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Culture of *Moina macrocopa* Using Different Types of Organic Wastes

Kamrunnahar Kabery[1], Md. Anisuzzaman[1], U-Cheol Jeong[1] and Seok-Joong Kang[1*]

[1]*Department of Marine Biology and Aquaculture, Gyeongsang National University, Tongyeong 53064, Republic of Korea.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors KK, MA and SJK designed the study. Author KK wrote the article. Authors KK, AM and UCJ manufactured the experimental feed, conducted the feeding trial and performed the analyses. Author SJK conceived and coordination and revised the manuscript. All authors read and approved the final manuscript.

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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 org mL-1 whereas pig manure treatment showed the lowest among all the culture medium. The highest population density was observed in low concentration treatments, on the contrary, higher concentrations showed an 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 a comparatively higher percentage of highly unsaturated fatty acids than the other treatments and could be an inexpensive and sustainable cultivation approach of *Moina macrocopa*.

**Corresponding author: Email: sjkang54@gmail.com;*

Keywords: Moina macrocopa; animal manure; food waste; vial test; life table demography.

1. INTRODUCTION

To feed the increasing human population, it is, therefore, impervious 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 a 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 the specific production and feed 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, the 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 a 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, a 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, the domestic policy of South Korea 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 phosphorus, nitrogen and carbon for natural food production [18]. Animal manure used as organic matter supplied to ponds can stimulate phytoplankton growth and 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 consume [24]. Some quantitative data are available on the fatty acid profiles of rotifers, copepods and cladocerans using algae as food, but there is a dearth of data on the fatty acid profiles of *M. macrocopa* using organic wastes as food. *Chlorella vulgaris* is commonly used in *M. macrocopa* culture [25]. 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* naturally 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 needs 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 the quality and quantity required for maximizing production. Determination of optimum concentration of each organic waste for the culture of *M. macrocopa* is 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 *M. macrocopa* started breeding overnight and baker's yeast was added at 1gL-1 to 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 mixed 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 of 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 dichlorination. Water temperature in the tanks was maintained at 25ºC. The temperature of the 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 the 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 tank 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].

Where,

T = Total number of *M. macrocopa* counted
A = Area of the grid in mm2
N = Number of grids counted
1000 = Area of counting chambers in mm2

2.3.2 Water quality parameters

Dissolved oxygen (mg/L) and pH 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 50 mL 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 24 hrs. 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 (AL) = ∑ n χ/n;

Gross Reproduction Rate (GRR) = ∑mχ;

Net Reproduction Rate (Ro)=∑l χ m χ;

Generation Time (T) = ∑l χ m χ χ /Ro

Where,

n χ =Number of individuals alive for each age class
m= The age-specific fecundity (number of neonates produced per surviving female at age X)
l χ =The proportion of individuals surviving to age χ
n = The number of replicates

Final Rate Population Increase (r) = (lnNt-lnN0)/t

Where

N0=initial population density
N t=population density after time t [29].

2.3.5 Population density of *M. macrocopa*

Twenty vials of 50 mL size were assigned with four treatments of five different concentrations: chicken manure, pig manure, cow manure, and food waste of 500; 1,000; 2,500; 5,000; and 10,000 ppm 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, which was carried out until finishing of this experiment. *M. macrocopa* were transferred into different culture dishes for quantification and after quantification, live *M. macrocopa* were returned to the culture vial, and the dead organisms were discarded. This experiment was carried out for 21 days.

