Culture of *Moina macrocopa* using different types of organic wastes

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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. 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 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, 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 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 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 [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 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 quality and quantity required for maximizing production. Determination of 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 *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 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 dichlorination. 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 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].

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

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.

Comment [JC1]: Why this parameter, the authors did not make salinity experiments with a freshwater organism

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].

Comment [JC2]: And gross reproduction rate definition???????

Average Longevity (AL) = $\sum n_x/n$;

Gross Reproduction Rate (GRR) = $\sum m_x$;

Net Reproduction Rate (Ro) $=\sum l_x m_x$;

Generation Time (GT) $= \sum l_x m_x x /Ro$

Where,

n_x =Number of individuals alive for each age class

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

l_x =The proportion of individuals surviving to age x

n = The number of replicates

Final Rate Population Increase (r) = $(\ln N_t - \ln N_0)/t$

Where

N_0=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 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 solvent using a rotary evaporator (R-114, BUCHI, Swiss) at 38 ℃. 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 ℃

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 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℃; injection temperature = 250℃; column temperature =180℃ for 8 min, programmed to increase at 3℃/min up to 230℃ 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 with reference to 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 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.

Comment [JC3]: Why with respect environmental parameters???? The physic-chemical parameters of water were used, and they do not change highly (only in high manure concentration). Salinity is not important.

In other think, the authors have two variables in their experiment: different manures and different concentrations. They need to make a two-way ANOVA test to know the significance of each variable in this experiment.

The One-Way ANOVA test can be used if authors wants to compare the final density from each treatment

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 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 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})	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

Comment [JC4]: This is not important, because the authors controlled it.

Comment [JC5]: This information is not relevant. Please eliminated

3.2 Population growth of M. macrocopa

Figure 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. 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.

Comment [JC6]: Please uniform this sign, because the authors change to cursive letter and not cursive letter with capital letter.

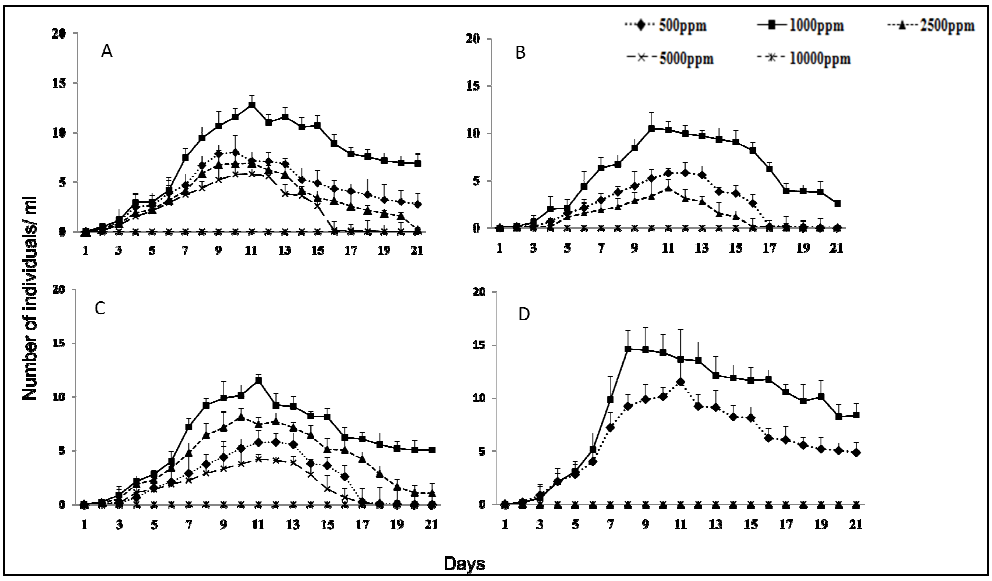

Fig. 1. Population growth of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste culture medium for 21 days experimental period~~. Error bars indicate means ± standard deviation.~~

