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REPORT OF MEETING

XXV scientific meeting of the Italian Association of Developmental and Comparative Immunology (IADCI), February 19-21, 2025, Department of Sciences and Technological Innovation - DISIT, University of Eastern Piedmont “Amedeo Avogadro”, Alessandria, Italy

Organizers: **F Dondero, A Calisi**

Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy

Award “Soci non strutturati” (Best presentation and *curriculum studiorum* for members under 35)

Immune responses in marine mussel to cross infections with *Malaciobacter marinus* and *Vibrio aestuarianus*

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Marine bivalves are increasingly subjected to mortality outbreaks in Europe. Although oysters have been historically more impacted, mortality episodes have been recently observed also in mussels (*Mytilus spp.*) populations. The consequences of these events can have important environmental and economic consequences, which highlights the need to identify factors and processes that could explain part of these mortalities. Although the causes are often considered polymicrobial and multifactorial, the dynamics involved in host-pathogen interactions, including those related to multiple infections with different pathogenic or opportunistic bacteria, are still unknown.

Among them, bacteria of the *Arcobacter-like* genus are being frequently detected in diseased oysters and stressed bivalve species, often in association with more pathogenic bacteria (e.g. Vibrios). This is the case of the present study, where strains of the member of the *Arcobacter* family, *Malaciobacter marinus,* were isolated together with *Vibrio aestuarianus* in adult oysters during a mortality event in 2019 in Spain. However, we have recently shown in both *in vitro* and *in vivo* studies that mussels (*Mytilus galloprovincialis*) after single infection with *M. marinus* showed their ability to mount an efficient immune response and consequent bacterial clearance. However, no data are available so far concerning the dynamics of infections caused by multiple pathogens in mussels. In this light, it is necessary to identify the role of each strain in the successive steps that lead to disease or mortality of the host.

In this context, cross exposure experiments were performed using strains of *M. marinus* (M.m) and *V. aestuarianus* (V.a) to study immune responses of *M. galloprovincialis*. Mussels were injected with either V.a or M.m (5×10^6 CFU/mL) and after 24h p.i the extracted hemolymph was cross-challenged *in vitro* with M.m or V.a, respectively. Functional immune parameters were measured: hemocyte lysosomal membranes stability (LMS) was evaluated as a proxy of cellular stress and extracellular responses (ROS production and Lysozyme activity) were measured. Finally, the bactericidal activity by hemocytes and serum components was assessed.

The results obtained after single infection with either V.a. and M.m. at 24h p.i showed that both strains reduced hemocyte LMS (V.a < M.m). However, whereas M.m significantly stimulated extracellular ROS production and increased lysozyme activity, no responses were elicited by V.a.

With regards to *in vivo/ in vitro* cross exposure experiments, in both conditions a large decrease in hemocyte LMS was observed. However, differences in cellular/lysosomal effects; when hemocytes from V.a. injected mussels were challenged with M.m. cell rounding and lysosomal fusion events were observed; in contrast, in samples from M.m injected mussels challenge adherent hemocytes with smaller lysosomes were observed. Moreover, *in vivo* infection with V.a interferes with subsequent *in vitro* extracellular immune defense toward M.m and bactericidal activity. In contrast, in M.m. injected mussels, stimulation of ROS production seems to prevail on subsequent inhibition by V.a, resulting in a faster activation of bactericidal activity towards V.a.

Overall, these data indicate that *in vivo* challenge with V.a increased cellular stress and resulted in lower immune responses to subsequent infection with M.m. In contrast, *in vivo* challenge with M.m did not prevent subsequent immune response towards V.a challenge.

These data are in line with a scenario where infection with *V. aestuarianus* could compromise mussel immune system, thus favoring the secondary colonization by opportunistic bacteria such as *M. marinus*. Such a weakening of immune defenses of the bivalve host may thus contribute to disease and mortality events. The approach proposed in the present work helps identifying the role of different bacterial species and strains in a multiple infection event in mussels and it could be successfully applied to other bivalves such as oysters.

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Award "Giovani laureati" (Best presentation and *curriculum studiorum* for members under 29)

A cell-free wound healing approach: the effects of human Dental Pulp Stem Cells-derived Conditioned Medium on tissue regeneration in *Hirudo verbana*

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Human Mesenchymal Stem Cells (*h*MSCs) have garnered significant attention in the field of regenerative medicine, due to their remarkable regenerative potential and ability to modulate various biological processes. These stem cells (SCs) secrete a variety of bioactive molecules, including cytokines and growth factors, known for their anti-inflammatory, anti-apoptotic, and pro-angiogenic properties. In this context, the secretome of human Dental Pulp Stem Cells (*h*DPSCs), known as conditioned medium (CM), has emerged as a promising candidate in regenerative medicine, since capable of enhancing cellular proliferation, differentiation, and tissue repair without the direct use of SCs.

To address ethical concerns surrounding the use of vertebrate animal models, we propose the medicinal leech *Hirudo verbana* as an alternative invertebrate organism to investigate the *in vivo* effects of *h*DPSC-CM on wound healing and tissue regeneration. Indeed, despite their relatively simple anatomy, leeches exhibit both cellular and tissue processes comparable to those found in more complex vertebrates, making them an ideal system to be used in biomedical research.

In the current study, medicinal leeches were wounded and subsequently treated with either starvation medium (SM), here used as control, or *h*DPSC-CM. The healing process was then monitored at 48 h, 72 h, and 1 week post-treatment, through morphological, histoenzymatic, immunofluorescence, and molecular assays. The obtained results revealed marked cellular invasion and vascular growth at the wound site, both of which are crucial processes for effective tissue regeneration and hypertrophic scar prevention. Furthermore, leeches treated with *h*DPSC-CM showed a notable improvement in the speed and quality of wound healing, thus suggesting that the bioactive molecules in CM not only accelerate tissue regeneration but also improve the functional integrity of the healed tissue. This highlights the potential of *h*DPSC-CM as a novel, cell-free therapeutic strategy for wound healing and tissue repair.

Our findings therefore suggest that *h*DPSC-CM could offer a promising alternative to conventional treatments, particularly for chronic wounds and a variety of other chronic conditions where tissue regeneration is impaired.

KEYNOTE LECTURE

Neuroendocrine and environmental control of early larval development in *Mytilus galloprovincialis*

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Marine bivalves living in intertidal and estuarine coastal areas are subjected to fluctuations of different environmental parameters, and thus most vulnerable to ongoing global changes. In order to survive these extremely variable environments, bivalves evolved efficient adaptation mechanisms to deal with a number of abiotic and biotic stressors. In particular during early larval stages, where neurogenesis, morphogenesis and shell biogenesis occur, neuroendocrine responses to stress have been described in which monoaminergic systems play central roles. Data are here summarized on neuroendocrine and environmental control of early larval development in the mussel *Mytilus galloprovincialis*. Early larval development from eggs to 24 and 48hpf after exposure to different emerging contaminants (EC) was evaluated as previously described (doi:10.1016/j.marenvres.2014.05.007). The effects of increasing temperature and of bacterial challenge were also investigated. Gene expression and confocal microscopy were performed as in Miglioli *et al*., 2021 (doi:10.1016/j.scitotenv.2020.144003).

