Asian J. Exp. Sci., Vol. 34, No. 2, 2020; 7-11

Online available on : www.ajesjournal.com, ISSN : 0971-5444

Mass Culture of Cladocerans, *Diaphanasoma sarsi* and *Ceriodaphnia cornuta* Using Chicken Manure

Sivakumar Kandasamy*, Muthupriya Palanichamy and Shree Rama Mani
Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology
GST Road, Chinna Kolambakkam, Padalam – 603308,
Madhuranthagam (Tk.), Chengalpet (Dt.), Tamilnadu
*Corresponding Author : ksivakumar76@gmail.com
Received: April 4, 2020; Revised: June 1, 2020; Accepted: June 05, 2020

Abstract : Cladocerans are important live food organisms for rearing of early stages of fishes and prawns. Considering this aspect, the present study was aimed to culture of *Diaphanasoma sarsi* and *Ceriodaphnia cornuta* using chicken manure carried out for 21 days. Microbial population (bacteria, algae and protozoa) of *D. sarsi* culture medium was high density on 11th day of culture period, whereas in *C. cornuta* culture medium bacteria (2453.00±52.51 cfu/ml), algae (3.92±0.06 x 10^4cells/ml) and Protozoa (48.00±1.00 nos./ml) 14th day, 7th day and 11th day, respectively. The densities of microbes compared with cladocerans were positively correlated except protozoan with *C. cornuta* density. The ranges of population of *D. sarsi* and *C. cornuta* recorded in the range of 2456.67 ± 57.02 nos./l – 5967 ± 60.56 nos./l and 971 ± 31.61 nos./l to 6247.33 ± 60.48 nos./l, respectively. DMRTs' test performance of *D. sarsi* and *C. cornuta* showed significantly difference in density during culture periods, except 7th day and 11th day and 7th day and 21st day respectively. Based on the present investigation, *D. sarsi* and *C. cornuta* are recommended as live food organisms to aquaculture industry.

Key words: Cladoceran, *D. sarsi*, *C. cornuta,* Chicken manure

Introduction

Zooplankton in general and rotifers, cladocerans and copepods in particular are potential organisms, which can serve as live-food for the early developmental stages of many commercially important cultivable species (Sorgeloos *et al*., 1980). Cladocera significantly contribute to the productivity and energy flow in aquatic ecosystem, due to their feeding on detritus and autotrophs as well as because of rapid turn-over rates. The cultures of cladocerans in most of the investigations have been restricted to two genera namely *Daphnia* and *Moina*.

Culture of *D. carinata* (Murugan, 1989) and *C. cornuta* (Jana and Pal, 1985) using different food media was reported and *D. pulex* was reared in the medium containing *Chlorella* (Harvey, 1972). Mass production of *Moina macrocopa was* attempted using artificial medium (Conklin and Provasoli, 1977), while *Daphnia carinata* and *Ceriodaphnia cornuta* (Paray and Al-Sadoon, 2016; Herman *et al.,* 2017), *Moina dubia* and *Moina micrura* were produced using agro-industrial wastes (Murugan and Moorthy, 1990; Pagano *et al*., 2000; Altaff and Sivakumar, 2002, Sivakumar, 2005; Sasikala *et al*., 2018).

The availability of live-food organisms in sufficient quantities is a major problem in the cultivation of early stages of shellfish and finfish. Despite the variety of natural food organisms, only a few have been used in hatcheries (Sivakumar, 2005; Kar *et al.,* 2017). The artificial culture of the copepods, like Acartia tonsa (Øie *et al*., 2017) and Centropages hamatus (Jakobsen *et al.,* 2016) and rotifers (Folkvord *et al.,* 2016) like Brachionus (Maehre *et al.,* 2013; Sharma *et al.,* 2018) are established to meet the demand of the live food for the fish larvae in commercial aquaculture. In aquaculture, an increasing demand exists for live-food organisms of suitable size and quality to serve as prey for crustaceans and fish larvae in spite of availability of *Artemia* nauplii (Pagano *et al*., 2000; Oladele and Omitogun, 2016) and rotifers (Pourriot, 1986). Hence, the present study was an attempt at culture of freshwater cyclopoid copepods and cladocerans for sustainable aquaculture.

