

## **Picophytoplankton community in a tropical estuary: Detection of *Prochlorococcus*-like populations**

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### **ABSTRACT**

In order to understand the influence of hydrography on the picophytoplankton (PP) abundance in estuaries, sampling was conducted along a salinity gradient for the first time in an Indian estuary. *Prochlorococcus*-like cells were detected at salinities ranging from 0.06 to 35, which otherwise is reported from offshore regions, thereby showing that this group is capable of surviving in estuarine waters. PP also comprised picoeukaryotes and two groups of *Synechococcus*, one rich in phycoerythrin and other in phycocyanin. Salinity played an important role in the picophytoplankton distribution. *SYN-PE* was represented by two sub-groups, one which was found only in saline waters (*SYN-PEII*) and the other throughout the salinity gradient (*SYN-PEI*). *SYN-PEI* and *SYN-PC* dominated downstream and upstream, respectively but were present throughout the salinity gradient unlike in other estuarine regions. Picoeukaryotes abundance showed an increasing trend from saline to brackish water and decreased in freshwater. The entry of sea water into the estuary regulated *SYN-PE* and *Prochlorococcus*-like cells downstream whereas their higher abundance in freshwater could be due to different strains of freshwater origin. The average contribution of picophytoplankton to the total photosynthetic biomass during spring and neap tides was 43% and 29%, respectively which highlights the importance of picophytoplankton in estuaries.

*Keywords:* Zuari estuary; Neap tide; Picophytoplankton; *Prochlorococcus*-like cells; Spring tide; *Synechococcus*

*Regional Index terms:* India, Goa, Zuari estuary

## 1. Introduction

It is a well known fact that picophytoplankton (PP; < 2 $\mu$ m), the smallest group representing the phytoplankton community forms an integral part of the marine microbial food web contributing substantially to primary production especially in oligotrophic ecosystems (Campbell et al., 1998). PP serves as food for many protists, large zooplankton and small invertebrates (Richardson and Jackson, 2007; Wilson and Steinberg, 2010). This acts as a pathway for the transfer of PP carbon to the higher trophic levels as well as its vertical flux to the deep sea. PP comprises of two cyanobacteria, *Prochlorococcus* and *Synechococcus* and the picoeukaryotes. Discovery of *Prochlorococcus* (Chisholm et al., 1988) was a breakthrough research in biological oceanography since this is the smallest known phototrophic organism that is capable of flourishing in the oligotrophic regions and the only dominant photosynthetic group found in the waters as deep as 150 to 200 m (Partensky et al., 1999). *Synechococcus* and picoeukaryotes are abundant in coastal waters as compared to oceanic regions (Pan et al., 2005). Although *Prochlorococcus* is most abundant in oligotrophic waters relative to the other photosynthetic populations, it is by no means restricted to nutrient depleted waters (Partensky et al., 1999). Also this group was thought to be a truly oceanic species, but there are few reports which show their presence in the estuaries, bay and riverine regions (Vaulot et al., 1990; Shimada et al., 1995; Shang et al., 2007; Mitbavkar et al., 2009). However, there is still a question about the existence/origin of this group in such areas as to whether they are passively advected from the open oceans or they are capable of growth in these areas.

While the importance of PP is well documented in open oceans, their role in coastal waters is not yet well established, especially in the tropical estuaries (Moran et al. 2007). Moreover, no information is available from the estuaries in the Arabian Sea, although a lot of information is available on the larger groups such as diatoms and dinoflagellates (Patil and Anil, 2008). The objective of this study was to characterize the PP in the Zuari estuary in order to develop an understanding of their prevalence and distribution along a salinity gradient.

## 2. Materials and methods

The Zuari estuary is a tide-dominated coastal plain estuary located in Goa, on the west coast of India and joins the Arabian Sea in the North Indian Ocean. The main channel of this estuary is about 50 km long and its cross-sectional area decreases towards the upstream regions (0.5 km) with a width of ~

5.5 km at the mouth (Shetye et al., 1995) and an average depth of 5 m (Sundar and Shetye, 2005). The main estuarine channel receives freshwater from a number of streams and rivers (Shetye, 1999). At about 11 km from the mouth, this estuary is joined by the Cumbarjua canal. The general flow pattern in this canal is regulated by the entry of seawater during the incoming tide and during the outgoing tide the flow in the canal is reversed (Qasim and Sen Gupta, 1981). In this way the estuarine system gets flushed. Compared to the monsoon season (June to September), November to May is a dry season when the estuary receives comparatively less river discharge and is vertically well mixed throughout the length and the tidal influence reaches up to 50 km from the mouth (Shetye et al., 1995). The increase in elevation of the estuarine channels prevents tides from propagating beyond this distance. Tides in these estuaries are of the mixed semidiurnal types which occur twice a day raising and lowering the water level by about 2 m and the tidal ranges are about 2.3 and 1.5 m during the spring and neap tides, respectively. Circulation in the channels is forced by oscillatory tidal flow at the mouth and by river discharge at the head (Shetye, 1999).

Ten stations along the estuary were sampled during December 2009 over spring tide (ST; 04 December) and neap tide (NT; 29 December) (Table 1). Samples were collected from the surface (S) and near-bottom (NB) from S1 in the morning subsequently proceeding towards S10. At each station a secchi disk was used to estimate the euphotic zone thickness. Temperature and salinity were recorded in the water column using a conductivity-temperature-depth (CTD) probe. Phytoplankton biomass was estimated as total chlorophyll *a* concentration by filtering 500 ml of seawater through GF/F which was then extracted in 90% acetone overnight and analyzed with fluorometry (Parsons et al. 1984). Water samples for PP analysis were fixed in paraformaldehyde (0.2% final concentration), preserved in liquid nitrogen and later stored at -80°C until analysis.