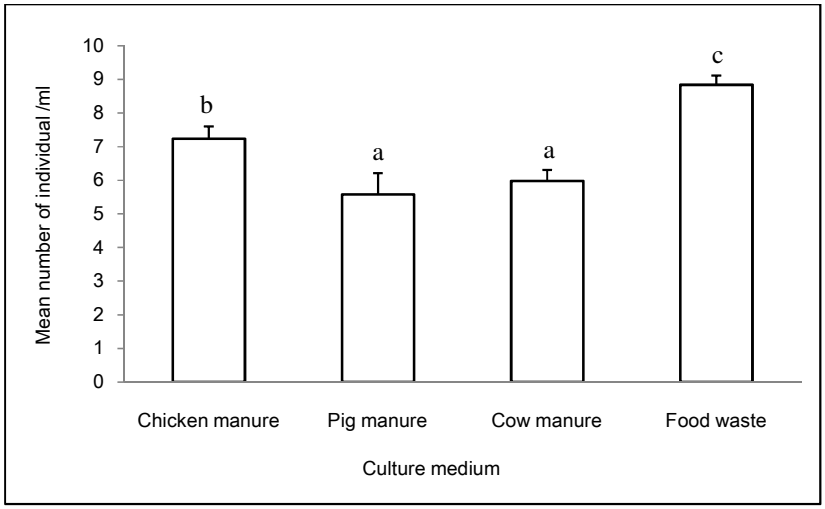

Fig. 2. Mean population growth of *M. macrocopa* cultured in 1000ppm concentration of four culture mediums. Values are the (mean ± SD).

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

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* in relation to 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 treatments. 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.

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 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	-	-	-	-

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.

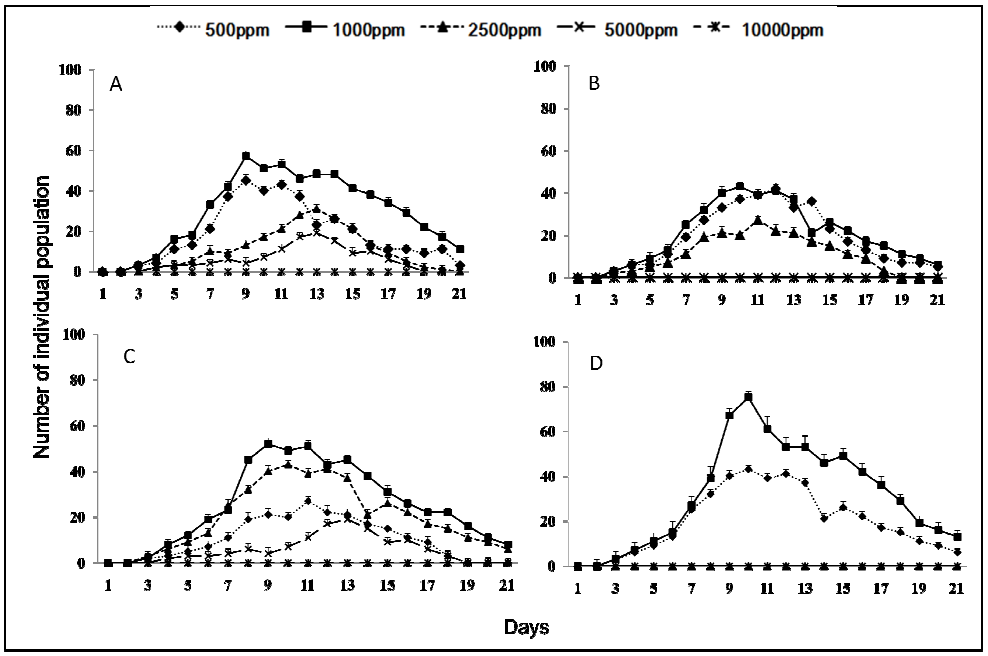

Fig. 3. Population density from a single neonate of *M. macrocopa* in different concentrations of (A) chicken manure, (B) pig manure, (C) cow manure, & (D) food waste treated medium for 21 days experimental period. Error bars indicate means ± standard deviation.

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 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 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

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.
Hyphen (-) indicates non- detectable fatty acids.

efficiency. Among the culture medium, the highest population growth was observed in food waste medium. In this study pH increased with the high concentration of animal manures but food waste showed 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. 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 became sexually mature at high diet concentration. In contrast, Loh [32] reported that, 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 growth efficiency of any species is largely depends on the high carbon/nitrogen ratio in the food source. Which clearly 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 has 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 of time, thus allowing more number of cultivation batches per cycle which is important for commercial live feed producers. After reaching the peak population day within second week, the population began to decrease from the starting of third week. Which could be caused by insufficient of 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 possibly 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.

