

## **Chapter 4.**

# **Relation of Thraustochytrids and Aplanochytrids to Bacteria and Transparent Exopolymeric Particles (TEPs) in the water column of the equatorial Indian Ocean**

## 4.1 Introduction

Information on spatial and temporal occurrence and population densities of any given group of organisms in different habitats provides an insight into their ecological niche. Early studies on thraustochytrids focused on their presence in coastal habitats, including marine algae, intertidal regions and nearshore waters ([Goldstein & Belsky, 1964](#); [Perkins, 1973](#)). Alwin Gaertner in Germany pioneered studies on the occurrence and population densities of thraustochytrids in offshore waters ([Gaertner, 1982](#); [Gaertner & Raghukumar, 1980](#); [Raghukumar & Gaertner, 1980](#)). Gaertner carried out extensive studies on the abundance of thraustochytrids in the North Sea waters and the Atlantic, using the Most Probable Number (MPN) culture techniques, using the pine pollen baiting technique. His studies showed that these protists were present in the water column in numbers ranging from below-detectable levels to a few hundreds per liter of sea water. Their numbers in the sediments were far higher. Although these studies unequivocally showed that these protists were always present in the water column, the actual population levels cannot be accurately determined using culture methods, as was shown in the case of bacteria ([Jannasch & Jones, 1959](#); [Watson et al., 1977](#)). The development of epifluorescence methods to directly enumerate bacteria in water ([Francisco et al., 1973](#); [Hobbie et al., 1977](#)) showed that culturable numbers are far less than the actual numbers present, a phenomenon that came to be termed 'The Great Plate Count

Anomaly' (Staley & Konopka, 1985). An epifluorescence method for counting thraustochytrids in the water column was developed by Raghukumar & Schaumann (1993) to provide a more accurate estimate of their abundance and to overcome the difficulty in distinguishing these protists from others of the same size and colour. This method, using acriflavine (the acriflavine direct detection or AfDD technique), allowed their detection in natural environment by distinguishing them from the other protists through differential staining of their cell wall and cellular contents.

Studies on their abundance and biomass using the AfDD technique, namely those of Raghukumar & Schaumann (1993) in the North Sea, Naganuma *et al.* (1998) and Kimura *et al.* (1999) in the Seto Inland Sea, Japan, Raghukumar *et al.* (2001) in the Arabian Sea and Bongiorno *et al.* (2005a) in the Mediterranean, have demonstrated that thraustochytrids often attain densities of a few hundred thousand cells per liter seawater.

Bacteria are the most abundant living organisms in the sea and play an extremely significant role in the biogeochemistry of the oceans (Azam *et al.*, 1983). Since the Labyrinthulomycetes, including thraustochytrids, aplanochytrids and labyrinthulids are also osmoheterotrophic in nutrition like bacteria (Porter, 1990; Raghukumar, 2002), they are likely to play a role similar to that of bacteria in remineralization of particulate and dissolved organic matter. However, the

relative roles of the two groups of organisms in such ecological processes is not known. Therefore, it is important to study the relation between populations of thraustochytrids, aplanochytrids and labyrinthulids *vis a vis* bacteria in order to understand their ecological role better.

[Kimura \*et al.\* \(1999, 2001\)](#), [Raghukumar \*et al.\* \(2001\)](#) and [Bongiorni \*et al.\* \(2005a\)](#) have compared thraustochytrid biomass to that of bacteria. Their studies demonstrated that thraustochytrids occasionally attained biomass values up to 50 % of the bacteria. These few studies, dealing with these protists in coastal waters or the land-locked Arabian Sea, have noticed varying relations between thraustochytrids and bacteria.

There seems to be a significant positive relation between thraustochytrids and particulate organic carbon ([Kimura \*et al.\*, 2001](#), [Raghukumar \*et al.\*, 2001](#)). Allochthonous material in the sea brought by riverine input seem to have significant influence on the abundance of these organisms which in turn greatly influences the coastal carbon cycling. The importance of autochthonous particles and substrates that thraustochytrids and aplanochytrids utilize in the water column is not clear.

Marine aggregates or marine snow are hot spots of microbial activity in oceanic waters. Marine aggregates comprise detrital and inorganic particles, faecal pellets and cadavers of zooplankton and microorganisms. These are entrapped in Transparent Exopolymeric Particles (TEPs) which act as the sticky matrix for marine snow ([Simon \*et al.\*, 1990](#); [Passow & Alldredge, 1994](#)). 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 (Aldredge *et al.*, 1993; Beauvais *et al.*, 2003). TEPs have been studied for many years and their importance in oceanic biogeochemical processes is well accepted (Simon *et al.*, 2002). TEPs too are potential substrates for microbial growth. Bacteria play a major role in remineralization of these particles (Kiorboe, 2001). Relation of thraustochytrids with POC suggests that marine aggregates could be one of the potential habitats of thraustochytrids in the water column. Raghukumar *et al.* (2001) noticed dense colonization of marine aggregates by thraustochytrids.

The occurrence and abundance of thraustochytrids and aplanochytrids in the oceanic water column removed from coastal influences have not been studied so far. Consequently, it is also not known how they are related to bacteria in this environment. Besides, the possibility of TEPs serving as a substrate for thraustochytrids in the water column has not been addressed so far.

As a step towards filling these lacunae, it was decided to carry out the following studies.

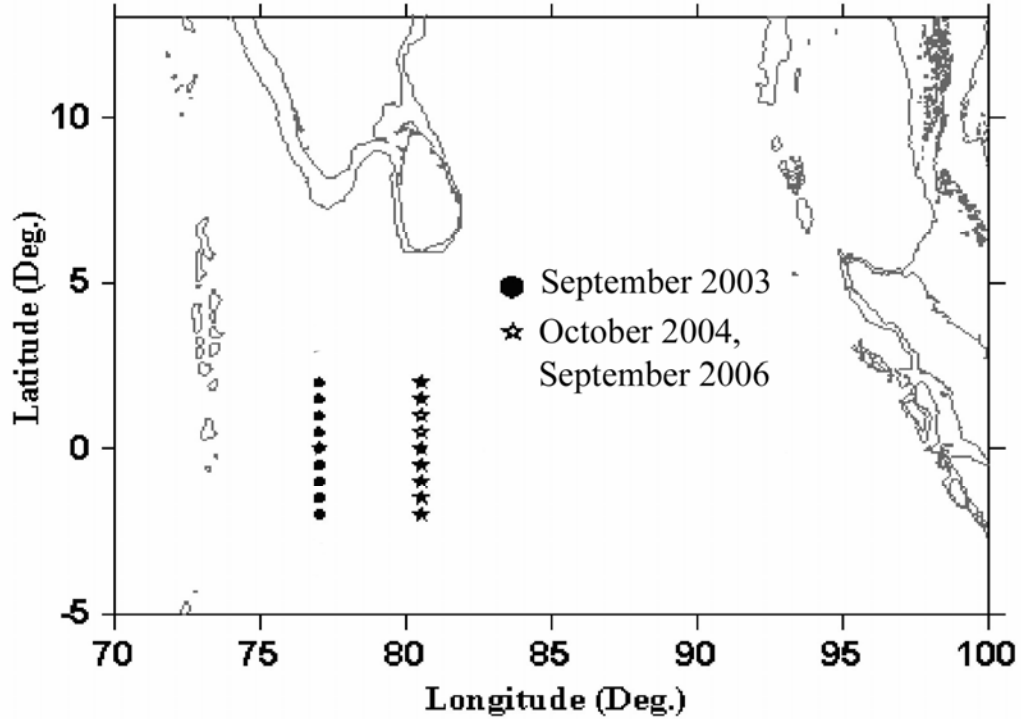
- (1) To enumerate thraustochytrids in the water column of the equatorial Indian Ocean in order to understand their distribution in an oceanic environment.
- (2) To enumerate bacteria from the water column to analyze the relationship between thraustochytrid and bacterial populations.
- (3) To enumerate TEPs from the water column in order to study their relation to thraustochytrids and bacteria.

## **4.2 Materials and Methods**

### ***4.2.1 Sampling stations***

Sampling 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.

