An experimental study of the culture of the water flea (*Moina micrura*) in different culture media

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Abstract

In ornamental fish culture, live feed may increase the growth and survival rate of the juveniles of many species, and the water flea (*Moina micrura*) has been shown to be an excellent natural food for raising economically important fish. Laboratory and field experiments were carried out to develop techniques for the mass production of water fleas with different media such as freshly cultured unicellular algae, *Chlorella* and organic manure.

The type of food ingested by *Moina* was observed to include bacteria, small protozoa, *Chlorella* sp. and decomposed organic matter. The production trials were carried out in round fibre glass tanks (600 liter capacity) which were enriched with inorganic fertilizers, *Chlorella* and cow dung. Water temperature and pH were measured daily. Initial stocking of 2000-3000 individuals of *Moina* with inorganic fertilizer and *Chlorella* produced 6.5±0.14 number/ml on the sixth day of the culture period which was significantly higher ($P<0.05$) than that which resulted with the loading of cow dung as organic fertilizer (3.05±0.07 number/ml). Surface water temperature was 27.5 - 29.0° C and 27.13 - 28.13 ° C and water pH was 7.25 – 8.45 and 7.30 – 8.10 in culture with *Chlorella* and organic manure, respectively, which were conductive for optimum growth of *Moina*. The pH of water in all the tanks declined towards the end of the culture period.

Propagation and growth of water flea – *Moina* was much higher after loading of inorganic fertilizers with inoculation of *Chlorella* when compared to loading cow dung as organic fertilizer ($P<0.05$). The quantity of *Moina* sp. produced using the *Chlorella* was more suitable for commercial production.

Keywords: Live feeds, *Moina*

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Introduction

The economic importance of tropical aquarium fish in Sri Lanka has been growing over the last decade. In aquaculture, the success of raising larvae is dependent largely on the availability of sufficient quantities of live food organisms (Zooplankton) such as *Daphnia, Moina, Brachionus* and *Artemia.* Studies on the nutritive value of artificial and natural foods for tropical fish have found that the water flea, *Moina,* is not only a good protein source for fish fry (Shim and Bajrai, 1982), but also an organism well suited for various biological experiments (Murachi and Imai, 1954). It is a frequently used food source in fresh water ornamental fish culture because of its small size (males 0.6-0.9 mm & female 1.0-1.5 mm) and the ability to be produced in relatively large quantities (Tamaru *et al.*, 1998). The type of food it consumes, include bacteria, small protozoa, *Chlorella* sp and decomposed organic matters (Tavarutmaneegul *et al.*, 2000). It should, therefore, be possible to culture *Moina* on agro-industrial wastes such as rice bran, soya bean meal, and other animal manures including chicken manure and cow dung (Shim, 1988). Contamination with other zooplankton and transmission of diseases are major disadvantages of using such wastes.

The mass culture techniques for *Moina* have been developed along with the culture of phytoplankton. *Chlorella* is a freshwater unicellular alga that can be used as feed in *Moina* culture. The present paper compares the use of micro algae with organic manure for the mass production of *Moina micrura* a popular live food organism used by the freshwater ornamental fish trade.

Materials and Methods

The experimental cultures were conducted during the month of November 2007. *Chlorella* was cultured in the laboratory using three 500 ml conical flacks each containing 250 ml of sterilized fresh water, 0.25 ml of culture medium (Walne, 1974), shown in Table 1 and 25 ml of a uniform suspension of *Chlorella* (cell density - 167×106 cells/ ml). The culture containers were inoculated under a fluorescent lamp and aerated for 5 days.

Table 1. Composition of culture medium (Conwy medium) used for laboratory culture of C*hlorella* (Walne, 1974).

$NaNO_3$	100.0 g
Na_2EDTA	45.0 g
H_3BO_3	33.6 g
NaH_2PO_4 $2H_2O$	20.0 g
$FeCl_3$ $6H_2O$	1.3 g
$MnCl_2$ $4H_2O$	0.36 g
*Trace Metal solution	1 ml
** Vitamin Mix	100 ml
Distilled water (to make)	1000 ml
*Trace Metal Solution	
$ZnCl_2$	2.1 g
$CoCl_2$ $6H_2O$	2.1 g
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	2.1 g
$CuSO_4$ $5H_2O$	2.0 g
Distilled Water	100 ml
(acidify with 1N HCl until solution is clear)	
** Vitamin Mix	
Vitamin B_1	20 mg
Vitamin B_{12}	10 mg
Distilled water	200 ml

Glass aquarium tanks with a capacity of 45 liter (size 75×30×20 cm) were used as containers for the mass culture of *Chlorella.* Three tanks were filled with filtered tap water to a depth of 20 cm and the nutrient medium, urea (4.5 g), Triple Super Phosphate (TSP) (0.45 g) and $FeCl_3$ (0.12 g) were added. The laboratory culture of *Chlorella* - 2.25 l containing 187×10^6 cells/ml – was then inoculated and the alga grown for six days with aeration in shaded sun light. Algal cell density, water temperature and pH were determined daily during the culture period using a haemo-cytometer, thermometer and a portable pH meter (Model HACH sensION1), respectively.