Materials and Methods

Culture of the cladocerans *D. sarsi* and *C. cornuta* were undertaken in the present study. Culture of the cladocerans *D. sarsi* and *C. cornuta* were carried out for 21 days in 25 L fibre tanks. For the culture of cladocerans, culture tanks were filled with 20 L of tap water and fertilized with 10gms of chicken manure. The culture medium was continuously aerated for 24 hrs. and then mixed algae (*Pennate* sp., *Eurastrum* sp. and *Stephanodiscus* sp.) were introduced into the culture medium at the rate of 4.25 x 10^4 cells/ml. For preparing the inoculums of the cladocerans, different species were collected from the ponds and were sorted out under binocular dissection microscope in the laboratory. They were maintained in the lab with yeast and mixed algal diet. Required densities of cladocerans were raised in the laboratory and they were inoculated at the rate of 40 nos./l in the culture tanks. The inoculum consisted of neonates and mature animals (Muthupriya *et al*., 2004).

Microbial population of the culture medium were analyzed on 5th, 7th, 14th and 21st day of culture. Bacterial populations of the culture media were analysed following Bergey's manual (1986) and expressed as cfu/ml. Algae of the culture media were identified following the description of Turner (1978) and Anand (1998) and were quantified following the method of Maeda (1999). Protozoans in the culture media were identified and quantified following the methods of Patterson (1996) and Maeda (1999).

Cladoceran populations were enumerated on 5th, 7th, 11th, 14th and 21st day of culture. For this purpose one litre of

culture medium was sampled after mixing it thoroughly for uniform distribution of cladocerans. Sub-samples of 100 ml was drawn from the sample, cladocerans were filtered using a plankton net cloth and their number was counted under binocular dissection microscope. Five such sub-samples were analysed to determine the population of the cladocerans. Experiments were conducted in triplicate, mean and standard deviation of the microbes and cladocerans were calculated. Correlation co-efficient was computed between microbial and cladoceran populations.

Results and Discussion

In the present study, some of the species such as *D. sarsi* and *C. cornuta* were cultured at laboratory level in culture medium fertilized with chicken manure and mixed algae. During culture period the media were phase fertilized regularly with the manure. In general, cladoceran culture has been attempted using variety of agro-industrial residues and live stock wastes (Tay *et al*., 1991). It is realized during the present study the proper doses of fertilization of the medium to provide optimum feeding is important for sustained culture. Over feeding invariably results in either contamination or depletion of the population. While culturing *D. magna* and *M. micrura*, high mortality due to over feeding was reported by DePauw *et al*. (1981) and Tay *et al*. (1991).

In the culture of Cladocera, the type of microorganisms and the algae produced by the initial and phased fertilization of organic and inorganic material is important as the food, influences growth, maturity and reproduction of the cultured species. In the present study, microorganisms produced with chicken manure promote higher density of *C. cornuta* population than *D. sarsi*. DeBernardi and Guissaini (1990) record high population of *Bosmina* sp. and *Ceriodaphnia* sp. in the presence of blue green algae. Such influence of food on the population density of Cladocera was also reported by Hebert (1978) and Boersma and Vijverberg (1996).

The population of *D. sarsi* ranged between 2456.67 ± 57.02 nos./l and 5967 ± 60.56 nos./l during the culture period of 21 days. Maximum density was observed on the 11th day of the culture (5967.00 ± 60.56nos./l), while minimum density (2456.67 ± 57.02nos./l) was observed on the 21st day of the culture (Fig. 1). In the culture system *D. sarsi* showed positive correlation with bacteria, algae and protozoans. Of the three micro-organisms, *D. sarsi* showed higher positive correlation with protozoa (r = 0.975) and bacteria (r = 0.868) than the algae (r = 0.616) (Table-1). Statistically significant difference was observed with regard to the density of protozoans and *D. sarsi* as indicated by the *P* value at 5% level.

