DOI: https://doi.org/10.36811/gjcee.2019.110002 **GJCEE: June-2019: Page No: 08-13**

Global Journal of Civil and Environmental Engineering

Research Article Open Access

Observational developments of the culture of big-belly seahorse, *Hippocampus abdominalis* (Lesson, 1827): A conservation effort for the future

Mohamad Saupi Ismail[1*] and Chee Kong Yap[2]

[1]Fisheries Research Institute, Batu Maung, 11960 Pulau Pinang, Malaysia
[2]Department of Biology, Faculty of Science, University Putra Malaysia,43400 UPM, Serdang, Selangor, Malaysia

***Corresponding Author:** Mohamad Saupi Ismail, Fisheries Research Institute, Batu Maung, 11960 Pulau Pinang, Malaysia, Email: saupi@rocketmail.com; saupi@dof.gov.my

Received Date: *May 08, 2019 /* ***Accepted Date:*** *June 04, 2019/* ***Published Date:*** *June 06, 2019*

Abstract

The objective of the present study was to document the growth and survival rates of *Hippocampus abdominalis*, beginning from captive-born to adulthood, and finally to the complete life cycle. The most significant finding from this culture study was that, after day 193, the life cycle of first generation of captive-born *H. abdominalis* in AkuaTAR was successfully observed. It is claimed that this is the first successful life cycle in captivity and production of the next generation of *H. abdominalis* in Malaysia. Our finding was obviously better because the survival rate was significantly higher when compared to those previously reported for this seahorse species. In short, the present observational study presented a simple culture technique that can produce optimum growth and survival through to adult stage of seahorse *H. abdominalis*. Hence, the present finding is important for conservation of seahorse H. abdominals in the future.

Keywords: Seahorse; *Hippocampus abdominalis*; Closure of life cycle; Conservation

Cite this article as: Mohamad Saupi Ismail, Chee Kong Yap. 2019. Observational developments of the culture of big-belly seahorse, *Hippocampus abdominalis* (Lesson, 1827): A conservation effort for the future. Glob J Civil Environ Eng. 1: 08-13.

Introduction

The big-belly seahorse *Hippocampus abdominalis* (Family: Syngnathidae; Figure1) is one of the largest seahorse species in the world. The adult of this species can reach as high as 35 cm. This is a temperate species and therefore its natural distribution is normally found in the waters of New Zealand and southeast Australia [1,2].

The seahorse natural distribution has been under ecological stress due to the loss of habitat, poaching, hunting and fishing for commercial trades mostly for traditional

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Chinese medicines [2,3]. Currently, all seahorses are listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) [2]. Therefore, captive-raised seahorse is important for conservation purpose [4]. This can cater for the ever-increasing supply demand of seahorse [5]. To achieve this goal, the best way is to develop the captive breeding technique and successfully closing the life cycle of seahorse species [6]. Nevertheless, the culturing of seahorse has encountered problems including scarce information on the breeding techniques [7]. There has been studies reporting the culturing techniques of *H. abdominalis* in New Zealand and Australia [8,9]. However, there have been no studies on the culture of *H. abdominalis* in Malaysia. Therefore, this study aims to document the growth and survival rates of *H. abdominalis*, beginning from captive-born to adulthood, and finally to the complete life cycle.

Figure 1: A male adult *Hippocampus abdominalis* in captivity.

Materials and Methods

Four matured *H. abdominalis* (2 males and 2 females) with total lengths (TL) ranging from 13 -16 cm were used in the present study. Taken from the public aquarium in Kuala Lumpur, these seahorses were transported to Tunku Abdul Rahman Aquarium (AkuaTAR) in Pulau Pinang. They were kept alive in a rectangular glass tank (90 cm (L) x 45 cm (W) x 50 cm (H)), and nourished with live mysid shrimps twice a day. The waste matters were removed and the water was changed up to 25% daily. After the acclimatization period of 30 days, one pair of seahorses, that exhibits the courtship behavior, was transferred to a brooder tank (60cm x 45cm x 75cm). The photoperiod was set at 12 h light and 12 h dark. Water quality parameters such as water temperature, salinity, dissolved oxygen, and pH were kept at 19°C, 33 ppt, 4.0 mg/l, and 7.6 respectively. Two batches of newborn seahorses were used in this study. All newborns were counted, before transferred to rectangular glass tanks (40cm x 25cm x 25cm) for 100 days. The maximum number of newborns in one tank was set to 100 individuals. For the first 20 days, a 24L: 0D photoperiod was given by employing 18W T5 fluorescent tubes with the light intensity of 3000 - 4000 lux. Once all juveniles showed settlement behaviors, the photoperiod was changed to 10L: 14D (lights on at 8 am and lights off at 6 pm).

