Estimation of Live Food Consumption in the Seahorses *Hippocampus barbouri* and *Hippocampus kuda*

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Abstract

Seahorses inhabit a wide range of marine ecosystems, including seagrass beds, coral reefs, mangroves, and estuaries. Among them, *Hippocampus barbouri* and *Hippocampus kuda* are two of the most heavily traded species in the ornamental and traditional medicine markets. Increasing interest in seahorse aquaculture has highlighted the need to improve larval rearing techniques, particularly feeding strategies during early developmental stages. This study investigated live food consumption by newborn, juvenile, and adult stages of *H. barbouri* and *H. kuda* under captive conditions. Both species initiated feeding immediately after birth. Newly hatched *Artemia* nauplii successfully supported the growth and survival of newborn and juvenile seahorses. The results demonstrated a positive relationship between seahorse age and body size (height) and the number of *Artemia* nauplii consumed per feeding event. At 3 days after birth (DAB), *H. barbouri* (14.24 ± 0.14 mm) and *H. kuda* (10.71 ± 0.13 mm) consumed an average of only 7 and 5 nauplii per feeding, respectively. *Artemia* nauplii were found to be suitable as live feed for *H. barbouri* from birth to 28 DAB and for H. kuda from birth to 42 DAB. Beyond these stages, larger prey items such as adult Artemia may be more appropriate, particularly for *H. barbouri* juveniles from 90 DAB onward. The findings provide useful information for optimizing feeding protocols and improving the culture of these commercially important seahorse species.

Keywords: Seahorse, *Hippocampus barbouri*, *Hippocampus kuda*, live food, *Artemia* nauplii, larval rearing, feeding requirements

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Introduction

Hippocampus is a genus of marine teleost categorized under the same family as pipefishes, pipehorses and seadragons (Lourie *et al*., 2004). Unlike most of the fish, they do not have scales, instead their bodies are made of little plates, covered by a thin layer of skin. Sexual characteristics in seahorses is distinctly different compared to other fishes in which female seahorses produces eggs and transferred them to male seahorses for fertilization during spawning (Foster and Vincent, 2004).

To date, this seahorse population is greatly affected by over fishing, evident with the declining catch. Thus, a control measure has been established under the Convention on International Trade in Endangered Species (CITES) effective from May 2004, whereby, catch of all seahorse species is regulated. Countries signing this international agreement will not cause negative impact on the wild population through exports (Perry *et al*., 2010).The few reasons of seahorse being over exploited are due to ornamental trade industry, traditional Chinese medicine (TCM) and habitat degradation (Foster *et al*., 2016).

Hippocampus barbouri also known as the Barbours`s seahorse often appear in different shades of coloration like white, yellowish, brown or may have dark bands across the dark lateral body with a striped snout. Thus this made it a very potential species for aquarium display. As for *Hippocampus kuda,* the yellow seahorse normally appear in black and brownish yellow or even orange often with dark spot on the body may not look as attractive as *H. barbouri* but due to its larger 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 sea grass or floating weeds. Due to low density existence, and together with poor mobility have added the pressure on wild populations as it does not require complicated skill in capturing the seahorse (Foster and Vincent, 2004). Concerns over the sudden declines in wild seahorse populations has 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 capture seahorse (Martin-Smith and Vincent, 2006). However problems arise in the effort to establish seahorse culture, mass mortality at the juvenile stage mainly due to lack of culture technique and suitable diet for culturing seahorse juvenile (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 feed 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 their thick snouts and jaws to suck in their food like a straw (Lourie *et al.,* 2004). Same goes to miniature seahorse which born at birth are unable to take in food that is larger than the snout opening. In seahorse aquaculture feeding becomes the major problem for seahorse keeper in aquaculture, hobbyist and researcher due to their complicated feeding behaviour which include the consumption amount, preference and nutritional value of diet (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* sp. 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 made it 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 larval rearing to prefer on using *Artemia* as early stages live food for cultured species. Therefore the use of *Artemia* as live food for *H. barbouri* and *H. kuda* at different life stages are being investigated in this study.

