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DIETARY EFFECT OF ENRICHED AND UNENRICHED *ARTEMIA* NAUPLII AND COPEPOD, *DIOITHONA RIGIDA* ON THE GROWTH, SURVIVAL AND N-3HUFA PROFILE OF *PENAEUS VANNAMEI* (BOONE, 1931)

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ABSTRACT

The present study evaluates the growth and survival of 21-days reared *Penaeus vannamei* Post larvae fed on enriched and un-enriched copepod *Dioithona rigida* and *Artemia* Nauplii. The 21 days larval rearing experiment reveals that the length and weight of the copepod *Dioithona rigida* fed larvae were significantly higher than that of *Penaeus vannamei* Post larvae fed on *Artemia* nauplii. The result of biochemical composition include moisture, lipids and fatty acids of shrimp larvae fed with different live feeds before feeding, the moisture and lipid contents of *Penaeus vannamei* larvae were; 82.01% and 21% respectively. But after feeding experiment the shrimp larvae fed with un-enriched *Artemia* nauplii and copepod, exhibitedthe final moisture and lipid contents of 77.69 %; 77.88 % and 14%;30% respectively. Further, the n-3 highly unsaturated fatty acidscontent was found to be higher in both enriched and unenriched

copepodfed Post larvaewhen compared to the *Artemia* nauplii fed Post larvae. The study concluded that the superior nutritional composition especially n-3 highly unsaturated fatty acidsof *Dioithona rigida* can enhance the growth and survival of the shrimp larvae.

Keyword: Copepod, *Dioithona rigida, Artemia, Penaeus vannamei*, Nutritional profile

1. INTRODUCTION

India is the third largest aquaculture producer and second peak populated country in the world with a coastline of 8129 km^2including Andaman and Nicobar and Lakshadweep islands and with 2.02 million km^2 EEZ. As far as India is concerned, aquaculture sector is considered to be an important industry because it contributes to food and nutritional security, employment support and which raises the socio-economic status of poor fishing communities [**12]**. However, unfortunately now a days the aquaculture failure especially in the hatchery phase is due to the lack of appropriate feed during early larval stage of fishes. Live feeds are a convenient and often essential food source for the larvae of some cultured species, especially the larvae that do not have fully developed digestive system. The two major concerns of the aquaculturists are; providing live feed organism's with appropriate size to the larvae at the first feeding stage and the supplying of the essential dietary nutrition required by larvae. The existing traditional live feed, *Artemia nauplii* can satisfy only the numerical requirement [**14, 22]**. And the dimensional and nutritional characteristics of *Artemia nauplii* do not fulfill the larval requirements. However, fortunately, copepods are able to naturally synthesize the essential HUFAs, and could maintain the appropriate DHA: EPA and EPA: ARA ratios required by marine fish larvae. In addition, as much as 90% of the total fatty acids present in copepods are in the more easily usable form of phospholipids. Therefore, unlike rotifers and Artemia, the copepods need not be enriched and they cannot lose their nutritional value quickly due to leaching or excretion. The biochemical profile of the widely used cultivated live food organisms such as *Artemia* sp. and *Brachionus plicatilis* is vastly different from that of copepods. Their content of n-3 HUFA is low are enriched with unless they diets rich in n-3 HUFA **[6, 8, 13, 19, 22, 25]**. *Penaeus vannamei* has been introduced and farmed in Asia since the mid-1990s, with good production in Mainland China being particularly significant. Hence in the present study an

attempt has been made to evaluate the live feed efficacy of enriched and un-enriched *Artemia* nauplii and marine copepod, *Dioithona rigida* on the larval growth, survival, moisture, lipid and fatty acids of *Penaeus vannamei.*

2. MATERIALS AND METHODS

2.1 Algal culture

The cultures of marine microalgae, *Chlorella marina, Nannochloropsis salina, Isochrysis galbana, Dunaliella salina* and *Tetraselmis* sp. Strain were maintained in special air conditioned room. Stock cultures were kept in 1 and 2 liters culture flasks, 5 and 15 liters plastic containers. The seawater filtered through filter bag (1micron), was sterilized by using autoclave and after cooling, the water was transferred to the culture flask. Culture flasks were covered by aluminum foil. All the vessels used in the algal culture were sterilized properly and dried in an oven before use. The Conway's medium was used for indoor culture. About 25 ml of inoculums in the growing phase was transferred to the culture flasks and culture was provided with 12:12 hrs. Light and dark cycle with 5000 lux by using two tube lights. After 7-10 days, the maximum exponential phase was obtained. The temperature and salinity were maintained in the ranges of 23-25^{o}C and 28-30 % respectively during the entire culture period and continuous aeration was provided for culture.

