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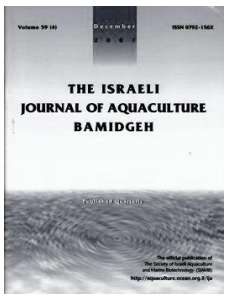

Impact of Potential Food Sources on the Life Table of the Cladoceran, *Moina macrocopa*

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Key words: *Moina macrocopa*, life history, fecundity, survivorship, population dynamics

Abstract

The zooplankton *Moina macrocopa* was cultured with three kinds of emulsified lipid media (Selco®, squid oil, canola oil) and two kinds of fish wastes (fish pellets and fish feces) to determine the impact of these food sources on its population dynamics. All diets were provided at five levels: 0.0625, 0.125, 0.25, 0.5, and 1.0 g/l. For all diets, life history parameters showed the highest values at the lowest concentrations, i.e., <0.125 g/l. Overall demographic performance was better in *M. macrocopa* fed the emulsified liquid diets than *M. macrocopa* raised in the control treatment (dechlorinated water; no diet) but not as good as in *M. macrocopa* fed the fish wastes. Fish feces was excellent for propagating *M. macrocopa*. In conclusion, using fish wastes to cultivate *M. macrocopa* could be an inexpensive and sustainable cultivation approach.

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Introduction

The water flea, *Moina macrocopa* Straus, is a freshwater cladoceran with excellent potential as a live food for larvae of finfish and crustaceans (Alam et al., 1993; Kang et al., 2006). The nutritional value of *Moina* is superior to commercially available newly hatched *Artemia* nauplii (Sorgeloos and Lavens, 1996; He et al., 2001). Although commercial-scale quantities of *Moina* are not readily obtainable from natural habitats, *Moina* can be farmed for use as live food. *Moina* can be easily mass cultivated under varying conditions including low oxygen and high ammonium content (Sarma et al., 2003), as these water fleas rapidly reproduce (Yamasaki et al., 2001) and rapidly grow on a range of food sources, e.g., agricultural wastewater, poultry and cattle waste, and even human urine (Nandini and Sarma, 2003; Golder et al., 2007; Loh et al., 2009; Patil et al., 2010). Hence, the relatively large amount of *Moina* zooplankton that are required for fish larvae cultivation can be produced from inexpensive, renewable waste materials. However, the use of animal fecal matter as a nutrient source in the production of food for human consumption raises hygiene and food safety concerns.

An alternative sustainable source of food for *Moina* is recycled wastes from aquaculture activities. Fish waste and fish feces are acceptable foods for zooplankton since they eliminate specific infection dangers associated with feces of domestic animals and constitute part of the natural diet of zooplankton in the wild. Further, aquaculture systems are generally under constant water quality assessment for environmental protection, which acts as an additional control of the fish wastes and feces produced in such systems. Aquaculture effluents, especially uneaten fish food and feces, contribute some 20-30% of the overall organic waste production from intensive aquaculture (Beveridge, 1987). Most fish farm effluents are discharged directly into waterways without further treatment. Such effluents contain high amounts of nutrients, e.g., phosphorus, nitrogen, and organic components (Kibria et al., 1997), which generally go to waste. The effluents may also contain fish oils and other lipid components, e.g., free fatty acids, polar lipids, and polyunsaturated fatty acids including highly unsaturated fatty acids (Henderson et al., 1997).

Highly unsaturated fatty acids (HUFA) enhance the essential lipid levels of *M. macrocopa* through oil emulsions, e.g., squid oil, canola oil, and commercial products (Loh et al., 2012), and these essential fatty acids promote the growth of other cladocerans (Müller-Navarra, 1995). This study determined the effects of aquaculture effluent materials (fish feces and uneaten fish food) on *M. macrocopa* propagation and population dynamics, in comparison with three commercially available emulsified lipid media (Selco®, squid oil, and canola oil).

