



COMMUNITY STRUCTURE OF PICOPLANKTON AND PROCESSES RELATED TO NITROGEN CYCLING OFF CENTRAL CHILE – COLIUMO BAY (~36,5 °S)

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The continental shelf region off central Chile is characterized by coastal wind-driven upwelling events during the spring/summer season. The Equatorial Subsurface Water (ESSW) is rich in nutrients and poor in dissolved oxygen (O₂). The upwelling of ESSW to the photic zone increases primary productivity and, as a consequence, organic particle sedimentation, which leads to micro-oxic (O₂ < 1 ml L⁻¹) to anoxic conditions from intermediate depths to the seafloor. The low O₂-supply influences the community structure and the metabolic behavior of the picoplankton at depth.

The aims of this study are (i) to describe the community composition of picoplankton (autotrophs and heterotrophs), (ii) to characterize the diversity of the bacterial groups and (iii) to evaluate the nitrogen transformation in the water column under oxic and suboxic (O₂ < 0,25 ml L⁻¹) conditions within a coastal embayment off central Chile (Coliumo Bay, figure 1).

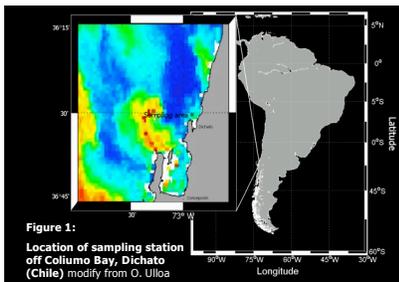


Figure 1: Location of sampling station off Coliumo Bay, Dichato (Chile) modify from O. Ulloa

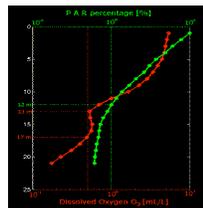


Figure 3: Vertical profiles of PAR (photosynthetically active radiation) normalized to surface irradiance and dissolved O₂ concentration. The photic depth, defined as the 1% surface light irradiance level, was at 12m. Hypoxic conditions (less than 0.5 mL O₂/L) prevailed below 13 m.

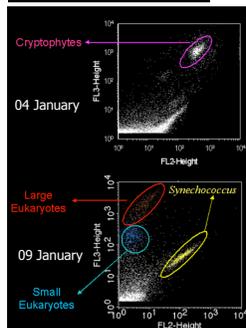


Figure 4: Dot plots of ultraphytoplankton auto-fluorescence measured by flow cytometry. January 4: Abundances are high for Cryptophytes and low for *Synechococcus* January 9: Changed community structure: The abundances of *Synechococcus*, small and large Eukaryotes were 1.04x10⁴, 7.20x 10³, and 3.81x10³ cells ml⁻¹, respectively.

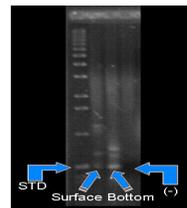


Figure 7: Electrophoresis gel of PCR products from ammonium oxidizing bacteria. Presence of ammonium-oxidizers was more evident in the bottom waters. Anammox-bacteria were not detected using PCR analysis, suggesting that this group was absent in the water column (data not shown).

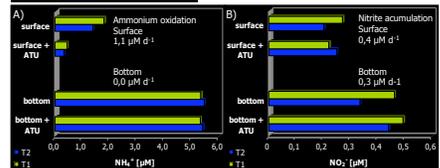


Figure 8: Results from the blockage experiments for samples with oxic and suboxic conditions; surface (photic zone) and bottom water, respectively. (A) NH₄⁺ and (B) NO₂⁻ concentrations were measured 1 (T1) and 6 (T2) hours after the addition of ATU (Allylthiourea).

METHODS

Sampling: Seawater samples were collected on January 04 and 09, 2006 at 0, 5, 10, 15, 20 and 25m depth.

Vertical profiles of temperature, salinity, dissolved oxygen, PAR and fluorescence were measured using a CTD equipped with the proper sensors (Figures 2 & 3).

