



Effect of nutrient level on phytoplankton population in zero water exchange shrimp culture farms

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ABSTRACT

Soil and water characteristics and their influence on phytoplankton abundance, at different stocking densities under zero water exchange were studied in two successive crops of tiger shrimp, *Penaeus monodon* at Mahabalipuram, Kanchipuram District in Tamil Nadu, India during 2007 and 2008. The shrimp ponds were categorised under two groups viz., low stocking density (8 no. m⁻²) and high stocking density (16 no. m⁻²). In pond water, physico-chemical properties and total nutrient content did not vary significantly between the stocking densities, whereas nitrate and phosphate significantly differed. All available and total nutrients viz., nitrogen, phosphorus and silicate increased significantly in pond water with days of culture. Water quality had an influence on the abundance of phytoplankton and it varied widely and significantly during culture period as well as with different stocking densities. Phytoplankton density had a positive correlation with nitrate ($r=0.87$), phosphate ($r=0.79$), silicate ($r=0.97$) and chlorophyll *a* content ($r=0.98$).

Keywords: Chlorophyll *a*, Culture practices, Nutrient level, Phytoplankton population, Shrimp stocking density

Introduction

Successful pond aquaculture largely depends on the overall aquatic environment. Scientific management of a water body is closely related to the acquisition of knowledge of the environmental factors, specially physico-chemical and biological factors that largely affect the aquatic productivity (Boyd, 1982; Hossain *et al.*, 2007; Rahman *et al.*, 2008). A combination of physical variables and biotic parameters has a direct role in maintaining the optimum water quality parameters in a shrimp culture pond (Jones *et al.*, 2001). Among the various biotic factors, phytoplankton plays a pivotal role in maintaining water quality by effectively maintaining oxygen levels, light regimes, bacterial numbers as well as zooplankton biomass and assimilates ammonia generated by shrimp excretion (Lorenzen *et al.*, 1997). The blooms also provide shade to shrimps, prevent the growth of benthic algae and provide food source for zooplankton and other invertebrates eaten by shrimps.

The production of natural food in pond depends chiefly on the water quality, availability of nutrients and primary productivity; and it varies depending upon the hydrobiological parameters and the primary producers present in shrimp culture ponds (Surianarayana Moorthy and Altaff, 2002). The phytoplankton population represents

a vital link in the food chain. The zooplankton forms the principal source of food for most of the fish (Prasad and Singh, 2003).

Both the qualitative and quantitative abundance of plankton in a culture pond are of great importance in managing successful aquaculture operations, as these vary from location to location and from pond to pond within the same location even with similar ecological conditions (Boyd, 1982). The biomass of the pond sustained/protracted from the stocking rate and in turn the management measures have a bearing on the plankton and nutrient dynamics in a shrimp pond. This study was conducted with an objective to understand the plankton dynamics in terms of abundance between the two farming systems with different densities and the role of nutrients on the plankton abundance therein.

Materials and methods

The study was carried out in *P. monodon* ponds each with an area of 0.62 ha and water depth of 1 to 1.5 m during winter (August to December) crop of 2007 (CR_1) and 2008 (CR_II) under two different stocking densities of SD8 and SD16 (8 and 16 no. m⁻²). The ponds were located at Mahabalipuram (12° 37' N 80° 14' E), Kancheepuram District, Tamil Nadu and the water source was from Edayur backwater connected with Buckingham canal.

The experimental ponds were allowed to dry after the harvest of shrimps cultured during January - May. In CR_I, scraping was done during pond preparation, whereas in CR_II, both scraping and tilling were done. Liming was done with lime stone powder ($\text{Ca}_2 \text{CO}_3$) in all the ponds at the rate of 750 - 800 kg ha⁻¹. Stocking was done with PL₁₈ of *P. monodon* with the required number of seeds at both the stocking densities. No water exchange and fertilization were done during the culture period in both the treatments. Aeration and probiotics were provided only to SD16 ponds. Aeration was provided with paddle wheel aerators (6 numbers per ha), at the rate of 6 h day⁻¹ from 60th day, 10 h day⁻¹ from 90th day and 12 h day⁻¹ from 105th day till the harvest. The shrimps were fed with commercial pellet feed throughout the crop in both the practices. Physico-chemical properties and nutrient levels of water, plankton biomass and chlorophyll *a* during the crop were estimated at fortnightly intervals.

