


Different dietary effects on growth and reproduction of freshwater zooplankton *Ceriodaphnia cornuta* (Sars, 1885) and its potential use in *Pangasius nasutus* larval rearing

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Abstract In an aquatic habitat, zooplankton organisms are an extremely valuable resource for aquaculture purposes. Potential zooplankton species (*Ceriodaphnia cornuta*) have been further explored to evaluate their applicability to feed fish larvae in freshwater aquaculture. There is a need to find more options other than *Artemia* as live food options in freshwater hatcheries. Thus, this study was conducted by enriching *Ceriodaphnia cornuta* with rice bran (F1), soybean meal (F2), *Chlorella vulgaris* (F3), *Spirulina* sp. (F4), and unenriched *C. cornuta* (control). The result showed that the best growth performance of *C. cornuta* occurred when enriched with an F1 and F2 compared to F3 and F4. Meanwhile, the best initial age of reproduction occurred when enriched with F1+F3 and F3+F4 (3.67 ± 0.57 days and 3.66 ± 1.15 days). Besides, the gross and net reproduction rate of *C. cornuta* differed with other enrichments where F1+F3 has the highest rates (67.66 ± 1.52 offspring/female and 56.32 ± 0.90 offspring/female) compared to other treatments. The generation time for *C. cornuta* was shorter when enriched with F2 (7.11 ± 0.88 days) and longer when enriched with F1+F3 (11.23 ± 0.66 days). Other than that, the best growth performance of *P. nasutus* larvae occurs when fed with F1+F3. This study indicates that F1+F3 constitutes better sources of enrichment to improve the growth performance and life history of zooplankton.

Keywords *Ceriodaphnia cornuta* . Life-table parameters . Zooplankton . Enrichment . *Pangasius nasutus* larvae

Introduction

Live food organisms are a precious aquaculture natural resource in an aquatic habitat. Larval fish in nature predominantly feed on phytoplanktonic and zooplanktonic tiny organisms (Das et al. 2012). Freshwater fish seed development typically hampered by a shortage of food availability. In the early stages of their lives, practically all fish consume plankton (Yannawar, 2022). Cladocera are the most important food component

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in freshwater aquaculture and are significant for fry feeding (Kandathil et al. 2020), either in a hatchery or harvested in the wild for their inherent food value as a natural food source (Gisbert et al. 2022). In the natural food chain, zooplankton is an important part of fish larvae's diet, and it is well known that Cladocera may meet their nutritional needs (Evjemo et al. 2003). Adequate and appropriate farming management depends on natural feed and optimum water quality in the hatchery (Nielsen et al. 2017). Planktons are microscopic organisms that constitute the foundation of all aquatic ecosystem's food chains and food webs (Trombetta et al. 2020). Enriched zooplankton directly impacts fish growth, particularly during the larval stage (Kandathil et al. 2020). Zooplankton is the starter feed for cultivated fish for contributing to growth performance. Fish larvae require small-sized of live food for feeding because it can easily digest and rich in biochemical content (Munirasu et al. 2016).

Among the Cladocera, *Ceriodaphnia cornuta* is found throughout the freshwater and could be considered a good replacement for *Artemia* (Gogoi et al. 2016). An investigation by Begum et al. (2015), states that *Ceriodaphnia* is an essential Cladocera used as fish food. *Ceriodaphnia* are effective consumers of protozoans, and feed on protozoans at higher rates in low-nutrient. *C. cornuta* has the opportunity of culturing in adverse condition (Farhadian et al. 2012). Study by Ajepe et al. (2014) found that *Ceriodaphnia* had higher moisture content and ash content hence, could help in better digestibility for *Ceriodaphnia* by the fish larvae. It was discovered that *Ceriodaphnia* has a great potential as natural food that can produce fast growth rate of *C. gariepinus*, *H. bidorsalis* and *Heteroclaris* spp. larvae as study showed that *Ceriodaphnia* were efficient in catfish larvae rearing and can enhance the aquaculture development in rural areas with little or no access to *Artemia* (Ajepe et al. 2014). Another study by Farhadian et al. (2012), found that the protein and lipid contents of *C. quadrangula* were 54% and 12.3% dry weight, respectively in which these amounts meet a major part of nutritional requirements of fish larvae. Based on the present study by Kandasamy et al. (2020), in aquaculture sector, used of *Diaphanasoma sarsi* and *C. cornuta* are recommended as live food organisms. Paray and Al-Sadoon (2016), concluded that microorganisms derived from organic basal diet induce a higher density of *C. cornuta* population. Moreover, it also had suitable larval growth and survival rate make it as a live food to use in aquaculture industry. This species has a high reproductive rate and the capacity to survive and thrive in crowded culture conditions, making it an excellent live feed for cultivable fish and crustacean larval rearing (Begum et al. 2015; Jusoh et al. 2020; Yuslan et al. 2021).

