CULTURE OF ZOOPLANKTON (*BRACHIONUS CALYCIFLORUS*, *MOINA MICRURA* AND *DAPHNIA PULEX*) AS LIVE FOOD FOR *HETEROBRANCHUS BIDORSALIS* HATCHLINGS.

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UNIVERSITY OF JOS.

FEBRUARY, 2014

DECLARATION

I hereby declare that this work is the product of my own research efforts undertaken under the supervision of Prof. P.C. Ofojekwu and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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CERTIFICATION

This is to certify that the research for this thesis and subsequent preparation of this thesis by Samuel Avengbe Okunsebor (PGNS/UJ/0160/04) were carried out under my supervision.

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This work is dedicated to my mother, Mrs. Iguehi Victoria Okunsebor and my wife, Mrs. Francisca Ebata Okunsebor.

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ABSTRACT

Live food Zooplankton population density in different zones of Awuma River at Shabu were investigated and *Brachionus calyciflorus, Moina micrura* and *Daphnia pulex* screened from littoral zone of the River at Shabu before the onset of rainy season was isolated into mono specific culture in the laboratory. Cow dung, chicken droppings, groundnut cake, soyabean cake, rice bran and single super phosphate were combined in different proportions of three, four and five combinations of manure in solution (3CM1...4, 4CM1...4 and 5CM1...4.) in three replicates for culture period of 9, 11 and 12 days for each of the zooplankton respectively. Effects of concentration, combination of manures and duration of production of the zooplankton were investigated. Dissolved oxygen, (mg/L) temperature (^{0}C), carbon dioxide (mg/L) and total alkalinity (mg/L) of the water used for the various treatments were monitored. *Chlorella vulgaris* was cultured using repeated subculture method. Effects of filtrate of the combined manure and *C. vulgaris* on population density of *B. calyciflorus* and *M. micrura* were determined. Influence of temperature on percentage fertilization, hatchability of *Heterobranchus bidorsalis* eggs and survival rate of hatchlings were investigated in Jos in a controlled temperature hatchery. The effects of *Artemia* shell free, live *B. calyciflorus, D. pulex, M. micrura, and a combination of M. micrura & B. calyciflorus* on percentage weight gain, total body length, condition factor and percentage survival rate were also determined. The fish eggs (500) were mixed with milt and spread on mosquito net kakaban in aquaria in 4 treatments (26^{0}C, 28^{0}C, 30^{0}C and 32^{0}C) in three replicates. The numbers of unfertilized eggs were counted after 6 hours from the time of mixing of the eggs with the milt. The kakabans were removed after the hatching and hatchlings were counted then and after 3days. Fry (50) of 3 day-old were placed in a 10 litre plastic bowl in five treatments of 3 replicates for 16 days. Initial average length (cm) and

weight (g) of the fry were recorded. Littoral zone of Awuma River had the highest population density of live food micro organisms. The highest population density of cultured *B. calyciflorus* (245 individual/ml of water) and of *D. pulex* (26,303 individual/L of water) were observed on day 5 and day 9 respectively in treatment 5CM1at concentration 4.00ml/L of water while *M. micrura* highest population density (55,184 individual/L of water) was observed on day 9 in 5CM2 at concentration 4.00ml/L of water. Beyond day 7 and day 9, a declining population density was observed in *B. calyciflorus, M. micrura/D. pulex* respectively. Population density of *B. calyciflorus* fed on filtrates of the manure was significantly ($p<0.05$) higher than those fed with *Chlorella vulgaris*. Water temperature range of 28-30°C was found to be significantly best for percentage fertilization, hatchability of eggs and 28°C for survival rate of catfish hatchlings. Mixture of *B. calyciflorus* & *M. micrura* significantly influence the highest percentage weight gain, total body length, survival rate and condition factor of *Heterobranchus bidorsalis* fry. These findings are highly recommended for zooplankton and fry production.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Naturally, fishes in the wild depend on plankton for the survival of their hatchlings throughout their fry growing seasons. Some zooplankton are essentially used to feed fry of fish species that do not accept artificial feeds (Bryant & Matty, 1980). Arrhenius and Hanson (1993) reported that some fish species feed exclusively on zooplankton during their entire life while about 90% foods of herring (*Clupea harengus)* consist of zooplankton. Sipaúba-Tavares and Bachion (2002) reported that zooplankton reproduction and growth rate increases availability of better food quality for subsequent trophic levels. Live food micro-organisms are important food sources for many fish species and the success of culturing zooplanktivorous fish fry depends primarily on zooplankton, their composition and density (Fernando, 1994).

Zooplankton, which most fry depend naturally upon as their live food, depends on phytoplankton that constitutes the major primary producer in the aquatic food web. 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 food sources in larviculture based on certain qualities such as purity, availability, acceptance, nutritional indicators (digestibility and organism nutrients/ energy), easily availability, easy reproduction and economically viability (Watanabe & 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. They are inexpensive and should serve as alternative to the brine shrimp which are expensive non-freshwater organisms. Okunsebor *et al.* (2008) reported that the survival rate and total body length of *Clarias gariepinus* fry fed on shell free *Artemia* were not significantly different ($p<0.05$) from those fed on cultured *Moina micrura* as starter feed.

Normally, fish fry grow in the wild where preys are readily available. In the hatcheries, where most of the activities are artificial, the survival of fry depends on availability of right food. Fry requires high protein food (42.0% and 52% for omnivorous and carnivorous fish respectively) for survival and growth (Tacon, 1990). It is important to note that not all zooplankton are suitable for fry rearing but live Rotifer, *Moina* and *Daphnia* species are reported to be good freshwater zooplankton that can enhance protein and other food content for the rearing of fry in our hatcheries (Olojo *et al.*, 2003).

Mass production of live food (*Brachionus calyciflorus, Daphnia pulex* and *M. micrura*) for the improvement of the hatchery production of *Heterobranchus* species *(H. longifilis and H. bidorsalis)* fry is yet to be addressed adequately. This form one of the main focus of this research work.

Heterobranchus species *(H. bidorsalis and H. longifilis)* are freshwater cultivable catfish. They are one of the desirable catfish species based on taste, colour, size and growth rate (Madu *et al.,* 1990). They are hardy and can withstand hatchery stress. With their accessory breathing organs, they are able to withstand adverse shortage of dissolved oxygen (Gupta and Gupta, 2006). They have high disease resistance and adults readily accept formulated feeds, but the fry accept mainly live food. Presently most hatcheries dwell on production of fry of *Clarias* species instead of *Heterobranchus* species due to higher mortality rates of the fry (Madu, 1995). The production of *Heterobranchus* fish fry

is still not able to meet the demand for the fish fingerlings even from the wild. The fingerlings from the wild are seasonal and can be accomplished by disease parasite into fish farm. Culturing of live food organisms to serve as starter feed may enhance the survival rate and increase production of *Heterobranchus* fry for high fingerlings production in our hatcheries.

1.2 STATEMENT OFTHE PROBLEM

Freshwater cat fish fry survive on freshwater zooplankton in the wild without the use of shell free artemia, or artemia nauplii. This implies that freshwater fish fry do survive on freshwater zooplankton. In Nigeria, most hatcheries depend only on artemia shell free for feeding of fry during the early stage of development. Sometimes artemia is not available and if available may not be in enough quantity, or may be found only in the cities where the local farmers rarely get access to them. Oftentimes, the artemia shell free (artificial feed) develops waste materials that pollute the water of fry culture. Owing to this limitation, farmers to depend on fingerlings produced in the wild for culture and fish prodection. In most cases, due to frustration as a result of poor survival rate of *Heterobranchus* fry, many interested fish farmers have to change from *Heterobranchus* fingerling production to other fish in their hatcheries.

The development of freshwater zooplankton (*B. calyciflorus, M. micrura* and *D. pulex*) as alternative to the imported artemia shell free for feeding fry may help the farmers immensely in rural and even in urban areas. The use of freshwater live food zooplankton to enhance production of *Heterobranchus* fry in our local hatcheries seems inevitable in the drive for food sustainability for human race.

1.3 JUSTIFICATION OF THIS STUDY

Many hatcheries (e.g Panyam indoor and out door hatcheries, Plateau State and National Institute for Freshwater Fisheries Research, Niger State) were built for fry production and fingerlings supply in Nigeria, yet there are no sufficient fry for fingerlings production. The survival of fry of planktivorous fishes that do not accept formulated diet is extremely difficult. When the yolk is exhausted, the fry starves to death as they habitually refuse inert diets.

In the culture of zooplankton through direct fertilization by Tech (1981), it was reported that some dominant species may be undesirable and some times larger than the mouth parts of fry. In some places where the ponds are naturally fertilized, dominant species (e.g. adult Cyclops) may be too large for the mouth parts of the fish hatchlings and some of the organisms intimidate fry while some, such as copepods even prey on fish fry. In some cases, zooplankton may be present in the pond but not enough to meet the nutritional needs of the fry or sustain 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 and seasonal, it encourages predation and disease infections on fry, this research work intends to culture monospecific culture of freshwater live food zooplankton to meet the demands of *Heterobranchus* fry production, and to provide information on how to feed them to the fish fry based on size, acceptance, and nutrient requirements for high survival rate and growth of the fish fry.

The production of the live food organisms are intended to serve as alternatives to artemia shell free and Artemia nauplii which are Marine, costly and not readily available locally.

1.4 AIMS OF THE STUDY

The aims of the study are:

i to examine the live food organisms and water quality in their natural environment for laboratory manipulations of successful culture,

ii to screen, isolate and culture mono specific strain of freshwater *B. calyciflorus, M. micrura* and *Daphnia pulex* live food zooplankton from the wild.

iii to ascertain the utilization of the freshwater zooplankton by *H. bidorsalis* hatchlings as alternative to shell free *Artemia* which is conventionally used as starter diet.

1.5 SPECIFIC OBJECTIVES OF THE STUDY

- To determine best culture media, optimum concentration of the combination of manure and duration of culture of mono specific local strains of *B. calyciflorus, M. micrura* and *D. pulex* live food organisms.
- To establish the effects of interaction of concentration of manure, different combinations of manure used and period of growth on population density of (*B. calyciflorus, M. micrura* and *D. pulex*) live food organisms.
- To investigate the effects of filtrate of combined manure / *Chlorella vulgaris* (phytoplankton) on population density of selected zooplankton.
- To determine the effect of temperature on fertilization, hatchability of catfish eggs and survival rate of the hatchlings.

- To determine the use of some cultured freshwater zooplankton (*B. calyciflorus*, *M. micrura* and *D. pulex*) on growth indices, survival rates and condition factor of *H. bidorsalis* fry.
- To determine the amino acid profile of the most useful cultured zooplankton fed to the fish fry.

1.6 NULL HYPOTHESES

- Concentration, combination of manure and duration of culture have no significant effect on population density of local strains of *B. calyciflorus, M. micrura* and *D. pulex* live food organisms.
- There is no significant interactive effect of concentration, combination of manure used and period of growth on population density of *B. calyciflorus, M. micrura* and *D. pulex* live food organisms.
- There is no significant difference in population density of selected zooplankton fed on filtrate of combined manure / *Chlorella vulgaris*.
- Temperature of water has no significant effect on fertilization, hatchability of catfish eggs and survival rate of their hatchlings
- The cultured freshwater zooplankton (*B. calyciflorus, M. micrura* and *D. pulex*) have no significant effect on on growth indices, survival rates and condition factor of *Heterobranchus* fry.
- Cultured *B. calyciflorus, M. micrura* and *Chlorella vulgaris amino acids* are not significant different from each other.

1.7 SCOPE AND LIMITATION OF THE STUDY

This research work investigated screened live food freshwater zooplankton (*B. calyciflorus, M. micrura and D. pulex* strains) that are found in our local freshwater, culture and manipulation of their population density by the use of organic, inorganic and food substances as manures suspension for mass production in batch culture system. The use of *C.vulgaris* (phytoplankton) as the selected zooplankton food was also investigated. Temperature range that favours high fertilization, hatchability of fish eggs and survival of hatchlings during yolk sac stage were also investigated. This research work also investigated the utilization of the cultured live food zooplankton by *H. bidorsalis* hatchlings. Continuous and semi continuous cultured freshwater zooplankton (*B. calyciflorus, M. micrura* and *D. pulex*) were not used because of frequent interferences of undesirable zooplankton, which would have made the culture non mono specific.

The disadvantages of frequent crash down in population density, infections, parasite load, the deterioration of water quality and quality of cultured organisms (Frank, 2002) make semi continuous or continuous cultured freshwater zooplankton (*B. calyciflorus, M. micrura* and *D. pulex*) unsuitable for this research work. The choice of *Heterobranchus bidorsalis* hatchlings was considered because they are highly carnivorous even from fry stage of life.

CHAPTER TWO
LITERATURE REVIEW

2.1 USE OF ZOOPLANKTON IN AQUACULTURE

Zooplankton is valuable source of crude protein, amino acids, lipids, fatty acids, minerals and enzymes for fry. Yurkowski and Tabachek (1979) reported that zooplankton satisfy all food requirements of fish and supported fry growth. Lysine and methionine, which are known to be the most limiting amino acids in feeds, are present in appreciable quality in zooplankton (Dabrowski & Rusiecki, 1983). High ratio of unsaturated fatty acids to saturated fatty acid of zooplankton shows that zooplankton is good quality food for rearing fish larva (Lokman, 1994). The polyunsaturated fatty acid (PUFA) contents showed high concentrations of eicosapentanoic acid (20:5ω3) and docosahexanoic acid (22:6ω3) with moderate amounts of linoleic acid (18:2ω6) in zooplankton. As ratios of ω3 to ω6 PUFA are high, the zooplankton is regarded as desirable food (Lokman, 1994).

It was reported that zooplankton is source of carotene and they improve flavor, colour and texture of fish fed on them (Spenelli, 1979). Live zooplankton is also reported to contain enzymes like amylase, protease, exonulease, esterase that play important roles in larval digestion (Munilla – Moran *et al.,* 1990). Mims *et al.* (1991) revealed that the exoskeleton of the live organism (as roughage) is necessary for food digestion in fish fry. Cladocerans have been found to be rich in essential nutrients, are easily ingested and digested by fish larvae, fulfill the larval dietary requirements and improve water quality by minimizing the need for artificial feeding (He *et al.,* 2001).

Ricardo (1981) reported that the *Moina* is ideal for aquaculture use because it is easy to handle, non selective filter feeder of bacteria in solution, reproduces fast, adaptable to wide range of environmental condition and has high nutritive value. *M.*

micrura often shows year-round presence in fish ponds, they are resistant to the handling involved in the culture system and are readily selected as food by fish larvae (Sipaúba-Tavares & Bachion, 2002). Furthermore, *M. micrura* is a highly productive, opportunistic species, well adapted to the low salinities (Saint-Jean & Bonou, 1994).

Watanabe *et al.* (1983) reported an excellent protein efficiency ratio (PER) value in Rainbow trout fed on *Daphnia* and *Moina* live organisms while Cheikyula and Ofojekwu (2003) observed that *Moina* has been extensively utilized as live food in many hatcheries in the maintenance and culture of aquarium fishes of commercial importance.

In the SEAFDEC Aquaculture Department, one of the popular zooplankton utilized for shrimps and fish seed production is Rotifer (*Brachiomus plicatilis)* (Tech, 1981). *Rotifera* an important component of freshwater zooplankton whose community dynamics is controlled by food availability (Devetter, 1998), presence of planktivorous fishes (Telesh, 1993), invertebrate predators and competitors (Nagata & Hanazato, 2006) has been reported. Notably, algae, heterotrophic nano-flagellates and bacteria are major food resources for rotifers and they are generally abundant in nutrient-rich environments (Yoshida *et al.*, 2003). Studies by Dhert (1996) revealed that live food had contributed to the successful fry production of at least 60 marine fish species and 18 species of crustaceans. However mass production of live food (*B. calyciflorus, Daphnia pulex* and *M. micrura*) for the improvement of the hatchery production of *Heterobranchus* species *(H. longifilis and H. bidorsalis)* fry is yet to be addressed. Rotifers are one of the smallest (*Rotifera*) metazoans, which served as perfect material for evolution theories and excellent food resources to larva in aquaculture (Haoyuan & Yilong, 2008). Chen *et al.* (2005) reported that food concentration significantly affects the reproduction of *B.*

calyciflorus. However, the food type significantly affected intrinsic rate of increase, net reproductive rate, generation time and the total percentage of mictic females in the offspring (Haoyuan & Yilong, 2008).

Madu and Ufodike (2001) recommended the feeding of hatchling with only *Artemia* nauplii during the first 10 days of life and a mixture of *Artemia*, zooplankton and fish meal thereafter, as the most satisfactory feeding protocol for indoor nursery management of *Clarias anguillaris* hatchlings. Madu (1995) reported high mortality rate of *Heterobranchus* fry in hatcheries. High mortality rate of *Heterobranchus* fry in few days after hatching is yet to be addressed properly since this fish is highly acceptable to many people. Conventionally, live food culture has been practiced by fertilizing pond with organic manure with the believe that live organisms will naturally appear but the problem of the un-screening and non isolating of the live organisms that might appear still reduce the survival rate of the fish fry as most of the dominant live food organisms might be larger than the mouth parts of the fish fry. In this regard, they might be present while the fish fry still starve to death. Madu *et al.* (1990) revealed that the problem of mortality is highly correlates with inadequate food in quality, quantity and in particle size. Rice bran, flour, ground shrimp, yeast, and formulated diet are some of the sources of food exploited for cultivation of rotifers (Arimoro & Ofojekwu, 2004). Chicken dropping with small quantity of inorganic fertilizer has been reported to produce favourable growth of algae for rotifer culture (Arimoro, 2006) while Gupta and Gupta (2006) recommended the use of organic manure (150ppm), oil cakes (75ppm) and super sulphate (30ppm) for the culture of Rotifers, *Moina* and *Daphnia* species.

Most of Rotifers, *Moina* and *Daphnia* species are indigenous in Nigerian freshwaters. Since these various live food zooplankton are diets for fish fry in Nigeria freshwater (Ovie, 1995), the culture and utilization of these potentials are vital in fish fry production in hatcheries. This research desires to bring to light these natural endowments, which only need articulation and simple application to provide sustainable fish fry production in Nigeria. This research work also focused on food availability for live zooplankton, since food significantly affect intrinsic rate of increase, net reproductive rate, generation time and the total percentage of mictic females in the offspring (Haoyuan & Yilong, 2008).

2.2 BIOLOGY OF FRESHWATER ROTIFER (*BRACHIONUS CALYCIFLORUS)*

A single rotifer can become thousand of rotifiers in few days when environmental condition is favourable to them (Arimoro, 2006). A female rotifer can reproduce up to 7 eggs simultaneously without any genetic imput from male and these eggs hatch in 12 hours and by 18 hours from hatching time the new rotifer is ready to start reproduction (Arimoro, 2006). Structurally, the organism is small in size, with low morbidity and high in amino acid profile (Arimoro 2006). Rotifers are well known in the scientific world for their contribution in live food web in freshwater and marine water communities (Morten, *et al.*, 2007).

2.2.1 Structure of *Brachionus calyciflorus.*

B. calyciflorus is a microscopic freshwater aquatic organism, which has nephridial system but lacks specialized circulatory and respiratory systems. The body shape is divided into three (the head, trunk and foot). The integument is thin and flexible. The thickened middle portion of the body forms a sort of shell called lorica. The lorica's

surface provides hollow ramifications branches or spines. The body wall consists of hypodermis (inner layer) which secretes an outer layer called cuticle (plate 1). The head carries the corona surrounded by cilia which is used for locomotion (Tech, 1981).

2.2.2 Classification of *Brachionus calyciflorus*

Kingdom: *Animalia*

Phylum: *Rotifera*

Class: *Eurotatoria*

Subclass: *Monogononta*

Superorder: *Pseudotrocha*

Order: *Ploima*

Family: *Brachionidae*

Genus: *Brachionus*

Species: *Brachionus calyciflorus* (Pallas, 1766)

2.2.3 Ecology and Water Quality Requirements of *Brachionus calyciflorus*

B. calyciflorus is one of the live food zooplankton species found in freshwater and they are approximately evenly distributed in the water column (Morten, *et al.*, *2007).* It is one of the most abundant rotifer in most hatchery operations and inland waters (Arimoro & Ofojekwu, 2004). They can also be of high density in shallower lakes with higher concentration of inorganic nitrogen and less herbivorous crustaceans such as *Sinocalanus dorrii* and *Daphnia* (Wang *et al.*, 2010).

It can tolerate temperatures between the ranges of 15-31^{0}C. The optimum pH is 6-8 at 25^{0}C and minimum dissolved oxygen level is 1.2 mg/L (Ludwig, 1993). *B. calyciflorus* feed successfully on micro algae like *Scenedesmus acuminatus,*

Ankistrodesmus convolutus and *Chlorella vulgaris* (Ovie & Eborge, 2002), yeast and artificial diet in both indoor and outdoor culture system. *B. calyciflorus* is small sized live food organism compared to *Moina* species. It moves very slowly and has the habit of staying in water column. In conducive habitat, it has high reproductive rate with high population density.

Some ways of obtaining pure cultures of *B. calyciflorus* was reported by Arimoro and Ofojekwu (2004). They recommended the use of Basudine, an organo phosphoric acid ester at the rate of 1.5mg/L. This concentration eliminates copepods, other cladocerans and aquatic insect larvae so that only rotifer is allowed to multiply and increase in population. Rotifers have been reported to be useful as indicator of trophic condition in the field and they form an important link in pelagic food web (Nogrady, 1993).

However, the progeny of the sexual phase produce fewer resting eggs. The switching from asexual to sexual reproduction is as result of reduction in the food supply leading to an increase in resting eggs production (Rottmann *et al.*, 2003). Rotifer has life span of 12 days and can get to peak reproduction level in about 3-5 days (FAO, 1996).

2.2.4 Life Cycle of *Brachionus calyciflorus*

Brachionus calyciflorus reproduce by asexual and sexual reproduction as shown in Figure 1. The asexual reproduction occurs when the environment is in good condition in term of food and less predating organisms. The asexual reproduction is the fastest reproductive method of this organism to colonize a given environment. Arimoro (2006) reported this mode of reproduction (parthenogenesis) is the primary mode of reproduction of the organism. In this mode of reproduction more females are reproduce parthenogenetically than the male. When condition is not favourable for this organism, it

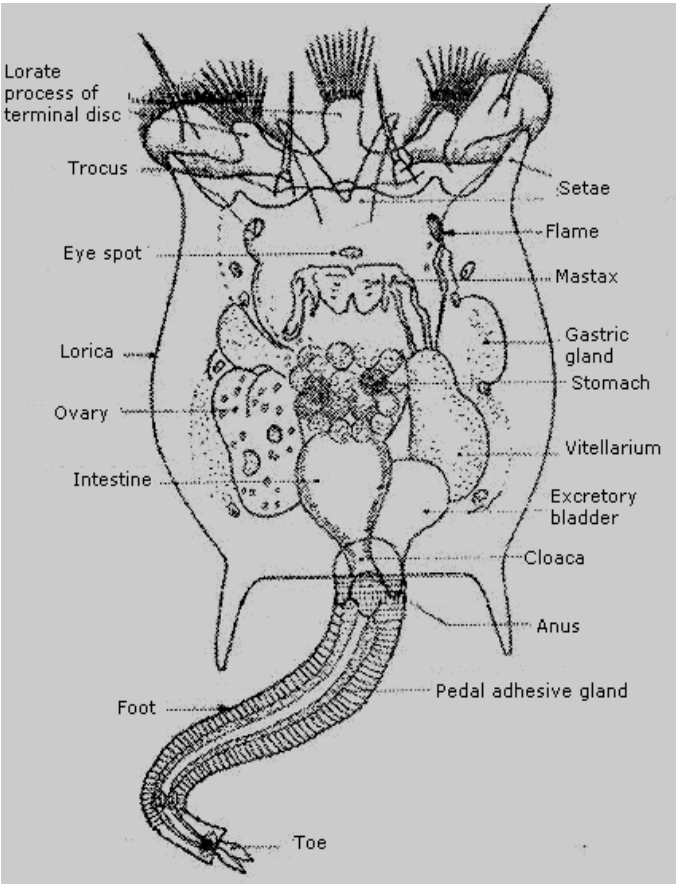


Plate 1: *Brachionus* External and Internal Morphology

Source: (Tech, 1981)

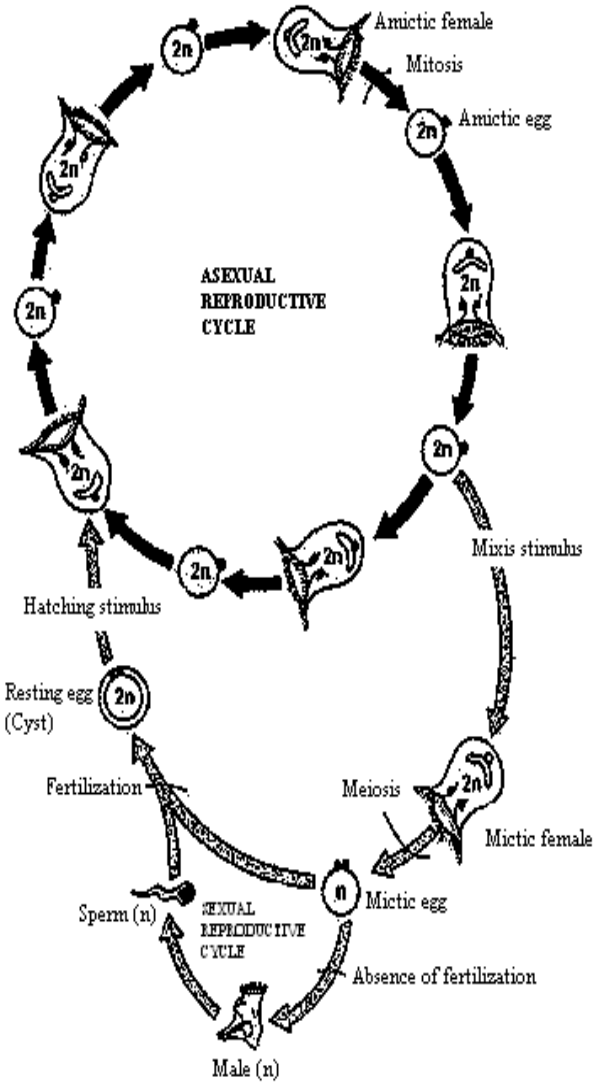


Figure 1: Life Cycle of *Brachionus calyciflorus*

Source: (Hoff & Snell, 1987)

switches over to the asexual reproduction where more males are reproduced as resting eggs (Hoff & Snell 1987).

2.2.5 Amino Acid Profile of *Brachionus calyciflorus*

Awaiss (1998) reported best growth rate, survival and biochemical composition of *Clarias gariepinus* food on rotifer in their report. Ovie and Ovie (2006) showed that *B. calyciflorus* had comprehensive and well distributed amino acid profile as documented in Table 1 but some of these amino acids are lacking in most conventional food.

2.2.6 *Brachionus calyciflorus* as Live Food Organism for Fish Fry

Kitto and Bechara, (2004) described rotifers as one of the dominant zooplankton species that are preferred by fish fry. *B. calyciflorus* as live food for fish was used as ideal starter feed for dwarf gourami (*Colisa lalia),* a tropical freshwater ornamental fish species with larvae that are too small to ingest *Artemia* nauplii or *Moina* at its first feed.

Rotifer as starter diet significantly improves the growth and survival of gourami larvae (2-12 days) (Lim *et al.,* 2003). Based on the stocking density, overall survival rates and volumes of rearing water, the yield of fry derived from larvae fed on rotifers are estimated to be 6,500 – 7,500 fry/m^3 of ornamental fish tanks (Lim *et al.,* 2003). *Symphysodon aeuifasciata* could be raised through feeding with *B. calyciflorus* for 4-7 days followed by *Artemia nauplii* for a week. Their growth and survival rates are reported to be comparable with those that rely on parental feeding (Lim & Wong, 1997). Rotifer could be kept alive in tanks so that only one feeding per day is needed (Lim *et al.,* 2003). Awaiss (1998) observed that the best growth, survival and biochemical composition were evident in *Clarias gariepinus* fry fed *B. calyciflorus* during the first week of larval feeding. Over 80% survivals in the toothed carp, *Aphyosemion gardneri*

Table 1**:** Amino Acid Profile of *Brachionus calyciflorus* (g amino acids per 100 g of protein)

Amino acid compositions	*Brachionus calyciflorus*
Alanine	4.00
Arginine	6.37
Aspartic acid	10.53
Cystine	1.55
Glutamine	12.22
Glycine	3.37
Histidine	1.83
Isoleucine	4.32
Leucine	8.95
Lysine	8.64
Methionine	0.93
Phenylalanine	5.20
Proline	6.03
Serine	3.45
Threonine	3.92
Tyrosine	2.82
Valine	4.83

Sources: Ovie and Ovie (2006)

larvae raised on freshwater rotifer from time they were 5 days old through 32 days of age was reported by Arimoro and Ofojekwu (2003).

Enriched rotifer fed to fry enhanced fish fry growth and survival rate. According to Watanabe *et al.* (1983), rotifer can be enriched using 7ml and 15ml of cod liver oil per 40L and 100L respectively for 12 hours every night with oxygen levels kept at 4-7 mg/L and enriched algae increased the free amino acids content of the rotifers.

2.3 BIOLOGY OF *MOINA MICRURA*

M. micrura is a cosmopolitan, cyclic parthenogenetic *Cladoceran* with ample morphological and ecological plasticity (Martínez-Jerónimo *et al.*, 2007). It is a small omnivorous species that is common in eutrophic water bodies (Wang *et al*., 2007a). They are live food organisms belonging to the *Cladoceran* crustaceans, which are usually called water flea. They are found as different strains all over the world.

2.3.1 Structure of *Moina micrura*

The size of *M. micrura* ranges from (700-1000µm) but a young *Moina* can be as small as 400µm (Rottmann *et al.*, 2003). It is thrice the size of adult Rotifers. *Moina* is approximately half the length of *Daphnia* and it does not die like brine shrimp in fresh water. Newly hatched fry of most freshwater fish species can ingest young *Moina* as their initial food. *M. micrura* is approximately adult Rotifers and smaller than newly hatched brine shrimp (Rottmann *et al.*, 2003). It has abdominal setae, Antenule and branched antenna. It also has powerful post abdominal claw (plate 2). The foods in the gut are visible under the microscope (Rottmann *et al*, 2003).

2.3.2 Classification of *Moina micrura*

Kingdom: *Animalia*

Subkingdom: *Bilateria*

Infrakingdom: *Ecdysozoa*

Superphylum; *Panarthropoda*

Phylum: *Arthropoda*

Subphylum: *Mandibulata*

Infraphylum: *Crustaceomorpha*

Superclass : *Crustacea*

Class: *Brachiopoda*

Family: *Moinidae*

Genus: *Moina*

Species *Moina micrura* (Kurz, 1874)

2.3.3 Ecology and Water Quality Requirements of *Moina micrura*

M. micrura requires an optimum temperature which is generally about 24^0C - 31^0C for survival and growth. High temperature reduces their growth (Rottmann *et al.*, 2003). Inoculation of 25/individuals/L encourages quick population growth and avoid over feeding. Over feeding can quickly cause problem in water quality when pH is greater than 9.5. The pH that is good for *M. micrura* culture is 7-8. *M. micrura* is tolerant to low dissolved oxygen from zero to super saturation. It has quick resistance to changes in oxygen concentrations (Rottmann *et al.*, 2003). The surviving ability in oxygen poor environment is due to their ability to synthesis haemagblobin. *M. micrura* feeds on bacteria, yeast and phytoplankton.

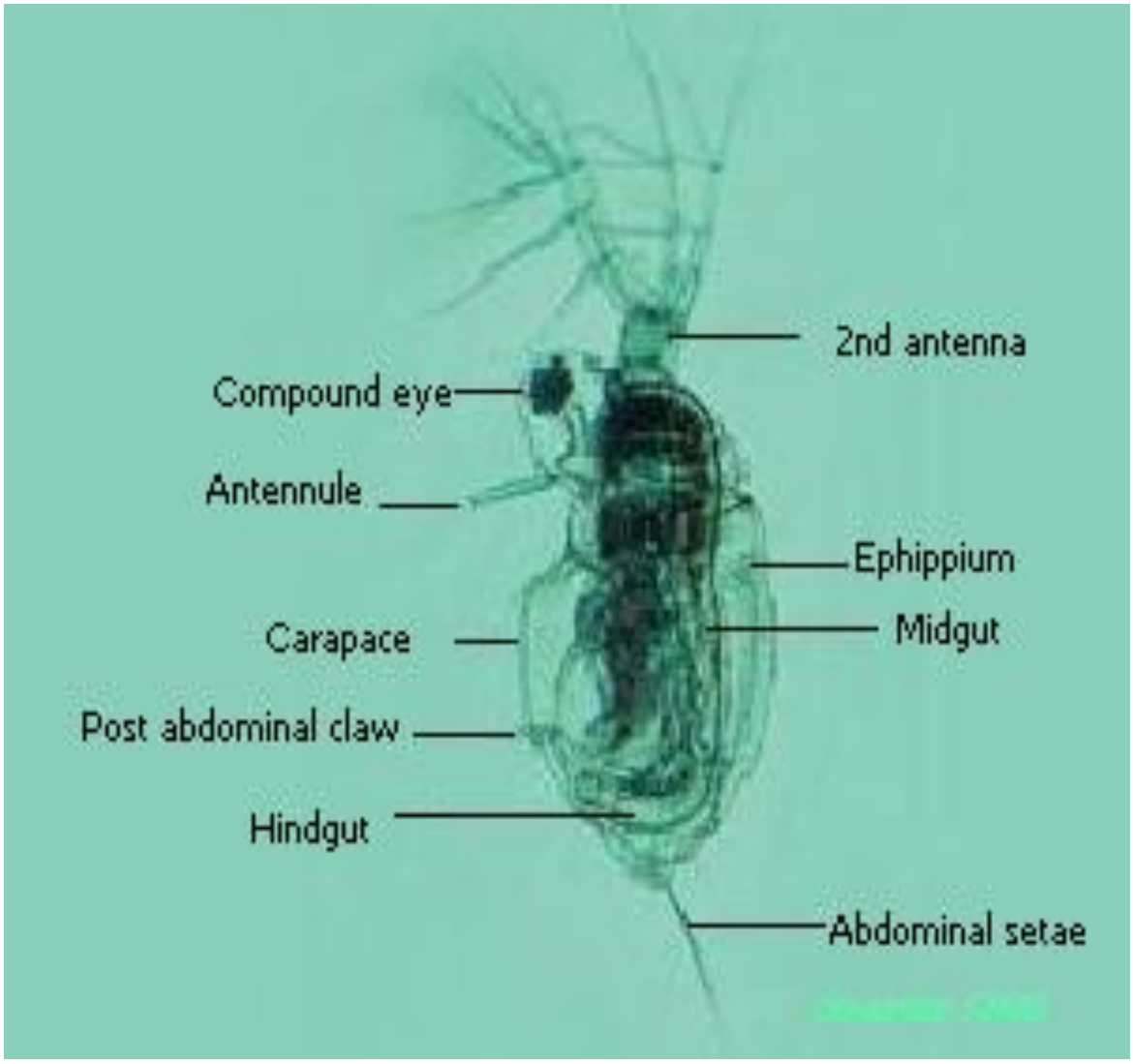


Plate 2: Structure of *Moina micrura*
Source: Koste and Shiel (1987)

Green or brown – red *Moina* with full intestinal tracks and active movement indicate a healthy culture. *M. micrura* with empty digestive tracts or *Moina* producing resting eggs are indication of suboptimum environmental conditions or insufficient food (Rottmann *et al.,* 2003).

