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Abundance of thraustochytrids and bacteria in the equatorial Indian Ocean, with relation to Transparent Exopolymeric Particles (TEPs)

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Abstract

Thraustochytrid protists are often abundant in coastal waters. However, their population dynamics and substrate preferences in the oceanic water column are poorly known. We studied the abundance and distribution of thraustochytrids, bacteria and transparent exopolymeric particles (TEPs) in the equatorial Indian Ocean waters during September 2003, October 2004 and September 2006. Thraustochytrids and bacteria were abundant, suggesting high biological productivity of the region. Thraustochytrids were positively related to bacteria during October 2004 but not at other times, suggesting overlapping or varying substrate preferences at different times. Thraustochytrid and bacteria were positively related to TEPs only in a few stations during October 2004, but were mostly positively related to TEPs generated from *in situ* water in a roller table experiment. TEPs from natural samples during October 2004 had a much greater affinity to the lectin Concanavalin A than to Limulin compared to those in September 2006 and from the roller tank experiments. Chemical composition of TEPs might explain their relationship to thraustochytrids. Thraustochytrids averaged higher biomass than bacteria in two of the three cruises, but were less frequent and more patchily distributed compared to bacteria.

Introduction

Thraustochytrids, the single-celled, marine stramenopilan protists, are widespread in the sea. Since they are similar to bacteria in their osmoheterotrophic mode of nutrition (Porter, 1990; Raghukumar, 2002), they are likely to play a role in remineralization of particulate and dissolved organic matter, as bacteria do. Within the context of this larger role, however, it might be expected that these heterotrophic protists occupy a distinct ecological niche in the sea, in order to avoid competition from the ubiquitous bacteria. Comparative studies on the relative abundance and biomass of bacteria and thraustochytrids in different habitats might throw light on this problem. Only a few studies, namely, those of Raghukumar & Schaumann (1993) in the North Sea, Naganuma *et al.* (1998) and Kimura *et al.* (1999, 2001) in the Seto Inland Sea, Japan, Raghukumar *et al.* (2001) in the Arabian Sea and Bongiorno *et al.* (2005) in the Mediterranean have dealt with the abundance and biomass of these organisms in marine habitats. These studies have demonstrated that (1) thraustochytrids in coastal waters or the land-locked Arabian Sea often attain densities of a few hundred thousand cells per liter seawater and (2) thraustochytrids may occasionally attain biomass values up to 50 % of the bacteria. Kimura *et al.* (1999, 2001) postulated that thraustochytrids biomass was related to riverine inputs of organic material. Therefore, it is not clear if these protists are prevalent also in oceanic waters and if so, what their relationships to bacteria are. One of the potential habitats of thraustochytrids in the water column are the Transparent Exopolymeric Particles (TEPs). TEPs are fibrillar mucopolysaccharides formed through coagulation of the increasingly refractory dissolved organic matter left behind after the action of heterotrophic bacterial processes on the biologically labile organic carbon of dissolved polysaccharide exudates released by phytoplankton and bacteria (Alldredge *et al.*, 1993; Beauvais *et al.*, 2003). They act as the sticky matrix for 'marine snow' or 'marine aggregates', comprising detrital and inorganic particles, faecal pellets and cadavers of zooplankton and microorganisms (Simon *et al.*, 1990, Passow & Alldredge, 1994).

The aim of this study was to examine the relative abundance and distribution of thraustochytrids with relation to bacteria and TEPs in open oceanic waters. We studied these aspects in the waters of the equatorial Indian Ocean along two parallel tracts during two cruises at the end of the southwest monsoon in September and transition period of the northeast monsoon in October. Only bacteria and thraustochytrids were studied in yet another cruise. The equatorial Indian Ocean comprises the southern limit of one of the three major circulation systems of the Indian Ocean, namely the seasonally changing monsoon gyre (Wyrski, 1973a & b; Reddy, 2001). This region has received little attention with regard to both bacterial and thraustochytrid dynamics, although several papers have examined bacterial dynamics in the adjacent northern Indian Ocean, namely the Arabian Sea (Ducklow, 1993; Ramaiah *et al.*, 1996; Wiebinga *et al.*, 1997; Pomeroy & Joint, 1999).

Materials and Methods

Sampling stations

This study was carried out in the equatorial Indian Ocean during three cruises on board *ORV Sagar Kanya* during September 2003 (Cruise # SK 196), October 2004 (Cruise # SK 212) and September 2006 (Cruise # SK 228) (Fig.1). Altogether 9 stations between 2° N and 2° S were studied for each cruise, at a longitude of 77° E during September 2003 and 80.5° E during October 2004 and September 2006.

Sample collection

Water samples were collected with clean 5 L or 10 L Niskin bottles attached to a Sea Bird CTD rosette sampler from surface, 10 m, 20 m, 40 m, 60 m, 80 m, 100 m, 120 m, and 200 m in all the 3 cruises (a total of 9 depths). Two additional depths of 500 m and 1000 m were sampled during October 2004 and September 2006 (a total of 11 depths).

Estimation of bacterial and thraustochyrid abundance

Water samples collected during both the cruises were preserved with formaldehyde at a final concentration of 2 % and stored at 5° C. Ten mL of water was filtered over 25 mm, 0.22 µm black polycarbonate Isopore membrane filters (Millipore, USA) and stained for bacteria by the acridine orange direct count (AODC) method (Parsons *et al.*, 1984). The filters were mounted on microscope slides in non-fluorescent immersion oil (Olympus Optical Co. Ltd) and observed under blue excitation light using BX60 Olympus epifluorescence microscope equipped with 100 W Hg lamp. Ten different fields were observed for counting.

Thraustochytrids were enumerated by filtering 25 mL of water over 0.4 µm black polycarbonate Isopore membrane filter (Millipore, USA). The filters were then stained according to the acriflavine direct detection (AfDD) technique (Raghukumar & Schaumann, 1993). Thraustochytrid cells were counted from 100 different fields under blue excitation filter and counterchecked for photosynthetic picoplankton under green filter.

Determination of biomass

Kimura *et al.* (1999) and Raghukumar *et al.* (2001) used a value of 20.6 pg of C per thraustochytrid cell, considering an average cell diameter of 5.0 µm. The average estimated size from 100 counts in our study corresponded to 4.7 µm. Therefore, our biomass estimations were also based on an average cell diameter of 5.0 µm as above. A factor of 20.0 fg of C per average cell from oceanic waters was used for bacteria (Ducklow, 2000).

Determination of TEPs concentration

Transparent Exopolymeric Particles (TEPs) were estimated in 2 of the 3 cruises (October 2004 and September 2006), following the method of Passow & Alldredge (1995). Ten to twenty five mL of water samples were filtered through 0.4 µm polycarbonate filter papers and stained with alcian blue (0.02 % wt/ vol in 0.06 %

acetic acid, pH 2.5). The filter papers were then transferred into beakers and soaked in 80 % sulphuric acid for 2 hours. The absorbance of the solution was read at 787 nm against distilled water as reference. Concentration of TEPs was determined in duplicates or triplicates for all the samples and were calculated using the formula described by Passow & Alldredge (1995) and expressed as milligram equivalent of alginic acid (AA) per liter [mg eq AA L^{-1}] (Ramaiah *et al.*, 2000).