Catfish species *Pangasius nasutus* has the potential to be grown in Malaysia. Numerous studies have been carried out to determine the efficacy of *P. nasutus* in the aquaculture environment. However, fundamental understanding about its diet and larval development is still rare. Although *P. nasutus* is a great food fish, it is still dependent on wild capture, which has resulted in a decline in natural resources (Iswanto and Tahapari 2011). In many instances, larval fish obtain their nutrition fully or largely from the zooplankton food supplies. Hence, regardless of the fish species, the cultivation of zooplankton is advocated (Fernandez-Jover et al. 2016). Various foods, including phytoplankton and organic base diet, can be fed to zooplankton to enhance their nutritional profile (Rasdi et al. 2020a). Consequently, microalgae and utilization of phytoplankton are too costly to be extensively used as enrichment as a protein source (Guedes and Malcata 2012; Ramlee et al. 2021). Nevertheless, organic by-products could also positively influence growth and dietary requirements (Altaff 2020). Feeding enriched zooplankton to fish boosts their growth performance while minimizing mortality (Chepurkina et al. 2014), promoting the faster development of the digestive tract as well as enzymatic activity (Kamaszewski et al. 2014), and enhancing stress tolerance (Adloo et al. 2012). The supplementation of feed with nutrients to improve the nutrition of zooplankton appears to be a useful approach in this regard (Parakrama et al. 2012; Rasdi et al. 2018).

A variety of enrichment were introduced in fish feeding as it is believed that those had beneficial effects on the growth, physiological activity, stress response and feed consumption (Ahmad et al. 2020; Han et al. 2019; Samat et al. 2021). Khani et al. (2017) and Abdel-Tawwab et al. (2018), study mention that *Chlorella* and *Spirulina* can lowered cholesterol levels indicating their involvement in lipid metabolism in fish, hence also can increase total protein and improve in weight gains. Thus, these enrichments were acquiring significant role in improving the growth and health zooplankton and fish. The enrichment of live food can improve the value and efficiency of aquatic species fed in hatchery conditions by enhancing their nutritional value and efficiency (Naman et al. 2021). The nutritional value of Cladocera can be boosted by enrichment by providing diverse culture media as established for *Artemia* nauplii and rotifers (Loh et al. 2016). It will



also raise the cost of production, although these live foods can be produced quickly and inexpensively in a hatchery (Barad et al. 2017).

This research is critical in developing a good enrichment media for the industrial application of *C. cornuta*, a less expensive alternative to other commercial fish feeds. Considering the increasing demand for live feed by aquaculture practice, it is imperative to develop a freshwater zooplankton culture technique and popular live food for fish larvae that is readily available (Evolubi et al. 2016). The quality of enrichment consumed is a key factor that governs zooplankton populations growth (Choi et al. 2014). This study evaluates the effects of using enrichment diets inferred the life table of *C. cornuta* and growth performance of *Pangasius nasutus*, which can be further used to enhance the live-food production for the aquaculture industry.

Materials and methods

Sampling and cultivation of *Ceriodaphnia cornuta*

Ceriodaphnia cornuta were collected live from Tanjung Mentong locality at Kenyir Lake in Terengganu (N 04°54.031'E 102°43.428') and cultured at the University Malaysia Terengganu Hatchery before being used in research. Zooplankton samples in the sampling region were taken with zooplankton nets (mesh size from 50 to 200 microns). A YSI Multiparameter (Pro1030 Water Quality Instrument, Xylem, USA) equipment was used to determine the water quality at the sampling location. The stock culture was sustained in natural freshwater (dechlorinated freshwater) at suitable temperatures and pH between 24–28 °C and pH 7–8, with slow aeration (Islam et al. 2017). The *C. cornuta* were cultured in the starter tank (100 L) and were fed with yeast (0.6 g/L). After that, half of them were separated into 500L tank once they bloom. The enrichment diets will be ground into small particle (<10µm) by using blender (model: Waring 7011HS) and will be dilute into the water. The *C. cornuta* were harvested and placed into seven different treatments; 0.6 g/L for dry diet and 6×10^7 cell/mL for microalgae (Paray and Al-Sadoon 2016; Wang et al. 2018) and for mix diets, the concentration used is based on 1:1 ratio (Rasdi et al. 2020b). The enrichments were used to enrich *C. cornuta* in 24 hours (Fereidouni et al. 2013).

