INFLUENCE OF YEAST, CHICKEN MANURE AND DAILY FEEDING OF *CHLORELLA ELLIPSOIDEA* IN THE POPULATION GROWTH OF *MOINA MICRURA*

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Abstract: The total number of broods produced, daily fecundity and the size-class distributions of *Moina micrura* were determined in a four-day culture experiment to evaluate the effectiveness of rearing *M. micrura* in six culture methods. This study shows that addition of yeast, chicken manure and daily feeding of *Chlorella ellipsoidea* are the effective protocols in culturing *M. micrura*, which produced a significantly higher number of individuals (708 ± 66 individuals) compared with no yeast, no chicken manure and non-daily feeding of *C. ellipsoidea*. Higher production of *M. micrura* in chicken manure could be attributed to bacteria proliferation, which serves as additional food for the zooplankters, as well as to the products synthesized by bacteria to enhance growth. Daily feeding of *C. ellipsoidea* also contributed to high brood production of *M. micrura*, which may be attributed to the presence of certain nutrients necessary for growth and development of cladocerans. The proportion of neonates, juveniles and adults did not differ significantly among treatments but the lengths of developmental stages were significantly different among the treatments. Knowing the size-class distribution of *M. micrura* is important since this would allow determination of appropriate feed for fish with various gape sizes.

Keywords: *Moina micrura, size-class distribution, culture method, population growth*

1. INTRODUCTION

Some organisms may provide nutrients such as essential proteins, lipids, carbohydrates and minerals to their predator (New, 1998). The growth and development of fish larvae is dependent on the nutrients provided by the live feeds, which should be acceptable to the larval fish in terms of size, shape and palatability. In larviculture, the use of live feeds had been recognized in catfish (Olurin and Oluwo, 2010; Faruque *et al.*, 2010), freshwater prawn (Indulkar and Belsare, 2004), finfish (Liao *et al.*, 2001), sand goby (Amomsakun *et al.*, 2003) and other ornamental fish (Dhert *et al.*, 1997). The amount and quality of live feeds should be amenable to supply the needs of rearing fish larvae. Thus, the stability and reliability of live feed culture are indispensable.

In aquaculture, cladocerans have been utilized successfully as food for larval fish (Qin and Culver, 1996) and preferred to be eaten by most fish larvae as they become visible through its jerky movement (Mayer and Wahl, 1997). Moina is a cladoceran that has been used as replacements live feed for *Artemia* (Alam *et al.*, 1993) but their availability is seasonal. It is therefore necessary to develop culture technology for *Moina* in order to ensure their availability at all times. Moreover, if the amount of food present in the medium is adequate as well as favorable environmental conditions are met, the

adult females undergo parthenogenesis (Innes, 1997), which increases the rate of reproduction and makes *M. micrura* suitable for mass culture production.

M. micrura can tolerate poor water quality, which increases its potential for monoculture in the laboratory. The success in developing monoculture depends on the degree of simulating the natural conditions of the target organism. A laboratory culture for *M. micrura* is more advantageous as decomposing organic matters (Rottman *et al.*, 1992) and other food sources critical to the growth and development of the organism could be provided. Moreover, optimal conditions of the abiotic factors can be maintained throughout the culture period such as dissolved oxygen, pH and temperature.

In this study, different protocols were explored to determine the effective culture conditions for *Moina micrura* in four day period. The idea of adding yeast and dried chicken manure in the culture medium as well as daily feeding of *Chlorella ellipsoidea* to the total broods produced, daily fecundity and class-size distribution were assessed after the four-day culture period.

2. METHODOLOGY

Moina micrura were harvested from a concrete pond at the University of the Philippines Los Baños (UPLB) Limnological Station in Mayondon, Los Baños, Laguna, Philippines. Subsequently, *M. micrura* was isolated and reared in the laboratory using chicken manure based medium (CMBM) at 3.5 g/L and fed daily with *Chlorella ellipsoidea*.

In this experiment, six protocols were used to culture *M. micrura* namely (Treatment 1) CNF (CMBM with addition of dried chicken manure (DCM) and no feeding of *C. ellipsoidea*); (Treatment 2) CWF (CMBM with addition of DCM and with daily feeding of *C. ellipsoidea*); (Treatment 3) CYNF (CMBM with addition of DCM and yeast suspension (YS) and no feeding of *C. ellipsoidea*); (Treatment 4) CYWF (CMBM with addition of DCM and YS and daily feeding of *C. ellipsoidea*); (Treatment 5) CWFNP (CMBM only with daily feeding of *C. ellipsoidea*); and (Treatment 6) CYWFNP (CMBM with addition of YS and daily feeding of *C. ellipsoidea*). The culture of *M. micrura* was carried out in 1L wide mouth bottle containing a total volume of 500 mL CMBM. A total of 20 mature individuals were carefully transferred to each bottle. The summary of the treatments used in this study is presented in Table 1.

