids fed HUFA-enriched plankton prey attain higher growth and survival rates than those fed prey with low HUFA contents (Payne et al., 1998; Payne and Rippingale, 2000; Woods, 2002).

The provision of HUFA-enriched nauplii or adult *Artemia* during hatchery rearing of juvenile seahorses aims to duplicate the contents of such fatty acids and other essential nutrients in wild zooplankton prey. However, the use of costly *Artemia* cysts, and enriching them in equally expensive HUFA formulations, add to the already high cost of operating a hatchery; even more so if *Artemia* is used as the only food supplement during grow-out culture.

Producing large amounts of copepods for the hatchery is likewise expensive and difficult to maintain (Støttrup and Norsker, 1997). Therefore, providing light-attracted zooplankton prey is an attractive and inexpensive alternative to *Artemia* feeding for growing zooplanktivore fishes like the seahorse.

Zooplankton consisting of 64-78% copepods (calanoids, cyclopoids, harpacticoids, and nauplii) are more abundant in illuminated sea cages than in non-illuminated cages at the Igang Marine Substation (Fermin and Seronay, 1997). Illuminated net cages attract marine plankton during the night, providing additional food to caged organisms. Recently, the seahorse *Hippocampus kuda* was reared in illuminated net cages (Garcia and Hilomen-Garcia, 2009). Rearing seahorses in illuminated cages minimizes the use of costly *Artemia* as a supplementary food during the grow-out period.

This investigation compares the growth and survival of juveniles of the seahorse (*Hippocampus barbouri*) reared in illuminated and non-illuminated sea cages with supplemental feeding of thawed mysid shrimp *Acetes* during the daytime.

Materials and Methods

Fish. Juveniles of *Hippocampus barbouri* (Teleostei: Syngnathidae, Jordan and Richardson, 1908) were obtained from several adult mating pairs and reared in concrete hatchery tanks for 1-3 months. The juveniles were fed live mysid shrimps and copepods daily, including the nauplii or adults of HUFA-enriched *Artemia* (Hilomen-Garcia et al., 2003).

At the end of hatchery period, equal batches of uniform sized juveniles were pooled to stock the cage experiments. The young seahorses (1.3-3.4 months, 0.18±0.01 g, 3.75±0.08 cm stretch height) were transported early in the morning (about 05:00) to the Igang Marine Substation on nearby Guimaras Island.

Experimental design. Treatment groups consisted of juvenile seahorses fed thawed *Acetes* in (a) illuminated cages or (b) non-illuminated cages, and (c) seahorses stocked in illuminated cages but not fed. According to preliminary trials, the fourth possibility, unfed seahorses raised in non-illuminated cages, die within 3-5 days post-stocking (Garcia and Hilomen-Garcia, 2009). Thus, this option was not included in the experimental design.

Cages. Floating net cages (1 × 1 × 3 m; 3 m^3; 2-3 mm mesh) were set in a 7 × 7 m bamboo frame, covered with a net of the same mesh, in a protected shallow (4 m deep at high tide) marine cove lined with mangrove trees and a muddy substrate with a dense cover of eel grass. Illuminated cages were lit from midnight until 05:30 by a 20-watt cool white electric bulb suspended a meter above the cage (Fermin and Seronay, 1997). Unilluminated cages were set in an identical bamboo frame about 500 m from the illuminated frames. Experimental cages were carefully replaced with clean units every 3-5 days. Ambient surface seawater temperature and salinity in the experimental cages ranged from 26°C to 30°C and from 32 to 34 ppt.

Stocking. Seahorses were stocked in three replicates for each treatment. Due to the limited availability of hatchery-produced seahorse juveniles, each cage was stocked with only 13-17 individuals (4-6 seahorse/m^3), a density so low that it could not have significantly affected performance in the experimental cages.

Feeding. Acetes were seined by local fishers from nearby coastal waters, washed thoroughly in clean fresh water, stored frozen (-4°C) in 2-3 kg aliquots, and thawed and finely chopped daily prior to *ad libitum* feeding at 10:00 and 14:00. Provision of thawed *Acetes* was stopped when seahorses refused to eat.