The relationship of bacteria, thraustochytrids and TEPs to each other during the cruises were analyzed using correlation matrix (Statistica 5.0).

Roller table experiment to study TEPs, bacteria and thraustochytrids

Surface seawater collected at 2° N 80.5° E during cruise # SK 228 in September 2006 was used to generate TEPs in a roller table based on the design of Shanks & Edmondson (1989). The water was filtered through a 200 μm mesh to remove mesozooplankton. Four bottles of 1 L each were filled with the water and incubated for 2 weeks at 24 rpm in the laboratory on board under diffuse light and at a temperature of 25 - 27°C. The bottles were divided into two sets of two each. Samples from one set were drawn on Day 0, 5 and 9 and from the other on days 3, 7 and 14. A total of 50 mL sample was drawn each time. The volume of the bottles was maintained constant by replacing the sample amount of 50 mL with 0.22 μm filtered seawater collected from the same station. The samples were fixed with formalin at a final concentration of 2 % and kept under refrigeration till enumeration of TEPs, bacteria and thraustochytrids was done in the laboratory on land. Samples were analyzed in duplicates.

Chemistry of the TEPs using lectins

Two lectins were used to examine the presence of different sugars in TEPs in water samples from representative stations, as well as the water sample in the roller table experiment. These were FITC-labelled Concanavalin A that binds to D-mannose, D-glucose and fructofuranose residues and FITC-labelled Limulin that binds to N-acetylneuraminic acid, glucuronic acid and phosphorycholine analogs. The lectins

were obtained from Sigma- Aldrich Chemicals Pvt. Ltd. A total 1 mL of water sample was taken in 2 mL Eppendorf tubes and 50 μl of Concanavalin A at a concentration of 2.5 mg mL^{-1} or 25 μL of Limulin at a concentration of 1 mg mL^{-1} was added separately and the samples were incubated for 30 minutes. They were then filtered over 0.22 μm black polycarbonate Isopore membrane filters and examined using an epifluorescence microscope at 450-490 nm (blue excitation filter). A total of 100 microscope fields were examined and the number of samples positive for each lectin were calculated.

Results

The isothermal mixed layer during September 2003 was present up to 40 m depth at the equator and north, becoming shallower (20 m) towards the south. The thermocline was deeper at all the locations during October 2004 and September 2006 (60 – 80 m during October 2004 and 45- 65 m during September 2006). A subsurface salinity maximum at a depth of 40 to 80 m was observed in the 3 cruises.

Distribution of thraustochytrids, bacteria and TEPs

Results on thraustochytrid and bacterial densities and concentration of TEPs for 6 out of 9 stations sampled during the cruises in October 2004 and September 2006 are presented in Figs. 2 and 4. Data for all 9 stations were included for the correlation analyses presented in Tables 1 and 2 and Figs. 3, 5 and 6. During October 2004, both thraustochytrids and bacteria showed a prominent peak of abundance both in the mixed layer between 0 – 40 m southwards of equator and below the mixed layer between 100 - 120 m northwards of equator. Thraustochytrids in the mixed layer up to 40 m ranged from below detection levels to $674.6 \times 10^3 \text{ cells L}^{-1}$ (Fig. 2), corresponding to a maximum biomass of $13.9 \mu\text{g C L}^{-1}$. Bacterial densities ranged from 1.84 to $759.0 \times 10^6 \text{ L}^{-1}$, corresponding to biomass values of 0.04 to $15.2 \mu\text{g C L}^{-1}$. The two groups attained maximum densities and biomass at 120 m at 1°N , corresponding to a thraustochytrid biomass of $15.8 \mu\text{g C L}^{-1}$ and bacterial biomass of $16.1 \mu\text{g C L}^{-1}$. The maximum thraustochytrid biomass contribution to the total of bacterial and thraustochytrids C occurred at 0.5°S (10 m),

where thraustochytrids comprised 99.4 % of the total. Thraustochytrids at this station numbered 276×10^3 cells L^{-1} , bacteria amounting to a total of 1.84×10^6 L^{-1} . Numbers of both thraustochytrids and bacteria were generally less below 120 m, the former ranging from negligible to 73.6×10^3 cells L^{-1} and the latter from 19.32 to 103.73×10^6 cells L^{-1} . Thraustochytrids and bacteria generally showed very similar trends of distribution in the water column and were positively related in 5 of the 9 stations (Table 1). There was an overall, highly significant positive correlation between the two groups (Fig. 3). TEPs ranged from 5.3 to 451.1 mg equivalent alginic acid L^{-1} . Peak TEPs values varied between the stations, occurring in the mixed layer (40 – 60 m), at 100 – 120 m or at 200 m. Their distribution did not relate with either bacteria or thraustochytrids on an overall basis (Fig. 3). However, a significant positive correlation of the two groups with TEPs was noticed at 2 stations (at 1° N and 2° S; Fig. 2; Table 1). TEPs from 6 of the samples examined from this cruise showed varying response to Concanavalin A and Limulin, percentage response to the latter ranging from 12 to 75 % of the samples (Table 3). TEPs from 1° N and 2° S, which showed a positive relationship with thraustochytrids as well as bacteria generally seemed richer in n-acetylneuraminic acid (Limulin positive) than those from the equator, where distribution of TEPs appeared to have a negative trend relative to both bacteria and thraustochytrids. The distribution trend of TEPs at 1.5° N from 0 to 120 m was similar to that of thraustochytrids and bacteria, but displaced downwards by 20 m (Fig. 2). A similar observation was also made at 2° N, the data of which are not shown in figure.

Abundance of both thraustochytrids and bacteria was much less during September 2006 (Fig. 4). Thraustochytrids in the mixed layer up to 80 m ranged from below detection levels to 255.6×10^3 cells L^{-1} , while bacteria varied from 0.81 to 183.4×10^6 cells L^{-1} . Thus, thraustochytrids contributed up to $5.3 \mu g$ L^{-1} , while bacterial biomass amounted to 0.016 to $3.6 \mu g$ C L^{-1} . The maximum thraustochytrid biomass contribution to the total of bacterial and thraustochytrid C occurred at 0.5° S (200 m), where thraustochytrids comprised 93 % of the total. Thraustochytrids at this station numbered 76.7×10^3 cells L^{-1} , bacteria amounting to a total of 6.4×10^6 L^{-1} . The maximum density of thraustochytrids was found at 10 m at 1° N. Bacterial

numbers at this station were also some of the highest noticed. Both were also often abundant at 100 to 1000 m, thraustochytrids ranging from below detection levels to 76.7×10^3 cells L^{-1} and bacteria from 2.6 to 104.9×10^6 cells L^{-1} . Unlike during October 2004, thraustochytrids and bacteria showed dissimilar trends of distribution in the water column during September 2006 and no positive relations between their numbers were found in any of the 9 stations (Table 2). TEPs ranged from 7.5 to 339.3 mg equivalent alginic acid L^{-1} (Fig. 4). Maximum levels of TEPs were often observed below 200 m depth. Their distribution in the water column did not correspond either with that of the bacteria or thraustochytrids and no significant relations were noticed in any of the sampling stations, or on an overall basis (Table 2; Fig. 5). TEPs from the 4 samples examined from this cruise showed a much greater response to Concanavalin A (mannose) than to Limulin (n-acetylneuraminic acid), the response to the latter lectin being in the range of 9 to 39 % (Table 3). However, the distribution trend of TEPs in the water column closely corresponded with that of thraustochytrids at the equator and with that of bacteria at 1° S, but displaced downwards by 20 m. When the analyses were carried out at these stations after shifting the values of these organisms 20 m downwards, a statistically significant relation was noticed between TEPs on the one hand and thraustochytrids and bacteria on the other.

