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REPORT OF MEETING

XXth scientific meeting of the Italian Association of Developmental and Comparative Immunobiology (IADCI), 13 - 15 February 2019, Department of Biology, Ecology and Life Sciences, University of Calabria, Rende, Italy

Organizers: **A Giglio[1], P Brandmayr[1], F Talarico[1], A Mazzei[1], S Marsico[2], A Naccarato[3], F Cavaliere[1], ML Vommaro[1], MC Granieri[1]**

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I met you when I was very young,
at the beginning of my professional life,
and I immediately understood
that you were reserving many surprises;
I followed you in silence
when your secrets have been revealed;
how do you always be the same and always different,
the "God problem" revealed.
I admired your anatomy,
simple, elegant,
based on harmonic couplings of a wonderful domain,
classic like a Doric temple;
I admired the flexibility of your body:
the movements of the hips and elbows,
your fingers that shape the socket.
I was entranced by the dynamics of your dance.

Then I had the pleasure
to be the only one to own you in the frost of polar ice,
where you appeared to me alone,
only wonder, generated by an evolutionary miracle.

It is true that I have been unfaithful to you;
I was attracted to other molecules,
from the aesthetics of the baroque forms of the Toll-like Receptors,
from the molecular labyrinth of the third factor of the Complement,
that is leafed in luminous fragments up to the small,
but multipotent, anaphylatoxin.

Now I will not see the many sure marvels
that you reserve to those
who will follow you in the future
but I have the memory
to have loved the most beautiful of the molecules:
Immunoglobulin.

By Umberto Oreste, past Researcher at Institute of Protein Biochemistry, National Council of Research, Naples (Italy)

Session 1. Chairmen: Magda de Eguileor, University of Insubria, Varese, Italy and Adriana Vallesi, University of Camerino, Camerino (MC), Italy
Cell-cell interaction

Mytilus hemocytes as a model to investigate the specificity of innate immunity in bivalve molluscs

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Invertebrates represent 97% of animal diversity and present in virtually any ecosystem. This implies that each species should be able to adapt and survive in its environment by only relying on innate immunity. The mechanisms of immune specificity (sophisticated recognition systems for a wide variety of non-self material) and immune training/memory (capacity to mount a faster and more effective response upon re-exposure to a stimulus) are therefore central to the invertebrate ability to survive in diverse environments.

Mytilus galloprovincialis hemocytes represent a powerful *in vitro* model to study the specificity of innate immunity in bivalve molluscs. Data obtained in response to both natural stimuli (different bacterial species and strains), and different types of nanoparticles-NPs, as environmental contaminants are summarized.

Moreover, a key role for soluble hemolymph proteins was identified. A case study is represented by the Extrapallial Protein precursor (EP), that specifically mediates the interactions between hemocytes and bacteria carrying mannose-sensitive ligands (i.e. *Vibrio aestuarianus 01/032*), resulting in efficient recognition, binding and killing. Moreover, this protein represents the sole component of the biocorona formed around PS-NH2 (amino modified polystyrene NPs), with consequent immunomodulatory effects on the hemocytes.

The interactions between hemocytes, hemolymph proteins, selected types of bacteria and NPs observed *in vitro*, at the basis of immune specificity, suggest that these mechanisms may also participate in immune training *in vivo*. To investigate this possibility, mussels were subjected to pulse exposure to PS-NH2 (10 μg/L; 24h, 48h depuration and further 24h). At each exposure time, several functional and molecular parameters were evaluated, including the bactericidal towards *V. aestuarianus*. After the 1st exposure, hemocyte lysosomal stability and mitochondrial potential were decreased, EP was upregulated, Lysozyme and MytB were downregulated, lysozyme activity was increased. However, killing of *V. aestuarianus* was unaffected. After the 2nd challenge, all stress parameters were recovered, immune-related genes were upregulated (EPp, MytB and FREP), and the bactericidal towards was increased up to 50%. The results indicate that repeated exposure to PS-NH2 results in immune training, where expression of selected genes may play a role in improving the defence against pathogens through common recognition mechanisms.

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The often overlooked coming out of ciliates: biological and experimental benefits from accepting genetically identical conspecifics as sexual partners

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Ciliates usually manifest sex in the form of conjugation, a unique phenomenon in which cells temporarily unite two by two in mating pairs to perform a mutual exchange of gamete-nuclei derived from meiotic products of their germinal micronucleus. The native view of conjugation as a spontaneous manifestation associated with environmental famine conditions was eventually denied by the milestone Sonneborn's finding (PNAS, 1937) that the most popular ciliate, *Paramecium*, actually controls conjugation through a genetic mechanism of mating types. Being only two in the *Paramecium* species studied by Sonneborn, these mating types were functionally equated to 'male' and 'female' sexes. And, as a consequence of this equation, conjugation was since thought of as a phenomenon committed to involve, as a rule, genetically distinct cells representing two 'complementary' mating types. However, this is a wrong tenet adverse the evidence that many ciliates conjugate with *no* discrimination between sex identity and diversity. And from this *no* discrimination both the ciliate biology and the students of ciliate biology draw benefit. The ciliate biology, because the homo-sexual pairs (yet ineffective to reshuffle the species gene pool) multiply the opportunity for cells to practice conjugation which, in every case, determines the initiation of a new life cycle and the replacement of the cell 'old' transcriptionally active somatic (macronuclear) genome with a completely new one generated from the permanently 'young' transcriptionally inert germinal (micronuclear) genome. The students of ciliate biology, because homo-sexual pairs form without requiring physical interactions between sexually/genetically different cells. They form as well in cultures of cells of the same identity previously suspended with filtrates from cultures of conspecific cells of different identity. Which immediately identifies species that interact sexually via water-borne mating signals (pheromones), and greatly facilitates the isolation and function-structure characterization of these signals directly from cell-culture filtrates

The pheromone genes of the self/non-self recognition mechanism of the ciliate *Euplotes crassus* generate multiple transcripts by an alternative splicing of 'matryoshka' introns

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In ciliates, cell-type distinctive protein pheromones control a self/non-self recognition mechanism responsible for the cell switching between the vegetative and sexual stages of the life cycle. These signaling molecules are encoded by genes (pheromone genes) that in the cell somatic nucleus (macronucleus) represent the transcriptionally active versions of transcriptionally silent genes allelic at the same genetic locus *mat* of the cell germinal nucleus (micronucleus). In the course of evolution, in *Euplotes*, the native single multiallelic *mat* locus underwent duplication among species which, such as *E. crassus*, form the latest branching clade of the phylogenetic tree. Because of this duplication, *E. crassus* expresses two distinct families of pheromone genes instead of a single family, as is the case in species of earlier branching clades.

