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Research Article

Effects of using enriched copepod with microalgae on growth, survival, and proximate composition of giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract

Copepods are suitable to be used as a starter feed for prawn or fish larvae in hatchery. Nowadays, farmers are still having problems sustaining growth of *Macrobrachium rosenbergii* production since the use of commercial diets such as micro-pallet. This study was carried out for 30 days to determine the best enrichment formula for copepods. Five dietary treatments with triplicates each were used in this study to test efficacy of enriched copepod (*Oithona* sp.) to *M. rosenbergii* larvae. The enrichments used were *Tetraselmis* sp., *Nannochloropsis* sp., mixed algae (*Tetraselmis* sp. and *Nannochloropsis* sp.), yeast (*Saccharomyces cerevisiae*) and micro-pallet (Dindings Shrimp Feed 2203) diet that acts as the control. Copepods were enriched twice per day (10:00 a.m. and 4:00 p.m.) at 500 mg L^{-1} concentration for each diet. Postlarvae were fed at 6-7% of body mass per day. The growth, survival and proximate analysis of *M. rosenbergii* larvae were conducted to compare effectiveness of each consecutive diet. Higher specific growth rate result of *M. rosenbergii* was observed when fed with copepod enriched with mixed algae 4.96±0.02% and the lowest was when fed with copepod enriched with *Nannochloropsis* sp. 4.58±0.03%. The greatest survival rate for *M. rosenbergii* was seen when fed with copepod enriched with mixed algae 95.16±1.04% compared to that fed with copepod enriched with micro-pallet 89.16±1.89%. Among used enrichments, mixed algae produced the best result compared with other enrichment, especially in the proximate analysis of the *M. rosenbergii* larvae. Mean value of protein, lipid, moisture and ash of *M. rosenbergii* were found to be 70.45±1.22%, 8.55±1.11%, 15.30±0.16%, and 9.05±0.59%, respectively. The results indicated that it is highly recommended to use copepod-enriched with mixed algae to feed giant freshwater prawn larvae (PL5 and above).

Keywords: Copepod, Giant freshwater prawn, Enrichment, Proximate analysis, Microalgae

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Introduction

About 80% of zooplankton represents copepods and it is food source for many marine fish larvae in the ocean. Due to several factors that are important for copepod growth and reproduction related to food quality and environmental conditions, copepods are difficult to be mass cultured. Food quality is the main factor regulating copepod nutrition and production (Drillet *et al.*, 2011). Copepods are zooplanktons that can be enriched by providing better nutrition and are very suitable to use as starter feed to marine or freshwater fish and shellfish in hatchery (Rasdi and Qin, 2018a; Rahmati *et al*., 2020). This makes copepods a suitable group as live feed in aquaculture.

Copepods are live foods containing high levels of fatty acids needed by many larvae of marine fish species since it is the best primarily consumed food by small larvae of aquatic organisms in the ocean. Copepod is superior in nutritional terms compared with other live foods species (Ajiboye *et al.*, 2011). Nutritional content of copepod largely depends on its culture medium, especially on the quality of feed provided to them (Agadjihouede *et al.*, 2014). The dry-weight concentration of protein is much higher in copepods than in other zooplankton species which is very important for growth and survival of larvae. Other live foods even after enrichment are still incomparable to copepods in terms of nutritional quality (Hamre *et al.*, 2013).

In general, *Artemia* gives low growth and survival rate compared to marine copepod that boost growth and survival of tiger shrimp *Penaeus monodon* (Ananthi *et al.*, 2011). Marine copepods are nutritionally superior for marine fish and crustacean larvae, and are valuable source of proteins, lipids, carbohydrates, amino acids, fatty acids and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval digestion (Kumar *et al.*, 2017). *Artemia* nauplii have a low nutritional content compare to copepod even though *Artemia* is easy to produce in large numbers. Previous studies have compared the growth and survival of seahorse fed with enriched-copepod and enriched-*Artemia* with Super Selco. Growth and survival of seahorses were significantly higher when fed copepod nauplii (Zhang *et al.*, 2015). Based on Payne and Rippingale (2000), *Artemia* nauplii showed a negative result and copepod nauplii were well digested by juvenile seahorses compared to *Artemia* nauplii. Enriching copepods with high HUFAs make them more suitable for feeding young fish or prawn larvae (Sargent *et al.*, 1997; Naman *et al.*, 2021). Hence, survival in *M. rosenbergii* may improve by feeding them enriched-copepod.

