Songklanakarin J. Sci. Technol.
43 (3), 864-870, May - Jun. 2021

http://www.sjst.psu.ac.th

Original Article

The impact of feeding algae and canola oil on the growth, survival and reproduction of *Moina* sp.♣

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Received: 11 February 2019; Revised: 24 October 2019; Accepted: 14 January 2020

Abstract

The increasing demand for fish produced by aquaculture industries has led to a high demand of live feeds, such as larvae. Further development of culture techniques is important to lower the production costs as well as to maximize the production of natural food for larval rearing. *Moina macrocopa* is a potential freshwater live feed that can replace *Artemia*, which originated from a saline environment. This study aimed to quantify the growth, survival, reproduction and overall life table parameters of *M. macrocopa* when using alternative diets of *Chlorella* sp., canola oil, or a mixed diet (*Chlorella* sp. mix with canola oil). *M. macrocopa* were cultured and enriched with the concentration of 2000 mg L^{-1} in triplicates for each dietary treatment. It was found that there was no difference in the specific growth rate of *M. macrocopa* (P= 0.05) between the alternative diets. The survival rate of *M. macrocopa* fed on the mixed diet (109.97 ± 32.85 %) was significantly higher (P< 0.05) than when fed with *Chlorella* sp. only or with canola oil only. The reproductive performance (e.g. average initial age of reproduction) did not differ from one dietary treatment to another (4.00 ± 0.00 days). For the generation time (54.42 ± 8.75 days) of *M. macrocopa* the mixed diet was the most favorable choice. The intrinsic rate (1.32 ± 0.14 ind L^{-1}) was the highest with *Chlorella* sp. feed. The findings from this study can be used to improve the performance of freshwater fish production in hatcheries by utilizing the highly nutritious *M. macrocopa* larvae as live food.

Keywords: freshwater zooplankton, *Moina*, enrichment, reproductive performance

♣Peer-reviewed paper selected from
The 8th International Fisheries Symposium 2018

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1. Introduction

Nowadays, some larval species' life cycles are under control due to significant changes in zootechnics, prey nutritional quality, and hygienic conditions in the rearing process (Agh & Sorgeloos, 2005). Over the past few years, survival, growth rates and fingerlings efficiency have improved significantly. Nonetheless, there is a need for further efforts to reduce the expense per produced juvenile fish (Planas & Cunha, 1999). Efforts should be directed at improving the rearing cycle of the larvae cultured for fish feed. Despite the recent progress in the production of inert diets for the larvae, feeding of most species of interest for aquaculture still relies on live feeds during the early life stages (Dong, Takao & Mitsushiro, 2006; Rasdi, Suhaimi, Yuslan, Sung, Ikhwanuddin & Yusoff, F.M., 2018).

Independent of their nutritional value, live feeds are easily detected and captured, due to their swimming movements in the water column, and are highly digestible, given their lower nutrient concentration (Bryan, 2008; Jusoh, Kasan, Hashim, Haris, M.F., Mohamed, Rasdi, Abd Wahid, Katayama & Takahashi, 2020). The necessity to use live feed is regulated by many aspects during fish ontogeny but is mainly attributed to the need for a behavioral stimulus to attack particles and for a developed gut to digest them. Live feed production plays an important role in the development of some aquatic organisms, principally larviculture, either as a total or complementary diet. Inert diets like pellets are not totally consumed by aquatic organisms, or do not completely fulfil nutritional requirements of the cultured organisms.

Cladoceran crustaceans, commonly called "water fleas", such as *Moina* sp., are small freshwater zooplankton. The genera of *Moina* sp. and *Daphnia* sp. are closely related. However, *Moina* sp. has special characteristics such as its small size; high reproduction rate; wide temperature tolerance; and being a transparent creature (Loh, 2011, Pronob Das, Sagar Mandal, Bhagabati, Akhtar, & Singh, 2012); with an attractive jerky hopping movement in water. Lately, *Moina* sp. has been considered a potential live feed, encouraged by its availability in most natural water resources (Loh, 2011) and its high nutritional value. This freshwater zooplankton is classified as a cladoceran and, in the aquatic food web, it occupies an important position especially in tropical countries, and inhabits several types of water bodies throughout the world.