Round fiber glass tanks (diameter 70 cm and height 50 cm) filled with unfiltered tap water up to 40 cm height were used for mass culture of *Moina* without organic manure. Inorganic fertilizer (urea 72g, TSP 6 g) , rice bran 150 g, soya bean 75 g and fish meal 75 g were added and the *Chlorella* was inoculated at a density of 20.00±0.80 cells/ml. Algae were allowed to grow for three days before 2000-3000 organisms of *Moina* were inoculated.

Fiber glass tanks (diameter 70 cm and height 50 cm) were used to culture *Moina* using organic manure. They were loaded initially with dried cow dung at the rate of 500 g/m^3, mixed with 600 l of fresh water and left for 3 days until the color of the water became dark green. The *Moina* was then inoculated (2000-3000 organisms) and 100 g/m^3 of dried manure was added every three days. One table spoon of the culture was taken every day and after adding a few drops of 70% alcohol, the number of organisms was counted to estimate the population density of *Moina*.

The two methods of culture were compared by subjecting the results to a one way ANOVA using a software package (SPSS).

Results and Discussion

The variation in the temperature and pH of water during the indoor culture period of *Chlorella* ranged from 27.00±0.00 to 28.17±0.29 ^{0}C and 8.90±0.09 to 10.00±0.00, respectively (Fig. 1). The pure culture of *Chlorella* achieved a density of 188.33±1.53 cells/ml after the six days of growth in laboratory culture (Fig. 2).

