Original Research Article

Effects of using different types of organic wastes for the mass culture of *Moina macrocopa*

ABSTRACT

Moina macrocopa was cultured with different animal manures (chicken manure, Pig manure and cow manure) and food waste to determine the impact of these food sources on its mass production. All diets were provided at five different concentrations: 500, 1000, 2500, 5000 and 10000 ppm. Gross and net reproductive rates were higher in 1000 ppm concentration of food waste medium and the highest average population growth was obtained of about 9 individual *M. macrocopa* per ml, whereas pig manure treatment showed the lowest among all the culture medium. Highest population density was observed in low concentration treatments, on the contrary higher concentrations showed adverse effect on *M. macrocopa* cultivation. The results of this study suggest that 1000 ppm concentration of food waste produces better results than other animal manures which showed the highest population density and exhibited comparatively higher percentage of highly unsaturated fatty acids than the other treatments and could be an inexpensive and sustainable cultivation approach of *Moina macrocopa*.

***Keywords*:** *Moina macrocopa*, Animal manure, Food waste, Vial test, Life table demography

1. INTRODUCTION

To feed the increasing human population, it is therefore imperious to upgrade aquaculture, including fish farming whose development goes essentially through the success of larval rearing which requires the availability of zooplankton [1,2,3,4]. Yet, the most used zooplankton for the feeding of the fish larvae was Artemia [5,6]. But, the utilization, mostly in developing countries is difficult because of these cysts hatching conditions, high cost and low availability on the local market [7]. It is then important to make an intensive production of zooplankton at low cost for the expansion of fish farming. *M. macrocopa* is increasingly used as food for larval and post larval rearing of crustaceans [8] and teleost

fish in culture [9,10,11,12]. It is a superior live food compared to Artemia due to its relatively high protein and nutrient content [13]. Although its culture technique is relatively simple but the specific production and fed technique knowledge for the commercial scale production was incipient in spite of its wide distribution from temperate to the tropical region.

Food resources play an important role in the production of *M. macrocopa* in natural systems [14,15]. In natural habitats, biotic and abiotic parameters such as water quality, quantity, quality level of food available and population density are one of the most important factors that interact in the population growth of the zooplankton. Among these factors, population density and food availability are the predominant factors affecting the growth of *M. macrocopa* [16]. Conventional food sources of *M. macrocopa* are very expensive and contains very low nutritive value. So, the scientists are now trying to find an alternative food source of *M. macrocopa* at low cost with high nutritive value.

Hence, relatively large amount of *M. macrocopa* that are required for fish larvae cultivation can be produced from inexpensive, renewable waste materials. However, different types of wastes generated day by day in extensive quantities, creating a significant problem in its management and disposal. Besides, domestic policy of South Korea totally banned the ocean dumping of all wastes from 2014, following this banning; Korean policy and industry have been tending to convert the waste into resources [17]. Animal manures have a long history of use as a source of soluble phosphorous, nitrogen and carbon for natural food production [18]. Animal manure used as organic matter supplied to ponds can stimulate the phytoplankton growth and also increases the biomass of zooplankton [19,20]. Animal wastes using for fertilization practices are popular in many countries to sustain productivity at low cost [21, 22]. But, only limited information is available on the utilization prospects of food waste as an alternative or additional protein source of *M. macrocopa*, which can lower the cost of fish farming and at the same time, conserve the ecological value of fish ponds.

When Cladocera is used as a food for larval fish, nutrient enrichment is necessary as is the case with Artemia [23]. Because the ingredients that compose their body change according to the food they intakes [24]. Some quantitative data are available on the fatty acid profiles of rotifers, copepods and cladocerans using algae as food, but no data are available on the fatty acid profiles of *M. macrocopa* using organic wastes as food. *Chlorella vulgaris* is commonly used in M. macrocopa culture [41]. But it needs to be enriched by a commercial enrichment diet before feeding to the fish larvae [25]. However,

it is necessary to improve the fatty acid composition of M. macrocopa in natural way by switching its diet to organic wastes. Highly unsaturated fatty acids (HUFA) enhance the essential lipid levels and these essential fatty acids promote the growth of *M. macrocopa* [26]. So, it need to be investigated because the synthesis and accumulation of fatty acids in zooplankton are related to the stage of the individual and the frequency of reproduction among others.

The present study was designed to test the effect of different animal manures and food waste in mass culture of *M. macrocopa* with a view of investigating quality and quantity required for maximizing production. Determination the optimum concentration of each organic waste for the culture of *M. macrocopa* are important for its mass cultivation. Development of a suitable culture media for commercial production of *M. macrocopa* will be an inexpensive alternative approach to live feeds needed for fish rearing.

