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Original Article

Effect of bio-organic fertilizer and agro-industrial residue on the growth and reproduction of cyclopoid copepod, *Oithona rigida* (Giesbrecht, 1896)

Amirah Yuslan[1], Hidayu Suhaimi[1], Norhidayah M. Taufek[2], Nadiah W. Rasdi[*1,3]

[1]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
[2]AquaNutri Biotech Research Laboratory, Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603, Kuala Lumpur, Malaysia.
[3] Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, Terengganu, Malaysia.

Abstract: Production of live feed for larval development of aquatic species is crucial in the aquaculture industry. The cultivation of cyclopoid copepod, *Oithona rigida* can enhance the growth performance and nutritional quality of fish and crustacean larvae. Hence this study was conducted to evaluate the different dietary regimes containing swiftlet waste, soybean meal, rice bran and microalgae (*Nannochloropsis* sp.) on the growth and productivity of *O. rigida*. The results showed that rice bran and *Nannochloropsis* sp. additives produced the best outcomes in terms of specific growth rate (0.109±0.002 and 0.104±0.001 day $^{-1}$) of *O. rigida*., protein content (66.83±2.25 and 72.08±2.02%), and lipid content (21.98±1.41 and 18.09±1.18%) respectively. A mixture of rice bran and *Nannochloropsis* sp. (41.62%) as dietary additives also improved the polyunsaturated fatty acids (PUFAs) content of *O. rigida* as compared with mono diet applied such as rice bran (28.16%) and *Nannochloropsis* sp. (31.35%). The use of rice bran as food additives for *O. rigida* has shown a comparable result with the *Nannochloropsis* sp., in terms of the growth, survival, reproduction and dietary value. Thus, rice bran and *Nannochloropsis* sp. was marked as the best feeding regime for the cyclopoid copepod, *O. rigida* as potential characteristics for mass culture in aquaculture hatcheries.

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Introduction

Primarily, zooplanktons are widely used as live food for crustacean larvae as they have a vital connection with the food system based on the larger targeted consumers (Gürbüzer et al., 2017). Live food organisms contain all required nutrients such as proteins, lipids, carbohydrates, vitamins, minerals, and amino acids, popularly regarded as 'living nutritious capsules' (Manickam et al., 2020; Radhakrishnan et al., 2020). Copepods have a high rate of generative capacity and can survive under adverse conditions for a short period (Loh et al., 2013; Rasdi and Qin, 2016). They are easier to ingest and digest, which is a crucial aspect of the growth and development of larval forms in aquaculture (Maquirang et al., 2020; Khoa et al., 2020). Production of live feed is vital for exogenous feeding of commercially cultured larvae such as blue swimming crab, *Portunus pelagicus* (Affandi et al., 2019), seabass, *Dicentrarchus labrax* (El-Sayed et al., 2021), common sole, *Solea solea* (Shawky et al., 2021), Japanese flounder, *Paralichthys olivaceus* (Khoa et al., 2021), and tiger shrimp, *Penaeus monodon* (Jaseera et al., 2021).

Copepods are common live feed found in marine, freshwater and brackish water, representing up to 80% of the zooplankton biomass in the natural environment (Mauchline, 1998; Piasecki et al., 2004; Kimmerer et al., 2018). Cyclopoid copepod, *Oithona rigida* has been reported to contain high content of HUFAs, comparable to *Artemia* and rotifer. Besides, Copepods vary in sizes for efficient capture, consumption, and optimise ingestion of food consumed by fish and crustaceans' larvae (Genodepa et al., 2004; Santhanam and Perumal, 2012a). *Oithona rigida* can swim freely in the water and is constantly accessible to newly hatched larvae due to their jerking movements in the water column that triggers the larvae's feeding

*Correspondence: Nadiah W. Rasdi
E-mail: nadiah.rasdi@umt.edu.my

reaction (Burbano et al., 2020).

Enrichment of live feed such as copepods and other zooplanktons has been widely applied using various media such as oatmeals and sunflower oil on free-living nematode, *Panagrellus redivivus* (Affandi et al., 2019) and agro-waste from Shochu distillery on *Brachionus plicatilis* rotifer (Khoa et al., 2021) as well as a waste by-product from defatted *Haematococcus pluvialis* meal on *Moina macrocopa* culture (Li and Liu, 2021; Rasdi et al., 2021). Although few studies are available on the utilization of agro-waste and by-products for the enrichment media, it is still considered very limited compared to those enrichments from the microalgae like *Nanochloropsis* sp. due to its high nutritional content (Manisali et al., 2019; Matsui et al., 2019; Rasdi et al., 2021).

Live feed containing desirable nutrients such as LC-PUFA is preferable since larvae require high levels of arachidonic acid (ARA, 20:4n-6), omega-6 (DPAn-6, 22:5n-6), eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) for their survival and growth (Basford et al., 2020). Enriching the live feed with high lipid sources ingredients, including lipid emulsions (Dhert et al., 2014) and microalgae (Ma and Qin, 2014; Rehberg-Haas et al., 2015) are common approaches in hatcheries. However, the form of neutral lipids and triacylglycerols present in the lipid emulsions is not as ideal as supplying polar lipids in the form of phospholipids contained in the microalgae (Basford et al., 2020).

The availability of different food sources for live feed enrichment is crucial in providing better quality live feed sources for aquatic larvae (Radhakrishnan et al., 2020). Various studies on the enrichment of live feed mainly focused on microalgae as the main and common food source (Latib et al., 2020; Ramlee et al., 2021). However, potential organic waste products and agro-industrial residue have been seen as possible food sources which could be explored to enhance the nutritional value of live feed culture in hatcheries (Kamrunnahar et al., 2019; Rasdi et al., 2020). Hence this work aimed to evaluate the different dietary regimes containing swiftlet waste, soybean meal, rice bran and microalgae (*Nannochloropsis* sp.) on the growth and productivity of *O. rigida*.

Material and methods

Sampling and experimental design of *Oithona rigida*: The zooplanktons were collected and isolated from the Setiu Wetland of Peninsular Malaysia using horizontal and vertical plankton net (50 μm). During the collection, the lagoon temperature was 24-30°C, salinity 22-34 ppt, and 8.0-9.5 pH at noon (Yuslan et al., 2021). Upon collection, the live samples were washed with freshly filtered seawater (1 μm) to avoid contamination and transported directly to the laboratory. Individual copepod was transferred to a new beaker containing filtered seawater for species identification following Dussart and Defaye (2001), Lopes et al. (2001) and Conway (2006). Enrichment diets, including rice bran, soybean meal, swiftlet waste and *Nannochloropsis* sp. were provided immediately after being transferred to the culture tank. *Oithona rigida* (Fig. 1) was isolated according to the morphological classification and gradually scaled up by standard protocols (Kasturirangan, 1963; Santhanam et al., 2015). All maintenance and feeding trials were conducted in Fisheries and Aquaculture Sciences Hatchery, Universiti Malaysia Terengganu.