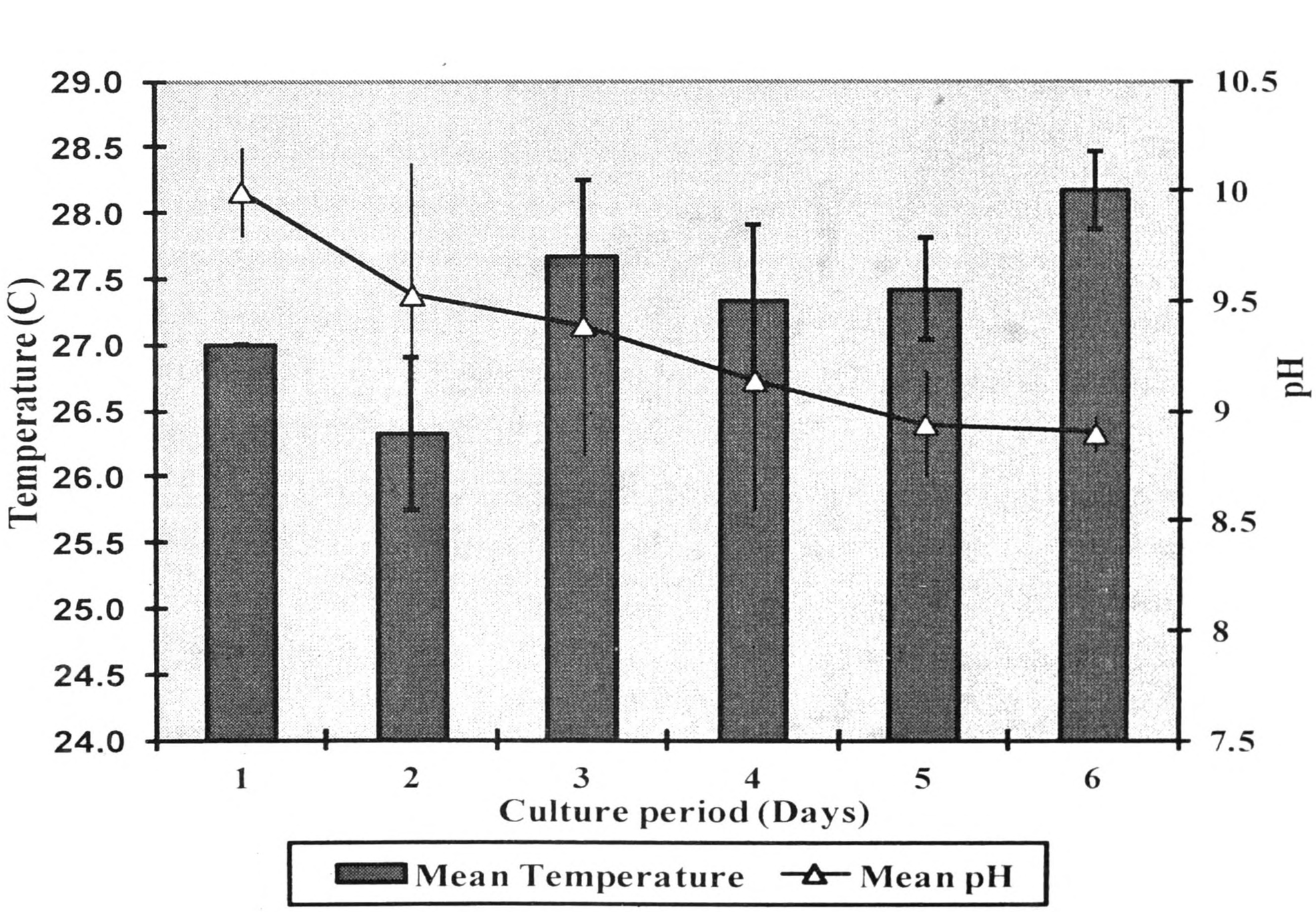

Fig. 1. Variation in mean water temperature and pH ± SD during the indoor laboratory culture of *Chlorella*.

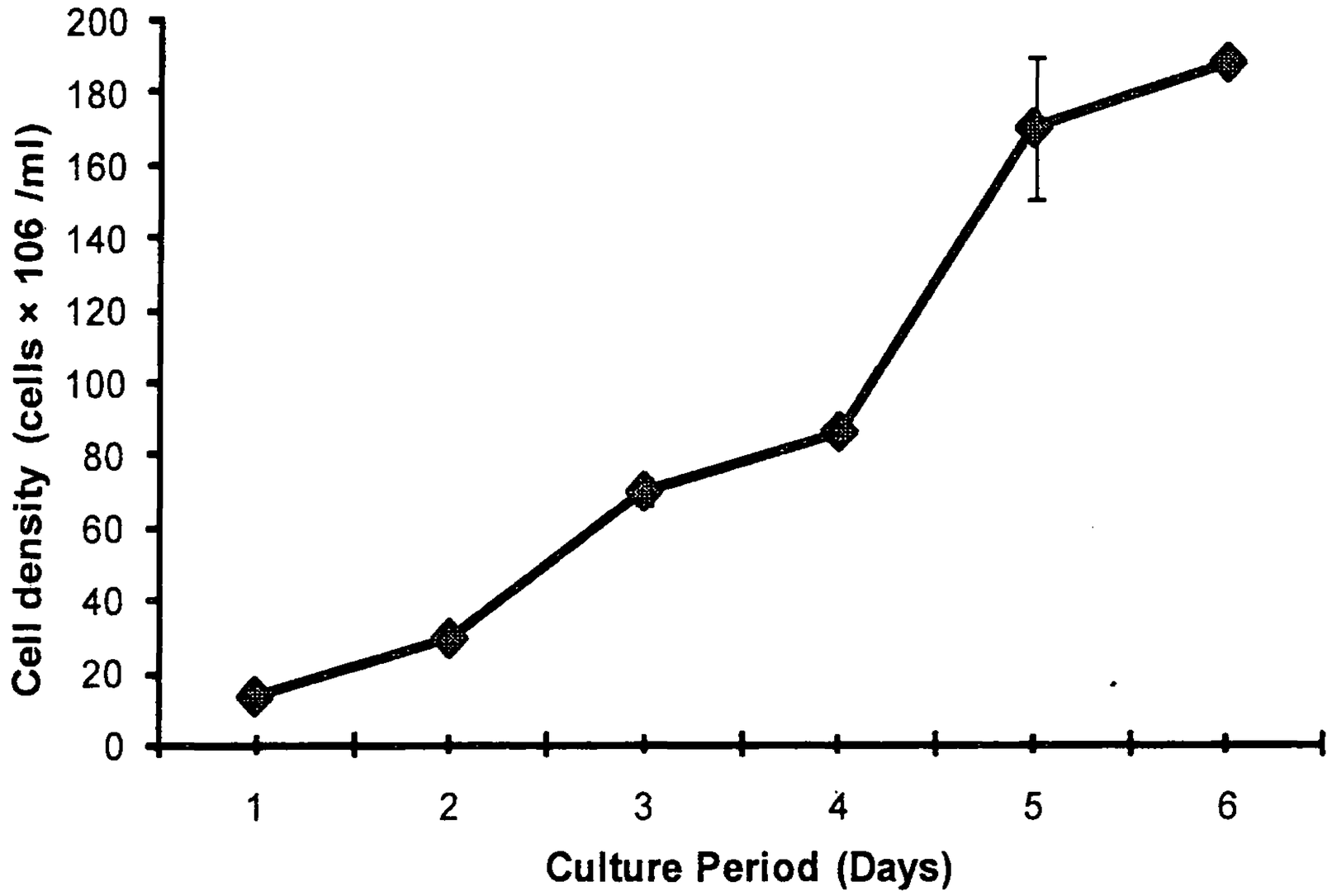

Fig. 2. The growth of *Chlorella* during the 6 days of indoor laboratory culture.

During the outdoor mass culture of *Chlorella,* the temperature and pH of water ranged from 26.33±0.29°C to 28.17±0.29 °C and 8.87±0.15 to 9.97±0.15(Fig.3), respectively; the density in the out door mass culture of *Chlorella* reached 20.00±0.80 10^6cells/ml (Fig. 4).

