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Alternative Methods for Marine Harpacticoid Copepod, *Macrosetella gracilis* Production in Marine Fish Larviculture

[1]N. Jeyaraj, [2]P. Santhanam, [2]P. Raju, [2]S. Ananth and [2]K. Jothiraj
[1]Department of Animal Science, School of Biological Sciences, Central University of Kerala, Kerala, India
[2]Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, 620 024, Tamil Nadu, India

Corresponding Author: N. Jeyaraj, Department of Animal Science, School of Biological Sciences, Central University of Kerala, Kerala, India

ABSTRACT

Sustainable aquaculture depends upon eco-friendly, economically and socially viable culture systems. The recycling of organic wastes for plankton culture serves the dual purpose of cleaning the environment and providing economic benefits. There has been no experimentation to measure the effect of organic manure for the aquaculture of copepods, it may be reduced time and labor cost. Hence, the present experiment was conducted to evaluate the mass culture feasibility of Harpacticoid copepod, *Macrosetella gracilis* using a different organic manures viz., cow dung, poultry manure, goat manure and mixture of these three (1:1:1 ratio) at 500 g for each tank manures were decomposed for twelve days before the inoculation of copepod. Twenty adult copepod *M. gracilis* were inoculated. The peak density was found on 10-14th day of at all doses, among these four doses, significantly higher numbers of organisms 4213.33±213.48 ind. L^{-1} were found in the cow dung followed by the poultry manure, goat manure and mixed doses 2350.66±148.20; 1573.60±121.41; 995±102, respectively. The number of organisms was ground very low in mixed doses than other doses. Water quality analyses of culture system was no significantly different among the treatments especially pH and salinity. Cow dung manure is therefore recommended for quick and high production of copepod, *M. gracilis* which invariably reduces the high cost of expensive algae feed for copepod.

Key words: *Macrosetella gracilis*, dissolved oxygen, organic manures, copepod abundance

INTRODUCTION

It is well known that crustaceans are importment in the diets of fishes and those copepods are among the principal food of several economically valuable fish species (Marshall *et al.*, 1939). In aquaculture, surrogate live feeds such as rotifers and *Artemia nauplii* are widely used as larval prey for marine finfish species (McKinnon *et al.*, 2003). However, *Artemia* and rotifer have very low n3-HUFA contents in their body (Leger *et al.*, 1986; Evjemo *et al.*, 1997). Marine fish larvae require food organisms which have relatively high concentrations of the long-chain, n-3 poly-unsaturated or Essential Fatty Acids (EFA) such as 20:5n-3 (EPA) and 22:6n-3 (DHA) (McKinnon *et al.*, 2003). Therefore the traditionally-used live foods cannot satisfy the requirement of n-3 HUFA for some fish species.

Harpacticoid copepods can synthesis several nutritionally important essential fatty acids that are extremely important for larval fish survival and growth (Olivotto *et al.*, 2008) and provide a broad spectrum of prey sizes suitable for developing fish larvae (Gee, 1989), clean the tank walls of detritus (Delbare *et al.*, 1996). Harpacticoids species are epibenthic organisms grazing organic debris from stable substrates they have high tolerance to a wide range of environmental condition and high reproductive capacity than calanoid and cyclopoids (Uhlig, 1984; Pinto *et al.*, 2001). Harpacticoids species can feed on microalgal diet but they are not dependent on constant supply. Most of the Harpacticoids are feed by scraping organic matter off surface and it is possible to culture these organisms in small volume with large surface area (Stottrup and Norsker, 1997). They can feed variety of inert feeds but it avoids due to hygiene problems (Uhlig, 1984). Moreover, Harpacticoids can be cultured in high densities and are apparently easier to culture than calanoid and cyclopoid copepods. However, large scale productions of copepod are more difficult. One factor that increases the time and money involved in rearing copepods is expense of growing live algae to feed them. The economic and labor cost of producing copepod may be reduced by using organic manure feed instead of cultured algae (Rhodes and Boyd, 2005).

There are very few studies about the usage of organic manure for zooplankton culture (Jana and Pal, 1985). Jana and Chakrabarti (1997) have developed *in situ* method for mass culture of *Daphnia* by using different organic manure. Ekelemu and Nwabueze (2011) found that organic manures are suitable feed for fresh water zooplankton production. Jha *et al.* (2004) have the same opinion both cow dung and poultry excreta gave better plankton production. Srivastava *et al.* (2006) found the dose of 2.104 kgs-3 to be optimum for the culture of zooplankton *Ceriodaphnia coronuta*. Golder *et al.* (2007) observed that the human urine was an excellent liquid waste that can be used for the mass production of zooplankton *Moina micrura*. Damle and Chari (2011) found that goat dung yield highest production of *Daphnia* sp. and Recently, Ak (2012) studied an organic fertilizer on growth of blue-green alga *Spirulina platensis*. Several authors are reported Copepods, copepodites and naupliar stages are good candidates in aquaculture sector (Fleeger, 2005). They are using algal as a feed for copepod. But no more literature has been found on the effect of the organic fertilizers on growth of Harpacticoids copepod, *M. gracilis*. Further, it has abundant in tropical waters (especially in India). In our laboratory we have maintain culture for the past two years and found to be nutritionally rich and could be successfully mass cultured (Ananth and Santhanam, 2011). Manures like cow dung, chicken manure and agricultural by-products like mustard oil cake are wastes in India. These can be used as potential organic manures to increase the productivity of water body, primary productivity and secondary productivity. Hence, the present investigation aims to determine the influence of the organic manures on growth and biomass composition of *M. gracilis*.