The results show that in larvae grown in physiological conditions significant changes occur across early development in expression of different gene groups related to estrogen and monoamine signaling, shell biogenesis and ceramide sphingolipid biosynthesis. These changes are accompanied by progressive neurodevelopment and shell formation. A number of ECs, including different pharmaceuticals, as well as increasing temperature and vibrio challenge significantly affected transcription of monoaminergic components, and the downstream mechanisms that control shell formation, leading to altered larval phenotypes at 48hpf. The results underline the susceptibility of neuroendocrine control of early larval development of mussels to environmental factors.

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Session I.1: Immunological response and disease of environmental disturbance and stress. Chair: Valerio Matozzo, University of Padua, Italy and Antonio Calisi, University of Eastern Piedmont, Italy

Physiological responses of early developmental stages of *Mytilus galloprovincialis* to ocean warming and pathogen infection in a global change scenario.

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Marine bivalves are sessile invertebrates that live in intertidal and estuarine coastal areas, where they are subjected to fluctuations of different environmental parameters. Bivalves evolved efficient physiological adaptation mechanisms to deal with a variety of stressors, including hypoxia, increased temperature, bacterial challenge, and exposure to pollutants. However, in a global change scenario, these stressors can be exacerbated and occur simultaneously in coastal environments, posing a threat to the health and survival of marine species, in particular of more sensitive larval stages. In this work, responses of early larvae (24 and 48 h post-fertilization) of the mussel *Mytilus galloprovincialis* to ocean warming and pathogen infection, alone and in combination, were evaluated. Normal development and survival were assessed at 48 hpf, and gene expression at both 24 and 48 hpf. Increases in T alone (18>20>22 °C) induced alterations of larval shell phenotypes and affected the time course of basal gene expression across development: genes related to shell biogenesis, ceramide metabolism, antioxidant and immune response and the serotonin receptor were most affected. Combined exposure to increasing T and concentrations of the emerging pathogen *Vibrio coralliilyticus* resulted in higher larval pathogenicity at 48 hpf, with interactive effects. In conditions that did not affect larval survival (T: 18-20 °C; *V.c.*: 104 CFU/mL), gene transcription revealed both common and distinct targets for the two stressors, with significant interaction at both 24 and 48 hpf. Although temperature is the main environmental driver affecting early development, sea warming may increase susceptibility to *vibrio* infection, with consequences on mussel populations.

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Investigating the Impact of PET Nanoparticles on the acute immune response in the medicinal leech *Hirudo verbana*

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In recent years, the spread of plastic pollution has risen as a critical environmental threat, significantly affecting ecosystems worldwide. Plastics are indeed one of the most employed synthetic materials globally, leading to the generation of tons of waste every year. Due to improper management of plastic objects, debris in the form of micro (MPs) and nanoplastics (NPs) can easily disperse in the environment, posing potential threats not only to vertebrate species but also to invertebrates, which play vital roles in ecological balance and food webs. Among the various types of plastics, polyethylene terephthalate (PET) stands out as one of the most common synthetic thermoplastics and the third most employed in the packaging sector. In this context, this project focuses on the evaluation of the effects, on the acute immune response, of different concentrations of PET NPs (0.05, 0.5, 5 mg/L) in aquatic settings, using the medicinal leech *Hirudo verbana* as a model organism. These studies have highlighted the potential toxicity of PET NPs, demonstrating their ability to activate the immune system, trigger inflammatory responses, and cause oxidative stress. The influence of these particles on such fundamental processes could have far-reaching implications, affecting not only the health and survival of these organisms but also the overall functioning of marine and freshwater ecosystems. Studying the interactions between PET nanoplastics and invertebrate immune responses is crucial for assessing the broader environmental impact of plastic pollution.

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Enviromental impact of two gadolinium forms on mussel immune response: a preliminary study

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Rare earth elements (REEs) represent an high environmental concern for their wide use in various applications. Particularly, gadolinium (Gd) is widely used in industrial applications, in the manufacture of electronic products and extensively employed in the medical field as a contrast agent, justifying its presence in aquatic environments. Several researches have focused on the harmful effect of Gd on aquatic organisms, probably related to its chemical affinity for Ca^{2+} causing interference in various calcium-dependent biological processes. Among the various biological pathways, impairments to the immune system appear reported in human cell lines, while limited knowledge exists on the impact of aquatic organisms immune system, particularly invertebrates. Aim of this work is to investigate the impact of two forms of gadolinium (gadolinium chloride $GdCl_3$; gadolinium oxide nanoparticles GdOx) on mussel *Mytilus galloprovincialis* using a multi biomarker (histological, enzymatic and molecular) approach. During a 28-day exposure to two concentrations (1 µg/L;10 µg/L) of GdCl3 and GdOx, an increase in haemocyte infiltration was observed immediately after 7 days of exposure (T7) as well as at 15 days (T15) and 28 days (T28) in the gills and digestive gland, associated with an imbalance in mucopolysaccharide secretions (gills). Gd-caused interference in immunity also appears to be supported by imbalances in ROS metabolism, alkaline phosphatase activity, lactate metabolism and neurotransmission, which are crucial in the regulation of immune activity in bivalves. These preliminary results support the potential impact of this pollutant on immune response of aquatic invertebrates.

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Toxic effects of tire particle-derived contaminants to *Lumbricus terrestris*

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Environmental discharge of additives from tire particles can pose serious hazard to ecosystems, since recent studies have demonstrated the toxicity of these molecules on different organisms. However, most of the research carried out so far focus on the aquatic biota, while the potential effects of these contaminants on the terrestrial environment is almost unexplored. In this perspective, this study investigated the effects of three tire particle-derived contaminants: N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), its main metabolite (6PPDquinone; 6PPD-q) and 1,3-Diphenylguanidine (1,3-DPG) to *Lumbricus terrestris*. Organisms were exposed for 28 days to environmental concentration (100 µg/kg) of the polymers. Growth rate and reproduction output were measured. The offspring was also maintained for another 28 days in contaminated soil. A suite of biomarkers related to cyto-genotoxicity, metabolism, oxidative stress, and damage and neurotoxicity was applied either in adults and juveniles. Organisms reared in the soil contaminated with all the three molecules showed a lower growth over time. In adults, 6-PPD and 1,3-DPG induced lysosomal membrane instability. The 1,3-DPG lowered SOD activity, while GSTs activity was significantly induced. The exposure to 6-PPDq increased significantly AChE activity. In juveniles, all additives reduced significantly the activity of ALKP and inhibited AChE activity. Moreover, juveniles exposed to all molecules showed lower content of reactive oxygen species and lower lipid peroxidation. Overall results highlight the vulnerability of soil biota to toxic compounds released from tire particles, calling for the need to monitor and regulate these emerging contaminants, to avoid unwanted consequences in the long term.