For the PP analysis, a BD FACSAria™ II flow cytometer equipped with a blue (488 nm) and a red laser (633 nm) which can distinguish the red fluorescence excited by blue light (produced by chlorophyll) and red light (produced by phycocyanin) was used. Emitted light was collected through the following set of filters: 488/10 band pass (BP) for right angle light scatter (RALS; proxy for cell size), 575/26 BP for orange fluorescence, 695/40 and 660/20 BP for red fluorescence from chlorophyll (blue laser) and phycocyanin (red laser), respectively. Fluorescent beads (2 µm for PP “Fluoresbrite”, polysciences) were used as internal standards and for calibration of the above parameters.

### **Insert Table 1 here**

Flow cytometry data was processed with the BD FACS Diva software. PP groups were discriminated according to their specific autofluorescence properties and RALS differences. The cellular carbon content for the different PP groups was based on the cell biovolume, estimated by the calibration method of Worden et al. (2004), assuming the PP cells to be spherical. A biovolume to carbon conversion factor of 254 fg C  $\mu\text{m}^{-3}$ , 240 fg C  $\mu\text{m}^{-3}$  and 239 fg C  $\mu\text{m}^{-3}$  for *Synechococcus* (Baudoux et al., 2007), *Prochlorococcus*-like cells and picoeukaryotes (Worden et al. 2004), respectively was used.

Linear regression was performed between the PP abundance, temperature and salinity. In order to determine the contribution of PP to the total phytoplankton biomass, the latter was calculated from chl *a* using a carbon-to-chlorophyll ratio of 40 (Gallegos 2001).

### **3. Results**

During ST and NT, sampling started at S1 during flood and ebb tide, respectively. During ST, by the time S5 was sampled the tide had started receding and water from the Cumbarjua canal entered the estuary resulting in a temperature and salinity change from this point onwards (Table 1). From S9, lowest water temperature and salinity were detected due to discharge from the Kushavati River. The estuary was occupied by saline, brackish and freshwater during ST from S1-S5, S6-S8 and S9-S10, respectively and during NT from S1-S3, S4-S8 and S9-S10, respectively.

PP were encountered throughout the estuarine transect during both tides and comprised of *Prochlorococcus*-like cells (*PRO*-like) identified based on their smaller RALS and red autofluorescence, two groups of *Synechococcus*, one rich in phycoerythrin (*SYN*-PE) and the other rich in phycocyanin (*SYN*-PC), identified based on their orange and red autofluorescence, respectively and picoeukaryotes (PEUK) identified based on their larger RALS and red autofluorescence. The group of cells with a higher red autofluorescence than the PEUK was identified as nanoeukaryotes (NEUK) (Annex Table 1; Fig. 1).

### **Insert Fig. 1 here**

*SYN*-PE group comprised of two sub-groups, one with a lower PE fluorescence (*SYN*-PEI) than the other (*SYN*-PEII). The latter group was observed only up to S3 or S4 (Fig. 1). Their contribution to the

total PP abundance during both the tides ranged from 7 to 39% (Fig. 2). *SYN-PEI* showed a decreasing trend up to S8 during both tides and increased from S9 to S10. A significant variation was observed between the surface and NB abundance during both the tides ( $P \leq 0.01$  and  $0.001$ , respectively). Higher abundance was observed in saline and freshwater. This group dominated during ST at S1, S2 and S4 and during NT at S1 to S5 and S10. Their contribution to the total PP abundance ranged from 35 to 75% in the saline waters during ST and 58 to 95% during NT. The percentage contribution decreased in brackish water and freshwater (5% to 39%) during both the tides except at S10 during NT. *SYN-PC* abundance increased from seawater to freshwater stations during both tides. The variations in abundance during both the tides were not significant and their contribution to the total PP abundance was low in saline waters (up to S5; 3 to 30%) and increased from brackish to freshwater (34 to 75%) except at S10 during NT (15%). A clear spatial pattern was evident in the distribution of *SYN-PC* and *SYN-PE* cells, where they were more abundant upstream and downstream, respectively. There was a sharp transition in dominance from *SYN-PE* to *SYN-PC* at salinities  $< 30$  during ST and at 25 during NT (Annex Fig. 2).

**Insert Fig. 2 here**

*PRO*-like cells showed significant variations between the surface and NB abundance ( $P \leq 0.025$  and  $0.005$ , respectively). During ST, their contribution to the total PP abundance ranged from 35 to 46% and 24 to 27% where salinity was  $>31$  and  $<0.5$ , respectively, except at S4 (6-5%). At S7 and S8 (salinity: 8-18), their concentrations were very low, representing  $<4\%$  of the total. During NT, their contribution to total was comparatively low in saline waters (0.7-2%) and in brackish waters (2.8-3.7%) whereas in freshwaters it was higher (10-14%). Although this group was present at salinities ranging from 35 to 0.06, for the marine samples, comparatively higher values of RALS (0.042-0.11) and chlorophyll fluorescence (0.02-0.21) were observed than for the brackish and freshwater (0.006-0.045 and 0.022-0.149, respectively).

PEUK abundance showed an increasing trend from saline to brackish water and decreased in freshwater. Highest abundance was found at S4, contributing 33% to the total PP abundance. The variations in surface and NB abundance during both the tides were not significant. NEUK abundance was higher in brackish waters than in saline and freshwaters. Their abundance in the surface waters was similar to that of PEUK. Highest abundance was found at S6, contributing 28% to the total PP

abundance. During ST, PP contribution to the total photosynthetic biomass ranged from 11 to 85% whereas during NT it ranged from 7 to 60% (Fig. 3).

