

Significance of water flow rate and period of nursing on the growth of juvenile seahorse, *Hippocampus barbouri* (Jordan and Richardson, 1908)

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Abstract

Drastic decline of seahorse population has been observed in the past decade. Evident shows that it is due to the over exploitation of this organism to fulfill demand for aquarium trades and Traditional Chinese Medicine industry (TCM). Considering this condition, seahorse farming is highly justified to ensure this species will not go extinct. In this study, effects of nursing period and water flow rate on the growth and survival of Barbour's seahorse, *Hippocampus barbouri* juveniles were investigated. Experiments were conducted in glass aquaria for a period of 8 weeks. Survival of seahorse juveniles was more than 80% when nursed for 9 days before being transferred into rearing tank. While the lowest water flow rate (0.056 ms⁻¹) resulted in better growth (height) of juvenile seahorse. These findings indicate that the length of nursing period and water flow rate can significantly affect the growth and survival of *H. barbouri* juveniles when cultured in captive condition.

Keywords: *Hippocampus barbouri*, Seahorse, Growth, Survival, Water flow rate, Nursing period

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Introduction

Over exploitation of seahorses is evident with the drastic decline of its population (Scales, 2010). Demand for seahorses comes mainly from the traditional Chinese medicine (TCM) industry which utilizes dried seahorses as part of the ingredient for treatment of health condition like sexual dysfunctions, kidney problems and baldness (Sinpetru, 2012). Cohen (2012) predicted that seahorse extinction will occur in the near future if the counter measure is not taken. More than a decade earlier, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2002) has foreseen this issue and taken precaution by listing all the seahorses under the genus *Hippocampus* in their conservation status which took effect in 2004. Among the 33 seahorses reported by Lourie *et al.* (2004), *Hippocampus barbouri* is one of the 8 species listed as vulnerable. This species is found distributed only in 3 countries, Indonesia, Malaysia and Philippines. This status strongly justified the need for sustainable seahorse supply through aquaculture. Presently, information leading to the establishment of suitable culture technique for seahorse is lacking. Particularly to ensure the survival of newborn seahorses. According to Lin *et al.* (2008), maintaining survival of newborn is a big challenge for seahorse farming. Preliminary observation found that new born juveniles reared in nursing system

had better survival. In this study, two factors, length of nursing period and water flow rate were studied to determine their effects on the growth and survival of *Hippocampus barbouri* juveniles.

Materials and methods

All experiments were conducted in the hatchery of Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Batches of *H. barbouri* juveniles used were from the same parents. Number of samples for each tank depends on number of juveniles produced per batch. Seawater supplies were from Port Dickson, Negeri Sembilan. All water sources were sterilized with UV and filtered using 0.1-micron filtration system. New born seahorse juveniles were reared in a glass nursing tank (40.0 x 23.5 x 30.0 cm) with approximately 30 liters of 30 ppt seawater which fitted with filtration systems (hang-on and gravel filters). As for rearing tanks (40.0 x 23.5 x 30.0 cm), it was fitted with hang-on biofilter. All tanks were equipped with nylon string as holdfast for the juveniles. All experimental tanks were filled with 30 liters of 30 ppt treated seawater. Both experiments were conducted for a period of 8 weeks.

Experiment on nursing period was conducted using seahorse juveniles from the same batch and introduced from nursing tank into rearing tank at day 3, 5, 7 and 9 after birth. Each of this treatment was triplicated with 4 seahorse juveniles per tank. Water

surface flow rate of the study was maintained at moderate level.

While, experiment on water flow rate was carried out by adjusting and maintaining the water flow rate from the hang-on filter prior to the initiation of the experiment. Water surface flow rate was determined by using a ping-pong ball tied with a string. The ping-pong ball was set at the hang-on filter outlet and flow for 30 cm. Time taken was recorded. Three water surface flow rates used were 0.056 (low), 0.077 (moderate) and 0.143 (high) ms⁻¹. Then, seahorse juveniles (12 day after birth) from the same batch were introduced from nursing tank into rearing tank. Each of this treatment was triplicated with 8 seahorse juveniles per tank.

