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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effects of concentrations, different combination of manures and period of growth on mass production of *Daphnia pulex*

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Abstract

To examine the effects of the concentration and combinations of manure on the population growth of the water flea *Daphnia pulex*, screened from Awuma River at Shabu before the onset of rainy season and isolated into mono specific culture in the laboratory. Cow dung, chicken dropping, groundnut cake, soybean cake, rice bran and single super phosphate were combined in different proportions of three, four and five combinations of manure solutions. Each combination solution (3CM, 4CM and 5CM) had five concentrations (1.00, 2.00, 4.00 and 8.00 and 16.0 ml/L) and each concentration had three replicates for a culture period of 12 days. Effects of concentration, combination of manure and duration of culture of on the water flea population growth were tested using analysis of variance. Dissolved oxygen, (mg/L) temperature (0ºC), carbon dioxide (mg/L) and total alkalinity (mg/L) of the water used for the various treatments were monitored. The results revealed that manure combination (5CM1) and concentration (2.00ml/L.) of water had the highest population density of *D. pulex* while day 9 proved to be the best period of the culture of the studied organism. The interactions of concentration, combination of manure and period of culture showed the highest population density of *D. pulex* (26,303 individual/L of water) was observed in 5CM1 at concentration of 4.00ml/L of water on day 9. Beyond day 9, a declining population density was observed in *D. pulex* in the treatment combinations. 5CM1 at concentration 4.00ml/L at day 9 is recommended for *D. pulex* in batch culture.

Keywords: *Daphnia pulex*; Density; Manure in solution; Population; Zooplankton

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Introduction

Live food micro-organisms are important feed sources for many fish species and the success of culturing zooplanktivorous fish fry depends primarily on zooplankton, its composition and density (Fernando, 1994). Sipaúba-Tavares and Bachion (2002) reported that zooplankton reproduction and growth rate increases the availability of good feed quality for subsequent trophic levels. Many species of live food organisms used in larviculture have superior and natural nutritional value than formulated diets. However, some live food zooplankton are selected as feed sources in larviculture based on certain qualities such as purity, availability, acceptance, nutritional indicators (digestibility and organism nutrients/ energy), easily obtainable, easy reproduction and economical viability (Watanab and Kiron, 1994). Sipaúba-Tavares and Bachion (2002) reported that the culture of Cladocerans offers the possibility of obtaining a large number of live food organisms within short periods of time under optimum conditions of temperature, food and water quality. These live food micro organisms are valuable source of protein, lipids, fatty acids, mineral and enzymes.

Gupta and Gupta (2006) reported the use of organic and inorganic manure for the culturing of zooplankton. The use of cow dung, chicken dropping, horse manure, rice bran and mineral fertilizer is encouraged by Rottmann et al. (2003). Under natural

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conditions, water fleas, rotifers and other zooplankton species generally feed on microscopic organic particles (bacteria, phytoplankton, fungi and protozoan) that are suspended in water (Rodolfo and Edmundo, 1980; Kim et al., 2008).

Normally, fish fry grows in the wild where prey items are readily available. In the hatcheries, where most of the activities are artificial, the survival of fry depends on availability of right food. The development of techniques for mass culture of live food organisms in hatcheries is becoming more popular for successful larval rearing of cultivable fish species. It is important to note that not all zooplankton are suitable for fry rearing but live Daphnia species were reported to be good freshwater zooplankton (Olojo et al., 2003) that can enhance protein and other food content for the rearing of fry in hatcheries. The biochemical compositions of Daphnia are: crude protein 70.1%, moisture 89.3%, crude fibre 13.07% (Creswell, 1993). The survival of fry of planktivorous fishes that does not accept formulated diet is extremely difficult. When the yolk is exhausted, the fry starves to death as they habitually refuse inert diets (Tech, 1981). In some cases where the ponds are naturally fertilized, dominant species of zooplankton are undesirable and some times larger than the mouth parts of the fish hatchlings and some of the organisms intimidate fry (Tech, 1981). In some cases, zooplankton may present in the pond but not enough to meet the needs of the fry. Direct fertilization of ponds with organic or inorganic manure methods for zooplankton production always cause heavy algal bloom which may initiate deleterious water quality that could lead to high mortality of fry in such ponds.

