BOOK OF ABSTRACTS

PRIMO22

26-29 MAY 2024
NANTES FRANCE

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How do contaminants (pesticides, microplastics, drug residues....) affect ecosystems, biodiversity and our health? What are the most reliable scientific sources for determining their effects, and what solutions can science provide?

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SCIENTIFIC PROGRAM OF PRIMO 22

Chemical exposome and new tools to track pollutant sources and transfers

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Identification of bioaccumulative emerging PFAS in bivalves by suspect and nontarget screening

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Per- and polyfluoroalkyl substances (PFAS) are a family of thousands of synthetic organofluorine compounds of high environmental concern, due to their persistent, bioaccumulative and toxic properties. Because of their stability and mobility in waters, oceans and marine ecosystems are widely contaminated with these compounds. Current monitoring practices generally focus on the analysis of a few dozen compounds, which may represent only a tiny fraction of the total PFAS burden. By applying a non-specific method for PFAS analysis in biota, we previously evidenced that unknown PFAS precursors were by far the major contributors to the total PFAS burden of bivalves from the French coasts. In this context, there is now a crucial need to identify the compounds responsible for this contamination. This work details i) a method using UHPLC-QToF that enables non-target analysis of PFAS, ii) the identification of these substances based on a non-target analysis workflow using a combination of data, e.g. exact masses, MS^2 class specific fragments and Kendrick mass defects, iii) PFAS contamination profiles in bivalves from various sampling sites. This work highlights the presence of several classes of PFAS, such as perfluoroalkyl sulfonamides (FASAs) and X:2 fluorotelomer sulfonamide betaines (X:2 FTABs), both classes detected in >90% of the investigated sites, confirming the ubiquity of such precursors in marine bivalves. Nineteen compounds from six different classes were identified and further identifications will allow a thorough characterization of the bivalve chemical exposome.

Key words
Per- and polyfluoroalkyl substances (PFAS), nontargeted analysis, LC-ESI-QTOF, French coasts, filter-feeding mollusks

Type of presentation
platform

Session
Chemical exposome and non-target screening approaches

Effect-based contaminant monitoring in the Baltic coastal ecosystem: a proof-of-concept

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Environmental contaminants induce adverse effects across biological levels, necessitating precise assessment tools. DNA adducts, which are chemical modifications to DNA, provide crucial insights into genomic effects and find broad applications in environmental toxicology and related fields. Liquid chromatography-mass spectrometry (LC-MS) with high-resolution mass spectrometry (HRMS) has emerged as the forefront technique for detecting and quantifying DNA adducts. HRMS allows comprehensive screening of adducts from diverse exposure classes, yielding detailed insights into modification types, chemical structures, and exposure diagnostics. This approach surpasses the constraints of classical assays, providing a superior omics perspective for understanding the impacts of contaminants on DNA modifications.

This paper delves into the cutting-edge field of environmental adductomics, highlighting the emerging role of DNA adductome analysis in the effect-based methods of environmental health assessment. Discussed are laboratory pipelines with target and non-target detection, supporting data monitoring and regulatory utilization, showcasing the transformative capacity of mass spectrometry in combination with bioinformatics approaches in advancing our understanding of the pollution impacts on DNA adductomes with reference to our experience and findings. We suggest that DNA adductomics is a technologically mature, mechanism-based novel approach ready for adoption in environmental research and monitoring to facilitate a comprehensive assessment of environmental impacts.

Key words
Environment, biomonitoring, effect-based, biomarker, contaminants

Type of presentation
Oral

Session
Chemical exposome and non-target screening approaches

Chemical eco-exposome in the Western Mediterranean Sea: a non-targeted study of contaminants of emerging concern in two bathing areas of the Côte Vermeille

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The development of advanced untargeted methodological approaches using the coupling of liquid chromatography to high-resolution mass spectrometry are necessary to provide a more comprehensive and nuanced exploration of the marine chemical eco-exposome. When combined with passive sampling strategy, these approaches offer a holistic perspective that is essential for effective environmental management and conservation efforts, and can considerably enhance the knowledge of marine CECs complementary to the targeted evaluation of prioritized substances. The aim of this study was to to analyze the composition of seawater collected from the bathing areas of Banyuls-sur-Mer and Collioure in the Côte Vermeille region of the French Western Mediterranean during the summer of 2022, using POCIS passive samplers. A non-targeted approach using UHPLC-HRMS/MS enabled the annotation of 22 contaminants, mostly tensioactive compounds, as well as insecticides, rubber and ultraviolet filter derivatives. To determine the sampling rate (Rs) and estimate the time-weighted average concentration of these pollutants in the sea, mesocosm experiments were conducted, resulting in the quantification of five specific pollutants. The risk quotient (RQ) was assessed by incorporating ecotoxicological data from the literature. This risk assessment will be used to present and discuss the potential environmental impact associated with the presence of these pollutants in the local area.

Key words
CECs, POCIS, HRMS/MS screening, untargeted metabolomics, risk assessment

Type of presentation
Platform

Session
t02: Chemical exposome and non-target screening approaches

First application of exposomics and metabolomics to detect impact of multiple anthropogenic stressors in two fin whale (*Balaenoptera physalus*) populations

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Mediterranean cetaceans are exposed to multiple stressors, such as marine litter, climate change, as well as bioaccumulation of contaminants. The main objective of this study was to apply for the first time a multi-diagnostic approach to demonstrate the use of exposomics and metabolomics, combined with gene expression analysis, for assessing the susceptibility of multiple stressors in fin whale (*Balaenoptera physalus*) from two semi-enclosed seas: the Mediterranean (Med) sea (Pelagos Sanctuary) and the Sea of Cortez (Mexico). Skin and blubber biopsies of fin whale were collected from the two areas: Med Sea (n=17) Sea of Cortez (n=11). Exposome profile was investigated in the blubber (by GC-HRMS) and 58 substances were confirmed with standardsand 120 were annotated. 41 substances were quantified, including PCBs, OC pesticides, PCDFs and PBDEs. A multivariate model significantly explained the variation in chemical exposures, with levels of PCBs higher in the whales from the Med than the Cortez. Phthalates were also detected by GC, and often higher in Med samples. A total of 386 compounds detected in skin by LC-HRMS were annotated. Several of these are included in the category of emerging contaminants, ranging from pharmaceuticals, plasticizers, PFAS, and UV-filters. In parallel, we carried out ^{1}H-NMR and MS metabolomics studies on biopsies. This is the first time in which metabolomics are used in cetaceans to discriminate between populations by a multivariate model. Finally, a correlation between transcriptomic and exposome data was detected in Med fin whale. In conclusion, we show a successful first application of exposomics combined with metabolomics and gene expression analysis in cetaceans inhabiting the highly anthropized ecosystem of the Med sea in comparison to Sea of Cortez.

Key words
Exposomics, metabolomics, multiple stress, fin whale

Type of presentation
Platform.

Session
Chemical exposome and non-target screening approaches

Mixture effects of pollutants

SCIENTIFIC PROGRAM OF PRIMO 22

Chemical exposome and new tools to track pollutant sources and transfers

(Posters)

PRIMO22

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Leveraging Multi-Omics Analyses to Explore the Toxicity of Urban Road Runoff Contaminants in Juvenile Salmonid Species

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A chemical derived from tires, 6PPD-quinone, has been correlated to pre-spawn mortality events in salmon populations. This study aims to clarify observed variations in 6PPD-q toxicity among different salmonid species and define the mechanisms of action. It is predicted that exposure to 6PPD-q will enhance salmonid sensitivity to other co-occurring contaminants, such as the polycyclic aromatic hydrocarbon 9,10-Anthraquinone (AQ). Sublethal concentrations of 6PPD-q were established in juvenile *Oncorhynchus* species (chinook, coho, and rainbow trout). A targeted method was developed in a triple quadrupole mass spectrometer, coupled to an ultra-high-performance liquid chromatography system to quantify 6PPD-q and AQ in water samples. Fish were exposed to sublethal concentrations of 6PPD-q and AQ separately and in combination for five days. Non-targeted mass spectrometry-based metabolomics analyses were performed on brain and liver samples. Using spectral libraries, 290 and 260 metabolites were annotated in the brain and liver tissues, respectively. More metabolites were significantly different in the liver tissues within each species when compared to the brain tissues. 6PPD-q and co-exposure groups resulted in more modulated metabolites compared to other exposure groups. Fatty acid biosynthesis was the pathway most affected by 6PPD-q exposure in coho salmon, as revealed by metabolic pathway enrichment analysis. Salmonid mortality and affected metabolites varied across species: coho > rainbow trout > chinook. Transcriptomic analysis will be integrated with the metabolomics data.

Key words
Metabolomics, Transcriptomics, 6PPD-quinone, Mass Spectrometry, Non-target

Type of presentation
Poster

Session
Chemical exposome and non-target screening approaches

Transfer of 7 organic UV filters from sediment to the ragworm *Hediste diversicolor*: bioaccumulation of benzophenone-3 and further proof of octocrylene metabolism

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The widespread use of organic UV filters, particularly in tourist coastal areas, results in their continuous release into aquatic ecosystems. Most of these UV filters have low solubility in water and tend to accumulate in sediment. This poses a potential risk of toxicity and bioaccumulation to sediment-dwelling organisms.

This study aimed to assess the potential transfer of seven UV filters including benzophenone-3 (BP3), bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT), butyl methoxydibenzoylmethane (BM), methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT), 2-ethylhexyl salicylate (ES), diethylhexyl butamido triazone (DBT), and octocrylene (OC) from artificially spiked sediment (10 $\mu g.g^{-1}$ dry weight) to sediment-dwelling worms (*Hediste diversicolor*).

After 28 days of exposure, all UV filters were detected in the worms, but only BP3 showed bioaccumulation, with a biota sediment accumulation factor (BSAF) of 12.4 ± 4.6. OC had a BSAF f 0.2 ± 0.0. Interestingly, metabolomic profiling revealed that OC was metabolized by the worms into 11 fatty acid conjugates. This demonstrates that OC also accumulated in the worms in the form of OC–fatty acid conjugates with a much higher factor compared to the calculation based on OC alone.

This study highlights the importance of not solely relying on the quantification of the parent organic UV filter to assess accumulation factors and organism exposure. It is crucial to complement conventional methods, such as BSAF calculus, with other techniques like non-targeted metabolomics. By doing so, a more accurate assessment of the potential bioaccumulation of xenobiotics, including transformed xenobiotics, can be achieved.

Key words
Hediste diversicolor; bioaccumulation; UV filters; marine sediments; emerging pollutants

Type of presentation
Poster

Session
Chemical exposome and non-target screening approaches

SCIENTIFIC PROGRAM OF PRIMO 22

Microorganisms as target and vector for chemical pollutants

(Oral talks)

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Effects of pharmaceuticals on marine phytoplankton and role of phytoplankton on pharmaceutical's fate

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Effects of organic contaminants (OCs) on phytoplankton physiology were extensively studied in the last years. However, a knowledge gap remains regarding the role of phytoplankton on OCs fate. Such information is required to better assess contaminants persistence and their trophic transfer, to identify biomarkers of contaminants exposure and to improve microalgae-based water remediation strategies. In our study the marine diatom *Phaeodactylum tricornutum* was exposed to three OCs, two pharmaceuticals Diclofenac (DCF) and Ethynilestradiol (EE2) and the herbicide Diuron (DIU). OC effects on cellular traits and physiology were studied together with OC removal from the exposure medium (HPLC-UV) and OCs biotransformation (HPLC-MS).
The lowest observed effect concentration (LOEC) varied considerably between pharmaceuticals (0.3-1 mg/L) and the pesticide (3 µg/L) and different modes of toxic action were described for the three contaminants. DIU inhibited cell growth and photosynthetic activity, DCF effected membrane integrity but not through generation of oxidative stress. Finally, EE2 induced oxidative stress and altered cell morphology with lipid droplets formation. In contrast to DIU, *P. tricornutum* cultures were shown to be able to influence pharmaceuticals fate. Significant removal from the exposure medium was observed for EE2 (up to 60% for the tested concentrations after 72h exposure) and transformation products were identified only for DCF.
Our results confirm that phytoplankton can influence the fate of contaminants in the water column when not strongly affected by their toxicity. However, this process depends on the OC and its modes of toxic action. Ongoing analyses on transcriptomic data will shed light on the activated pathways possibly involved to respond to pharmaceuticals exposure through biotransformation and/or compartmentalization processes.

Key words
Phytoplankton, organic contaminants, stress responses, biotransformation, trophic transfer.

Type of presentation
Platform presentation

Session
Microorganisms as target and vector of chemical pollutants
Biotransformation pathways and mode of toxic action (MOA) of chemical pollutants

Metal impacts on deep-sea microbial N_2O metabolism and diversity

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Deep-sea mining of minerals is expected increase. This activity may expose microbial communities to toxic concentrations of metals, such as cadmium (Cd) and copper (Cu). These microbes provide important ecosystem services, from genetic diversity to regulating the production of greenhouse gases, such as nitrous oxide (N_2O). Yet, the potential impacts from metal exposure on deep-sea microorganisms remain unknown. This work aims to investigate potential effects of Cd and Cu on the N_2O metabolism of deep-sea bacteria and on the community structure of N_2O producers and reducers in deep-sea sediments.

Cd and Cu exposure experiments with the deep-sea bacterial strain *Shewanella loihica* PV-4 were performed in bioreactors and net N_2O production was measured as well as the relative expression of N_2O-producing and reducing genes. Also, deep-sea sediments were used for shipboard 96h exposure experiments with different concentrations of Cd. Extracted DNA was used for high-throughput sequencing of the 16S rRNA gene and the relative abundance of N_2O-producing and N_2O-reducing microorganisms was estimated.

The results suggest that Cd and Cu have contrasting impacts on the N_2O metabolism of *S. loihica* PV-4, with the former inhibiting net N_2O production and the latter enhancing it. Gene expression patterns partially explain this observation, suggesting a transcriptional effect from metal exposure. The results from Cd exposure on sedimentary microbial communities suggest differential metal effects on different genera of N_2O-producing and N_2O-reducing groups, which will be discussed here.

Key words
Deep-sea mining; Microbial diversity; N_2O; Cadmium; Copper.

Type of presentation
Platform.

Session
Microorganisms as target and vector for chemical pollutants.

Contaminants from dredged sediments alter the transcriptome of Manila clam and induce shifts in microbiota composition

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In the Venice lagoon, sediment management is of crucial importance as sediments are often utilized to build-up morphological structures such as marsh lands. With the recent Decree 86/2023, new guidelines entered into force. Sediment quality classification must be based on both chemical characterization and ecotoxicity testing. In the frame of the Venezia2021 research project, the contribution to sediment quality assessment of additional investigations such as transcriptomic analyses has been tested.

In this study, we exposed for 14 days the Manila clam to sediments sampled from different sites within a Venice lagoon navigable canal connecting Porto Marghera to Venice. Moreover, we investigated the impacts of dredged sediments on clam's microbial communities.

While bioaccumulation of organic contaminants of industrial origin reflected sediments' chemical concentrations, metal bioaccumulation was not consistent with metal concentrations measured in sediments probably due to the activation of ABC transporters. At the transcriptional level, we found a persistent activation of the mTORC1 signaling pathway, which is central in the coordination of cellular responses to chemical stress. Microbiota characterization showed the over-representation of potential opportunistic pathogens following exposure to the most contaminated sediments, leading to host immune response activation. Overall, this study reveals important transcriptional and microbial changes of Manila clams after exposure to sediments, therefore the reuse of dredged sediments must be planned according to their quality to avoid potential risk for this species.

Key words

Venice lagoon, sediments, transcriptomics, microbiota characterization

Type of presentation

Platform

Session

Mixture effects of pollutants

Biomonitoring and assessment of integrative approach

New approach methodologies to assess pollutant toxicity

Microbial Mediation of the Embryonic Transcriptome: Impacts on Critical Developmental Pathways and the Chemical Stress Response

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Exposure to environmental pollutants during critical periods alters developmental trajectory, with emerging evidence suggesting the microbiome impacts organismal development and the host chemical stress response. While this knowledge predominantly focuses on post-embryonic development, there exists a gap in understanding host-microbe communication during the earliest stages of embryonic development, when organisms are most vulnerable to chemical exposure. We utilize zebrafish (*Danio rerio*) as a model to study embryonic host-microbe interactions, performing RNA sequencing on pre-hatched embryos at 32 hours post-fertilization reared GF and conventionalized with microbes. While microbes are too large to physically breach the chorion, our results reveal external microbes influence embryonic transcription of critical developmental processes including neurodevelopment, bioenergetics, and lipid metabolism. Furthermore, the external microbial community upregulates *cyp1a* involved in xenobiotic metabolism in the developing embryo. Physiological assays reveal the embryonic microbiome effects behavioral and bioenergetic responses to benzo(a)pyrene exposure, a *cyp1a* inducer. Traditionally viewed as an independent process driven by genetics, this research reveals a significant role of the surrounding aquatic microbial community in sculpting developmental gene expression and the chemical stress response during embryogenesis. These findings will transform understanding of host-microbe communication and emphasize the importance of considering the microbiome in toxicological studies.

Key words
Microbiome, development, toxicology, transcriptomics, exposure

Type of presentation
Platform (or poster)

Session
1. Microorganisms as target and vector for chemical pollutants

2. EDCs and Neuroendocrine effects

3. Mixture effects of pollutants

Effects of Polystyrene Nanoplastic Particles on Benthic Microbial Communities

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Marine sediments are rich habitats for microbial communities and act as a sink for many environmental contaminants including plastic particles, which may fragment into nanoplastic (NP) particles (<1 μm). Plastics can be substrates for microbial growth, but less is known about the interaction of NPs and microbes. The objective of this study was to use a 16S barcoding approach to investigate the effects of polystyrene NPs on microbial community diversity and structure. Sediment cores were collected from the Pettaquamscutt River estuary in Rhode Island (USA) and used as mesocosms for exposures to seawater-weathered 900 nm NP spheres at concentrations of 0, 0.1, 1, 10, or 100 mg/kg dry weight amended to a reference sediment for two weeks. DNA was extracted from the top 1 cm sediment layer, 16S rRNA gene marker was PCR-amplified, and amplicons sequenced on an Illumina MiSeq. A dose-dependent decreasing trend in α-diversity was observed. Additionally, the abundance of anaerobic, sulfur-reducing bacteria increased in higher NP treatments compared to lower treatments. Sulfur-reducing bacteria are known Hg-methylators, so total mercury and methylmercury (MeHg) concentrations were measured in the exposed sediment. A slight trend towards an increase in MeHg and the MeHg/total mercury ratio with increasing NP concentrations was observed. These results, and the findings of previous studies evaluating NP impacts to eukaryotic communities, contribute to the understanding of plastic particles directly and indirectly affecting environmental conditions leading to community-level impacts.

Key words
Nanoplastic, microbial community, marine, Environmental DNA

Type of presentation
Platform

Session
t12: Microorganisms as target and vector for chemical pollutants

t05: Particles, fibres, plastics and their additives

Comprehensive investigation of realistic microplastic hazards in zebrafish larvae: integrating multi-omic approaches to unravel bacterial-mediated alterations

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The present study investigated the impact of microplastics on the gastrointestinal microbiota and host gut of zebrafish larvae and the role of bacteria in shaping realistic microplastic hazards. Larvae (3 days post-fertilisation) were exposed to bottle micro-fragments and textile micro-fibres of polyethylene terephthalate (PET) for 5 days, concurrent with an *Aeromonas hydrophila* challenge. Zebrafish gut was collected for microbiome and proteome analysis. The molecular and cellular mechanisms by which microplastics may affect the establishment of host-microbiota relationships were investigated. Particular bacterial taxa were significantly affected, suggesting adaptive responses to altered conditions. The detection of distinct groups of significantly affected proteins provided insight into the physiological and pathological changes observed in larval gut following combined exposure. Overall, these findings provide novel knowledge on the realistic hazards of microplastics when combined with pathogenic bacteria to aquatic vertebrates at early life stages and highlight the need for further research to unravel the long-term consequences of such interactions on aquatic organisms and ecosystems.

Key words
Zebrafish larvae, Microplastics, Bacteria, Omics

Type of presentation
Oral

Session
Mixture effects of pollutants

Extrapolating sublethal responses to contaminant exposure between species: a case for Gadids

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We need to be able to quantify the effects of toxic substances in marine ecosystems. Even limiting the scope to higher trophic levels, it is a challenge to select the optimal model species.

The aim of the current study was to compare responses in acetyl cholinesterase (AChE) activity and cytochrome P4501A activity (measured as EROD), quantified in different tissues and for two seasons in four Gadids: Atlantic cod, whiting, Norway pout and haddock. Fish were collected by trawling in spring and autumn in the urban inner Oslofjord and at a location in the outer fjord with no known local pollution sources.

Results for PAH metabolites confirmed that fish in the inner fjord were exposed to higher level of pollution than fish in the outer fjord. There were clear seasonal differences and systematic patterns in responses for both biomarkers in the four species. Gill EROD was elevated in the inner fjord. AChE activity differed between tissues and also had a seasonal component. The patterns in the four species were similar, however, and there were no differences between fish collected in the two areas. The study has shown that although there is a clear urban contaminant signal in fish in the inner fjord, this was not strongly reflected in biomarker responses in the four species, although EROD was affected in some species and tissues. We need more understanding of the inducibility of responses, although the results from this study suggest monitoring programs can be adapted to use different species. The results will be discussed in view of results from other studies with the same species.

Key words
codfishes, species comparison, biomarkers, AChE, EROD

Type of presentation
Platform.

Session
Biomonitoring and development of integrative assessment approach.

SCIENTIFIC PROGRAM OF PRIMO 22

Microorganisms as target and vector for chemical pollutants

(Posters)

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Evaluating impact of chemicals on marine phytoplankton communities

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Phytoplankton communities provide key services to marine ecosystems. Chemical pollution is recognised as one of the five drivers for biodiversity loss and ecosystem change. Currently, the assessment of contaminant impacts on marine phytoplankton is primarily confined to standard toxicity testing often utilizing the model diatom *Skeletonema* spp.. However, our understanding of the broader effects of chemicals on phytoplankton communities remains limited. This work aims to address this knowledge gap by exploring the use of natural phytoplankton communities. Chlorine was selected as an example contaminant which is commonly used as antifouling agent in marine and estuarine environments. Different techniques were used to better understanding changes in the community. Thus, biomass was evaluated with chlorophyll *a*; diversity with functional diversity obtained with flowcytometry analysis and pigment composition obtained with HPLC analysis; finally, photosynthetic activity was evaluated with PAM fluorometry. Environmental relevant concentrations of chlorine had no impact on total biomass. Changes in size structure and functional diversity were instead quantified using flow cytometry with a reduction in smaller cells, particularly eukaryote picophytoplankton. Flow cytometry provided important additional information over more standard ecotoxicology methods. Effects are likely to be localised close to the discharges of chlorinated waters. Nevertheless, impact on coastal food webs and biogeochemical cycles should be further evaluated.

Key words
Community ecotoxicology, phytoplankton, chlorine, picophytoplankton

Type of presentation
Platform

Session
Microorganisms as target and vector for chemical pollutants

Microplastics in the Antarctic coastal environment of Potter Cove: zooplankton plastivory

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A major threat of microplastics (MP, <5 mm) lies in their bioavailability to marine organisms, leading to adverse health effects. The extent and consequences of MP ingestion by zooplankton, a key component of Antarctica's marine food webs, remain insufficiently studied. The ubiquity and the small size of MP (< 1 mm) in the coastal waters of Potter Cove (King George Island/25 de Mayo, South Shetlands, Antarctica; Antacli et al. 2024), raise concerns regarding their environmental risks. Here we assessed how plastivory (i.e. MP ingestion; <30 µm Nylon 6.6 and polypropylene irregular fragments, obtained by CryoMilling) affects the algal grazing rate of female *Calanus propinquus,* a key local copepod species. Grazing rates were assessed by acute exposure (72 h in 500 mL bottles, filtered seawater) under controlled natural temperature (1°C) and three feeding scenarios: MP only (T1), MP+Algae (T2), and Algae only (T3). MP ingestion was confirmed and varied across treatments. When starved copepods were fed with MP only (T1), ingestion was evident. Results from T2 and T3 treatments showed MP significantly decreased algal feeding and survival. Ingestion of MP was further confirmed by fecal pellets examination. We also observed MP adhered to the external carapace and appendages of exposed zooplankton, suggesting that MP pollution can potentially affect zooplankton and the entire food web via trophic transfer.

Key words
Microplastic pollution, Zooplankton, Coastal Antarctica, Plastivory, Faecal pellets

Type of presentation
Poster

Session t12: Microorganisms as target and vector for chemical pollutants
t05: Particles, fibres, plastics and their additives

Screening of antibiotic-resistant bacteria in different locations of the Gulf of Biscay

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The massive use of antibiotics leads to an increase in the presence of these drugs in natural aquatic environments, where they can generate noticeable ecological and health impacts. The increase in the number of microorganisms exposed to antibiotics favors the generation of resistances to these compounds.

We isolated and identified bacteria resistant to azithromycin, ciprofloxacin and erythromycin in samples collected monthly during 2023 from four locations in the Gulf of Biscay with different anthropogenic influences: Getxo Port, Astondo Beach, Butroi Estuary and Plentzia Bay. Most of the cultured bacteria belonged to the phyla *Actinomycetota, Bacillota, Bacteroidota* and *Proteobacteria,* where *Gammaproteobacteria* was the most represented class. There were differences in total bacterial abundance and culturability rates between the Butroi Estuary and the other three sampling points. In general, we found a high abundance of antibiotic-resistant bacteria, mainly associated with high rainfall, minimum salinity and high bacterial abundance.