Bio-Marine *Artemia* cyst was used for all the studies. One gram of *Artemia* cyst was weighed and mixed with 1 liter of 30 ppt of seawater. Strong aeration was provided to ensure good hatching rate. After 24 hours, newly hatched *Artemia* was siphoned out for feeding. All newborn and juveniles seahorses were fed to observed satiation twice daily (0900 and 1600) with newly hatched *Artemia* nauplii. In experimental tanks, feces were siphoned daily. Length (height, Ht) and weight of each seahorse juveniles were measured using digital caliper and microbalance at the initiation and the end of the experimental period. Height (Ht) was taken from the top of the coronet to the tip of the straightened tail (according to Lourie *et al.*, 1999) and

survival was noted throughout the study period. YSI Professional plus Multi-parameter and API salt-water test kit were used to measure pH, dissolved oxygen (DO), temperature (°C), salinity (ppt), ammonia (ppm), nitrate (ppm) and nitrite (ppm) once a week. Data from the experiments were analyzed using one way of Analysis of Variance (ANOVA) and Tukey test to determine the significant difference between treatments using SPSS software (Version 21.0).

Results

Nursing Period

Based on the growth measurements (Table 1), there was no significant different ($p>0.05$) for height and weight of seahorse juveniles with different nursing period (3, 5, 7, and 9 day after birth). However, the percentage of survival recorded the highest (83.3%) in juveniles nursed and transferred at 9 day after birth. While juveniles nursed and transferred at 3, 5 and 7 day after birth recorded survival of 41.7, 41.7 and 50% respectively. As for water quality (Table 2), ammonia level ranged from 0 to 0.5 ppm, nitrite 0 to 0.25 ppm, nitrate 0 to 10 ppm, dissolved oxygen (DO) 5.10 to 6.16 mgL⁻¹, and pH 7.42 to 7.99. While temperature and salinity fluctuate between 27.10 to 28.25°C, and 30.02 to 33.75 ppt, respectively.

Table 1: Growth and survival of *Hippocampus barbouri* juveniles at different nursing period.

Nursing period (day after birth)	N	Height (mm)		Weight (g)		Survival (%)
		Initial	Final	Initial	Final	
3	12	16.64±1.23	34.54±2.84 ^a	0.173±0.00	0.128±0.30 ^a	41.7
5	12	19.35±0.89	35.16±3.31 ^a	0.023±0.00	0.133±0.35 ^a	41.7
7	12	22.24±1.41	33.70±5.35 ^a	0.038±0.00	0.126±0.06 ^a	50.0
9	12	22.94±2.44	37.68±4.46 ^a	0.038±0.01	0.168±0.07 ^a	83.3

* Mean ± S.D. with the same superscripts within the same column are not significantly different.

Table 2: Range of water quality for all the treatments during the 8 weeks experimental period.

Nursing period (day after birth)	3	5	7	9
Temperature (°C)	27.10-28.12	27.21-28.25	27.13-28.21	27.18-28.21
Salinity (ppt)	30.46-33.21	30.63-33.02	30.02-33.34	30.76-33.75
DO (mgL-1)	5.13-5.76	5.18-6.16	5.33-6.10	5.10-6.05
pH	7.42-7.66	7.71-7.92	7.76-7.98	7.77-7.99
Ammonia (ppm)	0-0.50	0-0.50	0-0.50	0-0.50
Nitrite (ppm)	0-0.25	0-0.25	0-0.25	0-0.25
Nitrate (ppm)	0-10	0-10	0	0-5

Water Flow Rate

At the end of the experimental period, growth measurements in Table 3 showed significant different ($p < 0.05$) for height of seahorse juveniles cultured for two water flow rate, 0.056 and 0.077 ms⁻¹. However no significant different ($p > 0.05$) was observed when compared to the highest water flow rate, 0.143 ms⁻¹. As for weight of seahorse juveniles, no significant different ($p > 0.05$) was observed between all treatments. Percentage of survival of seahorse juveniles recorded the highest (54.2%) when cultured in tanks with the lowest water flow rate (0.056 ms⁻¹). Mortality occurs between weeks 5 to 8. Table 4 showed the range of water quality for the 8 weeks experimental period. Ammonia level ranged from 0 to 0.50 ppm, nitrite 0 to 0.25 ppm, nitrate 0 to 5 ppm, DO 4.70 to 5.61

mgL⁻¹, and pH 7.49 to 7.81. Low fluctuation of temperature and salinity were observed, between 26.91 to 28.01 °C, and 30.08 to 33.59 ppt, respectively.

