

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

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Received: 15 April 2011 / Accepted: 5 December 2011 / Published online: 27 December 2011
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Abstract The role of microzooplankton (MZP) in the pelagic trophodynamics is highly significant, but the responses of marine MZP 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 μatm) for 5 days in May and June 2010. A significant negative correlation was obtained between phytoplankton and MZP abundance which indicated that phytoplankton community structure can considerably be controlled by MZP in this region. The average relative abundances of tintinnids under elevated CO₂ levels were found to be significantly higher (68.65 ± 5.63% in May; 85.46 ± 9.56% in June) than observed in the ambient condition (35.68 ± 6.83% in May; 79 ± 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

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therefore further investigation on longer time scale needs to be done and might give us a better insight about phytoplankton and MZP 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 Calbet 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, dinoflagellates, 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 Anadón 2001; Godhantaraman and Uye 2001; Godhantaraman 2001; Landry et al. 1993; Landry and Hassett 1982). Phytoplankton mortality due to MZP 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 MZP grazing on marine phytoplankton after assimilating 788 paired data points from literature on phytoplankton growth and MZP grazing. The authors reported 75% of the phytoplankton mortality is due to MZP grazing. MZP 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 (Rassoulzadegan and Etienne 1981).

Protozoan MZP community forms a major food source for the early larval stage of marine fish larvae and hence has a direct impact on marine fisheries (Stoecker and Capuzzo 1990; Liu et al. 2002). MZP also contributes to DOC production (Strom et al. 1997) in the aquatic environment. MZP 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 MZP 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.

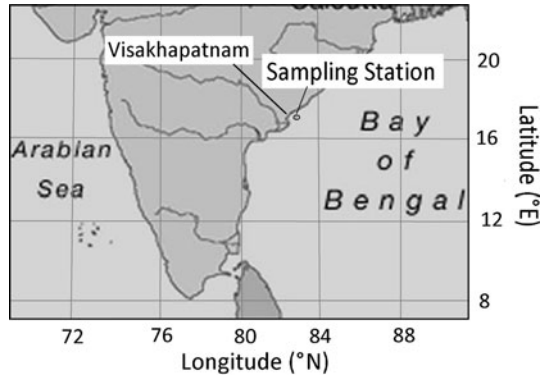
CO₂ as a primary greenhouse gas is contributing to global warming and climate change. The increasing rate of anthropogenic CO₂ emission in the atmosphere is not only intensifying global warming, but also changing surface seawater carbon chemistry which could influence different marine organisms in dissimilar ways and at different magnitudes (Wolf-Gladrow et al. 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; Hare et al. 2007; Yoshimura et al. 2009) and recently from a tropical

estuary by Biswas et al. (2011). However, the response of MZP 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 MZP 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 MZP 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 MZP responses to predicted changes from the other parts of the global oceans. Studying the responses of MZP 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 (Qasim 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 MZP are well documented from the Indian waters including Bay of Bengal (Jyothibabu et al. 2008, 2006a; Godhantaraman 1994; Godhantaraman and Krishnamurthy 1997; Godhantaraman 2001). While the MZP 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 MZP and phytoplankton community and their interactive effects under future predicted CO₂ level.

Methods

Between May 20th and June 1, 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. 200 µm mesh was used to pre-filter large mesozooplankton. A similar practice was also followed by Rose et al. (2009) while testing the responses of MZP under elevated CO₂ level. Salinity during the study period varied between 32 and 33 psu. The initial pCO₂ of the sample water were found to be 356 ± 9 µatm in May and 439 ± 7 µatm in June. The initial pH values were 8.21 ± 0.01 and 8.13 ± 0.003 in May and June, respectively. CO₂ levels were manipulated by adding base (NaHCO₃) followed by diluted acid (0.1 N 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 µatm and 711 ± 12 µatm in May and June, respectively. The final pH was 7.99 (for both May and June) after adjusting the pCO₂. 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).

Fig. 1 Map showing station location**Table 1** Initial and final (ambient condition and high CO₂ treatment) nutrient concentrations over the experimental period ($n = 3-4$, \pm SD)

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

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 (2003) using a 794 Basic Titrino from Metrohm. Partial pressure of CO₂ (pCO₂) was calculated from CO2SYS using Alkalinity, pH and DIC (Lewis and Wallace 1998). Nutrients were analysed following the spectrophotometric method by Strickland and Parsons (1972). ¹⁵N_{POM} was analyzed using the Delta V Plus, Isotopic Ratio Mass Spectrometer. 500 ml to 1 l 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:

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

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 2 ml Eppendorf tube and refrigerated after adding formalin until analysis. 250 μ l water was incubated with 40 μ l of 4,6-diamidine-2 phenyl indole (DAPI) for 10 min and filtered on a black polycarbonate 0.2 μ m filter paper. The entire filter paper was mounted

on a glass slide in oil immersion and examined under 1,500 magnification ($\times 1,500$) of the microscope (Olympus BX51 Epi-Fluorescence) using UV. Phytoplankton and MZP were analysed by using light microscopy. For phytoplankton and MZP quantitative estimation, 1-l water samples were preserved (Lugol's iodine solution plus buffered formaldehyde for phytoplankton and Rose Bengal and buffered formaldehyde (4%) was used for MZP) 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 Syversten (1997), Sykes (1981), Newell and Newell (1973) and Balasubramanian et al. (2009). Phytoplankton and MZP 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 MZP cells were measured with the help of a computerized program IMAGE J and standardized against a stage micrometer.