2. MATERIALS AND METHODS

2.1 Source of M. macrocopa

M. macrocopa were collected from a pond near Tongyeong, South Korea and the sample was taken to the laboratory immediately. *M. macrocopa* species were isolated from the collected sample by the micropipette and placed individually in petri dishes filled with dechlorinated tap water (10ml/plate) for breeding. Mature Moina started breeding overnight and baker's yeast was added at 1g/l to the petri dishes as a food source during the breeding period. Newborn *M. macrocopa* were collected for subsequent experiments.

2.2 Source of Organic Wastes

Three different types of animal manures such as pig manure, cow manure and poultry manure were sourced from the pig, cow and poultry production institute in Goseong, South Korea. The food wastes used in the present study included food processing waste (e.g., various types of fruit peels and leafy vegetables, rice bran, and soybean meal) and post-consumption waste (e.g., rice grain, spaghetti, beef, pork, and chicken) collected from local hotels and restaurants. The collected food wastes were transferred to the laboratory, for further processing. The food wastes were mixing together in a mixer machine, diced into small pieces, and excessive water was squeezed out by waste compressing equipment. Then the final leachate was used in this experiment.

2.3 Experimental design

2.3.1 Population growth experiment

The experiment was conducted in total 60 tanks with a water volume of 40 L. Three replicates were used for each treatment. The tanks were cleaned and dried for two days and filled with tap water and left for one day with aeration for dechlorination. Water temperature in the tanks was maintained at 25ºC. The temperature of water reservoir was regulated by a thermostat, which controlled the on / off switch of a 2000-W electric heater. Four treatments with five different concentrations: chicken manure, pig manure, cow manure, and food waste of 500, 1000, 2500, 5000, and 10000 ppm were assigned in the experiment. Twenty healthy individuals *M. macrocopa* were individually introduced into the tanks. The trial has repeated a total of three times and data pooled at the end of the period for each treatment.

Following initiation of different growth experiments, the number of living individuals of each tank was counted daily. The population of *M. macrocopa* was recorded by using the Sedgewick-Rafter counter cell which is 50 mm long, 20 mm wide and 1 mm deep. *M. macrocopa* cultured in each experimental tanks was recorded by using a tally counter under a dissecting microscope (10X to 40X magnification). The number (no./mL) was calculated according to the formula outlined by Boyd and Lichktoppler [27] :

$$\text{Number of } \textit{Moina macrocopa} \text{ /ml} = \frac{\text{T x 1000}}{\text{A x N x Vol. of concentrate in ml/Vol. of sample}}$$

Where,

T = Total number of *M. macrocopa* counted

A = Area of grid in mm^2

N = Number of grids counted

1000 = Area of counting chambers in mm^2

2.3.2 Water quality parameters

Dissolved oxygen (mg/L), pH and salinity(%) were measured by dipping into the water surface. Ammonia was measured by Palintest compact ammonia duo meter. Recordings were taken after tank inoculation and thereafter every 24 hours.

2.3.3 Vial test

Twenty vials of 50mL size were selected for this test and each was replicated three times. Four treatments with five different concentrations: chicken manure, pig manure, cow manure, and food waste of 500, 1000, 2500, 5000, and 10000 ppm were assigned in each vial. One healthy neonate of less than 24hrs old was transferred in each vial and the alive individual and offspring produced from it were quantified every 24hrs. The neonates produced by *M. macrocopa* were collected gently and transferred into a culture dish for quantification. This counting was also carried out using a tally counter. Then it transferred to the new test jars with appropriate culture medium and the dead adults and neonates were removed. Each vial test was discontinued after the last adult in each vial was died. Mortality and fecundity were recorded to calculate the life table demography of *M. macrocopa*.

2.3.4 Life table demography

Life table demographics is an important tool for describing the life cycle of zooplankton under continuously changing environmental conditions. The survival period, initial age of reproduction, average longevity, gross reproduction rate, net reproduction rate, rate of increase, and generation time were selected for life history variables for this study [28]. 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. The following formulae were used [29].

Average longevity = $\sum n_x/n$;

Gross Reproduction rate = $\sum m_x$;

Net reproduction rate (Ro)=$\sum l_x m_x$;

Generation time (T) = $\sum l_x m_x x /Ro$

Where,

n_x =Number of individuals alive for each age class

135 m=The age specific fecundity (number of neonates produced per surviving female at age X)

136 l_χ =The proportion of individuals surviving to age χ

137 n = The number of replicates

138 The final rate of population increase (r) was calculated over the 21 days experimental period. The rate
139 of population increase (r) was derived using the following equation; $r = (\ln N_t - \ln N_0)/t$, where N_0=initial
140 population density and N_t=population density after time t [29].