Oithona rigida stock culture was first cultivated in 1 L flask with an initial density of 8 ind/mL. The culture was upscale once the density increased where the cultures were transferred to 500 L tanks for stock culture. Adult *O. rigida* was isolated from the stock culture and transferred to a 1000 L mass culture tanks and supplied with the specified food for one generation before data were collected to remove any consequences of previous feed regiments (Kamrunnahar et al., 2019). *Oithona rigida* were maintained under controlled laboratory conditions as specified in Table 1. After six months, the new *O. rigida* were harvested and placed into the 100 L enrichment tanks for the feeding trial. The five enrichment tanks represent five experimental diets (one control) in triplicates for each treatment.

Enrichment of *O. rigida* with bio-organic fertilizer

Table 1. The parameters for *Oithona rigida* cultivation and enrichment.

	Parameters		References
Diets concentration	Swiftlet waste, rice bran and soybean meal	= 500 mg/L	Paray and Al-Sadoon (2016)
	Microalgae (*Nannochloropsis* sp.)	= 500 mg/L = 2 x 10^7 of algal cells/mL	Espinosa-Rodríguez et al. (2012) and Rasdi and Qin (2015)
Duration of enrichment	6 hours		Payne and Rippingale (2001) and Estévez and Giménez (2017)
Salinity	26±0.3 ppt		Santhanam and Perumal (2012b) and Santhanam et al. (2019)
Temperature	26 ± 0.3 °C		Milione and Zeng (2008) and Santhanam et al. (2019)

Figure 1. Dorsal view of adult *Oithona rigida* (left) and berried female *Oithona rigida* (left) under a compound microscope.

(swiftlet waste) and agro-industrial residue (rice bran B**and soybean meal):** Bio-organic fertilizer (swiftlet waste) was obtained from post-harvest facilities at Universiti Malaysia Terengganu. The agro-industrial residue (rice bran and soybean meal) was purchased from a fertilizer supplier in Kedah, Malaysia. Before swiftlet wastes were used as feed, they were first transformed from raw manure to bio-organic fertilizer by using mineralization technique to make the feed become more hygienic and safer to be used as feed for zooplanktons (Nagavardhanam, 2017). These organic feeds were dried at 105°C for 24 hours to remove the moisture and stored in plastic jars for further use (War and Altaff, 2011).

Prior to the feeding trial, all diets were grounded, and the micronized substances were reduced into powdered form (<0.1 mm) using a blender at maximum speed for approximately 3 minutes. The feeds were then weighed and dissolved in distilled water to achieve a heterogeneous suspension culture. Next, the feed mixture was sieved using a net of 50 µm before being poured into the *O. rigida* culture tank (Loh et al., 2013). The dietary suspensions were then used to fertilize the culture medium as the enrichment media for *O. rigida.*

Enrichment of *O. rigida* with microalgae (*Nannochloropsis* sp.): *Nannochloropsis* sp. pure culture stock strain was obtained from Universiti Malaysia Terengganu hatchery and cultured prior to the experiment. *Nannochloropsis* sp. was grown in Conway medium at temperature 29ºC, salinity 30 ppt with ratio of light: dark cycle of 12:12 h and continuous aeration (DeVille et al., 1995). Weekly algae preparation using the optimization algal growth process was appropriate for copepod growth. **Population density and specific growth rate of**

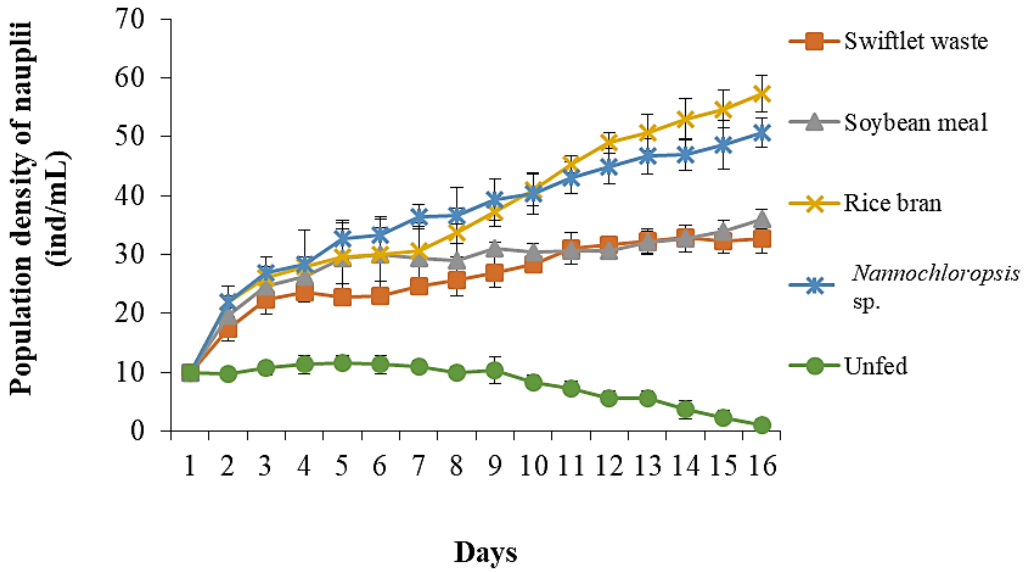

Figure 2. The population density of nauplii copepod, *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3).

