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Ecology and Diversity of Marine Microorganisms ECODIM V

ASSESSING THE DIVERSITY AND ABUNDANCE OF PICOEUKARYOTES IN THE UPWELLING SYSTEM OFF CONCEPCION, CHILE.

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The abundance and diversity of picoeukaryotes was analyzed by molecular and culture approaches in the upwelling system off Concepcion. This zone is characterized by a temporal oxygen minimum zone (OMZ) caused by upwelling events during the spring/summer season.
We were capable of found a fraction of eukaryotes cells that probably correspond to <3 µm. The station nearest the coast shows a mayor abundance of picoeukaryotes (~20%). Results show a good correlation between FISH counts and Flow Cytometry, but a discrepancy between DAPI and FISH counts. Culture approach, although was not efficient in the enrichment of picoeukaryotes, was useful to distinguish some picoeukaryotes individuals.

Introduction

Picoeukaryotes (cells of <3 µm in diameter) contribute significantly to marine plankton biomass and productivity, specially in oceanic zones. However, in coastal wind-driven upwelling areas, in which primary productivity is very high and strongly seasonal, little is known about the abundance and diversity of this kind of plankton. The continental shelf region off shore central Chile is characterized by upwelling events during the spring/summer season, which leads to organic particle sedimentation and micro-oxic (O_2<1 ml/L) to anoxic conditions from intermediate depths to the seafloor. This zone is known as the Oxygen Minimum Zone, OMZ. The OMZ contributes to the global sink of nitrogen in the ocean and acts as a source of the greenhouse gas N_2O to the atmosphere, and the low O_2-supply in this zone influences the community structure and the metabolic behavior of the picoplankton at depth. The main objective of this work was to assess the abundance and diversity of picoeukaryotes in the upwelling system off Concepcion, in the central Pacific area.

Methodology

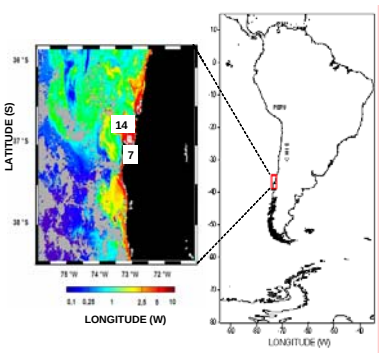

Figure 1: Satellite images of chlorophyll-a at the day of sampling in stations 7 and 14. The water samples were collected at 36º30'08''S 73º07'07''W, near Dichato Bay; at Stations 7 and 14.

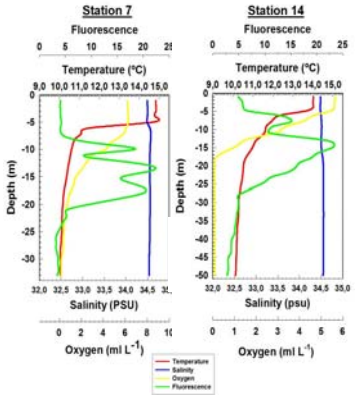

Figure 2: Oceanographic conditions (CTD-O profiles) of water column at the day of sampling in stations 7 and 14. In station 7, samples were collected at a depth of 5, 15 and 25 m. In station 14, samples were collected at 5, 20 and 50 m.

Abundance was achieved through Flow Cytometry, DAPI and FISH with probes against Eukarya. Diversity was achieved through PCR with Eukarya and functional primers, cloning and cultures (only for depth 15 and 20 m). PCR for bacteria was also done for control porposes.
Cultures were grown in medium f/2 supplemented with different carbon source with or without antibiotc.

