



Development of new bioassays to assess the quality of coastal and estuarine environments: the potential of the cosmopolitan single cell marine protozoa *Euplotes crassus*

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- The contamination of the aquatic environments by pollutants has become a serious problem in the recent years since thousands of organic and inorganic chemicals are continuously discharged in lakes and rivers by atmospheric deposition, runoff and as final effluents of domestic and industrial wastewaters
- The interest in the toxicity of complex mixtures of pollutants and their biological effects, has increased during the last decades as they enter waterways and may be transported to estuaries and coastal sites potentially causing harm to a large variety of non-target wildlife organisms
- Pollutant concentration in sediments and interstitial sediment water can be more than 10 - 100 times higher than that present in the overlying water column
- It is of increasing concern to identify a panel of organisms displaying sensitive responses to environmental perturbations
- An approach based on the estimation of sub-lethal stress indexes (biomarkers) can provide useful information for identifying those situations requiring a close investigation at an early stage

- The development of valuable bioassays to be exploit in standardized laboratory procedures for evaluating the toxicity of chemical compounds on polluted environmental matrices
- Identification of sensitive and reliable biological markers of stress for the evaluation of organisms health status
- Validate bioassay application by performing a pilot biomonitoring program
- Target species: the protozoa *Euplotes crassus*, Dujardin 1841 (*Ciliophora*, *Hypotricha*)

A single cell, free-crawling, interstitial marine ciliate



Why protozoa?...

- **Ecological relevance:** These microorganisms mediate the flow both of biological substances and energy from one trophic level to the next making them suitable models for predicting the effects of chemicals on aquatic communities.
- **Good biological model:** Due to their nature as a eukaryotic cell/organism, protists exhibit a relatively simple organization and a high degree of specialization.
- **Significance of responses:** these marine protozoa can be simply cultured in the laboratory under natural-like conditions, so that their biological responses are more reliable.

A biomarker is a xenobiotically, sub-lethal induced variation in cellular or biochemical components, processes or functions, that is measurable in a biological system

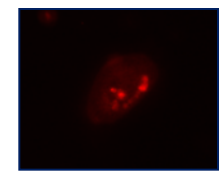
Being measurable at different levels of biological organization, they provide a correct snapshot of organisms health status anticipating changes at higher levels of biological structure

Biomarkers = 'early warning' tools in environmental quality assessment

The responses of two typical ecotoxicological high-level endpoint tests like **mortality** and **replication rate** were combined with sensitive, sublethal, early-warning biomarkers of stress like:

The **lysosomal membrane stability**: was estimated by the retention time of lysosomal dye Neutral Red

