E-ISSN 2477-6939 P-ISSN 0216-0749
AQUACULTURA INDONESIANA
An International Journal of Indonesian Aquaculture Society

Aquacultura Indonesiana, 26(1), 2025

On-line: 20https://aquacultura.id/index.php/ai/article/view/203

Enhancing Early Larval Performance of Penaeus monodon Using Red Microalga Porphyridium cruentum in a Combined Diet Strategy

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Submitted: June 15th, 2025; Revised: June 30th, 2025;
Accepted: July 15th, 2025; Available Online: July 29th, 2025

DOI: 10.21534/ai.v26i1.203

Abstract

The potential of the red microalga Porphyridium cruentum as a functional complementary live feed for black tiger shrimp (Penaeus monodon) larvae was evaluated through a series of controlled laboratory experiments. Larval ingestion rates were quantified at the zoea-3 and mysis-2 stages using graded cell densities of P. cruentum (2,500–7,500 cells mL^{-1}), while larval performance from nauplius to postlarva-1 (PL-1) was assessed under mono- and combined-feeding regimes. Additional trials examined the synergistic effects of P. cruentum when co-fed with selected diatom microalgae throughout larval development. Zoea-3 larvae exhibited significantly higher ingestion rates than mysis-2 larvae, confirming stage-specific feeding responses. Larvae fed P. cruentum alone at 15,000–30,000 cells mL^{-1} failed to reach the PL-1 stage, whereas combined feeding with artificial feed or diatoms markedly enhanced larval stage index and survival ($P < 0.05$). The highest survival (72.71 ± 3.82%) was achieved when P. cruentum was combined with Thalassiosira sp., exceeding that of conventional diatom combinations. These findings demonstrate that P. cruentum is not suitable as a sole live feed for P. monodon larvae but functions effectively as a complementary feed component, particularly in combination with diatoms. The study highlights the practical potential of integrating functional red microalgae into shrimp hatchery feeding strategies to improve larval performance and survival.

Keywords: Black Tiger Shrimp; Functional Microalgae; Larval Development; Porphyridium Cruentum; Survival

1. Introduction

Black tiger shrimp (Penaeus monodon) remains one of the most economically valuable aquaculture species in the Indo-Pacific region, including Indonesia. Despite its high market value, national production of P. monodon has stagnated over the past two decades, reaching only approximately 208,000 tons in 2020, compared with more than 700,000 tons of whiteleg shrimp (Litopenaeus vannamei). This stagnation has been largely attributed to limitations in hatchery performance, particularly the availability of high-quality seed and persistent disease problems.

Larval nutrition is a fundamental determinant of survival, growth, and robustness during early shrimp development. During the zoeal and mysis stages, shrimp larvae rely heavily on live feeds such as microalgae, which provide essential nutrients and contribute to digestive development, immune modulation, and stress resistance. Conventional hatchery systems commonly employ diatoms such as Skeletonema, Chaetoceros, Thalassiosira, Isochrysis, and Tetraselmis owing to their favorable size, digestibility, and nutritional profiles. However, increasing disease pressure and the need to reduce antibiotic dependence have driven interest in functional microalgae that provide not only basic nutrition but also bioactive compounds with antioxidant, immunostimulatory, and antimicrobial properties.

Porphyridium cruentum is a unicellular red microalga characterized by its small cell size, lack of a rigid cell wall, and high content of proteins, polyunsaturated fatty acids, phycobiliproteins, and sulfated polysaccharides. These attributes suggest that P. cruentum may serve as a functional complementary feed capable of enhancing larval physiological performance and resilience. Nevertheless, its application in P. monodon hatchery systems remains poorly documented, particularly with respect to ingestion dynamics, developmental outcomes, and interactions with conventional diatom feeds.

The novelty of the present study lies in the comprehensive evaluation of P. cruentum as a functional complementary live feed for P. monodon larvae, rather than as a sole dietary component. Specifically, this study aimed to (1) quantify larval ingestion of P. cruentum at different developmental stages, (2) evaluate its effects on larval development and survival when provided alone or in combination

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with artificial feed, and (3) assess its synergistic potential when combined with selected diatom microalgae commonly used in shrimp hatcheries. The results provide new insights into the role of red microalgae as complementary nutritional and functional components in shrimp larviculture.