MATERIALS AND METHODS

Stock cultures of copepods: Copepod samples were collected from the coastal waters of Muthupet (Lat. 10°20'N and Long 79°35'E) during early in the morning using plankton net with 158 µm mesh. The collected samples were provided with vigorous aeration by using battery aerators and the samples immediately transported to laboratory and thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *M. gracilis* was identified under microscope using the standard key (Kasturirangan, 1963). After the confirmation, 200 No. that including male and female *M. gracilis* were isolated and stocked separately in an oval shaped flat-bottomed fiberglass tank (0.54 m dia, 0.81 m length) filled with 100 L of filtered (1 µm) seawater and gentle aeration was given. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of 26-30°C; 28-32‰;

7.5-8.5; 5.0-.5 mL $^{-1}$, respectively (during rearing period) fed with a daily ration of mixed algae viz., *Chlorella marina*, *Dunaliella* sp., *Isochgrysis galbana* and *Nannochloropsis* sp., in the concentration of 20,000 cells mL $^{-1}$. The copepod cultures were harvested at every 12 (*M. gracilis*) days by gentle siphoning. The generation time of *M. gracilis* is about 10-12 days under optimal conditions at 26-30°C. Finally the adult male and female copepods were used to restart stock culture.

EXPERIMENTAL DESIGN AND SETUP

This study was done to compare the copepod, *M. gracilis* culture using a different type of organic manures viz., poultry droppings, goat manure, cow dung and mixture of these three in 1:1:1 ratio in 20 L plastic tanks were conducted in the Marine platonic logs+aquaculture laboratory, Bharathidasan University, Trichy, Tamil Nadu. The organic manures were used in 500 g each tank. Three replicates were used for each treatment. All the manures were decomposed for 12 days before the inoculation of adult copepod. This manure-water was mixed thoroughly throughout the study period. There was no additional feeding of *M. gracilis* and no aeration supplied in the copepod culture tanks. On 13th day, adult *M. gracilis* were inoculated at 20 individual L^{-1}. Water quality parameters such as temperature, salinity, pH and dissolved oxygen were measured at five days interval from each tank (Strickland and Parsons, 1972). Population density of *M. gracilis* was recorded daily by taking triplicate samples (1 mL each) from the culture media after stirring gently. The whole experiment was conducted for a period of fifteen days and the copepods were fed with the designated manure, all the contents of each replicate tank were drained through a 38 µm sieve and the total number of nauplii, copepodites and adults of *M. gracilis* retained were fixed in 5% formalin solution. The counting were made using a Sedwick Rafter counter under high-powered microscope.

RESULTS

Results of the study are presented in Fig. 1 shows the *M. gracilis* species abundance in the different culture media. Mean population density of *M. gracilis* after being fed a designated manure was highest for the cow dung with a final population density of (ind. L^{-1}) (4213.33±213.48) of these nauplii (1445.66±52), copepodites (2081±98), adults (686.66±62) followed by the poultry droppings (2350.66±148.20 ind. L^{-1}), Goat manure (1573.60±121.41 ind. L^{-1}) and mixed manure (1:1:1) (995±102 ind. L^{-1}). Population density of the cow dung treatment was significantly higher than all the other manures. The mixed manure produced the lowest population number at the end of the experiment.

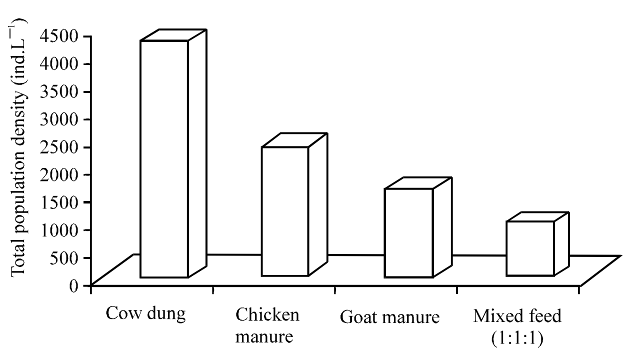

Fig. 1: Mean total population of *Macrosetella gracilis* (±SE) produced at the four organic manure cultured for 15 days from an initial population of 20 adult pairs