Effects of polyethylene nanoplastics (nPET) on immune responses of the colonial ascidian *Botryllus schlosseri*

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Plastic pollution represents an environmental problem, with an estimated 20 million tons of plastic waste entering the world's aquatic ecosystems annually. Plastic particles smaller than 1 µm are classified as nanoplastics (NPs). NPs are of particular concern as they have the capacity to enter cells and cross the blood-brain barrier, accumulating in vital organs and potentially influencing their physiology over long periods of exposure and accumulation. Ascidians are invertebrate chordates, closely related to vertebrates, and their phylogenetic position renders them ideal organisms for the study of the evolution of various biological processes, with particular focus on the invertebrate-vertebrate transition. *Botryllus schlosseri*, a colonial ascidian, is a widely used model in studies of innate immunity and the effects of pollutants on physiology. The focus of our research is on innate immune responses following exposure to polyethylene NPs (nPET). Haemocytes were exposed directly to nPET, or the latter were directly microinjected into the colonial circulatory system.

Our analysis shows that exposure of haemocytes to NPs has a negative influence on the phagocytosis of yeast (*Saccharomyces cerevisiae*) cells, and is accompanied by a modification of phagocyte morphology, probably related to cytoskeletal alterations. NPs have also a negative effect on the degranulation of morula cells, the immunocytes with cytotoxic activity that trigger the inflammatory response. In addition, we analysed the expression of genes involved in immunity and oxidative stress scavenging, observing a higher expression of C3, RBL and SOD in treated animals than in the control group under certain conditions.

Haemocyte responses in the blue crab *Callinectes sapidus* exposed to organic and inorganic contaminants

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The blue crab *Callinectes sapidus* is an invasive species whose population is increasing rapidly in the Lagoon of Venice. With the intention of beginning to study the adaptive capacity of this species, in this study we measured haemocyte parameters in crabs exposed for 7 days to Bisphenol S (BPS) and mercury, alone (300 ng/L) or as a mixture (MIX: 150 +150 ng/L). Effects on immunomarkers, such as Total Haemocyte Count (THC), haemocyte proliferation, cytotoxicity (LDH assay), haemocyanin and the activity of some immune response-related enzymes were assessed. Results demonstrated that exposure to the two contaminants affected significantly some haemocyte responses. THC reduced significantly following exposure of crabs to both BPS and mercury. Exposure to mercury and MIX reduced significantly haemocyte proliferation, whereas increased acid phosphatase activity in cell-free haemolymph. Alkaline phosphatase activity decreased significantly in cell-free haemolymph from crabs exposed to the MIX. Lastly, exposure to mercury and MIX reduced significantly haemocyanin concentrations. Summarising, the present study highlighted that exposure to traditional contaminants, such as mercury, more than the emerging ones, namely BPS, induced alterations in haemocyte parameters of crabs. In addition, results obtained suggested that *C. sapidus* can modulate its cellular parameters to cope with contaminants. This may explain - partially at least - the great adaptability of *C. sapidus* and its widespread out of the native area. In any case, further studies are necessary to better understand the capability of such invasive species to cope with stressors, such as changing environmental parameters.

Session II.1: Molecular and cellular process in comparative and developmental immunobiology of metazoans. Chair: Maria Maisano, University of Messin, Italy and Maria Rosaria Coscia, National Research Council of Italy, Naples, Italy

Flow cytometry analysis reveals variable DNA content in non-proliferating hemocytes of the invasive freshwater snail *Pomacea canaliculata*

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Invertebrate immunity relies on hemocytes which are essential for immune functions and tissue homeostasis; yet little is known about hemocyte renewal and differentiation in most invertebrates. In *Pomacea canaliculata*, hemocytes have been categorized into small and blast-like Group I (GI) and large Group II (GII), with phagocytic activity mainly detected in GII agranular hemocytes. The number of circulating hemocytes and the GII/GI ratio are stable but individual-specific, suggesting an individual-specific control of the turnover of the hemocyte population.

To provide a basis for investigating *P. canaliculata* hemocyte turnover, hemocyte number and GII/GI ratio were assessed by flow cytometry at 1.5, 3, 6, 9, 18, 24 or 48h after a first hemolymph collection. GII/GI ratio was the lowest at 18h and recovered at 24h, when the total hemocyte number was highest. It was not until 48h after the first collection that all conditions were restored. To assess whether mitosis was involved in the recovery of hemocytic parameters, cell cycle and DNA content were assessed using “Vybrant™ DyeCycle™” staining at 18h and 24h. Interestingly, flow cytometry analysis showed that hemocytes contain varying amounts of DNA, quantitatively unrelated to cell cycle phases but rather to cell endocycling, a mechanism observed in several organisms and cell types, including gastropod neurons, but to our knowledge not in molluscan hemocytes. A positive correlation between DNA-content and cell size was also observed. In addition, the percentage of high-DNA-content circulating hemocytes decreased significantly after 18h and returned to baseline after 24h, consistent with the observations on the GII/GI ratio.

These data support previous observations of the absence of active mitosis in circulating hemocytes and suggest that *P. canaliculata* hemocytes may exhibit localized DNA amplification, more evident in larger cells, as observed in gastropod neurons.

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DNA methylation analysis unveils a fine-tuned regulation of immune-related genes from the cold adapted teleost *Trematomus bernacchii*

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DNA methylation is one of the main epigenetic modifications in the vertebrate genomes, which regulates several biological mechanisms. DNA methylation occurs on cytosines in the CpG context, especially in the CpG islands (CGIs), which are mostly located at the gene promoter sequence, and the degree of their methylation status varies individually. Although recent advances in the knowledge of epigenetic mechanisms in teleost fish, to date the epigenetic regulation in Antarctic species is almost unknown. In a previous study on the characterization of the polymeric Ig receptor (pIgR) gene from the Antarctic teleost *Trematomus bernacchii*, we detected two CGIs, one located upstream of the 5' region of the gene, the other one located in the second intron, upstream of a DNA transposon motif. In the present work, we investigated the methylation status of these CGIs in mucosal tissues and in a lymphoid organ. Interestingly, we observed a slightly different degree of methylation in the first CGI, which suggests a tissue-specific regulation of the pIgR expression. The second CGI showed a high DNA methylation profile in all tissues, in line with the role of DNA methylation in controlling the transposable element activity. Since pIgR is involved in the transport of polymeric immunoglobulins (Ig), we extended our analysis to Ig heavy (H) and light (L) chain genes. We detected a CGI upstream of the 5' region of the IgL gene, whereas none was found in the IgH gene. These findings suggest a fine-tuned epigenetic regulation of immune genes under cold conditions.

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Assessment of antioxidant defenses and energy metabolism in neglected marine organisms: an integrated approach for *Sabellaria spinulosa* (Sabellariidae, Annelida)

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Anellid polychaetes represent perfect model animals for the study of marine biodiversity: they are pertinent markers of changes in ecosystem functioning and proxy for the complete macrozoobenthic community. Different functional and morphological categories are represented by these organisms frequently used in ecotoxicological studies.

Because polychaetes interact with sea-bottom substrates, they are typically utilized to investigate the toxicity of various pollutants occurring in sediments and pore water. However, little baseline information is available on biochemical and physiological parameters in marine polychaetes that may be affected by contaminant exposure.