Since the collection of zooplankton from the wild is a game of chance, seasonal encourages predation and disease infections on fry, this work intends to develop culture methods for the mass production of local freshwater strains of mono specific live food organisms (irrespective of season) to meet the demands of fry production. The production of the live food organisms are intended to serve as alternatives to shell free Artemia and Artemia nauplii which are marine, costly and not readily available locally. The study aimed at examining the effects of the concentration and combinations of manure on the population growth of the water flea *Daphnia pulex.*

Materials and Methods

Determination of water quality parameters used in laboratory

Laboratory examination of water quality

The water quality parameters were examined using APHA/AWWA/WPCF (1995) methods. The temperature of the water used for various treatments was measured using a mercury centigrade dry bulb thermometer. The readings were taken in few centimetres below the surface of the water. The mean of three readings were taken for each group.

The pH of water for each treatment was measured using a pH meter model 901. Three readings were taken in each case and their means was recorded. The free carbon dioxide dissolved in water was measured using phenolphthalein method. About 100 ml of water was taken from 25 cm below the water surface into a conical flask and 10 drops of phenolphthalein indicator was added. The sample was then titrated till the colour change was observed using N/44 NaOH. The free Carbon-dioxide (ml/L) was recorded as ten times the amount (ml) of N/44 NaOH used for the titration. The Alsterbeg (Azide) method was employed to determine dissolved oxygen. The water samples were collected using 250 ml stopper bottle at 25cm below the water surface. The bottles were corked inside the water to avoid any trapping of air. The water samples were then fixed by adding 2 ml of manganese sulphates and 2 ml of alkaline-iodide (Sodium Azide). The water was restored and a careful shaking was done for proper mixing. The samples were allowed to settle for few minutes and then 2 ml of concentrated Sulphuric acid was added. Careful mixing was done by shaking until a solution is formed. About 200 ml of the formed solution was transferred into a conical flask and titrated to pale yellow using 0.025N Sodium Thiosulphate. When 1 ml of 1% starch solution was added, the solution turned blue immediately. Titration was carried out until the blue colour first disappears. The volume of the 0.025N sodium thiosulphate used in the titration was recorded as the amount of oxygen in the water sample (mg/L). A sample of water of 100 ml was taken into a conical flask from below the water. About 4 drops of phenolphthalein indicator was added. The water sample was clear. Two drops of methyl orange indicator were added and titration was carried out until the greenish yellow colour turned pink-orange. Ten times the volume (ml) of the 0.02N sulphuric acid for titration was recorded as the alkalinity of the water (mg/L) of $CaCO_3$.

Screening and acclimatization of *Daphina pulex*

Collection of zooplankton from water was done with modified standard Clarke-Bumpus zooplankton sampler fixed with straining net and collection bottle (Ovie, 1991; Adigun, 2005). Water samples were collected from different locations of Awunma River at Shabu (Plate1) into a 4-litre container at 6:00-6:30 am in the morning. At the littoral zone, sampling was done from water among the weeds by filter using zooplankton harvesting net (Ovie and Sarma, 1993). Right at the river bank, the filtration was done (Ovie and Sarma, 1993) and debris, macrophytes, macroorganisms and fish fry were removed using a mosquito net (Adigun, 2005). The remaining micro-organisms were taken to the laboratory at the Faculty of Agriculture, Nasarawa State University, for microscopic examinations. Two mesh sizes were used (the upper 2000µm while the lower 50 µm sieve). The lower end collects the organism while the upper end collected the waste particles in water filled bowl (Garza-Mourino et al., 2005).

In the residues, automatic pipette was used to place some few drops of the water samples on a glass slide fixed on a microscope (Ovie et al., 1993). The sample was observed in x10 objective of the microscope (Javellana and Escritor, 1981) while the automatic pipette was used for the collection of micro organisms present into a separate 2-litre aquarium containing aerated water covered by mosquito net (Ovie et al., 1993). The isolated micro organisms were fed with green algae culture dominated by Scenedesmus acuminatus, Ankistrodesmus convolutus and Chlorella vulgaris at concentration 1.5 x 106 cells/ml and this was determined by the help of a haemocytometer (Ovie and Eborge, 2002). On the third day, the growth of population of this selected organism was noticed in each of the glass aquarium with some other zooplankton.

Isolation of *Daphina pulex* into mono specific Culture

Repeated subculture method was adopted with slight modifications (Villegas, 1981). The procedures were as follow:

A drop of plankton water was put into several places in a glass slide fixed on the microscope (in some cases, dilution of the plankton water was done to lower the number of organisms and kinds in the drop). With the help of automatic pipette, the desired organisms (*D. pulex*) from conical flask 'b' were collected and transferred into another glass conical flask 'c' containing aerated algal water at temperature 260ºC and pH 7.8. Incubation was done under controlled temperature, light intensities and illumination time. After 3 days under these conditions, a dominant desired species appeared in the conical flask 'c' then the process was repeated in conical flask 'd', 'e' and then 'f' to produce a mono specific culture of the desired Daphnia species. The cultured organisms were identified by Monogoonta invertebrate taxonomy (Koste and Shiel, 1987) and identification of Daphnia of low land (Fernando et al., 1987). The isolated mono specific culture of *D. pulex* were fed freshwater green algae culture as stated above (Ovie and Egborge, 2002; Sipaúba-tavares and Bachion, 2002) prior to treatment with various combinations of manures at various concentration. The microphotographs of cultured *D. pulex* were taken by Sony digital camera, cybershot model 7.2 fixed on Olympus binocular microscope.

