ISJ 23: 57-71, 2026 ISSN 1824-307X

REPORT OF MEETING

XXVI scientific meeting of the Italian Association of Developmental and Comparative Immunology (IADCI), February 11-13, 2026, University of Camerino URDIS, Ricerca e Formazione, San Benedetto del Tronto (AP)

Organizers: **A Vallesi, C Alimenti**

School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy

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

On the morpho-functional features of haemocytes from the blue crab *Callinectes sapidus*

J Fabrello[1], V Matozzo[2]
[1]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy
[2]Department of Biology, University of Padova, Padova, Italy

In this study, we present a morpho-functional characterization of haemocytes from the blue crab *Callinectes sapidus*. Three main haemocyte types were identified in the haemolymph: hyalinocytes, semigranulocytes, and granulocytes, representing approximately half, one quarter, and one third of the total haemocyte population, respectively. All haemocytes showed a predominantly round or oval shape, with no significant size differences among cell types (7-20 µm range). Cytochemical analyses further classified haemocytes into acidophil, basophil, and neutrophil subpopulations, which were present in similar proportions within the haemolymph. Neutral Red staining failed in revealing lysosomes, suggesting limited membrane permeability under the experimental conditions. Ultrastructural observations, using transmission electron microscopy, confirmed the presence of the three haemocyte types. Functional assays demonstrated that granulocytes and hyalinocytes could phagocytose yeast cells. However, the phagocytic index was low, indicating that phagocytosis may not represent the primary immune defence mechanism in *C. sapidus*. All haemocyte types exhibited superoxide anion production and showed activity of hydrolytic enzymes and phenoloxidase. Overall, our results confirm the presence of three distinct haemocyte types in *C. sapidus* and suggest that immune responses in this species may rely more strongly on alternative mechanisms, such as enzymatic activity and other non-phagocytic pathways. These findings provide a basis for future studies on degranulation processes and inflammatory responses in the blue crab.

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

The medicinal leech *Hirudo verbana* as an *in vivo* model to evaluate the efficacy of 3D patches in wound healing and tissue regeneration

G Costantini[1], L Pulze[1], N Baranzini[1], E Campodoni[2], M Sandri[2], M Sugni[3], A Grimaldi[1]
[1]Department of Biotechnology and Life Science, University of Insubria, Varese, Italy
[2]Institute of Science Technology and Sustainability for Ceramics (ISSMC), National Research Council (CNR), Faenza (RA), Italy
[3]Department of Environmental Science and Policy, University of Milan, Milan, Italy

Skin injuries can result from common causes such as surgery, burns, pressure sores, cuts, and disease, and they require a tightly regulated healing cascade to restore structural and functional integrity. Skin regeneration and the immune response are tightly interconnected processes that ensure tissue homeostasis after injury. While acute wounds normally progress through four phases: haemostasis, inflammation, proliferation, and remodeling; chronic wounds arise when this process fails, often due to persistent inflammation or infection. The limited availability of effective

treatment options, together with the growing challenge of antibiotic resistance, highlights the urgent need for innovative therapeutic approaches. In this context, biomaterials have emerged as promising tools to promote tissue repair, modulate inflammation, and prevent infection. Invertebrate models, such as the medicinal leech *Hirudo verbana*, offer unique advantages for dissecting these mechanisms due to their conserved innate immune pathways and remarkable regenerative capacity. In this study, *H. verbana* was employed to evaluate the biocompatibility and regenerative potential of two structurally and compositionally distinct 3D biomaterial patches: a porous gelatin–chitosan composite and a denser sea urchin–derived collagen matrix. Both materials were implanted into injured leeches, and tissues were analyzed at 72 hours and 1 week post-implantation, with an additional 2-week time point for the gelatin-based patch. Histological, immunohistochemical, and ultrastructural analyses were performed to assess biomaterial integration, cell infiltration, extracellular matrix deposition, and activation of the leech's innate immune components, which play a key role in wound healing and antimicrobial defense. The use of *H. verbana* thus provides a valuable comparative model to explore evolutionarily conserved links between tissue regeneration and immune function and to assess the biocompatibility and regenerative potential of novel biomaterials in a whole-organism context, while offering a cost-effective and ethically advantageous platform for their preclinical evaluation.

HISTORICAL PERSPECTIVE

Thirty years of IADCI: past, present and future of the Society

L Ballarin
Department of Biology, University of Padua, Padua, Italy

The Italian Association of Developmental and Comparative Immunobiology was founded in late 1996 in Palermo and in the following year the first Society meeting took place, within the annual meeting of the Italian Zoological Society (UZI). hold in Cattolica (RN). Until 2006, the meetings took place annually, with the exception of the year in which the IDCI had its triennial meeting. From 2007, the meeting takes place annually, organized by various national universities. That of this year is the XXVI meting and, for the second time, in San Benedetto del Tronto (AP). First president of the Society was prof. Nicolò Parrinello, University of Naples, followed by prof. Enzo Ottaviani, University of Modena and Reggio Emilia (passed away in 2017), prof. Loriano Ballarin, University of Padova, prof. Matteo Cammarata, University of Palermo and the current president, prof. Davide Malagoli, University of Modena and Reggio Emilia. During its thirty years of activity, the IADCI granted awards to young and early-career scientists, curated the publication of a book (Lesson in immunity, Academic Press, 2016) and a special issue of the journal: *Biology* entitled: *Ancient Immunity. Phylogenetic Emergence of Recognition-Defence Mechanisms*. Members of the Society also created networks with other members of the IADCI to write projects and apply to National Research Calls, in many cases successfully

MAIN LECTURE

Resilience in biological systems: adaptive cellular responses of mussels to environmental changes

M Maisano
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

Biological systems resilience denotes the capacity of living systems, ranging from individual organisms to populations and the entire ecosystems, to absorb disturbances, adapt to stresses, and reorganize while maintaining core functions, structure, and identity. It represents a system's ability to recover from perturbations, such as environmental change or injury, rather than merely resisting change. The Mediterranean mussels *Mytilus galloprovincialis* are considered powerful indicators of ecological resilience due to their ability to withstand, adapt to, and recover from severe environmental stressors, as documented in numerous laboratory studies on mussel adaptive cellular responses following exposure to emerging challenges. A first evidence is provided by exposing mussels to environmental concentrations of the anti-inflammatory dexamethasone, a Pharmaceutically Active Compound (PhAC), that induced in gills hemocyte infiltration, increased neutral and acid mucopolysaccharides, a general pro-oxidant effect witnessed by lipid peroxidation, and rise in protein turnover and energy demand. Moreover, treatment of mussels with environmentally relevant doses of gadolinium, a Rare Earth Element (REE), revealed changes in the reproductive tissues of both sexes, with induction of early and mass spawning events, hemocyte recall in response to gonadal atresia, and disorders in energy pathways including glycogen, essential for proper gametogenesis. Finally, it is demonstrated the high sensitiveness of mussel early life stages to emerging pollutants, namely polystyrene microplastics and bisphenol A that induced altered embryogenesis and metabolic disorders. Overall, these evidences highlight the need of using mechanistic effect models (MEMs) in ecocytotoxicology within a One-Health framework because of the close interactions among humans, animals, and ecosystems.

Session I/1. Defence mechanisms in response to environmental stresses. Chair: Tiziana Cappello, University of Messina, Messina, Italy and Nicolò Baranzini, University of Insubria, Varese, Italy

Immunotoxicological evaluation of a new generation PFAS mixture in the earthworm *Eisenia fetida*

D Gualandris, D Rotondo, V Bonetto, A Calisi, F Dondero
Università del Piemonte Orientale, Dipartimento di scienze e innovazione tecnologica, Alessandria, Italy

Soil pollution represents a major emerging environmental concern, particularly due to the widespread presence of per- and polyfluoroalkyl substances (PFAS), highly persistent compounds extensively used in numerous industrial applications. New-generation PFAS, introduced as alternatives to legacy compounds, remain poorly characterized from an ecotoxicological perspective, especially regarding their effects on the immune system of soil-dwelling organisms. The aim of this study is to investigate the potential immunotoxic effects of a PFAS mixture (PFBA, PFOA, HFPO-DA/GenX, PFMOBA and PFMOPrA) on the innate immune system of the earthworm *Eisenia fetida*, a key species for soil quality assessment. Sexually mature earthworms were exposed to different concentrations of the PFAS mixture (range concentration of 0.6-229 microM) using a filter paper contact test, in accordance with OECD guideline No. 207. Immune responses were assessed using an integrated approach combining enzymatic assays, and cytotoxic and genotoxic effects in earthworms' coelomocytes. Phenoloxidase activity, a central biomarker of innate immunity, was measured in tissue-derived samples, based on previous standardization studies demonstrating no significant differences compared to cellular assays and improved robustness in data acquisition. Coelomocytes were collected from the coelomatic fluid showing an increase in dimension accompanied by cell rounding with loss of pseudopods. Moreover, an increase of micronuclei frequency was also seen. This study provided novel insights into the combined effects of alternative PFAS on the innate immune system of soil organisms, and contributed to the development of an ecotoxicological database to support integrated environmental risk assessment and future regulatory strategies. Acknowledgements: This work was supported by Green Deal H2020 programme (European Grant n° 101037509- SCENARIOS)