Among marine polychaetes, the reef-building sabellarids, that are sessile sedentary organisms using sandy grains to build the arenaceous bioconstructions in the littoral environments of temperate coastal areas, play a fundamental role in maintaining ecosystem functions. Along Mediterranean and Atlantic coasts, the most significant reef-building species are *Sabellaria spinulosa* (Leuckart 1849) and *Sabellaria alveolata* (Linnaeus, 1767).

The species *S. spinulosa*, which is widely found along coasts of the Atlantic and the North Sea, as well as the Adriatic coasts of the Mediterranean, is notable for its ecological adaptability, as it can colonize both natural and artificial substrates.

In this integrated and multidisciplinary study, we provide the biochemical pattern and morphological (i.e., SEM and TEM) characterization of *S. spinulosa*, focusing on antioxidant defense system and energy metabolism. The investigation of the unknown physiological characteristics of this species using an integrated approach could provide baseline information to identify the potential sublethal effects induced by contaminant exposure.

Session II.2: Molecular and cellular process in comparative and developmental immunobiology of metazoans. Chair: Jacopo Vizioli, University of Lille, France and Giovanna Parisi, University of Palermo, Italy

Myticalins: a class of antimicrobial peptides and their role in mussel immunity

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Molecular effectors of the mussel innate immune system are encoded by multigene families subject to gene presence-absence phenomena, resulting in very high interindividual variability. This diversity likely evolved in response to the wide variety of microorganisms encountered in coastal environments by these filter-feeders. To date, the most detailed studies of mussel antimicrobial peptides (AMPs) have focused on molecules expressed by haemocytes, the circulating immune cells recruited to infection sites. However, recent evidence supports an equally important role played by mucosal surfaces, as they represent the major area of contact with the external environment in bivalve mollusks. Myticalins are one of the rare examples of mussel AMPs that are not expressed by hemocytes, showing instead a strong specificity for the gills.

We provide a detailed exploration of Myticalin diversity, greatly expanding the catalogue of previously reported molecules, and describing the new subfamily E, found exclusively in *Mytilus coruscus* and *Mytilus californianus*. Through careful estimation of gene expression, we show that Myticalins are produced at biologically significant levels even under normal physiological conditions. This suggests that these AMPs may be involved in the regulation of the gill-associated microbial community, with potential implications for the health status of these economically valuable bivalves.

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Insights into the immune response of *Ciona robusta*: The TLR-NFkB pathway and functional enzymes

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Ascidians (Tunicata) are valuable models for exploring the innate immune system due to their phylogenetic proximity to vertebrates. To elucidate the mechanisms and dynamics of immune responses to bacterial infections in Ciona robusta, we investigated the effects of lipopolysaccharide (LPS) stimulation through immunohistochemistry and functional enzymatic analyses. Our results demonstrated the involvement of a Toll-like receptor (TLR) and nuclear factor kappa B (NFκB) in the inflammatory response, with both molecules showing significant expression in the pharynx tissues 4 hours post-LPS injection. Notably, inflammatory nodules were observed in the pharyngeal vessel lumen, and the endothelium vessels also participated in the response. Histological analysis of naive and buffer-injected ascidians confirmed immuno-positive reactions. Additionally, enzyme activity assays revealed up-regulation of phenoloxidase, glutathione peroxidase, lysozyme, alkaline phosphatase, and esterase following LPS challenge. These enzymes could play pivotal roles in mediating and regulating the inflammatory process. Our findings underscore a robust and complex innate immune activation in *C. robusta* that parallels vertebrate immune mechanisms, providing new insights into the molecular and cellular networks involved in pathogen interactions. Future studies should aim to close existing knowledge gaps and further clarify the universal principles of immune regulation in tunicates.

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Immune responses of *Mytilus galloprovincialis* to challenge with a bacterial isolate from a mussel mortality outbreak in a Spanish aquaculture farm

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Bivalve host-pathogen interactions have been increasingly investigated in different species due to recurrent mass mortality events. Data are here reported on responses of *Mytilus galloprovincialis* to challenge with a vibrio strain (IRTA-r172) isolated from a mussel mortality outbreak occurred in Alfacs Bay (Spain) in 2022. A cohabitation experiment was first performed in order to investigate a potential horizontal transmission of bacteria among different individuals. Mussels injected with the vibrio strain (Donors-D, final concentration of 10^8 CFU/mL) and not injected mussels (Recipients-R) were put together in the same tank at a ratio 1D:3R. At different times p.i. (T0, T24h, T96h) samples of hemolymph for immune parameters (LMS and lysozyme activity) and different matrixes for PCR detection of IRTA-r172 (mantle, water) were collected. In addition, a bath infection experiment was run in order to simulate environmental exposure. Mussels were directly exposed to IRTA-r172 in seawater (10^5 CFU/mL) and samples were collected at T0, T24h, T48h, T72h and T96h as described above. The results show that in both experiments mussels were not able to activate immune defences, but stressful conditions were observed in hemocytes, confirming previous results obtained in vitro. The results of cohabitation experiments indicate that some horizontal transfer of bacteria may occur from D to R, inducing sub-lethal conditions in R. Bath infection experiments indicated mild cellular stress induced by infection with a full recovery after 72h. The results indicate that the bacterial isolate studied is not a primary pathogen for mussels but it may represent an opportunistic species.

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Cerebral ganglia of *Pomacea canaliculata* express immune-related mediators *Pc*AIF1 and *Pc*IL-17 in response to tentacle amputation

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The interplay between the immune system (IS) and the central nervous system (CNS) is critical for their proper functioning and is also based on a common pool of molecular mediators. In this respect, the early phases of regeneration of a complex sensitive organ, the cephalic tentacle of the freshwater snail *Pomacea canaliculata*, may provide a useful biological context to study the IS-CNS interplay. In this study, the expression of inflammation-related soluble molecules, namely allograft inflammatory factor 1 (*Pc*-AIF1) and interleukin 17 (*Pc*-IL17), was evaluated in cerebral ganglia of *P. canaliculata* 24 and 48 hours after amputation (hpa) of left cephalic tentacle. Gene expression levels were quantified in left (homolateral) and right (counter-lateral) cerebral ganglia. Results show that both genes were significantly up-regulated at 24 hpa in both left and right ganglia, indicating an immediate response and possible involvement in regeneration following tentacle amputation. More specifically, in the homolateral ganglia, *Pc*-AIF1 was significantly induced at 24 hpa and returned to control levels at 48 hpa, while *Pc*-IL17 expression was significantly induced at both 24 and 48 hpa. Conversely, in the contralateral cerebral ganglia, while *Pc*-AIF1 expression had a profile similar to that in the homolateral ganglia, *Pc*-IL17 induction was not found to be significant. Overall, similar to observations in other invertebrates, *P. canaliculata* cerebral ganglia respond to the amputation of a sensitive organ with the expression of immune-related mediators. Further morphological studies will help to understand whether microglial cells are involved in this neuro-immune response.