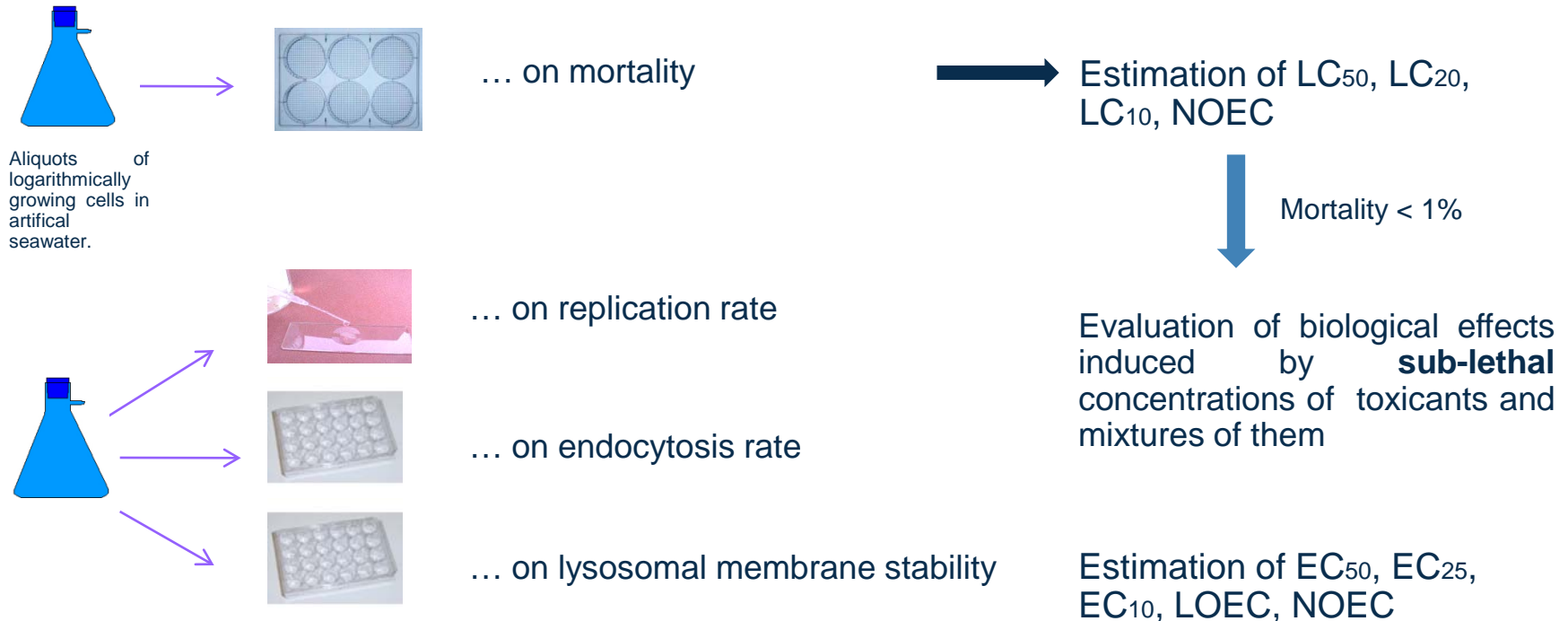
The **endocytotic rate**: was assessed by quantifying cellular intake of fluorescent bio particles Escherichia coli K-12 Strain



to provide a clear estimation of organism's health status

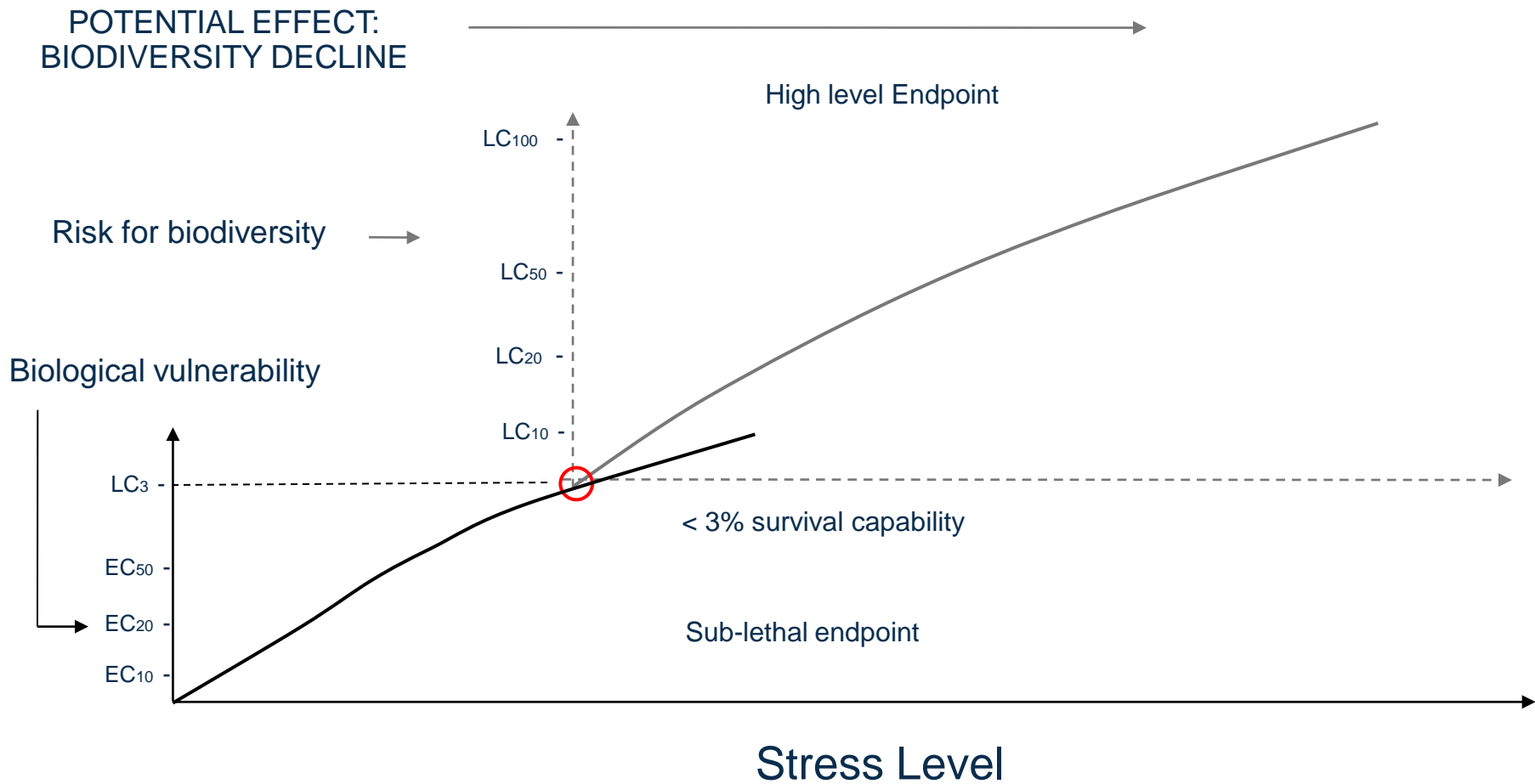
Three environmentally relevant chemicals tested: Cu (essential trace element)
 Hg (not essential trace element)
 Benzo(a)pyrene (organic pollutant model)