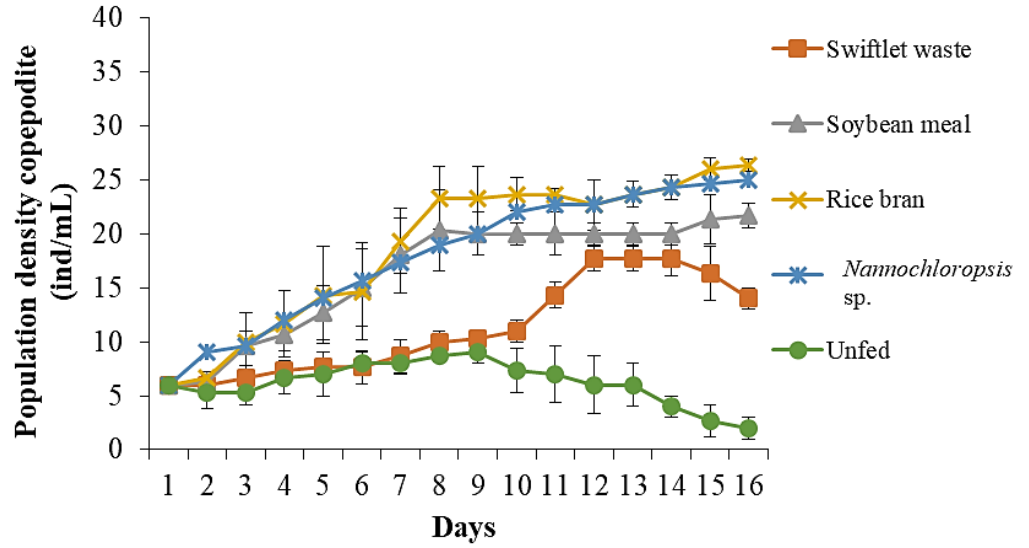

Figure 3. The population density of copepodites *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3).

***O. rigida*:** A culture setup consisted of 100 L tank of *O. rigida* with a population consisting of mixtures of 20, 10, 6, and 4 ind mL^{-1} adult was cultured in the 500 mL beakers with triplicates for each dietary treatment. The measurements of life-history parameters in *O. rigida* were monitored, and the data were recorded. The 3 mL subsample of *O. rigida* was taken and calculated daily from the 500 mL culture medium under dissecting microscope. Triplicate counting was performed from the subsamples. Optimum water quality parameters were maintained daily using a YSI meter, and gentle aeration was provided. The experiment was performed for 16 days until the entire cohort dies. The specific population growth rate (r) (Days^{-1}) has been derived from population density data using the following equation by Lee et al. (2013).

$$\text{The specific population growth rate (r)} = \left(\frac{\ln Ne - \ln Ni}{\text{t}}\right)$$

Where t = culture days, Ni = initial density of copepods and Ne = end density of copepods.

Hatching rate, hatching time, survival, and generation time of *O. rigida*: Fifty berried females were randomly collected from each beaker and placed in a 30 mL petri dish with three replicates for each treatment. Eggs were examined every 6 hours to count newly hatched nauplii over 24 hours. The nauplii were then transferred to a new petri dish with the same diet

Table 2. The specific population growth rate of *Oithona rigida* fed different enrichment diets. All values are mean ± standard deviation (n = 3). Different letters on the same column indicate significant difference (P<0.05).

Diets	Specific population growth rate (day^{-1})
Swiftlet waste	0.067±0.005[a]
Soybean meal	0.082±0.001[b]
Rice bran	0.109±0.002[b]
Nannochloropsis sp.	0.104±0.001[a]
Unfed	-0.084±0.015[c]

as the broodstock to test the following *O. rigida* survival from incubation to maturity. The number of unhatched eggs was counted after 48 hours, and the hatching rate was calculated (Pan et al., 2014).

$$\text{Hatching rate (\%)} = \left(\frac{\text{Total number of the unhatched eggs}}{\text{Total number of the eggs}}\right) \times 100$$

$$\text{Survival rate (\%)} = \left(\frac{\text{Total number of the sample taken}}{\text{Total number of initial density}}\right) \times 100$$

Subsequently, the hatched nauplii were monitored daily for mortality in each treatment to assess the average lifespan from hatching to maturity. Throughout the trial, food was applied regularly to each beaker using a plastic dropper to prevent over-feeding. The hatched *O. rigida* were then placed in a petri dish with three replicates for each dietary treatment to determine the developmental period from nauplii to the mature stage. The development period of *O. rigida* was monitored every 1 hour before the mature stage was reached, and the development time for each post-embryo stage was calculated from the mean triplicate for each treatment. The developmental stages were observed under a dissecting microscope to calculate the development time from nauplii I to VI, the generation time of copepodite I to adult and the generation time of nauplii I to gravid.

Offspring production, spawning, and lifespan: One mature female was taken from the preconditioning copepod stocks culture tank and placed into a 50 mL beaker, covered with aluminum foil with holes for ventilation for each diet treatment in triplicates. The females of *O. rigida* were fed with all the diets enrichment and the beakers were monitored daily for the occurrence of new eggs in the egg sacs. The individual products from the previous eggs were counted in the Sedgewick-Rafter chamber on a dissecting microscope using a 40 μm mesh. The daily development of offspring and offspring per egg sac was derived from the average of the triplicates of *O. rigida* for each diet. Full spawning in a lifetime was recorded and the longevity of females was reached by an average of all life expectancy within 30 days before the entire cohort died.

Lipid and protein analysis of enrichment diets, unenriched and enriched *O. rigida* and *Artemia*: The analyses have been done at the Faculty of Fisheries and Food Science laboratory in UMT. Enrichment diets and live feeds were analyzed using the Association of Official Analytical Chemists (AOAC, 1995) standard protocol in triplicates. All prepared samples were finely crushed using mortar and pestle and further blend into powder form (<0.1 mm) using a blender at maximum speed for approximately 3 minutes (Loh et al., 2013). The protein was analyzed using the Kjeldahl method with a conversion factor of 6.25 to transform total nitrogen to crude protein (Pearson, 1999). Samples (200 mg) were approximately placed into glass tubes to conduct the digestion procedures, followed by the addition of the digestion mixture according to the treatments (Silva et al., 2016). Lipids were extracted from the Soxhlet petroleum ether extractor. Approximately 100 mg of samples were applied using a modified Bligh and Dyer method (Branco-Vieira, 2018; Gorgich, 2020). After successful extraction, the residue was dried to a constant weight at 100°C.

Fatty acid methyl esters (FAME) analysis of enrichment diets, unenriched and enriched *O. rigida* and *Artemia*: Plankton net (100 μm) was used to sieve the samples of live feeds. The samples were washed with distilled water and taken to the

Table 3. Life table of *Oithona rigida* fed with different dietary regimes.