For treatments 1, 2 and 5,

Table 1. Summary of the various treatments performed in the experiment.

Treatment No.	Treatment Code	Addition of Yeast Suspension	Addition of Chicken Manure	Daily Feeding
1	CNF	(−)	(+)	(−)
2	CWF	(−)	(+)	(+)
3	CYNF	(+)	(+)	(−)
4	CYWF	(+)	(+)	(+)
5	CWFNP	(−)	(−)	(+)
6	CYWFNP	(+)	(−)	(+)

Legend:

- CNF = chicken manure based medium (CMBM) with addition of dried chicken manure (DCM) and no feeding of *C. ellipsoidea*
- CWF = CMBM with addition of DCM and daily feeding of *C. ellipsoidea*
- CYNF = CMBM with addition of DCM and YS and no feeding of *C. ellipsoidea*
- CYWF = CMBM with addition of DCM and YS and daily feeding of *C. ellipsoidea*
- CWFNP = CMBM only with daily feeding of *C. ellipsoidea*
- CYWFNP = CMBM with addition of DCM and YS and daily feeding of *C. ellipsoidea*

C. ellipsoidea was added at about 5.6×10^7 cells/mL. On the other hand, a concentration of 0.013 g/L yeast suspension was added to treatments 3, 4 and 6. All treatments were added with 1.75 g of dried chicken manure in pouch (CMP) except for treatments 5 and 6 (NP). Three replicates of each treatment were established and the culture bottles were distributed using Completely Randomized Design (CRD). The culture experiment lasted for 4 days in which the highest count of individuals had been observed in the laboratory.

The broods produced after 4 days of culture were harvested by collecting the broods using a 30 µm mesh nylon filter. The collected broods were re-suspended in 10 mL base medium (chicken manure) and immediately preserved using buffered formalin (pH 7). The preserved broods were transferred in a Sedgewick-Rafter chamber and the total number of individuals was counted under a light compound microscope (Bausch & Lomb). The developmental stages (neonate, juvenile and adult) of 100 individuals per replicate were determined by measuring the total length, from its head to the tip of post abdomen, using a calibrated ocular micrometer.

3. RESULTS AND DISCUSSION

The summary of broods produced by *M. micrura* after 4 day culture period for each treatment as well as the average number of broods produced by each female per day is presented in Figure 1. ANOVA revealed that there were significant differences ($p<0.05$) on the number of broods produced among treatments. CYWF produced the highest number of individuals (708.33 ± 66.031 individuals) after the culture period.

The broods produced in CYWF were 1.29 and 1.30 times higher than those cultivated in CWF and CYNF, respectively. Results also showed that *M. micrura* reared in CYWF were not significantly different (p>0.05) from those cultured in CWF and CYNF.

As compared with other treatments, addition of CMP in the culture medium yields higher productivity of *M. micrura* after 4 days culture. CMP may support bacterial proliferation during the culture period, which is comparable to the larval fish feed ration given by Sipauba-Taveres and Bachion (2002). Aside from the algae and treatments added with yeast, the bacteria that proliferated in the medium during the culture period can also be a food source for *M. micrura*, which was recognized to be of high food value (Rottman *et al.*, 2003). Moreover, it was reported by Yu *et al.* (1994) that the bacteria in the medium can synthesize cobalamin (vitamin B_{12}) and considered to be critical in enhancing the growth of some zooplankters. *Brachionus plicatilis* grown in bacterial-free medium but fed with vitamin B_{12}-enriched *Chlorella vulgaris* support the growth of the rotifers (Maruyama and Hirayama, 1993).