2. Materials and Methods

2.1. Preparation of animal test

Nauplii of P. monodon were obtained from wild broodstock collected in Cilacap, and subsequently reared on shrimp hatchery facilities at the Brackishwater Aquaculture Development Center (BADC), Jepara, Central Java, Indonesia. The nauplii were stocked at density of 100 individuals per liter in indoor 10 m3 concrete tanks filled with pre-treated seawater at salinity of 30 ppt. During the zoea and mysis stage larvae were fed Skeletonema costatum at approximately 5x104 cells/mL, administered twice daily. In addition, microencapsulated artificial feed (FRiPPAK #1 CAR and #2 CD, INVE Aquaculture) were provided six time a day. Larvae reared under this condition were subsequently used for ingestion rate experiments during the zoea and mysis stages.

2.2. Microalgal Culture

Microalga were cultured in sterilized seawater at a salinity of 28-30 ppt using 1-L Erlenmeyer flask under laboratory conditions. P. cruentum was grown in seawater enriched with KW21 fertilizer (Daiichi Seimo Co., Ltd., Kumamoto, Japan) at a concentration of 1 mL/L. The diatom species (Chaetoceros calcitrans, Skeletonema costatum and Thalassiosira sp.) were cultured using the same fertilizer, sumplemented with sodium silicate to support frustule formation. All cultures were maintained at temperature below 20°C under continuous illumination (3,000–3,500 lux), and gently aerated with filtered air to ensure proper mixing and gas exchange. Cell densities were routinely monitored using a hemocytometer to assess growth dynamics throughout the culture period.

2.3. Experimental Design

2.3.1. Experiment-1: Larvae Ingestion on P. cruentum

The larval ingestion rate on P. cruentum were assessed during zoea-3 and mysis-2 stages, respectively. The larvae were raised in 1-liter beaker glasses (salinity: 30 ppt; temperature: 29-30°C) housed in styrofoam containers, each with a single aeration unit. Each beaker held 50 larvae at the zoea-3 stage and 50 at the mysis-2 stage. P. cruentum was offered to the larvae at three level cell densities, namely: 2,500, 5,000, and 7,500 cells/mL, and further denoted as P-2500, P-5000, and P-7500 treatments, respectively.

Larval ingestion (zoea-3 and mysis-2) on P. cruentum was observed at 1 hour, 3 hours, and 6 hours intervals. Briefly, 1 mL of larval rearing water sample was taken by micro pipette and further add 1–2 drops of formalin before observing and counting their cell density. The larval consumption rate (LCR, cells/larvae/h) on P. cruentum was calculated using the following formula (Millán-Almaraz et al., 2021):

$$LCR = \frac{(C0 - C1)}{n\ x\ t} xV$$

where: C0 and C1 denote initial and final concentration of microalgae, respectively; V = volume (mL); n = number of larvae, and t = time (hours).

2.3.2. Experiment-2 : Larvae fed P. cruentum and artificial feed

Shrimp nauplii were stocekd at the density of 100 individuals per liter and reared in 30-liter containers. The culture was conducted at ambient temperature (27,2–28,6°C), with pH, dissolved oxygen, and salinity were within the ranges of 7.1–7.4 and 5.8–6.2 mg/L, 30-31 ppt respectively. At the zoea stage onwards, larvae were fed Porphyridium cruentum (PC) at three different concentrations: 15.000 cells/mL; 30.000 cells/mL and 45.000 cells/mL, either with and without artificial feed (AF, FRiPPAK, INVE Aquaculture). There were six treatments along with one control treatment, as follow: A (PC-15,000); B (PC-30,000); C (PC-45,000); D (PC-15,000 + AF) ; E (PC-30,000 + AF) ; F (PC-45,000 + AF) and K (Artificial feed only, as control). All treatments were conducted in triplicates, and the experiment continued until the larvae metamorphosed into the Pl-1 stage.

