



BOOK OF ABSTRACTS

SETAC Italian Language Branch

5th WORKSHOP | Chioggia

Edited by:

Valerio Matozzo, Marco Munari, Federica Cacciatore, Silvia Franzellitti,
Giovanni Libralato, Stefania Marcheggiani

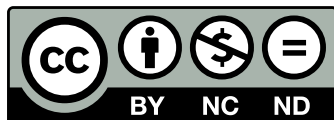
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ABSTRACT BOOK

SETAC Italian Language Branch

5th WORKSHOP | CHIOGGIA

28
29 September
2026



UNIVERSITÀ DI PADOVA
Dipartimento
di Biologia



Edited by:

**Valerio Matozzo, Marco Munari, Federica Cacciatore, Silvia Franzellitti,
Giovanni Libralato, Stefania Marcheggiani**

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SETAC Italian Language Branch

5th WORKSHOP | CHIOGGIA

28-29 September 2026

Palazzo Grassi • Chioggia (Venezia)



COMMITTEES

SCIENTIFIC AND ORGANIZING COMMITTEES

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 Vice-President: Giovanni **Libralato (UNINA)**,
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 Paulina **Perera Wirz (UAlg)**, Federica **Oselladore (ISPRA)**,
 Silvia Giorgia **Signorini (UNIMI-SZN)**.



SCIENTIFIC PROGRAM

28TH SEPTEMBER

12.30-13.00

REGISTRATION

13.00-13.20 Welcome greetings from the Director of DiBIO University of Padua, Prof. Luigi **Bubacco**,

Prof. Valerio **Matozzo**, University of Padova,

Prof. Sandrine **Andres**, President SETAC Europe

Opening of the workshop by Dr. Stefania **Marcheggiani** (Istituto Superiore di Sanità, President of SETAC ILB) and Prof. Giovanni **Libralato** (University of Naples Federico II, Vice President of SETAC ILB).

13.20-15.10

SESSION A - MEDITERRANEAN CLIMATE REGION FROM BIODIVERSITY TO HUMAN HEALTH

Chairs: Rossella **Boscolo Brusà** (ISPRA) and Ilaria **D'Aniello** (UNIPD-SZN).

13.20-13.30 Arianna **Bellingeri** (UNISI), Vulnerability of a Mediterranean Coral adult and larvae to Nanoplastics and Gadolinium.

13.30-13.40 Claudio **Bortot** (UNIPD), Blue crab in the North Adriatic Sea lagoons: extreme conditions and physiology of an invasive species.

13.40-13.50 Federica **Cacciatore** (ISPRA), Salinization trends and effects on macrozoobenthos community dynamics in Northern Adriatic transitional waters.

13.50-14.00 Silvia Giorgia **Signorini** (UNIMI-SZN), Can natural CO₂ vent systems provide generalizable insights into responses to Ocean Acidification? Two case studies from the Mediterranean Sea.

14.00-14.10 Mariachiara **Galati** (UNIME), Between vulnerability and resilience: "thermal priming" in *Mytilus galloprovincialis* as a strategy for climate change.

14.10-14.20 Andrea **Gaion** (ISPRA), Acidification as a toxicity amplifier: a ten-year causal analysis of climate-metal interactions in Mediterranean mussels.

14.20-14.30 Marco **Munari** (SZN), Investigating Ocean Acidification effects on contaminant dynamics and One Health risks at volcanic CO₂ vents.

14.30-14.40 Filippo **Drigo** (UNIPD), Heatwave responses in native and allochthonous *Sporobolus*: a multi-disciplinary perspective.

14.40-14.50 Lea **Di Massa** (UNINA), Environmental Safety and Ecotoxicological Assessment of Bioactive Extracts Derived from *Thymus serpyllum* L.

14.50-15.10 Open Debate

15.10-15.30 Digital Poster Session

15.30-16.20

SESSION: BEST YOUNG ITALIAN SCIENTIST AWARD

Chairs: Silvia **Franzellitti** (UNIBO), Camilla **Puccinelli** (ISS) and Antonio **Morgillo** (UNIS-SZN).

Candidates: Arnosti, Calattini, Guadagnin, Cortese, Kholidhaghighi, Lazzari, Maganza, Martinisi, Magnabosco, Mongera, Sgariboldi, Soriano.

16.20-17.10

SESSION B - REGULATORY ASPECTS OF ENVIRONMENTAL POLLUTION

Chairs: Roberta **Pedrazzani** (UNIBS) and Jacopo **Fabrello** (UNIPD)

16.20-16.30 Paolo **Rossini** (ISPRA), Chemical risk management in Europe: from end-of-pipe control to Safe and Sustainable by Design.

16.30-16.40 Emanuela **Pace** (ISPRA), Regulatory Challenges of PFAS Pesticides: Evidence from Italian Monitoring and Implications for Integrated Environmental Policy.

16.40-16.50 Giuseppe **Granato** (ISPRA), Environmental Risk Management of Hexavalent Chromium under REACH: From Authorisation to Restriction.

16.50-17.00 Daniele **Zatta** (UNIPD), Optimization of an Isotope Dilution GC-MS Method for PFAS Analysis in Water.

17.00-17.10 Open Debate

17.10-19.00

SESSION C1 - EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

Chairs: Valerio **Matozzo** (UNIPD-SZN) and Silvia Giorgia **Signorini** (UNIMI-SZN)

17.10-17.20 Giada **Caorsi** (UNIMI), Additives Matter: A Multi-tier Ecotoxicological Assessment of Commercial PVA-based Dishwasher Pods on *Danio rerio* Embryos.

17.20-17.30 Giuseppe **De Marco** (UNIME), Biological alterations, caused in *Mytilus galloprovincialis* by pharmaceutical active compounds, are partially attenuated by eco-friendly treatment with ulvans.

17.30-17.40 Cristina **Cremonesi** (UNIMI), Ecotoxicological Effects of Polymers Used in Wastewater Treatment Plants on *Daphnia magna*.

17.40-17.50 Ifra **Ferheen** (ISS), Italian case study of the BlueAdapt H2020 project: Health Risk Assessment of Antibiotic-Resistant Bacteria Spread into a Surface Water.

17.50-18.00 Giovanni **Cilia** (CREA), Use of a National Network of *Apis mellifera* L. Colonies for Environmental Surveillance of Antimicrobial Resistance Genes (ARGs).

18.00-18.10 Alexandra **Dal Lago** (UNIBO), Survival of *Apis mellifera ligustica* (Spinola, 1806) Under Combined Exposure to Thermal Stress, Humidity Variation, and Microplastic Ingestion.

18.10-18.20 Letizia **Iuffrida** (UNIBO), The Northwestern Adriatic Sea as a Dynamic Laboratory to Explore Multiple Stressor Effect on Marine Bivalves.

18.20-18.30 Marika **Bertoni** (SZN), Macroalgal habitat shapes physiological responses of canopy associated invertebrates exposed to legacy and emerging contaminants.

18.30-18.40 Paulina **Perera Wirz** (UNI Algarve), From Blow to Biomarkers: Small RNA Sequencing of Drone-Collected Cetacean Blow for Non-Invasive Biomarker Discovery.

18.40-19.00 Open Debate

19.00-20.00 DIGITAL POSTER SESSION AND FREE SCIENTIFIC APERITIVE

29TH SEPTEMBER

9.00-11.00

SESSION C2 - EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

Chairs: Marco **Munari** (SZN) and Monica **Colli** (Biotechnologie BT Srl).

9.00-9.10 Laura **Giovanetti** (UNISI), A multi-biomarker approach to evaluate the ecotoxicological status of great tit (*Parus major*) populations from areas with different anthropogenic impacts.

9.10-9.20. Lorenzo **Federico** (UNIMIB), From individuals to communities: soil arthropod responses to natural geogenic CO₂ emissions in a mofette.

9.20-9.30 Hanane **Maghrebi (UNIPD)**, Food-relevant exposure to okadaic acid triggers early transcriptomic responses in complementary porcine and human intestinal models.

9.30-9.40 Chiara **Marulli (UNI Stavanger)**, Linking genomic insights and experimental evidence to understand seaweed resilience to environmental changes.

9.40-9.50 Antonio **Morgillo (UNISI-SZN)**, Multilevel responses to emerging contaminants in the tunicate *Ciona intestinalis*: a comparative perspective.

9.50-10.00 **Michela Panni (UNIVPM)**, Ecotoxicological effects of the antidepressant sertraline in different non-target marine species.

10.00-10.10 Lara **Nigro (UNIMIB)**, Species-Specific Sensitivity to PFCA's and Chronic Effects in Cucumber: From Early Root Responses to Plant-Level Impairment.

10.10-10.20 Sara **Pacchini (UNIPD)**, Surviving contamination: energetic trade-off and redox disruption in PFAS-chronically exposed fish (*Squalius cephalus*).

10.20-10.30 Alessio **Marafini (UNIROMA1)**, A Combined Risk-LCA Approach for the PFAS REACH Restriction: A Case Study for Safe and Sustainable by Design.

10.30-10.40 Alessandro **Nardi (UNIVPM)**, From environmental occurrence to biological responses: a multi-level assessment of PFAS across Mediterranean waters, biota and toxicological models.

10.40-11.00 Open Debate

11.00-11.30 Digital POSTER Presentation and Coffee break

11.30-12.30

SESSION C3 - EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

Chairs: Giovanni **Libralato (UNINA)** and Tiziana **Cappello (UNIME)**.

11.30-11.40 Stefania **Pezzana (UNIMI)**, Micro- and Nanoplastics Disrupt Autophagy-Lysosome and Mitochondrial Homeostasis in Primary Human Monocytes.

11.40-11.50 Elisabetta **Piva (UNIPD)**, Oxidative stress and the gut-brain axis as emerging tools in environmental physiology.

11.50-12.00 Giacomo **Sabbadin (UNIPD)**, An approach to the study of underwater noise and nanoplastics and their effects on the colonial ascidian *Botryllus schlosseri*.

12.00-12.10 Andrea **Masseroni (UNIMIB)**, Effects of pristine and aged LDPE and PP microplastic leachates on behavioural responses of the soil arthropods *Folsomia candida* and *Porcellionides pruinosus*.

12.10-12.20 Open Debate

12.20-12.30

AWARD CEREMONY FOR THE BEST YOUNG ITALIAN SCIENTIST AND BEST POSTER

12.30-13.00

CLOSING CEREMONY

Wrap-up and closing remarks from Stefania **Marcheggiani (President of SETAC ILB, ISS)** and Giovanni **Libralato (Vice-President of SETAC ILB, UNINA)**.

INTRODUCTION

The SETAC Italian Language Branch (ILB) is one of the regional branches of SETAC Europe. Established about 20 years ago, the ILB promotes the development and application of scientific knowledge in environmental sustainability, ecosystem preservation, and ecosystem integrity. Its activities reflect SETAC mission and values and are based on a multidisciplinary and collaborative approach.

The ILB brings together Italian-speaking members of SETAC Europe living and working in Italy and abroad, including biologists, chemists, engineers, environmental scientists, policymakers, researchers, and other stakeholders. It provides a dynamic platform for scientific exchange, collaboration, and knowledge sharing on emerging and re-emerging issues at the interface between environmental science and human health, with a strong One Health perspective.

At the international level, the ILB actively contributes to the SETAC Europe Annual Meeting, giving its members opportunities to present and discuss scientific and technological advances. At the national level, it promotes dialogue and collaboration among universities, research institutions, industry, public authorities, and other stakeholders, helping to strengthen the connection between scientific evidence and environmental policy and practice.

A key component of the ILB activities is the organisation and support of training courses, conferences, workshops, and scientific networking events, with particular emphasis on students and early-career researchers. These initiatives provide opportunities to exchange ideas, develop professional networks, showcase innovative research, and encourage the development of science-based solutions to current and emerging environmental challenges.

The ILB currently comprises 123 members from universities, research institutions, and industry in Italy and abroad. Its activities include numerous free-of-charge, self-funded events organised through the active involvement of its members. This participatory approach reflects the collaborative, inclusive, and community-driven spirit of SETAC ILB.

Since 2009, the SETAC ILB has progressively expanded its national activities through a series of workshops hosted by leading Italian universities and research institutions:

1st National Workshop – University of Siena, 2022

2nd National Workshop – National Research Council (CNR), Rome, 2023

3rd National Workshop – University of Milan, 2024

4th National Workshop – Department of Biology, University of Naples Federico II, 2025

The 5th National Workshop held in Chioggia (Venice) featured a rich scientific program, with 36 oral presentations and 29 posters covering a broad spectrum of current environmental and health-related challenges. The scientific contributions were organized into three thematic sessions:

- Mediterranean climate region from biodiversity to human health
- Regulatory aspects of environmental pollution
- Exposure and effects of legacy and emerging contaminants, and multiple stressors

The event attracted 130 participants, demonstrating the growing interest in the ILB activities. Participants included representatives from academia, national research institutions and centres, and private companies.

A key component of the workshop was the Best Young Italian Scientist Award, established in 2018, that recognises and encourages outstanding research by early-career Italian scientists. In the 2026 edition, 12 young researchers participated in the competition. A panel of reviewers assessed their work according to SETAC Europe established evaluation criteria, based on both the submitted extended abstracts and a five-minute oral pitch presented during the workshop. The award recipient received free registration for the SETAC Europe 37th Annual Meeting, held in Geneva, Switzerland, 25–29 April 2027, providing an important opportunity to present their research and engage with the international SETAC community.

TABLE OF CONTENTS

Committees	3
Scientific program	4
Introduction	6
Oral presentations	8
Session A - Mediterranean climate region from biodiversity to human health	9
Session B - Regulatory aspects of environmental pollution	13
Session C1 - Exposure and effects of legacy and emerging contaminants, and multiple stressors	15
Session C2 - Exposure and effects of legacy and emerging contaminants, and multiple stressors	20
Session C3 - Exposure and effects of legacy and emerging contaminants, and multiple stressors	24
Best Young Italian Scientist Award	26
Posters	44

ORAL PRESENTATIONS

SESSION A

MEDITERRANEAN CLIMATE REGION FROM BIODIVERSITY TO HUMAN HEALTH

Vulnerability Of A Mediterranean Coral Adult And Larvae To Nanoplastics And Gadolinium

A. Bellingeri^{1,2}, S. Signorini^{2,3}, C. Spagnolo¹, T. Makaras⁴, C. Della Torre^{2,3}, I. Corsi^{1,2}

¹Department of Physical Sciences, Earth and Environment, University of Siena, Siena;

²National Biodiversity Future Centre NBFC;

³Department of Biosciences, University of Milan, Milan;

⁴Nature Research Centre, Vilnius Lithuania.

Corals are fundamental elements of rocky shore coastal ecosystems, which are threatened by several local and global stressors including wastewater and river discharges, tourism and climate change. Among these, the impact of contaminants of emerging concern (CECs) still needs to be thoroughly investigated. The aim of this study was twofold: *i*) monitoring the health status of the Mediterranean zooxanthellate solitary coral *Balanophyllia europaea* in the Tyrrhenian Sea and *ii*) assessing sensitivity to polystyrene nanoparticles (PS NPs, 50 nm) and rare earth elements (Gadolinium, Gd) at low concentrations (10 µg/L - 1 mg/L) on *B. europaea* adults (chronic, 28d) and larvae (acute, 48h, chronic, 28d). The natural population was monitored via mucus microbiome analysis and untargeted whole-organism metabolomics. Bench-studies on PS NPs and Gd ecotoxicity involved coral adults, evaluating mortality, predatory ability, photosynthetic efficiency, zooxanthellae count, pigment density and oxidative stress biomarkers, and coral larvae evaluating behaviour, mortality, metamorphosis and histological alterations. Results showed that both microbiome and metabolic profile were highly influenced by sampling times and environmental conditions. Bench-studies revealed no mortality in either larvae and adults, while acute Gd and PS NPs exposure caused a reversible alteration of larval swimming behaviour at 1 mg/L, and the chronic exposure caused structural depletion of surface cilia for PS NPs and 50% nonsense metamorphosis (metamorphosis without settlement) for Gd. Adults showed a mild reduction in photosynthetic pigments of zooxanthellae after 28 days with no alteration of photosynthetic efficiency and oxidative stress biomarkers. Overall data showed that larval swimming and adult mucus microbiomes provide effective, non-invasive indicators for early toxicity and wild coral health monitoring. Furthermore, these results emphasize the need to consider the vulnerability of early life stages in conservation strategies, highlighting how CECs can alter critical developmental processes in Mediterranean coral species.

Blue Crab In The North Adriatic Sea Lagoons: Extreme Conditions And Physiology Of An Invasive Species

C. Bortot¹, F. Piccardi^{1,2}, G. Guadagnin^{4,3,1}, C. Checchin¹, A. Fabbri^{1,2}, K. Previato¹, A. Duric¹, F. Baldessin^{1,5}, T. Patarnello², L. Peruzza², C. Mazzoldi^{1,4}, A. Barausse^{1,4}

¹Department of Biology (DiBio), University of Padua, via U. Bassi 58/B, 35131, Italy;

²Department of Comparative Biomedicine and Food Science (BCA), University of Padua, Legnaro, Italy;

³Department of Earth and Marine Sciences, Via Archirafi 22, 90123 Palermo, Italy;

⁴National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133, Palermo, Italy;

⁵ARPAV - Regional Agency for Environmental Prevention and Protection of Veneto, Regional Laboratories, Department, Veneto, Italy.

The blue crab *Callinectes sapidus* is a marine decapod crustacean native to the western Atlantic. During the 20th century, its transport through ballast waters allowed it to spread to the Mediterranean Sea, including the coastal areas and the lagoons of the Adriatic Sea, where its invasion is threatening biodiversity and aquaculture. The physiological plasticity and the wide tolerance ranges to temperature and salinity of this species play a key role in its invasiveness. Therefore, understanding its potential niche in these new environments is crucial to predict the future distribution of this species. In fact, there is a lack of physiological analysis related to the populations of Adriatic estuarine areas at summer and winter combined conditions. Since climate change is affecting these hydrological patterns, the ecophysiological effects need to be investigated. Thus, the goal of this study is to assess the response of this species to various temperature and salinity gradients to define its potential ecological niche under seasonal conditions at the extremes of the data series distribution, characteristic of northern Adriatic lagoons fluctuations. We focus on the aerobic oxygen consumption and heart rate frequency of adult crabs of both sexes collected in lagoons of the southern Po Delta. Adult crabs were exposed to nine treatments per season, resulting from the combination of temperatures and salinities, reflecting data recorded in the Venice and Scardovari Lagoons, especially in summer and winter. After a brief acclimation period under these conditions, oxygen consumption and heart rates were recorded. The results show that the oxygen consumption changes according to temperature, salinity and sex. A strong influence of temperature is recorded in both seasons, and its effect changes according to the salinity and sex. Considering the summer, a significant effect of salinity is observed, showing an increased heart rate at the highest temperature and salinity values. On the other hand, during winter a higher aerobic oxygen consumption has been found in females rather than males at the highest temperatures, with the lowest temperature showing a similar metabolism between sexes. This study highlights how the seasonal pattern of temperature and salinity influence the physiology of male and female individuals of *Callinectes sapidus*. These results provide important insights for assessing the potential niche of the population of the Northern Adriatic Sea, predicting its distribution and supporting more effective management strategies.

Salinization Trends And Effects On Macrozoobenthos Community Dynamics In Northern Adriatic Transitional Waters

F. Cacciatore¹, V. Bernarello¹, F. Oselladore¹, M. Cornello¹, M. Novello², A. Girolimetto², M. Lionello², A. Bonometto¹, F. Strazzabosco², R. Boscolo Brusà¹

¹ISPRA – Italian Institute for Environmental Protection and Research, CN-COS/Tides and Lagoons Area, Chioggia – Venice, Italy;

²ARPAV – Environmental Prevention and Protection Agency of Veneto Region, Padova, Italy.

Northern Adriatic lagoons, including the Venice Lagoon and the Po Delta system, represent some of the most extensive and ecologically significant transitional environments in the Mediterranean. These shallow ecosystems are characterized by a dynamic interplay between freshwater and marine inputs, which supports high biological productivity but also renders them uniquely vulnerable to climate-driven and anthropogenic stressors. Among these, salinization is a critical global threat, often misinterpreted as a natural feature despite being a major driver of changes in community composition and structure. Driven by climate change, sea-level rise, reduced freshwater inputs, tidal dynamics, evaporation, and anthropogenic alterations, such as dredging and canalization, rising salinity significantly impacts organism survival, growth, and metabolic rates. Macrozoobenthic communities are essential Biological Quality Elements (BQEs) for assessing ecosystem health under the Water Framework Directive (WFD). As predominantly sedentary organisms with relatively long-life spans, they respond sensitively to environmental shifts and effectively integrate disturbance events over time. Consequently, they serve as robust indicators for assessing the ecological integrity of coastal and transitional waters. This study integrated long-term monitoring data from the Venice Lagoon (2011–2025) and five lagoons of the Po Delta (2008–2025), leveraging the WFD monitoring network managed by ARPAV and ISPRA. In the Venice Lagoon, Seasonal Kendall tests revealed a significant increase in salinity ($p < 0.05$), driving a marinization process that led to progressive homogenization and, combining with other stressors, a marked decline in macrozoobenthic species richness and abundance. Key taxonomic groups, including Bivalvia and Polychaeta, were halved over the decade. BEST/BIOENV analyses confirmed that salinity is a primary variable explaining macrozoobenthic dissimilarities ($p < 0.01$). In the Po Delta, salinity trends appear more heterogeneous; a significant increase was recorded in the Canarin lagoon ($p < 0.01$), while others, such as Barbamarco, exhibited localized decreases ($p < 0.001$). These shifts, compounded by a significant increase in water temperature across the area ($p < 0.01$ in most lagoons), contributed to dramatic fluctuations in benthic density, which collapsed from 7716 ± 10564 ind./m² in 2023 to critical lows of 155 ± 86 ind./m² in 2025. The findings confirm that while macrozoobenthic communities are sensitive multi-scale indicators for detecting impairment, community-level changes often reflect late-stage ecological shifts. To enhance predictive capacity and adaptive management, it is necessary to advance research across biological levels, prioritizing the integration of ecological indices with specific physiological and molecular biomarkers capable of capturing early-stage osmotic stress in Mediterranean transitional waters.

Can Natural CO₂ Vent Systems Provide Generalizable Insights Into Responses To Ocean Acidification? Two Case Studies From The Mediterranean Sea

S.G. Signorini^{1,2,3}, A.A.-T. Weber³, M. Munari⁴, J. Santos³, F. Crocetta^{2,5}, M. Giulio³, A. Meda¹, M. D'Alessandro⁶, M.C. Gambi⁶, C. Cremonesi¹, A. Binelli¹, G. Lungo¹, C. Della Torre^{1,2}

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⁵NBFC, National Biodiversity Future Center, Palermo, Piazza Marina 61, 90133, Palermo, Italy;

⁶National Institute of Oceanography and Applied Geophysics – OGS, Trieste, Via Beirut 2, 34014, Trieste, Italy.

Ocean Acidification (OA) exerts negative effects across marine taxa and particularly on calcifying species due to alterations of seawater carbonate chemistry. Within this context, naturally acidified CO₂ vent systems provide valuable natural laboratories to investigate the effects of OA on organisms exposed to this environmental disturbance for their entire life cycle, allowing to investigate potential tolerance and adaptive strategies. The aim of this study is to evaluate whether these vent systems can be used to define generalizable hypotheses on OA impacts or organismal responses are site-specific and thus dependent on local environmental conditions. To answer this question, specimens of the calcifying gastropod *Patella caerulea* were collected from two CO₂ vent systems of the Mediterranean Sea: the Castello Aragonese vent at Ischia Island (San Pietro/N1: pH = 8.1; N2: pH = 7.7; N3: pH < 7.4) and the San Giorgio vent at Sicily Island (Patti: pH = 8.1; San Giorgio: pH = 7.84). On these samples, we assessed: (i) morphometric parameters (shell length, width, and height, and total weight); (ii) biochemical endpoints related to energy metabolism, oxidative stress, and neurotoxicity; and (iii) population genomics (PCA, FST, π) and epigenomics (DNA methylation) at adult and juvenile stages. Heavy shell corrosion was evident in both CO₂ vent systems. At the Ischia vent we observed a significant increase in shell sizes in acidified conditions, accompanied by a significant increase in energy resources (glycogen and proteins), potentially related to the different algal coverage along the pH gradient. A significant increase in glycogen content was observed also in limpets collected from the Sicily vent, even though no significant differences in shell sizes were found between ambient and low pH sites. A slight imbalance of the oxidative status was assessed at the Sicily vent, while no significant differences were detected at the Ischia one. At the molecular level, contrasting patterns emerged: at Ischia, strong genomic differentiation was detected in adult specimens from acidified sites, while DNA methylation changes were reduced, suggesting selection acting on standing genetic variation through a within generation response. In contrast, organisms from the Sicily vent showed no genomic differentiation, but more pronounced shifts in DNA methylation between ambient and low pH sites, indicating a potential stronger role for epigenetic mechanisms under more moderate pH conditions. Overall, our results suggest that, while natural CO₂ vents are powerful analogues for future ocean scenarios, site-specific environmental variability may play a fundamental role in shaping organismal biological responses.