Study of effects of toxicant-exposure:



To provide basic knowledge about the linkage

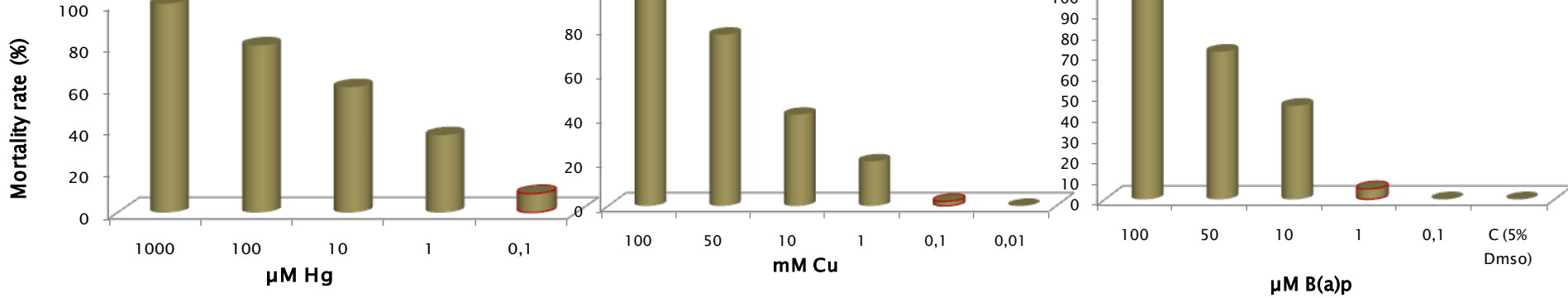
Pollutants toxicity ↔ Ecological relevance