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 *Hippocampus kuda* at Unit Training, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. Mature F2 *H. barbouri* which established from 3 pairs of wild brood stock bought from fisherman at Semporna, Sabah in 2013, while mature *Hippocampus kuda* broodstock were sourced from fisherman at various locations in Thailand or from Chatuchak Market, Bangkok, Thailand were used to produce juvenile for experiment. Broodstock were conditioned in a laboratory with constant feeding and optimum water parameters. Pairing brood stock with courtship behavior were then separate and placed in breeding tank fed with various diet comprised of post-larvae shrimp, frozen mysid, adult *Artemia*, fresh mysids, and freshwater shrimp depending on the pair preferences. After giving birth, the newly born seahorse will then transferred to a nursing tank.

Culture system

All brood stock tanks for *Hippocampus barbouri* were equipped with Ultraviolet (UV) and hang-on filter (containing bio filtration materials) for water recirculation, while all brood stock tanks for *Hippocampus kuda* were equipped build-in bio filtration unit containing bioball. Plastic chains tied to weigh stone were used as holdfast for brood stocks. Newly born seahorse were removed from the breeding tank and placed in juvenile nursing glass tanks measuring 40x25x30 cm (Fig. 1) equipped with under gravel sand bed and hang-on filter.

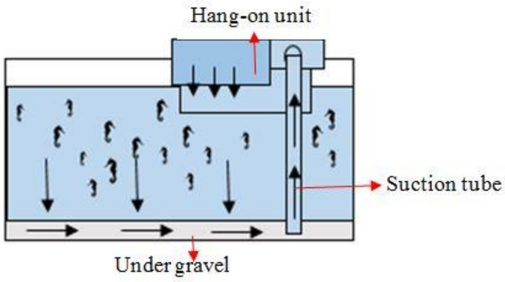

Figure 1: Seahorse nursing tank with arrows indicating recirculation of water in the tank system

Feed preparation

Artemia cysts (Bio-Marine, USA) was used throughout this study. Hatching of *Artemia* was carried out daily to provide newly hatched nauplii to the new born seahorse juveniles. *Artemia* cysts weighing 0.2g was incubated in 2L of seawater in a beaker with strong aeration. *Artemia* cysts hatched approximately 24 hours later with the release of nauplii. Harvesting was carried out by removing the aeration, to allow the nauplii to congregate at the bottom of the beaker, while the cyst shell floating at the surface. After 5 minutes, nauplii were siphoned from the beaker and ready to be used for feeding to seahorses. Artemia was used as live food for the juvenile seahorse up to 1 month-old. These Artemia were cultured to various sizes and used as live depending on the sizes of the seahorses in circular 0.5 tonne fiberglass tanks, provided with strong aeration and fed twice daily with *Chlorella* sp. obtained from Department of Aquaculture, UPM.

Feeding Experiment for early stage juvenile seahorse

The first experiment was carried out after successful in producing batch of new born seahorse. It aims to examine the number of consumption of new born seahorse. Experiments were carried out using *H. barbouri* of five different age groups, 3, 7, 14, 21 and 28 day after birth (DAB). Beakers of 2L were filled with seawater from nursing tank and provided with slow aeration. Feeding experiments were carried out at 0900 for all age groups, respectively. Ten seahorses of the same age group with similar height were randomly picked and placed into individual beaker. Ten *Artemia* nauplii were siphoned using tube and placed into each beaker. As soon as the seahorses finished the 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 groups were recorded at the end of each feeding experiment.