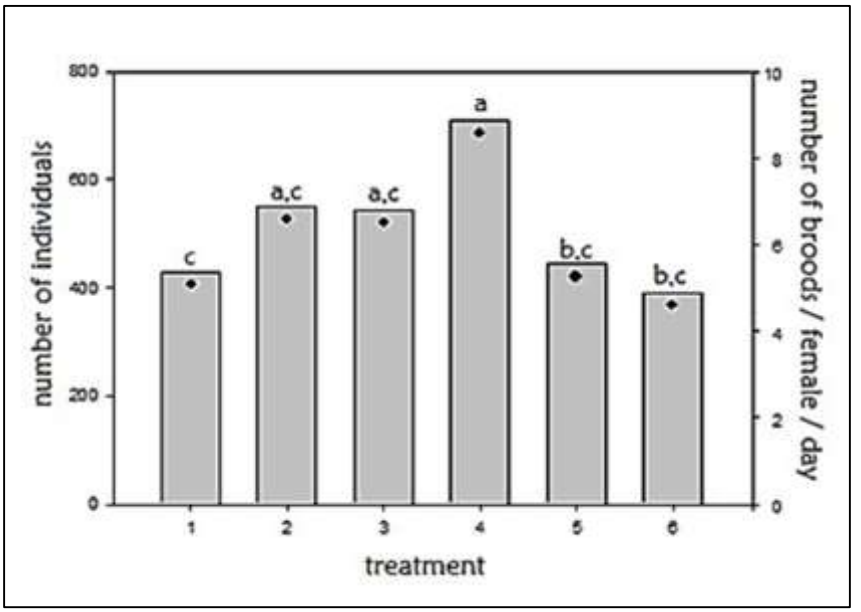

Figure 1. Summary of the total number of broods produced and number of broods produced by each *Moina micrura* female per day in each treatment after the 4 day culture period. Treatments with the same letter are not significantly different (p>0.05) from each other. Legend: 1=CNF; 2=CWF; 3=CYNF; 4=CYWF; 5=CWFNP; 6=CYWFNP

The food quality given to zooplankters cultured in the laboratory is based on its assimilation efficiency (Brett and Muller-Navara, 1997). *Scenedesmus quadricauda* was found to be of lower assimilation in cladocerans due to its colonial morphology (Macedo and Pinto-Coelho, 2001). In this study, the non-colony forming alga, *Chlorella*

ellipsoidea was used and its smaller size (<10 µm) than *Scenedesmus* probably gave higher assimilation in *M. micrura*. Pagano (2008) reported that *M. mic*rura has variable selectivity in terms of feeding and they are fed efficiently on a wide range of phytoplankton sizes (4 µm to 40 µm). In rotifers, feeding of live *Chlorella* in the laboratory has higher population growth as compared with the dried and processed algae (Mostary *et al.*, 2007). Their study also found out that live algal feeds were better than using baker's yeast alone. Thus, significant increase in the number of broods observed in CYWF may be due to the combination of *Chlorella* and yeast given at the start of the culture period and subsequent daily algal feeding. Natural diets are more advantageous to cladocerans since certain nutrition necessary for the development and growth of the fish larvae can be acquired and be transferred as they were eaten by the fish larvae (Sipauba-Taveres and Pereira, 2008).

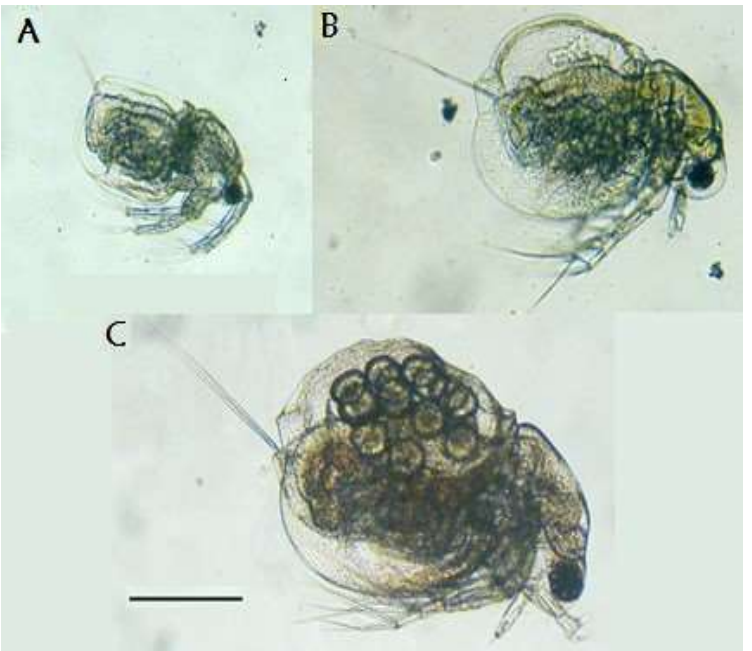

Figure 2. The different developmental stages of *Moina micrura* namely (A) neonate, (B) juvenile and (C) adult. The mean length (±S.E.) obtained for each stage after the 4 day culture period were: neonates, 327.05±1.70 µm; juveniles, 527.31±2.71 µm; and female adults, 755.64±7.35 µm. Bar = 200 µm.