Results

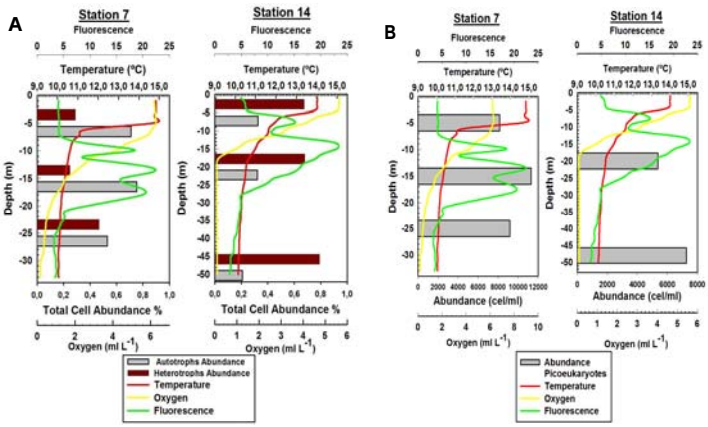

Figure 3: Relation between picoeukaryotes abundance and oceanographic conditions of water column at the two stations of sampling. A) Abundance was determined by DAPI, in which the criteria was counting cells between 1 and 3 µm. **B)** Abundance determined by FISH Hybridization with probes EUK1209, CHLO and NCHLO (Biegala, *et al*., AEM 2005, 69, 5519–29).

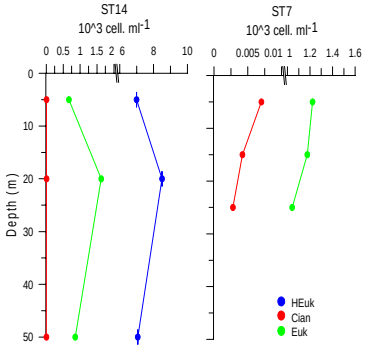

Figure 4: Abundance of picoeukaryotes determined by Flow cytometry . Station 14 show a higher abundance of heterotrophic picoeukaryotes compared with photosintetic fraction. For station 7, data for heterotroph picoeukaryotes are not available.

Table I. Cultures experiment for diversity and enrichment.

Plate #	Carbon Source	Cml 0,2 µg/ml	Light	Growth St 14 5m	St 14 20m	St 7 5m	St7 15m
1	/	-	+	-	-	++	-
2	/	+	+	-	-	-	-
3	/	-	-	-	-	-	-
4	/	+	-	-	-	-	-
5	Glucose	-	-	-	-	-	-
6	Glucose	+	-	-	-	-	-
7	Acetate	-	-	-	-	-	-
8	Acetate	+	-	-	-	-	-
9	Lactate	-	-	-	-	-	-
10	Lactate	+	-	-	-	-	-
11	LBm	-	-	-	-	-	+
12	LBm	+	-	-	-	-	-

Cultures were done in 24-multiwell plates and incubated at 16ºC for 12 days. Cultures were analysed by Phase contrast, Brightfield, Darkfield and Epifluorescence microscopy using a Zeiss microscope.

Results from table I show that carbon source amendment did not promote the growth of picoeukaryotes, most probably because of the overgrowth of bacteria.

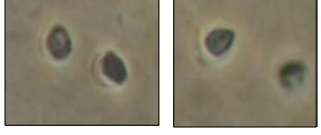

Figure 5: Light microscopy picture from picoeukayotes. Cultures were done in 100 ml cell cultures bottles and incubated at 16ºC for 12 days, with the same contidions shown in Table I. Microorganisms in the range of >1 and <3 µm were identified in the cultures samples. The growth of picoeukaryotes was detected in bottles from St.7 at 15 m of depth, cultured at light without carbon source and antibiotic.

Conclusions

- The mayor concentrations of picoeukaryotes were observed below the oxycline, at the same point of maximum fluorescence. The variation in the concentration of picoeukaryotes between the different depths is low (~8%).
- The station nearest the coast shows a mayor abundance of picoeukaryotes (~20%).
- The flow cytometry and FISH values are coincident, supporting the FISH hybridization techniques for discrimination of picoeukaryotes to determinate their abundance.
- DAPI and FISH values show a discrepancy, maybe because of an underestimation of the cell number by FISH and an overestimation by DAPI due to the difficulty to differentiate larger bacteria from picoeukaryotes with our experimental approach.
- We were unable to determine the diversity of the picoeukaryotic population in the Concepcion Bay, because of faillure in cultures experiment.

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