The Role of Superoxide Dismutase Across Organs and Time Following Chronic PFAS Exposure in the Veneto Region

M Cortese[1], S Pacchini[1], L Drago[1], E Piva[1], S Kholdihaghighi[1,2], A Bottacin-Busolin[3], P Irato[1], A Marion[3], G Santovito[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy
[3]Department of Industrial Engineering, Università degli Studi di Padua, Padua, Italy

Per- and polyfluoroalkyl substances (PFAS) are a class of persistent environmental contaminants widely distributed worldwide, raising concern due to their persistence and tendency to bioaccumulate in biological tissues. In 2013, a major PFAS contamination event was identified in the Veneto region (Italy); however, its long-term effects on local fauna remain insufficiently understood. PFAS exposure has been associated with increased production of reactive oxygen species (ROS), potentially leading to oxidative stress. Therefore, investigating the activation of antioxidant defence systems is essential for a better understanding of PFAS-induced toxicity. With this aim, specimens of *Squalius cephalus* were collected from three sites in the Vicenza area characterised by different PFAS contamination levels. Sampling was conducted twice over a one-year interval to evaluate temporal variations. Superoxide dismutase (SOD) activity was measured in the liver and kidney, two of the main organs associated with PFAS accumulation and detoxification, as a biomarker of the oxidative stress response. The results indicate that chronic PFAS exposure affects SOD activity, with variations depending on contaminant concentration, organ type, and time of exposure. These findings suggest that PFAS contamination can alter antioxidant defences in freshwater fish, highlighting the importance of temporal and tissue-specific analyses when assessing PFAS-related oxidative stress. Acknowledgements: This work was supported by the European Union (Next Generation EU) in the context of the Italian PNRR Programme 2021-2027, which funds the RETURN project (Grant Agreement n° PE0000005).

First assessment of the effects of copper and cadmium on *Clibanarius erythropus*

R De Luca[1], G Cammilleri[2], F Longo[1], I Restivo[1], V Ferrantelli[2], L Tesoriere[1], V Arizza[1], M Vazzana[1], M Mauro[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy
[2]Experimental Zooprophylactic Institutes of Sicily "A. Mirri" (IZS) Palermo, Italy

Heavy metal pollution is a long-standing environmental challenge, particularly in the Mediterranean basin, and recent reports indicate a worsening situation due to increasing human activities. Although efforts have been made to control toxic elements in marine ecosystems, traditional monitoring methods fail to capture their effects on biota, and for this reason, numerous studies have explored the responses to metals in aquatic organisms. Despite this, species-specific data are lacking for *Clibanarius erythropus* (Latreille, 1818). This hermit crab is widespread in the Mediterranean intertidal zones and, due to its detritivorous habits, is in close contact with contaminated sediments. In light of this, we present here the first evaluation of *C. erythropus* responses to copper (Cu) and cadmium (Cd). We performed acute toxicity assays (96-h LC_{50}), bioaccumulation analyses in the hepatopancreas (ICP-MS), and sublethal exposure experiments evaluating oxidative stress and immune-related biochemical responses. Mortality tests revealed significantly higher cadmium toxicity compared to copper, and bioaccumulation

data confirmed the hepatopancreas as the primary detoxification organ, showing dose-dependent accumulation for both metals. Furthermore, sublethal exposures triggered increased production of reactive oxygen species (ROS), elevated alkaline phosphatase, esterase, and peroxidase activities, and GSH depletion. This study provides the first evidence of how *C. erythropus* reacts to metal pollution. Although further research is needed to fully validate its use, our results suggest that this species could serve as a sensitive bioindicator for monitoring Mediterranean coastal ecosystems, potentially aligning with European assessment frameworks such as the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD).

Congener-Specific PFAS Modulate Lysenin-Mediated Humoral Cytolysis in the Earthworm *Eisenia fetida*

D Rotondo, S Repetto, D Gualandris, A Calisi, F Dondero
Università del Piemonte Orientale, Dipartimento di scienze e innovazione tecnologica, Alessandria, Italy

Per- and polyfluoroalkyl substances (PFAS) are increasingly implicated as immunotoxicants, yet their capacity to perturb extracellular effector mechanisms in soil invertebrates remains insufficiently characterized. Here, we investigated PFAS-driven modulation of the lysenin arm of humoral immunity in the earthworm *Eisenia fetida*. Lysenin is a soluble, sphingomyelin-specific β-pore-forming toxin released by coelomocytes and represents a major cytolytic effector of annelid innate defence. Earthworms were acutely exposed for 72 h under an OECD 207–style filter paper design to a panel of 31 legacy and emerging PFAS at two concentrations (0.6 and 229 µM). Decellularized hemolymph was subsequently assayed in an ex vivo sheep erythrocyte lysis test to quantify functional lysenin activity, and results were integrated with lysenin-targeted qPCR and, where available, label-free proteomics of the lysenin protein family.

Across the 31-PFAS panel, hemolysis revealed a marked congener-specific reshaping of lysenin-dependent cytolytic capacity. At the transcriptional level, lysenin mRNA was broadly above baseline across treatments, indicating effector priming. Proteomic readouts suggested isoform-specific reweighting within the lysenin family rather than uniform abundance shifts, with a strong increase of one lysenin-like entry under GenX exposure.

Collectively, functional, transcriptomic, and proteomic evidence supports a model in which PFAS modulate lysenin-mediated cytolysis predominantly through congener-dependent regulation of effector mobilization and isoform composition, rather than via a single dominant mechanism such as direct inhibition of lysenin activity. These findings identify lysenin-dependent hemolysis as a sensitive functional endpoint for PFAS immunomodulation in *E. fetida* and emphasize the vulnerability of extracellular humoral effectors to emerging PFAS chemistries.

Acknowledgements: This work was supported by Green Deal H2020 programme (European Grant n° 101037509- SCENARIOS)

Energetic and oxidative costs of chronic PFAS exposure in riverine fish *Squalius cephalus*

S Pacchini[1], M Cunha[2], M Cortese[1], E Piva[1], G Vanzan[1], L Drago[1], S Kholdihaghighi[1,3], P Irato[1], A Bottacin-Busolin[4], A Marion[4], R Freitas[2], G Santovito[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]CESAM, Department of Biology, University of Aveiro, Aveiro, Portugal
[3]Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Mestre (VE), Italy
[4]Department of Industrial Engineering, University of Padua, Padua, Italy

Fish exposed to chemical contamination must continually reallocate metabolic resources to survive, diverting energy from growth and reproduction toward detoxifying xenobiotics and controlling oxidative stress. Among emerging threats to freshwater ecosystems, per- and polyfluoroalkyl substances (PFAS) became emblematic due to their persistence, bioaccumulation, and long-range transport. This study investigated the physiological responses, in terms of energetic and redox costs, associated with chronic PFAS exposure, analysing the liver of *Squalius cephalus* from rivers with varying levels of contamination in the Veneto region (Italy). Results revealed a metabolic shift characterised by enhanced electron transport system (ETS) activity and depletion of glycogen (GLY) reserves, indicating increased mitochondrial respiration and energy mobilisation to sustain detoxification and antioxidant defences. Biotransformation responses showed an activation of phase I glutathione S-transferases (GSTs), followed by inhibition of phase II carboxylesterases (CbEs), suggesting an unbalanced detoxification process. Antioxidant defences were activated, both superoxide dismutases (SODs) activity and total antioxidant capacity (TAC), consistent with a compensatory response to elevated reactive oxygen species (ROS). Nevertheless, this was insufficient to prevent macromolecular damage at the highest PFAS level, as evidenced by the accumulation of advanced oxidation protein products (AOPP) but not protein carbonyls (ProC). In contrast, the reduction in lipid peroxidation (LPO) reflects an effective protection of lipids through the combined action of enzymatic and non-enzymatic scavengers. Overall, these findings demonstrate that chronic PFAS exposure imposes substantial energetic costs and oxidative stress on *S. cephalus*, primarily targeting mitochondrial metabolism and structural proteins, with potential long-term implications for fish health and ecosystem resilience. Acknowledgements: Supported by the European Union (Next Generation EU) in the context of the Italian PNRR Programme 2021-2027, which funds the RETURN project (Grant Agreement n° PE0000005)

