# Activities of Extracellular Enzymes and Substrate Specificity of Peptidases in Raritan Bay, NJ

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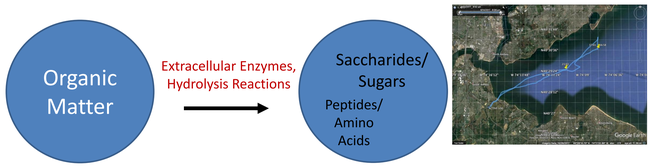
## Abstract

The Malcolm X Shabazz (MXS) Biogeochemistry Team in cooperation with Phil Sontag and Harold Ofori of the Reinfelder lab at Rutgers University have examined extracellular enzyme activity and tested inhibition in water from the Raritan Bay, NJ. Two sugar fluorescent substrates 4-methylumbelliferyl-α-D-glucopyranoside (MUF-α-glu), MUF-β-glu, and one peptidase L-leucine-7-amido-4-methylcoumarin hydrochloride (Leu-AMC) were tested in two sites in both filtered and unfiltered water. Filtered water showed higher enzymatic rates of activity than those in the unfiltered water for most of the substrates tested. A follow up study was conducted using L-arginine-AMC, arginine with a AMC fluorophore, EDTA and glutamic acid as inhibitors in filtered Raritan Bay water. The results showed potential for competitive inhibition of arginine hydrolysis in conditions with EDTA and glutamic acid present. More studies will have to be undertaken to better understand specificity of peptidases.

## Background

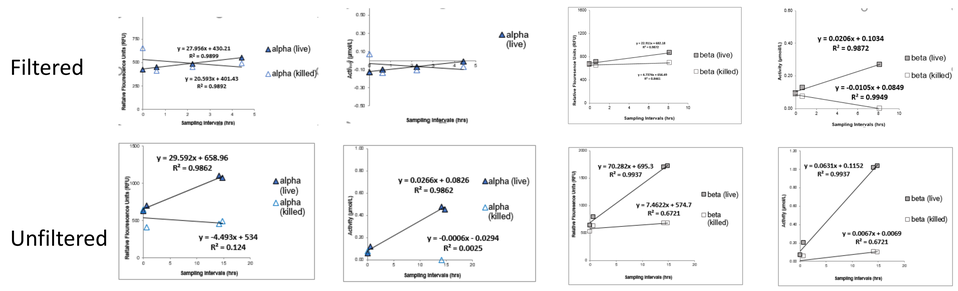
Since 2013, a group of students and teachers at Malcolm X Shabazz High School in Newark, NJ and researchers at the University of Tennessee, Knoxville, have been measuring activities of extracellular enzymes in diverse natural waters in order to better understand how microorganisms convert detrital organic matter into $CO_2$. Fluorescence measurements were made of model alpha and beta glucose sugar enzyme substrates and a peptidase enzyme, leucine-aminomethylcoumarin, which is intended to measure the activity of a single peptidase, leucyl aminopeptidase but the degree to which leucyl aminopeptidase reflects activities of other peptidases is not known. The research examined the role of extracellular enzymes such as leucine aminopeptidase (leu), α-glucosidase (alpha), and β-glucosidase (beta) on the carbon cycling in the Raritan Bay and its influence on organic matter remineralization and the bioavailability of contaminants in the Raritan Bay food web. Enzyme activities were compared in both filtered and unfiltered Raritan Bay waters. In most cases, enzyme activity was decreased in filtered water, which the team attributes to less organic material being available in the filtered water for the enzymes to act upon. In the filtered treatment, there are less particulates and aggregates, allowing free enzymes and dissolved substrates to interact.