Experimental design

Two discrete experiments were undertaken. The first experiment was designed to determine the life history stages of *C. cornuta* enriched with different enrichment. Seven treatments with triplicate were designed which is four treatments for single diets, two treatments for mix diets and control; Mono diets, (F1- Rice bran, F2- Soybean meal, F3- *Chlorella vulgaris*, F4- *Spirulina* sp., and Control); Mix diets, (F1+F3- Rice bran + *C. vulgaris*, F3+F4- *C. vulgaris* + *Spirulina* sp). Each group contained 340 ± 20 individuals for each replicate. The rice bran and soybean meal were bought from a fertilizer supplier, Sri Purta Sdn Bhd, at Kedah, Malaysia. The second experiment was designed to determine the growth performance of *P. nasutus* larvae. *P. nasutus* larvae were get from (Three Ocean Sdn. Bhd.) where the size about 1–2 mm (4 DAH). The larvae were randomly divided into 3 groups before the experiment. Each group obtained 3 treatments in triplicate (F1+F3- Rice bran + *C. vulgaris*, F3+F4- *C. vulgaris* + *Spirulina* sp, *Artemia* naupili) (40 individual for each) (Ferosekhan et al. 2020; Naman et al. 2021). The larvae were maintained in 50L aquarium with natural freshwater (dechlorinated freshwater) at suitable temperatures at 27–28°C, pH 6.5–8.5, dissolved oxygen (DO) 6–8 mg/L (Jaapar et al. 2021). The larvae were weighed initially, and the weight gain of larvae increase was measured separately every 10 days until 44 DAH (Thongprajukaew et al. 2019).

Evaluation of life table parameters

Using a zooplankton counting chamber, the average percentage survival of plankton live feed was evaluated every day. 20 neonates of *C. cornuta* were introduced into each of these vessels using a dropper under a measuring microscope (STM7-BSW, OLYMPUS, Olympus Scientific Solutions Americas Corp) at 40x magnification. The live table parameters of zooplankton in each treatment were calculated using the equation by Bergmans (1984) and Allan (1976) to measure the life table of *C. cornuta*. All data were collected at $15 \text{ days} \pm 1.00$, corresponding to the average lifespan (cycle) of *C. cornuta*.



Age of maturation (day) = age at which the first brood appears from female (1)

Longevity (day) = the average number of days of survival female (2)

Gross reproduction rate = $\sum m_x$ (3)

Net reproduction rate = $\sum l_x m_x$ (4)

Generation time (T) = $\sum \frac{l_x m_x x}{R_0}$ (5)

Life expectancy (e_x) = $\frac{T_x}{n_x}$ (6)

Where, l_x = Proportion individual survive; n_x = Number of individual alive; n = Number of zooplankton; m_x = fecundity of specific age (number of neonates spawn per survive female); R_0 = net reproduction rate. X = number of females; T_x = Generation time

Evaluation of growth performance of *Pangasius nasutus* larvae

The best enriched *C. cornuta* resulted from life-history parameters were fed to *P. nasutus* larvae. The parameters of growth performance were obtained according to the study of Thongprajukaew et al. (2019) and Ferosekhan et al. (2020) using the following equations:

SGR (% day⁻¹) = $\left(\frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{day}} \right) \times 100$ (7)

Weight gain (g) = Final weight (g) – Initial weight (g) (8)

Survival rate (%) = $\left(\frac{\text{Survival fish}}{\text{Initial fish stock}} \right) \times 100$ (9)

Fulton's condition factor (k) = $\left(\frac{\text{Weight of larvae (g)}}{\text{length of larvae (cm}^3\text{)}} \right) \times 100$ (10)

