

High Density Culture of a Cladoceran *Moina micrura* (Kruz, 1874) by Using Poultry Manure

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Abstract. The branchipod *Moina micrura* was mass cultured using three different concentrations (250 ppm, 500 ppm, and 1000 ppm) of chicken manure in the medium for 21 days. In the first concentration (250 ppm), the maximum density (1694.44 ± 9.68) of *M. micrura* was obtained on the 9th day of inoculation and the number of plankton declined gradually afterwards. In the second concentration (500 ppm), the maximum density (7296.88 ± 54.35) appeared on the 11th day while in the third concentration (1000 ppm), the maximum density (1510 ± 15.27) was obtained on the 14th day of inoculation. Temperature range of 27-31 °C and pH of 6-7 was conducive for optimal growth of *M. micrura*.

Keywords: live feed, *Moina micrura*, mass culture, poultry manure

Introduction

Zooplankton is important as food for both young and adult fish (Altaff and Mehraj, 2010; Srivastava *et al.*, 2006; Pangano *et al.*, 2000). Zooplankton collected from natural resources are used as diet for fish and prawn larval rearing in many hatcheries although this is an unreliable source for commercial use according to quality Wild zooplankton may introduce harmful organisms to the hatcheries and hence mass culture under controlled conditions is desirable (Altaff *et al.*, 2002).

M. micrura is a cosmopolitan, cyclic parthenogenetic cladoceran with ample morphological and ecological plasticity, inhabiting temperate, tropical and sub tropical regions worldwide (Martínez-jerónimo *et al.*, 2007). Organic fertilizers are usually preferred on mineral fertilizers because organic fertilizers provide bacterial and fungal cells, detritus as well as phytoplankton as food for *Moina* (Rottmann *et al.*, 2003). Earlier Jana and Chakrabarthy (1993a) reported the presence of detritus in the gut of *M. micrura*. Since then different culture techniques have been developed to increase the yield of cladocerans by employing different waste organic products as food sources (Mehraj and Altaff, 2010; Shrivastava *et al.*, 2006; Sivakumar, 2005; Pagano *et al.*, 2000). Among various species of zooplankton, the genus *Moina* of the Cladocera is known to be suitable as initial feed for larval stages of many fishes. The cost of *Artemia* cyst is very high and its use in developing

countries may not be appropriate as its production requires some specialized facilities (Hung *et al.*, 1999).

Young *Moina* are less than 400 µm in size, approximately of the same size as that of the adult rotifers and smaller than newly hatched brine shrimps, which die comparatively earlier in freshwater when fed to the fishes. *Moina* can be stored for longer periods by freezing in low salinity water and can be kept alive for several days in the refrigerator for use as live feed of fish larvae. High temperature tolerance of *Moina* is also of great advantage for both commercial fish farmers and hobbyists culturing live feed at home. *Moina* that are cultured in water enriched with organic manures are fed to bigger fish or brooders (Rottmann *et al.*, 2003). Unfortunately, there is little information available concerning practical mass culture methods of *M. micrura* and the literature on tropical and subtropical zooplankton feeding is very scarce (Pangano, 2008). Animal manures like, cattle manure, poultry droppings and agricultural by-products are wastes worldwide and can be used as potential organic manures to increase the productivity of water bodies.

Poultry manure has many advantages for fertilizing culture media when compared to other manures and algae (Altaff and Mehraj, 2010; Shrivastava *et al.*, 2006). Furthermore, the high price of *Artemia* cysts has increased the fish production cost, therefore cheaper alternative diets with comparable nutritional quality are needed to maintain the competitiveness of ornamental fish on global market (Altaff and Mehraj, 2010; Kumar *et al.*, 2005).

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In this context experiments were carried out on mass culture of *M. micrura* using poultry excreta, a waste which will make it a cheap and easily available food source to the fish culturists.

Materials and Methods

Chicken manure was collected from a local broiler chicken shop and was dried for 2 days to remove the moisture and stored in plastic jars for further use. Manure was micronized by grinding and the required quantity was dissolved in distilled water to get suspensions of 250, 500 and 1000 ppm for fertilizing culture medium. Micronisation of poultry waste is necessary for efficient filtration of the suspended particles. Zooplankton samples were collected from Chetpet freshwater pond, Chennai, India and were brought to the laboratory with the least disturbance. The adult *M. micrura* were identified and separated using binocular dissection microscope based on the key characters outlined by Altaff (2004).

Experimental aquarium tanks of 50 L capacity arranged in triplicate were filled with 40 L of filtered water and fertilized with chicken manure at the concentrations of 250, 500 and 1000 ppm. After 3 days, *M. micrura* were inoculated in each experimental tank at the density of 50 ± 5 Ind/L containing both adults and neonates. The culture experiment was conducted for 21 days. Water change was carried out at 3 day interval by removing 50% of the water throughout the culture period. Food was administered as a function of population density every 3rd day using the formula of Altaff and Mehraj (2010):

$$Y = [(\log 10^N/10) - 0.2] \times V \times d.$$

where:

- Y = quantity of chicken manure
- N = population density (Ind/L)
- V = volume of culture (L)
- d = no. of days for which the food was administered.