Diets	Swiftlet waste	Soybean meal	Rice bran	*Nannochloropsis* sp.	Unfed
Hatching time (days)	2.13±0.12^{a,b}	1.90±0.02^{b}	1.96±0.06^{b}	1.84±0.07^{b}	2.40±0.29 ^{a}
Generation time of Nauplii I to VI (days)	4.17±0.03^{b}	4.03±0.25^{a,b}	4.15±0.05^{a,b}	3.79±0.11^{c}	5.35±0.12 ^{c}
Generation time of Copepodite I to Adult (days)	3.53±0.10^{a}	3.21 ± 0.04^{a}	3.57±0.40^{a}	3.13±0.04^{a}	4.32±0.31^{b}
Generation time of Nauplii I to Gravid (days)	10.84±0.08^{b}	9.57 ± 0.07^{d}	9.81±0.03^{c}	9.17±0.05^{b}	13.32±0.14^{a}
Mean lifespan of copepods (days)	13.00±1.00^{a}	13.33 ± 1.53^{a}	14.00±1.73^{a}	14.67±2.31^{a}	11.67±1.15^{a}
No. of spawning /lifespan	9.00±1.73^{a,b}	10.67 ± 1.53^{a}	9.33±0.58^{a,b}	12.00±1.00 ^{a}	5.33±2.08^{b}
Daily offspring/ Female	5.60±0.69^{a,b}	6.62 ±0.95^{a,b}	7.53±0.50 ^{b}	8.67±0.70^{a}	4.58±0.54^{b}

All values are mean ± standard deviation (n = 3). Different letters on the same row indicate significant difference (P< 0.05). The parameters in bold letters have shown the best results achieved and fully described in the text.

laboratory to be stored in a deep freezer below -80°C for 48 hours. The sample was freeze-dried for 48 h prior to the preparation of a fatty acid analysis. Once each sample was thoroughly dried, it was finely ground prior to analysis. The combinations of the classical method (AOAC, 2000) and a one-step method (Atolani et al., 2015) have been used for preparing fatty acid methyl esters (FAME). All the chemicals used (hexane, chloroform, methanol, and 14% BF3 in methanol) were analytical reagent grades (GC-2010, Shimadzu, Tokyo, Japan). Supelco TM 37 Component FAME Mix, Sigma was used as an internal standard.

Statistical analyses: The data were shown as mean±standard deviation. All data were tested for normality (Shapiro-Wilk test), homogeneity, and independence (Levene's test) to fulfill the ANOVA assumptions (IBM SPSS Version 25.0, 2017). The data were analyzed using one-way analysis of variance (ANOVA) to see the effect of different diets on *O. rigida* population density, survival rate, and reproductive efficiency. The effects of selected diets on the nutritional compositions (protein, lipid and fatty acids) of *O. rigida* were compared with *Artemia*. The post-hoc Tukey's test comparisons were carried out, and differences were considered statistically significant at probability levels P<0.05.

Results

The total mean population density of *O. rigida* nauplii was highest when fed rice bran at 57.33±3.06 ind/mL in the final density (Fig. 2). The growth pattern showed that the density of nauplii was higher in copepods-enriched microalgae, *Nannochloropsis* sp. compared to other dietary treatments on day 0th until day 10. However, after 10 days of culture, the density of nauplii was drastically increased in copepods enriched-rice bran compared to other treatments. The abundance of *O. rigida* in copepodite stages was highest when fed rice bran (26.33±0.58 ind/mL; Fig. 3). The growth pattern of copepodite density fed rice bran on day 0 until day 6th was comparable with treatments fed *Nannochloropsis* sp. However, on the 7th day, the copepods enriched-rice bran diet showed drastically rising density compared to other treatments.

The population density of adult *O. rigida* was higher with rice bran as diet (31.00±1.00 ind/mL; Fig. 4). However, the concentration of unfed adult *O. rigida* reduced during the culture period and nearly died on 9th day. The highest total population of *O. rigida* occurred on day 16th when fed rice bran (77.46±27.93 ind/mL; Fig. 5). In contrast, all developmental stages of unfed *O. rigida* were declined and died on day 9th of the culture period. The specific population growth rate of copepods varied with the different dietary sources in 16 days of the culture period (P<0.05; P = 0.001; Table 2). *Oithona rigida* cultured in rice bran diet treatments achieved the highest specific growth rates (0.109±0.002 day^{-1}).

Table 4. Protein and lipid composition of different diets used for the enrichment.

Composition (%)	Swiftlet waste	Soybean meal	Rice bran	*Nannochloropsis* sp.
Protein	5.65±0.99[a]	31.08±0.69[b]	24.63±0.51[c]	53.46±0.94[d]
Lipid	3.09±0.71[a]	11.90±0.45[b]	22.58±0.72[c]	17.10±0.79[d]

All values are mean ± standard deviation (n = 3). Different letters on the same row indicate significant difference (P<0.05). The nutritional composition in bold letters has shown the best results achieved and fully described in the text.

Table 5. Protein and lipid composition of *Oithona rigida* fed different diets.

Composition (%)	*Oithona rigida*				*Artemia*	
	Swiftlet waste	Soybean meal	Rice bran	*Nannochloropsis* sp.	Unenriched	Unenriched
Protein	58.22± 1.30[a]	69.51± 2.7[b]	66.83±2.25[b]	72.08±2.02[b]	56.90±1.6[a]	50.34± 2.19[c]
Lipid	15.34±1.23[a,c]	16.86±0.63[a,c]	21.98±1.41[b]	18.09±1.18[a,b]	14.45±1.78[a]	18.90± 1.33b[b,c]

All values are ± standard deviation (n = 3). Different letters on the same row indicate significant difference (P<0.05). The nutritional composition in bold letters have shown the best results achieved and fully described in the text.

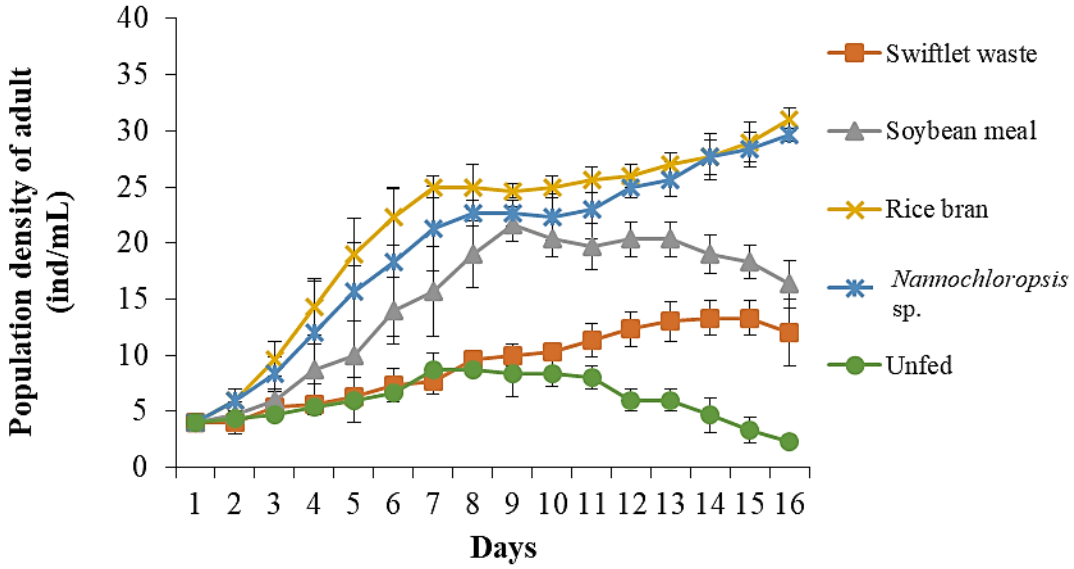

Figure 4. The population density of adults *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3).

