# DEVELOPMENT OF NEW BIOASSAYS TO ASSESS THE QUALITY OF COASTAL AND ESTUARINE ENVIRONMENTS: THE POTENTIAL OF THE COSMOPOLITAN MARINE PROTOZOA *EUPLOTES CRASSUS*

Alessio Gomiero [1]*, Lara Boatti [2], Cristina Nasci [1] and Aldo Viarengo [2]
[1] Thetis S.p.A. - alessio.gomiero@thetis.it
[2] Department of Environmental and Life Sciences, University of Eastern Piemonte "Avogadro", Via Bellini 25G, 15100 Alessandria, Italy.

## Abstract
Estuarine and coastal sediments represent the final destination of thousands of organic and inorganic pollutants which are continuously discharged into the environment as result of industrial, agricultural and domestic activities. Due to the complexity of this environmental compartment sole chemical characterization often fails to correctly estimate the toxicity of such complex matrix. In such a context, a multi-disciplinary, chemical-biological approach can overcome this lack providing useful information for identifying those situations which require a close investigation at an early stage. In this work, the possibility of utilizing the interstitial marine ciliate *Euplotes crassus* (Dujardin, 1840) was examined for the screening of polluted estuarine sediments, pore and interstitial water by laboratory and field experiments.
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Contamination of the aquatic environments by pollutants has become a serious problem in the recent years. Furthermore, it has been observed that the pollutant concentration in sediments and sediment's interstitial water can be more than 10-100 times higher than the one present in the overlying water column. Hence, the interest in the toxicity of pollutants occurrence in these matrices and their biological effects, has increased during the last decades as they enter waterways by runoff, atmospheric deposition etc., and may be transported to estuaries and coastal sites potentially causing harm to a large variety of non-target wildlife organisms. On this context, it is generally agreed that in the field of environmental biomonitoring biomarkers can provide useful information for pointing out those situations requiring a close investigation at an early stage [1]. From this point of view, it is of increasing interest to identify a panel of organisms displaying sensitive responses to sediment and pore water from coastal environments under anthropic pressure.

Thus, the possibility of utilizing *E. crassus*, an interstitial single-cell marine protozoa, was examined by applying a battery of tests including cell viability and growth as typical ecotoxicological high-level endpoints. This study was also addressed to detect more sensitive sublethal early-warning responses utilising biological parameters such as lysosomal membrane stability and endocytotic rate, which are typical stress biomarkers in eukaryotic cells [2]. The sensitivity of *E. crassus* assay has been assessed within a range of organic and inorganic chemicals concentration and mixtures of them. Two heavy metals species were selected, one essential (Cu) and a non-essential (Hg), since it has been suggested that heavy metals may block enzyme systems of microorganisms or interfere with some essential cellular metabolites of bacteria and protozoa [3]. Furthermore, benzo(a)pyrene was involved as organic pollutant model. A field application was also performed aiming to validate the proposed bioassay. Four sampling sites within the Venice Lagoon were chosen according to previously reported differences in both environmental conditions and level of anthropic pressure [4, 5].

Results of laboratory studies indicated that estimated sub-lethal index of stress presented significant differences between reference and treated animals even after exposure to nano/micro molar trace elements concentrations. The endocytotic rate data showed a particularly strong decrease of predation capability in organisms treated with Hg. Sub-lethal tests, like endocytosis rates and lysosomal membrane stability, were able to detect biological effects of n/µM concentrations of toxicants. Furthermore, a significant synergic effect was observed on lysosomal membrane stability and replication rate of ciliate protozoa exposed to binary mixtures of selected toxicants. The results of field samples were in agreement with the laboratory responses. The overall results confirmed the suitability of using *E. crassus* as sentinel organism for monitoring sediment toxicity and provided an ecotoxicological protocol based on cellular biomarkers for the early detection of biological disturbance [6].

## References
1 - Cajaraville M. P., Bebianno M. J., Blasco J., Porte C., Sarasquete C., Viarengo A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *The Sci. of The Tot. Env.*, 247, 2-3: 295-311.
2 - Moore M. N., Icarus A. J., McVeigh A., 2006. Environmental prognostics: An integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. *Mar. Env. Res.* 61, (3): 278-304.
3 - Morgan, G.B., Lackey, J.B., 1958. BOD determination in wastes containing chelated copper and chromium. *Sewage Ind. Wastes* 30: 283-286.
4 - Losso C., Arizzi Novelli A., Picone M., Marchetto D., Pessa G., Molinaroli E., Ghetti P. F., Volpi Ghirardini A., 2004. Evaluation of surficial sediment toxicity and sediment physico-chemical characteristics of representative sites in the Lagoon of Venice (Italy). *J. Mar Sys.* 51, (1-4): 281-292.
5 - Nesto N., Romano S., Moschino V., Mauri M., Da Ros L., 2007. Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice, Italy. *Mar. Poll. Bull.* 55, (10-12): 469-484.