Feed conversion ratio (FCR) = $\left(\frac{\text{Feed fed (g)}}{\text{live weight gain (g)}} \right)$ (11)

Data analysis

All the data was recorded and represented as a mean \pm standard deviation using IBM SPSS Version 25.0, 2017. *P. nasutus* larval growth performance was analyzed using one-way repeated measures ANOVA, and Shaffer's modified sequentially Bonferroni technique. All the data in this study were presented using one-way analysis of variance (ANOVA) to obtain the significant differences among enrichment treatments. Post hoc Duncan's multiple comparison tests were performed to analyze whether substantial variations in means between treatments ($P < 0.05$) were used as the significant difference level.

Result

Life history parameters of *Ceriodaphnia cornuta*

Survival rate of *Ceriodaphnia cornuta*

The survival rate of *C. cornuta* fed with different enrichment is shown in Fig. 1. The highest survival rate of



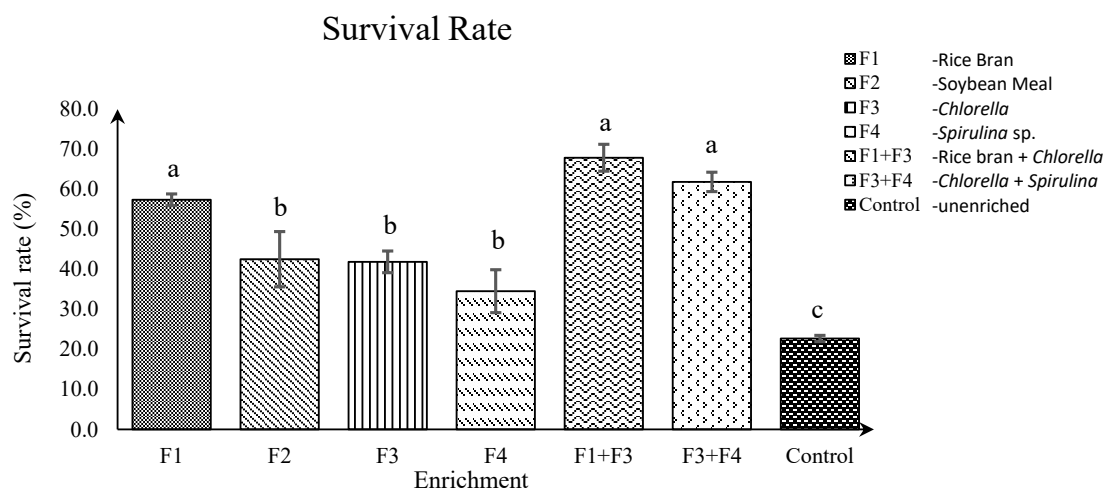


Fig. 1 Survival rate of *Ceriodaphnia cornuta* enriched with different enrichment. Small letters above bar indicate significant difference between treatments ($P < 0.05$)

C. cornuta occurs when enriched with F1+F3 (67.86 ± 5.81 %). In contrast, the lowest population density of *C. cornuta* occurs when no enrichment is given at control (22.71 ± 1.36 %, $P < 0.05$). Other than that, the single enrichment also influences the survival rate, where the result shown that F1 were higher survival rate (57.36 ± 2.51 %) compared to F3 (41.85 ± 4.69 %) and F4 (34.53 ± 9.29 %) at ($P < 0.05$), respectively.

Initial age reproduction and longevity

The first age of reproduction was considerably influenced by enrichment given. Female *C. cornuta* reproduces after being enriched with F1+F3 and F3+F4 (3.67 ± 0.57 days and 3.66 ± 1.15 days) compared to F4 and control (5.67 ± 0.58 days and 6.00 ± 1.00 days, respectively) has a long-delayed time to reproduce. Subsequently, *C. cornuta* enriched with F1, F2 and F3 also has a short initial age reproduction (4.00 ± 1.00 days, 4.67 ± 0.57 days and 4.33 ± 0.57 days, respectively). Apart from that, the highest longevity of *C. cornuta* was 21.00 ± 1.45 days when enriched with F1+F3. The shortest longevity occurs when fed with F2, F4 and control (17.11 ± 0.84 days, 16.89 ± 0.83 days and 15.99 ± 0.57 days, $P < 0.05$) respectively.