Between Vulnerability And Resilience: "Thermal Priming" In *Mytilus galloprovincialis* As A Strategy For Climate Change

M. Galati¹, M.A. Oueddi¹, M. Romeo¹, T. Cappello¹, M. Maisano¹

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

The overexploitation of natural resources combined with increased anthropogenic pollution has accelerated climate instability, impacting the degradation of many environments, particularly aquatic ones, and putting their biodiversity at risk. To assess the impact of current heatwaves and the possibility of a mitigation strategy, the effect of "thermal priming" on male and female gonads of the native Mediterranean species *Mytilus galloprovincialis* was investigated. Mussels were purchased from the S.A.Co.M. farm and pre-exposed separately to 28, 30, and 32°C for 2 h, returned to 17±1°C (acclimatization temperature) for 24 h, and then subjected to heat shock (36°C) for 2 h to verify the potential "thermal priming" effect. Furthermore, a negative control group (maintained constantly at 17±1°C) and a positive control group (directly exposed to 36°C) were also included. Histological results obtained by hematoxylin/eosin (H/E) staining demonstrated that direct thermal stress induces asynchronous spawning events with respect to the reproductive period in both sexes. Histochemical data obtained by the modified periodic acid-Schiff reaction (dPAS/PAS), supported by metabolomic data based on proton nuclear magnetic resonance spectrometry (1H NMR), highlighted a sex-specific divergence in stress adaptation related to energy management and a greater sensitivity to redox imbalance in female gonads, as shown by the trend of histidine, a constituent of ovothiol, a scavenger of reactive oxygen species. The May-Grünwald-Giemsa reaction, able to discriminate between haemocyte classes, and the Schmorl's method, used to detect phenoloxidase (an enzyme related to innate immunity), put in evidence an improvement in defence mechanisms following preadaptation. Overall, by estimating that temperatures will increase by 1.8-3.5°C over the course of the century, these results suggest that "thermal priming" may represent a promising strategy for improving the resilience of *Mytilus galloprovincialis*, a species of high importance as food source and as a biodiversity engineer in intertidal ecosystems. This study, by drawing attention to the impact of impending climate change on reproductive health, and therefore on reproductive fitness and survival of the species, highlights the close and vulnerable interconnection between biota, environment, climate, and humans. *This work was supported by the European Union - Next Generation EU, PNRR MUR – RAISE (CUP B73C24000590006) "TEC-SOS" Project.*

Investigating Ocean Acidification Effects On Contaminant Dynamics And One Health Risks At Volcanic CO₂ Vents

M. Munari^{1,2,3}, R. Ciampaglia⁴, J. Fabrello¹, I. D'Aniello^{1,2}, A. Capasso¹, A. Maraglino¹, S. Manzo⁵, S. Schiavo⁵, J. Rimauro⁵, L. Caiazza⁵, M. Simonelli⁴, C. Della Torre^{2,6}, S. G. Signorini^{2,6}, C. De Pittà¹, S. Franzellitti⁷, M. Motalipassi^{2,3}, A. Terlizzi^{2,3}, F. Micheli⁸, C. Knight⁸, T. Burnham⁹, A. Miccio¹⁰, C. Iacono¹⁰, F. Roviezzo⁴, V. Matozzo^{1,11}

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This study investigates how ocean acidification (OA) influences contaminant bioaccumulation and physiological stress in the Mediterranean mussel (*Mytilus galloprovincialis*). Recent research suggests that organisms living in naturally acidified environments experience significant stress, potentially impairing their ability to tolerate environmental contaminants. Leveraging OA gradients around Ischia, mussels were deployed for two months to assess how OA impacts contaminant uptake and toxicological responses. This research also involves quantifying contaminant concentrations in mussel tissues to evaluate potential risks within a One Health framework. Human cell cultures will be exposed to these detected contaminant levels, simulating realistic environmental exposure scenarios. Findings indicate that while acidified conditions can reduce contaminant accumulation, they also heighten physiological stress and mortality in mussels. These results underscore the complex interplay between OA, contaminant dynamics, with broader implications for ecosystem resilience and human health. Understanding the underlying mechanisms and long-term consequences of these interactions remains a critical area for future research.

Heatwave responses in native and allochthonous *Sporobolus*: a multi-disciplinary perspective

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The Venice Lagoon is a transitional environment, highly impacted by anthropogenic activities and global climate change. It is also considered a hot spot for biodiversity and biological invasion. Here, *Sporobolus maritimus* is a native halophyte, currently threatened by the spreading of the non-indigenous species *S. anglicus* and extreme climatic events, like heatwaves, which are reported with increasing frequency in the Venice Lagoon. We exposed *S. maritimus* and *S. anglicus* organisms to a simulated five-day heatwave and assessed the morphological, physiological and biochemical responses. These data were integrated with gene expression and metabolite profiling. To identify differentially expressed genes,

we also generated and annotated a *de novo* transcriptome for *Sporobolus* species. We observed a decline in the survival rate of *S. maritimus*, whereas *S. anglicus* showed no increase in mortality. Consistent with this pattern, morphological (structure of the photosynthetic apparatus), physiological (photosynthetic efficiency and pigment content) and biochemical assays (antioxidant enzyme activity) indicated that the native species is more susceptible to heatwave stress and exhibits limited recovery. In contrast, *S. anglicus* demonstrated greater tolerance and a clear capacity to recover for all analysed parameters. Gene expression analyses further suggest that the response of *S. maritimus* is slower and more severely impacted, whereas *S. anglicus* shows a faster and more effective response, highlighting the greater resilience of the non-indigenous species. Overall, our results indicate that the non-indigenous species *S. anglicus* responds more rapidly to heat stress, limiting damage to the photosynthetic apparatus and coping more effectively with prolonged stress, compared to the native species *S. maritimus*. Taken together, these findings indicate that *S. anglicus* is more resilient to heat stress than *S. maritimus*, raising serious concerns about its persistence in the Venice Lagoon under future climate scenarios and related ecological impacts.

Environmental Safety and Ecotoxicological Assessment of Bioactive Extracts Derived from *Thymus serpyllum* L.

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The identification of eco-safe natural compounds with biological activity represents a relevant challenge in environmental toxicology and sustainable biotechnology. Plant-derived secondary metabolites are increasingly investigated as low-impact alternatives to synthetic antimicrobial and antioxidant agents, whose extensive use may contribute to microbial resistance and environmental burden. In this context, Ischian Piperna (*Thymus serpyllum* L.), an aromatic plant traditionally associated with the island of Ischia, represents a poorly explored source of bioactive compounds. Moreover, Ischian thermal waters, characterized by peculiar physicochemical properties, may influence the biological performance of plant extracts. This study aimed to evaluate the environmental safety, cytotoxicity, antioxidant, antimicrobial and antibiofilm properties of Piperna extracts, alone and in combination with thermal waters, in order to explore their potential application in sustainable microbial control strategies. Piperna extracts were obtained using solvents of different polarities, including petroleum ether, ethyl acetate and ethanol, and were chemically characterized. The extracts dissolved in thermal water were first assessed for environmental toxicity using different bioindicators, including *Vibrio fischeri*, *Artemia salina* and *Mytilus galloprovincialis*. Cytotoxicity was evaluated by MTT assay on human immortalized keratinocytes. Total phenolic content and antioxidant activity were also determined. The biological activity was investigated against a broad panel of biofilm-forming bacteria and yeasts, including *Staphylococcus epidermidis*, *Rhodotorula mucilaginosa*, *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa*, selected as representative microorganisms associated with biotic and abiotic surfaces and as opportunistic pathogens of environmental and health relevance. Minimum Inhibitory Concentration values were determined, and the ability of the extracts to inhibit biofilm formation and to reduce preformed mature biofilms was evaluated in both single-species and mixed-species models. Anti-elastase and anti-collagenase activities were also investigated as indicators of the potential

modulation of biological processes related to enzymatic degradation of organic matrices. No significant adverse effects were observed in the selected ecotoxicological models, indicating a favorable environmental safety profile. Among the tested extracts, the ethanolic extract displayed the highest phenolic content and the most promising biological activity, including antioxidant, antimicrobial and antifungal effects. A marked reduction in biofilm formation and a partial effect on mature biofilms were observed. Interestingly, the combination with thermal waters enhanced the biological activities, suggesting favorable interactions between the mineral matrix and Piperna-derived bioactive extracts. Overall, these findings highlight Ischian Piperna as a promising natural source of eco-compatible bioactive extracts for sustainable biofilm control. Further ecotoxicological studies on non-target organisms and biodegradability will be essential to define its potential environmental and biotechnological applications.

SESSION B

REGULATORY ASPECTS OF ENVIRONMENTAL POLLUTION

Chemical Risk Management In Europe: From End-Of-Pipe Control To Safe And Sustainable By Design

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European chemicals governance is undergoing a structural transition from reactive, end-of-pipe risk management to preventive, design-stage intervention. This work examines the regulatory architecture driving this shift — REACH, the Ecodesign for Sustainable Products Regulation (ESPR), and the Safe and Sustainable by Design (SSbD) framework — and assesses their collective capacity to address the systemic chemical risks associated with the triple planetary crisis (climate change, biodiversity and pollution). An original analysis of EU Safety Gate alert data (2015–2025) was conducted to assess trends in chemical-risk notification across EU-27 Member States. The results show an increase from fewer than 750 alerts per year in the baseline period to 2,441 notifications in 2025, with Beauty and Personal Care products and Toys and Games accounting for the largest shares. This upward trend combined with the analytical limits of traditional customs screening, suggested that reactive monitoring — while necessary — may be structurally insufficient. A complementary case study on PFAS pesticides in Italian water bodies further illustrates the monitoring gaps and regulatory fragmentation inherent in substance-by-substance, end-of-pipe approaches. Against this evidence, the study argues that the integration of Risk Assessment and Life Cycle Assessment (LCRA) — the methodological core of the SSbD framework — provides the scientifically and regulatorily coherent pathway to genuinely cleaner processes. Key operational recommendations include the use of non-compensatory RA-LCA criteria in innovation governance; the application of simplified LCAs and in silico screening methods at the earliest Technology Readiness Levels; and the systematic inclusion of Substances of Concern mapping in Product Category Rules and Environmental Product Declarations; strengthening supply-chain data quality under FAIR principles (Findable, Accessible, Interoperable and Reusable). The study situates SSbD within the broader sustainability governance context, highlighting its potential to support more integrated approaches linking chemical safety, environmental protection, and life-cycle-based sustainability assessment in future policy frameworks.

Regulatory Challenges of PFAS Pesticides: Evidence from Italian Monitoring and Implications for Integrated Environmental Policy

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Per- and polyfluoroalkyl substances (PFAS) used as active ingredients in plant protection products (PPPs) represent a growing regulatory challenge due to their intrinsic extreme persistence, together with a variety of other properties as mobility, potential for bioaccumulation and (eco)-toxicity. This study investigates the occurrence, environmental fate, and regulatory status of PFAS pesticides in Italy, with particular attention to their contribution to water contamination and the inconsistencies within the current European regulatory framework. Based on the definition adopted in the European Chemicals Agency (ECHA) proposal for a universal PFAS restriction, 46 PFAS active substances have been identified in PPPs, 29 of which are still approved in the European Union. These substances are deliberately applied to agricultural soils, increasing the likelihood of their dispersion across environmental compartments. Analysis of Italian sales data shows a 39% increase in PFAS pesticide use between 2018 and 2023, reaching approximately 632 tons annually, indicating a significant and growing environmental pressure.

Water monitoring data collected within the Italian National System for Environmental Protection (SNPA) reveal widespread contamination. Between 2017 and 2021, 14–20 PFAS pesticides were detected in surface and groundwater, corresponding to up to 43% of the substances considered. However, this contamination is likely underestimated, as current monitoring programs include only a subset of PFAS compounds and generally exclude metabolites and co-formulants. Moreover, the regulatory threshold of 0.1 µg/L applied to pesticides does not account for the specific toxicological and environmental properties of PFAS. The study highlights a major regulatory inconsistency: while the proposed universal PFAS restriction under REACH emphasizes persistence as a key criterion for risk management, pesticide authorization remains based on substance-by-substance risk assessment. This divergence can result in the continued approval of PFAS pesticides despite their contribution to widespread, cumulative contamination. In line with the “One Substance One Assessment” (OSOA) approach, the findings underscore the need for a more harmonized regulatory strategy that consistently addresses PFAS across sectors. Strengthening monitoring frameworks, integrating metabolites into assessments, and prioritizing persistence in regulatory decision-making are essential steps toward reducing environmental and human health risks associated with PFAS pesticides.

Environmental Risk Management of Hexavalent Chromium under REACH: From Authorisation to Restriction

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Hexavalent chromium (Cr(VI)) is among the most hazardous substances regulated under the European Union's REACH Regulation due to its carcinogenicity, reproductive toxicity, and potential for widespread environmental contamination. Historically managed through the Authorisation process (Annex XIV), a Restriction proposal (Annex XVII) for Cr(VI) substances is currently under evaluation to establish a more harmonised framework for industrial uses, such as electroplating and metal surface treatment. This transition aims to ensure consistent environmental protection across Member States while reducing regulatory complexity. Environmental fate and behaviour are assessed focusing on emission sources, pathways, and risk management measures using regulatory evidence, fate data, and proposed emission limit values (ELVs). Industrial activities within the scope of Restriction proposal are estimated to release approximately 18.8 tonnes of Cr(VI) per year across the EU, distributed between air (5.6 tonnes/year) and water (13.2 tonnes/year). To address the heterogeneous emission profile across diverse installations, the proposal introduces risk-based ELVs complemented by monitoring requirements under maximum production capacities. This ensures that regulatory control captures temporal variability and potential peak release scenarios not reflected in average conditions. Following environmental release, Cr(VI) may undergo reduction to trivalent chromium (Cr(III)) in soil and aquatic compartments. Although this decreases toxicity and mobility, it does not eliminate ecological relevance; Cr(III) may still interact with suspended particulate matter and biota, contributing to adverse effects under specific physico-chemical conditions. Thus, risk assessment requires understanding chromium speciation dynamics rather than assuming that total reduction eliminates hazard. Overall, the transition from Authorisation to Restriction represents a substantive regulatory shift under REACH towards a more streamlined, enforceable, and

exposure-driven framework. This proposed system integrates emission control, life-cycle considerations, and a strict prohibition of direct soil releases to reduce environmental loading, improve ecosystem protection, and safeguard human health at the EU level. Importantly, by addressing emissions across the entire life cycle, the proposal strengthens management requirements for chromium-containing waste streams, creating a highly coherent and protective regulatory system for substances of very high concern (SVHCs). Towards a more streamlined, enforceable, and exposure-driven framework, aimed at reducing environmental burdens and improving the protection of ecosystems and human health at the EU level.

Optimization of an Isotope Dilution GC-MS Method for PFAS Analysis in Water

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Per- and polyfluoroalkyl substances (PFAS) are a large class of persistent organofluorine compounds widely used in industrial and consumer applications. Due to their extreme environmental persistence, and documented adverse health effects, PFAS are subject to increasingly stringent international regulations. In the European Union, Directive (EU) 2020/2184 establishes parametric values for PFAS in drinking water, including limits for PFAS Total (0.50 µg/L) and Sum of PFAS (0.10 µg/L), while some Member States, such as Italy, have implemented even stricter requirements, assigning 0.02 µg/L to the sum of PFOA, PFOS, PFNA and PFHxS. Liquid chromatography – tandem mass spectrometry (LC-MS/MS) is currently the reference technique for PFAS determination in water [3]. However, gas chromatography – mass spectrometry (GC-MS), when combined with appropriate derivatisation strategies, could represent an alternative approach that can offer advantages in terms of chromatographic resolution, selectivity, and accessibility. GC-based methods employing in-injector derivatisation with diaryliodonium salts have been previously reported for PFAS analysis, but their application has so far been limited in compound coverage and regulatory validation. In this work, we present a significantly improved GC-MS method based on in-injector diaryliodonium derivatisation combined with isotope dilution for the determination of PFAS in potable water. The method was expanded to include 30 PFAS of regulatory relevance. Isotopically labelled internal standards were employed to correct for derivatisation efficiency, matrix effects, and instrumental variability. A comprehensive validation was carried out in accordance with regulatory expectations for drinking-water analysis, including assessment of selectivity, linearity, limits of detection and quantification, trueness, precision, and robustness. Method performance was evaluated at concentration levels relevant to compliance monitoring, and applicability was demonstrated through the analysis of real drinking-water and wastewater samples. The validated method achieves limits of quantification compatible with current regulatory thresholds and provides reproducible and accurate results across the full target list. This study demonstrates that GC-MS with in-injector derivatisation can serve as a reliable and complementary alternative to LC-MS/MS for PFAS compliance monitoring in water.

SESSION C1

EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

Additives Matter: a Multi-tier Ecotoxicological Assessment of Commercial PVA- based Dishwasher Pods on *Danio rerio* Embryos

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Water-soluble polymers (WSPs) are a class of synthetic polymers characterized by unique physicochemical properties that support their widespread use in industrial, medical, and consumer applications. Despite their extensive use, the lack of specific regulation contributes to their uncontrolled release, especially into aquatic ecosystems, raising concerns about potential risks to organisms and human health. Although WSPs have generally been considered environmentally safe based on apical ecotoxicological assays, increasing evidence reports sub-lethal effects in various model organisms. Moreover, WSPs-containing consumer products are commonly combined with additives, which contribute to the final formulation released into the environment, raising additional concerns about their potential ecotoxicological impact. Among these products, liquid dishwasher pods represent a relevant source of environmental exposure, as they are enclosed in water-soluble films composed of polyvinyl alcohol (PVA) and formulation additives. The present study aims to investigate the potential effects of the different chemical components present in PVA-based pod film on *Danio rerio* embryos under environmentally relevant concentrations, adopting a multi-tier approach. Additives present in pod film were separated from PVA and chemically characterized using 1H-NMR spectroscopy and GPC. *Danio rerio* embryos were then exposed for 120 hpf to the whole pod film at the concentration of 0.1 mg/L, estimated for urban wastewater entering the Milan-Nosedo treatment plant (Italy), and to the corresponding concentrations of extracted PVA (0.07 mg/L) and additives (0.03 mg/L). Toxicity was evaluated through a multi-tier approach including metabolomic profiling, oxidative stress and neurotoxicity biomarkers, mitochondrial bioenergetics, cardiac activity, morphology, and behavioural analyses. Chemical analyses revealed that the film consisted primarily of PVA (70%), while additives accounted for the remaining 30% and were mainly represented by polyhydric alcohols and polyethylene-glycol oligomers. Exposure induced subtle yet significant biological responses across multiple levels of organization. PVA selectively affected mitochondrial respiration and morphology, whereas additives elicited broader effects, including metabolic reprogramming, neurotoxicity, behavioural alterations, and decreased heart rate. Notably, the whole film induced responses that could not be fully explained by the individual fractions, suggesting the occurrence of mixture-specific interactions. Overall, these findings highlight that additives, despite their lower exposure concentration, play a key role in driving the toxicity of PVA-based pod films and demonstrate the limitations of assessing the environmental safety of WSP-containing products solely from the pure polymer itself. These studies underline the importance of evaluating commercial formulations as complex mixtures to achieve a more realistic ecological risk assessment of WSP-based products.

Biological alterations, caused in *Mytilus galloprovincialis* by pharmaceutical active compounds, are partially attenuated by eco-friendly treatment with ulvans

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Emerging contaminants, due to the current lack of legislation relating their environmental release, represent a particular concern to aquatic ecosystems. Among them, the pharmaceutical active compounds (PhACs) are the contaminants attracting the most attention. Indeed, the inadequacy of wastewater treatment systems leads to the constant release of these compounds, able of interacting with various biological systems even in non-target organisms. Common effects induced by PhACs involve changes in the antioxidant and immune responses; therefore, the employment of antioxidant and immunomodulatory molecules could represent a potential mitigation strategy. In this context, ulvans, polysaccharides obtained from algae of the genus *Ulva*, exhibit important antioxidant and immunomodulatory properties. The aim of this study was to evaluate the impact of the PhAC dexamethasone (DEX), a globally used anti-inflammatory drugs, on *Mytilus galloprovincialis*, a species of ecological and commercial interest, and subsequently to test the beneficial effects of ulvans on specimens exposed to DEX. Accordingly, two 12-day experimental designs were carried out; in the first, mussels were exposed to four realistic concentrations of DEX (4 ng/L-2000 ng/L), whereas in the subsequent experimental design, the organisms were simultaneously exposed to 400 ng/L of DEX and 100 mg/L of ulvans, extracted by the invasive species *Ulva ohnoi*. The multi-biomarker approach carried out on mussel digestive glands, highlighted the ability of various concentrations of DEX to induce pro-oxidant effects, with increases in lipid peroxidation and in the activity of enzymes such as catalase and superoxide dismutase, accompanied by alterations in energy metabolism, such as an increase in lactate dehydrogenase. Disruption in digestive tubules organization and haemocyte infiltration was observed. Changes in the activity of enzymes such as phenoloxidase or acid phosphatase, supported the occurrence of impairments in the immune response. The use of ulvan partially mitigated the DEX effects. Indeed, histological analyses revealed an improvement in the structure of the digestive gland associated to ulvan treatment. Immune parameters such as acid phosphatase and phenoloxidase activity were found to be reduced in the combined DEX and ulvan exposure, confirming their immunomodulatory effect. The ulvan antioxidant effect was confirmed by the reduction in lipid peroxidation and the increase in catalase activity. Moreover, the absence of changes in lactate dehydrogenase activity suggested an improvement in energy metabolism. Overall, these results reveal the ability of ulvans to mitigate, albeit partially, the harmful effects of DEX, highlighting the need for further studies to optimise their employment in counteracting the effects of anthropogenic pollutants.

Ecotoxicological Effects of Polymers Used in Wastewater Treatment Plants on *Daphnia magna*

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Wastewater treatment plants represent essential infrastructures for the protection of aquatic ecosystems, as they enable the removal of some contaminants from domestic, industrial, and agricultural effluents before their discharge into the receiving water bodies. Wastewater commonly contains very fine suspended and dissolved solids, including inorganic and organic particles, whose removal represents one of the major challenges for industries. Among the available treatment technologies, coagulation and flocculation are considered among the most effective methods for colloid removal. These processes employ polymeric compounds of either synthetic or natural origin. Although only a small fraction of these polymers remains in the effluent, their extensive use raises concerns about potential impacts on aquatic organisms. To evaluate the ecotoxicological effects of flocculants and coagulants, the freshwater invertebrate *Daphnia magna* was selected as biological model. Since information on the environmental occurrence, fate, and toxicity of these polymers remains limited, a preliminary range-finding test was conducted. Based on the 48-h immobilization assay, EC10 concentrations were selected and subsequently applied in a 14-day chronic exposure experiment to simulate environmentally relevant exposures. The tested compounds were chosen based on a previous study identifying the most effective treatments for both organic matter and plastic particles removal from wastewater. Polyacrylamides, starch, tannin-based were selected as flocculants, while poly-aluminum chloride was chosen as coagulant. The study evaluates effects at different levels of biological organization, including biomarkers related to oxidative stress and damage, neurotoxicity, and energy metabolism, as well as many organismal endpoints such as immobilization, growth and behavioral alterations, reproduction, and feeding activity. Preliminary results indicate a markedly higher toxicity associated with synthetic flocculants. Even at environmentally relevant concentrations, chronic exposure induced immobilization rates ranging from 90% to 100%, highlighting potential long-term risks for freshwater organisms and overall ecosystem health. In contrast, natural-based compounds exhibited substantially lower toxicity while maintaining considerable efficiency in the removal of both chemical and physical pollutants, including plastic particles. These findings underline the importance of promoting already available sustainable alternatives in wastewater treatment processes, demonstrating that naturally derived flocculants may represent effective and environmentally safer solutions.

Italian case study of the BlueAdapt H2020 project: Health Risk Assessment of Antibiotic-Resistant Bacteria Spread into a Surface Water

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The proliferation of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in aquatic ecosystems represents an escalating global public health challenge, particularly in areas subjected to anthropogenic pressures including recreational activities, aquaculture, agricultural runoff, and inadequate wastewater management. This study was conducted within the framework of the EU-BlueAdapt project, presents a comprehensive, evidence-based Health

Risk Assessment of ARB and ARGs recovered from the Arrone River, Italy, during Italian bathing water season of 2023. Risk was evaluated through a structured framework integrating four analytical dimensions: phenotypic antimicrobial susceptibility profiling against eleven clinically relevant antibiotics; genotypic resistance profiling of 34 ARGs; multi-pathway human exposure assessment covering direct water ingestion, occupational contact, recreational exposure, and food chain contamination; and ecological risk evaluation addressing horizontal gene transfer (HGT), environmental resistome expansion, and downstream ecosystem impacts. Risk characterisation was based on the WHO Bacterial Priority Pathogens List (BPPL) 2024, which applies a Multi-Criteria Decision Analysis across eight weighted criteria including treatability, fatal burden, AMR trend, and communicability, and was further extended through a Hazard × Likelihood qualitative matrix consistent with established environmental health risk methodology. A total of 127 strains encompassing ESBL-producing Enterobacteriaceae and three pathogenic *Vibrio* species were recovered. All ESBL-producing Enterobacteriaceae were assigned to the Critical priority tier, alongside the top-ranked WHO pathogen, carbapenem-resistant *Klebsiella pneumoniae*, while *Vibrio* species were assigned to the High priority tier. Risk characterisation identified multiple concurrent exposure pathways, direct water ingestion, occupational contact at aquaculture and agricultural facilities, and food chain contamination via locally harvested seafood, placing riverine communities, aquaculture workers, farmers, and the consumers at significant and immediate risk. The ecological risk posed by ARB and ARGs in river and surface water extends beyond direct human health impacts as the long environmental persistence of ARGs in water and sediment serve as a genetic reservoir that can be acquired by indigenous bacteria through HGT. The sustained presence of Class 1 integrons at all three sites is the most significant ecological risk indicator, as integrons are the primary vehicles for multi-gene resistance cassette dissemination in aquatic environments. These findings demonstrate that the Arrone River constitutes a critical-risk environment for ARB and ARGs exposure and underscore the urgent need for coordinated, multi-sectoral intervention within a One-Health framework encompassing water safety enforcement, antibiotic stewardship in aquaculture, continuous surveillance of high priority organisms, and integrated environmental and clinical AMR monitoring.