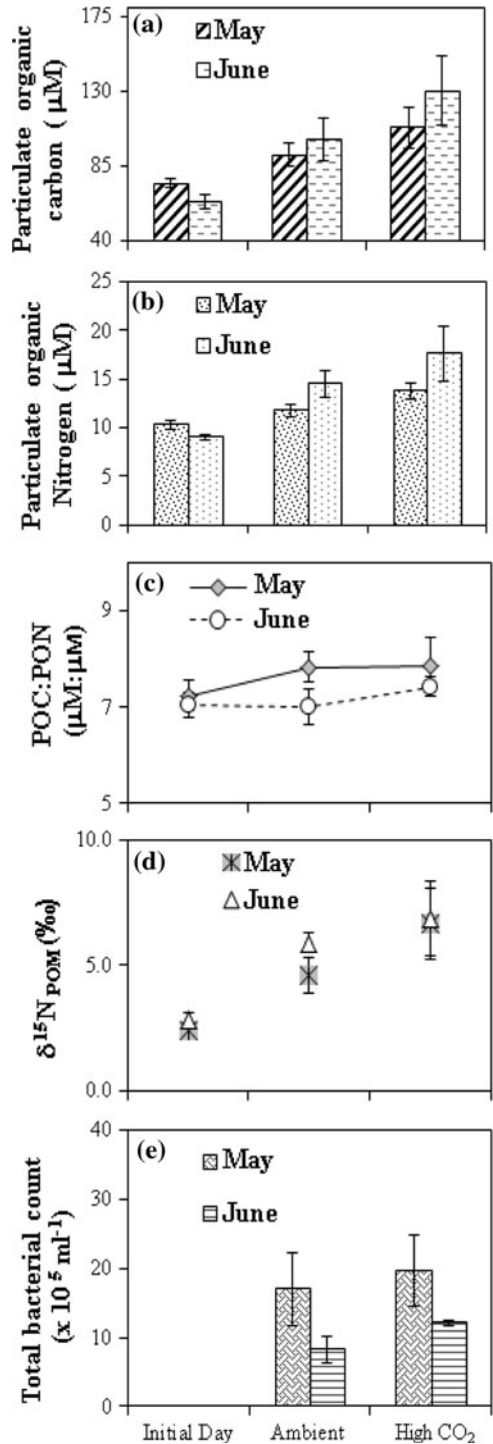
Statistical analysis

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, MZP, 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 $4.6 \pm 0.38\text{‰}$ (May) and $5.89 \pm 0.7\text{‰}$ (June) in the ambient condition. A further increase in $\delta^{15}\text{N}$ of particulate organic matter was observed ($6.68 \pm 1.4\text{‰}$ in May and $6.89 \pm 1.4\text{‰}$ in June) in the high CO₂ treatments. A significant positive correlation ($r^2 = 0.80$) was observed between $\delta^{15}\text{N}_{\text{POM}}$ and PON (data 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 CO₂

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



levels. 15% (May) and 49% (June) higher total bacterial count was observed under the elevated CO₂ 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 ± 27 μM) and high CO₂ treatment (265 ± 48 μM).

Phytoplankton cell number varied considerably over the experimental range (Fig. 3a, b). The initial cell number in May (8.6 × 10⁶ cells l⁻¹) was almost eight times higher than observed in June (5.5 × 10⁶ cells l⁻¹). In May the final cell counts were lower in both ambient condition and high CO₂ than the initial value. But 21% higher cell abundance was observed at the elevated CO₂ 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 × 10⁵ l⁻¹) with a reduced number (3.8 × 10⁵ l⁻¹) in the high