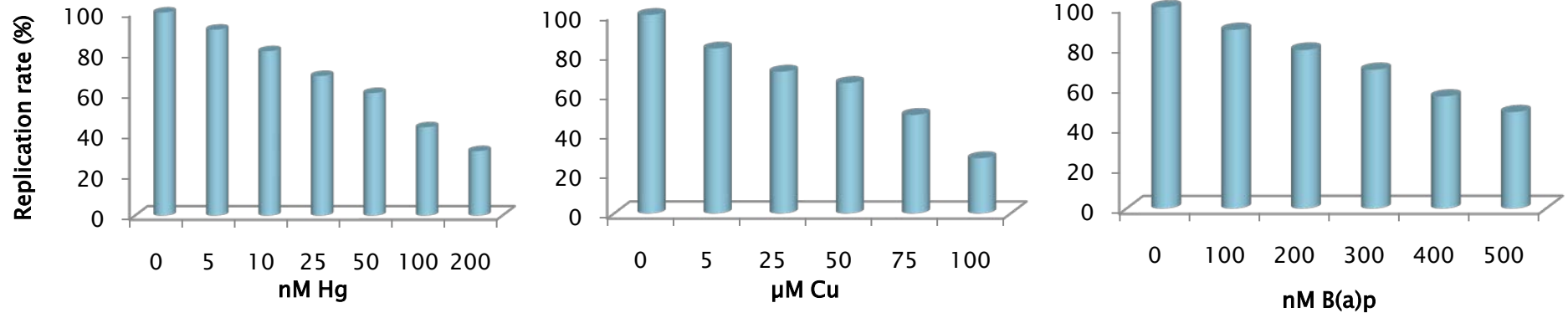
Enhanced compound-specific comprehension of dose / effects relationship

→ knowledge of changes at higher ecological levels

Mortality rate

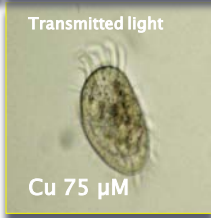
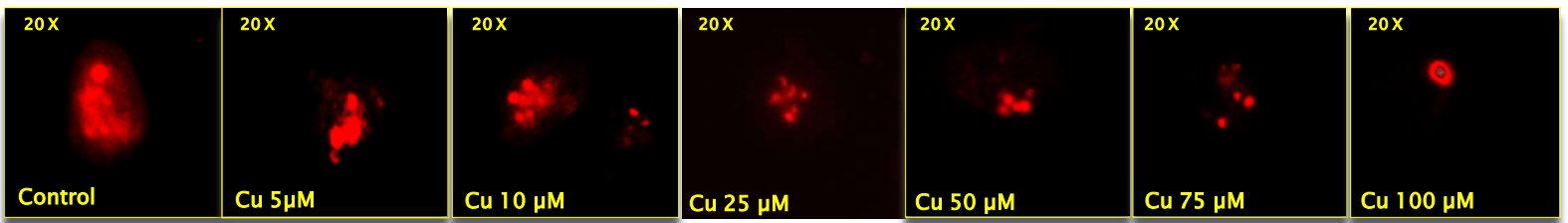
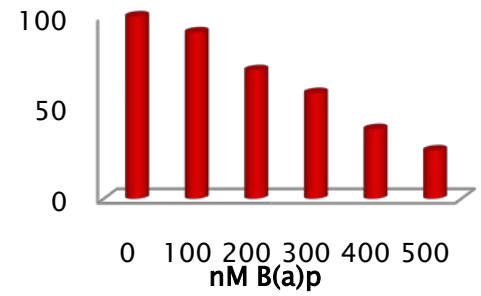
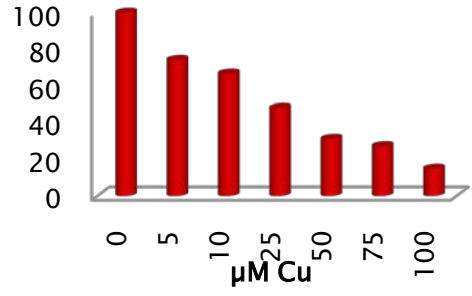
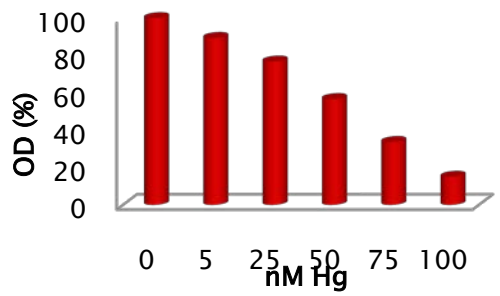