141 ***2.3.5 Population density of M. macrocopa***

142 Twenty vials of 50 mL size were assigned with four treatments of five different concentrations: chicken
143 manure, pig manure, cow manure, and food waste of 500; 1,000; 2,500; 5,000; and 10,000 ppm in
144 each vial. One healthy neonate of less than 24hrs old was transferred in each vial and the alive
145 individual and offspring produced from it were quantified every 24hrs, which was carried out until
146 finishing of this experiment. *M. macrocopa* were transferred into different culture dishes for
147 quantification and after quantification, live *M. macrocopa* were returned to the culture vial, and the
148 dead organisms were discarded. This experiment was carried out for 21 days.

149 ***2.3.6 Fatty acid analysis of M. macrocopa cultured in different organic wastes***

150 Total lipids of *M. macrocopa* were extracted according to the Bligh and Dyer method [30] by using
151 solvent mixture consisting of chloroform and methanol (2:1, v/v). After phase equilibration, the lower
152 chloroform layer was removed and total lipids were extracted by removing solvent using a rotary
153 evaporator (R-114, BUCHI, Swiss) at 38 ℃. 100 mg of extracted total lipid were put into a capped tube
154 and added 1.5 ml 0.5 N NaOH-methanol solution. The sample was mixed by vortex and heated 100 ℃
155 for 8 minutes for saponification. After cooling, methylation was done by using a fatty acid methyl ester
156 (FAME) with BF3-methanol. Then the sample was dissolved into 2 ml iso-octane and fatty acids were
157 analyzed using gas chromatography (Clarus 600, Perkin Elmer, USA) equipped with capillary column
158 (Omegawax-320, 30 m × 0.25 mm I.D., Supelco Co., Bellefonte, PA, USA). The operating parameters
159 were as follows: carrier gas =helium; detector (FID) temperature =270℃; injection temperature =
160 250℃; column temperature =180℃ for 8 min, programmed to increase at 3℃/min up to 230℃ with a
161 final holding time of 10 min; split injection at 1:50 ratio. Menhaden oil was used as standard. Each of

the specific fatty acid methyl ester peaks was identified by determining its equivalent chain length with reference to the known standard.

2.4 Statistical Analysis

The statistical analysis were carried out to evaluate the differences in the means of the derived individual number of *M. macrocopa* and environmental parameters of different treatments by using one-way ANOVA. Statistical significance among the different treatments was accepted at $p<0.05$ and the statistical package of SPSS- 16 (SYSTA, USA) was used to express the result.

3. RESULTS

3.1 Water quality

Table 1 shows the mean pH, DO and ammonia content of five various concentrations of four different culture medium over three weeks experimental period. The highest pH was recorded in the pig manure treatment which was in the range between 7.33 to 7.72 and the lowest was recorded in the food waste which was in the range of 5.50 to 6.46. pH increased with the increase concentration of animal manures, but in case of food waste it decreased. Food waste treated media showed significantly lowest ($P<0.05$) DO level throughout the culture period, which were found to be in the range of 0.4 to 1.08 mg/l. As the temperature was fixed from the beginning of the experiment, there is no significant difference was observed between the treatments. Ammonia contents increased with the increasing concentrations of organic wastes but showed no significant difference ($P >0.05$) among the treatments of all concentration.

Table 1. Water quality parameters for the *M. macrocopa* cultures at different concentrations of animal manures and food waste of the experimental duration

Culture medium	Concentration (ppm)	pH	DO (mg/l)	Temperature (°C)	Ammonia (mg/l)	Salinity (PSU)