During seven days of *Moina* culture, the pH of water in the experimental tanks declined from 8.45 to 7.25 and 8.10 to 7.30 in the two treatments, *Moina* culture with *Chlorella* (Fig. 5) and cow dung Fig. 6), respectively. The growth rates of *Moina* in the two treatments used in this experiment are shown in Fig. 7. The highest density of *Moina* (6.35±0.21 cells/ml) was recorded on the sixth day of culture in the tank with inorganic fertilizer and *Chlorella.* The corresponding value for the tank loaded with cow dung was by 3.05±0.07 cells/ml; this difference was statistically significant ($P<0.05$). After day 6, the density of *Moina* in the treatment tanks declined (Fig. 7).

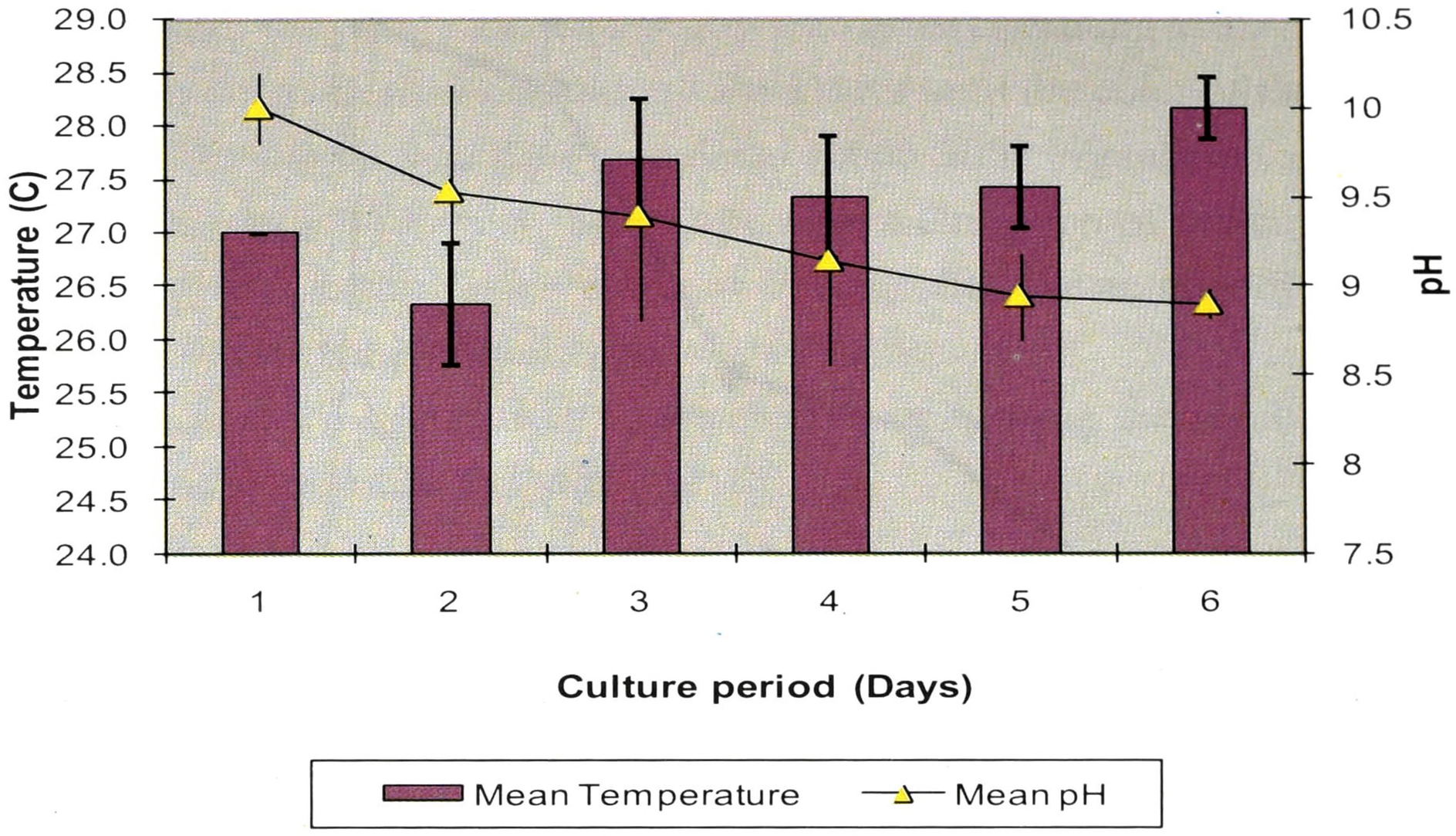

Fig. 3. Variation of mean water temperature and pH ± SD of outdoor mass culture of *Chlorella*.