According to Das, Tiwari, Venkateshwarlu, Reddy, Parhi, Sarma and Chettri, 2007, *Moina* sp. has been documented as a potential live feed to substitute for the more expensive *Artemia* sp. and other seasonal zooplankton, such as copepods, in larviculture. Moreover, since *Moina* sp. is half the size of *Daphnia* sp. it is suitable as feed for freshwater fish larvae, especially as fit for small and semi-developed mouth sizes of new-borns or newly hatched larvae. Furthermore, *Moina* sp. has a higher protein content than other live feeds, e.g. *Artemia*, thus making it suitable for feeding freshwater fish larvae (Loh, 2011; Sorgeloos & Lavens, 1996). Therefore, the survival of freshwater fish larvae reared with this zooplankton is better than with other natural foods (Shim & Bajrai, 1982). Martin, Arenal, Fajardo, Pimentel, Hidalgo, Pacheco, Carcia and Santiesteban, (2006), found that *Moina* sp. has also been extensively utilized as live feed in many hatcheries, and in the maintenance and culture of aquarium fishes of commercial importance.

At present it has been found that the production of *Moina* sp. in freshwater hatcheries remains scarce in Malaysia and in other countries. This is attributed to a poor capacity for mass production of this potential live feed for the aquaculture industry. Thus, without proper knowledge on successful techniques for *Moina* sp. production at affordable cost, it will not become widely used in hatcheries. Bryan (2008) notes that the enrichment data on *Moina* sp. using a mixed diet (*Chlorella* sp. and canola oil) has not been extensively studied. This means that there are still gaps in knowledge required for developing *Moina* sp. to a practical live feed food supply.

On the other hand, cladoceran culture often offers possibility for producing many individuals quickly under appropriate conditions of temperature, water quality and food availability (Sipaúba-Tavares, Truzzi, & Berchielli-Morais, 2014). In addition, in order to ensure the availability of a diet that can be consumed by the freshwater fish larvae in the future, the *Moina* sp. should be cultured. Moreover, based on a previous study by Rottmann, Graves, Watson, & Yanong, (2003), due to the available information of *Moina* sp. advantages in mimeographed documents, foreign journals, and other publications, the techniques and alternative culture methods must be assessed for *Moina* sp. enrichment to reduce the production costs for economic viability to local farmers.

In this study, we assessed suitable enrichment methods of *Moina* sp. with different dietary treatments. The low nutritional value of *Moina* sp. led us to seek for a better mode of enrichment for this zooplankton (Watanabe, 1993; Das *et al.*, 2007). Specifically, this study aimed to quantify the growth, survival and reproduction of *Moina* sp. fed with microalgae (*Chlorella* sp.), canola oil, or a mixed diet (*Chlorella* sp. mixed with canola oil) by using life table parameters. Therefore, these three alternative diets were used in order to select the optimal food source for the mass cultivation of *M. macrocopa.* Dietary treatments with *Chlorella* sp., canola oil, and the mixed diet were investigated as potential culture media for *M. macrocopa.*

2. Materials and Methods

2.1 Sampling and *M. macrocopa* sp. stock culture

M. macrocopa was sampled around the swamp near Universiti Malaysia Terengganu, Malaysia. The *M. macrocopa* was cultured and sustained with proper feed and daily observation in Universiti Malaysia Terengganu (UMT) hatchery since October 2017. The optimum range of temperature (26°C to 30°C) and water quality parameters, such as pH range from 7.2 to 8.1 and dissolved oxygen of 5.0 ± 0.1 ppm, were maintained. The average ambient temperature was 26°C to 30°C under natural photoperiod (12 h light :12 h dark) (Loh, 2011). The culture was upscaled and maintained for 150 generations. Water exchange was done at 20-30% level to avoid contamination and stress to the cultured *M. macrocopa.* The neonate stages of *M. macrocopa* were used in this study and different mesh size plankton nets were used to separate the adults and neonates of *M. macrocopa.*

