

Enhanced abundance of Tintinnids under elevated CO₂ level from coastal Bay of Bengal

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Abstract:

The role of microzooplankton in the pelagic trophodynamics is highly significant, but the responses of marine microzooplankton to increasing CO₂ levels are rather poorly understood. Hence the present study was undertaken to understand the responses of marine plankton to increasing CO₂ concentrations. Natural water samples from the coastal Bay of Bengal were incubated under the ambient condition and high CO₂ levels (703 -711 μ atm) for 5 days in May and June 2010. A significant negative correlation was obtained between phytoplankton and microzooplankton abundance which indicated that phytoplankton community structure can considerably be controlled by microzooplankton in this region. The average relative abundances of tintinnids under elevated CO₂ levels were found to be significantly higher (68.65 \pm 5.63% in May; 85.46 \pm 9.56% in June) than observed in the ambient condition (35.68 \pm 6.83 % in May; 79 \pm 5.36% in June). The observed dominance of small chain forming diatom species probably played a crucial role as they can be potentially grazed by tintinnids. This fact was strengthened by the observed high negative correlations between the relative abundance of major phytoplankton and tintinnids. Moreover, particulate organic carbon and total bacterial counts were also enhanced under elevated CO₂ level and can serve as additional food source for ciliates. The observed responses of tintinnids to increasing CO₂ might have multiple impacts on the energy transfer, nutrient and carbon cycling in the coastal water. The duration of the present study was relatively short and therefore further investigation on longer time scale needs to be done and might give us a better insight about phytoplankton and microzooplankton species succession under elevated CO₂ level.

Keywords: Microzooplankton, tintinnid, phytoplankton, Bay of Bengal, diatom, elevated CO₂

Introduction

Microzooplankton (MZP) comprises of primary grazers of phytoplankton and has great influence on the trophic dynamics of the marine food chain (Landry and Calbert 2004, Liu et al. 2002). These groups of microscopic zooplankton (size class between 20 and 200 μm) are taxonomically diverse and include mainly ciliates, flagellates, dianoflagellates, sarcodines and metazoans (Capriulo et al. 1991). They mostly feed on smaller phytoplankton (nano and pico plankton) and play an important role in transferring energy from low to high trophic level (Quevedo and Anadon 2001; Godanthoraman and Uye 2001; Godhanthoraman 2001; Landry et al 1993; Landry and Hassett 1982). Phytoplankton mortality due to microzooplankton grazing can be even as high as mesozooplankton in the marine environment (Gifford et al. 1995; Landry et al. 1997; Strom et al. 2001). Calbet and Landry (2004) showed the global impact of microzooplankton grazing on marine phytoplankton after assimilating 788 paired data points from literature on phytoplankton growth and microzooplankton grazing. The authors reported 75% of the phytoplankton mortality is due to microzooplankton grazing. Microzooplankton also efficiently consume heterotrophic bacterioplankton as well as detritus and establish a link with “microbial loop” (Sanders et al. 1992; Sherr and Sherr 1994). This makes them undoubtedly a major constituent of the marine trophic dynamics (Rassoulzadgan and Etienne et al. 1981).

Protozoan microzooplankton community forms a major food source for the early larval stage of marine fish larvae and hence has a direct impact on marine fisheries (Stocker and Capuzzo, 1990, Liu et al. 2002). Microzooplankton also contributes to DOC production (Strom et al. 1997) in the aquatic environment. Microzooplankton grazing can accelerate the nutrient regeneration in the surface water (Goldman et al. 1987) and can reduce the carbon export flux in the water column (Caron and Goldman 1990, Dolan 1997). Thus any alteration in microzooplankton abundance or physiology or community composition might exert a cascade impact to the entire food web dynamics (from the primary producers to higher fish population including the microbial loop) and biogeochemistry of the ambient water.

CO₂ as a primary greenhouse gas is contributing to global warming and climate change. The increasing rate of anthropogenic CO₂ emission in the atmosphere is not only intensifying global warming, but also changing surface seawater carbon chemistry which could influence different marine organisms in dissimilar ways and at different magnitudes (Wolf Gladrow 1999). Atmospheric carbon dioxide concentration has been predicted to be doubled (750 μatm) by the end of this century (IPCC 2007) and the responses of marine ecosystem to this change are highly unpredictable. Over the past decade, the responses of marine phytoplankton to increasing CO₂ levels have been studied

with sufficient attention (Riebesell 2004; Boyd et al 2010; Doney et al. 2009). Phytoplankton photosynthesis, calcification and nitrogen fixation were found to be affected by changing seawater carbon chemistry (Riebesell 2004; Feng et al. 2008; Ramos et al. 2007). Moreover, alteration in floristic composition of phytoplankton under elevated CO₂ levels have been reported from many areas of the global ocean (Feng et al 2009; Tortell et al. 2002; Kim et al. 2006; Tortell et al. 2008). However, the response of microzooplankton to increasing carbon dioxide level is still unknown and has not been well investigated except in a few recent studies by Suffrian et al. (2008) and Rose et al. (2009). The first study reported no alteration in microzooplankton community in response to different CO₂ levels during a nutrient enriched phytoplankton bloom in Norwegian waters. In the second study, the authors reported significant changes in microzooplankton community in response to different climate change variables (temperature and CO₂) from the North Atlantic spring bloom. Virtually no other reports are available so far regarding microzooplankton responses to predicted changes from the other parts of the global oceans. Studying the responses of microzooplankton to increasing CO₂ levels is of great concern and needs to be addressed with sufficient attention, because of its extremely important role in marine trophic dynamics and energy transfer.

The Bay of Bengal forms the northeastern part of the Indian Ocean and is a semi enclosed tropical sea characterized by its low surface salinity (22 -33 psu) as it receives a huge amount of freshwater discharge from the major monsoon fed rivers in India. This area has been considered as low productive despite receiving high riverine nutrient flux. Stratification caused by low salinity, less light availability and high turbidity during the summer monsoon are the factors thought to limit primary production in this region (Quasim 1977, Radhakrishna 1975). The phytoplankton community is mainly diatom dominated in this area (Paul et al. 2007; Ganapati and Subba Rao 1958). Seasonal abundance, ecology, food habit and biogeochemical significances of microzooplankton are well documented from the Indian waters including Bay of Bengal (Jyothibabu et al 2008; Jyothibabu et al. 2006a; Godhantaramana 1994; Godhantaraman 1997; Godhantaramana 2001). While the microzooplankton community from the Indian waters has been the subject of considerable attention, it is not known how future predicted CO₂ level will affect this biogeochemically important group. The present study was conducted to understand the responses of microzooplankton and phytoplankton community and their interactive effects under future predicted CO₂ level.

