Estimation of live food consumption for *Hippocampus barbouri* and *Hippocampus kuda*

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Abstract

Seahorse is found worldwide in marine habitats such as the seagrass beds, coral reefs, mangroves and estuaries. Among all the seahorses, *Hippocampus barbouri* and *H. kuda* are the most traded species. In recent year, worldwide attention strongly supports the establishment of seahorse aquaculture as to provide an alternative source of seahorse. However, the main bottleneck in succeeding seahorse aquaculture at the culture of early stage, is the diet. The selection of suitable diet will contribute to the success in the larval rearing of seahorse. In this study the consumption of live food by newborn, juvenile and adult seahorse were investigated. *H. barbouri* and *H. kuda* commenced feeding at birth. The used of live food, namely the newly hatched *Artemia* nauplii was able support the growth and survival of newborn and juvenile seahorses in specific tank systems. The results shows an increasing trend, with average numbers of *Artemia* nauplii consumed increase as the age and size (height) of seahorse. The minimum numbers of nauplii consumed by *H. barbouri* and *H. kuda* at 3 day after birth (DAB) with height 14.24±0.14 mm and 10.71±0.13 mm, were 7 and 5 nauplii per feeding respectively. This study showed that *Artemia* nauplii can be used as live food for newborn *H. barbouri* to 28 DAB, while for *H. kuda* from newborn to 42 DAB. At 90 DAB onwards, the used of adult *Artemia* instead of nauplii is highly recommended for *H. barbouri*,

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Introduction

The genus *Hippocampus* is categorized under the family Syngnathidae, the same as pipefishes, pipehorses and seadragons (Lourie *et al*., 2004). Unlike most of the fish, they do not have scales but instead bone structure that is made up of little plates and covered by a thin layer of skin. Sexual characteristics in seahorses are distinctly different compared to other fishes, whereby female produces eggs and transferred them to the male for fertilization and incubation (Foster and Vincent, 2004). The decreased numbers of seahorse along the coastal area show that the seahorse populations are greatly affected by over harvesting and habitat degradation. The ornamental trade and traditional Chinese medicine (TCM) are two main industry that's exploit these seahorses (Foster *et al*., 2016).

In order to prevent further exploitation, all seahorse species have been placed under the Convention on International Trade in Endangered Species (CITES) in May 2004. Countries signing this international agreement will not utilize wild seahorses for export purposes (Perry *et al*., 2010).

Hippocampus barbouri also known as Barbours`s seahorse, often appear in different shades of color like white, yellowish, brown with dark bands across the dark lateral body and striped snout. The uniqueness in shape has made seahorses suitable for aquarium display. As for *Hippocampus kuda,* it is normally appear in black and brownish yellow or even orange, often with dark spot on the body, but not as attractive as *H. barbouri*. Due its large size, it is often preferred in TCM trading (Job *et al*., 2002). This seahorse normally exist in shallow area of coastal bay and lagoons rich with seagrass or floating weeds. Poor mobility of seahorse made it an easy capture. Its low density existence in the wild has made the condition even worse (Foster and Vincent, 2004). Concerns over the sudden declines in wild seahorse populations have led to seahorse aquaculture. Establishment of seahorse aquaculture is by far the most suitable method to achieve both sustainable and conservation goals by providing an alternative source of seahorse hence reducing the dependence on wild stock (Martin-Smith and Vincent, 2006). However problems arise in the effort to establish seahorse culture, mass mortality at the early stage mainly due to lack of suitable culture technique and diet (Sheng *et al*., 2006; Planas *et al*., 2008).

Availability of suitable initial food for marine fish larvae is critically important in aquaculture to ensure the survival and continuous growth of cultured species. Food value for a particular fish species primarily affected by the size of food. Fish will take longer time and more energy spent to achieve satiation if fed with smaller size food, and this will subsequently results in poor growth due to insufficient feeding and energy wastage (Lim *et al*., 2003). *Hippocampus* spp. like other seahorses are predators, therefore only feed on moving prey like plankton and small crustaceans in the natural environment. They are slow and poor swimmers, often remain stationary by clinging to substrates with their prehensile tails (Choo and Liew, 2006). They are without teeth and feeding is through suction motion with their thick snouts and jaws (Lourie *et al.,* 2004). Newborn seahorses are unable to take in food that is larger than the snout opening. In seahorse aquaculture feeding becomes the major problem for seahorse hobbyist and researcher due to their complicated feeding behavior, with unknown consumption amount, prey preferences and nutritional requirements (Woods, 2002).

