

## Culture of *Moina macrocopa* Straus using Organic and Inorganic Inputs

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### Abstract

Five experiments were conducted over a five month period to investigate the feasibility of using buffalo manure, chicken manure, rice bran plus fish waste, septage and inorganic fertilisers in the culture of *Moina macrocopa* in outdoor concrete tanks. The loading of 5.7 g m<sup>-3</sup> and 11.4 g m<sup>-3</sup> of buffalo manure, chicken manure or rice bran plus fish wastes (1:2) did not yield a high production. An initial loading of 566 g m<sup>-3</sup> of rice bran plus fish wastes (1:2) followed by the subsequent loading of 75.5 g m<sup>-3</sup> resulted a higher density of *Moina* than that resulted with the loading of manure. Exposure to direct sunlight and aeration with the loading of 566 g m<sup>-3</sup> rice bran and fish wastes (1:2) gave a high production. Loading of rice bran, fish wastes and inorganic fertilisers with inoculation of *Chlorella* resulted a high yield of 292.6 g m<sup>-3</sup> of *Moina*.

### Introduction

*Moina macrocopa* Straus is a freshwater cladoceran, found in tropical and subtropical regions (Pennak 1978) and has a high food value as a protein source for the fish larvae (Watanabe et al. 1983; Villegas 1990). It is widely used as a larval food for many species of tropical food fish including *Clarias spp.* (Hecht 1981; Knud-Hansen et al. 1990), *Pangasianodon gigas* (Anon. 1988), *Oxyleotris marmoratus* (Tavarutmaneegul & Lin 1988) and various aquarium fish (Shim 1988).

The commercial culture practice of *Moina* in Thailand uses Monosodium glutamate waste (Ami Ami), urea, rice field fertiliser (N:P = 16:20) and superphosphate as inputs and achieves a relatively high yield. However, Ami Ami is not widely available in many Asian countries. Inorganic fertilisers are relatively expensive. Organic wastes such as animal manure and agricultural by-products are widely available cheaper sources of nitrogen and phosphorous and also serve as substrates for the growth of microorganisms (Tacon 1988) which is an important food of *Moina* (Dinges 1973; Pennak 1978; Norman et al. 1979; Tavarutmaneegul et al. 1989).

In the present study, *M. macrocopa* was cultured using several organic manure with a view of investigating quality and quantity required for maximising *Moina*

production. Attempts were also made to investigate the effects of shading and aeration and to evaluate the importance of the organic and inorganic inputs on *Moina* production.

### Materials and Methods

Outdoor circular concrete tanks of 120 cm diameter and 70 cm depth were used for all experiments conducted at the Asian Institute of Technology, Bangkok, Thailand.

All tanks were cleaned, drained and dried under sun for two days and filled with tap water upto 50 cm level and left for two days before commencing each experiment. *Moina macrocopa* and *Chlorella* required for the experiments were bought from Pathumthani Freshwater Fisheries Station.

#### *Water quality analysis*

Dissolved oxygen (Dissolved oxygen meter YSI 54A) at the 25 cm depth was measured during 0600 h - 0700 h. Water samples were collected from surface to bottom of each tank in 200 ml bottles using a bottle column sampler for the analysis of total Ammonia-N (Phenate method) and chlorophyll a content (Boyd 1979; APHA 1980) during 0600 h - 0700 h. Phytoplankton biomass was estimated by multiplying the chlorophyll a content by a factor of 67 (APHA 1980). Water pH (pH 96 microprocessor model) and temperature at 25 cm depth were measured during 1700 h - 1800 h.

These water quality parameters were measured during each experiment just before inoculation of *Moina* and subsequently in every two days.

#### *Experimental procedure*

##### Experiment 1:

This was a preliminary investigation of *Moina* culture using buffalo manure, chicken manure and rice bran plus fish wastes (1:2). A factorial experiment of randomised complete block design was conducted for one week period. Factor 1 was the type of organic matter and the other factor was the dry matter loading rate. Tanks were loaded with corresponding organic matter at the rates of  $5.7 \text{ g m}^{-3}$  and  $11.4 \text{ g m}^{-3}$ . Each loading had three replicates. *Moina macrocopa* was inoculated to the tanks (99/l) just after mixing the medium thoroughly. Tank water was sampled once in every three days for the estimation of the population density of *Moina*. Tanks were loaded at the same rates after three days.

##### Experiment 2:

A factorial experiment of randomised block design was conducted for 24 days using buffalo manure, chicken manure and rice bran plus fish wastes (1:2). Tanks were loaded initially with  $566 \text{ g m}^{-3}$  of corresponding organic matter and left until the colour of tank water was dark greenish. *Moina* was inoculated to the tanks (151/l) after three days. The population density of *Moina* was estimated once in every three days throughout the experimental period. Tanks were loaded with the corresponding organic inputs at the rates of  $37.7 \text{ g m}^{-3}$  and  $75.5 \text{ g m}^{-3}$  once in three days since the first sampling of *Moina*.