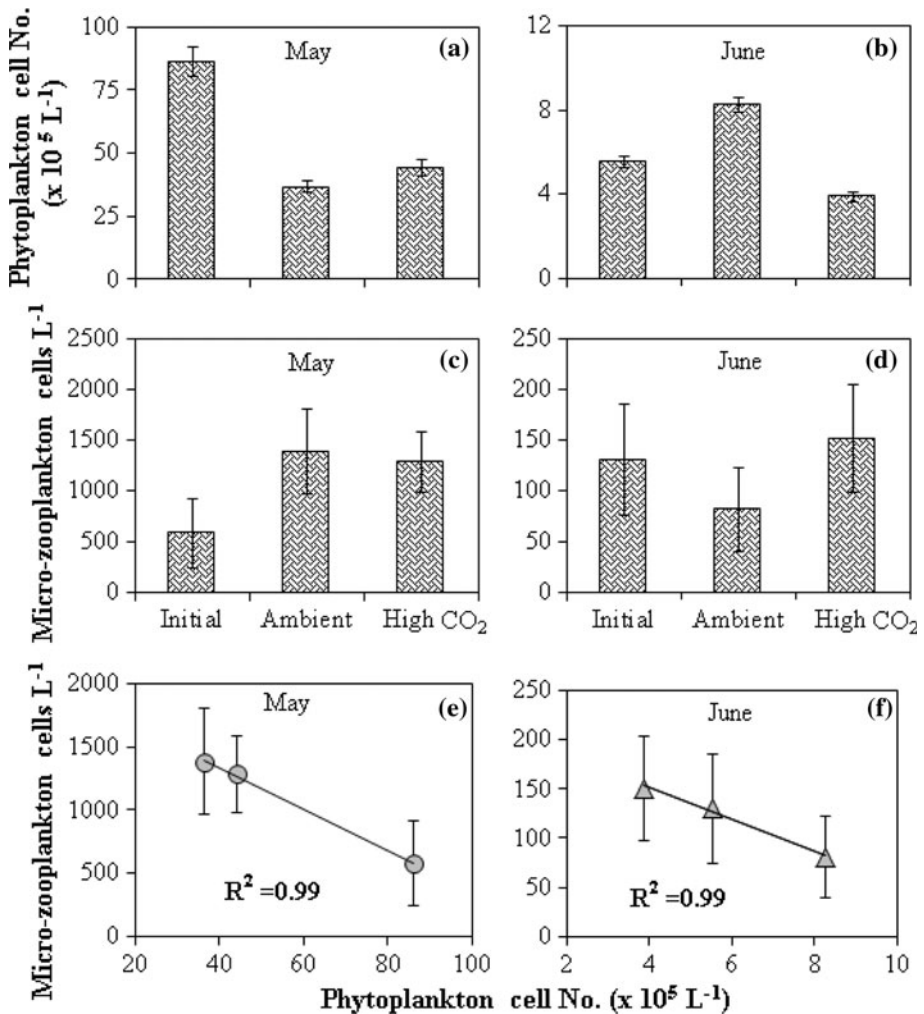


Fig. 3 Phytoplankton cell number in **a** May and **b** June; microzooplankton abundance during the experimental period **c** May and **d** June (*n* = 3–6, ±SD); correlations between phytoplankton and microzooplankton abundance during **e** May and **f** June

CO₂ treatment (Fig. 3a, b). Similarly, the average MZP abundance also showed reduced value in June than in May (Fig. 3c, d). In May the final count was 2.4 times (ambient condition) and 2.2 times (high CO₂) 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 CO₂ treatment (151 ± 53 cells l⁻¹). Phytoplankton and MZP abundance in both experiments were found to be negatively correlated with significant R^2 value (0.99) (Fig. 3e, f).

Phytoplankton community was mainly dominated by smaller diatoms. Few dinoflagellates 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 CO₂ treatment) samples in both experiments (Fig. 4a–f). 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 *S. costatum* decreased to 21.79% with three times enhancement of *T. decipiens* population (63.6%) (Fig. 4b), but *A. japonica* (2.44%) and *M. varians* (6.85%) did not show much change. The relative abundance of *N. closterium* got reduced to 0.26% from an initial value of 1.75%. In the high CO₂ treatment *S. costatum* and *T. decipiens* contributed almost equally (41–43%) whereas *Chaetoceros* sp. and *A. 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 *Chaetoceros* contributed 52% followed by 18% *N. closterium*, 12% *S. costatum* and 9.8% *T. 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 *T. 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). *A. 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 MZP 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, dinoflagellates, etc. (Fig. 4g, j). 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, h, k, l). But surprisingly, in the high CO₂ treatment, tintinnid became the major population contributing 70 and 85% of the MZP community in May (Fig. 4i) and June (Fig. 4l), respectively. In May, the average relative abundance of tintinnid was $68.65 \pm 5.63\%$ in the high CO₂ treatment which was almost 1.92 times higher than in the ambient condition (Student's *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 $79 \pm 5.36\%$ which was 8% less than the value observed in the high CO₂ condition $P < 0.05$.

When the relative abundance (initial, final ambient and final high CO₂) of tintinnid and five major contributing phytoplankton species (*T. decipiens*, *Chaetoceros* sp., *S. costatum*, *N. closterium*, *A. japonica*) were correlated, interesting correlations were obtained (Fig. 5).