2.2 *Artemia* culture

Artemia cysts (OCM Brand, USA) were purchased from the market. Required amount of cysts were inoculated into the containers having filtered seawater with continuous vigorous aeration and illumination for efficient hatching. The cysts were hatched out at a temperature of 30º C with salinity of 30 % in sterile water, after 16 to 24 hrs. Hatched out nauplii were harvested and used for enrichment study and fatty acids analysis.

2.3 Collection and identification of copepods

The zooplankton sampling was made from the Muthupet lagoon by using scoop (plankton) net with 158µm mesh. The collected samples were immediately transported to the laboratory by providing with vigorous aeration by using battery aerator. The zooplankton samples were thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *D. rigida* was identified under microscope using the key **[5, 11, 17]**.

2.4 Copepod culture

About 500 individuals of cyclopoid copepod, *D. rigida* were isolated and stocked in 100 liter of sea water in outdoor FRP tank.

The copepod cultures were fed with a daily ration of microalgae; *C. marina, N.oculata*, *I. galbana, D.salina* and *Tetraselmis* sp., at the concentration of 30,000cells/ml. The cultures were harvested at every 12 days by gentle siphoning. The generation time of *D. rigida* under optimal condition was about 10-12 days at 26-34^{o}C and by having 6 nauplii and 6 copepodite stages including the adults. Finally the adult gravid female copepods were used to restart the stock culture. Water quality parameters such as temperature, salinity, pH, Dissolved oxygen and the population density of nauplii, copepodite and adults of *D. rigida* were observed daily.

2.5 The enrichment of live feeds

50 ml of cod liver oil was taken into glass beaker and heated on hot plate at 50º C. The egg yolk and 50 ml of hot (50º C) tap water were added to the heated cod liver oil and mixed well. While mixing, 2.5ml of soya lecithin was added and mixing was continued until fluid turns into a creamy white solution. After mixing, the enrichment medium was poured in to 250 ml bottle and stored. The emulsion was used at a concentration of 0.5 ml per liter of sea water. The emulsion was aerated vigorously for 10 minutes to ensure good mixing before adding *Artemia*, and copepod. Around 300-400 numbers of *Artemia nauplii* and copepods were stocked separately in 1 liter beaker filled with 750 ml of filtered sea water. Continuous aeration was given to ensure proper uptake of emulsion by both the organisms. The experiment was extended for 6 hrs. Finally both of the enriched *Artemia nauplii* and *D.rigida* were harvested to feed the *P. vannamei* larvae.

2.6 Experimental setup for larval rearing

The PL 12 of *P. vannamei* was obtained from the Jay Jay Marine Hatchery, Marakkanam near Puducherry, Tamil Nadu, India. The larvae were stocked in 100 l capacity FRP tanks filled with 50 liters of filtered seawater and provide with vigorous aeration under the optimum temperature of 28-29^{o}C, dissolved oxygen of 6.5-7.5 ml/l and with the salinity of 32-34‰. Two feeding combinations (*Artemia*, and copepod) each in duplicate were used for 21 days larval rearing. The daily ration was given twice a day. The total length (TL), wet weight (WW), survival and lipid content of the larvae were used as parameters for understanding the impact of different live-food organisms and noted at the beginning and the end of the experiments. Feeding behavior and growth of larvae were determined by random sampling from various points at each tank. Survival of larvae was determined by counting the total

number of individuals harvested from each tank.

2.7 Larval rearing with *D. rigida* and *Artemia nauplii*

The effect of *Artemia* nauplii on the growth and survival of *P. vannamei* larvae was determined for 21 days. *D. rigida* and *Artemia* nauplii were given the rate of at 10ind./ml densities in each tank. The larvae were collected carefully for length and weight measurements at weekly intervals for 3 weeks. The mortality rate was monitored daily.