The culture water used in all experiments was filtered tap water, previously aerated for 24 h to dechlorinate the water. To avoid anaerobic conditions in the medium, the sediments (unconsumed food, faeces, and pseudo-faeces) were siphoned out from the bottom three times a week. Excessive fouling was also removed from the walls of the tanks. Wet weight of the animals was determined after draining 10 L of the culture medium

over a nylon gauze of 200 μ m mesh size and washed several times to remove other debris. The remaining water was absorbed using tissue paper and the animals were weighed on a digital balance with 1 μ g sensitivity. Population density was estimated by counting samples, taken at random with a 1 L beaker, after mixing thoroughly the culture volume. Sub-samples of 100 mL and then 10 mL were drawn from these samples. Samples were immobilized using alcohol and counting was carried out using Sedgwick Rafter cell under binocular dissection microscope. Results were expressed as number of individuals per litre (Ind/L).

Results and Discussion

In the first concentration (250 ppm), the peak density (1694.44 ± 9.68) of *M. micrura* was obtained on the 9th day of inoculation and the number of plankton declined gradually afterwards. In the second concentration (500 ppm), the peak density (7296.88 ± 54.35) appeared on the 11th day while in the third concentration (1000 ppm) peak density (1510 ± 15.27) was obtained on the 14th day of inoculation. The lowest density was observed in the tanks fertilized with 1000 ppm concentration (1510 ± 15.27). Thus the concentration of 500 ppm chicken manure was found to be more suitable for obtaining high density culture of *M. micrura* than the other concentrations used (Fig. 1). The density (Ind./mL) of protozoan population during different days in the culture medium fertilized with different concentrations of poultry manure is shown in Fig. 2. Higher wet weight of *M. micrura* biomass (mg) was obtained on the 11th day of the culture (Fig. 3).

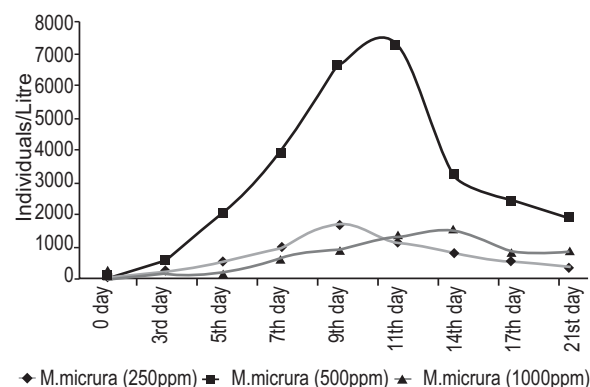


Fig. 1. Population density of *M. micrura* (Ind./mL) during different days in the culture medium fertilized with different concentrations of poultry excreta (Mean \pm SE).

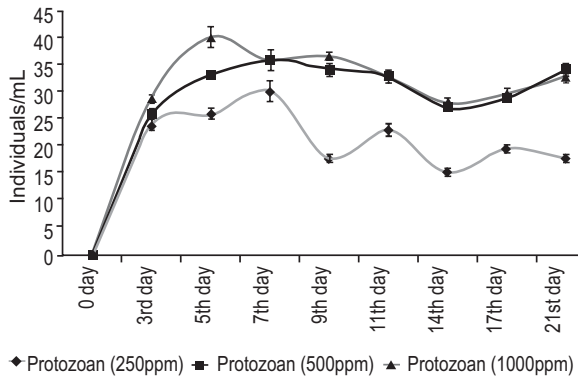


Fig. 2. Protozoan population density (Ind/mL) during different days in the culture medium fertilized with different concentrations of poultry manure (Mean \pm SE).

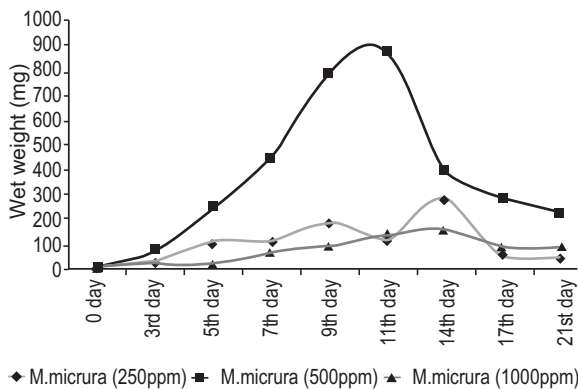


Fig. 3. Wet weight of *M. micrura* biomass (mg) during different days in the culture medium fertilized with different concentrations of poultry excreta (Mean \pm SE).