2.3.4 Life Cycle of *Moina micrura*

M. micrura is a high fecundity species of zooplankton in nature (fecundity varying from 2.2 to 7.7 eggs per adult female) (Saint-Jean & Bonou, 1994). *M. micrura* has both sexual and asexual phase of reproductive cycle. Under optimum conditions, *M. micrura* reproduce at only 4-7 days of age with brood size of female. Under adverse conditions, males are produced and sexual reproduction occurs resulting to formations of resting eggs (Rottmann *et al.,* 2003).

The switching from asexual to sexual reproduction is stimuli action as result of reduction in the food supply leading to an increase in resting eggs production. Asexual reproduction is better for high population density over a short period of time because the progeny of the sexual phase produce fewer resting eggs (Rottmann *et al.,* 2003).

2.3.5 Amino Acid Composition of *Moina micrura*

Ovie and Ovie (2006) showed that *M. micrura* had well distributed amino acid profile as documented in Table 2 and the amino acids of *M. micrura* are better than those in most conventional food for fry.

2.3.6 *Moina micrura* as Live Food Organism for Fish Fry

Fish fry fed on micro algae and rotifers followed by larger zooplankton, notably nauplii from brime shrimp (*Artemia franciscana)* was reported and the shrimp was collected as canned cysts from Great Salt Lake, Utah (Sorgeloos & Beardmore, 1995). The problem of importation, cost and availability, the size and life span of the shrime in freshwater necessitate the search for alternative in freshwater that will be available, cheap, smaller in size, and have better life span in freshwater. *M. micrura* may be

Table 2: Amino Acid Composition of *Moina micrura* (g amino acids per 100 g of protein)

Amino acid compositions	*Moina micrura*
Alanine	2.84
Arginine	8.17
Aspartic acid	9.84
Cystine	2.89
Glutamine	15.39
Glycine	3.90
Histidine	5.09
Isoleucine	4.18
Leucine	8.00
Lysine	10.73
Methionine	1.12
Phenylalanine	3.75
Proline	3.18
Serine	3.42
Threonine	2.93
Tyrosine	3.00
Valine	4.44

Source: Ovie and Ovie (2006)

considered promising species for feeding fish larvae and fingerlings in large-scale production as they have short life-span, small size, quick embryonic development, and abundant energy store (Sipaúba-Tavares & Braga, 1999; Sipaúba-Tavares & Bachion, 2002).

Cheikyula and Ofojekwu (2003) revealed that the best mean weight gain of goldfish fry (*Carassius auratus)* was recorded for fry fed with *Moina/cyclops*. They recommended the use of *Moina/cyclops* for rearing of gold fish fry instead of artificial diet. The use of *Moina* as fish larval feed has yielded commendable results with species indigenous to various regions of the world (Bryant & Marty 1980; Adeyemo *et al.*, 1994). Ovie *et al.* (1993) reported the use of *Scenedesmus* cell to enhance *M. micrura* population density and that *M. micrura* completely eliminate *Brachionus* from the mixture of zooplankton culture in spite of the initial density of rotifer as a result of competition over limited resources.

2.4 BIOLOGY OF *DAPHNIA PULEX*

Daphnia belongs to sub order cladocera which are crustaceans. It has close resemblance to real fleas (*Pulex iritans)* but real fleas are insects which share only an extremely distant common ancestry with *Daphnia*, since both crustaceans and insects are *Arthropods* (Clare, 2002). It is frequently used as food in freshwater larviculture (Delbare & Dhert, 1996). They are rich in amino acid profile (Dabrowski & Rusieki 1983) although they can be as small as 800micron in size. They undergo sexual reproduction and asexual reproduction depends on prevailing environmental condition (Clare, 2002).

2.4.1 Structure of *Daphnia pulex*

Daphnia is an organism with compound eye, two double branched antennae and leaf-like limbs from inside the carapace that produce water current and carry food and

oxygen to mouth and the gills. The body is transparent and the beating of the heart can be observed under the microscope. A carapace covers the body with pairs of thoracic appendages (4-6 pairs) (plate 3). The post abdomen bears two large claws used primarily for clearing debris out of the carapace (Delbare & Dhert, 1996). Species of *Daphnia* occur as different strains in different region or geographical locations. Some times the same species can look completely different both in terms of size and shape, depending on its origin and environmental factors at that location.

2.4.2 Classification of *Daphnia pulex*

Kingdom: *Animalia*

Phylum: *Arthropoda*

Subphylum: *Crustacea*

Class: *Brachiopoda*

Order: *Cladocera*

Family: *Daphniidae*

Genus: *Daphnia*

Species: *Daphnia pulex* (Muller, 1785)

2.4.3 Ecology and Water Quality Requirements of *Daphnia pulex*

D. pulex feeds on *Chlamydomonas* species, *Volvox species*, bacterial, fungi, protozoan and organic particles of suitable size. *Daphnia* dissolved oxygen requirement is zero to super saturation while pH level of 6.5 to 9.5 is acceptable by this organism (Rottmann *et al.,* 2003). *D. pulex* is sensitive to and become immobile or die in salt solution like Sodium, Potassium, Magnesium and Calcium (dissolved minerals). Low Phosphorus 0.5 ppm will stimulate reproduction but concentration of 1.00ppm is lethal to

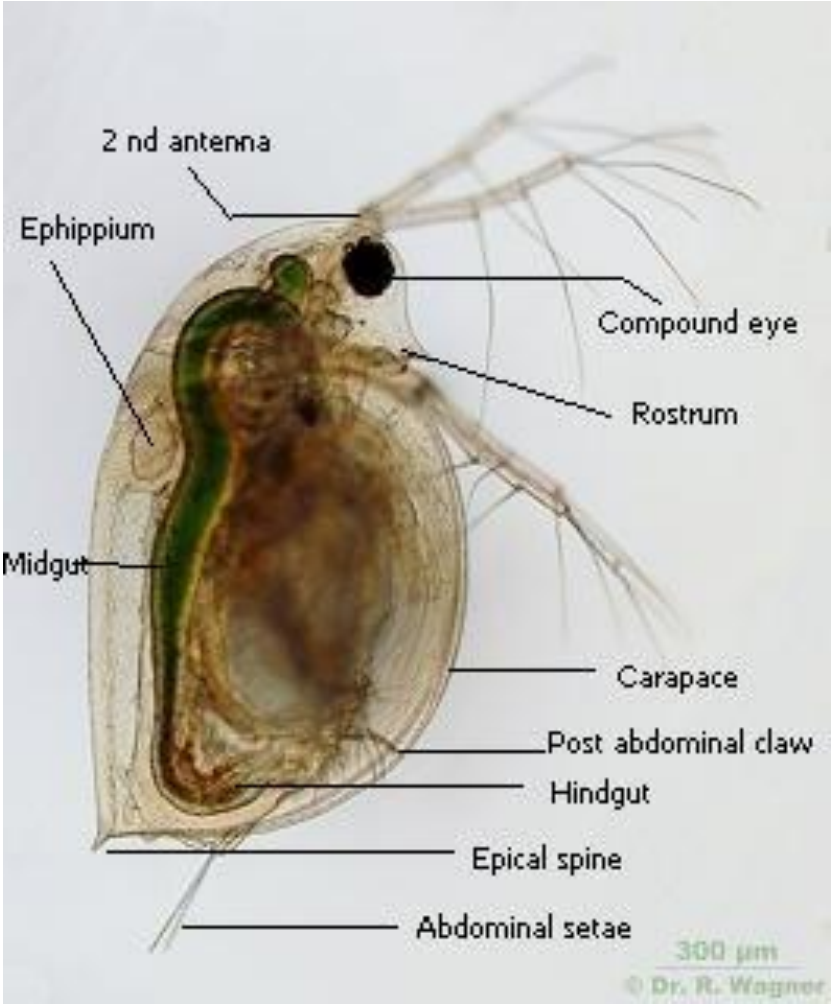


Plate 3**:** Internal and External Anatomy of *Daphnia pulex*
Source: (Wagner, 2007)

the young ones of the organism. *D. pulex* requires little water hardness (90mg carbonate) and does well in temperature range of 24-31^{0}C (Rottmann *et al.*, 2003).

2.4.4 Life Cycle of *Daphnia pulex*

Life span of *Daphnia*, from the release of egg into brood chamber until death of the adult is highly variable depending on the species and environmental conditions (Pennak, 1978). Generally, life span increases as temperature decreases due to lowered metabolic activities. *Daphnia* reproduces by parthenogenesis and the entire population is made up of females during this period. The developing embryos are often visible in the mother's body with the aid of microscope. Typically 6-10 eggs are released into the brood chamber. The eggs hatch in the brood chamber and the 7-8 juvenile are released between 1 to 2 days when the female molts. The organism reaches maturity within 6-10 days. *D. pulex* invests 67% of its energy for reproduction while the remaining 23% are for growth hence fast population density in favourable environment (Clare, 2002). The prolificity of this organism is due to their ability to replicate by parthenogenicity (i.e. the ability to self-replicate without fertilization of any form). The offspring is a replicate of the parent (genetic copy or clones) and if there is any difference in physical state of the cloves, it is likely to be due to environmental conditions (Clare, 2002).

2.4.5 Proximate Analysis of *Daphnia pulex*

Daphnia has protein content of 50% dry weight and fat content 20-27% for adult (Creswell, 1993). As most live food, they are what they eat. Vitamin and formulated food for *Daphnia* will give them food value. Creswell (1993) reported the biochemical composition of *D. pulex* (percentage of total organic matter on dry matter basis). Moisture 94%, Crude protein 50%, crude fat 16.66%, Ash 20.0% while Yurkowski and Tabachek (1979) reports recorded the following for *D. pulex* (Moisture 94%, Crude protein 49.7%, Carbohydrate 4.9%, Crude fat 16.3% and Ash 19.3%). The remaining percentages account for other dry organic matters

2.4.6 Amino Acid Compositions of *Daphnia pulex*

According to Dabrowski and Rusiecki (1983) *D. pulex* had good amino acid composition of ten amino acids. The amino acid profile of this organism is shown in Table 3. The position of this organisn is vital in food web and fish fry nutrition in aquatic habitat.

2.4.7 Uses of *Daphnia pulex*

In nature, Daphnia is a stable food for many fishes and fish fry. They are high in protein and essential nutrients, and their exoskeleton provides roughage necessary for good digestion. In the absence of live daphnia to offer fish, freeze-dried is the next best option (Rottmann *et al.*, 2003).

Daphnia has been proved to be very sensitive to poor water condition and a number of research and industrial groups used *Daphnia* to test water quality (Clare, 2002). They are sensitive to halides concentration like chloride or fluoride in tap water which are toxic to *Daphnia* more than fishes. *Daphnia* species are sensitive to metal ion concentration like Sodium, Potassium, Magnesium and Calcium whose increase in concentration can cause immobility and death. *Daphnia* species are extremely sensitive to Copper, Zinc and dissolved dichromate ions. They are used as bio water monitor of water quality so that only safe water is release to environment (Clare, 2002). According to Lynch (1980), larger *Daphnidae* invest less energy in growth and more in reproduction after they reach maturity.

2.5 HETEROBRANCHUS BIDORSALIS AND *HETEROBRANCHUS LONGIFILIS*

Heterobranchus species is one of the catfish with a very close resemblance to *Clarias* species but their differences are pronounced in some aspects. *Heterobranchus* species possess an adipose fin, four pairs of barbels on flattened strong depressed head

Table 3: Amino Acid Compositions of *Daphnia pulex* (g amino acids per 100 g of protein)

Amino acid compositions-	*Daphnia pulex*
Arginine	5.6
Histidine	2.1
Isoleucine	5.6
Leucine	8.2
Lysine	8.8
Methronine	2.3
Phenylalanine	4.7
Threonine	5.2
Tyrosine	-
Valine	6.4

Sources: Dabrowski and Rusiecki (1983)

and the flesh is less oily. It has rapid growth performance (grow up to 1.2m and weigh up to 30kg) and non-aggressive feeder (Reed *et al.*, 1967).

Heterobranchus species are among the most popular catfish in Nigeria market. The rearing of this fish fry is primarily from the wild because of the difficulty in handling the fry in the hatchling stage of their life. Madu *et al.* (1990) identified inadequate feeding as the highest single source of mortality at fry stage and that food must be adequate not only in quality and quantity but also in size.

2.6 USE OF MANURE FOR IMPROVEMENT OF ZOOPLANKTON POPULATION DENSITY

Jana and Chakrabarti (1993) reported the used manure (cattle manure, poultry droppings, and mustard oil cake, 1:1:1) on *M. micrura* and *Daphnia carinata* and found that the differences in offspring production of test cladocerans in five different treatments were directly correlated with gross or net primary productivity values. Srivastava *et al*. (2006) did a similar work on mass culture of *Ceriodaphnia cornuta* using a mixture of organic manures: cattle manure: poultry droppings: mustard oil cake (1:1:1) at four different doses: 0.263 kg/m^3 (first dose), 0.526 kg/m^3 (second dose), 1.052 kg/m^3 (third dose) and 2.104 kg/m^3 (fourth dose) in outdoor condition. They got the peak of *C. cornuta* on 12th day of inoculum in fourth dose which significantly ($P < 0.01$) had the highest numbers of the organisms (1,930 individuals/L).

The use of organic and inorganic manure for the culturing of zooplankton was reported by Gupta and Gupta (2006). The use of cow dung, chicken dropping, horse manure, rice bran and mineral fertilizer is encouraged by Rottmann *et al.* (2003). Under natural 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 & Edmundo, 1980; Kim *et al.*, 2008). A recent study by

Golder *et al.* (2007), demonstrated that human urine, cow urine, cow dung, poultry droppings and vermin-compost could be utilized for zooplankton cultivation. Loh *et al*. (2009) reported in their study that *Moina macrocopa,* fed with fish faeces, gave higher reproductive rates and produced a greater number of neonates within a shorter generation time while the peak population was reached on day 11.

2.7 TEMPERATURE, FERTILIZATION, HATCHABILITY OF *HETEROBRANCHUS BIDORSALIS* EGGS AND SURVIVAL OF HATCHLINGS

Temperature plays an important effect on hatching of eggs, and the growth rate of larvae increased at the optimum temperature (Brett and Groves, 1979). No egg of warm water fishes survived to hatch in water temperature of 20°C or 38°C (El-gamal, 2009). It is most likely that the temperature affects the tolerance level of viable eggs, (El-gamal, 2009). In addition, temperature is known to influence the efficiency of yolk utilization (Hamel *et al.*, 1997). It also was reported that the growth rate increase with increasing of water temperature, but when the temperature becomes superoptimal, it has a negative effect (Brett and Groves, 1979).

The temperature influences metabolic processes and it is the single most important factor that determines growth rates in fish (Brett, 1979). The temperature range of 27-30°C was reported be to be acceptable range of water temperature for warm water fishes (El-gamal, 2009).

2.8 *CHLORELLA VULGARIS* AND COMBINATIONS OF MANURE IN ZOOPLANKTON PRODUCTION

Chlorella vulgaris is small oval shaped cell which is within 2-15 µm in diameter. It divides into two, four non motile danghter cells enclosed in a little while, in an old wall. It occurs in vast quantity as green soup in cattle troughs (Belcher & Swode, 1978). Formulated diet for zooplankton in form of filterate of combination of manure both

organic and inorganic with other food substances like rice bran, fish faecal matters and oil cake has been reported for zooplankton production (Gupta & Gupta 2006, Arimoro 2006, Srivastava *et al.,* 2006). The richness of these organisms is directly related to what they feed upon and their usefulness to fry is likely based on what they feed on (Cho 2001; Castell *et al.,* 2003).

2.8.1 Classification of *Chlorella vulgaris*

Kingdom: *Protista*

Division: *Chlorophyta*

Class: *Trebouxiophyceae*

Order: *Chlorellales*

Family: Chlorellaceae

Genus: *Chlorella*

Species: *Chlorella vulgaris* (Beijerinck, 1890)

2.8.2 Usefulness of *Chlorella vulgaris*

Chlorella is the most cultivated eukaryotic green micro algae, as it is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry (Sharma *et al*., 2012). *Chlorella* is a green spherical single celled freshwater micro alga (smaller than a human red blood cell) and has been on earth for over 2.5 billion years since the Pre-Cambrian Period (Sharma *et al*., 2012). It contains proteins, carotenoids, lipids, immunostimulator compounds, polysaccharides, vitamins, antioxidants and minerals (Sharma *et al*., 2012). *Chlorella vulgaris* has 60% protein including 19 amino acids (Table 4) of which eight amino acids are considered to be essential for man, more than 20 bio-available vitamins and minerals rich in beta-carotene

Table 4: Amino Acids Content (g amino acids per 100 g of protein) of *Chlorella vulgaris* and Hens'egg

Amino acids	*Chlorella vulgaris*	Hen egg
Alanine	7.8	
Arginine	7.9	5.7
Cystine	0.27	10.5
Aspartic acid	9.70	2.3
Glutamic acid	13.1	12.6
Isoleucine	5.1	3.0
Leucine	2.0	8.8
Lysine	5.2	7.0
Methionine	9.1	9.2
Phenylalanine	8.4	7.4
Proline	2.4	3.0
Serine	5.2	5.1
Threonine	6.0	5.0
Tryptophane	4.0	8.4
Tyrosine	3.9	4.1
Valine	2.4	1.1
Glycine	5.8	4.2
Histidine	7.8	2.4

Source: (Khatun et al., 1994)

and vitamin, rich in unsaturated fatty acids (around 80% of its total fatty acids content) and highest known source of chlorophyll (Khatun *et al.* 1994*;* Sharma *et al*., 2012).

Hard wall of the *Chlorella* cell needed to be broken down for optimum digestibility by spray drying or disruption with mill process. Ultra-jet-sprayed dried process of *C. vulgaris* strain ensures maximum digestibility and availability of its nutrients (Khatun *et al.,* 1994).

2.8.3 Usefulness of Combinations of Manure

Srivastava *et al*. (2006) reported 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 in outdoor condition. They got the peak of *C. cornuta* on 12th day of inoculums. Chicken dropping with small quantity of inorganic fertilizer has been reported to produce favourable growth of algae for rotifer culture (Arimoro, 2006) while Gupta and Gupta (2006) recommended the use of organic manure (150ppm), oil cakes (75ppm) and super sulphate (30ppm) for the culture of *Rotifers, Moina* and *Daphnia* species.

CHAPTER THREE
MATERIALS AND METHODS

3.1 SITE WHERE ZOOPLANKTON WERE COLLECTED FOR MASS PRODUCTION IN LABORATORY

The map the site where the zooplankton were under study for laboratory manipulation is showed in Figure 2 The zones, some basic water quality parameters and some live food zooplankton of River Awuma at Shabu Town were studied for manipulations in the laboratory. The River Awuma is one of the main water bodies in Shabu community. The people use the water mainly for domestic purposes.

Shabu Town is a gateway of Lafia, the capital city of Nasarawa State of Nigeria. It is on the coordinate 8° 34 N /8567'N, 8.550 ^{o}E with elevation of 242M (794ft) while Nasrawa State is located between latitude 7° and 9° N and longitude 7° and 1° E.

3.2 DETERMINATION OF WATER QUALITY PARAMETERS AND AMINO ACID PROFILE OF CULTURED MICRO ORGANISMS

The water quality parameters were determined using APHA/AWWA/WPCF (1985) methods while amino acid profile of the cultured organisms were determined by using the standard methods of Spackman *et al.* (1958). The details of the methods are as follow:

3.2.1 Water Temperature

The temperature of the water of various experimental groups and sample sites in Awuma River at Shabu were measured using a mercury centigrade dry bulb thermometer. The readings were taken in few centimeters below the surface of the water. The mean of three readings were taken for each group (0-100°C).

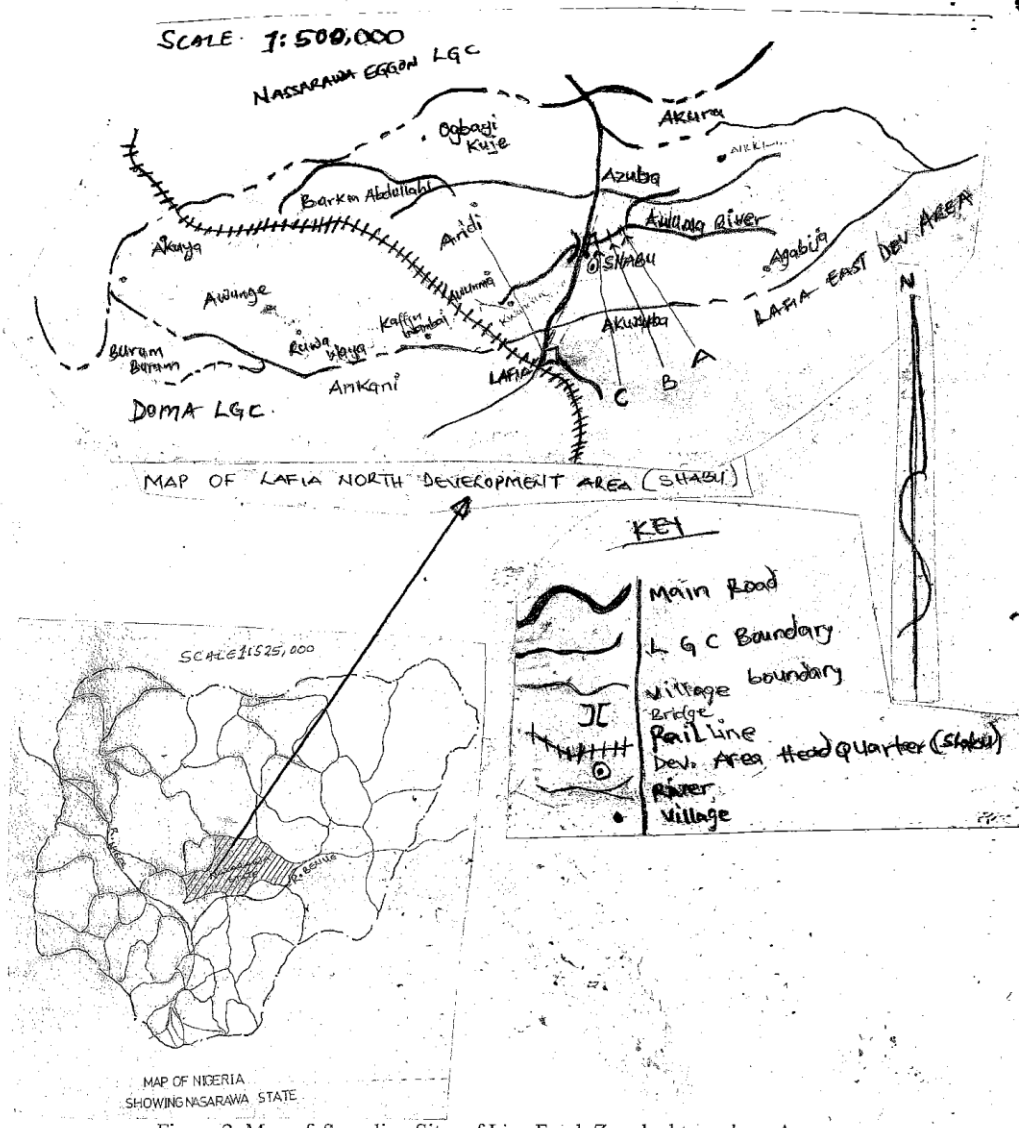


Figure 2: Map of Sampling Sites of Live Food Zooplankton along Awuma River, Shabu, Nasarawa State of Nigeria.

3.2.2 Water pH

The pH of water for each experimental group and sample site in Awuma River at Shabu were measured using corning pH meter model 901. Three readings were taken in each case and their means was determined.

3.2.3 Dissolved Free Carbon-dioxide

The free carbon dioxide dissolved in water was determined using phenolphthalein method. About 100ml of water was taken from 25cm below the water surface into a conical flask and 10 drops of phenolphthalein indicator was added. When colour change was not observed and the sample remained clear, titration was carried out 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. These procedures were repeated for the sampling zones in this study.

3.2.4 Dissolved Oxygen

The Alsterbeg (Azide) method was employed to determine dissolved oxygen. The water samples were collected using 250ml stopper bottle at 25cm below the water surface. The bottle was corked in side the water to avoid any trapping of air. The water samples were then fixed by adding 2ml of Manganese Sulphates followed by 2ml of Alkaline-iodide (Sodium Azide). The water was restoppered and a careful shaking was done for proper mixing. The samples were allowed to settle for few minutes and 2ml of concentrated Sulphuric acid was added. Careful mixing was done by shaking until a solution was formed.

About 200ml of the solution formed was transferred into a conical flask and titrated to pale yellow using 0.025N Sodium Thiosulphate. When 1ml 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 (mgL^{-1}) in the equation below (Boyd, 1979). These procedures were repeated for each of the sampling zones.

$$\text{Concentration of Dissolved Oxygen (mg/L)} = \frac{V(D)*N(D)*8*1000}{\text{Volume of sample}}$$

Where: V (D) = Volume of Sodium Thiosulphate used in tritration.

N(D) =Normality of Sodium Thiosulphate 0.025

(D) = Sodium Thiosulphate

3.2.5 Total Alkalinity

A sample of water of 100ml was taken into a conical flask from below the watersurface. About 4 drops of Phenopthalein indicator was added. When the water sample remained 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 (mgL^{-1}) of $CaCo_3$. These procedures were repeated for the different sampling zones.

Total alkalinity = (a) + (b) in mg/L as $CaCO_3$

(a) = Phenolphthalien Alkalinity
(b) = Methyl Alkalinity

3.2.6 Determination of the Amino Acid Profile of Cultured Micro Organisms

Samples were harvested with a 50 μm zooplankton net, and air-dried on Whatman no.1 filter paper. The caked sample was peeled off the filter paper and stored in dry marked screw-cap specimen bottles pending analysis. Samples were analyzed for amino

acid profiles according to Spackman *et al.* (1958). Determination of the amino acid profile included the following; samples were defatted, hydrolyzed, and evaporated in a rotary evaporator before being loaded into a Technicon Sequential Multi-Sample Auto-Analyser. The area under each peak was calculated using non leucine as an internal standard and molecular weight of each amino acid relative to the standard and percentage nitrogen in the sample, the concentration of each amino acid was calculated. Since acid hydrolysis destroys tryptophan and cysteine, these two amino acids were determined directly by the method of Gaitonde (1967). The determinations of the amino acid were done in two replicates for accuracy of results.

3.3 PREPARATION OF ALGAL CULTURE MEDIA

Mass culture of algae was achieved using the Venture and Enderez (1980) sack fertilization method. Previously dried chicken manure in a sack containing 20g/m^2 of water was placed in depth of 50cm concrete tank and the sack was submerged in water for 1-2 hrs after which it was transfered to productivity tank. Two litres of water that was screened through 50 µm net from sample site of Awuma River at Shabu was added. Dipping of the sack was carried out in 4 days and algae that appeared in the culture were identified using (Belcher and Swale, 1978).

3.4 SCREENING AND ACCLIMATIZATION OF *BRACHIONUS CALYCIFLORUS MOINA MICRURA* AND *DAPHINA PULEX* FOR THIS INVESTIGATION

Collection of zooplankton from the water body 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 Awuma River at Shabu (Plate 4) into a 4-litre container at 6.00 - 6.30am in the morning. At littoral zone, sampling was done from water among the weeds by filter using zooplankton

harvesting net (Ovie & Sarma, 1993). At benthic zone (1.2m deep), samples were collected with zooplankton harvesting net on the floor plain of the river while those of the pelagic were done in water column. Right at the river bank, the filtration was done and debris, macrophytes with macro-organisms and fish fry were removed using mosquito net (Ovie & Sarma, 1993; Adigun, 2005). The remaining micro-organisms were taken to the laboratory for microscopic examinations at Faculty of Agriculture, Shabu-Lafia, Nasarawa State University Keffi. Two mesh sizes were used (the upper 200µm 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 mounted on a microscope (Ovie *et al.*, 1993). The sample was observed in x10 objective of the microscope (Javellana & 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 10^6 cells/ml determined by the help of haemocytometer (Ovie & Eborge, 2002). On the third day, growth in population of this selected organism was noticed in each of the glass aquarium with some other zooplankton.

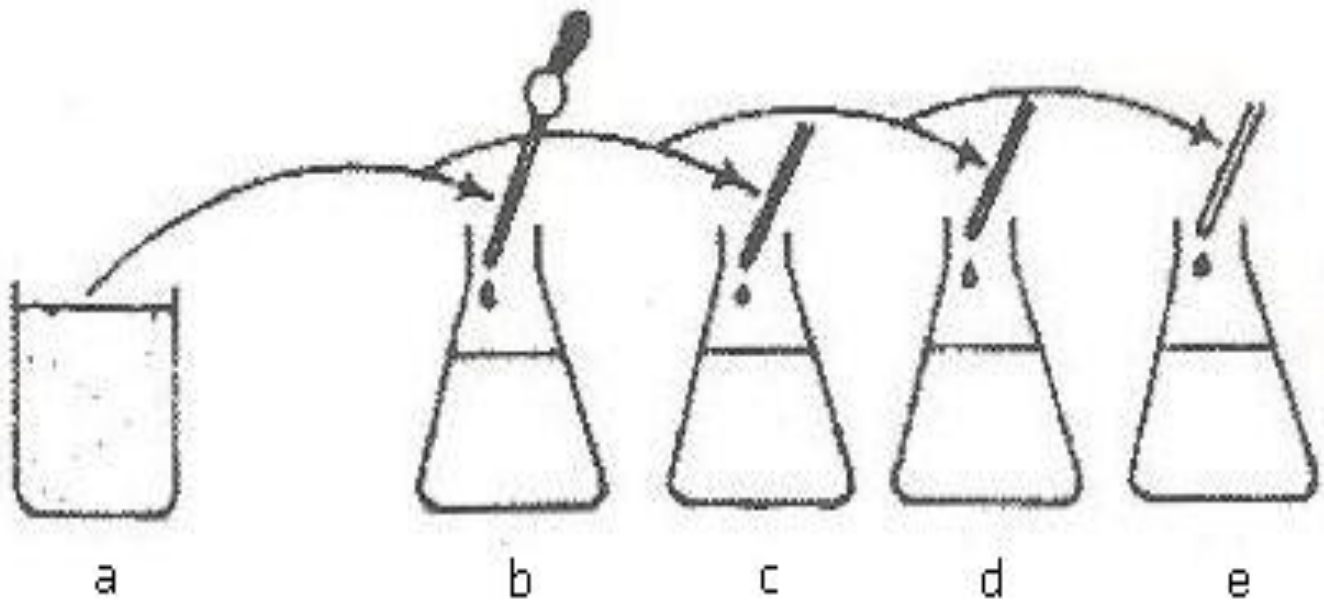
3.5 ISOLATION OF *BRACHIONUS CALYCIFLORUS* (ROTIFER), *MOINA MICRURA*, *DAPHINA PULEX* AND *CHLORELLA VULGARIS* INTO MONO SPECIFIC CULTURE

The essence of isolation is to have a desired mono specific culture of live food organisms in order to do away with undesirable zooplankton. Repeated subculture

(Figure 3) 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 mounted on microscope (in some cases, dilution of the plankton water was done to lower the number of organisms and kinds in a drop). With automatic pipette, the desired organisms (*M. micrura*, *D. pulex* and *B. calyciflorus*) from conical flask 'b' were collected and transferred into different glass conical flask 'c' containing aerated algal water at temperature 26^0C 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 each of the desired *Brachionus*, *Moina* and *Daphnia* species (Figure 3). The cultured organisms were identified by Pennak (1978); Koste and Shiel (1987); Fernando *et al.* (1987).

The isolated mono specific culture of *B. calyciflorus*, *M. micrura*, and *D. pulex* were fed using freshwater green algae culture as stated above (Ovie & Egborge, 2002; Sipaúba-tavares & Bachion, 2002) prior to treatment with various combinations of manures at various concentration. The microphotographs of cultured *B. calyciflorus*, *M. micrura*, and *D. pulex* were taken by Sony digital camera, cybershot model 7.2 mounted on Olumpus binocular microscope. Isolation of *Chlorella vulgaris* into mono specific culture was done by putting a drop of plankton water into several places in a glass slide mounted on microscope (in some cases, dilution of the plankton water was done to lower the number of organisms and kinds in a drop). With the help of automatic pipette, the desired organisms (*C. vulgaris*) from conical flask 'b' were collected and transferred into



` *a = Diluted original solution containing zooplankton*
b = Solution containing few species of zooplankton
c = Solution containing dominant species of desired zooplankton
d = Solution containing mono specific species of desired zooplankton
e = Solution containing comfirmed desired zooplankton

Figure 3: Diagram of Repeated Subculture System.

Source: Villegas (1981)

different glass conical flask ‘c’ containing manure filtrate at temperature 26^0C and pH 7.8.

Incubation was done under controlled temperature, light intensities and illumination time. After three 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 each of the desired *C. vulgaris*.

The isolated mono specific culture of *C. vulgaris* was fed using filtrates of manure. The microphotograph of cultured *C. vulgaris was* taken by Sony digital camera, cybershot model 7.2 mounted on Olumpus binocular microscope.

3.6 PREPARATION OF DIFFERENT COMBINATIONS OF THE MANURE SOLUTION

All the manures (organic and inorganic), cake (Gupta & Gupta, 2006) and rice bran (Rottmann *et al.,* 2003) were grind to powder, weighed in a scale, mixed together in the following proportions and combinations as shown in table 5 below. The combinations of manure were soaked in distil water for 36 hours in dark room (Jana & 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 dark room to avoid algal growth (Gupta & Gupta, 2006; Arimoro, 2006) for culturing of *B.* c*alycilflorus, M. micrura* and *D. pulex.*

3.7 SELECTED ZOOPLANKTON CULTURE USING DIFFERENT COMBINATIONS OF MANURE AT DIFFERENT CONCENTRATIONS AND DETERMINATION OF THEIR POPULATION DENSITY

B. calyciflorus, M. micrura and *D. pulex* were the zooplankton selected for this study. They were cultured in monospecific state on filterate of different manure combination at different concentrations. The duration of culture was also monitored.

Table 5: Manure Treatment Combinations.

Name	Combinations of manure (g/L of water)					
3CM1	Cowdung, (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	Cowdung, (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 (7.5)	Groundnut cake (1.25)	Soybean cake (1.25)	Ric 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)

CM = Combination of Manure
Figure in parenthesis = dried weight in g/L of water.

3.7.1 *Brachionus calyciflorus* Culture Using Different Combinations of Manure at Different Concentrations

Cultured *B. caiyciflorus*, a freshwater rotifer was treated with concentrations of different combinations of manure culture medium in 9 days duration of growth to determine the medium, concentration and duration for mass culture of the organism.

