UNIVERSIDADE ESTADUAL PAULISTA

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Instituto de Biociências - Campus do Litoral Paulista

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Amphipods as Effective and Viable Live Feed for Juvenile Seahorses

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Instituto de Biociência – Câmpus do Litoral Paulista

Praça Infante D. Henrique s/nº - CEP 11330-900 – São Vicente (SP) – Brasil Tel. (13) 3569-7100 – diretor.clp@unesp.br

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Trabalho de Conclusão de Curso (TCC) apresentado como parte dos requisitos para obtenção do título de Bacharel em Ciências Biológicas, com habilitação em Gerenciamento Costeiro, pela Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus do Litoral Paulista – São Vicente.

Orientadora: Profª Drª Tânia Marcia Costa
Coorientadores: Dr. Alexandre Luiz Arvigo e Dr. Caio Akira Miyai

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1. Abstract

In Brazil, populations of the seahorse *Hippocampus reidi* are declining due to habitat degradation and overcapture. Cultivation offers an alternative for conservation and commercial supply, but feeding remains a challenge. Amphipods are a natural prey item with good nutritional value, low production costs, and high potential as live feed. This study evaluated the effects of two amphipod species (*Cymadusa filosa* and *Parhyale hawaiensis*) as monospecific diets for juvenile *H. reidi*, compared with *Artemia salina*. Additionally, the influence of the diet for *C. filosa* (algae or formulated feed) on seahorse growth was examined. Despite having a consumption rate three times lower than *A. salina*, amphipods promoted six times higher weight gain and specific growth rate (SGR), as well as greater feed efficiency. No significant differences were detected between amphipod species or between the tested diets for *C. filosa*, indicating both are adequate. However, *C. filosa* stands out due to its higher fecundity, lower mobility, and ease of culture with formulated feed. These findings demonstrate that amphipods, particularly *C. filosa* reared on formulated feed, represent a viable and sustainable feeding strategy for *H. reidi*, supporting conservation-oriented aquaculture.

Keywords: aquarium trade; *Cymadusa filosa*; *Hippocampus reidi*; ornamental aquaculture; *Parhyale hawaiensis*.

2. Introduction

Aquaculture is among the fastest-growing food production sectors and is a major contributor to global fishery resource production, representing 51% of the total 185 million tons produced in 2022 [1]. Nevertheless, this growth has not been matched by the sufficient availability of essential dietary inputs, particularly live feeds, such as *Artemia* and rotifers, which are critical for the cultivation of numerous species [2–4]. This gap has driven the search for sustainable and nutritionally rich alternatives, especially in ornamental aquaculture, a rapidly growing sector that is supported by the development of new live feed options [5,6].

Seahorses (*Hippocampus* spp.) have gained prominence in aquaculture due to their high commercial value and growing market demand [7]. Their unique reproductive biology and limited mobility make them especially vulnerable to environmental disturbances and overexploitation [8]. In Brazil, *Hippocampus reidi* was the fifth most exported marine ornamental species between 2011 and 2018 [9], although its farming still faces several challenges that constrain sustainability and economic feasibility [6,10,11]. Since they are highly specialized visual predators that do not readily accept formulated diets, they depend almost exclusively on a continuous and reliable supply of live prey to meet their nutritional needs and achieve satisfactory performance [11–13]. Additionally, their diet must provide high levels of essential fatty acids, particularly long-chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are crucial for maintaining cell membrane integrity and proper development [12–14]. Although, the specific quantitative requirements for *H. reidi* regarding total lipid content remain undetermined; studies on other seahorse species have reported lipid levels ranging, values between 5.4 ± 1.2 g kg^{-1} for adults and 29.5 ± 2.2 g kg^{-1} for juveniles, although these may vary among species [15,16]. Therefore, identifying alternatives that are affordable, nutritious, and sustainable is increasingly essential for optimizing and ensuring the viability of their cultivation [17].

Commonly used live feeds, such as *Artemia salina*, are nutritionally deficient, especially in essential fatty acids, including EPA and DHA [18]. Thus, they must be enriched with lipid compounds to improve their quality, which substantially increases production costs [19,20]. Other options, such as mysids, are nutritionally superior but expensive to produce, as their culture requires constant feeding with *Artemia* nauplii, thereby constraining their large-scale use and economic viability [21–24]. However, one promising alternative is the use of amphipods, which exhibit great potential as live prey due to their high nutritional value (approximately 40% protein, 5.5% lipids, 27.4% carbohydrates, and 2.9% fiber, with variations among species and according to the diet used in their cultivation) and ease of cultivation [17,25–28]. These small crustaceans, often associated with algae, play a key ecological role as an important trophic link between primary producers and higher trophic levels [29,30]. They possess desirable traits for aquaculture production that facilitate culture under laboratory conditions and enable the maintenance of high population densities, including small body size in adults (generally under 20 mm), direct development, short life cycle (typically less than three months), and high fecundity [28,31–33]. Amphipods are nutritionally notable for their well-balanced nutrient profiles, being rich in long-chain polyunsaturated fatty acids (PUFAs), including omega-3 and omega-6, as well as arachidonic acid (ARA), which are essential for enhancing survival, growth, stress tolerance, and immune function in cultured fish [27,34–37]. Due to their naturally high nutritional value, amphipods generally do not require enrichment, which helps reduce production costs [17,25], although their fatty acid content can be further enhanced when reared with formulated diets [27,36].

Two amphipod species with significant potential for use as live feeds in aquaculture are *Parhyale hawaiensis* and *Cymadusa filosa* [27,36,37]. Both species occur in tropical and temperate regions and are commonly found on rocky shores associated with algae, where they constitute a natural dietary component for many marine organisms, including *H. reidi* and other species of seahorses [38–40]. Because they inhabit this ecological niche, they display nutritional profiles that are well-suited to the requirements of their predators, particularly due to their high PUFAs, which are essential for the growth, development, and health of marine fish [27,36,41]. *Parhyale hawaiensis* has already been successfully used as a model feed in studies with ornamental fish, while *C. filosa*, although less studied, exhibits similarly promising traits [27]. Thus, both species represent valuable options for diversifying the supply of live prey in ornamental aquaculture, with *C. filosa* standing out as an emerging candidate.

Currently used live feeds, such as *Artemia* and mysids, have limitations that increase both production complexity and cost [16,17,42,43]. This reliance on live feeds consequently hinders the development of efficient farming systems and may necessitate the capture of wild seahorses to offset challenges in aquaculture [44,45]. Therefore, the success of *H. reidi* aquaculture depends on the availability of live feeds that are nutritionally adequate, readily accepted, and cost-effective. Thus, this study aimed to evaluate two amphipod species (*P. hawaiensis* and *C. filosa*) that co-occur with *H. reidi* as alternative live feeds for seahorse aquaculture. We compared their performance as monospecific diets with the traditional live feed, adult *Artemia salina*. Additionally, we assessed how the culture diet of *C. filosa* - natural (algae-based) or artificial (commercial feed-based) - affects the growth performance of seahorses, given that the diet used during amphipod cultivation can modify their fatty acid content. We hypothesized that amphipods would promote better growth than *A. salina*, and that commercial feed-cultured *C. filosa* would further enhance performance due to an improved fatty acid profile. This study provides valuable insights for developing more efficient and sustainable feeding strategies for *H. reidi* aquaculture.

3. Materials and Methods

3.1. Ethical Approval

This study was conducted in compliance with Brazilian legislation regulated by the National Council for the Control of Animal Experimentation (CONCEA) and the Ethical Principles in Animal Research formulated by the Brazilian Society of Science in Laboratory Animals (SBCAL). It was approved by the Bioscience Institute (UNESP) Ethics Committee on Use of Animals (CEUA 01/2023).