In the present experiment, higher production of *M. micrura* was obtained compared to 1050-2600 Ind/L reported by Punia (1988) and 2500-3500 Ind/L, by Majumdar and Nandy (1989). The dynamic characteristics of zooplankton have led to the use of particular fertilization techniques and species-specific inoculation in culture ponds (Mehraj and Altaff, 2010; Shrivastava *et al.*, 2006). The food supply could be a limiting factor in this type of culture due to high filtering rate. Organic fertilization introduced a complex food web in the fish pond with higher intensity of bacterial activity through the detrital food chain (Safiullah and Altaff, 2002). The nutrients beyond the optimum level reduced population of *Moina*, which may be due to the rapid degradation of high nutrient content in the medium, resulting in increased production of ammonia and growth of pathogenic microbes resulting in unfavorable conditions

for the growth of population (Adeyemo *et al.*, 1994). Boyd and Doyle (1984) Anuta (1992) and reported that high input of chicken manure and commercial feed depressed dissolved oxygen for a prolonged time and water quality deteriorated in the culture resulting in suppressed growth and mortality of the cultured organisms. Similarly, concentration of nutrients below the optimum level also resulted in reduced populations of *M. micrura* which may be due to insufficient microbial and protozoan populations and lack of sufficient organic compounds, required for the growth and survival of live food organisms. Safiullah and Altaff (2002) also reported higher density of *M. micrura* at 500 ppm concentration using different nutrient media. Ray and David (1969) opined that chicken manure-fertilized medium produced a large population of cladocerans quicker than cattle manure dosages. The influence of food on the population density of Cladocera has been reported (Srivastava *et al.*, 2006; Jana and Chakrabarthy, 1997; Boersma and Vijverberg, 1996). Usually when environmental conditions are favourable, the progeny is constituted of parthenogenetic females which are clones of mother. When the environmental conditions become unfavorable, sexual reproduction becomes effective, leading to the production of males and females, eventually leading to the formation of resting eggs that enter into diapause, until the conditions become favourable again (Martinez-jerónimo *et al.*, 2007). Gradual decrease after attaining a maximum density may be due to the indirect effects caused by high population densities such as modifying feeding conditions by releasing and accumulating chemical substances or through behavioral indications.

Dissolved oxygen content of the culture medium is another important factor for population growth. In the present study in all culture media, dissolved oxygen level of 4 ± 1 mg/L was maintained through aeration and fertilization of the medium. Oxygenation of the medium was assured by air bubbling from the bottom of the culture; aeration also ensured a more regular distribution of the population over the whole volume and prevented stratification (Rottmann *et al.*, 2003). Several authors have stressed that aeration is an important culturing parameter and some studies showed that dissolved oxygen decreased with organic loads of the media (Sivakumar, 2005; Tay *et al.*, 1991). An absolute prerequisite to maintain cultures is to renew parts of the culture water at regular intervals, to ensure permanent good water quality, thereby providing a favourable environment for population growth in higher densities.

The culture results of Punia (1988) showed a higher density of 1050-2600 Ind/L using ten different raw materials. In the present study a higher density of *M. micrura* population was recorded compared to previous reports (7296.88 ± 54.35) on 11th day of culture in tanks fertilized with 500 ppm of chicken manure. Punia (1988) reported that *D. lumholtzi* in outdoor culture using small containers, produced about 6000 Ind/L. It appears that with proper standardization it is quite possible to culture high density of Cladocera with cheaper organic materials such as agro-industrial waste and manure. Bonou and Saint-Jean (1998) indicated significant correlation in biomass of *M. micrura* and the initial chlorophyll concentration, which suggests that *M. micrura* population cannot be predicted in the availability of algal food alone.

The physico-chemical parameters appear to play an important role in the successful culture of Cladocera. Tay *et al.* (1991) did not report any relationship between physico-chemical parameters and physiological processes in zooplankton. Shrigur and Indulkar (1987) had proposed a range of water temperature between 27-31 °C for optimum growth of *M. micrura*. In the present study, a temperature range of 27-31 °C produced optimum density of cladocerans. Tay *et al.* (1991) reported the upper lethal limit close to 40 °C where 50% mortality of cladocerans occurred.

Moina micrura can be grown in high densities on optimal quantities of chicken manure. Overfeeding causes high mortality due to unfavorable conditions for culture. This implies that zooplankton with similar feeding modes, can be limited by different factors of their food, which partly explains why so many different organisms are found in the plankton (Hutchinson, 1961). The feeding experiments conducted on *M. micrura* revealed that the feed concentration plays a decisive role in obtaining the maximum production and this is in accordance with the studies conducted by Safiullah and Altaff (2002); Okauchi (1991) and Yufera *et al.* (1983). Chicken manure is an excellent food and has many advantages in comparison to other foods, it is available in large quantities; it can be purchased easily at low price; can be used directly after drying; it can be stored for longer periods of time; and it has none of the problems involved in maintenance of algal stocks and cultures. Dried algae are in some cases also an excellent food but they are too expensive to be used at large scale (Altaff and Mehraj, 2010). In the present study high density of *M. micrura* was obtained, which is commercially viable, especially in the present scenario of organic farming where production of live feed organisms

using manures is of considerable importance in order to reduce the cost of seed production and to produce large number of quality seeds. Moreover, the densities obtained at different days at different concentrations are of aquacultural importance, the doses can be manipulated, depending upon the requirement of the aquaculturists.

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