Pure cultured of *B. calyciflorus* (10) were placed into 1.0 ml of aerated bore hole water (Delbare & Dhert, 1996; Ovie & Ovie, 2004; Arimoro, 2006) and the fourdifferent combinations of manure in 5 different concentrations each (1.00, 2.00, 4.00 and 8.00 and 16.0ml/L) in 3 replicates. The concentrations of the manure were fed every morning to the *B. calyciflorus* culture for nine days. Each concentration was aerated gently to distribute the nutrient in the culture (Ovie & Ovie, 2004). The population density of *B. calyciflorus* was observed using microscope and automatic pipette (Ovie, 1991). The automatic pipette (graduated) was used to collect samples randomly into subsampling bottle. The sampled bottle was inverted several times and individual *B. calyciflorus* in the subsample were determined using modified Sedgwick Rafter Counter (Ovie, 1991)

The population density of *B. calyciflorus* of known volume was counted under x10 objective of microscope (Javellana & Escritor, 1981) and the organisms were returned back to the stock. In the terminal counting (i.e. at the end of the experiment) especially when decline in population was noticed (Ovie & Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0g, Iodide crystals 1.0g and water 100ml) was used to paralyse the organisms in collected water sample so that counting will be easier and more accurate (Javellana & Escritor, 1981).

3.7.2 *Moina micrura* Culture Using Different Combinations of Manure

In this investigation, four different treatments of 3, 4, 5 combinations of manure solution were used at 5 different concentrations in 11 days period of growth of cultured *M. micrura* (plate 7). 20 pure cultured of *M. micrura* were placed into 1 litre of aerated bore hole water (Delbare & Dhert, 1996; Ovie & Ovie, 2004) and the 3 different sequence combinations of Manure in 5 different concentrations each (1.00, 2.00, 4.00 and 8.00 and 16.0ml/L) in 3 replicates. The concentrations of the manure were fed every morning to the *M. micrura* culture for eleven days. Each concentration was aerated gently to distribute the nutrient in the culture (Ovie & Ovie, 2004). The population density of *M. micrura* was observed using microscope and automatic pipette (Ovie, 1991). The automatic pipette (graduated) was used to collect samples randomly into sampling bottle.

The sampled bottle was inverted several times and individual *M. micrura* in the subsample were determined using modified Sedgwick Rafter Counter (Ovie, 1991). The population density of *M. micrura* of known volume was counted and the organisms were returned back to the stock. In the terminal counting (i.e. at the end of the experiment) especially when decline in population was noticed (Ovie & Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0g, Iodide crystals 1.0g and water 100ml) was used to paralyse the organisms in collected water sample so that counting will be easier and more accurate (Javellana & Escritor, 1981).

3.7.3 *Daphnia pulex* Culture Using Different Combinations of Manure

Pure cultured of *D. pulex* (10) were placed into one litre of aerated bore hole water (Delbare & Dhert, 1996; Ovie & Ovie, 2004) and the fourdifferent combinations of manure in 5 different concentrations each (1.00, 2.00, 4.00 and 8.00 and 16.0ml/L) 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 & 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 sampled bottle was inverted several times and individual *D. pulex* in the subsample were determined using modified Sedgwick Rafter Counter (Ovie, 1991). The population density of *D. pulex* of 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 decline in population was noticed (Ovie & Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0g, Iodide crystals 1.0g and water 100ml) was used to paralyse the organisms in water sample collected so that counting will be easier and more accurate (Javellana & Escritor, 1981).

3.7.4 Determination of Population Density of Live Food Zooplankton in the Study

Determination of *Brachionus calyciflorus, Moina micrura* and *Daphnia pulex* population density was generated from Ovie (1991) and used to determine the population density of the zooplankton:

$$P_d = \frac{1000 \times B_x}{V\ (ml)}$$

P_d = Population density of *B. calyciflorus* in 1000ml of water.
V = Average volume of water sampled using automatic pipette
B_x = Average Number of *Brachionus calyciflorus, Moina micrura* or *Daphnia pulex* counted in various random Samplings

3.8 MASS PRODUCTION OF SELECTED ZOOPLANKTON CULTURE IN THIS STUDY

D. pulex, M. micrura and *B. calyciflorus* were mass produced using filtrates of five combinations of manure/chlorella vulgaris. The population density of the various treatments as monitored and documented for the purpose of comparison.

3.8.1 Mass Culture of *Brachionus calyciflorus* Using Filtrate of Combinations of Manure /*Chlorella vulgaris*

Cultured *B. calyciflorus*, a freshwater rotifer (Plate 6) was treated with filtrate from combinations of manure/*Chlorella vulgaris* in 10 days to determine the population density of the of the organism.

Pure cultured of (10) *B. calyciflorus/ml* were placed into 1 litre of aerated bore hole water (Delbare & Dhert, 1996; Ovie & Ovie, 2004; Arimoro, 2006) fed with the filtrate from combinations of Manure solution from grind Cow dung (7.5g/L), single supper phosphate fertilizer(1.5g/L), groundnut cake (1.25g/L), soybean(1.25g/L) rice bran (1.25g/L) at 4.00ml/L / *Chlorella vulgaris* ($1.5X10^6$ cells/L of water) in 3 replicates. The concentrations of the manure were fed every morning to the *B. calyciflorus* culture for 10 days. Each treatment was aerated gently with battery aerator to distribute the nutrient in the culture (Ovie & Ovie, 2004). The population density of *B. calyciflorus* was observed using microscope and automatic pipette (Ovie, 1991). The automatic pipette (graduated) was used to collect samples randomly into sub sampling bottle. The sub sampled bottle was inverted several times and individual *B. calyciflorus* in the subsample were determined using modified Sedgwick Rafter Counter (Ovie, 1991)

The population density of *B. calyciflorus* of known volume was counted under x10 objective of binocular microscope (Javellana & Escritor, 1981) and the organisms were

returned back to the stock. In the terminal counting (i.e. at the end of the experiment) especially when decline in population was noticed (Ovie & Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0g, Iodide crystals 1.0g and water 100ml) was used to paralyse the organisms in collected water sample so that counting will be easier and more accurate (Javellana & Escritor, 1981).

3.8.2 Mass Culture of *Moina micrura* Using Filtrate of Combinations of Manure / *Chlorella vulgaris*

Cultured *M. micrura,* a freshwater *Cladoceran* was treated with filtrate from combinations of manure solution from grounded chicken droppings (7.5g/L), single supper phosphate fertilizer (1.5g/L), groundnut cake (1.25g/L), soybean (1.25g/L), rice bran (1.25g/L) at 4.00ml/L /*Chlorella vulgaris* ($1.5X10^6$ cells/L of water) in 12 days to determine the population density of the of the organism.

50 pure cultured of *M. micrura* were placed into 1 litre of aerated bore hole water (Delbare & Dhert, 1996; Ovie & Ovie, 2004; Arimoro,2006) fed with the filtrate from combinations of Manure (4.00ml/L) / *Chlorella vulgaris* ($1.5X10^6$ cells/L of water) in 3 replicates. The concentrations of the manure were fed every morning to the *M. micrura* culture for 12 days. Each treatment was aerated gently to distribute the nutrient in the culture (Ovie & Ovie, 2004). The population density of *M. micrura* was observed using microscope and automatic pipette (Ovie, 1991). The automatic pipette (graduated) was used to collect samples randomly into sub sampling bottle. The sub sampled bottle was inverted several times and individual *M. micrura* in the subsample were determined using modified Sedgwick Rafter Counter (Ovie, 1991)

The population density of *M. micrura* of known volume was counted under x10 objective of microscope (Javellana & Escritor, 1981) and the organisms were returned

back to the stock. In the terminal counting (i.e. at the end of the experiment) especially when decline in population was noticed (Ovie & Eborge, 2002), one drop of Lugol's solution (Potassium iodide 2.0g, Iodide crystals 1.0g and water 100ml) was used to paralyse the organisms in collected water sample so that counting will be easier and more accurate (Javellana & Escritor, 1981).

3. 8.3 Mass Production of *B. calyciflorus*, *M. micrura* and *Daphnia pulex* for Rearing Fry

The live zooplankton *(B. calyciflorus* and *M. micrura)* were collected from cultured strains developed for this work in Research Teaching Farm of Faculty of Agriculture Lafia Campus. Individual/L (50) of each zooplankton were distributed into two ponds measured at 2x2x1M pond and covered with mosquito net (Okunsebor and Ofojekwu, 2009). Each pond was fed every day at concentration of 4.00ml/L of the prepared manure filtrate from grounded chicken droppings(7.5g/L), single supper phosphate fertilizer(1.5g/L) Groundnut cake (1.25g/L) soybean (1.25) and rice bran (1.25g/L) (Okunsebor and Ofojekwu, 2012). The population growth of each treatment was monitored daily. Harvest was done according to (Rottmann *et al.*, 2003; Okunsebor and Ofojekwu 2009).

3.9 WATER TEMPERATURE AND HATCHING OF *HETEROBRANCHUS BIDORSALIS* EGGS AND FRY

Temperature of water and the effects on rate of hatching of *H. bidorsalis* eggs were investigated. The temperature of 28^0C was maintained for the broodstock in the broodstock tank. The stripped eggs were strictly treated according to the selected temperature treatments.

3.9.1 Hatching of *Heterobranchus bidorsalis* Eggs

Hatchery raised gravid brood stocks; *Heterobranchus bidorsalis* were selected and prior to injection, brood stock was kept singly in tank with 200 litres of aerated water. The brood stock was weighed with salter weighing balance and the amount of hormone used was 0.5ml Ovaprim for each 1kilogram of female brood stocks with 2ml syringe while injection was done intramuscularly above the lateral line at 10cm from posterior end of the fish. The injected fish was returned into their various tanks (Hamffa & Sridhav, 2002).

Stripping was done 9 hours after injection at 28°C while the male fish was sacrificed and the milt was prepared for fertilization of the eggs (that was stripped) using normal saline The eggs were carefully stirred with chicken feather for fertilization to effectively take place. The eggs were spread evenly on pretreated Kakaban in a bowls of aerated water at controlled water temperature of 28°C. After 24 hours, hatched eggs were collected. The numbers of fry for the study were not fed until the third day. The water was aerated continuously to increase the dissolved oxygen of the water; and one third of the water in each bowl was changed with clean water daily by siphoning in rubber hose. Each of the live zooplankton was collected from the culture using 50µm plankton net. These organisms were rinsed in clean water before they were used for the feeding of the fish fry according to treatments.

3.9.2 The Influence of Temperature on Percentage Fertilization Rate, Hatchability of Fish Eggs and Survival Rate of Fry

The influence of temperature on percentage fertilization rate, hatchability and survival rate of fry (within the first 3 days of eggs were investigated in University of Jos

in a controlled temperature hatchery (plate 4 & 5). The temperature was varied with the help of adjustable electric thermostat heater empowered by a generator.

Viable 500 eggs were spread on mosquito net kakaban in aquaria in 4 treatments of temperature (26^0C, 28^0C, 30^0C and 32^0C) in three replicates. The processes of hatching were restricted to a particular temperature according to treatments. The number of unfertilized eggs were collected after 6 hours from the time of treatment of the eggs with milt and spread on the kakaban. The kakabans were removed immediately after the hatching and hatchlings were counted then and after 3days. The percentage fertilization rate, hatchability and survival rate of fry were determined using the formula below (El-gamal, 2009; Naeem *et al*., 2011).

(i) Percentage fertilization rate of eggs = (No of fertilized egg/Total no of eggs spread on kakaban) x 100.

(ii) Percentage hatchability = (No of hatchings/fertilized eggs) x 100

(iii) Percentage survival rate = (survived hatchlings after 3 days/ total no of hatched egg) x 100.

3.9.3 Fry Treatments during the Experiment and Data Calculations

3.9.4 Individual fry (50) of 3 old days were placed in a 10-litre plastic bowls in five treatments of 3 replicates for 16 days. Initial average length (cm) and weight (g) of the fry was recorded.

The water in the plastic bowls was aerated continuously and ⅓ of the water was changed with freshwater daily. The following feeds were fed as treatments to the fry satiation in bowls were: *Artemia* shell free, live *Brachionus calyciflorus*, *Daphnia pulex*, *M. micrura, and* live *M. micrura & Brachionus calyciflorus.* Percentage weight gain, total body

Plate 4: A Controlled Temperature Hatching Laboratory in Jos

Plate 5: Hatching Tanks and Their Accessories in Controlled Temperature Laboratory.

length, the condition factor and percentage survival rate were collected using the formulae below.

(a) Percentage weight gain of *Heterobranchus bidorsalis* fry within the period of the experiment was calculated according to (Cheikyula and Ofojekwu (2003); Adewolu *et al.* (2008).

Percentage weight gain (PWG) ={ $(W_2-W_1) / W_1$ } x 100

Where W_2 = final mean body weight and W_1 = initial mean body weight

(b) Total body length of *Heterobranchus bidorsalis* fry fed from various treatments was measured in milimeter. The fry was placed with water into transparent glass dish to determine the total length with help measuring tape (Karl *et al*., 1977).

(c) The condition factor of *Heterobranchus bidorsalis* fry was calculated according to (Madu *et al*., 2003). Condition factor (CF) $K = 100w/L^{3.}$ Where w = weight of fish in (g), L = length of fish in (cm).

(d) The percentage of survival of *Heterobranchus bidorsalis* fry within the duration of the experiment was calculated the formula below (Cheikyula & Ofojekwu, 2003; Odedeyi , 2007))

$$\text{Percentage survival rate} = \frac{\text{No. of fry that survived}}{\text{Total No. of fry that start the treatment in each bowl}} \times 100$$

3.10 STATISTICAL ANALYSIS

The data obtained from water quality parameters of the source water for culturing of fry in each treatment, cultured zooplankton and distribution of live organisms in the sampling zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State were analyzed using descriptive statistics, general statistic of variance of GenStat

Discovery Edition 3 statistical packages. Statistical difference between various means was separated using Duncan multiple range at 95% confidence level.

Data collected from effects of concentrations, different combinations of manure culture medium, period of growth and their interactions on population density of cultured oganisms were analyzed using general statistic of variance of GenStat Discovery Edition 3 statistical packages. The collected data from effect of temperature on eggs fertilization, hatchability, survival rate of fry and utilization of the cultured organisms by fry were also analyzed using general statistic of variance of GenStat Discovery Edition 3 statistical packages. Statistical difference between various means was separated using Duncan multiple range at 95% confidence level.

CHAPTER FOUR
RESULTS

4.1 WATER QUALITY PARAMETERS

Results of water quality parameters of the zones where samples were collected in Awuma River at Shabu, those of the cultured live food organisms and those of source of water used for rearing the fry are presented in this section. Variations in the results of water quality parameters observed are stated in the subsections.

4.1.1 Water Parameters Distribution in the Sampling Zones of Awuma River

The results in the sampled zones of Awuma River at Shabu are presented in Figures 4 to 8 and Appendix 1. Results show that pH ranges of the three zones investigated were not significantly different ($p>0.05$) from each other at a particular week of the study. Fluctuations in the pH results were observed in the three zones studied as duration increases. These fluctuations were common to the littoral, limnetic and benthic zones. Results shows that week 4 had the highest pH value in the littoral and limnetic zones and they were significantly different ($p<0.05$) from other weeks in studied area. Results also show that pH values for all the zones were lowest in week 1 and week 5 in the sampling zones (Figure 4).

The temperature in the littoral were significantly different ($p<0.05$) those of limnetic and benthic zones of the water body.The temperature of the littoral zone of the river was significantly high compared with other zones. At the benthic and limnetic zones, water temperature was not significantly different (>0.05) from each other.The smallest value of temperature was recorded in the benthic zone of the water body.

Results show Carbondioxide was significantly different ($p<0.05$) in the littoral, limnetic and benthic zones of the River. The highest value of carbondioxide was recorded

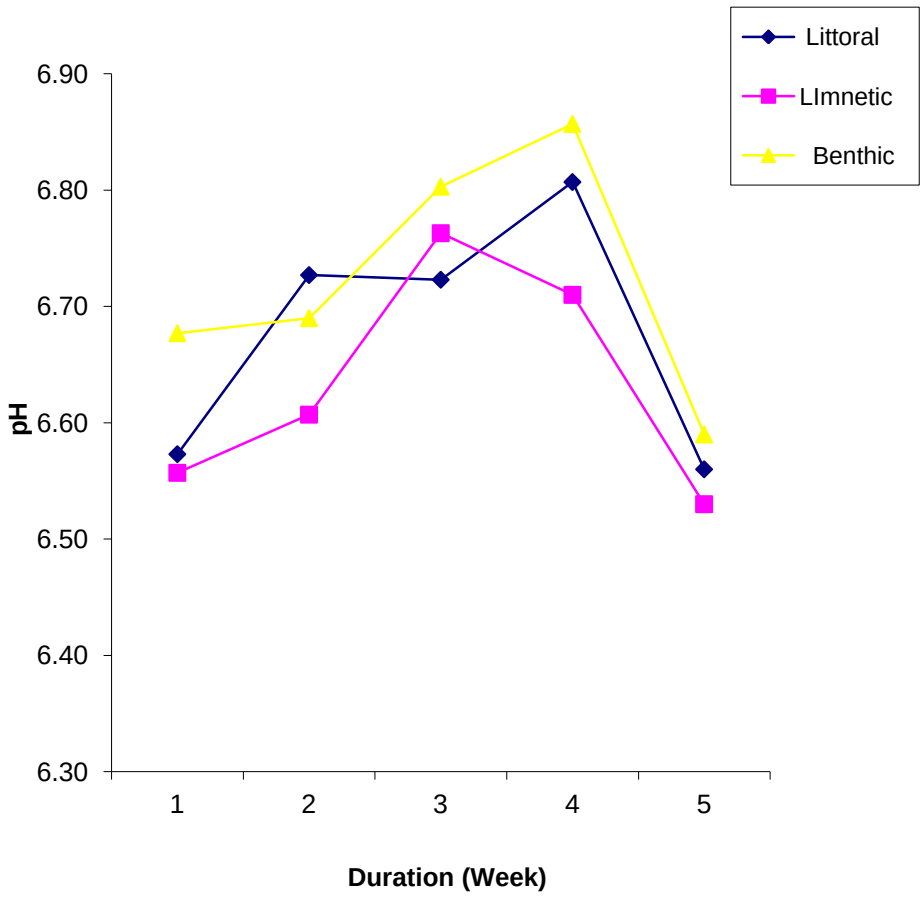


Figure 4: Hydrogen ion Concentration (pH) Distribution during the Period of Collection of Live Food Zooplankton in the Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

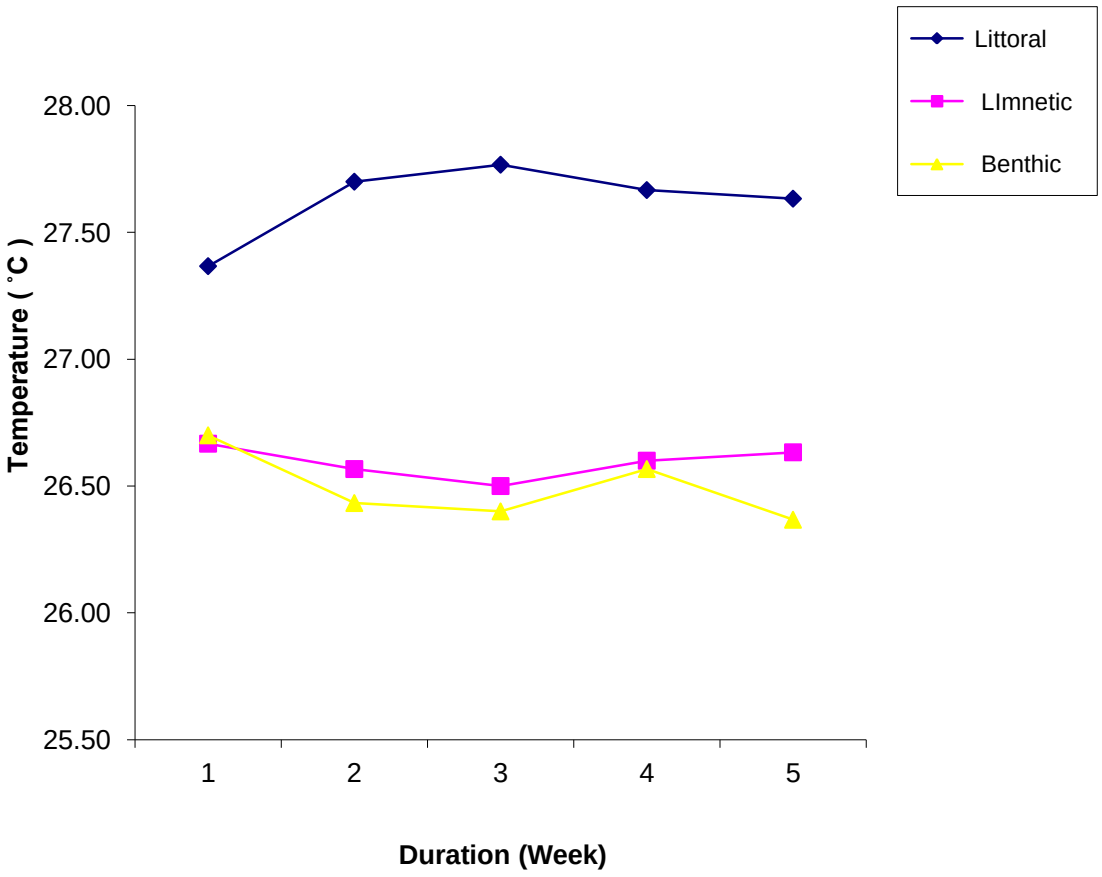


Figure 5: Prevailing Water Temperature Distribution during the Period of Collection of Live Food Zooplankton in the Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

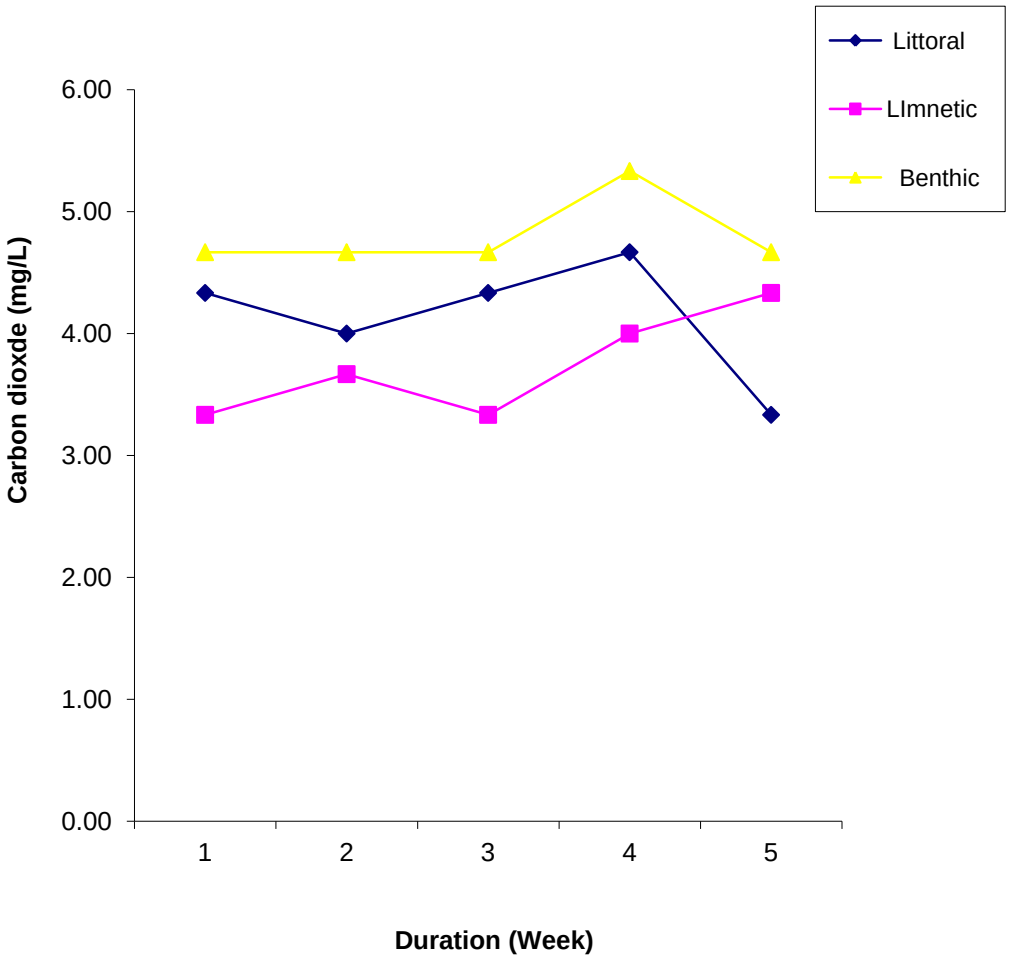


Figure 6: Carbon dioxide Distribution during the Period of Collection of Live Food Zooplankton in the Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

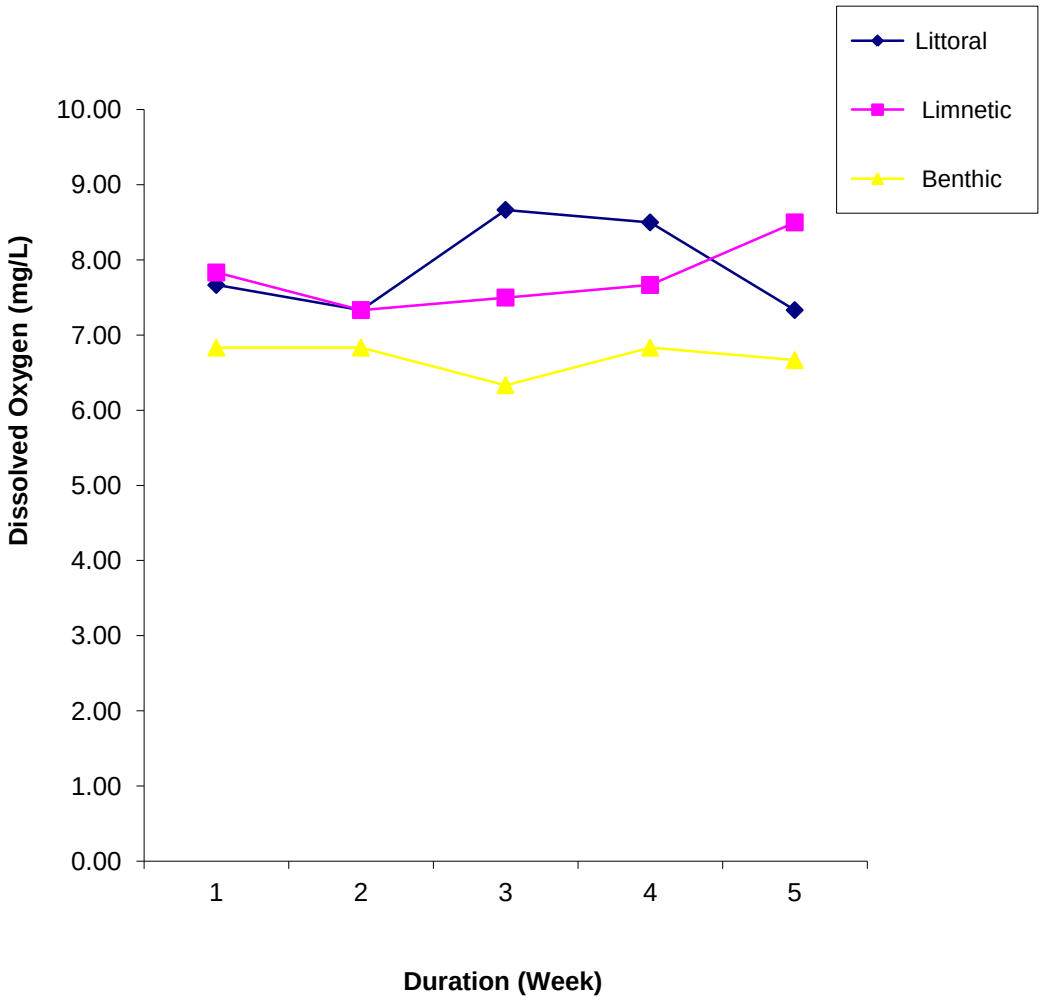


Figure 7: Current Dissolved Oxygen Distribution during the Period of Collection of Live Food Zooplankton in the Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

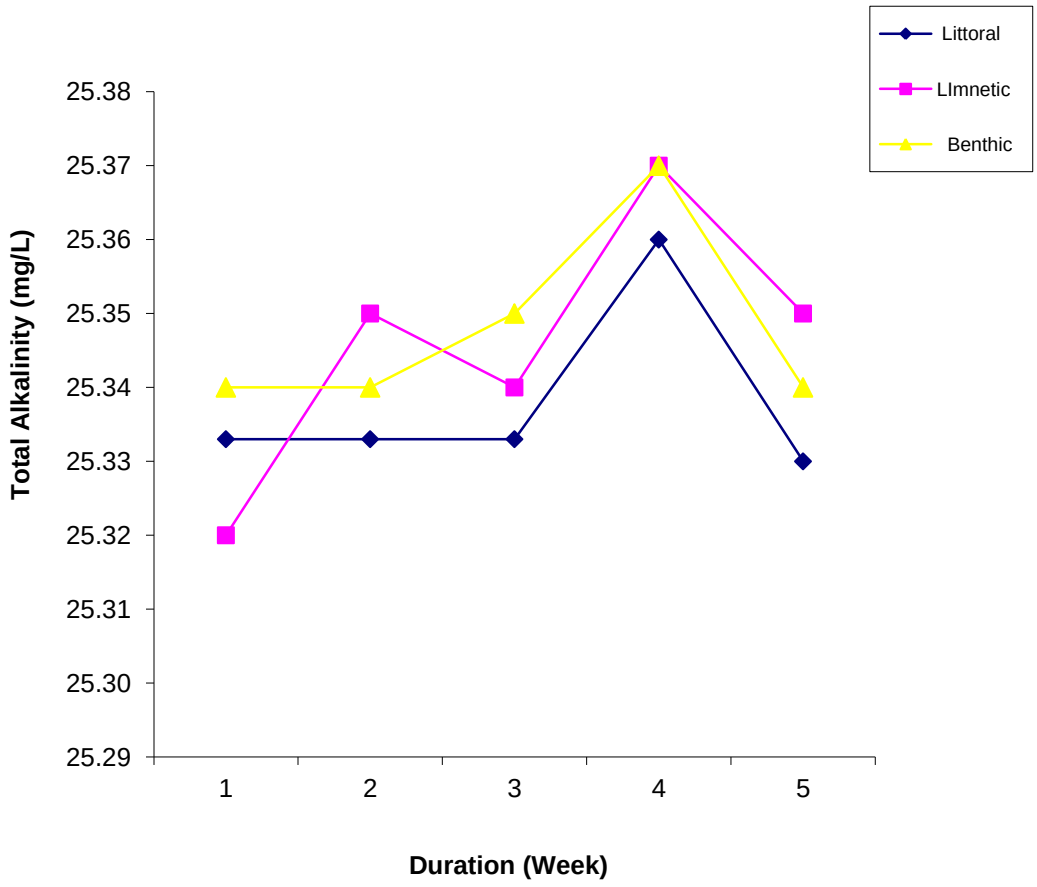


Figure 8: Prevailing Total Alkalinity Distribution during the Period of Collection of Live Food Zooplankton in the Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

in the benthic zone in all sampling area of the river while the smallest value was recorded in the limnetic zone of the water body.

Dissolved oxygen was observed to be lowest in the benthic zone of the investigated zones. The littoral and benthic zones of the water body were not significantly different ($p>0.05$) from each other but they were significantly different ($p<0.05$) from enthic zones in all places investigated in the river.

Results of total alkalinity in the water body of the studied areas of Awuma River were significantly higher in limnetic and benthic than the littoral zone. However, the list value of alkalinity was recorded in littoral zones of the water body.

Table 6 shows the results of water parameters throughout the duration of the samplings. Water parameter in a partcular zone of each week was not significantly different ($p>0.05$) from each other throughout the sampling periods of each zones investigated in this study. However there was significant different ($p<0.05$) in each particular water parameter in the different zones in each week. The results also show that fluctuation in temperature, total alkalinity and pH as duration of sampling increases were in each case not significantly different from each other ($p>0.05$).

4.1.2 Water Parameters Used for the Cultured Live Food Micro Organisms

The results of water parameters used for the culture of live food organisms are presented in Table 7. The water temperature ranged from 26.07 to 28.17 ^{0}C throughout the period of the study. However, the daily water temperature in each treatment throughout the period of investigations was not significantly different ($p> 0.05$) among the various treatments.

Table 6: Water Parameters Distribution in Littoral Zone of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

Water parameters	Duration (Week)				
	1	2	3	4	5
CO_2 (mg/L)	7.33 (0.04)	7.00 (0.05)	7.33 (0.03)	7.67 (0.05)	6.78 (0.04)
DO (mg/L)	7.67 (0.05)	7.33 (0.6)	8.67 (0.05)	8.50 (0.05)	7.33 (0.06)
pH	6.57 (0.01)	6.73 (0.01)	6.72 (0.02)	6.81 (0.01)	6.53 (0.02)
Temp (^{o}C)	27.37 (0.02)	27.70 (0.02)	27.77 (0.01)	27.67 (0.01)	27.63 (0.02)
T. Alk (mg/L)	25.33 (0.02)	25.33 (0.02)	25.33 (0.05)	25.00 (0.1)	25.67 (0.02)

Values in parentheses = standard error of mean.
pH = Hydrogen ion concentration, Temp = Temperature, CO_2 = Carbon dioxide, DO = Dissolved Oxygen, T. Alk. = Total Alkalanity.

Table 7: Water Parameters Used for the Cultured Live Food Organisms.

Treatments	Water pH	Total Alkalinity (mg/L)	Carbon dioxide (mg/L)	Dissolved Oxygen (mg/L)	Temperature (^{o}C)
3CM1	6.80	15.00	4.36	6.50	27.76
3CM2	6.80	15.00	4.33	6.33	27.62
3CM3	6.83	16.00	4.32	6.33	27.72
3CM4	6.90	15.33	4.31	6.50	27.59
4CM1	6.83	14.67	4.33	6.33	27.81
4CM2	6.80	15.00	4.30	6.33	27.61
4CM3	6.83	15.33	4.33	6.33	27.72
4CM4	6.90	15.00	4.35	6.50	27.77
5CM1	6.83	15.00	4.34	6.17	27.81
5CM2	6.83	14.33	4.37	6.33	27.77
5CM3	6.93	14.67	4.35	6.33	27.66
5CM4	6.87	14.67	4.34	6.50	27.70
Grand mean	6.85	14.92	4.34	6.38	27.05
SEM	0.04	0.23	0.02	0.14	0.33

CM= Combinations of manure
SEM. = Standard error of mean

The water pH, total alkalinity, free carbon dioxide, and dissolved oxygen varied slightly in some of the treatments but the variations were not significantly different ($p > 0.05$) from among the various treatments used in this study.

4.1.3 Water Quality Parameters of the Source Water for Culturing of Fish Fry in each Treatment

Water quality parameters monitored in the source water for the culturing of the fish fry in this study is shown in Table 8. The temperature for each of the treatment *M. micrura, B. calyciflorus and M. micrura, B. calyciflorus,* Artemia shell free and that of *D. pulex* in all the treatments were not significantly different ($p>0.05$) from each other.

