



## Research Article

# Early Success Using Larval Stages of White Shrimp *Litopenaeus vannamei* as Seahorse Feed: Indicative for a Simultaneous Mariculture?

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### Abstract

The seahorse *Hippocampus reidi* (Syngnathidae) is classified as “data deficient” on the IUCN Red List (International Union for Conservation of Nature) and as “vulnerable” on the official Brazilian list. In order to seek alternative live feed for captive seahorse cultivation, *H.reidi* newborns were fed for the first time using larval stages of sea shrimp *Litopenaeus vannamei* and the conventional *Artemia salina* and rotifer *Brachionus plicatilis* diets in Short-Duration experiments - SD (up to 11 days) and Medium-Duration MD (up to 60 days). Treatments used each food item separately or combined with another. The experiments were carried out in closed systems with recirculation and partial seawater exchange (salinity 30), 12 h photoperiod and controlled temperature (25°C±1). The crustacean nauplii and rotifers were fed with the microalgae *Chaetoceros gracilis*. For SD experiments, the use of *B.plicatilis* up to 10°C and the addition of *Artemia salina* nauplii from the 5<sup>th</sup> day of life obtained a good result with a survival rate of 72%. For the MD experiments, the combination of *L.vannamei* nauplii and rotifers had a survival rate up to 20 days of life of 89.2% and varied between treatments from 50 to 86% until 60 days of life. The development in weight and height reached the highest values in the treatments that used *L.vannamei* nauplii.

**Keywords:** Artemia; Culturing; Fish; Prawn; Rotifer

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### Introduction

Seahorses are bony fishes and all species are included in a single genus, *Hippocampus*, which belongs to the family Syngnathidae. Species of *Hippocampus* are listed by the IUCN Red List (International Union for Conservation of Nature) as Vulnerable (VU) or Data Deficient (DD) [1]. There are three species native to Brazil: *Hippocampus reidi*; *H.erectus* and *H.patagonicus* [2-4]. In Brazil, the three species have been listed as VU by the Ministry of Environment, through Ordinance 455/2014 [5].

According to Vincent [6], aquarists and people involved with traditional medicine, mainly traditional Chinese medicine, started to become interested in seahorses many decades ago. The earliest record of cultivating these fishes is from 1950 [7]. The first attempts to commercially cultivate seahorses were during the 1970s; in the 1990s, the aquaculture of seahorses began in Asia and, then in 1996, could be found in Australia and the United States [8].

In Brazil there are very few records regarding the true sale of live seahorses and nothing regarding the trading of dried seahorses. According to Baum and Vincent [9], 100 kg of seahorses were sold in 1990 in this country. It is also known that dried seahorses are used throughout Brazil by individuals practicing traditional medicine in, for example, “seahorse tea” which is used to treat asthma and fatigue [10,11].

In addition to the trade of dried seahorses in Brazil, the trade of live seahorses for aquaria has a quota of 250 seahorses/species/year/company [12]. According to Baum and Vincent [9], Brazil is the largest exporter of seahorses in Latin American. These are reasons that make seahorse aquaculture of vital importance for supplying the world market and for maintaining natural stocks.

A comprehensive review of seahorse aquaculture was compiled and summarized by Koldewey and Martin-Smith [8], in which the authors discuss the use of live and frozen diets. These authors found that mysids, brine shrimp, amphipods, copepods, rotifers and wild zooplankton are the most common food sources used. The authors also comment on the importance of economic feasibility studies in seahorse cultivation.

In the absence of a literary record on the use of sea shrimp nauplii as food for early stages of seahorses, the purpose of this manuscript was to draw attention to alternative living diets using *Litopenaeus vannamei* sea shrimp for this purpose.

### Materials and Methods

#### Short-duration experiment - 1SD- up to 11 days old

A short-term experiment was conducted with two treatments (A and B) in a climate-controlled environment (25±1°C), with newborns of the *H.reidi* from the same brood. The breeders were also born and raised in our laboratory. For the present cultivation experiment, a density of 10 newborn seahorses per liter was maintained.