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

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

2.4 Statistical Analysis

The statistical analysis was carried out to evaluate the differences in the means of the derived individual number of *M. macrocopa* and physic-chemical parameters of water of different treatments by using two-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 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 of 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 increasing concentration of animal manures, but in the case of food waste is decreased. Food waste treated media showed significantly lowest ($P<0.05$) DO level throughout the culture period, which was 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 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 (mgL^{-1})	Temperature (°C)	Ammonia (mgL^{-1})
Chicken manure	500	6.816±0.02	2.83±0.01	25±0.1	0.20±0.03
	1000	6.973±0.02	2.71±0.01	25±0.1	0.22±0.03
	25000	7.281±0.04	2.25±0.02	25±0.1	0.33±0.06
	5000	7.406±0.03	2.28±0.03	25±0.1	0.39±0.04
	10000	7.554±0.02	0.57±0.03	25±0.1	0.41±0.08
Pig manure	500	7.335±0.01	3.54±0.02	25±0.1	0.23±0.02
	1000	7.208±0.02	3.23±0.02	25±0.1	0.25±0.06
	25000	7.557±0.02	3.06±0.04	25±0.1	0.29±0.05
	5000	7.524± 0.03	2.86±0.07	25±0.1	0.41±0.03
	10000	7.729±0.06	1.03±0.03	25±0.1	0.44±0.05
Cow manure	500	6.719±0.02	3.45±0.01	25±0.1	0.19±0.05
	1000	7.083±0.01	3.11±0.03	25±0.1	0.22±0.04
	25000	7.159±0.03	1.76±0.02	25±0.1	0.26±0.04
	5000	7.230±0.02	1.53±0.05	25±0.1	0.36±0.03
	10000	7.592±0.04	0.61±0.03	25±0.1	0.37±0.08
Food waste	500	6.461±0.02	1.08±0.01	25±0.1	0.23±0.02
	1000	6.823±0.03	0.91±0.02	25±0.1	0.25±0.04
	25000	6.064±0.05	0.93±0.01	25±0.1	0.36±0.06
	5000	5.963±0.02	0.32±0.01	25±0.5	0.35±0.05
	10000	5.501±0.03	0.41±0.02	25±0.1	0.38±0.04

3.2 Population growth of *M. macrocopa*

Fig. 1 shows the population growth of *M. macrocopa* with four different organic waste treated medium for 21 days experimental period, with five concentrations each one. 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. Fig. 2 showed the mean population growth of *M. macrocopa* cultivated in 1,000 ppm concentration of four culture medium. The 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.

3.3 Life Table Demography

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

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 (Fig. 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 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.

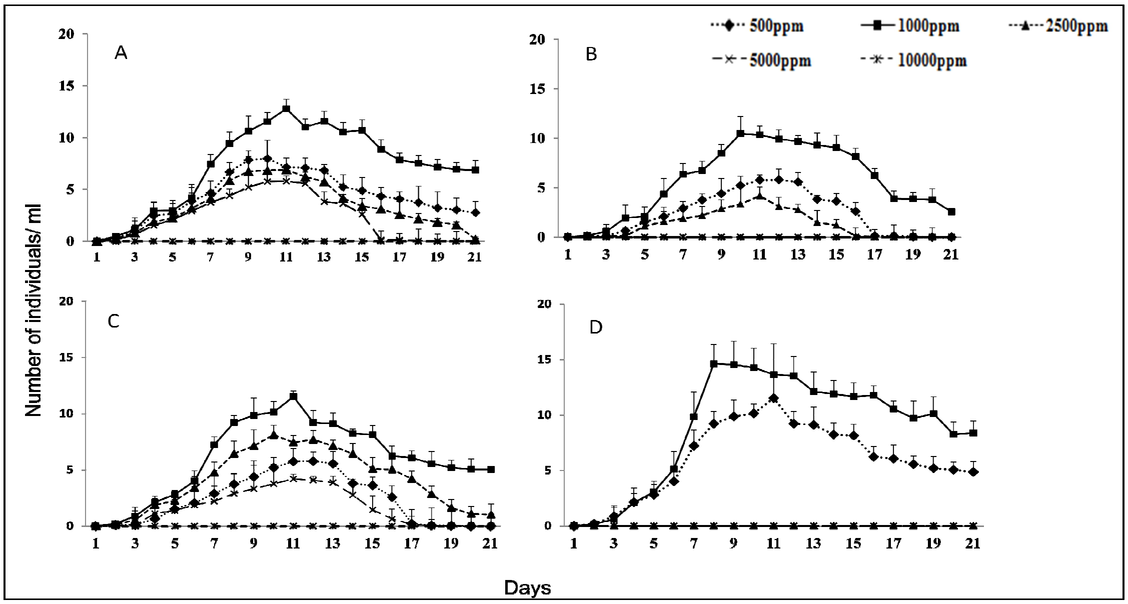

Fig. 1. Population growth of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste culture medium for 21 days experimental period