Methods

Between May 20th and June 1st 2010 surface seawater was collected in 20 L acid cleaned polycarbonate carboys (Nalgene) from coastal Visakhapatnam, (Fig 1) with the help of a mechanized

boat and immediately carried to the laboratory. 200µm mesh was used to pre-filter large mesozooplankton. A similar practice was also followed by Rose et al., 2009 while testing the responses of microzooplankton under elevated CO₂ level. Salinity during the study period varied between 32-33 psu. CO₂ levels were manipulated by adding base (NaHCO₃) followed by diluted acid (0.1N HCl) after the methodology of Riebesell et al. 2010. After manipulating the carbonate system by base and acid addition, the final CO₂ concentrations were 703±5 and 711±12 in May and June, respectively. 5.6 L borosilicate screw capped bottles were used for further incubation and incubated in triplicate for each CO₂ level in a water bath under natural day and light conditions for five days. A set of controls were also kept in triplicate without changing the carbon chemistry which represents the ambient conditions and mentioned thereafter as “ambient condition” throughout the text. Each bottle was mixed everyday very gently to avoid any sedimentation of phytoplankton biomass. Nutrients (nitrate and phosphate) were added at the beginning of the incubation to ensure phytoplankton growth was nutrient replete (Table 1). After the 5th day of incubation the bottles were taken to the laboratory for further analysis. DIC and alkalinity samples were taken in 40 ml glass bottles with screw caps fitted with Teflon septum and fixed with HgCl₂ until analysis. DIC was analysed with the help of coulometric acidification module (CM5 130) and total alkalinity was analysed according to Dickson et al. (2003) using a 794 Basic Titrino from Metrohm. Partial pressure of CO₂ (pCO₂) was calculated from CO2SYS using Alkalinity, pH and DIC (Lewis & Wallace 1998). Nutrients were analysed following the spectrophotometric method by Strickland and Person (1972). ¹⁵N_{POM} was analyzed using the Delta V Plus, Isotopic Ratio Mass Spectrometer. 500 ml to 1L sample water was filtered through the pre-combusted glass fiber filter (GF/F) followed by overnight drying at 40°C. Filters were exposed to HCl fume to remove the particulate inorganic carbon if any and then measured using the PDB standard following the formula:

$$\delta^{15}\text{N}_{\text{POM}} = \left[\left\{ \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \right\} - 1 \right] \times 1000$$

POC and PON were analysed using the elemental analyser attached to the Isotopic Ratio Mass Spectrometer Delta V Plus (Sharp 1975). The number of total bacteria was quantified following the method of Hobbie et al. 1977. Samples were collected from all the bottles in the 2ml Eppendorf tube and refrigerated after adding formalin until analysis. 250 µl water was incubated with 40 µl of 4, 6-diamidine-2 phenyl indole (DAPI) for 10 min and filtered on a black polycarbonate 0.2 µm filter paper. The entire filter paper was mounted on a glass slide in oil immersion and examined under 1500 magnification of the microscope (Olympus BX51 Epi-Fluorescence) using UV. Phytoplankton and microzooplankton were analysed by using light microscopy. For phytoplankton and microzooplankton quantitative estimation, 1-liter water samples were preserved (Lugol’s iodine

solution plus buffered formaldehyde for phytoplankton and Rose Bengal and buffered formaldehyde (4%) was used for microzooplankton) and allowed to settle for a complete day. After 24 h of sedimentation, the supernatant was rejected and the plankton counts and identifications were made from the settled material using a Sedgwick rafter counting chamber following Hasle and Syvertsen (1997), Sykes (1981), Newell and Newell (1977) and Balasubramanian et al. (2009). Phytoplankton and microzooplankton were also photographed under the microscope with the help of a camera (OLYMPUS IMAGING CORP E330-A-DU1 2x) fixed with the microscope. Phytoplankton and microzooplankton cells were measured with the help of a computerized program IMAGE J and standardized against a stage micrometer.

Statistical Analysis

Two sample t-test was performed using MINITAB version 15 to test the level of significance for the existing differences observed in control and high CO₂ treatment. The average relative abundance of tintinnid from the ambient and high CO₂ conditions were tested to find out whether they were significantly different. The level of significance was considered as $p = <0.05$ and <0.001 .

Results

Phytoplankton, microzooplankton, total bacterial count and particulate organic carbon were found to be comparatively lower in June than in May. Initial phytoplankton species compositions were also different in May and June. Nutrients became depleted after the 5th day of incubation in both ambient condition and high CO₂ treatments (Table 1). The final nutrient concentrations at the elevated CO₂ levels were lower than in the ambient condition indicating higher consumption. This drawdown of nutrients was reflected by a parallel increase in the particulate organic matter. POC (Fig 2a) and PON (Fig 2b) showed significant variations between the ambient condition and high CO₂ treatments. The initial POC values ($74 \pm 2.58 \mu\text{M}$ in May and $64 \pm 4.32 \mu\text{M}$ in June) were enhanced in the final samples (in the ambient conditions as well as in the high CO₂ treatment) indicating net growth of the plankton community. 18% (May) and 29% (June) higher POC was observed in the high CO₂ treatments in comparison to the ambient condition. PON showed more or less similar trend with 18% (May) and 21% (June) higher values under elevated CO₂ levels in comparison to the ambient condition. In May C:N ratio did not differ much (Fig. 2c) in relation to CO₂ levels (7.83 ± 0.32 in ambient condition and 7.85 ± 0.61 in high CO₂), whereas, in June 6% increase in C:N ratio was observed under elevated CO₂ condition (7.01 ± 0.36 in ambient condition and 7.43 ± 0.19 in high CO₂) (Fig. 2c). $\delta^{15}\text{N}$ of particulate organic matter (Fig 2d) was quite low in the initial samples ($2.38 \pm 0.34 \text{ ‰}$ in May and $2.8 \pm 0.25 \text{ ‰}$ in June). These values were found to be increased to

4.6±0.38 ‰ (May) and 5.89±0.7‰ (June) in the ambient condition. A further increase in $\delta^{15}\text{N}$ of particulate organic matter was observed (6.68±1.4‰ in May and 6.89±1.4‰ in June) in the high CO_2 treatments. A significant positive correlation ($r^2 = 0.80$) was observed between $\delta^{15}\text{N}_{\text{POM}}$ and PON (Fig. is not presented here) showing the close coupling between PON building and $\delta^{15}\text{N}_{\text{POM}}$. Total bacterial count showed significant response to increasing CO_2 levels. 15% (May) and 49% (June) higher total bacterial count was observed under the elevated CO_2 levels than in the ambient conditions (Fig. 2e). Dissolved organic carbon was only measured in June and did not show any variation between ambient condition (266±27µM) and high CO_2 treatment (265±48µM).