Thraustochytrids and bacteria, but not TEPs were estimated during the first cruise in September 2003 (SK 196). Thraustochytrids at this time ranged from below detection levels to 598×10^3 cells L^{-1} , contributing up to $12.3 \mu g L^{-1} C$, while bacteria ranged from 49 to 3533×10^6 cells L^{-1} , contributing a maximum of $67.5 \mu g L^{-1} C$. The maximum thraustochytrid biomass contribution to the total of bacterial and thraustochytrid C occurred at 60 m at 0.5° N, where thraustochytrids comprised 79.1 % of the total. Thraustochytrids at this station numbered 184×10^3 cells L^{-1} , bacteria amounting to a total of 50×10^6 L^{-1} . The two groups showed no correlations in terms of abundance (Fig. 6).

Roller table experiment to study TEPs, bacteria and thraustochytrids

Experimental results on the association of bacteria and thraustochytrids with TEPs using a roller table carried out on board during the cruise in September 2006 showed that TEPs increased steadily over time up to 12 days (Fig. 7). This was also accompanied by a steady increase of thraustochytrids, the maximum densities reaching up to 400×10^3 cells L^{-1} . These levels corresponded to those occurring in the natural water column. Bacteria showed a much more rapid increase in numbers up to 2 days, followed by a decline. Their numbers, however, increased substantially towards the end of the experiment when TEPs values were high, reaching values of about 200×10^6 cells L^{-1} , also similar to levels found in the water column. Thraustochytrids showed a positive trend with TEPs from day 5 onwards. TEPs from these experiments contained high amounts of mannose (Concanavalin positive), as well as n-acetylneuraminic acid (Limulin positive), the positive percentage response to Concanavalin A ranging from 32 to 67 and that to Limulin from 33 to 68 (Table 3).

Discussion

Kimura *et al.* (1999, 2001) suggested that thraustochytrids were associated with allochthonous particles in coastal waters, rather than phytoplankton- and bacterial-derived particulate organic carbon (POC). Raghukumar *et al.* (2001) showed that they were often related to chlorophyll 'a' and POC in the Arabian Sea, suggesting that thraustochytrids might be important in degradation of autochthonous oceanic material. This study unequivocally demonstrates the abundant presence of thraustochytrids in the oceanic waters of the equatorial Indian Ocean far removed from coastal influences, suggesting that they also play a role in the oceans, by utilizing autochthonous sources of nutrients.

Densities of thraustochytrids in the present study corresponded to earlier reports of up to a few hundred thousand cells per liter seawater (Naganuma *et al.*, 1998; Kimura *et al.*, 1999; Kimura *et al.*, 2001; Raghukumar *et al.*, 2001; Bongiorno *et al.*, 2005). Bacterial densities, likewise, corresponded to values reported for the

adjacent Arabian Sea (Ducklow, 1993; Ramaiah *et al.*, 1996; Wiebinga *et al.*, 1997; Pomeroy & Joint, 1999; Prasanna Kumar *et al.*, 2001). Such high densities suggest that the equatorial Indian Ocean may be a biologically productive region.

Each of the 3 cruises revealed different relations between bacteria and thraustochytrids (Fig. 8). (1) At one end of the spectrum, namely September 2006, thraustochytrids were detected only in 72 % of the samples and had the lowest average biomass value of the 3 cruises ($0.74 \mu\text{g C L}^{-1}$). This average biomass value was higher than that of the bacterial biomass of $0.53 \mu\text{g C L}^{-1}$. However, thraustochytrids showed a high variability in their populations and therefore, their biomass (SD 0.9; Fig. 8). Thraustochytrid and bacterial abundances were not related. (2) During September 2003, thraustochytrids were found in all the samples (100 % frequency of occurrence) and their average biomass in the water column was $2.5 \mu\text{g C L}^{-1}$. Bacterial biomass was nearly 10 times that of the thraustochytrids. Bacteria and thraustochytrids showed no relations between each other. (3) Thraustochytrids were found in 98 % of the samples during October 2004. Their average biomass was the highest of all 3 cruises ($3.8 \mu\text{g C L}^{-1}$) and again exceeded the average bacterial biomass of $3.4 \mu\text{g C L}^{-1}$, but with a high variability (SD 3.4; Fig. 8). Thraustochytrids and bacteria showed a highly significant positive relationship. The above results provide various clues and questions regarding thraustochytrids.

1) Thraustochytrids in the water column may occur in patches of very high density, as also reported by Raghukumar *et al.* (2001) for the Arabian Sea and Bongiorno *et al.* (2005) in fish farm impacted seagrass sediments. An average thraustochytrid cell of $5.0 \mu\text{m}$ diameter, $65.41 \mu\text{m}^3$ biovolume and 20 pg C (Kimura *et al.*, 1999; Raghukumar *et al.*, 2001) would correspond to 1000 bacterial cells, each of $0.5 \mu\text{m}$ diameter, $0.065 \mu\text{m}^3$ and 20 fg biomass C . Therefore, patches of thraustochytrids would constitute 'hot spots' of nutrition for microbivorous protists, particularly by providing the ω -3 fatty acid, docosahexaenoic acid (DHA), a key essential fatty acid in the growth and maturation of crustaceans (Veloza *et al.* 2006).

2) Kimura *et al.* (2001) reported a positive correlation between thraustochytrids and bacteria for waters of Seto Inland Sea of Japan. Bongiorno *et al.* (2005) also noticed a weakly positive correlation between bacterial and thraustochytrid abundance in fish farm sediments. However, the relationships between bacteria and thraustochytrids in the equatorial Indian Ocean waters was highly variable as found by Raghukumar *et al.* (2001) for the water column of the Arabian Sea. Therefore, thraustochytrids and bacteria may depend on the same or different nutrient sources in the water column.

What were the possible nutrient sources for thraustochytrids? The general patchiness of distribution of thraustochytrids in the water column, both in terms of their frequency of occurrence, as well as abundance, suggested that they were particle dependent. Raghukumar *et al.* (2001) had earlier noticed dense populations of thraustochytrids in association with diatom mucus particles in the water column at the end of the southwest monsoon in the Arabian Sea. Lyons *et al.* (2005) reported the dense presence of the thraustochytrid pathogen QPX in marine aggregates and suggested that aggregates might provide a means for survival and transport of the pathogen. Kimura *et al.* (2001) and Raghukumar *et al.* (2001) found that thraustochytrid abundance was significantly related to POC concentration in the water column. In view of the above, we were prompted to examine the relationship of thraustochytrids and bacteria with TEPs in the water column. We frequently observed the association of thraustochytrids with TEPs in the water column, these too staining with acriflavine used for direct detection of cell wall sulphated polysaccharides of thraustochytrids (Fig. 9). However, except for a few individual stations, thraustochytrids and TEPs did not show a positive relation. Therefore, we attempted to study them under experimental conditions using a roller tank. The results indicated a similar trend in changes among thraustochytrids, bacteria and TEPs, suggesting that these relationships are variable, probably depending upon the chemistry of TEPs.