M. rosenbergii post-larvae culture is still an important and recent activity in many countries, where there is little research on giant freshwater prawn nutrition and methods to improve the growth and survival rates through enrichment and production of live food. The latest study has shown that highly unsaturated fatty acids (HUFA) are very important for many fish species (Chang and Southgate, 2001). Nutritional composition and environmental conditions are factors that affect growth and survival rate of fish larvae in many cases. Production of digestive enzymes develops an efficient digestive capacity in the fish body (Ikpegbu *et al.*, 2011).

Availability of high nutrition, suitable consumed diets and efficiently digested diet would promote the success of larval rearing in a particular fish species (Giri *et al.*, 2002). In general, higher proteins, lipids and highly unsaturated fatty acids are required for early stages of *T. tambroides* for growth, survival and neural development. The study on the feeding of freshwater larvae using *Artemia*, *Moina* and *Daphnia* have been done due to their height in protein sources for larval development, and their broad spectrum of digestive enzymes and fatty acids (Macedo and Pinto-Coelho, 2001; Yuslan *et al.*, 2021). Independently of their high nutritional value, other than easy to digest, live feed is the best starter feed as it is easy to detect and capture by fish or prawn larvae due to its swimming movements in the water column (Conceição *et al.*, 2010).

It is important to use live food to improve the success of larval fish rearing. In most cases, live food such as copepod, *Artemia*, and rotifers provide adequate nutrients for finfish and crustacean larvae (Drillet *et al.*, 2008). However, for the good improvement of growth and survival of larvae reared in the hatchery, commercially live used food nutrition such as rotifer and *Artemia* are still not enough for the size and nutrition required by certain species of fish and prawn larvae to enhance their growth performance (Solgaard *et al.*, 2007). More specifically, low survival and high deformity will occur with inadequate nutritional supply provided during these early stages of development (Rajkumar and Kumaraguru Vasagam, 2006).

At present, live feeds such as *Artemia* and rotifers have been used widely in the culture of fish larvae where enrichment of *Artemia* and rotifers is commonly done using the oil emulsion method containing various amounts of DHA ethyl ester and EPA ethyl ester (Samat *et al.*, 2020). However, some fish larvae require specific nutrients such as a higher content of PUFA. Even after artificial enrichment using emulsified oils and other common enrichment products, rotifers and *Artemia* are still nutritionally inadequate and cannot provide adequate nutrition for some fish larvae compared to enriched copepod (Rasdi *et al.*, 2016).

The consumption of copepods can improve the survival of prawn larvae since copepod is better regarding its appropriate size for ease of consumption by prawn larvae (Drillet *et al.*, 2006). The nutritional content, digestibility, and size of the offered food are the factors for successful rearing of early development stages of fish or prawn larvae because proper

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nutrition is considered as the prime factor in providing better digestion, growth and survival of fish in the ocean (Lavens and Sorgeloos, 1996). The aim of this study was to determine the best enrichment formula for cyclopoid copepod (*Oithona* sp.) that could stimulate better growth and survival to *M. rosenbergii* post-larvae. Besides, this study was designed to evaluate the proximate composition of copepod and *M. rosenbergii* post-larvae after feeding with enriched-copepod.