**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).**

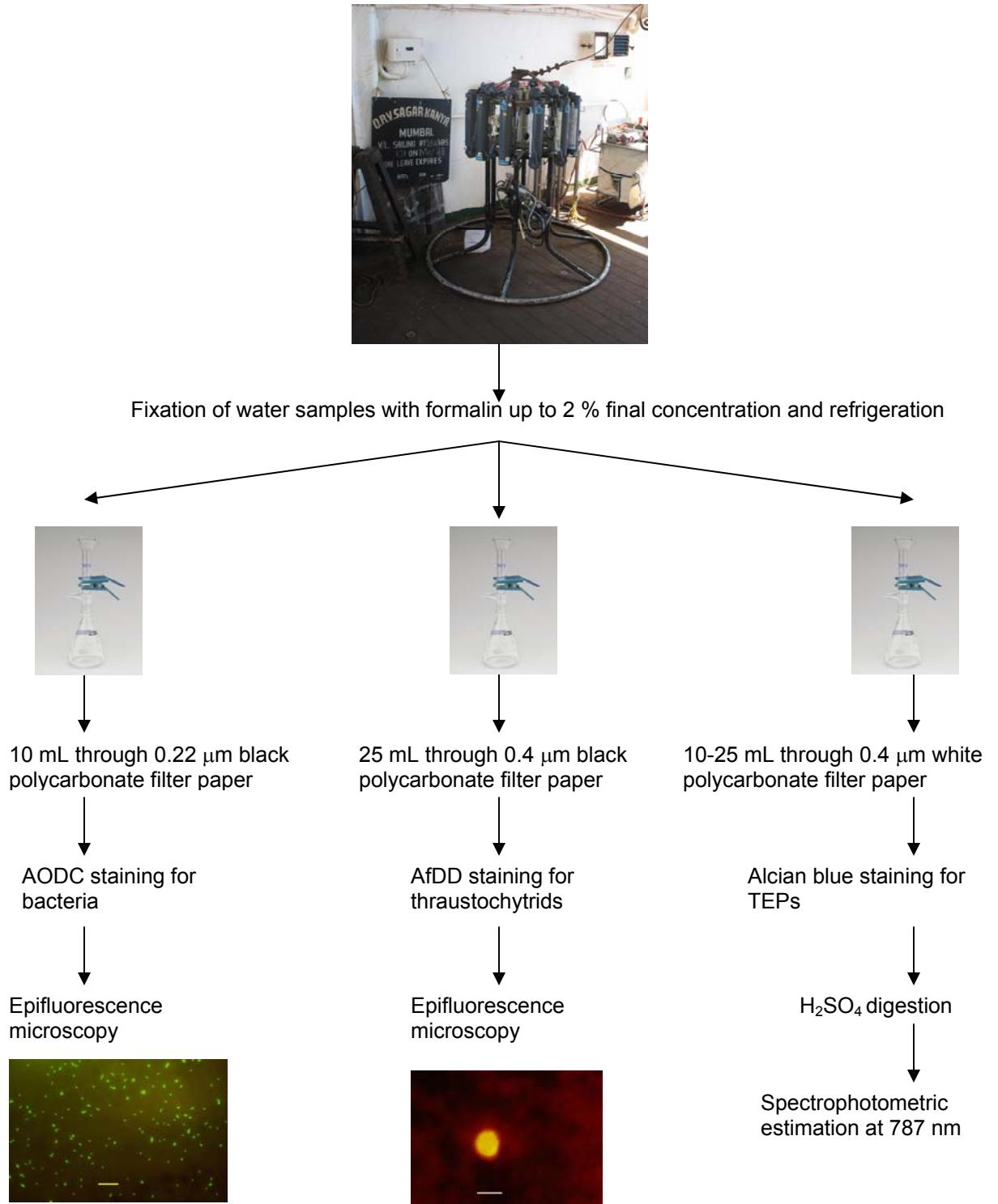


#### ***4.2.2 Sample collection***

A general scheme of collection and processing the water samples is given in Fig. 2. Water samples were collected with clean 5 L or 10 L Niskin bottles attached to a Sea Bird CTD rosette sampler from 9 depths in each cruise, namely surface (3 m), 10, 20, 40, 60, 80, 100, 120 and 200 m. Two additional depths of 500 m and 1000 m were sampled during October 2004 and September 2006 (a total of 11 depths). Temperature and salinity data were obtained from the CTD readings.

**Fig. 2. General scheme of collection of water samples from various depths by SeaBird CTD and processing them for analysis.**

Niskin bottles fixed to SeaBird CTD for collecting water samples from various depths.





#### **4.2.3 Estimation of bacterial and thraustochyrid abundance**

Water samples collected during the three 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). The filter paper was stained for bacteria by the acridine orange direct count (AODC) method ([Parsons et al., 1984](#)). A concentration of 0.01 % of acridine orange was overlaid over the filter paper and incubated in dark for 4 mins. After incubation, the filter paper was washed by passing sterile 0.22 µm filtered distilled water through it. 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](#)). After filtering the sample water through the filter, sterile 0.22 µm filtered distilled water was passed through it and later it was overlaid with 0.05 % acriflavine (working solution) and incubated for 4 mins in dark. After incubation, the stain was drained and the filter paper was overlaid with 70 % isopropanol for 1 min. The filter paper was mounted on microscope slide in non-fluorescent

immersion oil (Olympus Optical Co. Ltd) and observed under epifluorescence microscope. Thraustochytrid cells were counted from 100 different fields under blue excitation filter and they could be distinguished from the other protists by their red cell wall and green cellular contents and were counterchecked for photosynthetic picoplankton (fluorescing red) under green filter.

#### **4.2.4 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  $\mu\text{m}$ . The same value was used as factor for converting thraustochytrid cell densities into biomass. For bacteria, a factor of 20.0 fg of C per average bacterial cell from ocean waters was used ([Ducklow, 2000](#)).

#### **4.2.5 Determination of TEPs concentration**

Transparent Exopolymeric Particles (TEPs) were estimated in 2 of the 3 cruises (during 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  $\mu\text{m}$  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 \(1995\)](#) as follows.

$$C_{TEP} = (E_{787} - C_{787}) \times (V_f)^{-1} \times f_x$$

where  $E_{787}$  is absorption of the sample,  $C_{787}$  is the absorption of the blank,  $V_f$  is the volume filtered in liters and  $f_x$  is the calibration factor in  $\mu\text{g}$ .

$f_x$  is calculated by relating dry weight measurements of alginic acid particles retained on the filters to their staining capacity according to

$$f_x = W \times [(est_{787} - C_{787}) \times V_{st}^{-1}]^{-1}$$

where  $W$  is the dry weight of the standard ( $\mu\text{g L}^{-1}$ ),  $est_{787}$  is its average absorption,  $C_{787}$  is the absorption of the blank, and  $V_{st}$  is the volume in liters filtered for staining.

$C_{TEP}$  is expressed as milligram equivalent of alginic acid (AA) per liter [ $\text{mg eq AA L}^{-1}$ ] ([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).

## **4.3 Results**

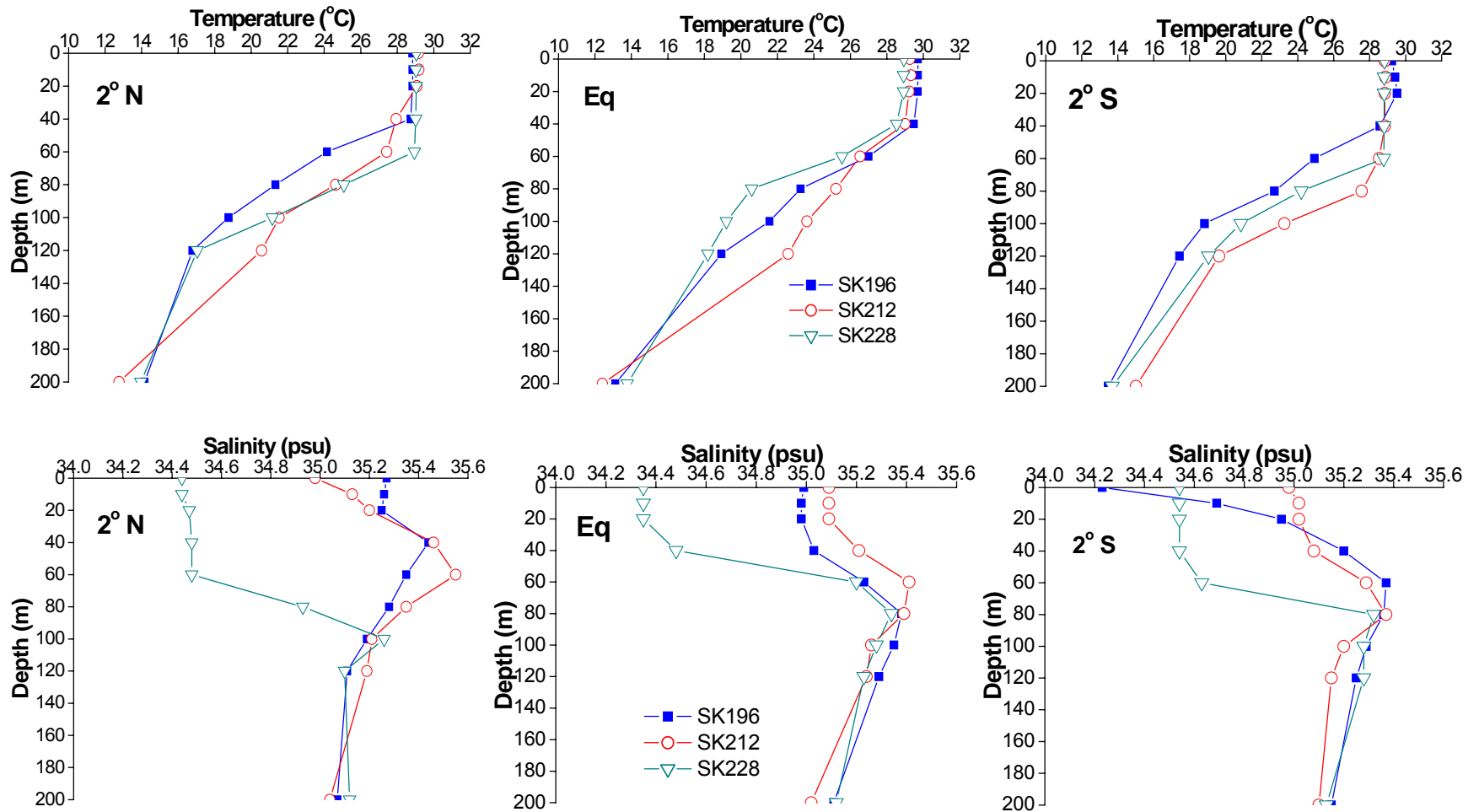
### ***4.3.1 Hydrography of study area***

During September 2003, the isothermal mixed layer was present up to 40 m depth at the equator and north, becoming shallower (20 m) towards the south (Fig. 3). The thermocline was deeper at all the locations in October 2004 and September 2006 as compared to September 2003 (60 – 80 m). Salinity profiles showed the presence of a halocline at 20 m depth in September 2003 and October 2004. The halocline during September 2006 was present at about 40 m at the equator and deeper at about 60 m on either side of it. A subsurface salinity maximum at 40 to 80 m was observed during the three cruises.