2.3.3. Experiement-3 : Larvae fed mixed microalgae

The rearing protocols followed the same procedures as in Experiment-2, with the exception that larvae were exclusively fed a combination of P. cruentum and selected diatom species. The microalgal density was maintained at 45,000 cell/mL throughout the culture period, using a 1 : 1 rasio between the two algal species in each treatment. No water exchange was conducted during the rearing period. The culture temperature followed ambient conditions, ranging from 27.4 to 28.5oC. Dissolve oxygen and pH levels were recorded within the ranges of 5.7-6.8 ppm and 7.9-8.2, respectively. The experimental treatments consisted of the following combinations: A (P. cruentum + S. costatum), B (P. cruentum + C. calcitrans), C (P. cruentum + Thalassiosira sp.), and D (C. calcitrans +Thalassiosira sp.). Each treatment was conducted in triplicate and the experiment lasted upon larval reaching Pl-1 stage.

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2.4. Larvae stage index

The Larvae Stage Index (LSI) was evaluated at the zoea-3, mysis-2 and postlarva-1 (Pl-1) stages. For this purpose, three 100-mL water samples were randomly taken from each rearing container to determine the number of larvae and their respected developmental stages. The LSI was then calculated using to the following formula, as described by Nguyen et al., (2021):

$$LSI = \frac{(N1\ x\ n1) + (N2\ x\ n2) + (Ni\ x\ ni)}{n1 + ni}$$

where, N represents the numerical value assigned to each larval stage: Zoea = 2, Mysis = 3, and PL-1 =4, while n refers to the number of larvae observed at respective stage.

2.5. Survival Rate

The experiment was carried out until the postlarva-1 (Pl-1) stage. At the end of the trial, the larvae were harvested using a fine mesh screen and subsequently counted individually to determine the survival rate (SR), which was calculated using the following formula:

$$SR\ (\%) = \frac{Number\ of\ PL-1\ harvested}{Number\ of\ nauplii\ stocked}\ x\ 100$$

2.6. Statistical Analysis

Survival and LSI data were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS software version 22. When significant difference were detected ($P<0.05$), Duncan's multiple range test was applid as a post hoc analysis to determine pairwise differences among treatment means at a confidence level of $\alpha = 0.05$.

3. Results and Discussion

3.1. Ingestion of P. cruentum by Shrimp Larvae

Microscopic observations confirmed that P. cruentum cells were ingested by P. monodon larvae, with visible red cells present in the digestive tract of zoea-stage larvae and red-colored fecal matter indicating digestion (Figure 1). The distinctive red pigmentation of P. cruentum, attributable to its high phycoerythrin content (Liberti et al., 2023), provides a clear visual marker for tracing ingestion and gut passage. The accumulation of intact cells in the foregut and partially digested material in the midgut suggested active mechanical and enzymatic breakdown within the digestive system. Furthermore, the presence of red-colored fecal strings provided strong evidence that the ingested cells not only passed through the gut but also underwent digestion and assimilation, supporting the physical and digestive suitability of P. cruentum as a feed component for early larval stages of P. monodon.

Given its rich content of essential fatty acids, proteins, and bioactive compounds (Safi et al., 2013; Ferreira et al., 2021; Bayu et al., 2023), the confirmed ingestion and digestion of P. cruentum highlight its potential to enhance larval nutrition, support robust development, and improve survival during critical early life stages.

In the ingestion trials, zoea-3 larvae demonstrated higher ingestion rates than mysis-2 larvae across all microalgal densities and time points (Figures 2 and 3). The P-5000 treatment consistently showed the highest ingestion across time intervals, suggesting an optimal density-to-larva ratio at this level. This trend indicates that increased microalgal density enhances encounter probability and ingestion, consistent with earlier findings (Farhadian, Yusoff and Arshad, 2007).