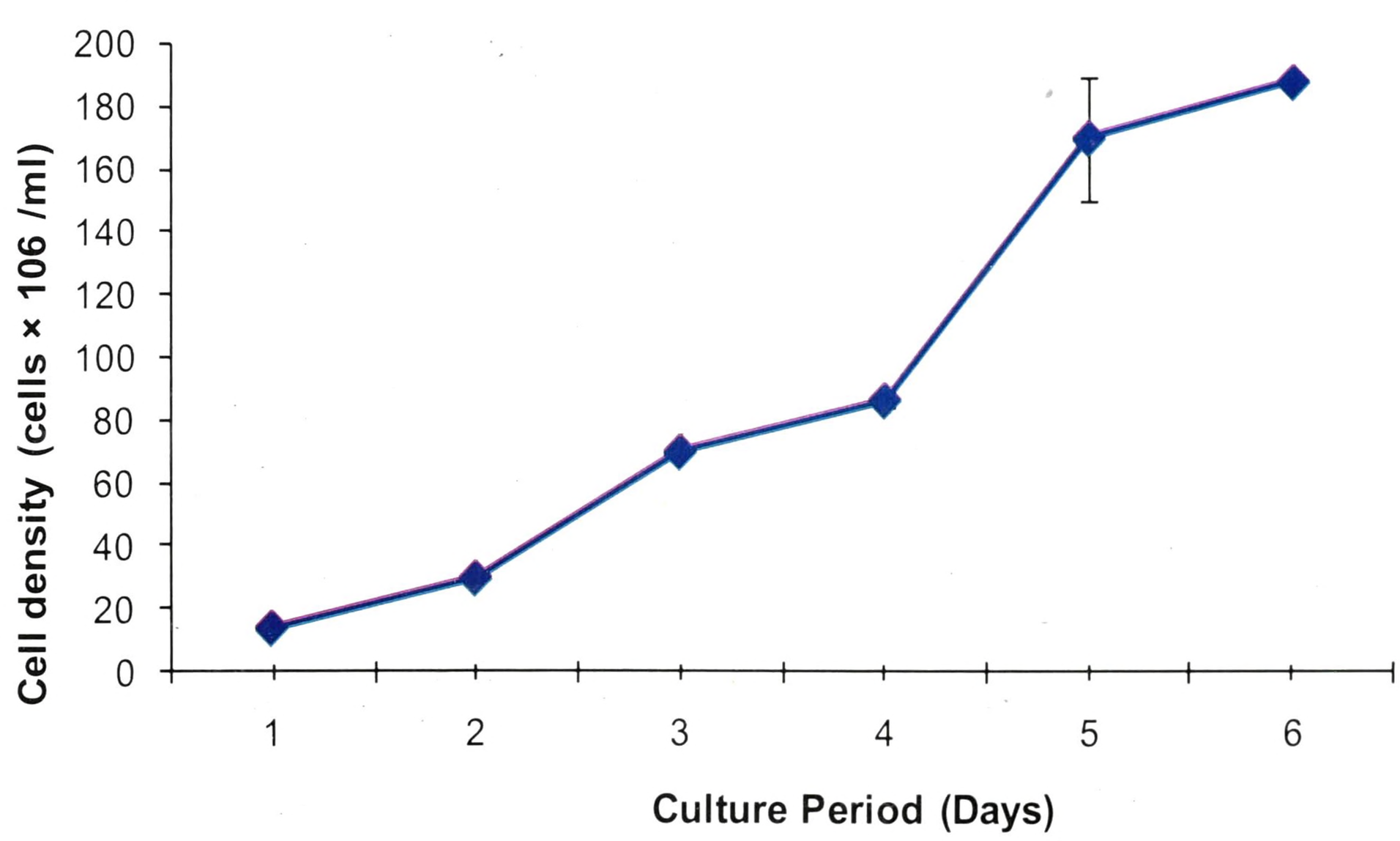

Fig. 4. The growth of *Chlorella* during outdoor mass culture.