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Experiment 3:

A randomised factorial experiment (2x2) was conducted for nine days using rice bran plus fish wastes (1:2) as inputs as these yielded the highest population density of *Moina* in experiment 2. Tanks were loaded initially with 566 g m<sup>-3</sup> of inputs and subsequently with 75.5 g m<sup>-3</sup> in every three days. The two factors were the level of shading (with shading and without shading) and the level of aeration (with aeration and without aeration) of the tanks. Six tanks were shaded using a black net material. Water in six tanks were aerated. Tank water was sampled once in every three days for the estimation of *Moina* population density.

Experiment 4:

A randomised block design experiment was conducted using eight types of inputs (Table 1). Blocking procedure (Ratnayake 1991) was employed in this experiment to counteract the effect of new tanks (Block 3) and the shading of some tanks due to the roof of the adjacent laboratory. Each treatment (Table 1) was in three replicates. All experimental tanks were aerated.

As a large amount was required, *Chlorella* was bought from Pathumthani freshwater fisheries station and cultured in two tanks using inorganic fertilisers and Ami Ami.

Initially, experimental tanks were loaded with corresponding materials in the rates or concentrations given in Table 1. *Moina* was inoculated (150/l) to the tanks after two days. Tank water was sampled once in every two days for the estimation of *Moina* density resulted with the different treatments.

Table 1. Inputs and their weights/concentrations used in experiment 4. Tr. = Treatment; TKN = Total Kjeldhal Nitrogen.

Tr.	Inputs	Dry weight (g)/ Concentration (ppm)	Algal inoculation (50 l/tank = 89 l m <sup>-3</sup> )
1	Rice bran and minced fish wastes (1:2)	300 g	<i>Chlorella</i>
2	-do-	300 g	None
3	Septage	30 ppm TKN	<i>Chlorella</i>
4	-do-	30 ppm TKN	None
5	Urea	127 g	<i>Chlorella</i>
	N-P-K rice field Fertiliser	64 g	
	Superphosphate	11 g	
6	Tr. 1 + Tr. 5	As used in Tr. 1 + Tr. 5	<i>Chlorella</i>
7	Tr. 3 + Tr. 5	As used in Tr. 3 + Tr. 5	<i>Chlorella</i>
8	Tr. 5	As used in Tr. 5	<i>Chlorella</i>
	Ami Ami	850 ml	
	Lime	127 g	

#### Experiment 5:

The best treatment selected from the experiment 4, the loading of rice bran and fish wastes (1:2), inorganic fertilisers and inoculation of *Chlorella*, was used in this experiment. There were three replicates. Experimental procedure was similar to that of experiment 4. However, *Moina* was completely harvested using a hand net (120  $\mu\text{m}$  mesh) while draining tanks on the eighth day after the inoculation. Wet weight and density (number/ml) of *Moina* in each tank were recorded.

#### Determination of *Moina* population density

A PVC pipe of 5 cm diameter and 1.5 m long was used to sample tank water from the surface to bottom at three random points during 0600 h - 0700 h. Two litres of water collected from each tank in this manner were passed through a sieve of 125 micrometer mesh to collect *Moina*. The samples were preserved with 4% buffered formalin and few drops of glycerine (APHA 1980). A Sedgewick Rafter counting chamber with marked grids was used to count *Moina* in each sample (Ratnayake 1991).

#### Statistical analysis

The actual number of counts of *Moina* were transformed into log scale and analysed statistically using Statgraphics software package. The highest population density of *Moina* obtained with different treatments in the experiments were analysed by Two-way ANOVA followed by Scheffe's test.

## Results

The population density of *Moina* observed with all the treatments used in experiment 1 was very low and the highest population density with each treatment was observed on the third day after inoculation (Fig. 1). The tanks loaded with rice bran plus fish wastes (1:2) at a rate of  $11.4 \text{ g m}^{-3}$  (RF2) among other treatments attained the highest population density on the third day. Water pH values of all treated tanks were observed to be low (4 - 5) until the fourth day of the experiment. There was no significant effect of type of input on the *Moina* density observed at the loading rates used in this experiment.