Preparation of the different combinations of the manure solution

All the manures (organic and inorganic), cakes (Gupta and Gupta, 2006) and rice bran (Rottmann et al., 2003) were grounded to powder, weighed and mixed

Table1: Manure treatment combinations

Name	Combinations of manure (g/L of water)					
3CM1	Cow dung, (7.5)	Soybean cake (3.75)	Single super phosphate (1.5)			
3CM2	Chicken droppings(7.5)	Soybean cake, (3.75)	Single super phosphate (1.5)			
3CM3	Cow dung, (7.5)	Groundnut cake (3.75)	Single super phosphate (1.5)			
3CM4	Chicken droppings(7.5)	Groundnut cake (3.75)	Single super phosphate(1.5)			
4CM1	Cow dung(7.5)	Soybean cake (1.88)	Rice bran (1.87)	Single super Phosphate (1.5)		
4CM2	Chicken droppings (7.5)	Soybean cake (1.88)	Rice bran (1.87)	Single super phosphate (1.5)		
4CM3	Cow dung(7.5)	Groundnut cake (1.88)	Rice bran (1.87)	Single super Phosphate (1.5)		
4CM4	Chicken droppings (7.5)	groundnut cake (1.88)	Rice bran (1.87)	Single super Phosphate (1.5)		
5CM1	Cow dung(7.5)	Groundnut cake (1.25)	Soybean cake (1.25)	Rice bran (1.25)	Single super phosphate (1.5)	
5CM2	Chicken droppings (1.5)	Groundnut cake (1.25)	Soybean cake (1.25)	Rice bran (1.25)	Single super phosphate (1.5)	
5CM3	Cow dung(7.5)	Chicken droppings (1.25)	Groundnut cake (1.25)	Soybean cake (1.25)	Single super phosphate (1.5)	
5CM4	Cow dung(3.88)	Chicken droppings (3.87)	Groundnut cake (1.25)	Soybean cake (1.25)	Rice bran (1.25)	Single super phosphate (1.5)

3CM, 4CM and 5CM = three, four and five ingredients Combinations of Manure; Figures in parenthesis = dried weight in g/L of water.

in the proportions and combinations shown in Table 1. The combinations of manure were soaked in distil water for 36 hours in a dark room (Jana and Chakrabarti, 1993). After the 36 hours of soaking, the solution was filtered through different mesh size and finally through 50µm mesh filter. The filtrates were kept in clean corked bottles in the dark room to avoid algal growth (Gupta and Gupta, 2006; Arimoro, 2006) for culturing of *D. pulex*.

Daphnia pulex culture using different combinations of manure

Ten pure cultures of *D. pulex* were placed into one litre of aerated borehole water each (Delbare and Dhert, 1996; Ovie and Ovie, 2004) in 3 series of 4 different combinations of manure (Table1) at 5 different concentrations (1.00, 2.00, 4.00 and 8.00 and 16.0 ml/L) each in 3 replicates. The concentrations of the manure were fed every morning to the *D. pulex* culture for 12 days. Each concentration was aerated gently to distribute the nutrient in the culture (Ovie and Ovie, 2004). The population density of *D. pulex* was observed using microscope and automatic pipette (Ovie, 1991). The automatic pipette (graduated) was used to collect samples randomly into sampling bottle. The bottle was inverted several times and individual *D. pulex* in the sub-sample were measured using modified Sedgwick Rafter counter (Ovie, 1991). The population density of *D. pulex* of a known volume was counted and the organisms were returned to the stock. In the terminal counting (i.e. at the end of the experiment) especially when population decline was noticed (Ovie and Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0 g, Iodide crystals 1.0 g and water 100 ml) was used to paralyze the organisms in the collected water sample so that counting was easier and more accurate (Javellana and Escritor, 1981).

Estimation of population density of live food zooplankton in the study

Estimation of *Daphnia pulex* population density was done according to Ovie (1991);

Pd = 1000 x Bd

V (ml)

Pd = Population density of *D. pulex* in 1000 ml of water.

