# VARIABILITY OF ENZYMATIC ACTIVITIES IN THE IONIAN SEA

Zaccone R. \*, Monticelli L. S., Zampino D.

Istituto per l'Ambiente Marino Costiero, CNR, section of Messina. Spianata S. Raineri, 86, 98122 Messina, Italy - \* zaccone@ist.me.cnr.it

### Abstract

The variability of aminopeptidase (LAP), ß-glucosidase (GLU) and alkaline phosphatase (AP) enzymatic activities in the water masses of the Ionian Sea was studied during two SINAPSI cruises. In the surface layer, we found a significant increase of all enzymatic activities during spring. The AP values were 1.5-2.9 times higher than LAP, indicating a faster regeneration of P during the productive season. In the intermediate and deep (DW) layers the values showed a sharp decrease. In the bottom layer (BBL) we observed an increase of LAP and AP enzymatic activities during winter. The winter high AP/LAP ratio (2.9 and 1.73, respectively) in the DW and BBL layers indicated that AP plays an important role for deep bacteria not only as P source.

Key words: Enzymes, Mediterranean Sea, deep bacteria.

## Introduction

The Ionian Sea represents a crossing region between the Western and the Eastern Mediterranean Sea and is one of the sites most affected by the outflow of Adriatic water and the changes in water composition and dynamics caused by the Eastern Mediterranean Transient. These changes have produced an adaptation of microorganisms related to nutrient sources and/or temperature increase.

In the euphotic zone, the heterotrophic microbial activity is particularly intense and is derived from adaptation of a very wide spectrum of nutritional sources produced by autotrophic processes. In the deep sea, life depends on the availability of reduced carbon in the form of particulate organic matter (POM) and dissolved organic carbon (DOC) (1).

Previous investigations have been carried out so far, on microbial activities in the Mediterranean Sea, mainly in coastal and euphotic zones. Information on the deep sea is very scarce and predominantly collected in the open ocean (2).

The aim of the work was to study the variability of the hydrolysis processes of aminopeptidase (LAP),  $\beta$ -glucosidase (GLU) and alkaline phosphatase (AP) enzymatic activities in the water masses of the Ionian Sea.

# Materials and Methods

The study was carried out during two SINAPSI (Seasonal Interannual and decAdal variability of the atmosPhere, oceans and related marine ecosystems) cruises (winter 1999 and spring 2002). Samples were collected at eleven pelagic stations, distributed at different depths (from surface to 4985 m) within a triangular area whose vertices are defined by the following coordinates (19.2°E, 40.3°N; 14.5°E, 35.3°N; 22.0°E, 35.3°N).

Microbial activities of aminopeptidase (LAP), ß-glucosidase (ß-GLU) and alkaline phosphatase (AP) were determined by the use of the fluorogenic substrates leucine-7-amido-4-methyl-coumarin (Leu-MCA), methylumbelliferyl-ß-glucoside (MUF- $\beta$ -GLU) and MUF-phosphate (MUF-AP) according to Hoppe (3), incubating the samples in the dark at in situ temperature. All bacterial extracellular enzyme activities were measured as potential activities (V<sub>max</sub>) (4).

We grouped the water masses into five layers, according to their distribution and characteristics. The surface layer (ISW) extended up to 100 m, the intermediate layer, mainly occupied by the Levantine Intermediate Water (LIW) spanned over the depth interval 200-600 m, the transition layer with depths from 700 to 1500 m, the deep layer (DW) for depths >2000 m and the benthic boundary layer (BBL) about 10 m from seafloor.

### **Results and Discussion**

The condition of the water column showed a beginning of stratification of waters with peaks of fluorescence at 50-100 m, although the spring mean values of temperature were similar to those in winter, varying from 15.30°C (ISW) to 13.97°C (BBL) in winter and from 15.33°C (ISW) to 13.32°C (BBL) in spring.