Figure 1. Zoea-1 stage larvae with P. cruentum feed visible in the stomach and intestine, along with fecal matter (indicated by the blue arrow) (Photo by: Abidin Nur).

In contrast, mysis-2 larvae exhibited comparatively lower ingestion percentages overall, which may be attributed to ontogenetic shifts in feeding behavior and dietary preferences during metamorphosis. Interestingly, the highest ingestion for mysis-2 was recorded in the P-2500 treatment, indicating that excessively high cell densities might reduce capture efficiency or feeding motivation in this stage. This could be related to changes in feeding appendage morphology and swimming behavior, as mysis larvae begin transitioning toward zooplankton-based diets (Jamali, Ahmadifard and Abdollahi, 2015).

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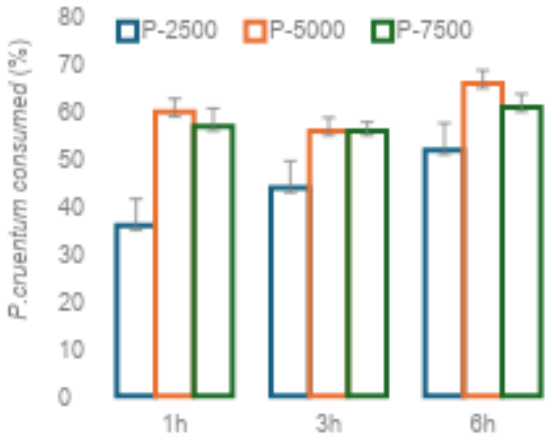

Figure 2. Percentage of P. cruentum consumption by P. monodon larvae at the zoea-3 stage after 1, 3, and 6 hours of rearing at various cell concentrations.

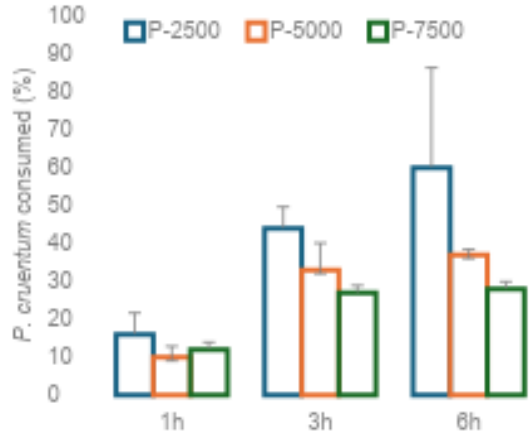

Figure 3. Percentage of P. cruentum consumption by P. monodon larvae at the mysis-2 stage after 1, 3, and 6 hours of rearing at various cell concentrations.

These results suggest that while higher densities of P. cruentum benefit earlier zoeal stages, moderate densities are more appropriate for mysis larvae to maximize feeding efficiency and prevent potential over-saturation of the culture environment.

The ingestion dynamics of P. monodon larvae exhibited clear stage-specific differences, reflecting physiological and behavioral adaptations during ontogeny. Zoea-3 larvae showed a pronounced surge in ingestion rate during the first hour (55 × 10^3 cells $larva^{-1}$ h^{-1}), followed by a logarithmic decline over the six-hour observation period (Figure 4). This rapid initial feeding response is likely driven by post-starvation hunger and the need to meet high metabolic demands associated with growth, organogenesis, and molting. The subsequent decline may be attributed to gut satiation, reduced algal cell density due to consumption, and changes in larval activity patterns as feeding shifts toward digestion and assimilation.

In contrast, mysis-2 larvae displayed a polynomial feeding pattern, with ingestion rates gradually increasing to a peak at the third hour (21 × 10^3 cells $larva^{-1}$ h^{-1}) before declining (Figure 5). This delayed peak and lower overall algal intake compared with zoea-3 larvae reflect an ontogenetic dietary transition from herbivory to omnivory, during which mysis-stage larvae increasingly incorporate larger prey items, such as Artemia nauplii, into their diet (Jamali, Ahmadifard and Abdollahi, 2015; Divya and Aanand, 2020).