Galaxolide (HHCB) exposure impairs innate immune homeostasis and alters metabolic pathways in the medicinal leech *Hirudo verbana*

A Rihan[1], L Pulze[1,2], A Grimaldi[1,2], S Tasselli[3], N Baranzini[1,2]
[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
[2]Italian Leech Farm (ILFARM) Srl, Varese, Italy
[3]Water Research Institute, CNR-IRSA, Brugherio, Italy

The exponential increase in synthetic chemical production has led to the emergence of numerous micropollutants (EMPs) that remain largely unregulated and poorly characterized in terms of their ecological and biological impacts. Among them, the synthetic fragrance Galaxolide (HHCB) has raised growing concern due to its widespread presence in aquatic environments, including rivers, lakes, and sediments. Its high lipophilicity promotes bioaccumulation in tissues, increasing the risk of persistence along the trophic chain. Consequently, establishing a robust ecotoxicological risk profile for HHCB, including its toxicity and mechanisms of action, is scientifically urgent. In this study, the aquatic leech *Hirudo verbana* was employed as an invertebrate model to investigate HHCB-induced effects using an integrated methodological approach. Quantitative analyses confirmed the accumulation of HHCB in leech tissues. Subsequent morphological, immunofluorescence, and molecular assays revealed significant alterations in the innate immune system, including modulation of angiogenesis and recruitment of macrophage-like cells. HHCB exposure also induced oxidative stress, as shown by increased reactive oxygen species (ROS) production and activation of antioxidant enzymes such as superoxide dismutase, glutathione S-transferase, and catalase. Additionally, HHCB affected GABA metabolism, with a marked upregulation of GABA transaminase compared to glutamate decarboxylase, suggesting a metabolic shift toward GABA catabolism. Overall, these findings demonstrate that even sub-lethal HHCB concentrations can disrupt immune and metabolic homeostasis, highlighting the value of alternative invertebrate models for assessing emerging contaminants.

Session I/2. Defence mechanisms in response to environmental stresses. Chair: Antonio Calisi, Università del Piemonte Orientale, Alessandria, Italy and Gianfranco Santovito, University of Padua, Padua, Italy

Hidden Impacts: Does Microplastic Accumulation Affect the Physiology of Reef-Building Organisms?

C Ciacci[1], L Canesi[2], N Mancin[3], G Lo Bue[3], F Frontalini[4]
[1]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy
[2]Department of Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy
[3]Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy
[4]Department of Pure and Applied Sciences, University of Urbino "Carlo Bo" – Urbino, Italy

Biogenic structures built by sabellariid polychaetes, which play a crucial role in the coastal environment retaining sediment, preventing erosion and creating new habitats for associated species, are increasingly threatened by anthropogenic activities. Recent findings indicate that sabellariid reefs passively trap microplastics (MPs) in abundances reflecting those in surrounding sediments; however, the biological and physiological consequences of the passive MPs accumulation in sabellariid reefs are still unknown. It is therefore important to assess how the accumulation of MPs could pose hidden risks to coral reef organisms and associated communities.

Our previous study evaluated several biomarkers on *Sabellaria spinulosa* providing reference values for both antioxidant and metabolic enzymes across different size classes.

Here, we report the results of a 28-day exposure of *S. spinulosa* to MPs (PET, Polyethylene terephthalate; particle size 100 µm). A worst environmental scenario (1,9 items ml-1) was tested to evaluate physiological responses. Enzymatic activities related to the antioxidant defense system and energy metabolism were assessed at different exposure times (0, 7, 14, and 28 days) analyzing male and female separately. Stimulation of oxidative pathway enzymes was more pronounced in females, while modulation of glycolytic enzymes and activation of the antioxidant response were observed in both sexes, particularly at intermediate exposure times.

Reef-building polychaetes of the genus *Sabellaria* are largely neglected from a physiological and ecotoxicological perspective. Although the basic physiology of *S. spinulosa* is still poorly known, this study provides the first evidence on sublethal effects of microplastic exposure, through an integrated and multidisciplinary approach.

Effects of polyethylene nanoplastics on immune- and stress-related genes of ascidians

G Sabbadin[1], T Sunanaga[2], S Fujiwara[2], L Ballarin[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Faculty of Science and Technology, Kochi University, Japan

The increasing levels of plastic pollution, particularly in the form of micro- and nanoplastics (NPs), represents a growing threat to aquatic ecosystems, as these particles persist in the environment and interact with multiple biological compartments. Despite their ecological relevance, the effects of these anthropogenic stressors on immunity, development, and physiological resilience remain poorly understood in sessile filter-feeding invertebrates such as ascidians, which can directly uptake NPs from the surrounding environment

during feeding. This work aims to study the effects of NP pollution on the expression of immunity- and stress-related genes in two species of ascidians *Polyandrocarpa misakiensis* and *Ciona robusta*. Animals were collected in the coastal area near the Usa Marine Biological Institute of Kochi University (Japan). Ascidians were exposed, in the laboratory, to NPs for a week at a 0.5 µg/mL concentration. After exposure, the gut and pharynx of Ciona as well as the buds and zooids of *Polyandrocarpa* were separately collected and the expression of the genes of interest was analyzed through qRT-PCR. Results show how NPs exposure can alter the expression of some of these genes; results indicate also a different effect depending on the type of tissue analyzed.

Histological and cellular alterations by dexamethasone affecting the defence mechanisms of *Mytilus galloprovincialis* and the promising use of ulvans as eco-friendly solution

G De Marco[1], M Galati[1], B Randazzo[2], A Cristaldi[3,4], G Maricchiolo[2], G Genovese[1], G Oliveri Conti[3], M Ferrante[3], T Cappello[1,5], M Maisano[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[2]IRBIM-Institute for Marine Biological Resources and Biotechnologies, CNR-National Research, Messina, Italy

[3]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy

[4]Department of Medicine and Surgery, University of Enna "Kore", Enna, Italy

[5]Universal Scientific Education and Research Network (USERN)

The current failure of wastewater treatment plants to retain many compounds released daily into urban sewage systems, lead to their flow into aquatic ecosystems. Dexamethasone (DEX), one of the most widely used anti-inflammatories, is regularly detected in both marine and freshwater environments. Few information is available on its potential effects on non-target organisms, especially on immune response of invertebrates. Aim of this work was to elucidate the detrimental effects of realistic DEX concentrations (C1: 4 ng/L; C2: 40 ng/L; C3: 400 ng/L; C4: 2 µg/L) to mussel *Mytilus galloprovincialis* during a 12-days exposure. The effects of DEX were evaluated on the digestive gland, chosen for its crucial role in the detoxification of xenobiotics, at different exposure times (T3; T6; T12). A relevant uptake of DEX in the treatment with the highest concentrations (C3, C4) was recorded. At tissue level, impairments in digestive tubule organization, hemocyte infiltration, and a rise in Tumor Necrosis Factor-α (TNF-α) positive cells were observed. Alterations in the antioxidant system, energy metabolism, level of thiol groups, and a general enhancement of lipid peroxidation confirmed the harmful effects of this contaminant at cellular level. These data highlight the need of innovative mitigation strategies against these emerging contaminants. Therefore, we also tested ulvans, polysaccharides with antioxidant properties extracted by algae of the genus *Ulva,* as feed additives in a combined exposure with the same DEX doses on mussels, obtaining promising results for using this algal extracts as eco-friendly solution to counteract the negative impact of DEX on non-target organisms.