In this investigation results recorded for Carbon dioxide, Total alkalinity, Dissolved oxygen and pH throughout the period of the experiment were not significant different ($p> 0.05$) in their various categories. Throughout the period of this work, the lowest value for DO was 8.20 mg/L while the highest CO_2 was recorded as 4.20mg/L. The pH values ranged from 7.45 to 7.47 throughout the period of investigation.The DO and pH values of water parameters used for the cultured live food organisms and that water parameters used for the cultured live food organisms slightly different from each other at the different time of the investigation.

4.2 DISTRIBUTION OF LIVE FOOD ZOOPLANKTON DURING THE PERIOD OF SAMPLINGS IN AWUMA RIVER AT SHABU OF LAFIA NORTH DEVELOPMENT AREA OF NASARAWA STATE

Littoral zone of the River was observed to harbour the highest population density of live food zooplankton throughout the period of the samplings (Figure 9). It was significantly different ($p<0.05$) from the population density of live food zooplankton found in benthic and limnetic zones of the River. The population densities of live food zooplankton of the limnetic and benthic zones of the River were not significantly

Table 8: Water Quality Parameters of the Source Water for Culturing of Fish Fry in each Treatment.

Parameter	***Zooplankton***				
	M. micrura	*B. calyciflorus &M. micrura*	*B. calyciflorus*	Artemia shell free	*D. pulex*
Temperature (^{o}C)	27.43 (0.04)	27.43 (0.06)	27.44 (0.02)	27.43 (0.05)	27.43 (0.06)
pH	7.45 (0.02)	7.46 (0.03)	7.47 (0.03)	7.46 (0.03)	7.47 (0.03)
Total alkalinity (mg/L)	15.21 (0.03)	15.21 (0.01)	15.21 (0.02)	15.21 (0.03)	15.21 (0.01)
Dissolved oxygen(mg/L)	8.20 (0.03)	8.21 (0.03)	8.20 (0.03)	8.20 (0.01)	8.22 (0.03)
Carbon dioxide (mg/L)	4.20 (0.01)	4.20 (0.03)	4.20 (0.02)	4.20 (0.03)	4.20 (0.03)

Values in parentheses = standard error of mean

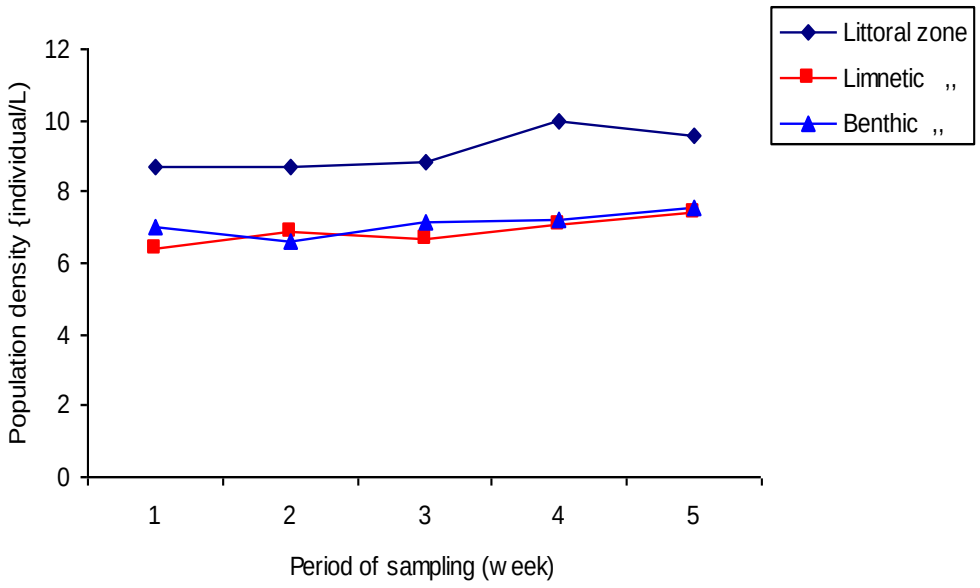
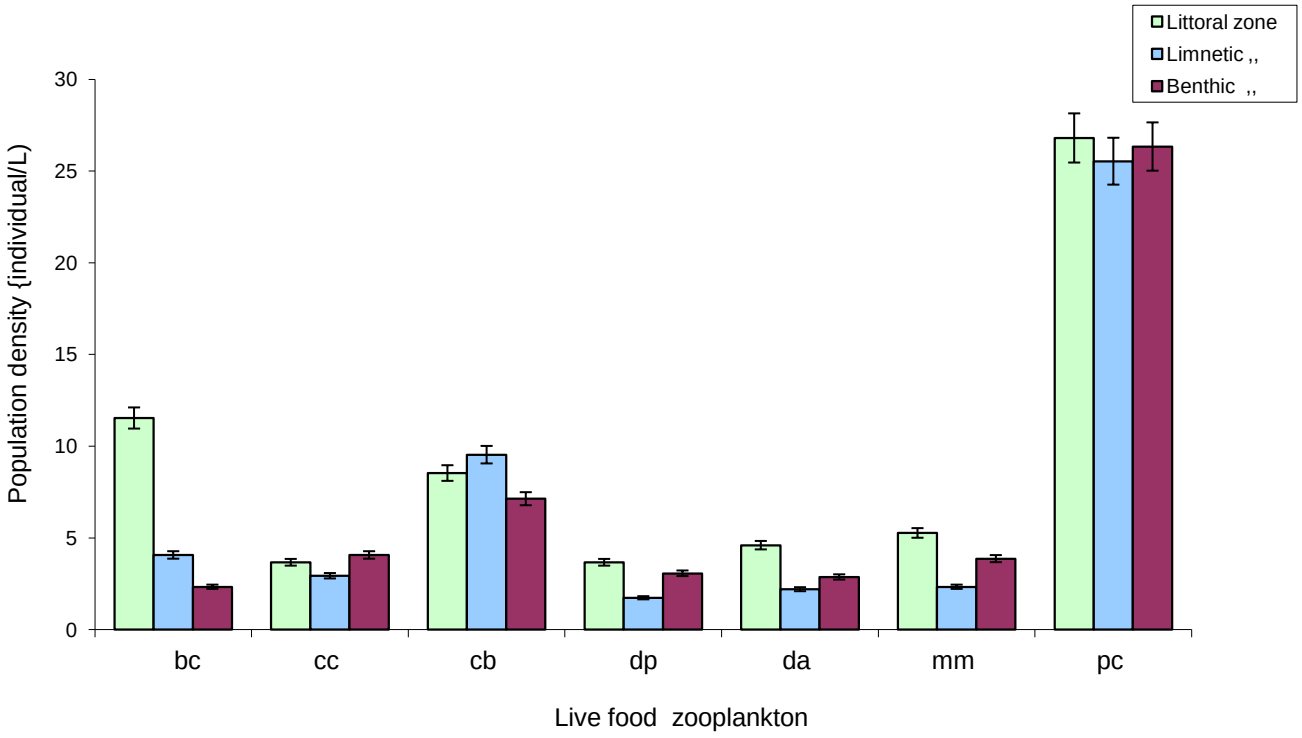


Figure 9: Distributions of Live Food Zooplankton during the Period of Samplings in Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

different ($p > 0.05$) from each other although the benthic zone was slightly populated with live food zooplankton.

Paramecium caudatum was found to be the most abundant live food zooplankton in all the zones of River studied (Figure 10). The next populous live food organism was *Cyclop bicuspidatus.* It was significantly ($p<0.05$) found in the Limnetic and littoral zones compared to benthic zone (Appendix 2). *B. calyciflorus* was significantly ($p<0.05$) common in the littoral zone of the river compared to other zones (Figure 10). *Ceriodaphnia cornuta* was found in the three zone of Awuma River at Shabu although that of benthic zone was significantly higher than other zones. *D. pulex, Daphanosoma aspinosum,* and *M. micrura were* found to be significantly less populous in the limnetic zones of Awuma River at Shabu. They were significantly found in higher population density in the littoral zone and in the benthic zone of the river.

The *B. calyciflorus* and *Daphnia pulex*, showed slight fluctuation in population density while *M. micrura* was not so in the weekly records (Table 9). The variation in the distribution of *D. pulex* and *M. micrura* in the limnetic and benthic zones in the period of sampling shows slight fluctuation in population density. *B. calyciflorus* population density was not significantly different from each other in benthic zone, except in week 4.



(Bc = *B. calyciflorus*, cc = *Ceriodaphnia cornuta*, cb = *Cyclop bicuspidatus*, dp = *Daphnia pulex*, da = *Daphanosoma aspiranum*, mm = *Moina micrura*, pc = *Paramecium caudatum.)*

Figure 10: Distributions of Live Food Zooplankton in Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

Table 9: Various Live Food Zooplankton Distribution in Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

Zone	Zooplankton	Duration (Week)					
		1	2	3	4	5	SEM
Littoral	bc	10^{bc}	11^{b}	12^{ab}	13^{a}	12^{ab}	0.50
	cc	4^{a}	4^{a}	3^{a}	4^{a}	3^{a}	0.24
	cb	10^{a}	8^{b}	7^{bc}	8^{b}	9^{ab}	0.55
	dp	3^{b}	2^{b}	3^{b}	5^{a}	5^{a}	0.51
	da	3^{b}	5^{a}	5^{a}	6^{a}	4^{ab}	0.39
	mm	5^{a}	5^{a}	5^{a}	6^{a}	6^{a}	0.16
	pc	25^{ab}	26^{b}	27^{a}	28^{a}	28^{a}	0.50
Limnetic	bc	4^{a}	4^{a}	4^{a}	4^{a}	4^{a}	0.19
	cc	2^{a}	3^{a}	3^{a}	3^{a}	3^{a}	0.19
	cb	9^{ab}	11^{a}	10^{a}	8^{b}	10^{a}	0.40
	dp	2^{a}	1^{a}	2^{a}	2^{a}	2^{a}	0.12
	da	2^{a}	3^{a}	2^{a}	2^{a}	3^{a}	0.23
	mm	2^{a}	1^{b}	3^{a}	2^{a}	3^{a}	0.33
	pc	24^{bc}	25^{b}	23^{c}	28^{a}	27^{a}	0.92
Benthic	bc	3^{a}	2^{a}	3^{a}	1^{b}	2^{a}	0.37
	cc	4^{a}	4^{a}	3^{ab}	4^{a}	5^{a}	0.29
	cb	7^{a}	8^{a}	7^{a}	7^{a}	7^{a}	0.13
	dp	2^{a}	2^{a}	3^{a}	4^{a}	4^{a}	0.31
	da	2^{a}	2^{a}	3^{a}	3^{a}	4^{a}	0.40
	mm	4^{a}	3^{a}	4^{a}	4^{a}	4^{a}	0.20
	pc	26^{ab}	25^{b}	27^{a}	27^{a}	27a	0.43

Mean within the same row with different superscript differs significantly.

Bc = *Brochionus calyciflorus*, cc = *Ceriodaphnia cornuta*, cb = *Cyclop bicuspidatus*, dp = *Daphnia pulex*, da = *daphanosoma aspiranum*, mm = *Moina micrura*, pc = *paramecium caudatum*, *SEM = Standard error of mean.*

4.3 POPULATION DENSITY OF ZOOPLANKTON (*B. CALYCIFLORUS* AND *M. MICRURA)* FED ON FILTRATES OF COMBINATIONS OF MANURE /*CHLORELLA VULGARIS*

The results of population density of *B. calyciflorus* fed on filtrates of manure in solution and *C. vulgaris* are showed in Figure 11. The population density of the *B. calyciflorus* on filtrates of manure in solution was significantly different (P<0.05) from those fed on *C. vulgaris* (Appendix 3). At the first 2 days of treatments, there were no significant difference in population density (P>0.05) but as culture duration increases the population density of the *B. calyciflorus* fed on filtrates from combination of manure were significantly higher up to day 8 before crashing of the population. Crashing in population density of the treatment group of *C. vulgaris* was gradual while the ones of filtrate from combination of manure were so sudden. In day 10, the population density of manure treatment group was the lowest (Figure 11).

Figure 12 shows the population density of *M. micrura* fed with filtrates of five combinations of manure / *C. vulgaris.* The results shows that population density of *M. micrura* was significantly higher (P>0.05) than those of *C. vulgaris* (Appendix 4) and the crashing of population density was more that those of *C. vulgaris.* The population density was at peak on day 8. While 48,906 individuals/L of water was recorded for *M. micrura* fed on filtrate of 5- combinations manure, 11,603 individuals/L of water was recorded for those of *C. vulgaris* at the peak of their population.The lowest poplation density was recorded on day 12 (644 individuals/L of water) in combined manure treatment group and it was significantly different (p>0. 05) from *C. vulgaris* treatment group (5,104 individuals/L of water) in this study. Microphotograph of Cultured *Chlorella vulgaris* is showed in Plate 6.

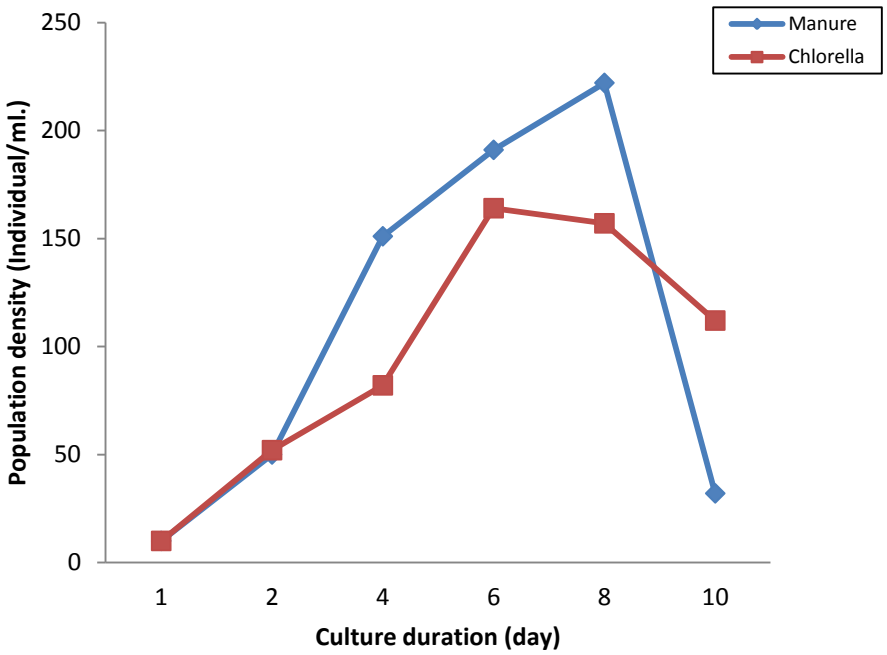


Figure 11: Population Density of *Brachionus calyciflorus* Fed on Manure in Solution / *Chlorella vulgaris*

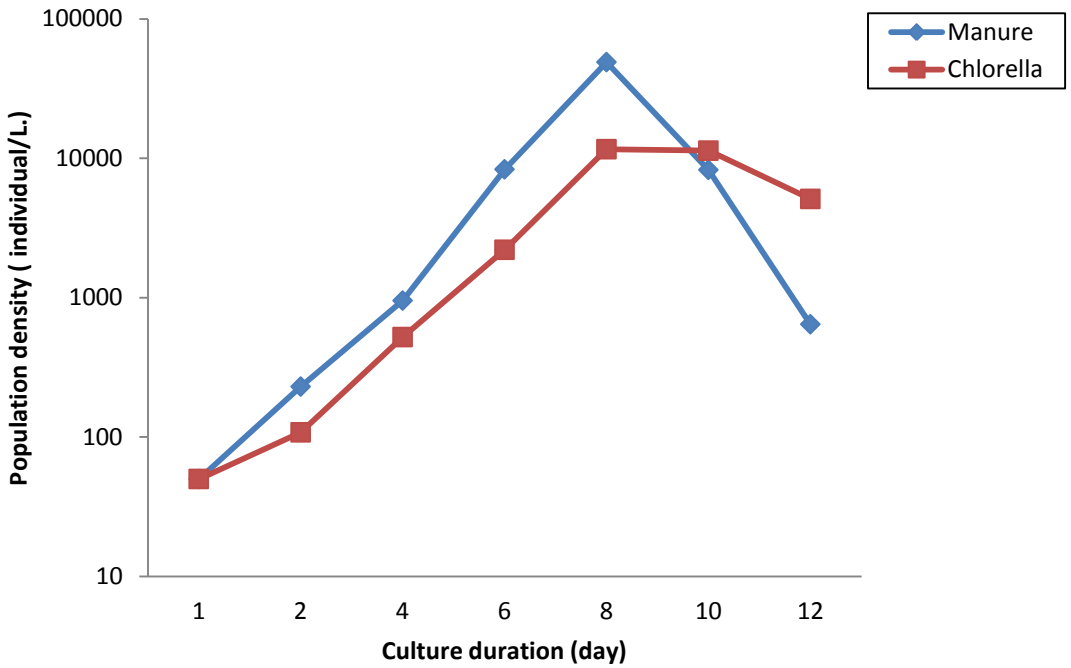


Figure 12: Population density of *Moina micrura* Fed on Manure in Solution / *Chlorella vulgaris*

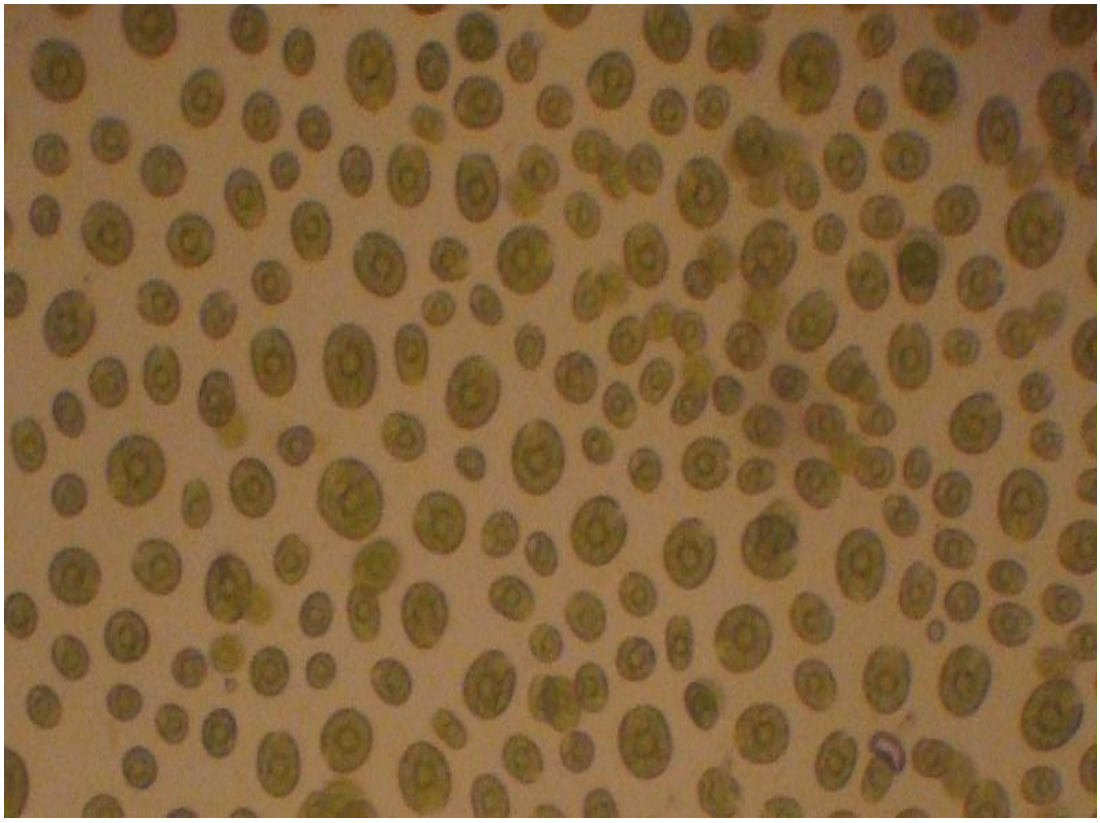


Plate 6: Microphotograph of Cultured *Chlorella vulgaris* **X384**

4.4 CULTURE OF *BRACHIONUS CALYCIFLORUS*

The results of the main effect of various concentrations, combinations of manure and period of growth on population density of the cultured organisms are shown in this section. The interaction of the manure combination, concentration and period of the culture distinctly shown that there was a significant pulled effects of the three factors.

4.4.1 Effects of Concentrations, Different Combinations of Manure Culture Medium and Period of Growth on Population Density of *Brachionus calyciflorus*

All the concentrations used supported population increase of the organism and the results of concentration on population density of *B. caylciflorus* are presented in Figure 13. Results showed that concentration 4.00ml/L and 8.00ml/L had the highest population density of *B. calyciflorus* (115 and 114 individuals/ml of water respectively) and they were significantly higher than the 1.00ml/L and 16.00ml/L of water used. Results of the different combinations of manure medium used for the culture of the organisms show that 5CM1 and 5CM2 significantly ($p<0.05$) increased population density of *B. calyciflorus* more than other combinations of manure medium used (Figure 14) although they were not significantly different from each other. The optimal population density was obtained on day 7 and it was significantly different from other days of the experiment (Appendix 5). The Microphotograph of the Cultured *Brachionus calyciflorus* is showed in Plate 7.

4.4.2 Effects of Interaction between Concentrations, Combination of Manure and Period of Growth on Population Density of *Brachionus calyciflorus*

The interaction between concentrations, combination of manure and period of growth on population density of *B. calyciflorus* is showed in Table 10. Results show that interaction between concentrations, combination of manure and period of growth were

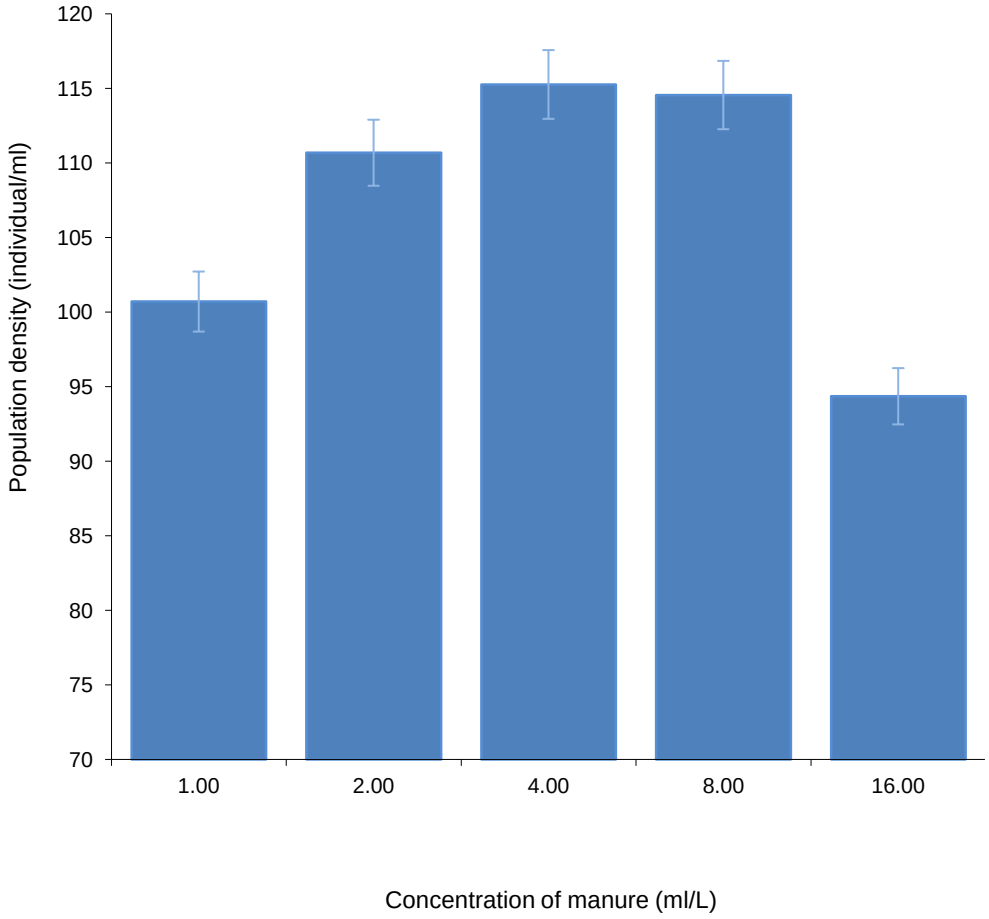
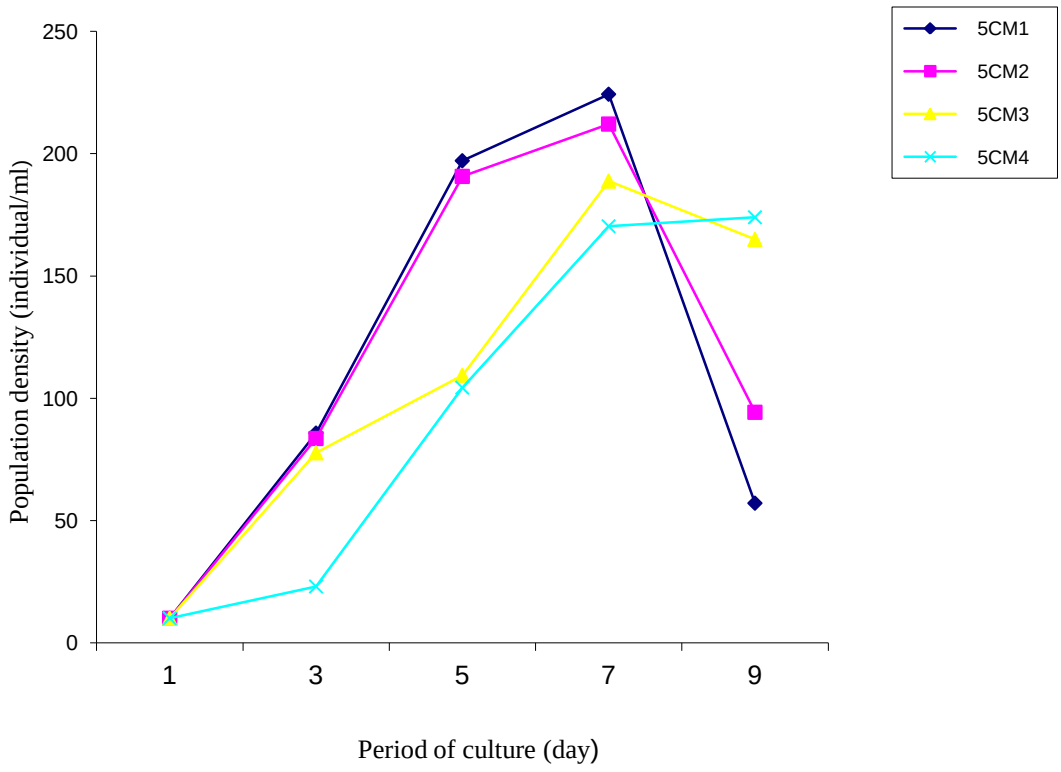


Figure 13: Effect of Concentration of Manure on Population Density of *Brachionus calyciflorus.*



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

Figure 14: Effects of Different Combinations of Manure and Duration of Growth on Population Density of *Brachionus calyciflorus*

Table 10: Effect of Interaction between Concentration, Combinations of Manure and Period of Culture on Population Density of *Brachionus calyciflorus*

Manure	Concentration (ml/L)	*Brachionus calyciflorus* population density (individual /ml)				
		Period of culture				
		1	*3*	*5*	*7*	*9*
5CM1	1.00	10	77	184	222	46
	2.00	10	86	222	234	54
	4.00	10	96	245	237	35
	8.00	10	95	172	225	65
	16.00	10	74	163	204	85
5CM2	1.00	10	74	180	220	54
	2.00	10	84	217	224	60
	4.00	10	96	236	233	83
	8.00	10	92	162	204	153
	16.00	10	71	158	180	122
5CM3	1.00	10	62	82	164	176
	2.00	10	74	124	187	193
	4.00	10	83	133	225	95
	8.00	10	86	132	215	196
	16.00	10	83	76	153	165
5CM4	1.00	10	11	132	163	162
	2.00	10	24	113	166	153
	4.00	10	31	123	213	182
	8.00	10	33	102	186	181
	16.00	10	15	52	124	191
	SEM		0.96	0.86	1.00	1.07
	LSD		2.77	2.48	2.89	3.11

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.

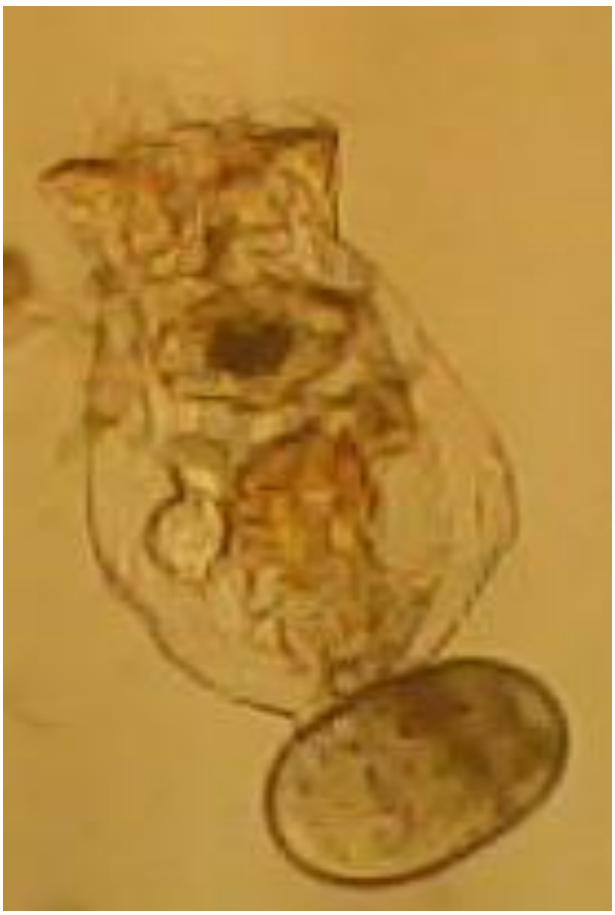


Plate 7: Microphotograph of Cultured *Brachionus calyciflorus* **X384**

highly significant ($p<0.01$). Treatment 5CM1 at concentration 4.00ml/L of water on day 5 significantly had the highest population density (245 individuals of *B. caylciflorus*/ml). Beyond day 7, a declining population density (237individuals *B. caylciflorus*/ml) of the cultured organisms was observed.

4.5 EFFECTS OF CONCENTRATIONS, DIFFERENT COMBINATION OF MANURES AND PERIOD OF GROWTH ON MASS PRODUCTION OF *MOINA MICRURA*

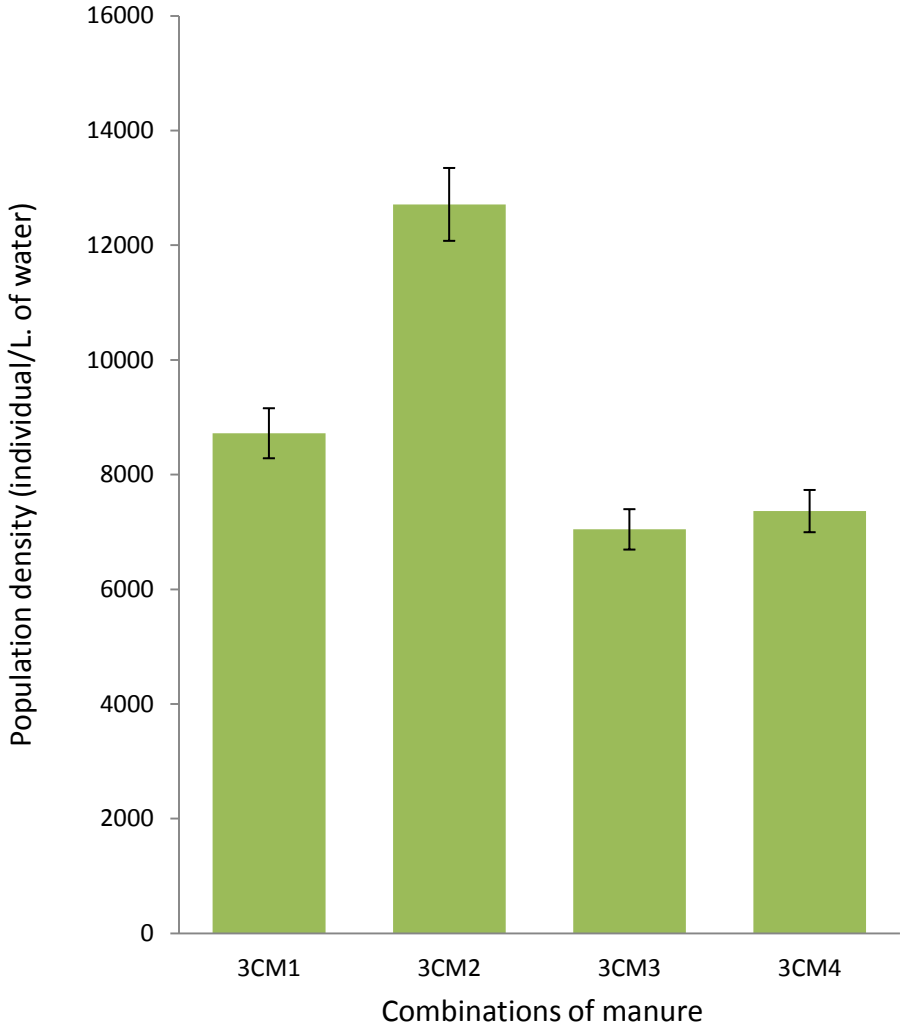
The results of the main effects of manure combination, concentration level of the manure and the period of the culture on *M. micrura* are presented in this section. The results show that manure combinations, period of culture and concentration of the manure individually influenced population density of the cultured *M. micrura* (Plate 8).

4.5.1 Effects of Combinations of Manure Solution on Population Density of *Monia micrura*.

Results (Figure 15-17 and appendices 6-8) show that all the 3,4,5 different combinations of manure increased population density of *M. micrura.* 3 combinations of manure solution 3CM2 had the highest population density of *M. micrura* with average population density of 12,711 individual *M. micrura*/L of water. It was significantly different ($p<0.05$) from all the other 3 combinations of manure used (3CM1: 8720, 3CM3: 7044. and 3CM4 7363 individual *M. micrura*/L of water) in the experiment.