The juveniles were provided with holdfasts from day 10 onwards starting with polyethylene lines (size 1 mm in diameter), before changing to cable ties (3 mm) from day 60. The holdfasts were substituted with plastic chains (5 mm) from day 100 onwards (Figure 2). Besides the holdfasts, there was no other substrate in the tanks. Approximately 25% of water were exchanged on daily basis. The water quality parameters of the tank including temperature (19-21°C), salinity (32-35ppt), dissolved oxygen (4-5mg/l) and pH (7.6-7.8) were maintained during the experimental periods. The juveniles were given live feeds as in Table 1.

Table 1: The types of different live diets which were fed on juveniles of seahorse H. *abdominalis* during the different periods of culture.

Diets	Periods of culture
Newly hatched *Artemia*.	Days 1 to 7
Artemia nourished with fish oil	Days 7 to 14
5-10 days old *Artemia* nourished with rice flour	Days 15 to 30
10-15 days old *Artemia*	Days 30 to 60
Mysid shrimps	Days 60 onwards

Figure 2: Polyethylene lines (left) and plastic chains (right) as holdfasts.

Mortality, if any, were recorded daily. For growth analysis, a minimum of 10 juveniles were randomly collected, every 10 days. Their body TL and wet weight (W) were determined according to Martinez-Cardenas and Purser [9]. From day 100, the juveniles were relocated to a bigger tank (90 cm x 45 cm x 50 cm). After 150 days, all remaining numbers of the seahorse were counted and their gender were recorded. The survivors were then kept together in a cubical 70cm x 70cm x 70cm fiber tank. This was to deliver potential broodstocks of the seahorse. According to (Indiviglio [1] and Lourie [2]), the typical pair-bonding behavior of seahorses has not been observed for this big belly seahorse. Therefore, multiple choices were given for these species to facilitate their partner preferences.

Results and Discussion

According to Wittenrich [3], to obtain suitable broodstock of seahorse is the first challenge in seahorse culture. However, in this study, adult seahorses already showed courtship and mating behavior after a few weeks of stocking in the brooder tank. Pregnancy in the seahorse was observed to be lasted for about 30 days, before delivering. Two batches of offspring with the brood sizes ranging from 74 to 189 individuals were obtained from the same broodstock. The present batch sizes were smaller than those reported by Woods [7] and Indiviglio [1]. They reported an average of 269 individuals of seahorses with a range of 300-700, respectively. The broodstock gave birth to rather large newborns, with a mean of 18.3 mm in TL and 0.019 g in W. The present study also discovered that the young seahorses were bigger in size, comparing to Woods [7] (15.6 mm TL and

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0.008 g W). The growth and survival of big-belly seahorse in captivity from the present study is presented in Table 2. The juveniles reached the mean heights of 52.0 mm, 88.5 mm, and 124.9 mm for 30, 60 and 100 days, respectively. Martinez-Cardenas and Purser [9] reported the mean growth rate of 48.3 mm in 6 weeks old, while [14] reported 32.2 mm and 60.0 mm in 30 and 60 days old, respectively in H. *abdominalis*.

Table 2: Growth and survival of big-belly seahorse in captivity from the present study.

	1 day	30 days	60 days	100 days	365 days
Mean size (TL), mm	18.30+0.80	52.00+4.45	88.50+7.21	124.85+9.18	185.90+9.47
Wet weight, g	0.02+0.01	0.26+0.07	1.72+0.44	5.24+1.34	18.80+3.06
Survival (%)	100	58	55	54	37

The present study witnessed comparatively rapid growth rates in the juveniles up to 60 days. The highest growth rate was observed at days 50 to 60. The growth rate was high up to the maturation stage but declined as the seahorses achieved sexual maturity status. After a year, the juveniles had reached a maximum height of 220 mm (mean: 185.9 mm) and a maximum weight of 25.65 g (mean: 18.8 g). This growth was significantly higher when compared to growth of 110.7 mm in 1 year as reported by Woods [7]. The seahorse could attain faster growth rates with higher food availability and nutritional diets [7]. We also found that female seahorses (mean: 188.1 mm) were significantly larger than the males (mean: 182.8 mm) and this is supported by the observation by Project Seahorse [10]. According to Woods [7], the first 15 days after birth is classified as critical stage during seahorse husbandry. This husbandry period is the period during which the juveniles of seahorse will change from pelagic to settlement phase. Suitable food supply with optimum amount are deciding factors in reaching the best growth and survival rate in the seahorse culture [6].