3.2. Animals, Collection, and Stocking Conditions

3.2.1. Adult Seahorses

Two adult pairs of *Hippocampus reidi* were obtained through a partnership with the Ubatuba Aquarium and transported to the laboratory in São Vicente/São Paulo State, Brazil. The animals were transported in sealed plastic bags filled with oxygen to minimize stress. Upon arrival, the seahorses were acclimated in a 310 L recirculating aquaculture system (RAS) equipped with biological filtration (biological media), mechanical filtration (acrylic fiber), a protein skimmer, and continuous aeration, under a 12 h light/12 h dark photoperiod. Water quality parameters (temperature at 26 °C, pH 8.3, and salinity 28) were monitored three times per week, and 10% partial water changes were performed twice a week [45,46].

The tank was enriched with polypropylene mesh (1 × 1 cm) and artificial plants to provide holdfast structures for the animals [27]. Feeding occurred twice daily, with small prey items of appropriate size for adult consumption, offered in the morning and afternoon, consisting of a varied diet of unenriched *Artemia*, mysids, and amphipods [11,47]. Seahorses were monitored daily for signs of stress, including changes in color, erratic swimming, and a reduced feeding response.

3.2.2. Juvenile Seahorses

Newborn *H. reidi* were initially maintained in 2 L Erlenmeyer flasks, flasks, totaling 40 individuals (20 ind./L), to reduce early mortality associated with gas bubble disease. After five days, the surviving juveniles were transferred to two 30 L tanks, ten individuals per tank, equipped with a recirculating aquaculture system (RAS), constant aeration, and a 12 h light/12 h dark photoperiod. Water quality parameters were maintained as described for adults. Polypropylene mesh structures were also provided to facilitate holdfast behavior [27]. Juveniles were fed *ad libitum* three times daily with zooplankton collected from Praia dos Milionários (23°58′27″ S, 46°22′20″ W, São Vicente, São Paulo) using a 180 µm mesh plankton net. Prior to feeding, the zooplankton underwent a depuration protocol, in which the collected seawater was gradually replaced with artificial seawater to ensure greater biosafety. Beginning in the fourth week of life, the juveniles were gradually weaned onto a diet consisting of *Artemia* and amphipods.

3.2.3. Amphipods

The amphipod species *Parhyale hawaiensis* (Dana, 1853) and *Cymadusa filosa* (Savigny, 1816) were selected as experimental diets due to their potential as live feed and their natural co-occurrence with *Hippocampus reidi* in algae-associated rocky shores, making them ecologically relevant prey. *Parhyale hawaiensis* has previously been identified as an effective food source for *H. erectus* [36], while *C. filosa* has been reported in the stomach contents of *H. patagonicus* [48] and is suitable for laboratory cultivation, being able to feed on both algae and formulated diets.

Parhyale hawaiensis and *C. filosa* were collected from the rocky shore at Praia de Cibratel (24°13′31″ S, 46°51′7″ W) in Itanhaém, São Paulo, by free diving at depths of 1 to 2 m. To capture the amphipods associated with algae, the algae were carefully enclosed in cloth bags to prevent the escape of fauna and then detached from the substrate using spatulas. The collected algae were placed in plastic trays, and amphipods were manually separated by successive seawater rinses. Both algae and amphipods were transported to the laboratory in thermos boxes with seawater and were then kept in separate under controlled conditions [40,49]. Any remaining associated fauna was immediately returned to the natural environment after collection.

In the laboratory, maintenance was carried out in four glass aquaria (40 × 25 × 20 cm; 20 L), each equipped with filters (Maxxi HF 240 model), constant aeration, and a controlled 12 h light/12 h dark photoperiod. One aquarium was used exclusively for the algae *Sargassum filipendula*, which served as the diet for *C. filosa*. Two aquaria were designated for amphipods fed with a Spirulina-based granulated feed (Tropical Spirulina Super Forte Granulat), one for *P. hawaiensis* and another for *C. filosa*. A fourth aquarium was used solely for *C. filosa* fed with algae *S. filipendula*. *Parhyale hawaiensis* was maintained only on formulated feed due to its detritivorous habits and good adaptation to this diet. In contrast, *C. filosa* was used as a model to evaluate the comparative effects of algal versus formulated diets, as its natural preference for algae allows for a more accurate assessment of how prey diet influences its nutritional quality and, consequently, the seahorse's performance.

3.3. Experimental Design

To assess the effects of different monospecific live feeds on the growth performance of juvenile *Hippocampus reidi,* a. 14-day randomized experimental design was conducted. One juvenile individual was housed per aquarium (20 × 20 × 20 cm; 8 L) and was fed daily with only one of the four treatments tested: (A) non-enriched *Artemia salina*; (B) *Cymadusa filosa* cultured with algae; (C) *C. filosa* cultured with formulated feed; and (D) *Parhyale hawaiensis*. Thus, our experimental design consisted of one fixed factor (diet) with four levels (treatments). Each treatment consisted of five replicates, totaling 20 experimental units (Figure 1). The duration of the experiment was based on Da Hora & Joyeux, 2009, [45], who reported rapid growth until approximately 100 days of age, and on a pilot experiment, confirming that this period was sufficient to detect significant differences in growth (full data are available in Supplementary Materials, Table S1).

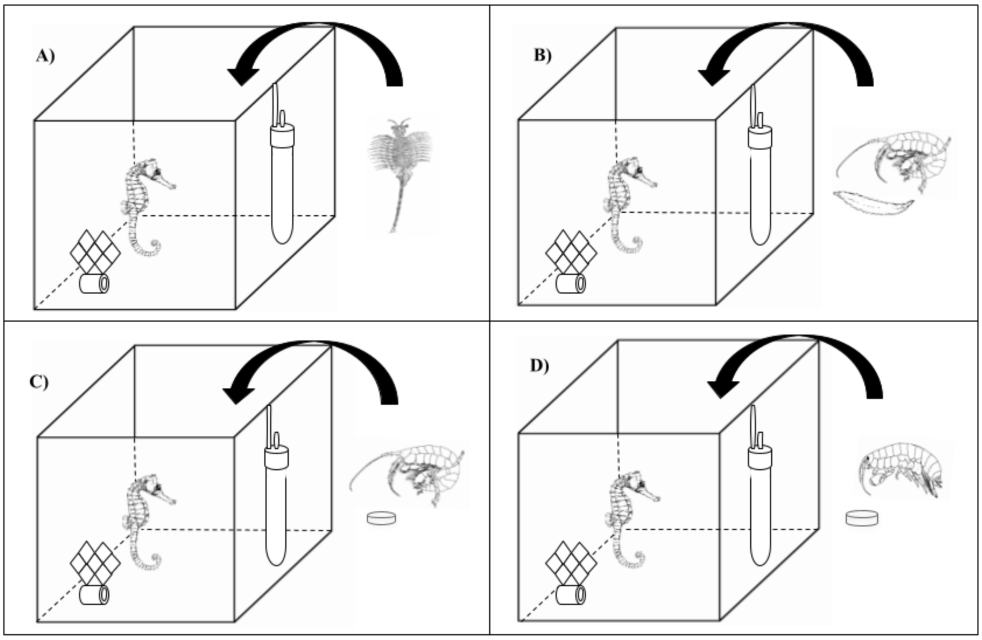

Figure 1. Experimental design for evaluating the effect of different live feeds on the growth performance of *Hippocampus reidi*. Each treatment included five replicates (one individual per tank). The treatments were as follows: (**A**) Seahorses fed with non-enriched *Artemia salina* (control); (**B**) Seahorses fed with *Cymadusa filosa* cultured with algae; (**C**) Seahorses fed with *Cymadusa filosa* cultured with formulated feed; (**D**) Seahorses fed with *Parhyale hawaiensis*.