2.2 Experimental design and preparation of treatment diets

In this study, microalgae (*Chlorella* sp.), canola oil (Sime Darby Edible Product Ltd., Singapore) and the mixed diet of *Chlorella* sp. and canola oil were used in experiments. The first treatment diet was *Chlorella* sp. The microalgae feed was prepared first prior to the experiment. Water enriched with the algal media (Conway media) (1ml media added to 1 L water) was cultured in 1.5L Erlenmeyer flask with aeration. During collection of samples, battery operated aerator was used to carry the sample from field to the laboratory for isolation. One loopful of *Chlorella* sp. from the best growth obtained above was inoculated in a sterile 15ml test tube with enriched medium (freshwater + modified Conway media: 1/2 ml media to 1L freshwater). After seven days, inoculated *Chlorella* sp. from the test tube was inoculated into 250ml Erlenmeyer flask. The mouth of the flask was closed with cotton wool bung and the flask was placed near a fluorescent lamp. In every stage, the cultures were examined under a compound microscope to observe any possible contamination. The pure stock of *Chlorella* sp. was upscaled to mass culture and maintained at UMT hatchery. This treatment also acts as a control in this study. The concentration of algal feed used is 1×10^6 cell ml^{-1} which is equivalent to 25.03 mg L^{-1}. In this experiment, 2000 mg L^{-1} of algal diet was used to fed *M. macrocopa*. once daily. The water samples with microalgae were collected using 30-micron mesh plankton net.

An emulsion of canola oil was prepared based on methods modified from Agh and Sorgeloos (2005). L-α-phosphatidylcholine (Sigma-Aldrich™, USA), was added to the oil at a ratio of 1:4 (w:w). L-α-phosphatidylcholine was added in order to enable the oil emulsion to become soluble in water so it can be fed to *M. macrocopa.* The mixtures were blended vigorously using an electric blender at its maximum speed for 3 minutes (Loh et al., 2012).

In the mixed diet treatments, the *Chlorella* sp. and the canola oil (that was initially prepared) were mixed together at a ratio of 1:1 (*Chlorella*: modified Canola oil) and homogenized. This produced a stable emulsion, i.e., one in which fats or oils will not rapidly separate from the other elements. Both canola oil and mixed diet were used at 2000 mg/l dose (Loh *et al.*, 2012) for feeding *M. macrocopa.*

The aeration was turned off during selection of neonates to reduce the water current in the tank. Only actively moving *M. macrocopa* (neonates) were selected for this study. *M. macrocopa* were harvested and collected with a suitable mesh size plankton net near the tank edge. Adults were separated in a glass beaker for quantification. For counting, test organisms from 1000ml beaker were transferred by syringe to a petri dish several times and the neonates were counted under a dissecting microscope. After counting, from a 1000ml beaker approximately 350 neonates were transferred to each 15L aquarium for the enrichment treatment. Mild aeration was provided, and dissolved oxygen was maintained at 5.0±0.1 ppm. The water temperature range was 26-30°C and pH range was 7.2-8.1. The experiments were conducted under the natural photoperiod (12 h light :12 h dark).

Subsequently, the survival, growth, reproduction and life table parameters were observed every day for all the treatment diets (Allan, 1976; Krebs, 1985; Loh, 2011; Lotka, 1913). There were three treatment diets with three replications for each. All treatment diets were prepared at concentrations of 2000 mg per liter. The experiment was repeated using the other two treatment diets and the life table parameters were observed.