Use of a National Network of *Apis mellifera* L. Colonies for Environmental Surveillance of Antimicrobial Resistance Genes (ARGs)

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Antimicrobial resistance (AMR) is a global public health priority. The environment acts as both a reservoir and a pathway for the dissemination of antimicrobial resistance genes (ARGs). Due to their wide foraging range and interaction with environmental matrices, *Apis mellifera* colonies can serve as bioindicators of microbiological contamination. This work aimed to evaluate the effectiveness of a national apiary

network for monitoring the main ARGs associated with the most widely used antimicrobials. Within the BeeNet project, 339 apiaries across Italy were sampled in April and June 2021. For each site, DNA was extracted from a pool of 30 bees and PCR analysis was performed to detect 31 non-chromosomal ARGs associated with eight antimicrobial classes (tetracyclines, macrolides, fluoroquinolones, aminoglycosides, β -lactams, sulfonamides, glycopeptides, and polymyxins). The spatial distribution of positivity for the different ARG classes was estimated using Bayesian geostatistical models. For the three most frequent ARG classes (tetracyclines, β -lactams, and polymyxins), multivariable models were fitted to assess possible associations with covariates related to soil composition, livestock density, apiary density, and human population density (per km²). The most relevant variables were selected using a Stochastic Search Variable Selection (SSVS) procedure. A total of 96.16% of samples tested positive for at least one ARG. Tetracyclines showed the highest average prevalence (30.4%), with the predominance of the genes tetM (61.7%), tetG (58.4%), and tetL (50.7%), particularly frequent in northern regions. Genes associated with colistin resistance (*mcr1*, *mcr2*, and *mcr9*) showed national prevalences ranging from 10% to 13%, with clusters observed in central and southern Italy. Among β -lactam resistance genes, blaNDM was the most widespread (15.3%), with peaks in Emilia-Romagna and Lombardy. The remaining ARGs showed lower prevalences but heterogeneous distributions. Positive correlations emerged between livestock density and the frequency of specific ARGs; in some areas, an association with human population density was also observed. The LASSO analysis highlighted positive associations between specific livestock categories and ARG classes: pig farms with polymyxin resistance genes (OR 1.135), horse farms with β -lactam resistance genes (OR 1.120), and apiary density with tetracycline resistance genes (OR 1.186). No relevant associations were found with soil covers. The results document the widespread environmental circulation of ARGs and support the use of *A. mellifera* colonies as a bioindicator at the national scale. Integrating this approach into epidemiological surveillance programs could contribute to the identification of territorial hotspots and strengthen strategies to combat AMR within a One-Health framework.

Survival of *Apis mellifera ligustica* (Spinola, 1806) Under Combined Exposure to Thermal Stress, Humidity Variation, and Microplastic Ingestion

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Microplastic (MP) pollution has emerged as a pervasive environmental threat, with particles now detected in worldwide ecosystems. Due to their persistence and widespread distribution, MPs can be incorporated into food webs and ingested by a broad range of organisms, including insect pollinators. Recent studies have shown that MP exposure can alter bee physiology, gut microbiota, immunity, and behaviour, raising concerns about their ecological consequences. At the same time, insect pollinators are experiencing global population declines driven by multiple stressors, including habitat loss, agricultural intensification, pesticide exposure, pathogens, and climate change. Rising temperatures and increasing frequency of extreme weather events are expected to further challenge pollinator health. Understanding how climatic factors interact with emerging pollutants is therefore crucial for predicting pollinator

responses under future environmental scenarios. This study investigated the effects of MP ingestion on honey bee (*Apis mellifera ligustica*) survival and food consumption under different temperature and humidity regimes. We further assessed whether environmental conditions could amplify MP toxicity and whether combined exposure to different MP types resulted in additive or synergistic effects. Newly emerged bees were exposed to polyethylene (PE) and polystyrene (PS) microspheres at concentrations of 0.5, 5, and 50 mg/L, administered individually or in combination through sugar syrup. Experimental assays were conducted under three environmental conditions: control (33 $\pm$ 2°C, 50% RH), elevated temperature (40 $\pm$ 2°C, 50% RH), and elevated humidity (33 $\pm$ 2°C, 80% RH). Survival and food consumption were monitored throughout a 29-day laboratory experiment. Both MP exposure and environmental conditions significantly affected bee survival. High MP concentrations produced marked reductions in lifespan, suggesting a dose-dependent toxic effect. Elevated humidity exerted the strongest impact, causing greater mortality than elevated temperature, particularly when combined with high concentrations of PE and PS. The PE-50 $\times$ PS-50 treatment under humid conditions showed the lowest median lethal time, approximately 8 days compared with 27.5 days in the control group. Food consumption also declined significantly under both climatic stress and MP exposure. Overall, the results demonstrate that environmental humidity can substantially amplify the detrimental effects of MP ingestion on honey bees, potentially representing a more critical stressor than heat under the tested conditions. Although no significant synergistic interactions between polymer types were detected, their effects were largely additive. These findings highlight the importance of considering multiple concurrent stressors when assessing pollinator health and suggest that interactions between climate-related factors and emerging pollutants may contribute to reducing pollinator resilience and the ecosystem services they provide.

The Northwestern Adriatic Sea as a Dynamic Laboratory to Explore Multiple Stressor Effect on Marine Bivalves

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Worldwide bivalve aquaculture is facing the effects of climate change and pollution, that are modifying the seawater chemistry and biotic parameters. Moreover, extreme weather events are projected to increase in frequency and intensity, posing significant threats to several economic and social aspects related to marine food production, from productivity and quality to safety and sustainability. Therefore, understanding how marine bivalves respond to interacting environmental stressors is critical for sustainable aquaculture under climate change. In Emilia Romagna (Italy), along the Northwestern Adriatic Sea, bivalve farming represents a relevant economic resource and is part of our cultural heritage since very ancient times. Therefore, the recent losses related to bivalve farming failure raise increasing public and social concerns and highlight the urgent need for effective mitigation measures. This collection of studies from our research group investigates the temporal dynamics of physiological status in Mediterranean mussels (*Mytilus galloprovincialis*) and striped Venus clam (*Chamelea gallina*) across productive sites along the Emilia Romagna coastline, integrating multi-year field data on biometric traits, physiological indices and molecular tools. Results highlight strong site-specific responses to environmental variability. During the 2022 heatwave, clams from low-productivity sites showed enhanced metabolism activity with the upregulation of digestive transcripts, whereas individuals from high productivity sites maintained stable

metabolism but exhibited increased antioxidant and immune activity. In mussels, the same heatwave induced the modulation of metabolic and cytoprotective pathways, while preserving biomineralization. Additionally, seasonal bioaccumulation of pharmaceuticals and pesticides altered transcriptional profiles, with increased metabolic activity and reduced detoxification and lysosomal functions, suggesting a different energy allocation under pollutants exposure. A reference gene analysis identified ribosomal RNA genes as the most stable under field analysis, while action and elongation factor-1 α were the most variables and their expression significantly correlated with seasonal and latitudinal changes of abiotic parameters and physiological indices, leading to consider them as molecular biomarkers. Overall, bivalve responses to multiple stressors are context and site dependent and shaped by local environmental conditions. These findings provide several early indicators of stress caused by the assessed alterations of water physics and chemistry, that can impact the productivity. Furthermore, these studies resulted in the development of molecular and physiological tools to support bivalve monitoring and development of climate-adaptive strategies for sustainable bivalve production in the Northwestern Adriatic Sea.

Macroalgal Habitat Shapes Physiological Responses Of Canopy Associated Invertebrates Exposed To Legacy And Emerging Contaminants

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Marine environments are highly dynamic, and organisms are adapted to this variability through physiological and behavioural mechanisms. Anthropogenic stressors, particularly when acting synergistically, can modify the environmental factors exceeding species' adaptive capacity. Among key global changes, pollution caused by both legacy and emerging contaminants and oxygen depletion are the main ones. Macroalgal habitats may modulate the energy balance, stress response, and resilience of associated fauna, which are naturally adapted to daily oxygen fluctuations. However, standard ecotoxicological experiments rely on constant conditions, potentially biasing toxicity assessments. To address this gap the present study investigates how the habitat provided by the macroalgal species *Gongolaria barbata* modulate the physiological and biomarkers responses of two invertebrate species usually associated with the algae, *Carcinus aestuarii* and *Ophiothrix fragilis*, to mercury and glyphosate contamination. The selection of the species was based on preliminary observations in the Venice Lagoon (Italy) suggesting the two invertebrates as good proxy of the environmental variability related to the canopy being respectively related to mobile lifestyle within the top portion of the macroalga and to a more sessile lifestyle at the bottom of the same. Once collected, the invertebrates faced a two-factor experimental design: acclimation (Accl vs NonAccl), contaminants (present vs absent). After a 24h exposure, samples went through both physiological and biochemical analysis evaluating respiration and excretion rates for *O. fragilis*; haemocytes parameters and acetylcholinesterase activity for *C. aestuarii*; total antioxidant capacity and lipid peroxidation (LPO) for both organisms. By the end of the experiment, *O. fragilis* exhibited contaminant specific

reactions with no acclimation combine effect, indicating a limited role of habitat-mediated effects in this species. In contrast, *C. aestuarii* displays a significant tissue-specific role of macroalgal prior acclimation. Specifically, macroalgal acclimation consistently reduced LPO in the hepatopancreas and modulated the antioxidant levels depending on the contaminant. Changes in haemocyte size further suggest the involvement of immune-related mechanisms in stress responses. Therefore, the results advise a priming effect of the algal habitat on oxidative balance and immune processes in crabs. Overall, this study supports the idea that macroalgal habitats can influence the sensitivity of associated fauna to contaminants, although this effect is strongly dependent on species, tissue, and contaminant type, disclosing the need to include biotic interactions in ecotoxicological assessments.

From Blow to Biomarkers: Small RNA Sequencing of Drone-Collected Cetacean Blow for Non-Invasive Biomarker Discovery

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Cetaceans are widely recognized as sentinels of marine ecosystem health, yet monitoring physiological and reproductive status in free-ranging individuals remains challenging because most approaches require direct interaction. The non-invasive collection of cetacean exhaled breath condensate ("blow") using unmanned aerial vehicles (UAVs) provides a promising alternative for obtaining biological material. MicroRNAs (miRNAs) are small regulatory RNAs with considerable potential as physiological biomarkers but their application in cetacean blow remains largely unexplored. As part of the BLOWOMICS project, this study evaluated the feasibility of combining drone-collected cetacean blow with small RNA sequencing for non-invasive biomarker discovery in free-ranging cetaceans. Seven blow samples from free-ranging short-finned pilot whales (*Globicephala macrorhynchus*) collected off El Hierro (Canary Islands, Spain) were analysed, including four pregnant and three non-pregnant females. Pregnancy status was used to assess whether biologically meaningful miRNA expression patterns could be detected in cetacean blow. Blow samples were collected using UAVs equipped with sterile petri dishes. Total RNA was extracted using a modified miRNeasy Serum/Plasma protocol and sequenced using Illumina small RNA sequencing. Raw reads were quality filtered using miRTrace, candidate miRNAs were identified using MirMachine and evaluated using MirMiner, and differential expression analyses were performed in R using DESeq2. Candidate biomarkers were prioritized using an integrated framework incorporating differential expression patterns, abundance, detection consistency, and reproducibility across multiple filtering strategies. Principal component analysis (PCA) of variance-stabilized expression data and heatmap analyses were used to investigate global expression patterns. Small RNA sequencing successfully recovered miRNA profiles from all seven blow samples. A total of 164,969,302 reads were generated, of which 136,900,842 passed quality control filtering, and more than 8.2 million reads were assigned to reference miRNAs. Exploratory analyses identified multiple candidate pregnancy-associated miRNAs, with several

candidates consistently recovered across independent analytical approaches. Heatmap analyses revealed expression patterns among pregnant and non-pregnant individuals, while PCA revealed variation in miRNA expression profiles among individuals, with PC1 and PC2 explaining 54% and 12% of total variance, respectively. Although larger sample sizes are required for validation, these findings demonstrate the feasibility of recovering biologically informative miRNA profiles from drone-collected cetacean blow and highlight the potential of this approach for non-invasive physiological and reproductive monitoring in free-ranging cetaceans.

SESSION C2

EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

A Multi-Biomarker Approach To Evaluate The Ecotoxicological Status Of Great Tit (*Parus major*) Populations From Areas With Different Anthropogenic Impacts

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Multiple anthropogenic pressures can lead to physiological alterations or damages to birds, potentially resulting in cascading effects on individual's fitness and population dynamics. Cavity-nesting birds, such as the great tit (*Parus major*), which readily use artificial nest boxes, represent interesting model species for investigating the impacts of environmental stressors on avian health. Their territorial behaviour and the accessibility of their nests make great tits particularly suitable for investigating the environmental conditions of their nesting locations. This study aimed to evaluate the ecotoxicological status of great tit populations inhabiting areas with different anthropogenic impacts, using a multi-biomarker approach targeting different physiological responses. During the breeding seasons of 2021 and 2022, blood and excreta samples were collected from 198 nestlings belonging to 44 nests across four different areas of the Veneto region, in northern Italy, representing wooded, urban and agricultural environments. Immunotoxicity, oxidative status, genotoxicity and neurotoxicity responses were evaluated on blood, while porphyrins levels on excreta. Results revealed significant physiological alterations in nestlings from urban and agricultural areas (oxidative status modifications, immune response impairments and genotoxic effects), likely associated with environmental stressors typical of anthropized ecosystems. Similar alterations were also detected in the wood area, where individuals exhibited porphyrin accumulation and compromised immune function. This multi-biomarker approach has proved to be a valid tool for assessing the ecotoxicological status of great tit populations exposed to different stressors, allowing it to be proposed at the managerial and regulatory level for the conservation of wild bird species.

Food-Relevant Exposure To Okadaic Acid Triggers Early Transcriptomic Responses In Complementary Porcine And Human Intestinal Models

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Harmful Algal Blooms (HABs) increase the risk of human exposure to marine biotoxins. Okadaic acid (OA), a phycotoxin produced by dinoflagellates and accumulated in shellfish, is the principal causative agent of Diarrhetic Shellfish Poisoning

(DSP). DSP symptoms can develop within a few hours (h) following seafood consumption and the intestinal epithelium represents the primary target of OA exposure. However, the early molecular events underlying OA intestinal toxicity remain poorly understood. Current knowledge is largely derived from conventional Caco-2 models, which may not fully reflect normal intestinal physiology due to their cancer-derived origin and limited metabolic competence. Therefore, we investigated two complementary intestinal in vitro models: the non-transformed porcine IPEC-J2 cell line and a metabolically competent human Caco-2 clone engineered to overexpress the Pregnane X Receptor (Caco-PXR). Cells were exposed for 6 and 12h to food-relevant OA concentrations corresponding to the European regulatory limit (20 and 80 nM), and higher concentrations approaching model-specific toxicity thresholds (160 and 600 nM for Caco-PXR). Transcriptomic responses were evaluated by RNA sequencing, followed by differential expression, Gene Set Enrichment Analysis (GSEA), and over-representation analyses. IPEC-J2 cells exhibited a rapid response to OA exposure. Although no significant differentially expressed genes (DEGs) were detected after 6h, GSEA revealed perturbations of pathways related to inflammation, hypoxia, stress responses, and epithelial remodelling. After 12h, exposure to 80 nM induced 181 DEGs and enrichment of biological processes associated with epithelial development, proliferation, immune regulation, and programmed cell death, suggesting progression from early stress signalling toward toxicity-associated responses. In contrast, Caco-PXR cells exhibited a progressive and dose-dependent response. Despite the detection of a few DEGs at food-relevant concentrations, GSEA demonstrated molecular perturbations. At 6h, OA (20 and 80 nM) induced adaptive responses characterized by metabolic reprogramming and activation of stress and inflammatory signalling. 160 nM promoted pathways related to apoptosis, tissue remodelling, and repair. In contrast, 600 nM induced extensive transcriptomic reprogramming accompanied by repression of metabolic and homeostatic functions. At 12h, the response became more pronounced and extended to lower concentrations, with food-relevant exposure inducing inflammatory and toxicity-associated signature. Despite their different sensitivities, both models converged on common OA-induced pathways related to inflammation, hypoxia, apoptosis, and epithelial remodeling. These findings demonstrate that food-relevant OA exposure can trigger early molecular perturbations before clear toxicity and highlight the value of complementary porcine and human intestinal models for improving the mechanistic understanding of OA intestinal toxicity.

Linking Genomic Insights And Experimental Evidence To Understand Seaweed Resilience To Environmental Changes

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Understanding seaweed responses to stressors, such as climate change and chemical pollution, is important for predicting future population dynamics. The present research studies combined physiological assessments and molecular approaches to investigate the response of the red seaweed *Palmaria palmata* to environmental changes. Physiological effects were evaluated in vegetative and sporulating thalli through exposure experiments simulating realistic environmental conditions, including marine heatwaves (+3°C, MHW), hyposalinity (-8 PSU), and a chemical compound mixture (i.e. wastewater treatment plant effluent, 100x

effluent), applied individually and in combination for 2 weeks followed by a recovery period. Growth (as mean of fresh weight and surface area) and photosynthetic efficiency (maximum quantum yield of PSII, Fv/Fm) were assessed. Final blade size did not differ significantly between exposures in vegetative blades despite temporarily increased growth rates. In sporulating blades, MHW and effluent led to 1.8-fold larger blade than controls, and the photosynthetic efficiency decreased due to combine exposure, indicating transient stress. A similar exposure study was also conducted in the spring period to assess the stressor effects on the synchrony of spore release. Sporulating thalli were exposed to MHW (+8°C), effluent (50x) and their combination for 10 days followed by 10 days of recovery. The effluent exposure significantly reduced both fresh weight (1.78 ± 0.26 % day⁻¹) and surface area (0.94 ± 0.11 % day⁻¹) growth rates compared to controls (4.27 ± 0.28 % day⁻¹ and 1.48 ± 0.28 % day⁻¹, respectively). The photosynthetic efficiency was higher after exposure to the effluent (0.33 ± 0.02) compared to the control (0.26 ± 0.02), with no differences after the recovery period. The spore release was observed after 5 days of exposure to the effluent, suggesting an effects of chemical pollution on reproductive timing. To provide a molecular insight, the available *P. palmata* genome was annotated, and transcriptomic analyses were performed across reproductive stages. 21,541 protein-coding genes were predicted, and differentially expressed genes were identified between vegetative, sporulating and sorus tissues. Functional analyses highlighted pathways associated with development, cellular signalling, oxidative stress responses and carbohydrate metabolism. Together, these results demonstrate the resilience of wild and cultivated *P. palmata*. Finally, the combination of physiological and molecular approaches provided a valuable foundation for stress-response investigations and mechanistic understandings.

Multilevel Responses To Emerging Contaminants In The Tunicate *Ciona intestinalis*: A Comparative Perspective

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The increasing presence of contaminants of emerging concern (CECs) in marine coastal waters necessitates urgent investigation into their impacts on marine biota and human health. Antidepressants and micro- and nanomaterials, particularly fibres, have gained increasing attention, as both ranked among the most widespread and hazardous classes, especially during and after the COVID-19 pandemic. Despite their increasing presence in the coastal environment, their effects on marine biota remain poorly resolved. Here, we investigated the acute and chronic effects, alone and in combination, of the antidepressant Venlafaxine (VEN) and two types of cellulose nanofibers, non-oxidized (CNF) and TEMPO-oxidized (TOCNF), at environmentally relevant concentrations and under hotspot scenarios, on the urochordate *Ciona intestinalis* from early development to the adult stage. To enhance the ecological relevance of the study, similar analyses were also carried out on the bivalve mussel *Mytilus galloprovincialis*, a widely recognized sentinel species in marine ecotoxicology. Using an integrated phenotypic,

molecular, and behavioural framework, juvenile stages of *Ciona* were investigated using phenotypic and transcriptomic analyses and larvae and adults were assessed using behavioural assessments. Our results show that single and combined exposure of VEN and CNFs induced significant, concentration-dependent, and stage/species-specific effects on developmental and, for *Ciona*, also behavioural endpoints. Computational analysis of *Ciona* larval swimming dynamics reveals changes in behavioural responses and their transitions. This comparative analysis allows us to identify conserved and species-specific responses to CEC exposure across marine taxa. Overall, these findings highlight for the first time the ecological risks of mixtures of an antidepressant and cellulose nanofibers, supporting the need for integrative, multi-level approaches in ecotoxicological risk assessment of CECs.

Ecotoxicological Effects Of The Antidepressant Sertraline In Different Non-Target Marine Species

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Sertraline (SERT), a selective serotonin reuptake inhibitor (SSRI) widely prescribed worldwide, is among the pharmaceuticals most frequently detected in aquatic environments due to its extensive consumption and continuous release through wastewater treatment plants. Despite its widespread occurrence in marine and freshwater ecosystems, current knowledge of its ecotoxicological effects on non-target aquatic organisms remains limited and fragmented. In this context, this study aimed at a multidisciplinary assessment of SERT's biological impact on marine organisms. An in vivo exposure was conducted in the Mediterranean mussel *Mytilus galloprovincialis*, which was exposed for 14 days to environmental realistic concentrations of SERT (from 0.2 to 500 ng/L). A wide panel of biochemical and cellular biomarkers associated with immune responses, oxidative stress, neurotoxicity, genotoxicity, and lipid metabolism was evaluated. In parallel, a battery of ecotoxicological bioassays was performed on species from different trophic levels, including *Aliivibrio fischeri* (bioluminescence inhibition), *Phaeodactylum tricornutum* (growth inhibition), and *Paracentrotus lividus* (fertilization and embryotoxicity), testing doses up to 100 µg/L. The results obtained from experiment on adult mussels indicated that the immune and cholinergic systems were the main targets of SERT exposure, as demonstrated by alterations in several haemocyte-based immune parameters, including lysosomal membrane stability (LMS), granulocyte-to-hyalinocyte ratio, and phagocytosis capacity, and by the inhibition of acetylcholinesterase (AChE) activity in gill tissue. Impairment in metabolic homeostasis were also observed, reflected by changes in biomarkers related to oxidative status, lipid peroxidation, and lipid metabolism. Ecotoxicological bioassays revealed different sensitivity of the tested species, confirming the low responsiveness of *A. fischeri* to pharmaceutical exposure. Notably, the highest tested concentration (100 µg/L) completely impaired embryo development in *P. lividus*, highlighting a high susceptibility of this species to SERT exposure. These findings suggest that environmental relevant levels of SERT may induce ecotoxicological effects in marine organisms, highlighting the need for further investigation into its chronic impacts in coastal environments.

Surviving Contamination: Energetic Trade-Off And Redox Disruption In PFAS-Chronically Exposed Fish (*Squalius cephalus*)

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Chronic exposure to environmental contaminants requires organisms to continuously invest energy in defence and maintenance mechanisms, potentially compromising other physiological functions. Among emerging pollutants, per- and polyfluoroalkyl substances (PFAS) are of particular concern due to their environmental persistence and bioaccumulation potential. In particular, the Veneto region (Italy) has experienced one of the largest cases of PFAS contamination in Europe, resulting in long-term pollution of freshwater ecosystems. Fish inhabiting watercourses are continuously challenged by these pollutants and must cope with their effects. Despite prolonged exposure, many populations persist in contaminated habitats, raising questions about the mechanisms that allow survival. In this context, this study evaluated biochemical responses associated with long-term PFAS exposure in the liver of wild *Squalius cephalus* from differently contaminated rivers in the Veneto region, focusing on oxidative damage, detoxification pathways, and energy metabolism. Results revealed oxidative impairment at the highest PFAS exposure level, as evidenced by the accumulation of advanced oxidation protein products (AOPP), indicating protein damage. Conversely, lipid peroxidation (LPO) decreased, suggesting that membrane lipids were effectively protected despite contaminant pressure, likely due to cellular defences. In fact, detoxification pathways were significantly modulated, with increased activity of glutathione S-transferases (GSTs). To cope with the increased production of reactive oxygen species (ROS), antioxidant defences were activated, as evidenced by increased total antioxidant capacity (TAC), reflecting the contributions of non-enzymatic antioxidants and the alternate activation of glutathione peroxidases (GPXs) and catalase (CAT). In contrast, superoxide dismutases (SODs) activity was inhibited, likely due to enzyme damage. Supporting these defence systems required a substantial metabolic investment, as evidenced by increased electron transport system (ETS) activity and depletion of glycogen (GLY) reserves, indicating enhanced mitochondrial respiration and energy mobilisation. Overall, these biochemical responses observed in the liver of *S. cephalus* reveal an adaptive strategy to chronic PFAS exposure. The activation of antioxidant and detoxification mechanisms, together with metabolic adjustments, contributes to the maintenance of physiological homeostasis under contamination pressure. However, at higher PFAS levels, these compensatory responses become less effective, as evidenced by oxidative damage to proteins. Therefore, survival in PFAS-contaminated environments comes at a measurable energetic cost, requiring the reallocation of resources from fitness-related processes toward defence, maintenance, and repair. Although *S. cephalus* exhibits considerable resilience through the activation of adaptive physiological responses, the persistence of this energetic burden may ultimately reduce individual fitness and represent an early warning signal of ecological vulnerability in freshwater ecosystems chronically affected by PFAS contamination.

A Combined Risk-LCA Approach for the PFAS REACH Restriction: A Case Study for Safe and Sustainable by Design

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Per- and polyfluoroalkyl substances (PFAS) are a class of chemicals of high concern in environmental management due to their persistence, potential for bioaccumulation, and toxicity. Within the European Union's Chemicals Strategy for Sustainability, moving towards a toxic-free environment requires a paradigm shift, operationalized through the Safe and Sustainable by Design (SSbD) framework. This study conducts a comprehensive Life Cycle Assessment (LCA) encompassing both PFAS production and individual downstream uses. The primary objective is to critically evaluate the current formulation of the ongoing universal PFAS restriction under the REACH Regulation (Annex XVII). To achieve this, the research introduces an advanced methodological framework based on a combined approach: integrating regulatory chemical risk assessment—derived directly from the European Chemicals Agency (ECHA) restriction dossiers and documents—with quantified LCA results. Inventory modelling and environmental impact assessments are conducted using openLCA software in combination with the Ecoinvent 3.11 database. This selection ensures a high level of scientific rigor, transparency, and maximum reproducibility of the developed models. The specific LCA models capture current industrial emission profiles and environmental burdens of PFAS manufacturing and specific end-use applications. They also provide the analytical basis to quantify and compare potential environmental impact reductions under proposed regulatory bans and emission limit values. Crucially, this combined REACH-LCA approach provides a concrete case study for the implementation of the SSbD paradigm. By integrating environmental fate assessment of highly persistent substances with standardized life cycle sustainability metrics, the approach demonstrates how ECHA regulatory data can actively support the design and selection of technological alternatives. This enables a systematic assessment of potential substitutes in terms of both human health protection and environmental sustainability across their full life cycle and is also relevant to identify possible burden-shifting effects, including increased resource use or dependence on critical raw materials associated with some technological alternatives. In conclusion, this work establishes a clear and replicable methodological framework for managing complex and persistent chemical classes, reducing regulatory uncertainty for the industrial sector, and guiding innovation toward effective and integrated sustainability.