Materials and Methods

Moina macrocopa were obtained from oxidation ponds of sewage treatment plants located within residential areas of Kuala Lumpur, Malaysia. Breeding females (1.1-1.6 mm) were isolated by suction using a blunt pipette and placed individually in petri dishes filled with dechlorinated tap water (10 ml/plate) for breeding. Mature *Moina* started breeding overnight after being introduced into the dish. Baker's yeast (Mauri-Pan®) at 1 g/l was added to the petri dishes as a food source during the breeding period. Newborn *M. macrocopa* were collected for subsequent experiments.

Canola oil (Sime Darby Edible Product Ltd., Singapore) and squid oil (Asia Star Laboratory Ltd., Thailand) were obtained in the form of commercial formulated emulsions. A1 DHA Selco® (ADS) was obtained from INVE (Belgium). Emulsified canola and squid oils were prepared based on modified *Artemia* enrichment methods (Estévez et al., 1998; Agh and Sorgeloos, 2005), whereby L-α-phosphatidylcholine (Sigma-Aldrich™, USA) was mixed with the oil products at a ratio of 1:4 (w/w) and the mixture was vigorously blended with warm dechlorinated water (40-60°C) in an electric blender (Waring Commercial®, USA) for 3 min. ADS emulsion was prepared according to the manufacturer's instructions.

Commercial freshwater fish pellets (18-20% protein, 6% fiber, 3% lipid, 11% moisture) were obtained from Cargill Feed Sdn. Bhd. (Malaysia) and used to mimic waste

uneaten fish food. The pellets were ground into fine granules with a mortar and pestle and further pulverized into powder (<0.1 mm) using a blender at maximum speed for 3 min. Fish feces were collected from a recirculating aquaculture system (RAS) containing red Nile tilapia (*Oreochromis niloticus*) in water with dissolved oxygen 6.89±1.04 mg/l, pH 7.36±0.17, temperature 26.60±0.16°C, total hardness 7-14 degrees of general hardness (dGH), ammonia-nitrogen 0.088±0.034 mg/l, nitrite 0.042±0.026 mg/l, and nitrate 23.85±13.48 mg/l. Excess water in the feces was removed by filtration and the semi-solids were heat-dried overnight in an oven at 55°C. Both pellets and feces were weighed, and then blended with dechlorinated water for 3 min at maximum speed.

All diets were prepared at 0.0625, 0.125, 0.25, 0.5, and 1.0 g/l in dechlorinated water. The highest concentration, 1.0 g/l, was based on the standard enrichment protocol for *Artemia* (Agh and Sorgeloos, 2005). Lower concentrations represented two-fold dilutions of this highest concentration.

In the subsequent experiment, *M. macrocopa* neonates less than 24 h old were harvested from the breeding females in the petri dishes. The experiments were carried out in four replicates. Neonates were distributed into petri dishes at 1 neonate/dish. Each dish contained 20 ml of the relevant diet, or dechlorinated water (control). The diets were replaced every two days with fresh media using a pipette. The petri dishes were maintained in well-ventilated ambient temperature (28-30°C) under natural light conditions (12 h light:12 h dark). Newborn *M. macrocopa* were counted and discarded daily throughout the experiment. Mortality and fecundity were recorded to calculate the life table. The experiments were carried out until all *Moina* individuals died.

Life table demographics is an important tool for describing the life cycle of zooplankton under continuously changing environmental conditions (Stearns, 1976). The survivorship, life expectancy, initial age of reproduction, average longevity, net reproduction rate, gross reproduction rate, generation time, and intrinsic rate of natural increase were selected as life history variables for this study (Smith and Smith, 2003; Molles, 2005; Chuah et al., 2007).