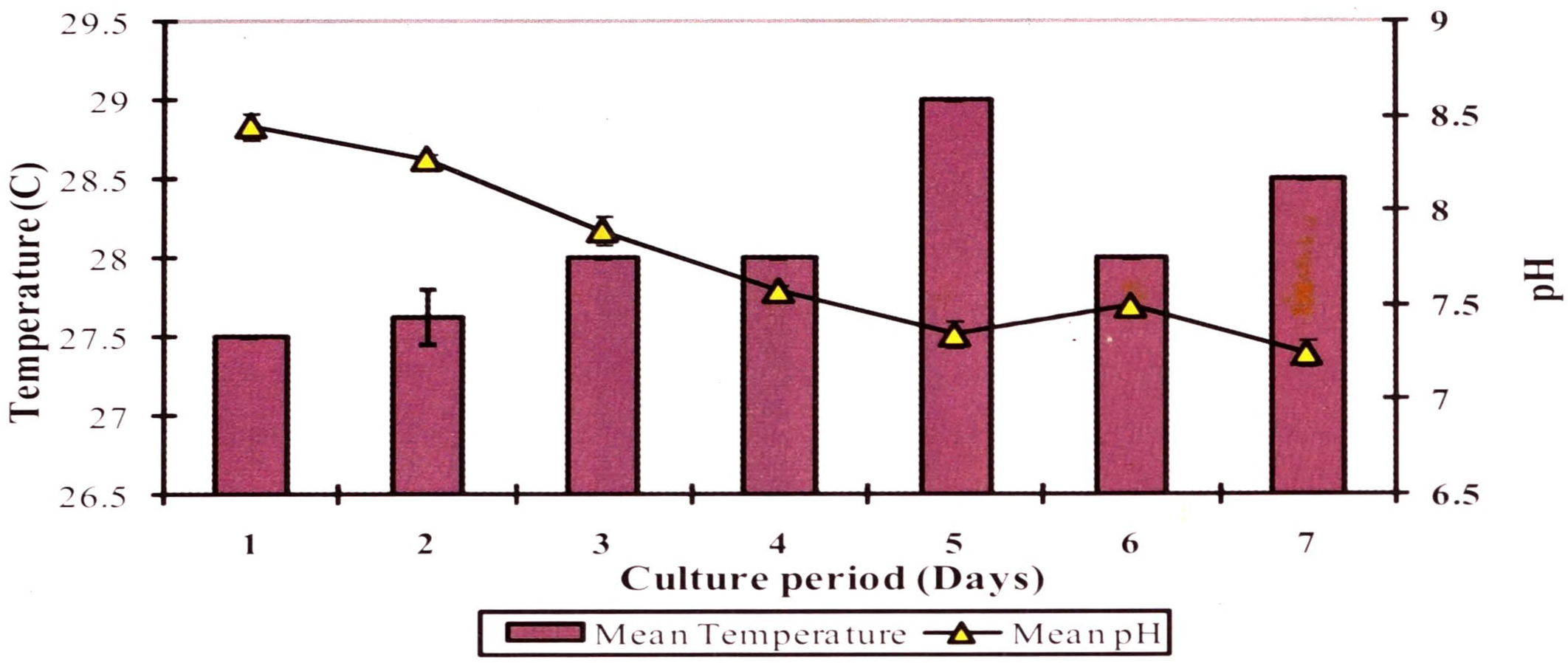

Fig. 5. Variation of mean water temperature and pH ± SD during 7 days culture period of *Moina* culture using micro algae-*Chlorella*.