### **Insert Fig. 3 here**

During ST, temperature showed a significant negative correlation with *SYN*-PEI ( $r^2 = 0.61$ ,  $P < 0.001$ ) and *PRO*-like cells ( $r^2 = 0.36$ ,  $P < 0.01$ ) while with PEUK ( $r^2 = 0.19$ ,  $P \leq 0.05$ ) and NEUK ( $r^2 = 0.24$ ,  $P < 0.05$ ) it showed a positive correlation. Salinity showed a significant positive correlation with *SYN*-PEII ( $r^2 = 0.27$ ,  $P < 0.02$ ) and negative correlation with *SYN*-PC ( $r^2 = 0.87$ ,  $P < 0.001$ ). During NT, salinity showed a significant negative correlation with abundance of *PRO*-like cells ( $r^2 = 0.66$ ,  $P \leq 0.001$ ), *SYN*-PC ( $r^2 = 0.59$ ,  $P < 0.001$ ), PEUK ( $r^2 = 0.81$ ,  $P < 0.001$ ) and NEUK ( $r^2 = 0.63$ ,  $P < 0.001$ ) whereas it showed a positive correlation with *SYN*-PEII ( $r^2 = 0.41$ ,  $P < 0.01$ ). Temperature also showed a significant negative correlation with *PRO*-like cells ( $r^2 = 0.63$ ,  $P < 0.001$ ), PEUK ( $r^2 = 0.3$ ,  $P < 0.01$ ) and NEUK ( $r^2 = 0.47$ ,  $P < 0.001$ ).

## **4. Discussion**

Since sampling at S1 started when the ST and NT were at its peak, the samplings at the subsequent stations were not in synchronization with the tide. Hence the samples, especially from upstream were not typical for the tide assigned. The estuarine circulation played an important role in structuring the community in estuaries because of their dynamic nature as compared to open oceans. Clustering of PP abundance data showed that under calm condition (NT) the estuary was less disturbed as compared to the ST (Annex Fig. 3). There was a clear spatial gradient in the distribution of the PE-rich and PC-rich cells with the former more abundant downstream and the latter upstream. Such a distribution pattern has also been reported in other estuaries. Murrell and Loes (2004) reported that the *SYN*-PC cells were an order of magnitude higher in abundance at the upstream end of the estuary to *SYN*-PE which decreased dramatically as salinity changed from  $\sim 20$  to 28. The PC:PE ratio of  $\sim 11$  observed in the Zuari estuary illustrates the importance of *SYN*-PC in estuarine systems. Although it is recognized that *SYN*-PC cells are not observed in oceanic systems, little attention has been accorded to the effect of salinity on the distribution of *SYN*. Waterbury et al., (1986) reported that *SYN*-PE cells have an obligate requirement for elevated concentrations of ions while marine *SYN* isolates that lack PE are halotolerant and grow equally well in seawater or freshwater. However, in this study we observed two sub-groups of *SYN*-PE, one which is found only in saline waters and the other throughout the salinity gradient, although in low

numbers in the brackish waters. Also, although a shift in dominance was observed between *SYN*-PEI and *SYN*-PC groups where each of these groups had a separate ecological niche, they were present throughout the salinity gradient and did not completely disappear as reported in some estuarine regions (Haas and Pearl, 1988). The PC:PE ratio shows that while the upstream end of the estuary is the source for *SYN*-PC, for *SYN*-PEI it is the downstream end. The significant negative correlation observed between *SYN*-PC and salinity was consistent with the observation of an increasing PC:PE ratio progressing upstream which showed that low salinity favored the growth of *SYN*-PC. However, such a significant negative relation was not observed between *SYN*-PEI and salinity because its abundance declined only in brackish water. Such a pattern can arise due to the presence of two different strains of *SYN*-PE abundant in saline and freshwater, which are physiologically/genetically different and cannot flourish in brackish water.

Only a few estuarine regions have reported the presence of *PRO*-like cells in low saline waters. Maximum surface *PRO*-like cell concentrations were similar to that found in the Rhone River of the Mediterranean Sea up to a salinity of 1.2 (Vaulot et al., 1990), Mississippi River plume (Jochem, 2003), Suruga Bay, Japan and Changjiang estuary, China at salinities > 20 whereas it was two orders of magnitude lower than that recorded in the Australian coastal lagoon within a salinity range of 1.77 to 3.54 and > 15 (Schapira et al., 2010). In the present study, although *PRO*-like cells were present at salinities ranging from 35 to 0.06, differences in their RALS signals and chlorophyll fluorescence is an indication that the cells could be taxonomically and/or physiologically different. Low temperatures are unfavorable for the growth of *PRO*-like cells, with its presence recorded at the lowest surface temperature of about 10°C and highest of 30°C in warm equatorial oceanic regions (Partensky et al., 1999). The temperature in the Rhone River, Suruga Bay, Changjiang estuary and in the present study area was within the range for its growth. Nutrient enrichment experiments in the Zuari estuary have shown that *PRO*-like cells can not only survive but also grow in these waters (S. Mitbavkar, unpublished data). These observations suggest that *PRO*-like cells possess capabilities to survive in high temperature and low saline estuarine waters. However, these results have to be confirmed through pigment analysis or genetic finger printing. Previous studies based on phytoplankton pigments along the southwest coast of India did not report *PRO*-like cells (Roy et al., 2006) which could be due to their low abundance compared to that in oceanic regions (Campbell et al., 1998). Another reason could be the type of filter paper used for sample collection where there is a possibility of losing the small sized cells. Earlier studies have reported 0.2 µm nylon membranes (Whatman) to be the most suitable for retention of

picophytoplankton (Knefelkamp et al., 2007). Taking this observation into consideration, in this study, the total phytoplankton biomass may be underestimated as GF/F was used.

The correlations between environmental parameters and PP abundance may not be a causal one. However, their distribution pattern could be influenced by the entry of water from the Cumbarjua canal into the estuary resulting in flushing out of *SYN*-PE and *PRO*-like cells and influx of *SYN*-PC and PEUK. The rise in *SYN*-PE, *SYN*-PC and *PRO*-like cells at S9 could be due to the Kushavati River discharge. A drop in the total abundance at S4 was a result of the high turbidity which impacted *PRO*-like cells the most.