Only up to 24% of the analyzed species corresponded to potentially pathogenic species, while the resistance to different antibiotics was widely distributed among environmental non-pathogenic species. The greater survival capacity of those non-pathogens in natural environmental conditions could entail a serious danger for the dissemination of the resistances among the entire microbial community. Future research on selected strains will involve the detection of the capacity to transfer antibiotic resistance genes from non-pathogenic resistant species to others.

Key words
Antibiotic-resistance, dissemination,

Type of presentation
Poster

Session
t12: Microorganisms as target and vector for chemical pollutants

SCIENTIFIC PROGRAM OF PRIMO 22

Biomonitoring and development of integrative assessment approach

(Oral talks)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

Decoding the relationship between fish gene expression and the environment

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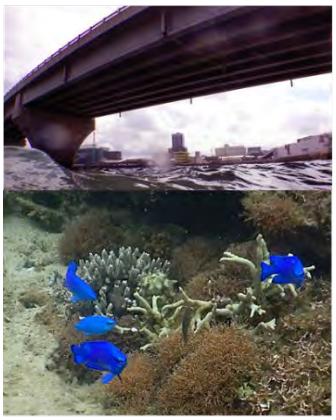
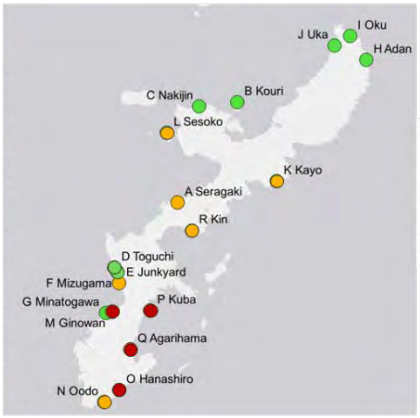
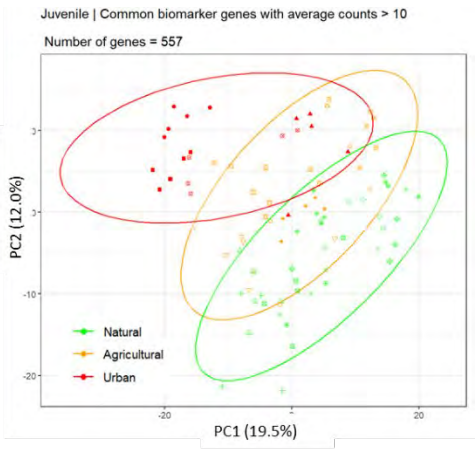
Traditionally, ecotoxicology studies expose an organism to a single contaminant over a limited duration and under controlled laboratory conditions. In natural environments, organisms are constantly exposed to varying levels of multiple stressors. Here, we obtained the transcriptomes of adult livers and whole-body juveniles from a coral reef fish, the blue damselfish *Chrysiptera cyanea*, living in different environments. The fish were collected in eighteen locations along the shoreline of the main island of Okinawa, Japan, from urban river mouths to pristine isolated beaches. The samples clustered depending on the site of provenance, and hundreds to thousands of genes significantly differed in terms of expression levels across the sites. The rich transcriptomic dataset was used to detect modified biological processes and associate the variation in the transcriptomes with biological and environmental characteristics, notably fish sex and size, nearby land use, and ecological fish communities. Through this study, we explored the potential of using wild-caught fish to characterise how different habitats with varying degrees of human presence can support biological processes such as fish growth, feeding, and reproduction. Can transcriptomics be used to identify key sets of markers genes for monitoring?







Key words

Transcriptomics, biomarkers, coral reef fish health, environmental assessment

Type of presentation

Platform (if not, poster)

Session

1) Biomonitoring and development of integrative assessment responses
2) Acclimation and adaptation to chemical stress
3) Chemical exposome and non-target screening approaches

Coupling environmental signatures and -omics tools on the European flounder (*Platichthys flesus*) to assess estuarine environmental health in an agricultural region (Brittany, France)

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This study aimed to establish a comprehensive, interdisciplinary method for evaluating the environmental health of eleven moderate-sized French estuaries. Data covering geographic metrics, hydrobiology, pollutant chemistry, and fish biology, were collected for each hydrosystem from watershed to estuary, accounting relevant anthropogenic factors affecting the environment.

Fish responses to anthropization was measured on European flounders (*Platichthys flesus*) gathered in September in the eleven estuaries; these fish showing a minimum residence time of five months in estuaries. Geographic metrics were produced using reference geographic information to characterize land use in each watershed. Water concentrations of nitrite and nitrate were assessed in the upper part of the estuaries, whereas levels of organic pollutants and trace elements were measured in sediment and biota; these metrics leading to the establishment of a first estuarine typology.

The fish metrics integrated targeted biomarkers and molecular data (liver shotgun proteomics), characterizing flounder responses to environmental stressors. Omics signatures in fish liver revealed a notable positive deregulation of different proteins linked to xenobiotic detoxification in systems with high population density, industrial activity, and predominantly agricultural watersheds affected by pesticides. Fish in the latter estuaries exhibited significant deregulation of the urea cycle, likely associated with a high nitrogen load. Molecular data also disclosed deregulation of proteins associated with hypoxia response, as well as potential endocrine disruption in particular estuaries.

The approaches carried out in this study (from larger scale: geography and land use, to finest scale: fish proteomics) allowed to produce an accurate typology of anthropization in the different hydrosystems.

A multibiomarker approach on the health assessment of European hake from NW Mediterranean fishing grounds

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Plastic pollution is a main environmental global problem, particularly in enclosed bodies of water, such as the Mediterranean Sea. To track the health status of the sea, the European hake (*Merluccius merluccius*), a main targeted commercial fish, has been proposed as a sentinel species for plastic pollution. Then, the high incidence of plastic litter in heavily urbanized regions such as the metropolitan area of Barcelona, may have negative consequences on the health and quality of fish, which would be a concern for human consumption. With this in mind, we studied pollution biomarkers, informative of chemical exposures, as well as the parasitic composition associated in hake sampled in three locations with different plastic pollution density in the Catalan coast. The multibiomarker approach here adopted showed that plastic litter occurrence did not impact the selected pollution related biomarkers nor the parasitic incidence (composition and diversity) of hake from the three sites. So far, it could be concluded that the health status from hake is similar in the 3 fishing grounds and the bulk plastic load was not correlated with biomarker responses or parasite assemblages. Thus, hake were not differentially impacted and the values here obtained could be considered as background guidelines. These results should be taken with caution since no chemical characterization was undertaken nor other biomarkers were measured that could significantly reflect an impact in hake from the most anthropogenic area.

Key words
plastic litter, biomarkers, carboxylesterases, cholinesterases, hake.

Type of presentation
platform.

Session

- Biomonitoring and development of integrative assessment approaches
- Particles, fibres, plastics and their additives
- Mixture effects of pollutants

Climate change enhance sensitivity of the key ice-associated Arctic species polar cod to oil spill events

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Arctic amplification of climate change is causing the sea ice to retreat at unprecedented rates, opening up large vulnerable Arctic areas for oil and gas exploration and new shipping routes. Extracting and transporting oil comes with the risk of accidental oil spills and are therefore strictly regulated. Oil exploration requires risk assessments by the authorities before licenses are granted. Risk assessments require knowledge of the spatiotemporal distribution of vulnerable marine resources and oil concentrations from spill scenarios to quantify ecosystem impacts. Polar cod (Boerogadus saida) is a key ice-associated Arctic species that is affected by both climate and oil spills near ice. In order to quantify adverse effects of oil spills we have developed a numerical data-driven individual-based (IBM) early life stage (ELS) polar cod model in combination with an ice-algae and *C. glacialis* model forced by a multi-ensemble ocean model combined with an oil spill and fate model. The IBM ELS polar cod model has parameterizations based on experimental work on the effect of various exposure concentrations and duration on eggs.

We show how climate change increases sensitivity of living organisms to oil pollution in high latitudes, and also to what extent potential oil spills affect polar cod year class strength. While oil spill scenarios with different origins in the northern Barents Sea display varying overlap with key polar cod early life stage habitats, the topographically steered Barents Sea Polar Front may to some extent act as a natural barrier to such impacts.

Key words
Polar cod, C. glacialis, oil spill scenario, modelling, impact assessment

Type of presentation
Oral

Session
1. New tools to track pollutant sources and transfers
2. New approach methodologies (NAMs) to assess pollutant toxicity
3. Biomonitoring and development of integrative assessment approach

Non-lethal tool for contaminant monitoring in fish: novel method validation for measuring emerging and legacy contaminants

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We report on advances to a non-lethal tool aimed at assessing emerging and legacy contaminants in salmonids (Atlantic salmon and sea trout). Contaminants such as perfluoroalkyl substances (PFAs) polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) have low water solubility and high affinity to particulate matter, therefore tend to accumulate via the food web. We have measured PBDEs and PCBs in adipose fins of sea trout returning to the river Tees (UK) and the detected congeners reflected the widely used commercial formulations. Overall, the concentrations where within or above previously reported levels on both salmonids (muscle and body homogenates) originating from North Sea, North Atlantic and Baltic Sea[1]. We are currently assessing these compounds, as well as PFAs and PAHs, in individually paired adipose fin-muscle samples collected from adult salmonids, returnig to UK rivers and from the West Coast of Greenland, to validate if the concentrations present in the adipose fin reflect the muscle contaminant load. Our results indicate that these fish could be bioaccumulating persistent organic pollutants via diet during their oceanic migratory routes[1]. Current evidence gathered in the North Atlantic indicates that the survival of salmon during the marine phase of their life cycle has declined in recent decades. The use of adipose fins has the potential to be further developed to monitor whether persistent contaminants are contributing to salmon mortality at sea, in populations showing declining trends.

[1] Assunção, M.G.L., M. Ives P.M., Davison, J.L. Barber, A. Moore and R.J. Law. 2020. Persistent contaminants in adipose fins of returning adult salmonids to the river Tees (UK). Marine Pollution Bulletin. 153:110945.https://doi.org/10.1016/j.marpolbul.2020.110945.

Key words
Non-lethal tool, persistent contaminants, salmonids

Type of presentation
Poster or platform

Session
T10 Biomonitoring and development of IEA

T03 New tools to track pollutants sources and transfer

Active mussel biomonitoring for the health status assessment of the Western Mediterranean Sea

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The Western Mediterranean coast is under the influence of differently sourced anthropogenic pressures such as extensive soil occupation, decreasing freshwater resources, increasing amounts of sewage, litter and dangerous waste, habitat destruction and contamination of marine resources. In response to these challenges, the French RINBIO network (http://www.ifremer.fr/envlit/) has implemented a monitoring program specifically dedicated to assessing the chemical contamination of coastal waters in the Mediterranean region. This network employs artificial caging to evaluate contaminant levels and bioavailability in mussel. Biological effects were also assessed in 2021 based on a battery of cell and tissue level biomarkers measured in mussels collected from 17 caging sites. The integration of chemical and biological results obtained from the 2021 campaign provides critical information for the adequate assessment of the ecosystem health status using mussels as sentinel species in the Mediterranean Sea. The potential influence of natural factors (i.e. trophic condition) and of the caging strategy on biological responses is considered. The campaign also allows to report a set of novel data available as reference values for biomarkers assessment in future large-scale monitoring campaigns of the area.

Key words
Monitoring program, Caging, Bivalves, Biomarkers, Histopathology.

Type of presentation
Platform.

Session
T10: Biomonitoring and development of integrative assessment approaches.

UV filters on seawater can pose a risk for coral reefs

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Coral reefs are among the richest and most productive ecosystems on the planet. They are home to an exceptional biodiversity of almost 100,000 species, with around a third of known marine species living in reefs. Unfortunately, these ecosystems are threatened by climate change and pollution generated by human activities.

Despite the importance of corals for biodiversity and the threats posed by chemical pollution, few studies have focused on the risk associated of chemical substances on coral species. This led the French Ministry of Ecological Transition and Solidarity to commission ANSES (French Agency for Food, Environmental and Occupational Health & Safety) and OFB (French Office for Biodiversity) for assessing the impact of chemical substances on coral reefs. A review of the scientific literature identified around a hundred chemical substances, including UV filters, pesticides, metals, hydrocarbons, etc., as potentially toxic to corals. A risk assessment focused on UV filters was carried out based on selected data. Sixteen substances were identified as potentially toxic for essentially two species of coral; toxicological reference values were calculated for eleven substances, most of them exceed existing $PNEC_{marine}$. The results of the risk assessment indicate that five substances octocrilene, enzacamene, octinoxate, oxybenzone and 2-ethylhexyl salicylate are present in the environment at concentrations exceeding toxicity thresholds.

Key words
Corals, UV filters, risk assessment

Type of presentation
Platform

Session
Biomonitoring and development of integrative assessment approaches

Whale shark (*Rhincodon typus*) exposure to legacy and emerging contaminants in La Paz Bay (Gulf of California, Mexico)

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Whale shark (*Rhincodon typus*) is a filter-feeder elasmobranch, classified as endangered worldwide by the IUCN due to several threats as by-catch, collisions with vessels and pollution. The whale shark population resident in the Gulf of California (Mexico) aggregates in the La Paz Bay for feeding during winter-spring months and might be exposed to legacy and emerging contaminants, including microplastics. Skin biopsies were used for the evaluation of the whale shark ecotoxicological status in this area. A total of 32 whale shark skin biopsies (12 samples in 2014 and 20 samples in 2018) samples were collected. The levels of organochlorine compounds (PCBs, DDTs), polybrominated diphenyl ethers (PBDEs), dechlorane plus (DPs), plastic additives (phthalate esters, PAEs), and related biomarkers responses by a gene expression approach using qRT-PCR (Cythocrome P450 1A and 1B, Estrogen Receptoy β, Stimulator of Interferon Genes, Retinoid X Receptor α) were investigated to correlate the exposure to the different classes of contaminants and alteration of mRNA levels. Data were compared among age classes and year of sampling. Statistically significant correlations resulted among gene expression analysis and some classes of contaminants (eg. CYP1B and Organochlorines). A PCA analysis was performed to evaluate a possible correlation among the size of the whale sharks, contaminants and molecular responses. The approach could be replicated in other whale shark populations worldwide contributing to the ecotoxicological risk assessment of this endangered species.

Cnidarian Models for Toxicology

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Coral reefs and tropical habitats are threatened worldwide by global warming and pollution stress. The purpose of these studies was to evaluate potential strategies for using Cnidarians for toxicological assessments. Laboratory studies were conducted with jellyfish and corals that were exposed to copper, and observational (pulsation rate in jellyfish and bleaching in corals) as well as biomarker responses (glutathione, lysosomal destabilization) were assessed. Jellyfish tended to be more sensitive than corals to Cu exposures. Jellyfish pulsation rate, lysosomal destabilization, and tissue Cu levels were significantly correlated. Glutathione (GSH) levels of jellyfish were lower than coral levels and may contribute to greater sensitivity between species. Field studies were conducted in Curaçao with adults and larvae of brain corals and other species to determine the baseline glutathione levels. The GSH levels of early life history stages of corals (eggs, embryos, larvae) were lower than adult levels, potentially indicating that these stages could be more sensitive than adults. The GSH levels of the younger coral stages were similar to the GSH levels of jellyfish adults. Glutathione levels of Cnidarians are much lower than those of more traditional bioindicators such as mussels or oysters. An important component of these studies is the partnerships with institutions such as Discovery Place (a public science exploration center) and CARMABI (Caribbean Research and Management of Biodiversity) that are actively engaged in the culture of Cnidarians.

Key words
Biomarkers, Jellyfish, Corals, Glutathione

Type of presentation
Platform

Session
T10 or possibly T4 , Or whatever session you think this would best fit in.

Chemical contamination and potential health risks to French seabirds: a multispecies and multisite study

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Toxic pollutants such as mercury (Hg) and perfluoroalkyl substances (PFAS), a group of man-made chemicals, require urgent monitoring across large geographical ranges. As apex predators, seabirds are relevant bioindicators for marine pollution but information on Hg and PFAS contamination in seabirds from France is very limited. Hg and 14 PFAS were analyzed in the blood of chicks of eight seabird species from 32 sites in metropolitan France (M-Fr) and of 16 species from the French Southern and Antarctic territories (TAAF). Associations between the concentrations of these contaminants and birds' trophic ecology and health (via biomarkers e.g. DNA damage) are currently being investigated. In M-Fr, mean Hg concentrations ranged from 0.1±0.1 to 2.9±1.3 μg g^{-1} dry weight (dw) in herring and great black-backed gulls, and from 0.2±0.1 to 3.9±1.6 μg g^{-1} dw in Cape petrels and Amsterdam albatrosses in TAAF. Most chicks from M-Fr exhibited low Hg concentrations, with 74% categorized at no risk, according to established toxicity thresholds. In M-Fr, concentrations of perfluorooctanesulfonic acid (PFOS), the dominant PFAS, ranged from 21.4±13.5 to 77.4±21.8 ng g^{-1} wet weight in herring gulls and Scopoli's shearwaters, respectively, while PFAS analyses in seabirds from TAAF are currently underway. Overall, M-Fr seabirds appear to be at low risk from Hg, while 88% of chicks were above PFOS toxicity thresholds. Recent studies have reported sub-lethal effects on seabirds carrying Hg and PFAS burdens considered to be safe, calling for further investigations on the impact of these pollutants.

Key words
Mercury, PFAS, blood, chick

Type of presentation
Platform

Session
Biomonitoring and development of integrative assessment approaches

Use of biomarkers to monitor the environmental status on the Atlantic Coast of Spain

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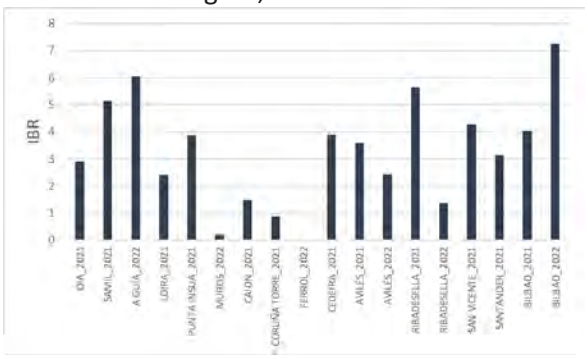
Descriptor 8 of the Marine Strategy Framework Directive (2008/56/EC) demands an approach based not only on the analytical chemistry of pollutants, but also on evaluating their effects on the ecosystems, to assess marine pollution in European seas. The assessment of the environmental status in the Spanish North Atlantic demarcation, conducted by the Instituto Español de Oceanografía, has been carried out by recording a series of biological responses in wild mussels (AChE, GST, CAT, GRx and GPx enzymatic activities and Micronuclei -MN-), imposex in gastropods, and bioassays with sea urchin embryos.

	AChE(nmol min^{-1} mg P^{-1})	**GST**(nmol min^{-1} mg P^{-1})	**GRx** (nmol min^{-1} mg P^{-1})	**GPx**(nmol min^{-1} mg P^{-1})	**CAT**(µmol min^{-1} mg P^{-1})	**% MN**
BAC	14.9	-	-	-	-	3.9
Mean 2021	22.4±5.07	-	37.9±5.34	13.9±3.15	57.5±8.94	4.4±1.2
Mean 2022	16.1±4.92	78.3±14.9	35.7±6.0	22.3 ±4.70	54.9±13.5	5.9±1.9

In the case of AChE activity, of the 18 sampling stations analyzed, 12 showed mean AChE activity levels higher than BAC (AChE=14.9 nmol min^{-1} mg P^{-1}) and 4 showed mean AChE activity levels between BAC and EAC (EAC=10.4 nmol min^{-1} mg P^{-1}). Therefore, the assessment of the Spanish North Atlantic demarcation according to the results obtained from the AChE activity does not comply with the GES, since less than 95% of the sampling stations in the demarcation present average values of the biological response higher than BAC. An Integrated Biomarker Response (IBR) was calculated according to Devin et al. (2014), by combining the different biological responses (GST, GPx, GRx, CAT, AChE and MN) to a single value, which can be used to describe the toxically induced stress level of populations in different stations. In the figure, it can be seen that the Bilbao station was the most contaminated in 2022.



With regard to Imposex, EcoQO values established by OSPAR were used. For *Nucella lapillus*, more than 95% of stations sampled in 2021 showed values lower than the EcoQO. However, the percentage of samples for *Nassarius reticulatus* from 2016 to 2021 with a VDSI value greater than EcoQO (63%) implied that the GES has not yet been reached. For the sea urchin larval bioassay, 86% of the sampled stations were classified as unpolluted and 12% were moderately polluted.

Key words
Biomonitoring, biomarkers, mussels and MSFD.
Type of presentation
Platform.
Session
t10: Biomonitoring and development of integrative assessment approach.

Tools to integrate contaminants and biomarkers in marine organisms

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Monitoring the biological effects of contaminants in marine sentinels faces numerous challenges, among which the selection of species and of parameters to be monitored, the lack of reference thresholds and the need to integrate contaminant and biological responses to inform on the environmental status.

Our aim is to propose a framework to integrate the components (biomarkers, contaminants) of a biological effects monitoring.

Several integrated approaches were tested: 1) indices informing on the distance to thresholds or other reference value types, 2) multivariate analysis to test the relationships among the different parameters and the possibility of a contaminant gradient effect and finally 3) percentages of threshold exceedance. They were applied to datasets issued from an ecotoxicological monitoring implemented in France since 2017 namely the SELI program (https://doi.org/10.18142/285). SELI took place in different bays receiving main and average size river waters (Loire, seine, Vilaine). A panel of biomarkers and contaminants, among the ICES recommendation list, were assessed in samples of mussels and flatfish used as sentinel species. The proposed frameworks are intended to support the implementation of an integrated approach in the context of the Marine Strategy Framework Directive (MSFD) and regional sea conventions (e.g. OSPAR and Barcelona convention in the case of France). This work supports the need for a long-term monitoring on mobile and sessile species to allow trends assessment.

Keywords

Integrated approach, MSFD, mussel, flatfish

Type of presentation

Platform

Session

Biomonitoring and development of integrative assessment approaches

Automated detection and quantification of histological lesions in whole slide images of Atlantic cod livers

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The histological assessment of tissue changes in aquatic organisms are commonly used for environmental monitoring of ecosystem health. However, traditional methods are often costly, time consuming, and prone to operator bias. Digital pathology allows the use of computer-based analyses which introduce the possibility of using artificial intelligence (AI) and high-throughput methodologies. These approaches have already been utilized in biomedical applications but have not been implemented in environmental studies.

In this proof-of-concept research, Atlantic cod (*Gadus morhua*) liver samples, collected during an environmental monitoring study in the North Sea, were utilised. A selection of histological lesions (steatosis, melanomacrophage aggregates, haemocytes infiltration, and granulocytoma) was evaluated by a trained histopathologist (traditional approach) and using an open-source software, QuPath (automated approach) in whole slide image (WSI) scans stained with haematoxylin and eosin. A subset of WSI was used to train a pixel classifier, and the classifier was then used to automatically detect and quantify the four lesions. Obtained results were compared to manually assigned severity scores. Comparative accuracies between the two approaches were 91.3% for steatosis, 95.0% for melanomacrophage aggregates, 78.8% for haemocytes infiltration, and 95.0% for granulocytoma. These results provide data for automated lesion detection and quantification methodologies for use in environmental monitoring studies.

Key words
Histopathology, fish, environmental monitoring, digital pathology

Type of presentation
Platform.

Session
Biomonitoring and development of integrated assessment approaches.

Biomarkers and contaminant burdens in marine tetrapod from coastal areas of southern and southeastern Brazil

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To comply with a condition imposed by the Brazilian environmental agency to carry out the production and outflow of oil and gas in Santos Basin, PETROBRAS conducts the Santos Basin Beach Monitoring Project (PMP-BS) and the Cetacean Monitoring Project (PMC-BS). The PMP-BS stands on samples from stranded marine mammals, birds, and sea turtles found in early stage of decomposition. In PMC-BS, biopsies are taken from cetacean with crossbows. This study aimed to standardize and quantify EROD, CYP1A, GST, and the transcription of genes in the liver of *Chelonia mydas*, *Larus dominicanus,* and *Puffinus puffinus*, and in the skin of *Tursiops truncatus*, *Stenella longirostris*, and *Balaenoptera brydei*. The biomarkers varied across the species, which may be related to the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) and organohalogen compounds, or other factors analyzed. Significant correlations were found between biomarkers and contaminants. For PAHs, few correlations were observed, possibly due to the capacity for biotransformation and excretion, as reflected by their low levels in the tetrapod. Polychlorinated biphenyls (PCBs) were found in all species, with higher levels of PCBs 131, 153, and 180, and were related to altered transcription of biotransformation and endocrine disruption genes. Season, year, sex, health condition, and life stage were identified as interfering factors. The continuation of this study is needed to provide a robust database that helps for assessing the risk of environmental exposure of tetrapod to oil and gas operations off the Brazilian coast.

Key words
Biomarkers, tetrapods, body burden, monitoring, PCB

Type of presentation
Platform.

Session
Biomonitoring and development of integrative assessment approaches.

Assessing the radioecological risk associated with the legacy discharges of oil and gas produced water in the offshore marine environment

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Produced water generated during offshore oil and gas (O&G) extraction activities is disposed of at sea and contains enhanced levels of radium-228 and radium-226 in the form of radiostrontiobarite ($Ba(Sr,Ra)SO_4$) particles. Due to limited access within the 500m safety zone established around any operating offshore O&G platforms, previous radioecological risk assessment studies have been conducted using data from the radiometric measurement of seawater samples collected further from the discharge point, dispersion modelling, and applying default transfer coefficients to estimate concentrations in sediment substrates and biota of interest.