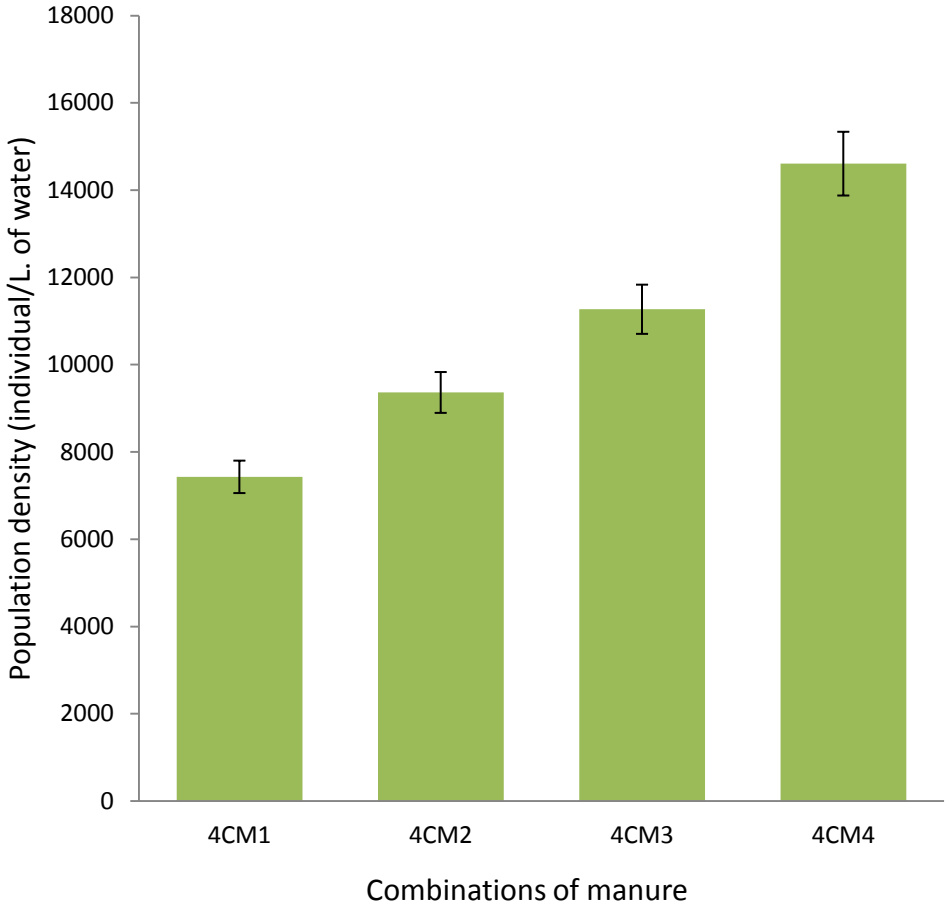
The 4 combinations of manure solution used in this experiment significantly increase the population density of *M. micrura* per litre of water (4CM1, 7430: 4CM2, 9364, 4CM3 11,270 and 4CM4: 14,607). 4CM4 combination significantly had the highest population density of *M. micrura* (14,607 individuals/L of water) (Appendix 6).

In the five combinations of manure, the population densities of *M. micrura* /L of water were observed as follows: 5CM1(10,336), 5CM2 (12,306) 5CM3 (10,268), and 5CM4 (8,290). Combination 5CM2 significantly ($p<0.05$) had the highest population density of *M. micrura* (12,306 individuals/L of water) while 5CM4 had the lowest



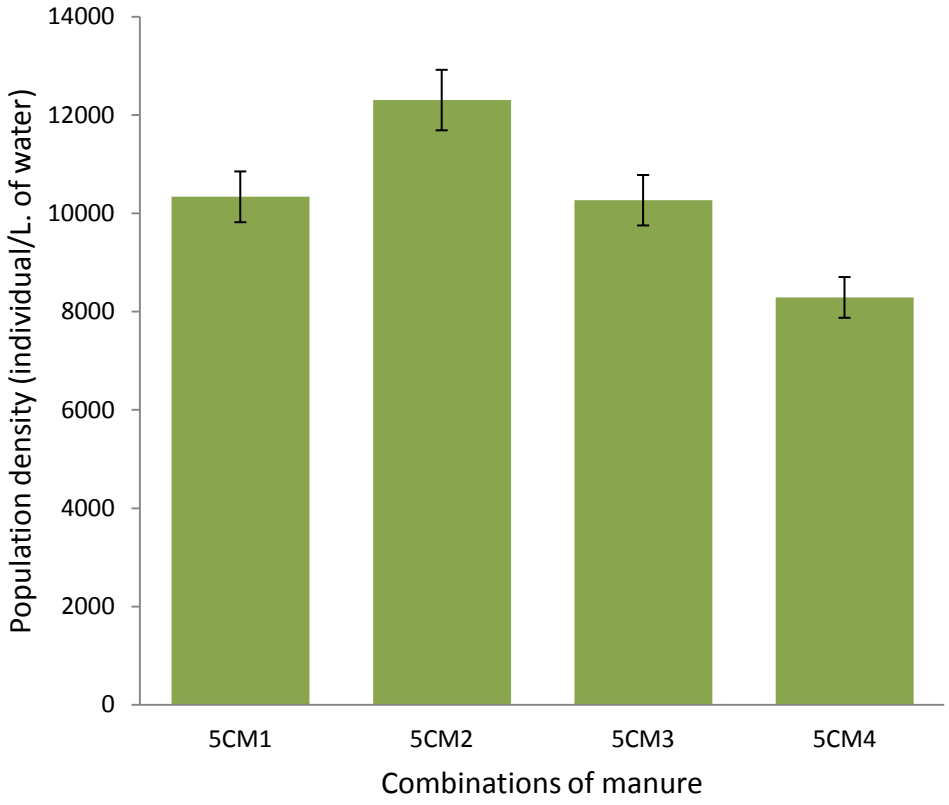
CM = combinations of manure

Figure 15: Effect of Three Different Combinations of Manure on Population Density of *Moina micrura*



CM = combinations of manure

Figure 16: Effect of Four Different Combinations of Manure on Population Density of *Moina micrura*



CM = combinations of manure

Figure 17: Effect of Five Different Combinations of Manure on Population Density of *Moina micrura*

Plate 8: Microphotograph of Cultured *Moina micrura* **X384**

population density of *M. micrura* in this investigation. The results of the investigation of 3, 4, 5 different combinations of manure solution show that 4CM4 significantly had the highest population density of individual *M. micrura* /L of water while 3CM3 had the lowest population density of individual *M. micrura* /L of water ($p<0.05$).

4.5.2 Effects of Different Concentrations of Manure Combination on Population Growth of *M. micrura*

Effects of different concentrations of manure combination on population growth of *M. micrura* are showed in Figure 18-20. Concentration 1.00, 2.00, 4.00, 8.00 and 16.00ml/L of water of the 3, 4, 5 different combinations of manure solution were used in this studies. The results show that all the different concentrations used are significantly different ($p<0.05$) from each other.

The results of the different concentrations of three different combination of manure solution showed that 4.00ml/L had the highest population density of *M. micrura* (12, 774 individuals/L of water and it was significantly higher in population density than others in the three combinations. The results of the effects of various concentrations of four combination of manure on *M. micrura* population density are as follow 1.00ml/L (9354, individuals/L of water), 2.00ml/L (12,732 individualss/L of water), 4.00ml/L (13,456 individuals/L of water) 8.00ml/L (10.932 individuals/L of water and 16.00ml/L (6,776 individuals/L of water). All the concentrations of the 4 combination of manure supported high population density of *M. micrura* with 4.00ml/L recording the highest population density of *M. micrura* followed by 2.00ml/L of water. The lowest population density of *M. micrura* was recorded in 16.00ml/L of water of the 4 combination of manure used and it was significantly different ($p<0.05$) from others. The results of concentrations of the 5 combination of manure used in the investigation are as follow: 7,676, 12,551, 14,046, 10,913, 6,313 *M. micrura* individuals/L of water for concentration

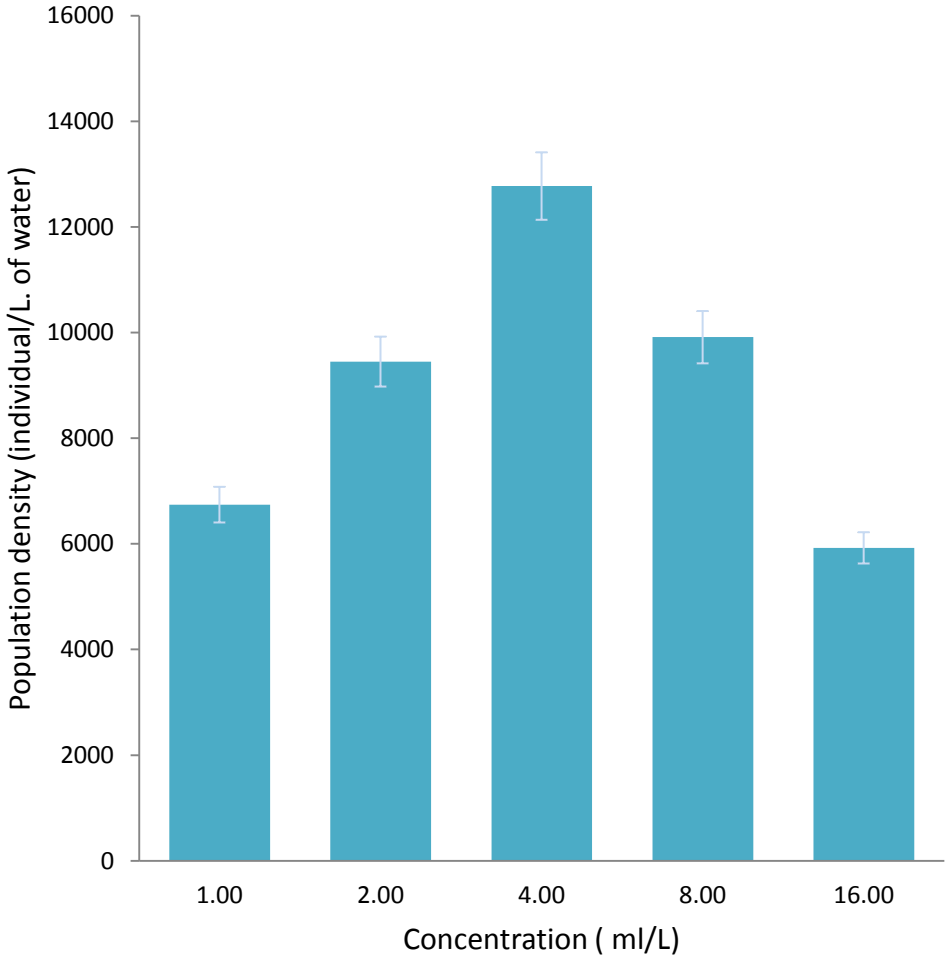


Figure 18: Effect of Concentrations of Three Different Combinations of Manure on Population Density of *Moina micrura*

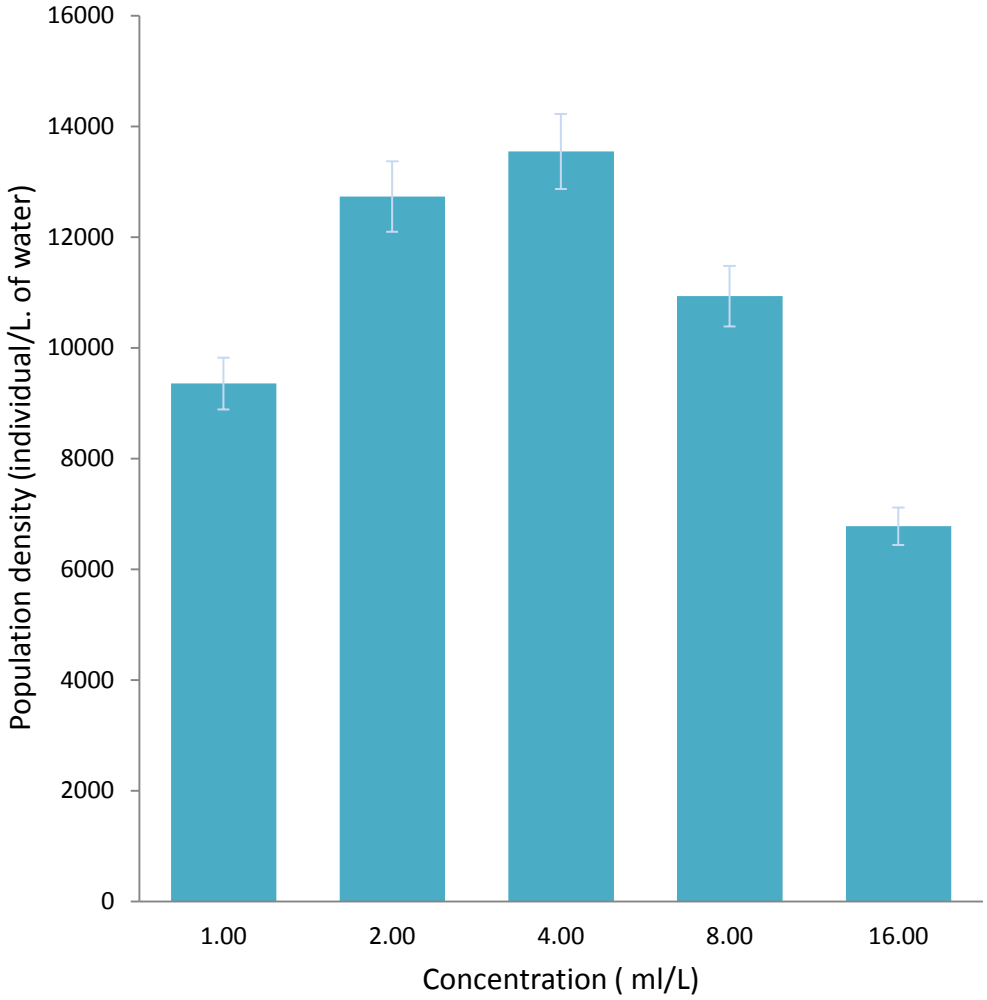


Figure 19: Effect of Concentrations of Four Different Combinations of Manure on Population Density of *Moina micrura*

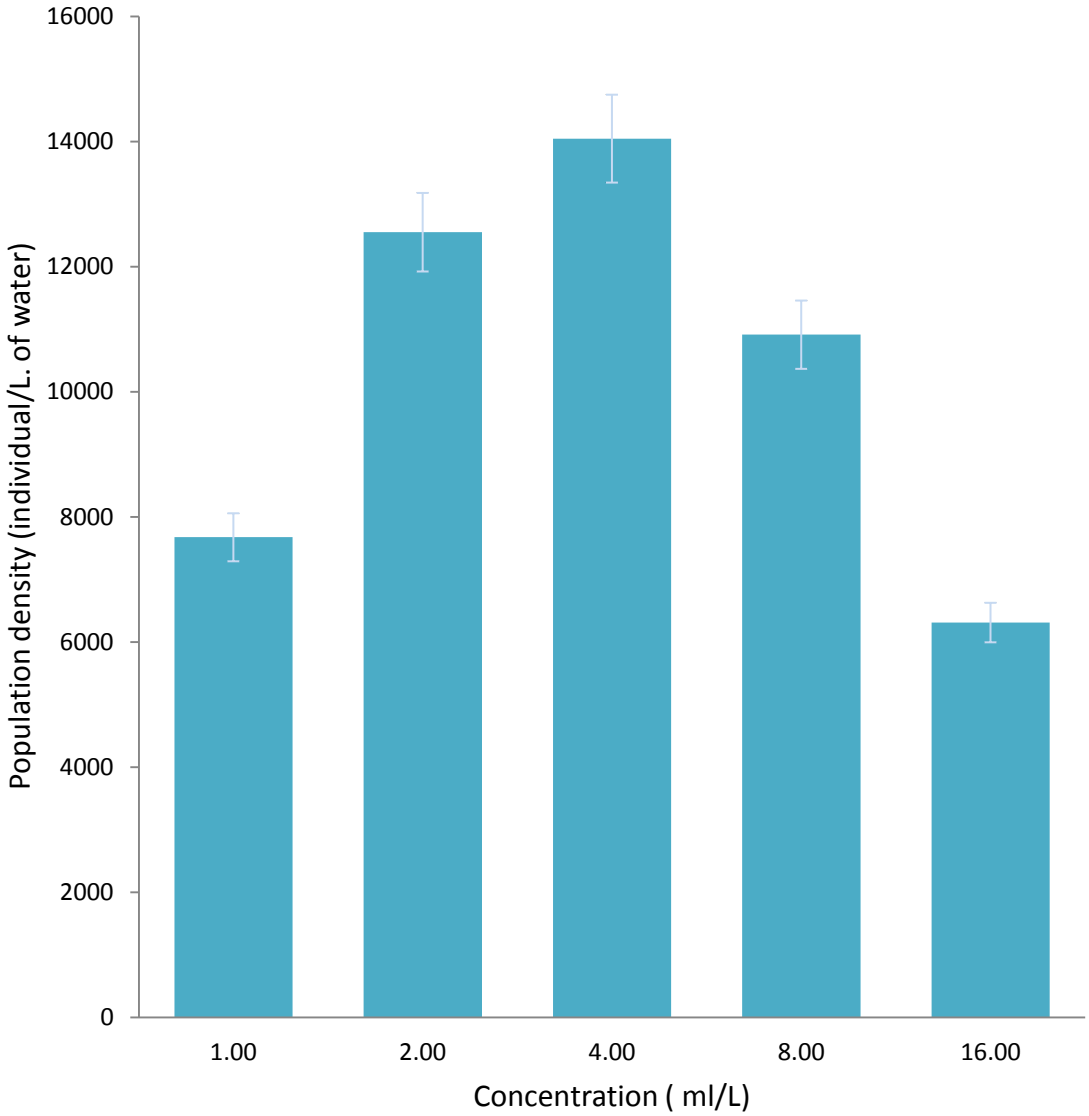


Figure 20: Effect of Concentrations of Five Different Combinations of Manure on Population Density of *Moina micrura*

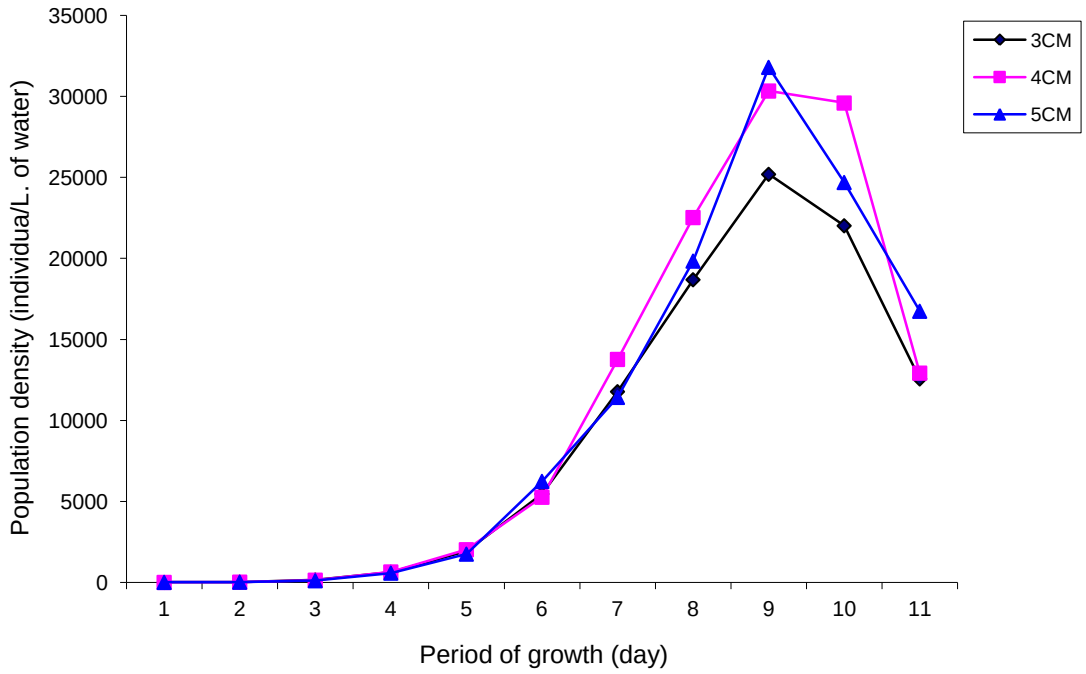
1.00ml/L, 2.00ml/L and 4.00ml/L 8.00ml/L and 16.00ml/L of water respectively. These various concentrations of the five combinations of manure are significantly different from each other. Concentration of 4.00ml/L of water had the highest population density of *M. micrura* and this was significantly better than other concentration of the five combinations of manure used. The results of the 3,4,5 concentrations of manure series showed that 4.00ml/L is most suitable for the culture of *M. micrura.*

4.5.3 The Effects of Period of Growth on Population Density of *M. micrura* Treated with 3, 4 and 5 Different Combination of Manures

The results of period of growth of *M. micrura in* this experiment are presented in Figure 21 and Appendix 9. Results show that *M. micrura* increased population from day 2 to day 9 before population decline was noticed. In the three combinations of manure, *M. micrura* increased its population from 20 individuals/L of water in day1 to 25,194 individuals/L of water in day 9. Population growth declined between day 9 and day 10 (22,023 individuals/L of water) and a sharp decline was observed in day 11 (12,574 individuals/L of water).

The period of growth in four combinations of manure shows that *M. micrura* increased population density from 20 individuals to 30,335 individuals/L of water before a decline in population density was observed in day 10 (20,595 individuals/L of water). Sharp decline in population density of *M. micrura* (12,930 individuals/L of water) was observed in day11 in period of growth.

Results of the period of the culture showed that *M. micrura* population density increased from 20 individuals/L of water (day1) to 31,794 individuals/L of water on day 9 in five combinations of manure. Population decline set in from day 10 (24,693 individuals/L of water) while day 11 experienced the highest rate of decline in population density of *M. micrura*.



CM = Combinations of Manure

Figure 21: Effect of Period of Growth on Population Density of *Moina micrura* treated with 3, 4, 5 Different Combinations of Manure.

The results of the period of growth of *M. micrura* treated with 3, 4, 5 different combinations of manure show a crashing population density from day 10. Day 9 of the period of growth showed the highest population density especially in five combinations of manure (31,794 individuals/L of water).

4.5.4 Interactions of Concentration, Combinations of Manure and Period of Growth on Population Density of *M. micrura*

The pulled effect of combination of manure, period of growth and concentration of the manure significantly influenced the population density of *M. micrura* in this study (Appendices 6-8)

4.5.4.1 Pulled effects of concentrations, three combinations of manure and period of growth on population density of *M. micrura*

The interaction between manure and concentration were highly significant through out the period of growth. Combination of manure 3CM2 in concentration 4ml/L had the highest population density of *M. micrura* (42,159 individuals/L of water) as shown in the Table 11. This was followed by 2.00ml/L concentration of water of that same manure combination, 3CM2 (40,127 individuals/L of water). These two concentrations of 3CM2 where significantly better than other interactions of 3 combinations manure on day 9 period of growth. However, they were significantly different ($p<0.05$) from each other. Day 10 and day 11 experienced crashing population density of *M. micrura* in this study. The crashing in population density in day 10 and day 11was sudden and significantly high in treatment groups with very high population density.

4.5.4.2 Pulled effects of concentrations, four combinations of manure and period of growth on population density of *M. micrura*

Interaction of concentrations, 4 combinations of manure and period of growth of day1 and day 2 were not significantly different ($p>0.05$) from each other. However there were significant interactions from day 2 to day 11 of the experiment (Table 12). The

highest interactive effects of concentrations and 4 combinations of manure were found in 2.00ml/L concentration of water of 4CM4 on day 9 of the experiment (51,678 individuals *M. micrura*/L of water).

Another close range of interaction (50,891 individuals/L of water) of population density *M. micrura* was found in 4CM4 on day 9 at concentration 4.00ml/L They were significantly different ($p<0.05$) from other interactive effects of this 4CM4 on day 9 of the period of growth. The crashing in population density was also sudden and significantly high in treatment groups with very high population density in day 10 and day 11.

4.5.4.3 Pulled effects of concentrations, five combinations of manure and period of growth on population density of *M. micrura*

There was no significant interactive effect of concentration and five combinations of manure of *M. micrura* on day 2. However, the interactive effects of other days were highly significant as shown in Table 13. Concentration of 4.00ml/L and five combinations of manure (5CM2) had the highest population density of *M. micrura* (55,184 individuals/L of water) on day 9 and it was significantly ($p<0.05$) better than others in same day 9 period of culture.

The next population density of *M. micrura* (54,353 individuals/L of water) was recorded in the same 4.00ml/L but for 5CM3 combination of manure. It was significantly different from others in day 9 period of growth. Day10 and day11 showed declining population density of *M. micrura*. The crashing in population density observed in day 10 and day 11 was not only sudden but significantly high in treatment groups with very high population density.

Interaction of concentration, combinations of manure and period of growth in the 3,4,5 series of manure treatments show that 5CM2 at 4.00ml/L had the highest population

Table 11: Pulled Effect of Concentration, 3 Combinations of Manure and Period of Growth on Population Density of *Moina micrura*

Conc. (ml/L)	Manure	*Moina micrura* population density (individual/L of water)										
		Period of Growth (day)										
		1	2	3	4	5	6	7	8	9	10	11
1.00	3CM1	20	39	155	713	1826	7205	10116	15312	20154	10657	7249
	3CM2	20	43	406	754	1449	4893	14525	22507	30124	29341	25275
	3CM3	20	30	88	283	1171	3584	4813	8109	10254	5750	3460
	3CM4	20	28	70	109	424	1407	4503	8126	15161	14675	11761
2.00	3CM1	20	37	115	623	1707	5216	10155	14133	20245	19650	11559
	3CM2	20	43	503	853	1590	6983	16908	32512	40127	35473	22665
	3CM3	20	33	122	561	1805	5628	12018	18190	30126	26775	7540
	3CM4	20	30	82	187	684	1838	5485	14124	20159	18451	10786
4.00	3CM1	20	44	128	906	3126	8124	16113	24016	35169	34182	17589
	3CM2	20	40	605	854	1749	9979	24011	34227	42159	41359	27523
	3CM3	20	39	126	964	2890	6577	19012	23202	30142	24561	6772
	3CM4	20	38	128	982	3508	6963	16608	23252	30164	27544	16579
8.00	3CM1	20	41	137	952	2714	8506	17507	25017	35012	29146	13641
	3CM2	20	28	104	816	1605	4811	13816	24352	30124	24680	18442
	3CM3	20	30	113	552	2640	4565	5985	16545	20091	15666	5758
	3CM4	20	35	126	579	2904	5245	15626	21470	30256	23757	12661
16.00	3CM1	20	29	127	689	1703	6409	8708	9815	10254	8346	4537
	3CM2	20	32	92	774	1704	4211	9514	21209	28511	28252	16485
	3CM3	20	34	104	523	2502	4807	5203	10141	15200	14645	7641
	3CM4	20	39	123	328	1157	2503	5103	7567	10464	7543	3557
SEM			2.66	10.39	4.94	14.27	11.37	8.14	20.66	21.73	12.81	15.55
LSD			7.63	29.75	14.13	40.87	32.55	23.32	59.14	30.72	36.69	44.52

3CM1 = Cowdung, Soybean and Single superphosphate, 3CM2 = Chicken droppings, Soybean and Single superphosphate
3CM3 = Cowdung, Groundnut cake and Single superphosphate, 3CM4 = Chicken droppings, Groundnut cake and Single superphosphate

Table 12: Pulled Effect of Concentration and 4 Combinations of Manure on Population Density of *Moina micrura*

Conc. (ml/L)	Manure	*Moina micrura* population density (individual/L of water)										
		Period of Growth (day)										
		1	2	*3*	*4*	*5*	*6*	*7*	*8*	9	10	11
1.00	4CM1	20	36	127	641	1857	3781	9556	15123	20257	19239	9545
	4CM2	20	32	148	443	1813	4969	9595	15299	20192	19269	9827
	4CM3	20	33	140	502	1795	4994	10530	14911	20187	20129	10267
	4CM4	20	32	136	610	2236	6541	22839	32538	40291	40152	20874
2.00	4CM1	20	39	126	682	1902	4107	12555	21651	30249	28635	12751
	4CM2	20	42	183	592	2184	5202	12567	24408	30160	28548	12650
	4CM3	20	35	168	687	2292	6906	14548	26656	35130	34960	14259
	4CM4	20	27	193	853	2262	6992	24530	35513	51678	50448	22751
4.00	4CM1	20	42	141	894	2483	5825	12636	22809	30355	29793	12819
	4CM2	20	40	173	678	2219	5303	12815	23904	30125	28870	12947
	4CM3	20	40	193	810	2622	7891	21571	31229	45140	44285	20450
	4CM4	20	36	213	788	2165	5890	21688	34530	50891	50130	20515
8.00	4CM1	20	41	137	909	2411	4915	8583	9303	10418	9363	5431
	4CM2	20	40	163	627	2236	5403	13204	23544	30025	29177	12767
	4CM3	20	35	183	762	1894	5665	11575	31213	40248	40130	10777
	4CM4	20	36	191	760	2071	4891	20202	31773	45166	45148	19501
16.00	4CM1	20	35	103	704	1225	3861	8404	9104	10126	9066	3758
	4CM2	20	40	139	385	1504	4803	9808	16503	20186	19627	9552
	4CM3	20	32	128	438	1765	4314	11522	15258	20582	19664	10226
	4CM4	20	33	124	512	1782	3211	6784	15291	25284	25258	6931
SEM		0	2.27	3.036	8.05	16.12	4.79	21.36	22.07	20.24	21.67	36.88
LSD		0	6.498	8.691	23.05	46.16	13.72	61.16	63.2	57.94	62.03	105.58

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

Table13: Pulled Effect of Concentration and Five Combinations of Manure on Population Density of *Moina micrura*

Conc. (ml/L)	Manure	*Moina micrura* population density (individual/L of water)										
		Period of Growth (day)										
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
1.00	5CM1	20	32	123	690	1763	6520	13528	19269	28146	26338	20378
	5CM2	20	31	126	681	2200	9560	14570	18054	20143	19450	16516
	5CM3	20	33	132	422	1264	5779	6930	8981	10280	8749	7901
	5CM4	20	32	112	360	1256	4976	5320	11320	21468	15690	8540
2.00	5CM1	20	35	138	761	1890	6986	12518	21929	30161	25460	18437
	5CM2	20	35	115	785	2955	9753	19255	25010	45135	40649	25794
	5CM3	20	35	105	533	1265	4984	12565	23515	50186	38765	24591
	5CM4	20	34	124	420	1247	4890	5485	20832	27319	24699	22775
4.00	5CM1	20	36	144	945	1861	6484	12654	31823	50174	30259	16733
	5CM2	20	37	110	794	2594	9979	22139	27539	55184	39717	22850
	5CM3	20	34	133	531	1978	4992	12926	26525	54353	34558	20770
	5CM4	20	35	126	460	1563	4991	5930	22558	52243	25471	15728
8.00	5CM1	20	40	144	973	1795	6358	13568	33564	50227	32464	15806
	5CM2	20	38	117	683	2528	9357	13574	18955	27423	27244	21459
	5CM3	20	40	124	424	1378	3860	12536	20593	28426	25778	18751
	5CM4	20	36	134	421	1491	4690	8553	16595	24422	20581	14957
16.00	5CM1	20	32	109	419	922	1956	3695	4042	5558	5369	5156
	5CM2	20	30	94	671	2678	8375	13419	18593	22526	22656	14550
	5CM3	20	34	125	435	1294	3970	11684	17292	20059	19453	14554
	5CM4	20	33	125	424	1361	6589	7856	9860	12446	10499	8752
SEM			2.16	3.27	8.17	12.78	10.85	16.71	19.21	18.47	38.53	24.80
LSD			6.18	9.36	23.39	36.60	31.06	47.85	54.99	52.87	110.31	71.01

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

density *M. micrura* (55,184 individuals/L of water) on day 9 of the period of the culture of the organisms. In all Interaction of concentration, combinations of manure and period of growth the 3,4,5 series of manure treatments, results show that population of the organism attained a peak on day 9 (Table 13).

4.6 EFFECTS OF CONCENTRATIONS, DIFFERENT COMBINATION OF MANURES AND PERIOD OF GROWTH ON MASS PRODUCTION OF *DAPHNIA PULEX* IN LABORATORY INVESTIGATION

The concentration as main effect significantly influenced the population density of the cultured *D. pulex*. Similarly, manure combination and period of the cultured of the organism individually influenced the population density of the cultured organism.

4.6.1 Effects of Combinations of Manure Solution on Population Density of *Daphnia pulex* Under Laboratory Investigation

Cultured D. pulex (Plate 9) treated with 3, 4, 5 different combinations of manure to determine the ones that will give the best population density of studied organism are shown in Figures 22 - 24. The results show that 3 combinations of manure 3CM1, 3CM2, 3CM3 and 3CM4 had the following mean population densities of *D. pulex*/L of water in 152, 325, 164 and 302 respectively. These combinations of manure were significantly different ($p<0.05$) from each other. Combination 3CM2 had the highest mean population density of *D. pulex* (325 individuals/L of water) and it was significantly different ($p<0.05$) from all other three combinations of manure used in this investigation.

The results of the four combinations of manure showed the mean values of population density of *D. pulex* as follow: 4CM1, (227), 4CM2 (750) 4CM3 (618) and 4CM4 (987.87 individuals *D. pulex* /L of water). Among this group, 4CM4 combination of manure had the highest population density of *D. pulex* and it was significantly different ($p<0.05$) from other combinations in this series.