We analyzed the structure and expression of a number of *E. crassus* pheromone genes representative of the two families. Like their orthologs of other *Euplotes* species, these genes show 5'-leader regions that are much more extended than the coding regions, lack canonical regulatory sequences for gene transcription, and synthesize multiple transcripts (in addition to the pheromone-specific one) through the activity of two distinct transcription start sites and a mechanism of alternative intron splicing. These *E. crassus* introns have been found to be unique with respect to introns of all the other *Euplotes* pheromone genes. They can be distinguished between 'matryoshka' introns, residing one inside the other like Russian Dolls, and 'non-matryoshka' introns. While the former possess canonical GTA/TAG splicing sites, the latter possess CTA/TAC splicing sites complementary to the canonical GTA/TAG splicing sites. This strongly suggests that both the DNA strands of the *E. crassus* pheromone genes can be used as template for transcription. We are currently attempting to verify this hypothesis and assign a function to the products of the multiple *E. crassus* pheromone gene transcripts.

Study of viral infections in bivalves as a tool to trace antiviral host pathways

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Viruses are the most abundant biological entity on Earth and the presence of an antiviral system in almost every living organism further supports their global distribution. Virus-induced selective pressure drive host population dynamics, can interfere with biological invasions and mediate evolutionary transitions. Actually, the gene flux from viruses to eukaryotic organisms is suggested to drive the long-term evolution of host genomes while the evolutionary pressure of host antiviral defenses shapes viral genomes in a never-ending arms race.

Although most of the molecular components of antiviral vertebrate pathways have been traced in bivalve genomes, evidence supporting their activation during viral infections is lacking. Aiming to obtain functional data on antiviral defenses in bivalves, we studied the behavior of different Malacoherpesviruses in different hosts by high-throughput DNA and RNA sequencing. Accordingly, we describe the virus-induced activation of different antiviral responses in bivalve and gastropod species. Among other findings, we showed that an Interferon Stimulated Gene mediates a significant editing of Malacoherpesviruses dsRNAs in the oyster *Crassostrea gigas* and abalone *Haliotid diversicolor supertexta*, and we provide evidence that, in the evolutionary time, such activity have shaped the genomes of these viruses.

Our results update the phylogenetic distribution of different antiviral pathways among invertebrates and support discussion on differences and commonalities in comparison to vertebrates.

This work was partially supported by the *Vivaldi* European project.

Two distinct lectin families with different glycan-binding specificity share a β-trefoil fold in mussels

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Metazoans possess a plethora of highly diversified lectins, which enable the specific recognition of sugar motifs exposed on the surface of target cells. These molecular interactions regulate diverse biological functions such as cell adhesion, fertilization, food particle selection and immune recognition. Over the course of evolution, marine invertebrates have recruited a number of different structural units to enable the recognition of glycans associated with invading pathogens. One of such units is the ricin B lectin domain, which adopts a characteristic β-trefoil three-dimensional folding and characterizes a number of carbohydrate-recognition proteins known as R-type lectins.

Through the combination of glycobiology, molecular biology and genomics approaches, we identified and functionally characterized two distinct families of β-trefoild lectins in mussels (Bivalvia, Mytilidae), named mytilectins and SeviL-like lectins. In spite of a shared three-dimensional structure, the two lectin types display no primary sequence homology and a markedly different glycan specificity, as shown by glycan-array assays. Indeed, while MytiLec-1 (isolated in *Mytilus*

galloprovincialis) recognized globotriose (Gal*α*1-4Gal*β*1-4Glc), SeviL (isolated in *Mytilisepta virgata*) could bind specifically to asialo-GM1 (Galβ1-3GalNAcβ1-4Galβ1-4Glc).

We further show that the two type of lectins display a peculiar taxonomic distribution and report the presence of members of the mytilecin family with an additional aerolysin-like pore-forming domain, which might endow such proteins with combined pathogen recognition and killing properties. While the role of β-trefoild lectins in the immune system of bivalves remains to be fully elucidated, we discuss the potential biotechnological application of these molecules. Indeed, the ability to specifically recognize glycans expressed on the surface of cancer cells, together with the ability to trigger cell death through the activation of the MAPK pathway and caspase-3/9 in a dose- and time-dependent manner, offer a great opportunity for the development of novel drugs or diagnostic tools based on mussel β-trefoild lectins. A first step in this direction has already been made with the computational design of Mitsuba, an artificial lectin based on MytiLec-1, able to recognize Burkitt's lymphoma cells.

Effects of a nematode-based molluscicide on survival and antimicrobial peptide expression in *Pomacea canaliculata*

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The freshwater snail *Pomacea canaliculata* is a highly invasive species with a robust innate immune system based on cellular and humoral components and only a few predators in nature. To date, no specific and lethal pathogens have been reported for the commonly known golden apple snails, although this information could be crucial for developing sustainable and eco-friendly approaches for controlling *P. canaliculata* diffusion.

In this context, we tested the effects on adult *P. canaliculata* of a commercial molluscicide developed against terrestrial slugs and constituted by the living nematode, *Phasmarhabditis hermaphrodita*. At the standard temperature of animal maintenance, *i.e.*, 25 °C, the molluscicide proved to be effective against adult specimens of *P. canaliculata* in a dose-dependent fashion. During one-week experiments, lethal effects have been observed at the highest concentration tested (17 g/L). After incubation at a sub-lethal concentration (1.7 g/L) the snails reduced the food intake and stopped eating while no evident effects were observed for the lowest concentration, 0.17 g/L. The molluscicide efficacy is also temperature-dependent since, especially for the 17 g/L concentration, significantly stronger effects have been observed at 18 °C whereas no effects on survival or feeding rate have been recorded after incubation at 30 °C.

Real time PCR experiments performed on animals exposed for 24 h at the concentration of 1.7 g/L revealed organ- and temperature-specific changes in the mRNA expression of the antimicrobial peptide (AMP) Bactericidal/Permeability-Increasing protein (BPI). More in details, BPI mRNA expression relative to control snails significantly dropped in the anterior kidney at 18 °C and in the gills at 25 °C. The other AMPs tested, Lipopolysaccharide Binding Protein (LBP) 1 and 2, did not change their expression in any of the tested organ at any temperature, as well as BPI after incubation at 30 °C.

On the whole, we have observed that at sub-lethal concentrations the molluscicide induced a reduction of feeding rate and negatively influenced the immune defense in key sentinel organs, namely anterior kidney and gills. Our results indicate that nematode-based molluscicides may represent an efficient solution for a sustainable control of *P. canaliculata* spread.