Comment [JC7]: Yes, but why??? The authors need to discuss this more.

Comment [JC8]: Why the presence of longer chain fatty acids modifies the water pH????

Comment [JC9]: But this is with phytoplankton food

Comment [JC10]: This is the important of this experiment. The manure and food waste particles were no important, the important was the bacterial production with these sources.

That's why highly particles in culture medium gave badly result. In other way, manure can increase phytoplankton concentration and many authors mentioned that high phyto concentration was badly to produce cladocerans culture.

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.

Comment [JC11]: Many works mentioned that manure can be used as fertilizer to produce phytoplankton

The important on cladocerans culture was type of phytoplankton and Vitamin B source.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

KK, AM and SJK designed the study. KK wrote the article. KK, AM and UCJ manufactured the experimental feed, conducted the feeding trial and performed the analyses. SJK conceived and coordination and revised the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

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

REFERENCES

1. Akodogbo HH, Bonou AC, Sossou SD, Adande R, Fiogbe DE. Comparison of two Techniques on the Optimization of Zooplankton Production from Pig Dung: Renewed and Non-Renewed Medium. International Journal of Multidisciplinary and Current Research. 2015; 3:200-205.

2. Legendre M, Teugels GG. Development and thermal tolerance of eggs in *Heterobranchus longifilis*, and comparison of larval developments of *H. longifilis* and *Clarias gariepinus* (Teleostei, Clariidae). Aquat Living Resources. 1991; 4: 227-240.

3. Legendre M, Teugels GG, Cauty C, Jalabert B. A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822), Heterobranchus longifilis Valenciennes, 1840, and their reciprocal hybrids (Pisces, Clariidae). Journal of Fish Biology. 1992; 40: 59-79.

4. Jha P, Barat S, Nayak CR. A comparison of growth, survival rate and number of marketable koi carp produced under different management regimes in earthen ponds and concrete tanks. Aquaculture International. 2006; 14: 615-626. doi: http://dx.doi.org/10.1007/s10499-006-9059-9

5. Sorgeloos P, Dhert P, Candreva P. Use of the brine shrimp, Artemia spp., in marine fish larviculture. Aquaculture. 2001; 200: 147-159. doi : https://doi.org/10.1016/S0044-8486(01)00698-6

6. Kang CK, Park HY, Kim MC Lee, WJ. Use of marine yeast as an available diet for mass cultures of *Moina Macrocopa*. Aquaculture Research. 2006; 37(12): 1227-1237. doi : http://dx.doi.org/10.1111/ j.1365 -2109.20 06.01553. x

7. Hyppolite A, Antoine C, Clement AB, Philippe AL. Survival and Growth of *Clarias gariepinus* and *Heterobranchus longifilis* Larvae Fed with Freshwater Zooplankton. Journal of Agricultural Science and Technology. 2012; 2: 192-197.

8. Alam MJ, Ang KJ, Cheah SH. Use of *Moina micrura* (Kurz) as an Artemia substitute in the production of *Macrobrachium rosenbergii* post-larvae. Aquaculture. 1993; 109 (3-4): 337-349. doi: https://doi.org/10.1016/0044-8486(93)90173-V

9. Sarah LP, Philipp D, Maik JL, Christian EW, Steinberg. Culture of the cladoceran *Moina macrocopa*: Mortality associated with flagellate infection. Aquaculture. 2013; 416–417: 374-379. doi : https://doi.org/10.1016/j.aquaculture.2013.09.029.

10. He ZH, Qin JG, Wang Y, Jiang H, Wen Z. Biology of *Moina mongolica* (Moinidae, Cladocera) and perspective as live food for marine fish larvae: review. Hydrobiologia, 2001; 457: 25-37.

11. Ingram BA. Culture of juvenile Murray cod, trout cod and Macquarie perch (Percichthyidae) in fertilized earthen ponds. Aquaculture. 2009; 287(1–2): 98-06. doi: https://doi.org/10.1016/j.aquaculture.2008.10.016

12. Aguado FP, Nandini S, Sarma. Functional response of *Ameca splendens* (Family Goodeidae) fed cladocerans during the early larval stage. Aquaculture Research. 2009; 40:1594-1604. doi: https://doi.org/10.1111/ j.1365 -2109.20 09.02259. x

13. Loh JY, Ong HKA, Hii YS, Smith TJ, Lock MW, Khoo G. Highly unsaturated fatty acid (HUFA) retention in the freshwater cladoceran, *Moina macrocopa*, enriched with lipid emulsions. The Israeli Journal of Aquaculture. 2012; 64: 637-646.