From Environmental Occurrence To Biological Responses: A Multi-Level Assessment Of PFAS Across Mediterranean Waters, Biota And Toxicological Models

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Understanding the environmental occurrence, fate and effects of per- and polyfluoroalkyl substances (PFAS) in marine ecosystems is essential to identify and support risks prioritization related to these “forever chemicals”. This study integrates a field-based assessment of PFAS occurrence in the Mediterranean Sea with in-vitro and in-vivo experimental approaches to investigate their effects. PFAS occurrence was assessed in surface waters of seven Mediterranean areas along spatial gradients extending from river and wastewater treatment plant discharges to coastal environments, as well as in wild marine invertebrate and fish species collected in the same areas. Results in water samples indicate that PFAS contamination follows anthropogenic pressure gradients, with higher detection in freshwater and wastewater treatment plants, and concentrations decreasing along nearshore-offshore gradients. In line with this spatial pattern, PFAS accumulation in marine organisms showed species- and site-specific patterns, largely driven by a distinct subset of PFAS congeners. In-vitro experiments were conducted using mussel haemocytes and human cell line models to investigate the cytotoxicity of selected PFAS, including perfluorooctanoic acid (PFOA), perfluoropentanoic acid (PFPeA) and hexafluoropropylene oxide dimer acid (HFPO-DA). Dose-dependent toxicity, clear chain-length-related differences, and compound-specific cellular sensitivity were highlighted. Organism-level responses were investigated through controlled laboratory exposures with in-vivo marine models to selected PFAS under both present-day and climate-relevant warming scenarios. Experimental evidence revealed alterations in cellular homeostasis, oxidative balance, lipid metabolism, and detoxification pathways, supporting the hypothesis that disruption of PPAR α -related metabolic processes may represent a key mechanism underlying PFAS toxicity in marine organisms. Finally, this multi-level assessment gathering environmental occurrence, bioaccumulation and biological effects represented a solid framework to validate integrative hazard profiling approaches based on weighted criteria able to support PFAS regulation and prioritization in Mediterranean marine ecosystems based on their presence, fate and effects.

SESSION C3

EXPOSURE AND EFFECTS OF LEGACY AND EMERGING CONTAMINANTS, AND MULTIPLE STRESSORS

Micro- and Nanoplastics Disrupt Autophagy-Lysosome and Mitochondrial Homeostasis in Primary Human Monocytes

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The detection of micro- and nanoplastics (MNPs) in human tissues is becoming increasingly frequent, prompting growing concern about their possible long-term effects on human health, especially on immune function. Monocytes, which play a key role in immune surveillance, efficiently take up these particles, representing a suitable cellular model to investigate how MNPs exposure influences phagocytic processing, autophagy-lysosome dynamics, and mitochondrial activity. However, little is known about the mechanisms of MNPs internalization and their effects on human cells, especially on intracellular organelle dynamics and functions. Here, we evaluated the impact of exposure to spherical polystyrene (PS) MNPs of different diameters, namely 0.5, 1, and 5 µm, on primary human monocytes at both ultrastructural and molecular levels, with particular attention to the autophagy-lysosome axis and mitochondrial respiration. Transmission electron microscopy (TEM) was used to investigate MNPs internalization by primary human monocytes and the resulting intracellular rearrangements at the ultrastructural level, with a specific focus on degradative organelles and mitochondria. Oxygraphy was used to assess metabolic alterations and mitochondrial stress, while RT-qPCR and Western blot analyses were performed to evaluate changes in gene and protein expression following MNPs exposure. The autophagic response was evaluated using MNPs concentration selected on the basis of IC50 values derived from concentration-response curves. Our results showed that MNPs are internalized by monocytes through phagocytosis and accumulate within lysosomes highlighting a multilamellar morphology, which was accompanied by massive autophagosome accumulation and expansion of the lysosomal compartment, indicating enhanced demand on intracellular degradative pathways. TEM imaging also revealed alterations in mitochondrial morphology, while oxygraphy indicated size-dependent changes in the respiratory chain. Consistent with these observations, genes and proteins associated with autophagy, such as LC3, and lysosome formation, such as LAMP1, were upregulated. Importantly, our data do not support a simple model of lysosomal collapse or defective degradation. Although p62/SQSTM1 accumulated after MNPs exposure, this increase was mainly associated with stress-induced transcriptional upregulation rather than impaired autophagic clearance. In conclusion, this study provides novel insights into the cellular and molecular responses triggered by MNPs in human monocytes. Our findings indicate that MNPs engage interconnected homeostatic networks involving autophagy, lysosomal activity, and mitochondrial metabolism, highlighting the potential cellular damage associated with human exposure to MNPs. These results contribute to a more

refined interpretation of MNPs toxicity and support further investigation into the long-term health consequences of MNPs exposure in humans.

Oxidative Stress And The Gut-Brain Axis As Emerging Tools In Environmental Physiology

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Antarctica is often perceived as pristine, yet contaminants of emerging concern are increasingly detected in polar ecosystems, where climate change may amplify their biological effects. Within this framework, POLAR-TOX investigates how endemic Antarctic fish respond to chemical stress, with a particular focus on host physiology, gut microbiota, and the gut-brain axis. The project focuses on *Trematomus bernacchii*, a cold-adapted notothenioid fish that evolved under stable environmental conditions and may therefore be particularly vulnerable to rapid environmental change and xenobiotic exposure. Adult specimens were experimentally exposed to bisphenol A (BPA; 25 µg/L), a widespread endocrine-disrupting compound, and brain and intestinal samples were collected to assess oxidative stress, molecular responses, and microbiota-associated changes. Preliminary results indicate that BPA exposure induces tissue-specific alterations in antioxidant defences. The most striking response was observed in the brain, where BPA strongly inhibited key antioxidant enzymes, including superoxide dismutase (SOD) and selenium-dependent glutathione peroxidase (Se-GPX), along with broad downregulation of their corresponding genes. This pattern is consistent with impaired neural protection against reactive oxygen species and supports the nervous system as a sensitive target of BPA toxicity, given BPA's ability to cross the blood-brain barrier. In the intestine, BPA exposure induced an unbalanced antioxidant response, characterised by increased superoxide dismutase and catalase activities but reduced total glutathione peroxidase activity, accompanied by increased oxidative damage to proteins and lipids. These findings suggest that BPA may reveal vulnerability points in the antioxidant defence network of this cold-adapted species. Ongoing complementary objective of POLAR-TOX is to characterise the mucosa-associated gut microbiota using a culture-based approach adapted to the limited biomass of archived Antarctic samples. Intestinal homogenates are plated on low-nutrient and marine agar media to promote the growth of cold-adapted bacteria; colonies are then purified and identified by 16S rRNA sequencing, enabling comparisons of microbial diversity and dominant taxa between BPA-exposed and control fish. By integrating oxidative stress biomarkers, gene expression, and microbiota profiling, POLAR-TOX aims to offer mechanistic insight into pollutant-microbiota-host interactions in Antarctic fish. This integrative pipeline may advance polar ecotoxicology and support future environmental risk assessment in a rapidly changing Southern Ocean.

An Approach To The Study Of Underwater Noise And Nanoplastics And Their Effects On The Colonial Ascidian *Botryllus schlosseri*

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Marine ecosystems are increasingly threatened by anthropogenic pollutants. While the effects of these stressors have been extensively investigated in vertebrates, some marine invertebrates remain relatively understudied despite

their key ecological roles. In this context, our research aims to investigate the effects of two emerging anthropogenic stressors, underwater noise and nanoplastics (NPs), on ascidians, a group sessile filter-feeding marine invertebrate. Our research focuses on the colonial ascidian *Botryllus schlosseri* (Pallas 1766), a well-established model organism that has been bred and studied for many years at the Department of Biology of the University of Padova. Ascidians are particularly suitable for investigating stress responses because they are widely distributed and amply tolerant to environmental conditions, being the major components of climax communities on hard substrates. To evaluate the effects of the two anthropogenic stressors, we investigate immunological, physiological, and developmental responses. Immune function is assessed through in vitro phagocytosis assays and flow cytometry (FACS) analyses, which measure the ability of phagocytic cells to engulf foreign particles. Inflammatory responses are evaluated by in vitro monitoring degranulation in morula cells and through phenol oxidase enzymatic assays. Physiological analyses include sensitivity tests on the oral siphon primary and secondary mechanoreceptors, heartbeat rate measurements, and filtration rate assays using spectrophotometric methods. In addition, stress-response pathways are examined through RT-qPCR on stress and immune related genes. Developmental investigations focus on the colonial blastogenetic cycle, including possible generational and transgenerational effects on zooids and buds. We also assess regenerative capabilities and stemness-related processes using FACS, RT-qPCR, and confocal microscopy. The results obtained so far indicate that both underwater noise and NPs negatively affect various biological functions. Underwater noise decreases mechanoreceptor sensitivity, reduces heartbeat frequency, and slows the progression of the asexual reproductive cycle. Exposure to NPs reduces phagocytic and inflammatory responses and impairs filtration activity. Overall, these findings suggest that anthropogenic stressors can compromise both immunological, physiological and developmental processes in *B. schlosseri*. Future perspectives are to investigate different treatments conditions and durations and evaluate the combined effects of the two stressors. Experiments conducted also in field settings will provide a more comprehensive understanding of their impacts on marine invertebrate health and ecosystem stability.

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Extended abstracts

Integrated mRNA and microRNA Transcriptomic Analysis of *Mytilus galloprovincialis* Exposed to Environmental Pollution in the Venice Lagoon.

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Introduction

The Venice Lagoon, a coastal ecosystem subjected to significant anthropogenic pressure from urban and industrial activities [1], provides an ideal natural setting for investigating the impacts of environmental pollution on resident organisms. In this context, *Mytilus galloprovincialis*, a bivalve species widely distributed along Mediterranean and Atlantic coasts, was selected as a sentinel organism due to its remarkable ability to bioaccumulate contaminants and its extensive use in environmental monitoring and ecotoxicological studies [2]. This study focuses on understanding how anthropogenic and industrial pollutants in the Venice Lagoon affect gene and microRNA expression profiles in mussels, aiming to reveal the post-transcriptional gene regulation mechanisms triggered by the contaminants released in this environment. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by promoting mRNA degradation or inhibiting translation [3]. Their expression rapidly responds to environmental stressors, making them promising biomarkers of pollutant exposure and biological effects [4]. Owing to their pleiotropic activity, individual miRNAs can regulate multiple target genes and pathways simultaneously [5]. Complementarily, mRNA expression signatures provide insights into the biological processes affected by contamination, including detoxification, oxidative stress, immune response, and metabolism. The integrated analysis of miRNA and mRNA transcriptomes offers a comprehensive view of pollutant-induced molecular responses, enabling the identification of regulatory networks and sensitive biomarkers of environmental health in sentinel species such as *M. galloprovincialis*.

Materials and methods

Mussels were deployed for 30 days at five selected sites across the Venice Lagoon: (1) Marghera Port, (2) the lagoon side of Pellestrina, (3) Chioggia Port, (4) The Chioggia wastewater outfall, and (5) Allunata, a site located outside the lagoon and used as a reference contro. Water samples were collected at both deployment (t0) and retrieval (t30), and a subset of mussel tissues was analyzed to characterize site-specific contamination by organic pollutants, including Polycyclic Aromatic Hydrocarbons (PAHs), as well as trace metals. Gills and digestive glands from a second subset of mussels were collected for integrated mRNA and microRNA transcriptomic analyses, using a *Mytilus* pangenome developed by the University of Trieste as the reference framework. In parallel, gills, digestive glands, and haemolymph from a third subset of individuals were analyzed for a suite of physiological and biochemical stress biomarkers.

Results and discussion

Chemical analyses of water samples revealed that Marghera Port exhibited the highest concentration of PAHs at both deployment (t₀) and retrieval (t₃₀). In contrast, the site located near the Chioggia wastewater outfall showed the highest levels of metal contamination at both sampling time points. Samples collected at the control site showed contamination levels comparable to those observed at the Chioggia Harbour site. This similarity could be due to the proximity of the two areas and the marine traffic of the vessels entering the channel. With regard to bioaccumulation in mussel tissues, individuals deployed at Marghera Harbour exhibited the highest concentrations of both organic pollutants and metals. In contrast, mussels from the control site showed the lowest levels of contamination, with organic pollutants being only marginally detected. Preliminary transcriptomic analyses indicated that site-specific variation was more strongly reflected in microRNA expression profiles than in mRNA expression, suggesting a greater sensitivity of microRNA-mediated regulatory responses to environmental conditions and contaminant exposure. This might be related to the rapid response of miRNAs to stressors [4], a process that could be delayed in protein-coding genes. Nonetheless, in both miRNAs and mRNAs analyses, the samples from Marghera Port, Pellestrina lagoon and Chioggia Wastewater outfall treatment showed subtle differentiation among each other, with a tendency to cluster together, and a higher differentiation from the other sites, which could be attributed to the contaminants concentrations. Preliminary biochemical analyses of mussel gill tissues revealed reduced acetylcholinesterase (AChE) activity in individuals collected from the Marghera Port and Pellestrina lagoon sites, suggesting

potential neurotoxic effects associated with contaminant exposure. In the same specimens, increased levels of lipid peroxidation were detected, indicating oxidative damage to cellular membranes induced by pollutants. This interpretation is further supported by a modest increase in catalase activity suggesting the activation of antioxidant defense mechanisms aimed at mitigating oxidative stress and limiting tissue damage.

Conclusions

In conclusion, the transcriptomic analysis revealed that *M. galloprovincialis* individuals collected from the most contaminated sites exhibited similar patterns of both microRNAs and mRNAs expression. A comparable trend emerged from the biochemical analyses, which highlighted evidence of neurotoxicity and oxidative stress, together with increased activity of antioxidant enzymes. Overall, these preliminary results suggest a correlation between contaminant exposure and the activation of antioxidant defense mechanisms. Furthermore, transcriptomic data suggest that environmental contaminants affect microRNA expression, potentially mediating mussel responses to pollutant-induced stress. Further analyses will clarify these relationships and evaluate the suitability of specific microRNAs as biomarkers of environmental contamination and stress.

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Multi-Compartment Assessment of Pharmaceuticals and Personal Care Products and Their Microbial Impacts in the Elsa River (Italy)

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Introduction

Freshwater ecosystems are among the most threatened globally, characterised by degradation due to multiple anthropogenic pressures [1]. Among these, the introduction of emerging contaminants, particularly pharmaceuticals and personal care products (PPCPs), is of critical concern, driven by widespread and increasing consumption [2]. These compounds continuously enter river systems primarily through wastewater treatment plant (WWTP) effluents, as well as diffuse sources such as agricultural runoff and the application of animal waste [3,4]. Because many PPCPs are designed to be biologically active, they persist in the environment, posing potential risks to aquatic communities and promoting bioaccumulation [5]. To comprehensively characterise the distribution, partitioning, and ecological fate of these contaminants, this study applied a multi-compartment approach. The aim was to evaluate the occurrence, environmental distribution, and associated ecological risk of 17 PPCPs across water, sediment, and fish muscle tissue (*Squalius squalus*). Concurrently, the potential impacts of these contaminants on the structure and abundance of the river's microbial community were assessed.

Materials and methods

The Elsa River is a medium-sized tributary of the Arno River in Tuscany (Italy). It was chosen as a case study due to the presence of diverse anthropogenic pressures, including agricultural, industrial, and urban activities, with 3 municipal WWTPs, and the presence of the Parco Fluviale dell'Alta Valdelsa, a hotspot of biodiversity, which is characterised by high calcium carbonate and travertine deposition. Seven sampling stations (ER0–ER6) were selected from upstream to downstream based on the river's hydromorphology and the anthropogenic pressures. At each station, surface water, bed sediment, and bioindicator fish (*S. squalus*) were sampled. Seventeen PPCPs were extracted and quantified across all collected matrices for each sampling point to evaluate environmental concentrations and bioaccumulation, as well as the associated ecological risk through risk quotient calculations. PPCPs were classified into six therapeutic groups: antibiotics (amoxicillin-AMX, ciprofloxacin-CIP, enrofloxacin-ENR, supfamethoxazole-SMX, N4- acetylsulfamethoxazole- N4SMX, erythromycin-ERY), anti-inflammatories (diclofenac-DCF, ibuprofen-IBU), anticonvulsant (carbamazepine-CBZ), stimulants (caffeine-CFF), insect repellents-DEET), analgesics/antipyretics (acetylsalicylic acid-ASP, paracetamol-PAR), and steroid hormones (17 β - estradiol-E2, estrone-E1, 17 α -ethinyl estradiol- EE2), disinfectant (triclosan-TCL). Concurrently, microbiological analyses of water and sediment were performed to assess the potential effects of these contaminants on the structure and abundance of the river's microbial communities.

Results and discussion

Occurrence and distribution of PPCPs

Ten PPCPs (i.e., DCF, IBU, AMX, CFF, DEET, PAR, ASP, E1, E2, EE2) were detected across the three environmental matrices, except for DCF, which was absent from sediments, while AMX was not detected in biota. Overall, steroid hormones and analgesics/antipyretics dominated all matrices and most sampling stations. A clear increase in contamination was observed downstream, particularly from ER4 onwards, where hormones and analgesics became increasingly prevalent, likely reflecting inputs from urban WWTPs. The antibiotic AMX occurred in water and sediments at all stations, with concentrations increasing downstream, especially from ER4, indicating the influence of WWTP discharges. Among the anti-inflammatory drugs, DCF was detected in water and biota but absent from sediments, likely due to its low affinity for particulate matter. Concentrations remained below the Environmental Quality Standard (EQS) established under the European Union Water Framework Directive (WFD). IBU was ubiquitous in water and biota and occurred in sediment at four sites. Its relatively high concentrations in fish and solid matrices suggest prolonged environmental persistence and bioavailability. The stimulant CFF was ubiquitous across all compartments, showing clear increases from upstream to downstream in water and sediments, with the highest concentrations in biota within the river park area. Although readily degradable,

continuous inputs make caffeine pseudo-persistent, supporting its use as an indicator of anthropogenic pressure. The insect repellent DEET occurred at low concentrations in water and biota throughout the study area, indicating widespread but limited contamination. Among analgesics/antipyretics, ASP and PAR were the most abundant compounds detected, showing an increase from upstream to downstream, consistent with the influence of the WWTPs. ASP reached its highest concentrations in water at the most downstream site, and was also elevated in fish muscle collected within the river park. PAR showed similar spatial patterns across all matrices, with accumulation in sediments becoming evident from ER4 downstream, further supporting the role of WWTPs inputs and progressive accumulation along the river course. Steroid hormones were consistently present in all matrices and showed pronounced downstream increases from ER4 onward, confirming WWTPs as their primary source. Their water and sediment concentrations increased markedly from upstream to downstream, exceeding the proposed WFD EQS at all sites. Elevated levels were also measured in fish muscle, indicating persistent exposure and potential for chronic endocrine effects, especially within the river park.

Ecological Risk Assessment

Risk assessment showed that most PPCPs in surface waters posed a low ecological risk ($RQ < 1$). The main exceptions were IBU and the steroid hormones, which consistently exhibited high risk values ($RQ \geq 1$) at nearly all sites. In sediments, AMX, IBU, CFF, E1, E2, and EE2 posed a high risk ($RQ \geq 1$) to benthic organisms at most stations, with CFF and steroid hormones particularly elevated from ER4. In biota, all steroid hormones exhibited high RQ values at every sampling station.

Conclusion

Overall, the Elsa River is affected by diffuse PPCPs contamination with accumulation particularly downstream from ER4, confirming WWTPs as the main sources of these contaminants. The frequent occurrence of PPCPs in biota, including upstream sites and especially within the river park area, suggests persistent exposure and potential long-term effects on aquatic organisms.

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Effects Of Environmental Variability On Thermal Tolerance And Cardiac Performance Of The Invasive Species *Callinectes sapidus* In The Venice Lagoon

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Introduction

The atmosphere is generally considered stable and predictably oxygenated, in contrast to aquatic systems, where variability is the norm, especially in coastal and highly productive zones. Furthermore, it is well established that coastal areas support high levels of biodiversity [1]. Taken together, these observations suggest that coastal zones host a wide range of species adapted to different degrees of environmental variability. For example, in-phase daily cycles of dissolved oxygen and temperature can reach extreme values in productive coastal areas [2-3]. Temperature peaks are typically recorded under hyperoxic conditions, whereas minimum values occur under hypoxic and/or anoxic conditions [1-4]. Although the mechanisms behind the occurrence of these fluctuations are well understood, their effects on the species inhabiting these environments remain to be investigated. In this context, Pörtner [5] highlighted the importance of considering the combined effects of multiple factors through the OCLTT hypothesis, which explains how high oxygen concentrations enhance tolerance to heat stress [1-4]. This study aims to assess the influence of environmental variability, represented by daily fluctuations in dissolved oxygen and temperature, on the invasive species *Callinectes sapidus* in the Venice Lagoon. This goal is achieved by investigating how exposure to different degrees of variability affects mortality and heart rate in this species across two different seasons following a thermal ramp.

Materials and methods

The experiments were conducted at the Hydrobiological Station "U. Ancona" (University of Padova, Italy), between November 2024 and June 2025. Adult male *Callinectes sapidus* (n = 24; WW = 198.73 ± 30.8 g; CW = 14.48 ± 1.11 cm; CL = 6.78 ± 0.39 cm) were caught in the southern Venice Lagoon and acclimated in the laboratory under two conditions: 1) Fluctuation Treatment (FT) reflected seasonal natural daily fluctuations in temperature and oxygen levels; and 2) Constant Treatment (CT) took the mean temperature and oxygen values of FT and kept them constant over the acclimation. Environmental data were recorded through two miniDOT Logger sensors (PME Inc. © 2021). Differences between treatments and variance patterns were assessed using t-tests and Levene's tests. After 6 days of acclimation (feeding stopped at least 24 h before trials [6]) individuals were exposed to a temperature ramp started from the average acclimation temperature, increasing by 1°C every 30 minutes (2°C h⁻¹) [7] until death of all individuals in normoxic conditions. Mortality was established by monitoring the heart rate fh using the heart frequency logger PULSE V2 (Electricblue © 2020), with data processed using the R package "heartbeats". TAB (temperature at which heart rate departs from its exponential increase, indicating the onset of cardiac limitation) was calculated from the Fh- temperature relationship. LT50 was calculated using the Quest Graph™ LC50 Calculator (<https://www.aatbio.com/tools/lc50-calculator>). Differences in LT50 between treatments were tested in each season using a binomial generalized linear model (GLM) with a logit link, assuming a common slope and including treatment as a fixed effect. A Linear Mixed Model (LMM) was applied to test the effect of Fluctuation Treatment and Constant Treatment on fh across temperature increase, acclimation treatment, and seasons. These elements were treated as fixed factors, while individual identity was included as a random factor to account for repeated measurements on the same crab. Model significance was assessed by applying ANOVA Type III in RStudio.

Results and discussion

Environmental data showed no significant differences in mean values between acclimation treatments within each season, while variability was significantly higher in FTs (p_{Levene} < 0.001) (Table 1).

Under rapid warming, FT individuals (turquoise line, Fig. 1) exhibited higher thermal tolerance than CT individuals (red line, Fig. 1), with significantly higher LT50 values in both seasons (spring: FT = 37.81°C, CT = 36.25°C; summer: FT = 40.35°C, CT = 39.50°C; p < 0.001). The larger ΔLT50 observed in spring compared to summer (ΔLT50 = 1.56°C vs 0.85°C) suggests that the effect of variability depends on seasonal conditions. A seasonal increase in LT50 further highlights the role of thermal history. fh increased with temperature up to a peak

(Fig. 1), with significant effects of treatment, season, and their interaction, revealing distinct cardiac responses depending on acclimation history.

Table 1: Acclimation results.

	FT		CT	
	mean	std	mean	std
Summer				
%air sat.	83.35	34.08	83.58	7.7
°C	23.86	1.58	24.04	0.2
Spring				
%air sat.	78.56	10.04	91.32	2
°C	11.6	1.51	11.7	0.3

Although TAB varied with treatment and season (spring: FT = 33.48°C, CT = 37.33°C; summer: FT = 37.70°C, CT = 38.81°C), higher TAB values in CT did not correspond to increased survival, suggesting that cardiac limits alone do not fully explain thermal tolerance. Overall, these results support the idea that environmental variability can be a key driver of physiological resilience.

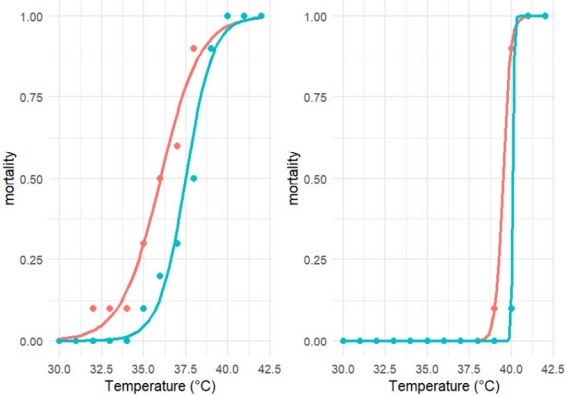


Figure 1. Thermal mortality curves (spring = left plot; summer = right plot; FT = turquoise, CT = red).

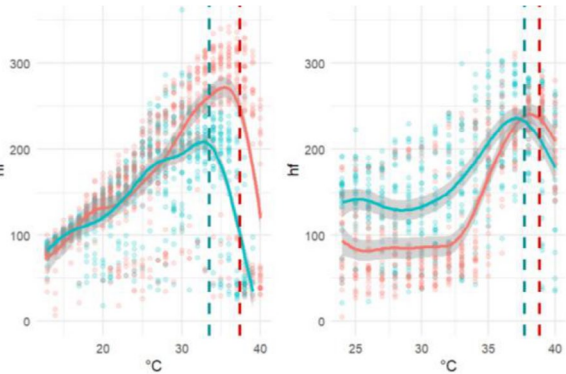


Figure 2. fh as a function of temperature (spring = left plot; summer = right plot; FT = turquoise, CT = red).

