

Dynamics and succession of phytoplankton communities with changing nutrient levels in tropical culture ponds of whiteleg shrimp

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Abstract

Optimal water quality is prerequisite for economic and environmental sustainability of shrimp aquaculture. The dynamics and succession of phytoplankton and microzooplankton assemblages and their inter-relationship with water quality parameters in two commercial ponds growing the whiteleg shrimp, *Litopenaeus vannamei* on the south-western coastal India were assessed through periodic sampling during 96 days of culture (DoC). Out of many centric diatoms that were encountered during the initial stages of the culture in nitrogen-rich condition, only two dominant species belonging to *Thalassiosira* persisted throughout the progression of the culture to produce a healthy bloom (up to 6×10^6 cells l^{-1}). Blooms of *Thalassiosira* spp. contributed significantly to the increased phytoplankton biomass towards the end of culture period with a concomitant decrease in concentrations of ammonia and nitrate. The succession of pennate diatoms such as *Nitzschia closterium*, *Pleurosigma elongatum* and *Thalassionema nitzschoides* in moderate abundance was also discernible. Results of canonical correspondence analyses revealed that the progression of diatom bloom, the emergence of dinoflagellates and occurrence of intermittent blooms of the mixotrophic flagellate, *Eutreptiella marina* were closely linked to factors such as higher temperature, salinity and phosphate concentration. The grazing by the herbivorous-bacterivorous ciliate communities could have controlled the blooms of undesirable groups of phytoplankton, thereby ensuring better shrimp growth, higher survival and lower food conversion ratio. Effective uptake of ammonium and nitrate by the blooming diatoms and phytoflagellates possibly prevented the nutrient concentrations reaching toxic levels and thereby generating an eco-friendly aquaculture water discharge into the adjacent ecosystem.

Keywords: Diatoms. Microzooplankton. Dinoflagellate. Nutrients. Diversity. Canonical correspondence analysis.

Introduction

Aquaculture of the whiteleg shrimp, *Litopenaeus vannamei* has expanded tremendously in the last decade with a reported worldwide production of about 4.16 million tonnes, which is 53% of total shrimp and prawn production in 2016 (FAO 2018). Most of this production is in tropical and subtropical areas of the world, mainly in Asian countries such as China, Thailand, Vietnam, Bangladesh and Indonesia including India. The obvious merits of *L. vannamei* such as high density tolerance, adaptability to varying environmental conditions (salinity and temperature) and relatively faster growth during shorter culture period (Ponce-Palafox et al. 1997, Argue et al. 2002, Roy et al. 2010) have made it the most sought-after shrimp species for commercial cultivation.

Water quality has been recognized as one of the important parameters that influence the optimal shrimp growth and yield and is a prerequisite for sustainable shrimp farming. With the progression of culture, however the water quality in shrimp ponds gradually tend to deteriorate due to higher shrimp biomass and accumulation of organic matter from excess/uneaten feed, feces and metabolites (Kumar et al. 2017, Ni et al. 2018). Generally, it has been reported that only 15% of the applied feed is transformed into shrimp biomass, whilst the remainder goes into the water and sediment (Briggs & Funge-Smith 1994). The deterioration in the water quality as a consequence of a build-up of nutrients has been identified as the potential cause of disease outbreak in shrimp ponds (Sánchez-Martínez et al. 2007, Joshi et al. 2014)

To improve economic sustainability of shrimp culture, high-intensity grow out systems with zero water exchange have been evolved (Boyd 1999). However, these systems generate effluents typically enriched in suspended solids, nutrients, chlorophyll *a*, and high biochemical oxygen demand (Paez-Osuna 2001a, 2001b). Shrimp farm wastewater discharges in conjunction with municipal and agricultural effluents have potential to contribute to eutrophication in the receiving coastal environment (Burford & Williams, 2001, Paez-Osuna et al. 2003, Lacerda et al. 2006, Mohanty et al. 2018). Shrimp farming industries therefore, are under increasing pressure to improve environmental sustainability. Since intensive shrimp aquaculture involves the input of various feeds, fertilizers and chemicals that compromise water quality, the use of bio-indicators in conjunction with physico-chemical variables to assess the water quality may be beneficial.

Phytoplankton are natural biota in shrimp aquaculture ponds and their abundance and composition is controlled by both biotic and abiotic factors. Phytoplankton are ingested by penaeid shrimps along with a variety of detrital aggregates (Dall 1968, Varadharajan & Pushparajan 2013). However, not all phytoplankton groups are desirable, as the development and blooming of harmful and toxin-

producing species of cyanobacteria and dinoflagellates c negatively affect shrimp growth, survival rate and net productivity (Alonso-Rodriguez & Paez-Osuna 2003, Songsangjinda et al. 2006, Case et al. 2008, Keawtawee et al. 2012). It has been postulated that diatoms enhance shrimp growth and a high proportion of diatoms in shrimp ponds is desirable (Boyd 1990). By virtue of their high nutritive value, particularly, the long-chain polyunsaturated fatty acids, many diatom species such as *Chaetoceros calcitrans*, *Skeletonema costatum*, *Thalassiosira pseudonana*, *Navicula* spp., *Nitzschia* spp. and *Amphora* spp. have long been used as live feeds in aquaculture (Brown et al. 1997, Becker 2004, Roy & Pal 2015).

Phytoplankton stabilize the whole pond ecosystem by minimizing the wide fluctuations in water quality and prevention of build-up of waste nutrients to toxic levels thereby ensuring better shrimp growth and yields (Buford 1997, Ziemann et al. 1992, Lemonnier et al. 2017). A positive effect on the water quality and productive shrimp performance during the co-culture of three species of microalgae and shrimp in zero-water exchange system has been recently demonstrated by Ge et al. (2016). Species succession is often observed in shrimp ponds, starting with some beneficial flagellates and diatoms species, followed by noxious dinoflagellates after a month of culture (Yusoff et al. 2002, Lemonnier et al. 2016, Lemonnier et al. 2017).

Phytoplankton are extensively grazed by microzooplankton (20–200 µm), which form a significant component of the natural biota of shrimp aquaculture ponds (Coman et al. 2003) and are live prey for the cultured shrimps (Calbet & Landry 2004, Cardozo et al. 2007). Therefore, microzooplankton form an important link between the phytoplankton and the shrimps (Rubright et al. 1981). Studies (Tacon et al. 2002, Izquierdo et al. 2006) showed that shrimps grow best and healthier in aquaculture systems that have high levels of algae and other natural biota. Owing to their sensitivity to subtle changes such as low dissolved oxygen levels, high nutrient levels, toxic contaminants, poor food quality or abundance and predation occurring in the environment, both, phytoplankton and microzooplankton are considered to be good bio-indicators of water quality of the ponds and shrimp health (Case et al. 2008, Vin 2017). In spite of key roles played by these plankton communities in regulating water quality and in shrimp diet, our knowledge on their composition, abundance, dynamics and succession in shrimp aquaculture ponds is fragmentary (eg. Burford et al. 2003, Case et al. 2008, Lemonnier et al. 2016). The majority of previous studies solely characterized the phytoplankton or environmental factors, while the inter-relationship, particularly water quality parameters in influencing the phytoplankton dynamics and succession has received limited attention.

Against this background, the present study was undertaken to assess the abundance, composition and succession of phytoplankton and microzooplankton communities and to understand their

relationship with the water quality parameters by ecological methods of classification and ordination. The significance of this study is that it demonstrated the role of abiotic factors that control the abundance and succession of plankton communities and the role of these communities in maintaining water quality in shrimp aquaculture ponds.

2. MATERIALS AND METHODS

2.1. Culture ponds and farm management

The present study was undertaken in two farm ponds located near Kumta town (Karnataka state) on the south-west coast of India (14.42° N lat; 74.40° E long.). The farm is tide-fed from an adjoining creek, about 3 km from the sea. Two identical ponds (P1 and P2) having an average depth of 1.2 m and water spread area of ~ 0.84 and 1.06 ha were chosen for the study. Management practices and inputs were similar for P1 and P2. Before stocking, the ponds were sun-dried for one month and limed (CaCO_3) at the rate of 500 kg ha⁻¹. After which, ponds were filled with dechlorinated seawater followed by 4 ppm inorganic fertilizer application (urea:single super phosphate:1:1). Organics (wheat bran, yeast and fruit/vegetable juices) were added for enhancing the growth of micro- and mesozooplankton and beneficial bacteria. Two weeks after pond preparation, stocking operation was carried out.

Healthy post larvae (PL₁₈) of *Litopenaeus vannamei* (Boone 1931) produced from SPF (specific pathogen-free) broodstock and negative to WSSV (white spot syndrome virus) as confirmed by PCR (polymerase chain reaction) procured from a nearby commercial shrimp hatchery (Skyline Aqua Hatchery, Kumta, Karnataka) were transported in oxygen-filled bags to the site of the ponds.. These bags were kept in pond water for about two hours for acclimatization and actively moving post larvae with no visible signs of disease or morbidity were stocked at a stocking density of 11 and 16 PL₁₈ m⁻²; respectively during early morning hours. Lime and pH fixers were added throughout the culture period to buffer the pH. The production cycle lasted 96 days (DoC = days of culture) with zero water exchange. However, prerequisite water depth (~1 m) was maintained on a fortnightly basis by adding fresh dechlorinated water to compensate the evaporation and seepage losses. Aeration was achieved using HOBAS aerators (Fernandes et al. 2010). The aeration protocol included eight hours aeration in a day up to 50 DoC, 12 h per day aeration during 51–80 DoC and 16 h per day aeration thereafter till harvesting (81–96 DoC).

