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

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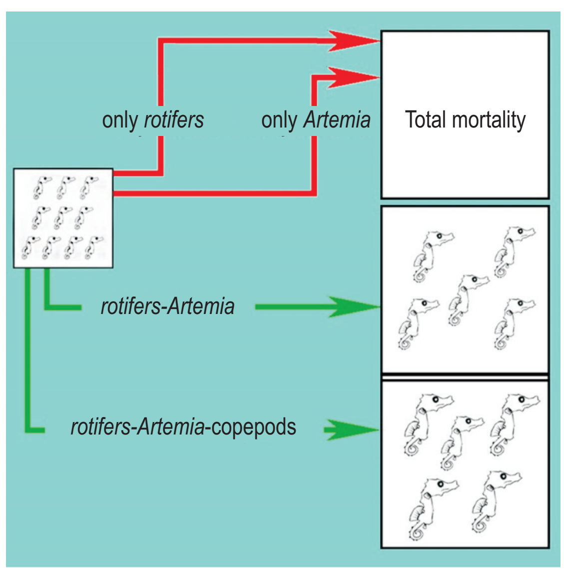
Abstract

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*.



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 (Ramaswamy *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 l 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 l 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., *Pseudoxomysis* 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*

Composition	Rotifers	Newly-hatched *Artemia*	Enriched *Artemia*	Copepods
Proximate (% wet weight)				
Moisture	92.5	91.4	92.3	91.2
Crude protein	3.8	3.9	3.7	5.1
Crude lipid	0.7	1.0	0.6	0.4
Carbohydrate [a]	2.2	3.1	2.9	2.8
Ash	0.8	0.6	0.5	0.5
Estimated gross energy (kJ g^{-1}) [b]	1.28	1.58	1.34	1.49
Individual dry weight	188 ng	1.68 µg	1.69 µg	5.59 µg
Individual gross energy ($\times 10^{-3}$ J ind^{-1})	0.24	2.65	2.26	8.33
Fatty acid (% total fatty acids)				
14:0	3.7	0.4	0.9	2.1
16:0	45.7	13.2	15.2	19.9
16:1	10.2	5.4	10.1	9.9
18:0	4.8	5.6	5.6	8.8
18:1 (n-9)	17.1	30.6	32.8	18.2
18:2 (n-6)	6.4	7.1	6.7	5.4
18:3 (n-3) ALA	2.2	32.9	10.8	1.2
20:4 (n-6)	2.6	1.7	5.9	3.7
20:5 (n-3) EPA	4.8	2.1	4.5	4.3
22:5 (n-3)	0.6	0.3	1.4	3.2
22:6 (n-3) DHA	1.9	0.6	6.1	23.3
ΣSFA	54.2	19.2	21.7	30.7
ΣMUFA	27.3	36.0	42.9	28.1
ΣPUFA n-3	9.4	35.9	22.7	32.1
ΣPUFA n-6	9.0	8.9	12.6	9.1
n-6 : n-3	1.0	0.2	0.6	0.3
DHA : EPA	0.4	0.3	1.4	5.4

[a] Carbohydrate = %dry matter – (%protein + %lipid + %ash); [b] 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 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 *et al.,* 2011; Zhang-y *et al.,* 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, 2.0×10^{6} 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 were then measured under a light microscope. The height (vertical distance from the tip of the coronet to the tip of the straighten tail) of juveniles was measured following the method of Lourie *et al*. (2004). During the later stages, three seahorses from each replicate were randomly selected every other day, photographed using a digital camera (Olympus TG-4) with a relative scaled ruler and then returned into their respective tank. The heights were measured using image processing software later. The mouth gape size (defined as the vertical distance of the snout at the narrowest point) from 0 and 9 DAB seahorses were measured under a light microscope following the method of Celino *et al*. (2012). The specific growth rate (SGR) for height was calculated by the formula given below :

$$\text{SGR \% d}^{-1} = \frac{\ln H_f - \ln H_t}{T} \times 100$$

where, H_f = final height of seahorse; H_i = initial height of seahorse and T = day of culture

Prey consumption per day per juvenile seahorse was measured every other day starting from 1 DAB. The initial prey density was measured by randomly obtaining three 10 ml replicate water samples from each tank. The final prey density was estimated from another three 10 ml water samples before the next feeding. A control tank, which contained similar prey densities but with no seahorse, was prepared daily, to account for possible changes in the prey densities, such as rotifer reproduction. Since all seahorses were fed twice daily, prey consumption was calculated based on the depletion of food through a modified formula given by to Willadino *et al*. (2012):

$$\text{Prey consumption} = [(D_{control} - D_1) + (D_{control} - D_2)] \times \frac{V}{n}$$

where, $D_{control}$ = the mean initial or control prey density; D_1 = final prey density after feeding session 1; D_2 = final prey density after feeding session 2; V = water volume (ml) and n= number of surviving seahorse

The biomass of the consumed prey was estimated by multiplying the total number of consumed prey by the dry weight of the respective live food organism.