Feeding experiment for mature seahorse
The third experiment fish were 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 were 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 new born to juvenile stage seahorses, only height of seahorse was measured due to fragile condition of these seahorse. While for experiment on adult seahorses, height and wet weight seahorses were taken. Height (Ht) of seahorse was measured to the nearest millimetre (mm) from the tip of coronet to the tip of the outstretched tail, with the head held at right angles to the body following (Lourie, 2003). Wet weight (Wt) was measured to the nearest gram (g) with seahorse gently dab on soft tissue to ensure water is absorb before taking measurement. Measurements were taken prior to the initiation of feeding experiment. Collected data of number of Artemia consumed were tabulated to observe the relation between size of seahorse and the consumption of one meal. All data were presented in mean ± standard deviation (S.D.).

Results

Consumption experiment for different age stages

Hippocampus barbouri of age groups of 3, 7, 14, 21 and 28 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. Result for the consumption experiment for early stage juvenile *H. barbouri* shows an increasing trend (Fig. 2). When the age of the seahorse is increase, the average number of Artemia nauplii consumed per feeding by seahorse n=10 also increased.

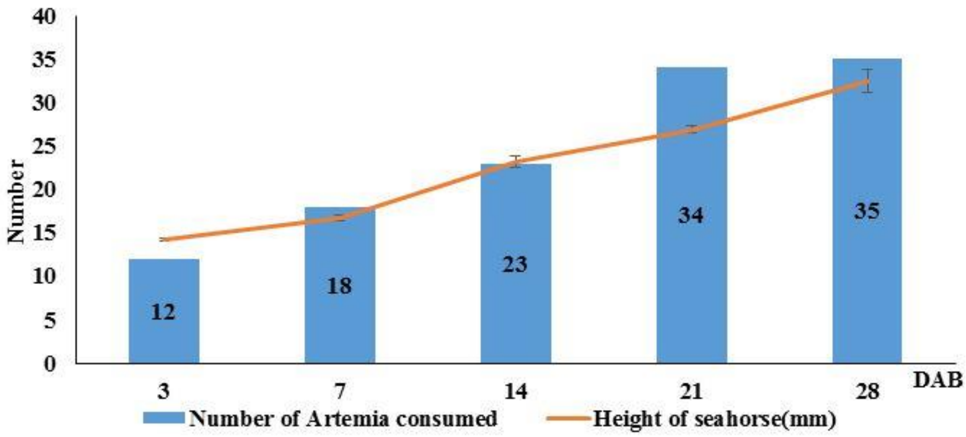

Figure 2: The average number of *Artemia* nauplii consumed by different age group of early Juvenile stage seahorse *Hippocampus barbouri.* Error bar indicates the standard deviation

The minimum number of Artemia nauplii consumed by 3DAB seahorse are 7 while the maximum can go up to 10 pcs per feeding. As for the 28 days old the minimum number of Artemia nauplii consumed were 27 while the maximum go up to 42 pcs. (Table 1). However, *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). Result for the consumption experiment for early stage juvenile *H. barbouri* shows an increasing trend (Fig. 2). However, *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±1.23mm, 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 numbers 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 prior to next experiment showed that more than half of the population of 90 DAB *H. barbouri* in culture tank reduced feeding on Artemia nauplii. This is possibly due to the small size of the nauplii, thus become less attractive for these seahorses to prey on. Thus, the introduction of adult Artemia, which are bigger in size.

Table 1: Numbers of Artemia nauplii consumed by *H. 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 *H. kuda* at early juvenile stage.