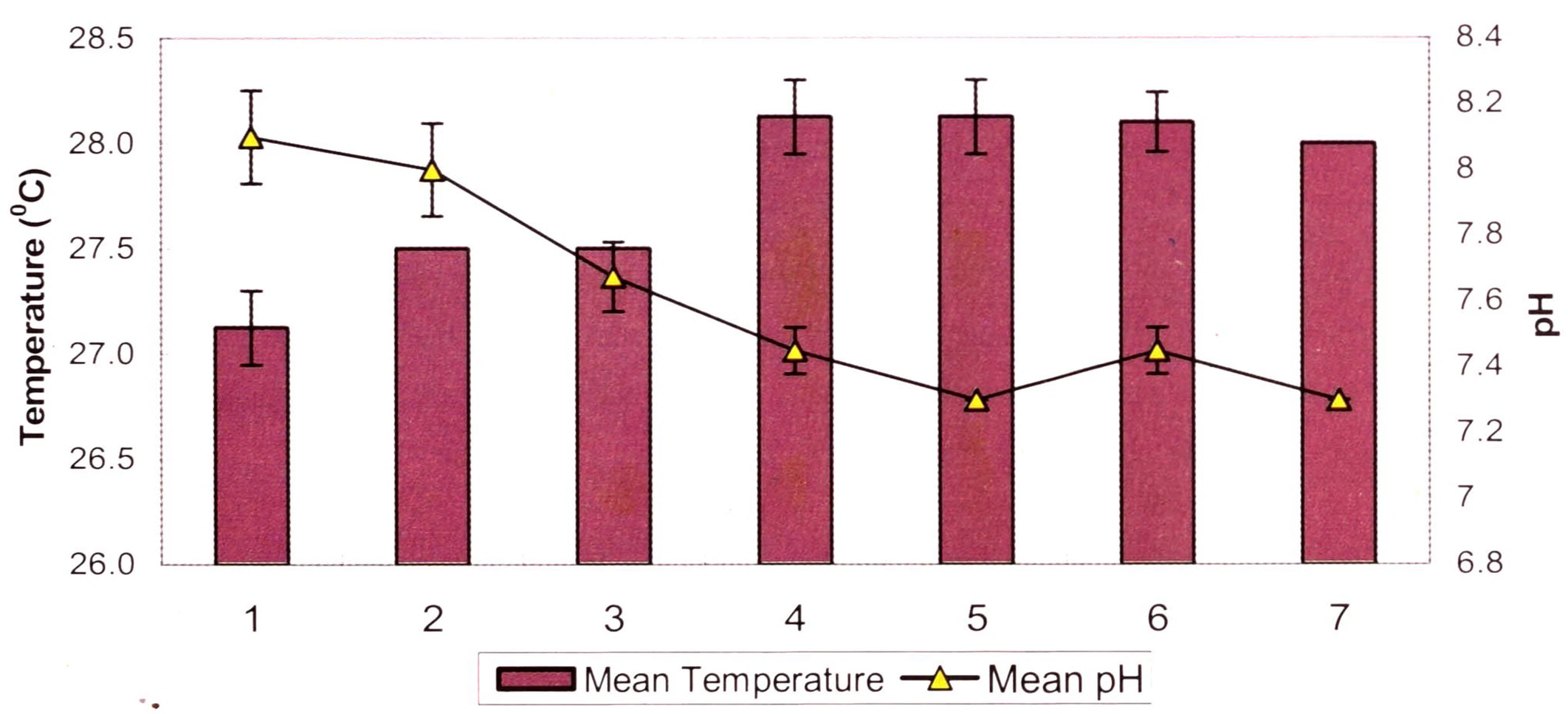

Fig. 6. Variation of mean water temperature and pH ± SD during 7 days culture period of *Moina* culture using cow dung.

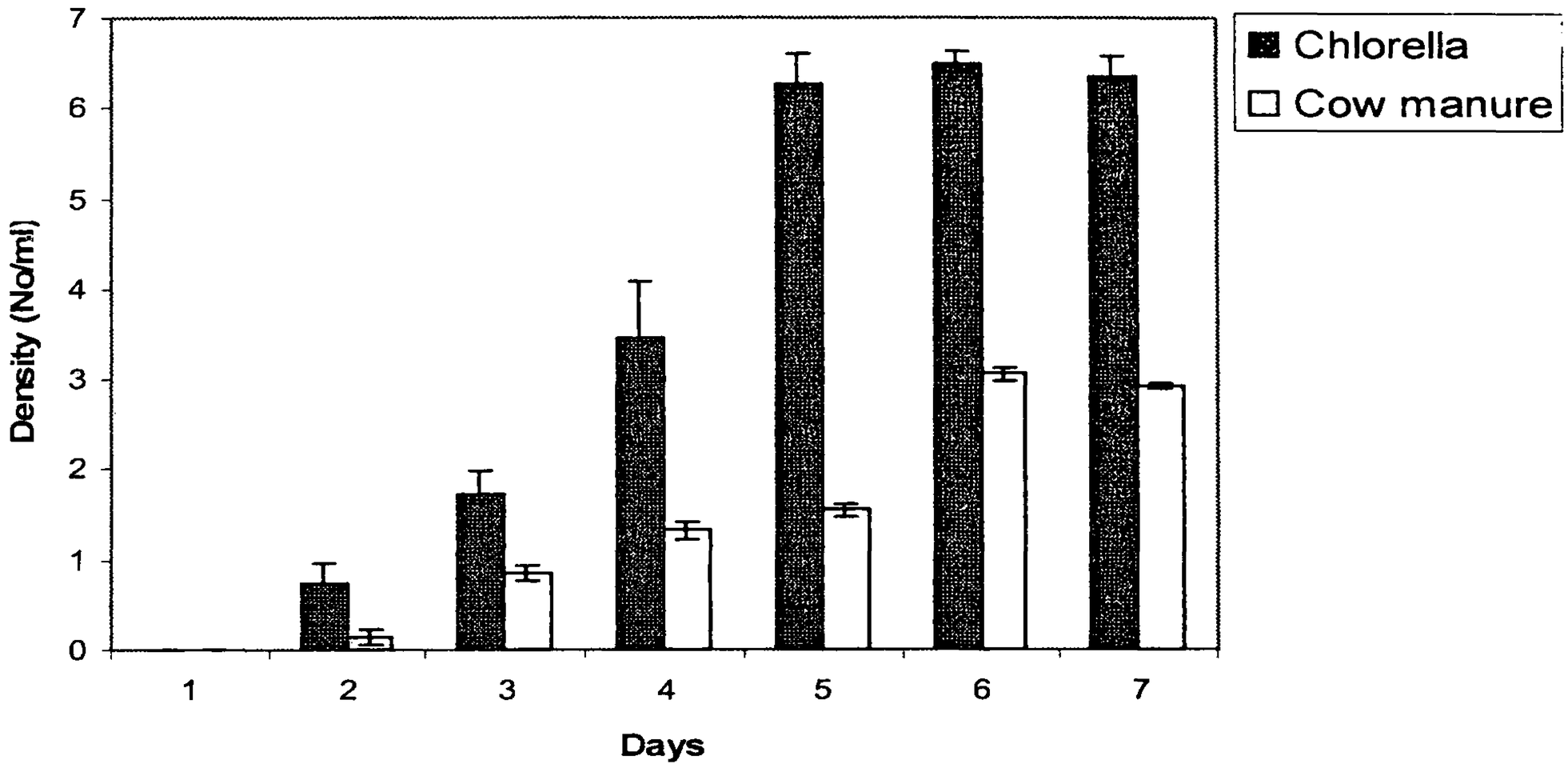

Fig. 7. The density (number/ml ± SD) of *Moina* during culture over the 7 day period with the two treatments; using micro algae-*Chlorella* or cow dung. Values with different superscript are significantly different (P<0.05).