Materials and methods

Sampling and copepod stock culture

Oithona sp. was sampled from the lagoon of Setiu Wetland, located in the state of Terengganu, Malaysia (5’37’’30.64N, 102’47’’15.08E). Habitat condition factors, such as temperature and salinity of sampling area were recorded. Normal habitat condition for optimal temperature being at 26-27°C and the optimal salinity at 24-26 ppt (Kassim and Busra, 2012) were maintained until the end of the experiment. The copepod was cultured and sustained at hatchery of Universiti Malaysia Terengganu (UMT). As a stock culture, the copepod was first cultured in a 1000L tank. Daily observation and feeding were provided. Apart from that, once in two days, 30% of the water was changed in order to avoid any contamination and toxicities from food given.

Experimental design and diets preparation

The diets for copepod were prepared. These adult copepods were isolated from stock and transported to other culture tanks (500 L) and fed for one generation with the specified food before data was collected to remove any consequences of previous feed regiments on copepods (Rasdi and Qin, 2018b). The copepod culture was closely monitored for egg reproduction and hatching of new nauplii. Next, the copepod was harvested and placed into four enrichment tanks for 6 hours before fed to the prawn larvae (Esmaeili Fereidouni *et al.*, 2013). Copepodites of the copepod were enriched using four different treatments. Four treatments consisted of *Tetraselmis* sp., *Nannochloropsis* sp., mixed algae (*Tetraselmis* sp., and *Nannochloropsis* sp.), yeast (*Saccharomyces cerevisiae*) and micro-pallet (Dindings Shrimp Feed, 2203) diet that acted as the control (Lananan *et al.*, 2013; Kasan *et al.*, 2020). Microalgae were cultured at plankton lab of UMT and were upscaled and maintained. The quality of the medium (Conway medium) used for cultivation was to determine the growth performance of microalgae (Lam and Lee, 2012). The concentration of the used algal diets was 500 mg L^{-1} which is equivalent to 2×10^{7} algal cells mL^{-1} (Kassim and Busra, 2012). For mixed algae, which are *Tetraselmis* sp. + *Nannochloropsis* sp., 1:1 ratio was used. Copepods were fed yeast at the concentration of 500 mg L^{-1} (Paray and Al-Sadoon, 2016).

Culture of M. rosenbergii post-larvae

Post-larvae of *M. rosenbergii* (PL5) were purchased from a hatchery in Aquatrope UMT, located in Kuala Terengganu, Malaysia. The post-larvae were transported to the hatchery for test feeding. For feeding experiments, 200 *M. rosenbergii* post-larvae (PL5) were cultured in each 25L aquarium with 20L of water. Gentle aeration was provided for each tank using the aeration system that was prepared in the hatchery. Five dietary treatments with three replicates each were used in this study to test efficacy of the enriched-copepods to the prawn larvae. 50 adult copepods (individual(s) /larvae) were fed twice per day (10:00 a.m. and 4:00 p.m.) with different diets for 30 days (Chakraborty *et al.*, 2001; Redzuari *et al.*, 2012). The water exchange was done for 50% every week and the water quality were maintained at the temperature of 26 -27°C, salinity of 0-6 ppt and pH of 3-5. The post-larvae were fed at 6-7% of body mass per day (Balaji *et al.*, 2002).

Growth and survival of M. rosenbergii post-larvae analysis

The body weight of post-larvae was measured every six days. Ten post-larvae were sampled randomly for every treatment and replicate. Samples were weighed to calculate the growth rate of post-larvae. Specific Growth Rate (SGR) was calculated using the formula: (ln Wt – ln Wi x 100) / t. Where, Wt=final weight (g), Wi=initial weight (g), t=time (days).

For the survival rate, mortality of post-larvae was recorded every day. Survival rate was calculated using the formula:

Survival rate = [(Total number of surviving fish) / Total number of initial prawn larvae density in the tank] x 100% (Mandal, 2010).