Hippocampus barbouri		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

DAB: day after birth
SD: standard deviation

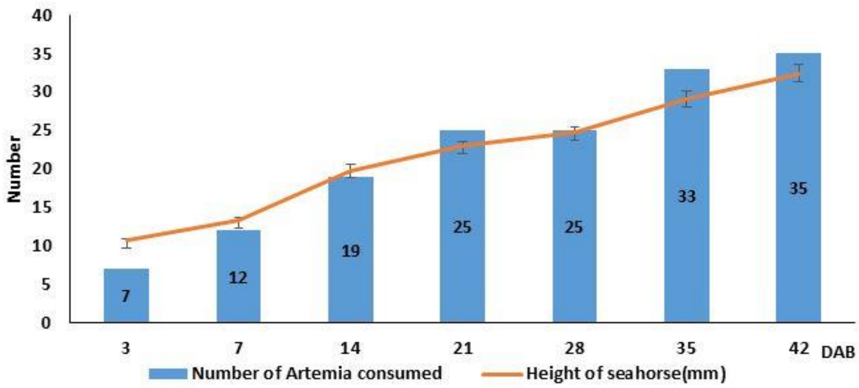

Figure 3: The average number of Artemia nauplii consumed by different age group of early juvenile stage seahorse *Hippocampus kuda.* Error bar indicates the standard deviation.

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.

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, affecting their food consumption. However, upon recovery, the numbers of Artemia consumed continue to increase as observed for seahorses at 270 DAB onwards.

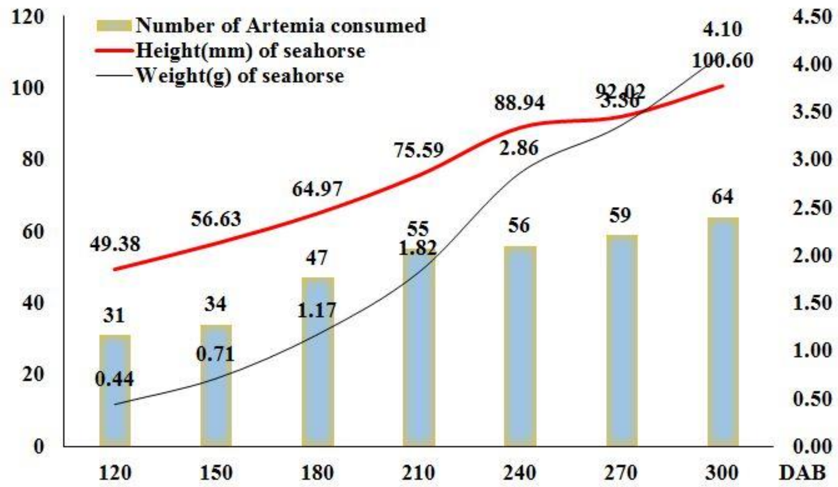

Figure 4: Average numbers of adult Artemia consumed by different age groups of adult *Hippocampus barbouri*.

Discussion

Active and stagnant are two types of feeding behaviors observed on *H. reidi* and *H. patagonicus* (Felicio *et al*., 2006; Storero *et al*., 2009). Active feeding observed whereby 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 were 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 in wild seahorse *H. abdominalis,* a big size seahorse (132-274 mm) consisted mainly of amphipod and Caridean shrimp. While the gut of *H. zosterae,* a small size seahorse (<35 mm) was consisted 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 *H. kuda* can give birth up to 500-900 new born at one time compared about 60-120 new born in *H. barbouri*. The size of new born *H. kuda* is comparatively smaller than *H. barbouri* (Payne, 2003; Cato and Brown, 2008). 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. Seahorses with bigger size 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) or age of the seahorses. Size of seahorses does influence the numbers of nauplii consumed.

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 away to 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 juvenile 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 problem 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 could swim against the water current (Qin *et al*., 2014). Therefore, the early juvenile 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, which 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 seahorses are symptoms of disease outbreak.

There are several tank designs and systems being recommended in order to improve the survival of newborn seahorse. Preliminary experiment was conducted using the pseudo-kriesel tank system. This type of tank uses principle on providing 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 under gravel system and hang on filter provide suitable condition for the nursing of newborn and juvenile seahorses. Water flow can be reduced to minimal speed, providing slow movement of water and at the same time allows the fragile seahorse to capture Artemia nauplii with much ease. With this type of tank system, gas bubble ingestion on newly born juvenile were 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 distress 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* newborn stage 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 to be fed to 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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