The following definitions apply: initial age of reproduction = the time when a female started to produce her first batch of offspring (number of days); longevity = the average number of days the female survived. Survivorship and reproduction were calculated using the standard procedures of Krebs (1985) and Molles (2005): survivorship = $\sum l_x$, where l_x = the proportion of individuals surviving to age χ; average longevity = $\sum n_x/n$, where n_x = number of individuals alive for each age class and n = the number of replicates; gross reproduction rate = $\sum m_x$, where m_x = the age-specific fecundity (number of neonates produced per surviving female at age χ); net reproduction rate (R_o) = $\sum l_x m_x$; generation time (T) = $\sum l_x m_x \chi / R_o$; and life expectancy (e_x) = T_x/n_x, where T_x = generation time at age χ. The intrinsic rate of natural increase (r) was calculated as $\sum l_x m_x e^{-r\chi} = 1$ (Lotka, 1913). The value r was calculated after 21 days and was indistinguishable from the r value of the entire life span of *Moina*. Due to the importance of early reproduction (Van Leeuwen et al., 1985), all calculations were based on 21 days of experimentation.

Results from the treatment diets and concentrations were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test), and were analyzed using two-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) comparison using Minitab (ver. 13) to determine the significance of values and interaction effects among the diet types and concentrations. Statistical significance was accepted at $p<0.05$ or <0.001.

Results

The highest fecundity (net and gross reproduction rate) was obtained with 0.0625 g/l fish feces (Table 1). Most of the *Moina* fed fish pellets consistently produced offspring by day 2 as compared to the other treatment diets (Fig. 1). Large numbers of neonates were produced by *Moina* fed fish pellets (0.125, 0.25, 0.5 g/l), fish feces (0.0625, 0.125, 0.25, 0.5 g/l), canola oil (0.0625, 0.125 g/l), squid oil (0.0625, 0.125 g/l), or Selco (0.0625 g/l). *Moina* fed 0.25 g/l canola oil began a second reproduction cycle on day 6. Fecundity declined at high concentrations for every diet while the highest concentrations

(1.0 g/l) of Selco, squid oil, and canola oil did not lead to the production of offspring. The intrinsic rate of population increase (*r* value) was positive for all treatment diets and concentrations except canola oil at 0.5 g/l, which showed a negative value.

Table 1. Life table of the water flea, *Moina macrocopa*, fed different diets at different concentrations (means±standard deviation, n = 4).

Diet type	*Life history variable*						
	Diet concentration (g/l)	*Avg initial age of reproduction (days)*	*Avg longevity (days)*	*Net reproduction rate*	*Gross reproduction rate*	*Generation time*	*Intrinsic rate of population increase*
Control (no diet)	0	3.00±0.00^{b}	4.50±1.66^{ab}	6.00	7.25±1.50^{b}	3.21	0.56
Selco®	1.0	–^{a}	1.00±0.00^{a}	–	–^{a}	–	–
	0.5	–^{a}	2.00±0.00^{ab}	–	–^{a}	–	–
	0.25	–^{a}	1.75±0.43^{a}	–	–^{a}	–	–
	0.125	2.00±0.00^{b}	2.50±0.50^{ab}	3.25	6.50±1.29^{b}	3.00	0.39
	0.0625	2.00±0.00^{b}	6.00±3.00^{bc}	11.50	11.50±1.73^{b}	2.65	0.92
Squid oil	1.0	–^{a}	1.00±0.00^{a}	–	–^{a}	–	–
	0.5	–^{a}	2.25±0.83^{ab}	–	–^{a}	–	–
	0.25	1.00±1.00^{b}	2.75±0.43^{ab}	1.50	2.00±2.45^{b}	3.00	0.14
	0.125	2.00±0.00^{b}	3.00±0.00^{ab}	10.25	10.25±3.30^{b}	3.00	0.78
	0.0625	2.00±0.00^{b}	3.75±0.43^{ab}	11.69	11.75±4.03^{b}	3.02	0.82
Canola oil	1.0	–^{a}	1.50±0.50^{a}	–	–^{a}	–	–
	0.5	2.00±2.00^{b}	3.00±1.22^{ab}	0.13	0.50±1.00^{b}	4.00	-0.52
	0.25	2.25±1.30^{b}	3.75±0.43^{ab}	2.00	2.00±1.83^{b}	3.00	0.23
	0.125	3.00±0.00^{b}	4.50±1.12^{ab}	15.56	16.50±2.06^{b}	3.06	0.90
	0.0625	2.00±0.00^{b}	5.00±1.22^{ab}	21.75	21.75±3.59^{c}	3.32	0.93
Fish pellets	1.0	2.25±1.30^{b}	2.75±0.43^{ab}	3.94	5.25±3.59^{b}	3.00	0.46
	0.5	2.00±0.00^{b}	3.50±0.50^{ab}	19.25	20.75±5.85^{c}	2.26	1.31
	0.25	2.00±0.00^{b}	4.75±1.79^{ab}	16.75	16.75±2.36^{b}	2.09	1.35
	0.125	2.00±0.00^{b}	5.00±1.22^{ab}	20.00	20.00±3.56^{c}	2.79	1.07
	0.0625	3.00±0.00^{b}	4.75±1.30^{ab}	3.25	3.25±1.71^{b}	3.00	0.39
Fish feces	1.0	1.50±1.50^{b}	2.50±0.50^{ab}	1.38	2.75±3.20^{b}	3.00	0.11
	0.5	3.00±0.00^{b}	11.25±5.80^{c}	13.00	13.00±1.83^{b}	4.00	0.64
	0.25	3.00±0.00^{b}	7.75±4.82^{bc}	10.94	11.75±3.10^{b}	3.45	0.69
	0.125	3.00±0.00^{b}	5.75±1.09^{bc}	25.25	26.50±7.68^{c}	3.54	0.91
	0.0625	3.00±0.00^{b}	7.75±1.09^{bc}	41.88	43.75±7.37^{d}	4.60	0.81