Conclusions

Environmental variability in oxygen and temperature enhances thermal tolerance in *Callinectes sapidus*, partially supporting OCLTT predictions. Overall, these results highlight variability as a key driver of resilience in coastal ecosystems.

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How Can Fish Face River Water Pollution? The Effects Of PFAS On *Squalius cephalus* And The Possible Recovery

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Introduction

The presence of contaminants in freshwater ecosystems poses a significant threat to the lives of the organisms that inhabit them. A clear example is the case of per- and polyfluoroalkyl substances (PFAS) pollution in the Veneto Region (Italy), where major contamination was discovered in 2013, although the contaminants were presumably present for many decades. PFAS are characterised by exceptional chemical stability and a tendency to bioaccumulate within organisms [1]. It has been estimated that they can induce lipid steatosis, alterations in cholesterol levels in the blood and other organs [2], and, finally, the activation of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase [3,4]. These features make PFAS particularly hazardous to the environment. Despite this, their environmental impact is still not entirely clear. The present study aims to evaluate whether freshwater fauna can recover following a reduction in PFAS contamination. This is a particularly interesting topic because it strengthens the influence our studies can have on environmental remediation policies.

Materials and methods

A variable number of *Squalius cephalus* individuals were collected from three sites in the Vicenza province, each characterised by a different total PFAS contamination level. Sampling was conducted during two monitoring campaigns separated by approximately one year (Table 1). Organisms were sacrificed, dissected, and all their organs were collected, frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Gene expression analysis

Total RNA was extracted from the liver using PRiMeZOL™ (Canvax) and purified with lithium chloride. A DNase treatment (Promega) was performed, and subsequently, cDNA was synthesised. qPCR analysis was performed using specific primers for *sod1* and *sod2*. *gapdh* was used as the housekeeping gene.

Advanced Oxidation Protein Products

Advanced oxidation protein products (AOPP) were measured using the Witko-Sarsat spectrophotometric method. The concentration of AOPP was expressed as micromoles/litre of chloramine-T equivalent.

Statistical analysis

Analyses were performed using GraphPad Prism 11.0.2. Parametric (ANOVA) and non-parametric (Kruskal-Wallis) tests were chosen based on data distribution.

Table 1: Description of sampling sites (data obtained from ARPAV database)

Site denomination	Total PFAS (ng/l) - mean 2023	Total PFAS (ng/l) - mean 2024
Control	3,5	6
Medium polluted	660,75	601
Highly polluted	1757,25	86

Results and discussion

sod1 and *sod2* expression

The higher expression of *sod2* in specimens collected during the first campaign at the highly polluted site suggests activation of the antioxidant defence system under high-exposure conditions. In particular, mitochondrial involvement appears relevant, given the difference observed between the two isoforms. This response was not detected after reducing contamination levels, suggesting a potential recovery of physiological conditions (Fig. 1).

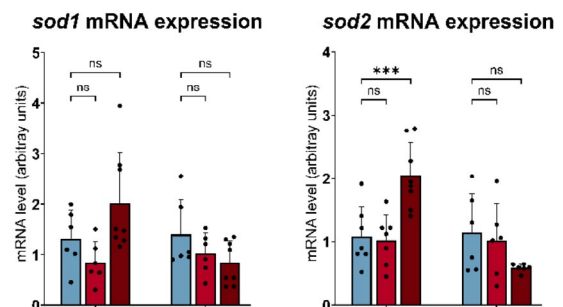


Figure 1. *sod1* and *sod2* mRNA expression in the liver of specimens from the three sites, among the two campaigns, with relative standard deviations (variable n). ($p > 0.12$ (ns), 0.033 (*), 0.002 (**); < 0.001 (***)).

SOD activity

Total SOD activity in the 2024 samples was lower at both contaminated sites than at the control site. This result, in contrast to the gene expression data, suggests a possible reallocation of energy towards other antioxidant enzymes. Indeed, previously published data from the same samples showed increased activity of catalase and glutathione peroxidase. Consistent with previous observations, the trend observed in the 2024 samples was no longer evident in the second-campaign samples from the highly polluted site, suggesting a recovery of physiological conditions following the reduction in PFAS contamination (Fig. 2).

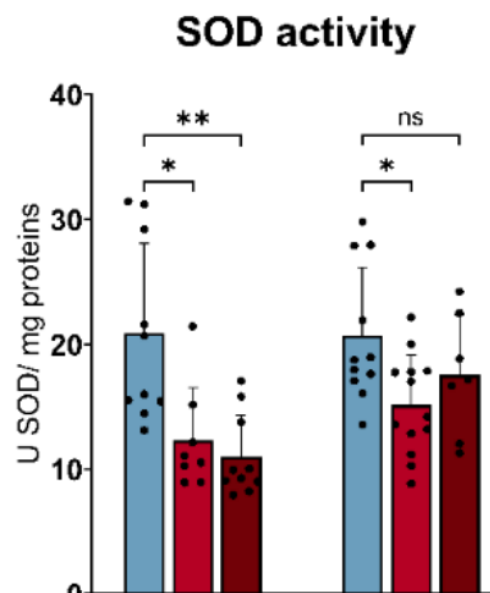


Figure 2. Means and standard deviation of SOD activity in the liver of specimens from the three sites, among the two campaigns (variable n). ($p > 0.12$ (ns), 0.033 (*), 0.002 (**); < 0.001 (***)).

Advanced oxidation protein products (AOPP)

The 2024 AOPP data showed a marked increase in protein oxidation levels in specimens from the highly polluted site, indicating oxidative stress. It has already been established that proteins are among the main targets of PFAS toxicity. Consistent with previous observations, the trend in the 2024 samples was no longer evident in the second-campaign samples from the highly polluted site (Fig. 3).

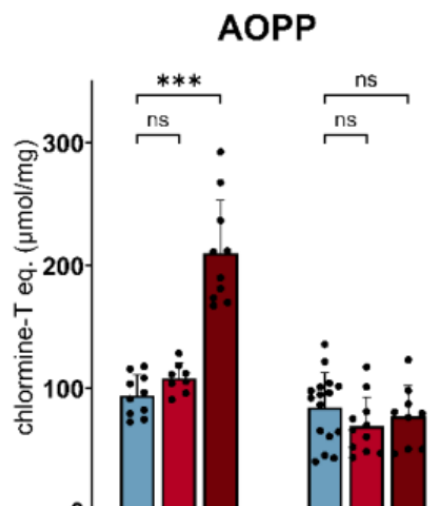


Figure 3. Means and standard deviation of AOPP in the liver of specimens from the three sites, among the two campaigns (variable n). ($p > 0.12$ (ns), 0.033 (*), 0.002(**); < 0.001 (***)).

Conclusions

The data showed that if environmental conditions improve and contamination levels decrease, *Squalius cephalus* appears able to recover. Future work should further investigate the gene expression and activity of other enzymes in the antioxidant system, along with analyses of lipid accumulation and pathways involved in lipid metabolism and cholesterol regulation.

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Beyond Oxidative Stress: Multi-Level Physiological Responses to Chemical Pollutants in the Antarctic Scallop *Adamussium colbecki*

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Introduction

Antarctic ecosystems, despite their geographical remoteness, are continuously exposed to chemicals [1]. Bisphenol A, cadmium, and lead — a mixture spanning emerging and legacy pollutants — reach polar areas mainly by long-range transport, where they bioaccumulate in aquatic organisms [2]. Among them, *Adamussium colbecki*, due to its direct pollutant uptake by filter-feeding, combined with its central role in Antarctic food webs, represents an exceptionally sensitive sentinel. Therefore, physiological disruptions in this species are ecologically consequential well beyond the individual level [3]. While oxidative stress is a recognized hallmark of pollutant exposure in polar bivalves, how antioxidant defenses, metabolic pathways, and tissue-specific responses interact under pollutant pressure remains largely unexplored. The current study addresses that gap by characterizing multi-level physiological responses in *A. colbecki* exposed to chemicals, offering new insight into homeostatic disruptions in Antarctic bivalves.

Materials and methods

Collected specimens of *A. colbecki* were acclimated for 5 days (−1 °C, pH 8.05). 10 individuals per treatment group were exposed for 10 days to: BPA (25 and 250 µg L^{−1}), Cd (10 and 100 µg L^{−1}), Pb (10 and 50 µg L^{−1}), and control (untreated). Biochemical analyses (LPO, AOPP, Se-GPx, CAT, GR, and GST) were performed on gills and digestive glands using established spectrophotometric methods. Transcript levels of related genes and isoforms were quantified by qRT-PCR using gapdh as a reference gene, and data were analyzed by GraphPad Prism 8.4.3, using one-way ANOVA with Tukey's post-hoc test or Student's t-test.

Results and discussion

Oxidative damages

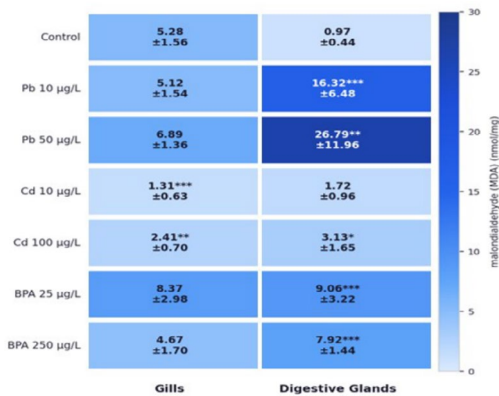


Figure 1. Malondialdehyde (MDA) levels (nmol/mg) in gills and digestive glands of *A. colbecki* following exposure to Pb, Cd, and BPA at two concentrations.

Lipid peroxidation (Fig. 1) illustrated contaminant-specific tissue responses. The digestive gland, due to its role in processing contaminants, was consistently more sensitive than the gills. While Pb revealed a dose-dependent pro-oxidant effect in the digestive gland, BPA exhibited a non-monotonic response —with greater damage at the lower dose. Cd comparatively subdued effect further highlights the mechanistic divergence among the pollutants. Protein oxidation levels (Fig. 2) confirmed the digestive gland as the main target. Both Pb and BPA showed a non-monotonic dose-response in gills, with stronger protein oxidation at lower concentrations — further supporting receptor-mediated rather than purely concentration-driven mechanisms. BPA has the most pronounced effect shifted toward the gills than its lipid peroxidation pattern, indicating pollutant-specific oxidative targets across tissues. Cd remained the least effective oxidant at both concentrations.

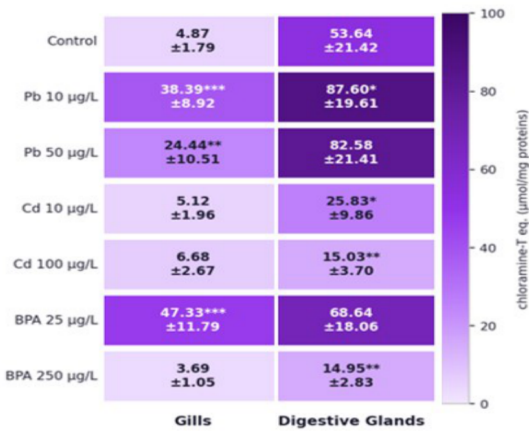


Figure 2. Advanced protein oxidation products (AOPP) levels (µmol chloramine-T eq./mg proteins) in gills and digestive glands of *A. colbecki* following exposure to Pb, Cd, and BPA at two concentrations.

Enzyme Activity

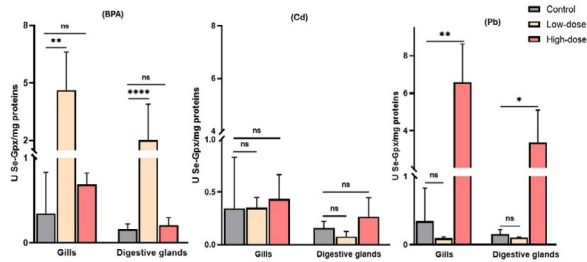


Figure 3. Selenium-dependent glutathione peroxidase (Se-GPx) activity (U/mg proteins) in gills and digestive glands of *A. colbecki* exposed to BPA, Cd, and Pb at low and high doses.

Low dose of BPA significantly upregulated Se-GPx activity (Fig. 3), showing an adaptive antioxidant response, and attenuation at higher dose suggests possible enzyme inhibition or exhaustion of antioxidant capacity under oxidative challenges. While in Pb, the activation of Se-GPx enzyme only occurred at higher concentration, and Cd elicited no significant effects in either tissue, further supporting its distinct mode of action.

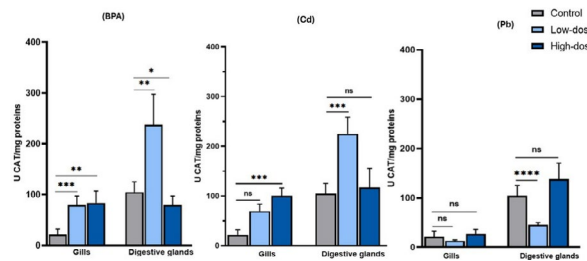


Figure 4. Catalase (CAT) activity (U/mg proteins) in gills and digestive glands of *A. colbecki* exposed to BPA, Cd, and Pb at low and high doses.

A different pattern of CAT activity (Fig. 4) was revealed compared with Se-GPx, with the digestive gland emerging as the primary responsive tissue among groups. Notably, BPA and Cd were more triggered at low doses, pointing to a non-monotonic pattern. While Pb followed a more dose-dependent response in digestive glands, where the highest dose showed no significant change, and had a reduction at the lower concentration.

Conclusions

The presented data are a subset of a comprehensive multi-biomarker dataset including enzymatic activities, gene expression, and metabolic endpoints. Together with all collected results, exposing *A. colbecki* to BPA, Cd, and Pb demonstrated that each contaminant could disrupt redox homeostasis, with non-monotonic tissue- and dose-specific responses, indicating receptor-mediated interference rather than

generalized cytotoxicity. The differential activation patterns of Se-GPx and CAT across groups emphasize the complex regulation mechanisms of oxidative stress in polar bivalves and the ecotoxicological importance of chemicals in Antarctic benthic ecosystems.

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Optimising Landfarming Remediation Technologies in the Framework of the Soil Monitoring and Resilience Directive

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Introduction

The environmental remediation of contaminated soils represents one of Europe's major challenges, as evidenced by the fact that over 60% of European soils are currently in poor health[1]. This is the context of the recent Directive (EU) 2025/2360 on soil monitoring and resilience, which introduces a new paradigm aimed not only at reducing contamination but also at restoring the functionality and health of the soil matrix[1]. Nowadays, to assess the effectiveness of a remediation technology, only the reductions in terms of contaminants is taken into account, leaving aside all the parameters, that, only together, can provide a representative evaluation of the health of the treated matrix and consequentially of the externalities coming from the technology applied. To increase the effectiveness of the landfarming remediation technology fertilizers are traditionally used, increasing the risk of pollution by leaching [2]. An effective alternative is represented by the addition to soil of two organic amendments, coming from the valorisation of waste products: compost and biochar. Previous research has shown that their application contributes to enhanced soil structure and water retention, while also increasing nutrient supply and soil organic carbon stocks [3], [4].

Materials and methods

To assess the actual contribution deriving from the application of these materials, a four months experimental activity was conducted on soil contaminated by heavy hydrocarbons (C>12) from the SIN of Sacco River Basin, characterized by an initial concentration of 9370 mg/kg dry soil. The lab-scale experiment compared three treatment lines, developed in reactors made of steel to avoid material-contamination interaction, with a 1% slope, a leachate collection system, and a weekly turning and moisture control, in order to reproduce the full-scale conditions of a landfarming system. The soil, coming from the plant of Itelyum Regeneration S.p.a., Ceccano (FR), where there is a remediation regime with operational safety measures, was quartered and manually mixed before being added into the reactors. The three experimental conditions tested—biostimulation using a mixture of compost and biochar (BSM), traditional biostimulation with chemical fertilizers (BS), and control (C)—were sampled weekly to assess both the effect on hydrocarbon biodegradation and on chemical parameters such as pH, nitrogen, phosphorus and organic carbon, all described in the Annex I of the Directive (EU) 2025/2360 [1].

Results and discussion

The results showed, for each of the treatment studied, a reduction in hydrocarbons exceeding 85%, indicating a significant intrinsic soil biodegradation capacity (Fig. 1). The concentration, after four months, has reached a steady state for the C treatment, 905 mg/kg dry soil, while for both BSM and BS treatments the measured value is below the threshold fixed by the law under which the real-scale remediation is taking place, respectively 580 mg/kg dry soil and 498 mg/kg dry soil. However, only for the BSM treatment, it is observed that for three successive measurements the value remained below the threshold, proving that the decontamination is effective and not affected by the intrinsic variability of the sample. Additionally, substantial differences emerged in the final health of the treated matrix, compared with the initial conditions T0. As shown in Table 1, for each of the parameters analysed, except for the pH that remains constant, the BSM treatment showed an improvement. Specifically, a significant increase in TOC from 3% to 5% was observed and an improvement in water retention capacity (WHC), from 36% to 43%. An evident improvement is also shown in terms of nutrients, both the nitrogen and the phosphorus increased, reaching values comparable with a potential reuse of the treated matrix as agricultural soil.

Table 1: Parameters comparison (T0: initial time; BSM: biostimulation with mixture; BS: biostimulation; C: control).

Treatment	WHC (%)	pH	TOC (%)	Ntot (mg/kg)	Ptot (mg/kg)
T0	36	7.6	3.0	600	338
BSM	43	7.6	5.1	2170	619
BS	36	7.6	1.7	1570	363
C	36	7.6	1.7	568	417

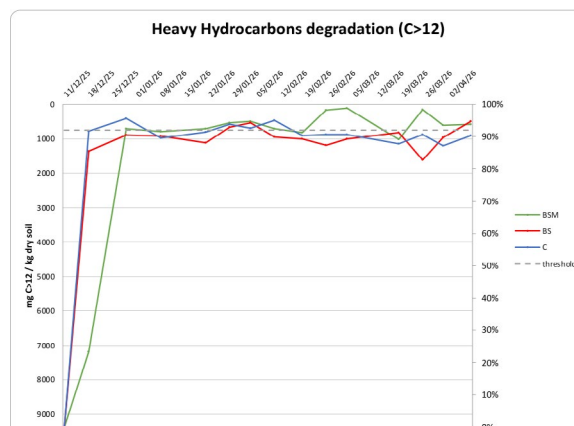


Figure 1. Heavy hydrocarbons degradation (C>12).

Conclusions

The results demonstrate that reducing contaminant concentrations alone is not a sufficient criterion for assessing the sustainability of a remediation intervention. The use of soil amendments, however, is a strategy capable of combining effective decontamination, increased organic matter, and restoration of overall soil health. The study therefore highlights the need to orient remediation interventions toward approaches consistent with the objectives set by Directive (EU) 2025/2360.

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From Ecological Threats to Environmental Sentinels: Invasive Aquatic Species as Models for Assessing Emerging Contaminants

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Introduction

Aquatic ecosystems are increasingly exposed to multiple anthropogenic stressors, including emerging contaminants, invasive species, and climate change, which may interact and compromise ecosystem health and resilience [1]. In this context, the present project explores the use of invasive aquatic species as bioindicators of contaminant exposure, highlighting their potential as experimental models for environmental monitoring rather than solely ecological threats [2]. Three invasive species were selected: the red swamp crayfish (*Procambarus clarkii*), the zebra mussel (*Dreissena polymorpha*), and the Atlantic blue crab (*Callinectes sapidus*).

Materials and methods

Specimens of *P. clarkii* and *D. polymorpha* were collected from the Avigliana Lakes Nature Park (Turin, Italy), acclimated under laboratory conditions, and exposed for 14 days to environmentally relevant concentrations of gadolinium (Gd; 0.1, 1, 10, and 100 µg/L), a rare earth element increasingly detected in aquatic ecosystems and used in medical applications. Bioaccumulation and oxidative stress responses were assessed through the analysis of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and lipid peroxidation (LPO) in different tissues. A second experiment focused on the pharmaceutical indomethacin, a non-steroidal anti-inflammatory drug. *P. clarkii* specimens were exposed for 28 days to concentrations of 2.5, 5, and 10 mg/L. Cell viability was evaluated in hemolymph and hepatopancreas tissues using Trypan Blue and Neutral Red assays, while oxidative stress biomarkers were analysed in hepatopancreas, gills, and muscle. Finally, the marine invasive crab *C. sapidus* was collected from the Sacca di Goro (Adriatic Sea, Italy) and exposed for 20 days to different concentrations of polyvinyl alcohol (PVA; 0.5, 5, and 25 mg/L), a synthetic water-soluble polymer increasingly found in aquatic environments.

Results and discussion

Cell viability and oxidative stress biomarkers were evaluated after 10 and 20 days of exposure. In both *P. clarkii* and *D. polymorpha*, Gd induced oxidative stress responses, although with species- and tissue-specific patterns. In *P. clarkii*, bioaccumulation occurred mainly in gills and hepatopancreas, with increased antioxidant enzyme activity and lipid peroxidation, particularly in the gills [3]. In *D. polymorpha*, oxidative biomarkers showed clearer dose-dependent trends, with stronger responses in the digestive gland and significant lipid peroxidation only at the highest concentration tested. Overall, Gd concentration emerged as the main driver of oxidative stress responses. Exposure to indomethacin produced significant cytotoxic and oxidative stress effects in *P. clarkii*. The hepatopancreas was the most sensitive tissue, showing reduced cell viability and altered antioxidant responses, while haemocyte viability remained largely stable. Biomarker patterns indicated both hormetic and classical dose-response effects, highlighting the complexity of physiological responses to pharmaceutical contamination [4]. PVA exposure in *C. sapidus* also elicited concentration and time dependent responses. Hepatopancreas cells were more sensitive than hemolymph cells, and oxidative stress increased with exposure duration and concentration, as shown by enhanced antioxidant enzyme activity and lipid peroxidation. Histopathological analyses revealed limited alterations in muscle and hepatopancreas tissues but evident structural changes in the gills, especially after prolonged exposure [5].

Conclusions

The three experiments revealed common features across freshwater and marine invasive species: tissue-specific sensitivity, activation of antioxidant defenses, increased oxidative damage at higher exposures, and a particular vulnerability of detoxification related organs such as the hepatopancreas. These findings support the use of invasive species as practical bioindicator models and highlight the value of multi-

biomarker approaches for assessing the ecological risks of emerging contaminants in aquatic ecosystems.

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Bioprecipitation as a One Health Primary Prevention Strategy for Metal and Metalloid Exposure

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Introduction

Heavy metals and metalloids, including arsenic (As), cadmium (Cd), chromium (Cr(VI)), lead (Pb), mercury (Hg), and nickel (Ni), are recognized environmental contaminants of major public health concern, associated with a broad spectrum of adverse outcomes, including cancer, chronic kidney disease, cardiovascular disorders, and neurodevelopmental impairment. Arsenic, cadmium, and hexavalent chromium compounds are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) [1]. Human exposure occurs mainly through contaminated drinking water, agricultural soils, and food chains [2]. Within the One Health framework, environmental contamination is interpreted as a continuum linking ecosystem integrity, animal health, and human wellbeing. Consequently, effective primary prevention strategies should target upstream environmental determinants rather than focusing exclusively on individual-level interventions. Bioprecipitation, defined as the microbially mediated immobilization of inorganic contaminants through carbonate, phosphate, and reductive pathways, may represent an innovative preventive approach capable of reducing contaminant bioavailability before human exposure occurs [3].

Materials and methods

This experimental study evaluated the effectiveness of a non-pathogenic bacterial strain, *Glutamicibacter bergereyi*, in promoting the immobilization/removal of dissolved Cd²⁺ through carbonate precipitation and/or co-precipitation with biogenic carbonates. The selected strain is capable of inducing localized increases in pH and carbonate ion concentration through ureolytic metabolism, thereby facilitating microbially induced carbonate precipitation (MICP). Bacterial cultures were grown in a liquid medium supplemented with urea and sodium chloride under controlled laboratory conditions (30°C, initial pH 7.0, continuous aeration). Cadmium was added at a concentration of 50 ppm to synthetic aqueous solutions simulating contaminated water. Cultures were incubated for eight days, and samples were collected after 2, 3, and 7 days. Residual cadmium concentrations were quantified by inductively coupled plasma mass spectrometry (ICP-MS), while precipitate formation was characterized by scanning electron microscopy (SEM) and mineralogical analyses. Statistical analyses were performed to assess significant differences among experimental conditions and sampling times.

Results and discussion

Previous studies have demonstrated that microbial bioprecipitation technologies can achieve removal efficiencies ranging from approximately 90% to over 99% for bioavailable fractions of As, Cd, Cr(VI), Cu, and Pb under laboratory and field conditions [3–5]. Carbonate-producing microorganisms promote the immobilization of divalent metals through the formation of stable mineral phases, whereas phosphate-mediated precipitation and microbial reduction processes effectively decrease metal mobility and toxicity in contaminated matrices [4,5]. The results of the present study demonstrate that *Glutamicibacter bergereyi* effectively removes dissolved cadmium through bioprecipitation mechanisms leading to the formation of insoluble metal-carbonate compounds. Under the tested experimental conditions, cadmium removal efficiency, calculated relative to the initial concentration of 50 ppm, approached 80%, highlighting the potential applicability of this biological process for pilot-scale remediation systems. From a One Health perspective, microbial bioprecipitation may provide benefits extending beyond environmental restoration by interrupting contaminant exposure pathways affecting livestock, wildlife, and human populations. However, current remediation assessments are largely limited to environmental performance indicators and rarely capture downstream health outcomes. Integrating environmental monitoring with human biomonitoring data, including urinary and blood biomarkers of metal exposure, could provide a more comprehensive evaluation of preventive effectiveness. Such an approach would enable estimation of avoided Environmental Burden of Disease (EBD) and Disability-Adjusted Life Years (DALYs), according to World Health Organization frameworks [6].