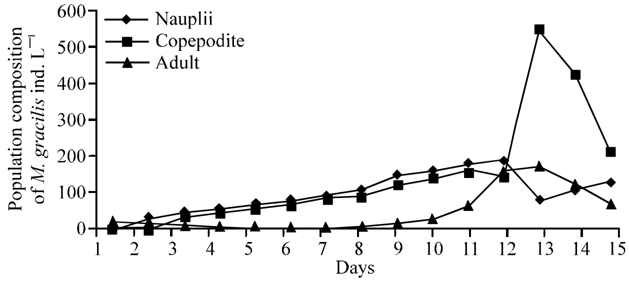

Fig. 2: Mean No. of life-stages (nauplii, copepodites and adults) within the population of *Macrosetella gracilis* cultured for 15 days at cow dung manure

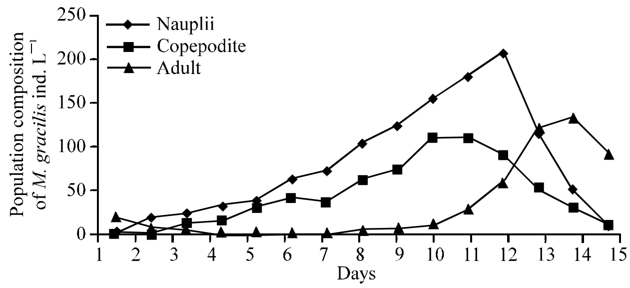

Fig. 3: Mean No. of life-stages (nauplii, copepodites and adults) within the population of *Macrosetella gracilis* cultured for 15 days at chicken manure

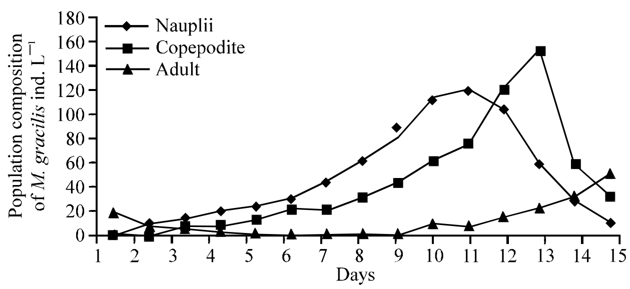

Fig. 4: Mean No. of life-stages (nauplii, copepodites and adults) within the population of *Macrosetella gracilis* cultured for 15 days at goat manure

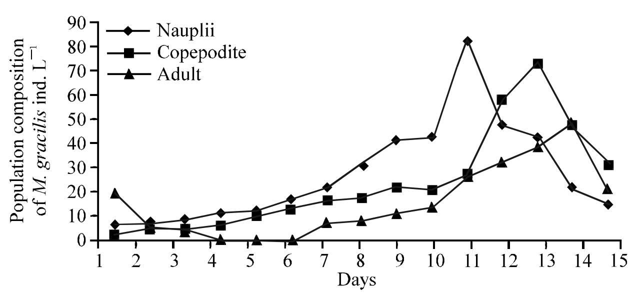

Fig. 5: Mean No. of life-stages (nauplii, copepodites and adults) within the population of *Macrosetella gracilis* cultured for 15 days at mixed manure (Cow dung 1: Chicken manure 1: Goat manure 1)

Table 1: Water parameters used for the cultured copepod, *M. gracilis*

Parameters	Days	Cow dung	Chicken manure	Goat manure	Mixed feed (1:1:1)
pH	1	7.2	7.0	7.5	7.0
	5	7.5	7.2	7.8	7.4
	10	7.6	7.2	8.0	7.4
	15	8.0	7.5	8.5	7.8
DO (mL L^{-1})	1	4.1	4.5	4.8	4.7
	5	5.0	5.1	5.2	5.0
	10	5.2	5.1	6.0	5.1
	15	5.5	5.2	6.1	5.8
Salinity (%)	1	30.0	30.0	30.0	30.0
	5	31.0	32.0	31.0	30.0
	10	31.0	33.0	32.0	30.0
	15	33.0	33.5	34.0	33.0

Figure 2-5 shows a breakdown of different life-cycle stages of the *M. gracilis* population fed various organic manure for 15 days. It showed that there were substantial differences in the numbers of the three life-stage categories, i.e., nauplii, copepodites and adults in different treatments. Most notably on 12th and 14th days the No. of nauplii (206 ind. L^{-1}) (Fig. 3) and copepodites (425 ind. L^{-1}) (Fig. 2) was markedly higher for the chicken manure and cow dung respectively. Remaining all diet evenly distributed nauplii, copepodites and adults. Interestingly, the peak density was found between 10 and 14th days at all treatments. The number of organism decreased rapidly at all treatments 15th days onward.