***In vitro* exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) impairs innate inflammatory response**

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Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that are added to numerous products to prevent accidental fires. Even though there is little information on the health effects of PBDE exposure, it is still of concern to humans because some types of PBDEs can build up in the fatty tissues of the several aquatic and terrestrial animals entering the food chain (EFSA Panel, 2011). A few surveys demonstrated that PBDEs bio-accumulate in human tissues with particular attention to human milk. Recently, their presence has been correlated to several pathologies but little is known about their effect on the human innate immune system activity. In this study we investigated the effect of the congener 2,2',4,4'-Tetrabromodiphenyl ether (PBDE-47) on the functional activity of the THP-1 human macrophages cell line and on ex vivo freshly isolated human basophils. Cytotoxicity and genotoxicity studies showed that PBDE-47 was able to induce toxic effects on the THP-1 cell line viability at concentrations ≥25 µM. Immune function of THP-1 was studied after stimulation with bacterial lipopolysaccharide (LPS) and PBDE-47 exposure at concentrations granting macrophage viability. Two dimensional electrophoresis showed modification of the proteome in the 3 µM PBDE-47 treated sample and Real Time PCR and ELISA demonstrated a statistically significant reduction in the expression of IL-1β, IL-6 and TNF-α cytokines. Furthermore, PBDE-47 was able to perturbate genes involved in cell motility upregulating CDH-1 and downregulating

MMP-12 expressions. Finally, basophil activation assay showed reduced CD63 activation in PBDE-47 treated samples. In conclusion, our study demonstrated that PBDE-47 may perturb the activities of cells involved in innate immunity dampening the expression of macrophage pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and genes involved in cell motility (MMP-12 and E-cadherin) and interfering with basophil activation suggesting that this compound can impair innate immune response.

Benthic foraminifera as a model to evaluate contaminant induced stress responses: a confocal microscopy approach

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Foraminifera are unicellular organisms enclosed in shell with a great abundance, and biomass in benthic marine habitat. Traditionally, the study of foraminifera has been the domain of palaeontologists however, their living counterparts has recently waked up the interests of foraminiferologists for biodiversity, ecological and biomonitoring studies.

Several investigations have demonstrated the value of benthic foraminifera in detecting environmental contamination and their possible application as bioindicators of pollution in marine and transitional marine settings. The response of benthic foraminifera to adverse environmental conditions may be investigated in terms of density and diversity, assemblage composition, reproductive capability, morphologic abnormalities, and cellular ultrastructure. Since very little is known about the cytological alterations of these organisms under stressful conditions, the ultrastructural changes induced by pollutant exposure are not fully understood.

Microscopy techniques have been traditionally used to study the diversity of benthic foraminifera in morphological, genetic, cytological and mineralogical perspectives. Most of these techniques(i.e., transmission electronic microscopy) produce high-resolution images but they require extensive sample preparation, and do not allow *in vivo* examination. Other approaches, such as Confocal Laser Scanning Microscopy (CLSM) using fluorescent and fluorogenic probes can be instead applied to living cells.

CLSM allows quali- and quantitative studies of the ultrastructural and biochemical organization of the cell both under natural conditions and in response to environmental stress. Furthermore, multifluorescence labelling permits simultaneous targeting of different organelles and physiological processes. In this work, CLSM is utilized to evaluate the effects of model contaminants (Hg and different types of nanoparticles-NPs) in the benthic foraminifera*, Ammonia parkinsoniana*. Data are presented on oxyradical production, lysosomal function and lipid accumulation utilizing selective fluorescent dyes. The results indicate that the CLSM approach can be successfully utilized to evaluate contaminant-induced stress responses in benthic foraminifera.

Session 2. Chairmen: Loriano Ballarin, University of Padua, Padua, Italy and Davide Malagoli, University of Modena and Reggio Emilia, Modena, Italy
Priming and immunity modulation

The evolution of specificity in immune priming

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Contrary to prior believe, recent studies indicate that all animals possess forms of acquired immunity, such as immune priming in insects, which can even be specific. However, we know little about the evolution of basic characteristics of these forms of immunity. We thus tested whether the specificity of immune priming can evolve rapidly and to what extent the evolved phenotypes are linked to transcriptomic changes. Using controlled evolution experiments, we selected the model beetle host *Tribolium castaneum* for either specific or unspecific immune priming towards pathogenic bacteria. After 14 host generations of evolution, specificity of priming was not universally higher in the lines selected for specificity, but rather depended on the bacterium used for priming and challenge. For instance, the insect pathogen *Bacillus thuringiensis* induced the strongest priming effect. These differences in priming specificity between the evolved populations were mirrored in the transcriptomic response, revealing an involvement of metabolic and transcription-modifying genes in immune priming.

***In vivo* isolation and characterization of telocytes using supplemented biomatrices**

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The leeches (*Hirudo verbana*) have proven to be a good model for deciphering basic biological processes for two main reasons: first of all, they have a reduced dimension and, despite a relative anatomical simplicity, share with vertebrates the complexity of immunological mechanisms and wound-healing processes; secondly, in animal kingdom there is a remarkable evolutionary conservation of biological responses, cell types, cellular mechanisms, and molecules. One of the most phylogenetically conserved system, from lower invertebrates to man, is the innate immune system that, strictly interacting with neuro-endocrine one, guarantee a powerful protection to organisms. In invertebrates, in addition to a

plethora of cytokines, a wide range of immunocytes such as macrophages, NK cells, granulocytes and a new type of cells, the telocytes, are involved.

Leech telocytes (TCs) are stromal cells engaged in surveillance and protection spread in various tissues and strategically localized among resident cells, nearby the capillaries and the nerve endings. These cells, contacting via gap junctions, are organized in an extensive three-dimensional network, and show cell-cell contacts with other cell types and interactions with collagen bundles of connective tissue.

The interactions among these and the other cells is obtained in two ways: physically, by direct cell–cell contacts and, chemically, via the release of microvesicles and exosomes, which can transport a variety of soluble factors involved in the regulation of different physiological processes.

Leech-TCs originate from circulating precursor cells and, once activated in response to chemical or physical stimuli, are able to change their morphology and behaviour, moving towards the injured area to participate in repair/regenerative processes.

To better characterize leech-TCs we have isolated and cultured these cells. The injection of an appropriate combination of Matrigel biopolymer, supplemented with selected factors in the leech *H. verbana*, has allowed to recruit these cells.

While few migrating cells are present in control Matrigel specimens lacking factors, an increased number of cells in relation to the time elapsed from the injection of the supplemented biomatrix, colonize Matrigel specimens containing: Monocyte Chemoattractant Protein-1 (MCP-1/ CCL2) or IL-8 or Ribonuclease T2 (RNASET2).