In this case study, sediment cores and benthic biota were collected in 2021 within and outside of the safety zone of a decommissioned O&G platform located in the UK North Sea. Concentration levels of radium-228, radium-226 and lead-210, in subsurface sediment materials were first determined by gamma spectrometry. Sediment aliquots, along with selected nematode and bivalve mollusc samples, were also digested for barium and strontium analysis by ICP-MS/MS to confirm the presence of $Ba(Sr,Ra)SO_4$ particles. In addition, full and micron-size slices of nematodes were prepared for Hard X-ray fluorescence analysis to investigate the different exposure routes involved. The subsequent dose rates were then estimated using the ERICA assessment tool and were found to be well below the threshold value of 10 µGy/h in both the control (3,200m) and exposed groups (50m), suggesting the radioecological risk to be negligible.

Key words
Radioecology, benthic biota, oil and gas produced water, Hard X-ray imaging

Type of presentation
Oral presentation

Session
New approach methodologies (NAMs) to assess pollutant toxicity

Contaminants of Emerging Concern in the Marine Environment: An Integrated Effects Assessment Approach (CONTRAST)

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Abstract

The Horizon Europe project, CONTRAST, will develop an integrated assessment and effect-based monitoring framework (IAF) to measure the impacts of contaminants of emerging concern (CECs) on the marine environment, which will contribute to the assessment of Good Environmental/ Ecological Status for application in EU policy (MSFD/WFD). The IAF will involve chemical measurements together with biological effects endpoints optimised to detect the presence and effect of CECs in the marine environment. Chemical prioritisation schemes will identify the CECs that pose the greatest threat to marine life and select which CECs to target in the laboratory experiments, where the effects on organisms and biodiversity will be assessed. *In silico*, *in vitro* and *in vivo* bioassays will be used to determine the mechanisms of toxicity of selected CECs. Providing information on how CECs interact with organisms at environmentally relevant concentrations and which biological effects tools should be used in the IAF to cover the range of toxicity mechanisms that CECs produce. A series of European-wide case studies will be used to test the suitability of the IAF to measure the effects of chemicals including CECs on indicator species and biodiversity. The knowledge gained from field testing and laboratory studies will form the basis for guidance documents and policy briefs on best practices for performing an IAF on CECs in the marine environment and help to provide the necessary protection of marine ecosystems.

Key words: Biomarkers, mechanisms of toxicity, biomonitoring, integrated assessment, EU policy.
Type of presentation: Platform.
Session: Biomonitoring and development of integrative assessment approaches.

Operational invertebrate behaviour videotracking for real-time wastewater surveillance and management

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As availability of water resources decreases, the apparition of new contaminants of concern (CEC) identified continues to increase. Current wastewater monitoring in treatment plants relies on interval based grab sampling measurements for a select few chemicals or for punctual effluent toxicity assessment using a restricted choice of bio-assays. Complementary innovative approaches to wastewater management are essential to avoid detrimental impact of CECs in aquatic ecosystems and water re-use.

ToxMate biomonitoring, whereby the behavioural video-tracking of bio-indicator invertebrates (Gammarus, Radix, and Erpobdella), has been shown to be effective in non-targeted effect-based screening of micro pollutant presence. Here, we illustrate results from long term surveillance in WWTPs to optimise operation, where the characterisation of effluents in real-time for the biomonitoring approach alerts operators, allows strategic sampling and provides indicators for re-use suitability, all leading to better a understanding treatment efficiency. Finally, continued laboratory testing has led to the accumulation of behavioral reaction analysis for over 40 micropollutants, leading to the discovery of behavioural fingerprints (Figure 1).

Coupling moments of concern with fingerprinting may help adapt wastewater management strategy in real-time. Thus, the potential is not only limited to the improvement of aquatic environmental quality through wastewater management, but also provides indicators of the suitability of re-use in various domains such as urban, agricultural and leisure use.

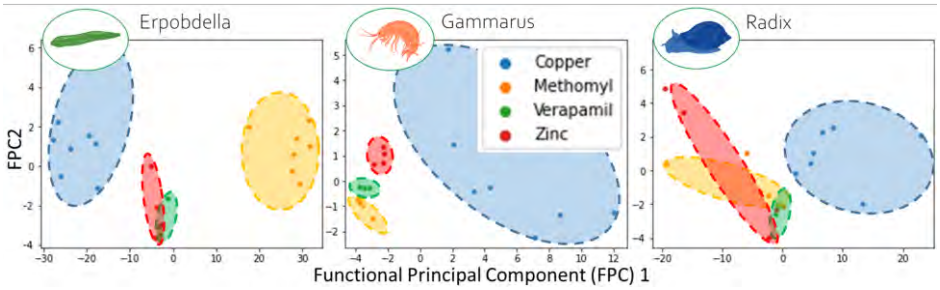

Figure 1 Functional Data Anlaysis to show behavioural differences in response to different micropollutants.

Key words

Biomonitoring, wastewater, micropollutants, ecotoxicology.

Type of presentation

Platform.

Session

1/ Biomonitoring and development of integrative assessment approaches 2/ New approach methodologies (NAMS) to assess pollutant toxicity 3/ New Tools to track pollutant sources and transfers

SCIENTIFIC PROGRAM OF PRIMO 22

Biomonitoring and development of integrative assessment approach

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Application of Environmental Indicator Organisms Using Genetic Engineering Techniques

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While the use of aquatic organisms to assess water environments has a long history, advancements in technologies such as genome editing have enabled a deeper understanding and application at the genetic level in recent years. Here, we report on the application of genome editing techniques to microcrustaceans used as environmental indicator organisms in aquatic environments for monitoring purposes.

Daphnia magna, a species of microcrustacean, is commonly used in environmental toxicity tests due to its sensitivity to chemicals, rapid growth, reproduction, and ease of cultivation.

By developing genome editing techniques for Daphnia magna, we have been able to create a system for monitoring water environments more conveniently.

One is the development of indicator organisms that visualize environmental responses. By introducing a gene encoding a fluorescent protein with the regulatory regions of environmentally responsive genes, we have succeeded to create strains that emit fluorescence in response to heavy metals or hormone-like chemicals, enabling real-time monitoring of changes in water environments.

We are also using genome editing techniques to prove the mechanisms of environmental response. For example, when the phase I p450 gene is disrupted by genome editing, sensitivity to specific chemicals increases, revealing the gene that is actually involved in toxin metabolism in Daphnia magna. Additionally, it has been revealed that sensitivity to chemicals increases when intestinal bacteria are removed. These approaches are shedding light on the mechanisms underlying sensitivity to chemicals.

These findings are crucial for assessing responses to and evaluating pollutants, including chemicals in aquatic environments, including marine organisms.

Key words
Genome editing, *Daphnia magna,* heavy metal,

Type of presentation
poster

Session
Biomonitoring, AOP

AROD activities and environmental contaminants in gray and Franciscana dolphins from brazilian coast: Is there any difference between species?

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Numerous studies have shown declines in odontocete populations. Polycyclic aromatic hydrocarbons (PAHs) can induce immediate and long-term toxic effects and are classically associated with the induction of cytochromes P450 (CYPs). Given their role in the xenobiotic biotransformation, CYPs are good biomarkers of exposure. *Pontoporia blainvillei* (Franciscana) is the most endangered cetacean in the Southwest Atlantic, while *Sotalia guianensis* (gray dolphin) is becoming threatened. These populations inhabit distinct regions influenced by human activities. Limited information is available about xenobiotic biotransformation in these species, and its characterization can contribute to the development of tools for conservation. The aim of this study was to assess the kinetic properties of CYP enzymes in the gray dolphin by measuring AROD activities (MROD, BROD, EROD) in liver microsomes. The samples were obtained during necropsy of stranded animals collected during the Beach Monitoring Project of Santos Basin (PMP-BS), carried out by PETROBRAS to satisfy environmental constraint of the federal environmental licensing of PETROBRAS. The results were compared with existing data on AROD activities in Franciscana. PAHs levels in both species were also compared. K_{mapp} and V_{max} were determined for MROD, BROD, and EROD activities in the gray dolphin. MROD and BROD activities exhibited significant differences between both species, suggesting different abilities of xenobiotic biotransformation possibly associated with CYP composition in Franciscana and gray dolphin.

Key words
AROD. CYPs. Dolphins. PAHs.

Type of presentation
Platform.

Session
Biomonitoring and development of integrative assessment approaches

Biotransformation pathways and mode of action (MOA) of chemical pollutants

French assessment of the good environmental status achievement for the descriptor 8 “Chemical contamination” of the marine strategy framework directive

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Within the marine strategy framework directive (MSFD), the descriptor 8 (D8) aims to assess the good environmental status (GES) achievement regarding chemical contamination and its biological effects. Data acquisition through monitoring programs allows the calculation of indicators for the periodical GES achievement assessment. Since the 2012 initial assessment, the monitoring and evaluation strategies have been enhanced, by adding new monitoring programs and developing new indicators on *i)* the chemical contamination in high trophic levels (fish, birds, marine mammals) and *ii)* the ecotoxicological status of fish and bivalves.

This presentation reports the D8 assessment strategy in France, with focus on the chronic chemical contamination and its effects on biota. The results showed a global contamination on the continental shelf, from the coast to the slope. Mercury and polychlorobiphenyls were the most frequent regulated substances with levels beyond the thresholds. Some emerging substances of concern, without environmental threshold to date, may also threat the environment. Moreover, flatfish were affected by the contamination and showed genotoxic and neurotoxic effects, leading to a non-achievement of GES for several indicators.

This work highlights the need for an extensive long term monitoring of the chemical contamination and its effects on the different environmental compartments, at different geographic scales and various trophic levels.

Key words
MSFD, Contaminants, Ecotoxicology, Monitoring, Assessment.

Type of presentation
Platform.

Session
t10: Biomonitoring and development of integrative assessment approach.

Assessment of trace elements contamination and its effect on *Paracentrotus lividus*

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Among the most common contaminants in marine ecosystems, trace elements (TEs) are considered as serious pollutants due to their toxicity, persistence and ability to accumulate in marine organisms. In Corsica, near an old asbestos mine, TEs from the leaching of mine residues have been discharged into the sea for many years. The aim of this study was to assess the levels of contamination in this area and the potential effects on *Paracentrotus lividus* using pollution indices, accumulation factors and biochemical tools. For this purpose, the concentration of 24 TEs was measured in sea urchins, macroalgae, seawater column and sediment collected at 12 stations nearby the old asbestos mine and at a reference site. TE Pollution Index revealed a higher level of contamination in the south of the old asbestos mine. This is mainly due to the dominant marine currents allowing the migration of mining waste along the coastline. This hypothesis was supported by TE Spatial Variation Index, which identified characteristic TEs in the southern area of the mine. High hydrogen peroxide content, associated with elevated catalase and glutathione-S-transferase enzyme activities, were also identified at these sites and at the reference site. TE contamination as well as several abiotic factors could explain these results. The results obtained in this study suggest that oxidative stress induced by contamination does not affect the health of *P. lividus*. This work has provided a useful dataset allowing better use of sea urchins and various tools for assessing TE contamination in coastal ecosystems.

Key words

Trace elements, Pollution Index, Oxidative stress, *Paracentrotus lividus*, Asbestos mine

Type of presentation

Poster

Session

Biomonitoring and development of integrative assessment approach

Other suggestions: New tools to track pollutant sources and transfers

Development of a multi-bioassay approach for dredged marine risks assessment before immersion at sea

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Harbor sediments are the receptacle of pollution from the watershed on which they depend and from the port activities. To maintain their activities, port authorities organize sediments dredging often dumped at sea, which can affect marine ecosystems by transferring their pollutions to the water column (Wilber et Clarke 2001).

In France, the environmental risk assessment for dumping at sea of port dredged sediments is based on chemical analyses using concentrations thresholds. Despite some guidance developed in the early 2000s, the ecotoxicological risk remains difficult to implement and apprehend. Only one of the bioassays recommended by GEODE[a] is generally carried out (Geode 2016) and its implementation and interpretation are not sufficiently framed. Different approaches are emerging for the assessment of dredged marine sediments, which recommend the use of a battery of bioassays covering different trophic levels coupled with chemical analyses (Anselmi et al. 2023).

The objective of the ECOSED[b] project is to propose a graded protocol for the ecotoxicological risk assessment adapted to marine dredged sediments to stakeholders, following the approach of the HP14 protocol for wastes management (Pandard 2016). Preliminary results from testing a battery of standardized bioassays (Microtox liquid phase test ISO 11348-3, marine algal growth inhibition test with *Phaeodactylum tricornutum* ISO 10253, acute lethal toxicity to marine copepods *Tisbee Battagliai* ISO 14669), on French port dredged sediments are presented.

[a] GEODE : Study and observation group on dredging and the environment
[b] ECOSED : Evaluation of ecotoxicological tools for diagnosing the quality of marine sediments

Anselmi, Serena, Paolo Pastorino, Francesca Provenza, et Monia Renzi. 2023. « Ecotoxicity of marine sediments: Sampling and laboratory artifacts and their impacts on risk classification ». *Journal of Environmental Management* 334 (mai): 117483. https://doi.org/10.1016/j.jenvman.2023.117483.
Geode. 2016. « Bonnes pratiques pour la caractérisation des matériaux en vue d'une opération de dragage et d'immersion en milieu marin et estuarien ».
Pandard, Pascal. 2016. « Classification réglementaire des déchets - Guide d'application pour la caractérisation en dangerosité ».
Wilber, Dara H., et Douglas G. Clarke. 2001. « Biological Effects of Suspended Sediments: A Review of Suspended Sediment Impacts on Fish and Shellfish with Relation to Dredging Activities in Estuaries ». *North American Journal of Fisheries Management* 21 (4): 855-75. https://doi.org/10.1577/1548-8675(2001)021<0855:BEOSSA>2.0.CO;2.

Key words
Ecotoxicological risk assessment, dredged marine sediments, Bioassays, MSFD

Type of presentation
Poster presentation.

Session
t10: Biomonitoring and development of integrative assessment approach
Field studies, methodology to integrate both chemical and biological data (biomarker responses). The link with reglementary (e.g MSFD D8 in Europe) and national and international surveys.

Do sampling methods influence stress measures on deep sea mussels?

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The implementation of research studies to assess the health status of marine environments relies on sentinel species and key biological parameters. While comprehensive knowledge regarding species biology and recommended procedures for biomarker assessments exists, detailed information on the effects of sampling methods on physiological processes is lacking.

When dealing with deep sea ecosystems, sampling procedures include the use of ROVs, which can induce several stress sources, like the long delays between samples collection, dissection and storage (≈12hrs), or the presence of broken mussels in the tank due to pliers sampling. This could affect the physiological responses used as proxies of the ecosystem health status. The best method for collecting deep-sea organisms while limiting physiological stress remains to be determined.

The present study aims to measure several stress markers at molecular and cellular levels on the deep mussel *Bathymodiolus azoricus*, in different sampling and storage conditions. Several endpoints related to global stress, oxidative stress, neurotoxicity,… were selected. The first step is to implement these endpoints on existing deep samples from previous campaigns.

The perspective of this work is to assess the same panel of physiological endpoints on the deep mussel *Bathymodiolus azoricus,* during future campaigns and to evaluate the impacts of various sampling modes. Lipid analyses and genetic tools will also be respectively used as proxies of cellular membrane integrity and molecular damages, and for a non-targeted screening of gene expression.

Key words
Ecotoxicology, bivalve, sampling, biomarkers.

Type of presentation
Platform.

Session
t10: Biomonitoring and development of integrative assessment approach.

Environmental Drivers and Antibody Responses Against *Streptococcus agalactiae* in Bottlenose Dolphins, *Tursiops truncatus*

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The bottlenose dolphin, *Tursiops truncatus*, is a sentinel species for evaluating and monitoring ecosystem health in critical coastal regions. For example, the Atlantic bottlenose dolphin health and environmental risk assessment project (HERA) has studied these animals over the past 20 years from the Charleston harbor in South Carolina, USA, and the Indian River Lagoon in Florida, USA, as part of an overall approach to understanding links between Oceans and Human Health. These samples were compared to the managed US Navy and GA Aquarium populations. Specifically, emerging infectious diseases and zoonotic pathogens have become a serious and complex threat to humans, animals, and environmental health. In this study, a monoclonal antibody (mAb KA-9) was generated against bottlenose dolphin IgG and used to calculate relative antibody titers against *Streptococcus agalactiae,* an emerging pathogen of concern, at a dilution of 1:200 and to compare these data to antibody activities calculated over a range of serum dilutions in serum samples. There were few differences between the groups in terms of relative antibody titers against *S. agalactiae*, but calculated antibody activity data demonstrate differences. Previously published studies from our group demonstrate differences in antibody responses against several other highly pathogenic bacteria when comparing the dolphin groups over the years. Compared to those data, it appears that bottlenose dolphins are not currently burdened by *S. agalactiae,* and the managed US Navy and GA Aquarium populations are very healthy.

Key words
Tursiops truncatus, bottlenose dolphin, *Streptococcus agalactiae*, mAb KA-9, Oceans and Human Health

Type of presentation
Poster

Session
Biomonitoring and Development of Integrative Assessment Approaches

Environmental Monitoring of Brodifacoum Residues After Rodent Eradication in Europa Island: a case study in a pristine environment

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Invasive rats are known threaten vulnerable native bird species in Western Indian Ocean islands. Brodifacoum (BDF), used to eradicate them, disrupts the vitamin K cycle, causing hemorrhages in target mammals. This study aimed to assess environmental contamination and BDF transfer to non-target marine species after a rodent eradication initiative in a pristine environment.

Rodent eradication was conducted in a small mangrove forest (17 ha) on Europa Island using broadcasted BDF pellets. This work focused on intertidal and coastal food web contamination, involving two sampling sites and three field campaigns (Figure 1). Sampling encompassed water and non-target organisms (4 invertebrates and 2 fishes).

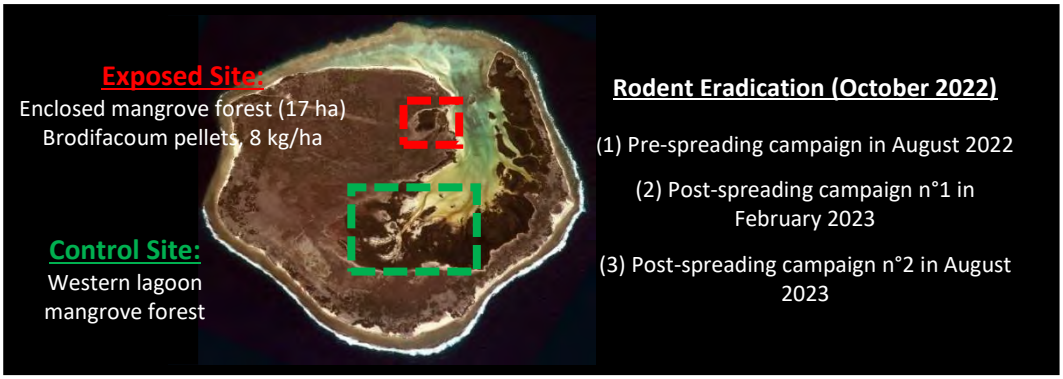

Figure 1: Map of Europa Island presenting the sampling sites

No BDF traces were detected in water samples. However, BDF was detected in 100% of biological samples from the exposed site, 4 months after spreading, with concentrations ranging from <1 to 165.2 $ng.g^{-1}$ dw. Primary exposure was observed for crabs and hermit crabs by camera traps, and likely linked to the highest concentrations quantified in the hepatopancreas of crabs (*Scylla serrata)*. BDF levels in fishes, *Gerres longirostris* and *Lutjanus argentimaculatus*, suggested secondary exposure via the marine food chain and/or the environment. Sessile oysters from the control site exhibited BDF traces, sing of potential contamination via tidal currents. Temporal trends were studied by comparing concentrations found in the two post-campaigns. This comprehensive investigation of punctual contamination in a pristine environment would support modeling of pollutant transfer mechanisms.

Key words
Brodifacoum, Rodent eradication, Environmental monitoring, Non-target marine species
Type of presentation: Platform
Session
1-New tools to track pollutant sources and transfers
2-Biomonitoring and development of integrative assessment approaches

Establishing standardized protocols for characterising marine litter in submarine canyons: implications for regulation and conservation

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Litter pollution is a global concern, occurring in all oceans, including deep benthic habitats, where the extent of the problem remains largely unexplored. Characterization of litter on river banks and beaches have been standardized in the OSPAR convention. However, to date, no such accurate protocol exists for the seabed, leading to the "opportunistic" quantification and categorization of benthic marine litter, whether by trawling or imaging (ROV), which makes temporal and geographical comparison difficult.

Submarine canyons host a wide variety of marine species, particularly with the presence of reef-building cold-water corals that provide habitat for many species. But canyons are also a major sink for waste debris that represent a threat to deep-sea biodiversity. Based on the OSPAR litter surveys, we propose a new protocol to standardize quantification of marine litter in canyon ecosystems, by conducting repeated 100-meter transects using ROV video capture.

This protocol was tested at three sites in the Lacaze-Duthiers Canyon (Gulf of Lion, Western Mediterranean Sea), along a longitudinal gradient from 300 to 600 m depth, where a high density of cold-water coral reefs occurs. A significant part of the marine litter found at these sites appeared to be fishing-related plastics and, to a lesser extent, bags, mostly entangled in coral reef structures.

This easy-to-implement deep-sea protocol is a promising tool for the assessment of the descriptor 10 of the European Marine Strategy Framework Directive (MSFD), which ensures that marine debris do not harm habitat condition.

Key words
Litter, pollution, canyons, deep-sea corals

Type of presentation
Poster

Session
Biomonitoring and development of integrative assessment approaches

Health status in amphipods and fish in the Baltic coastal ecosystem: effect-based contaminant monitoring

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Effect-based monitoring (EBM) is a valuable tool for understanding how contaminants impact ecosystems. However, successful implementation depends on reliable sentinel species and informative biomarkers accurately reflecting biological responses to pollutants. Our study assessed the usefulness of various biomarkers in fish and benthic invertebrates for EBM. This involved analyzing contaminant concentrations and biological responses in Baltic Sea coastal areas with different pollution levels.

Our findings revealed several contaminant groups exceeding safe levels, causing adverse effects in the amphipod *Monoporeia affinis* and fish (perch, *Perca fluviatilis*, and eelpout, *Zoarces viviparus*). In amphipods, trace metals, Hg, PBDD/F, PBDEs, HCB, and OTCs were linked to embryo aberrations and oxidative stress. In fish, HCBs, PFAS, and PCBs emerged as key predictors of multiple adverse effects and linked to e.g. slower growth, lower biomas index and oxidative stress; moreover, some of the effects in fish were sex-specific. We also developed a highly accurate logistic model (>90%) to assess exposure probability based on the observed biological responses.

This research highlights the significance of EBM in assessing the risk posed by environmental contaminants. Additionally, we present a classification tool leveraging regional sentinel species and biomarkers with established baselines to predict the impact of pollution in specific areas. Ultimately, our study contributes to advancing effective risk assessment strategies for environmental health.

Key words
Environment, biomonitoring, effect-based, biomarker, contaminants

Type of presentation
Poster

Session
Biomonitoring and development of integrated assessment approaches

Interspecies differences in lysosomal membrane stability in marine clams

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Lysosomal membrane stability (LMS) in bivalve hemocytes is a core biomarker of general cellular stress and is widely implemented in mussel biomonitoring programs for marine chemical pollution. However, the utility of this biomarker in other bivalve species has been less explored. The wedge clam (*Donax trunculus*) and the striped venus clam (*Chamelea gallina*) have a significant socio-economic value as fishing resources in the Western Mediterranean. Even so, their numbers have declined significantly over the past few decades in the Spanish Mediterranean Region.Using LMS biomarkers in these clam species may contribute to monitoring the natural populations' health status. Background knowledge of how LMS responds to abiotic and biotic factors in these species is necessary for a proper assessment. This work investigated the combined effect of the water temperature and salinity on the lysosomal membrane integrity in *D. Trunculus* and *C. gallina*. LMS was estimated in hemocytes by using the neutral red retention (NRR) assay. The animals were subjected to nine relevant environmental conditions for 21 days under controlled laboratory conditions. To this end, three water temperature ranges were combined with three salinity ranges. The results indicated interspecies differences in the background response of LMS under similar environmental conditions. Overall, the results pointed out that the adaptive capacity of the cellular processes in these species was reduced at the highest temperature tested (28ºC) and that *C. gallina* showed higher tolerance to water salinity and temperature changes than *D. trunculus. T*he environmental conditions that led to greater LMS (> 75 min) were those with medium temperature (20 °C) at the three studied salinity ranges (28, 33, and 38 PSU), supporting findings found by other authors that global warming will affect hemocyte functionality, which is directly related with bivalve immune defense capabilities.

Keywords
Chamelea gallina; Donax trunculus; Cytotoxicity; Global warming; Neutral red;

Type of presentation
Poster

Session

t10: Biomonitoring and development of integrative assessment approach

Metals in Manila clam (*Ruditapas philippinarum*) and Grooved carpet shell (*R. decussatus*) from the French English Channel and the French Atlantic coast

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Although metals are naturally present in the Earth's crust, anthropogenic activities (industry, mining, agriculture) increase their concentration in the environment, especially the marine one. They are well-known to lead serious ecotoxicological issues and to be toxic to marine organisms, as well as humans who consume them. Like many other intertidal filter-feeding bivalves, Manila clam (*Ruditapes philippinarum*) and Grooved carpet shell (*Ruditapes decussatus*) are both resistant to environmental physico-chemical variations and largely exposed to marine pollution. Then, they can easily bioaccumulate contaminants, such as metals. They are thus considered as good monitoring species. Moreover, they could be a risk for human health as these clam species are widely exploited. For the first time, spatial and seasonal variations of bivalve metal contents were studied in 8 areas from the French English Channel to the French South Atlantic coasts which correspond to the main French recreational fishing areas. Metal concentrations (Al, V, Cr, Mn, Ni, Cu, Zn, Cd, Pb, Hg, Fe, As) were determined in *R. philippinarum* and *R. decussatus* tissues using inductively coupled plasma - mass spectrometry (ICP-MS) after a mineralization step. Some differences of contamination were observed between the seasons, clam species, areas and the intensity of recreational fishing effort.