Impact of climate change on reproductive health of marine invertebrates: the case study of *Mytilus galloprovincialis*

M Galati, MA Oueldi, M Romeo, T Cappello, M Maisano

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

Increasingly invasive human activity has disrupted the precarious balance human-nature. Climate instability, leading to global warming, has subverted the chemical-physical conditions of many ecosystems, especially marine-coastal, which are more susceptible to multiple stressors. To assess the climate change impact and its bioremediation possibility in confined area, the "thermal priming" effect was evaluated on the reproductive health of the Mediterranean bivalve *Mytilus galloprovincialis*, choosing male and female gonads as target organ. Specimens from the farm S.A.Co.M. of both sexes were pre-exposed to 28-30-32 °C for 2 h and, subsequently, returned to 17±1 °C (acclimation temperature) for 24 h, and then subjected to heat shock (36 °C) for 2 h, to verify the "priming" phenomenon. A negative (no thermal variation) and positive (directly exposed to 36 °C) control group were added to pre-adaptation groups. Histological observation (H/E) showed early spawning events following the thermal increase in both sexes. Histochemical results (dPAS/PAS) highlighted a divergence in stress response between sexes, demonstrated by metabolomic data (1H NMR) on defence and osmotic pathways. The May-Grünwald Giemsa reaction (to distinguish between haemocytes classes) and Schmorl's method (to estimate the innate immunity related to phenoloxidase) indicate the susceptibility of both sexes to temperature variations. These data can help to better understand the impact of climate change on biota reproductive system and how "thermal priming" may be a useful strategy for enhancing the biota adaptive capabilities in mussel farms under appropriate and feasible conditions. Ultimately, this study summarizes the need to safeguard the close relationship between humans and environment. Acknowledgements: This work was supported by the European Union - Next Generation EU, PNRR MUR – RAISE (CUP B73C24000590006) "TEC-SOS" Project

Stress coping styles influence serum protein electrophoretic patterns in *Sparus aurata* following *Vibrio anguillarum* stimulation

M Staropoli[1], L Bisanti[1], M Dara[1], I Vazzana[2], P Carbonara[3], C La Corte[1], F Bertini[1], LT Marcianò[5], D Parrinello[1], MG Parisi [1], M Cammarata[1]
[1]Marine Immunobiology Laboratory, Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Palermo, Italy
[2]Experimental Zooprophylactic Institute of Sicily "A. Mirri", Italy
[3]Fondazione COISPA ETS, Via dei Trulli 18, 70126 Bari, Italy

Stress coping style (SCS) is increasingly recognised as a determinant of immune competence and welfare in breeding contexts. Behavioural phenotypes are closely associated with variability in immune and physiological responses to immune challenges, and may therefore represent a relevant criterion for welfare-driven selection in aquaculture. This study examined the impact of SCS on the physiological responses of *Sparus aurata*, a key species in Mediterranean aquaculture, following stimulation with the bacterial pathogen *Vibrio anguillarum*. Variations in fish status was assessed through serum protein electrophoresis using capillary electrophoresis, proposed here as a sensitive and innovative analytical tool. Fish were classified as *bold*, *intermediate*, or *shy* based on a risk-taking behavioural assay. Serum samples were collected before inoculation with inactivated *V. anguillarum* (day 0) and at 30- and 60-days post-stimulation. Electrophoretic profiles revealed a time-dependent increase in the β2-globulin fraction, likely linked to immunoglobulins and other components of the innate immune response against the bacterial stimulus, along with a concomitant reduction in the γ-globulin fraction over time. These shifts were most pronounced in *bold* individuals, whereas *shy* fish exhibited a similar but less intense response. Overall, the findings indicate that proactive behavioural types mount a stronger and more rapid responses to pathogen injection compared with reactive individuals. The modulation of serum protein fractions therefore emerges as a valuable biomarker for assessing responsiveness in *S. aurata*, highlighting the importance of integrating behavioural traits with biological parameters to improve welfare and management in aquaculture systems.

Session II/1. Basic and applied advances in comparative and developmental immunobiology. Chair: Jacopo Vizioli, University of Tuscia, Viterbo, Italy, Piero Giulianini, University of Trieste, Trieste, Italy

Immune priming in the colonial ascidian *Botryllus schlosseri*

A Peronato, N Franchi, L Drago, S Domenichi, P Venier, E Bortoletto, L Ballarin
Department of Biology, University of Padua, Padua, Italy

Immune priming is the capability of the innate immune system to use the information on the molecular structure of nonself, acquired in the course of a past experience, to give rise to an enhanced immune response in the case of a second exposure to related or unrelated molecule/cell. It can be considered a short-term immune memory, highly differing from the long-term, lymphocyte-based, immune memory of jawed vertebrates.

In the last decades, immune priming has been described in a variety of invertebrates. Nevertheless, studies on immune priming rely on a few parameters or the expression of a low number of genes involved in immune responses, such as those codifying pattern recognition receptors or complement factors. In addition, the molecular mechanisms at the basis of the phenomenon are, in many cases, far from being understood.

In the attempt to demonstrate the presence of immune priming in colonial ascidians, we microinjected colonies of *B. schlosseri* with bacteria or LPS from *Escherichia coli* and compared the obtained response, in terms of transcription of immune-related genes, to that obtained following a second challenge. The responses in various phases of the colonial blastogenetic cycle were also considered. Results indicate the capability of *B. schlosseri* to enhance the transcription of immune-related genes after a first challenge with nonself and pave the way for future analysis of the mechanisms underlying the process.

MAPK signaling integrates immune and stress responses during colonial blastogenesis in *Botryllus schlosseri*.

L Drago[1], F Frisoni[2], G Santovito[1], L Ballarin[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Biology, University of Konstanz, Konstanz, Germany

Mitogen-activated protein kinase (MAPK) pathways are evolutionarily conserved signaling cascades that regulate key cellular processes such as proliferation, differentiation, and stress responses. In the ascidian *Botryllus schlosseri*, the colony consists of repeated functional units called zooids, which are continuously replaced through blastogenesis, a cyclical developmental process requiring immune-mediated clearance of aging cells. We identified two MAPK genes, *bsmapk-1* and *bsmapk-8*, homologous to vertebrate *erk* and *jnk*, and examined their transcriptional dynamics throughout the blastogenetic cycle. The two genes showed distinct temporal patterns and differential responsiveness to immune stimulation and environmental stressors, suggesting pathway-specific roles. Functional inhibition of ERK and JNK signalling impaired hemocyte phagocytosis, altered the expression of genes involved in oxidative stress defense (*sod*, *gpx-5*) and stress granule dynamics (*tiar*, *ttp*), and disrupted normal blastogenesis, resulting in reduced zooid growth and developmental arrest. These findings demonstrate that MAPK pathways act as critical integrators of immune function, stress responses, and developmental renewal, ensuring the coordinated turnover and maintenance of colonial modules in a non-vertebrate chordate.

Revealing the unknown: Approaching oyster antiviral immunity using gene-centered network analysis

E Bortoletto[1], B Kaczmarek[2], R Frizzo[1], L Cendron[1], C Bernecky[2], P Venier[1], U Rosani[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Institute of Science and Technology Austria (ISTA), Vienna, Austria

Vast and morphologically diversified group of species, marine bivalves are particularly interesting because to allow their sessile lifestyle they should have developed effective strategies to cope with the abundance of microbes and viruses thriving in marine water. However, a comprehensive understanding of antiviral immunity in these species is limited, even in oysters which represented one of the most studied species due to exacerbation of Ostreid herpesvirus pathogenicity occurred in the last decades. Here, we have described the peculiar activity of Adenosine deaminases acting on dsRNA (ADAR) in editing viral RNAs, thus likely being a central player of antiviral immunity in oysters and related mollusk species. Using *Magallana gigas* ADAR1 (MgADAR1v) as a proxy of the activation of antiviral response, we have analyzed the global expression profiles in over 200 RNA-seq datasets and built a gene network analysis to identify co-occurring genes. Weighted gene co-expression network analysis identified gene modules positively associated with MgADAR1v expression, likely representing key antiviral signaling elements, including RLR/MAVS, cGAS/STING, JAK/STAT, and TLR/NF-κB genes. Notably, an average of 38% of these genes included in the different networks remained functionally uncharacterized. Using a comparative analysis we could reveal the taxonomic distribution of orthologs, revealing mollusc- or even bivalve-species gene groups. These genes, enriched in domains suggesting RNA metabolism, helicase, and nucleic acid processing activities, possibly represent novel antiviral signaling and effector molecules, suggesting the presence of previously unrecognized antiviral effectors. Based on our results we proposed the presence of a complex antiviral gene network whose elements have been only partially characterized so far, with ADAR1 possibly acting as a central node thanks to the ability to control antiviral activation as well as viral propagation.

From marine-derived peptides of *Actinia equina* to therapeutic antimicrobial potential

M Staropoli[1], C La Corte[1], M Dara[1], L Bisanti[1], A Jakas[2], M Roje[2], D Parrinello[1], MG Parisi[1], M Cammarata[1]
[1]Marine Immunobiology Laboratory, Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Palermo, Italy
[2]Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia

Sea anemones represent a resource of high interest for therapeutic research as they produce bioactive molecules associated with predation and defence mechanisms involving toxins and antimicrobial peptides. From the sea anemone *Actinia equina*, compounds exhibiting antimicrobial activity were extracted and isolated. Through purification by solid-phase reverse-phase chromatography and HPLC, the 40% acetonitrile fractions from acidic tentacle extracts were identified as responsible for broad-spectrum antimicrobial activity. Among these fractions, two peptides, 6.2 and 7.3, named Equinin A and Equinin B, respectively, were isolated and characterised. Both peptides displayed growth-inhibitory activity against Gram-positive and Gram-negative bacteria. In particular, *in silico* analyses highlighted the antimicrobial potential of Equinin B, a 34–amino acid peptide (GQCQRKCLGHCSKKCPKHPQCRKR CIRRCFGYCL), due to its strong similarity to antimicrobial peptides previously described in the literature. In order to gain insight into the mechanisms of action and to explore the relationship between peptide structure and antimicrobial activity, Equinin B was selected as a model for the development of new antimicrobial candidates. The resulting peptides were evaluated for their antimicrobial properties and showed promising activity against selected microorganisms.