14. Pagano M, Koffi MA, Cecchi P, Corbin D, Champalbert G, Saint- jean L. An experimental study on the effect of nutrient supply and Chaoborus predation on zooplankotn communities of a shallow tropical reservoir. Freshwater Biology. 2003; 48: 1379–1395.

15. Sayali S P, Andrew JW, Martin SK, Andrew SB. Utilizing bacterial communities associated with digested piggery effluent as a primary food source for the batch culkture of *Moina australiensis*. Bioresource Technology. 2009; 101 (10): 3371-3378. doi: https://doi.org/10.1016/j.biortech.2009.12.030

16. Nandini S, David AL, Sarma SSS, Pedro RG. The ability of selected cladocerean species to utilize domestic wastewater in Mexico city. Journal of Environmental Management. 2004;71(1) :59-65. doi : https://doi.org/10.1016/j.jenvman.2004.02.001

17. Il- ho K, Hyun-dong L, Jai-yeop L. Reduction treatment of food waste with malodor in Korea. Advanced Science and technology letters. 2016; 136 : 30-32. doi : http://dx.doi.org/10.14257/astl.2016.136.08

18. Jeremiah K, Joseph AB, Laura CH. Effect of using different types of organic animal manure on plankton abundance, and on growth and survival of *Tilapia Rendalli* in ponds. Aquaculture Research. 2006; 37: 1360-1371.doi: http://dx.doi.org/10.1111/ j.1365 -2109.20 06.01569. x

19. Prithwiraj J, Kropan S, Sudip B. Effect of Different Application Rates of Cowdung and Poultry Excreta on Water Quality and Growth of Ornamental Carp, *Cyprinus carpio* vr. koi, in Concrete Tanks. Turkish Journal of Fisheries and Aquatic Sciences. 2004; 4: 17-22.

20. Atay D, Demir N. The effects of chicken manure on the phytoplankton primary production in carp ponds. Acta Hydrobiologica. 1998; 40: 215 – 225.

21. Gupta MV, Noble F. Integrated chicken – fish farming. M. Halwart, J. Gonsalves and M. Prein (Eds.), Integrated agriculture – aquaculture : A primer, FAO Fisheries Technical Paper. 2001; 407: 49 – 53.

22. Majumdar S, Biswas S, Barat S. Abundance of ammonifying and heterotrophic bacterial populations in the water manured with cowdung and distillery sludge in outdoor model tanks. Asian Journal of Microbiology, Biotechnology and Environmental Science. 2002; 4: 229 – 233.

23. Yoshimatsu T, Imoto H, Hayashi M, Toda K, Yoshimura K. Preliminary results in improving essential fatty acids enrichment of rotifer cultured in high density. Hydrobiologia. 1997; 358: 153-157.

24. Olsen A.I, Jensen A, Evjemo JO, Olsen Y. Effects of algal addition on stability of fatty acids in enriched *Artemia franciscana*. Hydrobiologia. 1997; 358: 205-210.

25. Tomonari K, Hiroshi F, Aki M, Hiroshi F. Effects of feeding with frozen freshwater cladoceran Moina macrocopa on the performance of red sea bream Pagrus major larviculture. Aquacult. Int. 2016; 24: 183–197. doi: https://doi.org/10.1007/s10499-015-9918-3

26. Muller NDC, Brett MT, Liston. A highly unsaturated fatty acid predicts biomass transfer between primary producers and consumers. Nature. 2000; 403: 74-77

27. Boyd CE, Lichktoppler F. Water Quality Management in pond fish culture. International Centre for Aquaculture. Agriculture experimentation station Auburn University Research Development Series No. 22. Project AD/DSANG. 0039.