Gross reproduction rate and net reproduction rate

The highest gross reproduction rate of *C. cornuta* occurs when enriched with F1+F3 (67.66 ± 1.52 offspring/female, $P < 0.05$). The lowest gross reproduction rate for *C. cornuta* occur when enriched with control (25.00 ± 0.00 offspring/female) compare to others treatment. Furthermore, the highest net reproduction rate shown when *C. cornuta* enriched with F1+F3 and F3+F4 (56.32 ± 0.90 offspring/female and 41.98 ± 3.05 offspring/female) compare to others treatment at ($P < 0.05$).

Generation time and intrinsic rate of population increase

The generation time for *C. cornuta* shorter when enriched with F2 and F4 (7.11 ± 0.88 days and 7.81 ± 1.29 days) compare to F1, F3, F1+F3 and F3+F4 (10.68 ± 0.13 days, 10.26 ± 0.16 days, 11.23 ± 0.66 days and 9.20 ± 0.47 days), respectively. Table 1 showed the greatest intrinsic rate when enriched with F1 (0.14 ± 0.009 days) and F1+F3 (0.12 ± 0.013 days) and F3+F4 (0.12 ± 0.010 days), respectively. On the other hand, *C. cornuta* enriched with F2 and control shows the lowest intrinsic rate (0.07 ± 0.008 days and 0.05 ± 0.012 days), respectively.

Growth performance of *Pangasius nasutus* larvae

The growth performance of *Pangasius nasutus* larvae following a 40-day feeding test is summarized in



Table 1 Life history parameters of *Ceriodaphnia cornuta* fed with different enrichment

Enrichment	Life history parameters					
	IAR (day)	L (day)	GRR (offspring/female)	NRR (offspring/female)	GT (day)	IR (day)
F1	4.00±1.00 ^a	20.45±0.36 ^{ab}	47.00±4.58 ^{bc}	38.18±4.68 ^c	10.68±0.13 ^a	0.14±0.009 ^a
F2	4.67±0.58 ^{abc}	17.11±0.84 ^d	39.33±0.57 ^{cd}	26.07±3.79 ^{cd}	7.11±0.88 ^d	0.07±0.008 ^c
F3	4.33±0.57 ^{ab}	18.97±0.58 ^{bc}	39.32±4.04 ^{cd}	30.62±3.09 ^c	10.26±0.16 ^{ab}	0.11±0.010 ^b
F4	5.67±0.58 ^{bc}	16.89±0.83 ^d	32.67±5.03 ^{de}	22.29±1.53 ^d	7.81±1.29 ^{cd}	0.10±0.011 ^c
F1+F3	3.67±0.57 ^a	21.00±1.45 ^a	67.66±1.52 ^a	56.32±0.90 ^a	11.23±0.66 ^a	0.12±0.013 ^a
F3+F4	3.66±1.15 ^a	18.78±0.83 ^c	49.00±2.64 ^b	41.98±3.05 ^b	9.20±0.47 ^{bc}	0.12±0.010 ^b
Control	6.00±1.00 ^c	15.99±0.57 ^d	25.00±0.00 ^e	15.13±0.62 ^e	7.63±1.16 ^d	0.05±0.012 ^c

All values are mean of seven replicates ± SD. Small letters indicate significant difference between treatments (P<0.05)

Table 2 Development of *Pangasius nasutus* fed with different diets

Growth performance	Diet enrichment		
	F1+F3	F3+F4	Artemia
Weight gain (g)	0.169±0.012 ^a	0.129±0.004 ^b	0.133±0.005 ^b
Survival Rate (%)	56.67±6.29 ^a	51.67±8.03 ^a	29.16±6.29 ^b
SGR (% BW day ⁻¹)	7.47±0.22 ^a	6.80±0.23 ^b	6.89±0.21 ^b
Condition factor	7.60±1.07 ^a	7.77±0.05 ^a	7.86±0.15 ^a
FCR (g Feed g WG ⁻¹)	2.91±0.54 ^a	2.95±0.64 ^a	3.77±0.11 ^a

All values are mean ± SD. Small letters indicate significant difference between treatments (P<0.05)