Water parameters results are presented in Table 1. The salinity ranged from 30-34‰ throughout the period of the study. However, the daily water temperature in each treatment throughout the period of investigations was not significantly different from each other. The water pH and dissolved oxygen varied slightly in some of the treatments but the variations are not significantly different ($p>0.05$) from each other. The monitoring of water temperature, pH, dissolved oxygen are essential in the culturing of copepod because it enable laboratory manipulations of the particular strain in the locality.

DISCUSSION

Our results showed that organic manures such as poultry droppings, goat manure, cow dung and mixed diets (1:1:1 ratio) was significantly affected the total production of *M. gracilis*. In this experiment, organic manures were decomposed for initial 12 days before the inoculum of *M. gracilis*. In a study of life table responses of *M. micrura* and *Daphnia carinata*, Jana and Chakrabarti (1993) suggested that the interval between 2 and 10 days after manure application is a prerequisite for establishing suitable environmental conditions for plankton production. The same opinion was given in the earlier works by Srivastava *et al.* (2006) experimentally were made organic manures were decomposed for twelve days before the inoculation of *Ceriodaphnia cornutaorganic*. We used similar procedure to manure decompose for twelve days before the inoculation of *M. gracilis*. Among the manures tested total density of *M. gracilis* fed on mixed manure was lowers than those fed on poultry droppings, goat manure and cow dung. Reason is the mixed food items may not be accessible to nauplii stage of copepod.

Organic manure has been used as a source of fertilizer to supply nutrients needed for plankton production. Varies authors reported that using the manures as a feed for plankton and fish production. Fish production in ponds that were fertilized using organic manure such as cow dung and poultry droppings have been reported to almost double production than unfertilized ponds Boyd (1982). Jha *et al.* (2004) reported both cow dung and poultry excreta through maintenance of better water quality, greater abundance of plankton and better growth of Koi carp in the system. Present studies agree with this report high population density observed in cow dung fed copepod. Cow dung, poultry droppings and pig dung can be successfully used to fertilize fish ponds in fish culture, as they are capable of supplying the desired nutrients required for zooplankton production (Orji and Agunwa, 2005). Srivastava *et al.* (2006) noticed the mixture of cattle manure, poultry droppings and mustard oil cake in the ratio of (1:1:1) 2.104 kg m^{-3} dose of organic manure was optimum for the culture of *C. cornuta* in outdoor condition. Elnady *et al.* (2010) recommended that organic fertilizer should not be used as sole source in fertilizer programs and should be combined with chemical fertilizer in order to produce good algal growth necessary for the nourishment of farmed fish. Recently, Ekelemu and Nwabueze (2011) stated that poultry droppings as a better source of organic manure compared to cow and pig dung during zooplankton production. Damle and Chari (2011) observed that among the different animal waste goat manure as a suitable for highest production of *Daphnia* sp. (Jana and Chakrabarti., 1997) that the organic manure using excessive in culture tank that's can lead to water quality deteriorating.

In the present experiment we used 500 g of manure in the entire tank. In *M. gracilis*, culture tanks dissolved oxygen level was minimum and maximum in cow dung and goat manure, respectively. Dissolved oxygen was minimum at the beginning of the study and maximum at the end of the experiment in all these treatments. Morris and Mischke (1999) found that organic fertilizers may cause dissolved oxygen problems during initial decomposition. There was direct relationship between dissolved oxygen and number of organism. So, the production of *M. gracilis* was reduced when the concentration of dissolved oxygen was lower. As dissolved oxygen level increased in the culture system the number of organism such as nauplii, copepodite and adult also increased. Present study agree with the earlier study on population density of *Ceriodaphnia cornutaorganic* increased when the concentration of dissolved oxygen level increased.

Salinity and pH also on important factors to determine the zooplankton density in environment (Santhanam and Perumal, 2003). Salinity and pH also increased gradually as the days after manure application throughout the treatments on 10-15th day number of organism also increased rapidly. The same opinion was given in the earlier works in the initial days of manuring, the decomposition of manures required more dissolved oxygen which resulted in the depletion of dissolved oxygen in the culture system. Biological oxidation converts the organic matter in manure to carbon dioxide. This free carbon dioxide quickly combines in water to form carbonic acid, a weak acid. Therefore, at the initial days of plankton culture, pH and dissolved oxygen values were low.

CONCLUSION

This study was carried out to provide an inexpensive medium. For example, cost calculation of growth mediums showed that while the culture of thousands litters of algae to feed copepod costs more or five hundred rupees the tested manures would cost naught. Also, this manure is not only a low cost but also a high productive alternative. This alternative organic nutrient source can also reduce the cost and could be an economic improvement for large-scale cultivation of the *M. gracilis*.

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