In the treatment A, they were fed the rotifer *Brachionus plicatilis* for 10 days. In treatment B they were fed *B.plicatilis* for 11 days and newly hatched *Artemia salina* nauplii from day 5 to day 11. *Artemia salina* cysts (INVE) were obtained from local shops and enriched on a diet of the microalga *Chaetoceros gracilis* in 300-L tanks. The experiments were conducted in 1-L aquaria using 12 h photoperiod, natural seawater (salinity 30) that was sterilized with chlorine, and received moderate aeration. The water was replaced each morning, which was important for removing feces and old food from the aquaria and keeping the levels of ammonia and nitrite at zero and the pH at 8.2. In all experiments Ammonia and nitrite were monitored with colorimetric tests (Alcon®, Brazil), the pH with digital phmeter (Analyzer®, Brazil) and salinity with automatic temperature-compensated analogical refractometer (Instruterm®, Brazil) and temperature with digital thermometer.

The rotifers were fed the microalga *C.gracilis*. Each treatment was replicated nine times. The zooplankton used had a concentration of 80 ind/mL *B.plicatilis* and 50 ind/mL *A.salina*, and they were offered to the seahorses in volumes of 100 and 70 mL, respectively, three times per day (9:00 a.m.; 1:00 p.m. and 5:00 p.m.).

### Medium-duration experiments-MD- up to 60 days old

Two medium-term experiments were conducted using five 40-L aquaria each, equipped with an external filter and screens so the fish would not be sucked into the filter. The alevins of *H.reidi* used for each experiment were obtained from the same parents. The water used was seawater (30 ppm) sterilized with chlorine; 10% of the volume of the tank was changed each day. A 12-h photoperiod was used and the tanks were kept at a pH of 8.2, 0 mg.L<sup>-1</sup> ammonia and nitrite levels between 0 and 1.8 mg.L<sup>-1</sup>. When the nitrite concentration increased to more than 0.5 mg.L<sup>-1</sup>, 50% of the water was replaced. The experiment took place in a warm room, 25°C±1.

**Experiment 1MD-** Newborn *H.reidi* seahorses were stocked at a density of 1.25 fish per liter (50 seahorses in a 40-L aquarium). Each day, the seahorses were fed an exclusive diet of white shrimp (*Litopenaeus vannamei*) nauplii that were 1 to 2 days old. The nauplii were kept at an average concentration of 60 ind/mL, and given to the seahorses in 500-mL batches, twice a day (9:00 am and 2:00 pm.), for the first 22 days. Afterwards, the young seahorses were fed *A.salina* twice a day, which were up to 2 mm long and at a concentration of 10 ind/mL. The microcrustaceans were fed the microalga *C.gracilis* and the shrimp commercial liquid feed (rich in polyunsaturated fatty acids), Epifeed® LHF1 3 times per day.

**Experiment 2MD-** The newborn seahorses (n=100) were fed the rotifer *B.plicatilis* and nauplii of the white shrimp *L.vannamei* that were 1 to 2 days old. The rotifers were provided under the same conditions as in the short-term experiment until the seahorses reached 6 days of life, at which time the seahorses were fed white shrimp nauplii until day 20. After day 20 the seahorses were divided into three treatments (30 seahorses per 40-L tank):

- Shrimp treatment- 30 seahorses fed only *L.vannamei* nauplii (CAM).
- Shrimp+Artemia treatment- 30 seahorses fed *L.vannamei* nauplii (50%) and fed *A.salina* nauplii and juveniles (50%) (CAM+ART).
- Artemia treatment- 30 seahorses fed only *A.salina* nauplii and juveniles (ART).

As the medium-term experiment was dependent on external supply to our laboratory (supply of sea shrimp nauplii by the partner mariculture, far from our laboratory), they had no replication due to the difficulty of obtaining sufficient quantities of sea shrimp nauplii in the periods when necessary until the conclusion of the experiment.