Sampling. Seahorses were sampled every two weeks until the end of the ten-week experiment during which individual body weight, stretch height, and survival were recorded. The body weights of blotted-dry fish were recorded with a battery-powered weighing scale (±0.01 g). Stretch height, measured with a caliper (±0.1 mm), is the length of a seahorse from its coronet tip to the tip of its stretched tail. The instantaneous growth rates of body weight and stretch height were calculated every two weeks as [$\ln_{(BWt\ or\ SHt)}$ - $\ln_{(BWi\ or}$

$_{SHi)}$]/t, where $\ln_{(BWi\ or\ SHi)}$ is the natural logarithm of the parameter at the start of the experiment and $\ln_{(BWt\ or\ SHt)}$ is the natural logarithm of the same parameter at time t. The use of instantaneous growth rate (g or cm/day) rather than specific growth rate (SGR) is strongly recommended for reporting fish growth in aquaculture experiments (Hopkins, 1992), especially when small fish, such as seahorse juveniles, are studied in short-term aquaculture experiments because growth of small juvenile fish increases exponentially.

Data analysis. The homogeneity of variances of the data was tested by Levene's test prior to one-way analysis of variance. Where variances were not normally distributed, the Kolmogorov-Smirnov two-sample test was used to compare differences among treatment means. When appropriate, post hoc multiple comparisons of means were done with Duncan's range test. The null hypothesis (i.e., all treatment means are equal) was rejected at $p<0.05$ (Zar, 1984). Survival data were arc-sine transformed prior to statistical analysis. All statistical analyses were done with the Statistical Package for the Social Sciences (SPSS) Version 7.5 software (Chicago, Illinois, USA).

Results

Mean body weight and stretch height increased in all treatments as early as two weeks after initiation of cage rearing and peaked at the end of the test (Fig. 1). At week 10, the mean body weights in the illuminated cages were 1.88 and 2.24 g, higher than in the non-illuminated cages (0.88 g). Likewise, the mean stretch heights in the illuminated cages were 7.25 and 8.20 cm and only 6.32 cm in the non-illuminated cages.

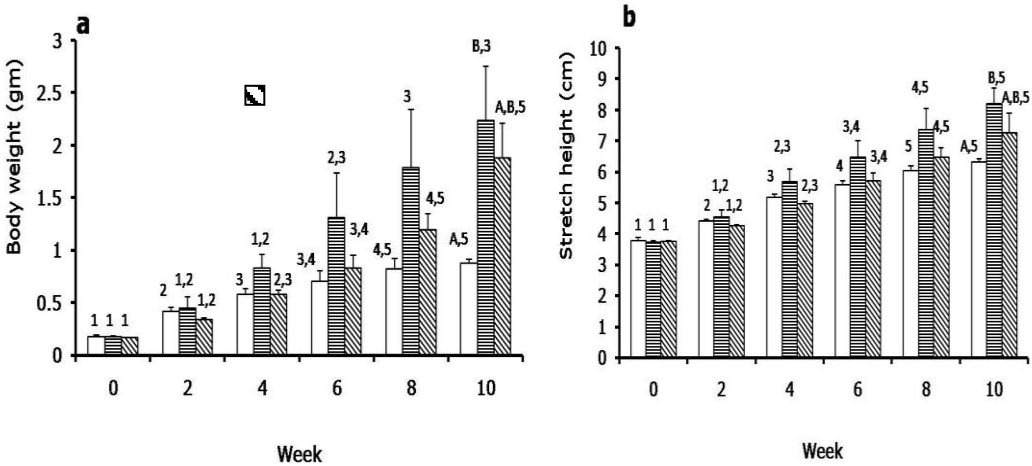

Fig. 1. Mean (a) body weight and (b) stretch height of juvenile *Hippocampus barbouri* grown ten weeks in sea cages. Seahorses were fed thawed *Acetes* and reared in non-illuminated (☐) or illuminated cages (▤), or not fed thawed *Acetes* and reared in illuminated cages (▧). Vertical lines above bars represent standard errors of means. Numbers above bars indicate significant differences between weeks within a treatment; letters above bars indicate significant differences between treatments during the given week; $p<0.05$.

The mean instantaneous growth rate of body weight significantly declined over the test period, from 0.056 to 0.023 g/day in non-illuminated cages and from 0.046 to 0.031 g/day in non-fed cages (Fig. 2). It also dropped in the fed-illuminated treatment (from 0.059 to 0.036 g/day), but did not differ significantly. Unlike the growth rate in body weight, the mean instantaneous growth rate for stretch height remained constant in all treatment groups. It was highest in the fed-illuminated treatment (0.011-0.015 cm/day) and significantly differed among treatments as early as week 4, when it was 0.015 cm/day in fed-illuminated seahorses, 0.010 cm/day in non-fed illuminated, and 0.011 in non-illuminated.

Survival of fed seahorses in non-illuminated cages (76-100%) and un-fed fish in illuminated cages (84-100%) did not significantly vary over the period. However, survival of fed seahorses in illuminated cages significantly declined to its lowest level (54%) at the end of the test (Fig. 3).