The recombinant *Hv*RNASET2 protein induces a connective tissue remodeling in the invertebrate model medicinal leech *Hirudo verbana*

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Recent studies demonstrated that the ribonuclease RNASET2 modulates inflammatory processes in both vertebrates and invertebrates. This protein chemoattracts macrophages in vivo and its expression significantly increases after bacterial infections. Moreover recent data obtained in our laboratory, demonstrated that the injection of the human recombinant protein RNASET2 in the leech body wall, a consolidated invertebrate model for studying both immune response and tissue regeneration, induces not only macrophages recruitment, but also a massive connective tissue remodeling. Based on these data, here we evaluated a possible direct or indirect role of the leech r*Hv*RNASET2, recently cloned in our laboratory in the synthesis of new collagen. The connective tissue reorganization and the cell types involved in this process were characterized in r*Hv*RNASET2 injected leeches by means of histochemical staining and morphological analyses at optical and electron microscopes. The expression of newly synthesized Pro-collagen1α1, together with that of fibroblast receptors (FGFb), was localized at tissue level by immunofluorescence. Moreover, to evaluate the expression profile of Pro-collagen1α1, western blot experiments were performed on protein extracts from leech body wall retrieved after different time elapse from r*Hv*RNASET2 injection. Double immunofluorescence assays were performed to correlate fibroblasts activation, collagen fibrils production and the consequent connective tissue remodeling with the expression of *Hv*RNASET2.

The data reported in this work provide compelling evidence in support of a pleiotropic role for RNASET2 in orchestrating an evolutionarily conserved cross-talk between inflammatory response and regenerative process, based on macrophages recruitment and fibroblasts activation coupled to a massive extracellular remodeling.

Towards decrypting stress response in tardigrade -Transcriptome survey of *Paramacrobiotus sideralis* during anhydrobiotic state

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Anhydrobiosis is a highly stable state of suspended animation in an organism due to its desiccation, which is followed by recovery after rehydration.

Tardigrades are water-dwelling, eight-legged, segmented animal ranging about 0.5 mm in length as adults and are among the most resilient known animals, because many species of this phylum are capable of anhydrobiosis and cryobiosis at certain environmental conditions. However, physiological and stress mechanisms at the bases of extreme desiccation remain elusive.

Using the anhydrobiotic tardigrade *Paramacrobiotus sideralis,* as a model organism, we have investigated stress-related transcripts. The transcriptomes of hydrated active animals and desiccated ones were compared. Anydrobiotic tardigrades were obtained after exposing them at 18 °C initially to 80% relative humidity (RH) for 4 h, then to 50 % RH for 4 h, and finally at room temperature to 0-3 % RH overnight.

A *de novo* transcriptome has been assembled through Trinity v2.4.0 and annotated with Trinotate v3.1.1. To identify high-quality and non-redundant transcripts, redundancy has been removed following EvidentialGene tr2aacds pipeline and evaluated with BUSCO. A total of 78832 contigs have been assembled and 2323 Differentially Expressed Genes (DEGs) resulted following dessication (Log_2 fold change >1 and a false rate discovery ≤ 0,01). About 74% of DEGs were up-regulated, and 26% down-regulated (1710 and 612 contigs, respectively). Among the

highest 50 up-regulated DEGs with an enriched 10- to 200-fold during desiccation relative to hydrated conditions, 1/6th resulted unknown and three of them are secreted. Within the highest induced and annotated transcripts immune-related are the most represented such as 5 Cytosolic-abundant heat soluble proteins (CAHS2s), 2 Cytochrome P450 proteins, 2 Apolipophorins, 2 Lysosome-associated membrane glycoproteins, and 1 Annexin.

Among the 50 highly suppressed transcripts, half of them are unknown and suppressed annotated categories are mainly related to translation activity, RNA silencing processes, transketolase activity, and structural arrangement. Secretory-abundant heat soluble protein 1, and chaperone proteins have been also found down-regulated.

Findings from these preliminary analyses confirm a huge suppression of metabolism following anhydrobiosis and confirm activation of Intrinsically Disordered Proteins, (as CAHS) to survive desiccation.

Susceptibility to entomopathogens and modulation of basal immunity in two insect models at different temperatures.

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In this work, we analysed the efficacy of different commercial bio-insecticides (*Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and *Bacillus thuringiensis*) by valuating the mortality induced on two insect models, *Galleria mellonella* (Lepidoptera) and *Sarcophaga africa* (Diptera) after exposure to different temperatures (10, 20 and 30 °C). Moreover, we investigated the effects of temperature on the basal humoral immunity of the two target insects; particularly, phenoloxidase (PO) and lysozyme activity.

Our results show that *G. mellonella* is susceptible to all bio-insecticides at all the examined temperatures, except when infected at 10 °C with *S. carpocapsae* and at 30 °C with *S. feltiae* and *B. thuringiensis*. *S. africa* is more susceptible at 30°C to all bioinsecticides; whereas, when infected at 10 and 20°C, *H. bacteriophora* is the most efficient. Temperature modulates PO activity of both *G. mellonella* and *S. africa*, otherwise variations in lysozyme activity is observed only in *G. mellonella*.

Except for a possible correlation between the increased lysozyme activity and the delayed *Bt* efficacy recorded on *G. mellonella* at 30 °C, a different resistance to bio-insecticides at different temperatures does not seem to be associated to variations of the host basal immunity, probably due to immunoevasive and immunodepressive strategies of these entomopathogens.

The immune response of *Hermetia illucens* larvae: preliminary evidence

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The dipteran *Hermetia illucens* (Diptera: Stratiomyidae), also known as black soldier fly, is a promising insect species for waste management, thanks to the ability of the larvae to grow on a wide variety of organic substrates. Moreover, the high nutritional value of the larvae makes this insect useful for the production of feedstuff. Despite the great interest on this species, information about the biology of this insect remain scarce, especially about the immune system.

In the present study we performed a preliminary investigation on the cellular and humoral response of *H. illucens* larvae. To this aim we conducted a morphological analysis of hemocytes that are involved in the cellular response. Moreover, we evaluated the main components of the humoral response after immune challenge with Gram-positive and Gram-negative bacteria. In particular, we analyzed phenoloxidase and lysozyme activity in the hemolymph of the larvae, and mRNA expression of antimicrobial peptides (AMPs) in the fat body.

Our results show an increase of phenoloxidase and lysozyme activity in infected larvae compared to controls. Moreover, we observed a modification of AMP expression 6, 12, and 24h after the immune challenge compared to naive larvae.

This study provides preliminary information on the immune system of *H. illucens*. This knowledge will hopefully open up the possibility to optimize the rearing procedures of *H. illucens* larvae and to obtain high quality larvae for feed production.

Characterization of the complement system in a colonial protochordate: C3 complement receptors and opsonic role of C3

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The complement system is one of the most ancient immune modulator mechanism of bilaterian metazoans, able to influence ancient cells and factors of both innate and adaptive immunity. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways: all of them converge on the cleavage of C3.