2.8 Moisture and lipid

100 mg of sample was taken and the excess moisture was removed by using a filter paper **[20]**. Then the sample was dried in a hot air oven at a constant temperature of 60º C until the wet sample dried completely. The moisture was estimated by subtracting the dry weight of the sample from the wet weight of the sample. Lipid content was estimated by the method of **[9]** and for which100 mg of dried sample was homogenized in 10 ml of chloroform-methanol mixture (2/1 v/v). The homogenate was centrifuged at 2000 rpm and the supernatant was washed with 0.9% saline solution (KCl) to remove the non-lipid contaminants and allowed to separate. The upper phase was discarded by siphoning. The lower phase was allowed to dry in an oven and the weight was noted.

2.9 Fatty acid estimation

For fatty acid analysis, the samples were homogenized with chloroform: methanol (2:1 v/v) mixture and they were extracted by following the method of **[2]**. After fat extraction, they were esterified with 1% H_2 SO_4 and fatty acid methyl esters were prepared by following the procedure of **[1]**. Identification and quantification of fatty acids were done using a Gas Chromatography (Hewlett Packard 5890 Model).

3. RESULTS AND DISCUSSION

3.1 Population density of *D. rigida*

The high total population density of *D.rigida* was obtained at the temperature of 26-34º C, salinity 26-35 ‰ and the algal food concentration of 30,000cells/ml with mixed algae. Over 12 day's culture, the culture system produced an average of 2126.54 nauplii L^{-1}, 1016.58 copepodites L^{-1} and 624.89 adults L^{-1}(on the 12^{th} days). The maximum mean density of *D. rigida* was recorded at 4524.9 nauplii L^{-1}, 2900.32 copepodites L^{-1} and 1906.23 adults L^{-1} on 12^{th} days of culture respectively. For the entire 45 days culture, the total mean production was 44,871.02 L^{-1}, comprising

19,026.54 nauplii, 14,256.89 copepodites and 11,589.64 adults L^{-1} **(Table 1, Figure 1)**.

3.2 Growth and survival of *P. vannamei* fed with different un-enriched live feeds

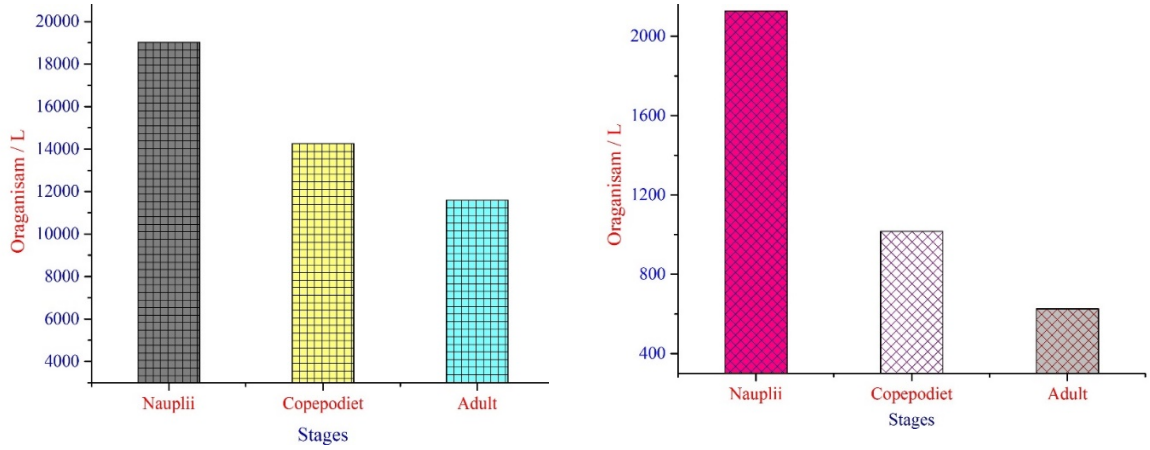
In the first experiment, highest survival was forum in shrimps fed with copepod (92.7%) and a survival of 82 % for shrimps fed with *Artemia* nauplii at the end of 21st days. The initial length and weight of the shrimp larvae were; 1.1 cm and 6mg, respectively. The average total length of *D. rigida* fed post larvae of *P. vannamei* was, 1.9, 2.2, 2.5 cm corresponding to 7th, 14th and 21st day respectively. The weight of copepod fed shrimp larvae were; 34, 78, 82 mg. on 7th, 14th and 21st day respectively. The average total length of *Artemia* nauplii fed post larvae was;1.5, 1.8 and 2.1 cm on 7th, 14th and 21st day respectively whereas the total of weight *Artemia* nauplii offered post larvae of *P. vannamei* was, 24,46, 59mg on 7th, 14th and 21st day respectively.