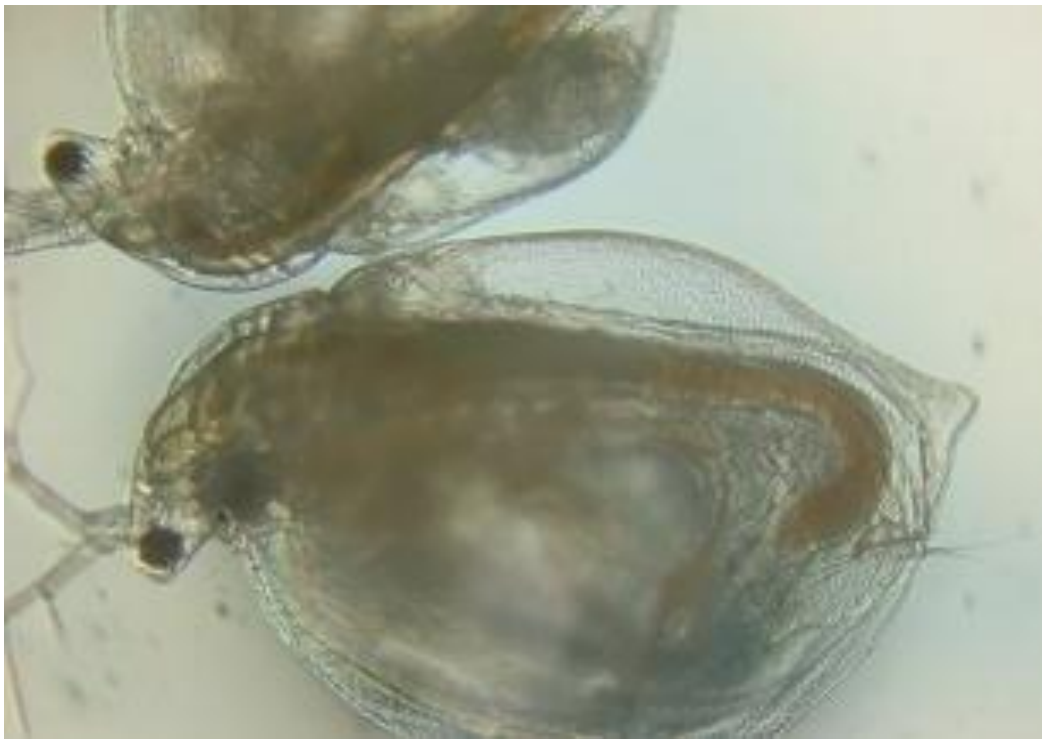
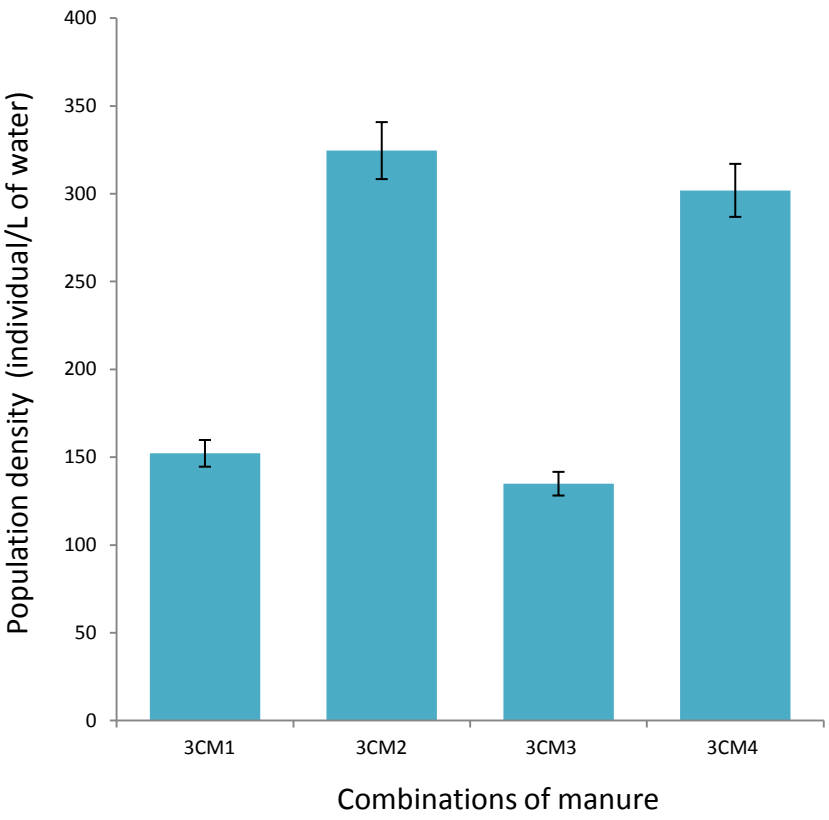
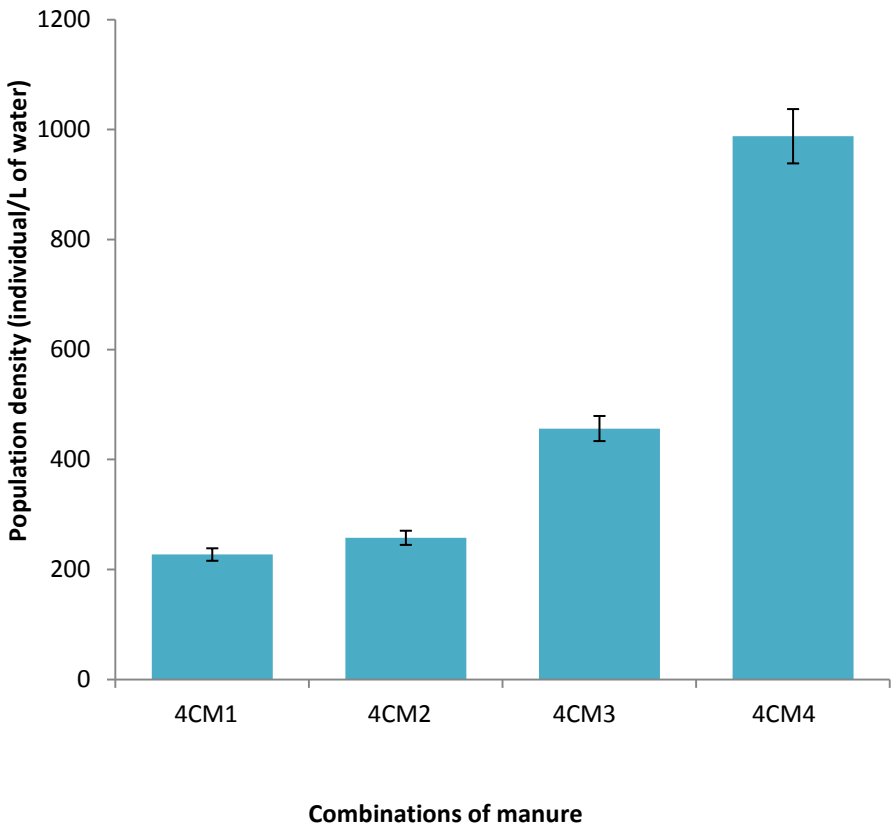


Plate 9: Microphotograph of Cultured *Daphnia pulex* **X 384**



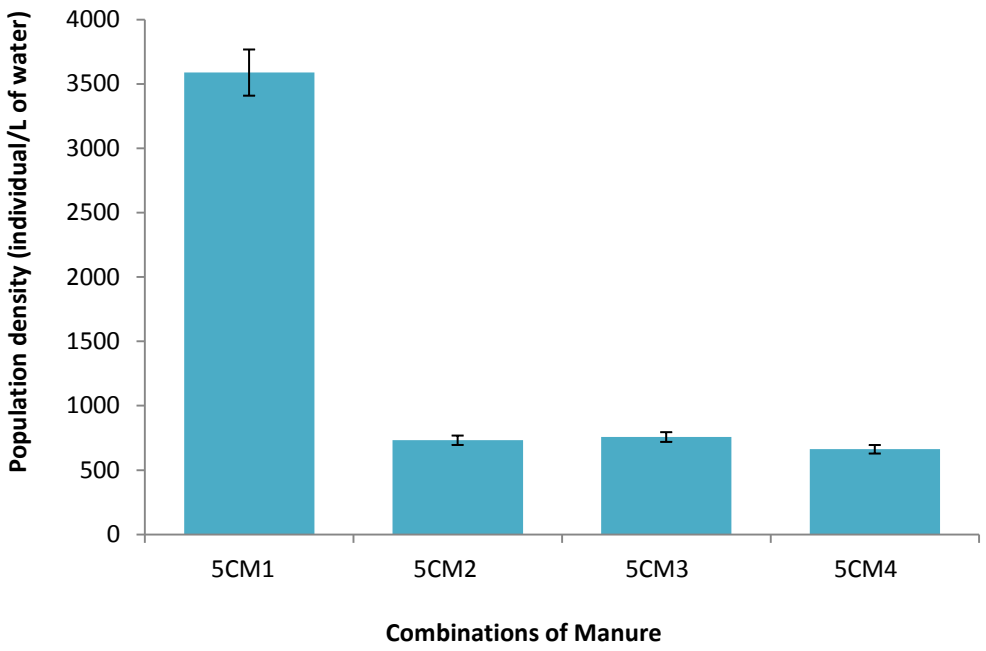
CM = combinations of manure

Figure 22: Effect of Three Different Combinations of Manure on Population Density of *Daphnia pulex*



CM = combinations of manure

Figure 23: Effect of Four Different Combinations of Manure on Population Density of *Daphnia pulex*



CM = combinations of manure

Figure 24: Effect of Five Different Combinations of Manure on Population Density of *Daphnia pulex*

The means population density of *D. pulex* from five combinations of manure were: 5CM1, (3, 588), 5CM2 (732) 5CM3 (757) and 5CM4 (662) individuals/L of water. These means were significantly different ($p<0.05$) from each other. 5CM1 had the highest *D. pulex* population density (Figure 24) while 5CM4 had the lowest *D. pulex* population density in these series of manure combinations.

4.6.2 Effects of Concentrations of 3,4,5 Different Combinations of Manure on Population Density of *Daphnia pulex*

The effects of concentrations of 3, 4, 5 different combinations of manure on *D. pulex* population density are present in Figures 25 - 27. The concentrations of the three combinations were as follows: 1.00ml/L (144) 2.00ml/L (364), 4.00ml/L (406) 8.00ml/L (166) and 16.00ml/L (62 individuals *D. pulex* /L of water). The results of concentration 4.00ml/L favoured the highest density of *D. pulex* studied while 16.00ml/L had the lowest in the group of 3 combinations of manure.

The results of concentrations of the 4 combinations of manure revealed that 2.00ml/L of the 4 combiantions of manures significantly ($p<0.05$) favoured *D. pulex* population growth more than other concentrations used in 4 combinations of manure of this experiment (Figure 26). The concentration of 1.00ml/L of water and that of 16ml/L of water significantly led to poor population density of the cultured organism in this study.

Concentration of the five combinations of manure had the following results 1.00ml/L (835), 2.00ml/L (2791), 4.00ml/L (2,429), 8.00ml/L (650) and 16.00ml/L (467 individuals *D. pulex* /L of water. The concentration 2.00ml/L encouraged highest population density of *D. pulex* although all the concentrations used significantly supported increase in population density of *D. pulex*. The concentration of 1.00ml/L of water and that of 16ml/L of water significantly resulted in poor population density of the cultured organism in this experiment.

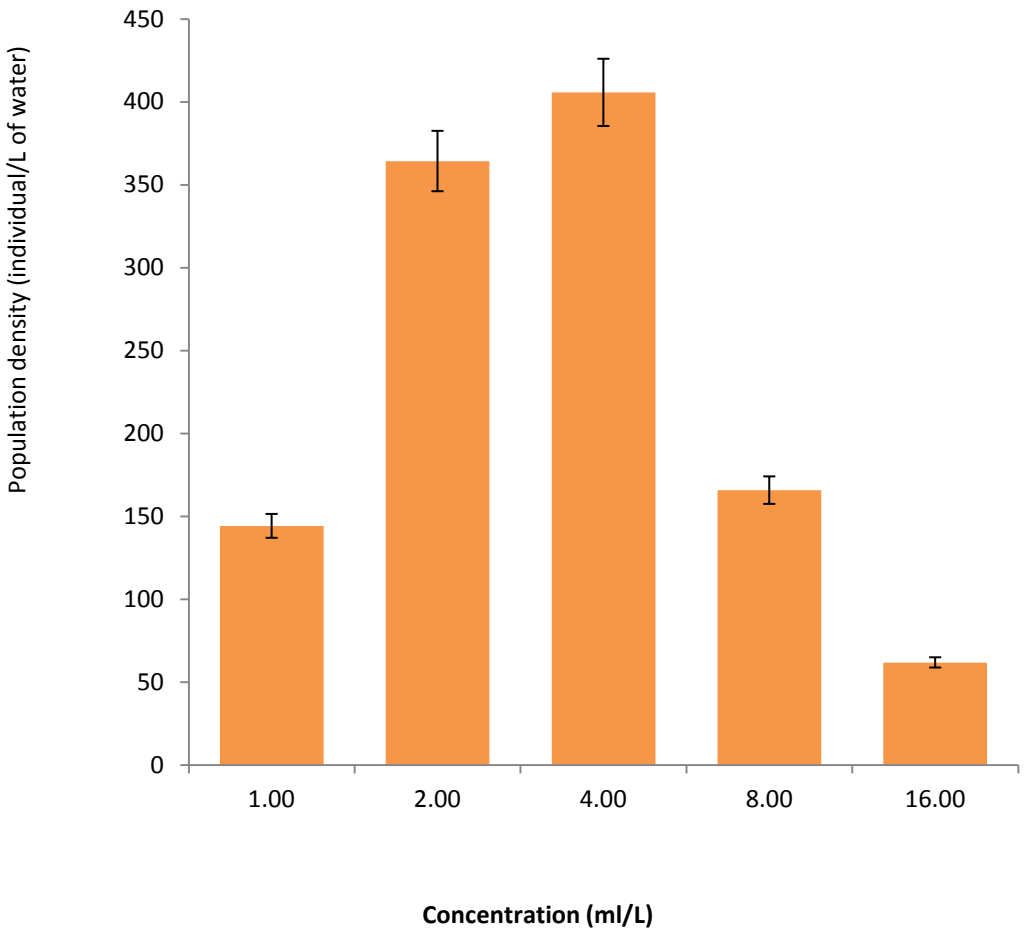


Figure 25: Effect of Concentration of Three Different Combinations of Manure on Population Density of *Daphnia pulex*

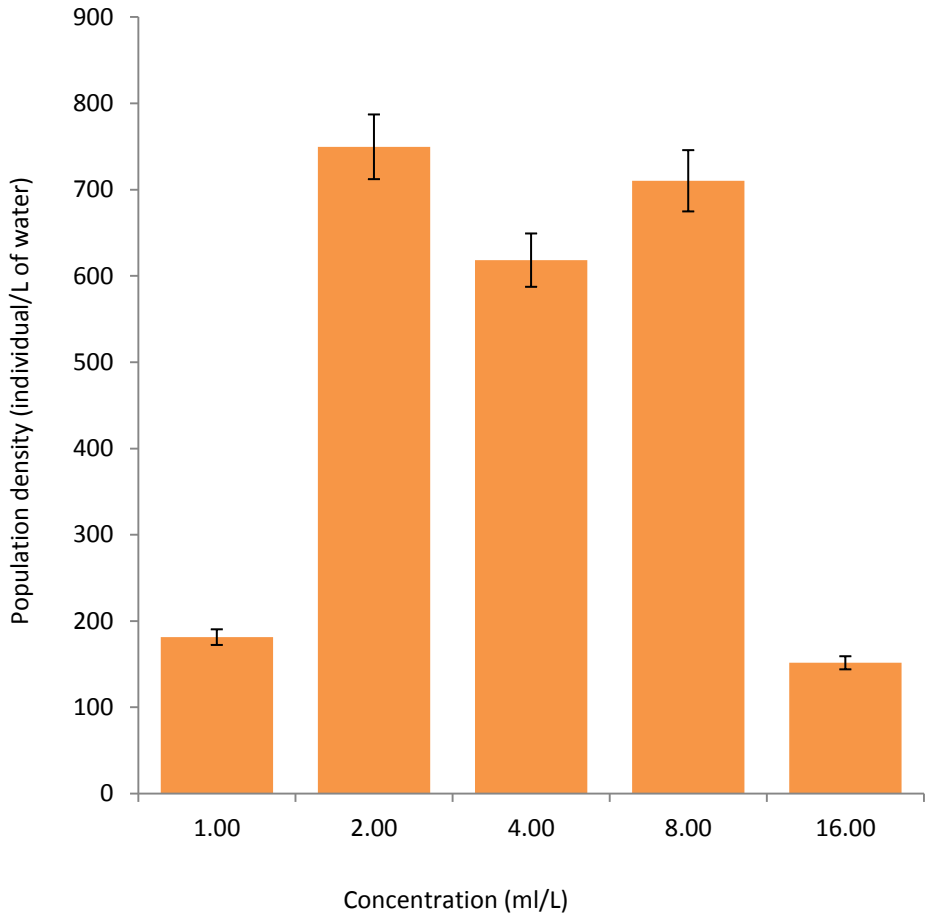


Figure 26: Effect of Concentration of Four Different Combinations of Manure on Population Density of *Daphnia pulex*

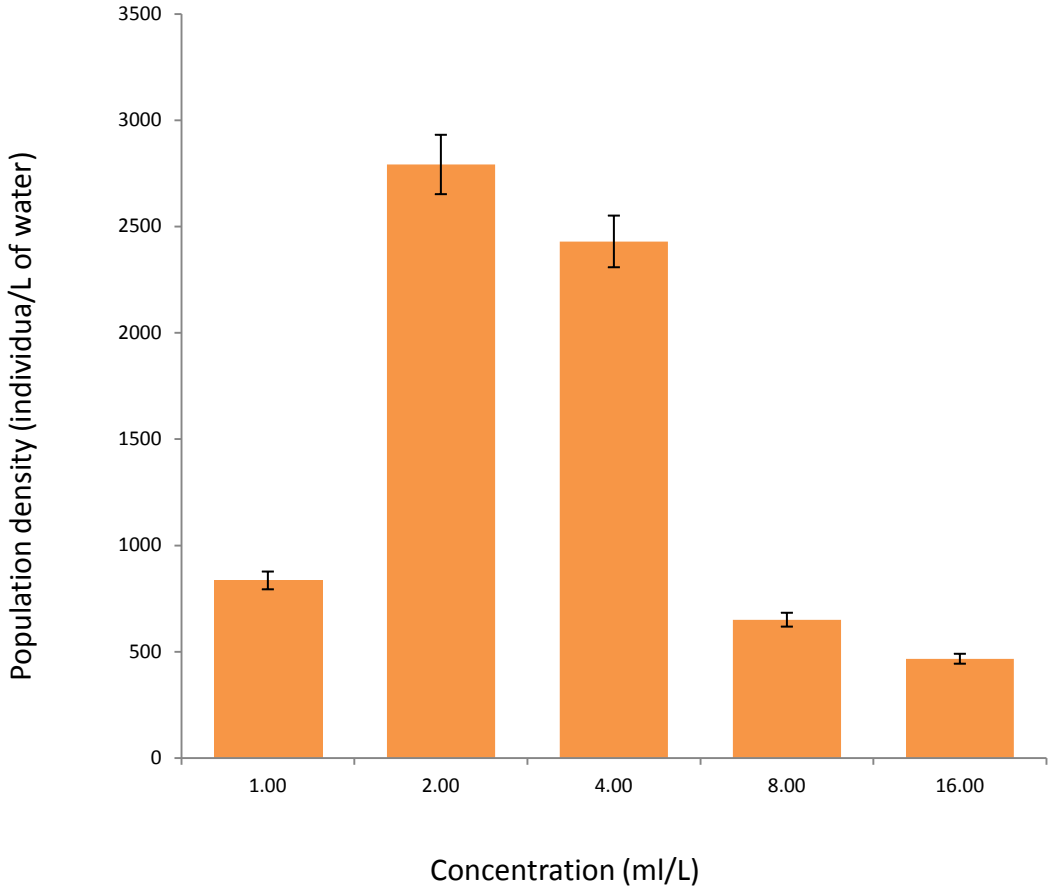


Figure 27: Effect of Concentration of Five Different Combinations of Manure on Population Density of *Daphnia pulex*

4.6.3 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 Figure 28 and appendix10. The results reveal 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 5combinations of manure (4,806 individuals *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.

4.6.4 Interactions of Concentration, Combinations of Manure and Period of Growth on Population Density of *Daphnia pulex*

The results show that the pulled effects of concentration, combination of manure and period of the culture significantly influence the population density of the D. pulex. The pulled effects were more pronounced to individual main effects in this study.

4.6.4.1 The pulled effects of concentrations, three combinations of manure and period of growth on population density of *Daphnia pulex*

The results reveal that interaction between concentration, three combinations of manure and period of growth were highly significant in *D. pulex* population density studied (Table 14). At 4.00ml/L concentration of 3CM2 on day 10 period of growth shows the highest population density of this group (1747 individuals *D. pulex*/L of water) and it was significantlly higher than others in the group. This was also followed by 3CM4 on of the same concentration but in 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

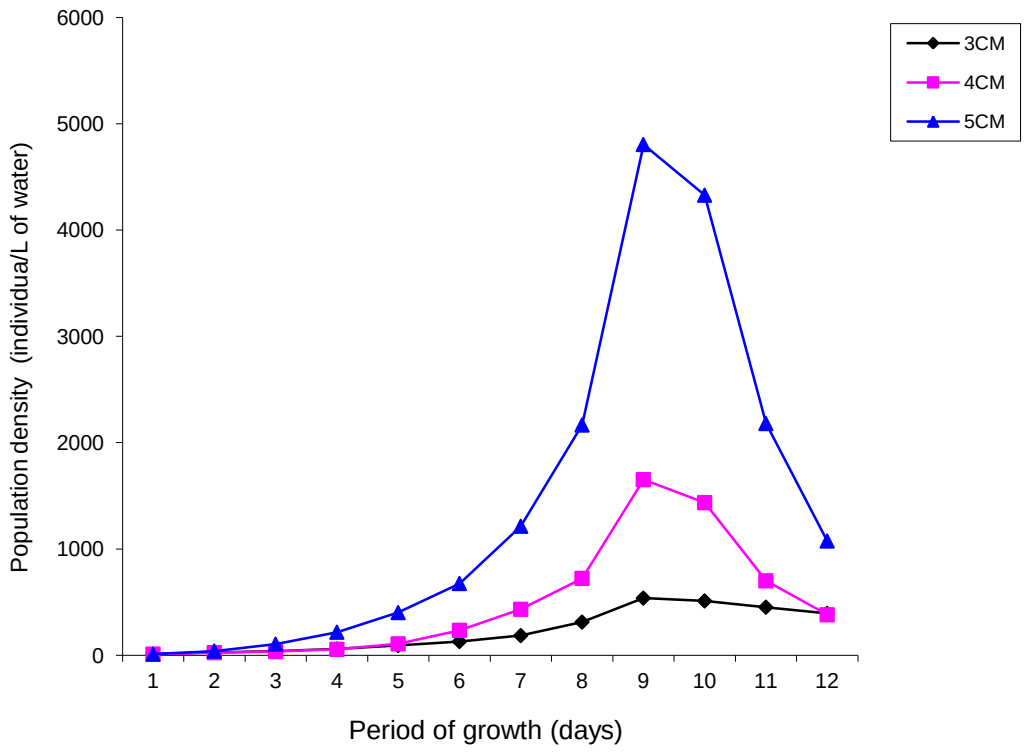
concentration 16.00ml/L could not support population increase of *D. pulex* from day 3 of the period of growth. In the results, it was observed that day 11 and 12 had high population density of *D. pulex* in some of concentrations of 3 combinations of manure but at a declining state.

4.6.4.2 The pulled effects of concentrations, four combinations of manure and period of growth on population density of *D. pulex*

The interactions between concentrations, four combinations of manure and period of growth are presented in Table 15. The highest population density of *D. pulex* (8,821 individuals/L) was recorded in 4CM4 at a concentration of 8.00ml/L on day 9 and this was significantly 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 (Table15). 4CM2 at concentration 16.00 could not support *D. pulex* population growth as well.

4.6.4.3 The pulled effects of concentrations, five combinations of manure and period of growth on population density of *D. pulex*

The interaction between the concentration, five combinations of manure used and the period of growth were highly significant (Table 16). The highest population density of *D. pulex* (26,303 individuals/L of water) were observed in 4.00ml/L concentration of 5CM1 at day 9 period of growth of the studied organism. This was closely followed by that of 5CM1 at concentration 2.00ml/L of water with population density of 25,012 individuals/L of water of day 9. Day 10 to day 12 of the experiment showed a declining population while 5CM2 did not support population increase from day 4 period of growth in concentration 16.00ml/L of water.



CM = Combinations of Manure

Figure 28: Effect of Period of Growth on Population Density of *Daphnia pulex* Treated with 3, 4, 5 Different Combinations of Manure.

Table 14: Pulled Effects of Concentration, 3 Combinations of Manure and Period of Growth on Population Density of *Daphnia pulex*

Conc. (ml/L)	Manure	*Daphnia pulex* population density (individual/L of water)											
		Period of Growth(day)											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
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

3CM1 = Cowdung, Soybean and Single superphosphate
3CM2 = Chicken droppings, Soybean and Single superphosphate
3CM3 = Cowdung, Groundnut cake and Single superphosphate
3CM4 = Chicken droppings, Groundnut cake and Single superphosphate

Table 15: Pulled Effect of Concentration and 4 Combinations of Manure on Population Density of *Daphnia pulex*

Conc. (ml/L)	Manure	*Daphnia pulex* population density (individual/L of water)											
		Period of Growth(day)											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
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	01	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

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

Table 16: Pulled Effects of Concentration, Period of Growth and Five Combinations of Manure on Population Density of *Daphnia pulex*

Conc. (ml/L)	Manure	*Daphnia pulex* population density (individual/L of water)											
		Period of Growth (day)											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
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

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

4.7 AMINO ACID PROFILE OF CULTURED MICRO ORGANISMS (*B.CALYCIFLORUS. M. MICRURA* AND *C. VULGARIS)*

The results of the amino acid profile of the *B.calyciflorus. M. micrura* and *C. vulgaris* are presented in Table 17. The number of each amino acid of the cultured zooplankton (*B. calyciflorus* and *M. micrura)* were not significantly different ($p>0.05$) from each other. In the case of *C. vulgaris,* the number of amino acids in the profile were significantly different ($p<0.05$) from those of the cultured live food zooplankton. The values of each of the amino acid in the cultured micro organisms were significantly different from each other. Tryptophane was absent in the cultured zooplankton but present in appreciable amount in *C. vulgaris*. Methionine was found with the highest values in *C. vulgaris* while lysine was highest in *M. micrura.*

4.8 EFFECT OF TEMPERATURE ON PERCENTAGE FERTILIZATION, HATCHABILITY *HETEROBRANCHUS BIDORSALIS* EGGS AND SURVIVAL RATE OF HATCHLINGS

The influence of temperature on fertilization of above named catfish eggs are shown in Figure 29. Temperature treatment 28^0C and 30^0C were not significantly different ($p>0.05$) from each other with regards to fertilization of the eggs while 26^0C and 32^0C were significantly the lowest ($p<0.05$) in percentage fertilization of the eggs. The results of influence of temperature on hatchability of the fish eggs are shown in Figure 30. The results showed that 28^0C and 30^0C were not significantly higher than each other although they were significantly higher than than those of 26^0C and those of 32^0C. The percentage survival rate of the hatchlings within the first 3 days of hatching is shown in Figure 31. Water temperature of 28^0C favoured significantly the survival rate of hatchlings more than the other treatments. The least percentage survival rate among the treatments was 32^0C while that of 26^0C was just within the range of 40% (Appendix 11).

Table 17: Amino Acid Profile of *Brachionus calyciflorus, Moina micrura and Chlorella vulgaris.* (g amino acids per 100 g of protein)

S/no.	Amino acid compositions	*Brachionus calyciflorus*	*Moina micrura*	*Chlorella vulgaris*
1	Alanine	4.01	2.82	7.80
2	Arginine	6.37	8.27	7.90
3	Aspartic acid	10.53	9.74	9.70
4	Cystine	1.56	2.87	0.27
5	Glutamine	12.22	15.35	13.00
6	Glycine	3.37	3.92	5.80
7	Histidine	1.83	5.06	7.80
8	Isoleucine	4.32	4.19	5.10
9	Leucine	8.95	8.01	2.00
10	Lysine	8.63	10.71	5.20
11	Methionine	0.92	1.12	9.10
12	Phenylalanine	5.20	3.74	8.30
13	Proline	6.03	3.17	2.40
14	Serine	3.45	3.40	5.20
15	Threonine	3.91	2.92	6.10
16	Tryptophane	ND	ND	4.00
17	Tyrosine	2.82	3.00	3.90
18	Valine	4.84	4.43	2.30

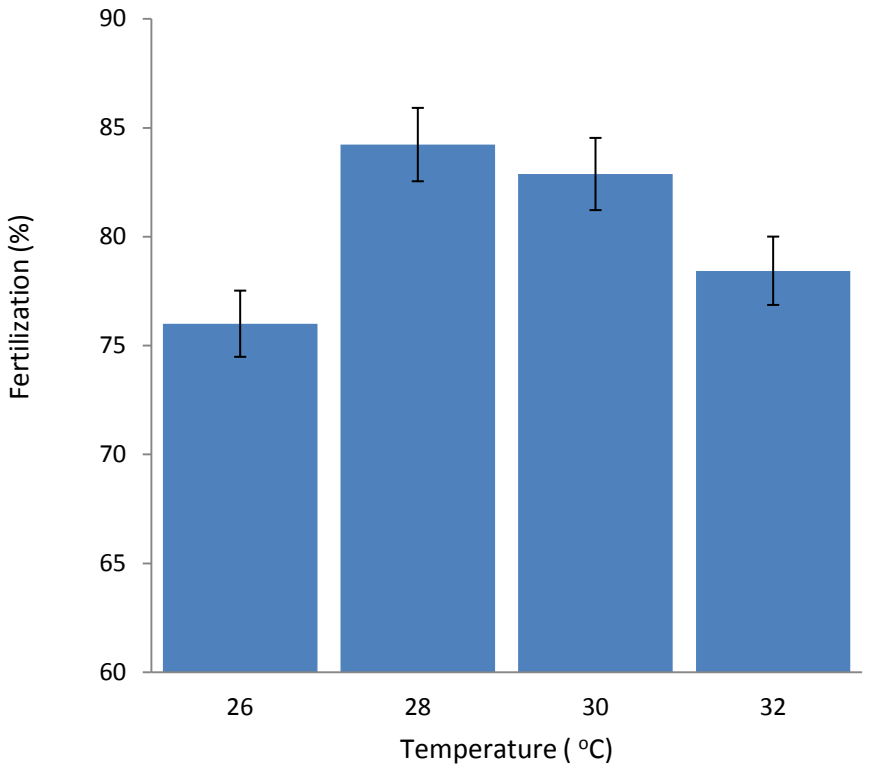


Figure 29: Effect of Temperature on Fertilization of *Heterobranchus bidorsalis* Eggs.

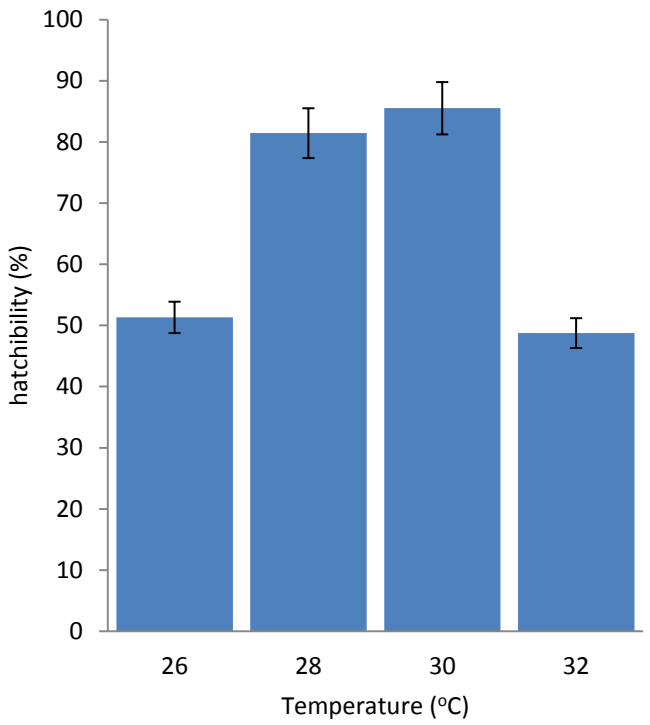


Figure 30: Effect of Temperature on Hatchability (%) of *Heterobranchus bidorsalis* Eggs.

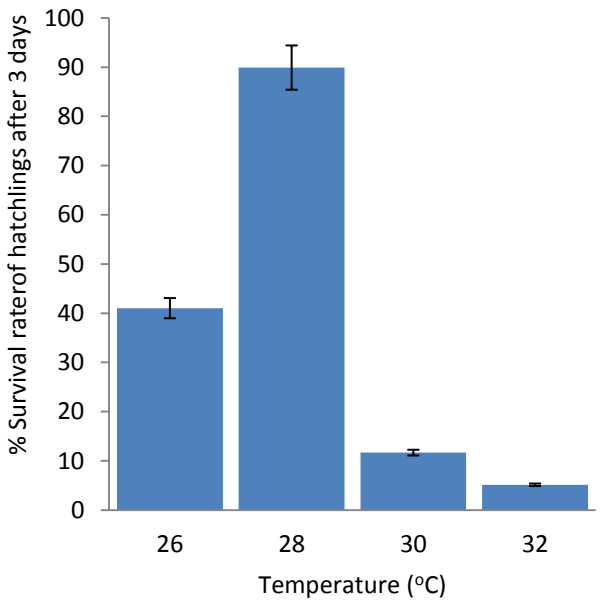


Figure 31: Effect of Temperature on % Survival Rate of Hatchlings within the First 3 Days of Hatching of *Heterobranchus bidorsalis* Eggs.

4.9 SOME GROWTH PARAMETERS OF *H. BIDORSALIS* FRY FED ON ZOOPLANKTON TREATMENTS FOR 16 DAYS

Table 18 shows the percentage weight gain of *H. bidorsalis* fry fed on zooplankton treatments for 16 days. All the treatments favour percentage increase weight gain of *H. bidorsalis* fry. However, the treatment of *B. calyciflorus* & *M. micrura* mixture significantly had the highest followed by *B. calyciflorus*. The least in the percentage weight gain was found in *D. pulex* and shell free artemia treatment groups. *M. micrura, B. calyciflorus* & *M. micrura*; and *B. calyciflorus* treatment groups favoured high percentage weigh gain of *H. bidorsalis fry* although they were not significantly the same (Appendix 12).

The results of total body length of *H. bidorsalis* are shown in Figure 32 and Appendix 13. The total body length of *H. bidorsalis* fry fed on *M. micrura* and those of *B. calyciflorus* were significantly longer in length than other treatment groups. The least result in the groups was recorded in *Daphnia pulex* treatment. However, *M. micrura B. calyciflorus* and mixture of both increase total body length within the 16 days of the experiments.

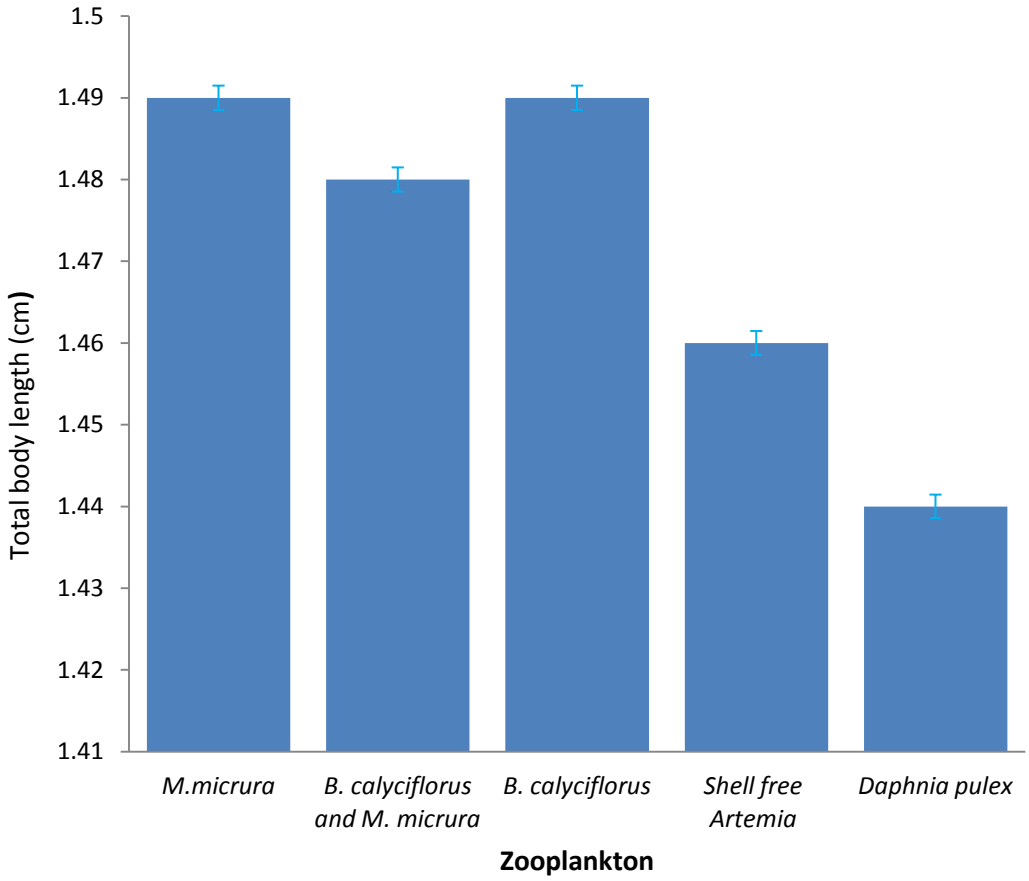
The survival rate of *H. biorsalis* fry in this experiment is shown in Figure 33 and appendix 14. The highest percentage survival rate of *H. bidorsalis* fry was recorded in *B. calyciflorus* and *M. micrura* treatments and it was significantly higher than the results obtained from other treatment groups.