Since TEPs may be formed as a result of bacterial activity on phytoplankton exudates (Sugimoto *et al.*, 2007), or even without bacterial intervention directly from

diatom exudates (Grossart *et al.*, 2006), the chemistry of TEPs may be important in understanding the relationship of thraustochytrids to them. TEPs are responsible for the formation of larger aggregates in the water column. We could not study the chemistry of the aggregates since none were detected in the natural water samples, probably owing to the sampling techniques. Nor were aggregates generated in the roller table experiment. Therefore, we examined the TEPs for a few sugars using lectins. Michael and Smith (1995) have used lectins to describe chemically complex carbohydrate moieties and heterogeneous structure of microbial biofilms on glass. Wigglesworth-Cooksey and Cooksey (2005) reported that extracellular polymeric substances (EPS) of diatoms stained with Concanavalin A and not with a lectin specific for fucose. Khandeparkar *et al.* (2003) noticed that EPS from bacteria stained positive with limulin for n-acetylneuraminic acid, glucuronic acid and phosphorycholine and not for n-acetyl-D-galactosamine, suggesting that the latter was more part of the bacterial capsular polysaccharides. We chose Concanavalin A and Limulin for studies based on the above. Our preliminary results suggest that thraustochytrids were generally positively related to TEPs concentrations that were relatively higher in n-acetylneuraminic acid (limulin positive) as in 2 stations during October 2004 and TEPs generated in the laboratory (Fig. 7). TEPs from the September 2006 cruise that were richer in mannose and glucose (Concanavalin positive) showed no definite relationship to thraustochytrids. Further studies along these lines might shed more light on thraustochytrids.

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Table 1. Results of correlation analyses ('r') values between abundance of thraustochytrids, bacteria and TEPs to each other during SK Cruise # 212 in October 2004. Significant values are given in bold.

Parameters	80.5° E Longitude								
	2°N	1.5°N	1°N	0.5°N	0°Eq.	0.5°S	1°S	1.5°S	2°S
Thraustochytrids vs. bacteria	0.17; p=0.63	0.70; p=0.02	0.9; p<0.001	0.54; p=0.11	0.82; p=0.01	0.28; p=0.4	0.92; p<0.001	0.13; p=0.75	0.8; p=0.003
Thraustochytrids vs. TEPs	-0.54; p=0.11	0.09; p=0.78	0.85 p<0.001	-0.11; p=0.75	-0.85; p=0.007	-0.27; p=0.42	-0.11; p=0.74	-0.2; p=0.6	0.67; p=0.02
Bacteria vs. TEPs	-0.14; p=0.69	0.29; p=0.42	0.93; p<0.001	-0.51; p=0.1	-0.75; p=0.03	-0.027; p=0.94	0.07; p=0.83	-0.19; p=0.63	0.73; p=0.01

Table 2. Results of correlation analyses ('r') values between abundance of thraustochytrids, bacteria and TEPs to each other during SK Cruise # 228 in September 2006.

Parameters	80.5° E Longitude								
	2°N	1.5°N	1°N	0.5°N	0°Eq.	0.5°S	1°S	1.5°S	2°S
Thraustochytrids vs. bacteria	0.19; p=0.58	0.5; p=0.15	0.56; p=0.07	-0.08; p=0.82	0.07; p=0.85	-0.27; p=0.42	0.09; p=0.80	0.41; p=0.22	-0.1; p=0.78
Thraustochytrids vs. TEPs	-0.22; p=0.52	-0.27; p=0.46	-0.09; p=0.79	-0.20; p=0.57	-0.31; p=0.35	-0.22; p=0.52	-0.57; p=0.09	-0.009; p=0.98	-0.23; p=0.5
Bacteria vs. TEPs	-0.2; p=0.57	-0.08; p=0.82	0.05; p=0.89	0.18; p=0.61	0.21; p=0.54	0.21; p=0.54	-0.51; p=0.13	0.59; p=0.06	0.29; p=0.4

Table 3. Percentage response of TEPs in different water samples to the lectins Concanavalin A and Limulin.

Period	Station	Depth (m)	% Positive response to ConA : Limulin	Thraustochytrid, TEPS relation
Oct 2004	1° N	10	88:12	Low TEPs levels; Few thraustochytrids
	1° N	120	50:50	Overall positive relation
	Eq	60	78:22	No overall correlation
	Eq	120	77:23	No overall correlation
	2° S	20	54:42	Overall Positive relation
	2° S	120	25:75	Overall positive relation
Sep 2006	0.5° N	500	83:17	No overall correlation
	1.5° N	40	71:29	No overall correlation
	1.5° N	1000	91:9	No overall correlation
	2° S	200	67:33	No overall correlation
Roller table experiment at different days during Sep 2006 (SK 228)	Day 3	-	56:44	Positive from Day 5 onwards
	Day 5	-	32:68	
	Day 7	-	54:46	
	Day 9	-	56:44	
	Day 14	-	67:33	

Legends to figures

Fig. 1 ORV Sagar Kanya station locations in the equatorial Indian Ocean during cruises SK 196 (September 2003), SK 212 (October 2004) and SK 228 (September 2006) where water samples were collected.

Fig. 2 Distribution of thraustochytrids, bacteria and TEPs in the equatorial Indian Ocean during October 2004.

Fig. 3 Regression analyses for thraustochytrid numbers against bacteria and TEPs as well as bacteria against TEPs in the water column during October 2004.

Fig. 4 Distribution of thraustochytrids, bacteria and TEPs in the equatorial Indian Ocean during September 2006.

Fig. 5 Regression analyses for thraustochytrid numbers against bacteria and TEPs as well as bacteria against TEPs in the water column during September 2006.

Fig. 6 Regression analyses for thraustochytrid numbers against bacteria in the water column during September 2003.

Fig. 7 Distribution of thraustochytrids, bacteria and TEPs with respect to time in the roller table experiment conducted on board cruise # SK 228 during September 2006.

Fig. 8 Thraustochytrid frequency and biomass with respect to bacterial biomass during three cruises. SD values (\pm) are given above the columns.

Fig. 9 Photomicrograph of acriflavine stained thraustochytrid cell (marked by arrow) from natural sample on a black filter paper along with other stained particles. Bar represents 10 μm .