The mean population density of *Moina* observed with different treatments used in the experiment 2 is shown in Fig. 2. The mean density of *Moina* in all treated tanks except that of tanks loaded at the rate of  $75.5 \text{ g m}^{-3}$  of rice bran and fish wastes was observed to be maximum on the third day after inoculation. The highest density of *Moina* was noticeable on the sixth day in the tanks loaded with  $75.5 \text{ g m}^{-3}$  of rice bran and fish wastes. The results of statistical analysis indicated that there were significant effects due to the type of organic matter and the loading rate. It also indicated that the most productive culture tanks were which loaded with rice bran plus fish wastes (1:2) at the rate of  $75.5 \text{ g m}^{-3}$  after the initial loading. Although the phytoplankton biomass in all treated tanks increased towards the third day (Fig. 3), the highest phytoplankton biomass was always apparent in the tanks loaded with rice bran plus fish wastes (1:2) at the rate of  $75.5 \text{ g m}^{-3}$  per 3 days. The mean temperature of all tanks increased until the fourteenth day and, then dropped towards the end of the experiment (Fig. 3a). The variation patterns of the dissolved oxygen concentration (Fig. 3b) were different in the different treatments. However, the tanks loaded with rice bran and fish wastes at the rate of  $75.5 \text{ g m}^{-3}$  showed the lowest dissolved oxygen values during the experimental period.

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The population density of *Moina* observed during the experiment 3 is shown in Fig. 4. The unshaded aerated tanks markedly bloomed with *Moina* on the third day after inoculation. The highest population density was observed on the third day in all treated tanks. The lowest third day *Moina* density was observed in shaded unaerated tanks. The statistical analysis indicated that levels of shading and levels of aeration had significant effect on *Moina* density in culture tanks. The unshaded tanks had higher temperatures (Table 2) during the experimental period. Dissolved oxygen concentration was observed to be always higher in unshaded tanks including the markedly different concentrations observed in aerated and unaerated tanks at the beginning of the experiment. This experiment lasted only for one week because rotifers infested the culture tanks towards the sixth day.

Table 2. Water quality characteristics in *Moina* culture tanks with ranges from minimum to maximum values throughout experiment 3.

Water quality indices	Treatment			
	Unshaded aerated	Unshaded unaerated	Shaded aerated	Shaded unaerated
Temperature°C	32.2 - 34	32 - 33.5	29.3 - 31	29.2 - 31
Dissolved oxygen mg l <sup>-1</sup>	4 - 11.4	0.9 - 4.5	4.3 - 7.2	0.6 - 1.0
pH	8.5 - 9.3	8.4 - 9.4	8.2 - 9.2	8.3 - 9.2
Total NH <sub>3</sub> -N mg l <sup>-1</sup>	0.75 - 2.5	0.75 - 2.1	1.2 - 2.6	1.6 - 3
Phytoplankton biomass mg l <sup>-1</sup>	1.2 - 79.0	14.2 - 67.6	17.8 - 56.7	12.8 - 47.0

Fig. 5 shows the mean *Moina* density observed in different days with the different treatments used in experiment 4 and the maximum mean *Moina* density resulted is presented in Table 3. The highest yield of *Moina* was observed in the tanks loaded with rice bran plus fish wastes (1:2) and inorganic fertilisers with the inoculation of *Chlorella*. The results of the statistical analysis showed that the effects of treatments were significant and the *Moina* density observed with the Treatment 1 was significantly lower than that observed with the Treatment 6. Furthermore, the densities of *Moina* observed with the Treatment 8 and the Treatment 1 were not significantly different from each other. During this experiment the values for water temperature observed in all treated tanks were slightly different from each other and high dissolved oxygen concentrations were recorded in all tanks on the day of *Moina* inoculation. Dissolved oxygen concentration of all tanks declined towards the end of the experiment. Water pH appeared to be in the alkaline range in all tanks. The highest values of total ammonia-N were observed in the tanks loaded with inorganic fertilisers. The phytoplankton biomass in culture tanks showed wide variations. The highest phytoplankton biomass at *Moina* inoculation was observed in the tanks loaded with rice bran, fish wastes and inorganic fertilisers with *Chlorella* inoculation.

**Table 3.** The mean maximum *Moina* density ( $\pm$  S. E.) observed in culture tanks in experiment 4. Note: Treatment means bearing different suffix letters (a-d) are significantly different ( $p < 0.05$ ).

Treatment	<i>Moina</i> density (No./l)
Tr. 1	14133 $\pm$ 246 <sup>h</sup>
Tr. 2	9406 $\pm$ 718 <sup>c</sup>
Tr. 3	3697 $\pm$ 173
Tr. 4	756 $\pm$ 66
Tr. 5	3097 $\pm$ 78 <sup>d</sup>
Tr. 6	17392 $\pm$ 1113 <sup>a</sup>
Tr. 7	261 $\pm$ 31
Tr. 8	15311 $\pm$ 1759 <sup>ab</sup>

The gross yield of *Moina* harvested at the end of experiment 5 is shown in Table 4. The mean yield of *Moina* was 390 g per tank which was 39.5% of the total yield. The rest of the total yield included sedimented *Chlorella*, accumulated organic inputs and chironomid larvae. Table 5 presents the extrapolated yield of experiment 5 in comparison with the yield from commercial culture system recorded by Anon. (1988). This shows that the production of *Moina* was higher in experiment 5 than that of the commercial culture system although the *Moina* harvested was only 39.5% of the total yield.