Phytoplankton cell number varied considerably over the experimental range (Fig. 3a and 3b). The initial cell number in May (8.6×10^6 cells L^{-1}) was almost 8 times higher than observed in June (5.5×10^6 cells L^{-1}). In May the final cell counts were lower in both ambient condition and high CO_2 than the initial value. But 21% higher cell abundance was observed at the elevated CO_2 level than in the ambient condition. In June the final cell counts showed quite an opposite trend where higher cell counts were observed in the ambient condition (8.24×10^5 L^{-1}) with a reduced number (3.8×10^5 L^{-1}) in the high CO_2 treatment (Fig. 3a and b). Similarly, the average microzooplankton abundance also showed reduced value in June than in May (Fig 3c, 3d). In May the final count was 2.4 times (ambient condition) and 2.2 times (high CO_2) higher than the initial value (580 ± 336 cells L^{-1}). But in June the initial count (130 ± 55 cells L^{-1}) was decreased in the ambient condition (81 ± 41 cells L^{-1}) and increased under the high CO_2 treatment (151 ± 53 cells L^{-1}). Phytoplankton and microzooplankton abundance in both experiments were found to be negatively correlated with significant R^2 value (0.99) (Fig. 3e, 3f).

Phytoplankton community was mainly dominated by smaller diatoms. Few diatoms were also observed but the relative abundance was negligible (<0.3%). Phytoplankton taxonomic structure showed large variations in the initial and final (ambient condition and high CO_2 treatment) samples in both experiments (Fig. 4a -4f). In May, the initial phytoplankton population was dominated by *Skeletonema costatum* (61%) followed by *Thalassiosira decipiens* (22.39%), *Melosira varians* (6.97%), *Asterionella japonica* (2.63%), *Chaetoceros* sp. (1.88%) and *Nitzschia closterium* (1.74%) (Fig.4a). After the 5th day in the ambient condition the relative abundance of *Skeletonema costatum* decreased to 21.79% with three times enhancement of *Thalassiosira decipiens* population (63.6%) (Fig.4b), but *Asterionella japonica* (2.44%) and *Melosira varians* (6.85%) did not show much change. The relative abundance of *Nitzschia closterium* got reduced to 0.26% from an initial value of 1.75%. In the high CO_2 treatment *Skeletonema costatum* and *Thalassiosira decipiens* contributed almost equally (41-43%) whereas *Chaetoceros* sp. and *Asterionella japonica* increased to

almost twice that of the initial and ambient condition (Fig.4c). In June the initial phytoplankton community structure was little different where *Chaetocers* contributed 52% followed by 18% *Nitzschia closterium*, 12% *Skeletonema costatum* and 9.8% *Thalassiosira decipiens* (Fig. 4d). On the final day, this pattern was found to be completely changed both in the ambient condition and high CO₂ treatment. As in May, here also significant enhancement in *Thalassiosira decipiens* population was observed. The initial relative abundance was 9.8% (Fig. 4d) which was increased to 73% in ambient condition (Fig. 4e) and 79% at the elevated CO₂ level (Fig. 4f). *Asterionella japonica* showed a similar trend with almost 4 times enhancement in the high CO₂ and 1.77 times in the ambient condition. *Chaetoceros* sp. population decreased almost 10 times in the ambient condition and became negligible in the high CO₂ (0.11%). *Skeletonema costatum* was also reduced to 8.75% in the ambient condition (Fig 4d) and further decreased to 2.06% in the high CO₂ treatment (Fig. 4f). *Thalassionema nitzschioides* was not observed initially, but appeared in the ambient condition (1.95%) and less in the high CO₂.

Initially, the microzooplankton population was mainly dominated by the crustacean larvae (74.86% in May and 45% in June), tintinnid (20% in May and 40% in June) and others (4 - 14%) including planktonic foraminifera, diatoms, etc. (Fig. 4g and 4j). After 5 days of incubation, different genera of tintinnid increased tremendously in the ambient condition as well as in the high CO₂ treatments (Fig. 4g, 4h, 4k 4l). But surprisingly, in the high CO₂ treatment, tintinnid became the major population contributing 70% and 85% of the microzooplankton community in May (Fig 4i) and June (Fig. 4l), respectively. In May, the average relative abundance of tintinnid was 68.65±5.63 % in the high CO₂ treatment which was almost 1.92 times higher than in the ambient condition (student t-test shows this difference was significant at 95% confidence level; $t = 8.12$, $DF = 6$, $p = <0.001$). In June a similar difference was also observed but the magnitude was reduced. The average relative abundance of tintinnid in the ambient condition was 79±5.36 % which was 8% less than the value observed in the high CO₂ condition ($t = 1.9$, $DF = 10$, $p = >0.05$).

When the relative abundance (initial, final ambient and final high CO₂) of tintinnid and five major contributing phytoplankton species (*Thalassiosira decipiens*, *Chaetoceros* sp., *Skeletonema costatum*, *Nitzschia closterium*, *Asterionella japonica*) were correlated, interesting correlations were obtained (Fig. 5). Significant negative correlations were observed between the relative abundance of *Skeletonema costatum* ($r^2 = 0.99$ in May, 0.66 in June), *Nitzschia closterium* ($r^2 = 0.54$ in May, 0.80 in June) and *Chaetoceros* sp. ($r^2 = 0.66$ in May and 0.06 in June) with tintinnid (considering the relative abundance from the initial, ambient final and high CO₂ final). Whereas, *Thalssiosira*

decipiens ($r^2 = 0.64$ in May and 0.63 in June) and *Asterionella japonica* ($r^2 = 0.99$ in May and 0.29 in June) were found to be positively correlated.

To get a better understanding about the tintinnids food preference, the lorica oral diameter of the major tintinnids were measured. The major tintinnids genera were observed during this experiment were *Eutintinnus*, *Favella*, *Acanthostomella*, *Dictycysta*, *Petalotricha* and *Tintinnopsis* with variable species number. The average diameter of the observed tintinnids genera was found to be $95.8 \pm 33 \mu\text{m}$ ($41 - 141 \mu\text{m}$). The average diameter of the major phytoplankton genera were also measured and were found to be $5.36 - 6.88 \mu\text{m}$ of *Nitzschia closterium*, $2.8 - 23.77 \mu\text{m}$ of *Skeletonema costatum*, $6.06 - 29.34 \mu\text{m}$ of *Chaetoceros* sp. Whereas, the cell diameter of *Asterionella japonica* ($28.45 - 75.31 \mu\text{m}$) and *Thalassiosira decipiens* ($9.8 - 40.1 \mu\text{m}$) were much larger.

Discussion and conclusions

The observed difference in the phytoplankton biomass and community composition between May and June can be attributed to the variation in the nutrients levels. June is the peak of pre-monsoon when high temperature combined with high salinity and low nutrients can limit phytoplankton growth. The observed nutrients concentration were significantly lower in June than in May (Phosphate $< 0.5 \mu\text{mol L}^{-1}$, Nitrate $< 4.8 \mu\text{mol L}^{-1}$, Silicate $< 5 \mu\text{mol L}^{-1}$) and in order to avoid the effects of low nutrients on phytoplankton growth, nitrate and phosphate were added to maintain the final nutrient concentration similar for both experiments. In general, the occurrences of nanophytoplankton ($< 20 \mu\text{m}$) during the nutrient poor high saline water is very common in the coastal water and also consistent with the present study.