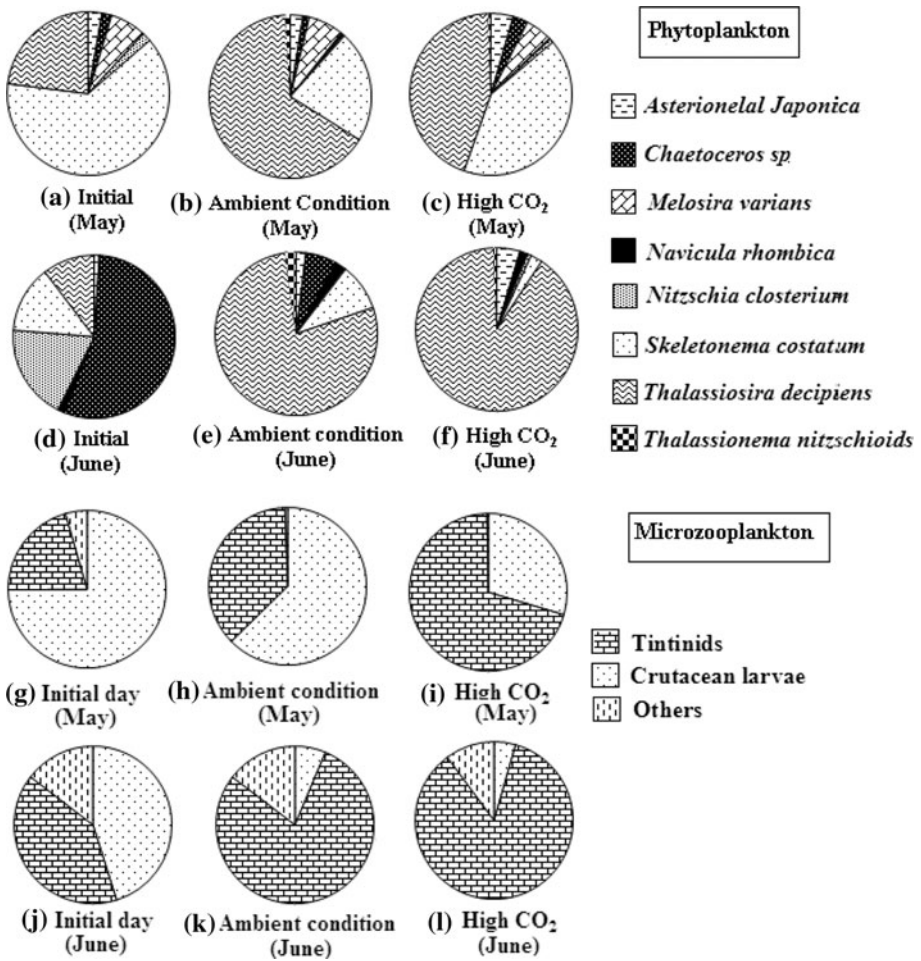


Fig. 4 Phytoplankton relative abundance (examined by microscopy) in the initial **a** May and **d** June; in the ambient condition **b** May and **e** June; in the high CO₂ treatments **c** May and **f** June; microzooplankton relative abundance (examined by microscopy) in the initial **g** May and **j** June; in the ambient condition **h** May and **k** June; in the high CO₂ treatments **i** May and **l** June

Significant negative correlations were observed between the relative abundance of *S. costatum* ($r^2 = 0.99$ in May, 0.66 in June), *N. 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, *T. decipiens* ($r^2 = 0.64$ in May and 0.63 in June) and *A. 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 μm). The average diameter of