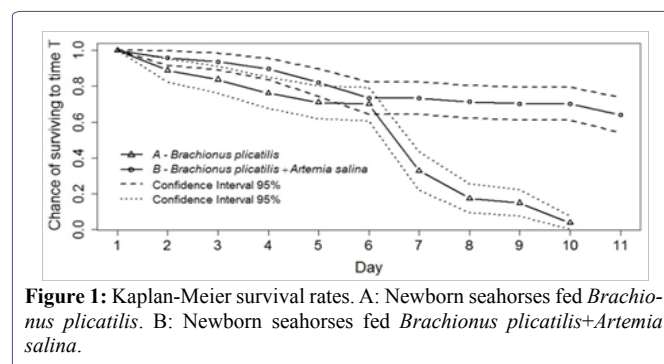
### Statistical Analysis

Short-term survival rates were evaluated at the end of the tenth day and eleventh day of Experiment 1SD. For medium-term Experiments 1MD and 2MD, survival rates were evaluated for the respective treatments at the end of the experiments, and the growth of the seahorses was evaluated in terms of height (cm) and weight (g) on the 30<sup>th</sup> and 60<sup>th</sup> day of age in only Experiment 2MD. The seahorses were measured for height according to Lourie et al. [1] and individually weighed using a semi-analytical balance (0.0001 g). To ensure that the seahorses newborns could eat their prey, 1 ml sample of each food item was measured against the size of the mouth of the sea horse, using an ocular micrometer attached to the binocular stereomicroscope, observing the size range of the prey. The evaluation of the survival rate was performed by the Kaplan-Meier method only in the short-term experiment [13]. The Wilcoxon test was used to evaluate the similarity in survival between the series generated [14]. Tukey's test was used for comparison of average weight and height of different treatments [15]. Proportions were compared using the binomial test, using the software R version 3.6.1. [16].

### Results and Discussion

#### Short-term experiments

In figure 1, it can be seen that treatments A and B of short-term Experiment 1SD had an average survival rate of 71% (±4.7%) and 75% (±4.6%), respectively, on the sixth day. The Kaplan-Meier assessment of survival rate in this case showed that on the sixth day, treatment A showed survival between 60-79%, and B 64-82%.



**Figure 1:** Kaplan-Meier survival rates. A: Newborn seahorses fed *Brachionus plicatilis*. B: Newborn seahorses fed *Brachionus plicatilis*+*Artemia salina*.

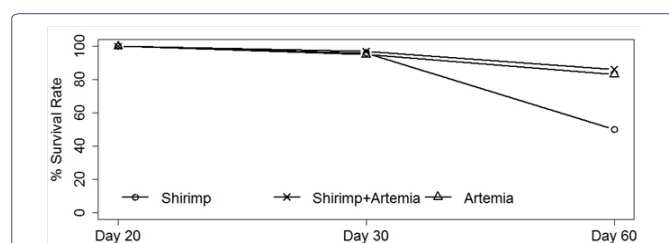
In treatment A, in which continued the use of only rotifers until the tenth day, there was a significant drop in survival rate relative to treatment B, between the sixth day (p=0.043) and the tenth day (p=0.007), at which point there was only a 1.6% survival rate. In treatment B, in which rotifers were used and then *A.salina* starting on day 5, the survival average rate was 72% at the end of the experiment (61-79%).

#### Medium-term experiments

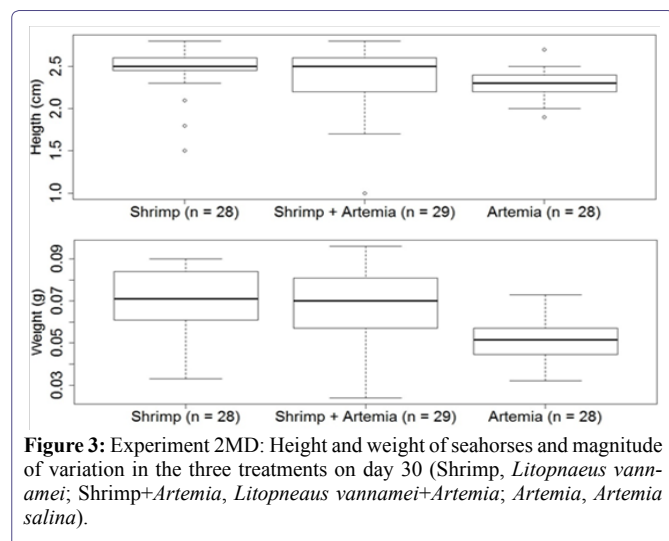
In Experiment 1MD, on the tenth day, 50% of the seahorses died. There were no deaths between day 10 and day 22, but the seahorses