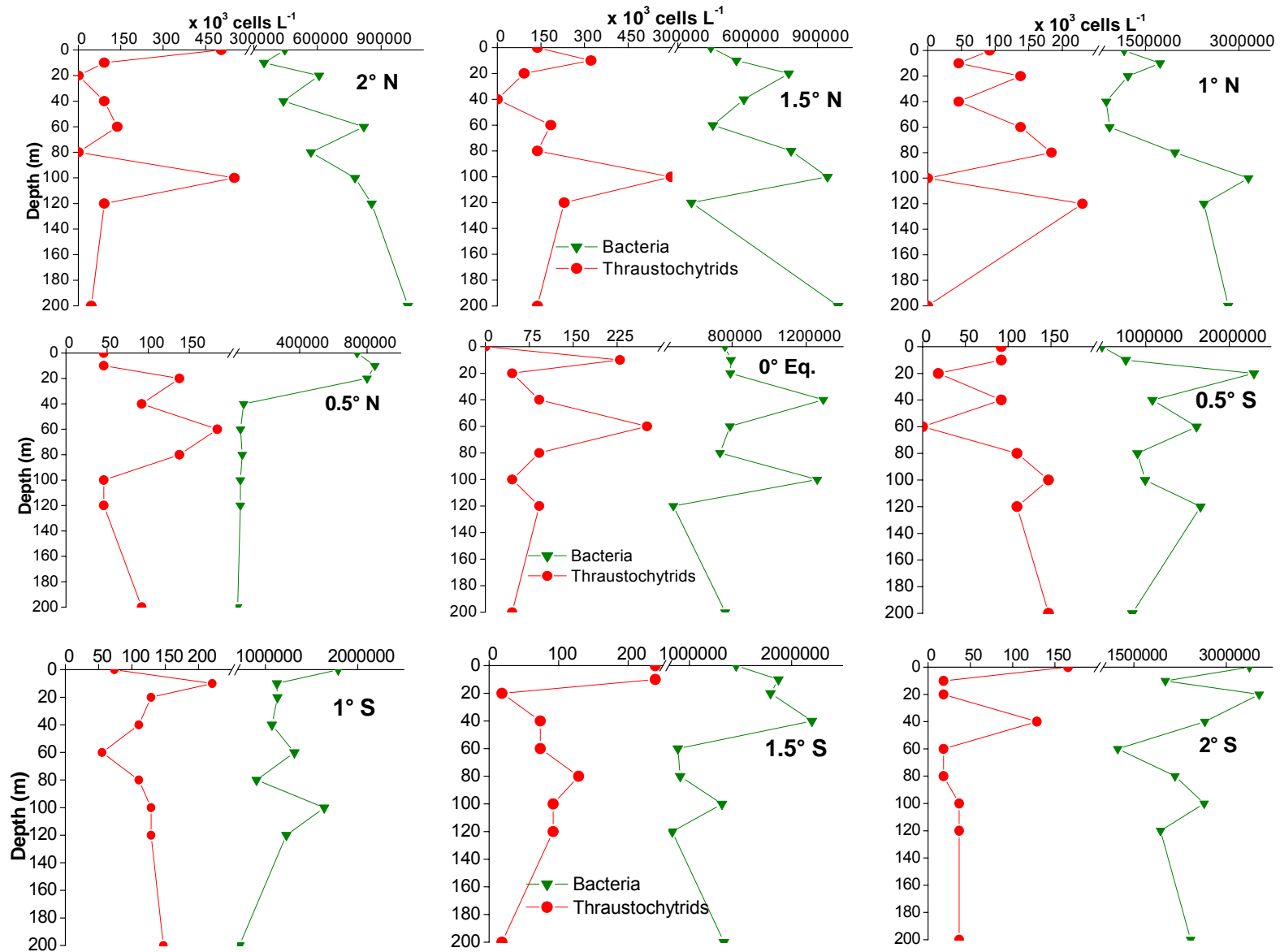
### ***4.3.2 Distribution of thraustochytrids and bacteria during SK 196 (September 2003)***

Thraustochytrid numbers showed clear vertical distribution profiles in 8 of 9 stations (Fig. 4). Two peaks of abundance were present, one above the thermocline in the surface waters from 0 to 20 m, and the other below it at 40 to 100 m. Likewise, bacteria were abundant at 0 to 20 m, but showed a prominent peak also at 100 m in most of the stations. Thraustochytrid numbers were low at the thermocline.

**Fig. 3. Thermohaline profiles of 3 stations during cruises September 2003 (SK 196), October 2004 (SK 212) and September 2006 (SK 228).**

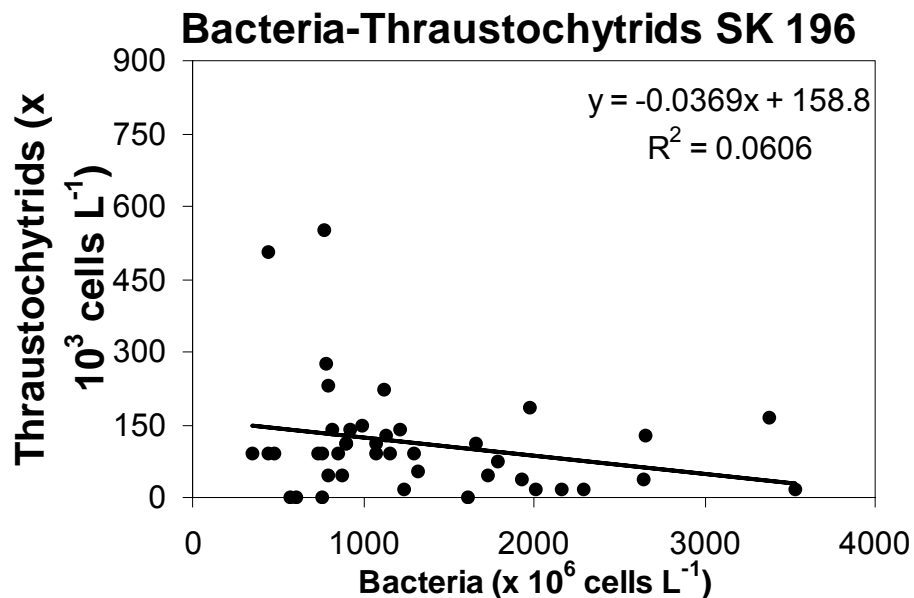


**Fig. 4. Distribution of thraustochytrids and bacteria with depth in equatorial Indian Ocean during September 2003 (Cruise SK 196).**



Thraustochytrid populations in the water column above the thermocline ranged from values below detection levels to  $506 \times 10^3$  cells  $L^{-1}$ , while bacteria ranged from 69 to  $3533 \times 10^6$  cells  $L^{-1}$  (Fig. 5). The maximum density of thraustochytrids was found at the surface at  $2^\circ N$ , while the highest bacterial density occurred at 20 m at  $2^\circ S$ . Thraustochytrid numbers below the thermocline ranged from values below detection to  $598 \times 10^3$  cells  $L^{-1}$  and bacterial numbers ranged from 33 to  $3151 \times 10^6$  cells  $L^{-1}$ . Highest density of thraustochytrids was found at 100 m at  $1.5^\circ N$ . No distinct relationship between bacteria and thraustochytrids were discernible when all the stations were considered (Fig. 5). However, when the relationship between the two were analyzed for individual stations, a statistically negative correlation was noticed in one station, namely at the latitude of  $0.5^\circ S$  (Table 1).

**Fig. 5. Scatter plots of thraustochytrids against bacteria during September 2003.**



**Table 1. Results of correlation analyses ('r') values between abundance of thraustochytrids and bacteria at different stations during SK Cruise # 196 in September 2003. Significant values are given in bold.**

Parameters	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	-0.07; p=0.85	0.26; p=0.5	-0.25; p=0.5	-0.22; p=0.6	-0.12; p=0.75	<b>-0.64;</b> <b>p=0.06</b>	-0.38; p=0.31	0.1; p=0.8	0.49; p=0.2