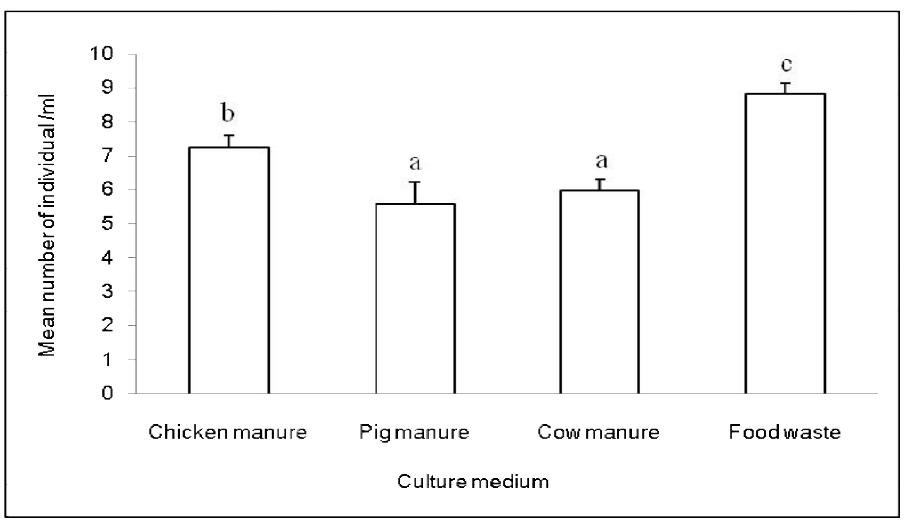

Fig. 2. Mean population growth of *M. macrocopa* cultured in 1000 ppm concentration of four culture mediums. Values are the (mean ± SD)

Note: Different letters shown significant differences (p<0.05)

Table 2. Life table of *M. macrocopa*, cultured with different organic wastes at different concentrations.Data are the means with the standard error of three replicates

Medium types	Medium Conc.(ppm)	Initial age of reproduction	Longevity	Net reproduction rate	Gross reproduction rate	Generation time	Rate of population 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	-	-	-	-