The compound ascidian *Botryllus schlosseri* is a reliable model organism for the study of immunobiology. As an invertebrate, *B. schlosseri* relies only on innate immunity for its defense and immunocytes. Recently, in the same species, we demonstrated the presence of homologues of mammalian C3, Bf, MBL and MASP1, referred to as BsC3, BsBf, BsMBL and BsMASP, respectively. All the complement components identified so far, are

expressed by morula cells, the most abundant circulating hemocytes.

In mammals, once the complement system is activated, a cascade of reactions that involves proteolysis and polymerization occurs resulting in the cleavage of the third complement component (C3) to C3a and C3b, the former exerting a chemokine–like activity, the latter acting as opsonin and, ultimately, activating the lytic pathway. The best-known receptor for C3a in mammals is C3aR, whereas CR1 is the receptor able to recognize and bind C3b on the microbial surfaces.

In the present work, we described, in *B. schlosseri*, two new genes showing homology with vertebrate C3aR and CR1, respectively, and studied their transcription in the course of the colonial blastogenetic cycle. Results indicate that their mRNAs are located in different immunocyte types suggesting the presence of an important cross-talk between phagocytes and morula cells. In addition, we continued our analysis of the role of C3 in *Botryllus* immunity by studying the modulation of BsC3 transcription during the colonial blastogenetic cycle and the effect of *bsc3* knockdown on immune responses.

Only morula cells, and no other immunocytes type, were labelled by the antisense probe for BsC3aR, whereas phagocytes and young, undifferentiated cells known as hemoblasts were the cells stained by the probe for BsCR1.

Both the *bsc3ar* and *bscr1* genes are constitutively transcribed as almost all morula cells and phagocytes, respectively, resulted labelled by the antisense probe in the ISH assay, independently of their previous challenge with zymosan, a known activator of *B. schlosseri* hemocytes. However, a modulation in the extent of transcription occurs during the colonial blastogenetic cycle as the amount of BsC3aR mRNA abruptly decreased at TO, whereas no differences were observed when EC and MC were compared. This is probably related to the renewing of circulating cells at TO, when 20-30% of hemocytes undergo cell death by apoptosis and are replaced by new, differentiating cells entering the circulation in the same period.

Tentacles regeneration in *Anemonia viridis* (Anthozoa, Cnidaria).

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The mechanisms for discriminating the "self" from "non-self" have evolved into a long history of cellular and molecular strategies, from damage repair to the co-evolution of host-pathogen interactions. Not all immune responses are due to the presence of genetically foreign entities, but to the emission of danger/alarm signals from injured cells, such as those exposed to pathogens, toxins and mechanical damage. In this sense, the cnidarian capacity for regeneration could be considered an additional arm of innate immune defense.

In this study, the immune responses and tissue regeneration in the temperate symbiotic sea anemone, *Anemonia viridis*, induced by cutting tentacles were investigated.

The experimental plan was carried out on groups of animals to which 10, 20 and 30 tentacles were respectively cut. Protein extracts of bodies and tentacles were prepared 7, 14 and 21 days after cutting.

Morphological observations on tentacles in state of regrowth, measurements of the expression of Proliferating Cell Nuclear Antigen (PCNA) as a regeneration marker and protease activities detection were carried out. Starting from previous knowledge on the natural seasonal variability in biometric traits and enzymatic biomarkers of *A. viridis*, the activity of enzymes involved in inflammatory response such as protease, peroxidase and esterase were analyzed in the tentacle and body extracts. Finally, haemolytic activity of the tentacle extracts was assayed against sheep erythrocytes.

The injury elicited a significant increase in the phosphatase and peroxidase activities of the tentacles, in contrast to esterase that seems not been affected. SDS electrophoretic analysis revealed a variable gelatinolytic and fibrinolytic activity in the tentacle extracts, while none fibrinolytic activity was observed in the body extracts. The immunoblot in both tentacular and body extracts showed a cross reaction with AntiPCNA antibody and a significant difference between treatment and control groups. The PCNA is more present in tentacles extracts than in the body samples. Particularly, high positivity was detected in tentacular extracts after 7 and 14 days from cut of 10 and 20 tentacles.

In perspective, we want to study at histological and molecular level how homeostatic tissues start the regeneration program while triggering immune response and their mediators of inflammation.

Selenoprotein T: an example of novel endocrine modulator in non-mammalian species

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Selenoprotein T (SELENOT) is a thioredoxin-like protein expressed in mammals and in non-mammalian vertebrates. It possesses a selenocysteine (Sec, U) within a CXXU motif, and this confers oxireductase functions. Studies in mammals proposed SELENOT as a humoral modulator with a role in endocrine homeostasis, neuroprotection, and in myocardial response to ischemia/reperfusion (I/R). Despite many recent research efforts, its endocrine/paracrine/autocrine functions are still to be fully described, particularly in non-mammalian species. In the goldfish (*Carassius auratus),* a hypoxia tolerance fish model, three

SELENOT transcripts (gfSelT1a, gfSelT1b and gfSelT2) have been detected. This study aimed to evaluate the cardiac expression of SELENOT, and its putative role as an endocrine/paracrine/autocrine modulator of the goldfish heart function under both normoxic and hypoxic conditions. By western blot and immunofluorescence we found that SELENOT is expressed in the heart, and the expression is increased under hypoxia. On *ex vivo* isolated and perfused goldfish heart preparations, under normoxia, exogenous PSELT, a SELENOT-derived peptide, dose-dependently enhanced myocardial contractility by involving a modulation of membrane and sarcoplasmic calcium, and a cAMP-dependent signaling. Under hypoxia, PSELT unaffected goldfish myocardial contractility but reduced myocardial nitrosative stress. These data propose SELENOT as an evolutionary conserved protein with a protective potential against cardiac hypoxia-dependent redox unbalance. They also pave the way to explore the role of the protein as a cardiac endocrine modulator in fish and a humoral intermediary of the communication between the heart and distal tissues and cells.