Conversely, unfed *O. rigida* showed a negative specific population growth rate and almost died after 12 days (-0.084±0.015 day^{-1}).

Reproductive performance of *O. rigida*: The hatching time of *O. rigida* depends on the enrichment diets (P<0.05; P = 0.011; Table 3). *Oithona rigida* fed with *Nannochloropsis* sp. (1.84±0.07 days) took the shortest time to hatch their eggs. The diets also influenced the hatching rates of *O. rigida* (P<0.05; P = 0.001). The highest hatching rate was recorded when *O. rigida* fed with rice bran (82.00±7.21%) (Fig. 6). Generation time from nauplii I to nauplii VI in *O. rigida* fed *Nannochloropsis* sp. had the shortest development time (3.79±0.11 days; Table 3). The maturation of copepodite I until the adult phase of *O. rigida* fed *Nannochloropsis* sp. (3.13±0.04 days) took a shorter time to develop. The generation time from newly hatched nauplii stage I to gravid *O. rigida* fed *Nannochloropsis* sp. had the shortest development time (9.17±0.05 day). The survival rate of *O. rigida* from hatching to the adult stage was significantly stimulated by the varieties of nutrients during the cultivation period (P<0.05; P = 0.001; Fig. 7). *Oithona rigida* fed rice bran produced the highest survival rate from hatching to adult stage compared to other dietary treatments.

The lifespan of *O. rigida* was not significantly affected by the types of food used (P>0.05; P = 0.098;

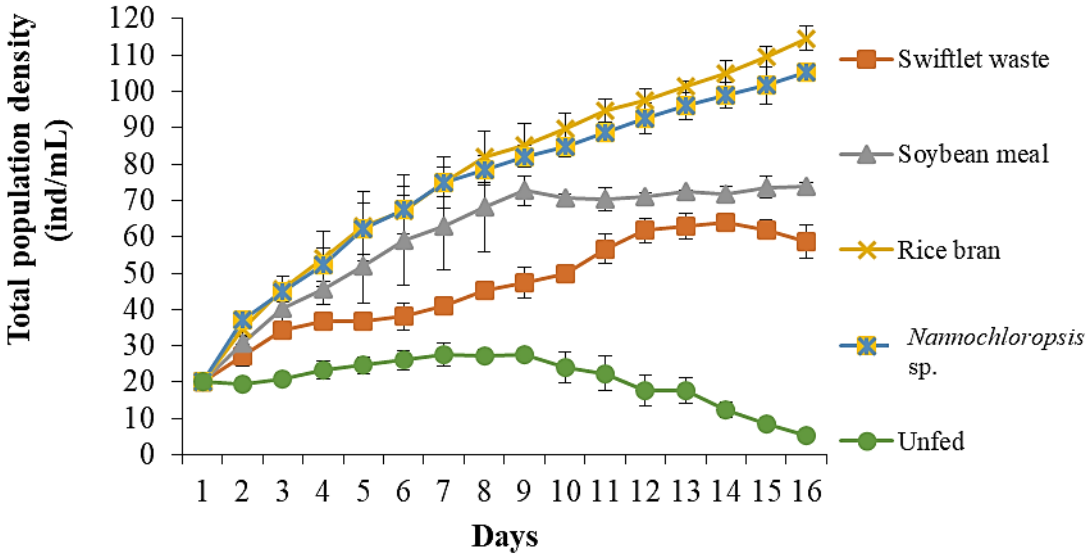

Figure 5. The total population density of *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3).

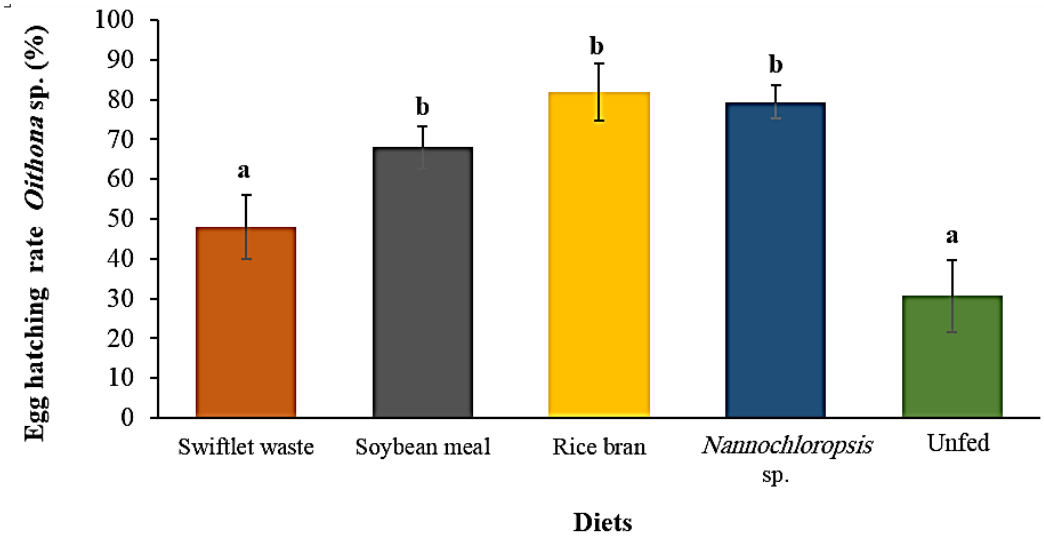

Figure 6. The egg hatching rate of *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3). Different letters on the bar indicate significant difference (P<0.05).

Table 3). A longer lifespan was observed in treatments with *Nannochloropsis* sp. (14.67±0.53 days). *Oithona rigida* had a high spawning rate when fed with *Nannochloropsis* sp. (12.00±1.53), while the lowest spawning was recorded in swiftlet waste (9.00±1.73). The different types of diets significantly affected the number of spawns per lifetime and the total number of offspring per female produced in 16 days (P<0.05; P = 0.003; Table 3).