**Table 4.** The gross yield of *Moina* ( $\pm$  S. E.) observed in experiment 5.

Repli cate No.	Wet weight g	Total number in tank	Weight of <i>Moina</i> * g	% Weight of <i>Moina</i>
1	432	8467200 $\pm$ 432000	171.0 $\pm$ 8.7	39.6
2	332	5776800 $\pm$ 464800	116.7 $\pm$ 9.4	35.2
3	407	8791200 $\pm$ 407000	177.6 $\pm$ 8.2	43.7
Mean	390 $\pm$ 30		155.1	39.5

\* Calculated on the basis of the biomass of *Moina* = 20.2 micrograms.

**Table 5.** Comparison of the yields of experiment 5 and commercial culture system in Thailand.

Culture system	Wet inoculated weight g m <sup>-3</sup>	Yield of <i>Moina</i> g m <sup>-3</sup>	Purity%
Experiment 5	3.02*	292.6 (= 23.3 kg)	39.5
Commercial culture	240	960	90

\* Calculated on the basis of the biomass of *Moina* = 20.2 micrograms and extrapolated to g m<sup>-3</sup>. Value in parentheses indicate the probable production if *Moina* was inoculated in the same rate as in commercial culture system.

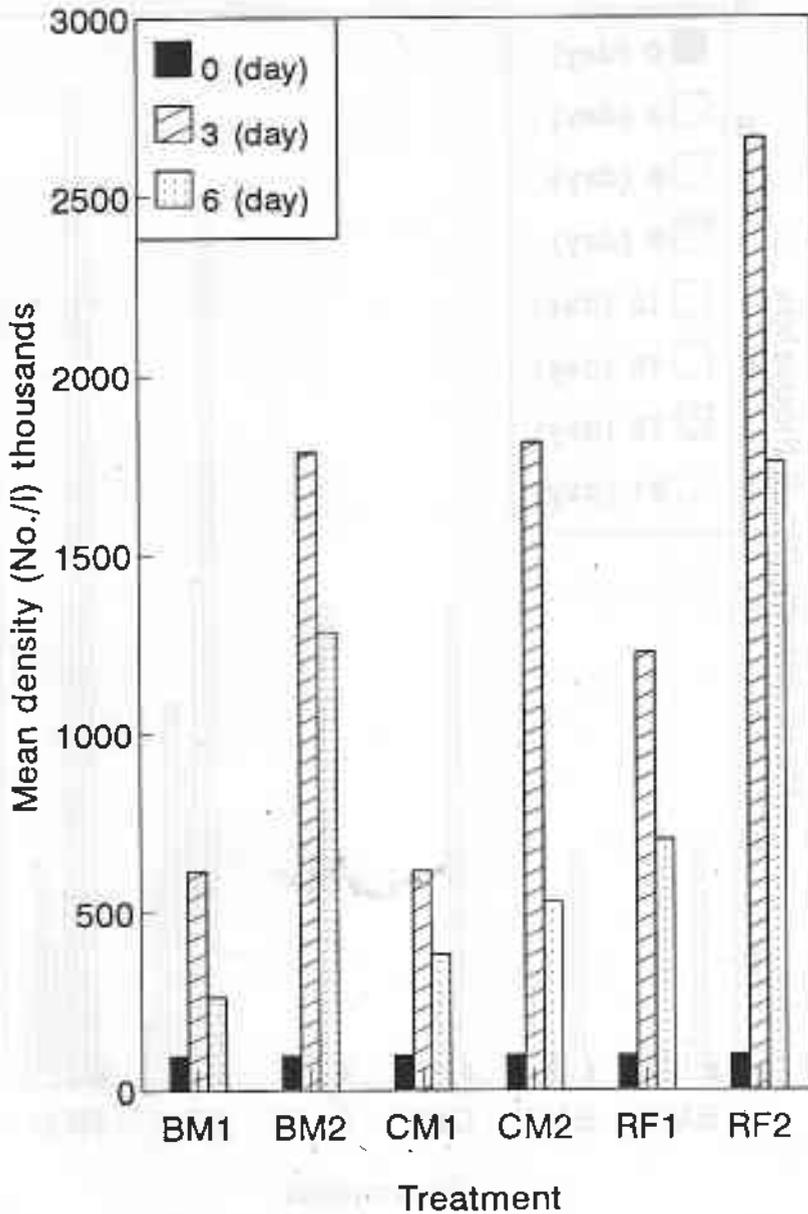
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Fig. 1. The *Moina* density observed with different treatments used in experiment 1. BM1 - 5.7 g m<sup>-3</sup> of buffalo manure, BM2 - 11.4 g m<sup>-3</sup> of buffalo manure; CM1 - 5.7 g m<sup>-3</sup> of chicken manure; CM2 - 11.4 g m<sup>-3</sup> of chicken manure; RF1 - 5.7 g m<sup>-3</sup> of rice bran and fish wastes (1:2); RF2 - 11.4 g m<sup>-3</sup> of rice bran and fish wastes (1:2).