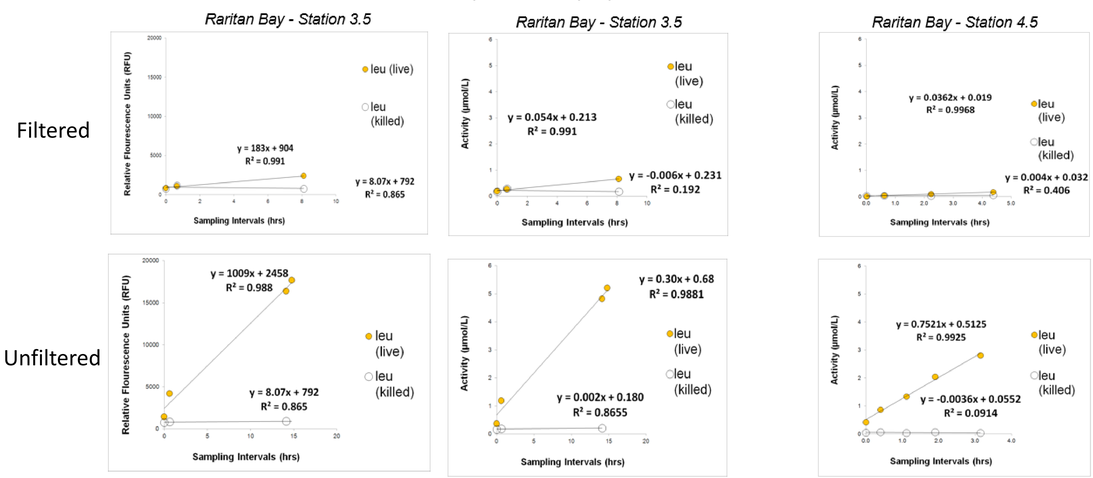

## Methodology and Data

In a first study, the team looked at relative fluorescence and the rates of enzyme activity at two sites in the Raritan Bay and compared these metrics in filtered and unfiltered samples. The conclusion was reached that the metrics were lower in unfiltered water due to the presence of cell-specific and free enzymes. Due to the fact that the solution was filtered, the smaller number of molecules/substrates that are left in the solution have less competition with the large macromolecules originally in the solution. Leucyl aminopeptidase showed higher activity than both of the glucosidase enzymes. This finding prompted the next step in the research, which was to add synthetic substrates to filtered Raritan Bay water with the addition of competitive inhibitors to observe and monitor enzymatic inhibition. Arginine containing the fluorophore 7-amido-4-methylcoumarin was used to measure its relative enzymatic activity in the presence of EDTA and Glutamic Acid.

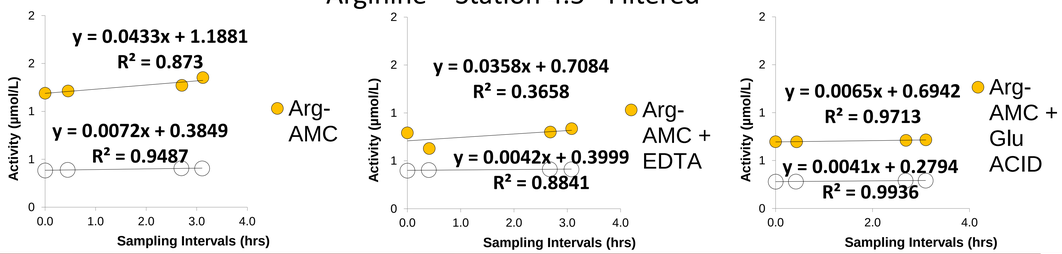
### Alpha and Beta Glucosidase – Station 3.5




### Leucyl Aminopeptidase




### Arginine – Station 4.5 - Filtered

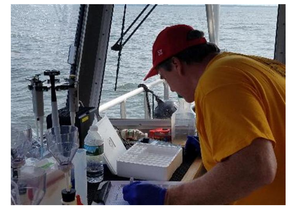


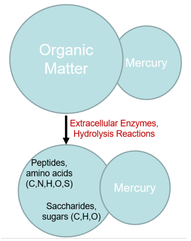

## Discussion

The addition of EDTA shows slightly lower enzymatic activity but yields a low correlation, meaning that there may not be notable inhibition. The addition of Glutamic Acid yielded much lower enzymatic activity which supports the idea that there was inhibition of arginine through hydrolysis reactions with Glutamic Acid.

## Conclusions and Directions for Future Research

The data gathered from the two experiments allude to a need for experiments to be undertaken in order to widen current knowledge on enzyme activity in aquatic systems. More specifically, how free free enzymes affect organic matter in its different states and qualities. Also, an area of interest is to characterize the organic matter in its dissolved phase. Subsequent experiments are underway that will have *in situ* data of enzyme activity on samples collected in the Raritan. Studies focusing on the relation between mechanism of mercury uptake and nitrogen demand will also be addressed.






## Acknowledgements

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## References

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Raritan River Consortium