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Stress granules as new potential biomarkers to assess PFAS toxicity in *Squalius cephalus*

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Squalius cephalus, a fish endemic to rivers in the Veneto region, has recently been used as an experimental model to assess the effects of exposure to perfluoroalkylated substances (PFAS) and related physiological responses. Previous studies have suggested that the responses against PFAS-induced oxidative stress are probably regulated by the assembly/disassembly of particular cytoplasmic complexes of proteins and mRNAs called stress granules (SGs). SG formation occurs with the overexpression of mRNA binding proteins, such as TIAR, G3BP and TTP, which temporarily block the translation of specific mRNAs stored inside SGs. In this way, eukaryotic cells can rapidly activate protein synthesis in the presence of acute stress.

In this study, we aimed to assess whether SGs play a role in the post-transcriptional regulation of the expression of the antioxidant enzymes glutathione peroxidase (GPx) and catalase (CAT). In the liver and the caudal kidney of *S. cephalus*, which are organs known to be involved in xenobiotic accumulation and detoxification processes, the transcription levels of SG-related genes, i.e. *tiar*, *g3bp,* and *ttp,* and genes for antioxidant enzymes, i.e. *gpx1*, *gpx4,* and *cat*, were evaluated by qRT-PCR. Biochemical assays evaluated GPx and CAT tissue activities. In addition, *in situ* hybridization and immunohistochemistry experiments were carried out to better assess the formation dynamic of SGs.

The obtained results allowed us to start understanding the possible interaction between SGs and the antioxidant system and the potential of SGs as biomarkers for environmental monitoring.

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Chair: Francesco Dondero, Università del Piemonte Orientale, Alessandria, Italy

KEYNOTE LECTURE

The ascidian phagocyte: a multitasking cell

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Ascidian professional phagocytes represent an important fraction of circulating hemocytes. Together with cytotoxic, proinflammatory cells, they represent the cellular component of the immune system and are collectively known as immunocytes. However, notwithstanding the increasing number of contributes dealing with ascidian immunity, ascidian phagocytes remains a neglected cell type, its study being mostly limited to a morphological level. In the last three decades, using the colonial ascidian *Botryllus* *schlosseri* as our reference species, we studied phagocyte behaviour and physiology under various experimental conditions aiming at revealing their true contribution to immune responses. We could demonstrate the molecular basis of the interaction with nonself, acquire new information on the receptors involved in the recognition of foreign and effete cells, clarify their involvement in the secretion of humoral factors able to improve phagocytosis and affect the activity of cytotoxic cells. The emerging scenario suggests an active and continuous cross-talk between phagocytes and cytotoxic cells able to modulate the immune response(s) to nonself.

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Session I.2: Immunological response and disease of environmental disturbance and stress. Chair: Nicola Franchi, University of Modena and Reggio Emilia, Italy and Annalisa Grimaldi, University of Insubria, Italy

Bivalve pangenomes: investigating the inter-individual diversity of immune-related molecules

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Recent genomic investigations have reveald that bivalve molluscs exhibit open pangneomes with numerous hemizygous regions associated with structural variation and gene presence/absence variation (PAV). By studying the available whole genome resequencing data of three commercially important farmed species (Mytilus spp. Crassostrea virginica and Crassostrea gigas), we show that each species harbors several thousand dispensable genes, even though PAV is more widespread in mussels than in oysters. Despite the presence of some interpsecific differences, dispensable genes are enriched in survival and immune functions, suggesting roles in local adaptation. In particular, we detected a significant over-representation of families encoding soluble immune receptors, molecules involved in immune signal transduction, and anti-microbial effectors in all species. C1q-domain containing proteins and AIG1 GTPases, in particular, appeared to be among the most expanded and hypervariable classes of immune-related genes enriched in the dispensable fraction of bivalve pangenomes. We also reported that gene PAV patterns could be used, in parallel with SNPs, to characterize the genetic structure of natural populations, which suggest that the differentiation among populations found in different geographical locations might be the result of local adaptation and past selection events linked with immune challenges.

Organ-specific accumulation of PET-nanoplastics in *Pomacea canaliculata* (Gastropoda) under chronic and acute exposure

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Plastic waste degrades into nanoplastics (NPs, <100 nm) infiltrating aquatic ecosystems and posing risks to organisms by crossing biological barriers and disrupting cellular functions. This study investigated the accumulation of Nile red-stained polyethylene terephthalate (PET) nanoplastics in the organs of the freshwater snail *Pomacea canaliculata* under chronic and acute exposure conditions.

Chronic exposure consisted of dietary administration of 5 mg/L PET-NPs via gelatin for seven days (0.08 μg/g of snail per day), while acute exposure consisted of a single 10 mg/L PET-NP injection (0.16 μg/g of snail) followed by organ collection after 24 h. For both exposures NP accumulation was assessed in hemolymph, anterior kidney (AK), posterior kidney (PK) and, for chronic exposure only, digestive gland (DG) and stomach (ST). Tissues are processed either by cryostat or paraffin embedding to assess NP presence and morphological signs of tissue damage.

No lethality was observed under either exposure. Cryostat processing preserved the accumulated NPs in the tissues, whereas paraffin embedding allowed a better evaluation of tissue morphology, while not preserving the accumulated NPs. Chronic dietary exposure resulted in minimal NP accumulation, limited to the stomach. Tissue morphology was intact and no signs of histological suffering were observed. Acute exposure resulted in significant intracellular accumulation of NPs in hemolymph, AK and PK. NPs were mainly internalized by hemocytes or hemocyte-like cells. No organ damage was observed. The uptake of NPs by hemocytes was confirmed by *ex vivo* incubation with 2.5, 5 and 10 mg/L PET-NPs.

These findings suggest that PET-NPs can accumulate in target tissues after gaining access to the circulation. Future research should evaluate evidence of bioaccumulation after longer intervals of NP exposure, explore gene expression changes and the effects of NPs on processes that rely on hemocytes.

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Expression analysis of Toll-like receptor genes in response to temperature challenge in the Antarctic fish *Trematomus bernacchii*

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The increasing rate of climate change, specifically in polar regions, has driven an urgent need to understand how Antarctic fish can cope with rising temperatures. Of particular interest is to investigate the impact on their immune system, given the acquisition of multiple molecular adaptations that enable it to function under cold conditions. Temperature increases can disrupt homeostasis, making organisms more susceptible to infections and diseases. Fish mainly rely on innate immunity, mediated by PRRs like toll-like receptors (TLR) and NOD-like receptors (NLR). The present study investigated the effect of temperature fluctuations on the expression of TLR genes in the Antarctic teleost *Trematomus bernacchii* for the first time. To this aim, fish specimens were exposed to acute thermal stress mimicking short-term temperature increases due to climate changes, to a controlled temperature up to +3 °C above the baseline environmental temperature of approximately -2.0 °C. The mRNA expression of selected TLR genes (*trl2* and *trl9*), whose encoding sequences had been previously determined in two Antarctic fish species, was analyzed in the spleen and liver. The obtained results highlight differences in the expression of TLR transcripts when *T. bernacchii* is subjected to thermal stress and confirm its sensitivity to such conditions. Furthermore, the genes are differentially expressed in the two organs analyzed, suggesting an organ-specific response. These preliminary findings point out the relative susceptibility of Antarctic fish to climate change, with important implications for ecosystem resilience and the conservation strategies that might be needed.