Dash (-) indicates no offspring was produced

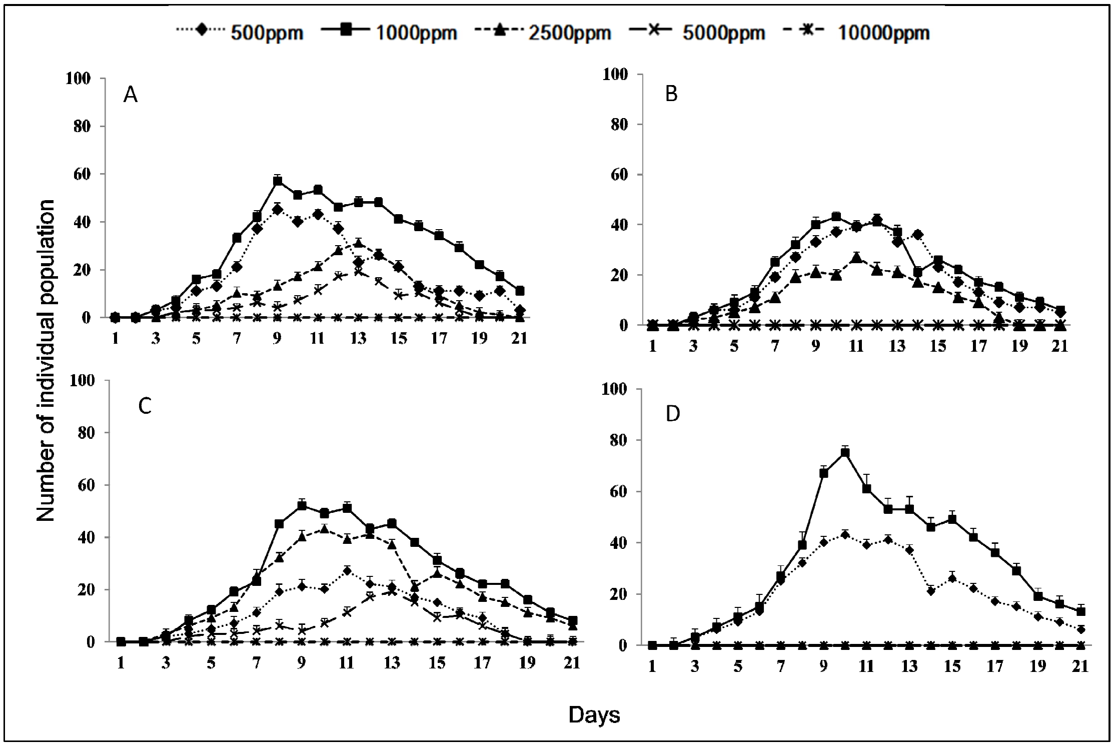

Fig. 3. Population density from a single neonate of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste treated medium for 21 days experimental period. Error bars indicate means ± standard deviation

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 is 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 cladocerans, *M. macrocopa* has been investigated most intensively regarding 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 efficiency of *M. macrocopa* using various concentrations of different organic wastes as a culture medium is different. Low population growth was observed in the 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 harm 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. High concentrations of all the diets produced suboptimal culture conditions. Burak [34] & Porter [35] described that a high concentration of particles can 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 the population of *M. macrocopa* declined in

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

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

Data of 1,000 ppm concentration of different organic waste are shown here.
The hyphen (-) indicates non- detectable fatty acids

using a 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 efficiency. Among the culture medium, the highest population growth was observed in food waste medium. *M. macrocopa* tends to ingest more bacteria, which are enriched by food waste. This might be the reason for the excellent performance of this food source. In this study, pH increased with the high concentration of animal manures but food waste showed a different trend where pH decreased with the increasing concentrations, might be the presence of higher amount longer chain fatty acids. Life table demography of *M. macrocopa* followed the same trend. The average lifespan was lowest in culture mediums with higher concentration. Food waste containing 1,000 ppm concentration showed the highest average lifespan and early reproduction ability. The present study showed that *M. macrocopa* needs time to become sexually mature at high diet concentration (phytoplankton food). In contrast, Loh [32] reported that the initial age of reproduction of *M. macrocopa* is earlier in high concentration diet than in low concentration. Different results are observed in this test, which indicates that diet type and concentration play a significant role in determining the initial age of reproduction. Gross and net reproduction rates were generally higher at lower treatment concentrations and highest in food waste medium than other diets. Jana and Pal [36] revealed in their study, high fecundity and gross reproduction rates suggest that the growth efficiency of any species largely depends on the high carbon/nitrogen ratio in the food source. Which indicates that food waste contained high C/N ratio than the other medium.

The highest population density was obtained in 1,000 ppm concentration of food waste medium. The good performance of this food source can be attributed to the feeding habit of *M. macrocopa* that tend to consume bacteria and filtered particles that are abundant in food waste when other food sources have limited. In terms of time and efficiency, *M. macrocopa* cultured in food waste reached its peak population on day 10 which was about 75 individual. This also means that *M. macrocopa* could be harvested by food waste within a shorter period, thus allowing more number of cultivation batches per cycle which is important for commercial live feed producers. After reaching the peak population day within the second week, the population began to decrease from the starting of the third week. Which could be caused by insufficient space, food availability, sexual transformation, and/or allelopathic effects [37,10,38,39,40]. Jiun [41] reported that *M. macrocopa* has a higher density adaptation in a captive culture environment compared to *M.*

micrura, because high stocking density may lead to a population collapse. According to Jana and Pal [36], the growth performance of *M. macrocopa* was limited at the density of 4 ind. and 20 ind./ 50ml. Results of this study showed that *M. macrocopa* had a better adaptation in food waste treatment at the density up to 75 individual per 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 number 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 polyunsaturated 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]. The 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 was fed algal diets in another study [10]. This result suggests that *M. macrocopa*, when the 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 the case of food waste, this contamination possibilities is comparatively low. Studies have 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]. Many works mentioned that manure can be used as fertilizer to produce phytoplankton. The important in cladocerans culture was the type of phytoplankton and Vitamin B source.

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 a higher percentage of n-3 HUFA , which may serve as effective, inexpensive and sustainable food sources for *M. macrocopa* cultivation.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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