The condition factor of the *H. bidorsalis* fry treated with zooplankton is shown in Figure 34 and appendix15. *B. calyciflorus and M. micrura* recorded the highest condition factor and this was significantly different from the results of other treatment in the experiment. In the case of the condition factor recorded in artemia treatment group, although the condition factor was high but it was still the lowest in all treatment groups.

Table 18: Percentage Weigh Gain of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days

Parameters	Zooplankton				
	Moina micrura	*B. calyciflorus and Moina micrura*	*B. calyciflorus*	Shell free *Artemia*	*Daphnia pulex*
Final weight (g)	0.11 (0.001)	0.14 (0.002)	0.12 (0 .001)	0.10 (0.001)	0.11 (0.003)
Initial (g)	0.01	0.01	0.01	0.01	0.01
Weight gain(g)	0.10 (0.001)	0.13 (0.002)	0.11 (0.001)	0.09 (0.001)	0.10 (0.003)
% Weight gain	1010 (5.77)	1250 (15.28)	1130 (5.77)	877 (3.33)	967 (33.33)

Values in parenthenses = Standard error of mean.



F igure 32: Total Body Length of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days

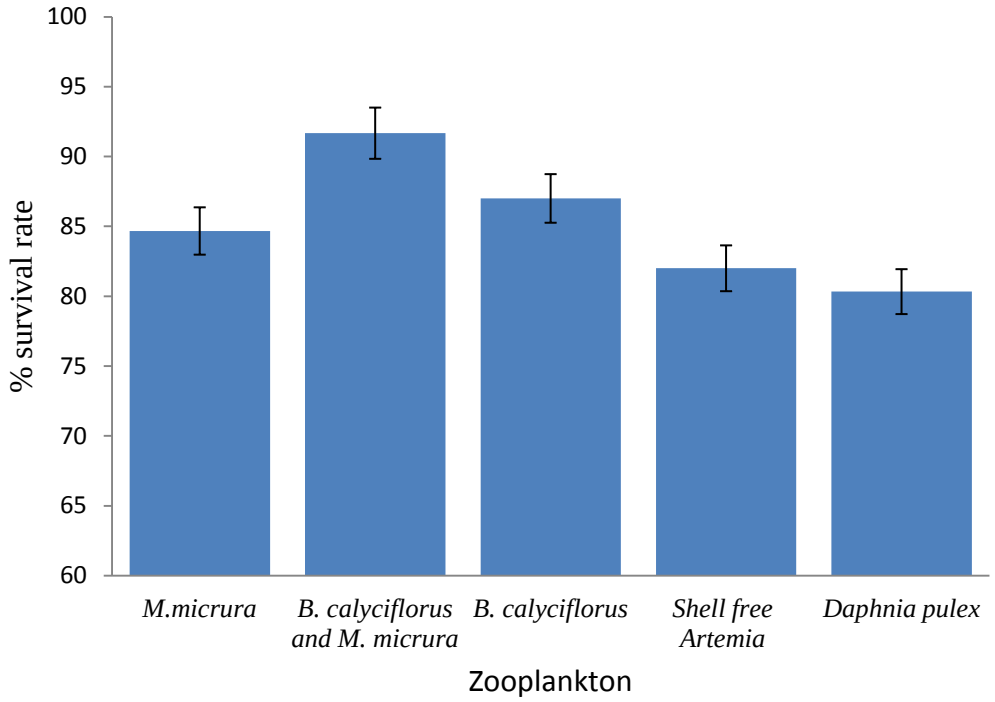


Figure 33: Percentage (%) Survival Rate of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days.

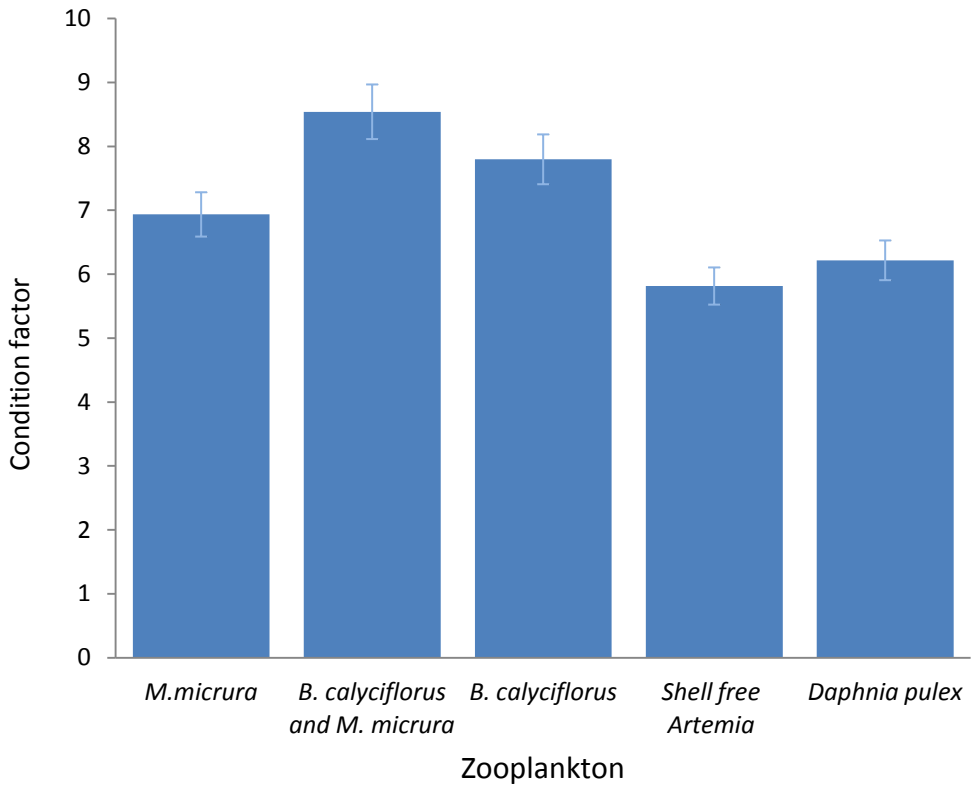


Figure 34: Condition Factor of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days.

CHAPTER FIVE
DISCUSSION

5.1 WATER PARAMETERS

Water quality parameters in the sampling zones were influenced by human activities, including the rate of water flow and decompositions of materials at the zones. The photosynthetic activities of the macrophytes also contribution to the differences observed in the water parameter. The water used for the culture organisms was from the same source and the water parameters investigated are within acceptable tolerable range (Boyd &Lichtkoppler, 1979; Rottmann *et al.*, 2003).

5.1.1 Water Quality Parameters in Live Food Zooplankton Sampling Zones of Awuma River at Shabu

The water parameters investigated were the same river of Awuma at shabu and the sampled zones were from the same water source. The pH and the alkalinity of the water body were within acceptable level and complementary as alkalinity functionally resist pH changes by its buffering effects (Murphy, 2008). The pH of 6.5 – 9 was recommended for aquatic life in the work of Boyd & Lichtkoppler (1979). The human activitives in the area were mainly peasant farming and pollution was not pronounced at that part of the River as the river is flows gently (Murphy, 2008). Although there is good mixing of the water, littoral zone where most of photosynthetic activities occur still have more advantages of dissolved oxygen than the limnetic and benthic zones especially during the day. Carbon dioxide was high while dissolved oxygen was low at the benthic zone because of the utilization of the latter by the zooplankton while the activities of zooplankton and decomposition in the river benthic zone increased the former (Carbon dioxide). Each water parameter at a particular zone of each week was not significantly different in the period of this study because the volume of water was not subjected to

much fluctuations at each of the zone. The significant variation in a particular water parameter in the three zones in each week may be due to physical and bioactivities in each zone.

5.1.2 Water Quality Parameters of Water Used for the Culturing of the Live Food Zooplankton and Fry

The monitoring of water temperature, pH, dissolved oxygen, carbon dioxide and total alkalinity are very essential in the culturing of zooplankton because it enables laboratory manipulations of the particular strain of micro organisms in the locality. The temperature for culturing *M. micrura* ranges from 24-31^{0}C and average water temperature (26.38^{0}C ± 0.41^{0}C) observed in this study falls within acceptable tolerable range of water temperature for culturing *M. micrura* as recorded by Rottmann *et al.*, (2003).

Average pH of water (6.80 ± 0.20), dissolved oxygen (6.29 ± 0.35mg/L), free carbondioxide (3.65mg/L) and total alkalinity (15.0 mg/L) obtained in this study are within tolerable ranges for the culture of the organism as reported by Delbare and Dhert (1996); Rottmann *et al.,* (2003). Water parameters in the treatments for the culture of live food zooplankton were not significantly different ($p>0.05$) from each other as the water supplied was from the same source. The water quality parameters for culturing of the fry were similar to these findings. The average temperature, dissolved oxygen, total alkalinity and carbon dioxide for the various treatment observed in this study were within acceptable range (Adewunmi, 2009). Therefore, the results of each treatment were not altered by water parameters in this experiment.

5.2 LIVE FOOD ZOOPLANKTON ENCOUNTERED AT SAMPLING SITES OF AWUMA RIVER AT SHABU

The highest population density of live food zooplankton encountered in the littoral zone of the Awuma River at Shabu in this study may be attributed to abundant of food in the zone, slow rate of water flow and cover provided by macrophytes which protected the zooplankton from predators and prevented unwanted ultra violent ray from direct sun light. These results agreed with the report of Lauridsen & Lodge, (1996) ; Burks *et al*. (2002) which stated that during the day live food zooplankton like daphnids take shelter among macrophyte stands, which protect them against predation from the presence of fish.

The limnetic zone not only had large volume of water but the movement of water in this zone was faster (relatively) than the littoral and benthic zones. Most of live food zooplankton are slow swimmers which make it difficult for them to benefit from any fast moving water. This factor must have reduced the population density of most of these organisms in the zones. This revelation can be clearly viewed in line with *Paramecium caudatum* which was found in abundance in all the zones of the River as they can swim very fast, water current of the limnetic zone could not resist them or localise them to a particular zone of the river. *Cyclop bicuspidatus* is also relatively good swimmer and large body size of live food zooplankton. They may not have been disturbed by the current of water at the limnetic zone of this water body.

B. calyciflorus are common in zones where there is enough light, sufficient food and a slow water flow (Arimoro, 2006). These could be attributed to their abundance in the littoral zones. The benthic (1.2m deep) and littoral zones were good enough for *D. pulex, Ceriodaphnia cornuta, Daphanosoma aspinosum,* and *M. micrura* because these

zones enable them to avoid high flowing water and direct ultra violent ray. They also provided availability of resting covers and enough food. These organisms seem to like low light intensity as evidenced in their appearance at the surface of the water only in early morning and late evening of the day. The abundance of live zooplankton at some different portion in the same zone may be due to live cycle of these organisms, prevailing predation at the zones and behavioural nature of these micro organisms.

5.3 POPULATION DENSITY OF ZOOPLANKTON (*B. CALYCIFLORUS* AND *M. MICRURA* FED ON FILTRATES OF COMBINATIONS OF MANURE/*CHLORELLA VULGARIS* AND THEIR AMINO ACIDS PROFILES

Manure fed to the culture organism was already in soluble form which make it easier for cultured micro organism to utilized them compare to *C. vigarus* which has hard cell wall (Khatun *et al.*, 1994). The Amino acid profiles of the cultured zooplankton were not significantly different from those reported by Ovie and Ovie (2006).

5.3.1 Population Density of Zooplankton (*B. calyciflorus* and *M. micrura* Fed on Filtrates of Combinations of Manure/*Chlorella vulgaris*

The nutrient requirements and available resources supported the population density of both treatments in the first 2 days of the experiments so there was no significant difference in their result. The filtrate of the five combinations manure and the phytoplankton increase conveniently to meet up with other nutrient requirements of the organisms (*B. calyciflorus* and *M. micrura*). As culture duration increases, the *C. vulgaris* could not supply enough of the requirements as treatment fed with filtrate of five combinations of manure for the dense population of *B. calyciflorus* (222 individuals/ml) so differences in the population growth in the treatments were noticed. 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. Hard wall of the *Chlorella* cell needed to be broken down for optimum digestibility by spray drying or disruption with mill process. The use of ultra-jet-sprayed dried process of *C.* vulgaris strain ensures maximum digestibility and availability of its nutrients (Khatun *et al.,* 1994). It can be inferred that even though *C. vulgaris* has 19 amino acids, 60% of protein, minerals and vitamins, they might not be available to the culture organisms except with good digestibility.

At day 8, the population density stressed the available resources and survival was threatened by age and shortage of available resources (FAO, 1996). The crash in population was high and sudden in treatment fed with filtrate of manure due to competition for space, available nutrient and pressure from waste products from the living organisms but crashing was less in the group of less dense population as pressure and competition was lesser in the group (Rottmann *et al.,* 2003).

M. micrura population density was observed to have similar pattern to that of *B. calyciflorus.* The filtrates of five combinations of manure encourage a very high population density (48,906 individuals/L of *M. micrura* compared to 11,603 individuals/L of water from the group fed with *C. vulgaris.* The peak in population density was noticed on 8 day and crash in population density of those organisms fed on filtrate from manure was more because culture duration, age and interference from environment by other organisms and by the amount of waste generated by the organisms. This must have hastened the peak of carrying capacity of the organism in the confined environment. Filtrate of five combinations of manure was reported to influence very high

population density of cultured *Brachionus calyciflorus, Moina micrura* and *Daphnia pulex* (Okunsebor, 2012; Okunsebor and Sotolu, 2012; Okunsebor and Ofojekwu, 2012)

The slight variation in the values of the amino acids of the cultured micro organisms from the ones reported (Khatun *et al.*, 1994; Ovie & Ovie, 2006) may be due to:

1. Region,
2. Food supply
3. Prevailing environmental factors of the habitat.

The numbers of each of the amino acids were significantly same because of the genetic compositions of the organisms. The high methionine and lysine values of the cultured micro organisms imply that they are most likely ones of the best live food micro organisms.

5.3.2 Amino Acid Profiles of Cultured Micro organisms

The results of amino acids profiles of the cultured micro organisms recorded in this experiment were similar to those reported by Khatun *et al. (*1994); Ovie and Ovie (2006) for *C. vulgaris, B.calyciflorus and M. micrura*. The methionine and lysine were not reduced in the cultured micro organisms. *M. micrura* had the highest value of lysine (10.71) while C. *vulgaris* was significantly higher than *M. micrura* in methionine value (9.1). These findings were consistent with the results reported by Khatun *et al. (*1994); Ovie and Ovie (2006)

5.4 EFFECT OF CONCENTRATIONS, COMBINATIONS OF MANURE CULTURE MEDIUM AND PERIOD OF GROWTH ON POPULATION DENSITY OF *BRACHIONUS CALYCIFLORUS*.

Concentration 2.00ml/L, 4.00ml/L and 8.0ml/L of water supported high population density of *B. cacyciflorus*. However, 4.00ml/L of water had the highest population density at day 5 possibly due to supply of needed nutrients at required level of

the cultured organisms without deleterious effect on the population of density of the cultured organisms. The results of the 2.00ml/L and that of 8.00ml/L clearly show that 4.00ml/L was likely accurate dose for optimal population growth of the organism.

Concentration of the combinations of the manure culture medium showed that 1.00ml/L and 16.00ml/L of water were too low and too high respectively for optimal production of *B. calyciflorus* in this investigation. The low concentration could not supply the needed amount of nutrients that could take the cultured organism to a very high population density. As the duration of growth of the organism increased from the early start off, population density was increasing in a very slow rate as high competition limts the chance of comfortable environment. Concentration of 16.00ml/L water on the other hand also increase population density of *B. calyciflorus* but the population increase was slow down because of high concentration due to daily load of feeding with this concentration of the manure combination making the water cultured environment unconducive enough to encourage a very high population density of the organisms.

The population density of *B. calyciflorus* increased in the following order in all the culture of manure combination (5CM1 > 5CM2 > 5CM3 >5CM4). This may suggest that as the selected combinations of manure may not have released the same amount of the needed nutrients to the organism and suppressive reactions must have occurred. In this investigation, 5CM1 encouraged population density of *B. calyciflorus* up to 245/ml of water at day 5 period of culture especially if the culture was started with 10 individuals/ml. 5CM1 and 5CM2 are likely to have contained more available nutrients leading to balanced nutrient requirements of *B. calyciflorus.* This investigation has

clearly shown that 5CM1 and 5 CM2 can be used conveniently for *mass* production of *B. calyciflorus* in hatcheries.

Declining population density observed in all the concentrations beyond day7 may be as results of sudden crash as population gets to the peak in small scale culture (Arimoro, 2006) and high quantity of dead decaying organisms observed with other zooplankton in the culture. These made the culture unconducive and non monospecific thereby encouraging predation, high competition, biotoxic build up and pollution which reduced the number of *B. calyicflorus* / ml of water. Arimoro (2006) noted that once there are appearances of copepods in a culture, the population density of rotifers can not be high. It can also be noted that any food that is not eaten can cause increase in the level of ammonia and Biological Oxygen Demand thereby reducing the water dissolved oxygen (Arimoro 2006) needed by the densed population of the organism

5.5 INFLUENCE OF CONCENTRATION, COMBINATIONS OF MANURE AND PERIOD OF GROWTH ON POPULATION DENSITY OF *MOINA MICRURA*

The pulled effects of the concentration, combination of manure and period of the culture of *M. micrura* show that each of the three factors must be consider in population study of the culture micro organism. The daily load of the concentration of the filtrate combined manure over the time of culture influenced the population density of *M. micrura.*

5.5.1 Effects of Different Combinations of Manure on Population Density of *M. micrura*

3CM2, 4CM4 and 5CM2 supported the highest population density of *M. micrura* in their various groups as a result of nutrient released by these manure combinations. Combination 3CM2, although highest in its group but was the lowest when compared to

those of 4CM4 and 5CM2 combiantions. 4CM4 combination of manure which had the highest population density must have released the highest amount of needed nutrient that favoured the increase of population of *M. micrura.* Comparing 4CM4, 5CM2 and 5CM4 combinations of manure, results showed that increasing the numbers of combinations do not necessarily increase population density of *M. micrura.* This finding is consistent with those obtained by Rottmann *et al.* (2003); Gupta and Gupta (2006), who suggested that organic manure, oil cake and super phosphate can be used for culture of Cladocerans.

5.5.2 Effects of Concentrations of Different Combinations of Manure on Population Density of *M. micrura*

Concentration 4ml/L of water of any of the 3,4,5 combination of manure supported the highest population growth of *M. micrura* because the nutrient supplied must have been adequate for the survival of the cultured organism. Concentration 1.00ml/L and 16.00ml/L of water were too low and too high respectively to support densed population density of *M. micrura*. The low population density may be as result of insufficiency of the nutrients needed for the growth while the 16.00ml/L must have caused overfeeding, pollution and toxic situation which discouraged the increase in population growth of *M. micrura.* The concentration (16.00ml/L) must have been too high for optimal population growth leading to suffocation of the cultured organisms. Ovie and Eborge (2002) reported a similar observation in *M. micrura* fed on a very high density of *Scenedesmus acuminatus.* 1.00ml/L of the all manure combinations was not able to sustain high population density of *M. micrura* before intruders of other zooplankton came in and suppressed their population density due to succession and survival of the fittest. 1.00ml/L of this manure combination must have led to under feeding of the cultured organisms. The *M. micrura* may have probably switched over to

reproduction of males which slow down the rate of increase in population density of the organisms. Rottmann *et al.* (2003) reported that sexual reproduction slow down population density when condition is not favorable especially when there is abrupt reduction in food supply.

5.5.3 Effects of Duration of Culture on Population Density of *M. micrura*

The increase of M. micrura population from 20 individuals to 25,194, 30,335, and 31,794 individuals/L of water of 3CM, 4CM and 5CM selected combinations of manure respectively is quite encouraging in the period of growth. In this study, the peak of the population density (31,794 individuals *M. micrura* /L of water of 5CM) obtained on day 9 is an indicator of good management practice of this organism. At a favourable environment with good nutrients as released by 4CM or 5CM, *M. micrura* was able to increase in population density up to 30,335, and 31,794 individuals/L of water respectively on day 9 of the culture. These are further improvements of the results of Ovie and Eborge (2002) who got peak of 11,000 individuals *M. micrura* /L of water using *Scenedesmus acuminatus* as live feed.

Beyond day 9, a declined population density observed must have been the results of competition over the available resources, toxic waste products accumulations from the cultured organisms and invations of intruders of other micro-organsims which are spread by air in the environment. As they are noticed, the culture was no long pure and the numbers of *M. micrura* /L of water were reduced as result of the unwanted organisms found in the culture. The culture environment experienced stressed condition and survival of the fittest, sucession of stronger and relatively environmental stress resistant micro-organims like Cyclops eventually came into play. Sexual reproduction may set in as

cladocerans usual phenomenon, induced by environmental factors associated with adverse conditions, including overcrowding (Martínez-Jerónimo *et al.*, 2007).

5.5.4 Interactive Effects of Concentration, Different Combinations of Manure and Period of Growth on Population Density of *M. micrura*

The effects as results of interations are well pronounced in this investigation. The concentrations, manure combinations and period of growth gave the resultant effect of improved population growth of *M. micrura*. The best resultant effects of 42,159, 51,678 and 55,184 individuals *M. micrura*/L of water recorded for 3CM2, 4CM4 and 5CM2 respectively revealed that concentration, combination of manure and period of culture must be considered when culturing this organism. When ever these factors are considered, the population density of *M. micrura* can be raised from 20 to 55,684 individuals/L of water of 5CM2 at concentrationof 4.00ml/L of water on 9 days period of growth. The resultant effects of these factors (encouraging growth) clearly show that enough of *M. micrura* can be produced within a short period of time in batch culture.

5.6 INFLUENCE OF CONCENTRATION, COMBINATIONS OF MANURE AND PERIOD OF GROWTH ON POPULATION DENSITY OF *DAPHNIA PULEX*

Daphnia is sensitive to water quality and as such, the daily load of the manure concentration over the period of growth may cause the crash in population density. The organism enjoyed the nutrient level until the aftermath of the manure daily load and waste build up as result of population density of the organisms before the crash of the population desnity.

5.6.1 Influences of Combinations of Manure on Population Density of *Daphnia pulex*

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

5.6.2 Influence of Concentrations of Manure on Population Density of *Daphnia pulex*

Although all the concentrations used in this investigation supported increase in population density, concentration 2.00ml/L of water of the 4CM and 5CM are the most populous treatment groups. Concentration 1.00ml/L of water of the various combinations was not able to provide the needed nutrients becasue it was too low to supply the needed nutrients while concentration 8.00 and16.00ml/L of water were too high constituting a sort of pollutant to the cultured organisms. 4.00ml/L of water of water of 3CM was good but could not support high population density of *D. pulex* like that of 2.00ml/L of water of 4CM and 5CM because this organism is highly sensitive to water quality (Clare, 2002).

5.6.3 Influence of Duration of Culture on Population Density of *Daphnia pulex*

Period of growth played a very vital role in the production of *D. pulex.* Day 9 from all the combinations of manure used in this study had the highest population density of *D. pulex.* This indicated that to have pure culture of the organism, one has to target within 9 days culture duration if the initial population density is as low as 10 individuals/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 in outdoor condition. The peak was obtained on 12th day of inoculum in fourth dose which significantly ($P < 0.01$) had the highest numbers of the organisms (1,930 individuals/L of water). *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 available nutrients in the culture. As the period of growth extends, algal 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.*

Declining population density of *D. pulex* noticed was probably as result of competititon over available resources. It can also be as result of accumulated waste products of the organisms which may lead to ammonia toxicity such that the environment became unfavourable for the cultured organisms.

5.6.4 Interactive Effects of Concentration, Combination of Manure and Period of Growth on *D. pulex*

The pulled effects of concentration, combinations of manure and period of growth revealed that concentration 4.00ml/L of 5CM1, (26,303 individuals/L of water) and 2.00ml/L of 5CM1 (25,012 individuals/L of water) on day 9 improved *D. pulex* population density in this investigation. With these population margins, it therefore

implied that good population density of this organism can be realized on the 9 day period of culture especially when the concentration and the manure combinations are considered. Clare, (2002) reported that *D. pulex* experieced fast growth rate when condition in favourable. 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* 3CM2 and 4CM4 combinations of manure may be attributed to poor release of nutrients.

5.7 EFFECT OF TEMPERATURE ON FERTILIZATION, HATCHABILITY OF *HETEROBRANCHUS BIDORSALIS* EGGS AND SURVIVAL RATE OF THE HATCHLINGS

Fertilization of *H. bidorsalis* eggs in temperature treatments of 28°C and 30°C was significantly different from each other and of the various treatments because this selected ranges of temperature support fertilization of fish eggs as one of the warm water fishes in the region. The results of hatchability also shown that selected temperature treatments significantly affected the hatchability of the fish eggs. Temperature has main effect on hatching of fish eggs. El-gamal (2009) reported that growth of larvae increased at the optimum temperature from 27-30°C and no eggs survived to hatch at 20°C or 38°C. It is most likely that the temperature affects the tolerance level of viable eggs, (El-gamal, 2009). The optimum range for good hatchability of catfish eggs was proved to be 28-30 ^{0}C as this temperature range is reported to be acceptable range of water temperature of warm water fishes by El-gamal (2009). The results indicated that the optimum temperature for hatchability of the fry lies between 28 and 30°C. This finding agrees with the results from other species (Nwosu & Holzlohnev, 2000). At 26^{0}C and 32^{0}C, hatchability was found highly reduced indicating that the temperature is very low or very high for about 90% hatchability of the fish egg in hatchery. In addition,

temperature is known to influence the efficiency of yolk utilization (Hamel *et al.*, 1997). It was reported that the growth rate increase with increasing water temperature, but when the temperature becomes super optimal, it has a negative effect (Brett & Groves, 1979).

The temperature affects physiological processes in fish hatchlings as low temperature or high temperature affects the survival rate of fry in the results of this experiments. In addition, the temperature influence metabolic processing and is the single most important factor that determines growth rates in fish (Brett, 1979). At 40% survival rate of hatchlings found in 26°C seem good but in this study 28^{0}C gave about 90% survival of hatchlings during yolk sac stage of life. Optimum temperature range from 28-30°C plays an important role for improvement hatchability but growth, food intake and defense mechanism during the period of the larval development are significantly improved. The temperature at 30 ^{o}C could not support survival as suspected because the it tend toward extreme temperature tolerance for the fry. This finding is very important as 50% mortality at this stage affects directly fish production. At 32°C, survival of most fry was affected by high temperature.

5.8 GROWTH PARAMETERS OF *H. BIDORSALIS* FRY FED ON ZOOPLANKTON TREATMENTS

The feeding of fry with any of the selected zooplankton cultured in the experiment positively increase percentage weight gain of the fry which shows that they are good for live food for the fry. The feeding of fry with any of the zooplankton culture in the experiment positively increase percentage weight gain of the fry. The *B. calyciflorus* and a mixture of *B. calyciflorus* & *M. micrura* significantly increase weight gain more than other treatment groups because of the size, slow swimming rate and nutrient availability in the freshwater zooplankton as live feed without significantly

increasing water pollution as compared to uneaten supplied artemia shell free in fry tank. Rotifers are one of the smallest metazoans, which served as perfect material for evolution theories and excellent food resources to larva in aquaculture (Haoyuan & Yilong, 2008; Chen *et al.,* 2005). The adult of daphnia is suspected to be too large and too fast for young fry to easily prey on and the stress of catching the prey affected the percentage weight gain of the fry.

The result of good specific growth rate observed was also influenced by the size and slow swimming rate, nutrient availability in the *B. calyciflorus* and *M. micrura.* The live feed zooplankton organisms which were not too large for the mouth part of the fry were caught and easily utilized as they were easily digested by the fry. *B. calyciflorus* as live food for fish was used as ideal starter feed for dwarf gourami *Colisa lalia,* a tropical freshwater ornamental fish species with larvae that are too small to ingest *Artemia* nauplii or *Moina* at its first feeding. Rotifer as starter diet significantly improves the growth and survival of gourami 2-12 days larvae (Lim *et al.,* 2003).

The total body length of the fry was also improved by the live food organisms and the results proved that *B. calyciflorus* and *M. micrura which* were easier for the fry to prey on were the highest in the treatment groups. Ovie and Ovie (2006); Watanabe *et al.* (1983) showed that *M. micrura* had well distributed amino acid profile. The influence of the total body length of the organisms is linked to the live food utilization for growth of the fry.

Artemia shell free and *Daphnia pulex* treatment groups were low in total body length because of size and digestibility which lead to low utilization in the fry. With water quality parameter on acceptable level, food influences percentage survival rate of the fry. Easy catch, nutrient content and easy digestibility of the food organisms help to

increase the survival rate of the fish fry. *B.calyciflorus* and *M. micrura* treatment group was significantly the best in percentage survival rate because it meets the nutrient requirement, as live food size and slow swimming rate for fry easy catch (Sipauba-Tavares & Bachion, 2002).

Condition factor which shows how the well being of the fry in a given treatment is positive in all the result but *B. calyciflorus* and *M. micrura* treatment group favoured the rearing of the fry more than other treatment groups in the experiment. *M. micrura* may be considered promising species for feeding fish larvae and fingerlings in large-scale production as they have short life-span, small size, quick embryonic development, and abundant energy store (Sipaúba-Tavares & Bachion, 2002). *Daphnia pulex* treatment group condition factor was low because of the size and the fast swimming ability of the live food organism.

CHAPTER SIX
SUMMARY OF RESULTS, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

6.1 SUMMARY OF RESULTS

1 Dissolved oxygen, temperature and carbondioxide each significantly varied at littoral, limnetic and benthic zones of Awuma River at Shabu.

2 pH and total alkalinity show no significant different in the littoral, limnetic and benthic zones of Awuma River at Shabu through the period of the study.

3 Littoral zone significantly had the highest population density of live food zooplankton in all the zones.

4 *B. calyciflorus* highest population density was found in 5CM1 at 4.00ml/L on day 5 while beyond day 7 a decline in population density was observed.

5 Among the different concentrations of the combinations of manure investigated, concentration 4.00ml/L favoured the highest population density of *M. micrura*/L of water in these investigations.

6 *M. micrura* population density increased from day 2 to day 9 while day 10 to day 11 experienced crashing of population density during the period of culture.

7 The highest interactive effect on population density of *M. micrura* was recorded in concentration 4.00ml/L of 5combinations of manure (5CM2) on day 9.

8 The results of the effects of period of growth on population density of *D. pulex* revealed that day 9 in all the combinations of manure used had the highest population density.

9 The highest population density of *D. pulex* (26,303 individuals/L. of water) was observed in concentration 4.00ml/L of 5CM1 at day 9 period of growth of the studied organism.

10. 5CM2 did not support population increase from day 4 period of growth in concentration 16.00ml/L of water while day10 to day12 of the experiment experienced a decline in population density.

11 Combination manure 5CM1 and 5CM2 increased population density of *B. calyciflorus* & *M. micrura* respectively more than *C. vulgaris* (phytoplankton).

12 Hatchability of the fish eggs and 90% survival of hatchling during yolk sac stage of life were supported significantly at 28 ^{0}C.

13 Mixture of *B. calyciflorus* & *M. micrura* significantly increase percentage weight gain, survival rate, total body length and condition factor of the fry.

6.2 CONCLUSION

- Zooplankton (*M. micrura, D .pulex, C. cornuta, B. calyciflorus)* are common at the littoral zones of the river and littoral zone significantly had the highest population density of live food zooplankton, therefore collection of live food zooplankton in good population density for research and for culture should be done in the littoral zone of river.
- Highest population density of *B. calyciflorus* was found in 5CM1 at 4.00ml/L on day 5 while beyond day 7 a decline in population density was observed. It is profitable to harvest *B. calyciflorus* on the 5th day of culture of the organism if good culture medium like 5CM1 is employed.

- The highest population density on *M. micrura* was recorded in concentration 4.00ml/L of five combinations of manure (5CM2) on day 9 (55,184 individuals/L of water). The use of 5CM2 at concentration of 4.00ml/L can give culturist high population density of *M. micrura*. Harvest of *M. micrura* on day 9 of culture duration is highly economical and advantageous to culturist.
- Population density of *D. pulex* (26,303 individuals/L of water) was observed to be highest in concentration 4.00ml/L of 5CM1 at day 9 period of growth in this study. Though *D. pulex* is very sensity to water quality, a culture medium like 5CM1 at 4.00ml/L can help to raise the organism within 9 days to population density of 26,303 individuals/L of water.
- There is high interaction between concentration, combination of manure and period of growth of all the culture live food organisms in this study. Therefore; these three factors must be considered when the need to culture the organisms arises.
- Filtrate of the combination manure 5CM1 and 5CM2 significantly increase population density of *B. calyciflorus* & *M. micrura* respectively more than *C. vulgaris* (phytoplankton). The use of formulated feed in form of liquid can release nutrient to live zooplankton than some algae with hard cell wall the organisms is not efficient in digestion.
- Hatchability of the fish eggs and 90% survival of hatchling during yolk sac stage of life were supported significantly at 28 ^{0}C. Hatchability of the fish eggs and high survival rate is achievable at 28 ^{0}C

- A mixture of *B. calyciflorus* & *M. micrura* significantly increase percentage weigh gain, survival rate, total body length and condition factor of the fry more than others treatment groups. All live food zooplankton improve growth, survival and condition factor of fry but the mixture of *calyciflorus* & *M. micrura* in ratio 1:1 in fry in hatchery enhanced the listed factors more than when they are used as in monospecific culture state.

6.3 RECOMMENDATIONS

1 Research should be intensified to exploit more live food zooplankton in our freshwater for rearing of fish hatchlings.

2 Endermic species of live food zooplankton in Jos Plateau should be given a base line study.

3 Processing and storage of the mass produced *M. micrura* and *B. calyciflorus* in batch for easy application by local farmers is highly needed to avoid the technicality involved in the culture

4 The use of Blended *Chlorella vulgaris* as amino acid and vitamin adaptive to fry feed should be investigated

5 Technology of water recirculatory system where temperature range of 28 ^{o}C-30 ^{0}C can easily be maintained for hatchlings survival to advance fry should be developed.

