genome integration by horizontal gene transfers from endosymbiont or microbiome communities. Analyzing dual RNA-seq data of the only host-virus combinations both encoding a FicD gene, we revealed a time-dependent expression for White Spot Syndrome Virus and Ostreid herpesvirus-1 FicDs, with higher expression levels than crab and bivalve genes. The frequent exchange of FicDs and the shift of this enzymatic ability from bacteria to pathogenic dsDNA viruses underlie complex host-pathogen-predator interactions in the marine environment and a possible role for AMPylation at the edge of host-virus interactions in mollusks.

Protein extractions from *Amphistegina lessonii*: a new approach for the evaluation of the effects of heavy metals on benthic organisms

C Ciacci[1], M Betti[1], S Abramovich[2], M Cavaliere[3], F Frontalini[3]
[1]Department of Biomolecular Science, Urbino University, Urbino, Italy
[2]Department of Earth and Environmental Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel
[3]Department of Pure and Applied Sciences, Urbino University, Urbino, Italy

Different stressing factors such as heavy metals, nanomaterials and others environmental contaminants can induce physiological and biochemical changes on foraminifera, unicellular organisms typically living in sediments. Research on protein content of foraminifera is currently limited and no comparative analysis on the efficacy and reliability of the different assays has been conducted on foraminifera so far. The first purpose of this study is to compare the results of different methods of lysis, protein assays, and protein staining on *Amphistegina lessonii* – a symbiont-bearing foraminiferal species and presents a new protocol. This protocol could be applicable to other benthic foraminiferal species and represents a new experimental approach for the application of *A. lessonii* as a potential biomarker. The second part of this study is related to the evaluation of short-term effects of Hg exposure (10 ppb, 24 h) on *A. lessonii*. Benthic foraminifera have been mostly utilized as pollution bioindicators in marine and transitional marine environments and mercury contamination is a global issue due to its significant toxic effects on human health, and cytotoxicity being higher than many other heavy metals. In this work, we have evaluated the activity of different enzymes involved in antioxidant defence (SOD, GST, GSR and GPx) and several proteins (HSP70 and p38MAPK) are analysed by western blotting. The results show that the activities of the antioxidant enzymes increased in Hg-exposed foraminifera, indicating that heavy metals can induce oxidative stress and stimulate the engagement of antioxidant enzymes as cellular defense mechanisms; moreover, Hg treatment induces expression of HSP70 and activation of p38MAPK. As previously demonstrated, these data support that Heat shock proteins (HSP) play a crucial role in maintaining protein homeostasis under stress conditions in both prokaryotes and eukaryotes and the mitogen-activated protein kinase (MAPK) pathway is an evolutionarily conserved signalling pathway existing in unicellular organisms to humans. The results can be used to obtain information on the environmental quality of marine sediments and the development of the cellular biomarkers might represent a complementary approach to the traditional biomonitoring.

***Hirudo verbana* as a freshwater invertebrate model to assess the effects of polypropylene nano and microplastic dispersion.**

N Baranzini, L Pulze, C Bon, L Izzo, A Grimaldi
Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

Plastics represent the most widely employed synthetic materials that, given their physical and chemical properties, are used to produce robust and above all economic objects. Although from their discovery these materials became indispensable, their worldwide distribution caused an uncontrolled accumulation of waste products followed by an indiscriminate release in the environment. In particular, millions of tons are reversed in waters every year, making the aquatic ecosystems the most affected by plastics pollution. Moreover, their degradation, due to biotic and abiotic events, leads to the formation of small-size particles, known as nano (NPs) and microplastics (MPs), that can persist inside organisms for a long time and can accumulate in the trophic chain.

To evaluate the possible tissue accumulation and harmful effects in freshwater dispersion, we have exposed the leech *Hirudo verbana* to fluorescent polypropylene NPs and MPs at different timings (1 and 6 hours, 1 week, 1 and 2 months). Optical and fluorescent analyses demonstrate that these particles penetrate inside the organism both through the integument and food and induce epidermal mucous cell proliferation, angiogenesis, release of the pro-inflammatory molecules *Hm*AIF-1 and *Hv*RNASET2, macrophage-like cell migration and amyloidogenesis. Moreover, qPCR analyses reveal an increasing expression of the specific oxidative stress enzymes superoxide dismutase and glutathione S-transferase. Taken together, these data suggest the medicinal leech *H. verbana* as an innovative environmental biomarker ideal for evaluating the possible harmful effects deriving from NPs and MPs on freshwater animals.

Study of immunotoxicity responses of *Sabella spallanzanii* exposed to copper sulphate

F Bertini, C La Corte, M Dara, D Parrinello, MG Parisi
Department of Earth and Sea Sciences, University of Palermo, Palermo, Italy

In the last decade, the growing use of heavy metals in human activities has inevitably altered the health of the aquatic environment. Marine pollution due to heavy metals is an issue that has now a global dimension and has interested many researchers because of their ability to persist,

bioaccumulate and biomagnify in the trophic chain leading to adverse effect also on human health. Copper sulphate is a very soluble xenobiotic, still used today into antifouling paint and in aquaculture like biocide, with high activity against algae, fungi and marine invertebrates. Its main component is copper that at high concentrations have immunomodulating effect on marine organisms.