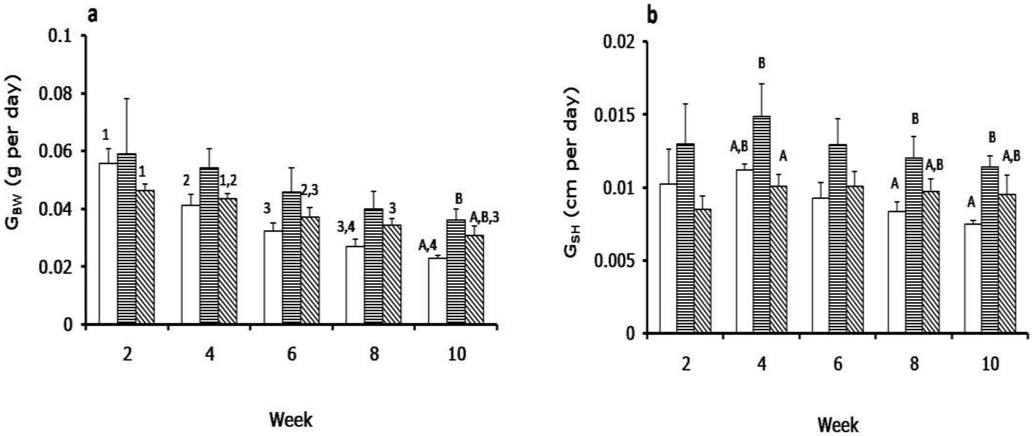

Fig. 2. Mean instantaneous growth rates of (a) body weight (g/day) and (b) stretch height (cm/day) of juvenile *Hippocampus barbouri* grown for ten weeks in sea cages. See Fig. 1 for explanation of symbols.

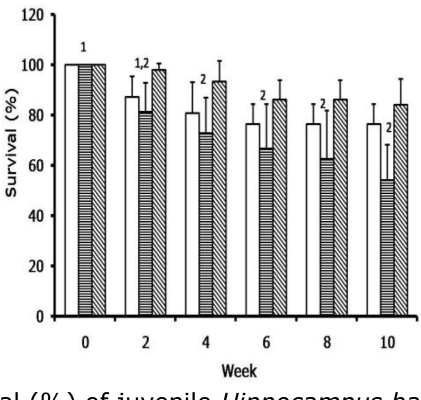

Fig. 3. Mean survival (%) of juvenile *Hippocampus barbouri* grown for ten weeks in sea cages. See Fig. 1 for explanation of symbols.

Discussion

In all treatments, the mean body weight growth rate declined and the mean stretched height growth rate did not vary. The growth rates were comparable to earlier reports on *H. barbouri* and other seahorses (Wilson and Vincent, 1998; Foster and Vincent, 2004; Garcia and Hilomen-Garcia, 2009). Although the seahorses gained weight and length, the declining growth rates indicate that incremental rates of increase of biomass drop as fish grow in size and age, as observed in juvenile *H. kuda* (Garcia and Hilomen-Garcia, 2009) and other fishes (Ricker, 1979).

At the end of the test, mean growth rates were generally higher in illuminated than in non-illuminated cages and *Acetes* feeding did not increase the growth rates in illuminated cages. This suggests that light-attracted zooplankton prey may be sufficient to maintain growth and growth rates in caged seahorses. The variety of wild zooplankton prey ingested by the seahorse juveniles in illuminated cages may possess enough essential fatty acids required for growth and development of young fish. Similarly, a diet consisting of live copepod nauplii improved the growth of captive pipefish (*Stigmatopora argus*) and seahorse (*H. subelongatus*) juveniles (Payne et al., 1998; Payne and Rippingale, 2000).

Mean survival rates were consistent with those in juvenile *H. kuda* grown under similar conditions (Garcia and Hilomen-Garcia, 2009). There was a disadvantage to feeding thawed *Acetes* to seahorses in illuminated sea cages. In addition to zooplankton, illumination attracts other marine and possibly terrestrial animals that may prey on cultured seahorse juveniles. Very likely attracted to the pungent odor of leftover *Acetes* at the bottom of the sea cages, grapsid crabs and small marine fishes such as balistids, monacanthids, tetraodontids, gobiids, and bleniids found their way to the cages at all hours and preyed on the slow-moving seahorse juveniles. Despite net covers, sea birds and rats were also able to catch the caged seahorses. Installation of hiding shelters for seahorses, manual removal of aquatic predators and carcasses from cage bottoms, and scare-tactics to fend off terrestrial predators may reduce such mortality.

