Dietary effects of rotifers, brine shrimps and cultured copepods on survival and growth of newborn seahorse *Hippocampus kuda* (Bleeker 1852)

**Abstract**

**Aim**: Low survival remains a significant obstacle during the early culture of *Hippocampus kuda*. To potentially develop a better feeding regime to newborn *H. kuda* without relying on wild zooplanktons, two separate feeding trials were performed using cultured live food items.

**Methodology**: Newborn *H. kuda* derived from the wild broodstock were reared in the captive conditions using different feeding regimes consisting of rotifers, *Artemia* and/or copepods *Oithona simplex*. The survival rate and growth performance were determined.

**Results**: In the first experiment, despite consumption of both rotifers and *Artemia*, a total mortality was observed among seahorses on 3rd and 5th day-after-birth (DAB), when only rotifers or *Artemia* were provided, respectively. Seahorses fed the mixed live foods have 60% survival with 6.3% per day specific growth rate (SGR). In the second experiment, the introduction of copepods had further improved the SGR of *H. kuda* to 8.1% d⁻¹ compared to 6.8% d⁻¹ without copepods, although the survival was similar.

**Interpretation**: Overall, the results indicated that in terms of survival, a feeding regime consisting of a rotifer and *Artemia* nauplii mixture was suitable for *H. kuda* juveniles during the first 10 days. To enhance growth, copepods *O. simplex* should be included in its diet since this live food was superior over enriched *Artemia* metanauplii. These findings are important to further develop an optimum feeding regime from reliable source of live foods for early culture of *H. kuda*.

---

**Authors Info**

J.C. Teh¹, Mohd. S. Kamarudin*, N. Romano¹, A. Arshad¹ and N.L.W.S. Wong²

¹Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

²Centre of Marine Science, Universiti Putra Malaysia, 71050 Port Dickson, Negeri Sembilan, Malaysia

*Corresponding Author Email : msalleh@upm.edu.my

---

**Key words**

Critical period
First feeding
Live foods
Newborn yellow seahorse
Prey consumption

---

**Publication Info**

Revised received : 06.01.2017
Re-revised received : 19.03.2017
Accepted : 28.12.2017
Introduction

Seahorses are renowned in the ornamental fish trade and are highly valued in oriental traditional medicine (Vincent et al., 2011; Kumaravel et al., 2012; Rosa et al., 2013). The World Conservation Monitoring Centre revealed that over 32 million seahorses (live and dried) were traded by nearly 70 different countries from 2004 to 2011 and over 96% of these were captured in the wild (AWI, 2013). Seahorse breeding and aquaculture can potentially offer an alternative solution to support the high market demand, which may help to reduce the seemingly uncontrollable harvest of wild animals and perhaps restore their natural populations (Job et al., 2002; Koldewey and Martin-Smith, 2010). The successful culture of seahorses has been reported in several studies, but high mortalities during the first 10 days after birth (DAB) remains a significant hurdle in the nursery culture of yellow seahorse Hippocampus kuda (Lin et al., 2006; Garcia and Hilomen-Garcia, 2009; Celino et al., 2012). Various reasons have been attributed to the early mortalities in seahorses including broodstock quality, infectious disease and poor nutrition (Olivotto et al., 2011; Blanco et al., 2014; LePage et al., 2015).

Wild caught zooplanktons, mostly comprise of copepods, are often used in the first feeding of newborn H. kuda (Job et al., 2002; Celino et al., 2012; Thuong and Hoang, 2015). It is well known that wild zooplankton such as copepods have a high nutritive value (Drillet et al., 2011; Rasdi et al., 2016); however, wild caught live food organisms may carry pathogens while the quantity and quality can be inconsistent from time to time (Buen-Ursua et al., 2011; LePage et al., 2015). Rotifers and Artemia nauplii are the more commonly used live foods in early larviculture of fish and crustacean species because of their small size and ease of mass culture (Dhont et al., 2013; Tsuji et al., 2015). Although these prey organisms are not natural foods for seahorses, they have been used in the feeding regime for several species of juvenile seahorses with some success (Otero-Ferrer et al., 2010; Willadino et al., 2012; Pham and Lin, 2013; Segade et al., 2015).