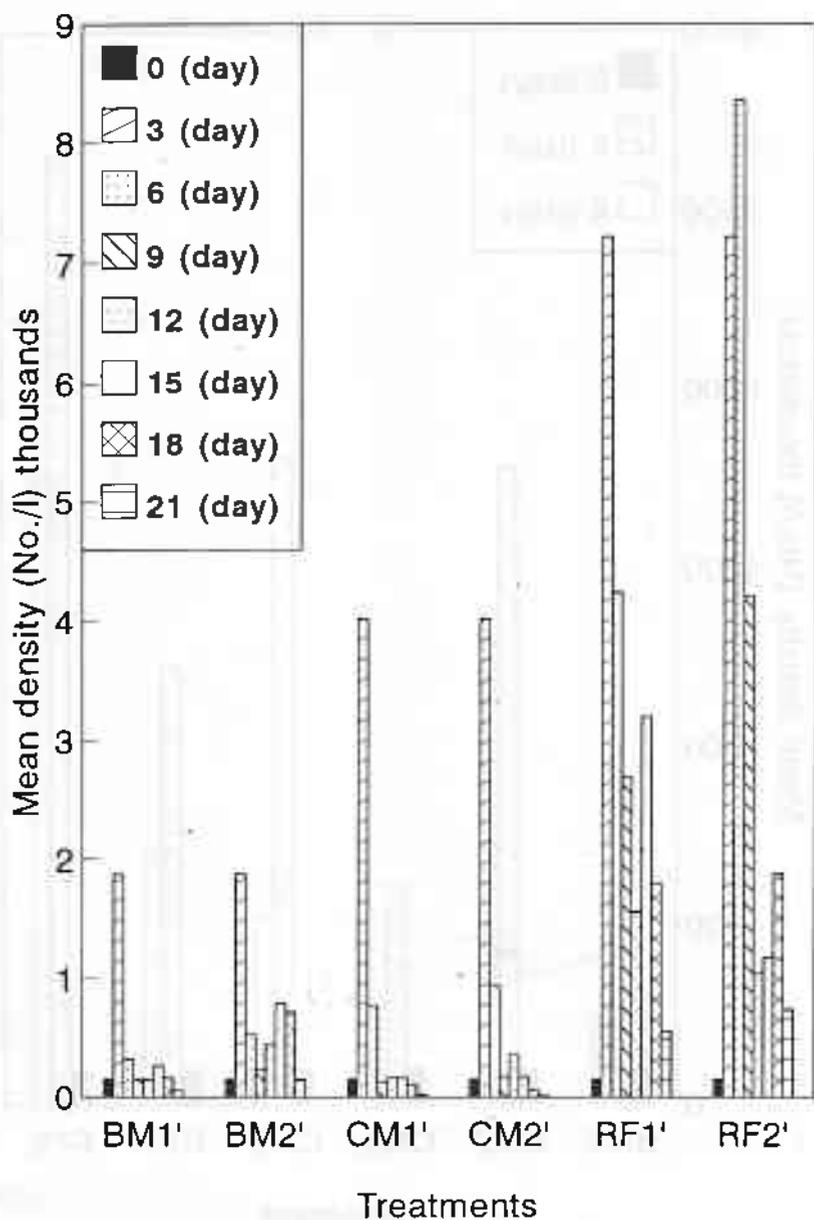


Fig. 2. The density of *Moina* observed with the different loadings used in experiment 2. BM1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of buffalo manure; BM2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of buffalo manure; CM1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of chicken manure; CM2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of chicken manure; RF1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of rice bran and fish wastes (1:2); RF2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of rice bran and fish wastes (1:2).

