

Spatial variability of microzooplankton in the central Arabian Sea during spring intermonsoon

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Abstract

Spatial variability of microzooplankton (MZP; organisms in size 20-200 μm) along with total heterotrophic bacteria (THB) and picophytoplankton in the upper 120m water column of the central Arabian Sea (AS) was studied during the spring intermonsoon period (March 2015). Six stations divided into 3 regions (northern, southern and central) were sampled along 68° E transect, between 9° N and 21° N. A total of 25 species of MZP were recorded corresponding to loricate ciliates (11 species), aloricate ciliates (2 species) and heterotrophic dinoflagellates (12 species). Along the transect, MZP total abundance that varied between 5 and 9 x 10³ org. m⁻³ was largely contributed by ciliates, especially loricate ciliates (30-62%) followed by heterotrophic dinoflagellates (23-26%). MZP counts were lower in the central region (SD \pm 0.4, 7-8 x 10³ org. m⁻³) than in the northern and the southern regions (SD \pm 0.7, 8-9 x 10³ org. m⁻³) of the Arabian Sea. Heterotrophic bacterial counts were higher in the northern (28-202 x 10³ cells ml⁻¹), Southern (16-92 x 10³ cells ml⁻¹) and lowest in the central (13-89 x 10³ cells ml⁻¹) region. In contrast, picophytoplankton showed higher counts in central (3- 54 x 10³ cells ml⁻¹) and southern (4-113 x 10³ cells ml⁻¹) regions and lowest in the north (1-45 x 10³ cells ml⁻¹). Strong positive coupling of MZP - THB (0.05 > p > 0.01) than that of MZP - picophytoplankton density and MZP-chlorophyll *a* concentration suggests the spatial scale variability and functioning of microbial loop in the central Arabian Sea during the spring intermonsoon period.

Keywords: Microzooplankton, Central Arabian Sea, Spring Intermonsoon, Picophytoplankton, Microbial loop.

Introduction

Microzooplankton (MZP) refers to a group of organisms defined by their size (20–200 μm), function and limited swimming ability (Dussart 1965). These are classified into two groups, mixotrophic and heterotrophic (Protozoans and Metazoans). Protozoans include dinoflagellates, ciliates, radiolarians and foraminifera, whereas metazoans contain rotifers, meroplankton and holoplankton larvae (Calbert and Alcaraz 2007). They are overall capable of exploiting picoplankton, which in turn are utilized by other large trophic components such as mesozooplankton (Stoecker and Capuzzo 1990). Their intermediary role between bacterioplankton and large mesozooplankton through grazing on bacterioplankton thus controls the bacterial community (Gifford and Dagg 1991; Azam et al. 1983; Bernard and Rassoulzadegan 1993). This is particularly important in stratified oligotrophic water, which is seasonally experienced in the study region in the central Arabian Sea. Majority of tropical ocean region experiences strong stratification seasonally and remains well mixed for the most part of the year (Cushing 1989). Thus, supporting two possible food chains in these waters (Gauns et al. 1996). Well mixed water column supports mesozooplankton through traditional food chain whereas in the strongly stratified oligotrophic water column, zooplankton switch over to “microbial loop” wherein MZP play a significant role (Gauns et al. 1996; Madhupratap et al. 1996). The central Arabian Sea is known to experience a variably stratified condition particularly during the intermonsoon (spring and fall) period; stronger in the northern than southern part (Prasanna Kumar 2000). The landlocked nature of AS in the north and opening of sea to the equatorial region in the south bring large scale seasonal variability in the dynamics of the mixed layer depth (MLD). During intermonsoon, this is a resultant of prevailing Rossby wave driven circulation in the southern part that lead to deep MLD and to the north increasing surface temperature and weak winds causes shallow MLD (Prasanna Kumar et al. 2005). Thus, in order to understand the response of microbial component (microzooplankton along with het. bacteria and picophytoplankton) to these physical changes, work was undertaken to study spatial pattern of MZP community composition and their numerical abundance in the upper 120m water column of central Arabian Sea during oligotrophic spring intermonsoon period of 2015.

Materials and Methods

Samples for phytoplankton biomass (chlorophyll *a*), microzooplankton (MZP), total heterotrophic bacteria (THB) and picophytoplankton were collected during the spring intermonsoon (March 2015) onboard R.V. Sindhu Sankalp (Cruise no. SSK79) covering transect between 9° and 21°N along 68°E. Upper 120m water column of this transect in the central Arabian Sea was sampled

at 6 stations; S1-9° N, S2-11° N, S3-13° N, S4-15° N, S5-17° N and S6-21° N (Fig.1). At every station, profiles of water temperature, salinity, dissolved oxygen (DO) and fluorescence were obtained with a Sea-Bird Electronics (SBE) 9plus CTD unit equipped with pre-calibrated sensors for DO and fluorescence.