began to die again after day 22 when the type of food was changed. Only 20% of seahorses (10 of 50 fishes) lived until the end of the experiment.

In Experiment 2MD, at the end of 20 days, the survival rate was 90%. This showed that the combination of *B.plicatilis* and *L.vannamei* was an effective feed. In the Experiment 2 treatments (Shrimp, Shrimp+Artemia and Artemia), the survival rate of the seahorses at 60 days was 50% for the Shrimp treatment and the seahorses that died had symptoms of loss of appetite, slow movement, body covered by a white mucus and necrotic spots on the tail. For the Shrimp+Artemia and Artemia treatments, the survival rates were 86 and 83%, respectively, showing that there was only a significant difference in the Shrimp treatment ( $Z=4.9439$ ,  $p<0.0001$ , figure 2). In addition to the survival rate, the height (cm) and weight (g) of the seahorses in the three treatments were also analyzed. The results are illustrated in figures 3 & 4.



**Figure 2:** Experiment 2MD: Survival rates of the *H.reidi* studied, between day 20 and day 60, using different diets. Shrimp, *Litopenaeus vannamei*; Shrimp+Artemia, *Litopenaeus vannamei*+*Artemia salina*; Artemia, *Artemia salina*.

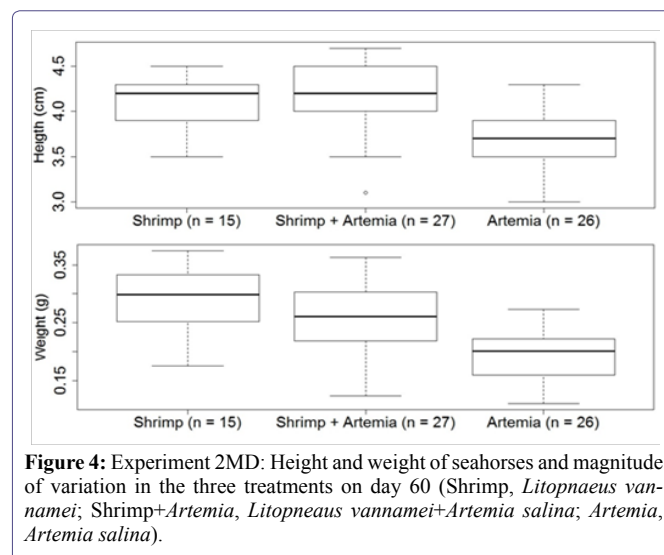


**Figure 3:** Experiment 2MD: Height and weight of seahorses and magnitude of variation in the three treatments on day 30 (Shrimp, *Litopenaeus vannamei*; Shrimp+Artemia, *Litopenaeus vannamei*+*Artemia salina*; Artemia, *Artemia salina*).

At the end of the first month, only the Artemia treatment showed a significant difference in comparison with other treatments, in the height and weight of the seahorses ( $p<0.01$ ). At the end of the second month, the Artemia treatment showed little development in height as well as weight ( $p<0.01$ ), even though the final survival rate was 83%.

The maximum and minimum measurements obtained for the size of the mouth opening of the newborn seahorses was 375-500  $\mu\text{m}$ , 150-170  $\mu\text{m}$  for length of rotifers and 420-540  $\mu\text{m}$  for the length of

marine shrimp nauplii (without considering the bristles, which increased the size to 730-750  $\mu\text{m}$ ). The newborn seahorses had great difficulty in feeding on the marine shrimp nauplii during the first 10 days of life because of their large size (1- to 2-day-old nauplii).