Replication rate



Good linear dose / effect relationship in both mortality and replication rates tests

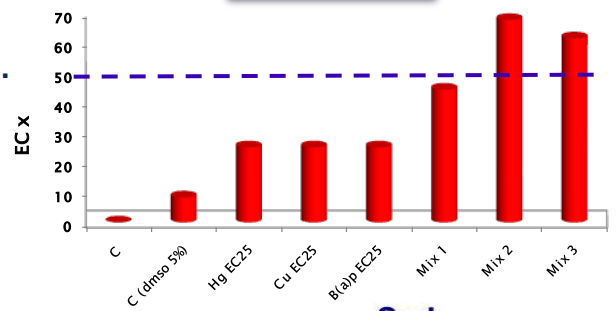
Endocytosis rate



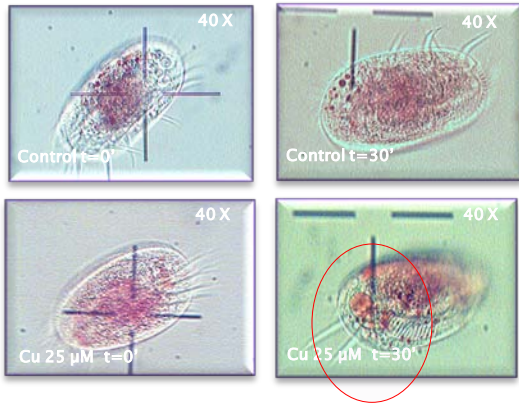
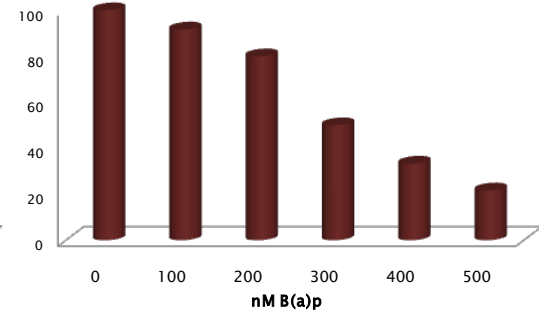
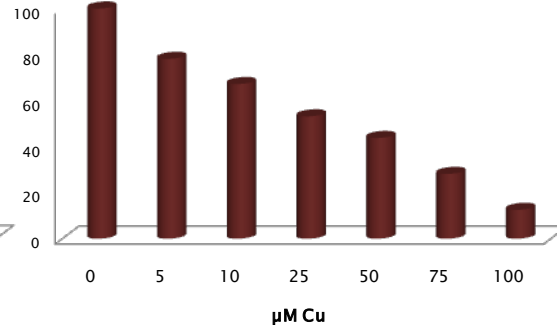
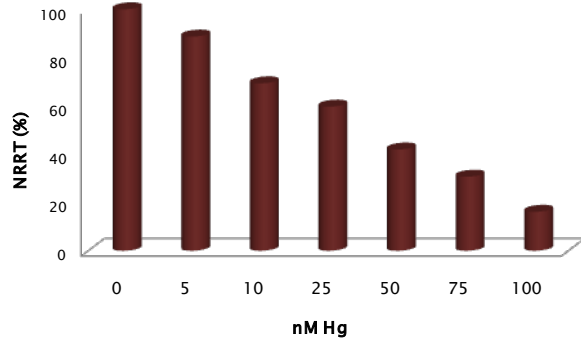
Study of binary mixtures..

- Mix 1 ⇨ EC₂₅Cu + EC₂₅ Hg • Exp EC₅₀ (?)
- Mix 2 ⇨ EC₂₅Hg+EC₂₅ B(a)p • Exp EC₅₀ (?)
- Mix 3 ⇨ EC₂₅Cu+EC₂₅ B(a)p • Exp EC₅₀ (?)

Additive, synergic or antagonistic effects ? ...