In two separate feeding trials, the effects of different live foods that included rotifers Brachionus sp., brine shrimps Artemia sp. and cultured cyclopoid copepods Oithona simplex, on the survival, growth and prey consumption of newborn H. kuda over the critical first 10 days were evaluated. In addition, the proximate and fatty acid compositions of the live foods, as well as their size and compatibility with the mouth gape size of the developing H. kuda juveniles were examined.

Materials and Methods

Live food preparation: Large (L-type) rotifers, Brachionus sp. were cultured with Nannochloropsis oculata (100–300 L) at 32.0 ± 3.0‰ salinity and 30.0 ± 2.8°C under an outdoor shed with a transparent roof. Cyclopoid copepods, O. simplex were collected, identified and isolated from the waters near Port Dickson. These copepods were then cultured under similar culture conditions as rotifers, but a mixture of yeast (2 mg l⁻¹), molasses (5 ml ton⁻¹) and rice flour (10 mg l⁻¹) was provided every other day (Ramassamy et al., 2013). The copepods harvested from the outdoor culture tank were disinfected in a 0.03 ml l⁻¹ formalin bath for 1 hr before use (Buen-Ursua et al., 2011). Meanwhile, newly hatched Artemia nauplii and metanauplii were prepared from Artemia cysts (Bo Hai Bay, China; Super Artemia Pte Ltd). The Artemia metanauplii (24–48 hr post-hatched) that were used in Experiment 2 were enriched for one hour with an enrichment made by blending 50 ml of cod liver oil (Scott’s, GSK group of companies, UK), one hard-boiled egg yolk and 10 g of baker’s yeast in one litre of distilled water (Dhert, 1996). The enrichment was stored in 4°C and used within 3 months.

Proximate and fatty acid composition: The proximate composition of the live foods was measured according to standard methods of AOAC (1997), while the gross energy contents were estimated following the Atwater general factor (FAO, 2003). Meanwhile, the samples were homogenized and the lipids were extracted in a chloroform: methanol (2:1 v/v) solution (Folch et al., 1957), and the fatty acid analysis was then performed according to Ebrahimi et al. (2012). Individual proximate and fatty acid composition of the live food organisms are shown in Table 1.

Size of live food organisms: The sizes of live food organisms were measured under a microscope (Olympus BX43F) attached with a digital camera (Olympus DP22). Rotifers were oval in shape and their mean (± SD) lorica length was 230 ± 45 µm (n=20). Meanwhile, the mean (± SD) length and width of Artemia nauplii were 567 ± 90 µm and 172 ± 31 µm (n=20), while for Artemia metanauplii these were 1014 ± 138 µm and 243 ± 51 µm (n=20), respectively. The average size of copepods O. simplex after being filtered through a 250 µm sieve was 1282 ± 21 µm in length and 305 ± 66 µm in width (n=20).

Source of experimental animals: All seahorses used in this study were handled in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes (IACUC, 2012). Pregnant male seahorse, Hippocampus kuda, was collected one at a time from Merambong Shoal, Johor, Malaysia and then transported in a 10 I container with an air supply to the Centre of Marine Science (COMAS), Port Dickson, Negeri Sembilan, Malaysia. The seahorse was slowly acclimated to the hatchery water conditions and then transferred into a 100-l circular fiber-glass tank filled with 80 I of filtered (1 µm) and UV-sterilized seawater. An artificial substrate made of nylon strings attached to a fishing weight was provided. The adult seahorse was fed twice daily on a mixture of small shrimps and mysids including Palaemon sp., Periclimenes sp., Pseudomysis sp. and Siriella sp. (collected from the sea off Port Dickson). After giving birth in the hatchery, the adult seahorses were returned to their original habitat.
Table 1: Proximate composition (% wet weight) and fatty acid composition (% total fatty acids) of rotifers Brachionus sp., newly hatched Artemia nauplii, enriched Artemia metanauplii and cyclopoid copepods Oithona simplex