2.2. Total food consumption, shrimp growth and survival

Shrimps were fed with commercial shrimp pellets (CP-Aquaculture, Chennai India; proximate composition, 38–40% crude protein; 5% lipids and 3% fibre) split across four different feed times (06:00, 11:00, 18:00 and 23:00 h) at a rate of 10% of their body weight during the juvenile stages, and the feeding was gradually reduced to 2% towards the end of the culture period. Feeding rates however were adjusted according to the shrimp biomass and survival. The amount of feed consumed by shrimp in each meal was recorded and calculated as total food consumption per day. Food conversion ratio (FCR) was calculated at the time of harvest (96 DoC) by dividing the total feed consumed on a dry weight basis (kg) by total shrimp production in terms of wet weight (kg). Average growth of shrimp at the time of harvest was calculated by measuring the body weights of 250 shrimps.

2.3. Water quality sampling and analysis

Water samples for physico-chemical and biological parameters were collected one day prior to the transfer of post-larvae (0 DoC) and thereafter at regular intervals (12, 24, 36, 48, 60, 72, 84 and 96 DoC) between January and April, 2014 at three pre-determined sampling locations in P1 and P2. A Niskin water sampler was used to collect pond water (30 cm above the bottom) and sampling time was fixed between 09:00 and 10:00 h. Samples were kept in an icebox and transported to the laboratory for analysis.

Temperature and salinity were measured *in situ* using Celsius thermometer and refractometer (Atago, Japan), respectively. Dissolved oxygen (DO) content was estimated by Winkler's method (Parsons et al. 1984). For estimation of dissolved nutrients such as ammonium ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$) and orthophosphate ($\text{PO}_4^{3-}\text{-P}$), pond water samples (250 ml) were collected in triplicate and preserved in icebox and transported to the laboratory for analysis. In the laboratory, the water samples for nutrient analysis were filtered through cellulose filters (pore size, 0.45 μm) using a vacuum pump (Millipore) for removal of unwanted organisms or other suspended particles. Concentrations of nutrients in water samples, ammonium by the salicylate method, nitrate by the cadmium reduction method, nitrite by the diazotization method and orthophosphate by the ascorbic acid method were analyzed following Parsons et al. (1984).

2.4. Plankton sampling and analysis

Similar to water quality sampling, plankton (phytoplankton and microzooplankton) samples were also collected. For the estimation of concentrations of chlorophyll *a* (chl *a*), a known volume of pond water sample (500 ml) was filtered through Whatman GF/F glass fibre filter papers (47 mm

diameter; nominal pore size, 0.7 μm), extracted in 90% acetone overnight at 5°C and the fluorescence measured in a calibrated fluorometer (Turner Designs 10 AU) following Parsons et al. (1984). For estimation of plankton abundance and composition, 500 ml of pond water was taken in clean polythene bottle and fixed with 1% Lugol's iodine and 1% formalin.

The samples were allowed to settle in the dark for 48 hrs, concentrated through 20 μm mesh and aliquots were counted in Sedgewick-Rafter cell at 100X to 400X magnification under a calibrated inverted microscope (Olympus, BH2 Japan). Phytoplankton were identified up to generic level using keys and illustrations by Tomas (1997), Desikachary & Ranjithadevi (1986), Desikachary & Prema (1987), Desikachary et al. (1987), Constance et al. (1985) Subramanyan & Sarma (1961) and Subramanyan (1968). Microzooplankton species were identified, according to Hada (1938) and Jyothibabu (2004). The species richness ('S') was estimated as the total number of species in a given sample. Shannon-Weiner diversity index ('H') and Pielou's evenness index ('J') were used to assess plankton communities according to Shannon & Weaver (1963) and Pielou (1966), respectively.

2.5. Statistical analysis

Temporal and spatial fluctuations in water quality and plankton abundances were assessed by analysis of variance (ANOVA, Underwood 1997) with time (DoC) and space (ponds) as sources of variation using analysis tool pack in Microsoft Excel. Significance in all statistical tests was judged at a $p = 0.05$ level. A canonical correspondence analysis (CCA) was used to evaluate the relationship between the water quality parameters and the groups of phytoplankton and microzooplankton using Canonical Community Ordination (CANOCO) software for Windows version 4.5 (Braak ter & Šmilauer 2002). The abundance data were square-root transformed and forward selection procedure of environmental variables was employed. All the canonical axes were used to evaluate the significant variables under analysis at 5% level by means of a Monte Carlo test (999 random permutations).

3. RESULTS

3.1. Water quality parameters

Results of ANOVA of water quality parameters between both the ponds are presented in Table 1. Except for higher concentration of dissolved oxygen (DO) and lower ammonia and phosphate observed in P2, most of the other parameters were similar between the ponds. Therefore, the results hereinafter are combined for both the ponds (mean \pm SD; $n=6$; Fig. 1). The water temperature was $28.0 \pm 0.92^\circ\text{C}$ at the beginning of culture (0 DoC), dropped to $26.4 \pm 0.07^\circ\text{C}$ on 24 DoC and then increased sharply to 32°C on 96 DoC ($p < 0.05$). Salinity increased significantly from 32 ± 0.71 ppt

at the beginning to 44 ± 0.71 ppt at the end of the culture period. Concentrations of DO decreased from 6.8 ± 1.3 (0 DoC) to 4.4 ± 0.9 mg l⁻¹ at 96 DoC, but a high value of 8.4 mg l⁻¹ in the middle of the culture period. The concentrations of ammonium and nitrate, respectively varied from 0.9 ± 0.07 to 11 ± 10 µM and from 4 ± 2.9 to 104 ± 94 µM, both decreasing towards the end of culture (96 DoC), whereas the concentration of phosphate increased from 2.7 ± 3.6 (0 DoC) to 10.4 ± 12 µM towards the end of the culture period (96 DoC). The range of the observed pH was narrow (7–8) and increased towards the end of the culture period.

3.2. Plankton biomass and abundance

The concentration of chlorophyll *a* (chl *a*) ranged from 0.6 ± 0.07 to 8.6 ± 3.5 mg m⁻³ (Fig. 2) and increased as the culture progressed. Phytoplankton cell abundance varied from 1000 ± 707 (24 DoC) to 640131 ± 297083 cells l⁻¹ (96 DoC; Fig. 2). Microzooplankton abundance was in the range of 51 ± 14 to 145180 ± 204830 ind. l⁻¹ and three uniform peaks in abundance could be discernible on 24, 48 and 84–96 DoC (Fig. 2).

3.3. Frequency of occurrence of plankton communities

Both centric and the pennate groups of diatoms made up the phytoplankton community structure. Centric diatoms showed almost 100% frequency of occurrence (% *FO*) of the diatoms during 48–84 DoC and reduced to a low of 22 at 96 DoC (Fig. 3a). Successive dominance by pennate diatoms was observed on 96 DoC (% *FO* = 78%). Overall, the ponds were dominated by centric diatoms. The microzooplankton comprised of ciliates, flagellates, heterotrophic dinoflagellates and larval stages of mesozooplankton (Fig. 3b). Ciliates were dominant on 0, 60 and 72 DoC, the heterotrophic dinoflagellates during 12–36 DoC and the flagellates on 48, 84 and 96 DoC.

3.4. Plankton community structure

Altogether, 59 phytoplankton species comprising mainly diatoms were recorded during the culture period (Table 2). Of these, only 16 species were centric diatoms. The major contributions to the bulk of the phytoplankton abundance throughout the culture period belonged to *Thalassiosira* spp which progressed into a bloom > 48 DoC. *Leptocylindrus minimum* was attained an abundance of 3700 cells l⁻¹ on 36 DoC, while the abundance of other species of centric diatoms was negligible on all DoC. As compared to the centric, more species of pennate diatoms (43) were found in the present study. They were mostly from the genera *Achnanthes*, *Navicula*, *Nitzschia* and *Pleurosigma*. Their numbers were low to moderate on most days except on 96 DoC where a massive abundance of 165000 cells l⁻¹ of *Nitzschia closterium* was observed. *Thalassionema nitzschoides* was also found in moderate abundance (15000 cells l⁻¹) on 36 DoC.

Microzooplankton comprised of 64 species/groups (Table 3). Of these, 14 were heterotrophic dinoflagellates, majority belonging *Dinophysis* sp. and *Protooperidinium* sp. An unidentified dinoflagellate species with a high abundance of 75000 cells l⁻¹ contributed to the microzooplankton abundance peak on 24 DoC (Fig 2). Although as many as 35 species of ciliates were recorded, their abundances, however were quite low. Moderate abundances of *Dadayiella acuta* and *Tintinnopsis acuta* were observed only on 74 DoC and *Euplotus balticus*, *E. crassus* and *Strombidium caudatum* on 96 DoC. Only two species of flagellates were recorded, with *Eutreptiella marina* being the most predominant component of the microzooplankton community. It was common in the community, but its exceptionally high abundance on 48 and 96 DoC contributed to the total microzooplankton peaks on these DoC (Fig 2). Seven mesozooplankton larval/juvenile groups comprising of *Arachnactis*, decapods, naupliar and copepodite stages of copepods, trochophore of polychaetes, invertebrate eggs, foraminifera and ostracods were recorded.

3.5. Dominant species

Thalassiosira spp. was the predominant diatoms that contributed to over 85% of the phytoplankton abundance (Fig. 4). Few pennate diatoms such as *Nitzschia closterium* (12.4%) contributed significantly and *Pleurosigma elongatum* and *Thalassionema nitzschoides* to a minor extent. Amongst the microzooplankton, the flagellate *Eutreptiella marina* was the most dominant and contributed 66% to the abundance. An unidentified species of dinoflagellate (UID2) contributed significantly (25%) to the abundance. A tintinnid, *Strombidium caudatum* and the copepod nauplii and copepodites were also recorded to a minor extent.