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Chronic PFAS Exposure in Terrestrial Oigochetaes (*E. fetida*) Unveiling sublethal Immune Responses

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Per-/polyfluoroalkyl substances (PFAS) are persistent organic pollutants (POPs) that significantly impact environmental matrices, with terrestrial ecosystems being particularly affected. It is well known that PFAS tightly bind to soil particles, making soil a reservoir for these pollutants. Understanding soil quality and pollution is critical, as soil supports human nourishment and agricultural productivity. Numerous studies highlight the toxic effects of PFAS on target organisms, including humans, with evidence of their ability to act as endocrine disruptors, and impair immune functions.

Limited information exists on the effects of PFAS on soil organisms, such as the terrestrial oligochaete *E. fetida*, which is essential for soil formation and fertility. This study evaluated immune biomarkers in *E. fetida* following 30 days of exposure to PFAS into soils samples at a firefighting drill site and surroundings according to the OECD guidelines (Test No. 222). We assessed morphological alterations in amebocytes, including micronuclei formation, expression of immune-related genes (*CCF-1* and *Lysenin*), and phenol oxidase activity, a critical enzyme in the organism's first line of defense.

The findings revealed significant immune system disruptions. In fact, amebocytes showed increases in area and perimeter, reduction of phenol oxidase activity, and impacts on gene expression of *CCF-1* and *Lysenin*. These results highlighted vulnerability of soil ecosystems to PFAS contamination.

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Session I.3: Immunological response and disease of environmental disturbance and stress. Chair: Tiziana Cappello, University of Messina, Italy and Nicolò Baranzini, University of Insubria, Italy

Anti-inflammatory drugs: an environmental threat to the reproductive health of non-target species?

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Dexamethasone (Dex), used in the treatment of various inflammatory conditions, from common allergies to autoimmune diseases such as lupus, it is a synthetic glucocorticoid, widely found in marine coastal areas, closer to the outflows of wastewater treatment plants (WWTPs) which, unfortunately, are unable to efficiently mitigate its release into the environment. This raises apprehension about the well-being of non-target organisms, as its involvement as endocrine disrupting compound (EDC) and immune modulator is known. The aim of the study was to evaluate temporal trend of biological responses (T0, T3, T6, T12) to realistic doses of Dex (range 0.004 µg/L - 2 µg/L) on male and female gonads in the species *Mytilus galloprovincialis* during

a 12-day exposure. Using a multi-bioindicator approach, an early hemocytic infiltration (T3) in both sexes was highlighted, accompanied in males by the activation of an apoptotic pathway mediated by Fibroblast-associated cell-surface-ligand (Fas-L), which over time was compensated by the modulation of the expression of the anti-apoptotic gene *B-cell lymphoma 2* (*Bcl2*), but also by other genes involved in antioxidant activity such as *Cooper/Zinc-Superoxide dismutase* (*Cu/Zn SOD*) and *5-histidylcysteine sulfoxide synthase* (*OvoA*) and implicated in the detoxification system, including the genes *Multi-drug resistance 1* (*MDR1*) and *Multidrug resistance-associated protein* (*MRP*). The altered glycogen reserves and branched chain amino acids (BCAAs), the latter energy substrates and involved in protein biosynthesis, question the possibility that Dex interferes with reproductive homeostasis in both sexes, inducing impact on spawning and, therefore, on the fitness and survival of a key species within intertidal ecosystems.

Exploring PFAS-induced Stress Responses: The Role of Prdx4 in Oxidative and Lipid Homeostasis in *Squalius cephalus* Kidney

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Given the increasing detection of perfluoroalkyl substances (PFAS) in different environmental matrices and their known persistence and toxicity, it is essential to investigate their impact on freshwater organisms and, consequently, the ecosystem. Studies show that PFAS exposure can lead to the risk of oxidative stress directly, affecting enzymes from the antioxidant defence system or indirectly, boosting reactive oxygen species (ROS) formation. ROS overproduction can cause cellular damage by oxidising macromolecules (lipids and proteins) if not well counteracted by the antioxidant system.

In this study, we evaluated how chronic environmental exposure to the two most harmful PFAS (PFOA and PFOS) affects the physiology of *Squalius cephalus*, a freshwater fish native to the Veneto region. We analysed the kidney, one of the main organs responsible for PFAS accumulation and elimination.

Results show that even low PFAS contamination can increase protein oxidation; conversely, higher PFAS levels do not determine lipid peroxidation.

We also examined the involvement of peroxiredoxin isoform 4 (*Prdx4*) in the physiological stress response. *Prdx4* is expressed explicitly in the endoplasmic reticulum, with two main functions: acting as a scavenger against peroxides and inhibiting lipid accumulation in the cell. This gene is down-regulated with increasing PFAS pollution, demonstrating the minor function of *Prdx4* as an antioxidant defence against oxidative stress. Instead, its down-regulation is essential in increasing lipid accumulation in the cell, creating a hydrophobic environment that limits PFAS bioaccumulation and their capacity to bind proteins, thus preserving them from further damage.

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Exploring acclimation responses to heatwaves-like events in the cold-adapted fish *Trematomus bernacchii*

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The exacerbation of climate warming is profoundly disrupting Antarctic ecosystems, with marine heatwaves (MHWs) emerging as critical stressors. Unlike gradual warming, MHWs are characterized by localized, short-term temperature spikes that challenge thermally sensitive Notothenioid fish, highly adapted to cold (about - 2 °C), stable environments. Characterized by limited thermal tolerance and slow metabolic rates, notothenioids could be particularly vulnerable to these acute warming events, which can disrupt physiological and immune homeostasis. This study investigates the molecular and physiological responses of a notothenioid species, *Trematomus bernacchii,* to thermal stress, focusing on how immune system adjusts to MHW-like events. Adult fish were sampled from the Ross Sea at Baia Terra Nova (74°42′S, 164°7′E) at depths of 60 to 100 meters and exposed to a gradual temperature increase from 0 °C to +3 °C, simulating acute warming scenarios. We analyzed by qPCR the expression levels of key genes of innate and adaptive immunity, in the liver, spleen, and red muscle. Our findings provide novel insights into the capacity of Antarctic fish to maintain immune defenses while coping with thermal stress. Preliminary results, combined with data obtained by analyzing oxidative stress responses in spleen, contribute to a deeper understanding of the acclimation mechanisms of Antarctic fishes. By shedding light on the challenges faced by *T. bernacchii* during MHWs, this study advances predictions on the resilience of Antarctic fish fauna in the face of accelerating climate change-related events.

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Histopathological and Molecular responses in Mussel Gills treated with Micro and Nanoplastics

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Microplastics (MPs) and Nanoplastics (NPs) are environmental pollutants that pose a significant threat to aquatic organisms, including bivalves such as *Mytilus galloprovincialis*.

M. galloprovincialis, is the most common mussel in the Mediterranean Sea, is defined as a biological sieve model and represent a good bioindicator and model organism for ecotoxicology and cytotoxicology studies.