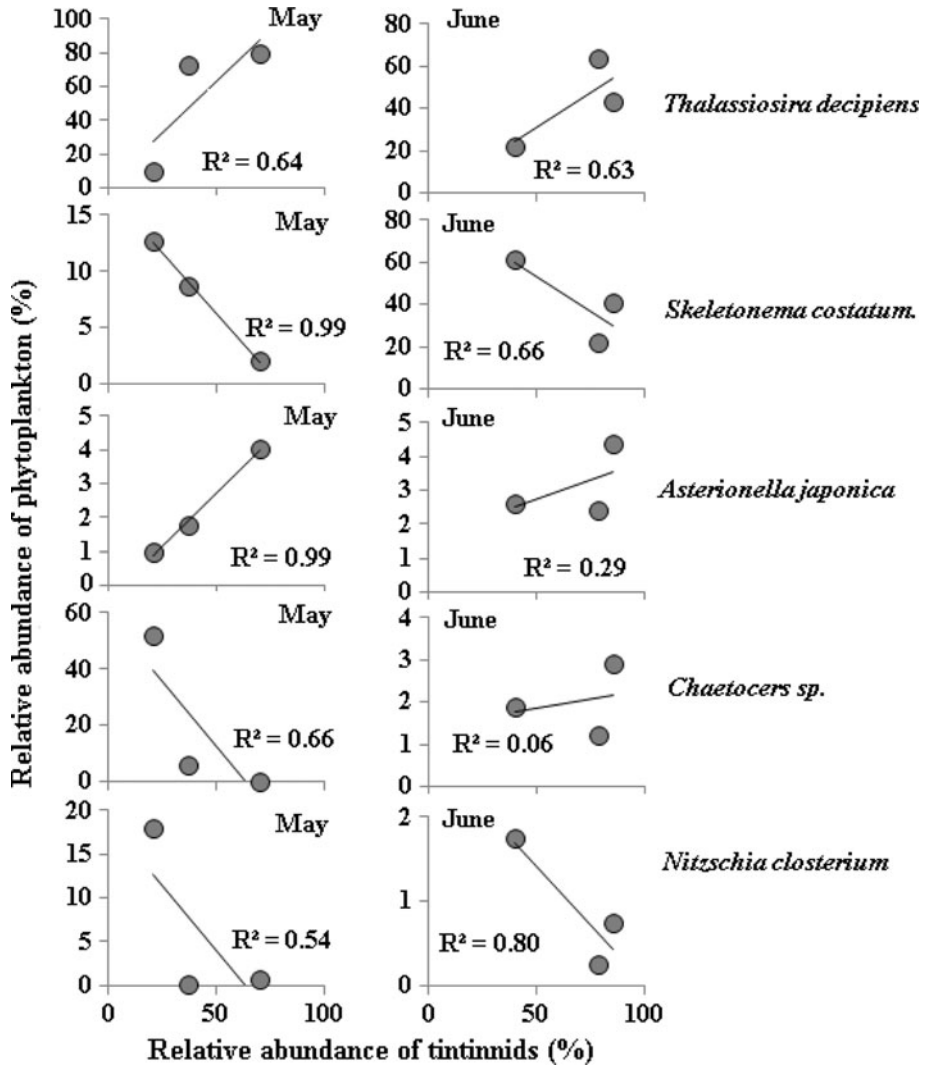


Fig. 5 Correlations between relative abundance of major contributing phytoplankton species and tintinnids during May and June. The values from the initial and final (ambient and high CO₂) samples have been considered here

the major phytoplankton genera were also measured and were found to be 5.36–6.88 μm of *N. closterium*, 2.8–23.77 μm of *S. costatum*, 6.06–29.34 μm of *Chaetoceros sp.* Whereas, the cell diameter of *A. japonica* (28.45–75.31 μm) and *T. decipiens* (9.8–40.1 μm) 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 (data 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 MZP community in the Bay of Bengal have been observed by many authors (Jyothibabu et al. 2006b, 2008; Gauns et al. 2005; Godhantaraman 2001) which is consistent with our observation. But the relative contribution of different groups of MZP may vary seasonally. Gauns et al. (2005) reported the dominance of dinoflagellates 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 dinoflagellate 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 MZP community with very few other groups like mixotrophic dinoflagellates (*Peridinium* sp., *Proocentrum* sp.), planktonic foraminifera, micro-gastropod, etc. The present study shows that in the coastal water of Bay of Bengal, MZP contributes a considerable part of phytoplankton grazing during the pre-summer monsoon. The observed significant negative correlations between phytoplankton and MZP cell abundance (Fig. 3e, f) clearly indicate a strong grazing pressure on the phytoplankton community exerted by MZP. 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 CO₂ treatment reflects the availability of more food material which could be small phytoplankton (nanoplankton <20 µm), 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 MZP 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 MZP 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 et al. 1987), nutritional value (Stoecker et al. 1995; Buskey 1997) and prey body size (Zhang et al. 2005). Available literature showed that the tropical tintinnid mostly feed on smaller size phytoplankton (<30 µm) (Godhantaraman 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 *S. costatum* and *N. 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 *T. 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 *S. 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 *N. closterium* < *Chaetoceros* sp. < *S. costatum* < *T. decipiens* < *A. 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). *T. decipiens* and *A. japonica* were found to be larger than the first three species and might not be preferred by the tintinnids. *A. 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 *A. 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 MZP 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 *N. closterium*, *Chaetoceros subtilis* and *S. 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 MZP 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 MZP community structure and on the other hand MZP 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 CO₂ the small diatom growth was enhanced, which influenced the MZP 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 CO₂ also can be used as a potential food sources for them. However, increased heterotrophic bacterial abundance under elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ level during a CO₂ enrichment experiment and which could be due to increased DOC production.

There are plenty of examples of MZP grazing from different parts of the global oceans. North Atlantic spring bloom is one of the biggest phytoplankton blooms in the global ocean where MZP (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 MZP 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 MZP 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 MZP 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 MZP 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 MZP 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 MZP 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 MZP behavior under elevated CO₂ levels. The author explained that the increasing CO₂ might not directly exert any change in MZP community. But high CO₂ and temperature which induced changes in the phytoplankton community during the experimental phase mainly seemed to influence the MZP 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 MZP 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 MZP also could respond in a different way which needs further investigation.

Implications

The present study shows that under the projected CO₂ level MZP (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 MZP mediated energy transfer in the marine environment. Moreover, enhanced MZP abundance in the marine environment could reduce the carbon flow in the water column. Since MZP serve as a food source for the larvae of many vertebrate and invertebrate species (Fenchel 1987) including commercially important fish larvae, enhanced population of MZP 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.

Acknowledgments This study was funded by the project SIP-1308 (CSIR funding). We acknowledge the financial support from the Indian Academy of Science to one of the co-authors Dr. Subhadra Devi Gadi for

conducting the study. We would like to express our sincere gratitude to the director NIO and the scientist-in-charge (RC, Visakhapatnam) for financial and moral support. We are thankful to Mr. Praven Kumar and V. Rajendra Prasad for analyzing POC/PON samples and dissolved organic carbon. We are thankful to all of our colleagues and students for their active cooperation during this study. The NIO contribution number is 5087.