3.6. Species diversity

The number of phytoplankton species on various DoC ranged from 6 to 32 (Fig. 5). The Maximum number of species was recorded on 12 DoC and the least on 72–84 DoC. The Shannon diversity of phytoplankton varied from 0.01 to 1.58 and was higher on 24, 36 and 96 DoC. A similar trend was witnessed in the evenness index, which was quite low and varied from 0.003 to 0.41. The diversity indices of microzooplankton were quite similar to that of the phytoplankton. The number of species ranged from 3 to 28, with the highest value on 12 DoC and the lowest on 72–84 DoC. Shannon diversity of microzooplankton varied from 0.002 to 3.0 with the higher value on 36 DoC corresponding to many species being recorded in very low abundances. Similarly, the lower diversity coincided with the occurrence of high abundance of UID2 (75000 cells l⁻¹) on 24 DoC and 145000 ind. l⁻¹ of *Eutreptiella marina* on 48 DoC (Table 3). The evenness ranged from 0.01 to 0.89 with higher values on 36, 60 and 72 DoC.

3.7. Canonical correspondence analyses

The effect of water quality on the phytoplankton and microzooplankton communities in P1 and P2 is shown in CCA ordination diagram (Fig. 6). The species represented by codes in the CCA diagram are listed in Tables 2 and 3. Forward selection of water quality parameters retained all the 7–8 variables that significantly explained the species distribution. In the first biplot (6a) for centric diatom species, the first two axes (Ax1 and Ax2) explained 83% of the total variance of species-environmental data. The Ax1 and Ax2 respectively explained 51% and 31% of the total variance. All the canonical axes were significant ($p < 0.05$). The Ax1 separated species found in a higher nitrate and ammonium environment from species corresponding to periods of higher temperature, salinity and pH. Following the Ax1, the species *Ceratulina pelagica*, *Cyclotella ocular*, *Dactyliosolen fragilissimus* and *Leptocylindrus minimum* were found in high nitrate and ammonium condition whereas the increase in abundances of *Thalassiosira* spp. corresponded elevated temperature, salinity and pH.

In the second biplot (6b) for pennate diatoms, the first two axes explained 50% of the total variance. Ax1 positively correlated with ammonium and nitrate and the species placed along this axis were *Achnanthes frigidus*, *Amphiprora paludosa*, *Diploneis suborbicularis* and *Thalasionema nitzschoides*. The same axis negatively correlated with phosphate, temperature and salinity and the species *Cylindrotheca closterium*, *Haslea trompii*, *Licmophora abbreviata*, *Navicula* sp., *Nitzschia closterium*, *Nitzschia sicula*, *Plagiotropis gausii* and *Nitzschia sigma* were associated with it. Along the negative side of Ax2 was the factor DO which showed good correlation with the species *Epithemia adnata*, *Navicula transitrans*, *Nitzschia longissima*, *Pleurosigma capense*, *P. elongatum*, *P. directum*, *Pleurosigma* spp. and *Synedra ulna*.

In the biplot for dinoflagellates (6c), the Ax1 and Ax2 explained 44% of the total variance. Most of the dinoflagellates were loaded along Ax2 at higher temperature and salinity and higher concentrations of phosphate and chl *a.*. On the other side of the same axis were DO and nitrate which correlated with most dinoflagellates, except an unidentified species.

In the biplot for ciliates, flagellates and copepod nauplii and copepodites (6d), the first two axes explained 46% of the total variance. Most of the species were separated along Ax2. Along the positive side of this axis, the species such as *Eutintinnus elongatus*, *Favella turaiakensis*, *Favella* sp., *Laboea strobila*, *Myrionecta rubra*, *Spirostomum* sp. and *Tintinnopsis fusus* correlated with nitrate and ammonium. Along the centre of the Ax2 were species *Actinosphaerium* sp., *Euplotes balticus*, *Euplotes crassus*, *Eutreptiella marina*, *Salpingacantha* sp., *Strombidium caudatum*, *Eutreptiella*

marina and copepod nauplii and copepodites indicating that they were influenced by factors on both sides of the axis including nitrate, ammonium, temperature, pH, chl *a* and phosphate.

3.8. Growth, survival and production of shrimp

Results of shrimp performance indices during 96 DoC of *L. vannamei* are presented in Table 5. Average shrimp production was $0.23 \pm 0.07 \text{ kg m}^{-2}$ and the final weight gain was $20.6 \pm 0.3 \text{ g shrimp}^{-1}$. The survival rate was $83 \pm 6.4\%$ and the FCR was 1.56 ± 0.14 .

4. DISCUSSION

In spite of differences in stocking densities between the two ponds, the variations in major water quality parameters in both the ponds were similar during the shrimp growth cycle. This may primarily be attributable to the common source of the inlet water and secondarily by good pond management practices. Being at the base of the food chain, autotrophic phytoplankton serve as a direct food source to the post larvae of penaeid shrimps (Coutteau 1996). Diatoms contain an average of 32 to 38% crude protein (Gordon et al. 2006), which are the major components in the natural food of penaeid shrimps. By contributing to dissolved oxygen, uptake of nutrients and supporting zooplankton grazers, phytoplankton play a pivotal role in maintaining water quality which is critical for sustainable shrimp production (Mohanty et al. 2018). The phytoplankton abundance varied widely from 10 to $10^5 \text{ cells l}^{-1}$ increasing significantly towards the end of the culture period. Among the many factors that affect the phytoplankton growth and abundance, light intensity, nutrients and zooplankton grazing are important (Chien 1992). But solar irradiance is not a limiting factor in the present study as the ponds are situated in a tropical marine region.

Influence of water temperature on the survival, growth, oxygen solubility and consumption and immune response of cultured shrimp has been documented (Guan et al. 2003, Abdelrahman et al. 2019). Initial temperature and salinity in ponds are dependent on the inlet water and are driven by the climatic conditions (Welch 1952). The production cycle of shrimp began in January (winter month) and harvested by end of April (summer month), with recorded seasonal warming with the progress of the culture. The temperature range ($26\text{--}32^\circ\text{C}$) recorded during the present study has been reported to be optimum for the growth of *L. vannamei* (Nuñez-Pastén 1988). The increase in salinity towards the end of the culture period was related to enhanced evaporation during the warmer months. In general, shrimps are euryhaline species and *L. vannamei* can easily adapt to varying levels of salinity (Ponce-Palafox et al. 1997).

Diatoms emerged as the major group in both ponds during 96 DoC. This is primarily because of their presence in the source water taken from the estuarine region off the west coast of India, which is rich in diatoms (Pednekar et al. 2014). The high nitrate concentration ranging from 4–105 μM prevailing in the ponds is favorable for the growth of diatoms (Malone 1980). The dominance of particular algal groups in aquaculture ponds is affected by abiotic and biotic factors such as salinity (Chien 1992), Light (Burford 1997), pond-flushing (Tseng et al. 1991), organic enrichment (Lemonnier et al. 2017), nutrient concentrations and their ratios (Boyd 1995, Paerl & Tucker 1995). Diatoms require a wide variety of inorganic nutrients for their growth, but the most important ones are nitrogen and phosphorus (Dawes 1981). The requirement for silica for the growth of diatom shells is mostly derived from shrimp pond sediments (Cremen et al. 2007).

Higher amounts of nutrients and metabolic wastes enter the water as the daily feed allotment increases in response to shrimp biomass during the progression of the culture. It has been reported as much as 80% of the nitrogen from the shrimp feed accumulates in water as excess (Sanders et al. 1987). Ammonia formed during the protein catabolism in shrimps and can account for 40-90 % of nitrogen excretion (Parry 1960). Nitrate is a product of nitrification in pond water and is dependent on the addition of fertilizers and feed. Remineralization of nitrogen from feed and from shrimp excreta might have led to a gradual increase in the availability of nitrate and ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) in the ponds up to 36 DoC. However, the concentrations of ammonium recorded during this study (1–11 μM) fell within the optimum range for the growth and survival of penaeid shrimps (Chien 1992). Higher concentrations of unionized ammonia (NH_3^-) have been reported to cause stress to the cultured shrimps (Burford & Lorenzen 2004). But the maintenance of optimum pH (7–8) in the ponds helped in regulating the ammonium levels, thereby keeping its unionized form under control. A dramatic decrease in the concentrations of nitrate and ammonium > 36 DoC and an increase in the phytoplankton abundance until 96 DoC were discernible (Fig. 2). As nitrate and ammonium are essential nutrients that encourage the growth of phytoplankton (Shan & Obbard 2001), the lower levels of nitrate and ammonium recorded > 36 DoC are possibly due to their uptake by phytoplankton. Thus, uptake by phytoplankton as well as adequate aeration and addition of probiotics as observed by Martinez-Cordova et al. (1998) and Lorenzen (1999) and Fernandes et al. (2010) might have prevented the build-up of ammonia to toxic levels.