Conclusions

Bioprecipitation should be reconsidered not only as an environmental remediation technology but also as a primary prevention intervention operating on upstream determinants of health. Within a One Health framework, its effectiveness should be assessed through integrated indicators encompassing environmental quality, contaminant bioavailability, animal health, human biomonitoring, and disease burden reduction. Achieving this objective requires multidisciplinary collaboration among environmental hygienists, microbiologists, toxicologists, veterinarians, and public health professionals. Such an integrated evaluation framework may facilitate the translation of technological remediation outcomes into quantifiable preventive health benefits and support evidence-based environmental health policies.

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Abiotic Degradation Of Biodegradable Plastics In Water: Chemical Characterization And Ecotoxicological Effects On *Daphnia magna* And Terrestrial Plants

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To reduce the ecological impacts of conventional plastics, the use of biodegradable plastics (BDPs) is increasingly spreading as alternative material. In the environment, BDPs are degraded by abiotic and biotic factors, resulting in the release of degradation products (DPs), including microplastics, potential toxic additives and polymers. This study investigates the impact of BDPs on freshwater environments, which are fragile ecosystems already affected by several anthropogenic pressures, including plastic pollution. To assess the chemical release from BDPs, abiotic degradation experiments were performed by exposing both bio-based and petrol-based BDPs to UV light, with a control sample kept in the dark. Experiments were carried out in both pure water and in water collected from Lake Maggiore and Lake Varese. The resulting BDP-DPs were analysed using different chemical techniques to identify their chemical composition. In addition, leachates were used in toxicity test to investigate the potential effects on biota. Target organisms included ecotoxicological model organisms, like the freshwater crustacean *Daphnia magna* and different species of terrestrial plants, *Sinapis alba*, *Lepidium sativum* and *Sorgo saccharatum*. The use of these model organisms allows for the comparison of toxicity using standardized methods. Moreover, including different trophic levels and multiple species within the same level provides a more comprehensive overview of the potential ecological impacts of BDP-DPs on freshwater ecosystems.

Biological Effects Of Leachates From Commercial And Weathered Plastics Using *In Vivo* And *In Vitro* Approaches

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Introduction

Plastic debris entering the marine environment can affect organisms through both mechanical and chemical pathways. From a chemical perspective, plastics act as vectors of complex mixtures of substances, including intrinsic additives incorporated during manufacturing and environmental contaminants adsorbed during their residence in seawater, forming hazardous chemical cocktails for marine biota [1]. The release and toxicity of these chemicals depend on polymer characteristics (e.g., crystallinity, molecular weight, polymer-additive interactions) and physical properties (i.e. porosity, permeability), but also concentration and properties of additives (molecular weight, polarity, concentration, affinity for the polymer). Environmental conditions—including temperature, salinity, UV radiation, hydrodynamics and biofilm formation—further modulate these processes, while weathering alters polymer structure and surface chemistry, often enhancing additive release [2]. Some of the most used plastic additives are flame retardants, plasticizers (e.g., phthalates), colorants, PFAS, antioxidants and stabilizers. Many of these substances are known to induce toxic effects across multiple biological levels of biological organization, from molecular and cellular to organism - and population-level [3]. Some are regulated by European agencies such as ECHA and EFSA, while others lack specific regulatory thresholds and are only recommended for monitoring. Understanding the composition of plastic-associated chemicals and the mechanisms driving their release is therefore essential for evaluating their potential hazard. In this context, the present study investigates the ecotoxicological effects of chemicals leached from commercial and naturally weathered plastics using complementary experimental approaches. We applied *in vitro* exposure to plastic leachates through standard ecotoxicological bioassays and hemocyte-based assays in *Mytilus galloprovincialis*, assessing multiple endpoints across different biological levels. In parallel, an *in vivo* tank-based experiment was conducted on *M. galloprovincialis* exposed to commercial and weathered plastic fragments to evaluate biochemical and cellular responses to leached chemicals. Results were integrated using a Weight of Evidence (WOE) model to provide a comprehensive hazard assessment posed by plastic-associated contaminants.

Materials and methods

Selection of plastic materials and leachate preparation

Four commercial plastic materials were selected: polystyrene (PS) sheets, polyurethane (PUR) tubes, polypropylene (PP) mussel-farming nets, and polyvinyl chloride (PVC) cables. Selection criteria included widespread industrial use, polymer properties, and frequent occurrence as marine litter. A subset of items was translocated near the port of Ancona (Central Adriatic Sea) for three months to evaluate how environmental weathering influences the leaching process. After this period, plastics were recovered, rinsed with ultrapure water, and gently brushed to remove fouling, following established protocols [4]. The same cleaning procedure was applied to commercial items to ensure analytical reproducibility. Environmental weathering was confirmed by FTIR-UATR analyses that revealed modified spectral profiles in weathered samples compared with the corresponding commercial materials, likely resulting from oxidation, biofouling and inorganic deposition. For the production of leachates, both commercial and weathered plastics were cut into ~1 cm fragments and mixed with pre-filtered artificial seawater at 100 g/L, excepting for PS items (30 g/L) due to lower density. Mixtures were agitated for 72 h and filtered (0.22 µm) to remove potentially generated microparticles. Although these concentrations exceed those typically found in the environment, they were necessary for the preparation of stock solutions used to generate serial dilutions. These dilutions were employed in a Toxicity Range- Finding Test through standard ecotoxicological bioassays to assess concentration-response relationships and determine the appropriate exposure concentration for the subsequent *in vitro* and *in vivo* studies with *M. galloprovincialis*.

Chemical characterization of plastic leachates

Commercial and naturally weathered PUR, PVC, and PP leachates (100 g/L), together with PS leachates (30 g/L), were analysed for the qualitative and quantitative determination of brominated flame retardants - BFRs (PBDEs: 9 congeners; HBCDs: 3 congeners; eBFRs: 9 congeners), PFAS (20 congeners), phthalates (11 congeners), metals (24 elements), selected as chemical classes commonly used as plastics additives and frequently detected as contaminants in the aquatic environments. After the purification, samples were analysed by GC-MS (phthalates, PBDEs, eBFRs), LC-MS/MS (HBCDs), and ICP-MS (metals). Due to their heterogeneous chemical nature, PFAS were processed separately, and extracts were analysed by LC-MS/MS.

In vitro approach: Standard Ecotoxicological Bioassays

Leachate toxicity was evaluated using undiluted leachates (100 and 30 g/L) and a series of serial dilutions (10, 1, 0.5, and 0.1 g/L), covering six exposure concentrations. A battery of ecotoxicological bioassays was employed, including the bioluminescence inhibition in *Aliivibrio fischeri* (UNI EN ISO 11348-3:2019), algal growth inhibition in *Phaeodactylum tricornutum* (UNI EN ISO 11348-3:2019), and embryotoxicity in *Crassostrea gigas* (ISPRA 11/2017).

In vitro approach: Hemocyte exposure to plastic leachates

In vitro exposure of *M. galloprovincialis* hemocytes followed standardized methodologies [5]. After incubation, the medium was replaced with the commercial and weathered PUR, PVC, PP leachates (10 g/L) and PS (3 g/L) reconstituted in BME. After 24 h exposure, cell viability, ROS production, and plasma membrane integrity were assessed following established protocols [5].

In vivo approach: Mussel exposure to plastic fragments

Adult mussels were acclimated for one week and then exposed for 21 days to fragments of commercial and weathered plastics. Organisms were distributed across nine treatment groups (n = 45 per 15 L tank): control, commercial PS, PUR, PVC, PP, and weathered PS, PUR, PVC, PP. Exposure concentrations were 10 g/L for PP, PUR and PVC, and 3 g/L for PS. At the end of exposure, target tissues were collected to exclude the uptake of microplastics potentially generated during the exposure period, and to assess BFRs, PFAS, metals bioaccumulation, and cellular and biochemical biomarkers. Analyses were conducted according to standardized protocols [6,7, 8,9].

Weight of Evidence Elaboration

Results from the Lines of Evidence (LOEs) ecotoxicological bioassays and *in vivo* exposure were elaborated and first evaluated separately using weighted criteria based on the number, magnitude, and toxicological relevance of the observed effects, yielding a LOE-specific hazard index. The resulting indices were then integrated to derive a single hazard assessment for each experimental condition, classified into five hazard categories ranging from Absent to Severe.

Results and discussion

Chemical Characterization of plastic leachates

Chemical analyses showed no detectable level of BFRs or PFAS in any leachate. Only metals and phthalates were found above the LOQ. Among metals, commercial PUR showed the highest levels for Cd (1 µg/L), Pb (2 µg/L), Hg (1 µg/L), exceeding MAC-EQS thresholds. Trace elements such as Fe, Sr and Zn were also detected in commercial PUR, while Al (~150 µg/L) was present in all leachates. Since metals were more readily leached from beached plastics than from commercial ones, these data may reflect environmental adsorption, as confirmed by other studies. Of the 11 phthalates analysed, six exceeded the LOQ, and Virgin PUR contained the highest phthalate levels. Commercial plastics showed a more heterogeneous profile in terms of phthalate content, whereas only DEHA was detected in all weathered samples, indicating loss of phthalates after marine exposure. This is consistent with their high mobility and solubility, which promote migration to the surface and gradual wash-out by seawater [10]. In contrast, DEHA showed an opposite pattern: it was detected in all environmental plastics at higher concentrations compared to the commercial ones.

This increase likely reflects absorption from seawater, where DEHA may originate from other plastic debris or from the degradation of different additives.

In vitro approach: Standard Ecotoxicological Bioassays

Virgin commercial leachates were more toxic than weathered ones across all assays (Figure 1). The strongest effects occurred at 30–100 g/L (Figure 1), especially for commercial polymers. However, the embryotoxicity test showed clear dose-dependent responses, with effects already detectable at 10 g/L, for most of the weathered and commercial polymers. Based on these results, 10 g/L was selected as the lowest effective concentration for the following in vitro and in vivo experiments.

In vitro approach: Hemocyte exposure to plastic leachates

Leachate exposure caused a reduction in cell viability and a marked increase in ROS in all treatments (Figure 2) compared to the control, indicating oxidative stress. Hemocytes exposed to weathered PVC and PP showed higher membrane integrity, suggesting activation of compensatory defence mechanisms under sub-lethal stress.

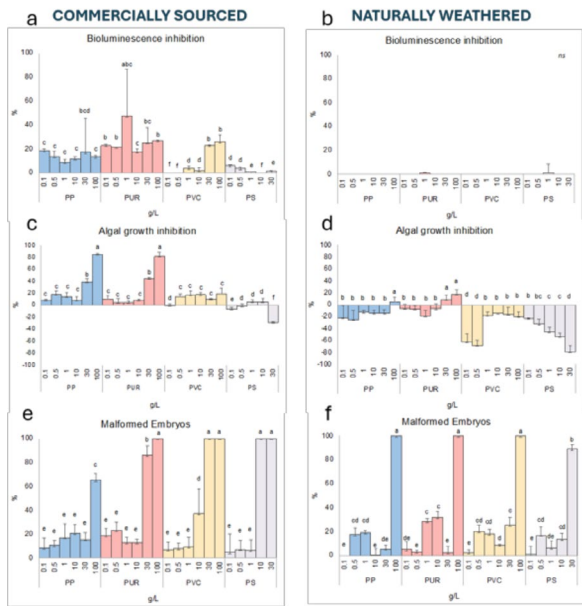


Figure 1. Percentage of bioluminescence inhibition (a -b), inhibition of algal growth (c -d) and embryotoxicity test (e -f).

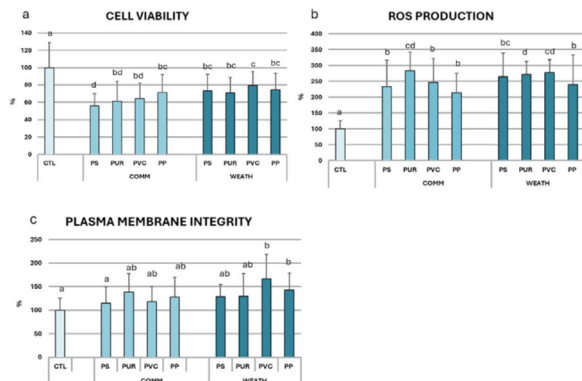


Figure 2. Percentage of cell viability (a), ROS production (b) and plasma membrane integrity (c) in hemocytes exposed to plastic leachates. Letters indicate statistically significant differences between CTL and treatment groups.

In vivo approach: Mussel exposure to plastic fragments

No plastic fragments were detected in mussel tissues, and metal levels were negligible. Bioaccumulation analyses revealed only PFDoA, PFTrDA, and PFTeDA (long-chain congeners) above LOQ. Among BFRs, only BDE-49 and BDE-99 were found, with higher levels (0.2 µg/kg) in mussels exposed to commercial and weathered PVC. The emerging BFR PBT showed elevated levels (0.1 µg/kg) in mussels exposed to weathered PVC. Biomarker analyses showed significant alterations in 11/17 parameters, mainly affecting immune, cholinergic and antioxidant systems. Commercial PS and PUR induced the strongest immune alterations (lysosomal destabilization, altered leukocyte populations, reduced phagocytosis), together with AChE inhibition, indicating disruption of neuro-immune regulation. Weathered plastics induced weaker but still detectable effects (Figure 3). Antioxidant responses showed decreased activity of CAT, GR and

GST in mussels exposed to commercial plastics, with weaker effects in weathered ones. Increased total antioxidant capacity in commercial exposures suggests activation of non-enzymatic defences. Elevated lipofuscin in mussels exposed to commercial PUR and PVC indicates enhanced lipid peroxidation (Figure 4).

Weight of Evidence Elaboration

WOE elaboration for LOE biomarkers and LOE bioassays confirmed the higher toxicity of commercial plastics. The integration of the results highlighted commercial PS and PUR as the most toxic polymers with a moderate hazard index (Figure 5).

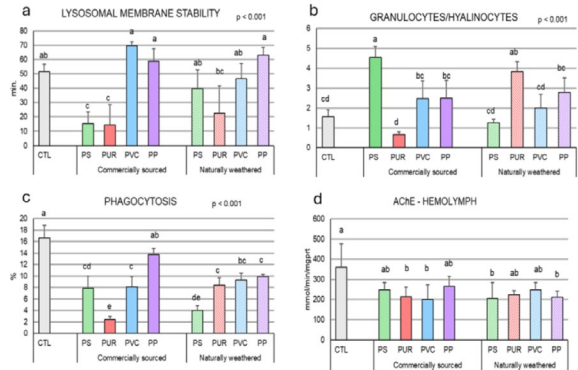


Figure 3. Results of immune (a, b, c) and cholinergic system (d) responses in mussels exposed to commercial and weathered plastic fragments. Values are expressed as mean ± standard deviation. Letters indicate statistical significant differences between the treatment groups and the control (p < 0.001).

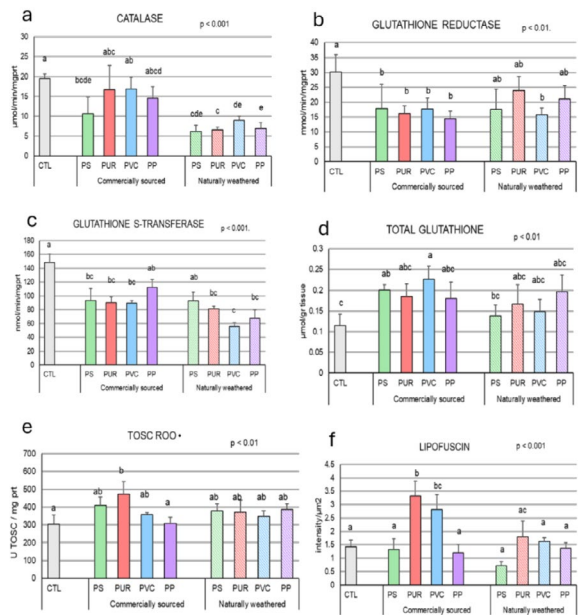


Figure 4. Enzymatic activity of single antioxidant enzymes (a, b, c), total glutathione (d), Total Antioxidant Scavenging Capacity (e) and Lipofuscin content (f) in mussels exposed to plastic fragments. Values are expressed as mean ± standard deviation. Letters indicate statistically significant differences between the treatment groups and the control (p < 0.001).

Treatments	LOE		WOE	
	Biomarkers	Bioassays		
COMMERCIALY SOURCED	PS	MODERATE	MODERATE	
	PUR	MODERATE	MODERATE	
	PVC	MODERATE	SLIGHT	
	PP	MODERATE	SLIGHT	
NATURALLY WEATHERED	PS	SLIGHT	ABSENT	
	PUR	MODERATE	ABSENT	
	PVC	SLIGHT	ABSENT	
	PP	MODERATE	ABSENT	

Figure 5. Weight of evidence Integration for data from LOE biomarkers and LOE bioassays.

Conclusions

Plastics acted as reservoirs of chemical additives, although not all released substances were bioaccumulated in organisms. Polymer structure strongly influenced the leaching profile: commercial PUR showed the highest hazard, likely due to its elastomeric nature and high additive content, while porous PS facilitated both additive release and uptake of environmental contaminants. Weathering modified polymer chemistry and surface properties, promoting additive loss during marine exposure, and reducing the residual load available for leaching. Nevertheless, both commercial and weathered plastics induced measurable biological effects, although commercial plastics consistently produced stronger responses, supporting a predominantly chemical-driven mode of action. Weathered plastics showed weaker but still detectable effects, indicating that three months of environmental ageing reduces, but does not eliminate, the toxic potential of leached substances. Given the complexity of plastic-associated contaminants, the integration of complementary approaches through a Weight-of-Evidence framework provided a robust hazard-focused evaluation. Across all analyses, PUR and PS consistently emerged as the polymers associated with the highest hazard.

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Prediction Of Climate Indicators To Screen And Design Safe And Sustainable Chemicals

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Introduction

Quantitative Structure-Activity Relationship (QSAR) models are widely used to support assessment of hazardous substances and to predict missing toxicological and ecotoxicological data. They are recognized within the REACH regulation and the Safe and Sustainable by design framework (SSbD), particularly in Tier 1 for hazard assessment. Their application to environmental sustainability metrics remains limited. Although machine learning and quantum chemical approaches have been proposed, only a few published models comply with the OECD validation principles for regulatory QSARs [1], raising concerns about their reliability and regulatory acceptance. Environmental impacts are commonly assessed through Life Cycle Assessment (LCA), with climate-related indicators such as Global warming potential and Ozone depletion potential, playing a key role in the Life Cycle Impact Assessment (LCIA) phase [2]. Data for these indicators are generally available only for substances included in international assessments, such as those published by the World Meteorological Organization (WMO) and Intergovernmental Panel on Climate Change (IPCC), leaving many existing and emerging chemicals without coverage. To address this data gap, we developed a framework for screening chemical substances and evaluating their potential environmental impact. Within this framework, a set of QSAR models compliant with the OECD principles for regulatory purpose was developed, enabling the reliable early environmental screening of existing and novel substances.

Materials and methods

We developed a framework for screening chemical substances and evaluating their potential impact on climate. The framework first considers the ability of a chemical to persist in the atmosphere and subsequently it assesses several climate indicators. To achieve this, we developed new QSAR models to predict the following endpoints (climate indicators): Radiative Efficiency (RE), Global Warming Potential over a 100-year time horizon (GWP100yr), and Ozone Depletion Potential (ODP). The dataset was derived from the latest report published by the WMO in 2022 [3]. For each endpoint, the data were divided into training and test sets, and new QSAR models were developed using R. Linear Discriminant Analysis (LDA) and Multiple Linear Regression by means of Ordinary Least Squares (MLR-OLS) were employed as modelling approaches. The most relevant molecular descriptors were selected using the step-up variable subset selection procedure. The best models for each endpoint were chosen based on the coefficient of determination for regression models and the misclassification rate for classification models. Model robustness and potential overfitting were evaluated using the bootstrap procedure. Finally, the applicability domain (AD) was assessed for the best performing models to define the reliability of their predictions. The final models were then applied to a large screening dataset containing more than 12000 potential refrigerant compounds using the QSAR-ME Profiler software (QSAR-ME Profiler 2025).

Results and discussion

The data curation procedure led to the creation of a final dataset consisting of 672 compounds. These chemicals are split into 20 different structural classes and are mostly halogenated. The procedures of data modelling led to the selection of four QSARs for the endpoints of interest, which are listed in Table 1 for the regression models and in Table 2 for the classification model. The application of the models to screen more than 12000 potential refrigerants was useful to identify a list of safer alternatives to these chemicals. Among these compounds, 538 substances were found to fall within applicability domain the three QSAR models, and 253 out of the 538 substances were predicted to have GWP values lower than 100 and are potential safe according to European Commission.

Table 1: summary of the statistical results of the MLR-QSAR. R^2 is the coefficient of determination, RMSE is the root mean squared error.

Endpoint	n° Training	n° Ext. test	R^2 Training	R^2 Ext. test	RMSE Training	RMSE Ext. test	RMSE Boost.	n° descriptor
Log ₁₀ GWP _{100yr}	397	132	0.75	0.70	0.45	0.51	0.51±0.002	4
Log ₁₀ RE	503	167	0.87	0.88	0.15	0.14	0.17 ± 0.001	4
Log ₁₀ ODP	214	71	0.84	0.80	0.20	0.22	0.34 ± 0.001	5

Table 2: summary of the statistical results of the LDA-QSAR to predict ODP values. ACC is the accuracy, MR is the misclassification rate, SN is the sensitivity, SP is the specificity, P is the precision.

Endpoint ODP	n	ACC	MR	SN	SP	P	n° descriptor
Training	438	0.99	0.01	0.98	0.99	0.99	1
External Test	145	1.00	0.00	1.00	1.00	1.00	1

Conclusions

The QSAR models developed in this study have proven to be reliable tools for predicting climate change indicators. The here proposed models are compliant with OECD requirements for the use of QSARs for regulatory purposes [1]. The new QSARs can be used to predict the potential impact on climate of new or untested chemicals, thereby reducing data gaps in these assessments. The proposed models are freely available in QSAR-ME Profiler 2025, making them easily accessible for practical applications. They can support the selection of environmentally preferable alternatives within sustainability assessments, including LCIA and the SSbD framework.

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Plastic Weathering And Leachates Toxicity

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Introduction

The ecological risk associated with plastic pollution in aquatic ecosystems has historically focused on the physical impacts of micro- and nanoplastics. However, emerging scientific evidence demonstrates that acute and chronic toxicity in biota is primarily driven by the leaching of complex mixtures of chemical additives that are not covalently bound to the polymer matrix. Current regulatory frameworks (e.g., REACH, Food Contact Materials regulations) exhibit structural limitations, as they assess chemical risk on a "substance-by-substance" basis using static migration tests on virgin polymers. Under realistic environmental conditions, the synergistic effects of UV-B radiation and mechanical stress (weathering) accelerate plastic degradation, generating a leachate rich in parent additives, photodegradation products, and Non- Intentionally Added Substances. Literature Studies on primary producers (phytoplankton) reveal significant inhibition of Photosystem II efficiency and a subsequent arrest in cell growth. In consumers (invertebrates and fishes), sublethal exposure induces intracellular oxidative stress, lysosomal membrane instability, neurotoxicity, and severe disruptions in embryonic development, particularly in the highly sensitive D-shell larval stage of bivalves [1]. Furthermore, recent approaches confirm that leachates derived from weathered commercial macroplastics exhibit higher toxicity compared to pure, virgin polymers, highlighting an omnipresent chemical threat. This work examines the ecotoxicological impact of conventional and biodegradable plastic leachates within diverse aquatic compartments in relation to aging conditions. To this aim we explored the effects of leachates before and after PE, PP, PS, PLA and Mater-Bi® weathering by in lab and natural exposure.

Materials and methods

PE, PP, PS, and PLA fragments exposure in the laboratory to 20, 30, and 40 °C (72 h), along with natural rooftop weathering for 3 months. Leachate samples, obtained in Milli-Q water (1 g/L) under continuous agitation for 24 h, followed by filtration at 0.45 µm, were tested by a battery included *Aliivibrio fischeri* (ISO 11348), *Raphidocelis subcapitata* (OECD 201), and *Daphnia magna* (OECD 202); PE, PP, PLA, and Mater-Bi® exposure for 3– 6 months at five coastal sites exhibiting different levels of natural (pH and T) and anthropogenic pressures (contaminant occurrence), located near the vents of Vulcano Island (Aeolian Islands). Leachates prepared in Artificial Seawater (ASTM 1994) at a 1:10 ratio (dw/v) were tested with *A. fischeri* (ISO 11348) and *Dunaliella tertiolecta* (ISO 10253). Where possible, Results were integrated into a synthetic index (TBI) of ecotoxicological risk [2].

Results and discussion

The results of the first experiment (Fig.1) show that plastic degradation, particularly under thermal and environmental stress, increases the release of harmful leachates and their toxicity to aquatic organisms. PS and PP exhibited low toxicity in their virgin state but became significantly more toxic after degradation, while PLA showed consistently high toxicity regardless of treatment, indicating a persistent release of bioactive compounds. In contrast, PE was the least toxic polymer under all tested conditions. Higher temperatures and outdoor weathering generally enhanced toxicity, especially for PS and PP, due to accelerated degradation and additive release. Among the tested organisms, algae were the most sensitive, highlighting their vulnerability to plastic- derived contaminants.

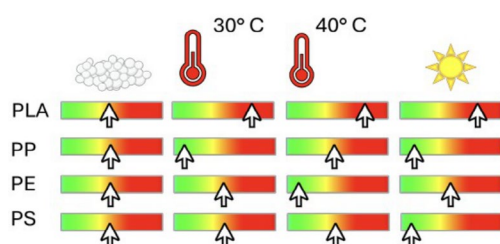


Figure 1. Leachate toxicity based on an integrated toxicity index in freshwater system.