Chicken manure	500	6.816±0.02	2.83±0.01	25±0.1	0.20±0.03	0.01±0.0
	1000	6.973±0.02	2.71±0.01	25±0.1	0.22±0.03	0.01±0.0
	25000	7.281±0.04	2.25±0.02	25±0.1	0.33±0.06	0.01±0.0
	5000	7.406±0.03	2.28±0.03	25±0.1	0.39±0.04	0.02±0.1
	10000	7.554±0.02	0.57±0.03	25±0.1	0.41±0.08	0.02±0.0
Pig manure	500	7.335±0.01	3.54±0.02	25±0.1	0.23±0.02	0.01±0.0
	1000	7.208±0.02	3.23±0.02	25±0.1	0.25±0.06	0.01±0.0
	25000	7.557±0.02	3.06±0.04	25±0.1	0.29±0.05	0.01±0.0
	5000	7.524± 0.03	2.86±0.07	25±0.1	0.41±0.03	0.02±0.0
	10000	7.729±0.06	1.03±0.03	25±0.1	0.44±0.05	0.03±0.0
Cow manure	500	6.719±0.02	3.45±0.01	25±0.1	0.19±0.05	0.01±0.0
	1000	7.083±0.01	3.11±0.03	25±0.1	0.22±0.04	0.01±0.0
	25000	7.159±0.03	1.76±0.02	25±0.1	0.26±0.04	0.01±0.0
	5000	7.230±0.02	1.53±0.05	25±0.1	0.36±0.03	0.01±0.0
	10000	7.592±0.04	0.61±0.03	25±0.1	0.37±0.08	0.03±0.1
Food waste	500	6.461±0.02	1.08±0.01	25±0.1	0.23±0.02	0.01±0.0
	1000	6.823±0.03	0.91±0.02	25±0.1	0.25±0.04	0.01±0.0
	25000	6.064±0.05	0.93±0.01	25±0.1	0.36±0.06	0.01±0.0
	5000	5.963±0.02	0.32±0.01	25±0.5	0.35±0.05	0.03±0.1
	10000	5.501±0.03	0.41±0.02	25±0.1	0.38±0.04	0.03±0.0

3.2 Population growth of M. macrocopa

Figure 1 shows the population growth of *M. macrocopa* with five various concentrations of four different organic waste treated medium for 21 days experimental period. Population growth was significantly higher in the treatments with low concentrations, on the contrary there is no population growth was observed in 10,000 ppm; the highest concentration used in this experiment. The highest growth was recorded in 1,000 ppm concentration for four culture medium and among these four mediums, food waste showed the best growth rate. Figure 2 showed the mean population growth of *M. macrocopa* cultivated in 1,000 ppm concentration of four culture medium. Highest mean population was found in food waste medium which was followed by chicken manure medium and showed a significant difference ($P<0.05$) among the other medium, where there is no significant ($P<0.05$) difference was observed between pig manure and cow manure treated treatment.

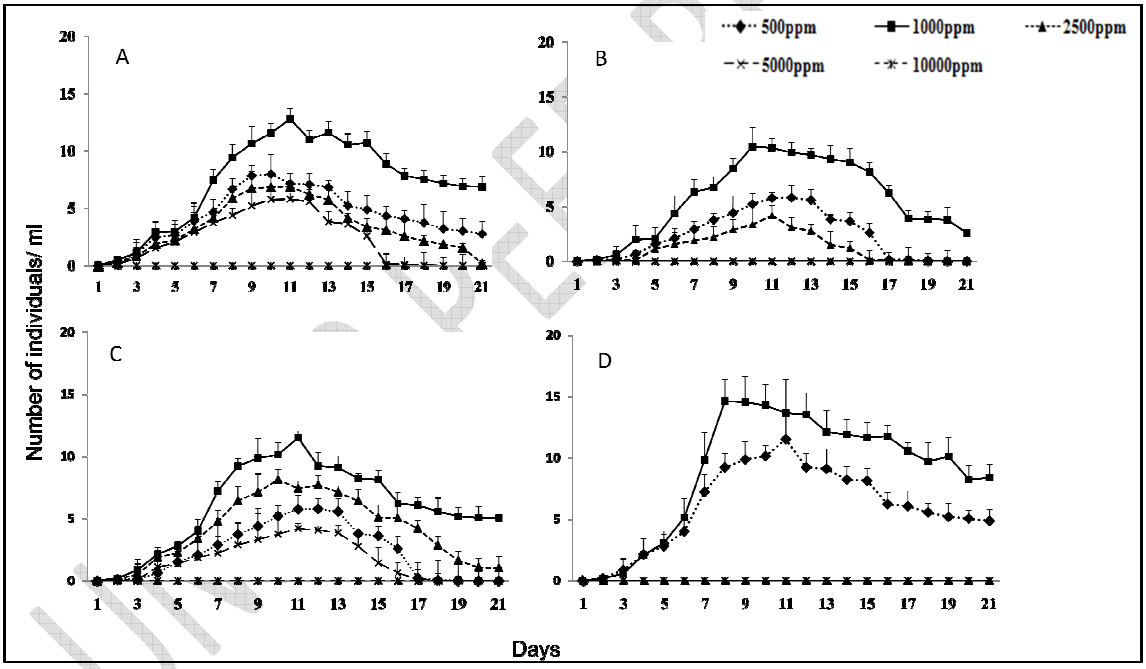
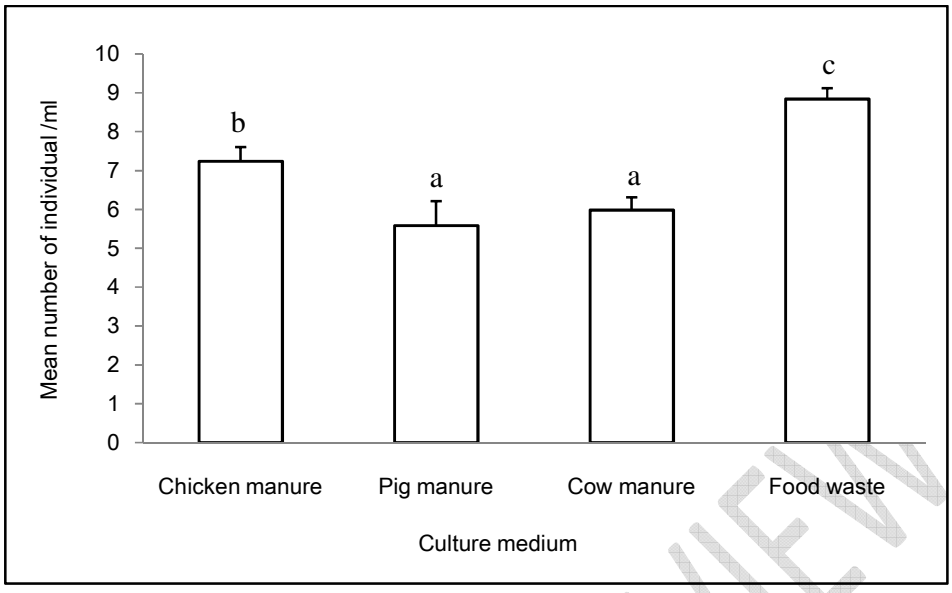