In the high CO_2 treatment, higher nutrient consumption is in agreement with the high POC production. Depleted silica could be indicative of diatom growth and also supports the microscopic observation. Initially nitrate, phosphate and silicate were added to allow phytoplankton to grow under nutrient replete condition and the values mentioned in the Table 1 are the final values. Nutrient addition seemed to promote phytoplankton growth during the experiment. Kim et al (2006) reported quite similar result from a mesocosm experiment in the Korean water where nutrients addition fueled particulate organic matter production considerably. The values of $\delta^{15}\text{N}$ of particulate organic matter suggest that the added nitrate was utilized potentially by phytoplankton and was evidenced by the increased $\delta^{15}\text{N}$ of POM. Richoux and Froneman (2009) reported a similar result from a subtropical convergence where PON and $\delta^{15}\text{N}$ of POM exhibited positive correlation. Kumar et al (2005) presented a series of PON and $\delta^{15}\text{N}$ of POM data from the Bay of Bengal and our results also found

to be alike. In general, nitrogen fixation is characterized by 0 to -2‰ of $\delta^{15}\text{N}_{\text{POM}}$ and the values well above this range are designated as nitrate utilization because $\delta^{15}\text{N}$ of nitrate is 4 - 6‰ (Liu and Kaplan 1989). Higher nitrate uptake under the elevated CO_2 condition is therefore in consistent with the increased $\delta^{15}\text{N}$ of POM (Fig 2d) and further confirmed by the significant positive correlation between PON and $\delta^{15}\text{N}$ of POM (Fig. not shown here). Although POC, PON and $\delta^{15}\text{N}$ of POM showed higher values in the high CO_2 treatment, but the responses of C:N ratio did not match in May and June. Increased C:N ratio in the high CO_2 treatment was observed in June whereas, in May it did not vary and the increase in POC production was probably stoichiometrically related to the increase in PON production resulting the C:N ratio to remain unchanged.

Dominance of different genera of tintinnids in the microzooplankton community in the Bay of Bengal have been observed by many authors (Jyothybabu et al. 2008, Gauns et al. 2005; Jyothibabu et al. 2006b; Godhantaraman, 2001) which is consistent with our observation. But the relative contribution of different groups of microzooplankton may vary seasonally. Gauns et al (2005) reported the dominance of dianoflagellates and tintinnids ($\approx 40\%$) from the central Bay of Bengal. Whereas Jyothibabu et al (2006a) observed high percentage of tintinnids (80%) followed by crustacean larvae and very little or no dianoflagellate in the Cochin back water system during the pre-summer monsoon. We observed quite similar trends on the initial day of the experiments where crustacean larvae and tintinnids dominated the microzooplankton community with very few other groups like mixotrophic dianoflagellates (*Peridinium* sp., *Proocentrum* sp.), planktonic foraminifera, micro-gastropod etc. The present study shows that in the coastal water of Bay of Bengal, microzooplankton contributes a considerable part of phytoplankton grazing during the pre-summer monsoon. The observed significant negative correlations between phytoplankton and microzooplankton cell abundance (Fig 3e, 3f) clearly indicate a strong grazing pressure on the phytoplankton community exerted by microzooplankton. Tintinnids increased in both ambient condition and high CO_2 treatment, but with higher abundance in the latter. The initial removal of mesozooplankton from the water sample could be the possible reason to allow tintinnids to grow exponentially during our experiment as they were not grazed simultaneously.

The observed higher abundance of tintinnid in the high CO_2 treatment reflects the availability of more food material which could be small phytoplankton (nanoplankton $<20\mu\text{m}$), detritus and bacteria (Strom 2002). Splitter (1973) reported that tintinnid are capable of consuming a food size which is 45-50% of their lorica oral diameter and consequently could feed on diverse food sources including detritus, small phytoplankton and bacterioplankton. Tintinnid can grow faster by simple cell division unlike the crustacean larvae which has comparatively slower developmental phase. In

general microzooplankton grazing is believed to be important during the post-bloom phase when the average phytoplankton size is small (Gifford et al. 1995). It has been proved by many studies that microzooplankton in general are very selective about their food and the selection is done through some mechanisms like biochemical properties (Monger et al 1999), quality (Burkill 1987), nutritional value (Stoecker et al. 1995; Buskey 1997) and prey body size (Zhang 2005). Available literature showed that the tropical tintinnid mostly feed on smaller size phytoplankton (<30µm) (Godhantaramana and Krishnamurthy 1997). Jyothibabu et al. (2006a) reported high abundance of tintinnid from a tropical backwater system in the West coast of India where high abundance of smaller diatom species like *Skeletonema costatum* and *Nitzschia closterium* were believed to serve as a major food source for tintinnid.

Interestingly, in the present study, decreased abundance of the above mentioned phytoplankton species including *Chaetoceros* sp was significantly correlated with increased abundance of tintinnid in both experiments (Fig. 5) and could be an indication of grazing by tintinnid. In a similar study by Rose et al. (2009), the highest abundance of a small ciliate was found to be associated with decreased number of picoplankton. In May on an average *Thalassiosira decipiens* showed increasing trends with increasing number of tintinnid while considering both initial day and final day values (Fig 5). But while considering only the final day values, the relative percentage was almost 1.5 times less in the high CO₂ than the ambient condition (Fig 4) and also could be a suitable prey for tintinnid. In June the simultaneous occurrence of decreased *Skeletonema costatum* and *Chaetoceros* sp. abundance was found to be well correlated with the enhanced number of tintinnid (Fig 5).

Phytoplankton cell diameter measurements showed that the average diameter of the dominant phytoplankton genera were as follows *Nitzschia closterium* < *Chaetoceros* sp < *Skeletonema costatum* < *Thalassiosira decipiens* < *Asterionella japonica*. The variation in the observed negative/positive correlations between tintinnids number and the nanoplankton species exactly showed a similar trend where the first three species in this series were negatively correlated with the significant r^2 values (Fig. 5). *Thalassiosira decipiens* and *Asterionella Japonica* were found to be larger than the first three species and might not be preferred by the tintinnids. *Asterionella Japonica* has a long apical part and could be as long as 150 µm which is larger than the average tintinnids oral diameter (95.8 µm). The observed positive correlations between *Asterionella japonica* and tintinnids are also consistent with this fact. Hence, the larger size might have given this phytoplankton species some advantage over the smaller phytoplankton to be escaped from tintinnid active grazing.