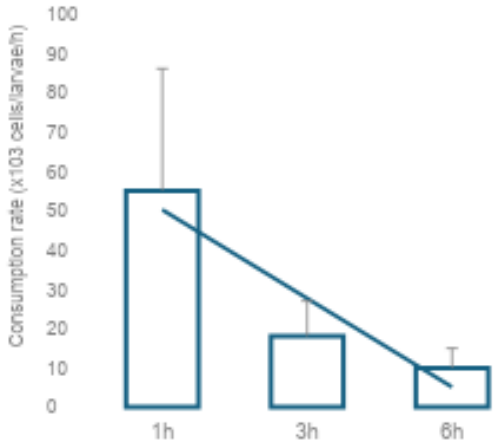

Figure 4. Effect of P. cruentum on the consumption rate of P. monodon larvae at zoea-3 after 1, 3, and 6 hours of rearing. Values are expressed as mean ± SD (n=3).

Morphological changes, including the development of feeding appendages and improved prey-capture ability, facilitate this dietary shift, thereby reducing reliance on microalgae as a primary food source (de la Peña et al., 2023). These findings underscore the importance of aligning live-feed provisioning with larval developmental stage, ensuring that microalgal supply is abundant during early zoeal phases while gradually transitioning to a mixed diet during the mysis stage to optimize nutrient intake, support immune development, and maximize survival in hatchery systems.

3.2. Larval Fed P. cruentum and Artificial Feed

The larval stage index (LSI) serves as an indicator of developmental progression and feed suitability (D'Souza and Kelly, 2000). Table 1 presents the LSI values and survival rates of shrimp larvae fed P. cruentum either as a sole diet or in combination with artificial feed.

The provision of microalgae during early larval stages plays a crucial role in influencing LSI outcomes, primarily through effects on digestibility, nutritional composition, and energy availability (Tang et al., 2020). These factors directly affect the ability of larvae to grow and transition efficiently through

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successive developmental stages (Hernández-Sandoval et al., 2022; Martínez Soler et al., 2023).

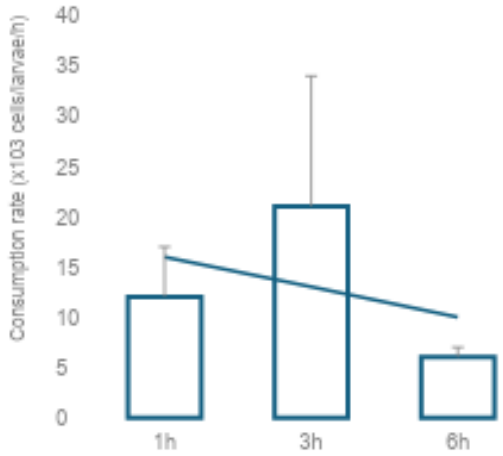

Figure 5. Effect of P. cruentum on the consumption rate of P. monodon larvae at mysis-2 after 1, 3, and 6 hours of rearing. Value are expressed as mean ± SD (n=3).

Table 1. Effects of feeding P. cruentum, with or without artificial feed, on the Larva Stage Index (LSI) and PL-1 survival of P. monodon.

	Treatments	LSI	Pl-1 survival (%)
a.	PC-15.000	1.00 ± 0,00c	-
b.	PC-30.000	1.00 ± 0.00c	-
c.	PC-45.000	1.31 ± 0.20c	10.97 ± 1.99e
d.	PC-15.000 + AF	1.73 ± 0.19b	31.59 ± 5.75c
e.	PC-30.000 + AF	1.77 ± 0.09b	48.02 ± 3.23b
f.	PC-45.000 + AF	3.11 ± 0.10a	68.16 ± 4.14a
K.	AF only	1.83 ± 0.03b	18.34 ± 2.02d

Mean values ± SD followed by different letters within the same column indicate significant differences (P<0.05).