Initial food for marine fish larvae normally does not includes commercial dry feeds due to the immobility of feeds and digestibility, therefore marine fish and crustacean hatcheries depend mainly on cultured or wild zooplankton such as rotifers, copepods and *Artemia* nauplii (Faulk *et al*., 2005). As such, *Artemia* became the most convenient live food since it can be hatched from market ready cysts (Sorgeloos, 1980; Léger *et al*., 1986). Upon hatching, the small sized nauplii is acceptable by most cultured fish larvae and shellfish, and subsequently gives a better growth and survival for aquaculture species (Dhont *et al*., 2013). In addition, *Artemia* at instar II stage can be enriched to temporarily cater for nutritional requirements of certain cultured species (Ohs *et al*., 2013). These have convinced most people involved in aquaculture specifically in larval rearing, to use *Artemia* nauplii as live food for cultured species at early stage. In this study, the use of *Artemia* as live food for *H. barbouri* and *H. kuda* at different life stages were investigated.

Materials and methods

Production of juvenile seahorse

Experiments were conducted at two different locations for two species of seahorses. *Hippocampus barbouri* at Hatchery unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia and *H. kuda* at Unit Training, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. Mature F2 *H. barbouri* line which established from 3 pairs of wild broodstock bought from fisherman at Semporna, Sabah in 2013. While mature *Hippocampus kuda* broodstock sourced from fisherman at various locations in Thailand, were used to produce juvenile for this study. Broodstock were conditioned in a laboratory with constant feeding and optimum water parameters. Pair of broodstock showing courtship behavior were then separated and placed in breeding tank. Feeds used were of *Macrobrachium* spp. post larvae (PL) caught from nearby freshwater pond, *Penaeus vannamei* PL (supplied by Asia Aquaculture Sdn Bhd, Malaysia), frozen mysid (Hikari, Japan) and adult *Artemia* (cultured from nauplii stage). Upon release, the newborn seahorses were transferred into a nursing tank.

Culture system

All broodstock tanks for *Hippocampus barbouri* were equipped with

Ultraviolet (UV) and hang-on filter (containing biofiltration materials) for water recirculation. While all broodstock tanks for *Hippocampus kuda* were equipped build-in biofiltration unit containing bioball. Plastic chains tied to weigh stone were used as holdfast for broodstocks. Newborn seahorses were removed from the breeding tank and placed in nursing glass tanks measuring 40x25x30 cm (Fig. 1) equipped with undergravel sand bed and hang-on filter.

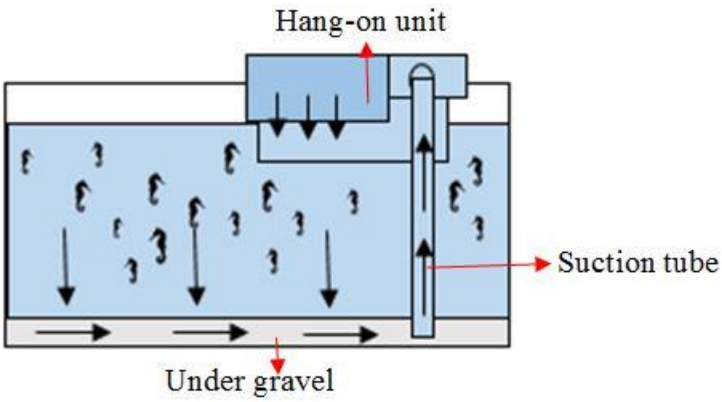

Figure 1: Seahorse nursing tank, arrows indicate recirculation of water in the tank system.