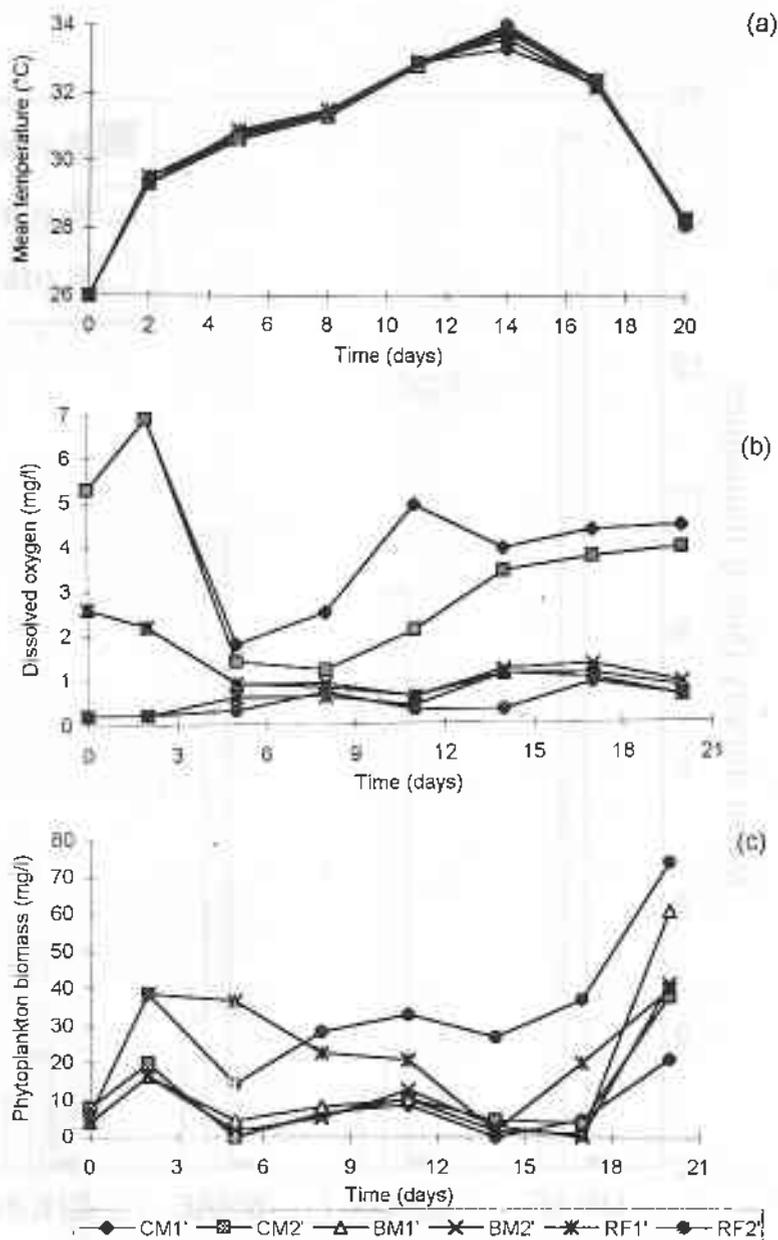
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Fig. 3. The fluctuations observed in major water quality parameters during experiment 2, (a) Water temperature (b) Dissolved oxygen (c) Phytoplankton biomass. BM1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of buffalo manure; BM2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of buffalo manure; CM1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of chicken manure; CM2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of chicken manure; RF1' -  $566 \text{ g m}^{-3}$  followed by  $37.7 \text{ g m}^{-3}$  of rice bran and fish wastes (1:2); RF2' -  $566 \text{ g m}^{-3}$  followed by  $75.5 \text{ g m}^{-3}$  of rice bran and fish wastes (1:2).