Fig. 1. Population growth of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste culture medium for 21 days experimental period. Error bars indicate means ± standard deviation.



205

206 Fig. 2. Mean population growth of *M. macrocopa* cultured in 1000ppm concentration of four culture
207 medium. Values are the (mean ± SD). Different subscripts denote significant differences at $P < 0.05$
208 ($a < b < c$) .

209

210 ***3.3 Life table demography***

211 Data on the selected life history variables (Table 2) of *M. macrocopa* showed that the average lifespan
212 was lowest in treatment with high concentration, about 1 to 2 days. The offspring production of *M.*
213 *macrocopa* in relation to the different concentrations of treatments showed a distinct shift towards early
214 reproduction with low concentration treatments. Gross and net reproductive rate also showed similar
215 trends. Large numbers of offspring were produced by *M. macrocopa* cultured in food waste (500;
216 1000ppm), chicken manure (500; 1000 ppm/l), cow manure (500; 1,000 ppm) and pig manure (500,
217 1,000 ppm) medium. Fecundity declined at high concentrations (2500 ppm and 5000 ppm), while the
218 highest 10,000 ppm concentration did not lead to the production of offspring for every treatments. The
219 rate of population increase was positive for all the culture medium, but 1000 ppm/l showed the
220 maximum. The highest rate of population increase (r) calculated for this experiment was 0.51 ± 0.08
221 obtained in the food waste treated treatment. This was followed by the chicken manure, cow manure
222 and pig manure medium with 'r' value of 0.47±0.23, 0.43±0.16, and 0.33±0.02 respectively.

223

224 **Table 2. Life table of *M. macrocopa*, cultured with different organic wastes at different**

225 **concentrations. Data are the means with the standard error of three replicates**