References

- Balasubramanian T, Murugesan P, Vijayalakshmi S et al (2009) Marine Plankton-A field guide, Campus program. Ministry of Earth Sciences, Government of India, pp 43–104
- Barcelos e Ramos J, Biswas H, Schulz KG, LaRoche J, Riebesell U (2007) Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*. *Global Biogeochem Cycles* 21:GB2028. doi:10.1029/2006GB002898
- Biswas H, Cros A, Yadav K, Venkata Ramana V, Prasad VR, Acharyya T (2011) The response of a natural phytoplankton community from the Godavari River Estuary to increasing CO₂ concentration during the pre-monsoon period. *J Exp Mar Biol Ecol* 407:284–293
- Boettjer D, Morales (2005) Microzooplankton grazing in a coastal embayment off Concepcion, Chile (36°S) during non-upwelling conditions. *J Plankton Res* 27(4):383–391
- Boyd PW, Strzepak R, Fu F, Hutchins DA (2010) Environmental ambient condition of open-ocean phytoplankton groups: now and in the future. *Limnol Oceanogr* 55(3):1353–1376
- Burkill PH, Mantoura RFC, Llewellyn CA, Owens NJP (1987) Microzooplankton grazing and selectivity of phytoplankton in coastal waters. *Mar Biol* 93:581–590
- Buskey EJ (1997) Behavioral components of feeding selectivity of the heterotrophic dinoflagellate *Protoperidinium pellucidum*. *Mar Ecol Prog Ser* 153:77–89
- Calbet A, Landry M (2004) Phytoplankton growth, microzooplankton grazing and carbon cycling in marine systems. *Limnol Oceanogr* 49(1):51–57
- Capriulo GM, Sherr EB, Sherr BF (1991) Trophic behaviour and related community feeding activities of heterotrophic marine protists. In: Reid PC, Turley CM, Burkill PH (eds) *Protozoa and their role in marine processes*. NATO ASI series, vol 25. Springer, Berlin, pp 219–265
- Caron DA, Goldman JC (1990) Protozoan nutrient regeneration. In: Capriulo GM (ed) *Ecology of marine protozoa*. Oxford University Press, New York, pp 283–306
- Dickson AG (2003) Reference materials for oceanic CO₂ analysis: a method for the certification of total alkalinity. *Mar Chem* 80:185–385
- Dolan JR (1997) Phosphorus and ammonia excretion by planktonic protists. *Mar Geol* 139:109–122
- Dolan J, Claustre H, Carlotti F, Plounevez S, Moutin T (2002) Microzooplankton diversity: relationships of tintinnid ciliates with resources, competitors and predators from the Atlantic Coast of Morocco to the Eastern Mediterranean. *Deep Sea Res I* 49:1217–1232
- Doney SC, Farby VJ, Feely RA, Kleypas A (2009) Ocean acidification: the other CO₂ problem. *Annu Rev Mar Sci* 1:169–192
- Egge JK, Thingstad TF, Engel A, Riebesell U (2009) Primary production during nutrient-induced blooms at elevated CO₂ concentrations. *Biogeosci Discuss* 4:4385–4410
- Engel A, Delille B, Jacquet S et al (2004) Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO₂ concentrations: a mesocosm experiment. *Aquat Microb Ecol* 34:93–104
- Eppley RW (1972) Temperature and phytoplankton growth in the sea. *Fish Bull* 70:1063–1085
- Fenchel T (1987) *Ecology of protozoa. The biology of free-living phagotrophic protists*. Springer-Verlag, Berlin
- Feng Y, Warner ME, Shang Y et al (2008) Interactive effects of increased pCO₂, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). *Eur J Phycol* 43:87–98
- Feng Y, Hare CE, Leblance K (2009) Effects of increased pCO₂ and temperature on the North Atlantic spring bloom: I. The phytoplankton community and biogeochemical cycles. *Mar Ecol Prog Ser* 388:13–25
- Ganapati PN, Subba Rao DV (1958) Quantitative study of plankton off Lawson's Bay, Waltair. *Proc Indian Acad Sci* 48:189–210
- Gatham IJ, Rhee GY (1981) Comparative kinetic studies of nitrate limited growth and nitrate uptake in phytoplankton in continuous culture. *J Phycol* 17:309–314
- Gauns M, Madhupratap M, Ramaiah N et al (2005) A comparative accounts of biological productivity characteristics and estimates of carbon fluxes in the Arabian Sea and Bay of Bengal. *Deep Sea Res II* 52:2003–2017

