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Protistan assemblages across the Indian Ocean, with a specific emphasis on the picoeukaryotes

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ABSTRACT

Protists, and among them the picoeukaryotes (cells $<3\,\mu m$), have been described as significant contributors to both carbon biomass and production in oligotrophic regions of the oceans. However, protist assemblages remain largely undescribed in pelagic ecosystems and in particular in the Indian Ocean. In the present work, we investigated protists along an eastward transect across the sub-tropical gyre of the Indian Ocean (from South Africa to Australia), with a particular focus on picoeukaryotes. We combined inverted and epifluorescence microscopy, flow cytometry, pigment analysis, denaturing gel gradient electrophoresis (DGGE), 18S rDNA clone libraries, and fluorescent *in situ* hybridization (FISH). Overall the picophytoplankton fraction contributed 88% and 90% of total Chl *a* at the surface and DCM, respectively, with picoeukaryotes accounting for 38% and 50% of total Chl *a* at the surface and DCM. Considering only the Indian South Subtropical Gyre (ISSG) province, we observed greater shifts in the picoeukaryotic assemblage throughout the upper 200 m of the water column than along the ca. 10,000 km cruise track. In terms of taxonomic diversity and contribution of each taxon to the picoeukaryotic community, prasinophytes were well represented at more coastal stations with the genus *Micromonas* reaching densities up to 750 cell mL^{-1} in coastal waters and less than 100 cell mL^{-1} at open ocean stations. Haptophytes (56% and 45% of picoeukaryotic pigments at surface and DCM, respectively) and possibly pelagophytes (28% and 40% of picoeukaryotic pigments at surface and DCM, respectively) appeared to be dominant at open ocean stations. Other groups and in particular organisms affiliated to chrysophytes, and to a lesser extent to cryptophytes, appear as clear targets for future qualitative and quantitative studies. Moreover, the occurrence of many sequences related to radiolarians (5% and 27% at surface and DCM, respectively) will require further investigation.

1. Introduction

Protistan assemblages can be categorized according to conventional taxonomic classification or to size fractions (Sieburth et al., 1978). The importance of picoplankton

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(defined here as cells $<3\,\mu m$) has been primarily emphasized in oligotrophic areas of the oceans, where they participate in nutrient remineralization (Azam et al., 1983) and may contribute up to 95% of the primary production (Raven, 1998). Among the three classically recognized groups of picophytoplankton, *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, the latter usually have the lowest numerical abundance. Nevertheless, the importance of picoeukaryotes in terms of biomass and primary productivity has been demonstrated for various marine pelagic ecosystems (Li, 1994; Marañón et al., 2001; Worden et al., 2004). Early studies of photosynthetic picoeukaryotic diversity in the open ocean were based mostly on pigment analysis. These investigations suggested haptophytes and pelagophytes as significant components of the community, while green algae and other groups were less frequently observed (Ondrusek et al., 1991; Letelier et al., 1993; Andersen et al., 1996). Nevertheless, green algae and more specifically prasinophytes can be significant in particular locations or under certain conditions (Suzuki et al., 2002). Since 2001, molecular techniques have been applied to qualitatively describe the diversity of picoeukaryotes in the open ocean (Díez et al., 2001b; López García et al., 2001; Moon-van der Staay et al., 2001). Our first insights into the abundance and distribution of particular taxa (i.e. *Micromonas pusilla*, Prasinophytes) were obtained in coastal environments (Biégala et al., 2003; Not et al., 2004; Countway and Caron, 2006), and few data (qualitative or quantitative) are currently available from oligotrophic regions of the open ocean.