<table>
<thead>
<tr>
<th>Composition</th>
<th>Rotifers</th>
<th>Newly-hatched Artemia</th>
<th>Enriched Artemia</th>
<th>Copepods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>92.5</td>
<td>91.4</td>
<td>92.3</td>
<td>91.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>3.8</td>
<td>3.9</td>
<td>3.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>0.7</td>
<td>1.0</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.2</td>
<td>3.1</td>
<td>2.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Ash</td>
<td>0.8</td>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Estimated gross energy (kJ g⁻¹)</td>
<td>1.28</td>
<td>1.58</td>
<td>1.34</td>
<td>1.49</td>
</tr>
<tr>
<td>Individual dry weight (ng)</td>
<td>188</td>
<td>1.68 µg</td>
<td>1.69 µg</td>
<td>5.59 µg</td>
</tr>
<tr>
<td>Individual gross energy (×10² J ind⁻¹)</td>
<td>0.24</td>
<td>2.65</td>
<td>2.26</td>
<td>8.33</td>
</tr>
</tbody>
</table>

Fatty acid (% total fatty acids)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Rotifers</th>
<th>Newly-hatched Artemia</th>
<th>Enriched Artemia</th>
<th>Copepods</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>3.7</td>
<td>0.4</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>16:0</td>
<td>45.7</td>
<td>13.2</td>
<td>15.2</td>
<td>19.9</td>
</tr>
<tr>
<td>16:1</td>
<td>10.2</td>
<td>5.4</td>
<td>10.1</td>
<td>9.9</td>
</tr>
<tr>
<td>18:0</td>
<td>4.8</td>
<td>5.6</td>
<td>5.6</td>
<td>8.8</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>17.1</td>
<td>30.6</td>
<td>32.8</td>
<td>18.2</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>6.4</td>
<td>7.1</td>
<td>6.7</td>
<td>5.4</td>
</tr>
<tr>
<td>18:3 (n-3) ALA</td>
<td>2.2</td>
<td>32.9</td>
<td>10.8</td>
<td>1.2</td>
</tr>
<tr>
<td>20:4 (n-6)</td>
<td>2.6</td>
<td>1.7</td>
<td>5.9</td>
<td>3.7</td>
</tr>
<tr>
<td>20:5 (n-3) EPA</td>
<td>4.8</td>
<td>2.1</td>
<td>4.5</td>
<td>4.3</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>0.6</td>
<td>0.9</td>
<td>1.4</td>
<td>3.2</td>
</tr>
<tr>
<td>22:6 (n-3) DHA</td>
<td>1.9</td>
<td>0.8</td>
<td>6.1</td>
<td>23.3</td>
</tr>
<tr>
<td>SFA</td>
<td>54.2</td>
<td>19.2</td>
<td>21.7</td>
<td>30.7</td>
</tr>
<tr>
<td>MUFA</td>
<td>27.3</td>
<td>36.0</td>
<td>42.9</td>
<td>28.1</td>
</tr>
<tr>
<td>PUFA n-3</td>
<td>9.4</td>
<td>35.9</td>
<td>22.7</td>
<td>32.1</td>
</tr>
<tr>
<td>PUFA n-6</td>
<td>9.0</td>
<td>8.9</td>
<td>12.6</td>
<td>9.1</td>
</tr>
<tr>
<td>n-6 : n-3</td>
<td>1.0</td>
<td>0.2</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>DHA</td>
<td>0.4</td>
<td>0.3</td>
<td>1.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* Carbohydrate = %dry matter – (%protein + %lipid + %ash); * At water general factor: protein = 17 kJ g⁻¹; fat = 37 kJ g⁻¹; carbohydrate= 17 kJ g⁻¹; SFA: saturated fatty acid; MUFA: monoene; PUFA: polyunsaturated fatty acid; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid and DHA: docosahexaenoic acid

All newborn seahorses were removed from the broodstock tank by a scoop net and disinfected in a 0.03 ml l⁻¹ formalin bath (Buen-Ursua et al., 2011). After disinfection, all seahorses were placed into a 100-l circular tank filled with 50 l of filtered (1 μm) and UV-sterilized seawater. Healthy newborn seahorses were then segregated into another tank based on their descriptive quality including relatively larger size, darker skin pigmentation and stronger swimming strength (Zhang et al., 2015). Only healthy newborns were used for experiments to eliminate the potential effects of poor intrinsic quality (Zhang, 2011; Zhang-y, 2015). Due to the small brood size and limited number of healthy newborns, seahorses used for each feeding trial were derived from different broodstock collected from the same seagrass area at Merambong Shoal.