Key words: Contamination, bioaccumulation, bivalve, recreational fishing

Neutrophils on the Move: Refinement of a Fish Neutrophil Migration Assay for Evaluating Immunotoxicity

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Environmental contaminants can adversely impact fish health by altering immune function. Despite the potential impacts of immunomodulation on populations, a standardized immunotoxicity screening method has yet to be developed leaving a gap in the hazard assessment toolbox. Alterations in the migration of neutrophils, the “first responders” of the immune system, may serve as an indicator of immune dysfunction. Neutrophil migration (NM) is typically evaluated by injuring the tail of a larval fish and counting the number of neutrophils that move to the injury site. This approach is time-intensive and requires a large number of fish due to high individual variation. This study aimed to refine a fathead minnow (fhm) NM assay to overcome these obstacles and improve its potential as an immunotoxicity screening tool. The specific objectives of this study were to determine: 1) if gene expression analysis can be used to evaluate NM and 2) the sensitivity of a gene-expression-based NM assays. To address the objective 1, the expression of several neutrophil-specific genes was measured in 7-day old fhms subjected to a tail injury and compared to that of uninjured fhms to identify biomarkers of NM. To address the objective 2, the sensitivity of the identified biomarkers (e.g., *mpo, elas2, etc.*) was evaluated by evaluating the dose-dependent expression of each in fhms exposed to dexamethasone (a known immunosuppressant). The results of this study, which will be presented in detail, demonstrate the potential utility of a fhm neutrophil migration assay for immunotoxicity evaluations.

Key Words
Synthetic Glucocorticoid, Biomarker, Immunotoxicity Screening Assay

Type of Presentation
Poster

Session
Biomonitoring and development of integrative assessment approach
Mixture effects of pollutants

Occurrence, biota distribution and trophic transfer of PFAS and toxicological responses in tropical aquatic food webs from the Cross River Estuary, Nigeria

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In this study, we have invested the occurrence, biota distribution, and trophic transfer of legacy and emerging PFAS in fish (Chrysicthys nigrodigitatus), fiddler crab (Uca tangeri), prawn (Macrobrachium vollenhovenii), periwinkle (Tympanotonos fuscatus) and sediment samples from three sites (Adiabo (control site), Obutong and Nsidung) representing different degrees of anthropogenic contamination along the Cross River Estuary. The sum ∑PFAS concentrations in sediment was significantly highest in Obutong (14.3 ng/g) and Nsidung (2.4 ng/g), compared with Adiabo (1.7 ng/g). PFHxA, EtFOSE, PFBS and PFPeS were the dominant PFAS congeners. Further, a significant increase in the concentrations of total ∑PFAS concentrations for C. nigrodigitatus, U. tangeri and M. vollenhovenii were measured at Obutong and Nsidung, compared to Adiabo. For T. fuscatus ∑PFAS (ng/g) concentrations was significantly higher at Obutong, compared with other sites. PFOA, PFOS, EtFOSE, PFBS in C. nigrodigitatus; PFOSA, EtFOSE, PFPeS in U. tangeri; PFOSA, EtFOSE, PFPeS in M. vollenhovenii and 6:2 FTS, PFBS, PFOS and EtFOSE in T. fuscatus were the dominant congeners. Significant accumulation of PFOS and PFOA was measured in the liver, compared with muscle tissues of C. nigrodigitatus. The BSAF showed the highest value in M. vollenhovenii (2.0), U. tangeri (1.9) and C. nigrodigitatus (1.0) at Nsidung, compared with other sites. The PFAS BMF shows site and species-specific differences. Using principal component analysis (PCA), we demonstrated a relationship between PFAS body burden and toxicological responses related oxidative stress (Gpx, Gr, Gst). Our data represent the first comprehensive reports on PFAS bioaccumulation across aquatic food webs from a developing country such as Nigeria, indicating possible biodiversity, human health risk and food safety concern.

Key words
PFAS, Bioaccumulation, Toxicological responses, Food webs, Cross-River Estuary

Type of presentation
Oral

Session
New tools to track pollutant sources and transfer.

One Health approach in a small marine bay: HOBE pilot project

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The Plentzia-Gorliz Bay represents a typical situation in the Atlantic European coast with strong seasonal tourism influence in a region with previous scientific data from various disciplines (marine ecotoxicology, microbiology etc.). We aim to understand how climate change modifies the impacts of coastal pathogens on human/animal/plant health using a One Health perspective. The novelty lies in grounding the framework on an "extended One Health" approach incorporating holistic concepts of nature of human, animal and ecosystem health (with a marine perspective). This requires bringing together i) an in-depth understanding of the physical, chemical and biological processes, ii) the complexities and interrelations arising as well as iii) the contextual knowledge of local agents from the social, economic and policy spheres. Water column was monthly filtered for chemical profiling, Vibrio content analysis, total bacteria activity (also for WWTP water), fito-, zoo-plankton and pigment content, and metagenome analysis. Moreover, seasonal sampling of oysters and squid provided pathogens, histopathology, contamination biomarker approach and chemical bioaccumulation info. Every hour pictures showed the general use of the beach; and social questionnaires provided info about the climate change knowledge and socioeconomic situation of the area. All the data will be included on a platform to facilitate the transparency to the stakeholders.

This is an opportunity to tackle some needs under the framework of the One Health paradigm.

This is part of the TED2021-132109B-C21research project funded by MCIN/AEI /10.13039/501100011033 and by the European Union NextGenerationEU/ PRTR

Key words
One health, pilot-study, pathogens, climate-change.

Type of presentation
poster.

Session
t10: Biomonitoring and development of integrative assessment approach.

Phthalate concentration in Mediterranean fish species from Ligurian Sea and Adriatic Sea using ultrasound assisted extraction methodology

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Plastic additives such as phthalates represent a significant concern for marine environment due to their widespread presence and their possible ecotoxicological impacts on marine organisms. The aim of this study is to provide a preliminary assessment of the occurrence and concentration of phthalates in muscles of four Mediterranean fish species (*B.boops, M.merluccius, M.barbatus, S.pilchardus*; N°=75) from the Adriatic and the Ligurian Sea. A new technique that coupled ultrasound assisted extraction, d-SPE clean-up and GC-MS analysis was applied for the evaluation of 11 different phthalates. Results showed that 9 out of 11 phthalates were detected. The most detected compounds were DEHP and DBP (97% occurrence), along with DIBP (93% occurrence), confirming the trends in the Mediterranean Sea, where these substances are among the most frequently observed. Regarding the sum of phthalates, results show different trends but not significant differences between the areas and species were highlighted. Concerning different compounds, the highest concentration of DEHP was found in a specimen of *M.merluccius* (246 ng/g w.w.), while for DBP and DIBP, the highest values were observed in a specimen of *M. barbatus* (361 and 455 ng/g w.w.). A statistically significant difference was found for DBP in *S. pilchardus* with concentrations of 116 ng/g in the Adriatic Sea and 30 ng/g in the Ligurian Sea. In conclusion these results provide a starting point to address the knowledge gaps concerning the distribution of phthalates in the mediterranean fish species and the potential toxicological effects.

Key words
Phthalates, emerging contaminants, plastic additive

Type of presentation
Poster

Session
Particles, fibres, plastic and their additives

Prevalence of PFAS Compounds in Commercial Alaska Fish

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Per-and polyfluoroalkyl substances (PFAS) are a ubiquitous class of contaminants commonly found in stain-resistant coatings, firefighting foams, and many other applications. Many PFAS have been linked to reproductive, developmental, and immunological toxicity. Due to their inert carbon-fluorine bonds, they do not readily break down and can bioaccumulate within organisms. Their persistence and toxicity have made them a chemical of concern. NOAA's NCCOS, along with the AFSC, are working to assess PFAS in juvenile and adult pink and coho salmon in Alaska, two species of ecological and economic importance to the region. A suite of 28+ PFAS are being analyzed in an outgoing juvenile cohort and then in returning, mature adults associated with the same cohort. Over a 3-year span, tissues are being collected in Sashin Creek at the Little Port Walter Marine Research station and Auke Creek Research Station, and analyzed for PFAS. The goal of the project is to better understand the transport potential of PFAS across industrial complexes to near-pristine environments, assess their bioaccumulation potential in coastal and pelagic environments using commercial fish stocks, and elucidate biomagnification pathways. This work will allow resource managers to better predict the effects of PFAS pollution and to develop appropriate mitigation policies. Results in outgoing juvenile cohort during the first year indicated elevated levels of PFOS in some coho salmon from Sashin Creek, some above established consumption guidelines. Analysis of samples from Year 2 and 3 of are currently underway.

Session (in order of preference):
1. t08: Mixture effects of pollutants
2. t10: Biomonitoring and development of integrative assessment approach
3. t01: Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

Key Words:
PFAS, Salmon, Fisheries, Monitoring

Presentation Type: Poster Preferred

Recent developments towards harmonised marine contaminant data reporting in the European Union

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Marine pollution by chemical contaminants is a major concern in European waters and is addressed by the EU Marine Strategy Framework Directive and Water Framework Directive, and also by Regional Sea Conventions (*e.g.*, Mediterranean Action Plan, HELCOM, OSPAR convention). Marine pollution assessment under these policies requires the collection and analysis of data from several sources (*e.g.*, national authorities, academic research). These data may be sparse and heterogeneous due to non-harmonised sampling protocols, analytical methods, quality assurance/control. The European Marine Observation and Data Network (EMODnet) was setup to provide harmonised, comparable and accessible data on multiple marine parameters. In this study, we analysed chemical contaminant data available through EMODnet Chemistry to evaluate coverage and coherence of monitoring efforts across European seas. Preliminary results show that more than 400 substances (*e.g.*, trace elements, legacy- and emerging pollutants, radionuclides) were measured in biota, water and sediment, at different spatiotemporal scales, with varying efforts depending on the marine region (Atlantic and Arctic oceans, Baltic, Black, Mediterranean and North Seas). This study will identify gaps in geographical and environmental media monitoring efforts and develop new recommendations to improve data reliability and comparability. Harmonised, efficient monitoring of marine contaminants across the EU is critical to inform policy makers and assist Member States in achieving Good Environmental Status with respect to marine pollution.

Key words
Assessment, Europe, data heterogeneity, monitoring, policy

Type of presentation
Platform

Session
Biomonitoring and development of integrative assessment approaches

Seasonal Profile of the Antidepressant Sertraline and its Derivative Norsertraline in Edible Bivalve Molluscs

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Sertraline is a highly prescribed selective serotonin reuptake inhibitor (SSRI) antidepressant which possesses high lipophilic properties that result in bioaccumulation. Pilot studies have shown that sertraline, and its metabolite norsertraline, had the highest concentration amongst 27 pharmaceuticals that were measured in bivalve molluscs (mussels and oysters) from England and Wales. The high levels of these antidepressants in bivalve molluscs are most likely due to their high prescription levels and their high potential for bioaccumulation. The levels of both sertraline and norsetraline demonstrated a seasonal and geographical pattern, with highest concentrations detected during the winter and spring at sites associated with high population densities. This study expanded upon the pilot study which investigated a broad range of compounds at 4 selected time points distributed over one year and provides the results of a three-year monitoring study on the levels of sertraline and norsertraline in bivalve molluscs from England and Wales. The strong seasonal pattern was confirmed during this period with highest concentrations during the winter and spring and maximal concentrations of 42.7 ng/g at one site and 111.5 ng/g at another. Similarly, the distinct link between human impact and sertraline concentrations was also confirmed. This study highlights the utility of bivalve molluscs as sentinel species for assessing sewage contamination in the coastal environment.

Key words
Bioaccumulation, uptake, antidepressant, mollusks, sertraline (5 key words at maximum)

Type of presentation
Poster

Session
t10: Biomonitoring and development of integrative assessment approach

Trace element levels and biomarker responses in a cold-water coral ecosystem, the Lampaul canyon within the Bay of Biscay

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Chemical contamination and its effects to marine organisms have been mainly studied in coastal environments. The deep sea remains largely unexplored, though few studies have reported contaminant levels sometimes close to those reported/found on the coast.

Our aim is to assess contaminant levels and biomarker responses in cold-water corals, their associated fauna and sediments in a submarine canyon of the Bay of Biscay (Lampaul canyon, NE Atlantic).

The Lampaul canyon is colonised by cold-water corals, which have been monitored by the Marley Observatory since 2021 at 780-m. During the ChEReef 23 sea campaign (10.17600/18003189), cold-water corals (*Madrepora oculata* and *Desmophyllum pertusum* (syn. *Lophelia pertusa*)), polychaetes (*e.g.* Eunicidae, Hesionidae) and bivalves (*Acesta excavata*) were sampled. Sediments were also collected from the canyon up to 4000-m depth.

Current state of knowledge regarding deep-sea contamination is first reviewed. Then, existing protocols for contaminant and biomarker analyses are adapted from existing protocols and applied to the selected samples. These protocols encompass trace elements, including rare earth elements as well as anti-oxidant enzymes (*e.g.* GST, SOD, CAT, …), metal detoxification (*e.g.* metallothionein), cellular stress (*e.g.* lysosomal membrane stability), neurotoxicity (*e.g.* acetylcholinesterase inhibition) markers and gene expressions associated to these functions. Overall, this study will provide first insights into chemical contamination levels and responses of marine organisms in deep-sea environment.

Key words
Deep-sea, trace element levels, biomarkers, Lampaul canyon

Type of presentation
Poster

Session

Biomonitoring and development of integrative assessment approaches

Uptake and Bioaccumulation of the Antidepressant Sertraline in Blue Mussels

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Sertraline is a highly prescribed and highly lipophilic SSRI antidepressant and thus has the potential for bioaccumulation in animal tissues. Previous studies have revealed that sertraline displayed the highest concentration amongst a range of measured pharmaceuticals in bivalve molluscs (mussels and oysters) from England and Wales. Here we describe an experiment that aimed at determining the toxicokinetic profile, uptake and depuration (via placement in clean water) of sertraline by adult blue mussels, *Mytilus* spp.

Mussels were exposed to sertraline at the single concentration of 100 µg/L in a flow-through system over a period of 4 days. Following this, exposure ceased, and mussels were depurated in clean water over 6 days. Samples were taken at regular intervals throughout the uptake and depuration periods and included 5 mussels per sampling point, as well as water samples. Concentrations of sertraline and its common metabolite norsertraline were quantified by LC-MS/MS, and the presence of other potential transformation products analysed by LC-HRMS. Both sertraline and norsertraline showed rapid uptake and accumulation in mussel tissue, reaching a plateau on day 2, which was maintained for the remaining exposure and depuration period. The study results demonstrated that sertraline is not only subject to rapid uptake by bivalve molluscs but importantly, it does not undergo depuration over a 6-day placement in clean water. Our data highlight the potential use of bivalve molluscs as sentinel markers of human pollution and call for further assessment of the chronic toxicity of antidepressants to molluscan species.

Key words
Bioaccumulation, uptake, antidepressant, mollusks, depuration

Type of presentation
Poster

Session
1st choice - t10: Biomonitoring and development of integrative assessment approach

2nd choice - t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

Validation of a Small Fish Model for Immunotoxicity Assessments: Bridging the Gap Between Transcriptional Responses and Organismal Health

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The presence of contaminants that adversely impact the health of fish poses a threat to aquatic ecosystems. The impacts of such contaminants on reproductive and developmental processes have been well studied; however, their immunotoxic potential has received less attention. Thus, there is a need to develop approaches for screening immunotoxic compounds. The goal of this study was to validate the fathead minnow (FHM) as a model for immunotoxicity testing. The specific aims of this work were to evaluate the impacts of dexamethasone (DEX) exposure on: 1) pathogen resistance and 2) pathogen-stimulated molecular immune responses. To accomplish this, FHMs were subjected to 28-d exposures to DEX at concentrations of 150 or 600 μg/L, alongside of a corresponding solvent control (SC) group. At 14-d, FHMs from each group were infected with *Yersinia ruckeri*. Of these fish, kidneys were collected from a subset of fish for transcriptomic analysis to identify pathogen-stimulated molecular immune responses; whereas, the remaining fish were monitored for survival to evaluate pathogen resistance. Significant reductions in pathogen resistance were noted in males exposed to 150 and 600 μg/L relative to the SC, and in females exposed to 600 μg/L. Transcriptomic analysis revealed between 95 and 621 differentially-expressed genes (DEGs), and the number of DEGs was dependent upon DEX concentration and sex. These findings validate the FHM as an immunotoxicity model by confirming the impacts of a known immunosuppressant and identify potential biomarkers for immunotoxicity assessments.

Key Words
Synthetic Glucocorticoid, Immunotoxicity, Screening Assay, Transcriptomics

Type of Presentation
Poster

Session
Biomonitoring and development of integrative assessment approach
AOP, System Biology approaches and other conceptual modeling tools
Mixture effects of pollutants

SCIENTIFIC PROGRAM OF PRIMO 22

Biotransformation pathways and mode of action (MoA) of chemical pollutants

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Flavin-containing Monooxygenases: The other xenobiotic metabolizing enzymes

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Flavin-containing Monooxygenases (FMOs) are a family of enzymes that are co-localized with cytochrome P450s (CYPs) in the endoplasmic reticulum of multiple cell-types, organs, and organisms. FMOs are also similar to CYPs in that they require NADPH and oxygen. However, the substrate specificities and catalytic reaction mechanism is significantly different in that soft-nucleophilic substrates attack peroxyl-flavins located in the active site of the enzyme. Numerous studies have shown an association with expression and functionality of FMOs in animals residing in saltwater or undergoing saltwater acclimation. Isolation and sequencing of salmonid FMO1 possessed Osmoregulatory response Elements (OsREs) in the promoter region of the gene, and expression of FMO mRNA was increased following NaCl treatment in isolated hepatocytes of rainbow trout. To determine the relevance of the enzyme in osmoregulation and xenobiotic metabolism, we targeted the *D.rerio fmo5* for functional disruption using direct microinjection of CRISPR-Cas9 Ribonucleoprotein (RNP) complexes into two-cell stage embryos.. We evaluated several small guide RNAs (sgRNAs) to the *fmo5* gene for editing efficiency and identified an sgRNA targeting the region encoding the functionally essential FAD binding site which resulted in high embryo editing efficiency and no embryo-lethality. We evaluated RNP and mock microinjected embryos at 7 days postfertilization, for sensitivity to NaCl, the organophosphate insecticide, phorate, nicotine, and thiourea. Genomic DNA was prepared from each embryo and editing efficiency was evaluated by direct PCR of the targeted region, sanger sequencing, and Inference of CRISPR edits (ICE) analyses. Importantly, all FAD targeted larvae were predicted to have 95% composite loss of function in all injected animals No significant differences were observed in thiourea ,nicotine, or NaCl treatments between mock injected and larvae confirmed to have predicted functional disruption. In contrast, while 100% mortality was observed in mock injected animals treated with phorate at all concentrations, only 42% mortality at the highest concentrations was noted in the larvae with *fmo5* disruption.

Massive gene loss and the making of xenobiotic-induced responses in Cetacea

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How animal species cope with chemical insults is a key question of the Anthropocene Era. This research avenue is specifically relevant for marine mammals (e.g., whales and dolphins). In fact, Cetacea hold record levels of environmental contaminant accumulation. To fight these external contaminants, or xenobiotics, animals rely on an integrated network of genes and pathways for their oxidation (cytochrome P450 (CYPs)), conjugation (UDP-glucuronosyltransferases (UGTs), glutathione S-transferases (GSTs) and sulfotransferases (SULTs)) and transport (organic anion transporters, multidrug resistance transporters, multidrug resistance associated proteins). The pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are nuclear receptors that, among other functions, are considered the master regulators of xenobiotic metabolism in mammals. Cetacea have been shown to lack functional PXR and CAR receptors and to possess a lower number of cytosolic GSTs when compared to terrestrial mammals. Thus, using knockout mice and comparative genomics, we sought to examine the conservation status of the Cetacea xenobiotic metabolism genes regulated by PXR and CAR. The analyses confirmed a drastic reduction in the number of functional genes for CYPs, UGTs and SULTs. These results bring forth some insight into how the evolution of genomes has shaped the chemical defense of whales and dolphins, which will benefit the preservation of such iconic species.

Acknowledgements
Part of this work was funded by the Research Council of Norway, project no. 334739, 2023-2027 (AG, OAK, BD, FC, RR).

Key words
Cetacea, PXR, CAR, xenobiotic metabolism

Type of presentation
Platform

Session
1. Biotransformation Pathways and Mode of Action (MoA) of Chemical Pollutants
2. EDCs & Neuroendocrine Effects

High throughput screening of a chemical library for zebrafish CYP1A and CYP2Y3 substrates

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Session topic: t07 Biotransformation pathways and modes of action of chemical pollutants

There are fish orthologs of the major mammalian CYP1, but not the CYP2, enzymes involved in xenobiotic metabolism. CYP2Y3 is highly expressed in the liver of adult zebrafish, suggesting a role in xenobiotic metabolism. We heterologously expressed zebrafish CYP1A and CYP2Y3 in bacteria and screened a large compound library for CYP enzyme activity. Each enzyme was expressed with human cytochrome P450-NADPH oxidoreductase. A clear P450 peak was evident from bacteriosomes and we assessed a variety of fluorogenic probe substrates for catalytic activity. For CYP2Y3, only pentoxyresorufin was oxidized, however this appears to be via reductase. CYP1A and CYP2Y3 were screened for activity with the Phase 2 ToxCast chemical library from the US Environmental Protection Agency. This library contains 1920 compounds, including pesticides, endocrine related compounds, anti-microbials, food additives, green chemistry alternatives, failed drugs, flame retardants, and toxicology reference compounds. The high throughput screening assessed NADPH consumption in reactions with 0.5% DMSO and 10 µM chemical. For CYP2Y3, 110 compounds were hits (>LOD); 180 compounds were hits for CYP1A. For CYP1A, 44 compounds exhibited ≥ 25% of the activity of ethoxyresorufin, a model substrate. Surprisingly, a number of hits overlapped between the two enzymes. Detailed kinetic studies will define the function of CYP2Y3 in fish and elucidate additional details of CYP1A xenobiotic metabolism. (EPA 84002901)

Intergenerational effects of bis(2-ethylhexyl) phthalate in *Danio rerio*: evaluation of histone modifications on testis and its role on transcriptomic changes in F1 embryos

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Bis(2-ethylhexyl) phthalate (DEHP) has been reported as an endocrine disrupting chemical inducing several adverse effects in spermatogenesis. Despite of several transgenerational effects reported in mice and the known impact of changes in histone modifications in gene transcription, these processes are still poorly understood for this compound. This study aims to evaluate the potential effects of DEHP during the gametogenesis of zebrafish (*Danio rerio*), its impact on their unexposed F1 embryos and whether these potential defects can be associated with changes on histone modifications (H3K4me3, associated to active gene expression, and H3K27me3, linked to gene silencing) in the DEHP-exposed sperm.

To achieve this goal, we exposed zebrafish to 0.4,2 and 10 µg/L DEHP during gametogenesis for 40 days. Reproductive effects in the parental generation were not observed. However, significant increase in mortality and developmental abnormalities were observed in F1 embryos. A detailed mechanistic evaluation was performed for the 2 µg/L DEHP treatment. Thus, RNA-seq was performed in F1 24hpf embryos to evaluate changes. Additionally, genome-wide distribution of histone modifications was assessed in the P0 sperm.

We identified 1808 differently expressed genes in the F1 embryos, involved in processes such as phagosome, nucleocytoplasmic transport, protein processing in endoplasmic reticulum, ribosome, proteome, cardiac muscle contraction, among others. Additionally, testes from exposed zebrafish showed a significant decrease in H3K27me3 and increased H3K4me3. The present study shows that the DEHP effects can be transmitted to the following generation, and the underlying mechanism(s) should be further disentangled.

Key words
Environmental risk assessment, inheritance, plasticizers, parental effects

Type of presentation
Platform

Session
t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants
t14: EDCs & Neuroendocrine Effects

Investigation of gene-environment interactions in medaka and identification of genes that influence individual pollutant responses

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Anthropogenic substances, including pharmaceuticals and environmental toxins, are increasingly penetrating aquatic ecosystems and are therefore posing a growing concern. Current risk assessment approaches typically neglect inter-individual differences in susceptibility to pollutants, relegating them to error effects.

Acknowledging the pivotal role of genetic diversity in driving individual responses to pollutants, we conducted a large-scale genome-wide association study (GWAS) in the teleost fish medaka (*Oryzias latipes*). To unravel gene-environment (GxE) interactions that lead to different susceptibilities, genetic polymorphisms were correlated to drug-induced heart rate changes, which served as a general physiological indicator. Using the Medaka Inbred Kiyosu Karlsruhe (MIKK) panel allowed us to precisely control for genetic background and environmental exposure.