Fig. 1

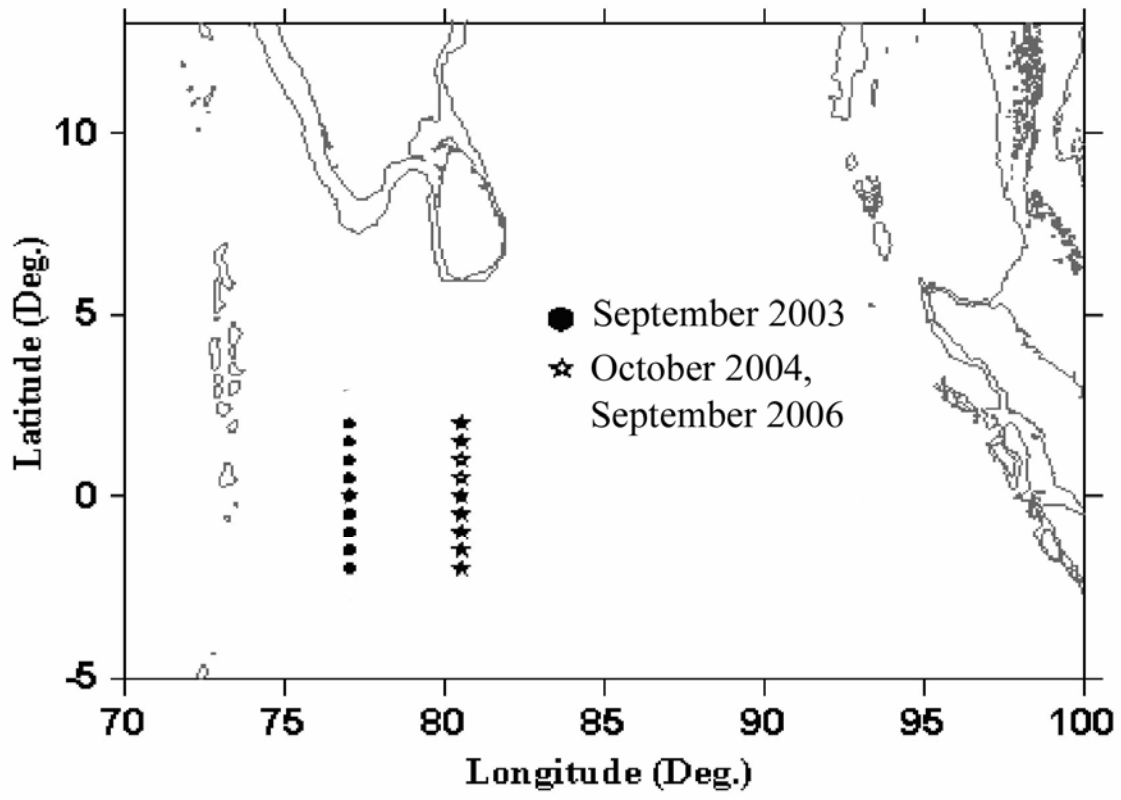


Fig. 2

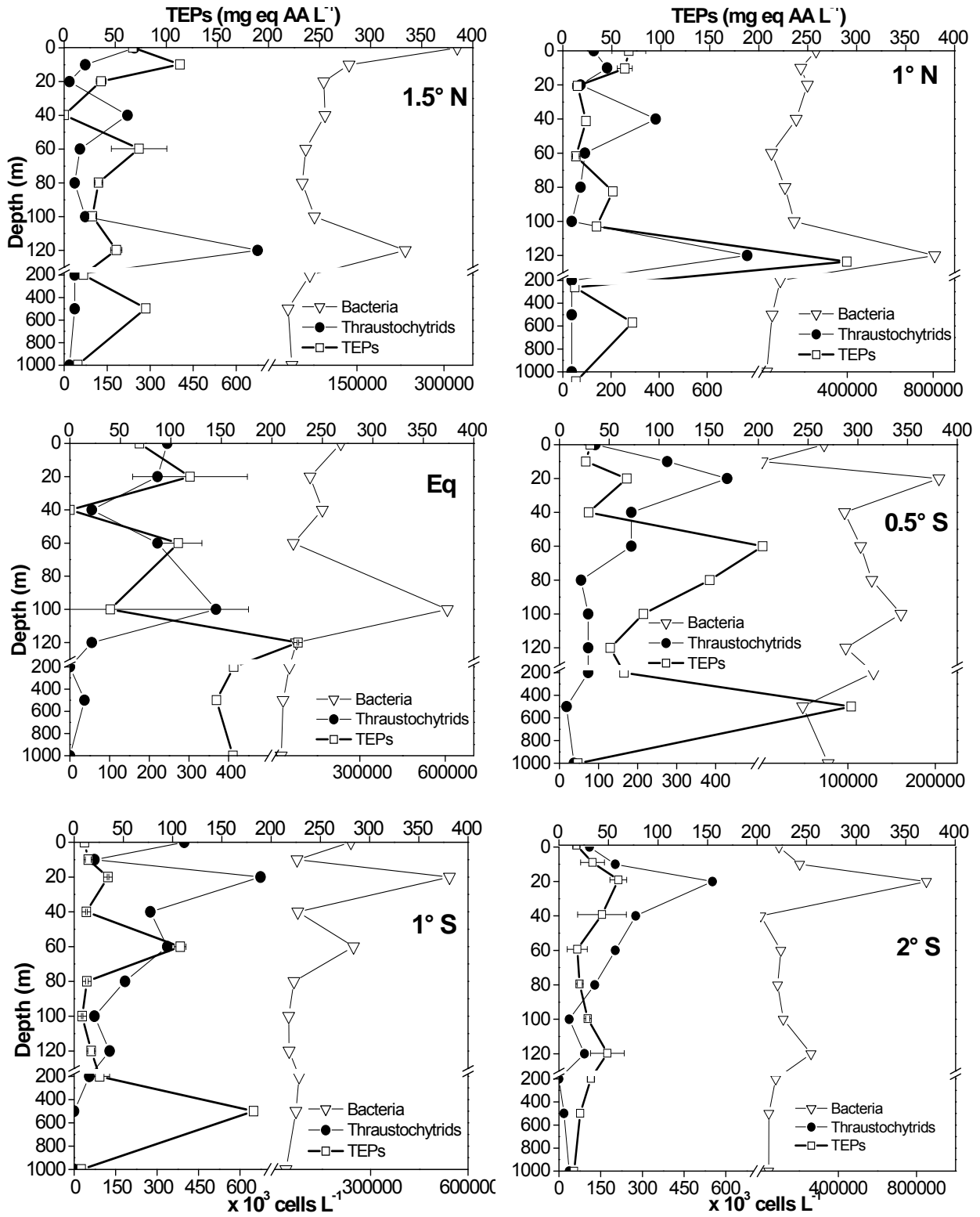


Fig. 3

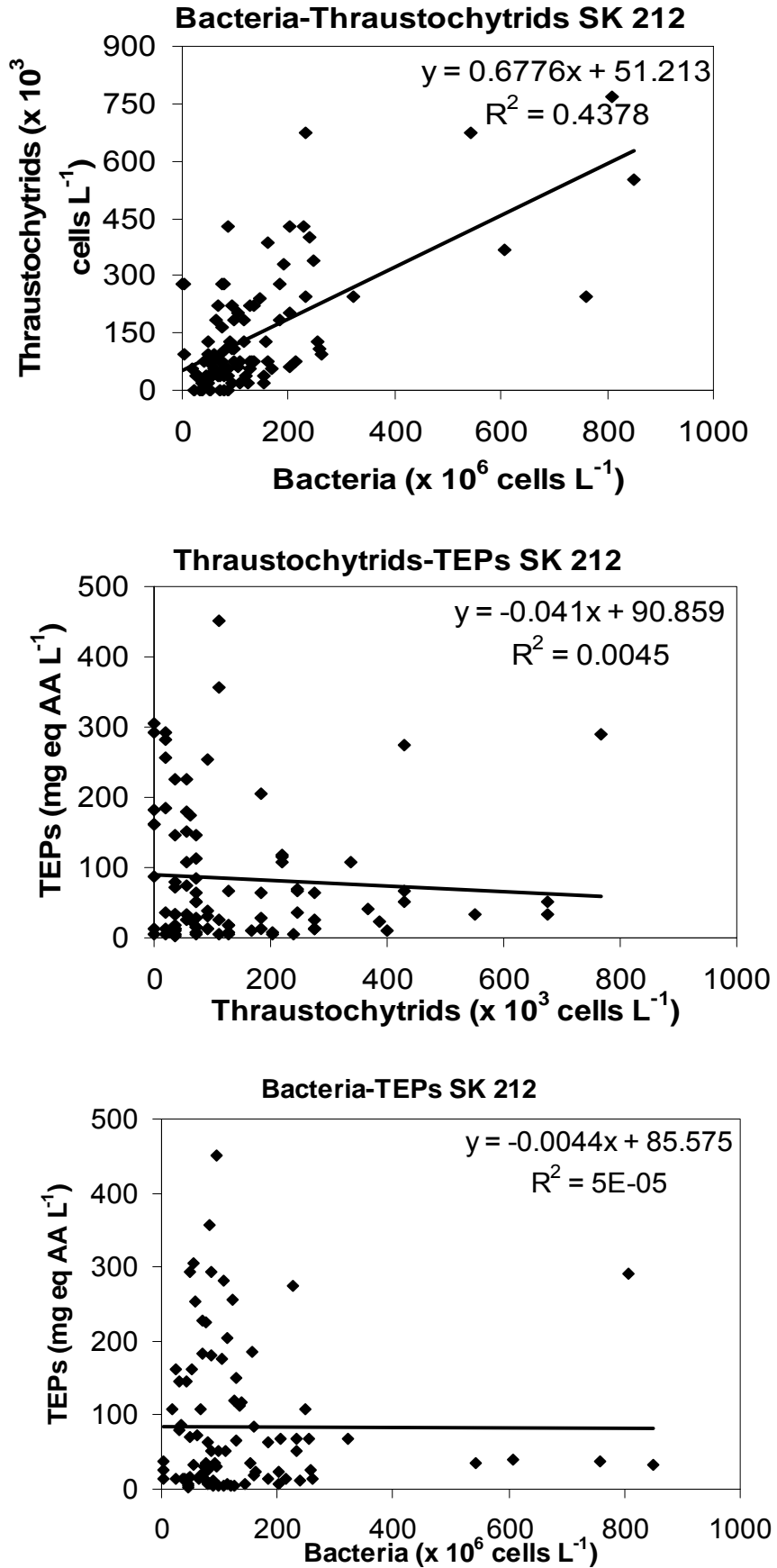