Although the Indian Ocean gyre is one of the largest oligotrophic areas of the world ocean, it has received far less attention than gyres of the northern Atlantic and Pacific Oceans. Only the Arabian Sea in the northwestern Indian Ocean (north of 10°S latitude), which is strongly influenced by monsoonal winds, has been subjected to several international interdisciplinary programs (e.g. the 1994–1996 Arabian Sea Expedition: Oceanic Response to Monsoonal Forcing; Smith et al., 1998). In those studies, picoplankton was shown to contribute 35–92% of phytoplanktonic chlorophyll *a* (Chl *a*) in transects from the coast towards the oligotrophic open ocean (Latasa and Bidigare, 1998; Brown et al., 1999). The abundance and contribution of picoeukaryotes to biomass was more important at coastal nutrient-rich stations than in oligotrophic areas, where *Prochlorococcus* dominated (Campbell et al., 1998; Latasa and Bidigare, 1998; Brown et al., 1999). Molecular approaches investigating the diversity of photosynthetic picoeukaryotes in the Arabian Sea demonstrated the prominent contribution of chrysophyte and prymnesiophyte algae (Fuller et al., 2006a, b). Extensive regions south of the Arabian Sea, including the oceanic gyre in the Southern hemisphere, remain poorly known.

In the present study we characterized the diversity of microbial eukaryotic assemblages along an eastward transect across the subtropical Indian Ocean gyre from South Africa to Australia. Because of the expected dominance of picoplankton in the planktonic community of oligotrophic areas, we focused our investigations on this size fraction and, more specifically, on the picoeukaryotes (both heterotrophs and phototrophs), for which assemblages in the open ocean remain poorly described. In order to achieve a comprehensive view of the system, we used a multi-technique approach, combining tools routinely used to assess eukaryotic microbial diversity: inverted and epifluorescence microscopy, flow cytometry, molecular fingerprinting (DGGE: denaturing gel gradient electrophoresis), 18S rDNA clone libraries, pigment analysis, and fluorescent *in situ* hybridization with taxon specific probes. Comparisons across techniques allowed us to observe both general patterns and the fine scale structure of assemblages.

2. Materials and methods

2.1. Sampling

The oceanographic cruise VANC10MV took place during late austral fall (15 May–13 June 2003) on board the oceanographic vessel R/V *Melville* (Scripps Institution of Oceanography, UCSD). Fourteen stations were sampled to assess the diversity of phytoplankton along an eastward transect from Cape Town (South Africa), through the subtropical Indian Ocean, to Port Hedland (Australia) (Fig. 1 and Table 1). Temperature, salinity, and *in situ* fluorescence profiles were obtained by CTD casts at each station (Sea-Bird Electronics 911 Plus, Bellevue, WA). CTD sensor data were processed according to standard Sea-Bird recommendations for each instrument, and subsequently included in a 10-m-depth binned final data file. Vertical profiles of *in vivo* fluorescence were corrected for background offset and converted to Chl *a* concentration using a regression of extracted Chl *a* concentrations as measured by fluorometry against *in vivo* fluorescence at all depths and stations sampled ($n = 83$, slope $= 0.66$, $R^2 = 0.77$). Seawater samples were collected using 15 L Niskin bottles mounted on a rosette. At each station, five to seven depths were selected for sampling based on real-time hydrological and fluorescence profiles obtained from the CTD sensors. Three levels were consistently sampled: surface (5 m), the deep chlorophyll maximum (DCM), and the layer below the DCM (200 m). One or two additional samples were collected between the surface and DCM, one between the DCM and 200 m and one mesopelagic sample (650–1000 m depth). Seawater was pre-filtered through a 200 μm mesh prior to further analyses.

2.2. Flow cytometry (FCM)

Seawater samples (1.5 mL, total and $<3\,\mu m$ size fraction) were fixed with a mix of glutaraldehyde and paraformaldehyde (0.1% and 1% final concentration, respectively). Triplicate samples for each size fraction were subsequently deep frozen in liquid nitrogen and stored at −80 °C for long-term storage. Samples were processed using a FACSsort flow cytometer (Becton Dickinson, San José, CA), and cells enumerated following the protocol described by Marie et al. (1999).