Discussion

Artemia nauplii were used as primary food for seahorse juveniles for both experiments. Proximate composition of nauplii and adult *Artemia* showed that they are rich in protein but low in lipid, carbohydrate and ash (Leger *et al.*, 1987). N-3 HUFA is essential for optimum growth of marine fish. Lipid produces higher energy as compared to protein and carbohydrate (Craig, 2009). Therefore, nutritional content of *Artemia* may not be sufficient to support the growth of seahorse juveniles.

Table 3: Growth and survival of *Hippocampus barbouri* juveniles at different water flow rate.

Water flow rate (ms-1)	N	Height (mm)		Weight (g)		Survival (%)
		Initial	Final	Initial	Final	
0.056	24	19.27±1.06	39.93±4.16 ^b	0.023±0.01	0.193±0.08 ^a	54.2
0.077	24	19.40±1.30	34.60±4.41 ^a	0.024±0.01	0.131±0.02 ^a	45.8
0.143	24	19.5±1.75	37.55±5.14 ^{ab}	0.023±0.01	0.153±0.06 ^a	45.8

* Mean ± S.D. with the same superscripts within the same column are not significantly different.

Table 4: Range of water quality for all the treatments during the 8 weeks experimental period.

Flow rate (ms-1)	Treatment 1 0.056	Treatment 2 0.077	Treatment 3 0.143
Temperature (°C)	27.20-27.39	26.91-27.93	27.05-28.01
Salinity (ppt)	30.08-33.31	30.42-33.24	30.49-33.59
DO (mgL-1)	5.07-5.21	4.70-5.20	5.30-5.61
pH	7.75-7.81	7.76-7.79	7.49-7.77
Ammonia (ppm)	0-0.25	0-0.50	0-0.25
Nitrite (ppm)	0-0.25	0-0.25	0-0.25
Nitrate (ppm)	0-5	0-5	0

Similar study on *Hippocampus reidi*, where nutritional value of the feed is the main factor affecting growth of seahorse (Melo *et al.*, 2015). Nur *et al.* (2016) reported that mysid shrimp as feed is better than *Artemia* to enhance the breeding performance of *H. barbouri*. Leger *et al.* (1987) suggested that enrichment can be done to overcome the nutritional deficiency of *Artemia*. While Thuong and Hoang (2015) recommended the use of copepod for better growth and survival of seahorse juveniles.

Length of nursing period definitely affected the survival of seahorse juveniles. It was observed that when seahorses are transferred at day 9 after birth from nursing tank to rearing tank, survival is more than 80%. Preliminary observation conducted on the nursing period of *H. barbouri* juveniles showed that mortality is higher when nursing

period is more than 14 days. This evident suggests that length of nursing period should be between 9-14 days for optimum survival of seahorse juvenile. At the same time, new born seahorses are weak, therefore need substrate or hold fast to support them in the water column. Therefore, providing thin nylon string as holdfast is a necessity.

Determination of suitable water flow rate for the culture of seahorse juvenile is crucial. Seahorses are known to be poor swimmer, often drifting, following the water current. At juveniles stage they are even weaker. The experiment on different water flow rate shows that at the lowest flow rate (0.056 ms-1) produces better height as compared to stronger water flow. At 0.056 ms-1 juvenile seahorses were able to feed better, thus the better growth (height). At faster water flow (0.077 ms-1), juvenile seahorse may not be able to

catch their prey since the strong water flow will keep pushing them forward, this explained the lower growth (height) of juvenile at the end of the experimental period.

Water temperature fluctuates less in an enclosed compartment. Salinity, dissolved oxygen and pH for both experiments were within acceptable range for the culture of marine organism Mitigating impact from aquaculture in the Philippines (PHILMINAQ) (2010). There is no specific data on ammonia, nitrite and nitrate level for marine water. However, these three parameters showed very low fluctuation. Water quality is crucial in aquaculture, however, the parameters measured in this study were within similar ranges therefore very unlikely to affect the growth and survival of *H. barbouri* juveniles. *Hippocampus barbouri* is sensitive to water quality changes, particularly ammonia. The addition of biofilter managed to reduce the excessive ammonia build up in the rearing tank. Findings of this study showed that it is possible to culture *H. barbouri* in laboratory condition. Recent studies showed the possibility of producing *H. reidi* (Fonseca *et al.*, 2015) and *H. abdominalis* (Martinez-Cardenas and Purser, 2012) through aquaculture.

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