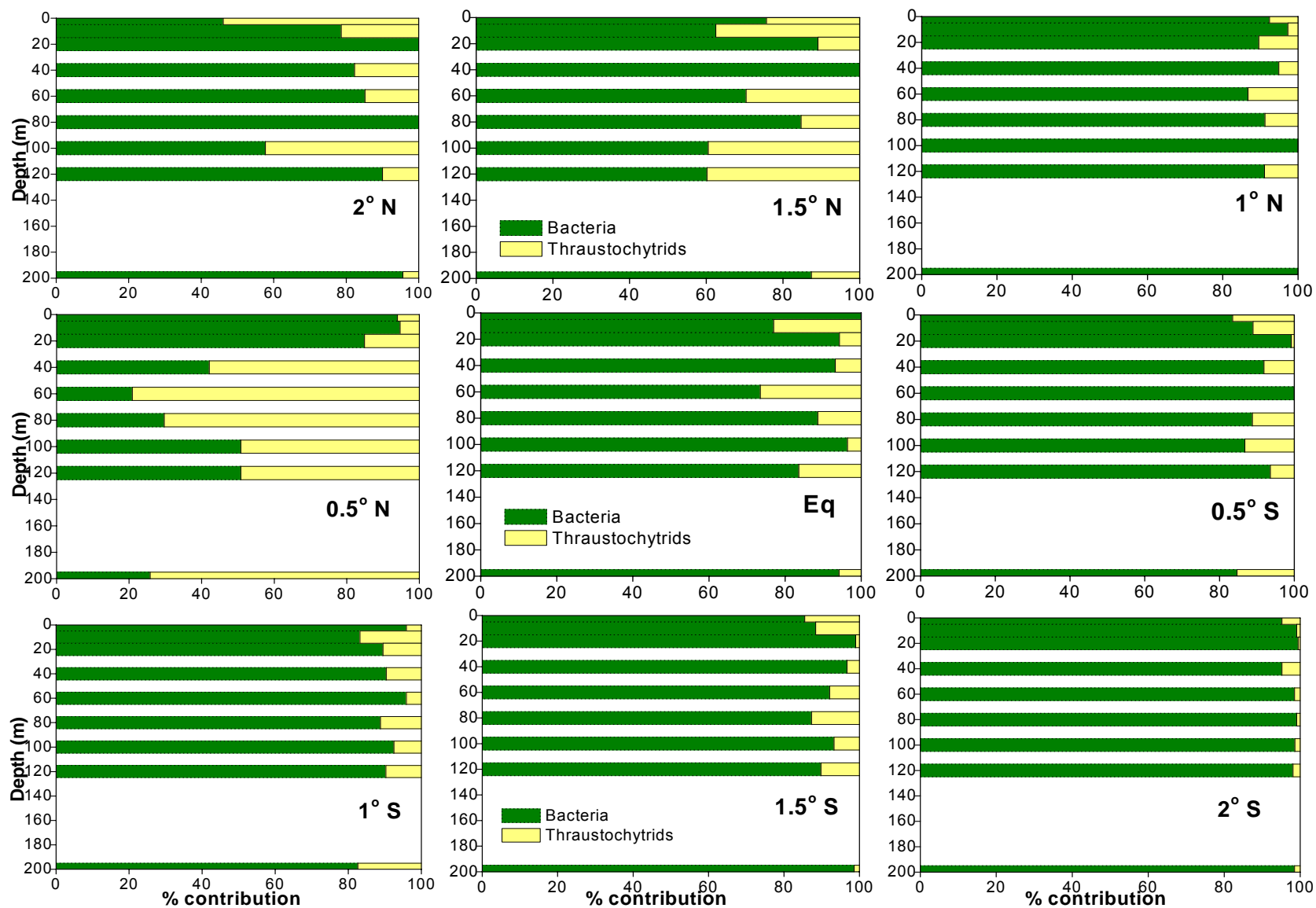
**Table 2. Contribution of thraustochytrid/ bacterial biomass to microbial carbon in terms of  $\mu\text{g C L}^{-1}$  in the water column of equatorial Indian Ocean during September 2003.**

Depth (m)	2°N	1.5°N	1°N	0.5°N	0°Eq	0.5°S	1°S	1.5°S	2°S
<b>0</b>	10.4/8.9	2.8/8.8	1.9/23.2	0.9/14.8	0.001/15	1.9/9.6	1.5/35.8	4.9/29.2	3.4/67.5
<b>10</b>	1.9/7	6.6/11	0.9/34.7	0.9/16.9	4.7/15.9	1.9/15.3	4.5/22.5	4.9/37.4	0.4/40.2
<b>20</b>	0.001/12.2	1.9/15.5	2.8/24.4	2.8/16	0.9/15.8	0.4/45.9	2.7/22.6	0.4/35.9	0.4/70.7
<b>40</b>	1.9/8.8	0.001/12	0.9/17.5	1.9/1.4	1.9/25.9	1.9/21.6	2.3/21.4	1.5/44	2.7/53
<b>60</b>	2.8/16.4	3.8/9	2.8/18.5	3.8/1	5.7/15.8	0.001/32	1.1/26.3	1.5/17.8	0.4/24.7
<b>80</b>	0.001/11.4	2.8/15.7	3.8/39.5	2.8/1.2	1.9/14.7	2.3/18	2.3/18.1	2.7/18.2	0.4/43.3
<b>100</b>	11.4/15.5	12.3/18.9	0.001/63	0.9/1	0.9/25.2	3/19.9	2.7/32.8	1.9/26.4	0.8/52.8
<b>120</b>	1.9/17.1	4.7/7.2	4.7/48.8	0.9/1	1.9/9.7	2.3/33.1	2.7/24.6	1.9/16.7	0.8/38.6
<b>200</b>	0.9/20.5	2.8/19.8	0.001/57	1.9/0.7	0.9/15.3	3/16.8	3/14.4	0.4/26.8	0.8/48.4

Thraustochytrids contributed 0 to 10.4  $\mu\text{g C L}^{-1}$  to the upper 40 m water column, while bacterial biomass amounted to 1.3 to 70.7  $\mu\text{g C L}^{-1}$  (Table 2). Similarly thraustochytrids contributed up to 12.3  $\mu\text{g C L}^{-1}$  below the thermocline and bacteria up to 63  $\mu\text{g C L}^{-1}$ . The relative contribution of thraustochytrid biomass to the total microbial biomass carbon (bacteria + thraustochytrids) amounted to 0.002 - 42.3%, except at one station (depth 60 m, 0.5° N, 77° E) where thraustochytrids contributed 79.1 % (Fig. 6).



**Fig. 6. Relative percentage contribution of the biomass of thraustochytrids and bacteria in the water column of equatorial Indian Ocean during September 2003.**



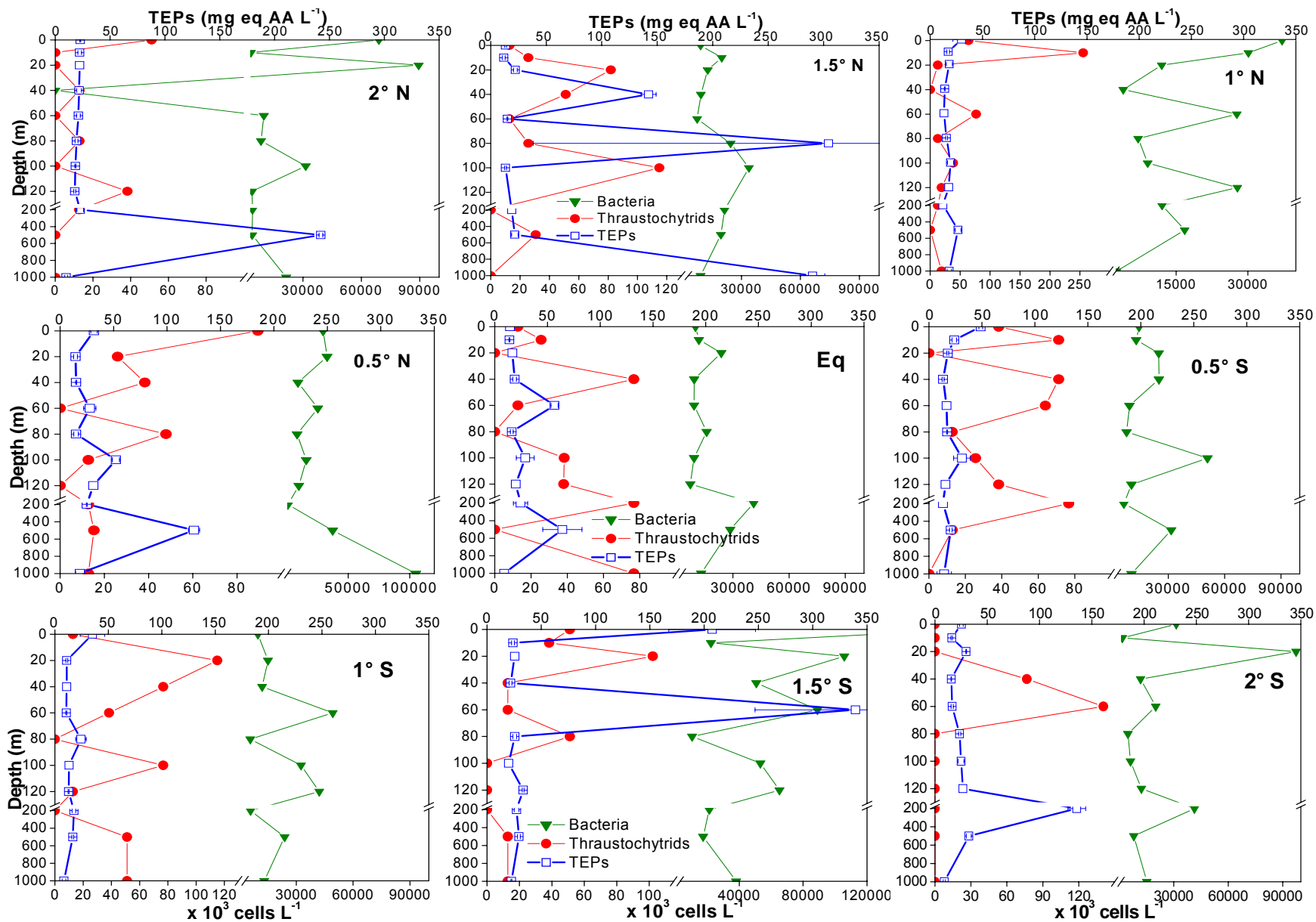
### **4.3.3 Distribution of thraustochytrids, bacteria and TEPs during SK 212**

Thraustochytrids and bacteria generally showed 2 peaks of distribution, one above the thermocline between 0 – 40 m and the other below it at 100 - 120 m from equator northwards. Maximum densities of the two groups was found below the thermocline at 120 m at 1° N. Both groups showed a prominent peak between 20 and 40 m southwards of the equator. TEPs showed several peaks in the water column, many of these occurring below 100 m.