Experiment 1: A total of 65 newborns were released from a male broodstock but only 50 newborns were considered ‘healthy’, based on the preliminary assessment. Among the healthy newborns, 45 individual seahorses were selected and randomly allocated to nine white-coloured circular tanks. All tanks were filled with 10 l of filtered (1 μm) and UV-sterilized seawater, while gentle aeration was provided. Three different feeding regimes were offered to the seahorses that included: (i) rotifers Brachionus sp. (20 ind ml⁻¹), (ii) Artemia nauplii (4 ind ml⁻¹) and (iii) a combination of rotifers (15 ind ml⁻¹) and Artemia nauplii (1 ind ml⁻¹). As the size of the Artemia nauplius was approximately five times bigger than a rotifer, one Artemia was compensated by five rotifers in the feeding regime. Approximately, 2.0 × 10⁶ cells ml⁻¹ of microalgae N. oculata was added into each culture tank (Mélo et al., 2016); faeces and debris at the bottom of each tank were siphoned out and approximately 20% of the water was changed twice daily (09:00 and 17:00 hrs). Water temperature, salinity, dissolved oxygen and pH were measured daily at 29.1 ± 1.3°C, 31.0 ± 0.5‰, 4.5 ± 0.6 mg l⁻¹ and 7.8 ± 0.2, respectively.

Observations were made twice daily for any mortality and dead seahorses were immediately removed and recorded. The initial heights of the newborn seahorses were taken from five healthy individuals, which were fixed in 10% formaldehyde and measured under a light microscope. The height
Experiment 2: A total of 133 newborns were released from a male broodstock in which 83 healthy newborns were segregated. Among the healthy newborns, 60 individual seahorses were selected and randomly allocated to six experimental tanks (effective volume = 10 l), each at one seahorse per litre stocking density. The experimental set-up was similar as Experiment 1 but two different feeding regimes were compared in triplicates: (i) a combination of rotifers (15 ind ml$^{-1}$) and Artemia nauplii (1 ind ml$^{-1}$) for the first three days and then from 3 to 9 DAB, enriched Artemia metanauplii (0.5 ind ml$^{-1}$) was added; (ii) the seahorses were similarly fed rotifers and Artemia nauplii, but copepods O. simplex (0.5 ind. ml$^{-1}$) were added from 3 to 9 DAB instead of enriched Artemia metanauplii. The initial heights of seahorses were measured under a microscope by sacrificing 18 newborn seahorses that were randomly selected from the healthy pool of newborn seahorses. The heights, survival and SGR for heights were also determined as described in Experiment 1.

Statistical analyses: All data were analyzed using a one-way ANOVA in Experiment 1 and Student’s t-test in Experiment 2 after prior confirmation of data variance homogeneity and normality. Survival data were arcsine square root transformed before analysis. If significant differences were found (p<0.05) when performing a one-way ANOVA, a Scheffe’s post-hoc test was performed to identify the differences among treatments. All statistical analyses was performed using STATA11.0 (StataCorp, USA).

Results and Discussion

Experiment 1: A total mortality occurred on 3 and 5 DAB among those exclusively fed rotifers and Artemia nauplii, respectively. In contrast, seahorses fed a combination of rotifers and Artemia had a final survival of 60%. The height of seahorses, fed on a combination of rotifers and Artemia was almost doubled after 10 days with a SGR of 6.3 ± 0.2 % d$^{-1}$ (Fig. 1), and similarly the mouth gape size of seahorses increased over two fold from 257 ± 35 μm on 0 DAB to 529 ± 77 μm on 9 DAB.