Values in a column with different superscripts differ significantly (p<0.05; Tukey's Honestly Significant Difference test).
Dash (-) indicates no offspring was produced.

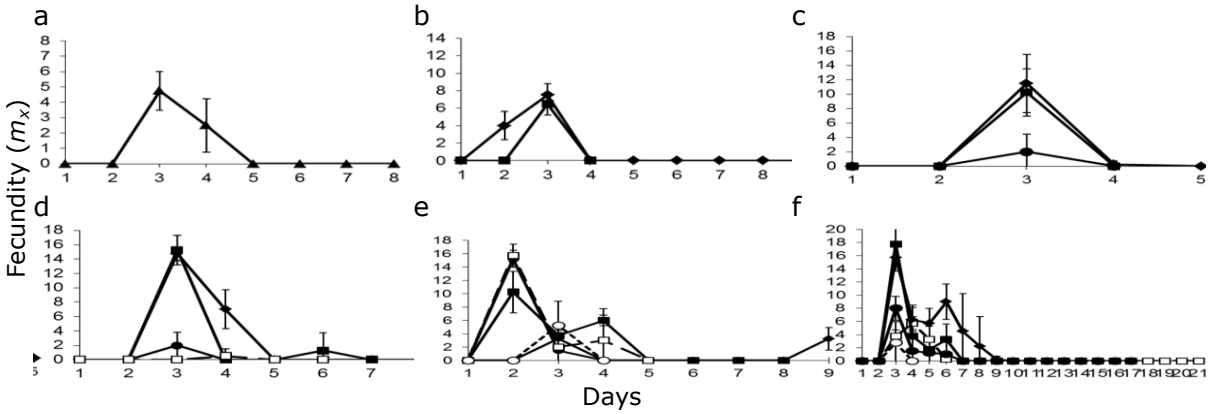

Fig. 1. Fecundity of *Moina macrocopa* in (a) control (no diet), and different concentrations of (b) A1 DHA Selco®, (c) squid oil, (d) canola oil, (e) fish pellets, and (f) fish feces. Error bars indicate means±standard deviation. = control, = 0.0625 g/l; = 0.125 g/l; = 0.25 g/l; = 0.5 g/l; = 1.0 g/l. Permutations of treatment diet type and concentration were tested. Only those that yielded offspring are shown here.