Thraustochytrids ranged from below detection levels to  $674.6 \times 10^3$  cells L<sup>-1</sup> (Fig. 7). Bacterial densities were lower, ranging from 1.84 to  $807.3 \times 10^6$  L<sup>-1</sup>. Numbers of both thraustochytrids and bacteria were less below 120 m, the former ranging from negligible to  $73.6 \times 10^3$  cells L<sup>-1</sup> and the latter from 19.32 to  $103.73 \times 10^6$  cells L<sup>-1</sup>. TEPs ranged from 5.3 to 451.1 mg equivalent AA L<sup>-1</sup> in the water column during this period (Fig. 7).

The combined data for thraustochytrids and bacteria at all depths for all the stations showed a strong positive correlation between the two groups (Fig. 11). Correlation analysis carried out between the two at the individual stations showed that bacteria and thraustochytrids were positively related in 5 of the 9 stations (Table 3). No significant overall correlation was found between TEPs and bacteria or thraustochytrids (Fig. 11; scatter diagram). However, when individual stations were analyzed, a

**Fig. 7. Distribution of thraustochytrids and bacteria with depth in equatorial Indian Ocean during October 2004 (Cruise SK 212).**



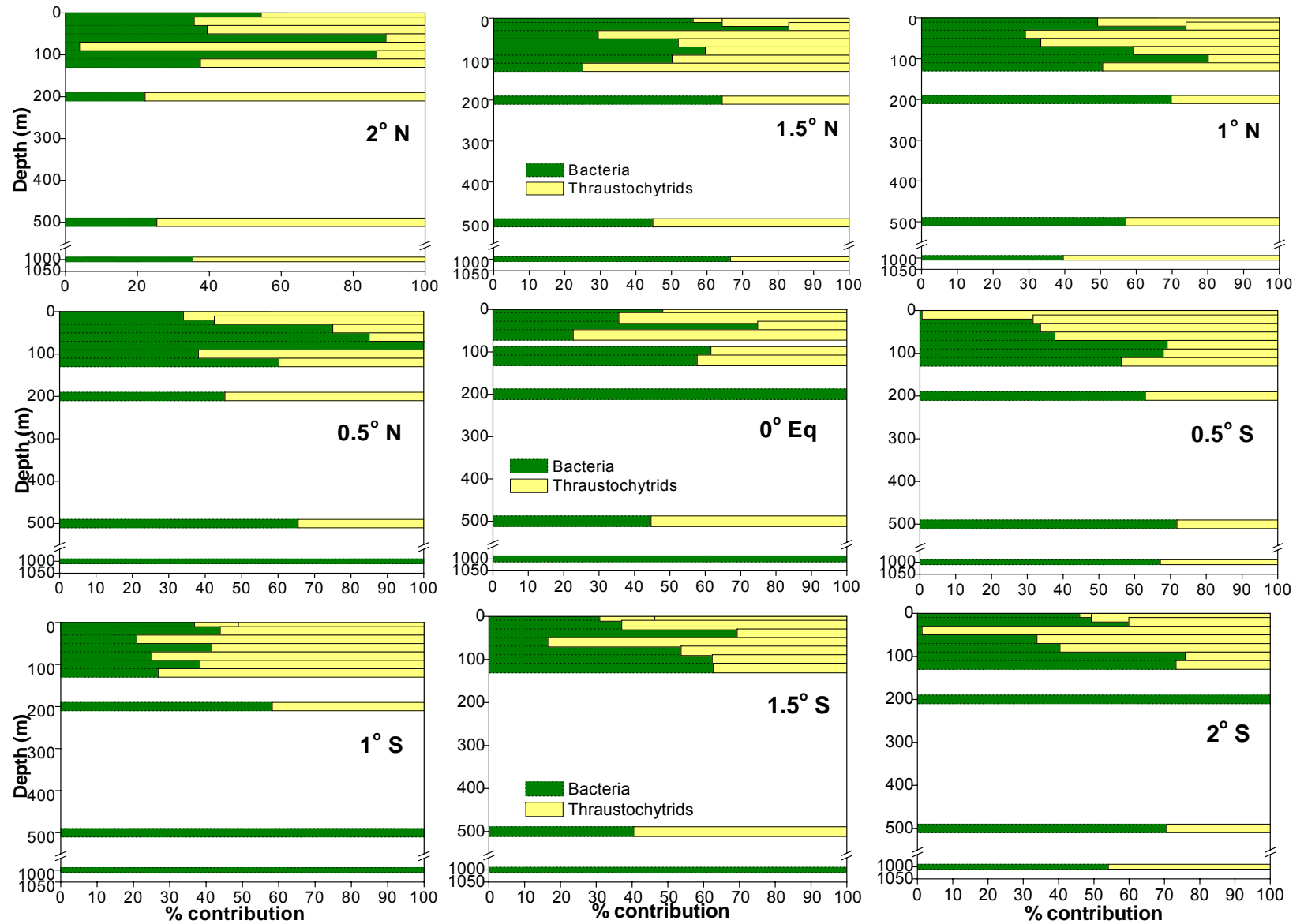
significantly positive correlation of TEPs to thraustochytrids and bacteria was noticed at 2 stations (at 1° N and 2° S) and a negative correlation at one (0° Eq; Fig. 3; Table 3). The vertical profiles of TEPs at many stations was very similar to that of bacteria and thraustochytrids but displaced by 20 m upwards or downwards.

The biomass carbon contributed by thraustochytrids ranged from negligible to 15.8  $\mu\text{g C L}^{-1}$  (Table 4). Organic carbon from bacteria ranged from 0.04 to 17.02  $\mu\text{g C L}^{-1}$ . Bacteria and thraustochytrids contributed almost equal amounts of carbon to the water column at most of the stations during this period. At some of the locations, the biomass carbon contributed by thraustochytrids exceeded that of bacteria, eg. at 2° N and 0.5° S. Thus, thraustochytrids contributed 0.1 - 99.4 % of the total bacterial and thraustochytrid biomass carbon during October 2004 (Fig. 8).

**Table 3. Results of correlation analyses ('r') values between abundance of thraustochytrids, bacteria and TEPs at different stations during SK Cruise # 212 in October 2004. Significant values are given in bold.**

Parameters	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	<b>0.70;</b> <b>p=0.02</b>	<b>0.9;</b> <b>p&lt;0.001</b>	0.54; p=0.11	<b>0.82;</b> <b>p=0.01</b>	0.28; p=0.4	<b>0.92;</b> <b>p&lt;0.001</b>	0.13; p=0.75	<b>0.8;</b> <b>p=0.003</b>
Thraustochytrids vs. TEPs	-0.54; p=0.11	0.09; p=0.78	<b>0.85</b> <b>p&lt;0.001</b>	-0.11; p=0.75	<b>-0.85;</b> <b>p=0.007</b>	-0.27; p=0.42	-0.11; p=0.74	-0.2; p=0.6	<b>0.67;</b> <b>p=0.02</b>
Bacteria vs. TEPs	-0.14; p=0.69	0.29; p=0.42	<b>0.93;</b> <b>p&lt;0.001</b>	-0.51; p=0.1	<b>-0.75;</b> <b>p=0.03</b>	-0.027; p=0.94	0.07; p=0.83	-0.19; p=0.63	<b>0.73;</b> <b>p=0.01</b>

**Fig. 8. Relative percentage contribution of the biomass of thraustochytrids and bacteria in the water column of equatorial Indian Ocean during October 2004.**



**Table 4. Contribution of thraustochytrid/ bacterial biomass to microbial carbon in terms of  $\mu\text{g C L}^{-1}$  in the water column of equatorial Indian Ocean during October 2004. NS= Not sampled.**