The protein and lipid compositions of different feeds used for enrichment: The protein and lipid content from the dietary treatments were recorded to be in the range of 6 to 54% and 4 to 22% of dry weight, respectively (P<0.05; P = 0.001; Table 4). However, these variations are more likely reflect variations in the nutritional composition of different diets, such as bio-organic fertilizer, agro-industry residue, and microalgae e.g. *Nannochloropsis* sp. (54.46±0.94%) had the highest protein content, whereas rice bran recorded the highest lipid composition (22.58±0.72%).

The protein and lipid compositions of unenriched (*O. rigida* and *Artemia*) and enriched *O. rigida*: The protein content in *O. rigida* was recorded at 56.90±1.61%, and it was higher than the commercial live feed, *Artemia* (50.34±2.19%). The protein and

Table 6. The FAME analysis of unenriched and enriched live feeds fed with different diets enrichment. All values are mean ± standard deviation (n = 3). Different letters on the same row indicate a significant difference (P<0.05).

Fatty acid (%)	*Oithona rigida*					*Artemia*
	Swiftlet waste	Soybean meal	Rice bran	*Nannochloropsis* sp.	Unenriched	Unenriched
Saturated						
C 12	0.18±0.02	0.54±0.06	1.46±0.55	1.05±0.43	0.16±0.05	0.00±0.00
C 14	6.90±0.08	8.80±0.09	9.44±0.12	9.76±0.15	5.47±0.09	1.63±0.34
C 16	20.24±0.08^{a}	21.48±0.18^{b}	26.44±0.27^{c}	23.54±0.27^{d}	19.40±0.15^{e}	11.67±0.44^{f}
C 18	5.52±0.14	7.59±0.21	9.48±0.25	8.48±0.24	4.55±0.37	4.38±0.16
C 24	0.74±0.01	0.85±0.03	1.16±0.02	1.03±0.01	0.63±0.02	0.29±0.07
SUM SAFAs	33.5	39.25	47.98	43.86	30.22	18.98
Monounsaturated						
C 16:1	4.87±0.65^{a}	7.61±0.39^{b}	9.89±0.09^{c}	9.31±0.28^{c}	4.61±0.39^{a}	3.06±0.50^{d}
C 18:1n9c[3]	1.38±0.21	2.18±0.82	4.47±0.24	5.46±0.20	1.61±0.39	1.31±0.33
C 20:1n9	0.34±0.01	0.54±0.02	0.98±0.01	0.75±0.01	0.17±0.06	0.54±0.01
C 22:1n9[3]	0.00	0.00	0.00	0.00	0.00	0.00
SUM MUFAs	6.59	10.33	15.34	15.52	6.38	4.90
Polyunsaturated						
C 18:2n6t[2]	0.44±0.03	0.64±0.03	0.85±0.02	0.54±0.01	0.00	0.00
C 18:2n6c[2]	0.36±0.01	0.52±0.04	2.54±0.30	1.37±0.12	0.58±0.17	0.00
C 18:3n6[2]	0.39±0.01	0.66±0.01	0.91±0.07	0.75±0.01	0.32±0.00	0.20±0.01
C 18:3n3 (ALA)[1]	0.35±0.06^{a,c}	0.54±0.02^{a}	0.79±0.01^{b}	0.55±0.19^{a}	0.13±0.03^{c}	15.4±30.01^{d}
C 20:4n6 (ARA)[2]	0.87±0.02^{a}	1.75±0.05^{b}	2.75±0.06^{c}	3.86±0.08^{d}	0.44±0.22^{e}	1.04±0.03^{a}
C 20:5n3 (EPA)[1]	5.52±0.15^{a}	8.42±0.12^{b}	10.53±0.19^{c}	14.620.33^{d}	6.61±0.39^{e}	3.70±0.21^{f}
C 22:6n3 (DHA)[1]	3.53±0.34^{a}	6.61±0.39^{b}	9.79±0.25^{c}	7.65±0.11^{d}	2.76±0.21^{e}	2.31±0.22^{e}
SUM PUFAs	13.46	19.18	28.16	31.35	10.84	22.68
Total fatty acid	53.55	68.76	91.48	90.73	47.44	46.56

A value of 0 indicates that FA was not detected. Different letters indicate significant differences between treatments: [1]ω-3 fatty acids, [2]ω-6 fatty acids, and [3]ω-9 fatty acids. ALA: alpha-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. The nutritional composition in bold letters is described in the results.

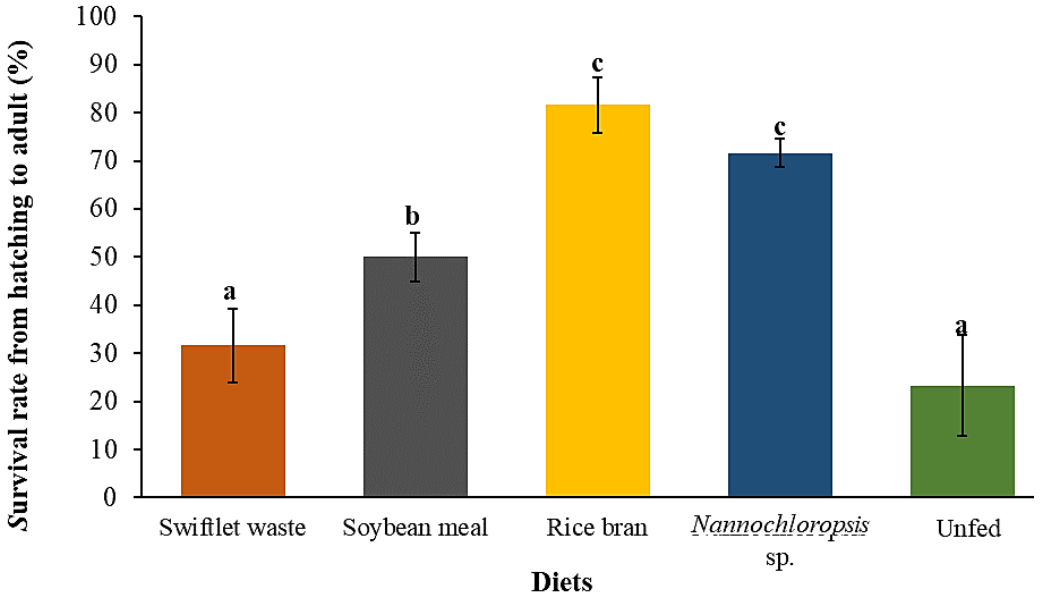

Figure 7. The survival rate of *Oithona rigida* fed with different diets. All values are mean ± standard deviation (n = 3). Different letters on the bar indicate significant difference (P<0.05).

lipid content in *O. rigida* were influenced by the enrichment given (P<0.05; P = 0.001; Table 5). *Oithona rigida* yielded higher protein content when enriched with *Nannochloropsis* sp. (72.08±2.02%) followed by soybean meal, rice bran and swiftlet waste yielded the lowest protein content (58.22±1.31%). The composition of lipid content in all the treatments gradually increased compared to unenriched *O. rigida* (14.45±1.79%), and *Artemia* (18.90±1.33%). The lipid content was significantly

higher in the *O. rigida* enhanced with rice bran (21.98±1.41%), than *Nannochloropsis* sp. (18.09±1.18%), soybean meal (16.86±1.63%) and swiftlet waste (15.34±1.23%).