Depending on the food type and treatment concentration, *M. macrocopa* showed Type-I or Type-II survivorship curves (Fig. 2). In the Type-I survivorship curve, there was low mortality in the early and middle life spans, whilst in Type-II curves the death rate was roughly constant with age. Survivorship of *M. macrocopa* appeared to be significantly dependent ($p<0.05$) on food type and concentration. *Moina* fed 0.5 g/l feces had the longest survival, almost three times that of the control that survived only until day 7. Survival of *Moina* fed squid oil, canola oil, or pellets did not significantly differ ($p>0.05$) from the control. Feces at 0.5 g/l was the only treatment in which survivorship was significantly better than that of the control. The same trends were observed in the life expectancy curves (Fig. 3). Except for *M. macrocopa* fed pellets, the cumulative birth rates of organisms fed 0.0625 g/l were higher than that of the control organisms (Fig. 4).

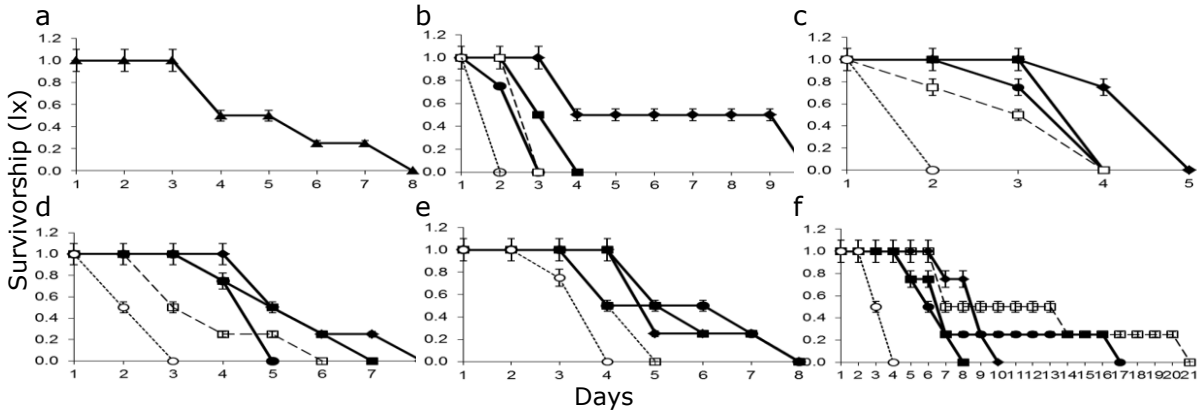

Fig. 2. Survivorship of *Moina macrocopa* in (a) control, and different concentrations of (b) A1 DHA Selco®, (c) squid oil, (d) canola oil, (e) fish pellets, and (f) fish feces. Error bars indicate means±standard deviation. ▲ = control, ◆ = 0.0625 g/l; ■ = 0.125 g/l; ● = 0.25 g/l; □ = 0.5 g/l; ○ = 1.0 g/l.