In the present study, different instar stages of cultured *Artemia* were fed to the settled juveniles of seahorse. After two months, live mysid shrimps were provided to the juveniles gradually. It was observed that there was a change of prey items which is subjected to the sizes of the juveniles. At the same time, the types of diets should be altered according to the growth of seahorse [5]. The present study showed that the seahorse juveniles had high survival rate of 64% if continuous photoperiod was given during the settlement period. This agreed with some previous study that showed positive effect of longer photoperiod on the survival or growth of seahorse juveniles [6,10]. Based on direct observation, when the juveniles of seahorse exhibiting settlement behaviors, we intentionally provided the holdfasts in the rearing tank. These holdfasts are acting as attachment points for the juveniles. According to Murugan *et al*. [6], the size of holdfast materials is important and critical in the seahorse culture because they function in reducing their energy expenditure and maximize growth. In this study, the settlement of seahorse juveniles on holdfasts was initiated from day 10 by using fishing lines. There was 100% of settlement of juveniles within 20 days. During the growth of juveniles, we increased the thickness of the holdfasts. On day 60, we replaced polyethylene lines by cable ties while on day 100 onwards, the holdfast was changed to plastic chains. The replacement of different types and thicknesses of holdfasts of seahorse juvenile rearing have been reported in *H. barbouri* that used plastic chains [11] and *H. kuda* that used fishing lines and plastic cables [12].

The present study observed higher mortality rate of *H. abdominalis* before the settlement phase. We found juvenile survival rates of 68%

and 64% at days 10 and 20 of culture, respectively. After 30 days of culture, 58% of the juveniles still survived and mortality rate declined. After 100 days of culture, the juvenile survival rate was 54%. There was only one mortality from days 100 to 150. At the end of culture at 5 months, the survival rate was maintained at 53%. This observation strongly showed that the mortality rate for the juvenile was only high during the first 20 days, indicating high sensitivity of the juvenile seahorse. The present finding was supported by some literatures [7,9,13] that higher mortality of juvenile seahorse in the pelagic phase (usually the first 20 days) while becoming more ecological adaptable on reaching the settlement phase with better survivorship. In this study, little disease problems were encountered although mortality could be caused by bacteria, parasites and viruses dominating during the pathological progress [5]. On day 80, the first development of male pouch was observed with an average length > 11 cm. By day 100, the juveniles already surpassed the mature size of 13 cm, and males have fully grown brood pouches. At this age, the number of percentages for females and males was 53% and 47%, respectively. According to [13], the males of seahorse reached maturity by the presence of brood pouch, while female also reached the maturity status at the same age. The seahorses were continued to rear for 1 year. On day 193, the life cycle of first generation of captive-born *H. abdominalis* in AkuaTAR was successfully completed. We claim that this is the first successful life cycle in captivity and production of the next generation of *H. abdominalis* in Malaysia. These *H. abdominalis* were kept for a period of 365 days and they finally grew up to reach maturity status. The average number of newborn juveniles was 37 individuals while the average survival rate of the first generation after 365 days was 37%. Our finding was obviously better because the survival rate was significantly higher when compared to that 10.6% as reported by Woods [7].

Conclusion

In conclusion, the present observational study presents a simple culture technique that can produce optimum growth and survival through to adult stage of seahorse *H. abdominalis*. After day 193, the life cycle of first generation of captive-born *H. abdominalis* in AkuaTAR was successfully observed. We claim that this is the first successful life cycle in captivity and production of the next generation of *H. abdominalis* in Malaysia. Hence, the closing of life cycle in captivity achieved in this study can reduce the dependency on wild brooders. This is an important finding for the conservation of seahorse *H. abdominalis* in the future.

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