Proximate analysis of copepod and M. rosenbergii post-larvae

Weight of the sample was measured. Samples were packed in separate polyethene bags, labelled and stored in freezer at -20°C for further laboratory analyses. Protein, lipid, moisture and ash were analysed at Department of Fisheries, laboratory of Universiti Malaysia Terengganu. Methods used are as follows:

Estimation of Protein:

Total protein content of fish was identified by Micro-Kjeldhal method (Kirk *et al.*, 1991).

Percentage of protein was calculated by multiplying percentage of N_2 (%) with an empirical factor of 6.25. Protein (%) = % of total amount of $N_2 \times 6.25$.

Estimation of Lipid:

Total lipid content of prawn was estimated by method of Bligh and Dyer (1959).

Percentage of lipid calculated by: Percentage of fat (%)=(weight of the extract/weight of the sample) × 100.

Estimation of moisture:

Moisture content of the prawn, *Macrobrachium rosenbergii* was estimated using hot air oven method (Jain and Singh, 2000).

Percentage calculated by: Percentage (%) of moisture=(weight loss/actual weight of the sample)×100.

Estimation of Ash:

Ash content of the prawn was evaluated by the method of Association of Official Analytical Chemists (AOAC, 1991).

Percentage of ash content was estimated by: Ash (%)=weight of ash/weigh of sample × 100. *Data analysis*

Data were presented as mean±standard deviation (SD). All the data were collected throughout the experiment and analysed using a one-way analysis of variance (ANOVA). This was in order to compare growth, survival and proximate composition of *M. rosenbergii* post-larvae and compare the effectiveness of each consecutive diet. Differences were considered significant at $p<0.05$ level. When the main treatment effect was significant, post-hoc comparisons were made using Tukey's test. All data were tested for normality, homogeneity and independence to satisfy assumptions for ANOVA.

Results

Specific Growth Rate

Figure 1 shows the overall specific growth rate of *M. rosenbergii* post-larvae in each treatment after being cultured for 30days. The highest growth rate of *M. rosenbergii* post-larvae occurred when larvae fed with copepod enriched with mix algae (4.96±0.02%), compared with other enrichments such as *Tetraselmis* sp. (4.75±0.16%), yeast (4.79±0.05%) and micro-pallet (4.66±0.16%). *Nannochloropsis* sp. (4.58±0.03%) showed the lowest larvae growth. However, all treatments were significantly different among each other ($p<0.05$).

Survival Rate

There was significant difference among the dietary treatments ($p<0.05$). *M. rosenbergii* post-larvae fed with copepod enriched mix algae showed the highest survival rate compared to other diets of *Tetraselmis* sp. (93.33±2.51%), *Nannochloropsis* sp. (93.00±1.03%) and yeast (90.66±1.75%, Fig. 2).

