INTERNATIONAL MARINE MICROBIOLOGY COURSE - DICHATO - CHILE - 2003

MICROBIAL DIVERSITY IN THE WATER COLUMN OF CONCEPCION BAY, CHILE

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OBJECTIVES

It was the aim of this study to assess the microbial diversity and its ecological determinants in a coastal upwelling area along the Pacific coast of Chile employing both molecular and conventional physicochemical methods.

STUDY SITE

Concepcion Bay (Fig 1) is located in central Chile. It is the largest (167.4 km²), and most enclosed ambayment along the Chilean coast. The bay is characterized by a strong hydrographic variability produced by seasonal upwellings of equatorial subsurface water (ESSW) in spring/summer. Upwelling waters are rich in nutrients (up to 80 µM NO_3^-, Fig. 3), poor in oxygen (<40 µM below 30 m depth, Fig. 2), and highly saline (>34.4ppm, Fig. 2). This fertilizes the bay, thereby increasing the phytoplankton biomass to 4 - 5 mg chl m^{-3} (Fig. 5) and primary productivity to values between 3.5 and 7.5 gC $m^{-2}d^{-1}$.

SAMPLING

Samples were collected from aboard the research vessel Kay-Kay on December 5, 2003 at stations 7 and 14 using an oceanographic rosette (Fig. 1). The environmental determinants at the site were characterized by CTD profiles (Fig. 2).

ANALYSES

The following determinants were analyzed in samples from three different water depths (0-6m, 35 m and 57 m) if appropriate:

I - Concentration of NH_4^+, NO_3^-, and NO_2^- employing standard colorimetric methods (Fig. 3)

II- Cell abundance of bacteria, cyanobacteria and picoeukaryota by flow cytometry (Fig. 4).

III- Chlorophyll a and phaeophytin pigment concentration in the total phytoplankton and from the size fraction < 5 µm by fluorometric methods (Fig. 5).

Concentrated samples from each depth were examined qualitatively by fluorescence microscopy, as well as by bright and dark field microscopy and by phase contrast microscopy where appropriate (Fig. 7). In order to determine the genotypic diversity of the bacterioplankton community in the surface layer, we amplified 16S rDNA genes from prokaryotes using primers which were specific for cyanobacteria and bacteria (OXY 107F-1313 R and 27F-1224 R, respectively and compared RFLP patterns to characterize community differences (Fig. 6).

Figure 1: Location of sampling stations at Concepción Bay, Chile. The stations marked in red were investigated in this study

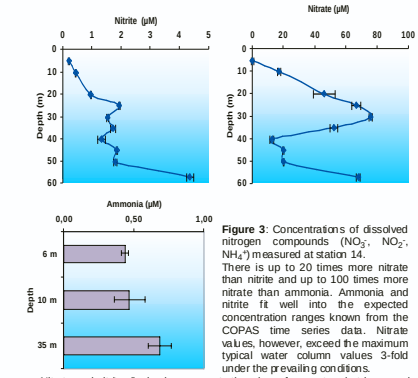


Figure 3: Concentrations of dissolved nitrogen compounds (NO_3^-, NO_2^-, NH_4^+) measured at station 14.

There is up to 20 times more nitrate than nitrite and up to 100 times more nitrate than ammonia. Ammonia and nitrite fit well into the expected concentration ranges known from the COPAS time series data. Nitrate values, however, exceed the maximum typical water column values 3-fold under the prevailing conditions.

Nitrate and nitrite display low concentrations in surface waters but increased ones at greater depths. The highest nitrate values were registered at 30m and 57m depth. The nitrate profile shows a consumption horizon between 40 and 50 m depth implying a denitrifying zone at this microoxic depth. The 3 ammonia measurements showed a gradual increase from suface to deep waters. The concentrations are low, however, such that the high nitrate values cannot stem from a high rate of nitrification.

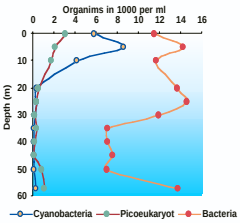


Figure 4 A: Abundance of cyanobacteria, picoeukaryotes, and bacteria. Values obtained by flow cytometry from samples collected at station 14 .

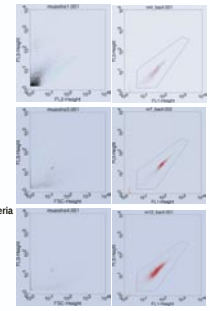
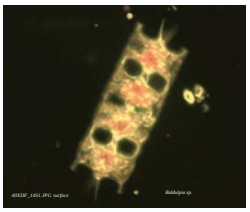
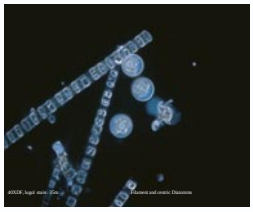
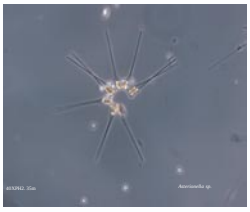
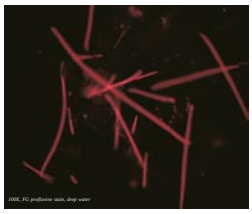


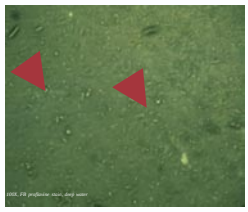
Figure 4 B: Flow cytometric patterns of samples from different depths. The pico-eukaryotes were distributed in the upper 20 m of the water column. The Cyanobacteria displayed their maximal abundance at 5m; they were virtually absent at depths below 20 m. Bacteria are present in large numbers at all depths.