V = Average volume of the sampled water using automatic pipette

Bd = Average Number of *D. pulex* counted in various random Samplings

Statistical Analysis

The data obtained were analyzed using descriptive statistics, general statistic of variance of Genstat 32.22 version statistical package. Statistical difference between various means was tested at 95% confidence level using Duncan Multiple Range Test.

Results and Discussion

Effects of different combinations of manure on population density of *D. pulex*

Cultured *D. pulex* (Plate 1) were treated with 3, 4 and 5 ingredients different combinations of manure to determine the one that will raise the best population density of the studied organism. The results (Fig. 2) showed that 3 ingredients combinations of manure (3CM1, 3CM2, 3CM3 and 3CM4) were significantly different ($P<0.05$) from each other. Combination 3CM2 had the highest mean population density of *D. pulex* (325 individual/L of water) and it was significantly different ($P<0.05$) from all other 3-combination manure in this investigation.

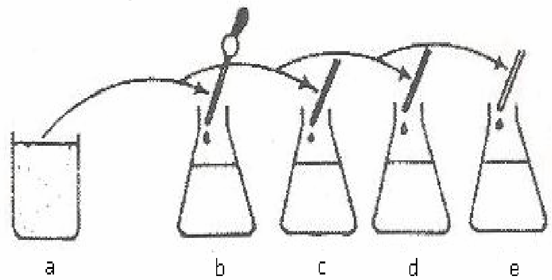

Fig. 1: Diagram of repeated subculture method

Source: Villegas (1981)

a = Diluted original solution containing zooplankton.

b = Solution containing few species of zooplankton

c = Solution with dominant species of desired zooplankton.

d = Solution with monospecific species of desired zooplankton.

e = Solution containing confirmed desired zooplankton

Plate 1: Microphotograph of the cultured *Daphnia pulex*

It further revealed that the 4 ingredients combinations of manure show the mean values of population density of *D. pulex* as follows: 4CM1, (227), 4CM2 (750) 4CM3 (618) and 4CM4 (987.87 individual *D. pulex*/L of water). Among this group, 4CM4 combination of manure had the highest population density of *D. pulex* and were significantly different (P<0.05) from other combinations in this series. Fig. 2 also showed that the population densities of *D. pulex* from 5 ingredients combinations of manure were: 5CM1, (3,588), 5CM2 (732) 5CM3 (757) and 5CM4 (662) individual/L of water. These values were significantly different (P<0.05) from each other. The highest population density of *D. pulex* was recorded in 5CM1 while 5CM4 had the lowest population density among the series of manure combinations.

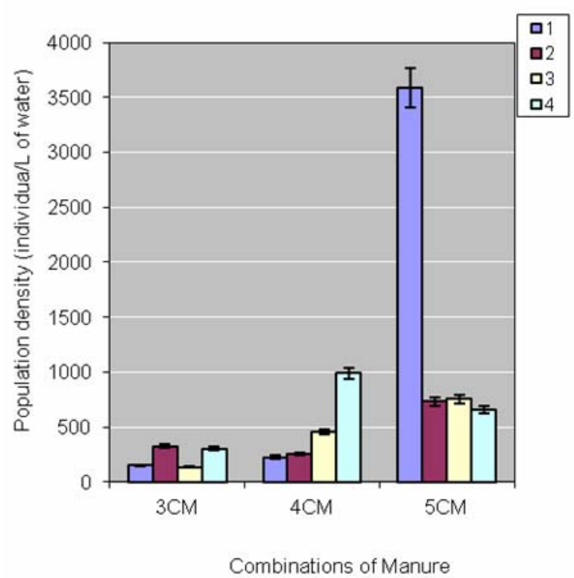

Fig. 2: Effects of combinations of manure on mass production of *Daphnia pulex*.

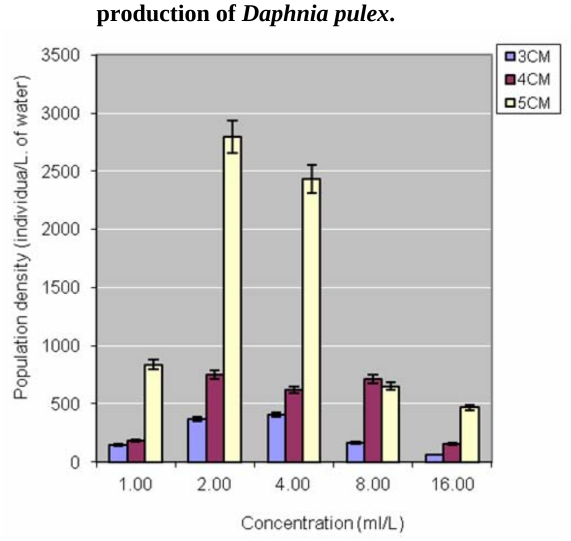

Fig. 3: Effects of concentrations of manure on mass production of *Daphnia pulex.*