Feed preparation

Artemia cyst (Bio-Marine, USA) was used throughout this study. Hatching of *Artemia* was carried out daily to provide newly hatched nauplii to the newborn seahorse. *Artemia* cysts weighing 0.2g was incubated in 2L of seawater in a beaker with strong aeration. Approximately 24 hours later, nauplii were released. Harvesting was carried out by removing the aeration, to allow the nauplii to congregate at the bottom of the beaker, while the cyst shells float at the surface. After 5 minutes, nauplii were siphoned from the beaker and ready for feeding to seahorses. *Artemia* nauplii were used as live food for the newborn up to 30 day after birth (DAB). These *Artemia* were cultured in 0.5 tonne of fibreglass tank, to various sizes depending on the sizes of the seahorses. *Chlorella* sp. (from SATREPS Project in UPM) was use as feed for *Artemia* throughout the study period.

Feeding Experiment for early stage juvenile seahorse

The first experiment was carried out to determine the numbers of *Artemia* nauplii consumed by newborn *H. barbouri* at 3, 7, 14, 21 and 28 DAB. Beakers of 2L were filled with seawater and provided with slow aeration. Feedings were carried out at 0900 for all age groups. Ten seahorses of the same age group with similar height were placed into individual beaker. Ten *Artemia* nauplii were siphoned using tube and placed into each beaker. As soon as the seahorses finished the first batch of nauplii, another batch of ten nauplii were subsequently added into the beaker. This step was repeated until seahorses were observed to be satiated. Then the total numbers of *Artemia* nauplii consumed were recorded. This feeding protocol was repeated for the rest of the age groups of *H. barbouri*.

The second feeding experiment for *H. kuda* was conducted with similar feeding protocol as to that of *H. barbouri*. Seven age group used were 3, 7, 14, 21, 28, 35, and 42 DAB. However, in this experiment, glass tanks measuring 10x10x20cm were used instead of beaker. Numbers of *Artemia* nauplii consumed by each age group were recorded at the end of each feeding experiment.

Feeding experiment for adult seahorse

The third experiment used late juvenile to adult stages of *Hippocampus barbouri*. Seven different age groups used were 120, 150, 180, 210, 240, 270 and 300 DAB. During the culture period the seahorses were fed thrice daily at 0900, 1300 and 1700, with mixed diets which include adult *Artemia*, frozen mysid, and *Penaeus vannamei* postlarvae. Feeding protocol was similar to the feeding experiment on juveniles *H. barbouri* and *H. kuda*. However, adults *Artemia* were used instead of nauplii since preliminary experiment showed that larger seahorses preferred large sized *Artemia*.

Data collection and analysis

In feeding experiments involving newborn to juvenile stage seahorses, only height of seahorse was measured due to the fragile condition of these seahorses. While for experiment on adult seahorses, height and wet weight seahorses were taken. Height (Ht) of seahorse was measured to the nearest millimeter (mm) from the tip of coronet to the tip of the outstretched tail, with the head held at right angles to the body (Lourie *et al*., 1999). Wet weight (Wt) was measured to the nearest gram (g) with seahorse gently dabbed on soft tissue to ensure water was absorbed before taking measurement. Measurements were taken prior to the initiation of feeding experiment. Collected data of numbers of *Artemia* consumed were tabulated to observe the relation between size of seahorse and the consumption per feeding. All data were presented in mean ± standard deviation (S.D.).

Results

Consumption experiment for different age stages

Hippocampus barbouri, age groups of 3, 7, 14, 21 and 28 DAB with corresponding average height of 14.24, 16.75, 23.18, 26.91 and 32.51 mm respectively (Table 1), were observed to consume on average of 12, 18, 23, 34 and 35 *Artemia* nauplii per feeding. Results from the consumption experiment for early stage *H. barbouri* shows an increasing trend (Fig. 2). As the age of the seahorse increased, the average numbers of *Artemia* nauplii consumed per feeding by seahorse n=10 also increased. The minimum number of *Artemia* nauplii consumed at 3DAB was 7 to 10 nauplii per feeding.