Session 3. Chairmen: Luigi Abelli, University of Ferrara, Ferrara, Italy and Giuseppe Scapigliati, University of Tuscia, Viterbo, Italy
Fish immunity

Immunity in the teleost digestive tract

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Teleost fish constitute the most abundant vertebrate group, exhibiting a high assortment of morphological variations of their gastrointestinal (GI) tract related to phylogeny, ontogeny, environment and feeding habits of each species. This variability is closely linked with several specialized functions, since the GI tract is involved not only in nutrient absorption and digestion, but also in water and electrolyte balance, and immunity. Teleosts moreover, possess a complex gut microbiota, that shapes every physiological system of the host and even effects the intestinal morphology. Because the GI tract is a main route for pathogen entry, its mucosa plays an effective defence against potentially harmful determinants of the environment, while it tolerates a diversity of harmless microbes and dietary antigens. The mechanisms allowing antigen uptake over the epithelial barrier play a crucial role for maintaining the gut homeostasis and regulate appropriate immune responses, and involve the presence of antigen sampling cells equivalent to mammalian microfold and dendritic cells. Teleosts possess an extensive system for immune activation, and responses to antigen uptake have usually been reported higher after anal than oral delivery. Gene expression profiling revealed functional specialization along the GI tract, and demonstrated that the establishment of mucosal immune responses is especially relevant in the posterior intestine. Generally speaking, teleost fish do not contain lymphoid aggregates in the mucosa, however their gut harbors high numbers of diffuse T ($CD8^+$ and $CD4^+$) and B cells. IgT^+ B cells outnumber the IgM^+ cells in teleost gut and the IgsT produced display a preponderant and specialized role in mucosal immunity, being the key players in the defence against pathogens. Importantly, commensal bacteria shape B cells and Igs responses and in a parallel way, mucosal Igs and secretory component allow the host to sculpt its microbial communities. Also T cells, mostly those bearing γδT receptors, play an essential role in intestinal cell-mediated immunity and it seems they are important in tolerance or attack against the microbiota. Studies evidenced that fish gut microbiota responds to dietary manipulations, and although the interplay between nutrition and immune system is well recognised, understanding the link between diet, gut microbiota and health in fish is only at the beginning.

Evolution of immune responses: similarities between fish lymphocytes and mammalian innate-like lymphocytes

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Innate lymphoid cells (ILCs) of Vertebrates are a cluster of innate immune cells that are classified in three groups for the expression of defined transcription factors, functional characteristics, and phenotype. The lymphocytes of Vertebrates are classically described as the cells responsible of adaptive responses, but experimental evidence suggest that subpopulations of mammalian lymphocytes may behave as ILCs, engaging non-self rapidly and without antigen restriction. The innate-like lymphocyte subpopulations have been mainly identified as γδ-T cells, mucosal associated invariant T cells (MAIT), and B1-B cells, are principally located in mucosal tissues, may be involved in human pathologies and their functions and tissue(s) of origin are not fully understood. The similarities in the morphology and immunobiology of immune system between fish and mammals have been established, but the homologies between fish lymphocytes and mammalian innate-like lymphocytes is an issue poorly considered in comparative immunology. Increasing experimental evidence suggests that main fish lymphocyte populations could have developmental, morphological, and functional features in common with innate-like lymphocytes of mammals. However, despite these similarities and with the hypothesis that mammalian innate-like lymphocytes could be evolutionarily related to fish lymphocytes, information on possible links between γδ-T lymphocytes and and B1-B cells of fish and mammals is missing, Our research is currently aimed to investigate these possible similarities in lymphocyte evolution.

Effects on immunity of exposure to microplastics in adult zebrafish

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It is now widely accepted that microplastics (MPs) represent a serious concern for aquatic environments, therefore assesment of biological pathways affected is crucially relevant.

This study focused on variations of liver transcriptome, histology of gastrointestinal tract and gills, and locomotor activity of exposed fish along various days after treatment.

Adult zebrafish (3 groups, N=12 each) were fed for 20 days with dry food alone (controls) or supplemented with a mix of pristine high-density polyethylene and polystyrene microplastics (0.1 or 1 mg/L), ranging in size from <25 to 90 µm.

The exposure to MPs resulted in differential transcription of 324 genes in total, already affected at the lower dose, mainly involved in cholesterol biosynthesis (fatty acid degradation) and immunity pathways.

Up-regulation of transcripts subserving response to extra-cellular antigens, and down-regulation of others involved in innate antimicrobial response, antiviral defense and maintenance of epithelial integrity highligted defective control of pathogen entry at epithelial barriers, confirmed by occurrence of histopathological signs in both intestine and gills. Furthermore, variations in energy utilization likely accounted also for alteration of circadian rhythm of locomotor activity.

Immunodetection of IgM, IgT and pIgR in mucosal tissues of Antarctic teleost

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We have previously investigated the immune response at hepato-biliary level in the Antarctic teleost *Trematomus bermacchii*, a species belonging to the Perciform suborder Notothenoidei, the most abundant component of the fish fauna living in the Antarctic ocean. By that time only the IgM isotype was known and well characterized at molecular and biochemical levels in Antarctic fish.

Over the past few years we have cloned and sequenced genes encoding other two key molecules of the mucosal immune system, IgT and polymeric Ig receptor (pIgR) of T. *bernacchii*. The present study aimed at investigating the localization in mucosal tissues of IgM, IgT and pIgR in an attempt to clarify the protein occurrence and transepithelial transport. Biochemical and immunohistochemical data provided convergent data about specific mechanisms operating apical release of IgT in exocrine way, as well as depicting peculiar (maybe ancestral) features compared with well-known mechanisms described for polymeric Igs transport in mammalian tissues.

F-type lectin from serum of *Trematomus bernacchii* (Boulenger, 1902): purification, characterization and bacterial agglutinating activity.

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Lectins belong to a protein family, present in almost all living organisms and involved in several biological processes, including immune responses. Peculiarity of these proteins is the ability to bind carbohydrates due to their carbohydrate-recognition domains (CRDs).

In fish, C lectin, F binding lectin (FBL), galectin, Rhamnose-binding lectin (RBL) and pentraxin have been identified in both cartilaginous and bony fish. In addition, selectins and other genes have been found in the currently available fish genomes.

The FBL, known as fucolectins, constitute the most recent lectin family identified and structurally characterized in teleosts. The FBL family is constituted by a large number of proteins exhibiting multiples of the F-type motif, either tandemly arrayed or in mosaic combinations with other domains.

In the present study, a FBL has been purified and characterized from serum of the Antarctic fish *Trematomus bernacchii* by affinity chromatography on fucose-agarose column. Assay of inhibition from carbohydrates in fact showed affinity of this lectin for the fucose. A convincing Hemoagglutinating activity (HA) was detected towards rabbits red blood cells (RBC) and at lesser extent towards sheep erythrocytes.

The HA activity was analyzed at different temperatures. It was maintained at temperature values comprised between 4 °C and 37 °C and was completely depleted after exposure at 50 °C. In SDS-PAGE analysis, the FBL exhibited an apparent Molecular weight of 30 kDa in non-reducing conditions and an increase to 32 kDa after reduction. This difference is recognized as a classical shrinkage of F-lectins, due to the present of internal disulfide bridges.