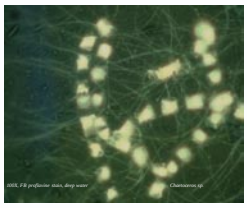












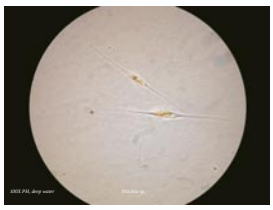


Figure 7
Selection of microorganisms present in the water column at stations 7 and 14 identified by Fluorescence, phase contrast and bright or dark field microscopy, respectively, using a ZEISS research Microscope.

RESULTS

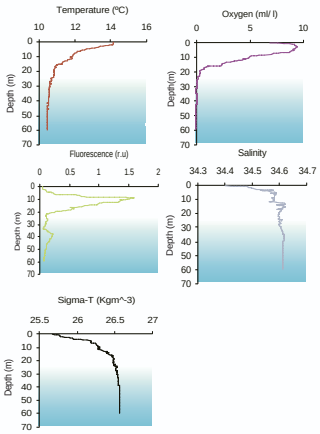


Figure 2: Profiles of hydrographic parameters obtained at station 14 on November, 5 2003.

The values and the shape of the profiles are as expected for this station and time, except for a second peak in the fluorescence profile at ±40m depth. The exceptional signal is probably due to an increased density of eukaryotic algae. The data are in acordance with the cytometer profile obtained at the same depth and at this station as well as at station 23.

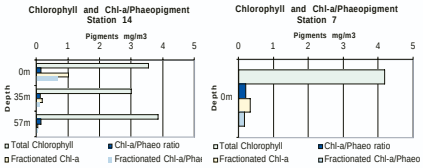
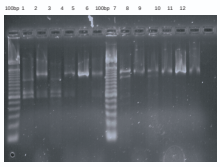


Figure 5

Chlorophyll-a and chlorophyll-pheophytin concentrations (mg/m³) from all organisms (total chlorophyll) and from organisms < 5µm (fractionated chl-a). The graphs also display the chl-a/phaeo-pigment ratio for the total and the < 5µm fraction. The main contribution to the total chlorophyll-a concentration stem from organisms with a size > 5µm. This holds for all depths sampled and for both stations.



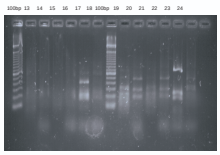


Figure 6

RFLP analysis of 16S rDNA fragments amplified by PCR with specific primers for cyanobacteria and with universal primers for eubacteria (OXY107F-1313R and 27F- 1224R, respectively). Total environmental DNA was extracted from samples collected at different depths of station 14 and from the surface at station 7. PCR products were digested with the restriction enzymes *AluI* (1-5), *HaeIII* (7-12), *EcoRI* (13-18) *and HindIII* (19-24). The digestion products were separated on 2% agarose gels. The figure assembles a number of RFLP patterns obtained from amplified 16S rDNA fragments of water column samples.

CONCLUSIONS

The hydrographic conditions observed in this study were similar to those previously obtained and reported for the long term observation stations located in Concepción bay (COPAS data). Although pigments from cyanobacteria and picoeukaryotic phototrophs were distributed troughout the entire water column, the presence of living phototrophic cells could be related to the hydrographic conditions: They were present mainly in the top 30m where the oxygen concentration was highest. The distribution of heterotrophic bacteria follows, basically, the abundance of primary producers in the top layers and those in the low light layer below 45m, but they are still half as numerous in the zone of the nitrate sink between 35 and 50 m. Multicellular filaments and unicellular diatoms with large sizes were found at all depths. According to the chlorophyll/phaeopigment -ratio, the increase of the fluorescence at 35m is due to a sedimenting accumulation of large cells containing chlorophyll-a and degraded chlorophyll pigments. The nutrient values measured corroborate those obtained previously by the COPAS project. The concentration patterns (nutrient depleted water at the surface and high concentrations in deeper water layers) is a common feature of this upwelling area. The RFLP patterns of the amplified 16S rDNA suggest that the population of cyanobacteria present in the surface layer do not differ markedly at the two stations. However, a high level of genetic variability through the water column was found for the 16S rDNA PCR products amplified by universal eubacterial primers at station 14.

Acknowledgements

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