2.3 Data collection

Survival, growth, and reproduction of *M. macrocopa* were recorded every day. The initial density and final density of *M. macrocopa* were recorded as their survival rate data. The growth of M. macrocopa was observed by measuring its body length, using a microscope profile projector, from the base of the caudal spine to the anterior edge of the head. Then, the growth of *M. macrocopa* was determined from the body length increase over time. In order to get detailed results on *M. macrocopa* reproduction, the life table parameters were recorded and analyzed for reproduction. The life table variables recorded were the initial age of reproduction; survivorship; average longevity; gross reproduction rate; net reproduction rate; generation time; longevity (days); and life expectancy. These life table parameters were used to estimate the net reproduction rate (Ro) and generation time (T), as shown below (Allan, 1976; Krebs, 1985):

1) Net reproduction rate (Ro) = $\sum lx\, mx$

2) Generation time (T) (days) = $\sum lx\, mx \times / Ro$

where;

lx = the proportion of individuals surviving to age, x (survivorship)

mx = the age specific fecundity (number of neonates produced per surviving female at age x)

x = days

The variables related to survival and reproductions were derived based on the collected data, using standard procedures (Krebs, 1985; Krohne, 2001; Molles, 2005) :

1) Survivorship (%) = $\sum l\chi$;

2) Average longevity (days) = $\sum n\chi / n$;

3) Gross reproduction rate (%) = $\sum m\chi$;

4) Life expectancy ($e\chi$) (days) = $T\chi / n\chi$

where;

$n\chi$ = actual number of individuals alive for each age class, n = total number of replicates $T\chi$ = generation time at age χ

The r (intrinsic rate of population increase) was calculated at the end of the experiment and all the data were recorded until the last individual of each cohort died. The data in this study are expressed as mean ± SD. The results of growth, survival, reproduction and life table of *M. macrocopa* were subjected to one-way analysis of variance (ANOVA) to test for significant effects of the alternative types of feed used to enrich *M. Macrocopa.* Further analysis was done using *post hoc* Tukey's multiple comparison test to confirm significant differences of means between treatments for each independent factor (Ferrão-Filho, Fileto, Lopes, & Arcifa, 2003). The threshold level for significant difference was set at ($P< 0.05$). All the data were tested for normality, homogeneity and independence to satisfy the assumptions of ANOVA.

3. Results and Discussion

The cladoceran species have been extensively

studied throughout the world for both fundamental and applied aspects over more than two decades (mid 1980s to 2004) (Nandini & Sarma, 2003). Among the cladocera, *M. macrocopa* has been extensively studied as regards the effects of food abundance, growth, and reproduction parameters (Burak, 1997; He, Qin, Wang, Jiang, & Wen, 2001). The quality and quantity of food are the most important factors determining the biomass production by *M. macrocopa* (He *et al*., 2001; Rasdi, Arshad, Ikhwanuddin, Hagiwara, 2020).

The results showed that *M. macrocopa* fed with the mixed diet had the highest population density and the longest lifespan (Figure 1, Table 1). This high rate of population density growth can be attributed to the feeding preference of *M. macrocopa*. *M. macrocopa* reared under usual pond cultivation conditions contained only 12% oleic acid, and the contents of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) were reported to be 10% and 0.2%, respectively (Lavens & Sorgeloos, 1996). However, according to Ravet, Brett & Muller-Navarra (2003), *M. macrocopa* enriched with canola oil gives the highest growth and fatty acid composition, while the microalgae with high lipid content promote growth and survival of *Daphnia magna*, and high zooplankton growth rates could be attainable when direct dietary HUFAs are available for fast growing cultured zooplankton (Fereidouni, Fathi, & Khalesi, 2013).

Mortality did not occur in any of the treatments during the first 6 days. *M. macrocopa* fed with *Chlorella* sp. began to die on day 9, but those fed with canola oil and mixed diet showed longer survivorship. Some *M. macrocopa* fed with *Chlorella* sp. began dying on day 8. Nonetheless, a stable population was achieved between day 10 and day 14, with complete mortality only on day 15 (Figure 2, Table 1).