On 1 DAB, juvenile seahorse fed solely on rotifers had an intake of 4070 preys juvenile$^{-1}$d$^{-1}$ which was almost two-fold more than those fed solely on Artemia (2080 to 2230 preys juvenile$^{-1}$d$^{-1}$) before a total mortality occurred (Fig. 2). In the mixed feeding treatment, the rotifer intake increased rapidly from 1 DAB to 3 DAB and reached the highest point on 5 DAB, which was approximately 12000 preys juvenile$^{-1}$d$^{-1}$ (Fig. 2). From 5 DAB onwards, the consumption of rotifers gradually decreased while the daily consumption of Artemia gradually increased from approximately 2000 preys juvenile$^{-1}$ on 1 DAB to 6000 preys juvenile$^{-1}$ on 9 DAB. Although the number of Artemia consumed by the seahorses was lower than the number of rotifers, the consumed biomass of Artemia was much higher than rotifers (Fig. 3). Overall, the total biomass of prey consumed by juvenile seahorse increased linearly from approximately 3.8 to 11.3 mg juvenile$^{-1}$d$^{-1}$.

Experiment 2: Within the first 5 days of culture, no significant difference in the mean height of juvenile seahorses between treatments was observed. However, by 7 and 9 DAB, the height of those fed on the rotifer–Artemia–copepod combination became significantly higher (p<0.05) than those fed on the rotifer–Artemia combination (Fig. 4). The SGR of seahorses fed rotifer–Artemia–copepod combination (8.1 ± 0.3 % d$^{-1}$) was also significantly higher (p<0.05) than of those fed on the rotifers–Artemia combination (6.8 ± 0.4 % d$^{-1}$). No significant difference in survival (43–50%) was detected between the treatments.

The size of live food is an important consideration during the early culture of aquatic animals, especially fish larvae or newborn seahorses that are unable to masticate their prey into
smaller pieces (Merchie, 1996; Olivotto et al., 2011). In this study, the mean size of rotifers was slightly smaller than the mouth gape size of the newborn H. kuda, which likely facilitated their ingestion by the newborn seahorses at higher rates compared to Artemia. No seahorses, however, survived past 3 DAB when fed exclusively on rotifers. Celino et al. (2012) had earlier reported that H. kuda juveniles fed only rotifers had the highest mortality by 4 DAB and a total mortality within one week. Rotifers are known to be deficient in certain essential nutrients for developing fish larvae (Conceicao et al., 2010; Olivotto et al., 2011; Nordgreen et al., 2013). Although the crude protein and crude lipid contents of rotifers were comparable with those of Artemia nauplii in this study, carbohydrate and n-3 PUFAs, especially the 18:3n-3 (ALA), were low in rotifers. Deficiencies in these essential nutrients could be compounded by relatively small body size of rotifers that necessitates a higher consumption to obtain a total energy equivalent to that of larger prey (Scharf et al., 2000; Olivotto et al., 2011). Indeed, despite the higher consumption of rotifers in the current study, the total biomass as well as the gross energy of this live food was substantially lower compared to Artemia.

The sole use of Artemia has been reportedly sufficient for the first feeding of several seahorse species including H. erectus and H. hippocampus (Lin et al., 2008; Otero-Ferrer et al., 2010). Zhang et al. (2015) reported that captive-bred H. kuda newborns...
are routinely fed only Artemia metanauplii (enriched with a fish oil emulsion) with survival ranging from 40 to 80% after 5 weeks. In contrast, this study showed that H. kuda early juveniles failed to survive beyond 5 DAB when only fed on newly hatched Artemia nauplii. A possible explanation for this discrepancy is that captively bred seahorse are larger at birth, and thus have larger mouths and more effective foraging abilities (Palma and Andrade, 2012) compared to juveniles that are obtained from wild broodstock, as in the present study. Moreover, in this study, the newborn seahorses were routinely observed to orientate themselves to a position that enabled them to strike and swallow the Artemia nauplii. This was presumably because of the Artemia being longer in length than the mouth gape size of the seahorse, but such a foraging behavior took some time. This likely contributed to the lower amount of Artemia consumed on an individual basis compared to the smaller rotifers, and this may have contributed to the total mortality on 5 DAB.

The survival and growth of the seahorses were substantially improved when using the mixed feeding regime. The consumption of Artemia continually increased over the culture duration, whereas the intake of rotifers peaked at 5 DAB and then decreased. These findings indicated that the larger Artemia nauplii were preferred over the smaller prey rotifers as juvenile seahorses grew. Celino et al. (2012) also observed that H. kuda juveniles ingest larger prey (adult copepods) compared to smaller prey (rotifers or copepod nauplii) as they develop. Similarly, the transition of prey selectivity has also been observed in H. reidi in which they initially prefer smaller copepod nauplii and rotifers but the consumption of Artemia nauplii increases as the seahorses increase in size (Souza-Santos et al., 2013). These findings are consistent with the optimal foraging theory in which a combination of live foods can offer greater flexibility in the feeding behaviors of aquatic animals as their situations change (Scharf et al., 2000; Storero and Gonzalez, 2009; Jobling et al., 2012).