An interesting observation by Sun et al (2007) shows that the nano-phytoplankton within the size range of 2-20 μm grows faster than the bigger microphytoplankton (>20 μm) and can be grazed on a high rate by microzooplankton in the Chesapeake Bay. The study showed that the dominance of tintinnid was accompanied by the presence of small diatoms with low carbon content like *Nitzschia closterium*, *Chaetoceros subtilis* and *Skeletonema costatum* which is quite similar to our observations. Madhu et al (2006) reported very similar phytoplankton community composition from the Bay of Bengal region. It has been observed often in the marine environment that flagellates like tintinnid selectively feed on the fast growing bacteria and phytoplankton at a high rate (Strom 2002) and therefore the smaller phytoplankton experience the highest grazing pressure. Phytoplankton community structure is believed to have a great control on microzooplankton community (Dolan et al. 2002). Thus on one hand the emergence of a particular size class of phytoplankton under a defined condition could control the microzooplankton community structure and on the other hand microzooplankton selective food habits greatly control the phytoplankton community structure (Sun et al 2007; Strom 2002). Smaller phytoplankton, having a higher surface area to volume ratio than the bigger ones, can grow faster (Gatham and Rhee 1981) and we assume under elevated CO_2 the small diatom growth was enhanced, which influenced the microzooplankton community structure significantly resulting in a tintinnid dominated community.

Since tintinnid also actively feed on heterotrophic bacteria (Boettjer and Morales 2005, Fenchel 1987), the observed increased abundance of heterotrophic bacterial abundance under the elevated CO_2 also can be used as a potential food sources for them. However, increased heterotrophic bacterial abundance under elevated CO_2 indicates the possibility of higher DOC production. Usually, in parallel with carbon fixation, a major part of the photosynthesized carbon can be leached out from the cell as a potential source of DOC and can be available thereafter as substrate for heterotrophic bacteria (Nagata 2000; Lopez-Sandoval et. al., 2010). Increased supply of CO_2 could enhance phytoplankton production and has been reported by several authors (Riebesell 2004; Hein and Sand Jensen 1997; Tortell et al. 2008; Feng et al. 2009), but we have not measured the rate of carbon fixation and DOC did not show any variation in relation to the CO_2 levels. It is also possible that DOC was simultaneously consumed by heterotrophic bacteria and grew faster, which is also reflected in the enhanced bacterial abundance. Under elevated CO_2 level, enhanced production of extracellular organic matter has been observed by Engel et al. (2004). Egge et al. (2009) reported increased bacterial production in the high CO_2 level during a CO_2 enrichment experiment and which could be due to increased DOC production.

There are plenty of examples of microzooplankton grazing from different parts of the global oceans. North Atlantic spring bloom is one of the biggest phytoplankton blooms in the global ocean where microzooplankton (small ciliates) were found to be the major grazer of the phytoplankton production (Gifford et al. 1995; Stelfox-Widdicombe et al. 2000, Karayanni et al. 2005). Up to 80% of phytoplankton mortality due to tropical tintinnid was reported from a tropical upwelling system (McManus et al. 2007). Boettjer and Morales (2005) studied the grazing rate of microzooplankton using chlorophyll *a* tracer in a dilution experiment and the observed rate was even more than 100% of the phytoplankton population during the non-upwelling time in a coastal embayment of Chile. Godhantaraman (2001) investigated the seasonal abundance of microzooplankton from an Indian estuary and mangrove system and the results showed overwhelming dominance of tintinnid species in the study area and their abundance was positively correlated with other environmental factors, chlorophyll *a* in particular. Our study also shows that microzooplankton grazing (tintinnid in particular) could be a major cause of phytoplankton mortality in coastal Bay of Bengal.

Phytoplankton community structure in the ambient condition and high CO₂ treatments clearly shows differential response, but this may not be only due to the change in carbon chemistry but also due to microzooplankton grazing. Phytoplankton species succession was earlier thought to be only controlled by abiotic factors (bottom up control), but the impact of zooplankton grazing (top down control) also could be equally important and has been considered recently (Strom 2002). The responses of microzooplankton to increasing CO₂ level were studied in two recent experiments (Suffrian et al. 2008; Rose et al. 2009). Suffrian et al. (2008) did not report any significant change in the microzooplankton community which could be because of no significant changes in the phytoplankton community in relation to different CO₂ concentrations. Ciliates, in particular, did not show any noticeable response to the increasing CO₂. But the study by Rose et al. (2009) reported some interesting understanding of microzooplankton behavior under elevated CO₂ levels. The author explained that the increasing CO₂ might not directly exert any change in microzooplankton community. But high CO₂ and temperature which induced changes in the phytoplankton community during the experimental phase mainly seemed to influence the microzooplankton community structure. Our result is also comparable with these findings.

Alteration in marine phytoplankton floristic composition has been considered to be controlled mainly by the major climate change variables like carbon chemistry of seawater, light, temperature and nutrients (Boyd et al. 2010). But the study by Rose et al (2009) and our results, clearly indicate not only physicochemical factors (bottom up ambient condition) under the climate change should be considered while accounting for phytoplankton taxonomic composition and abundance, but the

changes induced by microzooplankton could also play a fundamental role in this concern. In the present study those phytoplankton species which were found to dominate under elevated CO₂ probably were not preferentially grazed by the tintinnids. Whereas the other species which were still abundant in the ambient condition decreased largely in the high CO₂ level and seemed to be grazed by the tintinnids. This trend obviously tells us that the phytoplankton taxonomic composition in the future ocean will not only depend on the physicochemical variables (bottom -up control) but also on their potential grazer (top-down control).

However, the Bay of Bengal is largely influenced by the Indian summer monsoon and shows a large variability in the physicochemical parameters which affect phytoplankton production, growth and community structure (Kumar et al. 2010; Gomes et al. 2000, Madhu et al. 2006). During the monsoon, comparatively higher nutrient concentrations (contributed by river discharge) may promote the growth of larger phytoplankton. Thus far, their response to increasing CO₂ levels might not yield a similar result and microzooplankton also could respond in a different way which needs further investigation.

Implications

The present study shows that under the projected CO₂ level microzooplankton (tintinnids in particular) responded positively and can influence the trophic energy transfer in the study area. Higher abundance of heterotrophic bacteria, particulate organic matter and small phytoplankton could accelerate the microzooplankton mediated energy transfer in the marine environment. Moreover, enhanced microzooplankton abundance in the marine environment could reduce the carbon flow in the water column. Since microzooplankton serve as a food source for the larvae of many vertebrate and invertebrate species (Fenchel 1987) including commercially important fish larvae, enhanced population of microzooplankton can influence their larval development. They are also considered as the principal agent of nutrient mineralization and of major importance in sustaining nitrogen supply in the water column (Eppley 1972). Specific excretion rates of ammonia by tintinnids are 1-2 fold higher in order of magnitude than those of mesozooplankton. Hence, increased abundance of tintinnid species at the elevated CO₂ level can have great significance in the nutrient biogeochemistry of the coastal Bay of Bengal. The effects of elevated CO₂ thus could be propagated up to higher trophic level and also to the microbial loop mediated by the important microscopic protozoan groups. However, this experiment was a preliminary trial and further detailed study is required.