In seawater exposure, the ecotoxicological responses (Fig. 2) of plastic leachates varied across the different sites and exposure times (3 and 6 months), suggesting that both polymer type and local environmental conditions influenced toxicity. After 3 months, all polymers induced moderate effects on *D. tertiolecta* and *A. fischeri*, with PE and PLA showing consistently higher toxicity in all sites. Mater-bi generally exhibited lower effects, particularly in low-stress environments. After 6 months, the effects became more pronounced and site-dependent. In sites characterized by natural or anthropogenic stressors (acidification/anthropization and temperature/acidification), toxicity of bio-based and conventional plastics increased. The two bioassays showed distinct sensitivity patterns to plastic leachates. *D. tertiolecta* generally exhibited stronger inhibition than *A. fischeri*, particularly for PE and PLA after 3 and 6 months of exposure. This suggests that algal cells, due to their photosynthetic metabolism and larger surface area, may be more susceptible to compounds affecting membrane integrity, nutrient uptake, and photosynthetic pathways.

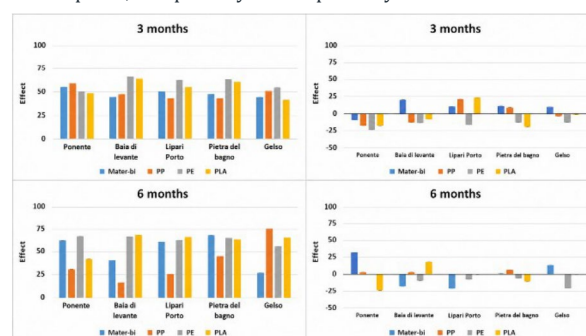


Figure 2. Toxic effects of plastic leachates (Mater-bi, PP, PE, PLA) after 3 and 6 months of field exposure at five coastal sites on *D. tertiolecta* and *A. fischeri*.

In contrast, *A. fischeri* responses were more variable and appeared less affected at early exposure times but became more pronounced after 6 months, especially at sites subjected to environmental stressors. This delayed rise in bacterial toxicity suggests that prolonged degradation may release compounds that interfere with bacterial luminescence systems, such as surfactants or oxidative by-products.

Conclusions

Overall, both polymer type and environmental conditions strongly influence ecotoxicological risk, emphasizing that degraded plastics, and even some bioplastics such as PLA, can pose significant threats to freshwater ecosystems. Marine algae respond more readily to early polymer degradation products, while bacteria are more sensitive to later-stage leachates enriched in reactive or soluble compounds.

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Streams as Vectors of Plastic Pollution in the Lake Orta Watershed (Northern Italy)

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Small streams can act as important connectors between terrestrial sources of pollution and receiving water bodies, potentially contributing to plastic fluxes at the catchment scale. Small freshwater tributaries remains largely overlooked. To fill this knowledge gap, this study examined plastic occurrence in six streams discharging into Lake Orta (Northern Italy): Fiumetta, Pescone, Qualba, Plesina, Lagna, and Pellino. Sampling was carried out at the mouth of each tributary using 100 µm plankton nets coupled with flowmeters to quantify filtered water volumes. Suspected particles were isolated and subsequently analysed through micro-Fourier Transform Infrared spectroscopy (µFT-IR) for polymer identification and particle characterisation. Overall, results indicate the widespread occurrence of plastics in the investigated tributaries. Particles smaller than 1 mm represented the majority of the identified plastics. Variability among tributaries suggests that local catchment characteristics and anthropogenic pressures may influence the transport and distribution of plastics within the Lake Orta watershed. Our findings highlight the relevance of small tributaries as pathways for the transfer of plastics from inland watersheds to lacustrine environments. These results also emphasise the importance of including smaller freshwater systems in monitoring strategies aimed at understanding plastic pollution at the basin scale. The study contributes to improving our understanding of plastic contamination in freshwater networks and provides a baseline for future investigations aimed at assessing seasonal variability within the Lake Orta basin.

Lead Poisoning From Hunting Ammunition: The Need For A Restriction

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The toxicity of lead has long been known. In humans, it can alter metabolic processes, impairing many body districts even at very low exposures. Children and women of child-bearing age are particularly at risk because lead hampers the development of the central nervous system, causing permanent dysfunctions. In elders, the main damage is to the renal and circulatory systems. The increasing impact of lead on the human population over the last century prompted health authorities to call for restrictions on its use. Consequently, lead has been banned from paints, gasoline, toys, inks and many other uses and products. Inexplicably, lead is still used to produce hunting bullets and gunshot, despite the wide consensus of the scientific community on the risks they pose to human health, wildlife and the environment. According to the European Chemicals Agency, in the European Union every year 14,000 tonnes of lead gunshot are dispersed into the environment. Over time, gunshot undergoes a slow chemical-physical alteration (which can last up to a century) involving the release of bioavailable lead compounds into the soil and water. This massive environmental contamination represents a heavy legacy for future generations. Conversely,

immediate damage occurs when lead ammunition hits prey. Upon impact against the animal's body, bullets and gunshot fragment, producing many tiny particles scattered across the meat even at a considerable distance from the wound. This makes game meat consumption a health risk for both humans and carnivorous animals. Humans are exposed because the cooking process, especially when acidic ingredients are used, facilitates the transformation of metallic lead into the ionic form, easily absorbed by the intestine. The European Agency for Food Safety found that frequent consumers of game meat are exposed to lead at levels six-fold higher than the overall population. Yet, a maximum level of lead was set by law for meat from domestic animals but not for game. Raptors have very acid gastric juices that solubilize the metallic lead they ingest along with their prey. A study revealed that 98 (47.8%) of 205 golden eagles and vultures found death or weakened in Italy had lead levels exceeding background values at least in one tissue. Preventing all this is possible by mandating the use of lead-free ammunition, as already done in some countries, such as Denmark. Furthermore, a maximum level of lead in game meat should be established. The solutions exist, now all that is needed is political will.

Bisphenol A in the deep south: gut oxidative stress responses in the Antarctic fish *Trematomus bernacchii*

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Antarctica is increasingly exposed to contaminants of emerging concern, yet the toxicological impact of many pollutants on native fauna remains unexplored. Among these, bisphenol A (BPA), a synthetic endocrine-disrupting compound widely distributed in polar environments, is of particular concern for species that evolved under stable, pristine conditions and may therefore have limited capacity for detoxification. The present study focuses on the notothenioid fish *Trematomus bernacchii*, a model species highly specialised to the cold, stable waters of the Southern Ocean. This species has evolved unique physiological adaptations including antifreeze proteins, modified buoyancy, and specialised antioxidant defences; yet these same adaptations may simultaneously increase its vulnerability to xenobiotics such as BPA. A further, largely unexplored dimension concerns the gut microbiota. The mucosa-associated intestinal microbiota of Antarctic fish is cold-adapted and functionally specialised, contributing to nutrient processing and xenobiotic biotransformation. Studies on temperate fish species suggest that BPA can disrupt microbial homeostasis, while microbiota composition may in turn modulate host responses to chemical stress. However, no study has yet investigated these reciprocal host-microbiome interactions under the extreme environmental conditions of the Southern Ocean. The POLAR-TOX project addresses this knowledge gap by investigating, for the first time, BPA-induced effects on *T. bernacchii* using a multi-level biological approach. Intestinal wall samples from fish experimentally exposed to BPA (25 µg/L) and from unexposed controls were collected during the 38th Italian Antarctic Expedition. Specifically, this project focuses on assessing oxidative stress responses through spectrophotometric quantification of total antioxidant capacity, lipid peroxidation, protein carbonylation and oxidation, and the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases

(GPXs). Gene expression of key antioxidant isoforms (sod1, sod2, cat, gpx1, gpx2, gpx4, bax) is also evaluated by qRT-PCR. Preliminary results indicate that overall BPA likely induce an unbalanced antioxidant response, by increased SOD and CAT activities but reduced total GPX activity. This imbalance may reveal a vulnerability in the antioxidant defence network and contribute to the observed increase in oxidative damage to proteins and lipids. Overall, these findings suggest that BPA induces oxidative imbalance in the intestinal wall of *T. bernacchii*, revealing a potential vulnerability point in the antioxidant defence network of this cold-adapted species. Ongoing microbiota analyses will further clarify whether these physiological alterations are associated with changes in the mucosa-associated gut microbial community.

Particulate Organic Carbon And Nitrogen And Relative Stable Isotopes Ratios In Arctic Environment

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Polar marine ecosystems represent some of the most vulnerable areas on the planet in the face of challenges posed by global climate change. Rising temperatures, the accelerated "freshening" of marine waters due to ice melt, and alterations in the seasonal cycles of sea ice formation are causing severe cascade effects on Arctic trophic webs. In this context, the characterization of the spatial distribution and isotopic composition of carbon and nitrogen in particulate organic matter (POM) serves as a powerful analytical tool. These parameters allow the identification of variations in crucial biogeochemical processes, such as nutrient utilization by phytoplankton, and provide an essential baseline to interpret dietary regimes, migrations, and movements of animals belonging to higher trophic levels at latitudes above 58°N. This research analyzed the isotopic composition of POM along the SDA route and within the sub-Arctic fjords of Greenland. Oceanographic sampling was conducted using Niskin bottles mounted on a high-precision CTD rosette system at various depths throughout the water column. Water samples were processed immediately on board via vacuum filtration through pre-ashed 47 mm GF/F glass fibre filters. In the laboratory, filters intended for POM analysis were treated with 100 µL of 1N HCl to completely remove inorganic carbonates and subsequently dried at 60°C. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) analyses were performed simultaneously using an isotope ratio mass spectrometer (IRMS Delta Plus, Thermo Scientific) coupled with a CHN elemental analyzer (Flash 2000, Thermo Scientific). To ensure data accuracy, reproducibility, and quality control, the certified reference materials were used. Sampling stations were divided into geographic groups: fjords, coastal zones, offshore areas, and stations south of the Faroe Islands. Preliminary results show relatively uniform baseline in terms of isotope ratios across most marine stations, with $\delta^{13}\text{C}$ values clustering around -24.5 ‰ to -22.0 ‰ and $\delta^{15}\text{N}$ ranging between 4.5 ‰ and 6.0 ‰. However, elevated $\delta^{15}\text{N}$ peaks up to +8.5 ‰ were recorded in specific offshore and coastal stations, indicating high nutrient utilization or localized trophic enrichment. Conversely, markedly depleted $\delta^{13}\text{C}$ values down to -28.0 ‰ in inner fjord stations suggest a significant terrestrial fingerprint from glacial and continental runoff. The next step of the research involves applying Bayesian mixing models through the SIMMR

package in the R environment. This approach will allow a precise estimation of the relative contribution of terrestrial and marine sources to POM, enhancing our understanding of global organic matter fluxes in a rapidly changing Arctic.

Immune Responses Of Human Monocytes To Pristine And UV-Aged Micro- And Nanoplastics

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The widespread presence of micro- and nanoplastics (MNPs) in the environment has raised increasing concerns regarding their potential impact on human health. Due to their small size, persistence and ability to interact with biological systems, MNPs may enter the human body through ingestion, inhalation or dermal exposure and potentially reach immune-relevant compartments. Chronic exposure to MNPs has been associated with inflammatory responses and immune system impairment, potentially increasing the risk of chronic inflammatory and autoimmune diseases. Although MNPs have been shown to affect immune cell function, the mechanisms underlying their effects, particularly after environmental aging, remain poorly understood. This study investigates the in vitro effects of pristine and UV-aged MNPs on primary human monocytes, focusing on inflammatory responses and immune-related pathways. Monocytes were exposed to MNPs of different sizes and concentrations, and cellular responses were assessed by flow cytometry and gene expression analysis by qPCR, targeting pathways involved in inflammation, inflammasome activation and antigen presentation. UV aging was considered to better mimic environmentally relevant exposure conditions, as photoaging can modify particle surface properties and potentially influence their biological reactivity. MNP exposure induced size-dependent immunomodulatory effects. Pro-inflammatory responses were markedly increased, as shown by the upregulation of IL-1 β , IL-8 and TNF- α , particularly following exposure to larger particles. NF- κ B was also upregulated, supporting enhanced inflammatory activation. In parallel, HLA-A expression was reduced, suggesting a potential impairment of antigen presentation pathways. Conversely, NLRP3 downregulation indicated a modulation of inflammasome-related responses, pointing to a possible decoupling between inflammatory signaling and inflammasome activation. Notably, UV-aged MNPs elicited stronger inflammatory responses compared with pristine particles, highlighting the role of photoaging in enhancing MNP immunotoxicity. These results suggest that environmental transformation may increase the capacity of plastic particles to interfere with innate immune functions. Overall, these findings demonstrate that MNPs can significantly affect immune homeostasis and that environmental aging represents a critical factor in modulating their biological impact. The inclusion of environmentally aged particles in experimental models may therefore improve the relevance of in vitro studies and contribute to a more accurate assessment of the immunotoxicological risks associated with plastic pollution.

Biochemical Responses Of The Solitary Tunicate *Ciona robusta* To Vanadium Exposure: Is Vanadium Tolerance A Biochemical Superpower Of Sessile Marine Invertebrates?

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In seawater, vanadium occurs naturally as a trace metal at dissolved concentrations of ~30–35 nM, yet increasing anthropogenic inputs — including fossil fuel combustion and industrial discharges — are elevating its environmental burden in coastal and transitional ecosystems. Despite its ecotoxicological relevance, vanadium remains a neglected contaminant compared to other metals of concern. Sessile marine invertebrates face disproportionate exposure risks due to their inability to escape contaminated habitats; paradoxically, certain taxa — most notably tunicates — exhibit extraordinary vanadium bioaccumulation capacity, up to 10,000-fold above ambient levels, implying the evolution of highly effective tolerance mechanisms. This study investigated the ecotoxicological effects of sodium orthovanadate on the solitary tunicate *Ciona robusta*, a cosmopolitan and ecologically relevant species, following 7- and 14-day exposures to environmentally relevant (100 µg L⁻¹) and challenge (1000 µg L⁻¹) concentrations. A multivariate biomarker approach was applied, targeting inflammation-related enzymes (phenoloxidase, arylsulfatase, acid and alkaline phosphatase), antioxidant and detoxification responses (catalase, superoxide dismutase, glutathione S-transferase), neurotoxicity (acetylcholinesterase), and oxidative damage endpoints (protein carbonylation, lipid peroxidation). Remarkably, most biomarkers remained unaffected across treatments, underscoring the capacity of *C. robusta* to withstand vanadium stress without eliciting broad physiological disruption. Alkaline phosphatase inhibition at the highest concentration after 7 days — consistent with vanadate interference with phosphate metabolism — was transient and fully recovered by day 14. A significant increase in superoxide dismutase activity at 14 days suggests a delayed but effective antioxidant mobilization. These findings highlight *C. robusta* as a valuable ecotoxicological model, not only for understanding vanadium dynamics in tunicates, but more broadly for investigating biochemical resilience strategies in sessile marine fauna exposed to metal contamination in coastal and transitional ecosystems.

From Regulatory Assessment to Harmonised Classification: Endocrine Disruptors under REACH, BPR, PPP and CLP

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Endocrine disruptors are an increasing priority in European chemicals regulation because of their potential to alter endocrine function and cause adverse effects on human health and the environment. Under Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Regulation (EU) No 528/2012 on biocidal products (BPR), and Regulation (EC) No 1107/2009 on plant protection products (PPP), the identification of endocrine-disrupting properties is a key element in hazard assessment and risk management. This regulatory framework has gained further relevance following the amendment of Regulation (EC) No 1272/2008 on the Classification, Labelling and Packaging (CLP) by Commission Delegated Regulation (EU) 2023/707, which introduced specific hazard classes for

endocrine disruption affecting human health (ED HH) and the environment (ED ENV). The objective of CLP classification is to incorporate the available evidence into a harmonised system of classification, labelling and hazard communication. The alignment between the available ED assessments and the new CLP classification is particularly relevant. The guidance, provided by European Chemical Agency (ECHA), indicates that active substances already concluded to have endocrinedisrupting properties under BPR or PPP procedures, as well as substances identified as substances of very high concern (SVHCs) and included in the REACH Candidate List because of their endocrine-disrupting properties, should be assigned to ED HH Category 1 or ED ENV Category 1 under CLP. This study analysed ECHA data on substances already identified as endocrine disruptors, including SVHCs in the Candidate List, substances proposed as endocrine disruptors in the Endocrine Disruptor List, and active substances identified as endocrine disruptors under the BPR and PPP assessment processes. The analysis aimed to describe the current status of assessments, regulatory outcomes, and the main chemical groups involved. The results indicate that environmental endocrine-disrupting concerns are more frequently reported than concerns relating to human health. The continuously evolving nature of ECHA regulatory lists suggests that additional substances may be assessed as endocrine disruptors in the future. The introduction of the ED HH and ED ENV hazard classes represents a major regulatory transition, as endocrine disruption is no longer addressed solely through case-by-case assessment but can now be formally communicated throughout the supply chain. In this context, the Candidate List may serve as an early regulatory indicator of future impacts on classification, labelling, and substitution strategies.

Biological Effects Of Tralopyril In *Calanus* spp. Under A Climate Change Scenario

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Antifouling agents, like Tralopyril, are compounds developed to prevent the settlement and growth of unwanted invertebrates on marine infrastructure. These substances might exert toxic effects on non-target marine species. At the same time, marine organisms are increasingly exposed to environmental stressors, including rising ocean temperatures related to climate change. The combined exposure to chemical contaminants and environmental stressors may lead to synergistic interactions, potentially amplifying adverse physiological effects in marine life. In order to investigate the role of temperature in modulating physiological and molecular responses to Tralopyril, wild and cultivated copepods *Calanus* spp. were exposed to Tralopyril (0.6 µg/L) at 10°C and 15°C for 96h. Copepods were collected to assess in vivo metabolic rates by measuring oxygen consumption using a multichannel microplate respirometry system and the expression of antioxidants, xenobiotic metabolism and general stress related genes. Results revealed different sensitivities of wild and cultured copepods to Tralopyril exposure, particularly under elevated temperature conditions. Cultured copepods exhibited increased oxygen consumption, whereas only minor changes were observed in the expression of stress-related genes. In

contrast, wild copepods showed a greater capacity to cope with chemical and thermal stress, potentially associated with enhanced antioxidant defenses and xenobiotic metabolism. These differences may reflect adaptive responses to environmental factors such as seasonal variability, temperature fluctuations, and food availability. Further studies are required to investigate lipid and metabolic biomarkers, seasonal changes in copepod physiology, and the degradation pathways of Tralopyril, including the identification and quantification of degradation products and the assessment of their potential ecotoxicological effects.

Nitrogen Transformation Test: Special Cases Observed In GLP Studies And Criticities Of OECD 216 Guideline

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The test described in OECD 216, applied in ecotoxicological field, aims to investigate the effect of chemicals on soil microorganisms, evaluating how a treated soil could transform nitrogen over time when compared with a control group. To trigger this process, soil is amended with lucerne, that will be consumed by microorganisms, releasing nitrates and increasing their concentration until a plateau. At the start of the test and after 14-days intervals, samplings are carried out in order to extract nitrates and to analytically quantify them. After a minimum period of 28 days, the test can stop or be prolonged up to 98-100 days, until deviation between nitrification rates is inside the limits of $\pm 25\%$. Some crucial factors can influence the course of the test, data elaborations and calculated endpoints. Soil used in tests must satisfy physico-chemical characteristics described in the guideline. Depending on the soil used, nitrates can follow a “rise” response (immediate increase) or a “dip” response (initial decline and subsequent increase), that appears to be soil-specific. The initial part of dip response is not mathematically considered in the formula when nitrogen formation rates are calculated from D0. Comparison between nitrate formation rates of different groups can be calculated referring to D0 (cumulative approach) or to the previous sampling date (incremental approach). Both are an approximation of nitrates production over time and are graphically represented by a straight line between initial and final concentrations examined. Cumulative approach represents a less representative approximation, especially in tests with dip response: as the dip part in the D0-D42 period of the test is not considered, high deviation values can be obtained, that, even if mathematically correct, are far from the actual condition of the test. After approximately 70 days, nitrate production gets closer to a plateau phase. In this part, increasing in nitrate concentration during the 14-days intervals can be minimal and the calculated rates can be near to 0. Using incremental approach, small differences between values of nitrate production rates can easily result in high percentage of deviations of treated groups from control. In these cases, deviation can get outside the limits $\pm 25\%$ even if it had been inside from the very start of the test. The two approaches have different limitations: the cumulative approach is more critical during the initial dip phase, while the incremental approach becomes less reliable during the final plateau phase.

First Evidence Of Persistent Organic Pollutant-Induced MicroRNA Signatures In Killer Whale Fibroblasts As Biomarkers For Marine Pollution And Cetacean Health

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As sentinels of ocean health, cetaceans are high-trophic predators that accumulate contaminants, making them valuable bioindicators of chemical pollution. Killer whales (*Orcinus orca*) are particularly vulnerable to emerging and legacy contaminants such as persistent organic pollutants (POPs), which are toxic bioaccumulative compounds known to disrupt endocrine, immune and reproductive functions, although their underlying mechanisms of action remain incompletely understood. Exposure to POPs may alter gene expression, including microRNAs (miRNAs), small regulatory molecules involved in development, immune responses and stress adaptation, making them promising biomarkers of environmental exposure. However, the effects of POPs on miRNA expression in cetaceans remain unexplored. Ecotoxicological research on cetaceans is constrained by ethical, technical and legal limitations, highlighting the value of ex vivo and in vitro models such as cetacean fibroblast cultures to investigate contaminant responses and pollution-induced cellular mechanisms. As part of the BLOWOMICS project, this study investigates the effects of POP exposure on miRNA expression in killer whale fibroblast cultures to identify candidate biomarkers of pollution. Early-passage skin fibroblast cultures derived from biopsies of four free-ranging killer whales (*Orcinus orca*) collected in the Kvænangen fjord near Skjervøy, northern Norway, in November 2023, were cryopreserved ($-80\text{ }^{\circ}\text{C}$) prior to experimental exposure. The sampling included three males and one individual of undetermined sex. After thawing, cells were exposed for 24 h to an environmentally relevant POP mixture reflecting the ten most abundant contaminants detected in killer whale blubber, at $1\times$ and $10\times$ environmental concentrations, alongside medium control and vehicle control (DMSO) treatments as previously described. Total RNA was extracted from culture supernatants using the miRNeasy Mini Kit and RNeasy MinElute Cleanup Kit (Qiagen) and sequenced with Illumina small RNA sequencing. Candidate miRNAs were predicted from the killer whale reference genome using MirMachine and used for expression quantification. Raw reads were quality filtered using MirTrace, and candidate miRNAs were evaluated using MirMiner. Differential expression analyses were performed in R using DESeq2, and results were visualised using volcano plots and heatmap analyses to identify POP-responsive miRNAs and global expression patterns. Differential miRNA response Small RNA sequencing of killer whale fibroblast cultures exposed to POPs revealed differential miRNA expression compared to control conditions, while no vehicle effects were observed. POP exposure induced upregulation of five miRNAs (miR-1, miR-203, miR-205, miR-140 and miR-192), although miR-1, miR-203 and miR-205 showed high inter-individual variability and inconsistent responses, suggesting limited robustness as biomarkers. Candidate biomarkers In contrast, miR-140 and miR-192 displayed low baseline variability and reproducible induction, identifying them as the most reliable candidate biomarkers. miR-140 is involved in regulation of cellular proliferation,

inflammation and tissue homeostasis, while miR-192 is linked to p53-mediated stress responses and toxicant-induced signaling pathways, consistent with activation of cellular stress mechanisms under POP exposure, as reflected by POP-associated differential expression patterns. A stronger transcriptional response under low-dose compared to high-dose exposure suggests a possible non-linear dose response pattern. Overall, these results identify miR-140 and miR-192 as robust and biologically relevant candidate biomarkers for cetacean ecotoxicological monitoring.

Microfibers Pollution In Antarctic Coastal Ecosystems: Evidence From Multiple Benthic Species From The Fildes Peninsula To The Ross Sea

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Microfiber (MF) and microplastic (MP) pollution is increasingly recognized as a pervasive threat to marine ecosystems, reaching even remote regions such as Antarctica through long-range transport and local anthropogenic activities. This work integrates multiple studies that investigate the occurrence, characteristics, and potential sources of MFs in marine benthic species across different coastal areas. The same methodological approach was applied, including thermo-oxidative digestion, density separation and μ -FTIR polymer identification. Furthermore, a preliminary assessment of MFs associated chemical additives has been applied in one of the organisms studied. The study encompassed key Antarctic species with different feeding strategies, including the gastropod *Nacella concinna* (grazer) from the Fildes Peninsula (King George Island) and the sea urchin *Sterechinus neumayeri* (grazer and scavenger) and scallop *Adamussium colbecki* (filter-feeder) from the Ross Sea. MFs and MPs were consistently detected across all investigated species, with 100% occurrence in sea urchins and scallops and 25% in the gastropods. Differences in particle abundance and size distribution were observed among species, with higher loads and longer fibers in the filter-feeding scallops compared to grazing organisms, suggesting a key role of feeding strategy in exposure and accumulation dynamics. Polymer characterization revealed a dominance of natural and semi-synthetic fibers, but also polyester and PET. Comparisons with reference materials from research station activities highlighted a strong similarity with textiles used on site. Furthermore, the co-occurrence of plastic additives, including phthalates and organophosphate esters, supports the potential role of MFs as vectors of chemical contaminants. Notably, additive concentrations in *A. colbecki* exceeded those reported for bivalves in other regions, suggesting a combined influence of local emissions and long-range transport. Partial characterization of particles indicates a likely underestimation of total contamination, and more studies are needed to confirm results. However, these findings provide evidence of MF pollution across Antarctic coastal ecosystems, highlighting the influence of biological traits on particle uptake and the relevance of local anthropogenic sources even in remote environments. The results support the use of Antarctic benthic invertebrates as bioindicators of MF contamination and

underline the need for improved monitoring and mitigation strategies in polar research infrastructures.