The average survival rate (Figure 2, Table 1) depended on choice of food type. *M. macrocopa* fed with the mixed diet showed a lower mortality rate in early and middle life spans, and the death rate shifted to a constant rate on day 14. Survivorship of *M. macrocopa* appeared to be significantly dependent ($P< 0.05$) on the food type. *Moina* sp. fed on mixed diet showed the longest survivorship rate among all the feeding treatments (Table 1). The average longevity of the *Moina* sp. enriched with *Chlorella* sp. and with the mixed diet (13.0 days) was higher than with the canola oil, which only provided survival until day 12.

The average number of neonates produced by the parthenogenetic females was significantly different ($P> 0.05$) between the two treatments and the control group. Females fed with mixed diet showed the highest average fecundity followed by those treated with *Chlorella* sp., and then with canola oil (Table 1). *M. macrocopa* fed with *Chlorella* sp., canola oil and mixed diet started to reproduce on day 4. The peak population was reached on days 7, 13, and 14, with *Chlorella* sp., canola oil and mixed diet, respectively. *M. macrocopa*, fed with the mixed diet, had the highest reproductive rates and produced the greatest number of neonates within the shortest generation time.

The fecundity of *M. macrocopa*, fed with mixed diet was the highest among all the treatment diets (22.81 ± 36.87 ind./day; Table 1), followed by canola oil (19.19 ± 33.71 ind./day), and *Chlorella* sp. (9.85 ± 21.69 ind./day). *M. macrocopa* fed with any of the three treatment diets started to produce offspring on day 4. Fecundity generally declined throughout the experimental period. The generation time of *M. macrocopa* fed with *Chlorella* sp. was slightly lower compared to the other dietary treatments (Table 1).

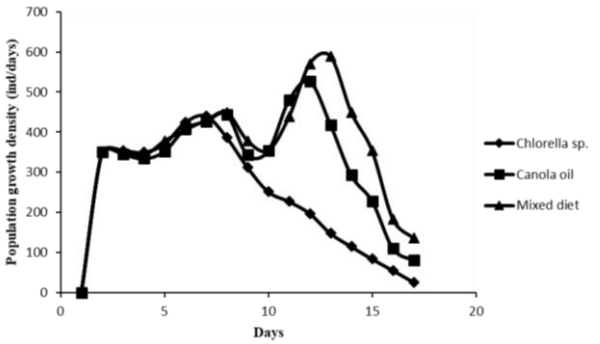

Figure 1. The population growth density of *M. macrocopa* fed with different treatment diets. Having the same character label after all curves indicates that the final states were not statistically significantly different.

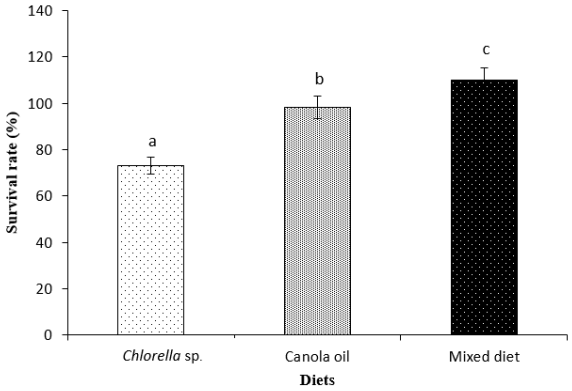

Figure 2. The survival rate of *M. macrocopa* fed with different treatment diets. Different superscripts indicate statistically significant differences ($P< 0.05$).

M. macrocopa showed the ability to reproduce well (net reproduction rate and reproduction span) when fed with the mixed diet (Table 1). The gross reproduction rate was the highest when *M. macrocopa* was fed with the mixed diet (65.33 ± 2.52, $P< 0.05$).