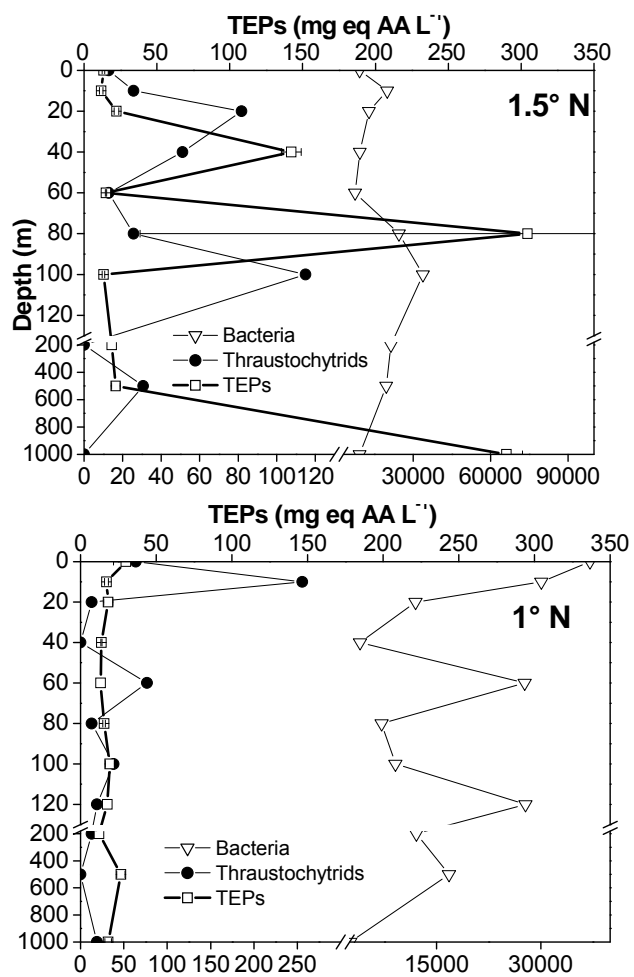
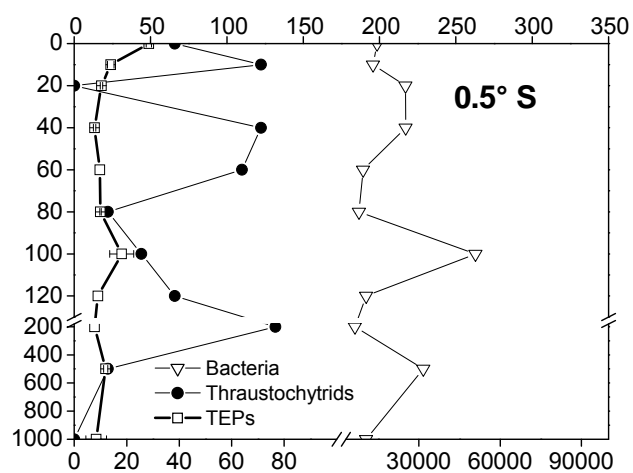
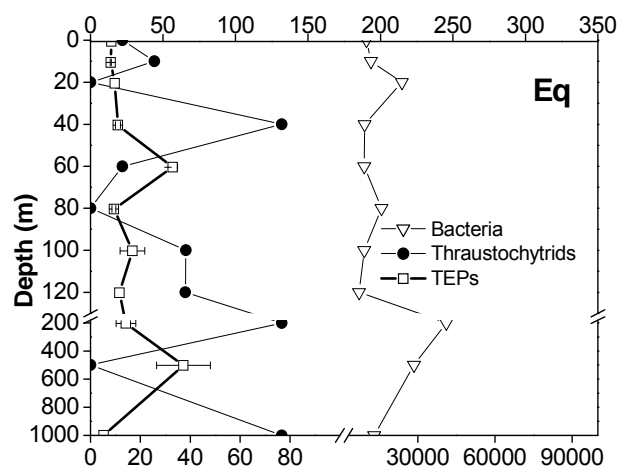