Medium types	Medium Conc. (ppm)	Initial age of reprod-uction	Longevity	Net reprod-uction rate	Gross repr-oduction rate	Generation time	Rate of populati-ion increase
Chicken manure	500	2.89±0.06	8.16±0.13	11.73±0.11	14.06±1.03	3.41±0.11	0.39
	1000	2.87±0.08	9.20±0.23	19.32±0.08	23.42±0.91	3.00±0.36	0.47
	2500	3.00±0.08	6.27±0.18	9.17±0.13	11.42±0.85	3.16±0.25	0.36
	5000	3.07±0.05	5.63±0.20	5.21±0.16	5.21±1.14	3.27±0.25	0.32
	10000	-	2.86±0.41	-	-	-	-
Pig manure	500	3.06±0.03	5.63±0.13	8.25±0.21	10.14±0.21	4.00±0.17	0.24
	1000	3.08±0.08	7.74±0.11	11.69±0.17	13.32±0.16	3.53±0.26	0.33
	2500	3.00±0.07	6.75±0.18	7.32±0.17	9.11±0.20	3.60±0.25	0.27
	5000	-	1.00±0.20	-	-	-	-
	10000	-	1.00±0.41	-	-	-	-
Cow manure	500	3.06±0.08	7.63±0.11	10.21±0.21	10.53±1.11	3.23±0.11	0.33
	1000	3.06±0.05	9.14±0.16	15.57±0.18	17.71±1.31	3.10±0.36	0.43
	2500	3.26±0.08	9.17±0.28	15.42±0.16	17.24±0.93	3.00±0.25	0.43
	5000	3.33±0.05	5.00±1.22	4.16 ±0.16	4.23±1.14	3.27±0.25	0.32
	10000	-	3.75±0.41	-	-	-	-
Food waste	500	3.00±0.07	8.71±0.11	12.42±0.18	15.35±1.00	3.00±0.10	0.43
	1000	2.88±0.06	9.82±0.26	28.16±0.18	33.71±0.21	3.00±0.16	0.51
	2500	-	1.75±0.08	-	-	-	-
	5000	-	1.00±0.01	-	-	-	-
	10000	-	1.00±0.00	-	-	-	-

226 Dash (-) indicates no offspring was produced

3.4 Populations density of M. macrocopa

Populations from a single neonate of *M. macrocopa* showed average growth rate until the first week in various concentrations of four different culture medium (Figure 3). It was growing continuously until the second week and after that, it started to decline and this same trend was observed in all treatments. Among all the concentrations highest population was found in 1,000 ppm concentration of all the culture medium. In case of food waste medium neonate is died in 2500, 5000 and 10000 ppm concentrations within 24hrs and 75 numbers of individual *M. macrocopa* were counted on day 10, in 1,000 ppm concentration, which was its peak population. Vials containing chicken manure, cow manure and pig manure medium showed moderate production with a peak population of 57, 52 and 43 individual on day 9 in 1,000 ppm concentration. After that the decreasing trend was started, which means that these peak populations are the maximum density of *M. macrocopa* for different culture medium.

