MICROBIAL DIVERSITY IN THE WATER COLUMN OF CONCEPCION BAY, CHILE

Anamar Britos[1], Silvana Collado[2], Rodrigo De la Iglesia[3], Paz Jopia[4], Francisco Santibañez[4], Natalia Trabal[1,4], Verónica Molina[4,6], Osvaldo Ulloa[4,6] & Kurt Hanselmann[5,6]

[1]Universidad de la República Oriental del Uruguay, [2]Universidad Católica de Valparaíso, [3]P. Universidad Católica de Chile, [4]Universidad de Concepción, [5]Universidad de Zurich, Suiza, [6]Course organizer.

Microbial diversity and ecological determinants were analyzed in the water column of Concepción Bay, an upwelling area on the Pacific coast of South America. The bay is characterized by seasonal upwellings of equatorial subsurface water (ESSW) in spring/summer.

Samples were collected on December 5, 2003 at stations 7 and 14 using an oceanographic rosette. The environmental determinants were characterized by CTD profiles. The following components were analyzed in samples from three different water depths (0-6m, 35 m and 57 m): i) concentration of NH_4^+, NO_3^-, and NO_2^- employing standard colorimetric methods; ii) cell abundance of bacteria, cyanobacteria and pico-eukaryota by flow cytometry and iii) by fluorometric methods chlorophyll *a* and phaeophytin pigment concentration in the total phytoplankton and from the size fraction < 5 µm.
Concentrated samples from each depth were examined by microscopy qualitatively for the organisms present. Total environmental DNA was extracted and 16S rDNA fragments were amplified by PCR with specific primers for cyanobacteria and with universal primers for eubacteria (OXY107F-1313R and 27F- 1224R, respectively). 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. RFLP pattern differences were used to characterize community variation.

Upwelling waters are rich in nutrients (up to 80 µM NO_3^{-2}), poor in oxygen (<40 µM below 30 m depth), and highly saline (>34.4ppm). They fertilize the bay, thereby increasing the phytoplankton biomass to 4-5 mg chl m^{-3} and primary productivity to values between 3.5 and 7.5 gC $m^{-2}d^{-1}$. 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. 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 micro-oxic depth. Nutrient depleted water at the surface and high concentrations in deeper water layers is a common feature of this upwelling area.

The pico-eukaryotes were distributed in the upper 20 m of the water column. The Cyanobacteria were most abundant at 5m; they were virtually absent at depths below 20 m. The distribution of heterotrophic bacteria follows, basically, the abundance of the primary producers in the top layers and those in the low light layer below 45m. The RFLP patterns of the amplified 16S rDNA suggest that the population of cyanobacteria present in the surface layer does not differ markedly at the two stations. However, a high level of genetic variability throughout the water column was found for the 16S rDNA PCR products amplified by universal eubacterial primers at station 14.

The main contribution to the total chlorophyll-a concentration stems from organisms with a size > 5µm. Although pigments from cyanobacteria and pico-eukaryotic phototrophs were distributed troughout the 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. Multicellular filaments and unicellular diatoms 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.