Table 2. The higher weight gain of *P. nasutus* larvae occurs when fed with *C. cornuta* enriched with F1+F3 (0.169 ± 0.012 g) compared to other treatments. The type of diets affected the survival of *P. nasutus* larvae (P < 0.05). *C. cornuta* supplemented with F1+F3 resulted in a higher survival rate of *P. nasutus* larvae (56.67 ± 6.29 %) compared to F3+F4 (51.67 ± 8.03%) and *Artemia* (29.16 ± 6.29 %), respectively. Hence, the type of diets provided determined the specific growth rate (SGR) of *P. nasutus* larvae. The *P. nasutus* larvae had the highest SGR when fed *C. cornuta* supplemented with F1+F3 (7.47 ± 0.22 % BW day⁻¹). Other than that, result shows that condition factor has approximately high in all treatment (F1+F3; 7.60 ± 1.07, F3+F4; 7.77 ± 0.05, *Artemia*; 7.86 ± 0.15). There is no significant different in condition factors. FCR was impacted when *P. nasutus* larvae, after analyzing one-way ANOVA of repeated measure, there was no significant difference between treatment in FCR over the feed type, $F_{2,7} = 30.334$; P > 0.05. The best ratio of FCR was obtained in the treatment of *P. nasutus* larvae fed with F1+F3 (2.91 ± 0.54 g Feed g WG⁻¹).

Discussion

Cladocera species have been widely examined for both fundamental and practical aspects worldwide. The impact of food abundance, growth, and reproductive characteristics on zooplankton has been investigated extensively (Yuslan et al. 2021). The food quality is available to zooplankton, the most critical element in determining reproduction performance. Among life-history variables, the type of food enrichment generally influences the survival rate. The lowest survival rate occurred when enriched with *Spirulina* sp., which was probably triggered by the dead algae (Ullimaz et al. 2020). Conversely, mixed enrichment results positively, where rice bran mixed with *Chlorella vulgaris* produces the highest survival rate. According to Mubarak et al. (2017) study, rice bran is a suitable feed as it provides high nutrients such as protein (12- 13%), lipid (16-205), linoleic acid (6.35-6.85%), acid α - linoleates (0.2-0.27%), vitamin B and minerals (6 - 9%) that can boost the nutritional content in Cladoceran. Moreover, *Chlorella* contains a beneficial phytonutrient called Chlorella growth factor (CGF), which is rich in nucleic acid associated substances such as peptides, proteins, amino acids, vitamins and vital sugars that attributed to the levels of supplementation above 12.5% brings progressive increase in fish performances (Ahmad et al. 2020). This study reveals that *C. cornuta* results showed a significant survival rate difference among the diets used.

On the other hand, enrichment profoundly impacts Cladocera reproductive variables. Gillooly and



Dodson (2000) established a positive correlation between generation time and initial age reproduction in various cladoceran species. The correlation between the reproductive rates of freshwater zooplankton in terms of generation time and initial age of reproduction is well-documented (Smyntek et al. 2008). It is possible to influence zooplankton by supplementing their diets with nutrients. Sarma et al. (2020) and Damayanti et al. (2020) agreed on this statement. The life history variables of rotifers fed with *Chlorella vulgaris* differed substantially from those of controls in roughly 46% (Sarma et al. 2020). Other than that, *M. macrocopa* culture with 29.30 mg/L fermented rice bran suspension had the maximum fertility and generation of offspring (20–24 ind/adult) (Damayanti et al. 2020). Female *C. cornuta* reproduces more rapidly after being supplemented with F1 (4.00 ± 1.00 days) than control (6.00 ± 1.00 days). In contrast, increasing productivity and reducing initial age reproduction by altering the quality and quantity of diet can further enhance the density of *C. cornuta*. The difference in enrichment influences the life history parameters of *C. cornuta*. Gross and net reproduction rates were greatly affected by the enrichments used. Malej et al. (2021) study concluded that *Ceriodaphnia* sp. maturation delays when fed with microalgae. According to Azuraiddi et al. (2013) study, illustrated that tropical Cladocera could reach maturity as early as day 3, resulting in an increased reproductive rate. The presence of different types of enrichment has a significant effect on cladoceran offspring reproduction. Enrichment type are factors strongly affecting the number of a zooplankton in a community and its life-table parameters (He et al. 1998). This study showed that, all enrichment type influenced on the reproduction of *C. cornuta*, which took a shorter time to become sexually mature before production of the first neonate. This state of affairs indicates that the enrichment type played vital roles in determining the initial age of reproduction. Previously, Alva-Martínez et al. (2007) discovered that most freshwater zooplankton species do not readily consume big colonial, filamentous, or spinous microalgal species. Most zooplankton rely on filamentous algae resulted in slow development and reproduction and has a higher mortality rate (Munirasu et al., 2016). The accelerated reproduction rate seen in cultures supplemented with *Spirulina* powder may be related to the comparatively large algal cells and their filamentous composition, making them unsuitable for *C. cornuta*. Study by Latib et al. (2020), showed the negative growth rate when *C. cornuta* fed with *Spirulina*. Most zooplankton species that feed on cyanobacteria alone tend to grow slower, delayed reproduction, and generally increased mortality (Munirasu et al. 2016).