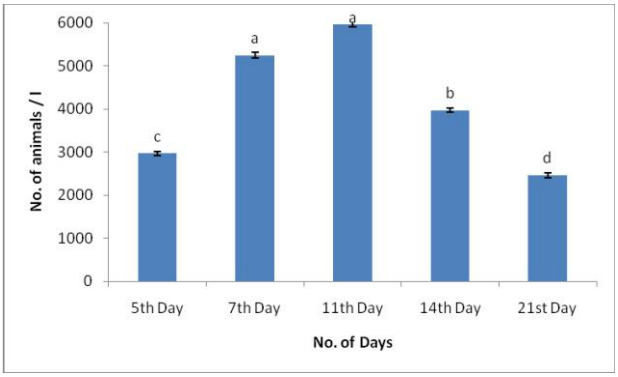

Fig. 1: Density of *D. sarsi* during different days

During the culture period, the density of algae fluctuated between 2.48 ± 0.04 to 3.92 ± 0.06 x 10^4 cells/ml. Protozoans number fluctuated from 29.67 ± 3.06 nos./ml to 48.00 ± 1.00 nos./ml during the culture period. In general, high density of protozoans was observed from the 7th day to the end of the experimental period. The population of *C. cornuta* ranged between 971 ± 31.61nos./l and 6247.33 ± 60.48nos./l during the culture period (Fig 2). Correlation co-efficient of *C. cornuta* indicated higher positive value (r = 0.891) with bacteria and moderate positive value (r = 0.537) with algae. While negative correlation co-efficient value (r = -0.220) was recorded between *C. cornuta* and protozoans (Table 2).

Table - 1: Microbial population in the culture system of *D. sarsi* (Mean ± S.D.)

Day	Bacteria (cfu/ml)	Algae x 10^4 cells/ml	Protozoa (nos./ml)
5th day	689.33±13.80	2.87±0.10	28.00±2.6 5
7th day	2468.00±42.30	3.30±0.10	35.00±2.65
11th day	3856.67±73.32	3.39±0.10	39.33±1.53
14th day	2942.00±60.30	3.20±0.08	33.67±4.03
21st day	1228.00±40.93	3.27±0.06	25.61±3.06
r value	0.868	0.616	0.975

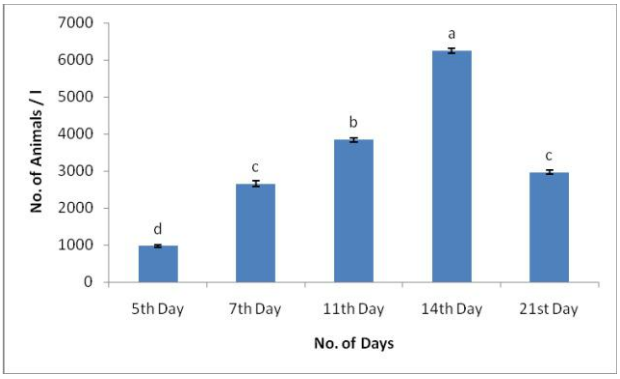

Fig. 2: Density of *C. cornuta* during different days

It is interesting to note that the population of bacteria, algae and protozoan increased during the course of culture period. In the culture media of *D. sarsi* and *C. cornuta*, high density of bacteria 3856.67 ± 73.32cfu/ml on 11th day and 2034.67 ± 60.74cfu/ml on 11th day, algae were 3.39 ± 0.10 x 10^{4} cells/ml on 11th day and 3.92 ± 0.06 x 10^{4} cells/ml on 7th day and protozoans were 39.33 ± 1.53nos./ml on 11th day and 48 ± 1nos./ml on 11th day, respectively. Further, utilization of ammonia by bacteria and phytoplankton might be the reason for low free ammonia during early period of culture. Uptake of large proportion of ammonia by phytoplankton and bacteria was also reported by Bouvy *et al*. (1998) in the *Moina* culture system. The decrease in bacterial population might have resulted due to development of protozoans, such a result is also reported earlier by Gurial *et al*. (1994).

In oilcake and buffalo dung manure fertilized medium Shirgur (1971) has recorded 500-700 nos./l of *Moina* density in culture condition. Ventura and Enderez (1980) have reported higher population density of 500-1000 nos./l of *Moina* with chicken manure. The culture results of Punia (1988) showed higher density of 1050-2600 nos./l using ten different raw materials. However, Nandy *et al*. (1977) have reported a density of 13825nos./l of *D. lumholtzi* with brewer's yeast. Punia (1988) have reported that *D. lumholtzi* outdoor culture in small container, which produced about 6000 nos./l. The present study showed that even in smaller container culture of *D. sarsi* and *C. cornuta* to the density of 2456.67 ± 57.02nos./l – 5967 ± 60.58nos./l and 971.00 ± 31.61nos./l – 6247.33 ± 60.48nos./l, respectively can be achieved during the culture period. 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.

Acknowledgement:

The authors are grateful to the Management, Principal and Head, Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology for providing infrastructure facility to conduct the experiment.

Table – 2: Microbial population in the culture system of *C. cornuta* (Mean ± S.D.)