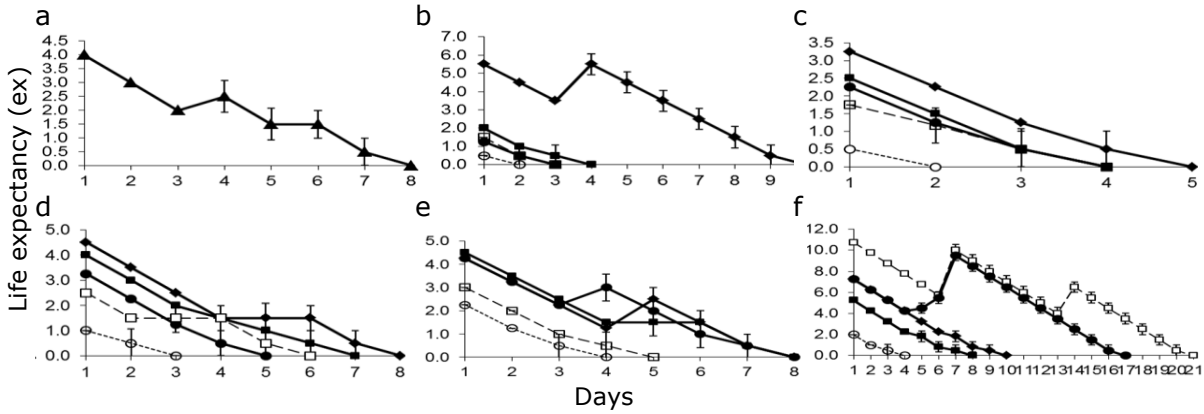

Fig. 3. Life expectancy of *Moina macrocopa* in (a) control, and different concentrations of (b) A1 DHA Selco®, (c) squid oil, (d) canola oil, (e) fish pellets, and (f) fish feces. Error bars indicate means±standard deviation. ▲ = control, ◆ = 0.0625 g/l; ■ = 0.125 g/l; ● = 0.25 g/l; □ = 0.5 g/l; ○ = 1.0 g/l.

Two-way ANOVA indicated that there were statistically significant, interacting effects between the nature and concentration of the treatment diet on the initial age of reproduction, average longevity, and gross reproduction rate (Table 2). The significance levels, including the interaction effects of both treatment diets and concentrations, ranged p = 0.001-0.01.

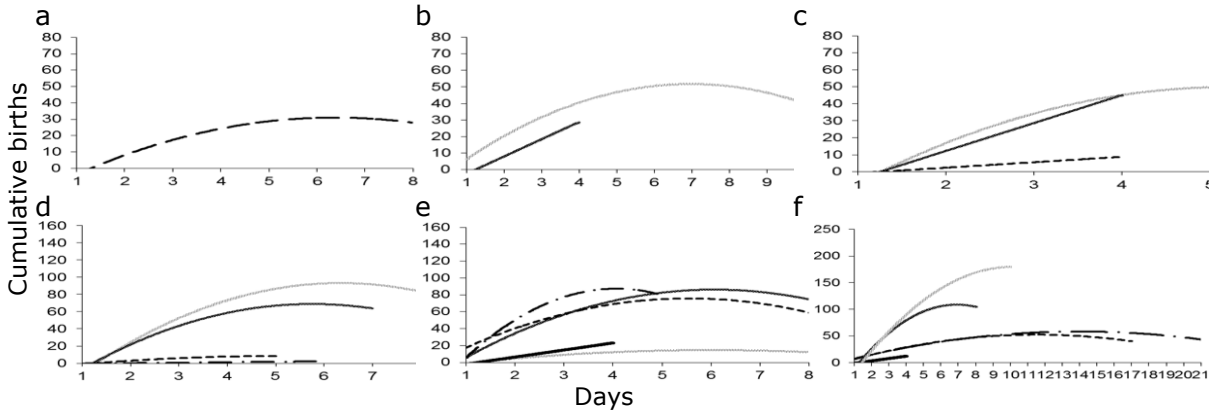

Fig. 4. Cumulative births (polynomial curves) of *Moina macrocopa* in (a) control, and different concentrations of (b) A1 DHA Selco®, (c) squid oil, (d) canola oil, (e) fish pellets, and (f) fish feces. = control, = 0.0625, = 0.125, = 0.25, = 0.5, = 1.0 g/l.

Table 2. of two-way analysis of variance (ANOVA): degrees of freedom (DF), sum of squares (SS), mean squares (MS), *F*-ratio (F), and *p* value (P).