Phenotypic examination of more than 16,000 embryos from 72 genetically distinct MIKK panel lines revealed clear, strain-dependent differences in heart rate response to three tested substances (ethanol, disulfiram and caffeine). In a subsequent F2 segregation analysis based on extreme line crosses, we identified several quantitative trait loci that exhibit GxE interactions and are associated with individual drug responses. Functional validation results of candidate genes with CRISPR/Cas9 and base editors will be presented.

Overall, this study lays the groundwork for future research, ultimately revealing a mechanistic understanding of individual pollutant responses from the molecular to the organismic level.

Keywords
gene-environment interaction, individual drug response, genome editing, medaka

Type of presentation
oral presentation is preferred

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants,
New approach methodologies (NAMs) to assess pollutant toxicity,
AOP, System Biology approaches and other conceptual modeling tools

Exploring fluoxetine bioaccumulation, metabolization and mode of action in Mediterranean mussels through omics

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The inadequate removal of emerging contaminants during wastewater treatment, coupled with the direct discharge of wastewater into aquatic ecosystems, poses a significant threat to non-target organisms. In marine ecosystems, pharmaceuticals warrant attention due to their potential to negatively impact marine organisms as already observed in bivalves. Our study aims to investigate the effects of exposure to a widely used antidepressant, fluoxetine (FLX), on the mussel Mytilus galloprovincialis by i) investigating FLX bioaccumulation, ii) characterizing its metabolites and iii) delving the molecular effects. The effects will be explored through the use of 3 omics approaches: transcriptomics, proteomics and metabolomics.

Exposure for 28 days to a nominal concentration of 3.1 µg/L FLX was monitored by sampling mussels on days 2, 7, 14 and 28. FLX and its metabolites were measured in gills, digestive glands and a composite of soft tissues to determine bioaccumulation. Metabolization was studied through non-target LC-HRMS analyses. The results showed continuous bioaccumulation over the 28-day period, although the steady state was not reached, and the production of various metabolites. Some of them were identified (i.e. N-demethylation, Hydroxylation, N-Formylation). The known mechanism of action of FLX involves serotonin up-modulation, and it was observed to also modulate the dopaminergic pathway, highlighting that FLX triggers molecular effects. The impacts at the individual level could lead to impaired reproduction and behavior.

Key words :

Fluoxetine ; metabolites ; LC-HRMS ; *Mytilus galloprovincialis* ; multi-omics

Type of presentation
Platform

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants.

New approach methodologies (NAMS) to assess pollutant toxicity.

Gene expression profiling and 16S rRNA microbiome characterization to determine the effects of ibuprofen and paroxetine on the mussel *M. galloprovincialis*

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Pharmaceuticals are continually introduced into aquatic environments raising increased concerns about their effects on the environment. These products are biologically active compounds designed to interact with specific physiological pathways, therefore their mode of action on non-target species requires to be investigated. In this study, *M. galloprovincialis* was exposed for 30 days to two widely used drugs in the aquatic environment: ibuprofen and paroxetine. Exposure was followed by a 14-day depuration phase. The transcriptome (RNA seq) and the microbiota (16S rRNA sequencing) were studied to assess the effects of these drugs and to understand their mode of action. Ibuprofen showed significant changes on molecular pathways involved in proliferation, stress response and immune system. Molecular changes were also observed after depuration, suggesting possible persistent effects. Concerning paroxetine, the functional analysis suggests that it interferes with various pathways connected with the serotonergic system (e.g. reproduction, immune system). A major risk was observed following combined exposure to ibuprofen and paroxetine (MIX), in particular in relation to DNA damage, immune system and stress response. Microbiota analysis revealed also major changes in microbial communities following exposure to MIX, confirming the importance of considering the effects of mixtures of pharmaceutical products. Overall, this study allowed to define the mechanisms of action of investigated pharmaceuticals as well as to establish for the first time possible host-microbiota interactions.

Key words
RNA-sequencing, 16S rRNA sequencing, mussel, Ibuprofen, Paroxetine

Type of presentation
Oral presentation

Session
Biotransformation pathways and mode of action (MOA) of chemical pollutants

Mechanisms of action of antidepressants in non target marine species: a case study with Fluoxetine in *Mytilus galloprovincialis*

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The antidepressant Fluoxetine (FLX) is one of the most studied and detected selective serotonin reuptake inhibitors (SSRI) in the aquatic environment, where it is found at ng-µg/L levels. FLX has been shown to exert a number of effects in aquatic species at environmentally relevant levels. However, most studies did not focus on the possible mode of action (MOA) of FLX in non-target species, marine invertebrates in particular.

In the marine bivalve, the mussel *Mytilus galloprovincialis*, FLX is bioaccumulated and partially metabolized into norfluoxetine. FLX exposure has been shown to induce several biomarker responses from cellular to organism level: in particular, recent studies indicate that lysosomes represent primary target of antidepressants, including FLX.

In this work, the mechanisms of action of FLX in *M. galloprovincialis* were evaluated. Data are presented on the short term *in vitro* effects of FLX on mussel hemocytes: immune responses, lysosomal, mitochondrial, and autophagic parameters were evaluated. *In vivo* exposure to FLX (7 d, 0.5-5-10 ng/L) induced changes in expression of serotonin receptor 5-HTR1, genes involved in biotransformation (GST, ABCB), autophagy (mTor), and ceramide metabolism (SP2, KSDR2).

The results indicate the environmental concentrations of FLX act on mussel cells through multiple mechanisms of action. As recently described in mammalian systems, FLX also in mussels may act as a lysosomotropic agent, and affect autophagic processes and ceramide metabolism. These data contribute to shed some light on the MOA of SSRI antidepressants in marine invertebrates.

Key words
Pharmaceuticals, SSRI, non-target species, bivalves, lysosomes

Type of presentation
Platform

Session
1) Biotransformation pathways and mode of action (MOA) of chemical pollutants
2) EDCs & Neuroendocrine Effects

Mode of action of stimulant alkaloids: cellular metabolism and effects of cocaine, caffeine and their mixture in *Mytilus galloprovincialis*

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The presence of alkaloids derived from human consumption, including stimulants and illicit drugs as caffeine and cocaine, is increasingly being reported in freshwater, wastewater treatment plants and coastal waters. Despite a solid knowledge on the effects of these substances in humans, their mode of action and biological effects in non-target aquatic species are virtually unexplored. In this study, mechanisms of cellular responses were investigated in the Mediterranean mussel *Mytilus galloprovincialis* exposed to 0.5 µg/L cocaine and 0.5 µg/L caffeine, dosed alone and in mixture. A 28-days exposure was followed by a 14-days recovery, and at the end of both these phases alkaloids uptake was evaluated along with whole transcriptome changes and a wide panel of subcellular functional endpoints, including xenobiotics biotransformation, cholinergic activity, immune system alterations, lysosomal damages, antioxidant defenses, lipid metabolism and genotoxic damages. Results highlighted a good capability of mussels to metabolize caffeine and cocaine, supported by several alterations measured at gene, catalytic and cellular level, either triggered at the end of the exposure or with a delayed onset. A different biological responsiveness toward cocaine and caffeine was observed, and interactive effects of the two compounds appeared in co-exposed organisms. Our findings provide novel insights to unravel the mode of action of these contaminants of emerging concern in non-target aquatic species, for a science-based environmental risk assessment approach.

Key words
Alkaloids, stimulants, xenobiotic metabolism, subcellular alterations, emerging contaminants.

Type of presentation
Platform.

Session
1. Biotransformation pathways and Mode of Action (MOA) of chemical pollutants.
2. Mixture effects of pollutants.

Effects of diclofenac treatment on zebrafish Oatp1d1 mutants

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Oatp1d1 transporter plays a dominant role in the uptake of organic anions in zebrafish. Previous *in vitro* characterization of Oatp1d1 showed its high affinity for pharmaceuticals and other xenobiotics. To further investigate the role of Oatp1d1 in cellular defense and elucidate its physiological and developmental role in zebrafish, we engineered a CRISPR-Cas9 Oatp1d1 mutant. The mutation of *oatp1d1* was confirmed at genomic and transcriptional levels and showed reduced expression of *oatp1d1* in mutant embryos and adult fish compared to wild-type fish. The lack of protein was confirmed using custom antibodies that showed a lack of localization of Oatp1d1 in the canalicular membranes of hepatocytes. To further characterize the phenotype, we exposed mutant and wild-type embryos to increasing concentrations of diclofenac, a frequently used anti-inflammatory drug and Oatp1d1 substrate. Exposure of wild-type embryos resulted in delayed development and other deformities manifested as spinal curvature, cardiac edema, and blood pooling in the heart, with lethal effects at higher concentrations. However, Oatp1d1-/- were less sensitive to diclofenac exposure, showing delayed onset and milder effects on development. Our results suggest that Oatp1d1 may be the main transporter for the uptake of diclofenac across hepatocyte plasma membranes, indicating that the lack of Oatp1d1 may result in less efficient entry of diclofenac into hepatocytes and slower biotransformation into potentially more toxic metabolites.

Key words

Zebrafish, membrane transporters, oatp1d1, diclofenac, CRISPR-Cas9 mutants

Type of presentation

Oral

Session

Biotransformation pathways and mode of action (MoA) of chemical pollutants

Toxicity and speciation of inorganic arsenics and their adverse effects on in vivo endpoints and oxidative stress in the marine medaka *Oryzias melastigma*

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Arsenic is a widely present metalloid that causes toxicity to organisms in an aquatic environment, and its effect varies depending on its form. This study investigated the acute and chronic exposure of two inorganic arsenic species, AsV and AsIII, in the marine madaka *Oryzias melastigma* to determine the effects of exposure on in vivo effects, bioaccumulation, biotransformation, and oxidative stress. The embryonic development investigation showed no effect on in vivo parameters, but the hatching rate increased in the AsIII-exposed group. In both acute and chronic exposure using juveniles, the highest concentration of arsenic was detected after AsIII exposure, and higher bioaccumulation was found during chronic exposure. In the case of arsenic speciation, the proportion of AsB in chronic exposure was high, ranging from 64.2 to 81.9%. On the other hand, in acute exposure, the ratio of AsV and AsIII was relatively higher than that of chronic exposure, indicating that bioaccumulation of inorganic arsenic induced oxidative stress. As ROS occurrence was induced in acute exposure, an increase in antioxidant enzymes SOD and CAT was observed, the highest increase in AsIII exposure, but no significant oxidative stress was induced in chronic exposure. Also, during acute exposure to AsV, GST enzyme activity increased twice as high and GSH decreased compared to other groups, suggesting that the role of GST in the initial detoxification process is important when exposed to AsV. In addition, RNA-seq-based ingenuity pathway analysis showed that inorganic arsenic affects various signaling pathways, particularly oxidative stress-related signal pathways. Also, MAPK signaling pathways were significantly activated in response to acute exposure to arsenic.

Key words
Inorganic arsenic, toxicity, speciation, oxidative stress, marine medaka

Type of presentation
Oral presentation

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants

Oxidative stress, biotransformation responses and bioaccumulation of polycyclic aromatic hydrocarbons in tropical estuarine food webs - Cross River Estuary, Nigeria

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In this study, we have investigated bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) and associated oxidative stress, biotransformation responses in some tropical estuarine food webs fish (*Chrysicthys nigrodigitatus*), fiddler crab (*Uca tangeri*), prawn (*Macrobrachium vollenhovenii*), periwinkle (*Tympanotonos fuscatus*) and sediment samples from three sites, namely - Adiabo (control site), Obutong and Nsidung representing different degrees of anthropogenic contamination along the Cross River Estuary. The hepatic enzyme activities for glutathione peroxidase (Gpx), glutathione reductase (Gr), glutathione S-transferase (Gst), uridine diphosphate glucuronosyltranferease (Udpgt), 7-ethoxy-, methoxy-, pentoxy-, and benzyloxyresorufin O-deethylase (EROD, MROD, PROD and BRDO) and concentrations of PAHs were analyzed. Our results showed species- and site-specific PAHs bioaccumulation patterns and oxidative stress, biotransformation responses in organisms and sediments from the contaminated sites (Obutong and Nsidung), compared with control site (Adiabo). The EROD, MROD, BROD, PROD activities in T. fuscatus and GPx, Gr, Gst, Udpgt in C. nigrodigitatus showed significant increases from contaminated sites (Obutong and Nsidung), compared with control site (Adiabo) and this paralleled high PAHs body burden from these sites. These findings suggest that the benthic species (C. nigrodigitatus and T. fuscatus) as more sensitive and reliable species for biological and chemical environmental monitoring, compared with the other species along the Cross River Estuary, Nigeria.

Key words
Oxidative stress, Biotransformation, Tropical estuarine food webs, PAHs, Cross River Estuary.

Type of presentation
Oral

Session
New tools to track pollutant sources and transfer.

Cytochrome P450 Genes in Giant Viruses: Functional, Environmental, and Evolutionary Questions

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Viruses are major infectious agents of ocean systems and may influence pollutant responses there, as targets for chemicals and by altering host responses. The view that chemical defense genes occur only in cellular life, and not viruses, changed with discovery of giant viruses (GVs) and sequencing of the Mimivirus genome in 2004. GVs occur in the oceans, in soils, and in fresh and waste waters, where they infect protists, phytoplankton and other eukaryotes. GV are up to 2 μm in size, with 1000s of genes, including for novel P450s, oxidoreductases, glutathione transferases, cytochrome b5, SOD, and catalase. *Our aim is to understand the implications of such genes for virus and host.* Mimivirus CYP5253A1 is a unique chimeric protein, with a P450 domain linked to a 2-oxoglutarate dehydrogenase (2-OGD) domain resembling an RNA repair enzyme (AlkB). CYP5253A1 protein is expressed when a host is infected, although the specific role is uncertain. Recombinant CYP5253A1 gives a P450 spectrum only as the chimera but not with the P450 domain alone, and the recombinant protein binds cholesterol, a potential substrate. While initially found only in GVs, CYP5253-like genes have now been found in a soil arthropod, the springtail *Folsomia candida*. Recombinant *F. candida* CYP5253 gives a typical P450 spectrum. The findings highlight the possibility for horizontal gene transfer of GV P450s to or from other organisms, potentially altering responses to chemicals, and with implications for the evolutionary origin of GV P450 genes and P450 genes as a whole. (Support: US NIH & NSF; Leverhulme, UK)

Key words
Cytochrome P450, viruses, P450 evolution, host response

Type of presentation
Platform

Session
t12: Microorganisms as target and vector for chemical pollutants

Induction of biotransformation enzymes in 3 trout species exposed to tire rubber particles

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Recent work has demonstrated that leachates of tire and road wear particles that enter aquatic environments as a constituent of road runoff can induce severe toxicity in aquatic species. Especially salmonid fish are susceptible to toxicity at low concentrations of toxicants like 6-PPD-quinone, polynuclear aromatic hydrocarbons and other components of tire rubber. However, significant differences have been found between fish species, even within the salmonids. The objective of this study was to further investigate if differences between biotransformation enzyme induction and activity could explain the observed species sensitivity differences. Rainbow trout, brook trout and brown trout were exposed to pulsed doses of tire crumb rubber over 96-h at environmentally relevant concentrations, after which gill, liver and intestinal tissues were collected. Activity of cytochrome P450-1A, glutathione transferases and phenol-type glucuronosyltransferase were measured in subcellular fractions. Results showed significant species differences for some of the enzymes, with overall a lower expression in rainbow trout. This could explain the higher sensitivity of this species to road runoff pollutants compared to the other ones. Differences were also observed between the tissues analyzed, which will have an effect on the toxicokinetics of different chemicals. The obtained information will be useful for risk assessment of tire wear particles as a subclass of the larger group of microplastics.

Key words
Tire wear particles, microplastics, 6-PPD, Cytochrome P450, glucuronosyltransferase

Type of presentation
Platform

Session
Biotransformation pathways

Particles, fibers, plastics and their additives

Mixture effects

Molecular toxicity pathways and behavioral alterations induced by per- and polyfluoroalkyls (PFASs) in *Scrobicularia plana*

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Thousands of per- and polyfluoroalkyl substances (PFASs) are used since the 1940s, due to their unique physico-chemical properties. Nevertheless, they are highly persistent and widespread in the aquatic environment. Recent studies proved their occurrence in the coastal water, with higher concentrations measured in the sediment. Their bioaccumulative and toxicity potential have been highlighted, especially for PFOS, PFOA and long-chain PFASs (> 8C), which has led to their regulatory prohibition or limitation. Nevertheless, data assessing their mode of action on aquatic biota is strongly lacking. Further, the toxicity of short-chain PFASs, alternatives of long-chain PFASs, is poorly documented.

This work compared the alterations induced by 3 PFASs of different chain length (PFBA ; PFOA ; PFUnDA) on the bivalve *Scrobicularia plana*, an endobenthic species, by sediments spiked at 20 ng/g dw, concentrations representative of polluted areas. The organisms were exposed for 24h or 14 days, to identify the early molecular toxicity mechanism, and to identify the acclimation mechanisms, respectively. The molecular approaches included the analyses of transcripts associated to cell proliferation, apoptosis, cellular stress and energy metabolism, biochemical markers related to antioxidant system as well as neurotoxicity, and lipidomics to characterize lipidome responses to PFASs exposures and to identify lipid markers specific to PFASs. This was complemented with clearance rate and burrowing activity measurements, to identify the physiological alterations induced by these contaminants.

Key words
PFAS, molecular toxicity pathways, bivalve, multi-omics, behavior

Type of presentation
Platform

Session
New approach methodologies (NAMS) to assess pollutant toxicity (1)

Biotransformation pathways and mode of action of chemical pollutants (2)

Chemical exposome and non-target screening approaches (3)

Effects of the benzophenone-3 on molecular biomarkers and nuclear receptors in the oyster *Crassostrea gigas*

Miguel Saldaña-Serrano[1]; Jacó Joaquim Mattos[2]; Daína de Lima[1]; Mariana Rangel Pilotto[1]; Luiz Otavio de Barros Vilas Bôas[1]; Tâmela Zamboni Madaloz[1]; Camila Lisarb Velásquez Bastolla[1]; Camila Pesenato Magrin[3]; Diego José Nogueira[1]; Carlos Henrique Araújo de Miranda Gomes[4]; Flávia Lucena Zacchi[4]; Guilherme Razzera[1]; Gustavo Amadeu Micke[3]; Afonso Celso Dias Bainy[1]

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Personal care products (PCPs), such as sunscreens, can be found in aquatic ecosystems. However, little information is available regarding the effects of these substances on marine bivalves. This study aimed to evaluated the sublethal effects caused by benzophenone-3 (BP-3) on *Crassostrea gigas* oysters exposed for 1 and 7 days at environmental concentrations (1 and 100 $\mu g.L^{-1}$). The accumulation of BP-3 in the oysters was determined, and the transcription levels of antioxidant (*SOD-like* and *CAT-like*), phase I biotransformation (*CYP356A1*, *CYP2AU2* and *CYP7A1*), phase II biotransformation: glutathione S-transferase omega (*GSTO.4A-like*), glutathione S-transferase pi (*GSTP.1.1-like*) and nuclear receptors: *CgNR0B-like, CgNR1P10-like, CgNR1P11-like, CgNR2E2-like* and *CgNR5A-like* genes were evaluated. The highest accumulation (34.58 ± 5.70 $\mu g.g^{-1}$) was observed in animals exposed for 7 days at 100 $\mu g.L^{-1}$. A higher transcript levels of *SOD-like* and *CYP356A1* genes were observed in animals exposed to both concentrations, and *GSTP.1.1-like* were higher in animals exposed to the higher concentration after 1 day of exposure. Furthermore, higher transcript levels of the *CgNR0B-like* and *CgNR5A-like* genes were observed in the animals exposed for 1 days at 100 $\mu g.L^{-1}$. The results show that oysters exposed to environmentally relevant levels of BP-3 accumulate this compound and exhibited enhanced transcript levels of genes related to antioxidant, phase I and II of biotransformation systems, and on the nuclear receptors in their gills, indicating potential toxic effects associated to BP-3 exposure.

Key words
Coastal pollution, mariculture, contaminant of emerging concern, oxybenzone.

Type of presentation
Platform.

Session
Aquaculture Environment Interactions.
Biotransformation pathways and mode of action (MoA) of chemical pollutants

Cellular transporters in zebrafish embryos – which ones are relevant for toxicokinetics?

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The zebrafish (*Danio rerio*) embryo is an important model system in (eco)toxicology. For the understanding of toxic effects it is important to consider factors influencing toxicokinetics (TK), such as action of xenobiotic transporters. So far, some TK-relevant cellular transporters have been shown to be present and active in the zebrafish embryo, however, there may be more relevant transporter candidates. Based on the vast available information on transporters relevant for TK/pharmacokinetics in mammals respective zebrafish orthologues can be considered candidates with a corresponding function. Based on available genome information, zebrafish orthologs of human ABC/SLC/SLCO efflux and uptake transporters were identified. It turns out that zebrafish do not in all cases have 1 to 1 orthologs of human transporters: in certain cases, there are either no or several gene orthologs. On the other hand, some toxicologically relevant transporters in zebrafish do not have a human ortholog. The transporter gene candidates were examined for expression in zebrafish embryos to obtain an insight, which ones could be relevant for TK in the embryo. Transcripts of only few transporter genes were found in the fertilized egg, indicating maternal origin of the mRNA of those transporters. There is indication from previous work that respective transporters are present as functional protein. With proceeding development, the number of expressed transporter genes increases and after 4 days post fertilization transcripts of almost all examined transporter candidates were present.

Key words
Xenobiotic cellular transporters, toxicokinetics, ADME, zebrafish embryo

Type of presentation
Platform.

Session
- Biotransformation pathways and mode of action (MoA) of chemical pollutants
- Chemical exposome and non-target screening approaches
- Acclimation and adaptation to chemical stress

SCIENTIFIC PROGRAM OF PRIMO 22

Biotransformation pathways and mode of action (MoA) of chemical pollutants

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Applications of a Zebrafish CYP1A-Targeted Monoclonal Antibody (CRC4) with Reactivity Across Vertebrate Taxa: Evidence for a Conserved CYP1A Epitope

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CYP1A is a heme-thiolate enzyme associated with the cytochrome P4501A1 monooxygenase system and is inducible by a wide variety of xenobiotics and endogenous ligands that bind and activate the aryl hydrocarbon receptor (AHR). The AHR-CYP1A axis is important for detoxification of certain xenobiotics and for homeostatic balance of endogenous sex hormones, amine hormones, vitamins, fatty acids, and phospholipids. Herein, we generated and described applications of a zebrafish CYP1A-targeted monoclonal antibody (mAb CRC4) that fortuitously recognizes induced CYP1A across vertebrate taxa, including fish, reptiles, chicken, mouse, rat, and human. We then demonstrated that mAb CRC4 targets a highly conserved epitope signature of vertebrate CYP1A. The unique complimentary determining region (CDR) sequences of heavy and light chains were determined, and these Ig sequences will allow for the expression of recombinant mAb CRC4, thus superseding the need for long-term hybridoma maintenance. This antibody works well for ELISAs, immunoblotting, immunohistochemistry (IHC), as well as whole mounted IHC in zebrafish embryos. Monoclonal antibody CRC4 may be particularly useful for studying the AHR-CYP1A axis in multiple vertebrate species and within the context of Oceans and Human Health research. By using archived samples, when possible, we actively promoted efforts to reduce, replace, and refine studies involving live animals.

Key words
CYP1A, AHR, zebrafish, monoclonal antibody, CRC4

Type of presentation
Poster

Session
Biotransformation Pathways and Mode of Action (MOA) of Chemical Pollutants; AOP, System Biology Approaches and Other Conceptual Modeling Tools

Assessing the effects of dietary exposure to PFOS and PFHxS in mummichogs (*Fundulus heteroclitus*)

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Per- and polyfluoroalkyl substances (PFAS) are a diverse class of persistent contaminants associated with a variety of adverse effects and found in aquatic ecosystems worldwide. There is an urgent need for data to assess the ecological risk from the universe of PFAS. Therefore, we are working to characterize mechanisms of toxicity, categorize and prioritize compounds, and inform predictive models. Herein, we exposed an estuarine fish (*Fundulus heteroclitus*, mummichog) to perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS). Reproductively active fish were fed control or diet amended with PFOS (1 or 10 µg/g dw) or PFHxS (0.3, 3, or 30 µg/g dw). Weight, length, and reproduction were measured throughout, and sacrificial endpoints obtained for individuals at 7 days or termination of exposure (51 days). Tissues were weighed and archived for molecular and chemical analyses. Whole organism results suggest reduced energy storage in PFHxS- but not PFOS-exposed female fish. Additionally, reproductive activity was somewhat reduced in PFHxS and high dose PFOS exposures. Assessment of bioaccumulation and multi-omic responses (transcriptomic, proteomic, metabolomic, and lipidomic) is ongoing; preliminary analyses show time- and dose-dependent changes in the hepatic metabolomic profile. These data support an integrated approach to identify mechanisms of toxic effects of PFAS in marine fish and will contribute to the development of pathways linking molecular perturbations to adverse apical outcomes to support extrapolation across compounds and species.