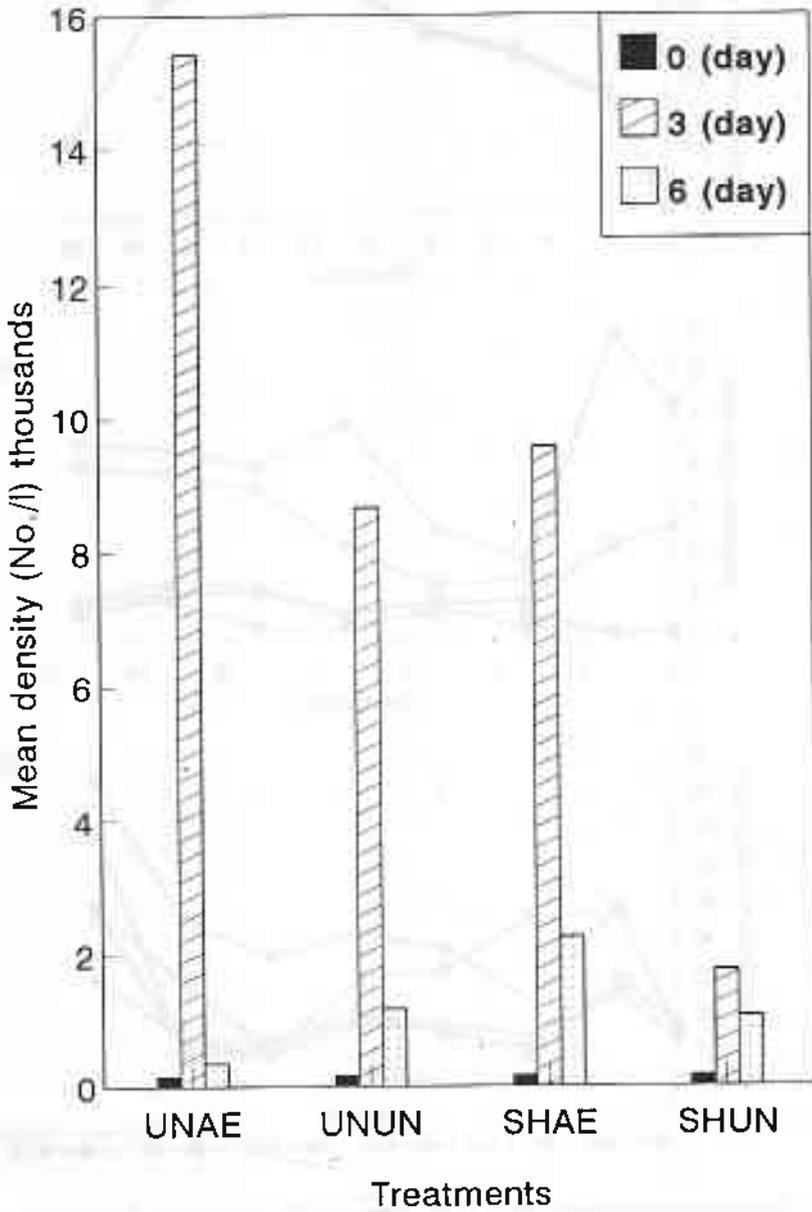


Fig. 4. The observed densities of *Moina* in experiment 3. UNAE - Unshaded aerated; UNUN - Unshaded unaerated; SHAE - Shaded aerated; SHUN - Shaded unaerated.

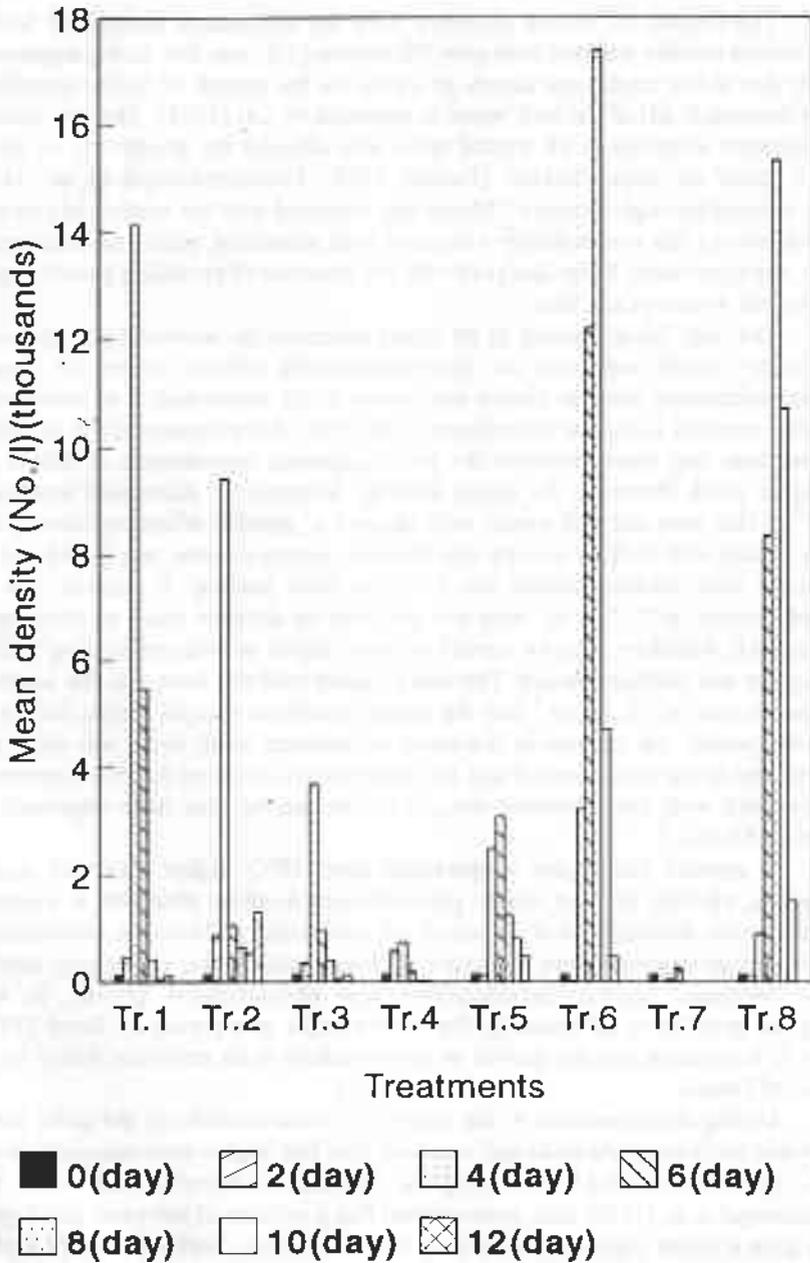


Fig 5. The *Moina* density observed with different treatments used in experiment 4. Tr. - Treatment. For a detailed description of treatments refer to Table 1.