Fig. 4



Thraustochytrids, bacteria and TEPs in the Indian Ocean



Thraustochytrids, bacteria and TEPs in the Indian Ocean

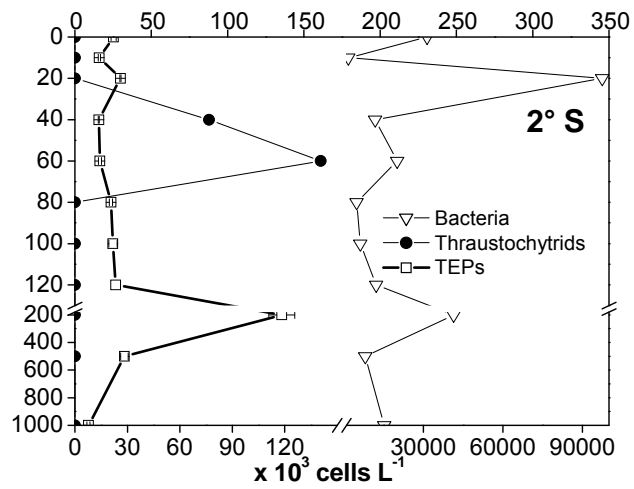
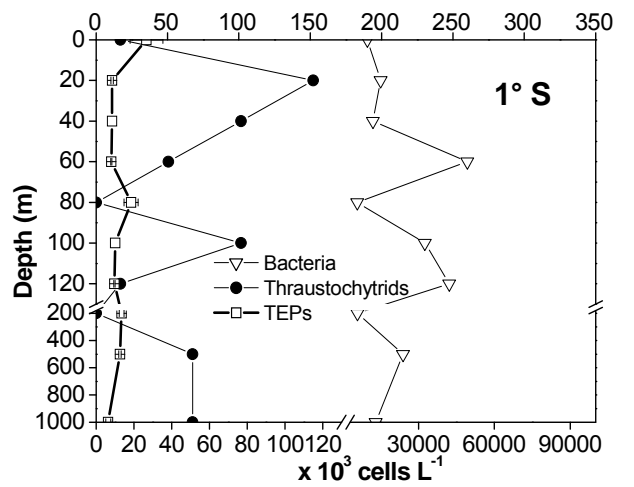