Higher concentrations of chl *a* observed towards the end of the production cycle may due to the significant increase in phytoplankton abundance mainly by two co-dominant species of the centric diatoms *Thalassiosira* sp., reaching nearly bloom proportions. Such an increase in abundance of plankton over the culture period has been reported earlier in shrimp cultures (eg. Alonso-Rodriguez & Paez-Osuna 2003). Since the bloom comprised of multi-species of *Thalassiosira* sp. as well as

pennate diatoms such as *Nitzschia closterium*, *Pleurosigma elongatum* and *Thalassionema nitzschioides* in moderate numbers, similar to observations of Smith (1985), the bloom persisted from mid to the end of the culture period. *Thalassiosira* sp. is a nanoplanktonic diatom which grows rapidly when nutrients are increased and has been reported as a major component of the spring diatom bloom (Guillard & Kilham 1977, Waite et al. 2005) and also in intensive shrimp ponds (Melo et al. 2010, Lemonnier et al. 2016). By virtue of their importance as live food for the prey of shrimps (Ryther & Officer 1981) and provision of adequate levels of the dietary requirements of PUFAs (polyunsaturated fatty acids), EPA (Eicosapentaenoic acid) and/or DHA (Docosahexaenoic acid), and ARA (Arachidonic acid) (Volkman et al. 2006), these centric diatom species, therefore are highly desirable in shrimp ponds

As also reported by Yusoff et al. (2002) and Lemonnier et al. (2017), phytoplankton blooms occur in response to the higher amounts of nutrients from metabolic wastes of shrimp. Smith (1983) suggested that in tropical areas where the temperature is high and light is abundant, the phytoplankton blooming could be due to the rapidly changing nutrient concentration and N: P ratios. The most favourable N: P ratio for blooming of diatoms in shrimp ponds has been reported to be 20:1 (Daniels & Boyd 1993). Ratios of N: P during 96 DoC varied from 0.3 to 61 and relatively higher during 0–36 DoC and decreased to < 12 with the further progression of the culture. Such a decrement in the N: P ratio might perhaps be related to the accumulation of phosphate towards the end of the production cycle. The build-up of phosphorus might have stimulated the peaking of diatom production (Fig. 2) in the presence of adequate nitrogen towards the end of culture as has also been observed by Stickney (2005) and Castillo-Soriano et al. (2013). Interestingly, few studies have reported a dominance of cyanophytes coupled with a high phosphate concentration (Yusoff et al. 2002; Shaari et al. 2011). Silicate to nitrogen ratios affecting the diatom dominance has been reported by Sommer (1989). Though silicate was not measured in this study, it is assumed from a similar study (Smith 1994) that amorphous silica present in the shrimp pond sediments was adequate enough in promoting diatom growth and apparently depressed the growth of cyanophytes. .

With an increase in phosphorus loading, a shift in communities from diatom to dinoflagellates has been observed by Hodgkiss (2001). In our study, few heterotrophic dinoflagellates belonging to the genera, *Dinophysis* and *Protoperidinium* were recorded intermittently during the production cycle. Additionally, the occurrence of UID2 in high abundance $>10^5$ cells l^{-1} at 24 DoC was recorded. But none of these dinoflagellate species belonged to the harmful category. Leaching of humic acids and other organic substances from feed is known to stimulate the growth of dinoflagellates (Prakash & Rashid 1968). The occurrence of *Protoperidinium* spp. is common along the west coast of India (eg.

D'Silva et al. 2011). These non-pigmented, phagotrophic dinoflagellates often occur in high abundance during diatom blooms and are significant consumers of blooming diatoms (Sher & Sher 2007). In the present study, the lower abundance of *Thalassiosira* spp on 24 DoC appears to be related to the grazing activity of UID 2.

Similarly, planktonic ciliates comprising mainly *Dadayiella acuta*, *Tintinnopsis minuta*, *Strombidium caudatum*, *Euplotes* spp. and *Eutintinnus elongatus* recorded in this study are known to be vigorous grazers of phytoplankton (Pierce & Turner 1993, Tillmann 2004). Their lower abundance throughout this culture period except for the high value of 12000 cells l⁻¹ on 96 DoC is probably related to the more abundant availability of phytoplankton, which many ciliates are known to feed upon (Liu et al 2017). Nearly all species of *Strombidium* are suspension feeders, feeding mainly on larger-sized diatoms (Fenchel 1968). A high abundance of *S. caudatum* and phytoplankton towards the end of shrimp culture is reflective of grazing activity of the former. Higher abundance of *Euplotes* spp. on 96 DoC could be related to bacterivory as they are known preferential grazers bacteria attached to the surfaces (Sieburth 1979), especially those associated with the decomposition of animal and plant tissues (Fenchel 1968). The higher abundance of ciliates at the end of the culture period might be in response to higher inputs of organic matter (Decamp et al. 2007, Melo et al. 2010), rapid reproduction rates, short generation times as well as their ability to use a large spectrum of food resources (Capriulo & Carpenter 1983, Capriulo et al. 2002, Urrutxurtu 2004)

Higher abundances of *Eutreptiella marina* are associated with nutrient enrichment (Urrutxurtu 2004) and the rapid changes in abundances could be due to the grazing impact of ciliates (Epstein et al. 1992). *Eutreptiella* sp. is a mixotroph which feed on eubacteria and *Synechococcus* sp (Yoo et al 2018) and employ a range of nutritional modes from osmotrophy to phagotrophy, enabling them to exploit both inorganic and organic resources (Mullner et al., 2001). The growth of *Eutreptiella* sp far exceeds the grazing pressure by mesozooplankton when nutrients are not limiting, as in case of shrimp ponds (Olli et al 1996). In coastal waters of China, the bloom of *Eutreptiella (gymnastica)* coincided with high phosphate concentration (Xu et al. 2012). Invertebrate metazoans such as copepod nauplii and copepodites, juvenile ostracods, decapod larvae and polychaetes which contributed 20% to the microzooplankton abundance in the present study are also known to be potential grazers of phytoplankton (Berggreen et al. 1988). Since microzooplankton grazing can remove up to 50% of the stocks of small phytoplankton (Zhang et al. 2011) and > 90% of dinoflagellate production (Epstein et al 1992), the preponderance of microzooplankton in our shrimp ponds would have exerted positive control on the phytoplankton bloom and prevented the latter reaching undesirable proportions. In addition, predation of the microzooplankton in turn by shrimps

(Coman et al. 2003) results in the transfer of a significant proportion of the nutrients from natural biota to the shrimp tissue (Anderson et al. 1987). Based on previous studies (Calbet & Landry 2004) and the abundances observed in this study (10^5 ind. l^{-1}), there is little doubt that microzooplankton formed a significant component of the natural biota of shrimp ponds and a key link between phytoplankton and the shrimps.

By virtue of their sensitivity to environmental changes plankton communities are often considered as excellent indicators of water quality (Li et al. 2009). During the culture period higher abundances of diatoms, dinoflagellates and ciliates coincided with very low species richness and evenness. Lower phytoplankton diversity (<1.6) observed in the shrimp ponds compared to the coastal waters is attributable to the chlorination of the seawater for the initial filling, zero-water exchange and dominance of three plankton species. In shrimp ponds adjoining the Hangzhou Bay, nitrogen and phosphorus load were found to strongly influence the proliferation of Chlorophyta blooms and low phytoplankton diversity (Ni et al. 2018).

CCA analysis which was performed to analyze the relationship between phytoplankton and environmental variables and to understand the main driving factors of phytoplankton community structure, suggested that many centric diatoms (*Ceratulina pelagica*, *Cyclotella ocular*, *Dactyliosolen fragilissimus* and *Leptocylindrus minimum*) and pennate diatoms (*Achnanthes frigidus*, *Amphiprora paludosa*, *Diploneis suborbicularis* and *Thalassionema nitzschoides*) were growing under high nitrate and ammonium condition during the first month of shrimp culture. Since biosecured zero-water exchange system of shrimp farming was followed in the present study, the increasing temperature led to a concomitant increase in salinity, triggering the bloom of the centric diatom *Thalassiosira* spp., and consequently causing a decrease in dissolved oxygen levels. As two species of *Thalassiosira* sp. were the main contributors to the high biomass of phytoplankton at the end of culture period and were negatively correlated with nitrate and ammonium, it indicates that the phytoplankton biomass was related to the removal of nitrogen at the end of the culture period. Though nitrogen is an essential element for phytoplankton growth and almost all chlorophyll-containing algae grow either on nitrate or ammonium (Syrett 1981), diatom growth is particularly promoted by inorganic nitrogen sources (Robert et al. 1986). Besides providing food, shade and increased oxygen levels and preventing the growth of undesirable benthic algae, reduction in toxic ammonia concentrations by the diatom blooms have been documented in shrimp ponds (Chien 1992, Burford 1997).

As the shrimp culture and the build-up of the algal biomass progressed, excess phosphate derived from the decomposition of shrimp feed favoured the bloom of a semi-heterotrophic euglenoid

unarmored flagellate, *Eutreptiella marina*, and few dinoflagellates. The rapid growth of *E. gymnastica* and dinoflagellates in enriched phosphate condition was also observed in other studies (Xu et al. 2012; Barcelos e Ramos et al. 2017). Increased turbidity due to higher suspended matter in shrimp ponds may stimulate picoplankton abundance which can even dominate the phytoplankton community (Burford 1997; Lucas et al 2010). However this aspect was not addressed in the present study but will definitely be considered in future investigations.

Low diversity of plankton communities and dominance of species indicates that these shrimp ponds often tend to become hypernutrified. However, this study has shown that effective uptake of nutrients by the increased abundance of desirable diatoms and their control by the microzooplankton with the progression of culture helped to maintain the water quality and consequently contributed to good shrimp yield (Table 5). Many studies have indicated that mixtures of diatoms and flagellates have produced good results in terms of shrimp growth and survival (Gaxiola et al. 2010). Also at harvest, the undesirable environmental impact due to the discharge of pond water into the coastal waters would be significantly lower.