Chlorophyll *a* was processed following JGOFS protocol (1994) wherein 1 L of water was filtered through 47-mm GF/F filters (0.7 µm pore size). The samples were incubated in dark for 24 h at -20° C for extraction purpose using 10 ml of 90% acetone and analysed by fluorometry (JGOFS 1994).

Sampling for MZP involved a slow vertical haul through a hydrographic winch to avoid cell damage using 20 µm mesh net (mouth diameter of 30 cm and length of 120 cm) within the upper 120 m covering euphotic depth of the water column. The volume of water filtered through the net was calculated using (Hydrobios - 438110) calibrated digital flow meter (Lasternas et al. 2011). Roughly ~ 3.7- 4 m³ of water was filtered through this process. Utmost care was taken while passing samples through 200µm to avoid/minimize damage to delicate forms of microzooplankton. Nonetheless, we caution reader that during this process there is fairly a good chance that some cells may have damaged leading to underestimation of total density. Processed sample was then immediately concentrated to ~500ml and preserved with 1% acid Lugol's iodine solution (Gauns et al. 1996). For MZP taxonomy, a known volume (1 ml from 500 ml) of concentrated sample was taken in a Sedgwick-rafter counting chamber and then the entire chamber was examined under an inverted microscope (magnification of 400X, Nikon Eclipse t-u). The MZP species were identified by using the standard identification keys (Hada 1938; Jyothibabu 2004).

Heterotrophic bacteria and picophytoplankton abundance were estimated using flow cytometer (BD FACS Calibur) equipped with a blue and a red laser, for which, 1.5ml of water samples were preserved with glutaraldehyde (0.2% final concentration) in cryovials, flash frozen in liquid nitrogen and stored at -80°C for laboratory analysis. The frozen samples were thawed to room temperature and then analysed on the flow cytometer. Autotrophic picophytoplankton were detected based on auto fluorescing and scatter properties of individual cells. While, heterotrophic bacteria were stained with SYBR green dye (1 part in 10000) which binds to the DNA and absolute counts were obtained using Cell Quest Pro software (Marie 1997).

Results

The hydrography of stations located in the central Arabian Sea along 68°E, transect between 9° N and 21° N implied variability in physicochemistry from north to south (Fig.2). The mixed layer depth (MLD) was relatively deep in the north (21° N; MLD ~30m) and shallow in the south (9° N;

MLD ~9m) region. The water temperature and salinity in upper 120 m column ranged between 21.6-27.3°C; 36-36.6 psu in the north, 16.3-28.4°C; 35.5-36.4 psu in central and 20.9-29.9°C; 36-38.6 psu in southern regions. A gradual increase in temperature was observed from north to south. However, the DO values decreased from north to south and ranged between 0.7-4.3 ml/L in the north, 0.05-4.2 ml/L in central and 0.96-4.1 ml/L in southern regions.

In addition, phytoplankton biomass (Chlorophyll *a*) in upper 120 m varied between 0.02 and 0.75 µg/L in the northern region, 0.01-0.65 µg/L in the central region and between 0.01-0.71 µg/L in the southern region. Overall, the trend observed in the chlorophyll distribution showed high concentration in north and least in the central region. Depth-integrated (0-120m) chlorophyll *a* ranged between 14 and 18 mg m⁻² (Fig.3). The sampling stations were hence grouped based on the above physicochemical conditions into three regions to emphasize biological variations (in MZP, THB and picophytoplankton): southern region (included stations S1 and S2; 9° to 11° N), central region (stations S3 and S4; 13° to 15° N) and the northern region (stations S5 and S6; 17° to 21° N).

Microzooplankton abundance

The abundance of MZP varied in the range of 5-9 x 10³ org. m⁻³ (Fig.3). The northern region displayed high abundance (8-9 x 10³ org. m⁻³; Avg. 9 x 10³ org. m⁻³) followed by southern region (7-8 x 10³ org. m⁻³; Avg. 8 x 10³ org. m⁻³). The lowest abundance of MZP was observed in the central region (5-6 x 10³ org. m⁻³; Avg. 5 x 10³ org. m⁻³).