Sampling and analysis

Water samples were collected in all the ponds at fortnightly intervals between 07:00 and 08:00 hrs in 500 ml polyethylene bottles for the analysis of pH, nitrate-N, soluble reactive phosphorus and silicate and were analysed immediately in the laboratory. *In situ* measurements were done for temperature, salinity and dissolved oxygen using thermometer, hand refractometer and winkler method respectively. Dissolved oxygen and pH were determined as per APHA (1998). Water nutrient parameters like nitrate-N, reactive P and silicate were analysed by standard methods (Strickland and Parsons, 1972). For chlorophyll *a*, water samples were filtered through glass fiber filter (Whatman GF/C) and algal pigments were extracted with 90% acetone and measured spectrophotometrically (APHA, 1998).

Water samples for phytoplankton analysis were collected at fortnightly intervals in 100 ml polyethylene tube using standard bolting silk number 25 plankton net

(mesh size 0.064 mm) and samples were preserved in 4% buffered formalin. Quantitative analysis of the samples was carried out in the laboratory following standard method (APHA, 1998).

Statistical analysis

Water parameters and plankton density variations with days of crop and between stocking densities were analysed statistically using ANOVA and Duncans Multiple Range Test (DMRT). Pearson's correlation analysis was done to determine which of the physicochemical and nutrient factors are related with phytoplankton density. All the statistical analyses were done using SPSS (V) 16 (Statistical Package for Social Sciences, Chicago, IL, USA) software package.

Results and discussion

Physico-chemical parameters

All metabolic, physiological activities and life processes are greatly influenced by temperature. It also affects the natural productivity of aquatic ecosystems, speed of chemical changes in soil and water. In this study, pond water temperature fluctuated between 29.1 and 32.2 °C during the first crop and between 27.0-30.2 °C in second crop (Table 1). The highest temperature was observed during the start of the culture period and it decreased as the rainy months commenced. The observed temperature range was optimal for mineralisation of organic nutrients into available form. This is supported by the findings of Wood *et al.* (1992) and Chakraborty *et al.* (2002). There was no significant difference in temperature between the two densities. However, the water temperature showed a high negative correlation with chlorophyll *a* ($r=-0.819$) and phytoplankton ($r=-0.74$) in both the densities as well as in both the crops.

In the present study, salinity of the pond water ranged between 16 (December) and 20.6 ppt (September). The reduction in salinity was due to dilution of pond water

Table 1. Water quality parameters during culture period (Mean \pm SE)

	1 st crop (CR_I)		2 nd crop (CR_II)	
	SD8	SD16	SD8	SD16
pH	7.92 \pm 0.081 ^a	7.75 \pm 0.078 ^b	8.27 \pm 0.048 ^a	8.22 \pm 0.043 ^a
DO (mg l ⁻¹)	6.01 \pm 0.135 ^a	5.64 \pm 0.147 ^a	6.03 \pm 0.078 ^a	5.38 \pm 0.086 ^a
Salinity (ppt)	18.5 ^a \pm 0.31	17.7 ^a \pm 0.23	19.3 ^a \pm 0.31	18.7 ^a \pm 0.37
Temperature (°C)	29.81 \pm 0.17 ^a	30.38 \pm 0.189 ^a	28.81 \pm 0.24 ^a	28.82 \pm 0.24 ^a
Silicate (mg l ⁻¹)	0.95 \pm 0.02 ^a	0.97 \pm 0.03 ^a	0.79 \pm 0.022 ^a	0.88 \pm 0.007 ^b
Total N (mg l ⁻¹)	0.85 \pm 0.058 ^a	0.91 \pm 0.055 ^a	0.81 \pm 0.057 ^a	0.83 \pm 0.057 ^a
Total P (mg l ⁻¹)	0.157 \pm 0.014 ^a	0.192 \pm 0.015 ^a	0.150 \pm 0.010 ^a	0.169 \pm 0.016 ^a
Chlorophyll <i>a</i> (μ g l ⁻¹)	42.9 \pm 0.85 ^a	44.7 \pm 0.99 ^a	39.5 \pm 1.07 ^a	42.8 \pm 0.72 ^a