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Legend to figures:

Fig. 1: Map showing station location

Fig. 2: Initial and final day values of particulate organic carbon (POC) (a); Particulate organic nitrogen (PON) (b); C:N ratio (c); $\delta^{15}\text{N}_{\text{POM}}$ (d) and total bacterial count (TBC) (e) in the ambient and high CO_2 treatments during May and June ($n=3-6, \pm\text{SD}$). Total bacterial count was not measured in the initial samples and hence left blank.

Fig 3: Phytoplankton cell number in a) May and b) June; microzooplankton abundance during the experimental period c) May and d) June ($n=3-6, \pm\text{SD}$); correlations between phytoplankton and microzooplankton abundance during e) May and f) June.

Fig. 4: Phytoplankton relative abundance (examined by microscopy) in the initial a) May and d) June; in the ambient condition b) May and e) June; in the high CO_2 treatments c) May and f) June; Microzooplankton relative abundance (examined by microscopy) in the initial g) May and j) June; in the ambient condition h) May and k) June; in the high CO_2 treatments i) May and l) June;

Fig. 5: Correlations between relative abundance of major contributing phytoplankton species and tintinnids during May and June. The values from the initial and final (ambient and high CO_2) samples have been considered here.

Table 1: Initial and final (ambient condition and high CO₂ treatment) nutrient concentrations over the experimental period (n=3 - 4, ±SD)

Parameters		Initial	Final	
			Ambient condition	High CO ₂
May	DIN (μM)	21.56±2.3	2.54±0.53	1.85±0.39
	DIP(μM)	1.11±0.084	0.24±0.11	0.18±0.02
	SiO ₄ (μM)	9.26±0.76	2.15±0.86	1.82±1.1
June	DIN(μM)	20.79±0.53	3.69±2	0.91±0.34
	DIP(μM)	1.9±0.22	0.87±0.21	0.94±0.11
	SiO ₄ (μM)	11.62±1.3	3.28±1	2.21±1.1