Day	Bacteria (cfu/ml)	Algae x 10^{4} cells/ml	Protozoa (nos./ml)
5th day	1750.67±39.00	2.48±0.04	29.67±3.06
7th day	1954.00±56.71	3.92±0.06	38.67±2.08
11th day	2034.67±60.74	3.42±0.04	48.00±1.00
14th day	2453.00±52.51	2.73±0.36	40.67±2.52
21st day	1693.33±75.64	3.39±0.03	45.33±4.04
r value	0.891	0.537	- 0.220

References

Altaff K. and Sivakumar K. (2002): High density production of *Moina micrura* using chicken manure and mixed algae. Journal of Aquatic Biology, 17(1): 75-78.

Anand N. (1998): Indian freshwater micro-algae. Published by Bishen Singh Mahendra Pal Singh, Dehradun. pp. 1-94.

Bergey's Manual of systematic bacteriology. (1986): (ed.). Sneath, P.H.A. Mair, N.S. Sharpe, M.E. and Holt, J.G. Williams and Wikins, Balitmore. pp. 1-1599.

Boersma M. and Vijverberg J. (1996): Food effects on life history traits and seasonal dynamics of *Ceriodaphnia pulchella*. Freshwater Biology, 35(1): 25-34.

Bouvy M. Arif R. Secchia P. Corbin D. Pagano M. Saint-Jean L. and Thomas P. (1998): Trophic coupling between bacterial and phytoplanktonic compartments in shallow tropical reservoir (Ivory Coast. West Africa). Aquatic Microbial Ecology, 15: 25-37.

Conklin D.E. and Provasoli L. (1977): Nutritional requirements of the water flea *Moina macrocopa.* The Biological Bulletin, 152: 337-350.

DeBernardi R. and Guissaini G. (1990): Are blue green algae a suitable food for zooplankton? an overview.

Hydrobiologia. 200 & 201: 29-41.

DePauw N. Deleenher L.Jr. Laureys P. Morales J. and Reartes, J. (1980): Cultures d' Algues et d' Invertebre's sur Dechets Agricols. In: Billard, R. (ed.). La pisciculture en Etang. INRA, Publ. Paris. 189-214.

Folkvord A. Koedijk R. Grahl-Nielsen O. Meier, S. Rydland Olsen B. Blom, G. Otterlei, E. Imsland, A.K. (2016). You are what you eat? Differences in lipid composition of cod larvae reared on natural zooplankton and enriched rotifers. Aquaculture Nutrition, 1-12.

Gurial D. Arif, R. Bouvy M. Pagano M. Saint-Jean L. and Thomas S. (1994): Ecological organization and succession during natural reorganization of a tropical pond. Hydrobiologia. 294: 229-242.

Harvey M. (1972): A semi-continuous culture technique for *Daphnia pulex*. Journal of Applied Ecology, 9(3): 331-334.

Hebert P.D.W. (1978): The population biology of *Daphnia* (Crustacea: Cladocera). Biological Reviews, 53: 387-426.

Herman H. Andriani Y. Sahidin A. Hidayat T. and Herawati T. (2017): Culture of *Daphnia* sp. (crustacean – cladocera): the effect of manure variation on the growth, natality, and mortality IOP Conf. Series: Earth and Environmental Science, 137 (2018) 012018.

Jakobsen H.H. Jepsen, P.M. Blanda E. Jørgensen N.O.G. Novac,A. Engell-Sørensen K. Hansen B.W. (2016): Plankton composition and biomass development: a seasonal study of a semi-intensive outdoor system for rearing of turbot. Aquaculture Nutrition, 22:1239-1250.

Jana, B.B. and Pal, G.P. (1985): Effects of inoculum density on growth, reproductive potential and population size in *Moina micrura* Kurz. Limnologica, 16: 315-324.

Kar S. Das P. Das U. Bimola M. Kar D. Aditya G. (2017). Culture of the zooplankton as fish food: observations on three freshwater species from Assam, India. AACL Bioflux, 10 (5): 1210-1220.

Maeda M. (1999): *Microbial process in aquaculture,* Japan. National Research Institute of Aquaculture, pp12-38.

Maehre H.K. Hamre K. and Elvevoll E.O. (2013): Nutrient evaluation of rotifers and zooplankton: feed for marine fish larvae. Aquaculture Nutrition, 19:301-311.

Murugan N. (1989): The mass culture of cladoceran, *Daphnia carinata* (King) for use as live food in aquaculture. In: Huisman, E.A., N. Zonneveld and A.H.M. Bouwmans (eds.). Proceedings of Asian Seminar on Aquaculture, 190-202.