3.4. Procedures

3.4.1. Feeding Test

Juvenile seahorses (*Hippocampus reidi*), aged 65 days and measuring 40.826 ± 3.629 mm in total length (all individuals were in good health, showing no signs of stress or color alteration, and feeding normally), were housed individually in glass aquaria (20 × 20 × 20 cm; 8 L; 0.125 ind./L), filled with artificial seawater (VEROMAR salt for corals), and equipped with a small anchoring structure (5 × 5 cm) made of polypropylene mesh (1 × 1 cm) fixed to a biological filter. Water quality in the experimental aquaria was maintained at a constant temperature of 26 °C (Roxin HT—1300 thermostat) and a 12 h light/12 h dark photoperiod throughout the 14-day trial. Aquaria were siphoned daily to remove debris and received partial water changes of approximately 1 L with aerated water to ensure stable conditions [45]. Physicochemical parameters were regularly monitored and kept consistent with those of the holding tank.

Seahorses underwent a 48 h acclimation period in the experimental aquaria and were fasted for 12 h prior to the start of the trials. During the experiment, animals were fed once daily with either 25 non-enriched *Artemia* (sourced from a specialized aquarium supplier) or 20 amphipods (kept in the laboratory as previously described), according to the assigned treatment. Feeding quantities were based on a pilot study, in which the maximum 24 h consumption averaged 18.21 ± 1.38 *Artemia* and 15.16 ± 1.70 amphipods (see Supplementary Materials, Table S1). A surplus was provided to ensure continuous food availability and to avoid feed limitation during the trial. Food remained in the aquaria for 24 h, after which uneaten prey items were removed and counted.

The wet weight of each feed type was determined prior to feeding using an analytical balance. Intake rates were calculated based on the number of prey items remaining after 24 h. Seahorses were measured (in centimeters) and weighed (in grams, using an analytical scale) at the beginning and end of the experiment. Length was determined from digital photographs of each individual placed alongside a reference ruler, and measurements were analyzed using ImageJ software with the scale calibrated to 5 mm.

3.4.2. Analyzed Variables

The following response variables were evaluated: (1) Intake Rate (IR), (2) Total Weight Gain (ΔWG), (3) Relative Weight Gain (WG%), (4) Specific Growth Rate (SGR), (5) Feed Conversion Efficiency (FCE), (6) Length Gain (LG), and (7) Condition Factor (CF).

Intake Rate (IR)

Intake Rate (IR) is the average daily consumption of crustaceans by seahorses in each treatment over the duration of the experimental period. This variable was used as an indicator of feed acceptance and feeding efficiency. IR was calculated (in g day^{-1}) by dividing the total amount of food consumed by the duration of the experiment:

$$IR = \text{Total weight consumed (g)/duration of the experiment (days)}$$

Total Weight Gain (ΔWG) and Relative Weight Gain (WG%)

Total Weight Gain (ΔWG) and Relative Weight Gain (WG%) were used as indicators of biomass conversion efficiency [50]. The ΔWG represents the absolute increase in body weight during the experiment, whereas WG% expresses this increase as a percentage of the initial body weight.

For Total Weight Gain (ΔWG), individuals were weighed at the beginning and end of the experiment using an analytical balance, after carefully removing excess surface water with absorbent paper. ΔWG was calculated as follows:

$$\Delta WG = FW - WI$$

Relative Weight Gain (WG%) was calculated using the following:

$$WG\% = ((FW - IW)/IW) \times 100$$

Specific Growth Rate (SGR)

The Specific Growth Rate (SGR) reflects the efficiency with which seahorses convert food into body mass over time. It was calculated using the logarithmic variation between initial and final weights, following Vargas-Abúndez et al. (2021) [27]:

$$SGR\ (\%\ day^{-1}) = ((\ln FW - \ln IW)/t) \times 100$$

where ln is the natural logarithm and t is the duration of the experiment (days).

Feed Conversion Efficiency (FCE)

Feed Conversion Efficiency (FCE) was used to assess how efficiently seahorses converted ingested food into biomass. It was calculated as follows:

$$FCE = \Delta WG/IR$$

Higher FCE values indicate greater feed utilization and superior overall performance.

Length Gain (LG)

Length Gain (LG) was used as a measure of linear growth and was determined by summing the lengths of three body segments: crown height, trunk length, and tail length. For measurements, individuals were gently placed in Petri dishes with sufficient water to keep them laterally submerged, which helped to minimize stress. A reference ruler was included in each dish. Images were taken using a digital camera (Nikon D3100, Nikon AF-S DX Nikkor 18–55 mm lens) mounted on a tripod positioned 50 cm above the specimen. The total length was later measured with ImageJ software.

Condition Factor (CF)

The Condition Factor (CF) was used as an indicator of health and physical robustness, allowing evaluation of nutritional status relative to body size. This metric helps identify differences in physiological condition across dietary treatments [50,51]. It was calculated as follows:

$$CF = (FW/FL^3) \times 100$$

where FW is the final weight (g) and FL is the final length (cm).

3.5. Statistical Analysis

The experiment followed a one-factor design (diet type), with four dietary treatments: (1) *Artemia salina* (control), (2) *Cymadusa filosa* reared on algae, (3) *C. filosa* reared on formulated feed, and (4) *Parhyale hawaiensis*. Data normality and variance homogeneity were tested using the Shapiro–Wilk and Levene's tests, respectively. When assumptions of normality and homoscedasticity were satisfied, treatment effects were assessed by one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. When assumptions were not met, the Kruskal–Wallis test was applied, followed by Dunn's test for pairwise comparisons. To complement the interpretation of p-values and better assess the magnitude of treatment effects given the relatively small sample size (n = 5 per treatment), effect sizes (Cohen's f) were calculated for each variable using the cohens_f() function from the effect size package in R [52]. Effect sizes were interpreted according to Cohen (1988), with f = 0.10, 0.25, and 0.40 corresponding to small, medium, and large effects, respectively [53]. To provide a more accurate estimate of the uncertainty associated with these effect sizes, bootstrap resampling (999 iterations) was performed using the boot() function [54]. This approach yields 95% confidence intervals (CIs) that represent the range of values within which the true effect size is expected to lie with 95% probability, based on the observed data. All statistical analyses were performed in R software [55], with a significance level set at 5% ($p < 0.05$).

4. Results

The provision of different live feeds had a significant influence on the growth parameters of *Hippocampus reidi*. Intake Rate was higher in individuals fed with *Artemia salina* (0.103 ± 0.004 g/day), approximately threefold higher than in amphipod treatments: *Cymadusa filosa* reared on algae (0.037 ± 0.007 g/day), on formulated feed (0.036 ± 0.003 g/day), and *Parhyale hawaiensis* (0.033 ± 0.008 g/day) (Kruskal–Wallis: $\chi^2 = 11.16$; df = 3; $p = 0.010$; Dunn's test, $p < 0.05$; Figure 2A). Among the amphipod treatments, no statistical differences were detected, indicating equal acceptance by the seahorses. The effect size for this variable was very large (Cohen's f = 5.69; 95% CI: 3.56 - 10.40), confirming that diet type had a strong influence on intake behavior.

Despite the higher consumption, the *A. salina* diet resulted in the lowest Total Weight Gain (0.009 ± 0.012 g), which was approximately one-sixth of the gains observed in the amphipod-fed groups: *C. filosa* reared on algae (0.055 ± 0.016 g), *C. filosa* on formulated feed

(0.058 ± 0.020 g), and *P. hawaiensis* (0.065 ± 0.028 g) (ANOVA: $F(3, 16) = 8.063$; $p = 0.002$; Tukey's test, $p < 0.05$; Figure 2B). The estimated effect size was large (Cohen's f = 1.23; 95% CI: 0.74 - 2.72), indicating a strong biological effect of diet on weight gain. These latter treatments did not differ significantly from each other. This same pattern was observed for Relative Weight Gain, with *A. salina* showing the lowest value (5.2 ± 6.3%), while the amphipod treatments reached values six times higher: *C. filosa* reared on algae (31.3 ± 8.8%), on feed (34.7 ± 13.6%), and *P. hawaiensis* (33.0 ± 11.6%) (ANOVA: $F(3, 16) = 8.97$; $p = 0.001$; Tukey's test, $p < 0.05$; Figure 2C). The effect size for this parameter was also large (Cohen's f = 1.30; 95% CI: 0.79 - 2.64).