Life table parameters are valuable in studies on the population dynamics of zooplankton. Table 1 shows the mean values (± S.D.) of *M. macrocopa* production fed with different dietary sources. The average initial age of reproduction did not differ between treatment diets tested on *M. macrocopa* (4 days). Average longevity varied between 12 and 13 days, with the maximum (13 days) achieved both with *Chlorella* sp. and mixed diet, and the minimum (12 days) with canola oil.

Diet type and concentration are factors strongly affecting the density of a species in a community and its life history parameters (He, Wang, Cui, Guo, & Qian, 1998, Lampert & Schoeber, 1980). In this study, both diet type and its nutritional value exerted significant influences on the maturation of *M. macrocopa*, which took a shorter time to become sexually mature with a high nutritional value diet before production of the first brood (Table 1). This phenomenon indicates that the diet type and the nutritional value of treatment diet played significant roles in determining the initial age of reproduction (Table 1). In this study,

Table 1. Life table of the cladoceran *Moina macrocopa* fed with different treatment diets. All values are given as mean ± standard deviation (n=3). Different superscripts indicate statistically significant difference within a column ($P< 0.05$).

Treatment diet	Avg Initial age of production (days)	Avg longevity (days)	Survivorship	Net reproduction rate	Gross reproduction rate	Generation time (days)	Life expectancy	Intrinsic rate	Fecundity (ind/day)
Chlorella sp. (control)	4.00 ± 0.00^{a}	13.00 ± 0.00^{a}	13.07 ± 0.19^{a}	73.20 ± 39.14^{a}	59.33 ± 4.93^{a}	5.90 ± 0.60^{a}	0.13 ± 0.06^{a}	1.32 ± 0.14^{a}	9.85 ± 21.69^{a}
Canola oil	4.00 ± 0.00^{a}	12.00 ± 0.00^{a}	14.05 ± 0.11^{b}	98.20 ± 34.48^{a}	60.00 ± 2.00^{a}	6.97 ± 0.17^{b}	0.12 ± 0.03^{a}	1.03 ± 0.03^{b}	19.19 ± 33.71^{b}
Mixed diet	4.00 ± 0.00^{a}	13.00 ± 0.00^{a}	14.56 ± 0.15^{c}	109.97 ± 32.85^{b}	65.33 ± 2.52^{b}	7.25 ± 0.15^{b}	0.13 ± 0.02^{a}	1.13 ± 0.05^{c}	22.81 ± 36.87^{b}

generation time seemed to be influenced by diet type (Table 1). However, population density was significantly affected by the dietary treatment and its nutritional value, where the total clusters of neonates produced by parthenogenesis showed an obvious difference between the treatments. The gross reproduction rate also increased with the low nutritional value diet namely *Chlorella* sp. Analysis showed that growth reproduction rate was statistically higher when *M. macrocopa* was fed with the mixed diet compared to *Chlorella* sp. or canola oil (Table 1).

The net reproductive rate (Ro) (109.97 ± 32.85) and the gross reproduction rate (mx) of *M. macrocopa* (Table 1) enriched with mixed diet were the highest among the treatments. The Ro values obtained for *M. macrocopa* enriched with canola oil (98.20 ± 34.48) or *Chlorella* sp. (73.20 ± 39.14) was distinctly lower than with the mixed diet. Average generation time (To) of *M. macrocopa* ranged from 5.90 to 7.25 days.

The intrinsic rate (r) of *M. macrocopa* ranged from 1.03 to 1.32 days, while the life expectancy (ex) varied between 0.12 and 0.13 days with the treatment diets. Both rates were higher for the mixed diet than for the other treatments.

This study showed that there is no significant difference ($P= 0.05$) in the average longevity of the cladoceran, *M. macrocopa*, among the three treatments, which gave 12.0 days for canola oil, and 13.0 days for *Chlorella* sp., and mixed diet (Table 1).