The combination of P. cruentum with artificial feed resulted in significantly higher LSI values ($P < 0.05$) compared with single-feed treatments. The highest LSI was recorded in Treatment F (PC-45,000 + AF), indicating enhanced larval development when P. cruentum was used synergistically with artificial feed. In contrast, feed treatments containing P. cruentum at 15,000 and 30,000 cells mL^{-1} (Treatments A and B) failed to support development to the PL-1 stage, suggesting insufficient nutritional density. These results are consistent with previous studies reporting poor larval performance under mono-algal feeding regimes in penaeid shrimp (Piña et al., 2006; Kiatmetha et al., 2011).

Survival to the PL-1 stage was significantly affected by dietary treatment. The highest survival rate (68.16 ± 4.14%) was observed in Treatment F (PC-45,000 + AF), which was significantly higher than that of all other treatments ($P < 0.05$). Transition to the PL-1 stage occurred approximately eight days after nauplius stocking. In contrast, larvae in Treatments A and B did not survive to the PL-1 stage, confirming that P. cruentum provided alone at lower cell densities was insufficient to support larval progression.

Similar outcomes have been reported previously, where mono-species microalgal diets such as Chaetoceros muelleri (10^5 cells mL^{-1}) and Thalassiosira weissflogii (10^4 cells mL^{-1}) resulted in reduced PL-1 survival of P. monodon (Tang et al., 2020). Likewise, Penaeus indicus larvae fed mono-species microalgae (Tetraselmis chuii and Skeletonema costatum) at densities below 20,000 cells mL^{-1} exhibited inadequate growth and survival up to the mysis-2 stage (Kumlu and Kumlu, 1998). The low survival observed in Treatment C (PC-45,000) in the present study is likely attributable to an insufficient concentration of P. cruentum to meet nutritional requirements at a stocking density of 100 nauplii L^{-1} (de Moraes et al., 2022; Baharuddin et al., 2024; Muthukrishnan et al., 2024). By comparison, artificial feed alone (Treatment K) resulted in lower survival than most P. cruentum + artificial feed combinations, possibly due to reduced palatability, limited digestibility, or physical constraints during early larval stages (Samat et al., 2020; Tang et al., 2020).

These results underscore the importance of feed diversity and appropriate dietary density in shrimp larviculture. Previous studies have shown that diatom genera such as Thalassiosira and Chaetoceros can promote high survival of P. monodon larvae when supplied at densities of 3–5 × 10^4 cells mL^{-1} (Kiatmetha et al., 2011; Abdelhay et al., 2025). In the present study, the performance of P. cruentum in combination treatments was comparable, reinforcing its potential value as a complementary component of natural larval diets capable of supporting survival and physiological development during early life stages.

3.3. Larvae Fed Mixed Microalgae

The use of microalgae in shrimp hatchery systems has long been recognized as fundamental to supporting larval health, growth, and survival. These benefits are largely attributed to microalgae serving as sources of protein, essential fatty acids, pigments, sterols, vitamins, and other bioactive compounds that are readily digestible and efficiently assimilated by larval digestive systems (Khumngern et al., 2025). Numerous studies have reported superior larval performance when diatom species such as Chaetoceros, Isochrysis, Thalassiosira, and

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Skeletonema are incorporated into feeding regimes, owing to their favorable fatty acid profiles (de Moraes et al., 2022; Ashour et al., 2025).

In the present study, P. cruentum was evaluated in combination with selected diatoms to assess potential synergistic effects on larval stage index (LSI) and survival to the PL-1 stage (Table 2).

Table 2. Effects of feeding P. cruentum alone and in combination with diatom microalgae on the larval stage index (LSI) and PL-1 survival of P. monodon..

Treatments	LSI	PL-1 survival (%)
A	2.56 ± 0.05^{b}	$26.72 \pm 3{,}34^{d}$
B	2.65 ± 0.19^{ab}	37.98 ± 5.70^{c}
C	2.77 ± 0.04^{a}	72.71 ± 3.82^{a}
D	2.72 ± 0.04^{a}	50.69 ± 3.25^{b}

Mean values ± SD followed by different letters within the same column indicate significant differences (P<0.05). Microalgae combination were : A (P. cruentum + S. costatum); B (P. cruentum + C. calcitrans); C (P. cruentum + Thalassiosira sp.); and D (Thalassiosira sp. + C. calcitrans).