Analyses:

- The abundance of various groups of picophytoplankton was measured using flow cytometry (Figure 4).
- The abundance of bacteria was quantified by counting DAPI-stained cells by epifluorescence microscopy (Figure 5).
- The genotypic diversity of bacterioplankton was estimated using T-RFLP (Terminal-Restriction Fragment Length Polymorphism) (Figures 5 and 6). The richness of the communities at the different depths was derived by a Non-Parametric Multidimensional Scaling (NMDS) method based on a Bray-Curtis similarity matrix.
- The presence of ammonium-oxidizers and anammox bacteria was analyzed by probing DNA extracts from natural samples with primers for characteristic genes employing the Polymerase Chain Reaction (PCR). In addition, blockage experiments for ammonium oxidation (Allylthiourea-ATU addition, 86µM) were conducted on oxic and suboxic waters. The concentrations of ammonium (NH₄⁺) and nitrite (NO₂⁻) were measured during the incubation period.

RESULTS

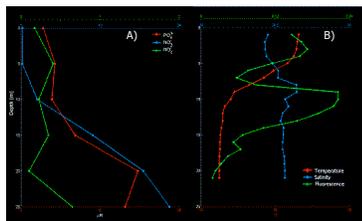


Figure 2: Vertical structure of the chemical and physical properties of the water column. A) Typical profiles of nutrients (Phosphate PO₄³⁻, Nitrate NO₃⁻ and Nitrite NO₂⁻). B) CTD profiles of temperature, salinity and fluorescence.

The fluorescence maximum was coincident with the upper nutricline, at a depth of ~10 m. A fluorescence minimum occurred at the depth of the pycnocline. The photic depth, occurs within the oxic layer (Figure 3).

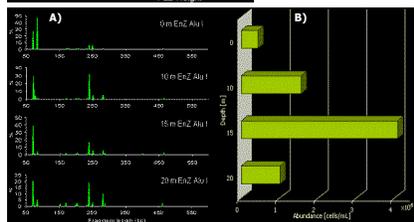


Figure 5: A) T-RFLP depth profiles of the bacterial community in the water column. PCR products were digested with AluI (restriction enzyme). Each peak represents one or several bacterial populations. B) Total bacterial cell abundance was obtained with the DAPI method. The abundance of cells increased with depth to 15m, which coincides with the onset of hypoxic conditions. Below this depth, cell abundance decreased again.

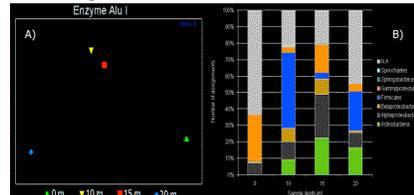


Figure 6 A: Two-dimensional NMDS plot of T-RFLP patterns of the bacterioplankton community. The communities found at 10m and 15m depths were more similar. B: Bacterial classes detected by T-RFLP. Actinobacteria, α-Proteobacteria, β-Proteobacteria, Firmicutes, and γ-Proteobacteria were the most abundant bacterioplankton groups in the water column at the site.

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DISCUSSION

The vertical structure of the water column was similar to observations reported for the time series stations situated close to the coast on the Continental Shelf off Concepción (COPAS, unpublished data).

The maximum of the bacterial cell abundance was below the fluorescence peak, and is likely the result of grazers feeding on the organic particles produced within the surface layer (Figures 2 and 5b).

The NMDS of T-RFLP plots revealed three different types of communities. Those at mid-depths are more similar than those at the surface and at the bottom (Figure 6).

The results of the blockage experiments (Figure 8) and the presence of the ammonium-oxidizers (Figure 7) suggests that ammonium-oxidation occurred in surface waters. Nitrification and assimilation by phytoplankton are also likely to be important NH₄⁺-sinks. In the bottom waters nitrogen compounds did not vary in the ATU treated assays, with the exception of a higher consumption of nitrite in the control (Figure 8 B). This, combined with the low O₂-concentration (Figure 3) and the absence of anammox-bacteria (Figure 7) suggests that denitrification might play a dominant role in the nitrogen cycling of the bottom waters.

CONCLUSIONS

Given the pronounced vertical structure in the physico-chemical variables, we conceptually divided the water column into three main layers: (1) upper (oxic / euphotic), (2) mid (dysoxic / dysphotic) and (3) bottom (hypoxic / aphotic) (Figure 3).

The community structure of picoplankton varied with depth due to the presence of radiation flux and chemical gradients. Experimental evidence suggests that the differences in the cycling of nitrogen and its associated metabolic pathways between surface and bottom waters might be caused by vertical changes in oxygen and nutrient levels.