Note: Values in the same row with different superscripts are significantly different in that crop

by rain during the period. There were numerically lower mean values in SD16 than SD8 (Table 1). The salinity levels observed during the study was conducive to normal growth of *P. monodon* (Chen, 1976; Chen, 1985). Pearson's correlation analysis showed that salinity had a highly negative correlation with chlorophyll *a* ($r = -0.872$) and with the density of phytoplankton ($r = -0.764$)

The pH observed under SD8 and SD16 in CR_I was 7.92 and 7.75 respectively whereas it was 8.27 and 8.22 in CR_II. No significant difference was noted between the treatments in CR_II, but there was a significant difference in CR_I (Table 1). pH was comparatively low at the end of crop than that at the initial period. The decrease in pH towards harvest time might be due to the decomposition of sediments that would liberate hydrogen ions (Praphrutham, 1985). The optimum range of pH kept all the metabolites especially unionized ammonia within limits. pH showed a negative correlation with chlorophyll *a* ($r = -0.184$), though not significant.

Dissolved oxygen is an important parameter for natural productivity and transformation of nutrients in soil, water and soil-water interface. The dissolved oxygen fluctuated during the course of the day. The average dissolved oxygen (DO) value under SD8 and SD16 throughout CR_I was 6.01 and 5.64 mg l⁻¹ respectively. In CR_II, the values were 6.03 and 5.38 mg l⁻¹ under SD8 and SD16 respectively (Table 1). In both the crops, the two densities exhibited different ranges but there was no significant difference. For example, DO in SD8 ranged between 5.5 and 6.8 mg l⁻¹, and SD16 from 5.0 to 6.7 mg l⁻¹. It is similar to the findings of Tookwinas and Songsangjinda (1999) and Cremen *et al.* (2007). In this study, dissolved oxygen was found within the optimal range in both SD8 and SD16 and aeration might have helped to maintain the optimum level in SD16.

Nitrogen and Phosphorus

Ammoniacal and nitrate forms of nitrogen constitute the ready source of available nitrogen to primary fish food organisms in food chain. Among the various forms of nitrogenous nutrients, nitrate is the most important, as it is the final form, being absorbed by plankton for their growth (Begum *et al.*, 2003). Nitrate concentration increased during the crop period and it ranged from 0.074 to 0.150 and 0.075 to 0.250 mg l⁻¹ at SD8 and SD16 stocking densities respectively in the first crop (Fig. 1). There was significant difference between the densities ($p < 0.01$) as well as between the months of crop ($p < 0.01$). The ponds with SD16 had higher concentration of nutrients than the one with SD8 due to higher feeding rates. The same trend of nitrate followed in the second crop also (Fig. 2).

Phosphorus is considered as an important nutrient for phytoplankton production, as it has been found to increase