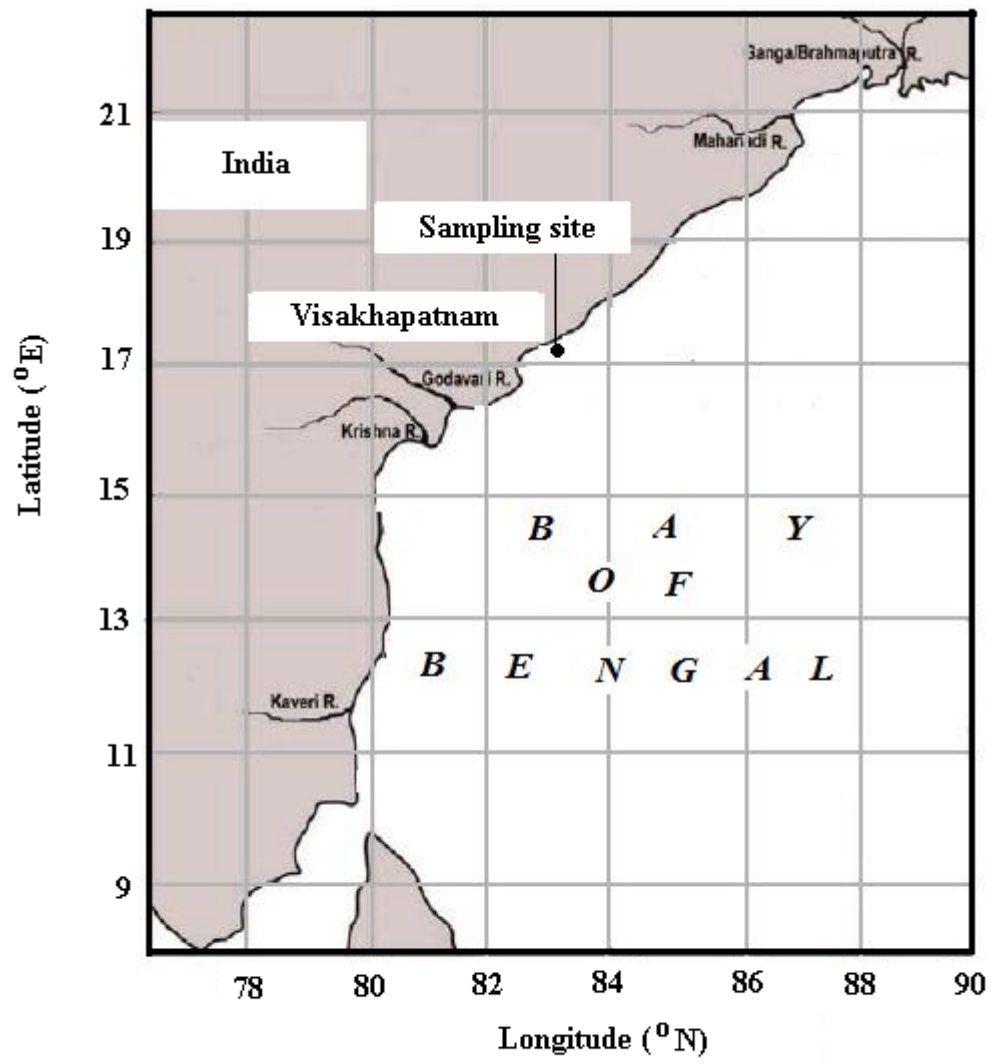


Fig. 1

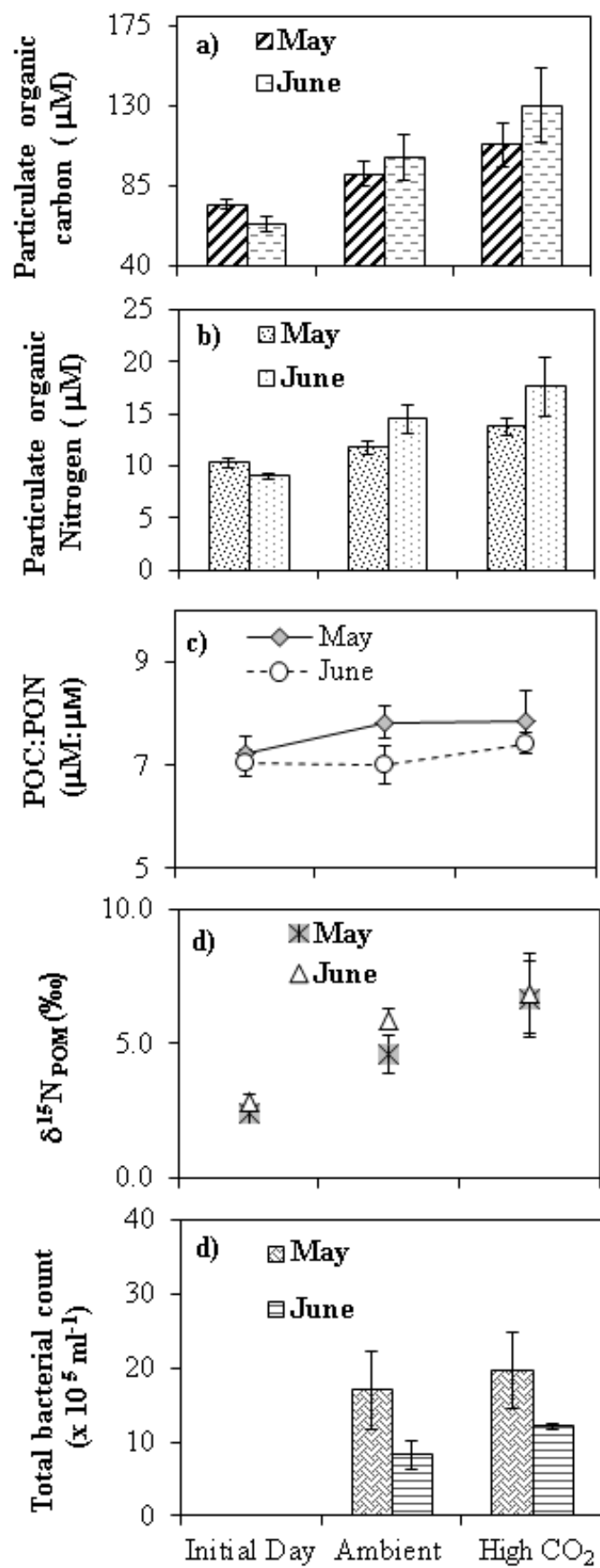


Fig. 2

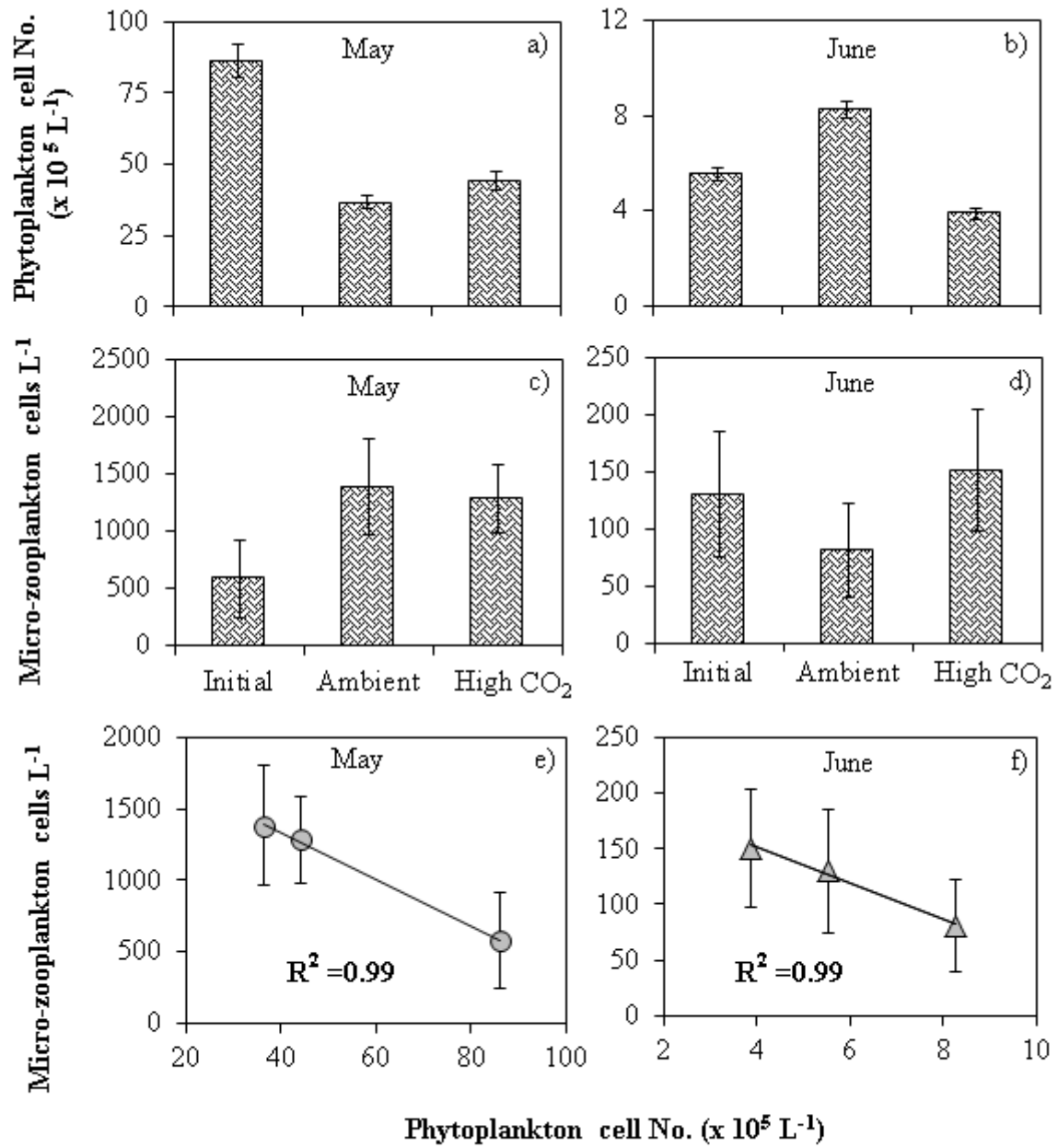