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 STATA 11.0 (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 were 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

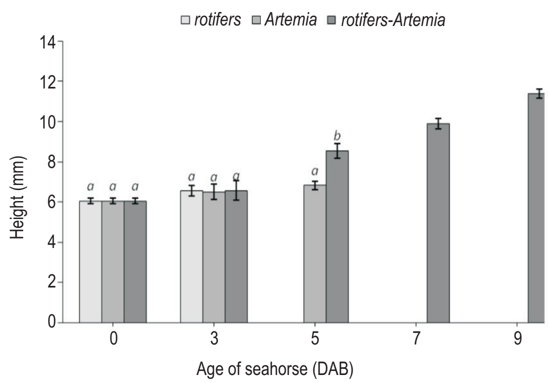


Fig. 1: Heights (mm) of juvenile seahorses, *Hippocampus kuda* at different feeding regimes. (i) only rotifers *Brachionus* sp., (ii) only *Artemia* nauplii and (iii) a mixture of rotifers and *Artemia* nauplii. Values are mean ± SD Bars with different letters on the same day are significantly different (P<0.05)

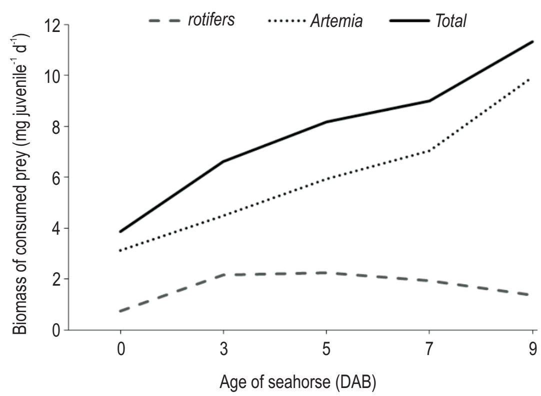


Fig. 3 : Biomass of prey consumed (mg juvenile^{-1} d^{-1}) by *Hippocampus kuda* juvenile fed a mixture of rotifers *Brachionus* sp. and *Artemia* nauplii

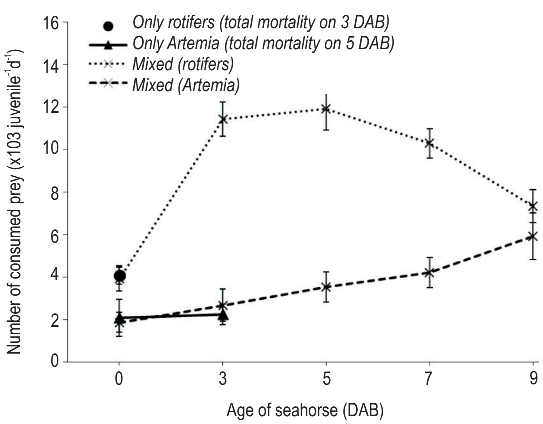


Fig. 2 : Number of prey consumed (×10^3 juvenile^{-1} d^{-1}) by *Hippocampus kuda* juveniles fed only rotifers *Brachionus* sp., only *Artemia* nauplii or a mixture of rotifers and *Artemia* nauplii. Total mortalities occurred in juvenile seahorses fed only rotifers and only *Artemia* nauplii on 3 DAB and 5 DAB, respectively. Values are mean ± SD

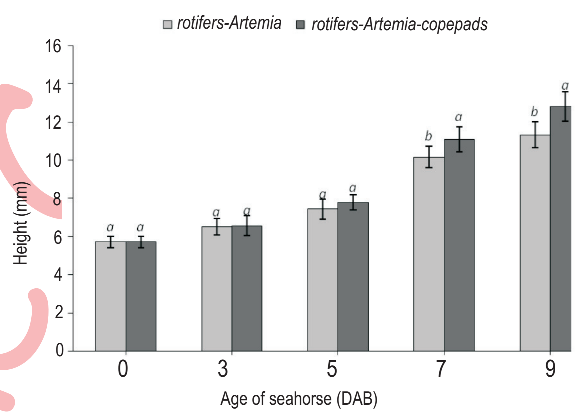


Fig. 4 : Heights (mm) of juvenile seahorses *Hippocampus kuda* fed combination of live foods that (i) copepods was not included and (ii) copepods *Oithona simplex* was included. Values are mean ± SD Bars with different letters on the same day are significantly different (p<0.05)

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 captively 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*, *Gladioferens 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.

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