The F lectin present on the *T. bernacchii* transcriptome show a very similar and congruent structure with a theoretical Mw 32.16 kDa and an isoelectropoint of 5.21.

Bacterial agglutinant activity (BA) of serum and purified fractions was tested towards *E. coli*. The serum showed high activity after incubation at room temperature (18 °C), as well as in the fractions. The sequence, structure, sugars specificity, fucose inhibition, molecular weight, protein shrinkage and activity against bacteria collocate this molecule on F-lectin family and thus suggesting its involvement in host pathogen interactions.

Mucosal immunity response of European eel (*Anguilla anguilla* L.) after bacterial infections

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Recently, mucosal surfaces of fish, in particular skin and its secreted mucus, have attracted significant interest among immunologists. The external mucus layer that covers fish skin contains numerous immune substances poorly studied that act as the first line of defence against a broad spectrum of pathogens and infections through the epidermis. For the first time, this study aimed to characterize and describe different humoral immune defence parameters in the skin mucus of the European eel (*Anguilla anguilla*) challenged by intraperitoneal injection with *Vibrio anguillarum* and *Tenacibaculum maritimum*. In order to do this, several immune-related enzymes as well as the bactericidal activity against fish pathogenic bacteria were evaluated in skin mucus of European eel at 24, 48, 72 hours post-challenge. The results demonstrated that European eel skin mucus showed significant increments in peroxidase and lysozyme activity at 48 and 72 hours after *V. anguillarum* injection respect to the other experimental groups (unchallenged and challenged with *T. maritimum*). In the case of antiprotease activity, an increase was observed at 24 hours in fish challenged with *V. anguillarum* respect to control group whilst this activity was not detected at 48 and 72 hours in any experimental group. Contrarily, protease activity decreased in challenged fish at 48 regarding control group whilst this activity only was diminished in skin mucus from fish challenge with *V. anguillarum* respect to unchallenged fish. Esterase activity showed only an increase at 72 hours in fish challenge with *T. maritimum* compared with the results obtained in skin mucus from the other experimental groups. Finally, European eel skin mucus revealed higher bactericidal activity against *Photobacterium damselae* than those observed against *V. anguillarum*. More concretely, bactericidal activity against *V. anguillarum* did not show any significant variations. However, the bactericidal activity, measured when the skin mucus was incubated with *P. damselae,* increased in skin mucus from fish challenged with *V. anguillarum* at 24 hours of trial. Interestingly, *T. maritimum* grew in presence of skin mucus from all experimental fish which could mean that this substrate serves as nutrient source for this bacterium. The present results could give new insights into the mucosal immune system of this primitive species with potential application to the aquaculture. FA Guardiola thanks the *Fundación Séneca de la Región de Murcia* (Spain) for his grant (Saavedra Fajardo program, Grant no. 20407/SF/17).

Session 4. Chairmen: Anita Giglio, University of Calabria, Rende (CS), Italy and Piero Giulianini, University of Trieste, Trieste, Italy
Microbiota and invertebrate immunity

Insect immunity as affected by stress factors and associated microorganisms

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Insects represent the most species-rich taxon of multicellular organisms. This biodiversity is paralleled by an equally rich diversity of associations between insects and microorganisms. The resulting metaorganisms are governed by complex molecular networks underlying their physiology and the intricate interactions they establish with other biological entities.

Insect immune barriers are suppressed by invading parasites and pathogens, which have developed effective virulence strategies, as a result of a long co-evolutionary process. However, the immune response is not only modulated by these biotic stress factors, but is also conditioned by insect associated microorganisms and several abiotic stressors. Among these, poor nutrition and pesticides play an important role. In particular, neurotoxic insecticides are able to interfere with the cross-talk between the nervous and immune systems, often separately considered. Unveiling the regulatory mechanisms of these physiological networks can be profitably done only at metaorganism level. This paves the way towards the development of new bioinspired strategies for pest control and pollinator protection.

Exposure to TiO_2 nanoparticles results in shift in hemolymph microbiome composition and immunomodulation in *Mytilus galloprovincialis*

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Increasing attention has been recently given to the microbiome and its complex dynamics maintained within the host (human, animal, plant), and on the different factors that could affect their natural equilibrium. Although invertebrates represent 95% of animal species, a minority of studies focus on this group. The interest in invertebrate-microbe interactions is also due to conservation of the mechanisms of innate immunity, and understanding cross talk between microbes and the immune system can lead to insights of broader relevance.

Marine invertebrates host a high microbial abundance and diversity, and alteration of the

microbiota due to stressful conditions and/or environmental changes has been linked with a compromised health status and susceptibility to disease. In particular, the presence of microorganisms in the hemolymph of healthy bivalves indicates that this ecosystem could contribute to host homeostasis.

The expansion of nanotechnology is raising concern on the potential biological effects of nanoparticles (NPs), including nano-oxides such as Titanium dioxide-nTiO_2. Its current addition to a variety of food attracted interest on the potential impact on mammalian gut microbiota. Moreover, nTiO_2 has been shown to alter the microbiota of fish and the composition of planktonic bacterial communities. Therefore, exposure to nTiO_2 may also affect the microbiota of marine invertebrates.

In this work, the effects of nTiO_2 exposure (100 µg/L, 96 h) on hemolymph microbiota of *Mytilus galloprovincialis* were investigated. The microbiome was analysed by targeted high-throughput sequencing of the 16S rRNA gene using Ion Torrent technology. The results show that although similar in composition, the hemolymph core microbiome was less diverse in nTiO_2-treated than in control mussels. The composition of the microbial population was unequally affected by nTiO_2, with decrease in abundance of some genera (*Kistimonas*, *Shewanella*, *Vibrio*) and increase in others (e.g. *Stenotrophomonas*). Moreover, determination of immune parameters revealed decreased hemocyte lysosomal membrane stability, increased serum lysozyme activity, and increased bactericidal activity of whole hemolymph. The results may partly explain the observed shift in microbiome composition induced by nTiO_2-exposure. These represent the first data on the effects of NPs on the microbiome of a marine invertebrate, and suggest an interplay between hemolymph microbiota and activity of the immune system.

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When immunity and extracellular matrix matter: repair and regenerative events after echinoderm arm injury

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Arm amputation in echinoderms is a traumatic event that removes differentiated body parts and damages all tissue types. Immediately after injury the repair phase begins. If phenomena typical of this phase, such as emergency reaction, inflammatory/immune response, wound closure and extracellular matrix (ECM) remodeling and deposition, do not properly occur, the following regenerative process may be prevented or ineffective.

In this study, the brittle star *Amphiura filiformis* (*Afi*) was used as model to investigate the main repair and regenerative events after arm injury, with a specific focus on the involvement of immune and ECM genes and proteins. In this perspective, both microscopy and molecular analyses were performed to highlight similarities and differences between regeneration-competent (*i.e.* echinoderms) and non-competent (*i.e.* mammals) animals.