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Table 1. Analysis of variance of water quality parameters between days of culture (DoC) and between the ponds. * $p < 0.05$.

Parameter	Source of Variation	SS	df	MS	F	F crit	p-value
Temperature	DoC	55.2	8	6.91	67.4*	3.44	0.010
Salinity	DoC	231	8	28.8	15.2*	3.44	0.010
Dissolved oxygen	DoC	21	8	2.6	14.6*	3.44	0.001
Ammonia	DoC	198	8	25	1.37	3.44	0.3
Nitrate	DoC	21699	1	2712	0.09	5.32	0.23
Phosphate	DoC	177	8	22	0.98	3.44	0.551
pH	DoC	1.62	8	0.2	8.7*	3.44	0.003
Chlorophyll <i>a</i>	DoC	166	8	21	7*	3.44	0.003
Temperature	Pond	0.18	1	0.18	1.76	5.32	0.22
Salinity	Pond	1.4	1	1.4	0.74	5.32	0.4
Dissolved oxygen	Pond	5.1	1	5.1	29*	5.32	0.001
Ammonia	Pond	14.2	1	14.2	0.79	5.32	0.016
Nitrate	Pond	193	1	193	0.12	5.32	0.23
Phosphate	Pond	208	1	208	9.22*	5.32	0.016
pH	Pond	0.08	1	0.08	3.7	5.3	0.09
Chlorophyll <i>a</i>	Pond	1.09	1	1.09	0.36	5.32	0.301

Table 2. Temporal variation in abundance (cells l⁻¹) of phytoplankton communities during the culture of *Litopenaeus vannamei* on the south-western coastal India. '–' denotes absence.

Code	Species	Days of culture (DoC)								
		0	12	24	36	48	60	72	84	96
	Centric Diatoms									
DC1	<i>Arcocellulus cornucervis</i>	-	-	2	-	-	-	-	-	-
DC2	<i>Cerataulina pelagica</i>	67	4	-	33	-	-	-	-	-
DC3	<i>Corethron criophilum</i>	-	4	-	-	-	-	-	-	-
DC4	<i>Cyclotella ocula</i>	-	-	-	13	-	-	-	-	-
DC5	<i>Cymatocira belgica</i>	-	-	2	-	-	-	-	-	-
DC6	<i>Dactyliosolen fragilissimus</i>	-	4	-	13	-	-	-	-	-
DC7	<i>Eucampia groenlandica</i>	-	4	-	-	-	-	-	-	-
DC8	<i>Eucampia zodiacus</i>	-	4	-	-	-	-	-	-	-
DC9	<i>Guinardia delicatula</i>	-	8	-	-	-	-	-	-	-
DC10	<i>Guinardia striata</i>	-	2	-	-	-	-	-	-	-
DC11	<i>Leptocylindrus danicus</i>	-	7	-	-	-	-	-	-	-
DC12	<i>Leptocylindrus minimum</i>	-	7	3	3700	-	-	-	-	-
DC13	<i>Odontella longicornis</i>	-	4	-	-	-	-	-	-	-
DC14	<i>Rhizosolenia hyalina</i>	-	4	-	-	-	-	-	-	-
DC15	<i>Thalassiosira</i> sp.	7300	12064	202	8673	1057	307565	125000	76	50010
DC16	<i>Thalassiosira</i> sp. 1	-	-	-	-	11667	-	515000	100250	-
	Pennate Diatoms									
DP17	<i>Achnanthes delicatula</i>	-	-	-	-	-	-	50	-	-
DP18	<i>Achnanthes exigua</i>	-	0.2	-	-	-	-	-	-	-
DP19	<i>Achnanthes frigidus</i>	-	-	-	33	10	-	-	-	-
DP20	<i>Achnanthes longipes</i>	-	49	-	-	-	-	-	-	-
DP21	<i>Amphora</i> sp.	-	-	-	-	-	-	50	-	-
DP22	<i>Amphiprora alata</i>	-	4	-	-	-	-	-	-	-
DP23	<i>Amphiprora paludosa</i>	-	-	-	33	-	-	-	-	-
DP24	<i>Cylindrotheca closterium</i>	-	-	-	-	-	-	-	-	360
DP25	<i>Cymbella marina</i>	-	-	-	-	-	-	-	-	-
DP26	<i>Diploneis suborbicularis</i>	-	7	-	25	20	-	-	-	-
DP27	<i>Epithemia adnata</i>	-	-	-	-	120	-	-	-	-
DP28	<i>Fragilaria ulna</i>	-	-	-	-	-	-	-	-	15
DP29	<i>Haslea trompii</i>	-	2	-	-	-	-	-	-	30
DP30	<i>Licmophora abbreviata</i>	-	-	-	-	-	-	-	-	1530
DP31	<i>Licmophora flabellata</i>	-	2	0.3	-	-	-	-	-	-
DP32	<i>Navicla subminiscula</i>	8	-	-	-	-	-	-	-	-
DP33	<i>Navicla transitrans</i>	-	-	-	-	-	20	-	25	-
DP34	<i>Navicula</i> sp.	0.4	-	2	-	-	5	-	-	60
DP35	<i>Navicula</i> sp.1	-	2	-	-	-	-	-	-	-
DP36	<i>Nitzschia closterium</i>	4	-	-	12	-	-	-	-	165000
DP37	<i>Nitzschia dissipata</i>	-	13	-	-	-	-	-	-	-
DP38	<i>Nitzschia longissima</i>	-	-	2	-	-	30	-	-	-
DP39	<i>Nitzschia sicala</i>	8	-	-	45	-	-	-	-	1500
DP40	<i>Nitzschia sigma</i>	-	7	-	-	4	35	-	-	1500
DP41	<i>Nitzschia</i> sp.	-	4	-	-	-	-	-	-	-
DP42	<i>Phaeodactylum tricornutum</i>	-	-	-	-	-	5	-	-	-
DP43	<i>Plagiotropis gausii</i>	-	-	-	-	-	-	-	-	30
DP44	<i>Pleurosigma angulatum</i>	-	7	-	-	-	-	-	-	-

DP45	<i>Pleurosigma capense</i>	8	18	-	-	26	-	-	-	-
DP46	<i>Pleurosigma elongatum</i>	1967	9	50	386	85	75	1	101	2325
DP47	<i>Pleurosigma directum</i>	-	52	-	25	30	5	-	-	-
DP48	<i>Pleurosigma normanii</i>	8	-	-	-	-	-	-	-	-
DP49	<i>Pleurosigma</i> sp.	8	8	-	-	-	-	-	-	-
DP50	<i>Pleurosigma</i> sp.1	25	8	-	-	-	-	-	-	-
DP51	<i>Pleurosigma</i> sp.2	-	2	-	-	-	-	-	75	-
DP52	<i>Pseudonitzschia delicatissima</i>	-	-	0.3	-	-	-	-	-	-
DP53	<i>Rhopalodia gibberula</i>	-	-	2	-	-	-	-	-	-
DP54	<i>Stauroneis constricta</i>	-	4	-	-	-	-	-	-	-
DP55	<i>Synedra formosa</i>	-	-	-	-	-	-	-	-	15
DP56	<i>Synedra ulna</i>	-	9	-	-	60	5	-	-	30
DP57	<i>Thalassionema nitzschoides</i>	-	-	-	15000	-	-	30	-	-
DP58	<i>Toxarium undulatum</i>	-	-	-	-	10	-	-	-	-
DP59	Unidentified species	-	-	-	13	-	-	-	-	-

Table 3. Temporal variation in abundance (ind. l⁻¹) of microzooplankton communities during the culture of *Litopenaeus vannamei* on the south-western coastal India. '-' denotes absence.