Specific Growth Rate followed the same pattern, being lowest in *A. salina* group (0.35 ± 0.42% day^{-1}) and sixfold higher in amphipods: *C. filosa* reared on algae (1.93 ± 0.50% day^{-1}), *C. filosa* on formulated feed (2.10 ± 0.70% day^{-1}), and *P. hawaiensis* (2.01 ± 0.63% day^{-1}) (ANOVA: $F(3, 16) = 10.561$; $p = 0.0004$; Tukey's test, $p < 0.05$; Figure 2D). The corresponding effect size was very large (Cohen's f = 1.41; 95% CI: 0.87 - 2.78), reinforcing the strong influence of diet on juvenile growth. These differences were also reflected in Feed Conversion Efficiency, with the lowest value recorded for the *A. salina* diet (0.006 ± 0.008), while the other treatments showed considerably higher values: *C. filosa* reared on algae (0.105 ± 0.023), *C. filosa* on feed (0.112 ± 0.032), and *P. hawaiensis* (0.136 ± 0.042) (ANOVA: $F(3, 16) = 19.13$; $p < 0.001$; Tukey's test, $p < 0.05$; Figure 2E). A very large effect size (Cohen's f = 1.89; 95% CI: 1.11 - 4.67) confirmed that diet had a marked impact on feed conversion efficiency. In contrast, body Length Gain did not differ significantly among treatments (ANOVA: $F(3, 16) = 0.9544$; $p = 0.438$), with values ranging from 2.69 ± 2.82 mm (*A. salina*) to 4.54 ± 1.31 mm (*C. filosa* fed with formulated feed). However, the moderate effect size (Cohen's f = 0.42; 95% CI: 0.20 - 1.78) suggests that minor biological trends may exist, even if not statistically significant.

Finally, the Condition Factor varied significantly between groups (ANOVA: $F(3, 16) = 3.8238$; $p = 0.031$; Figure 2F). The highest CF occurred in *P. hawaiensis* group (0.28859 ± 0.00002), the lowest in *A. salina* group (0.22835 ± 0.00004), while intermediate values were observed in *C. filosa*, regardless of diet (algae: 0.27419 ± 0.00003; formulated feed: 0.26095 ± 0.00002). The effect size was large (Cohen's f = 0.85; 95% CI: 0.51 - 1.66), indicating a meaningful biological difference in body condition across treatments.

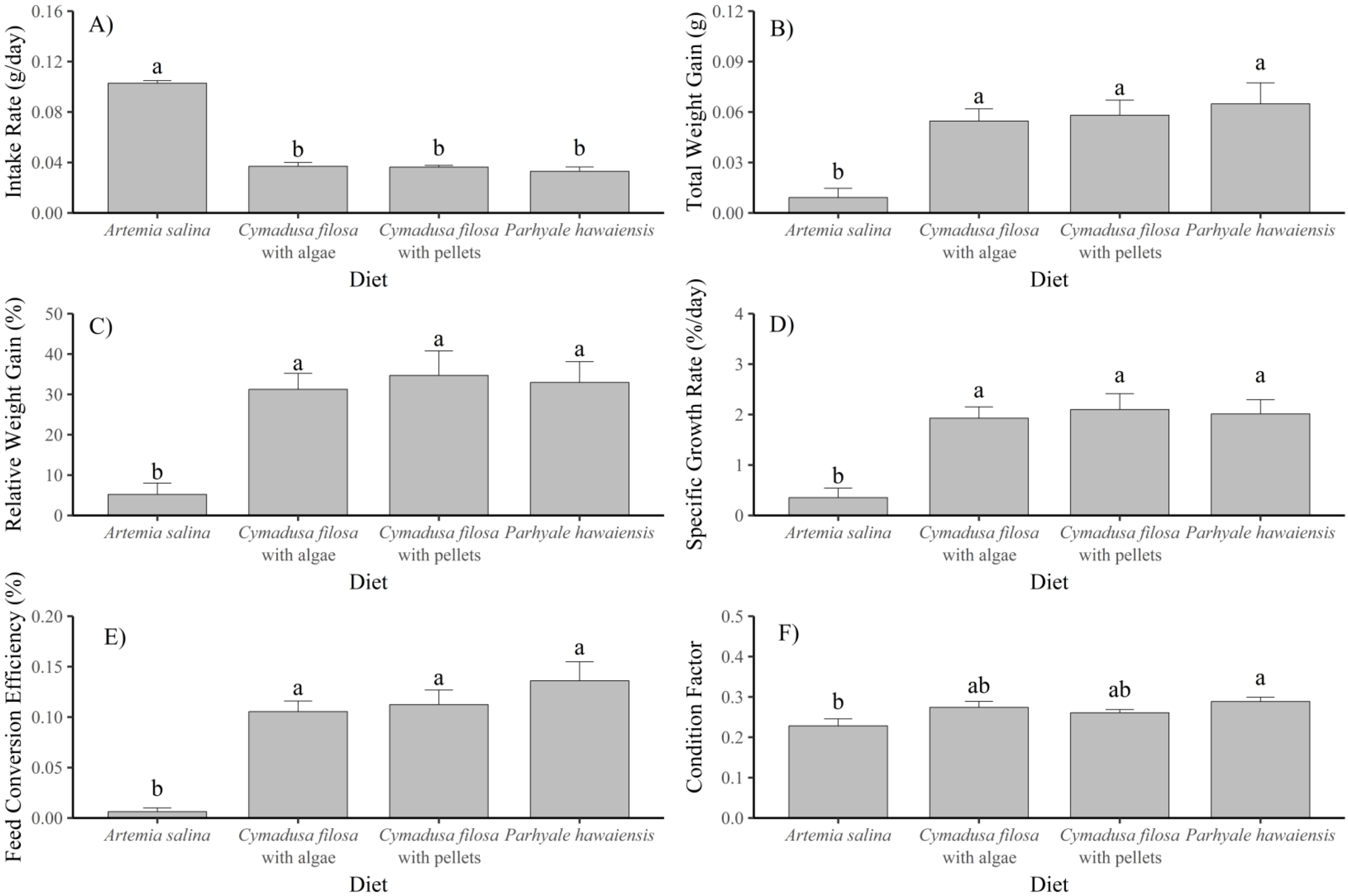

Figure 2. Growth parameters of *Hippocampus reidi* juveniles fed different live prey for a 14-day period: *Artemia salina*, *Cymadusa filosa* reared with algae, *C. filosa* reared with formulated feed, and *Parhyale hawaiensis*. (**A**) Intake Rate (IR); (**B**) Total Weight Gain (ΔWG); (**C**) Relative Weight Gain (WG%); (**D**) Specific Growth Rate (SGR); (**E**) Feed Conversion Efficiency (FCE); (**F**) Condition Factor (CF). Different letters indicate statistically significant differences among treatments (Dunn or Tukey test, $p < 0.05$). Bars represent mean ± standard deviation.

5. Discussion

Amphipods proved a highly promising live feed alternative for *H. reidi*, promoting superior growth performance compared to *A. salina*. Feeding with amphipods resulted in greater weight gain, specific growth rate, and conversion efficiency, even at lower ingestion rates. Furthermore, seahorses exhibited similar performance when fed with either of the amphipod species tested (*P. hawaiensis* and *C. filosa*), regardless of the diet used in their culture (algae or formulated feed). Although the number of replicates per treatment was limited ($n = 5$), the statistical analysis revealed large to very large effect sizes (Cohen's f ranging from 0.85 to 1.89; 95% CI within biologically meaningful ranges), confirming that the observed differences were strong and not artifacts of number of replicas. These results reinforce the superior growth performance of seahorses fed amphipods compared to *Artemia*, emphasizing their considerable potential for integration into more efficient and sustainable feeding strategies in seahorse aquaculture.