Microzooplankton composition

The MZP community was represented by three different groups corresponding to ciliates (loricate and aloricate), heterotrophic dinoflagellates and copepod nauplii. A total of 25 species of MZP were identified from the region, of which 11 species belonged to loricate ciliates, 2 species to aloricate ciliates and 12 species to heterotrophic dinoflagellates (Table.1). The dominant species were identified as loricate ciliates (*Streenostrupiella* sp, *Dadayiella gaymedes*, *Salpingella* sp and *Eutintinnus lusus undae*) and heterotrophic dinoflagellates (*Protopteridinium breve*, *Podolamphus* sp and *Protopteridinium latistriatum*).

In all the regions, MZP composition was majorly contributed by loricate ciliates (44-62%; Avg. 40-53%) followed by copepod nauplii (11-33%; Avg. 22-30%) and heterotrophic dinoflagellates (23-27%; Avg. 24-28%). Whereas the aloricates were found only in the central region with minor (3%) contribution to the MZP composition (Fig.4).

Diversity indices

In the upper 120 m water column, the number of MZP species (S) varied between 17 and 22. The number of species recorded in the southern region was 22, northern region was 20 and central region was 17. Species diversity (H') at southern region (2.7) was higher than north (2.5) and central (2.3) regions. Evenness (J) value at southern region (0.9) was higher than north and central (0.8) regions. Whereas, no variation in species richness (d) was observed between regions (0.1).

Heterotrophic bacteria and picophytoplankton

Total heterotrophic bacterial (THB) showed higher abundance in the northern ($28-202 \times 10^3$ cells ml^{-1}) region, lower in the southern ($16-92 \times 10^3$ cells ml^{-1}) and the central region ($13-89 \times 10^3$ cells ml^{-1}) regions. The column integrated (0-120 m) cell abundance of THB ($\times 10^{12}$ cells m^{-2}) followed similar trend wherein abundance varied in the range of 7-16 in the north, 5-8 in southern and 2-6 in central regions.

Picophytoplankton cell abundance in the water column varied from 1×10^3 cells ml^{-1} to 113×10^3 cells ml^{-1} . Cell counts were lower in the northern region ($1-45 \times 10^3$ cells ml^{-1}), higher in the southern ($4-113 \times 10^3$ cells ml^{-1}) and central ($3-54 \times 10^3$ cells ml^{-1}) regions. The column integrated (0-120 m) cell abundance of picophytoplankton ($\times 10^{12}$ cells m^{-2}) varied in the range of 2-3 in the north, 2-5 in southern and 1-3 in central regions.

Discussion

In the present study, MZP community in the upper 120 m of the central Arabian Sea was mostly dominated by loricate ciliates (30-62%) followed by heterotrophic dinoflagellates (23-33%). Loricate ciliates are known to dominate the MZP community in coastal and open ocean waters due to their euryhaline and eurythermal nature (Sanders 1987; Saab 1989; Edwards 1995; Leakey et al. 1996; Godhantaraman 2001). Likewise, the dominance of heterotrophic dinoflagellates is possibly due to their adaptive nature to different modes of feeding (mixotrophic) which compete with other predators for prey (Jyothibabu et al. 2003). Density profile of MZP revealed that southern and northern regions of central Arabian Sea were denser ($8-9 \times 10^3$ org. m^{-3}) than the central region ($5-6 \times 10^3$ org. m^{-3}). During the intermonsoon, the MZP biomass in the Arabian Sea ranged between 0.4 and 7.6 mg C m^{-3} (Stelfox et al. 1999) and the higher biomass found to be in the northern (0.43 g C m^{-2}) and southern (0.25 g C m^{-2}) regions (Dennett et al. 1999). The Arabian Sea is a tropical ocean which is characterized by a restricted circulation and seasonal changes by monsoon effect (Leakey et al. 1996). During intermonsoon, southern Arabian Sea harbours deep mixed layer due to Rossby

wave propagation (Prasanna Kumar et al. 2005), whereas the northern Arabian Sea experiences increasing sea surface temperature and weak winds which lead to shallow mixed layer depth and less primary production controlled by availability of nutrients in the surface water (Prasanna Kumar et al. 2005). However, in the present study, this trend was reverse with deep MLD in the north and shallow in the south suggesting a prolonged winter season during the study period.