The proposition that *PRO*-like cells can be brought to the coastal regions from the offshore regions may explain their higher numbers during ST (up to S5) than during NT. During ST, saline waters (>30 salinity) were present up to S5 whereas during NT, the tide was already receding. The difference in cell abundance between the two tides indicates that the entry of sea water regulates the population at the estuary mouth. However, the role of other factors such as nutrients and grazing, which were not monitored in the present study, cannot be overlooked. The higher abundance in freshwater during both the tides are probably represented by different strains having its origin from the freshwaters. The occurrence of *PRO*-like cells and *SYN*-PE groups along the salinity gradient may indicate presence of different strains which have specific salinity thresholds. This aspect should be confirmed through experimental studies on pure cultures in order to understand their physiology. The average contribution of PP to the total photosynthetic biomass was close to the values reported for other estuaries (Gaulke et al., 2010). However, unlike other estuaries, PP contribution was generally high when the total biomass was low. Similar results have been reported from the monsoonal Arabian Sea (Goericke 2002) where diatoms and PP dominated the phytoplankton biomass at high and low concentrations of total chl *a*, respectively.

## **Acknowledgements**

The authors are grateful to Dr. Satish Shetye, Director, National Institute of Oceanography for his support and encouragement. This is NIO contribution #.



## References

- Baudoux, A.C., Veldhuis, M.J.W., Witte, H.J., Brussaard, P.D., 2007. Viruses as mortality agents of picophytoplankton in the deep chlorophyll maximum layer during IRONAGES III. *Limnology and Oceanography* 52, 2519-2529.
- Campbell, L., Landry, M.R., Constantinou, J., Nolla, H. A., Brown, S. L., Liu H., Caron, D.A., 1998. Response of microbial community structure to environmental forcing in the Arabian Sea. *Deep Sea Research* 45, 2301-2325.
- Chisholm, S.W., Olson, R.J., Zettler, E.R., Goericke, R., Waterbury, J.B., Welschmeyer, N.A., 1988. A novel free-living prochlorophyte abundance in the oceanic euphotic zone. *Nature* 334, 340-343.
- Gallegos, C.L., 2001. A two-year time series of continuously monitored inherent optical properties in a eutrophic sub-estuary of Chesapeake Bay. *Estuarine Coastal Shelf Science* 64, 156-170.
- Gaulke, A.K., Wetz, M.S., Paerl, H.W., 2010. Picophytoplankton: A major contributor to planktonic biomass and primary production in a eutrophic, river-dominated estuary. *Estuarine Coastal Shelf Science* 90, 45-54.
- Goericke, R., 2002. Top-down control of phytoplankton biomass and community structure in the monsoonal Arabian Sea. *Limnology and Oceanography* 47, 1307-1323.
- Haas, L.W., Pearl, H.W., 1988. The roles of blue-green algae. McCoy SE (ed.) Chesapeake Bay. NOAA Estuary-of-the-Month Seminar Series No. 5 National Oceanic and Atmospheric Administration Estuarine Programs Office, Washington, D.C. pp. 99-113.
- Jochem, F.J., 2003. Photo- and heterotrophic pico- and nanoplankton in the Mississippi River plume: distribution and grazing activity. *Journal of Plankton Research* 25, 1201-1214.
- Knefelkamp, B., Carstens, K., Wiltshire, K.H., 2007. Comparison of different filter types on *chlorophyll-a* retention and nutrient measurements. *Journal of Experimental Marine Biology and Ecology*. 345, 61-70.
- Mitbavkar, S., Saino, T., Horimoto, N., Kanda, J., Ishimaru, I., 2009. Role of environment and hydrography in determining the picoplankton community structure of Sagami Bay, Japan. *Journal of Oceanography* 65, 195-208.
- Morán, X.A.G., 2007. Annual cycle of picophytoplankton photosynthesis and growth rates in a temperate coastal ecosystem: a major contribution to carbon fluxes. *Aquatic Microbial Ecology* 49, 267-279.
- Murrell, M.C., Lores, E.M., 2004. Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *Journal of Plankton Research* 26, 371-382.
- Pan, L.A., Zhang, L.H., Zhang, J., Gasol, J.M., Chao, M., 2005. On-board flow cytometric observation of picoplankton community structure in the East China Sea during the fall of different years. *FEMS Microbiology Ecology* 52, 243–253.
- Parsons, T.R., Maita, Y., and Lalli, C.M., 1984. *A Manual of Chemical and Biological Seawater Analysis*. Pergamon, New York.

- Partensky, F., Hess, W.R., Vaultot, D., 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews* 63, 106–127.
- Patil, J.S., Anil, A.C., 2008. Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. *Continental Shelf Research* 28, 2404-2416.
- Qasim, S.Z., Sen Gupta, R., 1981. Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuarine Coastal Shelf Science* 13, 557-578.
- Richardson, T.L., Jackson, G.A., 2007, Small phytoplankton and carbon export from the surface ocean. *Science* 315, 838-840.
- Roy, R., Prathihary, A., Mangesh, G., Naqvi, S.W.A., 2006. Spatial variation of phytoplankton pigments along the southwest coast of India. *Estuarine Coastal and Shelf Science* 69, 189-195.
- Schapira, M., Buscot, M.J., Pollet, T., Leterme, S.C., Seuront, L., 2010. Distribution of picophytoplankton communities from brackish to hypersaline waters in a South Australian coastal lagoon. *Saline Systems* 6, 2-15.
- Shang, X., Zhang, L.H., Zhang, J., 2007. *Prochlorococcus*-like populations detected by flow cytometry in the fresh and brackish waters of the Changjiang estuary. *Journal of the Marine Biological Association, UK* 87, 643-648.
- Shetye, S.R., 1999. Propagation of tides in the Mandovi and Zuari estuaries. *Sadhana* 24, Parts 1 & 2, February & April 1999, India, pp. 5-16.
- Shetye, S.R., Gouveia, A.D., Singbal, S.Y., Naik, C.G., Sundar, D., Michael, G.S., Nampoothiri, G., 1995. Propagation of tides in the Mandovi-Zuari estuarine network. *Proceedings of the Indian Academy of Sciences* 104, 682.
- Shimada, A., Nishijima, M., Maruyama, T., 1995. Seasonal appearance of *Prochlorococcus* in Suruga Bay, Japan in 1992–1993. *Journal of Oceanography* 51, 289-300.
- Sundar, D., Shetye, S.R., 2005. Tides in the Mandovi and Zuari estuaries, Goa, west coast of India. *Journal of Earth System Science* 114, 493-503.
- Vaultot, D., Partensky, F., Neveux, J., Mantoura, R.F.C., Llewellyn, C.A., 1990. Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea. *Limnology and Oceanography* 35, 1156–1164.
- Waterbury, J.B., Watson, S.W., Valois, F.W., Franks, D.G., 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In: Platt, T., Li, W.K., (Eds.), *Photosynthetic picoplankton*. Canadian Journal of Fisheries and Aquatic Sciences, Department of Fisheries and Oceans, Ottawa, pp. 71-120.
- Wilson, S.E., Steinberg, D.K., 2010. Autotrophic picoplankton in mesozooplankton guts: evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton. *Marine Ecology Progress Series* 412, 11-27.
- Worden, A.Z., Nolan, J.K., Palenik, B., 2004. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. *Limnology and Oceanography* 49, 168-179.