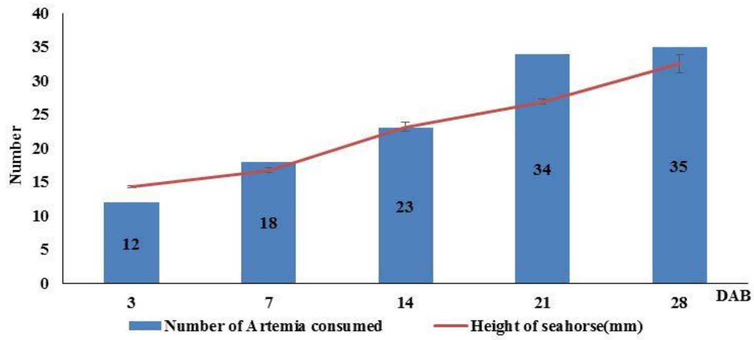

Figure 2: Average number of *Artemia* nauplii consumed by different age group of early stage *Hippocampus barbouri.* Error bar indicates the standard deviation.

While at 28 DAB, the numbers of *Artemia* nauplii consumed were 27 to 42 nauplii per feeding (Table 1). *H. barbouri* between the age groups of 21 to 28 DAB showed comparatively similar numbers of nauplii consumed.

Hippocampus kuda with age groups of 3, 7, 14, 21, 28, 35 and 42 DAB with corresponding average height of 10.71, 13.32, 19.83, 22.96, 24.76, 29.10 and 32.32 mm respectively (Table 2), were observed to consume on average of 7, 12, 19, 25, 25, 33 and 35 *Artemia* nauplii per feeding (Fig. 3) respectively. Results for the consumption experiment for early stage *H. barbouri* shows an increasing trend (Fig. 2). While *H. kuda* between the age groups of 21 to 28 DAB and 35 to 42 DAB showed comparatively similar numbers of nauplii consumed (Fig. 3).

In this study, it was observed that *H. barbouri* at 21 and 28 DAB, with height of 26.9±0.39 and 32.51±1.28 mm, and *H. kuda* at 35 and 42 DAB, with height of 29.10±1.08 and 32.32±1.23 mm, respectively, showed similar average numbers of nauplii consumed. This finding indicates that seahorses of different age groups and species, but of similar sizes, will be able to consumed similar amount of *Artemia* nauplii. In this case, feeding increases as the seahorses grow bigger in size, thus the numbers of nauplii consumed is dependent on the size of seahorses, regardless of age and species.

Preliminary feeding trials conducted on 90 DAB *H. barbouri* showed that more than half of the population in culture tank reduced feeding on *Artemia* nauplii. This is likely due to the small size of the nauplii, it become less attractive for these adult seahorses to prey on. Thus, the introduction of adult *Artemia*, which are bigger in size, as live food for adult *H. barbouri*. Age between 90 to 120 DAB is considered as transitional period for the change of prey size. Therefore, 120 DAB *H. barbouri* with average height of 49.38 mm and wet weight of 0.44 g were used as initial size to conduct feeding trial using adult *Artemia*.

Table 1: Numbers of *Artemia* nauplii consumed by *Hippocampus barbouri* at early juvenile stage.

Hippocampus barbouri		*Number of Artemia nauplii consumed*	
Age group (DAB)	**Height ± SD (mm)**	**Min**	**Max**
3	14.24±0.14	7	19
7	16.75±0.35	16	22
14	23.18±0.60	16	32
21	26.91±0.39	18	46
28	32.51±1.28	27	42

DAB: day after birth
SD: standard deviation

Table 2: Numbers of *Artemia* nauplii consumed by *Hippocampus kuda* at early juvenile stage.
DAB: day after birth

Hippocampus kuda		*Number of Artemia nauplii consumed*	
Age group (DAB)	**Height ± SD (mm)**	**Min**	**Max**
3	10.71±0.13	5	12
7	13.32±0.42	3	18
14	19.83±0.77	9	28
21	22.96±0.61	20	28
28	24.76±0.76	19	29
35	29.10±1.08	28	39
42	32.32±1.23	28	39