Mixed layer depths are known to control the biological productivity in the ocean through the availability of nutrients and light (Jang et al. 2011). In our study, depth-integrated chlorophyll *a* value showed a negative correlation with MZP density (Fig.3). The low density of MZP during high chlorophyll condition could be attributed to the phytoplankton species composition which is probably dominated by larger chain-forming and colonial diatoms such as (*Chaetoceros* sp.) followed by an inability of MZP to graze on this larger phytoplankton. A similar observation was recorded during diatom bloom in the East China Sea and Andaman Coastal waters (Lee and Kim 2010; Elangovan et al. 2012). In earlier studies, the phytoplankton composition from the same region are well reported with the chain forming species such as *Chaetoceros* sp, *Thalassiosira* sp, *Rhizosolenia* sp and *Bacillaria* sp are most dominant (up to 90%) in the coastal and open ocean of Arabian Sea during the intermonsoon season (Sawant and Madhupratap 1996; Devi et al. 2010; Padmakumar et al. 2017). MZP are known to feed on suspended detritus and its associated bacterial microflora (Moritz et al. 2006). On the other hand, ciliates consuming bacteria (several folds higher than flagellates) have been shown previously through grazing studies (Epstein and Shiaris 1992), a study from Eastern Bering Sea reported that in early spring season MZP do not increase in abundance until phytoplankton biomass is high enough to support their growth (Diane et al. 2014).

The present data shows that MZP density is positively correlated ($0.05 > p > 0.01$) with column integrated value of THB ($p=0.013$) and picophytoplankton ($p=0.011$) (Fig.5). Besides, Dennett et al (1999) reported that biomass of heterotrophic nanoflagellates (HNF) was higher in the northern (0.49 g cm^{-2}) and southern (0.43 g cm^{-2}) region during intermonsoon. The high bacterial abundance in the southern and northern regions could be due to the availability of total organic carbon (TOC) in the water column which ranged between $90 \mu\text{M}$ in the north and $77 \mu\text{M}$ in the south and lower ($60 \mu\text{M}$) in the central region (Suhas Shetye, unpublished data). Work of Hansell *et.al* (1998) earlier reported a similar trend of TOC during the spring intermonsoon in the range of $>80 \mu\text{M}$ from northern and $75\text{-}90 \mu\text{M}$ from the central and southern regions. Bacterial abundance is not related to seasons of high primary production but it depends on dissolved organic carbon (DOC) in the mixed layer depths (Ramaiah et al. 1996). DOC is present in the form of concentrated carbon for heterotrophic bacterial growth and respiration (Raymond and Bauer 2000 & 2001). Moreover, total

dissolved organic matter (DOM) also has a high ratio of carbon and nitrogen for DOC fluxes which could facilitate bacteria to compete with phytoplankton for inorganic N and P (Steelink 1985). In oligotrophic waters, a combination of picophytoplankton and heterotrophic bacteria biomass could signify a considerable part of the total particulate organic carbon (Li et al. 1992). In the surface layer, MZP play a major role in remineralisation of the particulate organic materials (Sanders and Wickham 1993) and act as a trophic intermediate between pico-nanoplankton and mesozooplankton (Gifford 1991).

Conclusion

During spring intermonsoon, the average abundance of THB community was higher in the southern and northern regions compared to the central region of the Arabian Sea and it correlated well with MZP density and picophytoplankton abundance. These results indicate a spatial variability in MZP community composition, food chain relationship and functioning of microbial loop in the Arabian Sea. Further, the interactions within the members of the microbial loop need to be studied for better understanding their spatial scale role in biochemical fluxes.