Our microscopy results showed that both emergency reaction and re-epithelialisation are faster in brittle stars than in mammals. Fibrosis, *i.e.* over-deposition of ECM due to an exaggerated inflammatory reaction, is not detectable in echinoderms as, instead, described for mammals, suggesting that immunity modulation may facilitate subsequent regeneration. Our molecular analyses showed that *Afi-ficolin* (an important gene in the immune response) is expressed in the first phase after injury, whereas almost all the selected ECM genes are not expressed at early stage of regeneration, suggesting an activation delay that may be directly connected to their regeneration efficiency, as proposed for other echinoderms and in contrast to most vertebrates. Moreover, at advanced regenerative stages these same genes are differentially expressed, suggesting that the molecular regulation of ECM deposition/remodelling is different throughout re-growth.

Overall, our brittle star model shows similarities in terms of repair and regenerative events and timing with other echinoderm species already studied. However, differences emerge between echinoderms and mammals: indeed, all phenomena should occur following specific signals and timing to ensure effective regeneration after severe wounds. Further quantitative analyses will allow a better understanding of immune system and ECM contribution to brittle star arm regeneration and of the evolutionary implications on the regeneration competence widespread in the animal kingdom.

Preliminary data on functional coelomocytes changes during *Echinaster sepositus* arm regeneration

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Echinoderms are known to have the greatest capacity for regeneration among Deuterostomes. The Mediterranean red sea star *Echinaster sepositus* is a suitable model for studying this phenomenon because it is able to regenerate arms following self-induced amputation (autotomy) or traumatic loss/damage. The overall regenerative process could be subdivided in three main phases: a repair phase, characterized by wound healing and edematous area formation; an early regenerative phase, characterized by first differentiation phenomena; and an advanced regenerative phase, with proper arm regrowth. Previous studies

evidenced that in sea star the regenerative process of arm tip is considered mainly morphallactic, involving either pluripotent progenitor cells such as coelomocytes or/and differentiated cells, which may undergo dedifferentiation or transdifferentiation. Moreover, coelomocytes are important elements involved in the repair phase of arm by forming a clot and in first immune response following the injury. We focused our attention on the role of coelomocytes, cells freely wandering in the coelomic fluid, during sea star arm regeneration.

In this work, we described the morpho-functional changes of coelomocytes occurring during the repair phase in the red sea star *E. sepositus* following traumatic amputation of the distal third of one arm. We evaluated ROS production and the AIF-1 protein expression in coelomocytes at different times after amputation. An increase in ROS production was evidenced 24h post amputation (p.a.), while the highest number of AIF-1 positive cells was detected 48 h p.a.

These preliminary data underline the interest of investigating the high regenerative capacities in this attractive model for further/future biomedical studies in regeneration.

Cellular immune response in *Harpalus (Pseudoophonus) rufipes* (De Geer, 1774) (Coleoptera, Carabidae)

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Carabid beetles are among the most important groups of beneficial arthropods in the agroecosystem food chain where they are predators of many pests (including aphids, lepidopterans, slugs and Diptera). Previous studies have been shown that they are good models to investigate the negative effects of agrochemical used in agricultural management practices on natural enemies of insect pests. In ecological immunology, variation on immune capacity of insects is an early warning, highly sensitive biomarker to monitor the sub-lethal effect of toxicants introduced into environment as a result of industrial or agricultural activity. However, morpho-functional data about immune system of carabid are scanty in spite of their ecological relevance. In this study, we have investigated the immune function of *Harpalus (Pseudoophonus) rufipes* for their use in eco-toxicological monitoring. This species is a omnivorous predator, very common in Calabrian (South Italy) agroecosystems and acts as a predator against insect pests. Tests performed on adult involve a general screen of cellular responses and include: characterization of circulating hemocytes and phagocytosis *in vivo*. The cellular population has been characterized by light and electron microscopy analysis and 4 morphotypes of circulating hemocytes were found: prohemocytes (0.55±0.11%), plasmatocytes (67±2.52%), granulocytes (29.32±2.35%), oenocytoids (0.73±0.20%). The phagocytosis assays were performed *in vivo* by injection of 0.9 µm carboxylate-modified polystyrene latex beads in order to identify the hemocyte types involved in phagocytosis. After 2h non-self challenge treatment, specimens showed a decrease of plasmatocytes and oenocytoid percentages (12.38±2.21% and 0.16±0.05%, respectively) and a non-specific immune response involving phagocytosis performed by granulocytes (82.74±2.54%). Moreover, hemocytes with mitotic figures and non-differentiate cells were found in the hemolymph (2.28±0.37), thus confirming a continuously turnover . Melanotic nodules have been found 2h after the immune challenge formed to immobilize the latex beads.

Herbicide exposure effects on cellular and humoral immunity of soil insects

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Herbicides used in agriculture have known negative effects in non-target organisms. Pendimethalin is a dinitroanaline herbicide used for pre-emergent control of annual weeds. This class of herbicides interfere with the structure and function of microtubules inhibiting the steps in the plant cell division responsible for chromosome separation and cell wall formation. Beneficial soil invertebrates, such as insects that inhabiting agricultural areas and provide ecosystem services, are potentially threatened by herbicides. This study has been designed to assess the effect of pendimethalin exposure on *Harpalus rufipes*, one the most common omnivorous predators in agricultural ecosystems involved in the pest control. Adults from an uncontaminated area (organic wheat field of about 6 ha in a biological farm, 39°17'10.28"N, 16°42'28.33"E, 1150 m a.s.l.; Macchia di Tuono Farm, San Giovanni in Fiore, Calabria, Southern Italy) were placed in plastic boxes contained soil sprayed with Activus® (a.i. pendimethalin; recommended field rate 4L per ha of active ingredient, from 330gr/L of commercial formulation). Tests lasted for 21 days and at 20 °C and 8:16 L:D cycle. To assess the sublethal effects of exposure over the time, the total hemocyte counts and the haemolymph phenyloxidase (PO) activity were measured after 48h, 1 week and 21 days of exposure as markers of immune system strength. Exposed animals showed a decrease of THCs compared to controls at all times. Basal and total PO activities were highly significantly lower in exposed animals compared to controls at all time. The negative impacts on these immune parameters demonstrate that the exposure to herbicides modify the susceptibility of this species to pathogens. As a result, we assume that the exposure of *H. rufipes* adults to commercial formulation of pendimethalin in field may altered other basic life history traits such as reproduction, dispersal activity and predation with effects on the adult fitness resulting in changes

of the population structure and in a reduction of the biocontrol activity for pest species in agroecosystem.