Code	Species	Days of culture (DoC)								
		0	12	24	36	48	60	72	84	96
	Heterotrophic dinoflagellates									
Hd1	<i>Ceratium dens</i>	0.2	4	-	-	-	-	-	-	-
Hd2	<i>Dinophysis acuminata</i>	-	-	-	-	-	-	-	-	1500
Hd3	<i>Dinophysis acuta</i>	40	-	-	-	-	-	-	-	1500
Hd4	<i>Dinophysis</i> sp.	-	-	-	25	-	-	-	-	-
Hd5	<i>Ornithocercus steinii</i>	-	-	0.3	100	-	-	-	-	-
Hd6	<i>Protoperidinium brevipes</i>	-	733	-	-	-	-	-	-	15
Hd7	<i>Protoperidinium conicoides</i>	-	-	-	-	-	-	-	-	60
Hd8	<i>Protoperidinium conicum</i>	-	20	-	-	-	-	-	-	-
Hd9	<i>Protoperidinium pellucidum</i>	-	-	-	-	-	-	-	25	-
Hd10	<i>Prorocentrum minimum</i>	-	-	2	-	-	-	-	-	-
Hd11	<i>Pyrocystis lunula</i>	-	-	2	-	2	10	-	-	-
Hd12	<i>Zygabikodinium lenticulatum</i>	-	-	-	-	8	-	-	-	-
Hd13	Unidentified dinoflagellate1	-	3	-	25	-	-	-	-	-
Hd14	Unidentified dinoflagellate2	-	-	75000	-	-	-	-	-	-
Hd15	Dinoflagellate cysts	-	-	-	-	-	-	50	-	-
	Ciliates									
C16	<i>Acineta</i> sp.	-	-	-	13	-	-	-	-	-
C17	<i>Acanthostomella norveigica</i>	-	-	-	13	-	-	-	-	-
C18	<i>Actinosphaerium</i> sp.	-	4	-	-	150	-	-	-	-
C19	<i>Amphorides minor</i>	-	0.3	-	-	-	-	-	-	-
C20	<i>Dadayiella acuta</i>	-	-	-	-	-	-	1000	-	-
C21	<i>Epiplocycooides reticulata</i>	-	4	-	-	-	-	-	-	-
C22	<i>Euplotes balticus</i>	-	-	-	-	-	-	-	-	1500
C23	<i>Euplotes crassus</i>	-	-	-	-	-	-	-	-	1500
C24	<i>Eschaneustyla</i> sp.	-	4	-	-	-	5	-	-	-
C25	<i>Eutintinnus elongatus</i>	120	0.3	-	-	-	-	-	-	15
C26	<i>Favella ehrenbergii</i>	-	2	-	-	-	-	-	-	-
C27	<i>Favella turaikaensis</i>	20	-	-	-	-	-	-	-	-
C28	<i>Favella</i> sp.	20	-	-	-	-	-	-	-	-
C29	<i>Keronopsis</i> sp.	-	-	-	-	-	30	-	-	-
C30	<i>Laboea strobila</i>	280	-	-	13	-	-	-	-	-
C31	<i>Myrionecta rubra</i>	-	4	-	25	10	-	-	-	-
C32	<i>Rhabdonella poculum</i>	-	2	-	-	-	-	-	-	-
C33	<i>Rhabdonella amor</i>	-	2	-	-	-	-	-	-	-
C34	<i>Rhabdonella spiralis</i>	-	0.3	3	-	-	-	-	-	-
C35	<i>Salpingacantha ampla</i>	-	2	-	-	-	-	-	-	-
C36	<i>Salpingella decurtata</i>	-	8	-	-	-	-	-	-	-
C37	<i>Spirostomum</i> sp.	-	-	-	33	-	-	-	-	-
C38	<i>Strombidium conicum</i>	-	0.01	-	-	-	-	-	-	-
C39	<i>Strombilidium strobilus</i>	0.2	-	-	-	-	-	-	-	-
C40	<i>Strombilidium reticulatum</i>	4	-	-	-	-	-	-	-	-
C41	<i>Strombilidium turicum</i>	-	-	-	-	-	-	-	-	-
C42	<i>Salpingacantha</i> sp.	-	7	-	-	-	-	-	75	-
C43	<i>Strombidium caudatum</i>	-	-	-	15	-	-	-	-	9000

C44	<i>Strombidium cornucopiae</i>	-	-	-	-	-	5	-	-	-
C45	<i>Tintinnopsis entzil</i>	-	-	-	-	-	10	-	-	-
C46	<i>Tintinnopsis aperta</i>	-	2	-	-	-	-	-	-	-
C47	<i>Tintinnopsis fusus</i>	-	-	-	33	-	-	-	-	-
C48	<i>Tintinnopsis minuta</i>	-	-	-	-	-	-	500	-	-
C49	<i>Trichocerca rousseleti</i>	-	4	-	-	-	-	-	-	-
C50	Unidentified	-	-	-	-	-	-	90	-	-
	Flagellates									
F51	<i>Campanoecca dilatata</i>	-	2	-	-	-	-	-	-	-
F52	<i>Eutreptiella marina</i>	20	-	-	13	145000	-	-	3000	45150
	Mesozooplankton larvae									
M53	<i>Arachnactis</i> larvae	-	3.5	-	-	-	-	-	-	-
M54	Decapod larva	0.2	-	-	-	-	-	-	-	-
M55	Foraminifera	0.2	-	-	-	-	-	-	-	-
M56	Invertebrate egg	1.2	-	-	-	-	-	-	-	-
M57	Nauplii & copepodites	4.8	27.8	-	11.7	-	-	-	-	6000
M58	Ostracod	-	-	-	12.5	-	-	-	-	-
M59	Polychaete trocophore	2.0	6.7	-	-	-	-	-	-	-
Total	Dinoflagellates	40	760	75004	150	10	10	50	25	3075
	Ciliates	444	43	3	144	160	50	1590	75	12016
	Flagellates	20	2	0	13	145000	0	0	3000	45150
	Mesozooplankton larvae	8	38	0	24	0	0	0	0	6000

Table 4. Summary for the two axes (Ax1 and Ax2) of CCA with eight selected environmental factors. % var sp-env: cumulative percentage variance of species-environment relation; Eigenvalues, sum of eigenvalues and sum of canonical eigenvalues are presented. Cil-fla-na-co: Ciliates, flagellates, nauplii, copepodites.

Variable	Centric diatoms		Pennate diatoms		Dinoflagellates		Cil-fla-na-co	
	Ax1	Ax2	Ax1	Ax2	Ax1	Ax2	Ax1	Ax2
Temperature	-0.77	-0.33	-0.88	0.31	0.18	0.86	-0.04	-0.59
Salinity	-0.65	0.23	-0.70	0.52	0.10	0.56	-0.03	-0.39
Dissolved oxygen	0.18	-0.22	0.49	-0.61	-0.04	-0.73	0.05	-0.01
Ammonium	0.66	0.65	0.90	0.06	0.01	0.39	0.07	0.43
Nitrate	0.91	0.33	0.91	-0.11	-0.20	-0.50	0.11	0.54
Phosphate	-0.43	-0.38	-0.89	0.18	-0.03	0.85	0.14	-0.53
pH	-0.63	0.32	-0.29	0.32	0.21	0.3	0.10	-0.57
Chlorophyll <i>a</i>					0.23	0.78	-0.18	-0.67
Eigenvalues	0.57	0.34	0.77	0.65	1.00	0.99	1.00	0.72
% var sp-env	51.3	82.5	27.2	50.1	22.0	43.8	26.7	46.0
Total inertia		1.34		3.34		4.56		3.74
Sum eigenvalues		1.34		3.34		4.56		3.74
Sum can eig.val		1.10		2.85		4.56		3.74

Table 5. Growth and production of *Litopenaeus vannamei* in two ponds (mean \pm SD).

Variable	Value
Total days of culture (DoC)	96
Net production (kg m ⁻²)	0.23 \pm 0.07
Final weight gain (g shrimp ⁻¹)	20.6 \pm 0.3
Survival rate (%)	83 \pm 6.4
Food conversion ratio (FCR)	1.56 \pm 0.14

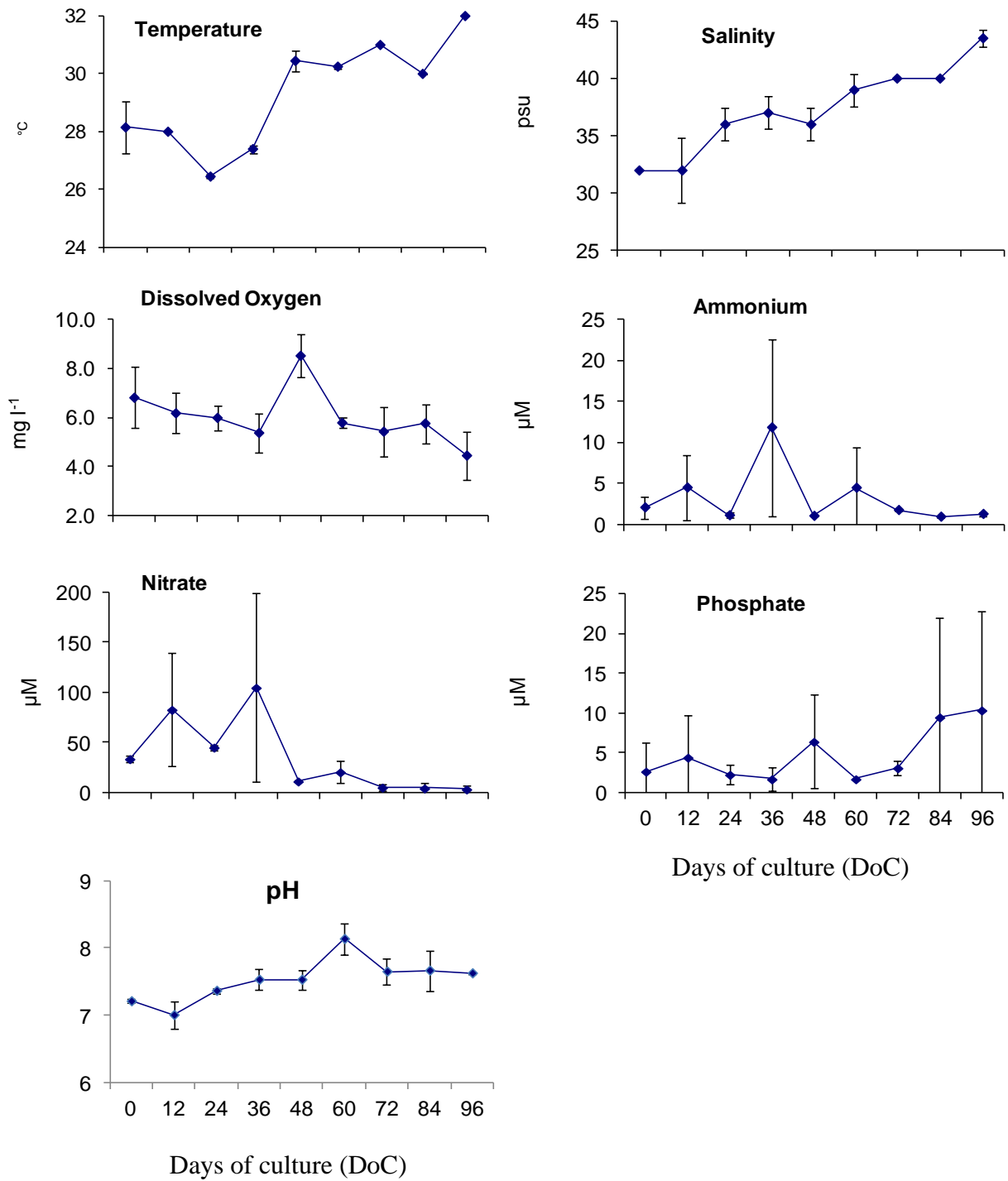


Fig. 1. Temporal variation in water quality parameters in during the culture of *Litopenaeus vannamei*.