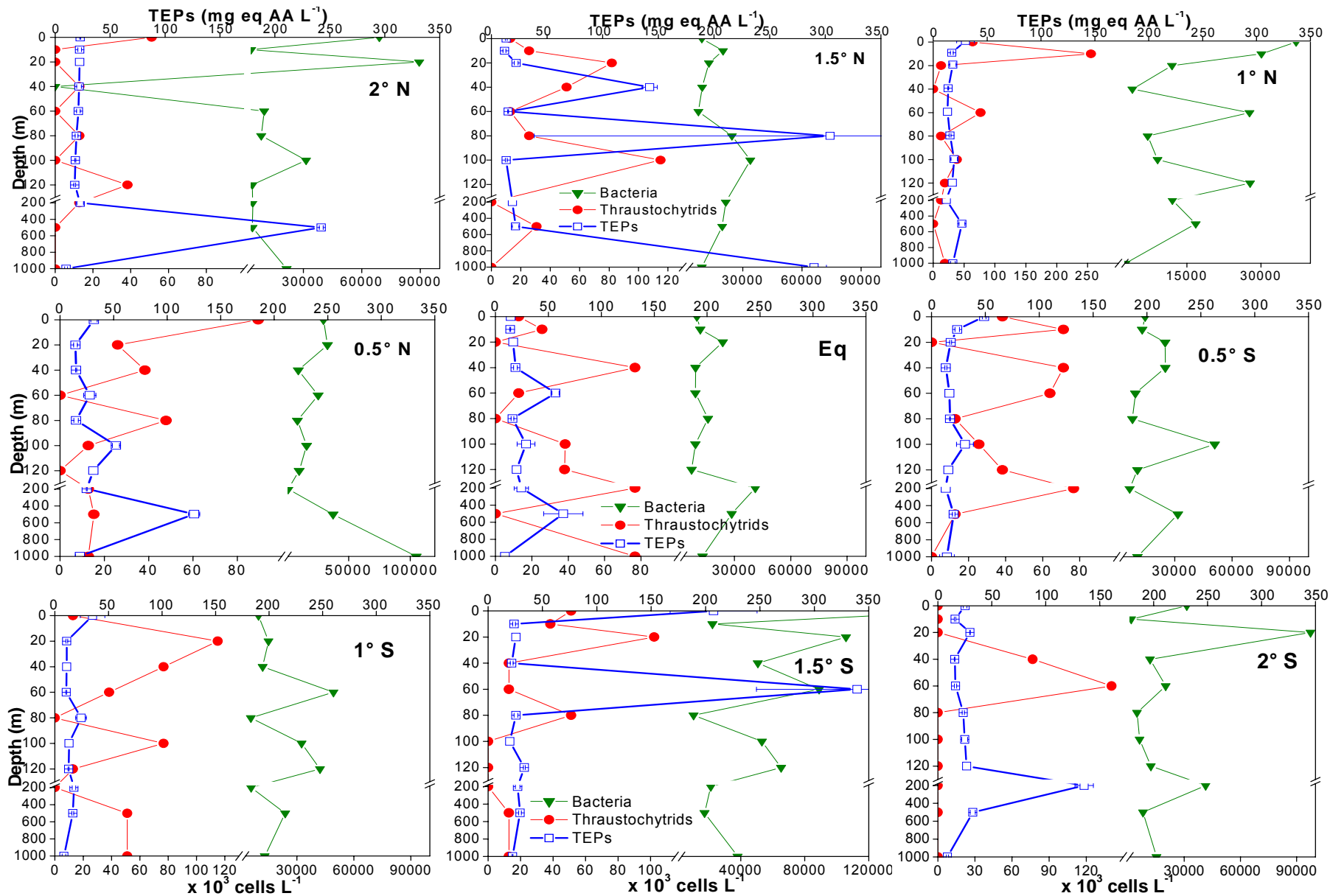
Depth (m)	2°N	1.5°N	1°N	0.5°N	0°Eq	0.5°S	1°S	1.5°S	2°S
0	1.3/2.0	5.1/6.5	2.7/5.1	1.1/1.6	5.1/4.7	1.9/1.5	8.2/4.8	3.4/1.5	2.3/1.9
10	2.7/3.2	1.5/2.7	3.8/3.7	8.8/4.6	NS	5.7/0.04	1.5/1.5	2.7/2.3	4.2/4.1
20	6.8/3.8	0.4/1.8	1.5/4.3	2.3/1.7	4.5/2.5	8.8/4.1	13.9/10.9	4.9/2.9	11.4/17.0
40	5.7/3.7	4.5/1.9	8.0/3.2	5.1/15.2	1.1/3.4	3.8/1.9	5.7/1.5	2.3/5.1	5.7/0.1
60	0.4/3.1	1.1/1.2	1.9/0.9	0.4/2.1	4.5/1.3	3.8/2.3	6.9/4.9	8.8/1.7	4.2/2.1
80	1.9/0.1	0.8/1.1	1.5/2.2	<0.4/1.1	NS	1.1/2.5	3.8/1.3	0.8/0.9	2.7/1.8
100	0.4/2.4	1.5/1.5	0.8/3.1	1.9/1.2	7.6/12.1	1.5/3.2	1.5/0.9	1.3/2.1	0.8/2.4
120	4.5/2.7	13.9/4.7	15.8/16.1	1.1/1.7	1.1/1.6	1.5/2.0	2.7/1.0	1.5/2.5	1.9/5.2
200	5.7/1.6	0.8/1.4	0.8/1.8	2.3/1.9	<0.4/1.1	1.5/2.6	1.1/1.6	NS	0/1.6
500	1.1/0.4	0.8/0.6	0.8/1.0	0.8/1.4	0.8/0.6	0.4/1.0	<0.4/1.4	1.5/1.0	0.4/0.9
1000	1.5/0.8	0.4/0.8	0.8/0.5	<0.4/1.7	<0.4/0.5	0.8/1.6	<0.4/0.8	<0.4/0.6	0.8/0.9

#### **4.3.4 Distribution of thraustochytrids, bacteria and TEPs during SK 228**

Thraustochytrids frequently revealed two peaks of distribution, one within and the other below the mixed layer, between 0 and 80 m at all stations (Fig. 9). High densities were also noticed at 200 m at the equator and 0.5°S. The vertical distribution of bacteria was very similar at stations south of equator, a prominent peak often occurring well below the mixed layer at 100 to 200 m in the high salinity maximum layer. High amounts of TEPs were noticed below 200 m at many stations.

Thraustochytrids in the mixed layer up to 60 m ranged from below detection levels to  $255.6 \times 10^3$  cells  $\text{L}^{-1}$ , while bacteria varied from 0.81 to  $183.4 \times 10^6$  cells  $\text{L}^{-1}$  (Fig. 9). Thraustochytrid abundance below the thermocline ranged from below detection levels to  $76.7 \times 10^3$  cells  $\text{L}^{-1}$

**Fig. 9. Distribution of thraustochytrids and bacteria with depth in equatorial Indian Ocean during September 2006 (Cruise SK 228).**



and that of bacteria from 3.9 to 104.9 x 10<sup>6</sup> cells L<sup>-1</sup>. 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 x 10<sup>3</sup> cells L<sup>-1</sup> and bacteria from 2.6 to 104.9 x 10<sup>6</sup> cells L<sup>-1</sup>. TEPs ranged from 7.5 to 339.3 mg equivalent AA L<sup>-1</sup> (Fig. 9). Maximum levels of TEPs were often observed below 200 m depth.

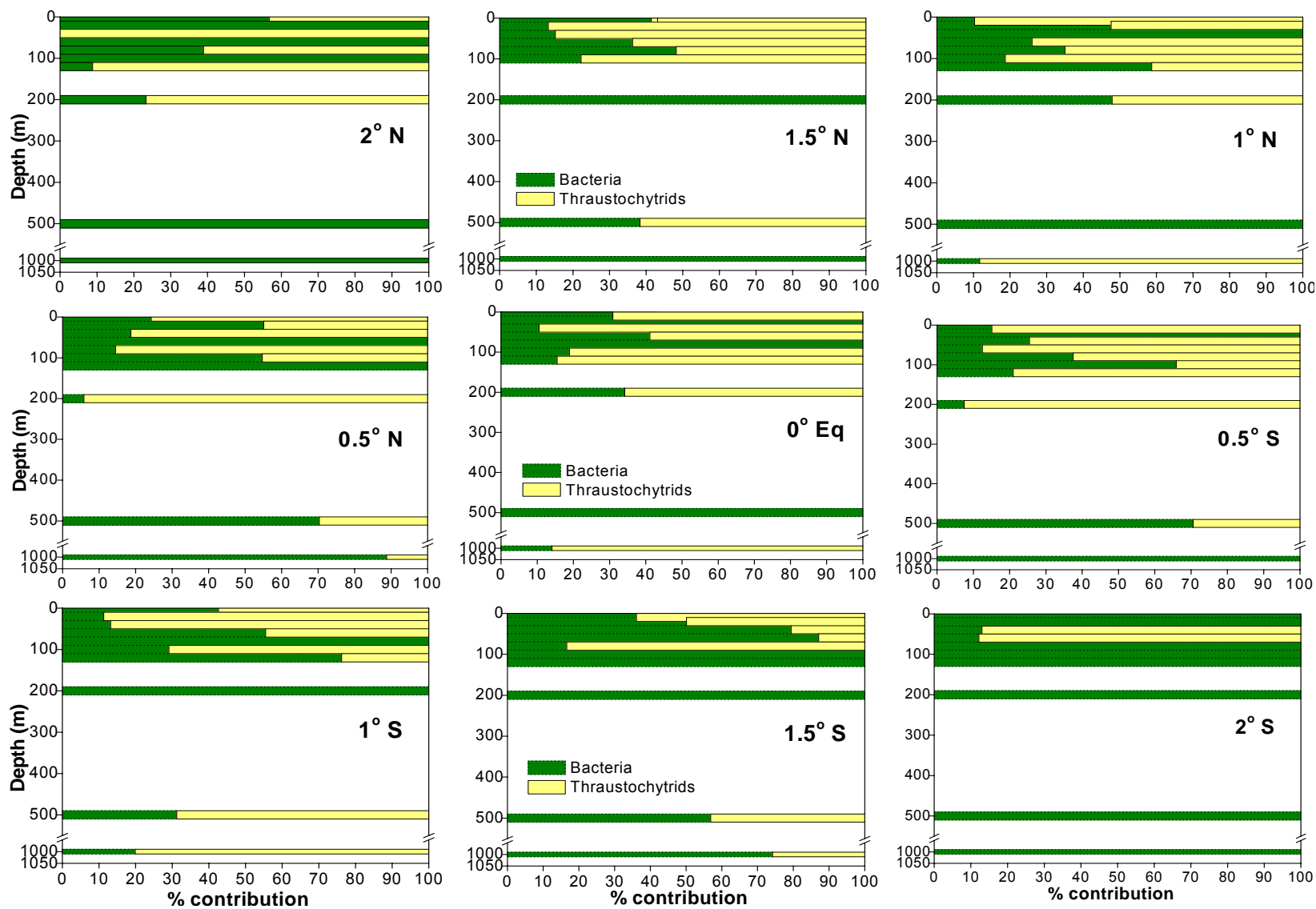
Thraustochytrids and bacteria showed dissimilar trends of distribution in the water column during September 2006 on an overall basis (Fig. 11). No significant relations between their numbers was found also when the 9 stations were analyzed individually (Table 5). Thraustochytrids contributed 0.26 to 2.9 µg C L<sup>-1</sup> biomass to the water column and bacteria contributed 0.03 to 2.1 µg C L<sup>-1</sup> (Fig. 10, Table 6). Photographs of thraustochytrid cell and bacteria as seen under the microscope after AfDD and AODC staining respectively are shown in Fig. 12.