Results showed a high variability and a significant increase during spring of all enzymatic activities in the surface layer (LAP and  $\beta$ -GLU: P<0.05, AP: P<0.01, ANOVA test).

The mean values of LAP in the surface layer (with the exception of the Adriatic station) ranged from 0.37 to 4.34 nmol  $l^{-1}$   $h^{-1}$  in winter 1999 and from 1.73 to 2.95 nmol  $l^{-1}$   $h^{-1}$  in spring 2002, indicating the

Rapp. Comm. int. Mer Médit., 37, 2004

influence of the Adriatic waters in the northern stations of the Ionian Sea. AP mean values in the surface layer ranged from 0.2 nmol l<sup>-1</sup> h<sup>-1</sup> to 6.44 nmol l<sup>-1</sup> h<sup>-1</sup> in winter 1999 and from 3.1 nmol l<sup>-1</sup> h<sup>-1</sup> to 10.35 nmol l<sup>-1</sup> h<sup>-1</sup>.  $\beta$ -GLU mean values ranged from 0.04 nmol l<sup>-1</sup> h<sup>-1</sup> to 0.32 nmol l<sup>-1</sup> h<sup>-1</sup> in winter, and from 0.11 nmol l<sup>-1</sup> h<sup>-1</sup> to 2.55 nmol l<sup>-1</sup> h<sup>-1</sup> in spring.

In the intermediate and deep waters the values showed a sharp decrease, with similar values in the two seasons and only in the deep waters, the LAP was significantly different between seasons (P<0.05, ANOVA test).

During winter, an increase of all enzymatic activities was observed at the BBL, in correspondence to the increase of bacteria. LAP and AP activities showed a high variability among stations. This trend was confirmed in the spring sampling, while ß-GLU showed opposite behaviour.

As observed in other temperate areas, LAP production always exceeded  $\beta$ -GLU activity showing a different LAP/ $\beta$ -GLU ratio among all layers from 1.2 to 17.1 in winter and from 1.5 to 29.4 in spring.

In the surface layer during spring, AP activity was higher (1.5-2.9 times) than LAP. This suggested that AP may be an indicator of fast regeneration of P during the productive season to sustain both bacteria and phytoplankton growth in oligotrophic waters. The potential remineralization rate of phosphorus was 5.01  $\mu$ g P dm<sup>-3</sup> per day.

In winter the potential remineralization rate of phosphorus (up to 1.14  $\mu$ g P dm<sup>-3</sup> per day), recorded at the BBL, was similar to the surface rate (1.55  $\mu$ g P dm<sup>-3</sup> per day). A high AP/LAP ratio (2.9 and 1.73, respectively) was observed in the DW and BBL layers. In these layers, despite of the availability of inorganic P, the high AP activity indicated that phosphatase plays an important role for deep bacteria not only as P source, but it also could represent an alternative C source for bacterial nutrition in accordance with Hoppe *et al.* (2). They suggested, in fact, that phosphatase was probably related more to C limitation of deep bacteria than P limitation and that the AP activity was a prerequisite for a more efficient C utilization. In conclusion the spatial and seasonal variability in microbial enzymatic activities, related to the different water masses of the Ionian Sea, could be considered an indicator of the main microbial processes in the oligotrophic waters.

## References

1 - Azam F., Smith D.C., Steward G.F. and Hagstrom A., 1993 Bacteriaorganic matter coupling and significance for carbon cycling. *Microb. Ecol.*, 28: 167-179.

2 - Hoppe H.G., Arnosti C., and Herndl G., 2002. Ecological significance of bacterial enzymes in the marine environment. Pp. 73-107. *In:* Burns R.G. and Dick R.P. (eds) Enzymes in the environment. Marcel Dekker Inc., New York.

3 - Hoppe H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.*, 11: 299-308.

4 - Zaccone R., Monticelli L.S., Seritti A., Santinelli C., Azzaro M., Boldrin A., La Ferla R., and Ribera d'Alcalà M., 2003. Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses. *J. Geophys. Res.*, in press.