In the second experiment, the introduction of copepods O. simplex with the rotifers and Artemia significantly improved the growth of juvenile seahorse, H. kuda during the first 10 days compared to only a mixture of rotifers and Artemia. Cyclopoid copepods Oithona spp. are generally small to intermediate in size, making them an ideal live prey for young marine fish (Dhont et al., 2013; Dahms et al., 2015). Moreover, it is known that copepods are the main food consumed by seahorses in the wild (Yip et al., 2015). Cultured copepods Tisbe biminiensis, Glaugiferens imparipes and Schmackeria dubia have been used in early feeding of temperate seahorse species H. reidi, H. subelongatus and H. erectus, respectively, with some improved growth and/or survival (Payne and Rippingale, 2000; Willadino et al., 2012; Zhang et al., 2015). Nevertheless, the provision of a mono-specific-diet during larviculture of fish has occasionally been linked to poor performance (Izquierdo, 1996; Tocher, 2010). A high mortality rate (>80%) among H. reidi juveniles on 2 DAB was reported when they were fed singly on harpacticoid copepod T. biminiensis. However, this improved when a mixture of copepods and Artemia was given during their first 14 days of culture (Willadino et al., 2012). Based on the results of Experiments 1 and 2 in this study, it seemed that a combination of different prey types might have enhanced the performance of juvenile seahorses by complementing the overall nutritional profile of their diets.

The copepod, O. simplex had a much higher DHA and 22:5n-3 (clupanodonic acid) compared to rotifers and newly-hatched Artemia. On the other hand, rotifers had higher 16:0 (palmitic acid), which has been reported to serve as the main energy source for H. guttulatus embryos (Faleiro and Narciso, 2010). Meanwhile, Artemia had a higher C18 polyunsaturated fatty acid content, which is also known to be essential fatty acids for various fish species (Glencross, 2009). Zhang et al. (2015) demonstrated that the growth performance of H. erectus juveniles is positively related to higher concentrations of DHA, EPA and n-3 PUFAs in Artemia following enrichments. However, H. erectus juveniles fed calanoid copepods, S. dubia, achieved an even better growth performance and survival rate than those fed on enriched Artemia (Zhang et al., 2015). The authors suggested this is due to the fact that copepods have not only a higher DHA content but also a more appropriate DHA: EPA ratio (Zhang et al., 2015). The higher growth achieved by H. kuda when supplemented with O. simplex that contained a much higher DHA seemed to support this notion.

In conclusion, the use of rotifers or Artemia singly as live foods was inadequate and likely failed to provide all the essential nutrients required for the early feeding of H. kuda juveniles. On the other hand, a mixed feeding regime consisting of both these live prey could sufficiently support the survival and growth of H. kuda juveniles over the first 10 days. Supplementing this feeding regime with, O. simplex from 3 DAB further improved the growth of H. kuda juveniles, but not survival. Further investigations on their nutritional requirements through live food enrichments, especially the longer chain unsaturated fatty acids, would likely be useful to further tailor a better feeding regime for the nursery culture of this seahorse species.

Acknowledgments

This study was funded by a grant from Universiti Putra Malaysia, project no. GP-IPB/2014/9440402. The authors are grateful to late Sarah Maidatul Mior Rosli for her dedicated assistance in this research. The authors would also like to acknowledge the Southeast Asia University Consortium for graduate studies in Agriculture and Natural Resources (UC), School of Graduate Studies UPM and Ministry of Higher Education Malaysia for their travel award grant (GCS16-2863), financial aid for overseas research attachment (UPM/SPS/GS37730) and scholarship (MyBrain15), respectively.

References

AOAC (Association of Official Analytical Chemists); Official Methods of Analysis of AOAC International. 16th Edn., AOAC International,
First feeding regime for yellow seahorse