Fig. 3

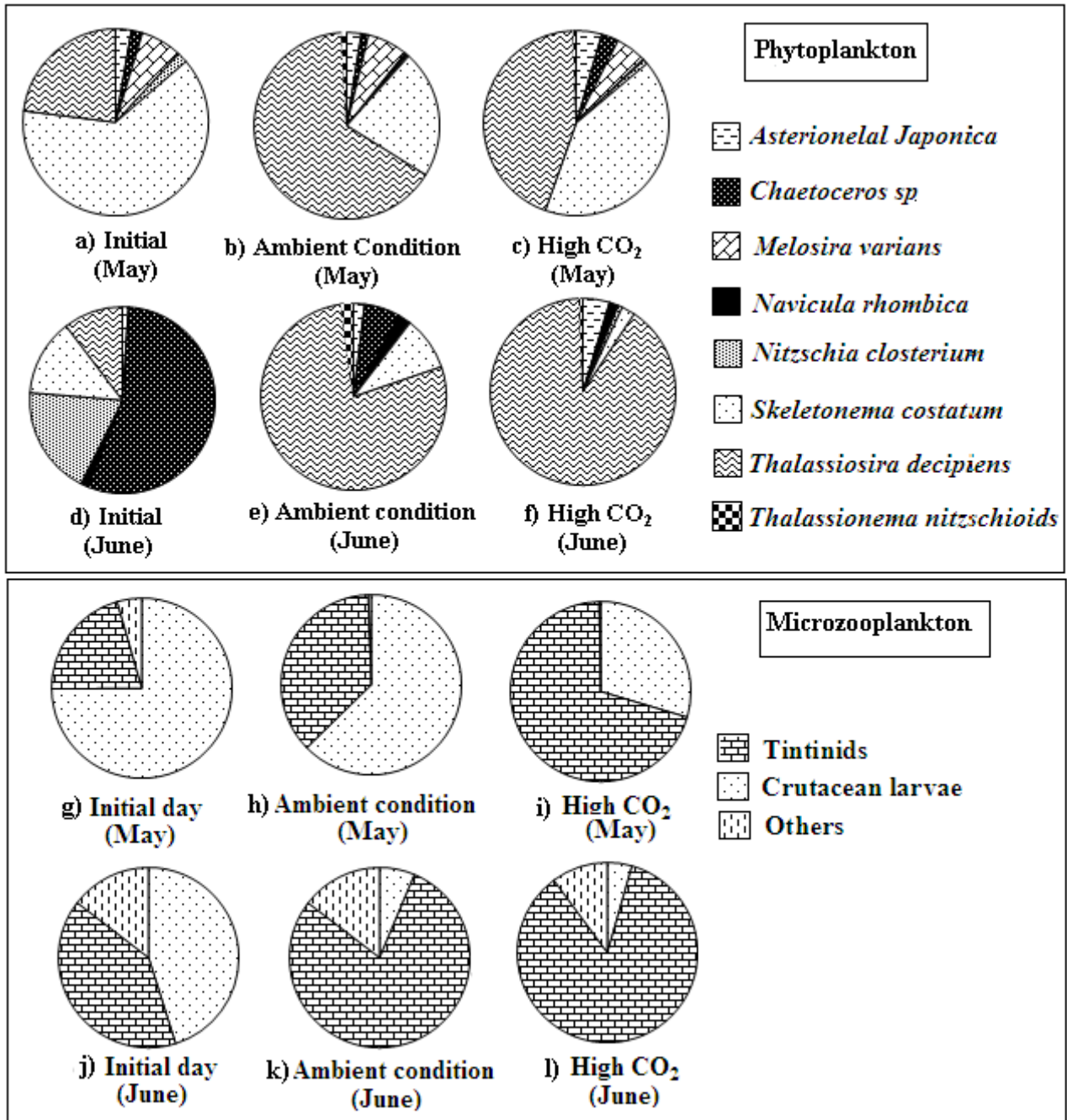


Fig. 4

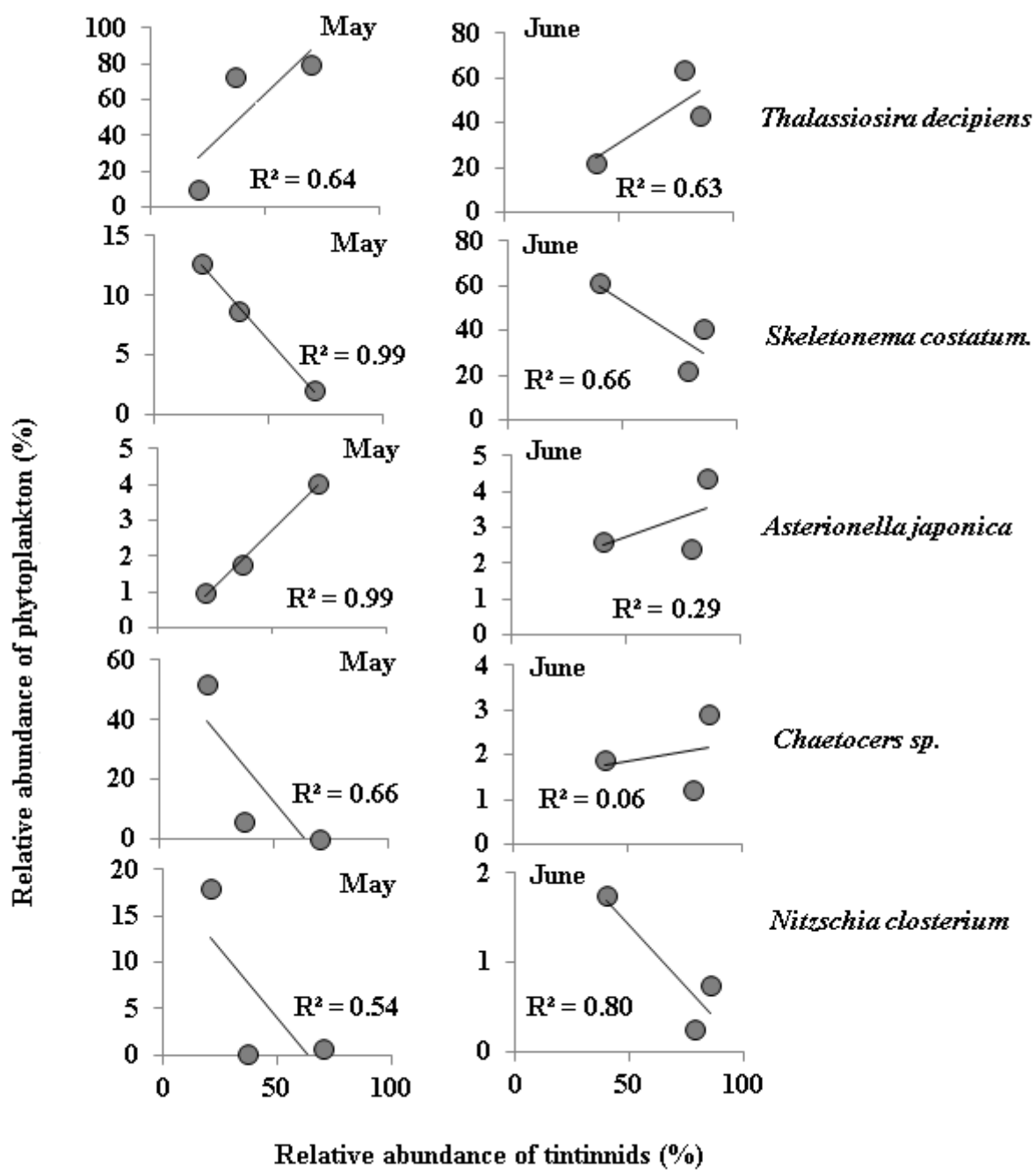


Fig. 5