Overall, these results highlight the role of marine invertebrates as models of innate immunity and demonstrate how molecules involved in their defence mechanisms can be exploited as a basis for the development of synthetic analogues with biological properties and potential therapeutic applications.

***Hirudo verbana*: a translational model for studying hDPSC secretome-enhanced angiogenesis in ECM-based biomaterials for wound healing**

G Marcolli[1], N Baranzini[1,2], L Pulze[1,2], S Zorzetto[1], L Barone[1], F Rossi[1], R Gornati[1], A Grimald[1,2]
[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
[2]Italian Leech Farm (ILFARM) S.r.l, Varese, Italy

The medicinal leech *Hirudo verbena* is an established *in vivo* model for vascular and regenerative studies, representing a valuable translational platform for investigating angiogenic mechanisms relevant to several processes, including chronic wound healing. In this study, *H. verbana* was used to evaluate a cell-free regenerative approach, based on the combination of a commercial collagen-based dermal substitute (MicroMatrix® UBM Particulate) with the secretome derived from human Dental Pulp Stem Cells (hDPSC-S), hypothesizing an enhanced pro-angiogenic potential of the biomaterial. Leeches were injected with MicroMatrix® resuspended either in serum-free medium (CTRL) or supplemented with hDPSC-S. Histological and immunohistochemical analyses, performed 1-week post-treatment, revealed effective biomaterial integration within host tissues in both experimental groups. However, hDPSC-S-supplemented scaffolds elicited a

significantly stronger regenerative response compared to CTRL in *H. verbana*. This was characterized by increased recruitment and infiltration of host regenerative cell populations, including telocytes and Hematopoietic Precursor Stem Cells, alongside robust stimulation of neovascularization, indicating a strong pro-angiogenic effect. These findings demonstrate that priming ECM-based biomaterials with stem cell-derived secretomes generates a bioactive microenvironment capable of recruiting endogenous regenerative cells and promoting angiogenesis without the need for direct cell transplantation. Overall, the use of *H. verbana* supports the potential of hDPSC-S-enriched MicroMatrix® as a promising off-the-shelf therapeutic approach for the treatment of complex wounds associated with impaired vascularization, such as diabetic ulcers and chronic ischemic tissues.

Cytosolic PCNA is localised in *Pomacea canaliculata* circulating hemocytes, independently from active cell proliferation

C Losi[1], A Ferri[2], M Montanari[2], S Sacchi[2], U Rosani[3], G Sansalone[2], D Malagoli[2,4]
[1]Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Italy
[2]Department of Life Sciences, University of Modena and Reggio Emilia, Italy
[3]Department of Biology, University of Padua, Italy
[4]NBFC, National Biodiversity Future Center, Palermo, Italy

In vertebrates, immune cells play essential roles in pathogen defense and tissue homeostasis and are maintained through continuous, tightly regulated turnover. In mollusks, these roles are mediated by circulating and tissue-resident hemocytes; however, hemocyte turnover and dynamics remain poorly understood. The laboratory-bred and genetically tractable gastropod *Pomacea canaliculata* (*Pc*) represents a suitable model to investigate hemocyte dynamics *in vivo*, as hemolymph can be collected repeatedly. *Pc*'s circulating hemocytes are morphologically classified as small, blast-like Group I (GI) cells and larger agranular or granular Group II (GII) cells.

To characterize hemolymph recovery and hemocyte dynamics following withdrawal, hemocyte number and morphotype composition were analyzed by flow cytometry at 1.5, 3, 6, 9, 18, 24, and 48h after an initial hemolymph collection. Model-based time-series analysis revealed non-directional changes in hemocyte composition, consistent with a dynamic recovery process. GI hemocytes peaked at 18h, total hemocyte number reached a maximum at 24h, and both parameters returned to baseline by 48h. Microscopic analyses did not reveal mitotic figures among circulating hemocytes.

Accordingly, Proliferating Cell Nuclear Antigen (PCNA), a marker of DNA replication and repair, was assessed via transcriptomic analysis and western-blot. RNA-seq data reveled *Pc*PCNA expression in circulating hemocytes, which was confirmed at the protein level. Preliminary observations suggest that cytosolic PCNA can be localized in a fraction of circulating hemocytes, a pattern previously described in terminally differentiated mammalian neutrophils and macrophages, where PCNA is uncoupled from DNA replication.

These data suggest that *Pc* regulates circulating hemocyte replenishment independently from active proliferation in circulation. Accordingly, PCNA expression was documented in circulating hemocytes both at transcript and protein levels, and initial evidence on its subcellular localization supports the view that these cells may represent differentiated immunocytes not directly involved in cell proliferation. Acknowledgements: This work was supported by National Recovery and Resilience Plan (NRRP), Mission 4, CUP E93C22001090001, Project title "National Biodiversity Future Center - NBFC"

Dynamic response of the posterior kidney to hemolymph withdrawal in *Pomacea canaliculata* involves hemocyte islet depletion and recovery

A Ferri[1], C Losi[2], U Rosani[3], S Sacchi[1], D Malagoli[1,4]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Italy
[3]Department of Biology, University of Padua, Padua, Italy
[4]NBFC, National Biodiversity Future Center, Palermo, Italy

In vertebrates, immune cells have a limited lifespan and must be continuously replenished through hematopoiesis to sustain immune competence, tissue homeostasis and organismal survival. In invertebrates, both larval and adult hematopoiesis indicate that hemocyte turnover occurs; however, the modalities of this process remain largely unexplored, particularly in lophotrochozoans, where molecular markers associated with hemocytes and their proliferation are still poorly characterized. *Pomacea canaliculata* represents a valuable gastropod model for studying hemocytic turnover *in vivo*, as increasing omics resources are available and hemolymph can be repeatedly collected without compromising animal survival. This enables controlled stimulation of hematopoiesis, temporal monitoring of hemolymph recovery, and expression analyses in relevant tissues and cells.

In this study, we identified reliable molecular markers associated with circulating hemocytes in *P. canaliculata* through transcriptomic mining. Transcriptomic analyses revealed the predominant expression of two conserved hematopoiesis-related genes, Hematopoietically expressed homeobox (*Pc*-Hhex) and Transglutaminase (*Pc*-TGase), in circulating hemocytes. Their expression profiles were subsequently analyzed by RT-qPCR, following hemolymph withdrawal, in the posterior kidney (PK), an organ containing resident hemocyte aggregates (hemocyte islets) proposed as potential hematopoietic sites. Fluorescent *in situ* hybridization

performed on control snails confirmed the constitutive expression of *Pc*-Hhex and *Pc*-TGase in hemocyte islets, but not in the renal parenchyma. The expression of hemocyanin (*Pc*-Hc), previously detected in circulating hemocytes of *P. canaliculata*, was used as an additional indicator of hemocyte presence.

Quantitative expression analyses in the PK revealed a significant decrease in *Pc*-Hc expression at 18 hours post-withdrawal (hpw), suggesting mobilization of resident hemocytes from hemocytic islets into the hemolymph. At 24 hpw, *Pc*-Hc expression significantly increased concomitantly with *Pc*-Hhex and *Pc*-TGase. Computer-assisted quantification of hemocyte islet density corroborated molecular data, showing an initial decrease followed by progressive recovery, although no mitotic figures were observed. Collectively, these results indicate that the PK responds to hemolymph withdrawal, supporting a functional link between circulating hemocytes and hemocytic islets. Acknowledgements: This work was supported by National Recovery and Resilience Plan (NRRP), Mission 4,CUP E93C22001090001, Project title "National Biodiversity Future Center-NBFC

Comparative genomic study of the Australian *Mytilus planulatus* to identify lineage-specific immune genes

D Kireta[1], DE Nerelli[1], A Sacco[2], E Bortoletto[3], U Rosani[3], A Pallavicini[1], M Gerdol[1]

[1]Applied and Comparative Genomics Group, Department of Life Sciences, Università di Trieste, Trieste, Italy; [2]Instituto de Investigaciones Marinas (IIM), Consejo Superior de Investigaciones Científicas (CSIC), Vigo, Spain; [3]Department of Biology, University of Padova, Padova, Italy

Blue mussels of the *Mytilus edulis* species complex are globally distributed, environmental and aquacultural foundation species. In Australia, blue mussels were previously assumed to be *Mytilus galloprovincialis,* consistent with human-mediated introductions and historic invasions from the northern hemisphere. However, recent SNP-based studies suggest that native Australian blue mussels are in fact *Mytilus planulatus* widely introgressed with *M. galloprovincialis.*

We recently generated the first *M. planulatus* reference genome and obtained resequencing data from three Australian populations, including a putatively pure *M. planulatus* population from Tasmania and a previously reported hybrid population from the east coast near Sydney. In contrast to recent SNP studies, our preliminary analyses suggest that while the Australian populations are closely related to northern *M. galloprovincialis,* they may represent comparatively pure *M. planulatus*.