28. Chuah TS, Loh JY, YS Hii. Acute and chronic effects of the insecticide-Endosulfan on freshwater cladoceran, Moina macrocopa Straus. Bull. Environment Contamination. Toxicology. 2007; 79: 557-561. doi : https://doi.org/10.1007/s00128-007-9234-3

29. Krebs CJ.. Ecology. In: The Experimental Analysis of Distribution and Abundance. Harper and Row. 1985; New York. 789 pp

30. Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 1959; 37: 911–917.

31. Savas S, Erdogan O, Cicek NL. Effects of L-carnitine on growth of individually cultured cladoceran, *Moina micrura.* Israeli Journal of Aquaculture. 2011; 63: 614.

32. Loh JY, Ong HKA, Hii YS, Smith TJ, Lock MW, Khoo G. Impact of Potential Food Sources on the Life Table of the Cladoceran, *Moina macrocopa*. The Israeli Journal of Aquaculture. 2013; 65:820.

33. Nandini S, Sarma SSS. Lifetable demography of four cladoceran species in relation to algal food (*Chlorella vulgaris*) density. Hydrobiologia. 2000; 435 (1–3): 117–126.

34. Burak ES. Life tables of *Moina macrocopa* (Straus) in successive generations under food and temperature adaptation. Hydrobiologia. 1997; 360:101-108.

35. Porter K.G., Gerritsen J. and J.D. Orcutt Jr. 1982. The effect of food concentration on swimming patterns, feeding behaviour, ingestion, assimilation and respiration by Daphnia. Limnol. Oceanogr. 27: 935-949.

36. Jana BB, Pal GP. Some life history parameters and production of *Daphnia carinata* (King) grown in different culturing media. Water Research. 1983; 17: 735-741. doi: https://doi.org/10.1016/0043-1354(83)90067-2

37. Fernando MJ, Jesus RE, Rafael VC. Effect of culture density and volume on *Moina micrura* reproduction, and sex ration in the progeny. Hydrobiologia. 2007; 594: 69-73. doi: https://doi.org/10.1007/s10750-007-9081-6

38. Hobaek A, Larsson P. 1990. Sex determination in Daphnia magna. Ecology. 2001; 71: 2255-2268. doi : https://doi.org/10.2307/1938637

39. Innes DJ, Singleton DR. Variation in allocation to sexual and asexual reproduction among clones of cyclically parthenogenetic *Daphnia pulex* (Crustacea: Cladocera). Biological Journal of Linnean Society. 2000; 71: 771-787. doi: https://doi.org/10.1006/bijl.2000.0474

40. Pagano M, Jean SL, Arfi R, Bouvy M, Shep H. Population growth capacities and factors in monospecific cultures of the cladocerans *Moina micrura* and *Diaphanosoma excisum* and the copepod *Thermocyclops decipiens* from Ivory Coast (West Africa). Aquatic Living Resources. 2000; 13:163-172. doi: https://doi.org/10.1016/S0990-7440(00)00152-2

41. Jiun Y L, Han KA, Ong YS, Hii GK. The effects of recirculating aquaculture system effluent water on the growth of Moina macrocopa. International Journal of Zoology Studies. 2016; 1(2): 1-8

42. Jose LGF, Maria EHS, Sarma SSS, Nandini S, Ricardo ZM, Ramesh DG. Temperature and age affect the life history characteristics and fatty acid profiles of *Moina macrocopa* (cladocera), Journal of Thermal Biology. 2015; 53: 135-142. doi : https://doi.org/10.1016/j.jtherbio.2015.10.005

43. Golder D, Rana S, Sarker D, Jana BB. Human urine is an excellent liquid waste for the culture of fish food organism. Ecological Engineering. 2007; 30(4): 326-332. doi: https://doi.org/10.1016/j.ecoleng.2007.04.002

44. Siebe C, Cifuentes E. Environmental impact of wastewater irrigation in central Mexico: an overview. International Journal of Environmental Health Research. 1995; 5: 161-173. doi : https://doi.org/10.1080/09603129509356845

45. Cheng Z, Lam CL, Mo WY, Nie XP, Choi YB, Man WM, Wong MW. Food wastes as fish feeds for polyculture of low trophic level fish: bioaccumulation and health risk assessments of heavy metals in the cultured fish. Environment Science Pollution Reseach. 2016; 23: 7195-7203. doi: http://dx.doi.org/10.1007/s11356-016-6484-9.