The developmental stage of *M. micrura* for the brood produced after the 4 day culture period was determined by measuring the length of each individual from the tip of its head to the end of post abdomen (Figure 2). The different lengths measured from the broods were grouped into three (3) classes that corresponds to adult, juvenile and neonate. The resulting classification of developmental stages were verified through discriminant analysis (eigenvalue = 3.932; canonical correlation = 0.893), which revealed that 95% of the original group cases were correctly classified. Misclassified values were then placed to their corresponding correct groupings. Thus, this study designated the individual as an adult female if the length varied from 675 µm to 950 µm.

These lengths were slightly shorter as compared to the report of Rottman *et al.* (2002), wherein the length of adult females ranges from 700 µm to 1,000 µm. Moreover, this study also considered the individual as juvenile and neonate when the measured length ranges from 440 µm to 660 µm and 200 µm to 437 µm, respectively. These length values in classifying the *M. micrura* individual as juvenile and neonate were slightly different from the claim of Sipauba-Taveres and Bachion (2002), in which the length of juvenile ranges from 608.53 µm to 653.66 µm while neonate lengthened from 438.16 µm to 475.56 µm. They reported that the sizes of zooplanktonic organisms were influenced by temperature, food concentration and food type. The differences in food type could explain the slight variations in the length of *M. micrura* for each developmental stage. In their study, *Ankistrodesmus gracilis* were used as algal diet while this study utilized *Chlorella ellipsoidea.*

The summary of the distribution at various developmental stages by the broods for each treatment is presented in Figure 3. After 4 days, *M. micrura* cultured in CYNF produced the highest number of neonates, which was about 1.1 times higher than those reared at CWF and CYWF, respectively. The number of neonates produced in CYWFNP was 49.12% lower as compared to CYNF. As compared with CYNF, the treatment with the least number of juveniles, CYWFNP produced 1.83 times more juveniles and 26.37 times more for female adults. However, the number of broods produced for each developmental stage were not significantly different (p>0.05) among treatments.

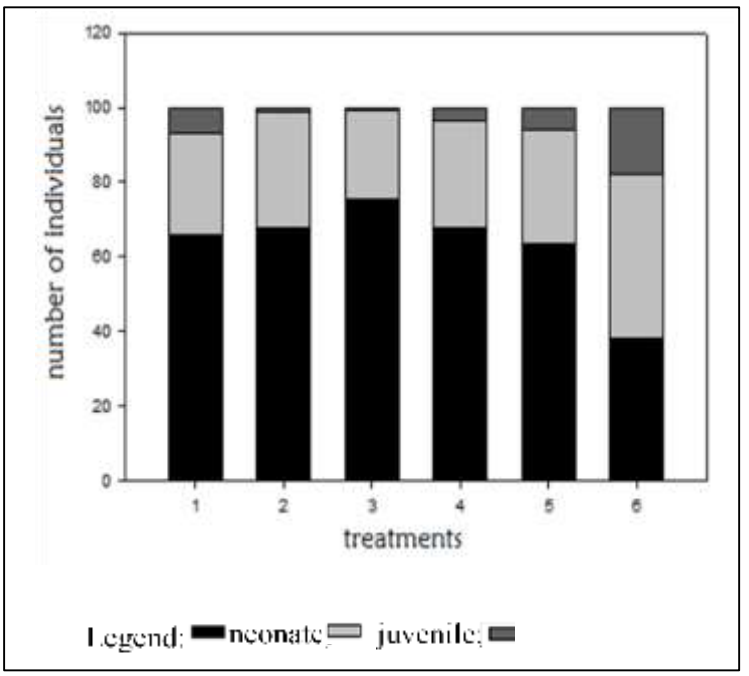

Figure 3. Distribution of broods produced at various development stages of *Moina micrura* for each treatment.

Figure 4 shows the summary of mean length per developmental stage among the treatments. For the three (3) developmental stages of *M. micrura*, the longest length was observed in CYWFNP while those cultured in CYNF had the shortest length. Results showed that the various length measurements per developmental stage were significantly different ($p<0.05$) among treatments. It is interesting to note that the highest productivity yielded among treatments (CYWF) was not significantly different ($p>0.05$) from the treatments with longest length (CYWFNP) at various developmental stages. This would give a better insight on choosing the best protocol in culturing *M. micrura* for 4 day period.