- Gifford DJ, Fessenden LM, Garrahan PR, Martin E (1995) Grazing by microzooplankton and mesozooplankton in the high latitude North Atlantic Ocean: spring versus summer dynamics. *J Geophys Res* 100:6665–6675
- Godhantaraman N (1994) Species composition and abundance of tintinnid and copepods in the Pichavaram mangroves (South India). *Cienc Mar* 20:371–391
- Godhantaraman N (2001) Seasonal variations in taxonomic composition, abundance and food web relationship of microzooplankton in estuarine and mangrove waters, Parangipettai region, Southeast coast of India. *Indian J Mar Sci* 30:151–160
- Godhantaraman N, Krishnamurthy K (1997) Experimental studies on food habits of tropical microzooplankton (prey-predator relationship). *Indian J Mar Sci* 26:345–349
- Godhantaraman N, Uye S (2001) Geographical variations in abundance, biomass and trophodynamic role of microzooplankton across an inshore-offshore gradient in the Inland Sea of Japan and adjacent Pacific Ocean. *Plankton Biol Ecol* 48(1):19–27
- Goldman JC, Caron DA, Dennett MR (1987) Nutrient cycling in a microflagellate food web chain: IV phytoplankton-microflagellate interactions. *Mar Ecol Prog Ser* 38:75–87
- Gomes HR, Goes J, Saino T (2000) Influence of physical processes and freshwater discharge on the seasonality of phytoplankton regime in the Bay of Bengal. *Cont Shelf Res* 20(3):313–330
- Hare CE, Leblanc K, DiTullio GR et al (2007) Consequences of increased temperature and CO₂ for phytoplankton community structure in the Bering Sea. *Mar Ecol Prog Ser* 352:9–16
- Hasle GR, Syversten EE (1997) Marine diatoms. In: Tomas CR (ed) *Identifying marine phytoplankton*. Academic Press, San Diego, pp 5–385
- Hein M, Sand-Jensen K (1997) CO₂ increases oceanic primary production. *Nature* 388:526–527
- Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* 33:1225–1228
- IPCC (2007) In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge
- Jyothibabu R, Madhu NV, Jayalakshmi KV, Balachandran KK, Shiyas CA, Martin GD, Nair KKC (2006a) Impact of fresh water influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters-India). *Estuar Coast Shelf Sci* 69:505–518
- Jyothibabu R, Madhu NV, Maheswaran PA et al (2006b) Environmentally-related of symbiotic associations of heterotrophic dinoflagellates with cyanobacteria in the Bay of Bengal. *Symbiosis* 42:51–58
- Jyothibabu R, Madhu NV, Maheswaran KV et al (2008) Seasonal variation of microzooplankton (20–200 μm) and its possible implications on the vertical carbon flux in the western Bay of Bengal. *Cont Shelf Res* 28(6):737–755
- Karayanni H, Chrustaki, Van Wambeke F, Denis M, Moutin T (2005) Influence of ciliated protozoa and heterotrophic nanoflagellates on the fate of primary production in the northeast Atlantic Ocean. *J Geophys Res* 110:C07S15. doi:10.1029/2004JC002602
- Kim JM, Lee KS, Kang K (2006) The effect of seawater CO₂ concentration on growth of a natural phytoplankton assemblage in a ambient controlled mesocosm experiment. *Limnol Oceanogr* 51:1629–1636
- Kumar S, Ramesh R, Sheshshayee MS, Sardesai S, Patel PP (2005) Signature of terrestrial influence on nitrogen isotopic composition of suspended particulate matter in the Bay of Bengal. *Curr Sci* 88(5):770–774
- Kumar SP, Narveka J, Nuncio M, Kuma A, Ramaiah N, Sardesai S, Gauns M, Veronica Fernandes V, Paul J (2010) Is the biological productivity in the Bay of Bengal light limited. *Curr Sci* 98(10):1331–1339
- Landry MR, Calbet A (2004) Microzooplankton production in the oceans. *ICES J Mar Sci* 61:501–507. doi:10.1016/j.icesjms.2004.03.011
- Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. *Mar Biol* 67:283–288
- Landry MR, Monge BC, Selph KE (1993) Time-dependency of microzooplankton grazing and phytoplankton growth in the Subarctic Pacific. *Prog Oceanogr* 32:239–258
- Landry MR, Barber RT, Bidigare RR et al (1997) Iron and grazing constraints on primary production in the central equatorial Pacific: an EqPac synthesis. *Limnol Oceanogr* 42:405–418
- Lewis E, Wallace DWR (1998) CO₂SYN₂_calc_DOS_Original: 1998. Program developed for CO₂ system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge
- Liu KK, Kaplan IR (1989) The eastern tropical Pacific as a source of ¹⁵N-enriched nitrate in seawater off southern California. *Limnol Oceanogr* 34:820–830