SD: standard deviation

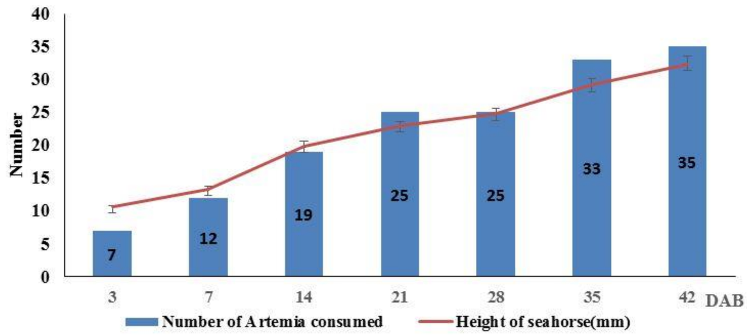

Figure 3: Average numbers of *Artemia* nauplii consumed by different age group of early stage *Hippocampus kuda*. Error bar indicates the standard deviation.

Hippocampus barbouri with age groups of 120, 150, 180, 210, 240, 270, and 300 DAB, with corresponding average height of 49.38, 56.62, 64.97, 75.59, 88.94, 92.02, and 100.60 mm, and weight 0.44, 0.71, 1.17, 1.82, 2.86, 3.36, and 4.06 g, were observed to consume on average of 31, 34, 47, 56, 56, 59, and 64 adult *Artemia* per feeding, respectively (Fig. 4). Increasing trend in numbers of adult Artemia consumed increases with the seahorse’s age group. The stagnant numbers of adult *Artemia* consumed for seahorses between 210 to 240 DAB was basically due to the occurrence of disease, which affected their food consumption.

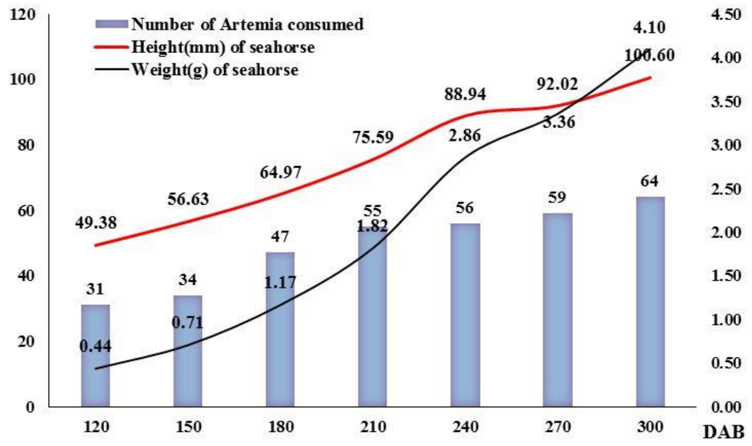

Figure 4: Average numbers of adult *Artemia* consumed by different age groups of adult *Hippocampus barbouri.*

However, upon recovery, the numbers of *Artemia* consumed continue to increase as observed for seahorses from 270 DAB onwards.

Discussion

Active and stagnant are two types of feeding behaviors observed on *H. reidi* and *H. patagonicus* (Felicio *et al*., 2006 and Storero *et al*., 2009). Active feeding coincides with early stage of seahorses, actively swimming to capture their prey. While stagnant feeding behavior occurs when seahorses become bigger and more stable, therefore hanging on substrate waiting the prey to come within range for them to capture. The shifting of from active to stagnant feeding behaviors was observed in 21 day-old *H. kuda* (Choo and Liew, 2006). In this study, similar behaviors were observed for *H. barbouri* and *H. kuda*. This could be the reason in the comparatively similar numbers of *Artemia* nauplii consumed by *H. barbouri* and *H. kuda* at later age groups.

Woods (2002) reported the gut content of wild *H. abdominalis,* which is extremely large in size (132-274 mm) consisted mainly of amphipod and Caridean shrimp. While the gut of *H. zosterae,* a small size seahorse (<35 mm) consists only of copepods (Tipton and Bell, 1988). Bigger seahorses may prefer larger prey. Thus this explained the comparatively similar numbers of *Artemia* nauplii consumed per feeding by 21 and 28 DAB juvenile seahorses in this study.