Data are mean \pm SD (n = 6).

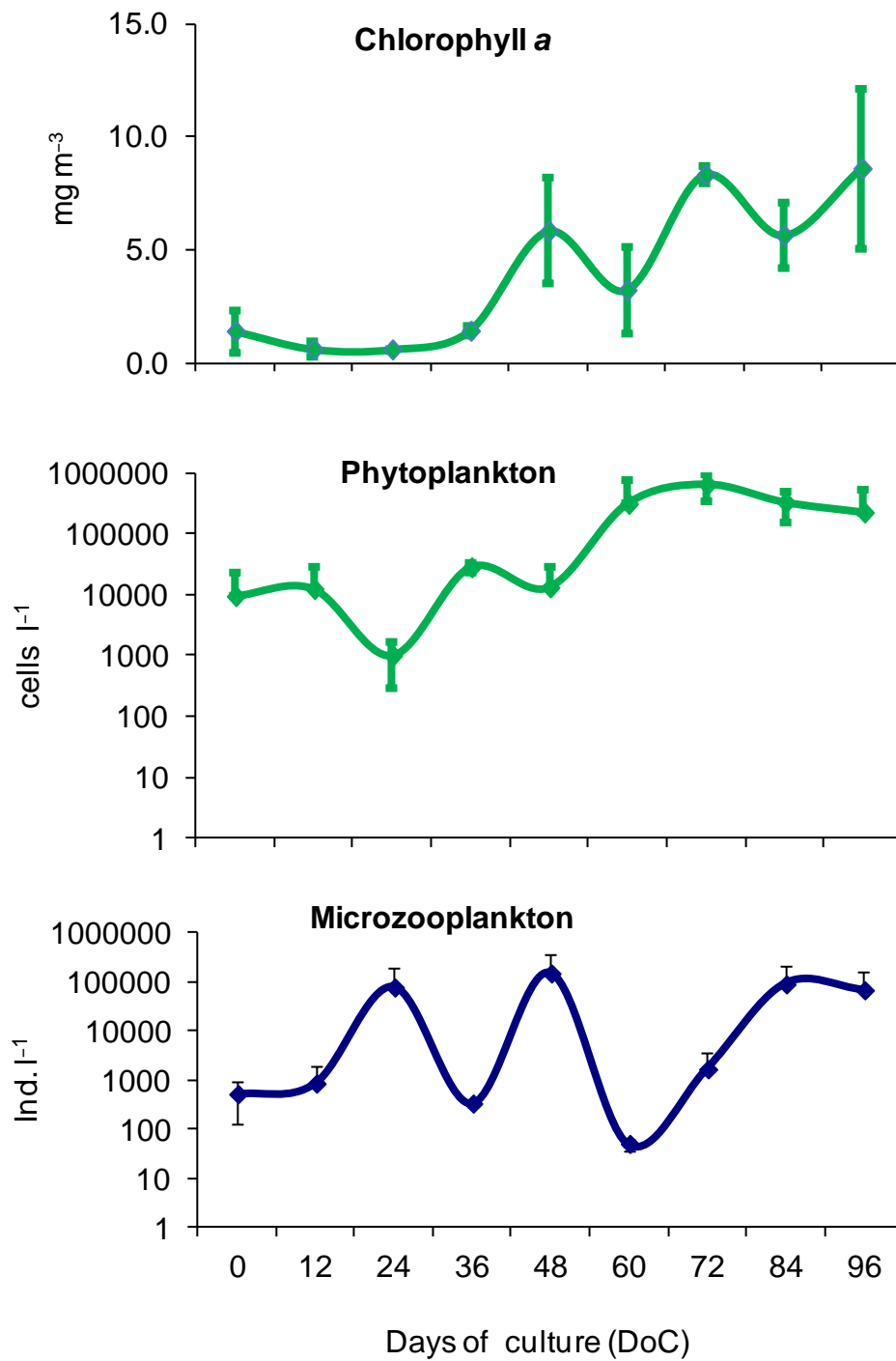


Fig. 2. Temporal variation in concentrations of chlorophyll *a* (mg m⁻³) and abundances of phytoplankton (cells l⁻¹) and microzooplankton (ind. l⁻¹) during the culture of *Litopenaeus vannamei*. Data are mean \pm SD (n=6).

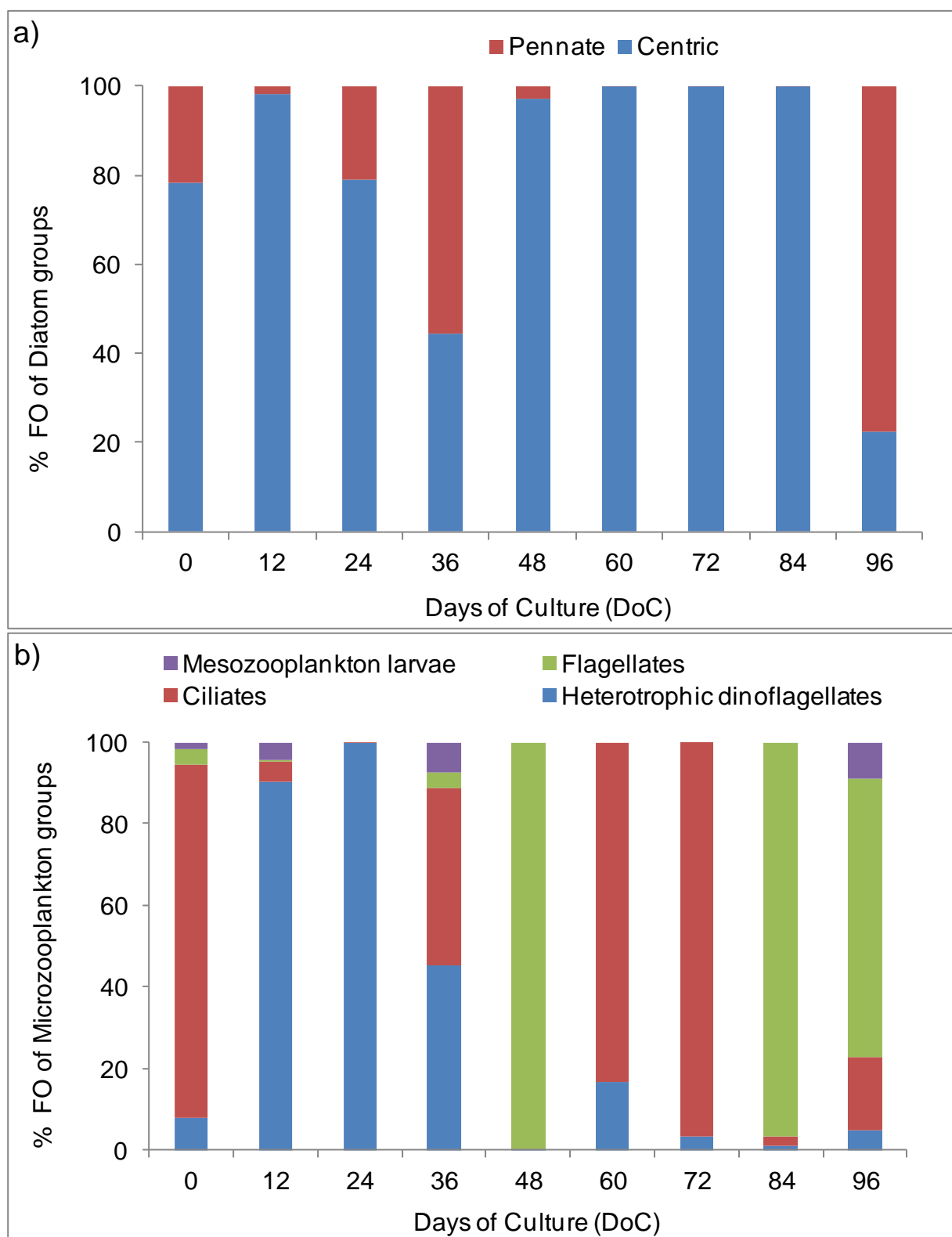


Fig. 3. Frequency of occurrence (% *FO*) of (a) diatom and (b) microzooplankton groups during the culture of *Litopenaeus vannamei*.

