Environmental Indicators: Ectoenzyme Activity in Different Size Communities

Annazette Sloley

ABSTRACT

Extra-cellular enzyme ("ectoenzyme") activities of aquatic microorganisms have been found to be effective determinants in evaluating nutrient deficiency. When a nutrient becomes limiting, it causes certain cells to synthesize ectoenzymes capable of increasing nutrient availability. Two of these enzymes are alkaline phosphatase, synthesized when phosphorus is limiting, and aminopeptidase, synthesized when nitrogen is limiting. This project examined phosphatase and peptidase activity overall and in what size fractions they each occur in the Gulf of Mexico during July 2004. During this period alkaline phosphatase activity occurred mostly in the <2µm size fraction. Aminopeptidase activity occurred equally among all three size fractions (>10µm, 10-2µm, and <2µm).

INTRODUCTION

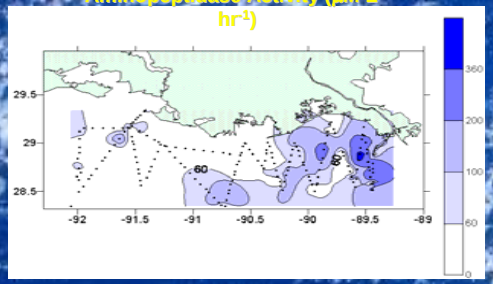
The Mississippi River is one of the world's top ten rivers in length, sediment delivery, and freshwater discharge. Increased nitrogen loading to the Gulf of Mexico causes increased primary productivity of the primarily nitrogen-limited waters. With plenty of nitrogen now present, organisms in the surface plume become phosphorus limited during the high flow season. The first response in the microbial community to environmental change is ectoenzyme activity. Ectoenzyme activities alone may not give the nutritional status of different trophic levels in the community because several different organisms synthesize the same enzymes. Ectoenzymes can be produced by many microorganisms, such as zooplankton and heterotrophic nanoflagellates. However, most activity is from bacteria and algae. Aminopeptidase has been commonly associated with heterotrophic bacteria and is generally found in bacterial size fractions (0.2-3µm). Alkaline phosphatase has been associated with both algae and bacteria and is therefore found in both bacterial (<2-3µm) and algal (3-150µm) size fractions.

METHODS

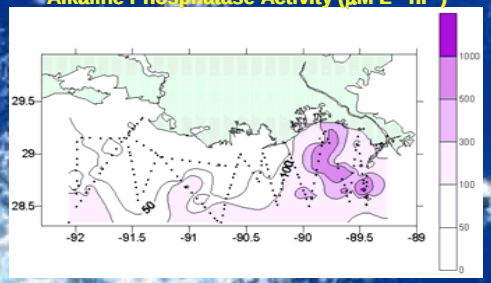
The cruise took place along multiple transects covering the Louisiana Shelf region from July 16th to July 20th, 2004. Sampling was done with the Multiple Instrument Data Acquisition System (MIDAS) aboard the *R/V* Pelican. MIDAS takes continuous measurements of salinity, surface temperature and other parameters while also pumping sample water into the ship. Alkaline phosphatase/aminopeptidase assays were done according to Ammerman (1993) with a Tecan Genios florescence microplate reader. The alkaline phosphatase assays were done with the fluorescent substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DifMUP). The aminopeptidase assays were done with the fluorescent substrate leucine 7-amino-4-methyl-coumarin (Leu-MCA). Size fractionation was done with a Whatman microplate vacuum manifold for 2 µm and 10 µm polycarbonate filters. In each case, the rate of substrate hydrolysis by the filtrate was measured and converted into activity.

RESULTS

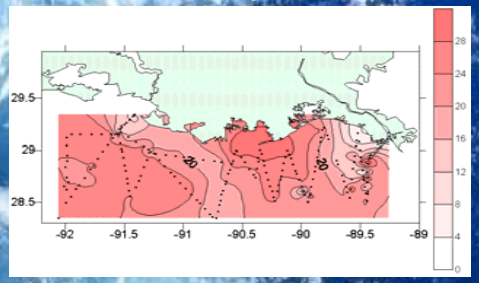
Aminopeptidase Activity (µM L^{-1} hr^{-1})



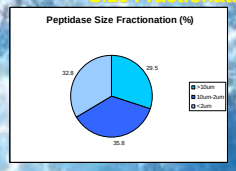
Alkaline Phosphatase Activity (µM L^{-1} hr^{-1})

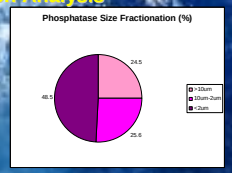


Salinity



Size Fractionation Analysis





	>10um	10um-2um	<2um
PHOSPHATASE			
AVERAGE:	24.5	25.6	48.5
STAND. DEV.:	3.7	3.8	7.2
N	45	45	45
PEPTIDASE			
AVERAGE:	29.5	35.8	32.8
STAND. DEV.:	4.1	5.0	4.6
N	52	52	52

Mississippi River Plume

Tecan Genios florescence microplate reader

Whatman microplate vacuum manifold

CONCLUSION

The contour map of aminopeptidase shows the highest activities at the Mississippi River mouth. This is assumed to occur because the low salinity in this region, and the increased nitrogen loading from the high flow rate of the river cause a substantial increase in biomass. Alkaline phosphatase activities are also highest in this region, providing evidence for phosphorus limitation. The size fractionation data shows that peptidase activity was somewhat equally distributed among the three size fractions. It is not yet understood why peptidase activities were found to occur equally. Phosphatase activity was highest in the <2µm size fraction. This fraction is where bacteria are found and so bacteria seem to be the most phosphorus limited. Past data suggests that in July, the system begins to move away from a phosphorus-limited environment to a nitrogen-limited environment. We propose that the decreasing nitrogen caused by the decreasing flow rate causes the population reduction of larger organisms. There is still enough nitrogen for the smaller organisms (i.e. cyanobacteria, which are prominent in the region in July) to survive. Therefore, phosphatase activities increase in the smaller size fractions.

Works Cited

Ammerman, J.W. (1993) Microbial cycling of inorganic and organic phosphorus in the water column. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 649-660.

Hoppa, Hans-Georg. (2003) Phosphatase activity in the sea. *Hydrobiologia*. 493: 187-200.

Rabalais, N.N., Turner, R.E. (2001) Hypoxia in the Northern Gulf of Mexico: Description, Causes and Change. In: Rabalais, N.N., Turner, R.E. (eds) Coastal Hypoxia: Consequences for Living Resources and Ecosystems. Vol 58. American Geophysical Union, Washington, p. 1-36.

Sala, M.M., Karner, M., Arin, L., Marrase, C. (2001) Measurement of ectoenzyme activities as an indication of inorganic nutrient imbalance in microbial communities. *Aquatic Microbial Ecology*. 23, 301-311.

Acknowledgements

Heartfelt thanks to Brian Gaas, Jason Sylvan, the RIOS team, John Quinlan, Judithe Grassle, and Jim Ammerman