- Liu H, Suzuki K, Saino T (2002) Phytoplankton growth and microzooplankton grazing in the Subarctic Pacific Ocean and the Bering Sea during summer 1999. *Deep Sea Res I* 49:363–375
- Lopez-Sandoval DC, Emiliomaran NI et al (2010) Particulate and dissolved primary production by contrasting phytoplankton assemblages during mesocosm experiments in the Ría de Vigo (NW Spain). *J Plankton Res* 32(9):1231–1240
- Madhu NV, Jyothibabu R, Maheswaran PA, Gerson VJ, Gopalakrishnan TC, Nair KKC (2006) Lack of seasonality in phytoplankton standing stock (chlorophyll a) and production in the western Bay of Bengal. *Cont Shelf Res* 26:1868–1883
- McManus GB, Costas BA, Dam HG et al (2007) Microzooplankton grazing of phytoplankton in a tropical upwelling region. *Hydrobiology* 575(1):69–81
- Monger BC, Landry MR, Brown SL (1999) Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. *Limnol Oceanogr* 44:1917–1927
- Nagata T (2000) Production mechanisms of dissolved organic matter. In: Kirchman DL (ed) *Microbial ecology of the oceans*. Wiley-Liss, New York, pp 121–152
- Newell GE, Newell RC (1973) *Marine Plankton: A practical guide* (Hutchinson biological monographs), Hutchinson Educational, London, 244 pp
- Paul TJ, Ramaiah N, Gauns M, Fernandes V (2007) Predominance of a few diatom species among the highly diverse microphytoplankton assemblages in the Bay of Bengal. *Mar Biol* 152(1):63–75
- Qasim SZ (1977) Biological productivity of the Indian Ocean. *Indian J Mar Sci* 6:122–137
- Quevedo M, Anadón R (2001) Protist control of phytoplankton growth in the subtropical North-east Atlantic. *Mar Ecol Prog Ser* 221:29–38
- Radhakrishna K (1975) Primary productivity of the Bay of Bengal during March April. *Indian J Mar Sci* 1:58–60
- Rassoulzadegan F, Etienne M (1981) Grazing rate of the tintinnid *Stenosemella ventricosa* (Clap and Lachm) Jorg. on the spectrum of naturally occurring particulate matter from a Mediterranean neritic area. *Limnol Oceanogr* 26:258–270
- Richoux NB, Froneman PW (2009) Plankton trophodynamics at the subtropical convergence, Southern Oceans. *J Plankton Res* 31(9):1059–1073
- Riebesell U (2004) Effects of CO₂ enrichment on marine phytoplankton. *J Oceanogr* 60:719–729
- Riebesell U, Fabry VJ, Hansson L, Gattuso JP (eds) (2010) *Guide to best practices for ocean acidification research and data reporting*. Publications Office of the European Union, Luxembourg
- Rose JM, Feng Y, Globler CJ et al (2009) Effects of increased pCO₂ and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. *Mar Ecol* 388:27–40
- Sanders RW, Caron DA, Berninger UG (1992) Relationship between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter ecosystem comparison. *Mar Ecol Prog Ser* 86:1–14
- Sharp JH (1975) Improved analysis for particulate organic carbon and nitrogen from seawater. *Limnol Oceanogr* 19:984–989
- Sherr BF, Sherr EB (1994) Bacteriivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microb Ecol* 28:223–235
- Splitter P (1973) Feeding experiments with tintinnids. *Oikos* 15:128–132
- Stelfox-Widdicombe CE, Edward ES, Burkill PH, Sleigh MA (2000) Microzooplankton grazing activity in the temperate and subtropical NE Atlantic: summer 1996. *Mar Ecol Prog Ser* 208:1–12
- Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. *J Plankton Res* 12:891–908
- Stoecker DK, Gallager SM, Langdon CJ, Davis LH (1995) Particle capture by *Favella* sp. (Ciliata, Tintinnina). *J Plankton Res* 17:1105–1124
- Strickland JDH, Parsons TR (1972) *A practical handbook of seawater analysis*, 2nd edn. Bulletin 167. Fisheries Research Board of Canada, Ottawa
- Strom S (2002) Novel interactions between phytoplankton and microzooplankton: their influence on the coupling between growth and grazing rates in the sea. *Hydrobiology* 480:41–54
- Strom SL, Benner R, Ziegler S, Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol Oceanogr* 42:1364–1374
- Strom SL, Brainard MA, Holmes JL, Olson MB (2001) Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. *Mar Biol* 38:355–368
- Suffrian K, Simonelli P, Nejstgaard JC et al (2008) Microzooplankton grazing and phytoplankton growth in marine ecosystems with increased CO₂ levels. *Biogeosci Discuss* 5:411–433
- Sun J, Feng Y, Zhang Y, Hutchins DA (2007) Fast microzooplankton grazing on fast-growing, low-biomass phytoplankton: a case study in spring in Chesapeake Bay, Delaware Inland Bays and Delaware Bay. *Hydrobiology* 589:127–139

- Sykes JB (1981) An illustrated guide to the diatoms of British coastal plankton. Field Study Council, Shrewsbury (journal offprint)
- Tortell PD, DiTullio GR, Sigman DM, Morel FM (2002) CO₂ effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. *Mar Ecol Prog Ser* 236:37–43
- Tortell PD, Payne CD, Li Y (2008) CO₂ sensitivity of Southern Ocean phytoplankton. *Geophys Res Lett* 35:L0460. doi:[10.1029/2007GL032583](https://doi.org/10.1029/2007GL032583)
- Wolf-Gladrow D, Riebesell U, Burkhardt S, Bijma J (1999) Direct effects of CO₂ concentration on growth and isotopic composition of marine plankton. *Tellus Ser B* 51:461–476
- Yoshimura T, Nishioka J, Suzuki K et al (2009) Impacts of elevated CO₂ on phytoplankton community composition and organic carbon dynamics in nutrient-depleted Okhotsk Sea surface waters. *Biogeosci Discuss* 6:4143–4163
- Zhang LY, Sun J, Liu DY, Yu ZS (2005) Studies on growth rate and grazing mortality rate by microzooplankton of size-fractionated phytoplankton in spring and summer in the Jiaozhou Bay, China. *Acta Oceanol Sin* 24:85–101