**Figure 4:** Experiment 2MD: Height and weight of seahorses and magnitude of variation in the three treatments on day 60 (Shrimp, *Litopenaeus vannamei*; Shrimp+Artemia, *Litopenaeus vannamei*+*Artemia salina*; Artemia, *Artemia salina*).

## Discussion

In the short-term experiment, both treatments (A & B) had similar and positive results when considering the survival rates (over 70%) of *H.reidi* on the sixth day of life (which is the time that fish farmers usually use rotifers). Murugan et al. [17] cultivated *Hippocampus trimaculatus*, which had a 29% survival rate when fed a diet of *B.plicatilis* until the sixth day. The authors comment that the rotifers were preferentially consumed by seahorses in the first two days old and that the nauplii of copepods promoted greater development, providing a survival rate of 73% on the sixth day. Hora and Joyeux (2009) [18], obtained high survival rates by using wild zooplankton as food until *H.reidi* was six days old. Similarly, Silveira and Santos [19] achieved 84% survival using wild zooplankton as the first feed of *H.erectus* until the 10<sup>th</sup> day of life. Melo et al. [20], achieved a survival rate of 76% by cultivating *H.reidi* from birth to the 15<sup>th</sup> day of life on a diet of copepods and Artemia enriched with seahorses kept in green water ( $2.02\pm 0.44 \times 10^6$  cells  $\text{mL}^{-1}$  *Nannochloropsis oculata*) against 25% survival of treatment maintained without microalgae.

Using a diet of rotifers and enriched *Artemia franciscana* for the newborn *H.abdominalis*, Thompson [21] did not observe growth or survival benefit with this diet, but this species is more than twice the average size at birth than *H.reidi* (15 mm and 7 mm, respectively). Seahorse species that produce small juveniles with fine snouts such as *H.kuda* and *H.subelongatus* are believed to be restricted to smaller prey as the first food (such as rotifers and small copepods) to increase growth and survival rates [22]. It would be the same case of *H.reidi* in this study, which obtained a good survival rate predated *B.plicatilis* and *A.salina* enriched until the sixth day. For seahorses that were fed rotifers only, after the fifth day the drop in survival rate can be seen as a reflection of nutritional deficiency or disinterest in a small food item that has become unattractive for the age and size of 6 days of life.

In medium-term Experiment 1MD, there was a high mortality (50%) during the first 10 days of cultivation, which was attributed to the larger size of the white shrimp nauplii (420-540  $\mu\text{m}$  long) in relation to the size of the mouth opening (375-500  $\mu\text{m}$ ) of the newborn *H.reidi*. The width between the lateral appendages with bristles of shrimp nauplii (750  $\mu\text{m}$ ) also represented an impediment, because the bristles although flexible gave the animals a larger appearance, making them look intimidating and precluding attack by the seahorse newborn. The variable size of the prey and the small size of the mouth opening of the seahorses resulted in 50% mortality during the first 10 days of the experiment, but the survival rate stabilized between day 11 and day 22. Seahorses that achieved mechanical ability or showed a larger oral perimeter were able to establish themselves in the aquarium. Seven days after the food type was changed (after day 22) from white shrimp nauplii to young *A.salina*, seahorses began to die again. The seahorses that adapted to this new food type (18.35%, nine fishes) remained alive until the end of the experiment (60 days). When cultivating *H.reidi*, Hora and Joyeux (2009) [18], always observed mortality after changing the food type, being essential that the substitutions happen gradually with a period of supply of both foods simultaneously, before the complete substitution, in order to reduce the food stress. Silveira and Silva [19] used the marine shrimp nauplii *L.vannamei* and *A.salina* for the cultivation of *H.erectus*, from the 11<sup>th</sup> day of life, obtaining 84% survival until adulthood, but in the first days of life used plankton as food until the 17<sup>th</sup> day.