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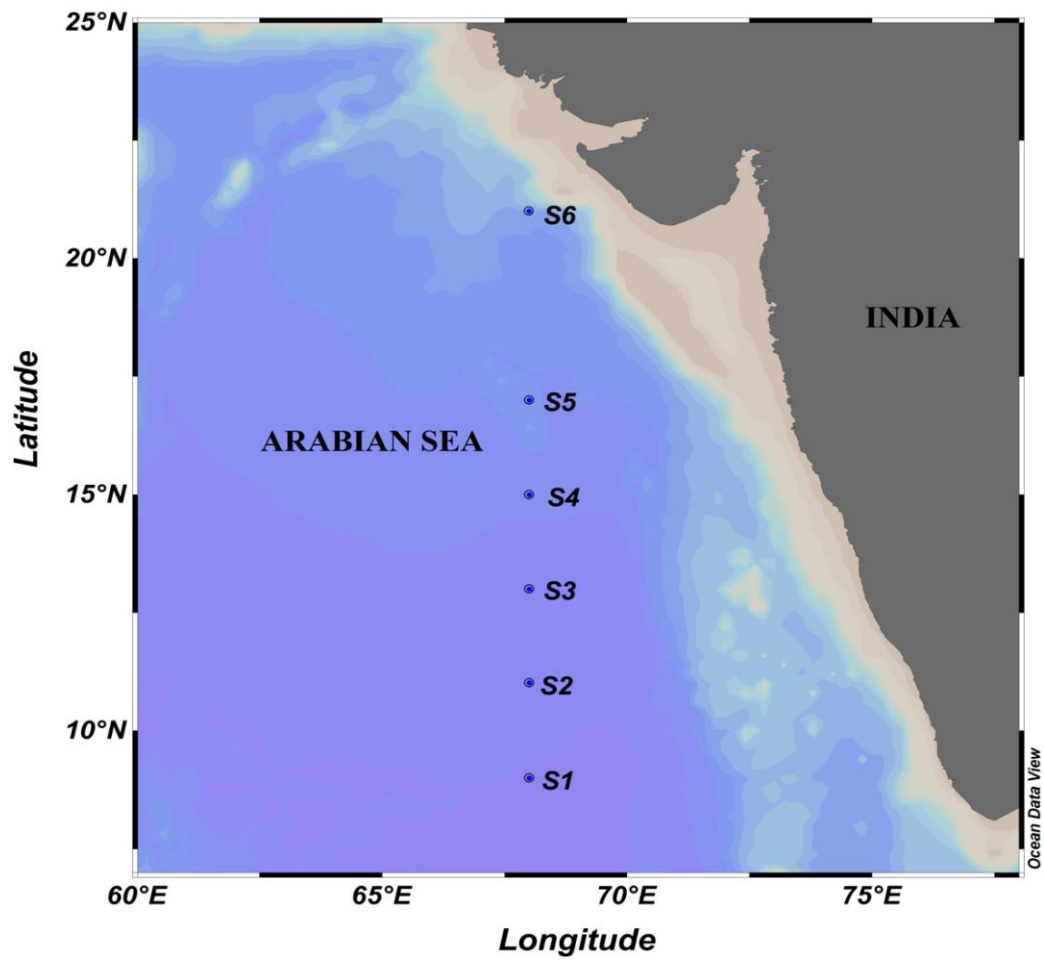