### **Figure legends**

Fig. 1. Distribution of picophytoplankton groups along the Zuari estuary during the spring and neap tide.

Fig. 2. Contribution (%) of the picophytoplankton groups to the total picophytoplankton abundance during the spring (a, c) and neap tide (b, d).

Fig. 3. Contribution of picophytoplankton (%) to the total photosynthetic biomass during the spring (a, b) and neap tide (c, d).

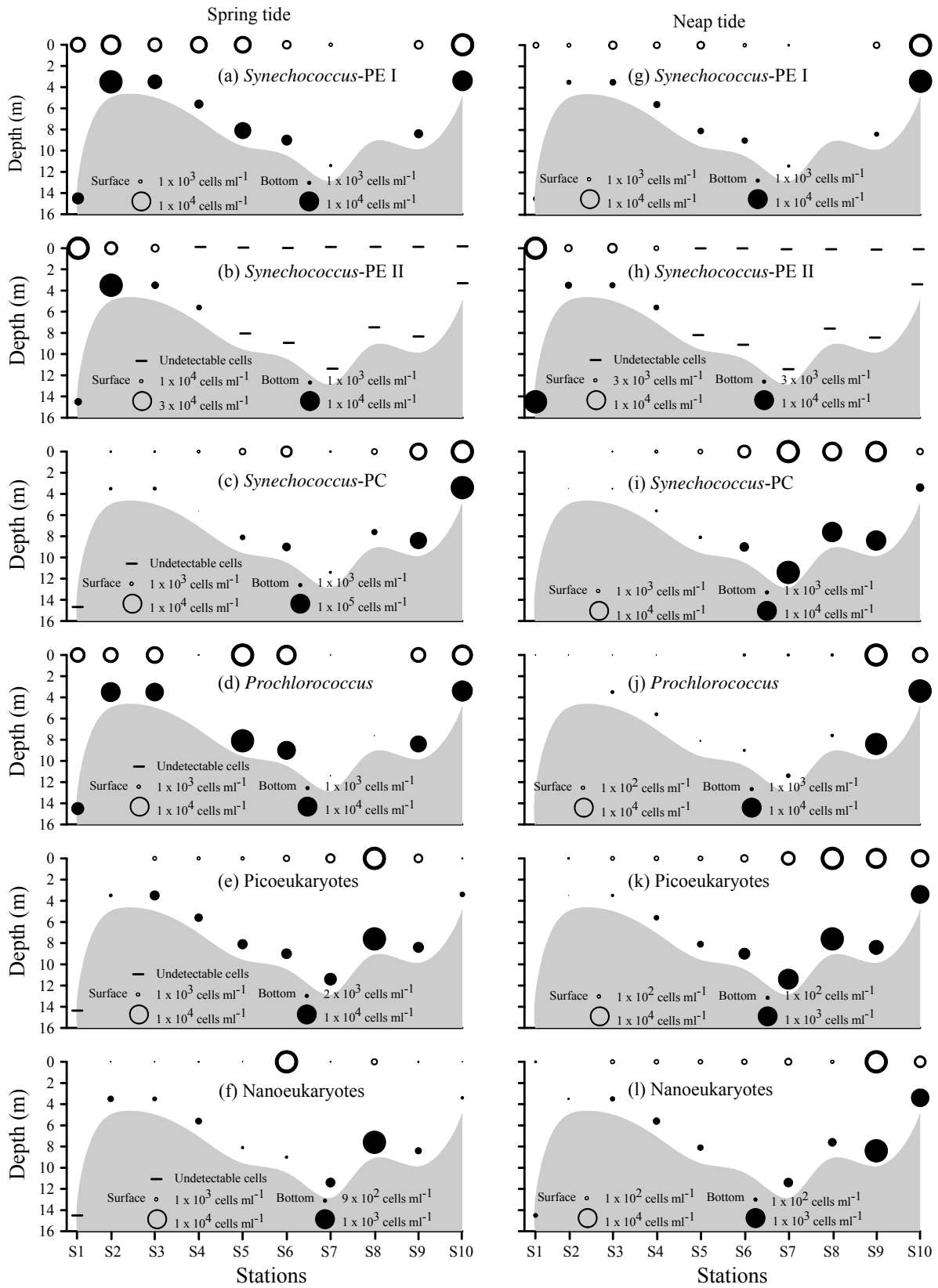


Fig. 1

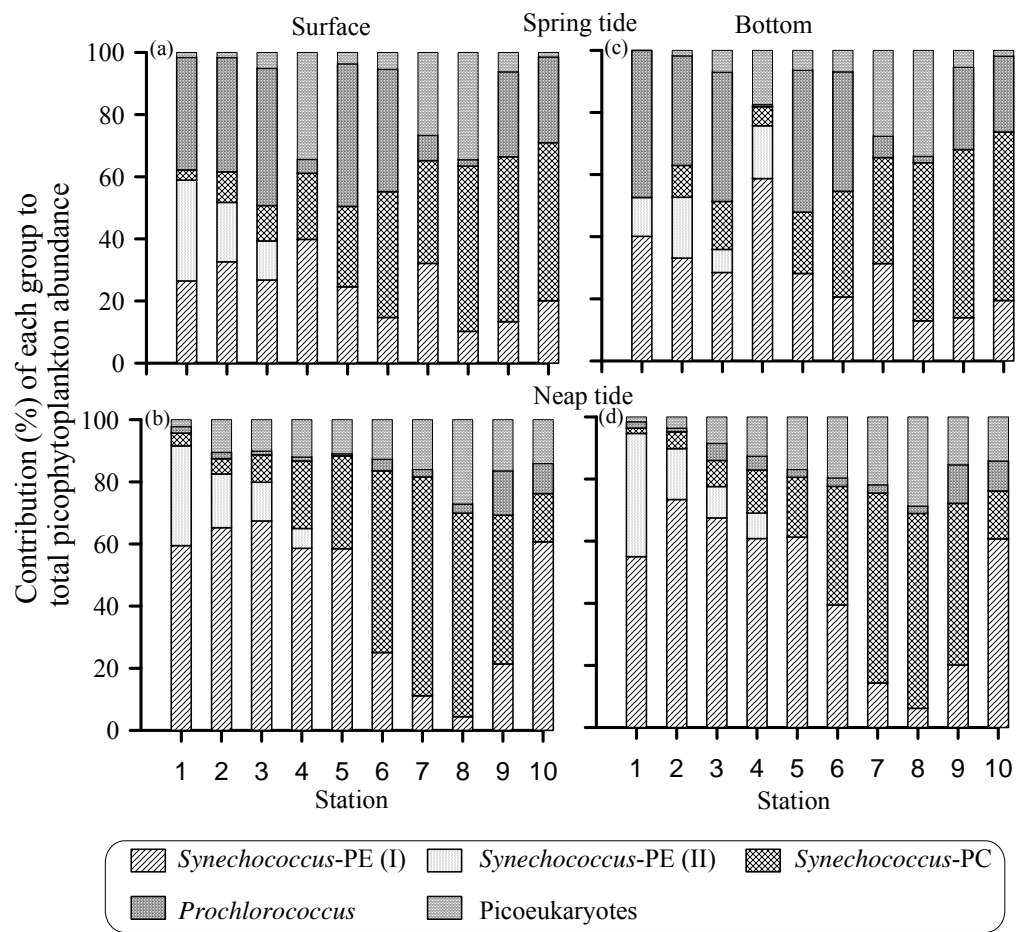


Fig. 2

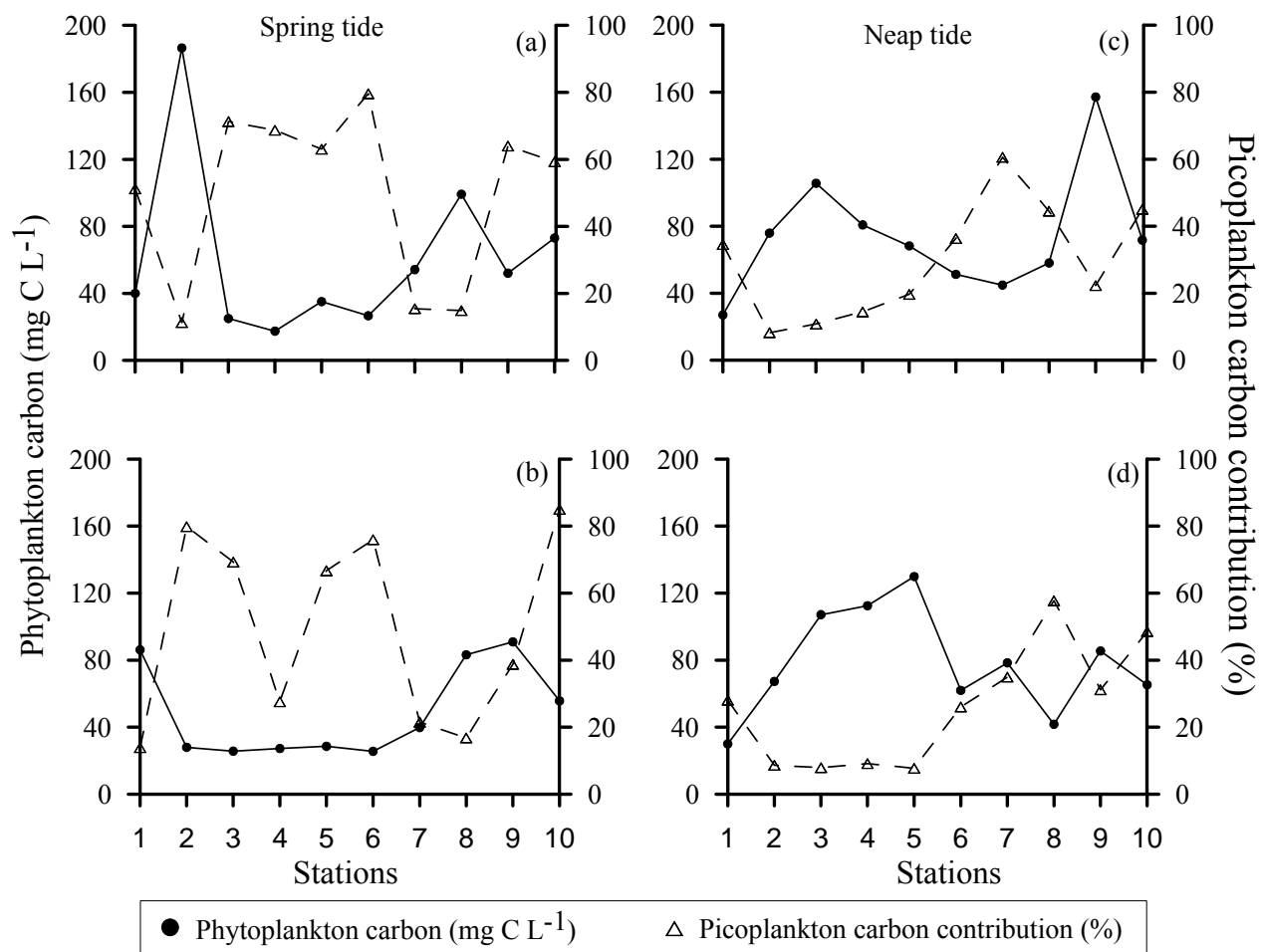


Fig. 3

**Table 1.** Sampling details for each sampling station in the Zuari estuary during a spring and neap tide.

Station	Sampling time (h)	Latitude;Longitude	Spring tide				Neap tide					