Juvenile seahorses fed with amphipods exhibited higher weight gain and growth despite lower ingestion compared to *A. salina*, suggesting a more suitable and highly digestible nutritional composition. This response seems related to their richness in essential fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are known to be crucial for seahorse growth, survival, and health [25,27,34,56]. The higher feed conversion efficiency and condition factor suggests that these nutrients were better assimilated by the seahorses, promoting not only growth but also improved physiological status, as evidenced by the superior body condition [50,51]. The nutritional quality of amphipods is well established in aquaculture, as they provide a balanced diet with high energy utilization [28,57,58]. Such a favorable nutritional profile is particularly advantageous during critical life stages, such as early development or reproduction, when nutritional requirements are elevated and strongly influence growth outcomes [6,12,34].

Juvenile seahorses presented similar performance irrespective of the amphipod diet. This finding has particularly relevant practical implications. The use of commercial feeds represents a more sustainable and cost-effective alternative by reducing reliance on natural algae collection, thereby lowering operational costs and increasing biosecurity in aquaculture systems [36,59–61]. Feeding with formulated diets may also reduce the risk of pathogen introduction, a major concern in aquaculture, while minimizing the environmental impacts associated with harvesting wild algae and amphipods, both of which play essential ecological roles as substrates and prey in coastal ecosystems [29,60]. Moreover, formulated diets can improve the biochemical composition of cultured organisms, potentially enhancing their essential fatty acids and amino acid content compared to wild specimens [27,41]. Contrary to our initial hypothesis, however, although *P. hawaiensis* was cultured exclusively with formulated feed and *C. filosa* with either formulated feed or algae, both species promoted similar growth performance in *H. reidi*, with no evidence of additional benefits linked to formulated-feed-based rearing. Nevertheless, the findings support the feasibility of using commercial formulated feeds in amphipod culture, as they did not compromise seahorse performance compared to naturally derived algae-fed amphipods. In addition to reducing dependency on natural resources, this approach contributes to the development of more efficient, safe, cost-effective, and environmentally sustainable protocols for ornamental aquaculture. This result may be linked to the lower sensitivity of juveniles to variations in fatty acid levels, compared to early life stages, where nutritional demands tend to be more critical [12,62,63].

Although both amphipods tested promoted similar performance in *H. reidi*, the behavioral and practical aspects suggest advantages in using *C. filosa* as live feed. This species is a generalist herbivore with low mobility that builds silk tubes and remains closely associated with substrates such as algae [49,59,64]. These traits facilitate predation by seahorses, which feed through a slow approach and suction strategy [47,65]. Additionally, *C. filosa* stands out for its ease of handling, capacity for high-density culture, and good adaptation to formulated feeds [49]. In contrast, *P. hawaiensis* is highly mobile, does not build tubes, and may exhibit intraspecific predation under high-density conditions, complicating large-scale cultivation [27]. Comparatively, *C. filosa* also shows faster growth than *P. hawaiensis*, a shorter generation time, and higher fecundity than *P. hawaiensis*, traits that favor its large-scale production [27,36,49,66]. These factors strengthen the potential of *C. filosa* as a viable live prey, with applicability not only in seahorse culture but also for other ornamental fish species.

In contrast, *A. salina* was consumed in greater quantities but yielded the poorest growth indicators, with the exception of body length. Although it is widely used in ornamental aquaculture due to its availability and ease of cultivation, its limited nutritional profile, especially in essential fatty acids, is insufficient to meet the metabolic demands of sensitive species or early developmental stages [3,20,34,67]. The higher ingestion of *Artemia* may be explained by the standardized feeding protocol, which ensured greater prey density and biomass. Additionally, being a pelagic species with constant movement, *A. salina* may have visually stimulated the seahorses, encouraging higher ingestion. It is also possible that the animals increased *Artemia* consumption to compensate for its lower nutritional quality. However, this higher consumption did not translate into improved weight gain or feed efficiency, reinforcing the nutritional limitations of *Artemia* as a sole diet. Similar findings have been reported for other seahorse species, even when using fatty acid-enriched *Artemia*, indicating that it’s use involves high costs and limited growth benefits [62,68,69].

Another alternative live feed for seahorse culture, besides *Artemia*, is the mysid species [63]. When tested as prey, these crustaceans showed growth performances equivalent to or even superior to enriched *Artemia* [51]. Long-term experiments also reported better reproductive performance, including larger brood size and higher spawning frequency [70,71]. These advantages are related to their higher nutritional quality, especially the elevated levels of essential fatty acids such as EPA and DHA [14,50,72]. Thus, both amphipods and mysids are considered promising natural prey for seahorses [13,27,70]. Although mysid species were not included in this study, available evidence indicates that both groups provide a suitable size (0.3–2.5 cm), good digestibility, and balanced nutritional composition, with satisfactory lipid and protein contents [17]. However, a practical difference lies in cultivation: while mysid culture still faces challenges such as high costs and dependence on *Artemia* nauplii, amphipods have shown greater feasibility, with the possibility of controlled supplementation through formulated diets, representing a promising path for large-scale production [17,25].

The results obtained reinforce the potential of amphipods as high-quality live feed for the cultivation of *H. reidi*. Regardless of the species or the diet used in amphipod culture, seahorses exhibited better growth performance compared to *A. salina*, highlighting their nutritional adequacy. Among the tested species, *C. filosa* reared on formulated feed stands out by combining nutritional, operational, and environmental advantages, making it more suitable for intensive culture. Incorporating live prey that aligns with the species' natural diet promotes not only growth and health but also more sustainable practices in ornamental aquaculture. Importantly, the inclusion of effect size estimates (Cohen's f) and bootstrap confidence intervals provides a robust statistical foundation, demonstrating that the detected differences are biologically meaningful and not compromised by the limited replication ($n = 5$). Nevertheless, further studies are required to characterize the detailed nutritional profile of amphipods and to validate their consistent use as an alternative to *Artemia*. Long-term experiments, including trials with adult seahorses, are also essential to deepen understanding of the growth effects of amphipod-based diets. These findings contribute to the development of more efficient feeding protocols that align economic viability with species conservation.

6. Conclusions

This study demonstrates that the amphipods *Parhyale hawaiensis* and *Cymadusa filosa* represent promising live feed alternatives for *Hippocampus reidi*, yielding superior growth, feed conversion efficiency, and body condition compared to *Artemia salina*, regardless of amphipod species or culture diet (algae or formulated feed). These findings confirm the high nutritional value of amphipods and their potential to support more efficient, sustainable, and economically viable feeding protocols for seahorse aquaculture. Among the species evaluated, *C. filosa* stands out due to its favorable behavioral traits, ease of culture at high densities, and compatibility with formulated feed, which reduces dependence on natural resources, enhances biosecurity, and lowers production costs. Incorporating nutritionally adequate live prey that aligns with the species' natural diet not only improves seahorse performance but also contributes to environmentally responsible practices and the conservation of wild populations. Furthermore, future studies should include comprehensive cost–benefit analyses of formulated diet-based amphipod culture systems, considering nutritional outcomes, production costs, biosecurity, and ecological sustainability, in order to validate and consolidate their use as a sustainable alternative to *Artemia* in seahorse aquaculture.

6. References

1. FAO. The State of World Fisheries and Aquaculture 2022. *Towards Blue Transformation*; FAO: Rome, **2022**;

2. Olsen, R.L.; Hasan, M.R. A Limited Supply of Fishmeal: Impact on Future Increases in Global Aquaculture Production. *Trends Food Sci. Technol*. **2012**, 27, 120–128, doi:10.1016/j.tifs.2012.07.001.