D. pulex treated with different levels of 3, 4 and 5 combinations of manure (Table 1) in the study showed improvement in the population density of the organism. The highest population density from results was recorded in 5CM1 (3,588 individual/L of water) among all the combination series (3CM, 4CM and 5CM). The nutrients released must have been optimal for maximal population density of the *D. pulex* and the organism must have preferred the combination of manure with cow dung to that of chicken manure because of the ammonia level. Clare (2002) stated that ammonia hinders Daphnia population growth especially in alkaline condition releasing toxicity that impairs reproduction. The neonates of Daphnia are destroyed by phosphorus at level greater than 1.00 ppm although the adults may survive it as reported by Clare (2002). Daphnia is highly sensitive to pollution and it is used in some places as indicator of good water quality (Clare, 2002).

Effects of concentrations of series of different combinations (3, 4, 5) of manure on population density of *D. pulex*

The results of the effects of concentrations of 3, 4, 5 different combinations of manure on *D. pulex* population density are presented in Fig. 3. Results of concentrations of the 3-combinations of manure were as follow: 1.00 ml/L (144) 2.00 ml/L (364), 4.00 ml/L (406) 8.00 ml/L (166) and 16.00ml/L (62 individual *D. pulex* /L of water). The results of concentration 4.00ml/L favoured the highest density of *D. pulex* in 3 combination of manure studied while 16.00ml/L had the lowest. The concentrations of the 4-combinations of manure revealed that in this series of experiment, 1.00ml/L, 2.00ml/L and 4.00ml/L had the following *D. pulex* population as 181, 750 and 618 individual/L of water respectively while 8.00 ml/L and 16.00 ml/L had 710 and 152 individual *D. pulex*/L. of water, respectively. The Concentration (2.00ml/L) of the 4 combinations of manure significantly (p<0.05) had *D. pulex* population growth more than the others in 4 combinations of manure of this experiment (Fig. 3). The concentration 2.00 ml/L. of water encouraged highest population density of *D. pulex* although all the concentrations used also supported increase population density of *D. pulex*.

Although all the concentrations used in the above investigations supported increase in population density, results of the concentration 2.00 ml/L of the 4 and 5 combinations of manure are the most populous groups. Concentration 1.00 ml/L. of the various combinations was not able to provide the needed nutrients because it was too low to supply the needed nutrients while concentration 8.00 and 16.00 ml/L. of water were too high constituting a sort of pollutant to the cultured organisms. 4.00 ml/L of 3CM was good but could not support high population density of *D. pulex* like that of 2.00 ml/L of 4CM and 5CM because this organism is highly sensitive to water quality (Clare, 2002).