3.3 Biochemical profile of *P. vannamei* fed un-enriched live feeds

The moisture, lipids and fatty acids composition of shrimp larvae fed with different live feeds are given in **Table 2**. The initial moisture and lipid content of *P. vannamei* larvae were; 82.01% and 21%. The final moisture content of shrimp larvae fed with un-enriched *Artemia* nauplii and copepods were; 77.69 and 77.88 % respectively. The final lipid content of the shrimp larvae fed on un-enriched *Artemia* nauplii and un-enriched copepod were; 14 and 30 % respectively **(Table 2, Figure 2)**.

3.4 Growth and survival of *P. vannamei* fed with different enriched live feeds

In this experiment, the highest survival was obtained in shrimps fed with copepods (96.7%) whereas 86 % survival was found in shrimps fed on *Artemia* nauplii at the end of 21st day. The initial length and weight of the shrimp larvae were; 1.1 cm and 6 mg, respectively. The average total length of shrimp PL enriched *Artemia* nauplii and copepod reared using were; 2.3, 2.9, and 3.8 cm on 7th and 14th and 21stday, respectively. The weight of copepod fed shrimp larvae were 37, 81, and 90.8 mg on 7th and 14th and 21st day respectively. The average total length of shrimp PL enriched *Artemia* nauplii reared using was, 1.7, 2.1, and 2.9cm on 7th and 14th and 21st day, respectively. The weight of copepod fed shrimp larvae were; 29, 70, and 82mg on 7th and 14th and 21st day respectively **(Table 3)**.



1a. Total population density of *D. rigida* **1b. Maximum population density of *D. rigida***

Figure 1: Population density of *D. rigida*

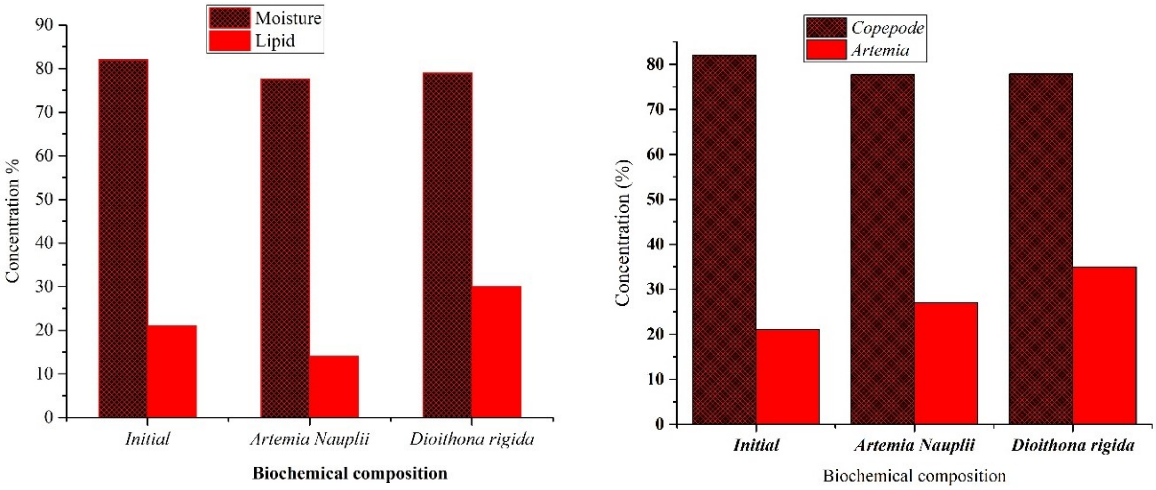
Table 1: Fatty acid profiling of *P. vannamei* larvae shrimp fed on enriched *D. rigida* and *Artemia* nauplii