### Discussion

The density of *Moina* observed with the continuous loading of buffalo manure, chicken manure and rice bran plus fish wastes (1.2) was low during experiment 1 probably due to the inadequate supply of inputs for the growth of *Moina* population and the unfavourable pH of the tank water as recorded by Lai (1988). The low biomass of phytoplankton observed in all treated tanks also affected the production of *Moina* because it feeds on phytoplankton (Pennak 1978; Tavarutmaneeagul et al. 1989). However, a relatively high density of *Moina* was observed with the loading of rice bran and fish wastes and this was probably a result of high nutritional quality of these inputs. Therefore, the experiment 2 was designed with the intention of providing enough supply of inputs for the *Moina* production.

The high initial loading of all inputs enhanced the available phytoplankton biomass in all culture tanks and the photosynthetically elevated water pH made a favourable environment prior to *Moina* inoculation in the experiment 2 as recorded by several other workers in similar experiments (Lai 1988; Tavarutmaneeagul et al. 1989). These conditions may have enhanced the parthenogenetic reproduction of *Moina* and gave a higher yield. However, the initial loading followed by subsequent loading of  $75.5 \text{ g m}^{-3}$  of rice bran and fish wastes only showed a positive effect on *Moina* yield. The tanks loaded with buffalo manure and chicken manure became less turbid and the tank bottoms were visible towards the fifth day after loading. It appears that the subsequent loading of  $75.5 \text{ g m}^{-3}$  was not adequate to develop algae in these tanks. Further research, therefore, may be carried out with higher subsequent loading rates of buffalo manure and chicken manure. The tanks loaded with rice bran and fish wastes at the continuous rate of  $75.5 \text{ g m}^{-3}$  had the lowest dissolved oxygen values during the experimental period. An increase in the mean temperature in all tanks was noticeable towards the end of the experiment 2 and this observation indicated that the temperature increase coupled with low dissolved oxygen concentrations may have depressed the production of *Moina*.

It appears that higher temperatures upto  $33^\circ\text{C}$ , higher dissolved oxygen concentrations, alkaline pH and higher phytoplankton biomass observed in unshaded aerated tanks were favourable and enhanced the production of *Moina* in experiment 3. The relatively higher temperature, exposure to direct sunlight and continuous aeration may have increased aerobic decomposition and phytoplankton growth, in turn, increasing the production of *Moina* in the culture tanks as reported by Boyd (1979). Therefore, it is apparent that the growth of phytoplankton is an important factor for the production of *Moina*.

During the experiment 4, the observed *Moina* densities in the tanks loaded with inorganic fertilisers, *Chlorella* and rice bran plus fish wastes were comparable with those in the tanks loaded with inorganic fertiliser, *Chlorella* and Ami Ami. Tavarutmaneeagul et al. (1989) also demonstrated that a mixture of inorganic and organic fertilisers gave a higher yield than when they were used alone. Furthermore, the loading of rice bran, fish wastes and *Chlorella* was equally effective as loading of Ami Ami, inorganic fertilisers and *Chlorella* for *Moina* production. The observed high levels of phytoplankton biomass in those tanks and the high nutritional dry matter content found in rice bran and fish wastes probably lead to a high yield of *Moina*. The tanks inoculated with *Chlorella* with the loading of  $566 \text{ g m}^{-3}$  of rice bran and fish wastes gave a higher yield of *Moina* than those tanks without the inoculation of *Chlorella* indicating a

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positive effect of the *Chlorella* inoculation. This may be due to the fact that *Chlorella* is a major food of *Moina* (Tavarutmaneegul et al. 1989). The *Moina* density observed with all loadings except rice bran plus fish wastes and Ami Ami treatments were relatively low. The high total ammonia-N levels and the lack of nutritional dry matter other than phytoplankton probably lead to a low production in the tanks loaded with inorganic fertilisers alone. The *Moina* production observed with the loading of septage was comparable with that reported by Lai (1988). The low nutritional quality of septage may have depressed the production as *Moina* is known to derive nutrients not only from phytoplankton but also from available suspended solids (Dinges 1973; Pennak 1978; Norman et al. 1979; Tavarutmaneegul et al. 1989).

In all experiments, the population density of *Moina* increased initially and then declined. The initial increase of density may be due to parthenogenetic reproduction which occurred when the food was abundant and the water quality was favourable (Hutchinson 1967; Pennak 1978). The decline of the *Moina* density was probably due to several factors such as the crowding of females, the deterioration of water quality due to accumulation of *Moina* waste products, the low food supply, a change in algal species and the development of predators (e.g. *Cypris*) and parasites in the tanks (Hutchinson 1967; Pennak 1978; Ventura & Enderez 1980; Jullie 1986; Lai 1988).

The high *Moina* yield resulted in the present system was comparable with that of the existing commercial culture system in Thailand. As Ami Ami is not available, the present system will be useful in the production of *Moina* in Sri Lanka. However, further research is encouraged to develop a sustainable *Moina* culture system with the partial harvesting at the maximum *Moina* density followed by water replacement and subsequent loading of the inputs.

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