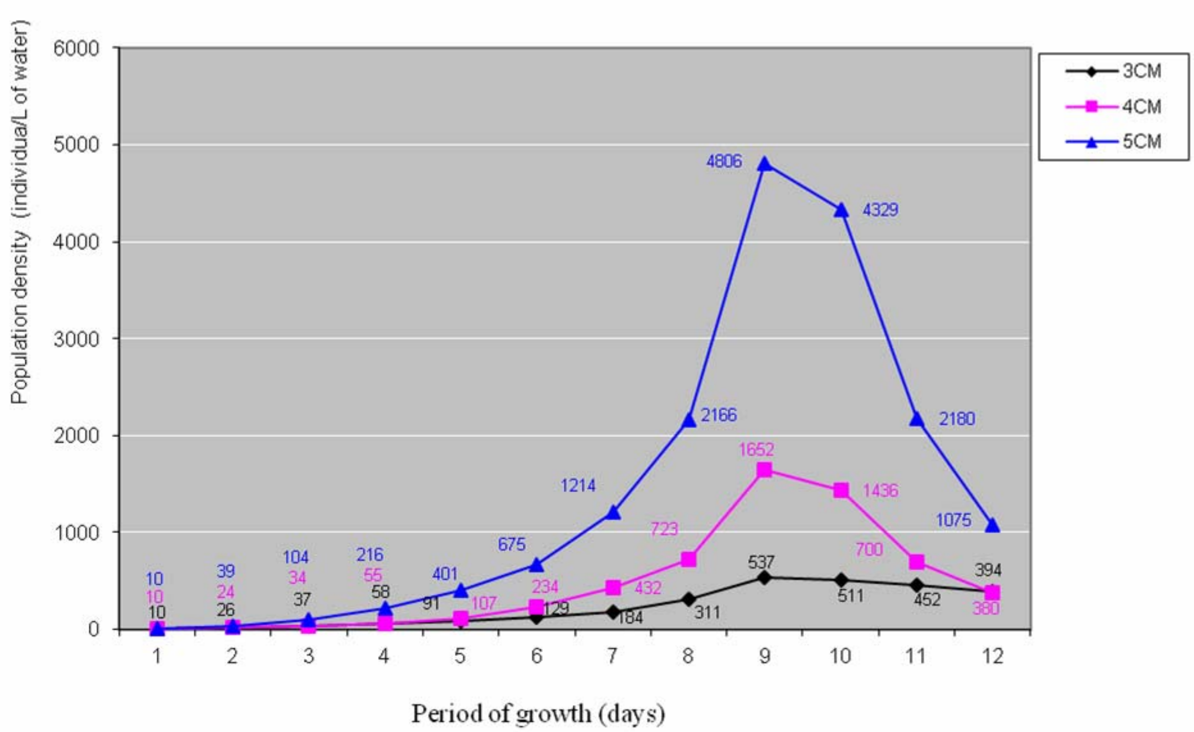

Fig. 4: Effects period of growth on mass production of *Daphnia pulex*.

Table 2: Interactive effects of concentration, 3 combinations of manure and period of growth on population density of *Daphnia pulex*

Conc. (ml/L)	Manure combinations	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	day 10	Day 11	Day 12
		Daphnia pulex population density											
1.00	3CM1	10	21	21	24	24	61	75	79	0	0	0	0
	3CM2	10	33	54	58	58	207	243	435	1033	1143	1022	947
	3CM3	10	20	21	50	50	71	99	111	230	240	184	155
	3CM4	10	20	11	9	9	0	0	0	0	0	0	0
2.00	3CM1	10	30	70	74	74	179	201	342	599	601	551	522
	3CM2	10	41	48	57	57	170	234	441	1130	1119	932	922
	3CM3	10	41	81	100	100	179	221	261	277	360	215	148
	3CM4	10	30	33	111	111	250	520	1160	1228	1251	1131	974
4.00	3CM1	10	60	70	74	74	130	181	241	478	501	457	336
	3CM2	10	40	50	61	61	201	239	351	1721	1747	1727	1536
	3CM3	10	31	59	120	120	181	201	216	245	256	194	124
	3CM4	10	11	32	179	179	328	497	1170	1295	1361	1092	1071
8.00	3CM1	10	40	49	55	55	71	81	83	254	241	236	228
	3CM2	10	22	13	31	31	92	147	123	232	248	137	126
	3CM3	10	20	43	50	50	135	141	163	222	240	183	154
	3CM4	10	8	10	12	12	97	358	760	776	963	515	276
16.00	3CM1	10	20	11	21	21	90	112	128	259	238	246	210
	3CM2	10	3	0	0	0	0	0	0	0	0	0	0
	3CM3	10	20	60	80	80	130	141	161	231	240	217	145
	3CM4	10	1	0	0	0	0	0	0	0	0	0	0
SEM			1.25	1.86	7.82	6.02	8.23	15.68	9.33	1.46	5.76	2.81	3.77
LSD			3.54	5.33	22.40	17.23	23.55	44.80	26.70	4.19	16.49	8.05	10.79
Conc. X manure		NS	**	**	**	**	**	**	**	**	**	**	**

3CM1 = Cow dung, Soybean and Single superphosphate; 3CM2 = Chicken droppings, Soybean and Single superphosphate; 3CM3 = Cow dung, Groundnut cake and Single superphosphate; 3CM4 = Chicken droppings, Groundnut cake and Single superphosphate

Table 3: Daily interactive effect of concentration, 4 combinations of manure and period of growth on population density of *Daphnia pulex*