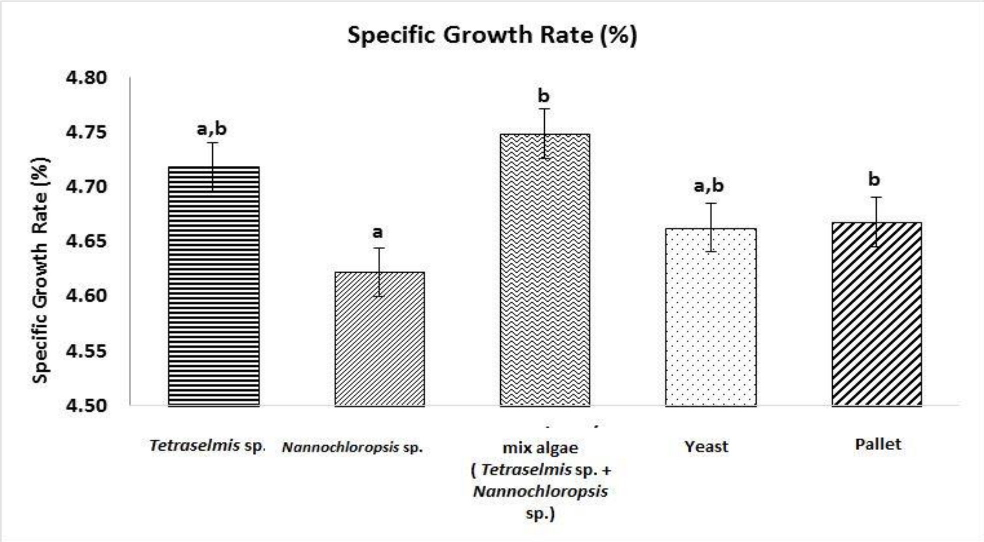

Figure 1: Specific Growth Rate (SGR) of *Macrobrachium rosenbergii* post-larvae fed with different enriched copepods and diets. Different small letters indicate significant difference among treatment, error bars show standard deviation.

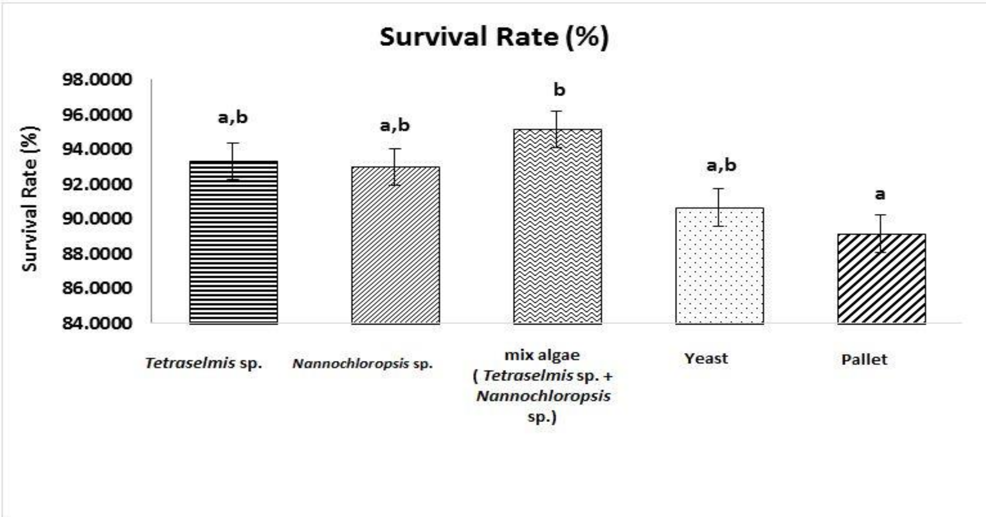

Figure 2: Survival rate of *Macrobrachium rosenbergii* post-larvae fed with different enriched copepods and diets. Different small letters indicate significant difference among treatments, error bars show standard deviation.

However, the lowest survival rate was recorded when *M. rosenbergii* being fed with copepod enriched micro-pallet (89.16±1.89%). Therefore, all treatments were significantly different among each other (p<0.05).

Proximate Analysis of M. rosenbergii Post-larvae

Table 1 shows the results for proximate analysis of *M. rosenbergii* post larvae. Post-larvae fed with copepod enriched with mix algae showed the highest content of protein, lipid, moisture and ash (70.45±1.22%, 8.55±1.11%, 15.30±0.16% and 9.05±0.59%), respectively.

Table 1: Body composition of *M. rosenbergii* fed with different enrichment. Different small letters indicate significant difference among treatments.