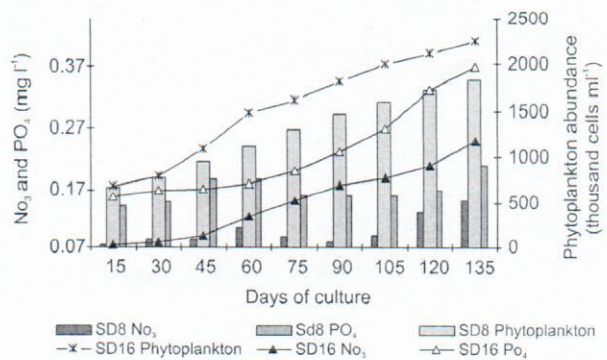


Fig. 1. Phytoplankton abundance in relation to nutrients during CR_I

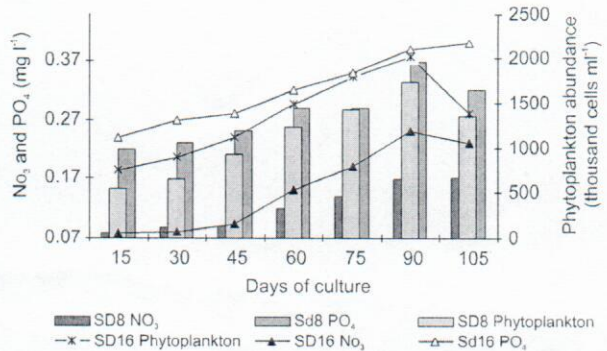


Fig. 2. Phytoplankton abundance in relation to nutrients during CR_II

the abundance of phytoplankton than nitrogen alone (Daniels and Boyd, 1993). Similar finding was reported by Hossain *et al.* (2006), wherein phytoplankton abundance is more in the ponds treated with poultry manure than with cowdung alone or combined with mineral fertilizers. It is due to the presence of more phosphorus (1.5%) and efficient release from poultry manure. Phosphate-phosphorus concentration ranged from 0.142 to 0.210 mg l⁻¹ and 0.157 to 0.370 mg l⁻¹ in SD8 and SD16 stocking density respectively in first crop (Fig. 1). The availability of phosphorus in the second crop exhibited the same trend (Fig. 2). The Phosphate-phosphorus levels were significantly lower in SD8 compared to that in SD16 and it was significantly different among the different months of crop ($p < 0.05$). The trends observed in the present study are similar to the findings of Shah *et al.* (2008).

Increase in silicate increases the proportion of diatoms as compared to green algae in the phytoplankton communities (Kilham, 1986; Schelske *et al.*, 1986) and 0.16 mg l⁻¹ of silicate is required to produce sufficient amount of plankton. In the current study, silicate concentration ranged from 0.72 to 1.15 mg l⁻¹ Si (Table 1), which is much higher than the recommended minimum value. There was a significant difference during the crop

period ($p < 0.05$), whereas no significant difference was observed between the densities ($p > 0.05$). Tookwinas and Songsangjinda (1999) also observed similar results and recorded a mean value of 0.76 mg l^{-1} .

Phytoplankton abundance

Phytoplankton plays a pivotal role in maintaining water quality by effectively maintaining oxygen levels, light regimes, bacterial numbers and zooplankton biomass. It has an important role in nitrogen cycling and effectively assimilates ammonia generated by shrimp excretion (Lorenzen *et al.*, 1997). Phytoplankton is the base of the food chain in pond cultures to support crustacean production. Phytoplankton abundance recorded in shrimp ponds in the present study are depicted in Fig. 1 and 2. Phytoplankton abundance varied widely and significantly during the crop, having a higher abundance in advanced stages of the crop cycle. In the first crop under stocking density of 16 m^{-2} , the phytoplankton density was recorded at $7,00,000 \text{ cells ml}^{-1}$ at 15 days of crop period and it steadily increased to $22,41,750 \text{ cells ml}^{-1}$ by the end of the crop period. Such increase in abundance of plankton over the crop cycle has been reported by Alonso-Rodriguez and Paez-Osuna (2003). Similar trend was observed in SD8 ponds as well.

During the second crop, phytoplankton abundance increased up to 90 days of culture at both the densities of shrimp crop but reduced thereafter, by 22% and 31% in SD8 and SD16 ponds respectively and recorded comparatively low value at the end of crop (Fig. 2). This unusual phenomenon was due to heavy rain and consequent drop in salinity.