Fig.1: Map showing station locations in the central Arabian Sea

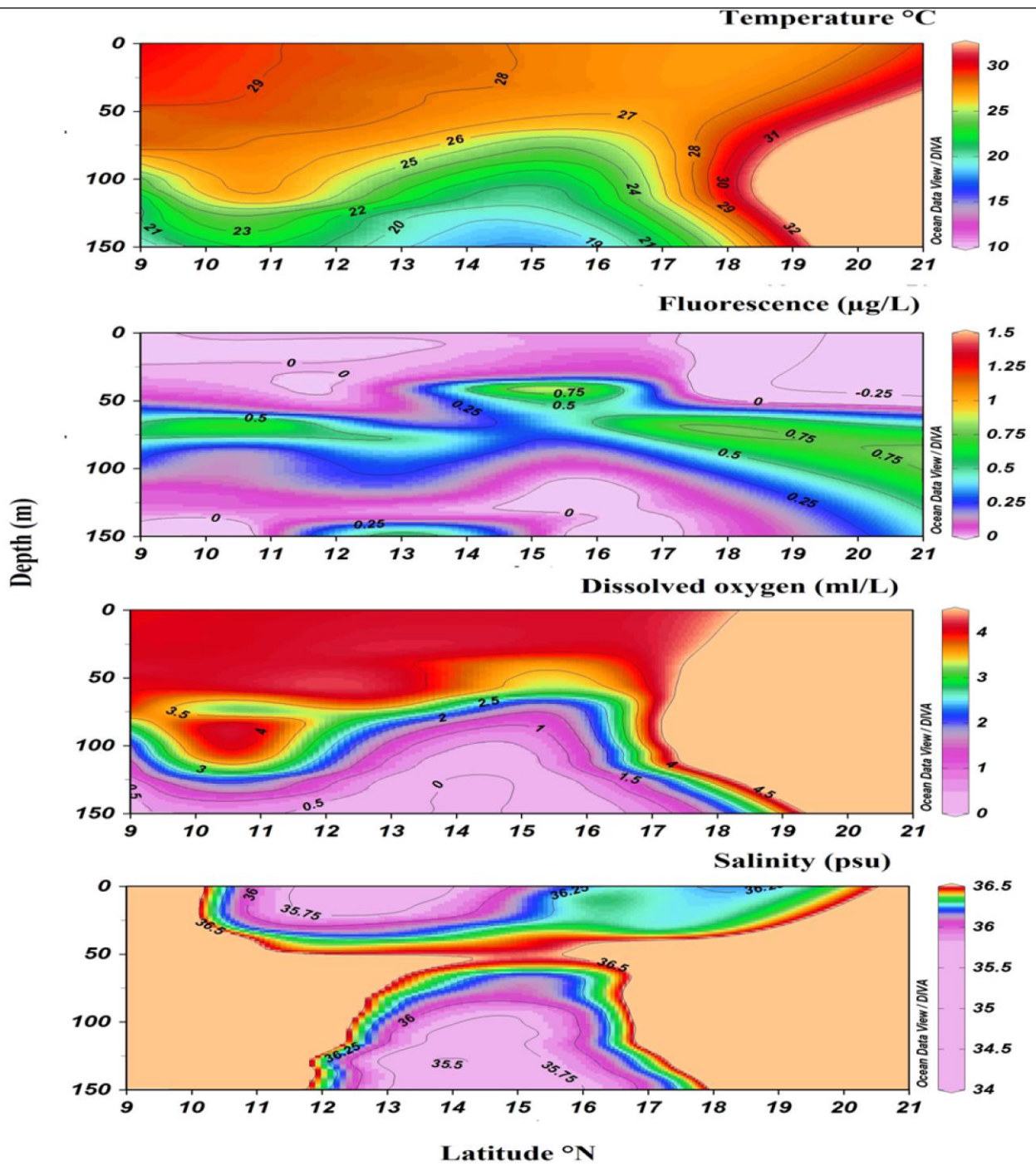


Fig.2: Vertical profiles of temperature (°C), salinity (psu), Do (ml/L) and fluorescence (µg/L) along the longitude 68° E transect (9° and 21° N).