Body Composition	Enrichment	Mean±SD (%)
Protein	*Tetraselmis* sp.	65.66 ± 1.09 [a]
	Nannochloropsis sp.	67.62 ± 0.78 [a,b]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	70.45 ± 1.22 [c]
	Yeast	66.28 ± 0.92 [a]
	Micro pellet	69.97 ± 2.18 [c]
Lipid	*Tetraselmis* sp.	7.82 ± 1.00 [a, b]
	Nannochloropsis sp.	6.21 ± 0.63 [a]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	8.55 ± 1.11 [b]
	Yeast	6.53 ± 0.45 [a,b]
	Micro pellet	8.43 ± 0.52 [b]
Moisture	*Tetraselmis* sp.	10.54 ± 1.08 [a]
	Nannochloropsis sp.	13.85 ± 6.48 [a]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	15.30 ± 0.16 [a]
	Yeast	10.53 ± 0.92 [a]
	Micro pellet	14.42 ± 0.97 [a]
Ash	*Tetraselmis* sp.	8.60 ± 0.32 [a]
	Nannochloropsis sp.	8.15 ± 0.64 [a]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	9.05 ± 0.59 [a]
	Yeast	8.44 ± 0.24 [a]
	Micro pellet	8.86 ± 1.44 [a]

Proximate Analysis of Diets, Unenriched Copepod, and Enriched Copepod.

Based on previous studies, the proximate of different used feeding were tabulated in order to compare with the results of the present study. According to Table 2, micro pellet (53.8%) contained the highest protein content compared with other diets, followed by yeast (46.11%), *Nannochloropsis* sp. (35.8%), mix diet of microalgae (33.7%), and *Tetraselmis* sp. (25.7) respectively. Previous results on the proximate analysis of *Oithona* sp. (Table 3), showed greater variations in protein content with 69.24%. The lipid content was 15.36% and moisture and ash contents were 83.07% and 3.96% respectively (Santhanam and Perumal, 2012). However, in our observation (Table 4), protein and lipid contents of enriched copepod were comparatively higher than unenriched copepod. The proximate analysis of copepod observed that copepod enriched with mix algae contained the highest content of protein and lipid (71.54±1.03% and 10.48±2.22%) respectively. Compared to other diets, micro pellet showed the lowest content of protein, 63.15±0.52%, and lipid, 6.05±4.55%. Therefore, the body composition of copepod depends on the type of provided enrichment (p>0.05, Table 4).

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Table 2: Proximate analysis of different enrichments used to enrich copepod, *Oithona* sp.

Body composition	Protein	Lipid	Moisture	Ash	Reference
Tetraselmis sp.	25.7	9.4	nr	nr	
Nannochloropsis sp.	35.8	11.7	nr	nr	Arkronrat *et al*., 2016
Mix diet (*Tetraselmis* sp. + *Nannochloropsis* sp.)	33.7	10.8	nr	nr	
Yeast	46.11	1.51	4.37	10.20	Olvera-Novoa *et al*., 2002
Micro pellet	53.8	30.5	nr	8.4	Rust *et al*., 2015

*nr: data was not recorded

Table 3: Body composition of unenriched cyclopoid copepod, *Oithona* sp.

Body Composition	Mean ± SD (%)	Reference
Protein	69.24 ± 0.070	Santhanam and Perumal, 2012
Lipid	5.36 ± 0.103	
Moisture	83.07 ± 0.075	
Ash	3.962 ± 0.081	

Table 4: Body composition of copepod-enriched with different enrichments. Different small letters indicate significant difference among treatment (*p*<0.05).

Body Composition	Enrichment	Mean±SD (%)
Protein	*Tetraselmis* sp.	66.36 ± 2.47 [a,b]
	Nannochloropsis sp.	67.89 ± 1.20 [b,c]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	71.54 ± 1.03 [b,c]
	Yeast	68.95 ± 0.52 [c]
	Micro pellet	63.15 ± 0.31 [a]
Lipid	*Tetraselmis* sp.	8.55 ± 1.11 [a]
	Nannochloropsis sp.	7.06 ± 6.90 [a]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	10.48 ± 2.22 [a]
	Yeast	6.52 ± 0.45 [a]
	Micro pellet	6.05 ± 4.55 [a]
Moisture	*Tetraselmis* sp.	10.54 ± 1.08 [a,b]
	Nannochloropsis sp.	13.85 ± 6.48 [a,b]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	8.99 ± 3.58 [a]
	Yeast	10.53 ± 0.92 [a,b]
	Micro pellet	14.42 ± 0.97 [b]
Ash	*Tetraselmis* sp.	11.90 ± 5.59 [a]
	Nannochloropsis sp.	4.08 ± 0.31 [a]
	Mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.)	9.13 ± 0.93 [a]
	Yeast	7.25 ± 3.08 [a]
	Micro pellet	9.08 ± 1.30 [a]