Cladocerans normally have 4 to 6 instar stages through a series of 4-5 molts when growing from nauplii to maturation. The time taken depends primarily on temperature and the availability of food. Factors such as a change in water temperature or food deprivation as a result of increases in the population, may induce the production of males. Their nutritive value depends on their age and the chemical composition of their food source (Rottmann, 1992; Habib *et al.*, 2003).

During the culture period, density of *Moina* population observed in the tanks loaded with cow dung was low probably due to the inadequate supply of inputs for their growth. However, a relatively high density of *Moina* was observed with the loading of inorganic fertilizers with *Chlorella* and this was probably due to increased availability of nutrients in this treatment. Sipaba-Tavares & Bachion (2002) reported that a diet containing algae and vitamins showed better results for intrinsic rate, fecundity, and embryonic & post-embryonic development of *Moina micrura.* The highest density that has been reported (17 individuals/ml), was attained when the tanks were loaded with rice bran, fish wastes and inorganic fertilizers, and inoculated with *Chlorella* (Dias and Yakupitiyage, 1998). This value cannot be compared with the results in the present study, however, as the initial density of *Moina* in the culture tanks was not available. Tamaru *et al.*, (1996)

reported that when fresh *Chlorella* is used to culture the *Moina*, the amount of food no longer is a limiting factor, as the slurry has approximately 5 billion cells/ml and can also be added continuously to the culture.

In commercial culture of *Moina* in Thailand, Monosodium glutamate waste (Ami-Ami), urea, rice field fertilizer and superphosphate (TSP) are used as major inputs and relatively high yields have been achieved (AIT, 2001). Some studies on the food quality of freshwater phytoplankton for the production of cladocerans showed that detritus and benthic food can be an important food source, especially when the food concentration falls below a certain threshold. Nevertheless, cladocerans seem to be non-selective filter feeders and high concentration of suspended materials can interfere with the uptake of food particles. There is an additional advantage of culturing *Moina* with phytoplankton since there is less chance of contamination with competitors such as protozoans, rotifers, copepods and predators such as fish larvae, back-swimmers, diving beetles and dragonfly larvae.

During the experimental period, pH, water temperature and dissolved oxygen levels were maintained within the optimal range reported in several other studies (Rottmann, 1992; He *et al*., 2001; Rojas *et al*., 2000). Rottmann (1992) reported that *Moina* are resistant to extremes in temperature and easily withstand a daily variation of from 5 -31 °C; their optimum temperature being 75 to 88 °F (24-31 °C). The number of young per female, the number of broods per female, the number of young per day of reproductive life, and the number of young per brood of *Moina salina* were increased up to a temperature of 25 °C (Gordo *et al*., 1994). Further he concludes that the temperature range 20-25 °C is optimal for the development and reproduction of *Moina salina*. He *et al*., (2001) recorded that the optimum temperature for *Moina mongolica* is between 25 °C and 28 °C, but it can tolerate high temperatures between 34.4 °C and 36.0 °C. Rojas *et al*., (2000) reported that optimum hatching conditions were pH 5-9, temperature 25 °C, photoperiod eight or more hours light per day and light intensity equal to or greater than 850 Lux. Boyd (1979) reported that relatively high temperatures, exposure to direct sunlight and continuous aeration may increase aerobic decomposition and phytoplankton growth, in turn, increasing the production of *Moina* population. Therefore, it is apparent that the growth of phytoplankton is an important factor for the production of *Moina* in aquaculture.

During the culture period, the population density of *Moina* increased initially, became stable 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 favorable as reported by Hutchinson (1967). The subsequent declined of the *Moina* density was probably due to several factors such as the crowding of females, deterioration of water quality, low food supply, a change in algal species, predation and parasites. Nevertheless Abramo (1980) and Martinez-Jeronimo *et al.*, (2007) reported that sexual reproduction in cladocerans is a phenomenon induced by environmental factors, mainly associated with adverse conditions, including crowding such as starvation, accumulation of excretory metabolites, or increased intra-specific encounter. During culture, increases in carbon dioxide levels due to respiration resulted in low pH values.

Conclusion

A significantly ($P<0.05$) higher density (6.5 cells/ml) of water flea – *Moina micrura*- was obtained in media containing the unicellular micro alga – *Chlorella* sp – when compared to organic manure, showing in this seven day study, that this medium is more suitable for rapid reproduction and growth of the *Moina* than organic manure.

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