Key words
PFAS, fish, multi-omics, energetics
Type of presentation
Poster
Session
Biotransformation pathways and mode of action of chemical pollutants
AOP, system biology approaches and other conceptual modeling tools

Bioconcentration of PFOS in two distinct aquatic taxa and different salinity conditions

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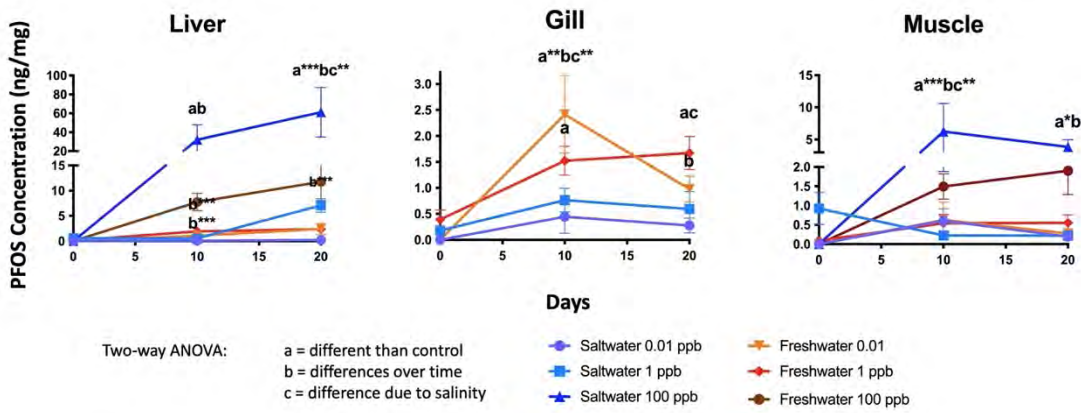
Perfluorooctane sulfonate (PFOS) is a persistent organic pollutant that is known for its resistance to degradation and bioaccumulation. In this study, we compared the bioconcentration of PFOS between the aquatic invertebrate, *Daphnia magna* and the estuarine fish species, *Fundulus heteroclitus*, and between saltwater and freshwater-acclimated *F. heteroclitus*. The estuarine fish species *F. heteroclitus,* known for its adaptation to both hypertonic and hypotonic environments, makes an ideal model to determine differences in the bioconcentration of PFOS in salt- and freshwater because the experiments can be performed in the same species. *F. heteroclitus* were acclimated to both saltwater (23-26 ppt) and freshwater conditions for 30 days before being treated with PFOS at 0.1 to 100 ppb 0,10, or 20 days to determine bioconcentration in the liver, gills, and muscle. PFOS bioconcentration was higher in saltwater than freshwater in the liver and muscle of *F. heteroclitus*. Expression changes of GR, CFTR, and OAT1 may play a role in the increased retention of PFOS in the gills of freshwater-acclimated fish. In exposures with *D. magna*, PFOS bioconcentration was similar in daphnids as mummichogs with the exception in the liver of mummichog at 100 ppb. This indicates previous reports of decreased sensitivity of daphnids to PFOS is not due to lower bioconcentration. In conclusion, PFOS accumulation in mummichogs is exacerbated by salinity at high concentrations in liver and muscle, but lower in gills of *F. heteroclitus* and PFOS is readily bioconcentrated in *D. magna*.



Keywords - PFOS, *Fundulus, Daphnia*, salinity, bioaccumulation
Type of presentation - Poster
Session
t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants
t09: AOP, System Biology approaches and other conceptual modeling tools

Characterization of acetylcholinesterase and carboxylesterases in the mangrove oyster *Crassostrea gasar* as biomarkers of exposure to pesticides

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Brazil is one of the world's leader agricultural pesticide–consuming countries, raising the need for identifying responsive biomarkers as diagnostic and prognostic tools to monitor aquatic pollution. Acetylcholinesterase (AChE) and carboxylesterases (CbEs) are ubiquitously expressed proteins that are involved, respectively, in neural transmission in the cholinergic synapses and detoxification of organophosphorus pesticides. As compared to mammals, AChE and CbEs activity has not been extensively characterized in bivalves, a suitable taxon for sentinel purposes. In the present study, AChE and CbEs activity was characterized in the mangrove oyster *Crassostrea gasar* collected in an estuarine system close to rice cultures in Southern Brazil. The enzymatic activities were compared between the oysters' gills and digestive gland revealing differences in terms of CbEs dependent activities using different substrates: p-nitrophenyl acetate (pNPA), naphthyl acetate (NA) and naphthyl butyrate (NB). While the CbEs activities were higher in the digestive gland, there were no significant differences of AChE activity between the tissues, suggesting the role of digestive gland as the main metabolic organ in *C. gasar*. The enzymatic activities of AChE and CbEs were characterized in gills and digestive gland using the selected substrates as well as the kinetic parameters V_{max} and K_{mapp}. The results of this study may provide insights into differences in neurotoxicity potential in oysters, but further validation is needed to confirm their use for monitoring pesticide pollution of coastal waters.

Key words
Bivalve, organophosphorus pesticides, coastal pollution, acetylcholinesterase, carboxylesterases.

Type of presentation
Poster

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants

Comparative Transcriptomic Analysis of the Effect of Three Emerging Contaminants on Marine Species

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Personal Care Products (PCPs) are one of the largest classes in the emerging contaminants group. PCPs are widely used in modern human life and contribute to improve its quality. Many of these compounds are not efficiently removed by conventional sewage treatment plants and thereby they are discharged into the environment making these chemicals ubiquitous in many aquatic ecosystems, including oceanic and coastal settings. This, together with their physicochemical properties, could suppose a risk for wild organism exposed to them.

Three PCPs were considered for this study: triclosan (TCS), N,N-diethyl-meta-toluamide (DEET), and Sulisobenzone (BP-4). These three pollutants are commonly detected in aquatic ecosystems.

In the present work, the Japanese clam (*Ruditapes philippinarum*), and the gilthead seabream (*Sparus aurata*) were exposed to a nominal concentration of 10 µg L-1 of TCS, BP-4 and DEET in separate tanks using a continuous flow-through seawater system under controlled laboratory conditions for 22 days. Concentration of pollutants in the water was monitored during the whole experiment as well as in organism tissue. Digestive glands and liver were dissected for transcriptomic analysis.

Data processing revealed that 834, 430 and 993 genes were significantly expressed in the digestive gland of clams exposed for 22 days to BP-4, DEET and TCS, respectively. Whereas in the liver of fish 371 and 250 genes were differentially expressed after exposure to BP-4 and DEET respectively and no differentially expressed genes were found in sea bream after exposure to TCS.

Key words
Transcriptomics, DEET, BP4, Triclosan.

Type of presentation
poster

Session
t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

Crude oil triggers invertebrate planktonic larvae to undergo rapid metamorphosis.

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Metamorphosis is a critical process in the life cycle of most marine benthic invertebrates, determining their transition from plankton to benthos. It affects dispersal and settlement, and therefore decisively influences the dynamics of marine invertebrate populations. An extended period of metamorphic competence is an adaptive feature of numerous invertebrate species that increases the likelihood of finding a habitat suitable for settlement and survival. We found that crude oil and residues of burnt oil rapidly induce metamorphosis in two different marine invertebrate larvae, previously unknown sublethal effects of oil pollution. When exposed to environmentally realistic oil concentrations, up to 84% of tested echinoderm larvae responded by undergoing metamorphosis. Similarly, up to 87% of gastropod larvae metamorphosed in response to burnt oil residues. This study demonstrates that crude oil and its burned residues can act as metamorphic inducers in marine planktonic larvae, short-circuiting adaptive metamorphic delay. Future studies on molecular pathways and oil-bacteria-metamorphosis interactions are needed to fully understand the direct or indirect mechanisms of oil-induced metamorphosis in marine invertebrates. With 90% of chronic oiling occurring in coastal areas, this previously undescribed impact of crude oil on planktonic larvae may have global implications for marine invertebrate populations and biodiversity.

Key words: metamorphosis, crude oil, planktonic larvae, pollution, benthic recruitment

Type of presentation
Platform.

Session
Mixture Effects of Pollutants.

Disruptions in energy metabolism can alter feeding behavior in Atlantic cod

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Energy homeostasis is essential for organism functioning and its disruption is detected by glucose-sensing mechanisms in the liver and the hypothalamus. In the liver, the response to altered glucose levels leads to a metabolic switch between glucose utilization and production whereas specialized neuronal populations in the hypothalamus regulate energy expenditure and food intake. However, if and how contaminants that disrupt glucose homeostasis affect feeding behavior in fish, remains entirely unknown. To start to understand this connectivity we use a key species of North-Atlantic marine food webs – the Atlantic cod (*Gadus morhua*) – and expose them to the emblematic environmental contaminant dexamethasone which is known to increase blood glucose in human by binding to the glucocorticoid receptor (GR). Our results show that active site residues of the GR are conserved between human and Atlantic cod and dexamethasone can activate the Atlantic cod GR *in vitro*, albeit at lower potency. Initial analysis of Atlantic cod exposed to 5, 50, and 500 µg/L dexamethasone for six hours shows alterations in plasma cortisol, cholesterol, and glucose levels; transcriptional changes of genes related to glucose metabolism in the liver; and feeding behavior. The immunohistochemical analyses of nutrient-sensing peptides (NPY and α-MSH) in the hypothalamus are currently underway. Collectively, our study suggests that a disruption in energy metabolism in a keystone species can alter feeding behavior which may be relevant for the survival and the role in the food web of contaminant-exposed cod.

Key words
Glucocorticoid receptor, Atlantic cod, feeding behavior, glucose metabolism

Type of presentation
platform

Session
t14: EDCs & Neuroendocrine Effects

t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

Embryos of lesser sandeel (*Ammodytes marinus*) are robust to crude oil exposure

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The oil industry's expansion and increased operational activity at older installations contribute to rising cumulative pollution and a heightened risk of accidental oil spills. The lesser sandeel (*Ammodytes marinus*) is a crucial prey species in the North Sea and coastal systems, with eggs that adhere to the seabed substrate upon spawning. We evaluated the sensitivity of lesser sandeel embryos to crude oil by exposing them to dispersed oil at concentrations of 0, 15, 50, and 150 µg/L oil from 2 days post-fertilization (dpf) to 16 dpf. Our analysis included water and tissue concentrations of total hydrocarbons (THC) and total polycyclic aromatic hydrocarbons (tPAH), expression of cytochrome P450 1A (*cyp1a*), lipid distribution in various tissue compartments, and assessment of sublethal morphological and functional deformities. In all oil treatment groups, we observed oil droplet accumulation on the eggshell, followed by a dose-dependent increase in *cyp1a* expression. Nevertheless, the exposure resulted in minor sublethal issues in the upper jaw and otolith, potentially affecting settling, feeding and communication abilities in later life stages. Additionally, we conducted a pioneering study on the specific fatty acid profiles in the eyes, head, and trunk of 4-day-old larvae. The oil exposure had no discernible impact on the fatty acid dynamics in these tissues. Our findings suggest that lesser sandeel embryos demonstrate robustness and relative resistance to crude oil exposure. The lowest observed effect level in this study was 36 µg/L THC and 3 µg/L tPAH.

Key words
Key prey species, oil exposure, sublethal deformities, fatty acid profile, lesser sandeel

Type of presentation
Platform.

Session
AOP, system biology approaches and other conceptual modeling tools
Biotransformation pathways and mode of action of chemical pollutants

Fish eggs with a sticky surface experience prolonged exposures during an oil spill due to aggregation of oil droplets

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During an oil spill, oil is present in the water column in both dissolved and droplet form causing toxicological effects for exposed fish eggs. Addressing this, the focus is usually on the dissolved oil by establishing laboratory-based threshold concentrations for effects. However, the amount of oil droplets could be several orders of magnitude higher than that of dissolved oil. Usually, oil droplets are either assumed to give no toxic contribution or are only implicitly considered by applying a lower threshold for dissolved oil. For most species this is a sufficient approach, but droplets should be addressed specifically for species with a sticky egg surface where droplets can aggregate. To do so, we develop a model for collisions between pelagic fish eggs and oil droplets in the ocean. Using this and a well-established impact assessment model for haddock, we simulate a large oil spill and show that the eggs experience a significant amount of oil droplets. Applying a known threshold of 0.1 µg/L dissolved TPAH as reference, 24% of the eggs experience above this. Equivalently, 24% of the eggs experience oil droplet concentrations above 137 µg/L and collide with at least 22 µg droplet oil, or about 1.4% of the egg mass. When it comes to the exposure duration, it could increase from hours when considering only dissolved oil up to about seven days when including collisions with oil droplets. Hence, oil droplets are toxicologically important for sticky eggs and further experimental effort should be put into establishing reliable effect thresholds.

Key words
Fish eggs, oil droplets, oil spill, modelling, impact assessment

Type of presentation
Platform

Session
1. New approach methodologies (NAMs) to assess pollutant toxicity
2. Particles, fibres, plastics and their additives
3. Mixture effects of pollutants

Oxidative stress response to organic UV filters in the coral *Pocillopora damicornis*

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The marine ecosystem is significantly affected by the presence of ultraviolet (UV) filters in personal care products, which have been proven to have detrimental effects on coral reefs. However, our knowledge of how these compounds specifically impact corals and the underlying mechanisms involved is still limited. It is crucial to characterize the coral's response in both the animal and symbiotic algae compartments, as a disturbance in the latter can lead to coral bleaching. The main objective of this study is to develop and analyze biomarkers that indicate the effects of UV filters on the animal tissue and symbiotic algae of the tropical coral *Pocillopora damicornis*.

To achieve this objective, laboratory-cultured colonies of *P. damicornis* will be exposed to increasing concentrations of three UV filters known for their toxicity to corals: octocrylene, oxybenzone, and octinoxate. At the end of the 7-days exposure period, the host and symbiont tissues will be separated using differential centrifugation, which will allow for the measurement of biomarkers at different levels. Biomarkers of oxidative stress will be developed by measuring enzymatic activity and assessing cellular damage through fluorescence. Additionally, the transcription levels of corresponding genes will be evaluated using qRT-PCR at the molecular level. This research aims to enhance our understanding of the impacts of sunscreen on corals.

Key words
Sunscreen, corals, biomarkers

Type of presentation
Platform

Session
t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

Phenotype changes in zebrafish Oatp1d1 mutants

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Oatp1d1 transporter is a member of the OATP/Oatp superfamily and plays an important role in ADME processes. It is ubiquitously expressed in zebrafish, with the highest expression in liver and brain, and has previously been suggested that it plays a significant role in the hormone balance. To better understand the role of Oatp1d1 *in vivo*, we developed a CRISPR-Cas9 mutant of Oatp1d1 and used it to evaluate possible changes in the phenotype of CRISPR-Cas9 knockouts in both embryos and adults. Comparing with the wild-type (WT), we observed differences in pigmentation. First, a lack of embryo coloration during embryonic development was clearly visible in comparison to WT embryos. In addition, the methanol extract of 5-10 embryos (24 - 120 hpf) or 1-2 larvae (12 - 18 dpf) showed a significantly lower signal at the excitation/emission wavelengths for pteridines. We were also able to detect the absence of pteridines in embryos by mounting the embryos in methylene blue, which labelled them in the nasal epithelium, inner ear and along the body. The difference begins at about 25 hpf, which coincides with the onset of pigment cells formation from cranial neural crest cells. The opposite was true for the adult mutant fish, which were darker and have a higher number of xanthophores. To confirm that the observed phenotype is due to the Oatp1d1 mutation, we crossed a mutant female with a WT male and obtained a rescue of the WT phenotype. We hypothesize that the Oatp1d1 could have a role in the transport of pigment cell precursors or hormones that coordinate their formation.

Key words

Zebrafish, membrane transporters, oatp1d1, phenotype changes, CRISPR-Cas9 mutant

Type of presentation

Poster

Session

Biotransformation pathways and mode of action (MoA) of chemical pollutants

Proteomic Analysis in the Brain and Liver of Sea Bream (Sparus aurata) Exposed to the Antibiotics Ciprofloxacin, Sulfadiazine, and Trimethoprim.

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Antibiotics, frequently detected in aquatic ecosystems, can negatively impact the health of resident organisms. Although the study on the possible effects of exposure to antibiotics on these organisms has been increasing, there is still little information available on the molecular effects on exposed marine non-target organisms.

Hypothesis: Environmentally relevant levels of ciprofloxacin (CIP), sulfadiazine (SULF) and trimethoprim (TRIM) are able to alter the protein expression in exposed specimens of the gilthead seabream, *Sparus aurata*.

Methods: Individuals of sea bream were exposed to single compounds (5.2 ± 2.1 µg L^{-1} CIP, 3.8 ± 2.7 µg L^{-1} SULF, 25.7 ± 10.8 µg L^{-1} TRIM) for 21 days and differential protein expression was evaluated using a label free proteomic approach.

Results: The number of differentially expressed proteins in the liver was 39, 73 and 4 for CIP, SULF and TRIM respectively. In the brain, there was no alteration of proteins after CIP and TRIM treatment, while 9 proteins were impacted after SULF treatment. The differentially expressed proteins were involved in cellular biological, metabolic, developmental, growth and biological regulatory processes.

Discussion: Overall, our study evidences the vulnerability of *Sparus aurata* to environmentally relevant concentrations of the major antibiotics CIP, SULF and TRIM and that their chronic exposure could lead to a stress situation, altering the proteomic profile of key organs such as brain and liver. The involved mechanism of action of SULF and CIP may be related to their effects produced in bacterial cells, as SULF acts on folic acid metabolism and CIP acts at the DNA level by inhibiting bacterial DNA gyrase and topoisomerase IV.

Key words
Antibiotics; Proteomics; *Sparus aurata*, folic acid metabolism, DNA synthesis.

Type of presentation
Poster.

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants.

Screening of exoenzymes for polymers biodegradation in activated sludge, soil and seawater

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To date, global production of polymers, has reached a total of 9 billion tonnes (Geyer et al., 2017). The majority (78-79%) accumulates in the environment giving rise to challenges related to environmental contamination and potential threats to both ecosystems and human well-being (Balla et al., 2021).

Biodegradable polymers offer a promising solution for improving waste management practices. These materials are specifically engineered to decompose naturally when exposed to the environment, thanks to the activity of microorganisms such as fungi and bacteria (Bher et al., 2022).

Biodegradation occurs only after fragmentation, a process involving the decomposition of polymers into smaller units called oligomers and monomers produced by exoenzymes found outside of cells, within their surrounding environments.

The aim of this work was to focus on the screening of exoenzymes from activated sludge, soil and seawater in order to evaluate the potential of fragmentation of natural polymers. In a first step different methods were used to recover exoenzymes and to demonstrate their presence in different environments. In a second step a shotgun metagenomic approach was utilized to directly analyze the genetic material extracted from the environments. This method unveiled a diverse spectrum of genes responsible for encoding a variety of exoenzymes.

Among these three environments, a high enzymatic activity was observed in the activated sludge, while no enzymatic activity was detected in seawater. Additionally, among these enzymes, esterases were the most abundant.

Key words
Environments, Biodegradation, Fragmentation, exoenzymes.

Type of presentation
Poster.

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants.

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Synthetic phenolic antioxidant exposure impairs early zebrafish development

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Synthetic phenolic antioxidants such as 2,4-di-*tert*-butylphenol (2,4-DTBP), 2-*tert*-butyl phenol (2-BP), and 4-*tert*-butyl phenol (4-BP) are used in personal care products, food packaging, and water pipes (PEX and HDPE). They have been receiving increased attention due to their high-production, prevalence in the environment, and detection frequency in human biological samples. Previous studies using human iPS cells have shown that 2,4-DTBP can impair their differentiation into somites and osteoblasts. Therefore, the goal of this study is to determine if 2,4-DTBP, 4-BP, or 2-BP affects the development of bone, cartilage, and muscle *in vivo*, using zebrafish as a model organism. Zebrafish embryos were exposed to 2,4-DTBP, 2-BP or 4-BP until 5 days post fertilization (dpf). 2,4-DTBP, 2-BP, and 4-BP decreased spontaneous tail coiling at 24 hpf and decreased hatching rates. At 5 dpf, the 10 µM 2,4-DTBP exposure group had a 73% increase in spinal curvatures, the 20 µM 2-BP exposure group had an 11% increase in spinal curvatures, while 20 µM 4-BP had no changes. qPCR was used to assess specific transcripts indicative of muscle, bone, and cartilage formation. The highest concentration of 2-BP significantly increased expression of *sox9a*, a transcript required for cartilage formation and *myod1*, a gene involved in muscle development. Overall, this study shows that 2,4-DTBP, 2-BP, and 4-BP differentially alter the early development of zebrafish larvae.

Key words
Synthetic phenolic antioxidant, zebrafish, osteogenesis, chondrocyte, development

Type of presentation
Poster

Session
Biotransformation pathways and mode of action (moa) of chemical pollutants
AOP, system biology approaches and other conceptual modeling tools

Unveiling the combined effects of temperature and polycyclic aromatic hydrocarbons in the trout RTL-W1 cell line

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In addition to the presence of pollutants, aquatic ecosystems are nowadays subjected to increasing temperatures due to global warming. However, the effects arising from simultaneous exposure to xenobiotics and temperature increase have been almost non-explored. Liver in vitro models are vital tools to unveil the pollutants' effects at the molecular and cellular levels. Accordingly, the trout liver RTL-W1 cell line has been used to study the impact of polycyclic aromatic hydrocarbons (PAHs), mainly on cell viability and enzymatic activity. To test if monolayer-cultured (2D) RTL-W1 cells are suitable to delve into effects of PAHs and raised temperature, we exposed the cell line (72 h) at 18 °C and 21 °C to benzo[a]pyrene (B[a]P) and benzo[k]fluoranthene (B[k]F), at 10 nM and 100 nM. Cell density and viability were assessed through trypan blue exclusion and lactate dehydrogenase (LDH) assays. The expression of a selection of detoxification genes (cytochrome P450 (CYP)1A, CYP3A27, glutathione S-transferase omega 1 (GST), uridine diphosphate–glucuronosyltransferase (UGT), catalase (CAT), and multidrug resistance-associated protein 2 (MRP2)), was analysed using RT-qPCR. Both PAHs reduced cell density, and cell viability and LDH leakage were impacted by temperature. Both PAHs increased the expression of the detoxification targets. Temperature also influenced gene expression, especially the phase II detoxification genes. In summary, our results suggest that RTL-W1 line cultured in 2D should help us unravel the effects of PAHs and potentially other toxicants in the context of global warming.

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Keywords: detoxification; global warming; liver in vitro models; PAHs; RTL-W1.

SCIENTIFIC PROGRAM OF PRIMO 22

EDCs & Neuroendocrine Effects

(Oral talks)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

Estradiol-17β and bisphenol A differentially affect growth and mineralization at early life stages of seabass

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Natural or synthetic estrogens are pollutants found in aquatic ecosystems at low concentrations reaching ng.L-1 to µg.L-1. At these concentrations, (xeno-)estrogens are able to interfere with fish endocrine system. If water-borne exposure occurs at early life stages, when blood estrogens concentrations are low, this may have significant consequences for estrogen-sensitive functions such as skeletal development. To better understand how (xeno-)estrogens may affect skeletogenesis, 12 days post-hatch (dph) and 16 dph larvae of the European sea bass Dicentrarchus labrax were experimentally exposed to the natural estrogen Estradiol-17β (E2, 0.4 and 40 ng/L) and to the xenoestrogen bisphenol A (BPA, 1.6 and 160 µg/L). Morphological characteristics of the larvae (growth, developmental abnormalities) were recorded together with bone mineralization levels using alizarine red staining. RNA expression levels of several genes playing key roles in skeletogenesis and estrogen signaling pathways were also quantified. When exposure to E2 and BPA started before initiation of bone mineralization, several osteoblast, chondrocyte and osteoclast marker genes were transcriptionally overexpressed. Interestingly, this was correlated to an increase in bone mineralization in larvae head exposed to 0.4 and 40 ng/L E2 or to 1.6 µg/L BPA. In constrast, after the initiation of bone mineralization, exposure to E2 0.4 ng/L had a negative effect on head, vertebrae and tail fin bone mineralization and few genes were differentially transcriptionally regulated. This study brings new insights into the regulatory mechanisms of skeletogenesis by estradiol and into the effects of waterborne exposure to (xeno-)estrogens on the early skeletal development of teleost fishes.

Key words
Bone mineralization; Bisphenol A; Estradiol-17β; Estrogen signaling; Seabass Dicentrarchus labrax

Type of presentation
Platform presentation

Session
EDCs & neuroendocrine effects

Estrogen immunomodulation in teleosts: Comparative assessment of 17α-ethinylestradiol and estetrol on immune parameters of juvenile zebrafish (*Danio rerio*)

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Estrogens can modulate immune regulatory functions in teleosts through estrogen receptors (ERs) on lymphoid cells and organs. The synthetic estrogen 17α-ethinylestradiol (EE2), widely used in combined oral contraceptives (COCs), disrupt the normal endocrine function of aquatic organisms and may generate immunotoxic effects on fish. The natural estrogen estetrol (E4) was recently approved for use in a new COC. Potential immunomodulatory effects of EE2 and E4 has been investigated in zebrafish. Zebrafish embryos were exposed from fertilization to 30-days post-fertilization to E4 and EE2 at 10, 100 and 1.000x the predicted (for E4) or detected (for EE2) environmental concentrations. Nominal concentrations were: 320, 3,200 and 32,000 ng E4/L and 1, 10 and 100 ng EE2/L. The highest EE2 concentration induced significant mortality, reduced body length and delayed thymus development characterized by a decreased absolute thymus size, compared to the control. Within the thymus, the medulla zone displayed a reduction in surface area. In contrast, all E4 concentrations did not display any thymus development impairment. By disrupting the ER signaling pathway, EE2 induced morphological disturbances in the medulla, potentially caused by a reduction of thymocyte maturation. The absence of thymus disruption in E4-exposed fish suggests that this natural estrogen is less toxic than EE2 for developing lymphoid organs. Immunotoxicity biomarkers are currently explored to assess the fish immunocompetence, relevant for environmental risk assessment of estrogen-active compounds in aquatic environments.