Conc. (ml/L)	Manure combinations	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	day 10	Day 11	Day 12
		Daphnia pulex population density											
1.00	4CM1	10	23	31	43	51	51	42	10	11	0	0	0
	4CM2	10	21	24	28	41	53	168	190	539	760	723	326
	4CM3	10	22	31	33	72	151	192	222	423	455	345	154
	4CM4	10	10	10	13	29	60	170	285	980	853	675	345
2.00	4CM1	10	30	42	53	83	90	152	363	1262	1236	1066	674
	4CM2	10	24	54	100	161	180	194	346	1362	1338	1026	542
	4CM3	10	43	50	99	202	398	825	2547	4403	3436	1591	786
	4CM4	10	11	31	43	122	242	348	1221	3064	2984	2357	765
4.00	4CM1	10	62	42	20	80	104	121	132	920	872	865	780
	4CM2	10	32	123	161	180	361	441	583	1500	1461	942	673
	4CM3	10	42	46	61	85	201	399	644	2501	1654	875	546
	4CM4	10	31	39	51	213	557	791	1561	4081	4025	438	343
8.00	4CM1	10	1	4	6	40	121	142	121	727	657	630	623
	4CM2	10	9	10	21	15	24	50	99	182	173	87	76
	4CM3	10	23	11	18	31	99	122	150	277	277	275	210
	4CM4	10	31	67	250	539	1624	3951	5151	8821	6785	1246	275
16.00	4CM1	10	16	5	3	40	120	140	100	243	194	187	147
	4CM2	10	1	0	0	0	0	0	0	0	0	0	0
	4CM3	10	19	25	32	61	121	132	149	661	528	343	234
	4CM4	10	36	40	68	85	125	259	581	1081	1026	325	107
SEM			1.19	1.16	1.08	1.35	1.42	2.21	1.56	1.48	2.69	13.52	3.59
LSD			3.42	3.31	3.06	3.87	4.07	6.32	4.48	4.24	7.70	38.70	9.71
Conc. X manure		NS	**	**	**	**	**	**	**	**	**	**	**

4CM1 = Cow dung, Soybean, Rice bran and Single superphosphate; 4CM2= Chicken droppings, Soybean, Rice bran and Single superphosphate; 4CM3 = Cow dung, Groundnut cake, Rice bran and Single superphosphate; 4CM4 = Chicken droppings, Groundnut cake, Rice bran and Single superphosphate**.**

Effects of period of growth on population density of *D. pulex* treated with 3, 4, 5 combinations of manure

The results of the effects of period of growth on population density of *D. pulex* are present in Fig. 4. The results reveals that day 9 in all the combinations of manure used had the highest population density: 3-combinations of manure (512), 4-combinations of manure (1652) and 5-combinations of manure (4,806 individual *D. pulex)* respectively. The population increase from day 2 to day 9 before a decline was observed in the population growth. The crash of the highly populated group was more sudden compared to those with low population density.

Period of growth played a very vital role in the production of *D. pulex*. The present results indicated that to have high density of pure culture of the organism, one has to target within 9 days culture duration if the initial population density is as low as 10 individual/L of water. Srivastava et al. (2006) did a similar work on mass culture of *Ceriodaphnia cornuta* using a mixture of organic manures such as cattle manure, poultry droppings and mustard oil cake (1:1:1) at four different doses: 0.263 kg/m3, 0.526 kg/m^3, 1.052 kg/m^3 and 2.104 kg/m^3 in outdoor condition. They got the peak of *C. cornuta* on 12th day of inoculum in the fourth dose which had significantly highest numbers of the organism (1,930 individuals/L). *D. pulex* life span can be up to one month (Rottmann et al., 2003) but the medium of the culture often accommodate other organisms which may bring competition over the space and the available nutrients in the culture. As the period of growth extends, algae growth occurred leading to overfeeding of *D. pulex*. Ovie and Eborge (2002) reported a similar observation in *M. micrura* fed on a very high population of *Scenedesmus acuminatus*. The noticed declining population density of *D. pulex* was probably a result of competition over available resources. It can also be a result of the accumulated waste products of the organisms which may lead to ammonia toxicity in such a way that the environment became unfavourable for the cultured organisms.

Interactions of concentration, combinations of manure and period of growth on population density of *Daphnia pulex*

The results reveal that interaction between concentration, 3-combinations of manure and period of growth were highly significant in *D. pulex* population density studied. Table 2 shows the results of the interaction. At concentration 4.00ml/L of 3CM2 on day 10 period of growth shows the highest population density of this group and it is significantly higher than others (1747) individual *D. pulex*/L of water. This was

Table 4: Interactive effects of concentration, 5 combinations of manure and period of growth on population density of *Daphnia pulex*