This study examines the cytotoxic effects of MPs and NPs on the gill tissue of mussels.

Mussels were exposed to environmental concentrations of MPs and NPs for 1, 3, and 11 days. Histopathological analyses revealed considerable cellular damage in gill tissues, including changes in cell morphology and structural alterations.

Immune system activation was observed, with a marked increase in haemocytes, melanin, and mucus-producing cells. Additionally, alterations in skeletal rod structures, sugar content, and PCNA expression were detected, indicating cellular stress and impaired cellular functions.

The results also demonstrated an increase in reactive oxygen species (ROS), oxidative damage to lipids, as well as heightened susceptibility to oxidants in vitro. Furthermore, total antioxidant capacity increased on days 3 and 11, reflecting a compensatory response to oxidative stress. These findings underscore the potential of MPs and NPs to induce oxidative damage and activate inflammatory pathways in aquatic organisms, potentially leading to long-term ecological consequences. This study is essential to understanding of the mechanisms underlying plastic-induced toxicity in marine invertebrates and emphasizes the need for further research to assess the impacts of micro- and nanoplastics in aquatic environments.

Chair: Francesco Dondero, Università del Piemonte Orientale, Alessandria, Italy

KEYNOTE LECTURE

Advancing comparative immunogenomics: insights into immune system evolution through -omics approaches

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In recent years, advancements in DNA sequencing technology have led to a significant development of -omics approaches in non-model organisms, enabling the expansion of molecular analyses from single targets to the entire genomes, transcriptomes or proteomes of a species. These technological developments have undoubtedly contributed to the emergence of a new branch of immunology, comparative immunogenomics, which is providing an increasingly comprehensive understanding of the evolution of the innate and adaptive defense systems in different metazoan lineages. Initially based on simple sequence homology detection methods, this discipline has matured considerably over time. Thanks to the integration of gene expression data and the application of deep learning models able to detect otherwise challenging molecular signatures, these approaches may allow the discovery of novel key players in immune response, providing an answer to some long-standing questions in comparative immunology, such as the nature of invertebrate cytokines. Recent progress has also brought renewed focus on the inter-individual diversity of invertebrate immune receptors and effectors, filling another significant knowledge gap. Nevertheless, the vast opportunities afforded by big data analysis come with limitations, which should be acknowledged by the scientific community. As artificial intelligence becomes increasingly utilized in scientific research, it is important to resist the temptation to view these strategies as replacements for experimental validation. Instead, they should be recognized as powerful supportive tools that, when combined with rigorous experimental methods, can further advance the field of comparative immunogenomics and deepen our understanding of immune system evolution across diverse lineages.

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Session III.2: Big Data analysis and innovative methodologies in comparative immunology. Chair: Gianfranco Santovito, University of Padua, Italy and Adriana Vallesi, University of Camerino, Italy

Effects of environmental pollutants on innate immune response and cell to cell communication

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Pollutants disrupt immune function by inducing chronic inflammation and oxidative stress, impairing cellular physiology and intercellular communication and contributing to the development of non-communicable diseases in human. Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that are present in the environment, they bio-accumulate in human and animal tissues entering the food chain (EFSA Panel, 2011). We previously demonstrated that the flame retardant BDE-47 modulates macrophage innate immune responses both in vitro and in vivo assays. However, its effects on the biogenesis, cargo composition, and functional impacts of macrophage-derived extracellular vesicles (EVs) remain poorly characterized. Extracellular vesicles (EVs) are categorized into subpopulations, often referred to as "small" (sEVs) and "large" (lEVs) EVs. In this study, sEVs (sEVsBDE) and lEVs (lEVsBDE) were isolated from BDE-47-treated M (LPS) THP-1 macrophages using differential ultracentrifugation (dUC). The concentration, size distribution, and specific markers of the EVs were analyzed using DLS, NTA and western blotting. Additionally, the miRNA cargo profiles of sEVsBDE and lEVsBDE were characterized by means of microarray. Our findings reveal that BDE-47 interferes with the miRNA sorting process in both EV subtypes altering their cargo profiles. Bioinformatic analysis identified the modulated miRNAs as potential regulators of genes involved in cell cycle checkpoints. Functional assays further demonstrated that sEVsBDE and lEVsBDE exhibit distinct effects on LNCaP cells proliferation and cell cycle regulator genes. These results underscore the significance of macrophage-derived EVs in toxicology and exposure science, highlighting their potential role in mediating the effects of environmental pollutants on cellular communication and disease progression.

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Exploring the Shared Molecular Mechanisms of Immunostimulation and Heavy Metal Exposure in Mussel Hemocytes

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The innate immune system of invertebrates, including the marine mussel *Mytilus galloprovincialis*, relies on conserved signaling pathways such as Toll-like receptors (TLRs) and the nuclear factor kappa B (NF-κB) transcription factor to respond to pathogens and environmental stressors. This study aimed to elucidate whether exposure to immunomodulatory agents and heavy metals activates overlapping signaling pathways and molecular responses in *M. galloprovincialis* hemocytes, thereby suggesting a co-evolution of immune and stress responses. Primary hemocyte cultures were treated with lipopolysaccharide (LPS), nickel (Ni), or cadmium (Cd) at concentrations determined to be non-lethal. Gene expression dynamics of NF-κB/Rel and IkB were assessed using quantitative real-time PCR, revealing transient modulation of NF-κB expression across treatments. High-throughput RNA sequencing (RNA-seq) identified thousands of differentially expressed genes in response to each treatment, with significant overlap in DEGs between LPS and nickel exposures. Functional analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) mapping highlighted enriched pathways related to immune response, signal transduction, and cellular processes. Both LPS and nickel treatments significantly influenced the TLR-NF-κB pathway, suggesting that heavy metal exposure can engage immune signaling mechanisms traditionally associated with pathogen recognition. Cadmium exposure exhibited distinct modulation of NF-κB-related genes, indicating differential effects among heavy metals. The results demonstrate that NF-κB serves as a central mediator in both biological stress and chemical responses in hemocytes. The overlapping transcriptomic responses to LPS and nickel support the hypothesis of a shared evolutionary basis for immune and stress response pathways in this species. These findings enhance our understanding of the molecular mechanisms underlying mussel resilience to environmental challenges and may inform ecotoxicological assessments.