In both the crops and densities, chlorophyll *a* levels tended to increase towards the end of cycle (for example, concentration ranged from 30.2 to $50.1 \mu\text{g l}^{-1}$ under SD8 ponds and it ranged from 33.8 to $50.7 \mu\text{g l}^{-1}$ under SD16 ponds). As daily feed allotment increase in response to shrimp growth throughout the culture, higher amounts of nutrients and metabolic wastes enter the water. Thus the availability of nutrients would correspondingly increase, promoting phytoplankton abundance.

In the first crop, significant difference in the phytoplankton abundance was observed both between the densities and between the months of crop ($p < 0.05$). Similar to the first crop, there was a significant difference in the abundance of plankton between the months and densities ($p < 0.05$) of the second crop. While comparing phytoplankton abundance between the two stocking densities, higher abundance was recorded in the SD16 ponds. The relationship between phytoplankton abundance and days of culture is depicted in Fig. 3.

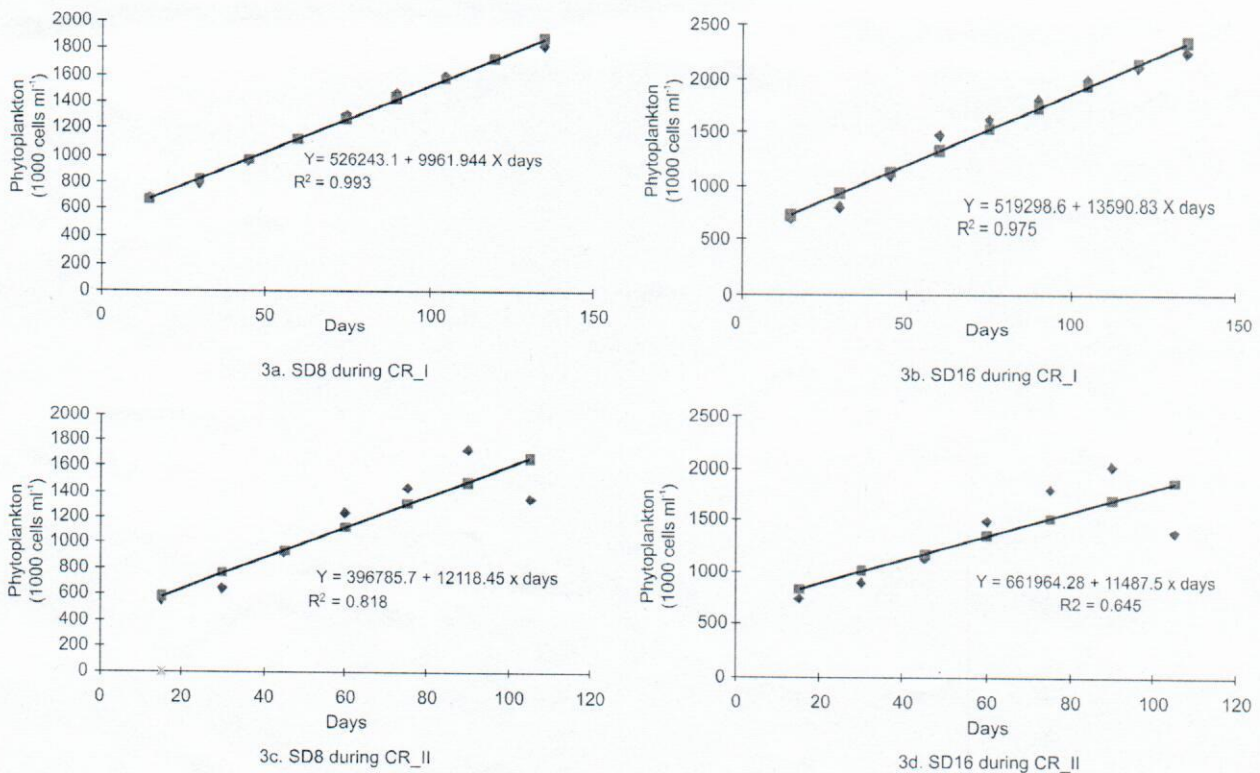


Fig. 3. Relationship between phytoplankton abundance and days of culture under different stocking densities and culture practices

The abundance of phytoplankton highly correlated with nitrate, phosphate and silicate with a correlation coefficient of 0.96. The regression equation was:

Phytoplankton abundance for SD8 under CR_I = $-1008808 + (3600065 \times \text{nitrate}) + (-2854551 \times \text{phosphate}) + (2540083 \times \text{silicate})$.