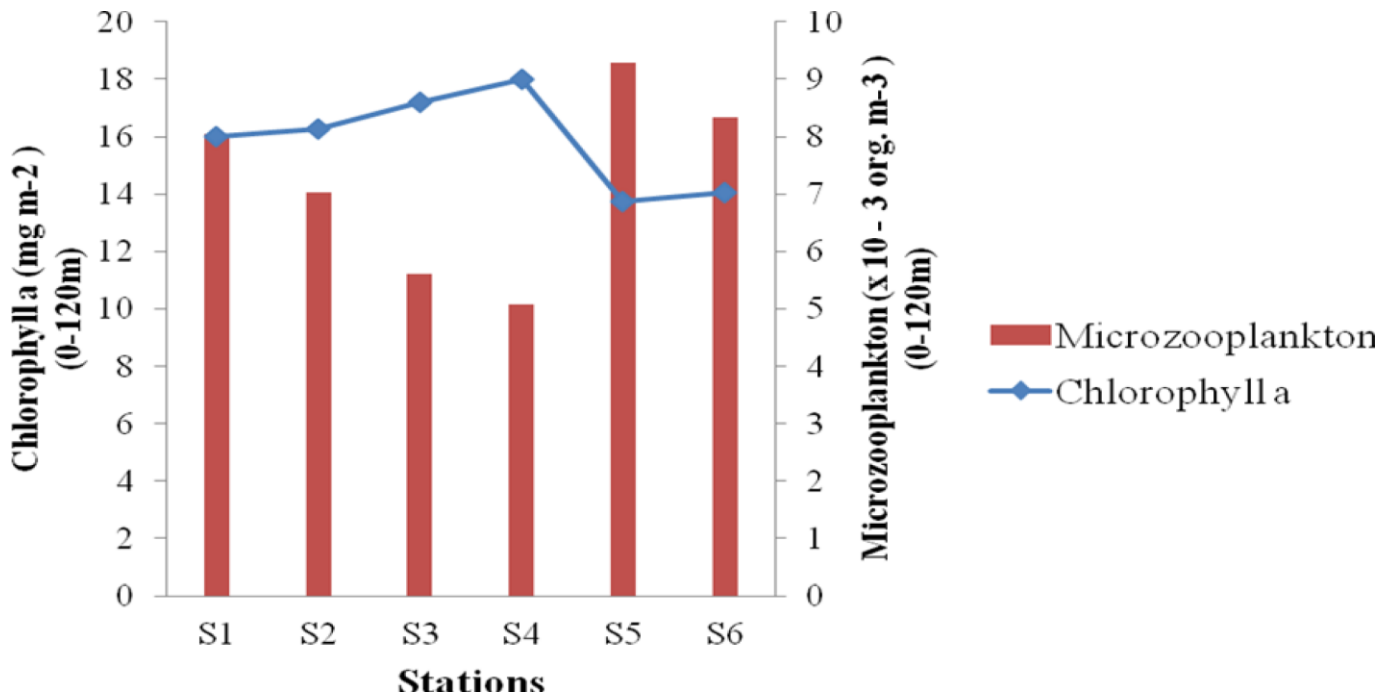


Fig.3: Spatial variation in microzooplankton density in abundance and depth integrated Chlorophyll

a.

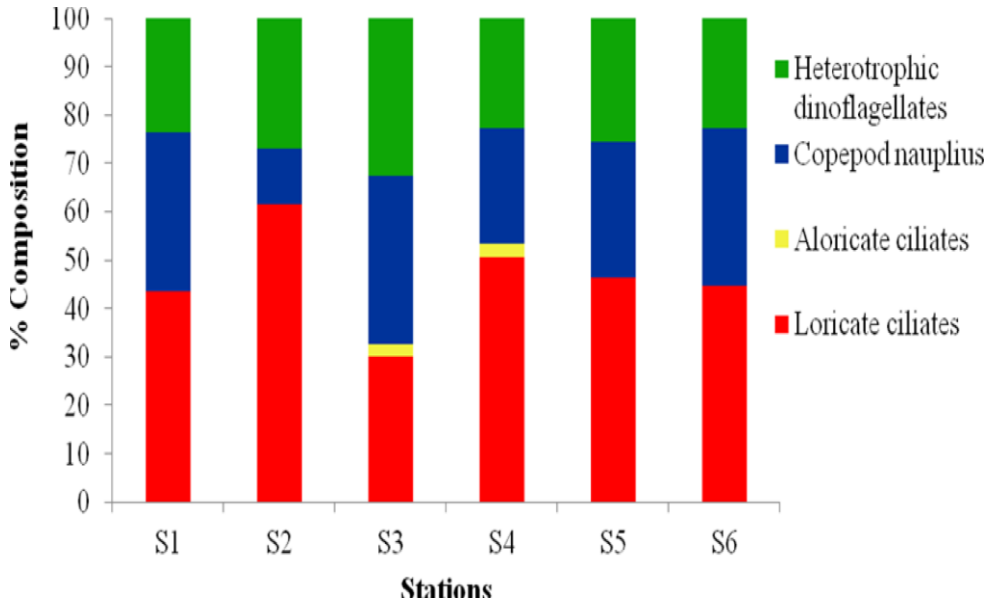


Fig.4: Percentage composition of Microzooplankton in the central Arabian Sea.