Fatty acids	Initial larvae	Un-enriched *Artemia* fed larvae	Enriched *Artemia* fed larvae	Un-enriched *D. rigida* fed larvae	Enriched *D. rigida* fed larvae
14:00	0.14	-	2.96	8.9	3.9
16:00	9.76	7.38	8.25	11.16	9.28
17:00	6.89	9.08	9.92	4.66	11.76
18:00	2.91	9.17	9.42	7.53	10.13
20:00	-	-	-	0.8	1.56
21:00	0.64	3.94	-	0.92	1.54
22:00	-	0.14	-	0.23	2.18
16:01	4.03	6.78	-	1	2.43
18:01	0.21	-	2.88	8.67	8.67
18:1-n 9	4.51	8.97	12.62	8.97	11.54
18:3-n 6	0.26	0.18	12.15	1.5	3.26
18:2-n 6	6.64	6.91	-	0	0.56
20:4-n 6 (ARA)	1.24	2.56	1.03	5.06	7.1
20:4-n 5	-	0.86	-	0.02	1
20:5-n 3 (EPA)	2.01	3.37	4.53	6.34	7.97
22:6-n 3 (DHA)	1.52	0.37	1.57	8.09	9.44
22:01	1.04	-	5.98	0.12	0.78

Table 2: Growth and survival of *P. vannamei* fed with different un-enriched live feeds

Feeding regimes	0 day (Initial)			7^{th} day			14^{th} day			21^{st} day (Final)		
	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)
Artemia nauplii	1.1	6	100	1.5	24	93.6	1.8	46	86.3	2.9	59	82
D. rigida	1.1	6	100	1.9	34	97.5	2.2	78	95.4	2.5	82	92.7

Note: L- Length, W- Weight, S- Survival



2a.Un-enriched live feeds fed PL **2b.Enriched live feeds fed PL**

Figure 2: Biochemical composition of *P. vannamei* PL fed on different live feeds

Table 3: Growth and survival of *P. vannamei* fed with different enriched live feeds

Feeding regimes	0 day (Initial)			7th day			14th day			21st day (Final)		
	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)	L (cm)	W (mg)	S (%)
Artemia nauplii	1.1	6	100	1.7	29	95.6	2.1	70	88.6	2.9	82	86
D. rigida	1.1	6	100	2.3	37	99.5	2.9	81	96.1	3.8	90.8	96.7

The ability of penaeid post larvae to acquire energy from diverse resources and the effective utilization of the obtained energy towards the maintenance and growth mainly depends on the environmental factors. During the early life stages, shrimp migrate to estuarine-nursery areas where they experience processes involving behavioral and physiological responses to changing environmental conditions. Salinity is a variable which strongly influences post larvae metabolism although early post larval stages seem to be characterized by a marked euryhalinity. Osmo regulatory capability varies during the development and penaeid adapt to consecutive biotopes. Penaeid post larvae have been shown to develop a strong hyper-/hypo-osmotic regulation. Hyper-osmo conforming larvae could rely on intracellular regulation as they remain at the surface as planktonic stages, whereas, the higher density hyper-osmotic post larval stages would be adapted to seek and settle on the bottom. Therefore, the osmo regulatory response of the shrimps varies according to the medium, from hypo- to hyper-regulation, and the energy requirement for osmo regulation is thus quite different depending

on the external salinity. Moreover, in penaeid shrimp, osmo regulation and ecology are closely linked, although other factors such as bottom nature food availability, and presence of predators may interfere with the choice of the biotope **[4]**. In the present study efforts were made to maintain the salinity of the water within range of 23-25 PSU.

The present experiment showed better survival rates of *P. vannamei* in all treatments copepod diet showed better treatments. Moreover, even after enrichment *Artemia* nauplii, if could not show better survival rate with un-enriched copepods. These findings do support the previous results obtained with penaeid post larvae by **[13, 16]**. However, it has also been shown that even if growth performance of post larvae was not improved by feeding enriched *Artemia* sp., it considerably enhanced their ability to withstand stressful environmental conditions like hypo-osmotic shock. This better resistance could possibly result from higher incorporation of (n-3) HUFA in cell membranes and from the improvement in the total physiological condition of the post larvae. While, research has been devoted to the role of dietary (n-3) HUFA in penaeid post larvae, not many studies have dealt with the effect of individual (n-3) HUFA, such as EPA and DHA. Although found that *P. monodon* post larvae may eventually concentrate DHA in preference to EPA post larval *P. japonicas* have a greater ability to bioconvert18:3 (n-3) to 20:5 (n-3) and 22:6 (n-3), there is little understanding of the biological value and essential fatty acid efficiency between EPA and DHA in penaeid post larvae. The present study showed that *P. vannamei* larvae fed with copepods showed higher survival, weight increment throughout the experiment period. It was very clear that even the un-enriched copepod performed better than enriched *Artemia* in terms of length, weight and survival. Further enriching *Artemia* is another tedious process and also while escalates the cost of production and other nutritional risks.