Phytoplankton abundance for SD16 under CR_I = $-3200847 + (5008036 \times \text{nitrate}) + (-5507340 \times \text{phosphate}) + (5418657 \times \text{silicate})$.

Phytoplankton abundance for SD8 under CR_II = $-5853758.918 + (7316098.055 \times \text{nitrate}) + (2197940 \times \text{phosphate}) + (6905710.003 \times \text{silicate})$.

Phytoplankton abundance for SD16 under CR_II = $-9398885 + (6587079 \times \text{nitrate}) + (-6521848 \times \text{phosphate}) + (13427920 \times \text{silicate})$.

This study showed greater abundance of phytoplankton in ponds with SD16 (Fig. 1 and 2), probably relates to increase in availability of nutrients like inorganic nitrogen and phosphate content in water because of higher nutrient input through feed (Boyd, 1989) and mineralisation of residual nutrients available from previous culture, excess feed and the favorable physical and chemical parameters.

Factors affecting phytoplankton

The growth of phytoplankton is influenced by several factors: light, temperature, nutrients and grazing (Burford, 1997). Seasonal variations in phytoplankton are related to a variety of environmental factors in the aquatic environment (Cetin and Sen, 2004) and are a major determining factor in phytoplankton growth and development. In the present study, the seasonal variations of total phytoplankton density may therefore be attributed to a wide range of physico-chemical parameters such as temperature (Mosisch *et al.*, 1999; Morales-Baquero *et al.*, 2006), salinity, nitrate-N and phosphate-P (Gouda and Panigrahy, 1996). Correlation analysis of the data showed that the phytoplankton density had a highly significant positive correlation with nitrate ($r=0.87$), phosphate ($r=0.79$), (Gouda and Panigrahy, 1996), silicate ($r=0.83$), total ammonia nitrogen ($r=0.89$) and chlorophyll *a* ($r=0.98$) (Cremen *et al.*, 2007). Chlorophyll *a* positively correlated with phytoplankton density. Hence, analysis of either of the parameters gives the overall picture of natural productivity in the ponds.

The growth, type and dominance of a particular phytoplankton (algal groups) in aquaculture ponds is affected by physical, chemical, biological factors, nitrogen to phosphorus ratio (Daniels and Boyd, 1993; Paerl and Tucker, 1995; Cremen *et al.*, 2007) and form and availability of nutrients (Boyd, 1995). Terry *et al.* (1985) indicated that N/P ratio can be used to determine which

nutrient is limiting but cannot be used to determine relative growth rates of species. Stickney (2005) observed that phosphorus regulates the phytoplankton production in the presence of nitrogen. N:P ratio can vary from <1.5:1 in nitrogen deficient algae to >15:1 in phosphorus deficient algae. In this study, average N:P ratio was around 3.50 in SD8 and 2.75 in SD16 and the higher values were observed at the end of the crop cycle which was optimal for primary production.

The present study has clearly shown that there is very high correlation between the phytoplankton abundance and the level of nitrate, phosphate and silicate in water. The increase in phytoplankton abundance with the progression of the crop, irrespective of the level of stocking, proves that the increase in nutrients in water is effectively used by them. The study has conclusively shown that in a tropical brackishwater shrimp culture pond, the plankton abundance is dependent on the nutrient dynamics which is influenced by the management practices followed in congruence with the level of stocking and feeding. Further, the nitrogen and phosphorus and their ratios act as indicators of the pond condition and the health and growth of stocked shrimps.

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