The fatty acid composition of unenriched (*O. rigida* and *Artemia*) and enriched *O. rigida*: Fatty acid compositions such as SAFAs, MUFAs and PUFAs of enriched *O. rigida* showed improvement after being supplemented with different dietary treatments (Table 6). *Oithona rigida* reached the higher fatty acid content after enriched with rice bran followed by *Nannochloropsis* sp., soybean meal and lower fatty acid content when enriched with swiftlet waste at 91.48, 90.73, 68.76 and 53.55%, respectively. The ARA content in *O. rigida* also varied between all dietary treatments ($P<0.05$). ARA content was found highest in *O. rigida* enriched with *Nannochloropsis* sp. (3.86±0.08%). On the other hand, EPA and DHA in *O. rigida* were significantly impacted by the different dietary treatments ($P<0.05$; Table 6). The highest content of EPA was observed in *O. rigida* enriched with *Nannochloropsis* sp. (14.6±0.33%), whereas the highest DHA was recorded in rice bran enrichment (9.79±0.25%).

Discussion

The present study revealed significant variations in population and peak densities at different days of life stages in copepod (nauplii, copepodites and adults). These findings are in accordance with studies conducted by Hyder et al. (2014) where a high density of *Thermocyclops decipiens* and *Mesocyclops aspericornis* was attained when chicken manure was used as diet. Previous research has shown the intricacies of mass-cultivation of copepods due to the wide varieties of food quality and environmental conditions that are prerequisites for their development and reproduction (Evjemo et al., 2003; Punnarak et al., 2017). The production of copepods is highly influenced by the biochemical composition of food consumed, which will impact population growth and reproductive ability (Khatoon et al., 2013). The highest population density and growth rate of *O. rigida* were observed in rice bran and microalgae-enriched treatments. The growth rate in both dietary treatments was higher than the previously documented growth rate of cyclopoids copepods such as *Apocyclops royi* (0.096±0.013 day^{-1}) (Lee et al., 2006) and *Tigriopus japonicas* (0.08±0.014 day^{-1}) (Lee et al., 2013). This indicates that *O. rigida* can be grown at high densities as live food for fish and crustaceans' larvae. Furthermore, the use of rice bran as fertiliser has shown increasing the growth and development of copepods such as *M. dussarti* and *T. neglectus* and cladocerans such as *Moina micrura* and *Diaphanosoma excisum* (Amian et al., 2018). The increase in these zooplanktons' production is closely linked to feeding decomposition, promoting the acceleration and maintenance of zooplankton abundance and survival (Amian et al., 2018; Rahmati et al., 2020).

Soybean meal also provides an outstanding result in sustaining copepod culture density and survival rate, owing to the good nutritional content digested by the copepods (Paray and Al-Sadoon, 2016). According to El-khodary et al. (2020), the highest population density was observed in *Cyclops abyssorum divergens* after feeding with soybean diet, and this may be attributable to the desirable food size. Hyder et al. (2014) suggested that it is possible to maximise the viability of a mass culture of two freshwater cyclopoid copepods, *T. decipiens* and *M. aspericornis,* with a sufficient dosage of organic waste. Animal waste such as swiftlet waste could be a potential enrichment diet for high-density mass cultivation of zooplankton culture. It could serve as an efficient, cheap and sustainable food supply to cultivate copepods (Kabery et al., 2019). Furthermore, manure contains numerous organic matters and bacteria that can be transformed into carbohydrates, proteins, pigments, oils, alcohols, and aldehydes, which could be filtered by zooplankton to promote their growth (Ansa and Jiya, 2002; A'tirah et al., 2016).

The hatching rate is a critical factor for measuring copepod production as these were closely correlated with maternal nutrition as egg quality dictates the progress of hatching (Rasdi and Qin, 2018). The

results indicated that efficient egg hatching and the quantity of spawns in copepods during a lifetime relied on different dietary treatments. Rasdi and Qin (2018) and Milione and Zeng (2007) have published comparable findings on *Cyclopina kasignete, Acartia sinjiensis* and *Bestiolina similis.* However, contrary to *Parvocalanus crassirostris*, there is no variation in egg hatching success under various algal treatments (Alajmi and Zeng, 2015). In short, the productivity achievement of copepods depends on the quality of foods and species cultured (species-specific). Therefore, the need to evaluate each copepod species' dietary requirements was emphasized, especially for copepods with the capacity for aquaculture as a live feed.

Many copepod species are inadequate for large-scale mariculture due to certain copepod species' long generation period and limited understanding of their nutritional requirements (Conceição et al., 2010). Besides, egg production and the maturity of live feed also depend on the consistent feed (Ullimaz et al., 2020). The rice bran and *Nannochloropsis* sp. produced more offspring, fast hatching rate, shortest developmental period and longer female lifespan than soybean meal and swiftlet waste. These results were consistent with previous studies, whereby rice bran suspension had accelerated the reproductive cycle by enhancing the *M. macrocopa* fecundity rate (Mubarak et al., 2017; Ullimaz et al., 2020). In addition, vitamin B, particularly thiamine and pyridoxine derived from rice bran, might contribute to the rising offspring production (Mehdipour et al., 2011).