Discussion

Post-larval stages of many shrimp species require feed with high nutritional content for development and continuous rearing in a hatchery and it is important to produce live food with high nutritional quality for feeding. The nutritional requirements of the copepods as a starter diet (Singh *et al.*, 2019) for *Macrobrachium rosenbergii* have to be fully understood in order to sustain growth and ability to be produced on massive scale. The results showed that microalgae were the best enrichment for copepod to improve the growth and survival rate of *Macrobrachium rosenbergii* post-larvae.

From the results (Fig. 1), it is observed that the Specific Growth Rate (SGR)

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and survival rate of post-larvae of *M. rosenbergii* were better fed with copepod-enriched with mix algae compared with other enrichments and diets such as *Tetraselmis* sp., *Nannochloropsis* sp., and yeast. The provided diets affected Specific Growth Rate (SGR) of *M. rosenbergii* post-larvae (*P*=0.03, Fig. 1). Apart from all treatments, micro pellet showed the lowest growth of post-larvae. To improve the nutritional content of copepod, algae are primary food for the advancement of copepod (Rasdi *et al.*, 2018). Several reports have shown *P. monodon* larvae fed with a mixed diet of *Chaetoceros muelleri* and *Thalassiosira suecica* performed well (D'Souza and Blackburn, 2013). Consistent with the literature, this research found that the survival and development of *M. rosenbergii* post-larvae were better on copepod-enriched mixed algae as a diet.

From the results (Fig. 2) it is clear that post-larvae of *M. rosenbergii* had high survival rate when fed with copepod-enriched mix algae. Both marine algae *Tetraselmis* sp. and *Nannochloropsis* sp. are generally high in EPA that can enhance growth and survival rate of many species (Rasdi and Qin, 2015). This study supports evidence from previous observations by De Araujo and Valenti (2007) that suggest that use of *Artemia*-enriched with microalgae during larval development of *M. americanum* can enhance growth and improves survival due to better nutrition after enrichment. As stated by Rasdi and Qin (2018a), improvement of the nutritional content of copepod enriched with microalgae can be used to increase the survival rate of post-larvae in aquaculture. Besides, the lowest result for survival rate was post-larvae feed with micro-pallet. The digestibility and nutritional quality of formulated diets were not as efficient as a starter food for fish larvae (Carneiro *et al.*, 2003). The nutritional composition in pellet was not enough to cover the required levels by larvae for growth. The protein composition in a micro-pallet was lower than the content of protein in enriched-copepods such as *Tetraselmis* sp. and *Nannochloropsis* sp. Therefore, use of pallet as starter feeding to prawn larvae will also indirectly increase ammonia level in the water (Coutteau and Mourente, 1997). High ammonium content in the water can be toxic for aquatic animals. It is difficult for aquatic organisms to sufficiently excrete the toxicant, leading to toxic build up in internal tissues and blood that can cause mortality.

Proximate analysis was done to compare the body composition of *M. rosenbergii* post-larvae after fed with differently enriched copepod. Post-larvae fed with copepod that was enriched with mix algae showed highest percentage of protein compared to other enrichments. Then, the post-larvae fed with copepod-enriched *Tetraselmis* sp. showed the lowest percentage of protein. Previous early studies on the rearing of *M. rosenbergii* showed an optimum dietary protein level ranged from 15 to 80% (Mai *et al.*, 2009). It is supported by Ben-Amotz *et al.*, (1987) that studied on *Penaeus japonicus*. They concluded that the diet of less than 10% protein was low in HUFA content that supported growth and survival of *Penaeus japonicus* larvae. Protein content below 15% can cause mortality during rearing of post-larvae as protein is the first and foremost factor that has to be considered for formulating a fish feed. However, excess protein in the diet may also inhibit growth (Lim *et al.*, 2003). The excess protein would be metabolized by the shrimp as a source of energy, and nitrogen will be excreted as ammonia. These studies are in agreement with those obtained by the previous, 70% content of protein in body composition of *M. rosenbergii* is regarded to be the best result when post-larvae fed with copepod-enriched mix algae.