6 Mixture of more live food zooplankton to enhance survival and growth of fish hatchlings should be highly encouraged.

6.4 CONTRIBUTION TO KNOWLEDGE

1. Provision of baline data on the live food zooplankton of Awuma River which was not avilaible in scientific documentations.

2. *Brachionus calyciflorus*, *Moina micrura* and and *Daphnia pulex* are most abundant in the littoral zone of freshwater body which can be exploited for mass production.

3. *Moina micrura* has well distributed and the best amino acid profile especially in methionine (1.12), lysine (10.73) and glutamine(15.39) in the studied live food zooplankton.

4. The successful mass production of the local stains of *Brachionus calyciflorus*, *Moina micrura* and and *Daphnia pulex* in this work, solved the existing cost-related and constrains associated with importation of brime shrimp (Artemia)

5. This work revealed the needed combinations of manure (5CM2), concentration (4.00ml/L) and duration of culture (day 9) for the highest population density of *M. micrura* (55,184 individuals/L of water)

6. Combination of manure (5CM1) at 4.00ml/L of water on day 9 and day 5 significantly supported mass production of *D. pulex* and *B. calyciflorus* respectively (Appendix B).

7. Concentration, food combinations and period of culture are very vital in mass production of the studied live food zooplankton.

8. Optimal temperature range for hatching of *H.bidorsalis* eggs was found to be $28^{o}C$

9. Mixture of *B. calyciflorus* & *M. micrura* in ratio 1:1 offer the best feeding strategy for high percentage survival rate, condition factor and growth rate of the fish fry.

REFERENCES

Adewunmi, A.A. (2009). Catfish culture in Nigeria: prospects and problems. *Aquaculture Research, 36*: pp. 479-585.

Adigun, B.A. (2005). *Water quality management in Aquaculture and Freshwater zooplankton production for use in fish Hatcheries*. National Institute for Freshwater Fisheries Research, New Bussa. 25pp.

Adewolu, M. A., Ogunsanmi, A.O. and Yunusa, A. (2008). Studies on growth performance and feed utilization of two *Clarias* catfish and their hybrid reared under different culture systems. *European Journal of Scientific Research, 23(* 2): pp. 252-260.

Adeyemo, A.A., Oladosu, G.A. and Ayinla, A.O. (1994). Growth and survival of fry of African catfish species, *Clarias gariepinus (*Burchell), *Heterobrachus bidorsalis (*Geoffrey) and *Heteroclarias* reared on *Moina dubia* in comparison with other first feed sources. *Aquaculture, 119:* 41-45.

AOAC **(**1984) *Official Methods of Analysis of the AOAC,* 13th ed. Association of Official Analytical Chemists,Washington, D.C. 1285 pp.

APHA/AWWA/WPCF (American Public Health Association, American Water Work Association, Water Pollution Control Federation 1985) Standard methods for the examination of water and waste water, 16th Edition, Washington, DC, American Public Health Association, 1268p.

Arimoro, F.O. and Ofojekwu, P.C. (2003). Incidence of feeding, growth and survival of the toothed carp, *Aphyosemion gairdneri* larvae reared on the freshwater rotifer, *B. calyciflorus. Tropical Freshwater Biology, (12, 13):* 35-43.

Arimoro, F.O. and Ofojekwu, P.C. (2004). Some aspects of the culture, population dynamics and reproductive rates of the freshwater rotifier, *B. calyciflorus* fed selected diets. *Journal of Aquatic Sciences, 19 (2):* 95-98.

Arimoro, F.O. (2006). Culture of the freshwater rotifer, *B. calyciflorus* and its application in fish larviculture technology. *African Journal of Biotechnology, 5 (7):* 536-541p.

Arrhenius, F. and Hanson, S. (1993). Food Consumption of Larval, Young and Adult Herry and Sprat in the Baltic Sea. *Marine Ecology Progress Series 96*: 125-137.

Awaiss, K. (1998). Feeding sequences (rotifer and dry chemical composition of African catfish, (*Clarias gariepinus)* Burchell (Pisces: Clariidae) Larvae. *Aquaculture Research, 29(10):* 731.

Belcher, H. and Swale, E. (1978). *A beginner's guide to freshwater algae.* Culture Centre of Alge and Protozoa. Institute of Terrestial Ecology. Natural Environment Research Council, London. Pp: 1-48.

Boyd, C.E. and LIchtkoppler F. (1979). Water quality management in pond fish culture. (R.andD. Series; 22) Auburn University Alabama, Department of Fisheries and Allied Aquacultures. 30pp

Brett, J.R. (1979). Environmental factors and growth. In fish physiology. Vol. 8. W.S. Hoar, D.J. Randall and J. R. Brett (Eds.). Academic press, London, U. K.. pp: 599-675.

Brett, J.R. and Groves, T.D.D. (1979). Physiological energetic in W.S., Hoar, D.J. Randall and J.R. Brett (Editors). Fish physiology *Vol. 3* . Bioenergetics and growth. Academic Press. New York, NY, pp: 279-352.

Bryant, P.L. and Matty, A.J. (1980). Optimisation of Artemia feeding rate for carp (*Cyprinus carpio* L.). *Aquaculture, 21*: 203-212.

Burks, R. L., Lodge, D. M. Jeppesen, E. and Lauridsen T. L. (2002). Diel horizontal migration of zooplankton: Costs and benefits of inhabiting the littoral. *Freshwater Biology 47*: 343–365.

Castell, J., Blair, T., Neil, S., Howes, K., Mercer, S., ReidYoung-Lai, J., Gullison, B., Dhert, P., Sorgeloos, P. (2003). Effect of different HUFA enrichment emulsions on the nutritiona lvalue of rotifers (*Brachionus plicatilis*) fed to larvae haddock (*Melanogrammes aeglefinus*). *Aquaculture International, 11(1-2):*109-117.

Cheikyula, J.O. and Ofojekwu, P.C. (2003). Growth responses and survival of the gold fish, *Casassius auratus (Cyprinidae)* fry reared on *Moina (Cladocera)* and Cylops (*copepod). Journal of Aquatic Sciences, 18(1):* 43-46.

Chen, F., Xi, Y.L., Gc, Y.L. and Xu, X.P. (2005). Life history traits of mictic in two strains of *Brachionus calyciflorus* (Rotifera). *Journal of Freshwater Ecology, 20:* 321-326.

Cho, S.H., Hur, S.B., Jo, J.Y., (2001). Effect of enriched live feed on the survival and growth rates in the larval Korean rockfish, *Sebates schlegeli. Aquaculture Research, 32(3):* 199-208.

Clare, J.P. (2002). *Daphnia, Aquarist guide.* Pennak Robert Freshwater Invertebrates of United States of America. 20pp.

Creswell, R.I. (1993). *Aquaculture Desk Reference*. AVI Book, New York. 206pp.

Dabrowski, K. and Rusiecki, M. (1983). Content of total and free amino acids in zooplanktonic food of fish larvae. *Aquaculture, 30:* 31-42.

Delbare, D. and Dhert, P. (1996). Cladocerans, Nematodes and Trochophora larvae. In manure on the production and use of live food for aquaculture (Lavens, P. and Sorgeloos, P., eds) *FAO Fisheries Technical paper Number* 361, 295pp.

Dhert, P. (1996). Use of On-grown artemia in nursery culture of tiger shrimp. *Aquaculture International. 1*:170-177.

Devetter, M. (1998). Influence of environmental factors on the rotifer assemblage in an artificial lake, *Hydrobiologia, 387–388***:** 171–178.

El-gamal, A.E. (2009). Effect of Temperature on Hatching and Larval Development and Mucin Secretion in Common Carp, *Cyprinus carpio* (Linnaeus, 1758). *Global Veterinaria 3 (2)*: 80-90

FAO (1996). Manual on production and use of live food for aquaculture. *FAO Fisheries Technical Paper* No. 361. Rome. 295p.

Fernando, C., Paggi, J. and Rajapaksa, R. (1987). *Daphnia* in tropical lowlands.—In: R. H. Peters & R. De Bernardi (eds.), *Daphnia, vol. 45*, 107–141.

Fernando, C.H. (1994). *Zooplankton*, Fish and Fisheries in the Tropical Freshwater. *Hydrobiologia, 272:* 105-123.

Frank, M. (2002). The Rotifer and Rotifer Home Culture. The Breeder's Net. Advance Aquarists On line Magazine. http://www.advancedaquarists.com/index.htm on 26th March, 2008.

Gaitonde, M.K. (1967). A spectrophotometric method for the direct determination of Cysteine and Tryptophan in protein. *BiochemistryJournal, 104*: 627 - 1633.

Garza – Mourino, G., Silva-Briano, M., Nandini, S., Sarma, S.S.S. and Castellanos – Paez M.E. (2005). Morphoogical and Morphometrical variations

of selected brachionid species from Lake Xochimilo (Mexico) *Hydrobiologia, 546*:169-179.

Golder, D., Rana, S., Sarkar, D. and Jana, B.B. (2007). Human urine is an excellent liquid waste for the culture of fish food orgamism, *Moina micrura. Ecological Engineering, 30*: 326-332.

Gupta, S.K. and Gupta, P.C. (2006). *General and Applied Ichthyology (Fish and Fisheries).* S. Chand and Company Ltd. 7361, Ram Nagar, New Delhi, 110055. 1130 Pp.

Hagiwara, A. (1989). Recent studies on the rotifer *(Brachionus plicatilis* as a live food for the larval rearing in marine Fish. *Lake Marine*. *27*:116-121.

Hamel, P., Mangon, P., East, P., Lapointe, M. and Laurendeau, P. (1997). Comparison of different models to protect in the situ embryonic development rate of fish with special reference to white sucker (*Catostomus commersoni*): *Canadian Journal of Fisheries Aquatic Sciences, 54*: 190-197.

Hamffa, M.A.K. and Sridhar, S. (2002). Induced spawning of spotted murrel (*Channa funtatu*) and catfish *Heteropneoustes fossilis* using human chorionic gonadotropin and synthetic hormone (ovaprim) *Veterinary Archives 72 (1)*: 77.

Haoyuan, H. and Yilong, X. (2008). Demographic parameters and mixis of three *Brachionus anguillaris* Gosse (Rotatoria) strains fed on different algae *Limnologica - Ecology and Management of Inland Waters, 38, (1)*: *56-62.*

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

Hoff, F.H. and Snell, T.W. (1987). *Plankton Culture Manual*. First edition, Florida Aqua Farms, Inc., Florida, U.S.A. 126pp.

Jana, B. B. and Chakrabarti, R. (1993). Life table responses of zooplankton (*Moina micrura* and *Daphnia carinata*) to manure application in culture system. *Aquaculture, 117*: 273-285.

Javellana, S. and Escritor, F. (1981). Culture of *Brachionus plicatilis* SE.AFDEC Aquaculture Department, Natural Fish Food Project, Tigbaum, Iloilo Philippines. Paper No. SCS/GFO/81/PE-8. 23-28pp.

Karl, F.L., John, E.B., Robert, R.M. and Dora, R.M.P. (1977). *Ichthyology*. 2nd Edition John Wilery and Sons. Inc. pp 403

Khatun, M., Huque, K.S., Chowdhury, S.A. and Nahar, Q. (1994). *Chlorella* and *Scenedesmus* : Isolation, identification and mass cultivation for feeding cattle. In : A Report on The Use of Algae as Potential Feed supplements For Cattle. Bangladesh Livestock Research Institute, Savar, Dhaka 1341, Bangladesh, pp 1-8.

Kim, D., Kim, T.S., Ryu, H.D. and Lee, S.I. (2008). Treatment of low carbon-to-nitrogen waste water using two-stage sequencing batch reactor with independent nitrification. *Process Biochemistry, 43*: 406-413.

Kitto, M.R. and Bechara, G.P. (2004). Business Agriculture in Kuwait, challenges and solutions. *World Aquaculture, 35(2)*: p56.

Koste, W. and Shiel, R.J. (1987). Rotifera from Australian Inland waters 2 *Ephiphanidae* and *Brachionidae* (*Rotifera*: Monogononta Invertebrate) *Taxonomy* **7**:949-1021.

Lauridsen, T. and Lodge, D. M. (1996). Avoidance by *Daphnia magna* of fish and macrophytes: Chemical cues and predator-mediated use of macrophyte habitat.

Limnology and Oceanography 41: 794–798.

Lim, L.C., Dhert P. and Sorgeloos, P. (2003). Recent Developments in the application of live feeds in the freshwater ornamental fish culture. *Aquaculture, 227*: 319-331.

Lim, L.C. and Wong, C.C. (1997). Use of the rotifer, *Brachionus calciflorus* Pallas, in freshwater ornamental fish larviculture *Hydrobiologia, 358 (1/3)*: 273 (5).

Loh, J.Y., How, C.W., Hii,Y.S., Khoo, G. and Ong, H.K.A. (2009). Fish faeces as a food Source for cultivating the water flea, *Moina macrocopa* potential. *Journal of Science and Technology in the Tropics, 5*:5-9.

Lokman, H.S. (1994). Lipid and fatty acid composition of indigenous zooplankton from Terengganu Waters of the South China, *Journal of Aquaculture Tropical.* 9: 291-196.

Ludwig, G.M. (1993). Effects of Trichlorfon, Fenthion and Diflubenzuron on the zooplankton community and on the production of the reciprocal-cross hybrid stripped bass fry in culture ponds. *Aquaculture, 110*: 301-319.

Lynch, M. (1980). The evolution of *Cladoceran* life histories. *Quaterly Review of Biology. 55*: 23-42.

Madu, C.T. (1995). The Status of Fish hatcheries and fish seed (fingerlings) production in Nigeria. In: Report of National Aquaculture Diagnostic Survey. May-July 1994 (J.S.O Ayeni E.D.) National Institute for Freshwater Fisheries Research, New Bussa, Nigeria pp. 13-34.

Madu, C.T. and Ufodike, E.B.C. (2001). Growth and Survival of Mudfish (*Clarias*

anguillaries) hatchling fed various natural and artificial feeds as test starter diets for indoor nursery management. *Journal of Aquatic Sciences, 16*: (2), 127-131.

Madu, C.T., Ufodike, E.B.C. and Ita, E.O. (1990). Food and Feeding habit of hatchling of the Mudfish *Clarias angullaries* (L.) *Journal of Aquatic Sciences, 5*: 27-31.

Madu, C.T., Okwuego, C.C. and. Madu, I.D. (2003). Optimum Dietary Protein Level for growth and gonadal maturation of female *Heterobranchus longifilis* (Velencciennes, 1840). Broodstock. *Journal of Aquatic Sciences, 18 (18)*: 29-34.

Martínez-Jerónimo, F., Rodríguez-Estrada, J. and Villaseñor-Córdova, R. (2007). Effect of culture density and volume on *Moina micrura* (Kurz, 1874) reproduction, and sex ratio in the progeny. *Hydrobiologia, 594, (1)* 69-73

Mims, S.D., Webster, C.D., Tidwell, J.H. and Yancey, D.H. (1991). Fatty acid Composition of *Daphnia pulex* cultured by two different methods *Journal of Aquacultural Society, 22 (2)*: 53-156.

Morten, O. A., Torodd, T., Jo, A. A. and Gunvor, O. (*2007).* Automatic measurement of Rotifer *Brachionus plicatilis* densities in first feeding tanks. *Aquacultural Engineering, 36*: 115-121

Munilla – Moran, R., Stark, J.R. and Barbour, A. (1990). The role of exogenous enzymes in digestion in cultured larvae (*Scophthalmus maximus* (L.) *Aquaculture, 88*: 337-350.

Murphy, S. (2008). Water quality parameters. Retrieved in August 12 , 2009 from htt://www.bcn.boulder.co.us/basin/natural/wqterms.html

Naeem, M., Zuberi, A., Salam, A., Ashraf, M., Elahi, N., Ali, M., Ishtiaq, A., Malik1,

T., Khan, M. J., Ayaz, M. M., Iqbal, M. J. and Ahmad, B. (2011). Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim–C at a fish hatchery Faisalabad, Pakistan. *African Journal of Biotechnology 10(53)*, 11048-11053.

Nagata, T. and Hanazato, T. (2006). Different predation impacts of two cyclopoid species on a small-sized zooplankton community: an experimental analysis with mesocosms, *Hydrobiologia, 556*: 233–242.

Nita, T. (1981). Work on the Food Organisms at Sukabumi Freshwater Aquaculture Development Centre and other freshwater in Indonesia. Training Course on Growing of Live Food Organisms, 14-15.

Nogrady, T. (1993). Rotifera. *SPB Academic Publishing*, The Hague, The Netherlands. 13-46.

Nwosu, F.M. and Holzlohnev, S. (2000). Influence of temperature on eggs hatching, growth and survival of larvae of *Heterobranchus longifilis*. (Teleostei: Clariidae). *Journal of Applied Ichthyology*, *16*: 20-23.

Odedeyi, D.O. (2007). Survival and growth of hybrid (female) *Clarias gariepinus* (B) and male Heterobranchus longifilis (val) fingerlings: Effect of broodstock sizes. *American-Eurasian Journal of Scientific Research 2(1)*: 19-23.

Okunsebor, S.A. (2012). Effects of Concentrations of Different Combinations of Manure Culture Medium and Period of Growth on Population Density of *Brachionus calyciflorus. International Journal of Science and Advanced Technology*, 5 (2): 8-16.

Okunsebor, S.A. and Ofojekwu, P.C. (2009). Effects of Different Concentrations of Manure Combinations and Period of Growth on Mass Production of *Moina micrura. Production Agriculture and Technology 5(1) 103-115.*

Okunsebor, S. A. and Ofojekwu, P. C. **(**2012**).** Mass Production of *Moina Micrura* through Manipulation of Concentration, Combinations of Manure and Period of Growth in Laboratory. *European Journal of Scientific Research 83 (4)*: 576 - 589

Okunsebor, S.A. and Sotolu, A.O. (2012). Effects of concentrations, different combination of manures and period of growth on mass production of *Daphnia pulex* . Research Opinions in Animal and Veterinary Sciences, *2(4)*: 299-308.

Okunsebor, S.A., Kigbu A. and Isa H. (2008). Survival and Total Body Length of *Clarias gariepinus* hatchlings fed cultured *Moina micrura* /Shell Free *Artemia* as starter feed. *Production Agriculture and Technology, 4(2)*: 220-227.

Olojo, E.A.A., Olurin, K.B. and Osikoya, O.J. (2003). Food and feeding habits of *Synodontis nigrita* from the Osun River, South West, Nigeria. *NAGA World fish Centre Quarterly* volume *26(4)* Oct.-Dec. 2003. pp. 21-24.

Ovie, S.I. (1991). Inter and intra specific zooplankton predation: some evidence from Field analysis of the zooplankton community of Round Valley Reservoir Hunterdon Country, New Jersey, U.S.A. *Journal of Aquatic Sciences 6*:39-43

Ovie, S.I. (1995). Natural live fish food production and the use in fish hatcheries in Nigeria. In: Report S.O. Ayeni, (ed.) May-July 1994. *National Institute for Freshwater Fisheries Research, New Bussa, Niger State Nigeria*. pp 35-40.

Ovie, S.I., Adeniji, H.A. and Olowe, D.I. (1993). Isolation and growth curve characteristics of a freshwater zooplankton for feeding early larval and fry stages

of fish. *Journal of Aquaculture in the Tropics, 8*: 187-196.

Ovie, S.I. and Eborge, A.B.M. (2002). The effect of different algal densities of *Scenedesmus acuminatus* on the population growth of *Moina micrura* Kurz *Hydrobiologia, 477*: 41-45.

Ovie, S.I. and Ovie, S.O. (2004). Effect of aeration on brood production and size of *Moina micrura* (Kurz, 1874) (Crustacea; *Anomopoda*). *Journal of Aquatic Sciences. 19(2)*: 107-112.

Ovie, S.I. and Ovie, S.O. (2006). Moisture, protein and amino acid contents of three freshwater zooplankton used as feed for aquaculture larvae and post larvae. *The Israeli Journal of Aquaculture – Bamidgeh, 58(1)***:** 29-33.

Ovie, S.I. and Sarma, S.S.S. (1993). Rotifer Fauna (*Rotifera*) of Asa Lake Nigeria, West Africa. *Environment and Ecology, 11(4)*: 842-849.

Pennak, R.W. (1978). *Freshwater Invertebrates of the United States,* Second Edition. John Wiley and Sons, New York. 803 pp.

Reed, W.J., Burchard, A.J., Hopson, A.J. and Yaro, I. (1967). *Fish and Fisheries of Northern Nigeria*. Published by Ministry of Agriculture, Ist Ed. 226p

Ricardo, C.D. (1981). A programme on growing food organisms for Leyte Freshwater Fish Hatchery Babatngon, Leyte, Philippines. Training Course on Growing Food Organisms for Fish Hatcheries. Pp 23-24.

Rodolfo, F.V. and Edmundo M.E. (1980). Preliminary studies on *Moina* species Production in freshwater tanks. *Aquaculture, 21***:** 93-96.

Rottmann, R.W., Graves, S. J., Watson, C. and Yanong, R. P.E. (2003). Culture

Techniques of Moina. Circular 1054, Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida. 1-9pp.

Saint-Jean, L. and Bonou, C.A. (1994). Growth, production and demography of *Moina micrura* in brackish tropical fishponds (Layo, Ivory Coast) *Hydrobiologia, 272 (1-3)* : 125-146

Sharma, R., Singh, G.P. and Sharma, V.K. (2012). Effects of Culture Conditions on Growth and Biochemical Profile of *Chlorella Vulgaris*. *Journal of Plant Pathology and Microbiology*, *3*:131. doi:10.4172/2157-7471.1000131.

Sipaúba-Tavares, L. H. and Bachion, M. A. (2002). Population growth and development of two species of Cladocera, *Moina micrura* and *Diaphanosoma birgei*, in laboratory *Brazilian Journal of Biology, 62(4)*: 20pp.

Sipaúba-Tavares, L. H. and Braga, F. S. (1999). Study on feeding habits of *Piaractus mesopotamicus* larvae in fish pond. *Naga. The ICLARM Quarterly, 22*: 24- 30.

Sorgeloos, P. and Beardmore, J.A. (1995). Correct taxonomic identification of Artemia species. *Aquaculture Nutrition, 1*: 67.

Spackman, D.H., Stein, E.H. and Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids . *Analytical Chemistry, 30: 1190-1191*.

Spenelli, J. (1979). Preparation of Salmonid diets containing zooplankton and their effect or organoleptic properties of pen-reared salmonids. *In:* J.E. Halver and K. Tiews (Eds) *Proceedings of the World Symposium on finfish Nutrition and fishfeed Technology,* Hamburg 20-23 June 1978, Berlin *2*: 383-392

Srivastava, A., Rathore, R. M. and Chakrabarti, R . (2006). Effects of four different

doses of organic manures in the production of *Ceriodaphnia cornuta*. *Bioresource Technology, 97*: 1036-1040.

Tacon, A.G.J. (1990). *Standard methods for the nutrition and feeding of farmed fish and shrimp,* Washington DC, Argent Laboratories Press, 454 pp.

Tech, E. (1981). Culture of zooplankton (*Brachionus* and *Moina)* Tigbauan Research Station, SEAFDEC, Aquaculture Department Tigbaun, Iloilo, Philippines. Pp. 22-26.

Telesh, I.V. (1993). The effect of fish on planktonic rotifers, *Hydrobiologia, 255/256*: 289–296.

Venture, R.F. and Enderez, E.M. (1980). Preliminary studies on *Moina* species Production in freshwater tanks. *Aquaculture, 21*: 93-96.

Villegas, C.T. (1981). Culture and screening of food organisms as potential larval food for finfish and shellfish. Tigbauan Research Station, SEAFDEC, Aquaculture Department Tigbaun, Iloilo, Philippines. Pp. 1-12.

Wagner, R. (2007) Water flea. http://www.dr_wagner. De/wasserfloehe-english.html

Wang, S., Xie, P. and Geng, H. (2010*).* The relative importance of physicochemical factors and crustacean zooplankton as determinants of rotifer density and species distribution in lakes adjacent to the Yangtze River, China *Limnologica - Ecology and Management of Inland Waters, 40(1)*: *1-7.*

Wang, S., Xie, P., Wu, S. and. Wu, A. (2007a). Crustacean zooplankton distribution patterns and their biomass as related to trophic indicators of 29 shallow subtropical lakes, *Limnologica, 37 (3)*: 242–249.

Watanabe, T., Kilajima, T.C. and Fujita, S. (1983). Nutritional values of live organisms

used in Japan for Mass propagation of fish. A review. *Aquaculture, 34:* 115-143.

Watanabe, T. and Kiron, V. (1994). Prospects in larval fish dietetics. *Aquaculture, 124*:223-251.

Yoshida, T., Urabe, J. and Elser, J. (2003). Assessment of "top-down" and "bottom-up" forces as determinants of rotifer distribution among lakes in Ontario, Canada, *Ecological Research., 18*: 639–665.

Yurkowski, M. and Tabachek, J.L. (1979). Proximate and amino acid composition of some natural fish foods. In J.E. Halver and K. Tiews (Eds). *Proceeding of World Symposium on Finfish Nutrition and Fish Feed Technology, Hamburg* 20-23 June 1978 Berlin *2*: 435-448.

Appendix 1: Water Parameters Distribution in Sampling Zones of Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

		Weeks					
pH	Zones	1	2	3	4	5	SEM
,,	Littoral	6.57	6.73	6.72	6.81	6.56	0.05
,,	LImnetic	6.56	6.61	6.76	6.71	6.53	0.05
,,	Benthic	6.68	6.69	6.80	6.86	6.59	0.03
		^{o}C	^{o}C	^{o}C	^{o}C	^{o}C	
Temperature	Littoral	27.37	27.70	27.77	27.67	27.63	0.07
,,	LImnetic	26.67	26.57	26.50	26.60	26.63	0.03
,,	Benthic	26.70	26.43	26.40	26.57	26.37	0.15
		mg/L	mg/L	mg/L	mg/L	mg/L	
Carbondioxide	Littoral	4.33	4.00	4.33	4.67	3.33	0.23
,,	LImnetic	3.33	3.67	3.33	4.00	4.33	0.19
,,	Benthic	4.67	4.67	4.67	5.33	4.67	0.13
		mg/L	mg/L	mg/L	mg/L	mg/L	
Desolved oxygen	Littoral	7.67	7.33	8.67	8.50	7.33	0.29
,,	Limnetic	7.83	7.33	7.50	7.67	8.50	0.20
,,	Benthic	6.83	6.83	6.33	6.83	6.67	0.10
		mg/L	mg/L	mg/L	mg/L	mg/L	
Total alkalinity	Littoral	25.33	25.33	25.33	25.36	25.33	0.11
,,	LImnetic	25.32	25.35	25.34	25.37	25.35	0.23
,,	Benthic	25.34	25.34	25.35	25.37	25.34	0.12

pH = Hydrogen ion concentration, Temp = Temperature, CO_2 = Carbon dioxide, DO = Dissolved Oxygen, T. Alk. = Total Alkalanity.

Appendix 2: Various Live Food Zooplankton Distribution in Awuma River at Shabu in Lafia North Development Area of Nasarawa State.

		Weeks					SEM
Zones	zooplankton	1	2	3	4	5	
Littoral	bc	10	11	12	13	12	0.50
	cc	4	4	3	4	3	0.24
	cb	10	8	7	8	9	0.55
	dp	3	2	3	5	5	0.51
	da	3	5	5	6	4	0.39
	mm	5	5	5	6	6	0.16
	pc	25	26	27	28	28	0.50
Pelagic	bc	4	4	4	4	4	0.19
	cc	2	3	3	3	3	0.19
	cb	9	11	10	8	10	0.40
	dp	2	1	2	2	2	0.12
	da	2	3	2	2	3	0.23
	mm	2	1	3	2	3	0.33
	pc	24	25	23	28	27	0.92
Benthic	bc	3	2	3	1	2	0.37
	cc	4	4	3	4	5	0.29
	cb	7	8	7	7	7	0.13
	dp	2	2	3	4	4	0.31
	da	2	2	3	3	4	0.40
	mm	4	3	4	4	4	0.20
	pc	26	25	27	27	27	0.43

Bc = *Brochionus calyciflorus*, cc = *Ceriodaphnia cornuta*, cb = *Cyclop bicuspidatus*, dp = *Daphnia pulex*, da = *daphanosoma aspiranum*, mm = *Moina micrura*, pc = *paramecium caudatum*, *SEM = Standard error of mean.*

Appendix 3: Anova on Population Density of *Brachionus calyciflorus* Fed on Manure in Solution / *Chlorella vulgaris*.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.56	0.78	0.08	
Rep.*Units* stratum					
Zooplankton	1	1613.36	1613.36	157.21	<.001
Day	5	153411.14	30682.23	2989.71	<.001
Zooplankton.day	5	22559.14	4511.83	439.64	<.001
Residual	22	225.78	10.26		
Total	35	177810.97			

Appendix 4: Population density of *Moina micrura* Fed on Manure in Solution / *Chlorella vulgaris*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.078E+02	1.039E+02	0.70	
Rep.*Units* stratum					
Zooplankton	1	5.562E+08	5.562E+08	3.754E+06	<.001
Day	6	4.293E+09	7.155E+08	4.829E+06	<.001
Zooplankton.day	6	1.664E+09	2.773E+08	1.872E+06	<.001
Residual	26	3.853E+03	1.482E+02		
Total	41	6.514E+09			

Appendix 5: Population Density of *Brachionus calyciflorus* Fed on five combinations of Manure Solution

	MANURE COMBINATIONS			
	5CM1	5CM2	5CM3	5CM4
1	10	10	10	10
3	85.73	83.54	77.67	23
5	197.13	190.73	109.4	104.33
7	224.33	212.12	188.8	170.33
9	57.07	94.25	164.93	174

Appendix 6: Anova on Effect of Concentration, three different combinations of Manure and

Period of Growth on Population Density of *Moina micrura* on Day 11.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.056E+02	5.282E+01	0.07	
Rep.*Units* stratum					
Manure	3	2.033E+09	6.777E+008	9.34E+05	<.001
Conc.	4	5.013E+08	1.253E+08	1.73E+05	<.001
Manure.Conc	12	3.777E+08	3.147E+07	4.34E+04	<.001
Residual	38	2.757E+04	7.225E+02		
Total	59	2.912E+09			

Conc = concentration.

Appendix 7: Anova on Effect of Concentration, fourdifferent combinations of Manure and
Period of Growth on Population Density of *Moina micrura*.

Source of variation	d.f.	s.s.	m.s	v.r.	F pr.
rep stratum	2	4.820E+03	2.410E+03	2.44	
rep.*Units* stratum					
conc	4	3.892E+09	9.730E+08	9.85E+05	<.001
manure	3	4.631E+09	1.544E+09	1.56E+06	<.001
days	10	8.646E+10	8.646E+09	8.75E+06	<.001
conc.manure	12	1.513E+09	1.261E+08	1.28E+05	<.001
conc.days	40	5.281E+09	1.320E+08	1.34E+05	<.001
manure.days	30	6.878E+09	2.293E+08	2.32E+05	<.001
conc.manure.days	120	2.552E+09	2.127E+07	2.15E+04	<.001
Residual	438	4.327E+05	9.880E+02		
Total	659	1.112E+11			

Conc = concentration.

Appendix 8: Anova on Effect of Concentration, five different combinations of Manure and Period of Growth on Population Density of *Moina micrura*.

Source of variation	d.f.	s.s.	m.s.	v.r	F pr.
Rep stratum	2	7.948E+03	3.974E+03	4.14	
Rep.*Units* stratum					
Manure	3	1.331E+09	4.437E+08	4.62E+05	<.001
Conc.	4	5.578E+09	1.395E+09	1.45E+06	<.001
Days	10	7.807E+10	7.807E+09	8.13E+06	<.001
Manure.conc.	12	2.010E+09	1.675E+08	1.74E+05	<.001
Manure.days	30	1.506E+09	5.019E+07	5.23E+04	<.001
Conc.days	40	1.175E+10	2.937E+08	3.06E+05	<.001
Manure.conc.days	120	4.364E+09	3.637E+07	3.79E+04	<.001
Residual	438	4.206E+05	9.603E+02		
Total	659	1.046E+11			

Conc = concentration.

Appendix 9: Effect of Period of Growth on Population Density of *Moina micrura* Treated with 3, 4, 5 Different Combinations of Manure.

Combination of manure	Days										
	1	2	3	4	5	6	7	8	9	10	11
3CM	20	36	173	650	1943	5473	11787	18691	25195	22023	12574
4CM	20	36	155	664	2036	5273	13776	22528	30335	29595	12930
5CM	20	35	123	592	1764	6253	11435	19842	31794	24693	16750

CM = Combinations of Manure

Appendix 10: Effect of Period of Growth on Population Density of *Daphnia pulex* Treated with 3, 4, 5 Different Combinations of Manure.

Combination of manure	Days											
	1	2	3	4	5	6	7	8	9	10	11	12
3CM	10	26	37	58	91	129	184	311	537	511	452	394
4CM	10	24	34	55	107	234	432	723	1652	1436	700	380
5CM	10	39	104	216	401	675	1214	2166	4806	4329	2180	1075

CM = Combinations of Manure

Appendix 11: Effect of Temperature on Percentage Fertilization, Hatchability *Heterobranchus bidorsalis* Eggs and Survival Rate of Hatchlings.

Parameters	Total eggs	Fertilized eggs	unfertilized eggs	% fertilized	Hatched eggs	% Hatched eggs	Survived hatchlings	% survived hatchlings
32 ^{o}C	500	380	120	76	195	51.31579	80	41.02564
30 ^{o}C	500	421	86	84.2	343	81.47268	308	89.79592
28 ^{o}C	500	414	79	82.8	354	85.50725	41	11.58192
26 ^{o}C	500	392	108	78.4	191	48.72449	10	5.235602

Appendix 12: Anova of Percentage Weigh Gain of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	853.3	426.7	0.44	
Rep.*Units* stratum					
Zooplankton	4	254800.0	63700.0	66.35	<.001
Residual	8	7680.0	960.0		
Total	14	263333.3			

Appendix 13: Anova on Total Body Length of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00001333	0.00000667	0.12	
Rep.*Units* stratum					
Zooplankton	4	0.00466667	0.00116667	20.59	<.001
Residual	8	0.00045333	0.00005667		
Total	14	0.00513333			

Appendix 14: Percentage (%) Survival Rate of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.933	0.467	0.41	
Rep.*Units* stratum					
Zooplankton	4	237.733	59.433	52.44	<.001
Residual	8	9.067	1.133		
Total	14	247.733			

Appendix 15: Condition Factor of *Heterobranchus bidorsalis* Fry Fed on Zooplankton Treatments for 16 Days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.05487	0.02743	0.81	
Rep.*Units* stratum					
Zooplankton	4	15.04434	3.76109	110.69	<.001
Residual	8	0.27183	0.03398		
Total	14	15.37104			

APPENDIX B

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Mass Production of *Moina Micrura* through Manipulation of Concentration, Combinations of Manure and Period of Growth in Laboratory

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Effects of Concentrations of Different Combinations of Manure Culture Medium and Period of Growth on Population Density of *Brachionus calycflorus.*

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