			Distance from mouth (km)	Local depth (m)	Sampling depth (m)	Z <sub>eu</sub> (cm)	Distance from mouth (km)	Local depth (m)	Sampling depth (m)	Z <sub>eu</sub> (cm)		
1. Marmugao	10:02	15° 25' 16.9"; 73° 47' 36.9"	0	16	14.5	215	09:28	15° 25' 17.5"; 73° 47' 36.6"	0	16	14.5	265
2. Chicalim	10:49	15° 25' 08.5"; 73° 50' 22.4"	5.8	5	3.5	186	10:04	15° 25' 03.1"; 73° 50' 25.1"	5.8	5	3.5	200
3. Island	11:24	15° 24' 57.4"; 73° 51' 57.0"	8.6	5	3.5	144	10:33	15° 24' 55.4"; 73° 51' 56.3"	8.6	5	3.5	157.2
4. Sancoale	11:50	15° 24' 45.1"; 73° 53' 30.6"	11	7.1	5.6	76	11:01	15° 24' 43.8"; 73° 53' 37.4"	11	7.1	5.6	201.3
5. Cortalim	12:10	15° 24' 32.0"; 73° 54' 50.2"	13	9.6	8.1	101	11:18	15° 24' 36.3"; 73° 54' 45.0"	13	9.6	8.1	185
6. Loutulim	12:53	15° 22' 54.0"; 73° 57' 24.4"	19.7	10.5	9	104	12:54	15° 22' 43.0"; 73° 57' 24.3"	19.7	10.5	9	136.8