The effects of different types of diet on the life history variables of *M. macrocopa* indicated that 2000 mg L^{-1} of *Chlorella* sp., canola oil, or mixed diet produced optimal growth and reproduction performance (Table 1). The decline in the neonate production with increasing canola oil concentration was presumably caused by the increased effort associated with food gathering due to the active filtering of the food particles (Nandini & Sarma,2000; Samat, Yusoff, Rasdi, Karim, 2020). High concentrations of the treatment diets did not provide an optimal culturing condition. Therefore, such high levels of particles could lead to the starvation of cladocerans as they are unable to clean their thoracic limbs once they are clogged by high particulate concentrations (Burak, 1997; Nandini & Sarma, 2000). These results corroborate those of Burak (1997), where a decline in the cultivated *M. macrocopa* population at high concentrations of algal diet (*Scenedesmus* sp.) was observed.

The r values ranged from 1.03 to 1.32 and did not seem to differ by the diet type (Table 1). The r values could be explained by the change in population size over a particular period of time and were elevated by the short lifespan and high fecundity of *M. macrocopa* (Stearns, 1976). This trend was clear in this study, particularly with the mixed diet (Table 1). Nandini and Sarma (2003) discussed the population dynamics of some cladoceran genera with regard to the life history variables. They proposed that the r values should be in the range from 0.01 to 1.50. The ratio in the present study ranged from -0.52 to 1.35, which reaches slightly lower than the proposed range. This may be a consequence of different *Moina* species, food type, and temperature used in the previous studies (Deng & Xie, 2003; Nandini & Sarma, 2003).

Alva-Martínez, Sarma & Nandini, (2007) who worked with *C. dubia* fed with an exclusive diet of *Chlorella vulgaris* ($1.0\text{-}1.5 \times 10^{6}$ cell mL^{-1}), obtained r values of 0.1 to 1.5, unlike this experiment which obtained r values of 1.03 to 1.32. Peña-Aguado *et al.* (2005), found different abundance picks in culture medium of many cladocerans, when using mixed diets of microalgae and yeast. In this study, *M. macrocopa* grows better with the mixed diet. This is due to the presence of different nutritional components in the feed for different stages of this *M. macrocopa*. Nevertheless, there was no significant difference between the use of a single microalgae diet or a diet of microalgae mixed with canola oil.

The oil emulsions were not good feed type, as anticipated, for *M. macrocopa* because their high concentrations resulted in a negative effect on the population dynamics of *M. macrocopa* (except for 2000 mg L^{-1} of canola oil, $p< 0.05$). It is therefore essential to balance between the concentration of the oil emulsion needed for an optimum level of enrichment uptake and the maintenance of a stable population of *M. macrocopa* during enrichment. This consideration would ensure effective enrichment of *M. macrocopa* for larval feeding.

In summary, several studies suggest that lipid emulsions can improve the fatty acid content in *M. macrocopa*. Animal and plant-based oil emulsions, such as squid and canola oils, have a competitive advantage in optimizing fatty acid distribution and increasing this zooplankton's n-3: n-6 lipid ratio. Oil emulsions are less expensive than the commercial enrichment diet, A1 DHA Selco®, thus making them a viable alternative for enriching *M. Macrocopa.* Canola oil and *Chlorella* sp. were applied for the enrichment of *M. macrocopa* to be used as a live feed for larviculture. In this study, the life table data for the different diets allowed elucidation of their effects on the life history variables of *M. Macrocopa.* This study demonstrates that mixed canola oil and *Chlorella* sp. may serve as effective and sustainable food source in *M. macrocopa* cultivation. The results of *M. macrocopa*. enrichment were mostly promising for use in the larviculture industry, to maximize the potential of live larval feed in culturing larval fish and crustacean species.

Acknowledgements

The Malaysian Ministry of Higher Education (MOHE) Fundamental Research Grant Scheme (FRGS) with vote number 59530, endorsed this study with the aim of creating new ideas and methods for aquaculture growth in Malaysia.

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