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Structural evolution of the water-borne protein pheromone families ruling self/not-self recognition in the ciliate *Euplotes*: an updated scenario

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In ciliates, self/not-self recognition mechanisms (usually reported as “mating-type mechanisms”) regulate the cell switching from the reproductive/growth stage to the sexual/mating stage of the biological cycle. Species of *Euplotes* are all characterized by multiple mating-type systems, in which indefinite numbers of cell types are chemically distinct from one another for producing type-specific pheromones, each determined by one of a series of multiple single-locus alleles. The three-dimensional conformations of various members of the *E. nobilii*, *E. raikovi* and *E. petzi* pheromone families have previously been determined by NMR spectroscopy and X-ray crystallography, prompting an initial picture of their degrees of intra- and inter-specific structural polymorphisms. Using these determined structures as templates in association with the AI predictive model AlphaFold 3, we have now resolved (with high degrees of reliability) the folding of pheromone amino acid sequences deduced from their coding genes cloned in species branching in key positions of *Euplotes* phylogenetic tree. An updated picture of the structural evolution of *Euplotes* pheromones suggests, first and more in general, that the course of this evolution may properly be synthesized by quoting the popular dictum “the earlier [structure], the smaller and simpler”. Second and more circumstantially, it identifies a regular alpha-helix as the secondary structure which is more tightly conserved at both intra- and inter-specific level. In addition to well accounting for the central role that this helix plays in driving and stabilizing the self (autocrine) and not-self (heterologous) pheromone/receptor interactions on the cell surface, this identification also provides a parsimonious explanation for common observations of pheromone-mediated inter-species mating inductions.

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When Immune Meets Neural: The Hidden Power of the Complement System in Gastropod Brain Development and Degeneration.

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The Complement System (CS), traditionally recognized for its role in humoral innate immunity in vertebrates, is now understood to extend beyond immune defense and participate in key developmental processes. Growing evidence indicates that CS can influence whether certain cells survive or undergo elimination during development, although the precise molecular pathways behind this phenomenon remain unclear.

A recent hypothesis proposes that complement regulators govern cell fate in both developmental and homeostatic contexts, employing a strategy similar to 'non-self' recognition. In the absence of these regulators, the CS component 3 (C3) may serve as the main effector of cell removal by promoting interactions with phagocytes. Consequently, pinpointing the distribution of C3 and its regulators in developing tissues becomes crucial for clarifying the true developmental role of the CS.

In this study, we focus on the most evolutionarily conserved CS elements in the gastropod *Pomacea canaliculata*. By examining tissue-specific Nanopore transcriptomes, as well as the expression and localization of C3 in selected tissues at different developmental stages, we investigate the presence of potential non-canonical CS functions in gastropods. Our data reveal variation in the sequence of C3 regulators and confirm that C3 is active outside of immune-related contexts during the development of *P. canaliculata*. Collectively, these findings support the notion that the proposed 'non-canonical' role of the CS may in fact represent its original function, driving its evolutionary persistence.

Session III.3: Big Data analysis and innovative methodologies in comparative immunology. Chair: Francesco Dondero, University of Eastern Piedmont, Italy and Davide Malagoli, University of Modena and Reggio Emilia, Italy

Impacts of bioplastic leachates on early larval stages and adult mussels: cellular, biochemical and physiological responses

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The potential release in the environment and biological effects of chemicals by conventional plastics and bioplastics is still a largely overlooked issue. In this work, the effects of leachates from common bioplastics in sea water were evaluated using the model marine invertebrate *Mytilus galloprovincialis*. Leachates were obtained by polylactic acid (PLA) plates, polybuthylene succinate and polylactic acid (PBS/PLA) spoons and glasses, polypropylene and starch-based polymer (PP/PSM) forks, supermarket (Sbs) and waste (Hera bag) bags in starch and polybuthylene adipate cotereftalate (A/PBAT) (Capolupo *et al*., 2022, 10.1016/j.envpol.2022.120951).

Non target chemical analysis allowed for the identification of the main organic additives present in different leachates: these include plasticizers, lubricants, dyes, fragrances, surfactants, solvents, and Non Intentional Added Substances-NIAS. Short term in vitro experiments, carried out on isolated mussel hemocytes show that only Sbs leachates induced lysosomal destabilization-LMS and enlargement from 10% dilution, whereas immune parameters (ROS and lysozyme) were unaffected. All leachates induced minor effects on fertilization, but significant impacts were observed on larval development at 48h from low concentrations (0.6%). In adults, exposure to different leachates (7 days, 0.6%) significantly decreased hemocyte LMS. In contrast, no effects were observed on digestive gland lysosomal biomarkers (LF, LN, LYS/CYT, LMS) or tissue enzymatic biomarkers (CAT, GST, AChE), except for a decrease in gill GST activity induced by PLA Plates leachate. Overall, the results show little impact of bioplastic leachates on mussel physiology, compared with previous data obtained with those from conventional plastics or other bioplastic leachates, indicating advances towards more ecofriendly bioplastic production.

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The highly dynamic pangenome of basal chordates is enriched in defence and immunity genes and is inherited following the Mendelian law

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Analysis of the pangenome, the sum of the genetic information of a species, can shed light on intra-species diversity and uncover phylogeographical gene patterns. Although the existence, extent and functional significance of animal pangenomes is still unclear, the reports of open bacterial-like pangenomes in some bivalve species pose important questions about the molecular mechanisms generating haplotype diversity, making the analysis of this phenomenon in basal chordates particularly interesting.

We investigated the pangenomic architecture of basal chordates by the analysis of 100 and 41 whole genome resequencing datasets of *Branchiostoma belcheri* and *B. floridae*, respectively. We evaluated the impact of genomic regions affected by presence-absence variations (PAV), the presence of conserved intra- and inter-specific patterns and the transgenerational transmission of PAV genes.

The pangenomes of both species revealed more than a thousand dispensable genes that display a Mendelian inheritance from parents to offspring. PAV preferentially impacted certain protein families, most notably those involved in defence, immunity and signalling, including GTPases of immunity-associated protein (GIMAP), caspases, toll-like receptors and apextrin C-terminal domain (APEC) containing pattern recognition receptors.

The dynamic nature of the immunity genes observed here in cephalochordates mirrors the prevalence of diverse immunity genes in the open bacterial pangenomes, suggesting that general principles governing genome evolution and innovation across domains of life are rooted in the host-pathogen interactions.

Exploring *Mytilus edulis* immune response using Raman vibrational spectroscopy

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Hemocytes play a pivotal role in the immune response and physiological homeostasis of marine bivalves. They release several bioactive compounds in circulating hemolymph upon immune stimulation. In this study, we present preliminary results obtained with Raman spectroscopy in order to characterize hemolymph and hemocytes from the mussel *Mytilus edulis*. Raman vibrational spectroscopy is a rapid, label-free and non-destructive technique, that, combined with confocal microscopy, allows the obtention of spectral fingerprinting of biological samples, providing insights into their biochemical composition and physiological status.

This work explores the potential of Raman spectroscopy for mussel spectral profiling. Hemolymph was collected from *M. edulis* and immediately analyzed to investigate spectral signatures indicative of chemical bonds assigned to nucleic acids, proteins, lipids and carbohydrates. If this technique is well known as a powerful tool in marine environmental analyses, notably on pollutant nano- and microparticles characterization, it is rarely used for immune response studies.

In a first attempt, our results demonstrate the relevance of Raman spectroscopy to describe the spectral variations among non-treated and LPS immune-stimulated hemolymph. In the future, particular attention will be paid to differentiate hemocyte populations offering new perspectives on their role in immune defense mechanisms. It will also help to monitor mussel health in response to environmental challenges.

This study underscores the importance of integrating advanced micro-spectroscopic methods like Raman in immunology research on aquatic invertebrate species, paving the way for innovative approaches in environmental monitoring and aquaculture management.