3. Conceição, L.E.C.; Yúfera, M.; Makridis, P.; Morais, S.; Dinis, M.T. Live Feeds for Early Stages of Fish Rearing. *Aquac. Res*. **2010**, 41, 613–640, doi:10.1111/j.1365-2109.2009.02242.x.

4. Division, P.L. Manual on the Production and Use of Live Food for Aquaculture. **1996**.

5. Tlusty, M. The Benefits and Risks of Aquacultural Production for the Aquarium Trade. *Aquaculture.* **2002**, 205, 203–219, doi:10.1016/S0044-8486(01)00683-4.

6. Planas, M.; Olivotto, I. Sustainable Aquaculture: Nutrition Studies in Early Developing Finfish, Ornamentals and Experimental Model Fish. *Animals.* **2022**, 12, 1384, doi:10.3390/ani12111384.

7. Foster, S.J.; Justason, T.; Magera, A.M.; Vincent, A.C.J. CITES Makes a Measurable Difference to the Trade in Live Marine Fishes: The Pioneering Case of Seahorses. *Biol. Conserv*. **2022**, 272, 109653, doi:10.1016/j.biocon.2022.109653.

8. Foster, S.J.; Vincent, A.C.J.J. Life History and Ecology of Seahorses: Implications for Conservation and Management. *J. Fish Biol*. **2004**, 65, 1–61, doi:10.1111/j.1095-8649.2004.00424.x.

9. Carvalho, L.M.; Mies, M.; Inagaki, K.Y.; Sanches, E.G.; Souza, M.R.; Longo, G.O.; Tomás, A.R.G. The Marine Ornamental Market in Brazil (Southwestern Atlantic) Frequently Trades Prohibited and Endangered Species, and Threatens the Ecosystem Role of Cleaning Mutualism. *Mar. Policy*. 2022, 146, 105305, doi:10.1016/j.marpol.2022.105305.

10. Cohen, F.P.A.; Valenti, W.C.; Planas, M.; Calado, R. Seahorse Aquaculture, Biology and Conservation: Knowledge Gaps and Research Opportunities. *Rev. Fish. Sci. Aquac*. **2017**, 25, 100–111, doi:10.1080/10426914.2016.1245009.

11. Willadino, L.; Souza-Santos, L.P.; Mélo, R.C.S.; Brito, A.P.; Barros, N.C.S.; Araújo-Castro, C.M.V.; Galvão, D.B.; Gouveia, A.; Regis, C.G.; Cavalli, R.O. Ingestion Rate, Survival and Growth of Newly Released Seahorse *Hippocampus reidi* Fed Exclusively on Cultured Live Food Items. *Aquaculture*. **2012**, 360–361, 10–16, doi:10.1016/j.aquaculture.2012.06.025.

12. Schubert, P.; Vogt, L.; Eder, K.; Hauffe, T.; Wilke, T. Effects of Feed Species and HUFA Composition on Survival and Growth of the Longsnout Seahorse (*Hippocampus reidi*). *Front. Mar. Sci*. **2016**, 3, 53, doi:10.3389/fmars.2016.00053.

13. Manning, C.G.; Foster, S.J.; Vincent, A.C.J.J. A Review of the Diets and Feeding Behaviours of a Family of Biologically Diverse Marine Fishes (Family Syngnathidae). *Rev. Fish Biol. Fish*. **2019**, 29, 197–221, doi:10.1007/s11160-019-09549-z.

14. Izquierdo, M.S.; Fernández-Palacios, H.; Tacon, A.G.J. Effect of Broodstock Nutrition on Reproductive Performance of Fish. *Aquaculture*. **2001**, 197, 25–42, doi:10.1016/S0044-8486(01)00581-6.

15. Segade, Á.; Robaina, L.; Novelli, B.; Otero-Ferrer, F.; Molina Domínguez, L. Effect of the diet on lipid composition and liver histology of short snout seahorse *Hippocampus hippocampus*. *Aquac. Nutr*. **2016**, 22, 6, 1312-1319, doi: 10.1111/j.1365-2109.2010.02505.x

16. Otero-Ferrer, F.; Molina, L.; Socorro, J.; Herrera, R.; Fernández-Palacios, H.; Soledad Izquierdo, M. Live Prey First Feeding Regimes for Short-Snouted Seahorse *Hippocampus hippocampus* (Linnaeus, 1758) Juveniles. *Aquac. Res*. **2010**, 41, e8–e19, doi:10.1111/j.1365-2109.2010.02505.x.

17. Suhaimi, H.; Abdul Rahman, M.I.; Ashaari, A.; Ikhwanuddin, M.; Wan Rasdi, N. Adaptation and Potential Culture of Wild Amphipods and Mysids as Potential Live Feed in Aquaculture: A Review. *PeerJ*. **2024**, 12, e17092, doi:10.7717/peerj.17092.

18. Sorgeloos, P.; Coutteau, P.; Dhert, P.; Merchie, G.; Lavens, P. Use of Brine Shrimp, *Artemia* spp., in Larval Crustacean Nutrition: A Review. *Rev. Fish. Sci*. **1998**, 6, 55–68, doi:10.1080/08920759809362306.

19. Sales, J.; Janssens, G.P.J.J. Nutrient Requirements of Ornamental Fish. *Aquat. Living Resour*. **2003**, 16, 533–540, doi:10.1051/alr:2003061.

20. Sorgeloos, P.; Dhert, P.; Candreva, P. Use of the Brine Shrimp, *Artemia* spp., in Marine Fish Larviculture. *Aquaculture*. **2001**, 200, 147–159, doi:10.1016/S0044-8486(01)00656-6.

21. Xiao, W.; Song, C.; Luo, Y.; Jiang, X.; Han, Q.; Jiang, M. Nutritional requirements and reproductive performance of *Neomysis orientalis*: Evaluating the effects of different diets. *Aquac. Rep*. **2024**, 36, 102129, doi: 10.1016/j.aqrep.2024.102129.

22. Domingues, P. M.; Turk, P. E.; Andrade, J. P.; Lee, P. G. Effects of enriched *Artemia* nauplii on production, survival and growth of the mysid shrimp *Mysidopsis almyra* Bowman 1964 (Crustacea: Mysidacea). *Aquac. Res*. **2021**, 32, 599-603, doi: 10.1046/j.1365-2109.2001.00608.x

23. Herrera, A.; Gómez, M.; Molina, L.; Otero, F.; Packard, T. Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain). *Aquac. Res*. **2011**, 45, 677-683, doi: 10.1111/j.1365-2109.2010.02786.x

24. Kitsos, M.S.; Tzomos, T.; Anagnostopoulou, L.; Koukouras, A. Diet Composition of the Seahorses, *Hippocampus guttulatus* Cuvier, 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. *J. Fish Biol*. **2008**, 72, 1259–1267, doi:10.1111/J.1095-8649.2007.01789.X.

25. Woods, C.M.C. Caprellid Amphipods: An Overlooked Marine Finfish Aquaculture Resource? *Aquaculture*. **2009**, 289, 199–211, doi:10.1016/j.aquaculture.2009.01.018.

26. Harlıoğlu, M.M.; Farhadi, A. Importance of Gammarus in Aquaculture. *Aquac. Int*. **2018**, 26, 1327–1338, doi:10.1007/s10499-018-0322-z.

27. Vargas-Abúndez, J.A.; Martínez-Moreno, G.L.; Simões, N.; Noreña-Barroso, E.; Mascaró, M. Marine Amphipods (*Parhyale hawaiensis*) as an Alternative Feed for the Lined Seahorse (*Hippocampus erectus*, Perri 1810): Nutritional Value and Feeding Trial. *PeerJ.* **2021**, 9, e12288, doi:10.7717/peerj.12288.

28. Baeza-Rojano, E.; Guerra-García, J.M.; Hachero-Cruzado, I.I.; wa Kangeri, A.K.; Jansen, J.M.; Barkman, B.R.; Donker, J.J.A.; Joppe, D.J.; Dankers, N.M.J.A.; Calero-Cano, S.; et al. A Preliminary Study of the *Caprella scaura* Amphipod Culture for Potential Use in Aquaculture. *J. Sea Res*. **2013**, 83, 146–151, doi:10.1016/j.seares.2013.06.001.