**Table 5. Results of correlation analyses ('r') values between abundance of thraustochytrids, bacteria and TEPs to each other during SK Cruise # 228 in September 2006.**

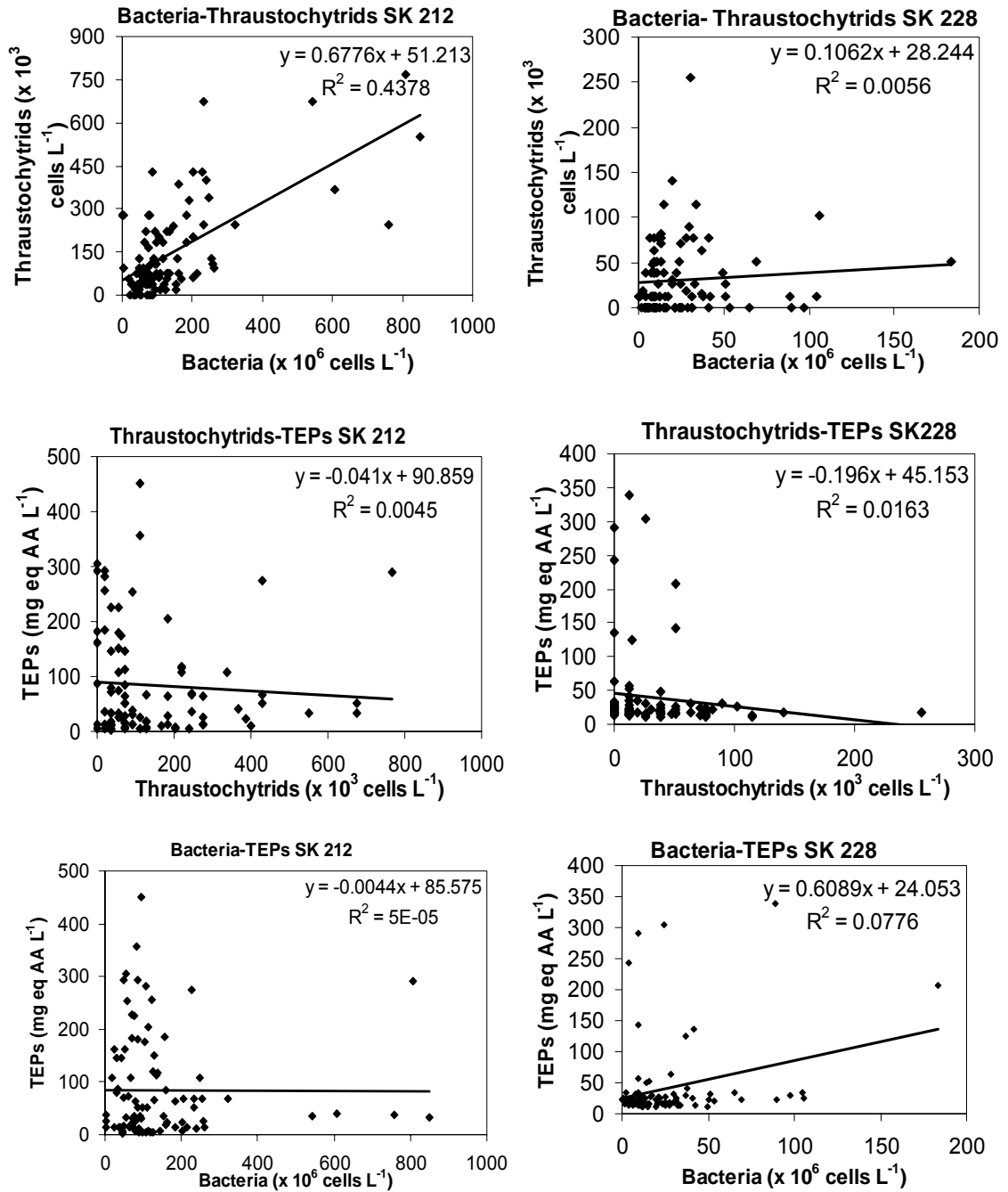
Parameters	2°N	1.5°N	1°N	0.5°N	0°Eq.	0.5°S	1°S	1.5°S	2°S
Thraustochytrids vs. bacteria	0.19; p=0.58	0.5; p=0.15	0.56; p=0.07	-0.08; p=0.82	0.07; p=0.85	-0.27; p=0.42	0.09; p=0.80	0.41; p=0.22	-0.1; p=0.78
Thraustochytrids vs. TEPs	-0.22; p=0.52	-0.27; p=0.46	-0.09; p=0.79	-0.20; p=0.57	-0.31; p=0.35	-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



**Fig. 10. Relative percentage contribution of the biomass of thraustochytrids and bacteria in the water column of equatorial Indian Ocean during September 2006.**



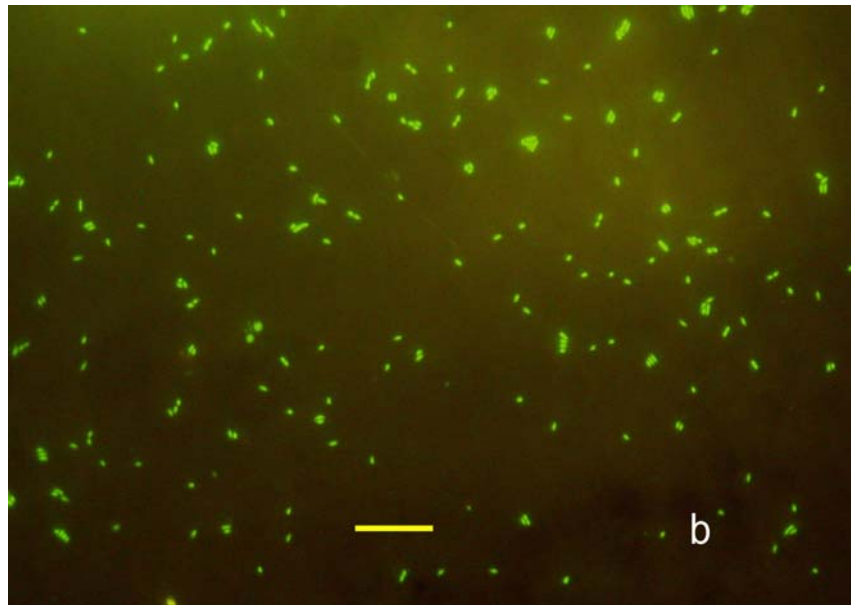
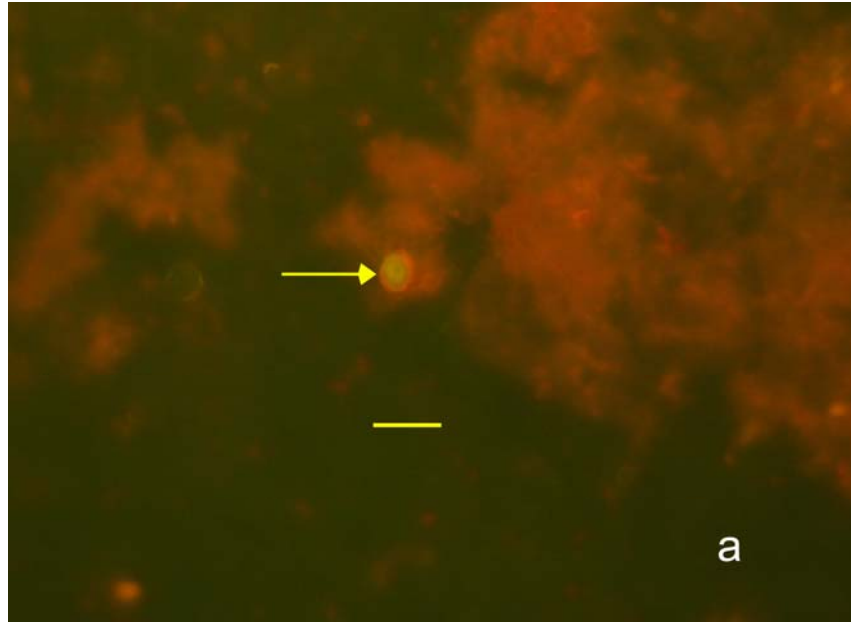
**Fig. 11. Scatter plots of thraustochytrids, bacteria and TEPs during October 2004 (SK 212) and September 2006 (SK 228).**



**Table 6. Contribution of thraustochytrid/ bacterial biomass to microbial carbon in terms of  $\mu\text{g C L}^{-1}$  in the water column of equatorial Indian Ocean during September 2006. NS= Not sampled, ND= Not determined.**