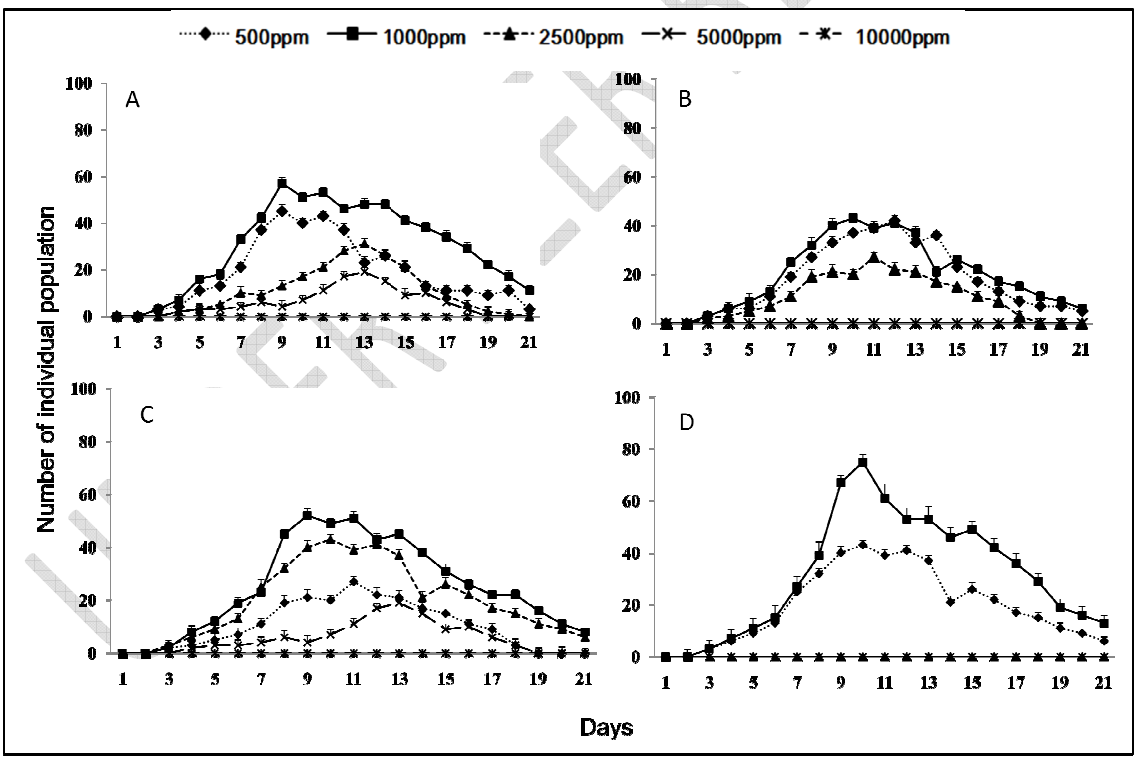


Fig. 3. Population density from a single neonate of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste treated medium for 21 days experimental period. Error bars indicate means ± standard deviation.

3.5 Fatty acid composition of M. macrocopa cultured in different organic wastes

Table 3 shows the average percentage of fatty acid composition of *M. macrocopa* cultured in different organic waste. Among the saturated fatty acids, 14:0, 16:0 and 18:0 comprised of about 33% of the total fatty acids and 16:1n-7, 18:1n-7, 18:2n-6 are the most dominant unsaturated fatty acids. The fatty acid composition of *M. macrocopa* cultured in cow manure, food waste, chicken manure and pig manure was dominated by, 16:00, 18:00, 18:1n-9, 18:2n-6. The level of EPA and DHA was significantly higher in *M. macrocopa* that cultured in food waste medium than those other wastes.

4. DISCUSSION

Among the cladocereans, *M. macrocopa* has been investigated most intensively with regard to the effects of food abundance on its growth and reproduction [10,31,32]. Quality and quantity of food are the most important factors in determining biomass production of *M. macrocopa* species. The results of this study indicate that the growth performance of *M. macrocopa* using various concentrations of different organic wastes as a culture medium are different. Low population growth was observed in high concentration of culture medium, while the highest concentration used in this study did not lead to the production of offspring in all the culture medium. This phenomenon has been attributed by various workers that the presence of high concentrations of animal manure significantly reduced the water quality, deplete the plankton population and cause adverse effect on the culture [4]. Nandini and Sarma [33], revealed that, the decline in neonate production that accompanied increasing concentrations of culture medium were presumably caused by the increased effort associated with food gathering due to active filtering of the food particles. In fact, high concentrations of all the diets produced suboptimal culture conditions. Burak [34] & Porter[35] described that, high concentration of particles can actually lead to starvation of cladocerans as they are unable to clean thoracic limbs that are clogged by high particulate concentrations. Savas [31] also found that, population of *M. macrocopa* declined in using high concentration of algal supplement. In this study, 1000 ppm concentration showed the optimal concentration of all the culture media in terms of growth and reproduction

271 **Table 3. Fatty acids composition (%) of *M. macrocopa* cultured in different organic wastes**
272

Fatty acids	Chicken manure	Pig manure	Cow manure	Food waste
14:0	3.59±0.12	3.13±0.08	3.8±0.15	4.2±0.18
16:0	18.3±1.20	22.1±2.50	18.6±1.50	17.11±2.71
16:1n-7	6.9±0.03	14.12±0.11	9.8±0.08	6.83±0.09
18:0	12.88±0.51	10.6±0.40	8.8±0.72	11.94±0.26
18:1n-9	17.7±0.08	16±0.91	16.3±0.18	13.14±0.28
18:1n-7	20.9±2.11	11.3±0.57	8.3±1.20	9.6±0.90
18:2n-6	13.4±0.07	16.6±0.08	24.2±2.74	20.5±1.25
18:3n-3	3.2±0.04	3.8±0.01	4.6±0.24	7.3±0.14
18:4n-3	0.17±0.01	-	2.6±0.09	-
20:00	-	-	0.13±0.08	1.5±0.07
20:2n-6	0.03±0.01	0.03±0.01	0.07±0.01	0.11±0.05
20:3n-6	0.82±0.05	0.69±0.04	0.7±0.01	0.87±0.09
20:4n-6	0.69±0.03	0.82±0.09	0.54±0.08	0.87±0.04
20:3n-3	-	-	0.13±0.02	2.11±0.06
20:5n-3	1.06±0.06	0.33±0.04	0.29±0.08	1.8±0.07
22:5n-3	-	-	-	0.06±0.01
22:6n-3	0.26±0.09	0.13±0.03	0.08±0.05	0.81±0.01
ΣSFA	34.77	35.83	31.33	34.75
MUFA	45.5	41.42	34.4	29.57
PUFA	19.63	22.4	33.21	34.43