29. Duffy, J.E.; Hay, M.E. Strong Impacts of Grazing Amphipods on the Organization of a Benthic Community. *Ecol. Monogr*. **2000**, 70, 237–263, doi:10.1890/0012-9615(2000)070[0237:SIOGAO]2.0.CO;2.

30. Ritter, C.J.; Bourne, D.G. Marine Amphipods as Integral Members of Global Ocean Ecosystems. *J. Exp. Mar. Bio. Ecol*. **2024**, 572, 151985, doi:10.1016/j.jembe.2024.151985.

31. Baeza-Rojano, E.; others Life History of *Caprella grandimana* (Crustacea: Amphipoda) Reared under Laboratory Conditions. *Mar. Biol. Res*. **2011**, 7, 85–92, doi:10.1080/17451000.2010.536712.

32. Abo-Taleb, H.; others; A. Abo-Taleb, H.; F. Zeina, A.; Ashour, M.; M. Mabrouk, M.; E. Sallam, A.; M. M. El-feky, M. Isolation and Cultivation of the Freshwater Amphipod *Gammarus pulex* (Linnaeus, 1758), with an Evaluation of Its Chemical and Nutritional Content. *Egypt. J. Aquat. Biol. Fish*. **2020**, 24, 69–82, doi:10.21608/ejabf.2020.78232.

33. Shahin, S.; Okomoda, V.T.; Ishak, S.D.; Waiho, K.; Fazhan, H.; Azra, M.N.; Rahim, A.A.; Wongkamhaeng, K.; Abualreesh, M.H.; Rasdi, N.W.; et al. Life History Traits of the Marine Amphipod *Cymadusa vadosa* under Laboratory Conditions: Insights on Productivity and Aquaculture Potential. *Aquat. Sci*. **2023**, 85, 103, doi:10.1007/s00027-023-00984-y.

34. Faleiro, F.; Narciso, L. Lipid Dynamics during Early Development of *Hippocampus guttulatus* Seahorses: Searching for Clues on Fatty Acid Requirements. *Aquaculture*. **2010**, 307, 56–64, doi:10.1016/j.aquaculture.2010.07.005.

35. Rufchaei, R.; Khara, H.; R., Z.; K., A.; Zhang, H.-W.; Huang, Y.; Man, X.; Wang, Y.; Hui, K.-M.; Yin, S.-W.; et al. Dietary Administration of *Pontogammarus maeoticus* Extract Affects Immune Responses, Stress Resistance, Feed Intake and Growth Performance of Caspian Roach

(*Rutilus caspicus*) Fingerlings. *Fish Shellfish Immunol*. **2017**, 63, 196–200, doi:10.1016/j.fsi.2017.02.015.

36. Vargas-Abúndez, J.A.; López-Vázquez, H.I.; Mascaró, M.; Martínez-Moreno, G.L.; Simões, N. Marine Amphipods as a New Live Prey for Ornamental Aquaculture: Exploring the Potential of *Parhyale hawaiensis* and *Elasmopus pectenicrus*. *PeerJ.* **2021**, 9, e10840, doi:10.7717/peerj.10840.

37. Laramore, S.; Albright, E. Influence of Temperature on Production of the Amphipod *Parhyale hawaiensis*. *BMC Zool*. **2025**, 10, 6, doi:10.1186/s40850-025-00078-x.

38. Dubiaski-Silva, J.; Masunari, S. Natural Diet of Fish and Crabs Associated with the Phytal Community of *Sargassum cymosum* C. Agardh, 1820 (Phaeophyta, Fucales) at Ponta Das Garoupas, Bombinhas, Santa Catarina State, Brazil. *J. Nat. Hist*. **2008**, 42, 1907–1922, doi:10.1080/00222930802102179.

39. Alegretti, L.; Umbuzeiro, G. de A.; Flynn, M.N. Population Dynamics of *Parhyale hawaiensis* (Amphipoda: Hyalidae) Associated with an Intertidal Algal Belt in Southeastern Brazil. *J. Crustac. Biol*. **2016**, 36, 785–791, doi:10.1093/jcbiol/ruw026.

40. Machado, G.B. de O.; Siqueira, S.G.L.; Leite, F.P.P. Abundance, Performance, and Feeding Preference of Herbivorous Amphipods Associated with a Host Alga-Epiphyte System. *J. Exp. Mar. Bio. Ecol*. **2017**, 486, 328–335, doi:10.1016/j.jembe.2016.12.013.

41. Zaid, M.M.A.; Razek, F.A.A.; Hamed, E.A.E.; Mohamed, T.A.; Sea, R.; Ahmed, H.O. Biochemical Composition and Fatty Acid, Amino Acid Analysis of *Cymadusa filosa* (Amphipod; Gammarids) in the Red Sea –Egypt. *Int. J. Innov. Stud. Aquat. Biol. Fish*. **2016**, 2, doi:10.20431/2455-7670.0201002.

42. Olivotto, I.; Planas, M.; Simões, N.; Holt, G.J.; Avella, M.A.; Calado, R. Advances in Breeding and Rearing Marine Ornamentals. *J. World Aquac. Soc*. **2011**, 42, 135–166, doi:10.1111/j.1749-7345.2011.00453.x.

43. Palma, J.; Bureau, D.P.; Andrade, J.P. Effect of Different Artemia Enrichments and Feeding Protocol for Rearing Juvenile Long Snout Seahorse, *Hippocampus guttulatus*. *Aquaculture*. **2011**, 318, 439–443, doi:10.1016/j.aquaculture.2011.05.035.

44. Koldewey, H.J.; Martin-Smith, K.M. A Global Review of Seahorse Aquaculture. *Aquaculture*. **2010**, 302, 131–152, doi:10.1016/j.aquaculture.2009.11.010.

45. Da Hora, M.D.S.C.; Joyeux, J.-C.C.; Hora, M. dos S.C. da; Joyeux, J.-C.C. Closing the Reproductive Cycle: Growth of the Seahorse *Hippocampus reidi* (Teleostei, Syngnathidae) from Birth to Adulthood under Experimental Conditions. *Aquaculture*. **2009**, 292, 37–41, doi:10.1016/j.aquaculture.2009.04.004.

46. Tseng, C.C.; Chien, J.H.; Chu, T.W.; Cheng, A.C.; Shiu, Y.L.; Han, T.W.; Liu, C.H. Effects of Food Type, Temperature and Salinity on the Growth Performance and Antioxidant Status of the Longsnout Seahorse, *Hippocampus reidi*. *Aquac. Res*. **2020**, 51, 4793–4804, doi:10.1111/are.14826.

47. Blanco, A.; Planas, M.; Felício, A.K.C.; Rosa, I.L.; Souto, A.; Freitas, R.H.A.A. Feeding Behavior of the Longsnout Seahorse *Hippocampus reidi* Ginsburg, 1933. *J. Ethol*. **2006**, 24, 219–225, doi:10.1007/s10164-005-0189-8.

48. Storero, L.P.; González, R.A.; VanderLugt, K.; Cooney, M.J.; Lechner, A.; Lenz, P.H. Cultivation of the Paracalanid Copepod, *Bestiolina similis* (Calanoida: Crustacea). *J. World Aquac. Soc*. **2009**, 40, 394–401, doi:10.1111/j.1749-7345.2009.00282.x.

49. Machado, G.B.O.; Ferreira, A.P.; Leite, F.P.P.P. Testing the Importance of Predation Refuge vs. Food Quality in Determining the Use of Macroalgal Hosts by a Generalist Marine Mesograzer. *Mar. Biol*. **2019**, 166, 55, doi:10.1007/s00227-019-3502-8.