Conc. (ml/L)	Manure	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	day 10	Day 11	Day 12
		Period of Growth — *Daphnia pulex* population density											
1.00	5CM1	10	22	51	203	544	843	1245	2202	4841	4783	3769	2427
	5CM2	10	32	27	29	54	82	94	153	180	174	175	104
	5CM3	10	53	101	202	322	426	609	1201	2006	1983	1253	991
	5CM4	10	51	100	360	531	892	1002	1351	1802	1250	983	544
2.00	5CM1	10	44	98	351	586	1542	4429	10256	25012	24127	10351	4574
	5CM2	10	31	157	542	1141	1880	2190	4201	6601	5731	5454	2250
	5CM3	10	56	126	352	421	582	823	1624	3160	3127	960	880
	5CM4	10	51	301	380	624	883	1270	1506	1901	1526	1268	572
4.00	5CM1	10	79	252	569	1258	2660	6963	10694	26303	24534	7325	2427
	5CM2	10	31	146	242	298	364	657	1325	4061	2548	1474	679
	5CM3	10	51	60	122	252	333	522	1321	2101	1566	1135	1155
	5CM4	10	51	351	422	826	1125	1542	2116	3361	1825	990	456
8.00	5CM1	10	20	51	123	219	426	563	1026	4903	4972	2675	1574
	5CM2	10	41	13	17	26	31	55	68	122	126	98	92
	5CM3	10	43	52	84	167	322	498	1124	2081	1750	1256	754
	5CM4	10	20	51	102	355	456	768	964	1261	942	544	343
16.00	5CM1	10	31	42	126	255	297	460	1113	4083	3646	2455	792
	5CM2	10	31	30	1	0	0	0	0	0	0	0	0
	5CM3	10	21	40	60	100	251	422	890	2041	1636	1224	672
	5CM4	10	18	32	42	45	101	166	186	301	326	213	213
SEM			0.97	1.65	1.09	9.03	1.28	2.55	1.19	0.97	2.24	2.67	4.37
LSD			2.79	4.72	3.12	25.86	3.67	7.29	4.00	2.77	6.42	7.69	13
Conc. X manure		NS	**	**	**	**	**	**	**	**	**	**	**

5CM1 = Cow dung, Groundnut cake, Soybean, Rice bran and Single superphosphate; 5CM2 = Chicken droppings, Groundnut cake, Soybean, Rice bran and Single superphosphate; 5CM3 = Cow dung, Chicken droppings, Groundnut cake, Rice bran and Single superphosphate; 5CM4 = Cow dung, Chicken droppings, Groundnut cake, Soybean, Rice bran and Single superphosphate

also followed by 3CM4 on of the same concentration but on day 9 of the period of growth. A declining population was observed beyond day 9 of concentration 4.0ml/L. Most of the concentrations of 3-combinations of manure show pronounced population decline from day 9. However, 3CM4 at concentration 1.00ml/L, 3CM2 at concentration 16.00ml/L and 3CM4 at concentration 16.00ml/L could not support population increase from day 3 of the period of growth.

The results of effect of interactions between concentrations, 4-combinations of manure and period of growth are presented in Table 3. The highest population density of *D. pulex* (8,821 individual/L) was found in 4CM4 at concentration 8.00ml/L on day 9 period of growth and it was significant different (P<0.05) from others in the group. Most of the combinations of manure in the different concentration experienced a declining *D. pulex* population from Day 10-12 (Table 3). 4CM2 at concentration 16.00 could not support *D. pulex* population growth as well.

The result of interaction between the concentration, 5-combination of manure used and the period of growth were highly significant (Table 4). The highest population density of *D. pulex* (26,303 individual/L. of water) was observed in concentration 4.00ml/L of 5CM1 at day 9 period of growth of the studied organism. This result was followed by that of 5CM1 at concentration 2.00ml/L of water with population density of 25,012 individual/L. of water of day 9. Day10 to day12 of the experiment showed a declining population while 5CM2 did not support population increase from day 4 of growth in concentration 16.00ml/L. of water.

The pulled effects of concentration, combinations of manure and period of growth revealed that concentration 2.00 ml of 5CM1, (26,303 individual/L of water) and 4.00ml/L of 5CM1 (25,012 individual/L of water on day 9 improved *D. pulex* population in this investigation. With these population margins, it therefore implied that good population of this organism can be realized on 9 days period of culture especially when the concentration and the manure combinations are considered. The pulled effects of the above interactions in 3CM2 (1,747) and 4CM4 (8,821) were low compared to that of 5CM1 reported above for the production of pure *D. pulex*. The cause of low population density of the *D. pulex* in these combinations of manure may be attributed to poor release of nutrients.

Conclusions

Population density of *D. pulex* (26,303 individual/L of water) was observed to be highest in concentration 4.00ml/L of 5CM1 at day 9 period of growth in this study. Day10 to day12 of the experiment experienced a decline in population density. 5-combination of manure (5CM1) at 4.00ml/L of water on day 9 and day 5 can be highly recommended for mass production of *D. pulex*

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