The results showed that the combination of P. cruentum and Thalassiosira sp. (Treatment C) produced the highest PL-1 survival (72.71 ± 3.82%), which was significantly higher ($P < 0.05$) than all other treatments. The LSI value in this treatment (2.77 ± 0.04) also indicated superior developmental progression, although it did not differ significantly ($P > 0.05$) from Treatment D (2.72 ± 0.04), representing the conventional diatom combination (Thalassiosira sp. + C. calcitrans). Treatment D was used as a reference because microalgae from the genera Chaetoceros and Thalassiosira have been widely reported as the most effective combination for rearing black tiger shrimp larvae (Penaeus monodon) (Kiatmetha et al., 2011; Tang et al., 2020), whiteleg shrimp (Litopenaeus vannamei) (Abdelhay et al., 2025), and Penaeus indicus (Rajamanickam et al., 2025). The LSI values exhibited a consistent trend with survival, suggesting a positive association between developmental progression and larval viability under different microalgal feeding regimes.

The enhanced performance observed in Treatment C may be attributed to the complementary nutritional and biofunctional attributes of the two microalgal species. Thalassiosira sp. is a well-documented source of long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Hernández-Sandoval et al., 2022; Sharma et al., 2025), which are known to play important roles in improving larval survival and supporting metamorphosis (Martínez Soler et al., 2023; Abdelhay et al., 2025). In addition to its relatively high content of arachidonic acid (ARA) and EPA (Bayu et al., 2023), P. cruentum provides antioxidant protection through phycobiliproteins and sulfated polysaccharides, which may contribute to enhanced immune function and stress resistance (Ferreira et al., 2021; Kim et al., 2021; Liberti et al., 2023). Consequently, this combination likely offers not only balanced nutrition but also improved physiological resilience, ultimately contributing to increased larval survival.

Interestingly, although the conventional diatom mixture (Treatment D) performed well, consistent with previous reports (Kiatmetha et al., 2011), the inclusion of P. cruentum in place of C. calcitrans appeared to confer additional benefits. This finding suggests that replacement or partial substitution strategies may be feasible in shrimp hatchery feeding protocols. Such an approach has practical implications for feed formulation, as it may facilitate diversification of microalgal production, reduce dependence on a single diatom species, and improve year-round stability of live feed supply.

Overall, the findings of this study indicate that the combined use of P. cruentum and Thalassiosira sp. represents a promising alternative to traditional diatom-based feeding regimes in P. monodon larviculture. By providing both high-quality nutrients and bioactive compounds, this combination has the potential to enhance larval performance, strengthen physiological robustness, and ultimately improve production efficiency in hatchery operations. Further studies are recommended to evaluate its application under commercial-scale conditions and to examine its effects on larval microbiota composition, immune-related gene expression, and resistance to common bacterial pathogens such as Vibrio spp.

4. Conclusion

The present study demonstrates that Porphyridium cruentum is not suitable as a sole live feed for Penaeus monodon larvae but functions effectively as a complementary dietary component when combined with artificial feed or diatom microalgae. In particular, the combination of P. cruentum with Thalassiosira sp. significantly enhanced larval development and survival to the PL-1 stage. These findings highlight the potential application of functional red microalgae in shrimp hatchery feeding strategies to improve larval performance and reduce reliance on single-species microalgal feeds. Further research is recommended to optimize feeding ratios and evaluate the applicability of this combined diet strategy under commercial hatchery conditions.

Acknowledgment

The author would like to express their sincere gratitude to the Rumah Program Purwa Rupa dan Hasil Riset dan Inovasi untuk Hilirisasi Sumberdaya Akuatik, 2025, under the Research Organization for Earth Sciences and Maritime, National Research and Innovation Agency (BRIN), for the financial support provided (Decree No. 3/III.4/HK/2025, dated January 6, 2025), which enabled the successful implementation of this research.

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