Depth (m)	2°N	1.5°N	1°N	0.5°N	0°Eq	0.5°S	1°S	1.5°S	2°S
0	1.05/1.38	0.26/0.19	1.32/0.74	1.8/0.59	0.26/0.2	0.79/0.29	0.26/0.2	1.05/3.7	<0.2/0.63
10	<0.2/0.06	0.53/0.4	5.3/0.6	NS	0.53/0.24	1.47/0.26	NS	0.79/0.45	<0.2/0.03
20	<0.2/1.8	1.7/0.26	0.26/0.24	0.54/0.66	<0.2/0.48	<0.2/0.5	2.4/0.3	2.1/2.1	<0.2/1.95
40	0.26/ND	1.1/0.19	<0.2/0.08	0.79/0.18	1.6/0.19	1.5/0.5	1.6/0.24	0.26/1.0	1.6/0.24
60	<0.2/0.2	0.26/0.15	1.6/0.55	<0.2/0.5	0.26/0.18	1.32/0.19	0.79/0.99	0.26/1.8	2.9/0.4
80	0.26/0.17	0.53/0.49	0.2/0.14	0.99/0.17	<0.2/0.32	0.26/0.16	<0.2/0.12	1.05/0.21	<0.2/0.1
100	<0.2/0.63	2.4/0.68	0.79/0.18	0.26/0.32	0.79/0.18	0.53/1.02	1.6/0.65	<0.2/1.1	<0.2/0.12
120	0.79/0.08	NS	0.39/0.56	<0.2/0.2	0.78/0.14	0.79/0.21	0.26/0.84	<0.2/1.3	<0.2/0.24
200	0.26/0.08	<0.2/0.43	0.26/0.24	0.26/0.02	1.6/0.82	1.6/0.13	<0.2/0.12	<0.2/0.4	<0.2/0.83
500	<0.2/0.08	0.63/0.39	<0.2/0.34	0.32/0.75	<0.2/0.57	0.26/0.63	1.05/0.48	0.26/0.35	<0.2/0.16
1000	<0.2/0.43	<0.2/0.18	0.39/0.05	0.26/2.1	1.6/0.26	<0.2/0.21	1.05/0.26	0.26/0.76	<0.20/0.3

**Fig. 12. Photomicrographs of thraustochytrid cell (arrow) in water sample stained by acriflavine direct detection (AfDD) technique (a) and bacteria stained by acridine orange direct count (AODC) method (b) as seen under blue light. Bar represents 10  $\mu\text{m}$ .**



#### 4.4 Discussion

The surface mixed layer of the ocean is a biologically dynamic region. When adequate mineral nutrients and illumination are available, primary productivity by photosynthetic organisms in the mixed layer is high, microbial activity is considerable and vigorous nutrient recycling takes place within it (Sarkar *et al.*, 2004). Microbial populations are therefore much higher in the mixed layer compared to deeper layers. Correspondingly, both thraustochytrid and bacterial abundance were high in the mixed layer of all the stations in the 3 cruises studied. The highest bacterial count of  $3533 \times 10^6$  cells L<sup>-1</sup> amongst all the three cruises was seen in this layer during September 2003 (cruise # SK 196). Thraustochytrids also reached values up to as high as 506 to  $674.7 \times 10^3$  cells L<sup>-1</sup> during September 2003 and October 2004 respectively.

The present study demonstrates the abundant occurrence of thraustochytrids in oceanic waters. Most of the earlier studies had addressed these protists in coastal waters or land-locked seas, such as the Arabian Sea. (Raghukumar *et al.*, 1990; Raghukumar *et al.*, 2001; Ramaiah *et al.*, 2005). Kimura *et al.* (1999) even hypothesized that thraustochytrids might be more dependent on allochthonous nutrients that are washed into the sea, more than autochthonous material. This study clearly shows that thraustochytrids are present abundantly in waters far removed from any land influence.

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.*, 2005a). Their numbers reached a maximum of  $766.6 \times 10^3$  cells L<sup>-1</sup>, a value that was almost equivalent to those found in the adjacent Arabian Sea by Raghukumar *et al.* (2001). These values exceeded those observed by Kimura *et al.* (1999, 2001) and Naganuma *et al.* (1998) in the Seto Inland Sea, Japan. Likewise bacterial densities corresponded to values recorded by Wiebinga *et al.* (1997) for the North West Indian Ocean ( $300-1600 \times 10^6$  cells L<sup>-1</sup>), Prasanna Kumar *et al.* (2001) in the Northern Arabian Sea during the North East Monsoon ( $66-1555 \times 10^6$  cells L<sup>-1</sup>), Pomeroy & Joint (1999) in the Arabian Sea during the southwest monsoon ( $620-1630 \times 10^6$  cells L<sup>-1</sup>), Ramaiah *et al.* (1996) in the central and eastern Arabian Sea at the end of Southwest Monsoon ( $340-1500 \times 10^6$  cells L<sup>-1</sup>) and Ducklow (1993) in the Northwest Indian Ocean ( $>1000 \times 10^6$  cells L<sup>-1</sup>). The occurrence of high densities of thraustochytrids and bacteria in this study suggests that the equatorial Indian Ocean might be a biologically productive region.

Thraustochytrids in the equatorial Indian Ocean often contributed significantly to the organic carbon pool like that of bacteria (Fig. 6, 8 and 10). Their biomass exceeded that of bacteria at certain stations. A similar observation was made by Raghukumar *et al.* (2001), who noticed that thraustochytrid biomass occasionally exceeded that of the bacterial

biomass during certain seasons and locations in the Arabian Sea. [Ramaiah et al. \(2005\)](#) also observed that thraustochytrid exceeded bacterial biomass during winter in Northern Arabian Sea.

Abundance, biomass and productivity are important indicators of the ecological role of bacteria in the water column and have been extensively studied for numerous geographical regions ([Ducklow, 2000](#); [Morán et al., 2004](#)). High densities of these organisms would result in enhanced rates of organic matter remineralization and will support active feeding and multiplication of microbivorous protists, thus sustaining the microbial loop ([Azam et al., 1983](#); [Lenz, 1992](#)). Thraustochytrids being similar to bacteria in their mode of nutrition may also play a role in remineralization of organic matter. They may also often play a major role as a feed for microbivorous protists. Thraustochytrid cells are characterized by the  $\omega$ -3 fatty acid, docosahexaenoic acid, a key essential fatty acid in the growth and maturation of crustaceans ([Veloza et al., 2006](#)). Zooplankton are believed to obtain their DHA from their prey and several studies have attempted to trace the source of DHA in the food web ([Veloza et al., 2006](#); [Alonzo et al., 2005](#)). Hence, the involvement of thraustochytrids in the microbial loop may have far reaching implications in the food web ([Naganuma et al., 1998](#); [Kimura et al., 1999](#)).

Standing biomass of microorganisms is the end result of their production and grazing by microzooplankton. One of the present constraints in understanding the ecological role of thraustochytrids is the

lack of a suitable technique to measure their productivity in natural samples. In contrast to the thymidine-incorporation method for bacteria (Fuhrman & Azam, 1982) and ergosterol synthesis rates in fungi (Newell, 2001; Newell *et al.*, 2000), no specific biochemical targets for estimating productivity of thraustochytrids has been found to date. Future studies are required to understand their productivity in natural environment.

Thraustochytrids were patchily distributed in the water column of the equatorial Indian Ocean. Unlike bacteria, which were always present in the water column, thraustochytrid populations varied widely, from levels below detection to hundreds of thousand cells per liter even at a single location. This suggests that they might be associated with particles or aggregates. 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. This prompted the study of TEPs in the equatorial Indian Ocean during October 2004 (cruise # SK 212) and September 2006 (cruise # SK 228). The two groups were occasionally, but not always related to TEPs in the water column. Bacteria and thraustochytrids, which



were significantly related to each other in 5 of the 9 stations during October 2004 also showed a positive relation to TEPs; thraustochytrids in 4 of the 9 and bacteria in 3 of the 9 stations (Table 3). Since thraustochytrids produce a variety of degradative enzymes and may decompose refractory organic substrates (Bongiorni *et al.*, 2005b), they might act upon or survive on the refractory material present in TEPs and marine aggregates. Even in those stations where no positive relation to TEPs was observed, the distribution patterns of bacteria and thraustochytrids were similar, however being displaced by about 20 m downwards in the water column (Fig. 6). This could be explained as follows. Although bacteria attached to marine snow cause a rapid enzymatic hydrolysis of the material, their carbon demand is low. Most of the dissolved organic matter (DOM) therefore diffuses into the surrounding waters as a plume trailing the increasingly refractory sinking particles (Aldredge & Youngbluth, 1985; Cho & Azam, 1988; Simon *et al.*, 1990; Smith *et al.*, 1992; Kiørboe, 2001; Engel *et al.*, 2004). High abundance of bacteria and thraustochytrids nearly 20 m above peak TEPs concentrations at some of the stations might reflect their active growth in such plume.

In contrast to October 2004 (SK 212), no relation between TEPs on the one hand and bacteria and thraustochytrids on the other were seen during September 2006 (SK 228). Such varying relationship between TEPs, bacteria and thraustochytrids suggests that the chemistry of TEPs

might be important, rather than the total amount of it. 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). These processes would have an influence on the chemistry of TEPs, which will have to be addressed to gain a greater understanding of the role of microbes attached to them.

#### **4.5 Conclusions**

Thraustochytrids were present in high abundance in the oceanic waters of the equatorial Indian Ocean. Their numbers frequently reached levels of several hundred thousands of cells per litre water, similar to those found earlier in the Arabian Sea and Japan Sea. The high densities of thraustochytrids and bacteria in the equatorial Indian Ocean suggest that the equatorial Indian Ocean region might be biologically productive. Since their population varied considerably during different seasons, they appear to be seasonally variable. Bacteria and thraustochytrids showed distinct vertical stratification in their abundance, mostly being confined to the mixed layer. However, high numbers were also occasionally seen below 200 m. Bacteria and thraustochytrids revealed varying relationships between them. They were highly positively related during October 2004, but not at other times. Thraustochytrids were distributed patchily in the water column, suggesting their possible dependence on particulate organic matter or TEPs. TEPs were studied in two cruises

during October 2004 and September 2006. Thraustochytrids and bacteria were positively related to TEPs in some, but not all the stations sampled. This varying relationship with TEPs suggest that the chemistry of TEPs might be an important factor that decides the growth of thraustochytrids and bacteria on them.