Key words
Endocrine disruption, Estetrol, Ethinylestradiol, Immune System, Zebrafish

Type of presentation
Platform

Session
EDCS NEUROENDOCRINE EFFECTS

Biological effects in fathead minnows exposed to per- and polyfluorinated chemicals

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Per- and polyfluoroalkyl substances (PFAS) have become an important emerging concern worldwide, as these substances constitute potential hazards to environmental and human health. They are known endocrine disruptors, causing infertility and hormone disruption in addition to immunotoxicity, hepatotoxicity and cancer. Long chain PFAS have been phased out and replaced by shorter chained PFAS, which may be less toxic. The aim of this work was to compare the biological effects on fathead minnows from shorter and longer chained PFAS. The hypothesis was that exposure to shorter chained PFAS would adversely alter embryonic development and cause endocrine related changes in adults. To test the hypothesis, we treated fathead minnow dechorionated embryos and adults with 50 ug/L perfluorobutane sulfonic acid (PFBS), perfluorooctyl sulfonate (PFOS), and perfluorooctanoic acid (PFOA) for 7 days. In embryos, we saw depressed heartbeat, heart edema, and alteration of lipid metabolism. In adults, we observed dysregulation of lipids in the optic lobe, telencephalon, and cerebellum from all three tested PFAS. From in vitro cell based assays, PFAS primarily target PPARs, but in addition can target estrogen receptors, and alter the expression of thyroid hormone receptors. These data complement previous RNAseq data in liver that showed alterations of lipid related pathways, hormone biosynthesis, glucose metabolism and respiratory chain, among others. This work builds on growing evidence that PFAS are persistent environmental hazards that can perturb the endocrine system of aquatic organisms.

Key words
PFBS, PFAS, lipidomics, heartbeat, neuroendocrine

Type of presentation
platform

Session preference

- New approach methodologies (NAMs) to assess pollutant toxicity
- EDCs & Neuroendocrine Effects

PRIMO-22, May 26-29,2024, Nantes, France

Characterization of the Atlantic cod (*Gadus morhua*) retinoid X receptor (Rxr) subtypes and their sensitivity to organotin compounds

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Organotins, and in particular tributyltin (TBT), were used as a biocidal ingredients in marine antifouling products until their use was globally banned in 2008 due to their adverse effects on non-target organisms. Although the levels of TBT have significantly declined in seawater, TBT can accumulate and persist in marine sediments for several decades. Sediment pollution monitoring surveys have revealed a number of TBT hot spots along the Norwegian coast, exhibiting TBT levels that are comparable to the most contaminated TBT sites internationally. TBT is especially known for causing imposex in neogastropods where females develop a non-functional male-like sexual tissue that covers the female sexual organ. Imposex results in female infertility and severe declines in neogastropod populations occurred worldwide as a result of TBT exposure.

The mode of action underlying imposex is believed to be inappropriate activation of the retinoid X receptor (Rxr) signaling pathway causing an increase in the concentration of endogenous free retinoid that promotes development of imposex phenotypes. However, less knowledge exists on the interaction between organotins and Rxr in teleost species. Here we present the molecular and functional characterization of the Rxra, Rxrb1, Rxrb2, and Rxrg from Atlantic cod (*Gadus morhua*), including the ligand activation patterns from five different organotins and the endogenous ligand 9-cis retinoic acid (9-cis RA). The Rxr subtypes demonstrated distinct tissue specific expression, as well as different activation profiles and sensitivities towards both organotins and 9-cir RA, where Rxrb1 and Rxrb2 were not activated by any of the compounds. The lack of activation of these subtypes is most likely due to a 14 amino acid extension of helix 7 in the ligand-binding domain. Moreover, RNAseq analyses of TBT-exposed Atlantic cod precision-cut liver slices revealed that the top affected cellular pathways include cholesterol metabolism/biosynthesis, which is largely regulated by the liver X receptor (Lxr). Notably, Rxr is an obligate partner of Lxr, suggesting that TBT modulates the function of the permissive Lxr/Rxr heterodimer through direct binding to Rxr.

This study is part of the iCod 2.0 project (project no. 244564), the dCod 1.0. project (project no. 248840), and the Xenosense project (project no. 342186) funded by the Research Council of Norway.

Key words
Retinoid X receptor, TBT, Atlantic cod, ligand activation

Type of presentation
Platform

Session
Biotransformation pathways and mode of action (MoA) of chemical pollutants
EDCs & Neuroendocrine Effects

Investigating the Influence of Ethinylestradiol and Estetrol on Zebrafish Metamorphosis: A Comparative Approach

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The synthetic steroid 17α-ethinylestradiol (EE2), a key component in combined oral contraceptives (COCs), has notable endocrine-disrupting effects on aquatic species. Estetrol (E4), a natural estrogen synthesized by the fetal liver during pregnancy, is an estrogenic component of a new COC and is in development for hormone replacement therapy. Although prior research suggests a lower environmental impact of E4 compared to EE2, their effect on non-reproductive biological function remain unexplored. This study compares the impact of EE2 and E4 on zebrafish (*Danio rerio*) metamorphosis, a pivotal process in the fish life cycle. Zebrafish were exposed to four concentrations of these substances: 1, 10, 100 and 1,000 ng EE2/L (detected at 0.1 ng EE2/L in surface waters) and 0.32, 3.2, 32 and 320 µg E4/L (environmental concentration predicted at 0.032 µg E4/L). Sampling occurred prior to, during, and post the metamorphic climax (at 14, 22, and 30 dpf). Assessment parameters covered various morphological traits related to metamorphosis, including body size, pigmentation, skeleton ossification, maturation, and lateral line/swim bladder remodeling. Larvae exposed to EE2 at 100 and 1,000 ng/L displayed concentration and time dependent alterations. In contrast, E4 exhibited no significant effects. The study findings suggest that E4 is more environmentally friendly and less likely to disrupt fish metamorphosis than EE2. A high-throughput transcriptomic analysis is also in progress to explore and gain a detailed understanding of the mechanisms of estrogenic hormones in metamorphosis.

Key words
Metamorphosis, Estrogens, Zebrafish, Endocrine disruptor, Development

Type of presentation
Platform

Session
EDCS & Neuroendocrine effects

Oocytes “For a Few ribosomes More”: “The Small, the Non-Coding, and the Ugly” in xenoestrogenic waters

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A unique morphological feature of teleost perinucleolar primary oocytes (PNOs) is the presence of many nucleoli, indication of rRNA production and ribosomal subunit assembly. Oocytes contribute to fertility by supplying enough ribosomes for protein synthesis in the new embryo. PNOs produce large amounts of 5S rRNA and tRNAs, while 45S rRNA maturation products accumulate during secondary growth. We developed 5S rRNA/18S rRNA and tRNA/5,8S rRNA indexes that numerically identify fish oogenic stage; high values in PNOs and low in vitellogenic oocytes and in somatic and male germ cells. Indexes identify also presence of PNOs in intersex testes (IT) of *C. labrosus* exposed to xenoestrogens with values between ovaries (Ov) and testes (Te). Then, we characterised the non-coding RNAs in Ov, Te and IT of *C. labrosus* through miRNA-Seq. Ov and IT show higher levels of 5S rRNAs, tRNAs and snoRNAs. 5S annotation reveals specific expression of ovarian 5S rDNA paralogues in both. In the subset of snorRNAs required for nucleolar 45S pre-rRNA ribonucleolytic cleavages, U3 (*snord3*) is upregulated in IT vs Te, with levels similar to Ov. A total 247 miRNAs were annotated. Levels of 47 distinguish Ov from Te and, while only 14 miRNAs differ in IT vs Ov, 47 (17 up, 30 down) vary IT vs Te. The high levels of tRNAs and specific rRNAs and snoRNAs pinpoint their upregulation as a consequence of Te feminisation under xenoestrogen exposure and flag that ribogenesis-control is crucial during oocyte differentiation.

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Key words
Intersex, xenoestrogens, ribogenesis, non-coding RNAs, miRNA-Seq

Type of presentation
platform

Session
T14 EDCs and Neuroendocrine effects

T08 Mixture effects of pollutants

T10 Biomonitoring and development of integrative assessment approaches

Endocrine disruption via nuclear receptors in mollusks: a case study with the Mediterranean mussel *Mytilus galloprovincialis*

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Despite being banned in the 1990s, the endocrine-disrupting chemical tributyltin (TBT) still represents a matter of great concern as one of the so called “legacy pollutants”. In addition, its mode of action, at least in invertebrates, remains to be defined. In this light, this work presents the first thorough characterization of the adverse effects and mode of action of environmental concentrations of TBT in developing larvae of a model invertebrate, the Mediterranean mussel *Mytilus galloprovincialis*.

Multiplexed *in situ* hybridization of tissue-specific marker genes was used to evaluate the effects of TBT on the development of ciliated epithelium, larval shell, musculature, and nervous system. Potential molecular targets of TBT were tested by *in cellulo* transactivation assays, and their relevance with respect to the observed phenotypes *in vivo* was evaluated by pharmacological rescue experiments and co-expression analyses with tissue markers.

The results show that, at environmental concentrations, TBT strongly affects *M. galloprovincialis* shell formation, neurogenesis, and muscle system development. These effects are induced by disruption of the transcriptional activity of several members of the nuclear receptor (NR) superfamily, including the retinoid X receptor (RXR). Pharmacological blockage of RXR prevented the majority, but not the totality, of TBT-induced phenotypes in *M. galloprovincialis* larvae, indicating that this NR is an important, but not the exclusive, target of TBT in developing bivalve mollusks.

Key words
“tributyltin”, “development”, “Mediterranean mussel”, “nuclear receptor”, “legacy pollutant”

Type of presentation
Platform presentation

Session
t14: EDCs & neuroendocrine effects

t04: New approach methodologies (NAMs) to assess pollutant toxicity

t09: AOP, systems biology approaches and other conceptual modeling tools

Mechanistic Investigation of the effects of Vertebrate Steroid Hormones on Sex Reversal and Differentiation in Blue Mussels

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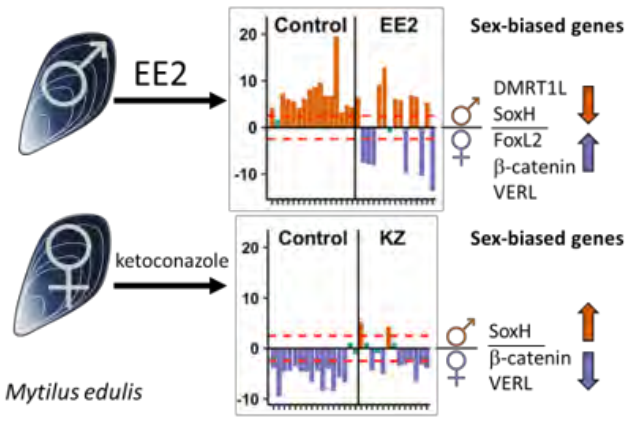
Mollusks in every part of the world have experienced a range of impacts from endocrine disrupting compound (EDC) pollution. In the blue mussel, *Mytilus edulis*, EDC exposure is correlated with feminized sex ratios in wild and lab mussels, but sex reversal is difficult to verify. Additionally, information about molluscan endocrinology and sexual differentiation is limited, making it difficult to understand and monitor the impacts of EDCs in mussels. Aim: We conducted a series of EDC exposures and RNAi treatments to characterize the mechanisms of sexual differentiation in blue mussels and their disruption by EDCs. Methods: We developed a non-destructive qPCR assay to identify the sex of *M. edulis*, and used this method to identify males and females prior to experimentation. We exposed male mussels to 17α-ethinylestradiol and female mussels to ketoconazole, EDCs that interfere with steroid synthesis and signaling in vertebrates. In a following experiment, we treated male and female mussels with RNAi targeting key genes proposed to be involved in sexual differentiation. Results: EDC exposures changed the sex of individual mussels, interfered with gonadal development, and disrupted gene expression of the sex differentiation pathway. Ongoing work will evaluate the effects of the RNAi gene knockdowns and investigate steroid hormone levels in exposed mussels. Discussion: This study expands the possibilities for laboratory and field monitoring of key mollusk species around the world and provides key insights into endocrine disruption and sexual differentiation in bivalves.



Key words
17α-ethinylestradiol, ketoconazole, steroids, Mytilus edulis, gonadal development

Type of presentation: platform
Session: (1) EDCs & Neuroendocrine effects, (2) Biotransformation pathways and mode of action (MOA) of chemical pollutants

Amphipod Nuclear Receptors: Underexplored tools for endocrine disruption assessment

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Wildlife is severely impacted by off-target effects of environmental chemical buildup. Of particular concern are endocrine disrupting chemicals (EDCs), substances that interfere with fundamental endogenous processes that regulate an organism's development and reproduction. In this sense, understanding the underlying mechanisms behind adverse physiological responses is essential for risk assessment (RA). Many nuclear receptors (NRs), as ligand-induced transcription factors involved in endocrine signaling, are prevailing EDC targets, and thus, key modulators of adverse responses. They are contemplated in multiple environmental guidelines for EDC RA (e.g., OECD), but their study is not equally representative across metazoans and is especially overlooked in invertebrates. The ecdysone receptor (EcR) is a clear example: Responsible for molting regulation, this NR has been wrongly regarded as insect-specific and used as a target for the development of insecticides. However, the elucidation of EcR orthologues on off-target species (e.g., amphipods like *Gammarus fossarum* and *G. locusta*) renders insecticides as potential EDCs. These crustaceans, who play critical roles in marine and freshwater ecosystem dynamics, are sentinel species greatly used in ecotoxicology. Yet, EcR function of both species is still uncharacterized. By using *in vitro* luciferase-based bioassays, we explored the function of this and other NRs and addressed the effects of environmental contaminants. Ultimately, our results can contribute towards the establishment of molecular tools for inclusive RA strategies.

Key words
Endocrine disrupting chemicals, Risk assessment, Invertebrates, Nuclear receptors, luciferase-based bioassays

Type of presentation
Oral presentation

Session
T14 EDCs & Neuroendocrine Effects

Endocrine disrupting antidepressants

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The residues of Antidepressants accumulate to µg/L concentrations in surface waters with negative effects on steroidogenesis and steroid hormone signaling in fish as well as on neuroendocrine signaling in invertebrates. Our studies provide further evidence for endocrine disruption by antidepressants both, in vertebrates and invertebrates.

Fluoxetine (FLX) was tested on seabass estrogen receptors with a reporter gene assay. Fish scales were exposed to FLX *in vitro* and the scale proteome from FLX-exposed fish was established. Expression of crustacean neuropeptide hormones were assessed in crabs exposed to FLX and glycaemia and ecdysone-levels were quantified. Juvenile crabs and cuttlefish were exposed to a mixture of antidepressants and behavioral endpoints were analyzed.

Antiestrogenic activity of FLX was observed with nuclear receptors, whereas estrogenic activity was detected with membrane receptors. Serotonin receptor transcripts were activated in fish scales. In crabs, FLX stimulated neuropeptide synthesis, increased glycaemia as well as locomotor and burying activity, whilst ecdysone levels decreased. Exposure of juvenile crabs to antidepressants reduced color change capacity. In post-hatch cuttlefish, antidepressants negatively affected feeding and burying behavior and reduced food intake and growth whilst background matching was affected.

Our results confirm endocrine disruption of steroid signaling by antidepressants for fish. Antidepressants also modulated neuronal and neuroendocrine signaling in crustaceans and mollusks by altering their neurotransmitter levels.

Key words
Selective serotonin reuptake inhibitors, neuroendocrine disruption, *Dicentrarchus labrax*, *Carcinus maenas*, *Sepia officinalis*

Type of presentation
Platform.

Session
EDC & Neuoendocrine effects

Effects of antibiotics and benzophenone-3 on the metabolism and behavior of the European seabass (*Dicentrarchus labrax*)

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UMR-I 02 INERIS-URCA-ULH SEBIO, Normandie Univ, UNIHAVRE, FR CNRS 3730 Scale, Le Havre, France

Estuary and coastal environments are heavily impacted by pollutants associated with anthropogenic activities. Among them, benzophenone-3 (BP-3), a UV filter widely used in cosmetic products and antibiotics are frequently detected in aquatic ecosystem, raising concerns about their potential impact on organisms. In this study we aimed at evaluating the effects of BP-3 or antibiotic exposure on juvenile European seabass (*Dicentrarchus* labrax). Fishes were exposed to BP-3 (0,35 µg/L and 35 µg/L), a mix of antibiotics (50 µg/L), solvent control (DMSO) or seawater during 7 days. Different analyses were conducted following exposure, i.e. behavioral tests, biometrics measurements and energy metabolism analyses. Primary results indicate that exposure to antibiotics affect biometric parameters of juveniles inducing a decrease of body weight and of hepatosomatic index, while BP-3 had no effects on these parameters. Regarding behavior, increased anxiety and decreased social interactions? were observed after antibiotic exposure. In the case of BP-3 exposure, a slight decrease in locomotion was observed in exposed fish suggesting an increase in anxiety. However, the other proxies of anxiety were not affected. These results, together with gene analyses, still in progress, will be discussed.

Key words
UV filter – Antibiotics – Liver toxicity – Lipid metabolism – Behavior

Type of presentation
Poster

Interests and constraints towards unravelling endocrine disruptors mechanisms using dose-response transcriptomic data: application to the impact of di-n-butyl phthalate on zebrafish

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Transcriptomic dose-response (DR) data has recently emerged, enabling the exploration of mechanistic exposure-effect relationships of endocrine disruptor compounds (EDC). This gives us access to discriminating independent mechanisms that could co-occur with distinct DR relations. However, unravelling these intricate signals along the dose gradient represents a real challenge, as revealed by our DR transcriptomic dataset derived from embryo-larval exposure of zebrafish (*Danio rerio*) to di-n-butyl phthalate (DBP).

To characterize the relationships between DBP exposure and effect, we adopted the DRomics workflow, computing transcript benchmark doses (BMDs) and depicting response trends. To address the complexity of transcriptomic DR data and its biological interpretation, we developed a reproducible workflow that harmonizes DR modelling metrics with bioinformatic techniques such as Markov Clustering and Over-Representation Analysis (ORA), leveraging complementary functional databases. This approach provides objective arguments from biological and functional databases to optimize disrupted hormone signalling pathways discovery. Comparing our workflow with more conventional methods for biological interpretation, we demonstrate its ability to reveal biological pathways that would otherwise have been overlooked.

Our analysis uncovers the disruption of the retinoid signalling pathway as one of the most sensitive mechanisms, providing an unprecedented sensitivity dimension to this pathway. Albeit the disruption of this pathway by DBP has been suggested in a handful of mammal studies, this mechanism has yet to be considered in an ecotoxicological context.

Key words
di-n-butyl phthalate, RNA-seq, dose-response framework, functional enrichment, clustering

Type of presentation
Platform

Session

SCIENTIFIC PROGRAM OF PRIMO 22

EDCs & Neuroendocrine Effects

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Ribosomal RNAs, tRNAs and RNA polymerase III regulating factors as molecular markers of xenoestrogen induced intersex condition in the mullet *Chelon labrosus*

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C. labrosus mullets from polluted sites in the Southern Biscay Bay display high prevalence of intersex condition. Oocytes (Oo) are unique cells in metazoans as they accumulate molecules to be used by another individual; the embryo. One of the energetically most demanding processes in a cell is protein synthesis and fish Oo do part of the job for the embryo by contributing the necessary ribosomes. In this sense, we compared the RNA profiles in ovaries (Ov), testes (T) and intersex testes (IT) of mullets from Gernika estuary, and identified 5S rRNA and tRNAs, as very powerful markers of the presence of perinucleolar oocytes in both Ov and IT. Their analysis through a simple electrophoresis of total RNA, and quantification relative to the presence of 45S rRNA maturation-products, serves to rank IT gonads according to the severity of the condition. Both 5S rRNA and tRNAs are produced by RNA pol-III, and some of the genes (*gtf3aa*, *gtf3ab*, *brf1a*, *brf1b*, *brf2a*, *brf2b)* that code the polypeptides of its 3 activating transcription factors, and also its inhibitor (*maf1a* and *b*), are duplicated in fish genomes. One of the paralogues in each case is oocyte-specific with strong transcription in mullet Ov and IT. Therefore, we conclude that activation of ribogenesis (RIB) is tightly controlled in fish Oo. Molecular tools based on qPCR analyses of target RIB genes preceded by analysis of total RNA through capillary electrophoresis are proposed as useful to study feminization in xenoestrogens exposed fish.

Acknowledgements: Basque Gov (IT1743-22), MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100)

Key words
Intersex, xenoestrogens, 5S rRNA, tRNA, RNA polymerase III.

Type of presentation
poster

Session
T14 EDCs and Neuroendocrine effects

T08 Mixture effects of pollutants

T10 Biomonitoring and development of integrative assessment approaches

Deleterious effects of exposure of rainbow trout embryos and larvae to sodium fluoride – the project SUSPECT

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Endocrine disruptors (EDs), ubiquitous in terrestrial and aquatic environments, have come under increased public and scientific scrutiny and have been classified as substances of very high concern for human health and the environment. The present study is part of the European collaboration project SUSPECT, the main objective of which is to determine the potential role of sodium fluoride (NaF), a potential ED, on the thyroid hormone system (THS)- immune system (IS)-microbiota triptych of rainbow trout. The aim here is to show the relation between disruption of the THS, the IS and the development/behavior on early life-stages. Embryos were exposed for 15 days to concentrations of 0-32.6 mg/L NaF. After 15 d, larvae were divided into 3 batches (1) to be NaF-exposed 8 more days to the same concentrations, (2) to be infected with the Infectious Haematopoietic Necrosis (IHN) virus or (3) to be held in clear water. The fish were monitored daily and sampled at different times to evaluate effects on the THS, including TH-dependent eye development, and the IS (health status, morphometry, behavior, histology, RNAseq). First results indicate impaired growth and a strong impact on behavior at the highest concentration tested. The intermediate concentration (10.8 mg/L) caused a significant delay in hatching, a decrease in virus-related mortality, an increase of thyroid follicle numbers and an increase of photoreceptor layer thickness. Further analyses are underway to determine the immunotoxicity of this potential ED.

Project funded by the National Environment-Health-Work Research Program of Anses with the support of the Ministries of Environment, Agriculture and Labor (ANSES-22-EST-050).

Key words
endocrine disruptors, immune system, thyroid, behavior, development

Type of presentation
platform

Session
1 – EDCS & neuroendocrine effects

Developmental expression of serotonin signalling components in *Mytilus galloprovincialis* as possible targets for SSRIs in bivalve embryos

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Marine bivalves need to cope with a variety of biotic and abiotic stressors and to do so they have evolved sophisticated stress response mechanisms, in which neuroendocrine regulation plays an important role[1]. Different neuroendocrine components in oysters and mussels' larvae have been shown to represent a target for environmental stressors and exposure to endocrine disrupting chemicals and pharmaceuticals[1,2].

Antidepressants are raising attention due their increasing prescription worldwide and consequent release into the aquatic compartment. In particular, selective serotonin reuptake inhibitors (SSRIs) are often detected in coastal marine environments. However, their mechanisms of action in non-target marine species are still unknown[3]. Recent data showed that SSRIs cause embryotoxicity in *Mytilus galloprovincialis*[4].

In this work, in order to investigate possible targets for SSRIs (e.g.: fluoxetine FLX) in bivalve larvae, different genes of the serotonergic system (receptors, metabolism, transporters, FLX resistance gene) were identified in *M. galloprovincialis* genome. Orthology assessment was performed by Maximum Likelihood phylogenetic analysis and potential binding domains were identified using Multiple Sequence Alignment. Gene expression dynamics across early larval development (from 0 to 72 hpf) were investigated on the genome guided transcriptome assembly and by HCR imaging[5]. The results shed some light on possible mechanisms of action SSRIs and the most critical steps of the serotonergic system that may be affected during *Mytilus* development.

Key words
bivalve larvae, SSRIs, serotonin signalling, RNAseq, HCR

Type of presentation
Poster

Sessions

- New approach methodologies (NAMS) to assess pollutant toxicity
- EDCS & neuroendocrine effects

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Autophagocytosis and lysosomal biogenesis in oocytes of fish exposed to xenoestrogens downstream a wastewater treatment plant

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Fate of oocytes during oogenesis depends on interconnected genetic, metabolic, physiological and environmental clues. The mechanistic target of rapamycin (mTOR) could integrate of the nutritional, hormonal and stress status activating either oocyte growth or follicular atresia. Indeed, mTOR controls ribogenesis during oocyte growth for protein synthesis in the early embryo or alternatively triggers autophagocytosis (AP) for resorption. Under starvation or other stresses, mTOR can activate ULK1/2 kinase to initiate AP. mTOR also regulates TFEB, transcriptional regulator of lysosomal (LYS) biogenesis genes such as *lamp2*. Firstly, *Chelon labrosus* ESTs coding for *mtor*, *ulk1*, *tfeb* and *lamp2* were obtained through gonadal RNA-Seq analysis, and then, the transcription of the four genes was studied through qPCR in different tissues, including gonads of mullets exposed to xenoestrogens downstream the Gernika WWTP. Similar analyses were conducted on control *D. rerio* tissues. Transcription of the four genes was ubiquitous in both species, with the exception of *mtor* that was highest in ovaries. In mullets intersex testis showed transcription profiles in between ovaries and testes in all genes. A β-GUS histochemical technique was validated granting the microscopic visualisation of large lysosomes in the ooplasm. In conclusion, the qPCR and histochemical methods validated will grant the study of AP and LYS activity in oocytes, both in ovaries and intersex testes of fish under nutritional/chemical stress.

Acknowledgements: Basque Gov (IT1743-22), MCIN & EU-FEDER/ERDF (PGC2018-101442-B-100)

Key words
Intersex, xenoestrogens, mTOR, lysosomes, autophagocytosis.