Variable	*DF*	*SS*	*MS*	*F*	*P*
Initial age of reproduction					
Treatment diets	4	33.38	8.35	17.67	0.000***
Concentrations	5	39.37	7.87	16.67	0.000***
Diets x Conc.	20	64.22	3.21	6.80	0.000***
Error	90	42.50	0.47		
Average longevity					
Treatment diets	4	218.45	54.61	12.77	0.000***
Concentrations	5	152.08	30.42	7.11	0.000***
Diets x Conc.	20	185.05	9.25	2.16	0.007**
Error	90	384.75	4.28		
Gross reproduction rate					
Treatment diets	4	2894.53	723.63	78.85	0.000***
Concentrations	5	4021.27	804.25	87.63	0.000***
Diets x Conc.	20	4416.57	220.83	24.06	0.000***
Error	90	826.00	9.18		

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Discussion

Cladoceran species have been extensively studied at fundamental and applied levels. Among the cladocerans, *Moina* has been investigated most intensively with regard to the effects of food abundance on its growth and reproduction (Burak, 1997; He et al., 2001; Savas et al., 2011). Quality and quantity of food are the most important factors in determining biomass production of *Moina* species (He et al., 2001).

The optimal concentration of canola oil, fish pellets, and fish feces, in terms of growth and reproduction performance, was 0.0625 g/l. Longevity of *M. macrocopa* fed fish feces at 0.0625 g/l was higher than the maximum achieved when *M. macrocopa* were propagated using the green alga *Chlorella vulgaris* (Nandini and Sarma, 2000). The net reproduction rate with feces was also much greater than achieved during culture with *C. vulgaris* (26.20±2.63; Nandini and Sarma, 2000). The decline in neonate production that accompanied increasing concentrations of pellets and feces was presumably caused by the increased effort associated with food gathering due to active filtering of the food particles (Nandini and Sarma, 2000). In fact, high concentrations of all the diets produced suboptimal culture conditions. High levels of particles can actually lead to starvation of cladocerans as they are unable to clean thoracic limbs that are clogged by high particulate concentrations (Porter et al., 1982; Burak, 1997; Nandini and Sarma, 2000). Our results corroborate studies on other *Moina* species, where the cultivated population declined at high concentrations of an algal diet of *Scenedesmus sp.* (Burak, 1997) or L-carnitine supplement (Savas et al., 2011).

In this study, *M. macrocopa* became sexually mature earlier at high diet concentrations, indicating that diet type and concentration play a significant role in determining the initial age of reproduction. Spawning intervals ranged 2.1-4.6 days for all treatment diets and concentrations, and generation time did not seem to be influenced by diet concentration or type. On the other hand, fecundity was significantly affected by diet type and concentration, and the total number of batches (cluster of

offspring per cycle) of neonates produced by parthenogenesis differed between concentrations and types. Gross reproduction rates were generally higher at lower treatment concentrations and higher in *M. macrocopa* fed canola oil, fish pellets, or fish feces than other diets. High fecundity and gross reproduction rates suggest that growth performance in this cladoceran is largely due to the high carbon/nitrogen ratio in the food source (Jana and Pal, 1983).

There was no obvious trend in the intrinsic rate of population increase (r). Elevated r values may occur with shorter generation times or increases in fecundity (Stearns, 1976). This inter-relationship was clearly observed in our study, particularly in the pellet diet. The positive r values for reproducing cultures of *Moina* were within the range previously proposed for cladoceran populations (Nandini and Sarma, 2003).

Moina macrocopa fed the fish pellet or fish feces diets had the highest population growths and longest lifespans. The good performance of these food sources can be attributed to the feeding preference of *Moina* that tend to consume bacteria and filtered particles that are abundant in fish wastes when other food sources, such as phytoplankton, are limited or unavailable (Abrantes and Gonçalves, 2003). The oil emulsions did not appear to be as good food sources for *M. macrocopa* cultivation as anticipated since all the diet concentrations, except 0.0625 g/l canola oil, resulted in negative effects on the population dynamics ($p<0.05$). Oil emulsions could, however, serve as beneficial additives to *Moina* diets in combination with waste materials, and thus contribute to *Moina* production despite the unfavorable effects observed in this study.

In conclusion, the life table experiments carried out using a range of concentrations of different diets allowed elucidation of their effects on the life history variables of *M. macrocopa*. This study demonstrates that aquaculture wastes may serve as effective, inexpensive, sustainable food sources for *M. macrocopa* cultivation for fish larviculture.

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