In this study, it was recorded that *H. kuda* can give birth up to 500-900 newborn at one time compared about 60-120 newborn in *H. barbouri*. The size of newborn *H. kuda* is comparatively smaller than *H. barbouri* (Cato and Brown, 2008; Payne, 2003). At 3 DAB, the average height of *H. barbouri* was 14.24mm (Table 1) compared to *H. kuda* 10.71mm (Table 2) at the same age group. Bigger seahorses will consumed more nauplii compared to smaller ones. Thus, numbers of *Artemia* nauplii to be fed to seahorses per feeding can be estimated based on the size (height) of the seahorse.

Feeding behaviour has long been known as a bottleneck for the feeding of seahorses. Newborn seahorses basically unable to control their movement and most of the time swim near water surface following the water flow in their upright position (Choo and Liew, 2006). Providing live food, such as *Artemia* nauplii as initial feed for newborn and juvenile seahorses is crucial. Feeding using live *Artemia* nauplii can ensure the seahorses will be able to feed continuously (Woods, 2000, 2003). At the same time, live food will not cause the deterioration of water quality. Often uneaten artificial food will cause the increased of ammonia and may give rise to bacterial population and microorganisms in the water (Datta, 2012).

Gas bubble ingestion is one of the major problems faced by newborn seahorse (Sanaye *et al*., 2013). During their pelagic phase, newborn seahorse *H. erectus* with limited swimming ability can only follow the water current, however when the seahorse gets older they can swim against the water current (Qin *et al*., 2014). Therefore, at early stages, seahorse tends to ingest air while preying on their food when they miss strike. It has no ability to expel out the air bubbles from their body, and this may lead to gas bubble problems. The presence of bubbles in seahorse body will subsequently cause the loss of balance and inability to swim and prey on food, leading to starvation and eventually mortality. Whereas for adult seahorses, physical changes in terms of strange swimming behavior reduced feeding, bloated abdomen, visible patches and lesions on seahorse body are symptoms of disease outbreak.

There are several tank designs and systems being recommended in order to improve the survival of newborn seahorses. Preliminary experiment was conducted using the pseudo-kriesel tank system. This type of tank provides balance distribution of water in circular tank (Job *et al*., 2002; Koldewey, 2005; Koldewey and Martin-Smith, 2010). Study on the effect of water flow rate showed that slow water movement provide better condition for the nursing of *H. barbouri* juveniles as compared to strong water flow (Er *et al*., 2017). Initially, it was able to provide circular movement of water and movement of newborn and juvenile *H. barbouri*. However, it causes problem for newborn to feed, since the food items were also recirculated following the water movement. In addition to the complicated setup of this pseudo-kriesel tank, it is also quite costly. In this study, the combination of undergravel system and hang on filter provide a suitable condition for the nursing of newborn and juvenile seahorses. Water flow can support these fragile seahorses to capture *Artemia* nauplii with much ease. With this type of tank system, the occurrence of gas bubble ingestion by newborn seahorses is not as much as reported in previous study.

Continuous water parameters monitoring is important to ensure the conducive water conditions for growth and survival of seahorses. A slight increase in ammonia or decrease of pH may cause physiological stress to seahorses. Based on the study by Nur *et al*. (2016), *H. barbouri* was able to tolerate levels of dissolved oxygen, temperature, ammonia and nitrite from 4.94 to 7.46 ppm, 26.78 to 28.03°C, 7.95 to 8.93, 0.04 to 0.20 ppm, 1.15 to 4.03 ppm, respectively. Long term water quality deterioration will usually cause mortality to seahorse.

This study showed that *Artemia* nauplii can be used as live food for *H. barbouri* from newborn to 28 DAB, while from newborn to 42 DAB for *H. kuda*. For optimal feeding, the findings of this study can be used as guideline for the numbers of *Artemia* for the different age stages of *H. barbouri* and *H. kuda*, based on their sizes (height) and weight. The used of adult *Artemia* is recommended for feeding of *H. barbouri* from 90 DAB onwards. Nur *et al*. (2018) reported some positive results in the use of *Artemia* enriched with thyroxine, potassium iodide and cod liver oil as live food for *H. barbouri*. Since *H. barbouri* and *H. kuda* showed ready acceptance to *Artemia*, application of nutrient enrichment through this live food may results in improve growth and survival of these seahorses.

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