In medium-term experiment 2MD, whose diet combined *B.plicatilis* with *L.vannamei*, the survival rate was 89% at the end of 20 days. From then on, these animals arranged in the three treatments (CAM; CAM+AERT; ART) and cultivated up to 60 days of life had survival rates of 50, 86 and 83%, respectively. Job et al. [23], achieved a 92% survival rate for *H.kuda* at 42 days of life, using a species of enriched Artemia. Bustamante and Salas [24], obtained an 84% survival rate for *H.igens* until the 63 days of life, while Woods [25], obtained a survival rate of 68 to 82% using the same species.

When we look at the height and weight performance provided by the treatments of the 2MD, we see that while the ART treatment had a good survival rate (83%), CAM and CAM+ART were significantly superior and similar to each other in height and weight at the end of the first month and the second month. These facts are possibly attributes to the use of *L.vannamei*, though extensive comparisons with the available literature become difficult, since the cultivation of seahorses with *L.vannamei* nauplii was seen only in Silveira and Silva [19], cultivating *H.erectus*. Although they presented a similar height at birth of 7 mm (*H.erectus*, Silveira and Silva [19] and *H.reidi*, in this experiment), the first reached 53 days with 4.36 $\pm$ 0.31 cm and weight of 0.377 $\pm$ 0.074 g, while *H.reidi* reached 60 days (seven more days) with 4.17 $\pm$ 0.413 cm and 0.265 $\pm$ 0.064 g. Both used the same type of food (CAM+ART) and the same temperature range, suggesting that perhaps the first species might have a faster development. Animals whose adult body size is smaller often have a faster metabolism than larger ones [26,27]. In this case, *H.erectus* has a maximum observed size of 12 cm in height, while for *H.reidi*, the maximum observed size was 18.7 cm in height, both in Brazilian waters [4].

Captive growth data are diverse for different species, and dependent on the variables to be considered in crops such, as type and quantity of feed, temperature, photoperiod, tank sanitation, water quality, and biological variables such as specific growth rate [8,24,28-33].

In Experiment 2MD' treatments (CAM; CAM+ART and ART), the survival rate at the end of 60 days was 50% for the CAM treatment. For CAM+ART and ART treatments, rates were similar, between 86 and 83%, respectively, resulting in a significant difference only in CAM treatment (p-value=0.0147, figure 4).

In the 2MD, the three treatments proceeded in a similar way until the seahorses were 30 days old. We expected the Shrimp treatment to have the highest seahorse survival rate because of the nutritional value of marine shrimp [34]; however, it had the lowest. In searching for an explanation for this situation, we talked to the technicians of Netuno (supplier of marine shrimp nauplii) and found out that shrimp mortality in their tanks was attributed to rod-shaped bacteria, which could be related to the mortality we observed in the treatment with only marine shrimp nauplii. Balcázar et al. [35] identified two species of *Vibrio*, which killed individuals of *Hippocampus guttulatus* that were being cultivated in Spain. Symptoms reported by these authors and Planas et al. [36], included lethargy, loss of appetite, white spots on the skin and necrosis of the tail; this was similar to what we observed in the medium-term Experiment 2MD. Although it was not possible to categorically determine the cause of death of the seahorses, we observed that poor nutrition (only *A.salina*) and the size of the prey caused food shortages, growth deficit, which probably made the seahorses more vulnerable to diseases.

Overall, the results of this study suggest that the breeding of seahorses using rotifers for the first 5 days and *L.vannamei* nauplii afterwards is promising for this type of aquaculture. This could be attributed to the higher nutritional value of white shrimp compared to *A.salina* nauplii [34,37]. Out of curiosity, after the end of the experiment (60 days of life of the seahorse), cultivation continued successfully using the other life stages of *L.vannamei* and adult brine shrimp, but with different objectives than those described here. For large maricultures, it would even be possible to cultivate *L.vannamei* and seahorses at the same time. It is necessary to conduct a study of economic viability recommending (or not) an experimental plot of seahorses for commercial purposes using the various stages of white shrimp as food. The findings of this study suggest a new alternative food source for farmed seahorses with the possibility of a type of consortium or simultaneous cultivation with *L.vannamei* and optimization techniques, considering commercial and conservation proposals.

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