The nutrient feasibility of copepods as feed for aquatic larvae can be manipulated by copepod-fed algal species (Molejón and Alvarez-Lajonchere, 2003; Jeyaraj and Santhanam, 2013). Rajkumar and Rahman (2016) have shown that *Nannochloropsis oculata* is a microalgae preferred for *A. erythraea* and *Oithona brevicornis*, resulting in the best growth result. El-Tohamy et al. (2021) also declared that microalgae, *Isochrysis galbana* proved to be the best algal diet for the best growth efficiency in the cultured calaniod copepod, *Gladioferens imparipes.* These results were also proportional to the outcomes in microalgae diet treatments reported in the current study. Santhanam and Perumal (2012b) disclosed that *O. rigida* cultivated in four dietary algae species has a high reproductive ability. Alajmi and Zeng (2015) have suggested using microalgae *Tisochrysis lutea* as an effective diet to achieve a greater number of progeny and a faster time of growth for the paracalanid copepod, *P. crassirostris. Isochrysis* sp. and *A. bilobata* (Lee et al., 2006; Pan et al., 2014). *Tisochrysis lutea* was also a superior food to increase *Cyclopina kasignete* offspring production (Rasdi and Qin, 2018). However, as pointed out by Payne and Rippingale (2001), the hardness and indigestion of its cell wall was the key explanation for the weak success of *Nannochloropsis* sp. in copepod cultivation compared to other species of microalgae, which was also observed when fed to *Artemia*.

The growth, fertilization, maturity and longevity may be enhanced by exploiting feed ingredients and their nutrients (Wacker and Creuzburg, 2007; Mehdipour et al., 2011; Nagaraju, 2011). Our results showed that all the dietary treatments used could promote the growth and development of *O. rigida*. Nevertheless, rice bran and *Nannochloropsis* sp. were highlighted to contribute to a successful cyclopoid copepod-feeding regime for *O. rigida*. Friedman (2013) explored rice bran as food to enhance the nutritional efficiency of aquatic animals as a live feed enhancement to improve fish and shellfish larvae development and survival, which indirectly contributed to the food quality management. Faria et al. (2012), Abbas et al. (2011), and Mubarak et al. (2017) have concluded that rice bran is ideal for live feed cultivation that provides several nutrients and energy sources, including protein (12-13%) and lipid (16-20%).

The results of nutritional properties of rice bran reported by Choi et al. (2011) is approximately is in agreement with the amount obtained, which was a protein of 12-21% and lipid of 4-20%. The levels of protein and lipid in *Nannochloropsis* sp. also recorded in the range of the previous research documented (50-55%) protein and (24-27%) lipids (Hulatt et al., 2017; Tibbetts et al., 2017). The protein and lipid content in

unenriched *O. rigida* rise with the increasing nutrient elements in the dietary treatments. The quality of dietary enrichments, such as protein and lipid, and the amount of the diets, promote the production of copepod eggs and influence the population size with regards to the copepod diet manipulation (Mitra and Flynn, 2005; Gusmão and McKinnon, 2009; Jónasdóttir et al., 2009; Finiguerra et al., 2013; Manklinniam et al., 2018).

A report by Mubarak et al. (2017) resulted in a high RNA/DNA ratio and protein concentration in live feed culture with rice bran suspension, specifically in *M. macrocopa*. Protein and lipid nutritional contents in rice bran were improved due to the catabolic bacterial interaction or break down of the feed's complex components such as complex proteins into amino acids to produce enzyme protease that utilized by microbes to increase the crude protein content indirectly and provide better digestion of zooplankton during cultivation (Damayanti et al., 2020). Therefore, rice bran is considered the best option for feed culture, which increased population fertility, broodstock percentage, and biomass of *M. macrocopa* (Mubarak et al., 2017). El-khodary et al. (2020) found that the most remarkable differences in total protein and lipids in *C. abyssorum divergens* were obtained when enriched with *Tetraselmis chuii* than soybean meal. The result was supported by the fact that protein providing the body with the energy required for the growth, enzyme metabolism, hormones and antibodies (El-khodary et al., 2020). The present works' findings regarding the nutritional composition of the *Nannochloropsis* sp. diet were in line with El-khodary et al. (2020) and Macias-Sancho et al. (2014), who stated that microalgae have high protein and amino acid profiles relative to other food proteins.

A study by Buttino et al. (2009) showed some of the critical issues in copepod mass cultivation where different copepod species have different dietary requirements in maintaining high populations. Copepod typically relies on live microalgae and yeast in most mass culture conditions (Zaleha et al., 2012; Rasdi and Qin, 2016). However, copepods fed yeast resulted in low PUFAs content relative to fresh algae, which indicates the unfavorable diet for copepods (Hazel et al., 2011). In the current study, *O. rigida* was given different dietary ingredients from different sources equipped with sufficient protein, lipid, and fatty acids for the diet consistency evaluation. As a result, the total fatty acid content of *O. rigida* observed from the experiment was higher than *Artemia* nauplii. Similarly, the same findings were reported by Santhanam and Perumal's (2012b) and Rasdi et al. (2015).

Different types of food used significantly impacted the concentrations of fatty acid in *O. rigida* PUFAs such as ALA, ARA, EPA, and DHA in copepod were essential and influenced by their feed (Chen and Chen, 2012). PUFAs are a vital nutrient implicated in many important marine copepod production and performance, such as growth and reproduction (Nielsen et al., 2021). Kabery et al. (2019) also revealed that SAFA and PUFA content of live feed fed different diets such as poultry manure and food waste would result in variance outcomes.

Interestingly, the EPA and DHA in copepod can be synthesized from ALA as documented in other copepod species such as *Tachidius discipes* and *Tisbe* sp. (Caramujo et al., 2008). Furthermore, Rasdi and Qin (2016) suggested that the cyclopoid copepod *C. kasignete* can convert ALA to EPA and DHA when fed green marine microalgae, similar to the *O. rigida* in the current study. The results showed that *O. rigida* was better supplemented with rice bran and *Nannochloropsis* sp. than other diets. Nevertheless, the challenge of sustaining a sufficient supply due to the algal culture collapse is another problem (Mostary et al., 2007). A good diet for copepods is an essential way that nutrient can be transferred to the larvae, which indirectly increases survival and development. Therefore, there is a need to consider further use of these diet types in the cultivation of copepod.

Conclusions

The findings of this work will contribute crucial information on copepods nutrition and valuable knowledge to guide management, technique and protocols for the effective development of copepods

as live food for fish and crustacean larvae. The growth and life history parameters of *O. rigida* were pretentious by different feeding used in this study. *Oithona rigida* fed or enriched with inadequate nutrients resulted in slower developmental performance. On the contrary, *O. rigida* provided good nutrient content such as high lipid and protein content successfully improving their growth and reproductive performance. The outcomes from this research indicated that all the diet types used could enhance the production of copepod. However, rice bran and *Nannochloropsis* sp. were the recommended diet that facilitates the growth and reproduction in copepods either in a single or a combined diet. In addition, rice bran as a substitution for microalgae can maintain copepod growth and reproduction to overcome the shortage of microalgae supply in copepod culture

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