Furthermore, Zhou et al. (2018) state that, different feeding characteristics influence generation times. Table 1 reveal that the type of enrichment utilized greatly altered the average generation time and intrinsic rate. Rapidly reproducing species had shorter generation times and were less susceptible to population stress. Longer generation times lead to a low intrinsic rate, which can prolong the life of Cladocera (Smyntek et al. 2008). *C. cornuta* had a higher intrinsic rate, which resulted in more neonates. Rasdi et al. (2020) have proven that the nutritional content of zooplankton enrichment is also crucial in ensuring the long-term reproductive rates of the continuous culture across numerous generations. Peña-Aguado et al. (2005) studied abundance levels of various tropical zooplankton using a mixture of microalgae and organic enrichment in the culture medium. The evolution of enrichment methods towards enhancing larvae growth and survival rate requires the exploration of novel potentials to produce live food in fish production. The research outcomes reveal that rearing *P. nasutus* larvae are important and substantially influences their growth and survival. This study shows that *P. nasutus* fed with rice bran mixed with *Chlorella vulgaris* have expressed the best weight gain and survival rate compared to other treatments ($P < 0.05$, Table 3). These findings are similar to Mischke et al. (2021) study, which reported that catfish larvae fed with enriched zooplankton exhibited good growth performance and survival. According to Vu and Huynh (2020) suggestions, feeding pangasius larvae with high nutritional feed resulted in enhanced growth performance and survival of *Pangasius* larvae. High nutritional food for *Pangasius* larvae grown in aquaculture also can increase survival rates. However, when compared to single diets such as microalgae, mix diet such as rice bran mix with *Chlorella vulgaris* is an excellent enrichment for enhance the nutritional in zooplankton. Zooplankton enriched with mix diet can improve the physiological condition of larvae subsequently feeding on the zooplankton by enhancing their nutritional content. Other than that, mix enrichment may have additional benefits such as increasing the concentrations of other trace nutrients in zooplankton, which may further improve growth and survival of aquatic animals (Rasdi et al. 2021). Microalgae were excellent for the live feed but were too pricey for large-scale use. Aquaculture needs were not being met by microalgae growing in mass cultures (Ramlee



et al. 2021). Cost-effective feed is required to reduce the cost of producing zooplankton (Drillet et al. 2011).

Conclusion

This experiment demonstrates that an organic base product mix containing microalgae can be an excellent supplement for the development and sustainability of *P. nasutus* larvae in the aquaculture industry. It can be used as a source of nutrients. *C. cornuta* enriched with rice bran mix with *Chlorella vulgaris* have been demonstrated to positively influence the life history parameters of *C. cornuta* and boost the growth performance of *P. nasutus* larvae depending on the enrichment provided to *C. cornuta*. Additional research is needed to comprehend organic fully- products as enrichments in a live feed to achieve excellent quality. It will also allow the future adoption of various supplement formulas for live feed in hatcheries to ensure fish larvae production.

Authors contributions N.A.-IGSS, data collection, data analysis and writing, review and editing; A.B.A.B., H.J.L. and M.S.K.-GPCS, formal analysis and writing, review and editing; M.M.H. and A.H.M.K.-CVFSC, formal analysis and writing, review and editing, A.A.- data interpretation and manuscript revision, N.W.R.-AOG, review and editing, designing, conceptualizing and supervision.

Ethical statement No animal was intentionally harmed during this experiment were conducted. This experiment was done for aquaculture purpose.

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Competing interests The authors declare that they have no conflict of interest.

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