In this study we have used the polychaete worm *Sabella spallanzanii* to investigate its immune response after exposure to copper sulphate and the combinate effect with the inoculation of *Escherichia coli*, to provide an overview of effect biomarkers for monitoring chemical and bacterial stressors. We aimed also to validate the species as model organism in marine-coastal biomonitoring and to investigate the modulation of TLR-4 as response useful to evaluate environmental alterations. Polychaetes were subjected to five treatments: 1. Control (naïve), 2. Filtered sea water + TBS injection, 3. Filtered sea water + *E. coli* injection, 4. Copper sulphate + TBS injection, 5. Copper sulphate + *E. coli* injection. The exposure to xenobiotic was chosen in relation to the specificity of the animals' response and the bacterial injections were made after the exposure. The immune markers evaluated in the total body extract of the animals were inflammatory and antioxidant markers. Therefore, were investigated the activity of: esterase (EST), alkaline phosphatase activity (ALP), cytotoxicity and detoxifying/antioxidant enzyme such as glutathione peroxidase (GPx). In addition, toll-like receptor (TLR), allograft inflammatory factor-1 (AIF-1), lysozyme (LYS) and hemagglutinating and inhibition activity have also been considered to highlights possible modulations.

The analysis of results indicated a significant activation of TLR-4 and AIF -1 in specimens inoculated with bacteria and treated with combinate exposure factors. The results regarding lysozyme and peroxidase enzyme as well as the hemagglutination activity have well highlighted the differences between the different environmental stimulations. Overall copper sulphate had an immunomodulating effect of the species under investigation, varying the immune response especially after bacterial inoculum.

Herbicide exposure alters the expression of antimicrobial peptide patterns in the mealworm beetle infected with the natural entomopathogen *Beauveria bassiana*

ML Vommaro[1,2], C Zanchi[2,3], A Giglio[1], J Kurtz[2]
[1]*Department of Biology, Ecology and Earth Science, University of Calabria, Arcavacata di Rende (CS), Italy*
[2]*Institute for Evolution and Biodiversity, University of Münster, Münster, Germany*
[3]*Institute for Biology, Freie Universität Berlin, Berlin, Germany*

Herbicide treatments are an integral part of agricultural practice. However, repeated applications lead to soil, water, and food contamination and adverse effects on non-target organisms. In this study, a pendimethalin-based commercial formulation (PND) was tested on the beetle *Tenebrio molitor* Linnaeus, 1758. The effects of herbicides on interspecific relationships, such as host-pathogen interaction are poorly studied. In the laboratory, adults were exposed to two different doses, the concentration corresponding to the contamination of the treated soil and the maximum residue level allowed by the EU in cereals. The beetles were then exposed to an inoculum with the entomopathogenic fungus *Beauveria bassiana* (Bb), commonly used as a bioinsecticide. Survival, sporulation of the fungus in cadavers, and expression levels of antimicrobial peptides (AMPs) Tenecin 1, 2, and 3 were examined. Although the survival rate did not change significantly between control and herbicide-exposed beetles, an alteration in the expression pattern of inducible AMPs was recorded. PND-treated beetles showed up-regulated Tenecin 1 and Tenecin 2 after inoculation with Bb. In addition, a slight increase in Bb sporulation on cadavers was recorded at the highest dose of the herbicide. Our results showed a potential alteration of the host-pathogen interactions, raising the question of bioinsecticide compatibility with synthetic pesticides and the effects of herbicides on interspecific relationships in wild species.

Organ-specific accumulation of PLGA nanoparticles injected into *Pomacea canaliculata* snails: preliminary *in vivo* observations

A Ferrari[1], J Duskey[2], G Bergamini[3], A Rinaldi[2,4], R Fiorino[1], B Ruozi[2], G Tosi[1], D Malagoli[1]

[1]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[2]*Nanotech Lab, Te.Far.T.I., Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[3]*Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[4]*Clinical and Experimental Medicine PhD Program, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*

Nanoparticle-delivered drugs are attracting growing interest for their potential to minimize side effects and create "patient personalized" therapeutic applications. Currently, preliminary animal testing of nanodrug systems must be performed following the European directives and national laws, which are mainly centered on vertebrates. However, *Pomacea canaliculata*, a freshwater amphibious snail from South America, is emerging as a possible substitute of vertebrates in preliminary bio-accumulation and bio-safety studies of nanodrug systems. *P. canaliculata*, which is easy to breed, manipulate, and presents a similar weight and longevity to mice, has already been proposed as a potentially model suitable for studying the distribution and organ accumulation of Superparamagnetic Iron Oxide Nanoparticles (SPIONs, size 14-80 nm). Here the accumulation of the Cy5 fluorescently labeled FDA approved Polylactic-co-glycolic acid nanoparticles