273
274 Data of 1,000 ppm concentration of different organic waste are shown here.
275 Hyphen (-) indicates non- detectable fatty acids.
276

277

278 performance. Among the culture medium, the highest population growth was observed in food waste
279 medium. In this study pH increased with the high concentration of animal manures but food waste
280 showed different trend where pH decreased with the increasing concentrations, might be the presence
281 of higher amount longer chain fatty acids. Life table demography of *M. macrocopa* followed the same
282 trend. Average lifespan was lowest in culture mediums with higher concentration. Food waste
283 containing 1,000 ppm concentration showed the highest average lifespan and early reproduction ability.
284 The present study showed that, *M. macrocopa* needs time to became sexually mature at high diet
285 concentration. In contrast, Loh [32] reported that, initial age of reproduction of *M. macrocopa* is earlier
286 in high concentration diet than in low concentration. Different results are observed in this test, which
287 indicates that diet type and concentration play a significant role in determining the initial age of
288 reproduction. Gross and net reproduction rates were generally higher at lower treatment
289 concentrations and highest in food waste medium than other diets. Jana and Pal [36] revealed in their
290 study, high fecundity and gross reproduction rates suggest that growth performance of any species is
291 largely depends on the high carbon/nitrogen ratio in the food source. Which clearly indicates that food
292 waste contained high C/N ratio than the other medium.

293 The highest population density was obtained in 1,000 ppm concentration of food waste medium. The
294 good performance of this food source can be attributed to the feeding habit of *M. macrocopa* that tend
295 to consume bacteria and filtered particles that are abundant in food waste , when other food sources
296 has limited. In terms of time and efficiency, *M. macrocopa* cultured in food waste reached its peak
297 population on day 10 which was about 75 individual. This also means that *M. macrocopa* could be
298 harvested by food waste within a shorter period of time, thus allowing more number of cultivation
299 batches per cycle which is important for commercial live feed producers. After reaching the peak
300 population day within second week, the population began to decrease from the starting of third week.
301 Which could be caused by insufficient of space, food availability, sexual transformation, and/or
302 allelopathic effects [37,10,38,39,40]. Jiun [41] reported that, *M. macrocopa* has a higher density
303 adaptation in a captive culture environment compared to *M. micrura*, because high stocking density
304 may possibly lead to a population collapse. According to Jana and Pal [36], the growth performance of
305 *M. macrocopa* was limited at the density of 4 ind. and 20 ind./ 50ml. Results of this study showed that,
306 *M. macrocopa* had a better adaptation in food waste treatment at the density up to 75 individual per
307 50mL which is more higher than that previous study.

Muller et al., [26] revealed that, population growth and reproduction of the species depends on the quantity of reserve lipids (14:0, 16:0 and 18:0). *M. macrocopa* cultured in this study comprised of 33% of saturated fatty acids in each treatment. *M. macrocopa* exhibited a fatty acid profile of poly unsaturated fatty acids (18:1n-9, 18:2n-6 and 18:3n-3) constituting 45.1%, 37.6%, 36.4% and 34.3% when grown in cow manure, food waste, pig manure and chicken manure treatment respectively. It could be that a decrease in PUFA, reduces the capacity of animals to withstand in the environment as reflected in adverse changes in demography [42]. Comparatively higher percentage of EPA and DHA was found in *M. macrocopa* grown in food waste treatment and the levels of EPA (1.8%) were comparable with those in rotifers or Artemia that were fed algal diets in another study [10]. This result suggests that *M. macrocopa*, when culture in food waste treatment, has the potential to be a suitable diet containing essential n-3 HUFAs for fish larvae.

Our study demonstrated that *M. macrocopa* can be cultured using animal manure and food waste. This is also in agreement with Nandini [33] and Golder [43]. However, Siebe C [44] reported that, Moina sp. cultivation using domestic wastes as a food source posing a high risk of pathogen contamination or toxicant pollution. But, in case of food waste this contamination possibilities is comparatively low. Studies has shown that food waste can replace part of the fish meal used in fish feeds to produce quality fish and no health risk was observed in the health risk assessment test [45].

5. CONCLUSION

In conclusion, the results of this experiment suggest that *M. macrocopa* could be cultivated using 1000 ppm concentration of different animal manure and food waste. Results indicate that, the food waste appeared to be more effective compared to all other treatments for successful mass culture of *M. macrocopa* to high density and higher percentage of n-3 HUFA , which may serve as effective, inexpensive and sustainable food sources for *M. macrocopa* cultivation.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL

All experimental protocols followed the guidelines of the Institutional animal Care and Use Committee of the Gyeongsang National University.

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