The physical parameters of the medium used during the culture period can affect the productivity of *M. micrura*. The daily variation of pH (8.34±0.02) and temperature (28.23±0.04°C) observed were not significantly different ($p>0.05$) among treatments. *M. micrura* embryos had a maximum hatching efficiency between pH 5 and pH 9 (Rojas *et al.*, 2001). On the other hand, hatching of eggs and increased growth performance were observed at optimum temperature between 24°C and 31°C (Rottman *et al.*, 2002; Rojas *et al.*, 2001). Temperature was also observed to play a vital role in controlling the duration of embryo in *Daphnia gessneri* (Hardy and Duncan, 1994). In this study, the temperature and the length of the broods at the end of culture period showed no significant correlation ($\alpha_{0.05}<0.220$).

This study intended to come-up with a protocol that would produce high yield of broods after the 4 day culture period. It is worth to mention that the number of broods at neonate and juvenile stages must also be considered in choosing the best protocol since the sizes at these stages are more contexts, *Moina micrura* is best cultivated in the medium supplemented with dried chicken manure in pouch and initially fed with yeast, which subsequently given daily algal diet.

4. CONCLUSIONS

In this study, the effective rearing method during four-day culture period of *Moina micrura* at laboratory condition is the addition of dried chicken manure and yeast solution in chicken manure based medium. Moreover, daily feeding of *Chlorella ellipsoidea* increases the fecundity of this cladoceran. Therefore, the type and amount of food sources during the cultivation affects the brood production and fecundity of *M. micrura*.

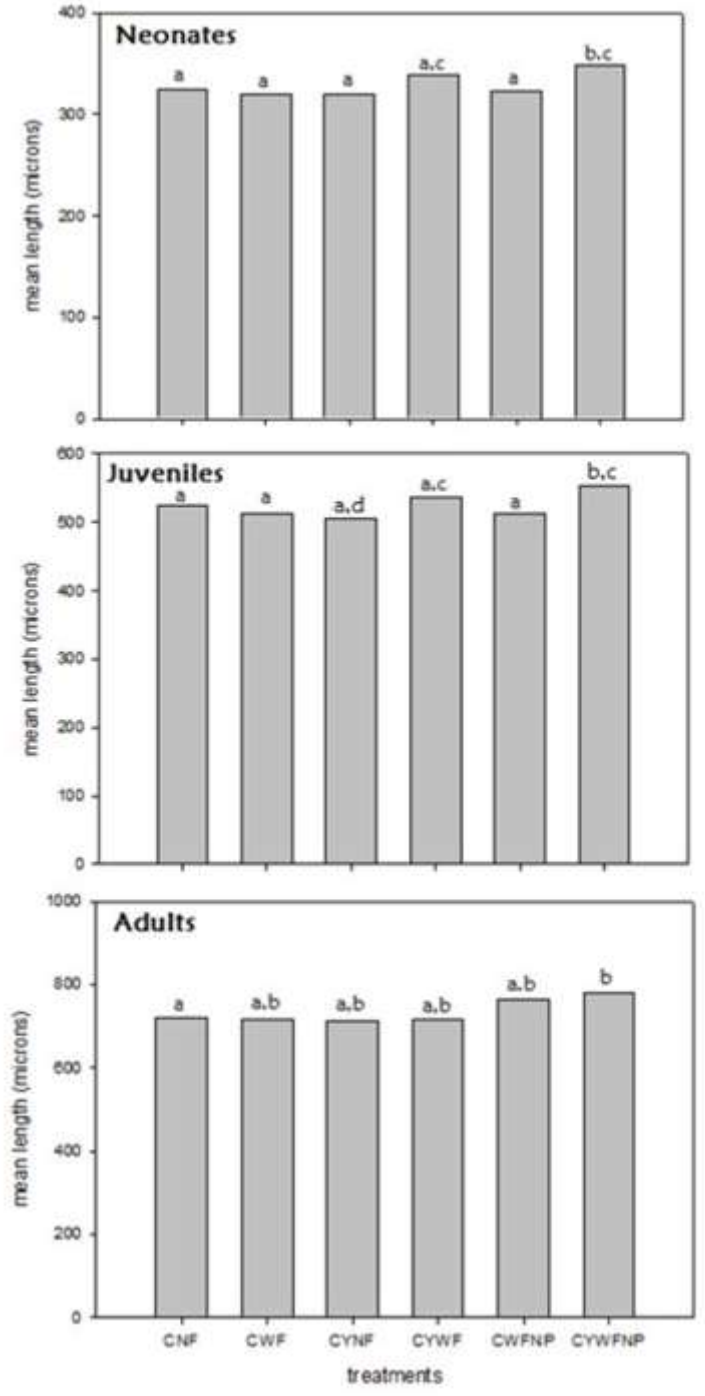

Figure 4. Summary of the mean length observed in each developmental stage of *Moina micrura* after 4 day culture period. Treatments with the same letters are not significantly different (p>0.05) from each other.

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