50. Palma, J.; Stockdale, J.; Correia, M.; Andrade, J.P.P. Growth and Survival of Adult Long Snout Seahorse (*Hippocampus guttulatus*) Using Frozen Diets. *Aquaculture*. **2008**, 278, 55–59, doi:10.1016/j.aquaculture.2008.03.019.

51. Woods, C.M.C. Growth of Cultured Seahorses (*Hippocampus abdominalis*) in Relation to Feed Ration. *Aquac. Int*. **2005**, 13, 305–314, doi:10.1007/s10499-004-3100-7.

52. Ben-Shachar, M. S.; Lüdecke, D.; Makowski, D. Effectsize: Estimation of effect size indices and standardized parameters. *J. open source softw*. **2020**, 5(56), 2815. doi: 10.21105/joss.02815.

53. Cohen, J. Statistical Power Analysis for the Behavioral Sciences (2nd ed.). Lawrence Erlbaum Associates. **1988**.

54. Efron, B.; Tibshirani, R. J. An Introduction to the Bootstrap. Chapman & Hall/CRC. **1993**.

55. R Core Team R: A Language and Environment for Statistical Computing **2023**.

56. Murugan, A.; Dhanya, S.; Sreepada, R.A.; Rajagopal, S.; Balasubramanian, T. Breeding and Mass-Scale Rearing of Three Spotted Seahorse, *Hippocampus trimaculatus* Leach under Captive Conditions. *Aquaculture*. **2009**, 290, 87–96, doi:10.1016/j.aquaculture.2009.01.033.

57. Baeza-Rojano, E.; García, S.; Garrido, D.; Guerra-García, J.M.; Domingues, P. Use of Amphipods as Alternative Prey to Culture Cuttlefish (*Sepia officinalis*) Hatchlings. *Aquaculture*. **2010**, 300, 243–246, doi:10.1016/j.aquaculture.2009.12.029.

58. Vargas-Abúndez, J.A.; Simões, N.; Mascaró, M. Feeding the Lined Seahorse *Hippocampus erectus* with Frozen Amphipods. *Aquaculture*. **2018**, 491, 82–85, doi:10.1016/j.aquaculture.2018.02.043.

59. Machado, G.B.O.; Ferreira, A.P.; Bueno, M.; Siqueira, S.G.L.; Leite, F.P.P. Effects of Macroalgal Host Identity and Predation on an Amphipod Assemblage from a Subtropical Rocky Shore. *Hydrobiologia.* **2019**, 836, 65–81, doi:10.1007/s10750-019-3941-8.

60. Tacon, A.G.J. Biosecure Shrimp Feeds and Feeding Practices: Guidelines for Future Development. *J. World Aquac. Soc*. **2017**, 48, 381–392, doi:10.1111/jwas.12406.

61. Aly, S.M.; Fathi, M. Advancing Aquaculture Biosecurity: A Scientometric Analysis and Future Outlook for Disease Prevention and Environmental Sustainability. *Aquac. Int*. **2024**, 1–27, doi:10.1007/s10499-024-01235-6.

62. Randazzo, B.; Rolla, L.; Ofelio, C.; Planas, M.; Gioacchini, G.; Vargas, A.; Giorgini, E.; Olivotto, I. The Influence of Diet on the Early Development of Two Seahorse Species (*H. guttulatus* and *H. reidi*): Traditional and Innovative Approaches. *Aquaculture*. **2018**, 490, 75–90, doi:10.1016/j.aquaculture.2018.02.029.

63. Woods, C.M.C.C.; Valentino, F. Frozen Mysids as an Alternative to Live Artemia in Culturing Seahorses *Hippocampus abdominalis*. *Aquac. Res*. **2003**, 34, 757–763, doi:10.1046/j.1365-2109.2003.00882.x.

64. Cruz-Rivera, E.; Hay, M.E. Can Quantity Replace Quality? Food Choice, Compensatory Feeding, and Fitness of Marine Mesograzers. *Ecology*. **2000**, 81, 201–219, doi:10.1890/0012-9658(2000)081[0201:CQRCFC]2.0.CO;2.

65. Gemmell, B.J.; Sheng, J.; Buskey, E.J. Morphology of Seahorse Head Hydrodynamically Aids in Capture of Evasive Prey. *Nat. Commun*. **2013**, 4, 2840, doi:10.1038/ncomms3840.

66. Siqueira, S.G.L.; Jacobucci, G.B.; Leite, F.P.P. Population Biology of *Cymadusa filosa* (Crustacea: Amphipoda) Associated with *Sargassum furcatum* (Phaeophyceae) Beds in a Coastal Area of Brazil with Petrogenic Hydrocarbon Pollution. *Iheringia. Série Zool*. **2024**, 114, e273063, doi:10.1590/1678-4766e2024103.

67. Chen, J.Y.; Zeng, C.; Jerry, D.R.; Cobcroft, J.M. Recent Advances of Marine Ornamental Fish Larviculture: Broodstock Reproduction, Live Prey and Feeding Regimes, and Comparison between Demersal and Pelagic Spawners. *Rev. Aquac*. **2020**, 12, 1518–1541, doi:10.1111/raq.12394.

68. Payne, M.F.; Rippingale, R.J. Rearing West Australian Seahorse, *Hippocampus subelongatus*, Juveniles on Copepod Nauplii and Enriched Artemia. *Aquaculture*. **2000**, 188, 353–361, doi:10.1016/S0044-8486(00)00346-6.

69. Blanco, A.; Planas, M. Mouth Growth and Prey Selection in Juveniles of the European Long--snouted Seahorse, *Hippocampus guttulatus*. *J. World Aquac. Soc*. **2015**, 46, 596–607, doi:10.1111/jwas.12240.

70. Otero-Ferrer, F.; Molina, L.; Socorro, J.; Fernández-Palacios, H.; Izquierdo, M.; Herrera, R. Effect of Different Live Prey on Spawning Quality of Short-Snouted Seahorse, *Hippocampus hippocampus* (Linnaeus, 1758). *J. World Aquac. Soc*. **2012**, 43, 174–186, doi:10.1111/J.1749-7345.2012.00550.X.

71. Buen-Ursua, S.M.A.; Azuma, T.; Arai, K.; Coloso, R.M. Improved Reproductive Performance of Tiger Tail Seahorse, *Hippocampus comes*, by Mysid Shrimp Fed Singly or in Combination with Other Natural Food. *Aquac. Int*. **2015**, 23, 29–43, doi:10.1007/S10499-014-9795-1/METRICS.

72. Izquierdo, M. Essential Fatty Acid Requirements in Mediterranean Fish Species. *Cah. Options Mediterranéennes*. **2005**, 63, 91–102.

PARECER FINAL DO TRABALHO DE CONCLUSÃO DE CURSO

Discente: EDUARDO KENYU OKIDO MATSUMOTO

Título: *"Amphipods as Effective and Viable Live Feed for Juvenile Seahorses"*

Orientador: Profa. Dra. Tânia Marcia Costa

Curso/Habilitação: Bacharelado em Ciências Biológicas/Gerenciamento Costeiro

COMISSÃO EXAMINADORA	CONCEITO
Profa. Dra. Tânia Marcia Costa	APROVADO
Dra. Ana Paula Ferreira	APROVADO

PARECER:

O discenta apresentou um dominio do tema durante a apresentação do trabalho e na escrita do texto. O Trabalho de conclusão de curso apresenta um desenho experimental adequado, análises robustas, conclusões pertinentes e que reportam aos objetivos do trabalho. O texto é bastante claro, completo e com linguagem cientifica adequada. Portanto, considero o trabalho como aprovado.

CONCEITO FINAL:

A Comissão Examinadora abaixo assinada conclui que o discente **Eduardo Kenyu Okido Matsumoto** obteve o seguinte conceito:

[X] APROVADO   [ ] REPROVADO

São Vicente, 01 de dezembro de 2025.

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