Lysosomal membrane stability

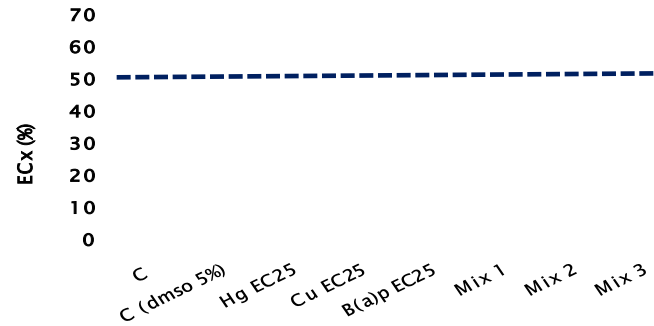


LMS Estimated as time to observe the release of NR in the 50% of scored protozoa...

Good linear responses among lysosomes permeability and toxicants concentrations

Same binary mixtures tested..

- Mix 1 \Rightarrow EC₂₅ Cu + EC₂₅ Hg • Exp EC₅₀ (?)
- Mix 2 \Rightarrow EC₂₅ Hg + EC₂₅ B(a)p • Exp EC₅₀ (?)
- Mix 3 \Rightarrow EC₂₅ Cu + EC₂₅ B(a)p • Exp EC₅₀ (?)

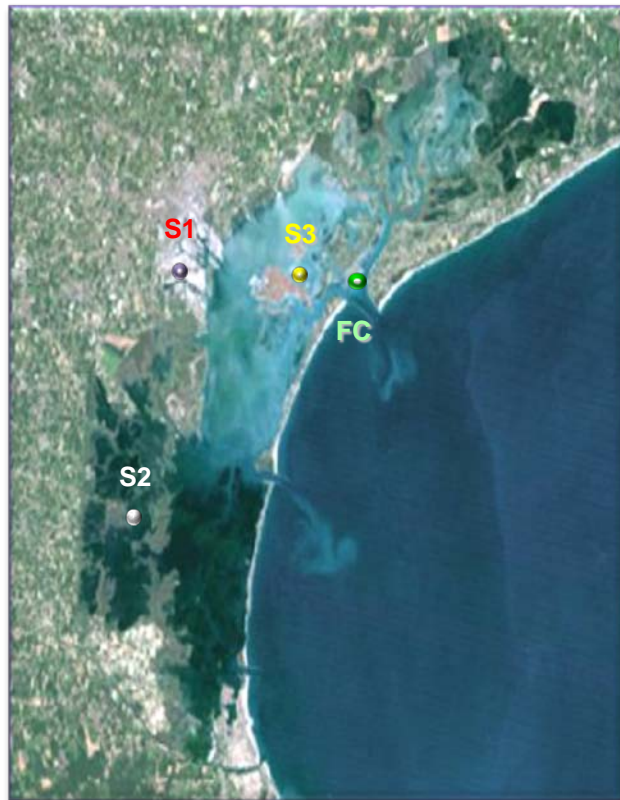


- Both endocytosis rates and lysosomal membrane stability were able to detect biological effects of n/μM concentrations of Hg, Cu and B(a)p
- Results of binary mixtures reported additive and/or synergic toxic effects on protozoa
- Developed biomarkers were easy to perform and provided rapid and objective results
- All available evidence indicates that the here presented new bioassay is fast, sensitive and easy to handle

Suitability for field biomonitoring programs?...

Represents coastal area with a peculiar ecosystem characterized by an high ecological and economic value

Sediments from 4 sites selected according to well known degrees of anthropic pressure were examined



S1

- Located close to the industrial district of Porto Marghera
- High levels of environmental contamination



S2

- Located in the southern part of Venice lagoon
- Site under moderate agriculture run-off



S3

- Located close to the historic centre of Venice
- Site affected by household wastewater