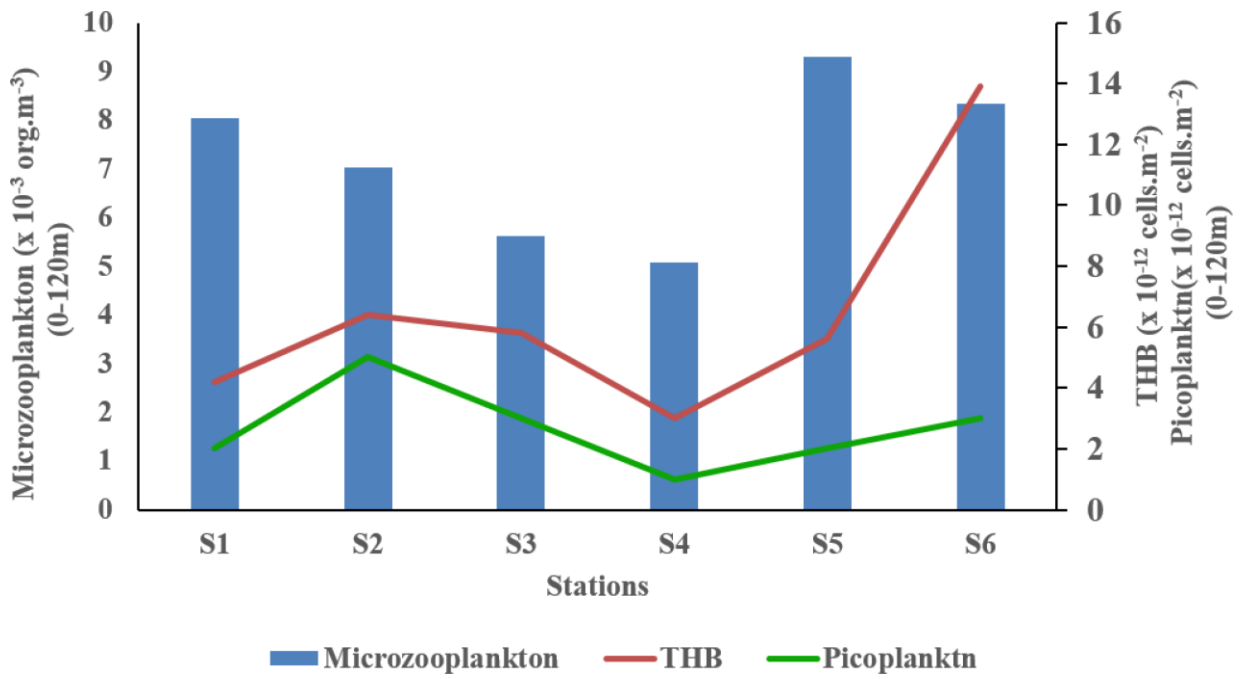


Fig.5: Depth integrated value of Microzooplankton, THB and Picophytoplankton in the upper 120m water column of central Arabian Sea.

Table.1. List of Microzooplankton species from the central Arabian Sea (0-120m) during spring intermonsoon

Microzooplankton (Org.m⁻³)	S1	S2	S3	S4	S5	S6	
Loricata ciliates							
<i>Amphorella acuta</i>	68	541	68	68	757	108	
<i>Ascambelliella</i> sp	0	0	0	0	0	162	
<i>Codonellopsis</i> sp	68	135	0	0	0	162	
<i>Codonellopsis morchella</i>	0	0	0	0	0	54	
<i>Codonellopsis ostenfeldi</i>	270	0	0	0	0	0	
<i>Dadayiella gaymedes</i>	338	541	68	135	973	865	
<i>Dictyocysta</i> sp	0	135	68	0	216	162	
<i>Epiplocyclus undella</i> sp	203	270	0	0	0	54	
<i>Epiplocyclus</i> sp	135	0	0	0	0	0	
<i>Eutintinnus lusus undae</i>	135	811	68	811	216	54	
<i>Metacylis</i> sp	68	135	0	68	0	432	
<i>Proplectella perpusilla</i>	338	135	0	0	0	108	
<i>Protorhabdonella simplex</i>	405	135	203	203	324	486	
<i>Rhabdonella elagans</i>	135	811	68	68	108	0	
<i>Rhabdonella poculum</i>	0	0	0	0	0	216	
<i>Rhabdonella spiralis</i>	68	135	0	0	432	270	
<i>Salpingella</i> sp	676	270	338	473	216	108	
<i>Stenosemella</i> sp	0	0	0	0	0	162	
<i>Streenostrupiella</i> sp	405	270	811	473	432	0	
<i>Xystonellopsis</i> sp	203	0	0	270	649	324	
Aloricata ciliates							
<i>Lohmaniella spiralis</i>	0	0	0	135	0	0	
<i>Strombidium conicum</i>	0	0	135	0	0	0	
Heterotrophic dinoflagellates							
<i>Dinophysis apicata</i>	405	270	68	0	216	0	
<i>Dinophysis caudate</i>	135	0	0	0	0	0	
<i>Gymnodinium</i> sp	0	0	68	0	0	0	
<i>Ornithocerus magnificus</i>	135	0	68	135	324	0	
<i>Ornithocerus thumii</i>	0	0	0	135	0	0	
<i>Podolamphus</i> sp	473	541	541	338	216	162	

<i>Protopeperidinium breve</i>	405	270	135	270	757	865	
<i>Protopeperidinium depressum</i>	0	0	0	0	0	270	
<i>Protopeperidinium divergens</i>	135	0	0	0	216	0	
<i>Protopeperidinium granii</i>	68	0	68	0	0	216	
<i>Protopeperidinium latistriatum</i>	135	270	676	270	0	108	
<i>Protopeperidinium longicollum</i>	0	135	0	0	0	0	
<i>Protopeperidinium ovatum</i>	0	270	0	0	0	216	
<i>Protopeperidinium stenii</i>	0	135	203	0	649	54	
Copepod nauplii	2635	811	1959	1216	2595	2703	