Fig. 5

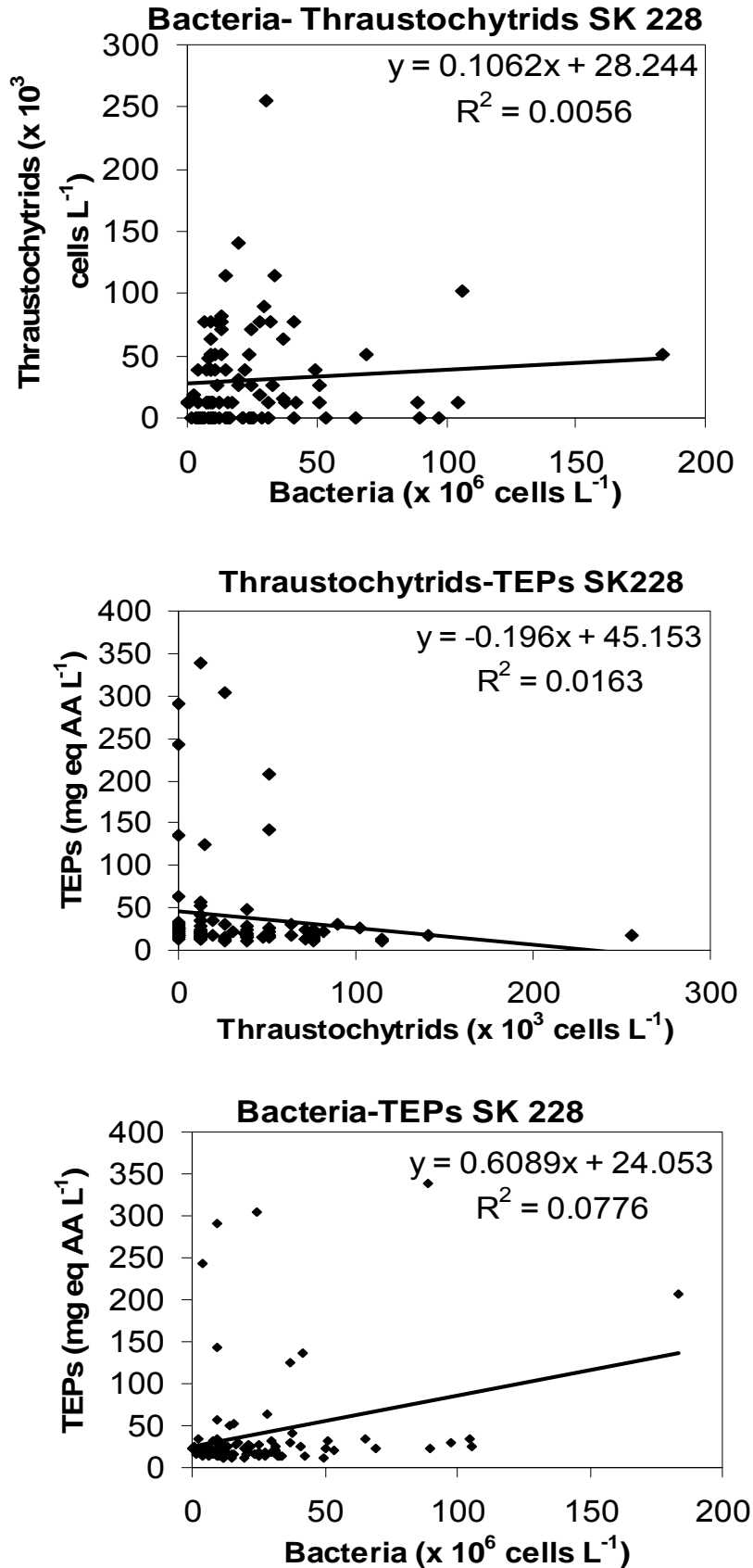


Fig. 6

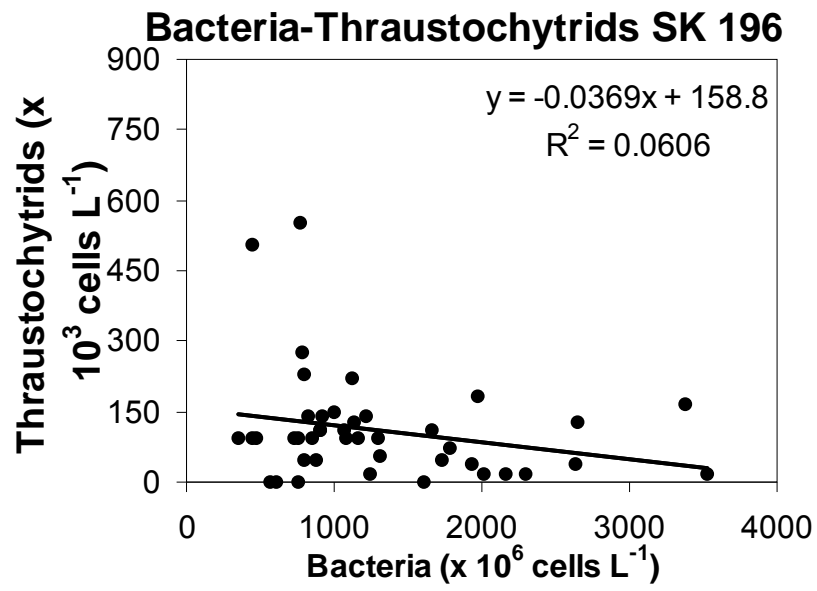


Fig. 7

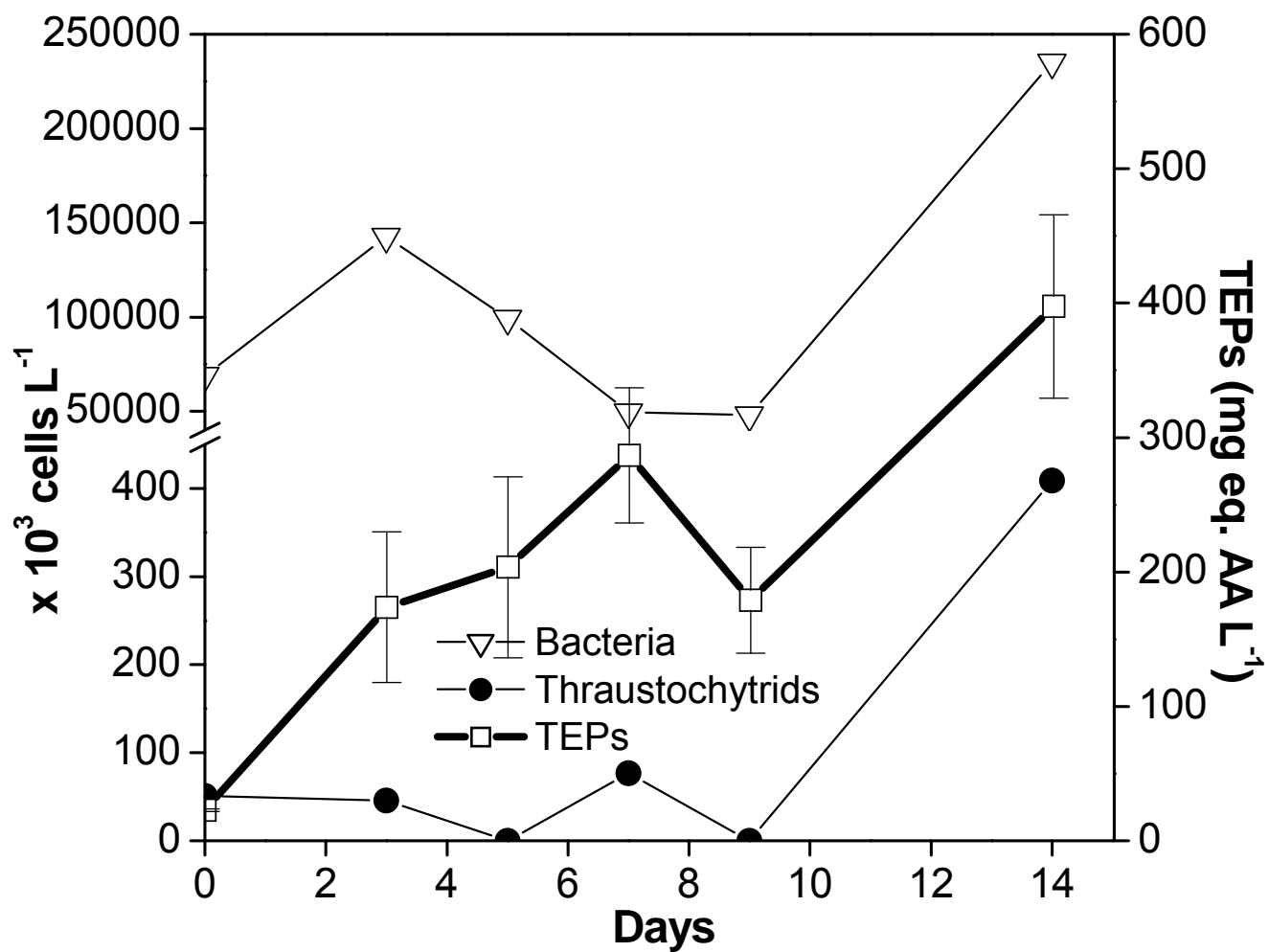


Fig. 8.

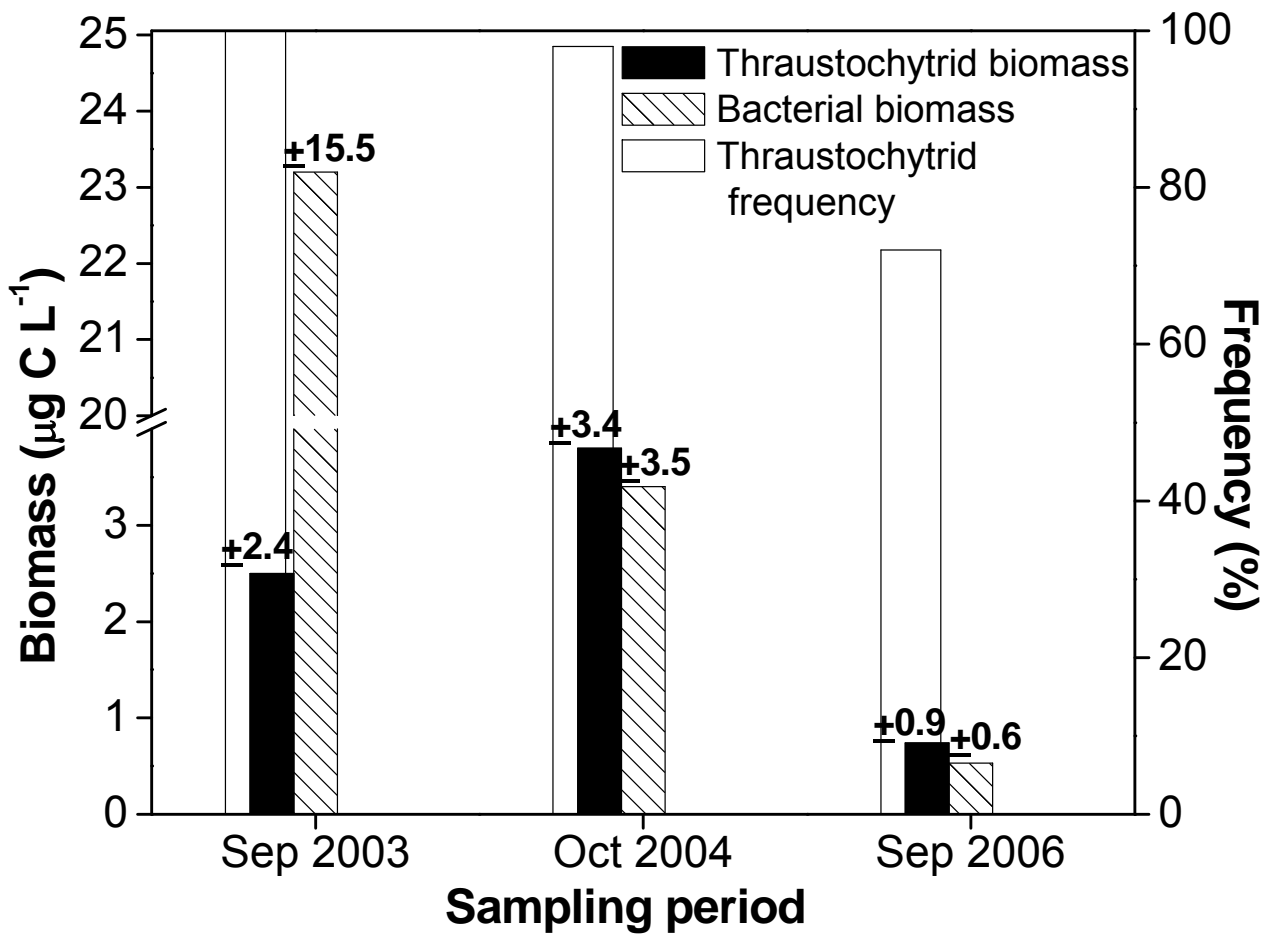


Fig. 9