FC

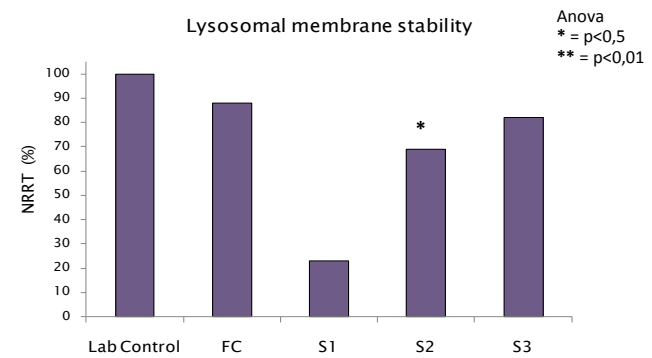
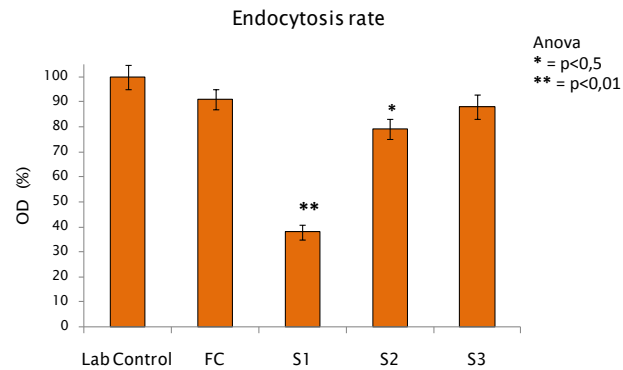
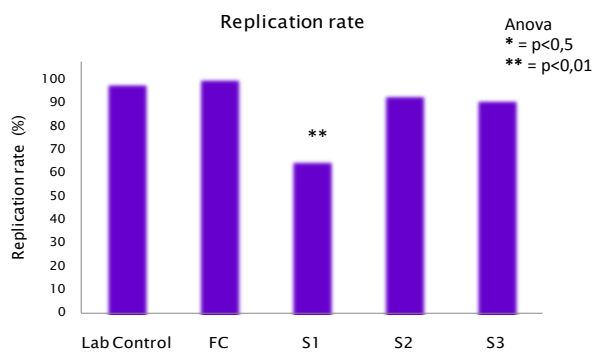
- Located close to "Lido" inlet
- Low levels of environmental contamination

Sediment samples were collected and processed (elutriated) according to standardized US-EPA methods

Moderate increments of mortality were recorded only in site S1 ($\cong 7-10\%$)

Significant effects on chronic high-level endpoint tests \rightarrow Reduction of replication rate

Average higher alterations were recorded by sub lethal biomarkers of stress



- The approach based on a battery of biomarkers in conjunction with high-level endpoint tests may help follow the pollutant-induced stress syndrome in the organisms, from early sub lethal effects to more deleterious effects (i.e. death)

From biological vulnerability

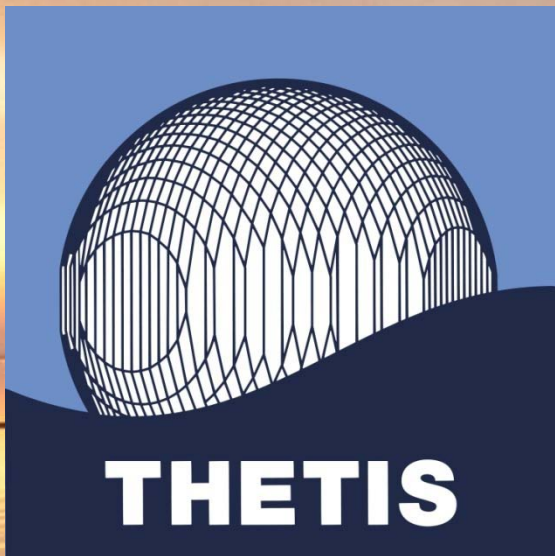


To risk for biodiversity

- The combination of all tests were able to discriminate a gradient of stress within the sampling sites
- The presented bioassay based on protozoa appears suitable for environmental biomonitoring purposes



...This leads to consider the future application of *E. Crassus* assay for the screening of sediments toxicity of coastal sites



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