In conclusion, the present study shows the feasibility of growing *H. barbouri* in illuminated sea cages, as described for *H. kuda* (Garcia and Hilomen-Garcia, 2009). Juvenile *H. barbouri* grew better in illuminated cages than in non-illuminated cages. However, as with *H. kuda*, daytime feeding of thawed *Acetes* to supplement light-attracted zooplankton did not improve the survival or growth rates; growth in illuminated cages without supplementary *Acetes* was comparable to growth in illuminated cages with supplementary *Acetes* feeding. Thus, juvenile seahorse culture may require only attraction of zooplankton by illumination of seahorse cages. Improvements in raising *H. barbouri* in illuminated cages, particularly elimination of predators, may reverse the low survival rates obtained in this study. Our results may help to up-scale grow-out operations for seahorse seed for trade and rehabilitation of wild stocks depleted by fishing. This study adds to the list of seahorses grown in sea cages as an inexpensive alternative to land-based enclosures.

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References

Fermin A.C and G.A. Seronay, 1997. Effects of different illumination levels on zooplankton abundance, feeding periodicity, growth and survival of the Asian sea bass, *Lates calcarifer* (Bloch), fry in illuminated floating nursery cages. *Aquaculture*, 157:227-237.

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. and G.V. Hilomen-Garcia, 2009. Grow-out of juvenile seahorse *Hippocampus kuda* (Bleeker; Teleostei: Syngnathidae) in illuminated sea cages. *Aquac. Res*., 40:211-217.

Hilomen-Garcia G.V., Reyes R.D. and C.M.H. Garcia, 2003. Tolerance of seahorse *Hippocampus kuda* (Bleeker) juveniles to various salinities. *J. Appl. Ichthyol*., 19:94-98.

Hopkins K.D., 1992. Reporting fish growth: a review of the basics. *J. World Aquac. Soc*., 23:173-179.

Job S.D., Do H.H., Meeuwig J.J. and H.J. Hall, 2002. Culturing the oceanic seahorse, *Hippocampus kuda*. *Aquaculture*, 214:333-341.

Kendrick A.J. and G.A. Hyndes, 2005. Variations in the dietary compositions of morphologically diverse syngnathid fishes. *Env. Biol. Fish*., 72:415-427.

Kitsos M.S., Tzomos T., Anagnostopoulou L. and A. Koukouras, 2008. Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier, 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. *J. Fish. Biol*., 72:1259-1267.

Payne M.F. and R.J. Rippingale, 2000. Rearing West Australian seahorse, *Hippocampus subelongatus*, juveniles on copepod nauplii and enriched *Artemia*. *Aquaculture,* 188:353-361.

Payne M.F., Rippingale R.J. and R.B. Longmore, 1998. Growth and survival of juvenile pipefish *Stigmatopora argus* fed live copepods with high and low HUFA content. *Aquaculture,* 167:237-245.

Ricker W.E., 1979. Growth rates and models. pp. 677-743. In: W.S. Hoar, D.J. Randall (eds.). *Fish Physiology*, vol. VIII. Academic Press, NY.

Sheng J., Lin Q., Chen Q., Gao Y., Shen L. and J. Lu, 2006. Effects of food, temperature and light intensity on the feeding behavior of three-spot juvenile seahorses, *Hippocampus trimaculatus* Leach. *Aquaculture,* 256:596-607.

Støttrup J.G. and N.H. Norsker, 1997. Production and use of copepods in marine fish larviculture. *Aquaculture*, 155:231-247.

Thangaraj M. and A.P. Lipton, 2008. Survival and growth of captive reared juvenile seahorse (*Hippocampus kuda*) fed live feeds and fishmeal. *Isr. J. Aquac. - Bamidgeh,* 60:185-189.

Watanabe T., Kitajima C. and S. Fujita, 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture,* 34:115-143.

Wilson M.J. and A.C.J. Vincent, 1998. Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aquac. Sci. Conserv*., 2:179-196.

Wong J.M. and J.A.H. Benzie, 2003. The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus whitei* (Bleeker, 1855), from Australia. *Aquaculture*, 228:107-121.

Woods C.M.C., 2002. Natural diet of the seahorse *Hippocampus abdominalis*. *N.Z. J. Mar. Freshw. Res.*, 36:655-660.

Woods C.M.C., 2003. Effects of varying *Artemia* enrichment on growth and survival of juvenile seahorses, *Hippocampus abdominalis*. *Aquaculture,* 220:537-548.

Woods C.M.C and F. Valentino, 2003. Frozen mysids as an alternative to live *Artemia* in culturing seahorses *Hippocampus abdominalis*. *Aquac. Res*., 34:757-763.

Zar J.H., 1984. *Biostatistical Analysis*, 2nd ed. Prentice Hall, Inc., Englewood Cliffs, NJ. 718 pp.