Environmental Conditioning Of Glitter Microplastics Modulates Elemental Accumulation In *Procambarus clarkii*

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Glitter particles are primary microplastics commonly used in cosmetics, arts and crafts products, special coatings, and fishing baits. They typically consist of a polymeric core, usually made of polyethylene terephthalate (PET) or polyvinyl chloride (PVC), coated with metals such as Al, Ti, Bi, and Fe, which confer their characteristic reflective properties. Environmental degradation of glitter may promote the release of these metallic components, potentially increasing their ecological impact. In this study, 52 specimens of the red swamp crayfish *Procambarus clarkii* collected from Lake Trasimeno (Umbria, Italy), were exposed for 30 days to pristine or lake-water-conditioned glitter at three concentrations: 0.067, 0.134, and 0.268 g/L. The accumulation of selected metals and metalloids, including glitter-associated elements such as Ag, Al, Ti, Bi, Rb, Tl and Cd, as well as environmentally relevant trace elements, was evaluated in the hepatopancreas and abdominal tissues. To highlight tissue-specific and conditioning-related patterns, data were pooled across sex and exposure levels, and differences among groups were assessed using the non-parametric Kruskal-Wallis test. Almost all elemental concentrations were generally higher in the hepatopancreas than in abdominal tissues, regardless of glitter condition. No statistically significant differences were observed in abdominal tissues between crayfish exposed to pristine and conditioned glitter. Conversely, significant differences were detected in the hepatopancreas between the two exposure conditions for six elements, namely Si, Cr, Cu, Zn, Cs e Tl, suggesting that environmental conditioning may alter the bioavailability of glitter-associated elements and promote tissue-specific accumulation in *P. clarkii*.

Flexible, Machine Learning-Based Automated Digital Histopathology Increases Throughput And Provides New Insights

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Histopathology is an essential tool for assessing the impact of pollutants at the tissue and organism levels and provides a key link between mechanistic and physiological data for adverse outcome pathways. Despite its importance, the use of pathology in environmental studies is diminishing due to heavy workloads and a decreasing trained workforce. Current methods also remain largely qualitative and are prone to observer bias. Recent advances in whole slide scanning technologies and machine learning (ML) based image recognition and segmentation provide opportunities to facilitate the use of histopathology and strengthen experimental outcomes. This research aims to develop a two-step process to a) reduce pathologists' workload; (b) increase

sample turnover, and c) provide objective, fully quantitative results to improve the reliability and power of analyses. Two sets of whole-slide images (WSIs) were used from animals collected during ocean monitoring exercises in 2021: fish liver tissue (268 individuals from 5 North Atlantic species), and blue mussel (*Mytilus edulis*, 293 individuals) were graded by 2 trained pathologists for 8 and 6 lesions, respectively. These grades were used to train weakly supervised models using the Clustering-constrained Attention Multiple Instance Learning (CLAM) framework to predict the presence (pathologist grade > 0) or absence (grade = 0) of lesions. Using the output from CLAM models and QuPath-generated annotations, a PyTorch-based segmentation model was trained for each tissue type. Quantification of the segmentation results was obtained based on characteristics of different lesions (e.g., total area coverage or number of cells in large aggregates). CLAM models were successful for most lesions, with a receiver operating characteristic (ROC) AUC > 0.8, indicating high true-positive-to-false-positive classification performance (e.g., parasites, 0.906; hepatocyte vacuolisation, 0.89). This was also reflected in validation and test errors < 0.25. Heatmaps showing high attention image areas also suggested that models accurately learned features associated with specific lesions (e.g., steatosis, haemocyte infiltration), but not with others (e.g., necrosis, parasites). Early segmentation results show successful learning and efficient quantification of various lesions. These methods are accessible and require limited expertise and training, paving the way for a more widespread and potent use of histopathology in environmental monitoring.

Physiological Responses of the Antarctic Bivalve *Adamussium colbecki* to Cadmium Exposure: Biochemical and Molecular Approaches

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Cadmium (Cd) is a non-essential and highly toxic heavy metal that represents a significant environmental contaminant in marine ecosystems. Although Antarctica is often considered a remote and relatively pristine environment, long-range transport processes and increasing human activities have contributed to the presence of trace metal pollution in Antarctic coastal areas. Among Antarctic marine organisms, the scallop *Adamussium colbecki* is recognized as a valuable biomonitor species due to its sedentary lifestyle, ecological relevance, and high capacity to accumulate metals in its tissues. The present study aimed to investigate the physiological and molecular responses of *A. colbecki* following exposure to increasing concentrations of cadmium. Particular attention was given to two target tissues, the digestive gland and the gills, which play key roles in metal accumulation, detoxification, and environmental exchange. Specimens collected in Terra Nova Bay (Ross Sea, Antarctica) during the "XXXVII Italian Antarctic Expedition (2021–2022)" were acclimated under laboratory conditions and subsequently exposed for ten days to two cadmium concentrations (10 and 100 µg/L), alongside an untreated control group. To assess cadmium-induced stress responses, biochemical biomarkers associated with oxidative damage were evaluated, including lipid peroxidation (LPO) through malondialdehyde (MDA) quantification, and protein oxidation assessed by Advanced Oxidation Protein Products (AOPP). In addition, antioxidant

defence mechanisms were investigated through the analysis of glutathione S-transferase (GST) and glutathione reductase (GR), two enzymes involved in cellular detoxification and redox homeostasis. Molecular analyses were performed by extracting total RNA from gill and digestive gland tissues, synthesizing complementary DNA (cDNA), and preparing samples for quantitative gene expression analyses targeting genes involved in antioxidant defence pathways, and normalized the values by a housekeeping gene. This study is based on the hypothesis that cadmium exposure induces oxidative stress and activates adaptive defence mechanisms in *A. colbecki*. By integrating biochemical and molecular approaches, the research provides a framework for understanding the effects of heavy metal contamination on Antarctic marine organisms and contributes to the development of sensitive biomarkers for environmental monitoring in polar ecosystems. Furthermore, the results may improve our understanding of physiological responses to pollution-related stressors in marine species living under extreme environmental conditions.

Assessment Of The Environmental Conditions Of Lake Orta Using *Unio elongatulus* As Sentinel Organism

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Lake Orta has historically been affected by severe industrial pollution, which has led to the acidification of its waters and to the disruption of its biological communities. Remediation measures, including liming, have significantly contributed to ecological recovery. However, the ecosystem has not yet been fully restored and the lake remains potentially vulnerable to the introduction of new sources of pollution. In this scenario, the INTEGRARE project aims to assess the presence of emerging contaminants of industrial and domestic origin and their biological effects. To this aim, an active biomonitoring was conducted using as sentinel organism the bivalve *Unio elongatulus*, a native species that has recently recolonized the lake. The monitoring was carried out by transferring organisms from a population of Lake Maggiore into cages, placed at five sites of the Cusio area: Imolo, San Maurizio d'Opaglio, Pella, Omegna and Bagnera, characterized by different levels of anthropogenic pressure. The bivalves were sampled 2 and 6 months after the beginning of the experiment and morphological and physiological (heart rate) measurements were conducted. At the same time, we investigated a suite of biochemical biomarkers related to: energy metabolism (electron transport activity), oxidative stress (levels of reactive oxygen species, SOD, CAT and GST enzyme activities), oxidative damage (lipid peroxidation), and neurotoxicity (acetylcholinesterase activity). The translocation procedures and the analyses of biological responses applied in this study confirmed the suitability of using *U. elongatulus* as a model organism for assessing the quality of lake ecosystems. The parameters analyzed showed no significant differences among the different sites, suggesting the absence of point sources of contamination in the lake. The chemical analyses currently underway will integrate the biological data obtained. Funding: "This research was funded by Cariplo Foundation Bandi Emblematici Provinciali Novara".

Species Sensitivity Distribution-Based Assessment Of Polypropylene And Polylactic Acid Microplastics In Marine Organisms

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Plastic pollution is an escalating global environmental issue, with significant implications for marine biodiversity, ecosystem functioning, and potentially human health. The increasing presence of micro- and nanoplastics in marine environments calls for integrated assessment frameworks capable of linking environmental compartments with human exposure pathways under a unified risk perspective. In response to this need, the BioPlast4Safe project (Biomonitoring of biodegradable micro- and nanoplastics: from the environment to humans in a One Health perspective), funded under the National Plan for Complementary Investments (PNC) to the National Recovery and Resilience Plan (PNRR), has been developed to address the complexity of plastic pollution through a multidisciplinary One Health approach. The project brings together expertise from ecology, ecotoxicology, chemistry, physics, microbiology, and human health sciences to improve current risk assessment methodologies for both conventional and biodegradable micro- and nanoplastics, with a specific focus on marine environments. By integrating multiple disciplines, BioPlast4Safe aims to generate a more comprehensive understanding of exposure dynamics, biological interactions, and potential ecological consequences associated with plastic particles of different origins. Ecotoxicological investigations conducted within the project assessed the effects of polypropylene (PP) and polylactic acid (PLA) microplastics across a range of marine species. The results indicated that both polymer types can induce adverse biological responses in marine invertebrates, whereas no significant effects were observed in primary producers or decomposer organisms under the tested conditions. Toxic responses were found to be strongly dependent on exposure duration, suggesting time-related dynamics in biological effects. In addition, particle size and polymer composition appeared to influence toxicity outcomes, highlighting the relevance of physicochemical properties in determining hazard potential. Evidence also suggests that chemical additives associated with microplastic particles may contribute to observed toxicity through combined mixture effects. Overall, biodegradable PLA and conventional PP particles exhibited broadly comparable levels of toxicity across the tested biological endpoints. To extend the assessment to the community level, experimental findings were combined with published literature data using a Probabilistic Species Sensitivity Distribution (PSSD) approach. This method enabled the derivation of Hazard Concentration values (HC₅), representing concentrations expected to affect 5% of species while protecting the majority of the community. The analysis indicates that high-quality ecotoxicity data suitable for the hazard assessment of PLA MPs in the marine compartment remain still limited, with key taxonomic groups insufficiently represented. Within these constraints, the exploratory results collectively suggest that biodegradable plastics may not necessarily represent a reduced-risk alternative to conventional plastics in marine environments.

Investigation Of The Biochemical Responses Of *Patella caerulea* Inhabiting Two CO₂ Vent Systems Of The Mediterranean Sea

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Ocean acidification (OA) represents one of the major threats to marine biodiversity and to the proper functioning of coastal ecosystems. This phenomenon has particularly relevant effects on calcifying organisms, impairing the formation and maintenance of calcareous structures. Nevertheless, some species are able to persist under acidified conditions through the activation of compensatory strategies, even though such mechanisms are still largely unclear. Within this context, the limpet *Patella caerulea* represents a suitable biological model for studying the responses of calcifying organisms to OA, due to its ability to survive and persist in areas naturally acidified by volcanic CO₂ emissions. In order to unravel the mechanisms underpinning their tolerance to OA, we collected populations of *P. caerulea* from two vent systems of the Mediterranean Sea: San Giorgio (pH = 7.84) and Vulcano (pH = 7.88). Marina di Patti and Marina di Vulcanello were considered the respective controls sites with ambient pH (pH = 8.1). For each individual (N = 10 x site), morphological parameters were measured and the gonadosomatic index (GSI) was calculated. The same organisms were subsequently subjected to biochemical analyses to characterize their metabolic conditions and assess the potential onset of stress responses. In particular, a set of biomarkers related to: energy metabolism (electron transport system ETS, glycogen and protein content), oxidative stress (reactive oxygen species level ROS, and the activity of antioxidant enzymes SOD, CAT, GST), oxidative damage (lipid peroxidation), and neurotoxicity (activity of Acetylcholinesterase) was applied. Preliminary results show heavy shell corrosion in individuals from both acidified sites; however, no significant difference among sites was observed in relation to shell size. Moreover, a decrease in the gonadosomatic index (GSI) was assessed in both sexes, being significantly lower at the San Giorgio vent. Biochemical analyses revealed a relevant modulation of the oxidative status with a significant reduction of ROS, CAT and SOD occurring in limpets collected at San Giorgio, with respect to controls. No neurotoxicity was observed at either vents. Overall, results suggest that limpets living at low pH/high pCO₂ are experiencing a stressful condition. Nevertheless, contrasting results emerged between the two CO₂ vent systems considered in this study, with more negative effects at the San Giorgio vent, potentially related to the different local environmental conditions.

Integrating New Approach Methodologies for Mechanistic Mixture Risk Assessment: A Case Study on European River Pesticides

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The advancement of next-generation risk assessment (NGRA) requires the development of mechanistically based approaches capable of integrating new approach

methodologies (NAMs) while addressing the challenges posed by exposure to complex chemical mixtures. In this work, we introduce an enhanced component-based mixture risk assessment (CBMRA) framework that links quantitative adverse outcome pathways (qAOPs) with high-throughput screening (HTS) data to enable biologically informed evaluation of mixture toxicity. The framework leverages HTS-derived evidence to identify chemicals interacting with a selected qAOP and to estimate their contribution to overall mixture effects through relative potency scaling within the qAOP domain of applicability. To illustrate its application, the framework was tested using an environmentally relevant case study involving pesticide mixtures reported in European river systems. The assessment was based on a previously published probabilistic Bayesian Network qAOP model associated with AOP-Wiki AOP#245 ("Reduction in photophosphorylation leading to growth inhibition in aquatic plants"). Pesticide occurrence data were obtained from the European Environment Agency's WISE Statistics – Pesticides database covering the period 1990–2021. Relative potency values for individual pesticides were estimated using HTS information from the ToxCast and Tox21 programs and supplemented by in silico prediction methods to address data gaps. Application of the framework indicated that although many of the assessed mixtures contained chemicals identified as active within the selected qAOP, the cumulative impact of these active chemicals was predicted to remain below the threshold necessary to induce growth inhibition in the model aquatic plant *Lemna minor*. Overall, the study demonstrates how NAM-derived data, mechanistic pathway information, and existing toxicological resources can be integrated into a coherent framework for mixture risk assessment. The robustness of the approach is influenced by the quality and availability of HTS data, uncertainties associated with relative potency estimates, and the suitability of the selected qAOP for the assessment context. As NAMs continue to evolve and expand, their integration into CBMRA frameworks is expected to improve the efficiency and scientific basis of mechanistic screening and prioritization of chemical mixtures.

Mason Bees Toxicity Test (OECD 254): Test Unit Configurations And Influence Of Experimental Conditions

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Pollinators may be exposed to pesticides and other substances dispersed in the environment either via contact or consumption of residue-containing food. To address the potential contact toxicity of a test substance (item), an acute contact study can be conducted in the laboratory by exposing adult bees to the test item. Pesticide risk assessment for bees have relied on *Apis mellifera* for many years. However, most of the bee species worldwide are solitary, with different ethology from social bees. In recent years, growing interest has been directed toward the red mason bee (*Osmia bicornis*, syn. *rufa*). In response to pollinator decline, *Osmia bicornis* has emerged as both a promising crop pollinator and a model species for acute contact toxicity testing. In 2025 the OECD 254 guideline was adopted to regulate the mason bees (*Osmia* sp.) acute contact toxicity test GLP-conducted. The guideline indicates several factors which can improve solitary bee conditions in the tests: ground-level feeders generally perform better than suspended ones, while reservoir feeders fitted with a wick or cotton are preferred; the use of coloured feeders can enhance attraction; in addition, larger cages with multiple feeders, or a lower bee density per cage combined with more replicates, may improve results. Test groups consisting of 5, 6, or 10 bees per cage have been shown to be suitable. However, substantial differences in mortality were observed between cages

containing 10 *Osmia bicornis* individuals and cages in which enrichment was provided and the number of bees was reduced to 5. For example, a 30-bees group treated with 1000 µg test item/bee showed 50% mortality at 48 hours when 10 bees/cage were used, while the same dosing gave a 20% mortality when the bees were 5/cage. Bees in the control groups showed a reduced sensibility as well, from a pooled value of 11.7% in the 10 bees/cage configuration to 3.3% in the 5 bees/cage configuration at 48 hours. Moreover, food-grade paperboard was used to enrich the cages and the feeder was replaced. Although both tests were carried out in accordance with the OECD 254 guideline, the bees appeared more stressed in the first case than in the second, suggesting a possible increased sensitivity to treatment-related effects. The number of mason bees/cage and the enrichments of cages are crucial factors that may affect the experimental conditions of the test (e.g. 10 bees probably increase the internal humidity of the cage) and the results' reproducibility.

Assessing Excavated Conditioned Spoil As By-Product: Human Health Considerations And Land-Use Recommendations

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The reuse of excavated materials as by-products is a key lever for circular economy goals in large infrastructure projects. In Italy, Presidential Decree 120/2017 regulates the management of excavated soils and rocks, permitting their reuse when protection of human health and the environment is ensured. Typical end-uses include backfilling, land reclamation, morphological restoration, embankments, and agricultural improvement. A specific challenge arises with Earth Pressure Balance Tunnel Boring Machines (EPB-TBM), where conditioning with foaming agents introduces anionic surfactants into the excavated ground, raising concerns about potential risks to human health and the environment associated with its subsequent reuse. Most of these conditioning products contain anionic surfactants as their primary active component, particularly Sodium Lauryl Ether Sulfate (SLES). Since the current legal framework does not define substance-specific acceptance criteria for the anionic surfactants introduced by conditioning, dedicated assessments are required to verify the compatibility of conditioned spoil with its intended reuse. In this context, the Istituto Superiore di Sanità (ISS) is responsible for evaluating the human health aspects of the technical documentation submitted by project proponents, while The Istituto Superiore per la Protezione e Ricerca Ambientale (ISPRA) is responsible for assessing environmental quality requirements. The assessment integrates: (i) verification against national contamination thresholds (Legislative Decree 152/2006); (ii) review of conditioning product information, including composition, hazard classification, persistence and biodegradation; (iii) chemical and ecotoxicological testing on conditioned soils: total anionic surfactants and their persistence and biodegradation behaviour in elutriates; bioassays with *Vibrio fischeri* and *Daphnia magna* on elutriates, and *Lepidium sativum* and *Heterocypris incongruens* on bulk soil; (iv) site-specific exposure analysis considering intended end-use, proximity to surface and groundwater, and drainage and infiltration pathways. Based on this assessment, project-specific recommendations are provided on the admissibility of conditioned spoil reuse and defining appropriate management conditions. These may include surfactant degradation periods prior to reuse, restrictions for sensitive land uses, water management requirements, and environmental restoration measures — all aimed at minimizing residual risks to human health. Drawing on case studies, this contribution illustrates the methodological approach, highlights critical factors

influencing land-use compatibility, and discusses recurring challenges encountered in practice.

Regional Risk Assessment of Diffuse and Point-Source Soil Contamination Using a Source-Pathway-Receptor Framework

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The recently approved Soil Monitoring and Resilience Law introduces a harmonized, risk-based system for identifying contaminated soils, requiring regional screening to support stepwise investigation. To address this need, two complementary regional risk assessment methods were developed within the HEU ISLANDR project, and applied in a European metropolitan area (ISLANDR Test Area nr.3). Both approaches follow a Source-Pathway-Receptor logic and use geospatial data to identify areas where soil contamination may pose risks to human health or the environment. The first method targets diffuse contamination originating from widespread sources such as agricultural activities, atmospheric deposition and urban emissions. Soil concentration data from European and regional monitoring programs are harmonized and compared with threshold values to identify elevated levels. Factors influencing contaminant mobility soil properties, hydrology, topography, land use and climate are used to characterize relevant pathways. Vulnerability analyses for humans, groundwater, surface water and protected areas enable the calculation of a regional risk index that highlights diffuse-contamination hotspots. The second method focuses on potentially contaminated sites linked to industrial or commercial activities. Sites are identified using historical records, land-use information and administrative sources. Each site is characterized through a structured hazard assessment and by evaluating key pathways and receptor sensitivities, producing a prioritization index suitable for regional-scale screening. Applied together, the two methods provide a coherent framework for identifying both dispersed pollution patterns and specific sites that may warrant further investigation. The results from the case-study area demonstrate that the approach delivers consistent screening-level risk indicators and supports proportionate decision-making in line with the stepwise logic of the forthcoming legislation. While data availability remains a constraint and boundary effects may influence some pathway analyses, the framework offers a robust and scalable basis for prioritizing monitoring needs and guiding more detailed assessments where justified.

Monitoring Antimicrobial Resistance Using *Apis mellifera* in South Tyrol

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Antimicrobial resistance (AMR) is a global challenge impacting human, animal, and environmental health. Within the One Health framework, attention is directed towards environmental reservoirs of antimicrobial resistance genes (ARGs). Honey bees (*Apis mellifera*), with their wide foraging range and interaction with air, water, plants, and soil, represent effective bioindicators of environmental microbial

contamination. This study investigated the presence of antimicrobial-resistant bacteria and ARGs associated with honey bees in South Tyrol (Northern Italy). Samples were collected from apiaries located in urban, agricultural, natural, and livestock-influenced areas. Sampling occurred across two seasons over two consecutive years to evaluate temporal shifts in AMR patterns. Microorganisms isolated from both the surface and gut of honey bees were identified using MALDI-TOF mass spectrometry. A total of 171 bacterial species were detected (98 and 115 in the first and second year, respectively), reflecting a high microbial diversity. Among the isolates were potential plant, animal, and human pathogens, including *Pantoea agglomerans*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. Antimicrobial resistance was evaluated phenotypically through Minimum Inhibitory Concentration (MIC) tests and genotypically using PCR assays targeting 32 ARGs belonging to six major antibiotic classes. Overall, 24 ARGs were detected in 92.1% of the samples. Tetracycline resistance genes (tetA, tetM, tetL, tetW) were the most frequently detected, followed by β -lactam resistance genes (blaTEM, blaSHV, blaCMY2, blaOXA48). Genes associated with resistance to fluoroquinolones (qnrB, qnrS), aminoglycosides (strA, aacA1), sulfonamides (sul1), and colistin (mcr-1) were also found. The observed co-occurrence of multiple ARGs indicates that honey bees reflect the environmental resistome, supporting their potential use as bioindicators for environmental AMR surveillance.

A Novel Shiny Framework for PBT/PMT Screening Through Integration of Experimental and In Silico Data

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Persistent, Bioaccumulative and Toxic (PBT) substances, together with Persistent, Mobile and Toxic (PMT) substances, have emerged as a major concern for environmental and human health due to their long term persistence in the environment, potential for bioaccumulation and ability to reach several water compartments. In 2023, the Classification, Labelling and Packaging (CLP) regulation introduced new hazardous classes including for PBT/vPvB and PMT/vPvM substances. Both REACH and CLP regulation promote the use of alternative methods, including Quantitative Structure-Activity Relationship (QSAR) models and other in silico approaches, particularly when experimental data are unavailable. Consequently, the assessment of PBT and PMT properties may rely on experimental evidence, computational predictions, or weight-of-evidence approach. In this work we developed an R shiny application designed to support the assessment of PBT/vPvB and PMT/vPvM properties according to a structured workflow. The workflow is developed according to the CLP regulation. The application allows users to upload compound lists containing identifiers and SMILES, together with experimental values, predicted values or pre-existing property assessments. Dedicated modules guide the user through the evaluation of persistence, mobility, bioaccumulation and ecotoxicity. For each endpoint, the app supports different input scenarios, including experimental data, in silico predictions and already available classification. When in silico predictions are used, the application allows users to upload outputs generated by selected QSAR tools. The software automatically extracts the relevant information, evaluates applicability domain and applies specific decision criteria to support regulatory assessment. For example, BLOWIN outputs (Models available through EPISuite tools) can be imported and analyzed to support persistence evaluation. By combining experimental evidence, computational

predictions and applicability domain analyses, the platform provides a transparent and reproducible framework for chemical screening. This Shiny application serves as a decision-support framework designed to integrate and interpret outputs from experimental and in silico sources according to predefined regulatory workflows for screening potential PBT/PMT chemicals. Future developments will encompass additional modules for evaluating the environmental impact of chemicals, as evaluated by Life Cycle Assessment, including the assessment of atmospheric persistence, mobility, and potential environmental impact. Furthermore, a similarity analysis against the ECHA list of PBT and PMT substances is envisioned as a prospective extension of the framework.

From Stress to Recovery: Physiological and Molecular Responses of *Squalius cephalus* to Declining PFAS Contamination in the Veneto Region

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals that have long been recognised as contaminants of global concern, owing to their exceptional persistence and tendency to bioaccumulate. Once accumulated, PFAS can disrupt mitochondrial function and stimulate the overproduction of reactive oxygen species, leading to oxidative stress responses. At the organ level, PFAS exposure has been associated with hepatic steatosis. Yet, little is known about the extent to which freshwater organisms can recover once PFAS concentrations begin to decline. The Veneto Region (Italy), one of the most severely PFAS-contaminated areas in Europe, offers a unique opportunity to investigate this question. The present study aimed to assess the recovery of *Squalius cephalus* following a reduction in PFAS contamination. Individuals were sampled from three sites with contrasting PFAS concentrations during two monitoring campaigns conducted one year apart, a period during which contamination levels at the most polluted site dropped considerably. The Hepatosomatic Index (HSI) and Gonadosomatic Index (GSI) were calculated to assess overall physiological conditions, while liver prdx4 gene expression, which encodes a key antioxidant defence enzyme, was quantified as a molecular marker of oxidative stress. prdx4 was also investigated due to its reported involvement in lipid metabolism, as its downregulation under moderate PFAS exposure has been associated with increased intracellular lipid accumulation. In both 2024 and 2025, results indicated that *S. cephalus* from the medium-polluted site had higher HSI values and lower prdx4 expression than the control. This suggests, as previously observed, that under medium PFAS exposure, this enzyme may be downregulated, potentially favouring lipid accumulation within cells. In 2024, at the highest contamination level, HSI, GSI, and prdx4 expression were higher than in the control, indicating hepatic enlargement, gonadal alterations, and upregulation of antioxidant defences. Under these conditions, the severity of contamination may have exceeded the threshold at which prdx4 regulation is associated with lipid accumulation, instead leading to an enhanced transcriptional response to counteract increased ROS production. Following the decline in contamination, the differences between sites were no longer evident in 2025. HSI, GSI, and prdx4 expression returned to levels comparable to those of the reference population. In

conclusion, these findings suggest that *S. cephalus* can recover from PFAS-induced stress at both the physiological and molecular scales when contamination declines. Further studies incorporating additional antioxidant biomarkers and lipid metabolism analyses would better characterise biological recovery in PFAS-contaminated freshwater systems.

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