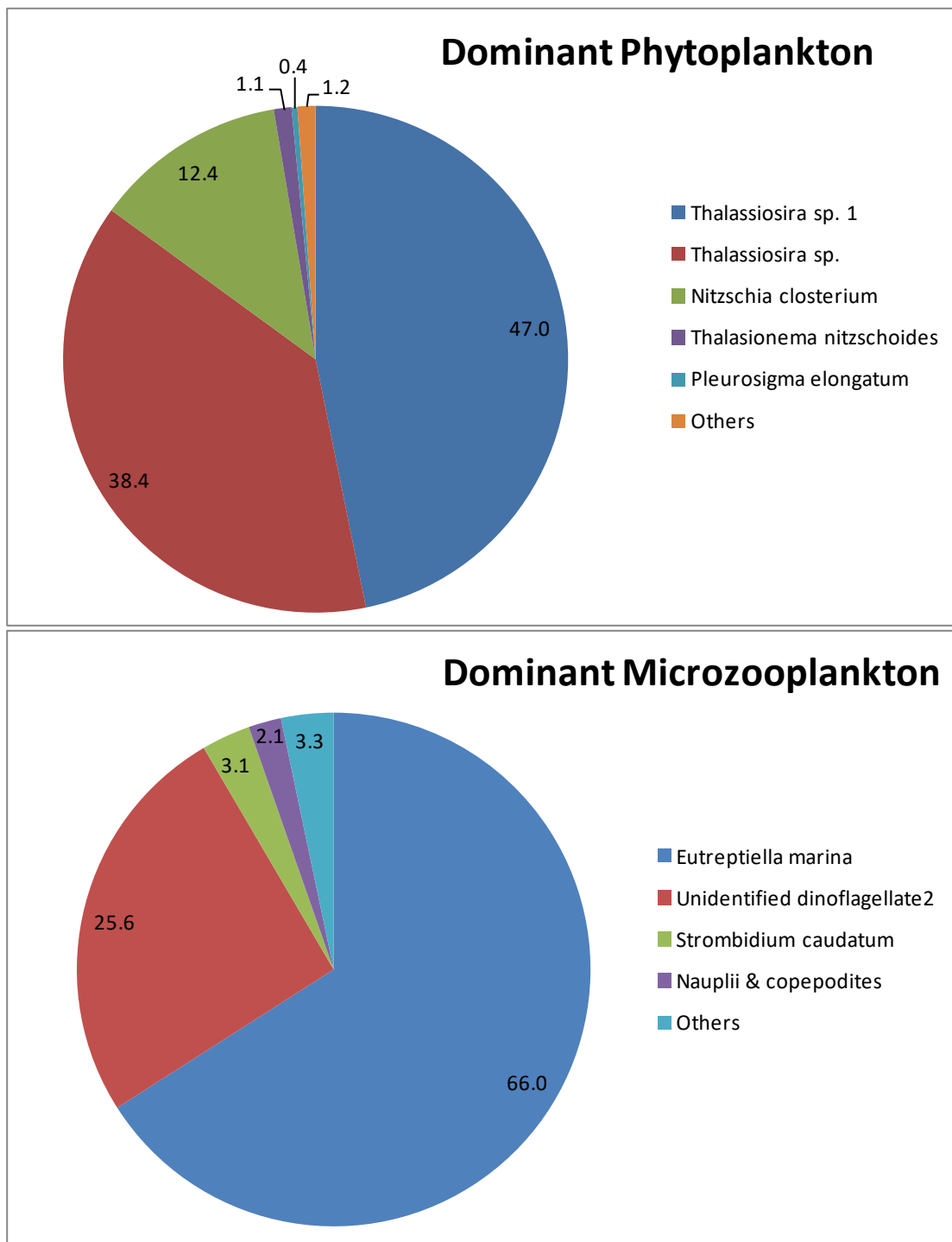


Fig. 4. Percent contribution of major phytoplankton and microzooplankton species during the culture of *Litopenaeus vannamei*

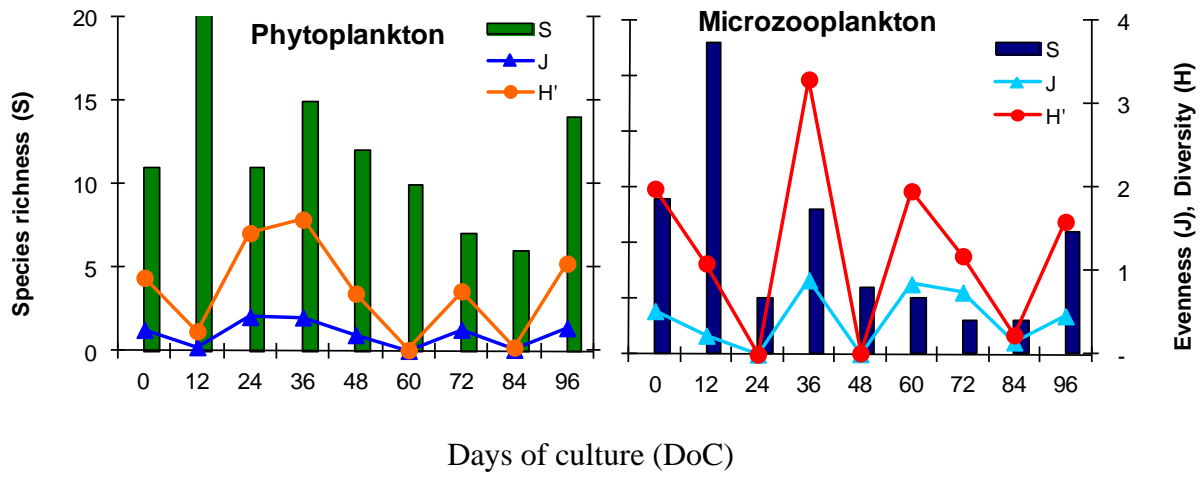


Fig. 5. Temporal variation in species diversity indices of phytoplankton and microzooplankton during the culture of *Litopenaeus vannamei*.

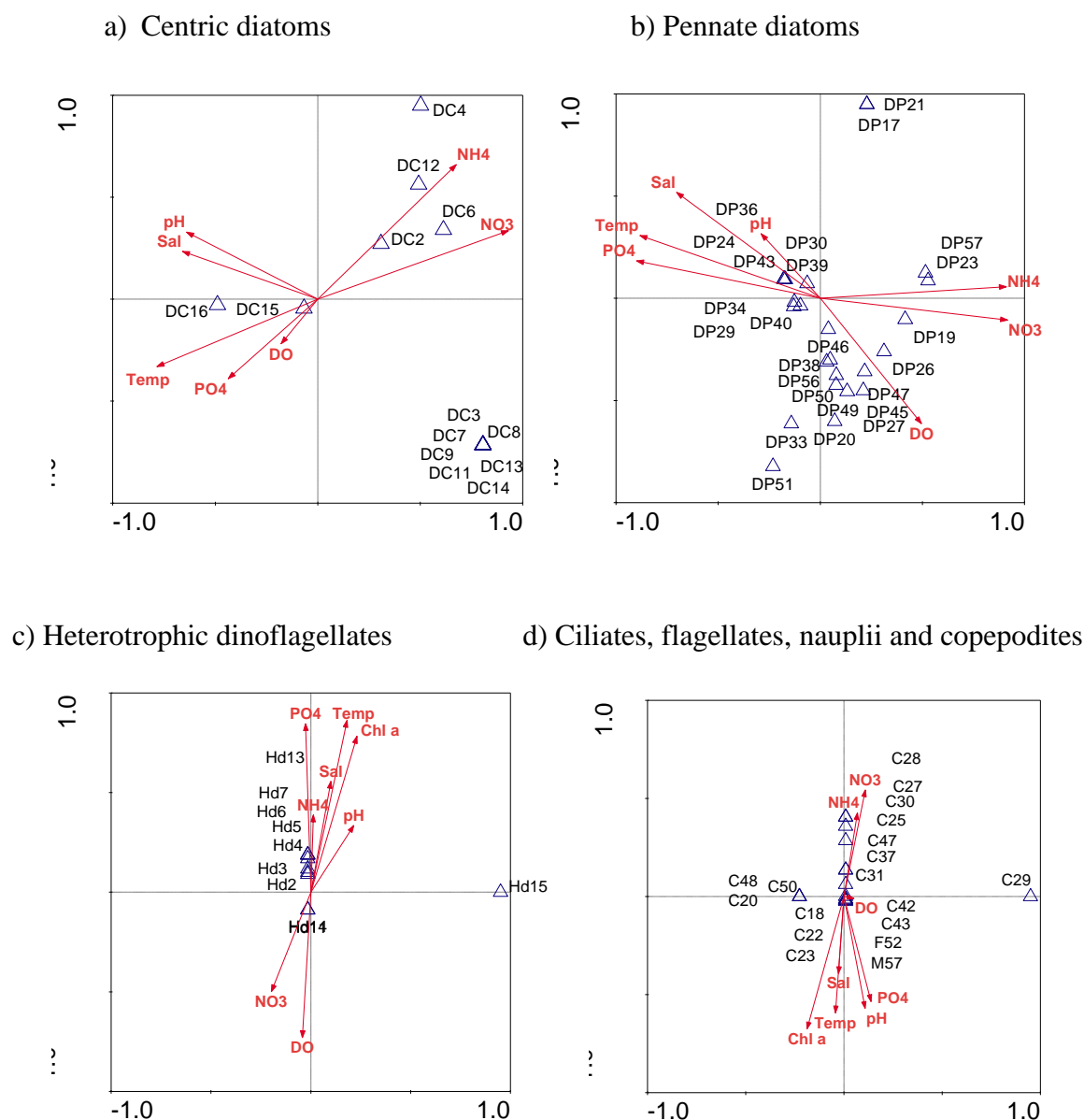


Fig. 6. Canonical correspondence analysis ordination diagram of water quality parameters with species/genus/groups of phytoplankton (a, b) and microzooplankton (c and d) during the culture of *Litopenaeus vannamei*. The results are for axis 1 (horizontal) and axis 2 (vertical) and the arrows represent forward-selected water quality variables (Temp: temperature; Sal: salinity; NH₄: Ammonium; NO₃: Nitrate; PO₄, phosphate; Chl *a*: chlorophyll *a*; DO: dissolved oxygen). The length of arrows indicates the strength of that variable in explaining the species distribution during the culture and the direction of the arrows shows approximate correlation to the ordination axes. For the groups/species codes, refer to Tables 2 and 3.