Lipidis the major source of metabolic energy during the embryonic and pre-feeding larval stages of fish. At the yolk-sac larvae have high levels of these energy sources, but they are dramatically reduced during the endogenous feeding stage. Thus, start-feeding larvae require a live feed that provides sufficient levels of these energy sources. Studies have shown that essential fatty acids (EFA), like docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n - 3), and arachidonic acid (ARA, 20: 4n - 6) are also important in larval fish nutrition **[7, 15, 21, 23, 24]**. The fatty

acid profiling aptly proved the nutritional superiority of copepods over *Artemia* (both enriched and un-enriched). The EPA, DHA and ARA content of *Artemia* were inadequate, though there was considerable increase after enrichment. But the factis that enriched *Artemia* cannot be stored for a longer period and proper balancing of nutrients cannot be done manually makes it less desirable.

The essential fatty acids, as components of phospholipids (PL), are critical structural and physiological components of the cell membranes of most tissues. However, the live feeds rotifers and *Artemia* commonly used for the first-feeding larval stages are naturally poor in these fatty acids, enrichment of live foods with lipids rich in EFA is necessary to achieve better growth and survival through metamorphosis **[18]**. Recently, absolute and relative levels of DHA, EPA, and ARA in the diets of marine fish larvae have received considerable attention [**21]** Several studies have been carried out on the fatty acid sources of commercial diets as well as plant and animal fats or oils to the larval stages of cultivable finfish and shellfish species through bio encapsulation process in live feeds like *Artemia* and rotifer.

Growth and survival of shrimp larvae is greatly influenced by their nutrition and infectious diseases **[24]**. Hence the nutritional research on the cultivable species of aquatic animals especially shrimps has been receiving great attention all over the world. Live feeds such as *Artemia franciscana* and *Brachionus plicatilis* are main food sources for larval forms of crustaceans, in particular for shrimp. Till date, *A. franciscana* is considered to be the best diet for feeding zoophages organisms that are provided as live food to over 85%. Such live-feed organisms are filter feeders and have been used successfully as biological carriers for transferring essential nutrients to predator larvae, bio-encapsulation technique **[3, 10, 13]**.

The un-enriched *Artemia* fed shrimp larvae possessed EPA, DHA and ARA values of 3.37, 0.37 and 2.56 respectively. On the other hand the un-enriched copepod fed shrimp larvae had values of 6.34, 8.09 and 5.06 for EPA, DHA and ARA respectively. The analysis of fatty acids of enriched *Artemia* showed 4.53, 1.57 and 1.03 for EPA, DHA and ARA respectively. The EPA, DHA and ARA values of enriched copepod were; 7.97, 6.44 and 7. 1, respectively. Hence it is well understood that the un-enriched copepods serve as better live feeds than

Artemia nauplii enriched with cod liver oil. With these results, the nutritional status of copepods and their efficacy in improving the growth, survival and weight of the *P. vannamei* has been well established.

4. CONCLUSION

The presently cultured estuarine copepod, *D. rigida* was tested for its efficacy as live feed for pacific white shrimp, *P. vannamei* in enriched and un-enriched form. The population density of *D. rigida* increased when the temperature was maintained in a certain limit with the range of thermal tolerance. The density of copepod *D. rigida* increased when the temperature was maintained in the range of 23^{o}- 25°C. The shrimp larvae being small, a food of suitable size is critical for their healthy development. Copepod nauplii are small enough for first feeding, and they offer a higher nutritional content to shrimp PL. The content of long-chain PUFA, especially DHA, EPA, and ARA, is the main factor in live food evaluation because the deficiency of these fatty acids is usually manifested in shrimp post larvae with the altered metamorphosis, molting processes, and mortality. The recorded low growth and survival in *Artemia* fed shrimp larvae may be a consequence of inadequate nutritional content. Moreover, un-enriched copepod performed better than even enriched *Artemia* nauplii. Further, enrichment of *Artemia* nauplii is cost and a tedious process. Hence with all these facts, the present study strongly prefers the use of copepods over enriched *Artemia* for successful and sustainable fish larval production.

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