Australia offers a distinctive opportunity to explore how immune repertoires have evolved across *Mytilus* lineages, and whether lineage-specific immune genes may have contributed to local adaptation. Immune effector genes in the *Mytilus edulis* species complex are highly dynamic, with rapid sequence divergence and gene family expansions, and significant gene presence/absence variation. The myticalin antimicrobial peptide family is a clear example of this dynamic. These proline/arginine-rich antimicrobial effectors are restricted to *Mytilus,* showing impressive diversity in copy number and sequence composition, including lineage specific sub-families - consistent with strong selective pressures imposed by local pathogens.

Here, we aim to identify unique *M. planulatus*-associated genes, and to test whether these genes are enriched for immune functions. The outcomes could provide implications for biosecurity surveillance, and aquaculture-relevant disease-resilience research. Acknowledgements: This work was funded under the National Recovery and Resilience Plan (NRRP) –NextGenerationEU – Project Title Prot. 2022ZRZBZ7, and by European Union – NextGenerationEU – Project Title Prot. P2022JEEMT

Integrated omics analyses of the Central Nervous System and gut immune responses in RGNNV-infected European seabass

V Pianese[1], A Kosakyan[2,3], P Moreno[4], E Garcia-Rosado[4], AM Fausto[1], G Scapigliati[1], S Picchietti[1]

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy

[2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

[3]National Biodiversity Future Center (NBFC), Palermo, Italy

[4]Instituto de Biotecnología y Desarrollo Azul (IBYDA), Departamento de Microbiología, Facultad de Ciencias, 29071, Universidad de Málaga, Malaga, Spain

Red-spotted grouper nervous necrosis virus (RGNNV) is a neurotropic pathogen and the etiological agent of viral encephalopathy and retinopathy, severely affecting marine fish species and leading to considerable losses in aquaculture. Although the central nervous system (CNS) is the main site of viral replication and pathology, the molecular and cellular mechanisms driving host defence within the brain, as well as the contribution of systemic immunological network to infection outcome, remain poorly understood. Recent advances in comparative neuroimmunology have challenged the traditional view of CNS immune privilege, demonstrating that the brain can mount both innate and adaptive immune responses during viral invasion. In European sea bass, RGNNV infection triggers interferon-mediated antiviral responses in the brain, while the evidence of lymphocyte involvement has only recently begun to emerge. In parallel, the gut and its associated microbiota represent key immunoregulatory hubs within the microbiota–gut–brain axis, yet their contribution to neurotropic viral infections in fish remains largely unexplored. With the present study, we provide the first integrated omics characterization of brain and gut responses to acute RGNNV infection in European sea bass. By

combining transcriptomics and 16S rRNA metabarcoding, we observed robust immune activation in the CNS and widespread antiviral transcriptional reprogramming. In contrast, the intestinal response was modest, with limited local immune modulation despite significant shifts in microbial community composition associated with the infection. Overall, these findings provide interesting insights into the RGNNV pathogenesis and contribute to a better understanding of the integrated responses to NNV infection in a marine fish model.

Session II/3. Basic and applied advances in comparative and developmental immunobiology Chair: Valerio Matozzo, University of Padua, Padua, Italy and Maria Giovanna Parisi, University of Palermo, Palermo, Italy

Controlling complement from development to immunity

F Bertolasi[1], S Sacchi[1], A Vezzi[2], G Sales[2], A Ferri[1], D Malagoli[1,3], N Franchi[1]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Biology, University of Padua, Padua, Italy
[3]National Biodiversity Future Center (NBFC), Palermo, Italy

The complement system is classically regarded as a key effector of innate immunity, whose activity must be tightly regulated to avoid self-damage. Beyond this canonical role, complement components are increasingly recognized as active players in developmental processes, where controlled activation may contribute to selective cell elimination and tissue remodeling.

Here, we investigate the RCA gene repertoire and expression dynamics in the gastropod *Pomacea canaliculata*, focusing on adult hemocytes and early development. We identified seven distinct RCA genes, several of which generate up to four alternative splicing variants, revealing a notable molecular complexity. Transcriptomic analyses show that RCA genes are predominantly expressed in hemocytes, consistent with their role in immune regulation, but are also detectable in other tissues such as the ampulla, posterior kidney, and ganglion. In adult hemocytes, expression levels of all PcRCAs are broadly comparable and similar to those of PcC3, suggesting a coordinated regulatory framework controlling complement activity.

The most informative patterns emerge during early development, within the first 20 days post-fertilization (dpf), prior to hatching. During this period, PcC3 expression progressively increases, while specific RCAs display divergent trends. PcRCA4 is gradually down-regulated, suggesting a reduced protective role and a potential permissive condition for complement-mediated cell elimination. Conversely, PcRCA6 closely follows the PcC3 expression profile, supporting a protective function against C3 activation.

These results, obtained through bioinformatic analyses of Nanopore-generated transcriptomes complemented with publicly available Illumina datasets, highlight a finely tuned and developmentally regulated complement control system in *P. canaliculata*, linking immune regulation to non-canonical roles during development.

Borrowed blueprints: tracing the emergence of hyaluronic acid in invertebrates

U Rosani[1], N Altan[1], P Venier[1], E Bortoletto[1], N Volpi[2], C Bernecky[3]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[3]Institute of Science and Technology Austria, Vienna, Austria

Hyaluronic acid (HA) is a key extracellular matrix component of vertebrates, where it mediates cell adhesion, immune regulation, and tissue remodeling through its interaction with specific receptors containing a structurally conserved *Extracellular link protein* (XLINK) domain. Although HA has been detected in a few invertebrate species, the lack of fundamental components of the HA molecular pathway poses relevant objections about its genuine presence and functional role in these species.

Using biochemical approaches to quantify HA we were able to reveal the presence of HA in several tissues of *Mytilus galloprovincialis*, definitively supporting the presence of HA in an invertebrate.

Moreover, we explored the distribution of XLINK genes beyond chordates, revealing hits in the genomes of anthozoans, bivalves, a tardigrade, an annelid, and an arthropod species, as well as in 15 bacteria, 12 viruses, and one archaeon. The predicted structures further showed that these XLINK domains are likely topologically similar to known HA binding domains, suggesting that invertebrate XLINK domains may indeed function as HA receptors.

Focusing on bivalves, we revealed that Mytilidae species encoded two XLINK gene loci, likely originating from an ancestral eukaryote-to-eukaryote horizontal gene transfer. These two were found to be active in developmental stages of three mussel species.

In conclusion, the presence of both HA and of an active gene with the potential to bind this molecule suggests that mussels, taken as an example of the few invertebrates encoding this gene, have likely acquired the ability to synthesize and to use HA from a protochordate ancestor.

First insights into the repertoire of secretory lectins in Ctenophora

DE Nerelli[1], Y Ozeki[2], H Fujita[3], Y Fujii[3], M Gerdol[1]
[1]Università degli studi di Trieste, Trieste, Italy
[2]Graduate School of NanoBio Sciences, Yokohama City University, Yokohama, Japan
[3]Graduate School of Pharmaceutical Sciences, Nagasaki International University, Sasebo, Japan

Ctenophora (comb jellies) are an ancient phylum of metazoans whose position in the tree of life, as the second-earliest branching animal lineage after Porifera, is fundamental for understanding the evolution of multicellularity. Despite their importance for evolutionary biology studies, ctenophores have so far been largely overlooked in immunological investigations. In this study, transcriptomic assemblies were generated for four species of comb jellies, namely *Bolinopsis mikado*, *Ocyropsis fusca*, *Coeloplana willeyi* and *Beroe mitrata*, in order to detect the presence of expressed transcripts encoding secreted proteins with putative lectin functions, characterized by the presence of one or more carbohydrate-recognition domains. Determining the families of lectin-like molecules present in this basal animal lineage has the potential to clarify the timeline of emergence of self/non-self recognition molecular systems commonly used across different animal phyla. The results, also confirmed at the genomic level in *B. mikado,* revealed C-type lectins as the most widespread and diversified group, with several proteins in which the domain was tandemly duplicated or associated with an immunoglobulin domain. Also noteworthy is the presence of galectins which, unlike those found in most metazoans, possess a signal peptide for canonical secretion, as well as several beta-trefoil (R-type) lectins. On the other hand, although several domains used for pathogen recognition in other invertebrates (e.g. C1q FreD, H-type, etc.) are completely absent, the presence of some lectins that are unusual in animals was detected, characterized by F-type and B-type domains.