Murugan N. and Moorthy R.K. (1990): Some trends in the culture of Cladocera. The 2nd Indian Fisheries Forum Proceedings, 33-37.

Muthupriya P. Sivakumar K. and Altaff K. (2004): *Mass culture of cladocerans*. A Manual of Zooplankton (Ed. K. Altaff), National Workshop on Zooplankton, from 5th to 10th July, 2004, Department of Zoology, The New College, Chennai, pp146-148.

Nandy A.C. Das P.R. and Majmdar S.K. (1977): Technique to obtain sustained culture of a cladoceran, *Daphnia lumholtzi* Sars. Proceedings of Symposium on Warmwater Zooplankton. Specl. Publ., 540-542.

Øie G. Galloway T. Sørøy M. Holmvaag Hansen M. Norheim, I.A. Halseth C.K. Almli M. Berg M. Gagnat M.R. Wold P.A. Attramadal K. Hagemann A. Evjemo J.O. Kjørsvik E. (2017): Effect of cultivated copepoda (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*) larvae. Aquaculture Nutrition, 23:3-17.

Oladele A.H. and Omitogun O.G. (2016): Raising zooplankton as a substitute for artemia for feeding the larval and fry stages of african catfish (*Clarias gariepinus*). *Livestock Research for Rural Development. Volume 28, Article #186.* Retrieved May 5, 2020, from http://www.lrrd.org/lrrd28/10/olad28186.html

Pagano M. Saint-Jean L. Arfi R. Boury M. and Sheap H. (2000): Population growth capacities and regulatory factors in monospecific culture of the cladoceran *Moina micrura* and *Diaphanasoma excisum* and the copepod, *Thermocyclops decipiens* from cote d' Ivoire (West Africa). Aquatic Living Resources, 13: 163-172.

Paray B.A. and Al-Sadoon, M.K. 2016. Utilization of organic manure for culture of cladocerans, *Daphnia carinata, Ceriodaphnia cornuta* and Copepod, *Thermocyclops decipiens* under laboratory conditions. Indian Journal of Geo-Marine Sciences. 45(3); 399-404.

Patterson D.J. (1996): *Free living freshwater protozoa.* John & Wiley & Sons, New York. pp. 9-220.

Pourriot R. (1986): Les rotifers. In: Barnabe, G. (ed.). Biologie. *Aquaculture Coll. Techn. Doc.*, 201-222.

Punia P. (1988): Culture of *Moina micrura* on various organic waste products. Journal of Indian Fishereis Association, 18: 129-134.

Sasikala P Indhu B. Pugazhendy K. and Sattanathan G. 2018. Phytochemical Study and In Vitro Evaluation of the Antifungal Activity of Hunteria Eburnea Pichon (Apocynaceae) on Trichophyton Mentagrophytes, Indo American Journal of Pharmaceutical Sciences, 05 (07):. 6275-6282.

Sharma J.G. Masuda R. Tanaka M. and Chakrabarti R. (2018): The Continuous Culture of Rotifer Brachionus plicatilis with Sea water. Madridge Journal of Aquaculture Research & Development. 2(1): 40-42. doi: 10.18689/mjard-1000108

Shirgur G.A. (1971): Observation on rapid production of zooplankton in fish nurseries by intensive phased

manuring. Journal of Inland Fisheries Association, 1(2): 25-35.

Sivakumar K. (2005): Freshwater fish and prawn larval rearing using indigenous live-feed. Ph.D. thesis, University of Madras, Tamil Nadu, India,

Sorgeloos P. Baeza-Mesa M. Bossuyt, E. Bruggeman E. Boddleir J. Versictrele D. Lavina E. and Bernandino A. (1980): Culture of *Artemia* on rice bran: conversion of waste product into highly nutritive animal protein. Aquaculture, 21(4): 393-396.

Tay S.H. Rajbanshi V.K. Ho W.H. Chew J. and Yap E.A. (1991). Culture of the cladoceran *Moina micrura* Kurz using agro industrial wastes. In: De Silva, S.S. (ed.). Fish nutrition research in Asia. *Proc. 4th Asian Fish Nutrt. Workshop. Asian Fisheries soc*., Spec. Publ., 5: 205.

Turner W.B. (1978): Freshwater algae of East Asia. Royal Swedish Academic of Science. pp1-87.

Ventura F. and Enderez M. (1980): Preliminary studies on *Moina* sp. production in freshwater tanks. Aquaculture, 21: 93-96.