7. Borim	13:44	15° 21' 03.6"; 73° 59' 58.0"	23.9	12.9	11.4	98.5	13:28	15° 21' 01.2"; 74° 00' 03.1"	23.9	12.9	11.4	170.1
8. Shiroda	14:36	15° 18' 12.3"; 74° 00' 55.5"	31.4	9.1	7.6	112.6	14:04	15° 18' 09.6"; 74° 00' 59.9"	31.4	9.1	7.6	162
9. Kushavati	15:42	15° 16' 31.7"; 74° 04' 28.3"	38.4	9.9	8.4	138	14:54	15° 16' 33.0"; 74° 04' 26.6"	38.4	9.9	8.4	156.3
10. Sanvordem	16:22	15° 16' 01.1"; 74° 06' 36.0"	42.2	4.9	3.4	119	15:31	15° 16' 02.4"; 74° 06' 31.6"	42.2	4.9	3.4	123

## **Appendix. Supplementary data**

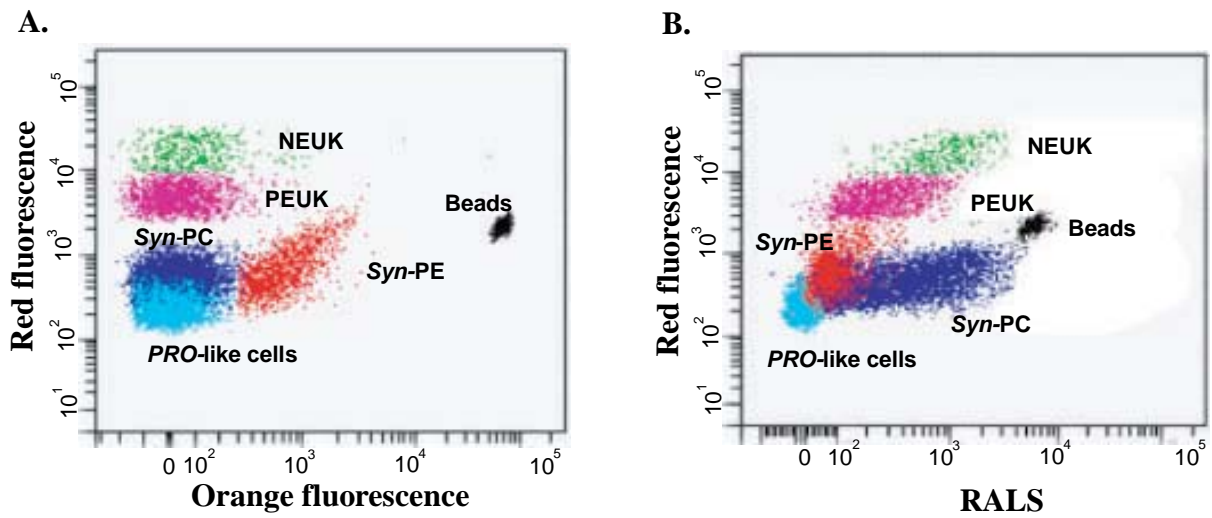
### **Figure legends**

Annex Fig. 1. Examples of flow cytometry cytograms. Data of samples taken from the surface waters of Zuari estuary on December 4, 2009. A. Orange fluorescence (phycobiliprotein derived) vs. red fluorescence (chlorophyll derived); B. RALS vs. red fluorescence.