Characterization of the interleukin-17 gene family in the gastropod *Pomacea canaliculata*

S Sacchi[1], A Ferri[1], C Losi[2], N Franchi[1], U Rosani[3], D Malagoli[1,4]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Italy
[3]Department of Biology, University of Padua, Padua, Italy
[4]NBFC, National Biodiversity Future Center, Palermo, Italy

Interleukin-17 (IL-17) represents a family of cytokines conserved across metazoans involved in neuroimmune interactions. Although IL17 have been functionally characterized in some bivalve species, their molecular interactions and functional roles remain poorly investigate in mollusks.

Pomacea canaliculata represents a valuable model for comparative neuro-immunobiology studies and several studies have investigated the expression of neuroinflammatory genes, such as allograft inflammatory factor-1 (*Pc*AIF-1) often associated with IL-17 expression in mollusks. However, little is known about IL-17 gene diversity, tissue-specific expression or potential roles in the nervous system of *P. canaliculata*.

Here, genome analysis combined with conserved domain searches was used to identify 11 IL-17 genes in the *P. canaliculata* genome. Transcriptomic profiling across multiple organs revealed distinct tissue-specific expression patterns. Notably, *Pc*IL17n was exclusively expressed in the ganglia and lacked a signal peptide, suggesting a non-canonical, potentially intracellular role. In contrast, *Pc*IL17a and *Pc*IL17b were expressed in most of the other tissues. *Pc*IL17a was the most expressed isoform and contained a signal peptide, while *Pc*IL17b showed lower expression and lacked a signal peptide. Expression of these three *Pc*IL17 sequences in ganglia and in different organs was confirmed by RT-PCR. Moreover, *Pc*IL17n expression was analyzed in nervous system of *P. canaliculata* juveniles.

In contrast to previous evidence on *Pc*AIF-1, RNA sequencing during tentacle regeneration did not reveal significant modulation of IL-17 isoforms. This suggests that IL-17 signaling is not directly linked to the regenerative process in *P. canaliculata* ganglia but may instead contribute to neural homeostasis. Overall, these findings provide the first comprehensive characterization of the IL-17 family in *P. canaliculata* and support the evolutionary diversification of IL-17 functions at the interface between the nervous and immune systems. Acknowledgements: This work was supported by National Recovery and Resilience Plan (NRRP), Mission 4,CUP E93C22001090001, Project title “National Biodiversity Future Center-NBFC

MAIN LECTURE

Symbiotic microbes and mosquito vectors: impact on vector biology and disease control

G Favia
School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy;

Mosquito-borne diseases impose a major burden on human and animal health, with a persistent risk of emerging and re-emerging epidemics. Current control strategies, including vaccines and pharmacological treatments, are often limited in availability or effectiveness. In this context, increasing attention has been directed toward the mosquito microbiota as a promising avenue for innovative disease control. Recent advances have highlighted the critical role of microbial communities in shaping mosquito vector competence. Both direct and indirect effects of microbiota components on pathogen transmission have been demonstrated, opening new perspectives for intervention. In particular, symbiotic bacteria such as *Wolbachia*, *Asaia*, and *Serratia* have shown considerable potential in reducing pathogen transmission and are already being explored in applied control strategies. Beyond their role in pathogen interactions, components of the mosquito microbiota have also been implicated in processes such as insecticide resistance and even host speciation, suggesting a broader influence on vector biology and evolution. A deeper understanding of microbiota–insect vector interactions will not only advance fundamental knowledge of insect biology but also provide a

foundation for the development of innovative, sustainable approaches to control mosquito-borne diseases. Acknowledgements: This work was supported by Miur-PRIN-PNRR 2022 (J53D23013770001)

Session III. Immunity and Environmental Adaptations: Immunological Plasticity in Extreme Habitats. Chair: Maria Rosaria Coscia, CNR, Naples, Italy and Simona Picchietti, University of Tuscia, Viterbo, Italy

Novel case of infection by Syndiniales parasite in Antarctic amphipoda *Orchomenella rinamontiae*

C Bidoli[1], S Greco[1], H Small[2], PG Giulianini[1], M Gerdol[1]
[1]Department of Life Sciences, University of Trieste, Trieste, Italy
[2]Virginia Institute of Marine Science, Gloucester Point, VA, United States

Parasitic dinoflagellates of the order Syndiniales are ubiquitous marine pathogens, known to infect decapod crustaceans worldwide. Thus, these parasites represent a significant threat to the marine ecosystem dynamics and the aquaculture industries. While Syndiniales have been reported in the Southern Ocean, infections in Antarctic amphipods remain undocumented. This study investigated the presence of parasitic infection in Antarctic amphipod *Orchomenella rinamontiae*, using a transcriptomic approach.

Nine individuals sampled in Terra Nova Bay (Ross Sea) underwent total RNA extraction and sequencing. Transcriptome assembly was performed using the Oyster River Protocol multi-assembler. Preliminary analyses revealed a substantial contamination of not-crustacean transcripts, which was later identified as a potential parasite infection. Taxonomic identification of a putative parasitic 18S rRNA transcript was conducted using BLAST and phylogenetic inference.

To discriminate host and parasite transcripts, orthology-based approaches, including BLAST and Orthofinder, were applied. Differential gene expression analysis was subsequently conducted to identify host genes potentially affected by parasite infection and involved in host immune response.

The assembly yielded 247924 contigs, including 14082 protein-coding sequences, identifying 3458 *O.rinamontiae* genes and 5560 parasite genes. Four individuals exhibited high infection levels, with parasite reads accounting for 5%-40% of the total reads. Phylogenetic analyses placed the parasite between *Syndinium* and *Ichtyodinium* clades, suggesting a previously undescribed Syndiniales lineage.

Overall, this study provides first evidence of a Syndiniales parasite infecting Antarctic amphipods, offering new insights into host-parasite interactions and immune mechanisms in *O.rinamontiae*. Acknowledgements: This work was supported by the Programma Nazionale di Ricerche in Antartide (PNRA0000059, PNRA0000016)

Epigenetic plasticity of the polymeric immunoglobulin receptor gene in an Antarctic teleost under simulated marine heatwaves

A Ametrano[1], E Piva[2], G Santovito[2], MR Coscia[1]
[1]Institute of Biochemistry and Cell Biology, National Research Council of Italy, Naples, Italy
[2]Department of Biology, University of Padova, Padova, Italy

Antarctic teleosts are extreme stenotherms whose immune systems have evolved to function under remarkably constant sub-zero temperatures. As marine heatwaves become more frequent, understanding the molecular bases of their immune plasticity is therefore essential for predicting their resilience in a warming Southern Ocean. We have previously shown in an Antarctic fish species that the gene encoding the polymeric immunoglobulin receptor (*pIgR*), a key mediator of mucosal immunity, carries distinct epigenetic marks and exhibits tissue-specific DNA methylation patterns under physiological conditions. In particular, the *pIgR* gene promoter contains a highly methylated CpG island whose regulatory relevance in cold-adapted species remains unexplored. Based on these findings, the present study investigates whether CpG promoter methylation profiles change in response to experimentally simulated heatwave conditions in Antarctic fish. Using the Antarctic teleost *Trematomus bernacchii* as a model species, we combined DNA methylation analyses with gene-expression profiling in the spleen and liver. Our results reveal temperature-dependent changes in promoter methylation levels, accompanied by differential modulation of *pIgR* expression in the spleen and liver. These observations suggest that DNA methylation contributes to short-term immune plasticity during heat stress. Acknowledgements: This work was supported by the Programma Nazionale di Ricerche in Antartide (PNRA0000059)

Oxidative Stress Signatures of Chemical Pollution in the Antarctic Scallop, *Adamussium colbecki*

S Kholdihaghighi[1,2], E Piva[1], S Pacchini[1], M Cortese[1], M Gerdol[3], D Pellegrino[4], P Irato[1], G Santovito[1]
[1]Department of Biology, University of Padova, Padova, Italy
[2]Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy
[3]Department of Life Sciences, University of Trieste, Trieste, Italy
[4]Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy

Chemical pollutants, which are transported to polar regions by long-range atmospheric and oceanic circulation, are increasingly affecting Antarctic marine organisms. Lead (Pb) and bisphenol A (BPA) represent two of these that pose new and ongoing risks, but little is known about how they influence Antarctic organisms physiologically. To examine tissue-specific oxidative stress

responses and the underlying defense mechanisms, the Antarctic scallop *Adamussium colbecki* was exposed to BPA and Pb at two environmentally relevant concentrations. The digestive gland and gills, two significant target tissues for pollutant uptake and metabolism, have undergone biochemical and molecular analyses. Oxidative damage biomarkers, including lipid peroxidation and advanced oxidation protein products, antioxidant enzyme activities (catalase, superoxide dismutase, and selenium dependent glutathione peroxidase) were measured. Furthermore, transcriptional modulation of genes involved in redox homeostasis and antioxidant defense was evaluated. Although there were variations in the trends of biochemical and transcriptional responses between treatments and tissues, both pollutants triggered a notable oxidative imbalance. Changes in oxidative damage markers, redox-related gene expression, and antioxidant enzyme activity reveal that BPA and Pb disrupt cellular homeostasis through distinct yet convergent oxidative stress pathways. These results highlight *A. colbecki*'s susceptibility to chemically induced stress. Overall, this study emphasises potential ecological risks for polar marine ecosystems under ongoing global change by providing integrated biochemical and molecular evidence that chemically diverse pollutants can disturb Antarctic bivalve physiology through oxidative stress-mediated processes. Acknowledgements: This work was funded by the Italian National Program for Antarctic Research (PNRA), project No. 2018/B2Z1.01

Molecular and cellular bases of immune and stress adaptation in the Antarctic Sea anemone *Urticinopsis antarctica*

C La Corte[1], G Scapigliati[2], M Gerdol[3], L Bisanti[1], M Dara[1], M Cammarata[1], MG Parisi Maria[1]
[1]Marine Immunobiology Laboratory, Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Palermo, Italy
[2]Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Viterbo, Italy
[3]Department of Life Sciences, University of Trieste, Trieste, Italy

Antarctic sea anemones are key benthic predators, yet the molecular bases of their adaptation to polar conditions remain largely unexplored. *Urticinopsis antarctica* is among the most common Antarctic actiniarians, but until now it has lacked an integrated molecular and physiological description. This study presents the first comprehensive characterization of this species by combining *de novo* transcriptome sequencing with histological and cytological analyses to examine immune function and stress-response mechanisms in this azooxanthellate anthozoan. Functional annotation based on Gene Ontology and Pfam domains revealed a diverse and highly conserved innate immune toolkit, including components of the Toll-like receptor, NF-κB, JAK-STAT and NOD-like receptor signalling pathways, as well as expanded families of pattern-recognition domains such as TIR, NACHT, LRR and C-type lectins. Coupling transcriptomic data with signal peptide prediction showed a strong enrichment of immune- and stress-associated genes within the predicted secretome, highlighting a predominant role for extracellular defence. Histological observations identified a typical trilaminar body plan with numerous cnidocytes, mucous cells and migratory amoeboid cells, supporting the presence of an epithelial-based immune system. The lack of algal symbionts was confirmed at both molecular and tissue levels. Overall, our results demonstrate that *U. antarctica* possesses a complex and adaptable immune and stress-response framework despite the stable, resource-limited Antarctic environment. This work establishes a critical molecular and cellular baseline, positioning *U. antarctica* as a valuable model for studying cold adaptation, resilience, and the evolution of immunity in polar anthozoans, and for assessing benthic responses to future environmental change. Acknowledgements: This work was supported by the Programma Nazionale di Ricerche in Antartide (PNRA22-0000059; DIMANT)

Life in the dark: molecular adaptations of the cave shrimp *Troglocaris planinensis*

S Radonic, M Gerdol
Department of Life Sciences, University of Trieste, Trieste, Italy

The absence of light in subterranean environments constitutes an extreme condition, precluding visual perception and photosynthetic energy acquisition. The most common traits observed in cave species are regression of eyes and depigmentation, often resulting from convergent evolutionary events. Although the production of pigments for protection from UV light is no longer necessary in cave habitats, pigmentation pathways can have pleiotropic roles, as in the case of melanization in arthropods. In this context, melanin synthesis is important in the immune system response, such as the encapsulation of pathogens, wound healing, clot formation and production of cytotoxic intermediates that kill pathogens. Prophenoloxidase, a key enzyme in arthropod immune melanization, is retained in several cave species, allowing wound melanization. However, previous studies showed that the genus *Troglocaris* is unable to produce melanin even in response to tissue damage, suggesting the loss of this ancestral trait, common to all arthropods. In this study, we generated combined genomic and transcriptomic resources for *T. planinensis*, with the aim of identifying putative gene loss or pseudogenization events linked with adaptation to darkness. In particular, we found loss of function in genes related to the phototransduction pathway (opsins and arrestins) and to the repair of UV-induced DNA damage (deoxyribodipyrimidine photolyase). Despite the apparent loss of melanization in *T. planinensis*, our investigations allowed the recovery of expressed sequences orthologous to prophenoloxidase and other key components of the melanization cascade previously described in other

crustaceans. Therefore, the molecular factors underpinning depigmentation in this species remain to be fully elucidated.

Oxidative imbalance induced by Bisphenol A in *Trematomus bernacchii*: evidence from brain and liver

E Piva[1], F Baroni[1], S Pacchini[1], S Kholdihaghighi[1,2], M Cortese[1], P Irato[1], D Pellegrino[3], G Santovito[1]
[1]Department of Biology, University of Padua, Padua, Italy
[2]Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Venice, Italy
[3]Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy

In the Antarctic ecosystem, emerging contaminants, transported through long-range atmospheric and oceanic currents, are increasingly being detected. This poses a serious threat to the native fauna, which evolved under exceptionally stable and pristine conditions for millions of years. Among the most widespread, bisphenol A (BPA) is of particular concern due to its endocrine-disrupting properties, yet its toxicological effects on polar species remain unexplored. In this study, we show that exposure to BPA at 25 µg/L disrupts redox homeostasis in the emerald rockcod, *Trematomus bernacchii*, causing organ-specific alterations in antioxidant defences. Biochemical and transcriptional analyses revealed that catalase (CAT) activity and expression were significantly suppressed in the liver. At the same time, genes encoding selenoprotein glutathione peroxidases (Se-GPXs) and peroxiredoxins (Prdxs) were upregulated, indicating possible compensatory responses and partial tissue plasticity. In sharp contrast, the brain exhibited pronounced vulnerability, with marked inhibition of superoxide dismutase (SOD) and Se-GPX activities, accompanied by general downregulation of antioxidant genes, reflecting impaired neural protection against reactive oxygen species. These findings confirm that BPA, due to its ability to cross the blood–brain barrier, can weaken neural cellular defences in Antarctic fish. By linking molecular and enzymatic disruptions to potential ecological consequences, this study shows that BPA poses a significant toxic threat to key Antarctic fish species. Moreover, contaminant-induced oxidative disruption is a significant component of the multiple stressors that threaten polar fish in the context of climate change. Future studies will extend this work by investigating intestinal responses to the aim of opening new perspectives on *T. bernacchii* under contaminant exposure. Acknowledgements: This work was funded by the Italian National Program for Antarctic Research (PNRA), project No. 2018/B2Z1.01

The polar ciliate, *Euplotes euryhalinus*, uses recycled canthaxanthin to withstand UV and oxidative damage

C Alimenti[1], G Guella G[2], P Luporini[1], A Vallesi[1]
[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy
[2]Department of Physics, University of Trento, Trento, Italy

Species of the ciliate *Euplotes* are common components of polar marine microbial communities, as they have independently evolved very effective adaptive mechanisms to face unique environmental conditions. Cells of one of these polar species, *E. euryhalinus*, isolated from Antarctic seashore pools that are particularly exposed to rapid variations in salinity, oxygen concentration and UV light radiation- attracted the most attention for an unusual bright orange pigmentation of their cytoplasm. The pigment molecule was purified and structurally characterized as canthaxanthin, a keto-carotenoid that is commonly synthesized by bacteria, algae and fungi, and well known for potent antioxidant and anti-inflammatory properties. No enzyme specific to canthaxanthin biosynthesis has been identified in *E. euryhalinus* transcriptomes implying that *E. euryhalinus* cells, which largely depend on green microalgae as food source, acquire the pigment from the diet. Exposed to strong oxidative and UV radiation stresses, *E. euryhalinus* cells answered with unmatched levels of resilience. It thus appears likely that their successful adaptation to live in polar seashore pools critically relies on filling the cytoplasm with granules of recycled canthaxanthin. This *E. euryhalinus* behavior is quite eccentric with respect to the generality of *Euplotes* species which regularly scatter canthaxanthin as a waste product. Acknowledgements: This work was supported by the Programma Nazionale di Ricerche in Antartide (PNRA0000059)