Based on a previous study of Hilton *et al.* (1984), the freshwater prawn requires 6% to 10% lipid. Furthermore, based on that study, they also observed that lower lipid content (>10%) can causes reduction in growth for post-larvae of *M. rosenbergii*. Therefore, based on proximate analysis that is done, the shrimp that was fed with high lipid content food sources had significantly more lipid and carbohydrate than others. In addition, this is also supported by findings, which report that proximate analysis of *P. monodon* larvae fed with mix algae that contain high lipid content produce highest lipid and carbohydrate content (D'Souza and Loneragan, 1999). Protein and lipid were found to be the major biochemical component in the cultured copepod as a feeding for larvae and crustaceans (Santhanam *et al.*, 2004). Copepods fed with manipulated diets were found to contain remarkable biochemical characteristics (Evjemo *et al.*, 2003) and were capable of stimulating appetites and feeding in larvae (Olivotto *et al.*, 2011). Based on the result (Table 4), copepod fed with single diet of *Nannochloropsis* sp. showed the lowest percentage of lipid content, relatively produce low population growth and reproductive performance, which was mainly due to derived low DHA and other HUFAs content of copepods (Rasdi and Qin, 2015). Therefore, the best result for lipid is prawn fed with copepod-enriched mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.). Copepod-enriched mix algae contain high in lipid. Thus, it makes prawn post-larvae absorb enough lipids for their energy and growth. Roughly the enrichment of copepod using mix algae suitable to use in aquaculture, increased the lipid content for improvement of growth and survival.

In the study, the moisture and ash content were maximum in body composition of post-larvae fed with copepod-enriched mix algae. Meanwhile, the minimum percentage of moisture was obtained in post-larvae fed with copepod-enriched yeast. Therefore, for metabolic process, minerals such as, calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur are inorganic elements required in the body. Calcium is required for exoskeleton formation, muscle contraction, and osmoregulation (Moss *et al.*, 2019). Shrimp are able to absorb calcium directly from the water, and shrimps living in seawater do not need calcium supplements in the diet (Davis and Gatlin, 1996).

In conclusion, growth, survival and proximate composition of *M. rosenbergii* post-larvae were affected by enrichment of copepod and provided mix diets. From the result, mix algae (*Tetraselmis* sp. + *Nannochloropsis* sp.) were the best enrichment for copepod to increase the growth and survival rate of *M. rosenbergii* post-larvae, and for obtaining a better feed conversion ratio for larvae rearing. It is followed by *Tetraselmis* sp., *Nannochloropsis* sp., micro-pallet and yeast which showed the slowest growth and survival for *M. rosenbergii* post-larvae. The micro-pallet showed the lowest survival rate on *M. rosenbergii* post-larvae among the dietary treatments. In general, feeding on live microalgae and yeast can improve the mass culture of copepods. However, selection of suitable microalgae species that are high in nutrients is important, as the nutritional profile differs among algal species. Many types of algae have been offered to copepods as food, but there is a need to identify algae with high content of essential nutrients, such as fatty acids (Rasdi and Qin, 2018c). Copepods fed microalgae have shown positive results on growth, reproduction and biochemical composition of copepods depending on the algal nutritional content fed to copepods. The mix algae used as enrichment to copepod can reduce the cost in aquaculture since it can increase the growth and survival of larvae rearing. However, other enrichment and diet still can be used in larvae rearing culture.

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