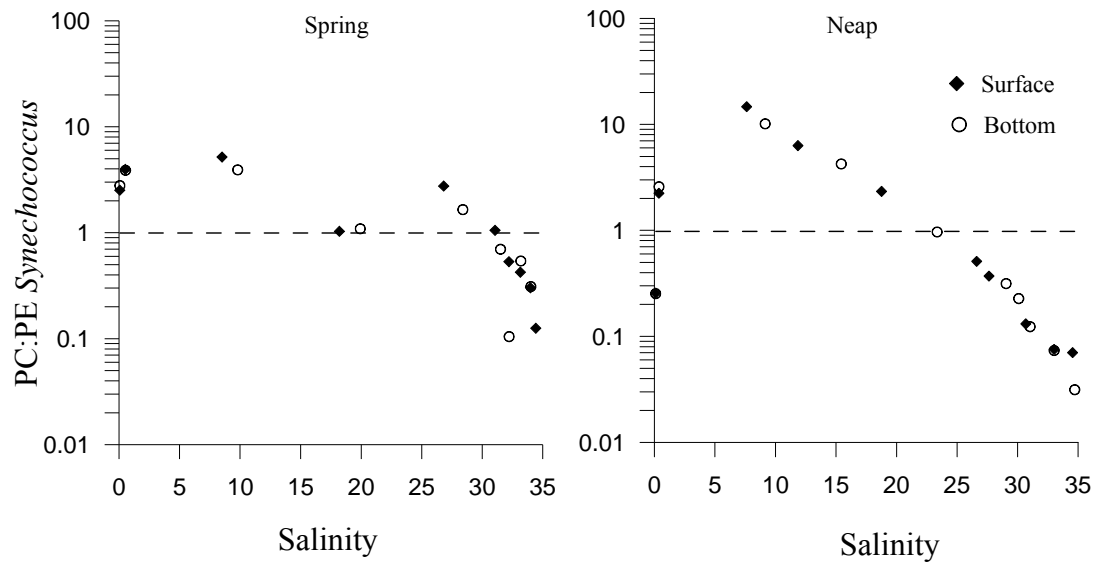
Annex Fig. 2. Ratio of *Synechococcus*-PC to *Synechococcus*-PEI along the Zuari estuary during the spring and neap tide.

Annex Fig. 3. Cluster analysis of the picophytoplankton abundance along the Zuari estuary during the spring and neap tide. The abundance data (cells ml<sup>-1</sup>) was used to construct a similarity matrix using Bray-Curtis coefficients which was subjected to clustering analysis using the group average method (PRIMER software version 5). Two major groups of stations were identified during ST, Group I (S7, S8 and S4) and Group II (all the other stations except 1NB and 4NB). During NT, four clusters were observed, Group I (S1 and S2), Group II (S3 to S5), Group III (S6 to S8) and group IV (S9 and S10). (S= Surface; B=bottom)

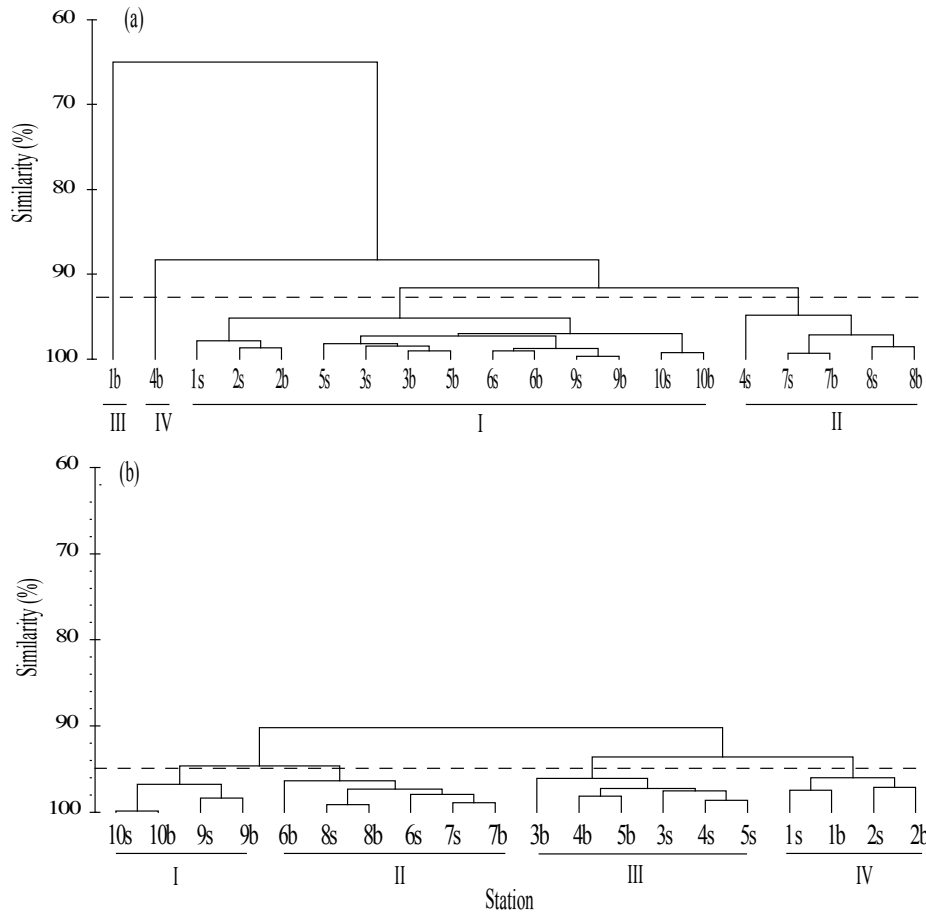




Annex. Fig. 1



Annex. Fig. 2



Annex Fig. 3

Annex Table 1. Flow cytometric characteristics of different picophytoplankton groups (arbitrary units)

Picophytoplankton groups	Forward light scatter	Right angle light scatter	Chlorophyll fluorescence	Phycoerythrin fluorescence	Phycocyanin fluorescence
<i>Synechococcus</i> -PEI	0.022	0.016	0.35	0.030	
<i>Synechococcus</i> -PEII	0.251	0.018	0.71	0.136	
<i>Synechococcus</i> -PC	2.329	0.407	0.38		942
<i>Prochlorococcus</i> - like cells	0.128	0.004	0.61		
Picoeukaryotes	2.24	0.37	4.09		