Type of presentation
poster

Session
T14 EDCs and Neuroendocrine effects

T08 Mixture effects of pollutants

T10 Biomonitoring and development of integrative assessment approaches

SCIENTIFIC PROGRAM OF PRIMO 22

New approach methodologies(NAMs) to assess pollutant toxicity

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

A Cell Line-Based RNA- and RIBOseq Approach Towards Understanding Chemically Induced Growth Inhibition in Fish

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Fish are a highly diverse group of species that suffer from both acute and chronic effects caused by their exposure to various chemicals. Since chronic effects prevail, regulatory studies often assess growth as pivotal endpoint. However, the tests conducted are not only under ethical scrutiny but are also time and resource demanding. A potential solution to this challenge are cell line-based assays that exploit the close link between organism growth and cell proliferation. While investigating the potential of the permanent gill-derived RTgill-W1 cell line, researchers from our group could not only demonstrate the feasibility of such an assay but also showed that a set of structurally and functionally different chemicals can cause the same effect: A reduction in cell proliferation and thus growth. However, the underlying molecular mechanisms and especially the points at which the initially different pathways converge and connect to the cell cycle remain unclear. In order to investigate this, we selected six chemicals and conducted concentration response experiments to determine an exposure concentration for time-resolved RNA- and RIBOseq experiments. The obtained concentration response data indicate that most of the effects happen before five days of exposure and that the shape of the curve does not change much after seven days. The results obtained will not only lead to a better understanding of reduced cell proliferation upon chemical challenge, but also further demonstrate the potential of cell lines as a tool to reduce, refine and replace conventional animal experiments.

Key words
chronic toxicity, cell proliferation, alternatives to animal testing

Type of presentation
Platform

Session
New Approach Methodologies (NAMs) to Assess Pollutant Toxicity

AOP, System Biology Approaches and Other Conceptual Modelling Tools

Biotransformation Pathways and Mode of Action (MOA) of Chemical Pollutants

Characterization of zebrafish liver spheroids: insights into 3D cell culture responses to toxicants

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In recent years, there has been increasing interest in 3D cell cultures as a more realistic model for investigating the effects of toxicants on aquatic organisms. However, few studies have thoroughly examined the molecular signature of 3D models. This study aims to characterize zebrafish liver cell (ZFL) spheroids compared to 2D cultures, by analyzing gene expression patterns, cell lipidomic signatures, and responses to model toxicants. The viability of ZFL spheroids was notably influenced by cell quality and the composition of the culture medium. ZFL cells, seeded at three densities (5000, 10000, 20000 cells/well) in ultra-low attachment plates, formed spheroids of various sizes. Post day 5, their size remained constant for at least 14 days, maintaining high viability. By day 7, spheroids (450 µm) showed an accumulation of storage lipids (cholesterol esters), increased expression of genes involved in cholesterol metabolism (*lcat*) and the urea cycle (*asl*). In comparison to cell monolayers, spheroids had decreased levels of membrane lipids and lower expression of genes related to fatty acid synthesis and elongation (*fasn*, *elovl6*). β-naphthoflavone exposure resulted in 100-fold higher *cyp1a* expression in spheroids than in cell monolayers. Differential responses were also observed when assessing the toxicity of bisphenol A derivatives using live-dead staining. Overall, this study emphasizes the importance of optimizing 3D culture conditions and highlights the more realistic nature of the 3D culture model for chemical toxicity assessments.

Key words
Liver cell spheroid, 3D, lipidomic, gene expression, response to toxicants.

Type of presentation
Platform

Session
NEW APPROACH METHODOLOGIES (NAMS) TO ASSESS POLLUTANT TOXICITY

A new generation of cell-based approaches in acute toxicity determination rely on subcellular responses to sewage effluents.

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Determination of acute toxicity is essential for ecological risk assessment. Traditionally, acute toxicity testing requires substantial numbers of animals and uses death as an apical end point which sacrifices large number of experimental animal and takes days to obtain the result. Application of fish cell line can provide a possible alternative to whole animal toxicity test. Since cells are more consistent with each other which makes the result more repeatable. However, cell-based assay may show several orders of magnitude less sensitive than the animal-based results. Some changes in cellular organelles could have higher sensitivity in responding to pollutants. For this reason, a new generation of cell-based assay was developed using rabbitfish fin cells as model and fluorescent probes to visualize the subcellular responses. The subcellular responses under sewage effluents exposure were captured by confocal microscopy and quantified. Through visualization of cellular responses, we further screened several cellular indexes including structure of cellular organelles and activity of essential cellular enzymes. Among these cellular indexes, lysosomal number and mitochondrial size which had a good linear relationship with sewage effluents content and these subcellular indexes were used to represent the toxicity. Besides, these cellular indexes had a good agreement between *in vivo* and *in vitro* results, demonstrating the accuracy of cellular parameters in representing the acute toxicity of sewage effluents. The developed cell-based testing assay presented here has the characteristics of a faster and cheaper method, which does not require complex facilities and large amount of testing samples. The developed assay may be further applied in predicting the acute toxicity to sewage effluents and replacing traditional animal testing.

Keywords
Toxicity determination, Subcellular fluorescent visualization, Sewage effluents

Assessment of transactivation potencies of killer whale (*Orcinus orca*) estrogen receptor alpha (ERα) by DDTs using *in vitro* and *in silico* approaches

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Killer whales (*Orcinus orca*), as apex predators, face risks from the high accumulation of persistent organic pollutants (POPs), particularly dichlorodiphenyl trichloroethane and its analogs (DDTs). Estrogen receptor alpha (ERα) plays a critical role in reproductive health. The functional analysis of killer whale ERα (kwERα) is essential for evaluating the impact of DDTs on killer whale populations, as there are interspecies differences in ERα ligand preference. This study aimed to assess kwERα transactivation potencies in response to DDTs. The 10% relative effective concentration (REC_{10}) to 17β-estradiol (E_2) was evaluated using an *in vitro* reporter gene assay with kwERα expression plasmid transfected into COS-1 cells. Additionally, *in silico* approaches including molecular docking and protein-ligand interaction fingerprint (PLIF) were applied to elucidate molecular mechanisms. *In vitro* results revealed a trend of E_2 > *o,p'*-DDT > *o,p'*-DDE > *o,p'*-DDD > *p,p'*-DDD > *p,p'*-DDOH > *p,p'*-DDT > *p,p'*-DDE (inactive) (1.92 x 10^{-6} µM, 0.02 µM, 0.04 µM, 0.14 µM, 0.48 µM, 1.20 µM, and 1.79 µM, respectively). *In vitro* findings are accounted for by *in silico* docking scores, suggesting that the stronger affinity of *o,p'*-isomers for kwERα may be attributed to *o,p'*-chlorine atoms facilitating stronger van der Waals interactions with the hydrophobic ligand binding site. PLIF highlighted Glu357 and Phe441 as critical residues for DDTs binding. These findings underscore the importance of *in vitro* and *in silico* approaches to assess the reproductive health of killer whales.

Keywords
Killer whale, Estrogen receptor alpha, DDTs, *In vitro*, *In silico*

Type of presentation
Oral

Session
1. New approach methodologies (NAMs) to assess pollutant toxicity
2. Biomonitoring and integrative assessment approaches
3. EDCs & Neuroendocrine effects

The importance of physioxia to *in vitro* research using marine cell models

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In vitro cell research is critical for enhancing assay uniformity, quickly assessing chemical toxicity, and reducing the use of animals in research. *In vitro* toxicity screens have been widely used in the field of ecotoxicology and risk assessment programs. However, traditional *in vitro* culture settings under high O_2 levels (~18-21 kPa O_2; hyperoxia) fail to mimic the cells' real-life microenvironment inside the organ (0-15 kPa O_2; physioxia). For the first time using a fiber-optic O_2 micro-sensor, we gained information on the physioxia levels in several tissues of the Pacific oyster Crassostrea gigas. Depending on the filtering activity, O_2 levels ranged from 0-12 KPa (physioxia) in all tissues, including in the blood (hemolymph). Thanks to these results, we performed toxicity assays on oyster blood cells (hemocytes) under hyperoxia (classical methods; 21 KPa O_2) versus physioxia (mimicking real-life microenvironment; 5 KPa O_2). Physioxia increased the toxicity (2-fold lower LC50) and the inhibition of mitochondrial O_2 consumption induced by the antifouling compound DCOIT. Likewise, cells were more vulnerable to the herbicide rotenone under physioxia. These early findings highlight the potential bias in toxicity testing and mode of action discovery under traditional marine cell culture conditions. Thus, conducting *in vitro* chemical hazard prediction in physioxia settings is crucial to increasing the biological relevance of *in vitro* methods for ecotoxicology, which is fundamental for chemical regulation and preservation of our marine ecosystems.

Keywords

New approach methodologies, *in vitro* toxicity testing, physiological oxygen, antifouling, pesticide.

Type of presentation
Platform.

Session
t04: New approach methodologies (NAMs) to assess pollutant toxicity

Validation and Ecotoxicology Applications of a 3D Culture Model for Primary Hepatocytes of Brown Trout

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The use of alternative models for in vivo assays is currently a recommended practice. The goal has been to search for models as close as possible to their in vivo counterparts. Evidence from distinct mammal studies (and to a lesser extent with fish) showed that three-dimensional (3D) hepatocyte cultures, namely spheroids, preserve the morphological features of hepatocytes and several target functions. In our ongoing work, we aim to develop and characterise brown trout primary hepatocyte spheroids and test their potential to assess ecotoxicological effects.

Hepatocyte spheroids were obtained from juvenile brown trout and maintained at 18°C for one month under constant agitation. Hepatocytes were cultured at $5x10^5$ cells/mL in a supplemented DMEM/F-12 medium. For characterization purposes of spheroids and comparisons with the in vivo model (liver from the same fish donors), the spheroids were collected on distinct days, and cell viability, morphology, and gene expression of selected pathways were tested. In the next step, the spheroids were tested under different culture temperatures (18°C and 21°C) and/or hormonal stimuli (including different 17α-ethinylestradiol concentrations).

The spheroids reached morphological and functional stability mostly after the 12^{th} day of culture. Specific responses to temperature were observed, as well as, for example, dose-dependent EE2 effects on estrogenic targets. Brown trout spheroids are a promising alternative model as they mimic in vivo responses and remain functional in culture longer than 2D hepatocyte cultures.

Key words
Spheroids, 3D cultures, primary hepatocytes, brown trout.

Type of presentation
Platform.

Session
t04: New approach methodologies (NAMs) to assess pollutant toxicity

t14: EDCs & Neuroendocrine Effects

t13: Acclimation and adaptation to chemical stress

PrecisionTox: Using phylogenetic relationships to predict interspecies differences in toxicity pathways

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Cross-species extrapolation is a critical component of chemical safety testing, yet, predicting the human health and environmental effects of chemicals is challenging, in part because toxicology has largely excluded evolutionary knowledge of how genes are functionally bound to one another. To address these needs, PrecisionTox was developed to better protect the health of people by establishing New Approach Methodologies for chemical safety testing using a mix of toxicogenomics approaches (e.g., comparative genomics, metabolomics), evolutionary theory, quantitative genetics, data science, toxicology, and law. PrecisionTox employs six model species/cells, i.e., human cell lines, embryos of zebrafish and frog, fruit fly, water flea, worm, which together represent major branches of animal evolution and are recognized biomedical model systems. Our approach leverages the shared genetic legacy of toxicity response with other animals, including invertebrates, to uncover molecular toxicity pathways shared across the animal kingdom. We demonstrate that over 70% of gene families associated with disease/health status are shared among the greatest variety of animal species through evolution. Pathway conservation between invertebrates and humans is based on the degree of conservation within vertebrates and the number of interacting genes within the human network. Human gene sets that already serve as biomarkers are enriched by evolutionarily conserved genes across the animal phylogeny. These discoveries are foundational to interpreting results of biomolecular data generated by chemical screens. Presented on behalf of the PrecisionTox project (https://precisiontox.org/)

Key words
evolutionary toxicology, cross-species extrapolation, toxicogenomics, model species, new approach methodologies

Type of presentation
I prefer platform.

Session
New Approach methodologies (NAMs) to predict toxicity

Development of a new cell culture model for ecotoxicological studies in marine bivalves

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A better understanding of organism adaptation to biotic and abiotic factors and the role of microbiota in health and disease are crucial focal points for ecosystem sustainability on a changing planet. To address this challenge, our group is developing novel *in vitro* methodologies using two bivalve species: the Pacific oyster *Crassostrea gigas* and the Manila clam *Ruditapes philippinarum*. Bivalves are well-recognized as excellent models for studying environmental health and host-microbe-environment interactions. One of our strategies aims at better understanding pollutant impacts on the health of aquatic invertebrates at the cellular level, under acute and sub-chronic conditions. To achieve this goal, we established a well-standardized primary immune cell culture model together with a toolbox of cellular tests for *in vitro* ecotoxicity assessments. We optimized the conditions to maintain *R. philippinarum* hemocytes viable and functional in culture for at least 15 days, which is unprecedented for clam hemocytes. During this period, our culture conditions allowed us to keep a diversity of morphotypes, as shown by phase-contrast microscopy and cytochemical assays used for hemocyte characterization. They have also maintained a constant viability level, phagocytic activity, and some motility which is representative of a good cell culture health status. Finally, a cytotoxicity screening of sponge extracts using our primary clam hemocyte cultures revealed the potential of our model for further marine ecotoxicological analyses.

Keywords

Bivalve, hemocytes, immunology, *in vitro* toxicity testing.

Type of presentation
Platform

Session
t04: New approach methodologies (NAMs) to assess pollutant toxicity

Integration of RNA-seq and 16S analyses to detect molecular effects of pharmaceuticals on the non-target species *M. galloprovincialis*

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Type of presentation Oral presentation

Session New approach methodologies (NAMS) to assess pollutant toxicity

Other suggestions: Biotransformation pathways and mode of action (MOA) of chemical pollutants; Mixture effects of pollutants

Key words: RNA-sequencing, 16S rRNA sequencing, mussels, pharmaceuticals

High-throughput transcriptomic analyses may provide information on molecular endpoints useful to be included into the environmental risk of compounds of emerging concern, including pharmaceuticals. In this regard, due to the filter-feeding nature, the use of marine bivalves in ecotoxicological studies may inform on consequences of drugs on non-target species. In this study transcriptomic (RNA-seq) and microbiota (16S rRNA sequencing) analyses were applied on mussels exposed for 30 days to environmental concentrations of different drugs (Gemfibrozil, Metformin, Ramipril, Venlafaxine) and after a recovery period of 14 days. Effects in cell proliferation, stress responses and DNA damage were commonly observed among treatments. In addition, treatment-specific responses were also detected mirroring effects and mode of action described in model species. Among them, changes in fatty acid metabolism in response to the cholesterol reducer Gemfibrozil, mTOR pathway following Metformin, ions regulation to the antidepressant Venlafaxine were seen. Despite the risk associated with the different compounds decreased after the recovery period, the impairment of immune system was still observed in all groups even after the depuration. This result can be also related to changes in microbiota composition observed, including the over-representation of potential opportunistic pathogen species. As conclusion, the application of analyses methodologies adopted in this study allowed to investigate consequences of several pharmaceuticals detected in the environment on non-target bivalve species.

Modeling Azole Fungicides' Endocrine Disruption: A Physiologically Based Kinetic Model of the Hypothalamus-Pituitary-Gonads Axis (PBK-TD) in Zebrafish

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This work aims to develop a physiologically based kinetic model, linked to a mechanistic toxicodynamic model of the hypothalamic–pituitary–gonadal (HPG) axis in zebrafish (PBK-HPG model), that could link the dose to adverse effects and be used for risk assessment of endocrine disruptors (ED). It was applied to two ED-suspected azole fungicides: prochloraz (PCZ) and imazalil (IMZ), both known for their aromatase inhibition potency.

The proposed PBK-HPG model comprises twelve compartments representing diverse tissues and adapts to various exposure scenarios. It considers vitellogenin (VTG) and steroid hormones (estradiol, testosterone, 11-ketotestosterone), focusing on key aspects such as brain feedback loops, liver VTG synthesis, and gonadal steroid hormone synthesis. Model predictions were compared to original data from OECD TG229 and a time-dependent measurement of steroid concentrations.

The model accurately predicted internal concentrations of PCZ and IMZ in key organs. It faithfully replicated the HPG axis baseline physiological conditions. Simulations of PCZ and IMZ effects on the HPG axis showed good results. Overall, the model was able to reproduce all the different experimental conditions.

To conclude, our model provides valuable insights into the kinetics of the HPG axis, offering an understanding of the mechanism underlying the impact of azole fungicides on the HPG axis in zebrafish. The novel PBK-HPG model holds promise for integration into a quantitative adverse outcome pathway framework, offering a comprehensive approach for assessing the toxic effects of ED.

Key words
PBK-TD Model, qAOP, Endocrine Disruption, Azole Fungicides, Zebrafish.

Type of presentation
Platform presentation.

Session
1. New approach methodologies (NAMs) to assess pollutant toxicity
2. AOP, system biology approaches and other conceptual modeling tools
3. EDCs & neuroendocrine effects

Effects of chemicals on intestinal CYP3A65 expression in transgenic *tg(cyp3a65:GFP)* zebrafish embryo

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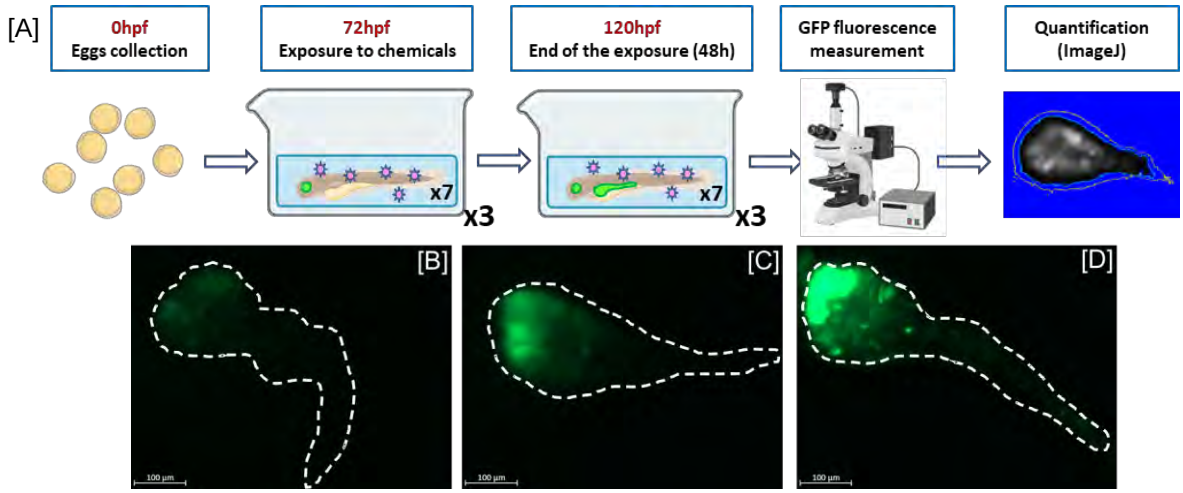
We recently developed a new transgenic zebrafish model expressing Green Fluorescent Protein (GFP) under the control of the zebrafish promoter of *cyp3a65* gene, coding for the CYP450 3A65, an ortholog of human CYP3A4. In *tg(cyp3a65:GFP)* zebrafish embryos, we showed that GFP is i) exclusively localized in intestine ii) dynamically expressed from 0 to 5 days post-fertilization iii) induced by specific PXR and AhR ligands alone or in combination. We subsequently developed a *tg(cyp36a5:GFP)* zebrafish embryo-based assay to screen the biological activity of several chemicals that were selected either due to their potential metabolic endocrine disrupting activity or known to disrupt the expression of *cyp3a4* in human. The data obtained revealed that several tested chemicals were inactive in the bioassay while others were able to disrupt the intestinal expression of *cyp3a65* in a concentration dependent manner. Their precise mode of action (MOA) on *cyp3a65* expression is not known but may reflect direct interaction and/or crosstalk with different nuclear receptor-regulated signaling pathways such as PXR, AhR and GR as shown in human. Altogether, our study showed that the use of the *tg(cyp3a65:GFP)* zebrafish embryo model provides novel data to characterize the hazard of chemicals on a poorly investigated tissue. Although the toxicological consequences of such perturbations in term of metabolic disorders need to be further explore, this zebrafish-based bioassay represent a relevant alternative model to investigate the MOA and effects of metabolic endocrine disruptors in a vertebrate model.



[A] Tg(cyp3a65:GFP) zebrafish embryo-based assay to screen activity of chemicals on intestinal cyp3a65 expression.. GFP expression in 120-hpf zebrafish embryos [B] control, [C] PXR ligand = clotrimazole 1.5µM [D] AhR ligand = TCDD 0.05 nM [D]. The dashed lines delineate the intestine.

Key words: Zebrafish, transgenic, intestine, cyp3a65, metabolic endocrine disruptors
Type of presentation: Platform presentation
Session: t04 - New approach methodologies (NAMs) to assess pollutant toxicity

SCIENTIFIC PROGRAM OF PRIMO 22

New approach methodologies(NAMs) to assess pollutant toxicity

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

A Whale of A Tale: One Environmental Health to Study the Impacts of Hexavalent Chromium Exposure on Human and Whale Lung Metabolomes

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Hexavalent chromium (Cr[VI]) is a carcinogen present in the marine environments. Bowhead whales are known to live to at least 200 years and not get cancer and recent reports indicate bowhead whale lung cells have a better DNA repair response to prolonged (120 h) exposure to Cr(VI) than human lung cells, while acute (24 h) responses are the same. This study aimed to determine if the metabolism of bowhead whales and humans responded differently to Cr(VI) exposures, as dysregulated cellular energetics is a hallmark of cancer but unexplored in Cr(VI)'s carcinogenic mechanism. We exposed human and bowhead lung fibroblasts to various concentrations of Cr(VI) for 24 h and 120 h and performed metabolomic experiments. We analyzed changes in the metabolites through differential metabolomics, pathway enrichment analysis, and upstream regulators analysis. In both cell lines, we observed acute Cr(VI) exposure resulted in a distinct metabolic response. In human cells, we observed a metabolic response with time (e.g., decreased amino acid biosynthesis and metabolite enrichment of sphingolipids), while in the bowhead cells, we observed no differences with time. Our results show Cr(VI) induces a metabolic response in human and bowhead whale lung cells. Our data indicate energy metabolism responses are key to Cr(VI) carcinogenic mechanism in humans, but whales are better adapted to prolonged exposure to Cr(VI). Future studies will further investigate these key differences to understand why bowhead whales have different responses with time compared to humans.

Key words

Hexavalent Chromium, Bowhead whales, lung cells, metabolomics

Type of presentation
Poster

Session
1. Acclimation and adaptation to chemical stress

2. *In silico* approaches, and *in vitro* assays including high-throughput (multi-omics) and high-content techniques.

Assessing the use of *Mytilus edulis* Digestive Cells for Contaminant Evaluation in the Arctic: A Flow Cytometry Approach

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Fish cell cultures are commonly used in toxicity testing with xenobiotics; however, less is known about primary cells derived from aquatic invertebrates. Digestive glands play a key role in the metabolism and biotransformation of xenobiotics in mussels, making digestive cells relevant *in vitro* models for studying pathways and toxic mechanisms of xenobiotics. Flow cytometry (FCM) can be used as a high-throughput screening method to evaluate the effects of contaminants in individual cells close to an *in vivo* state. So, the aim of this study was to investigate the sensitivity of FCM to quickly screen the effects of model chemicals in digestive cells isolated from *Mytilus edulis*. The morphological and physiological characteristics of digestive cells were first determined combining light and fluorescence microscopy and FCM, for which two main cell populations with different sizes and complexity were identified. The viability and biochemical functions of digestive cells 24 hrs post-isolation were determined, showing that cells maintained cellular integrity and biochemical functions over time. Exposure experiments with model contaminants are currently ongoing, for which mortality, cell viability, metabolic activity, neutral lipids and lysosomes presence, ROS formation, membrane potential, lipid peroxidation and DNA content will be determined by FCM. Overall, FCM seems to be a fast, accurate and reproducible method to assess a wide array of cellular functions in mussel digestive cells that can be integrated into next generation risk assessment of contaminants present in the Artic.

Acknowledgements
This work was supported by the EXPECT Project (#315969) funded by the Research Council of Norway.

Key words
Flow Cytometry, primary cell cultures, mussel digestive cells.

Type of presentation
Poster presentation

Session
New approach methodologies (NAMs) to assess pollutant toxicity.

Biochemical and Molecular Responses of Blue Mussel (*Mytilus* spp.) to Trophic PFAS Exposure: Implications for Coastal Monitoring and Environmental Risk Assessment

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Per- and polyfluoroalkyl substances (PFAS) encompass over 4000 non-natural chemicals classified as emerging contaminants due to their widespread environmental presence. Regulatory bans target some PFAS such as PFOS and long-chain PFCAs due to their persistent, bioaccumulative, and toxic traits. Despite this, the use of alternative compounds with unknown impacts persists.

This study aimed to investigate the biochemical and molecular effects of PFAS on the blue mussel (*Mytilus* spp.), a widely used sentinel organism in ecotoxicology for coastal monitoring. Mussels were exposed to PFAS mixtures representing environmental contamination via water and food in controlled laboratory tanks for four weeks, followed by a two-week depuration phase. Distinct ^{13}C isotopic labeling traced contributions of water and food contamination.

Comparative analysis of control and PFAS-exposed mussels from the different tank conditions was conducted. Health effects were evaluated using oxidative stress (CAT, SOD, LOOH), detoxification (GST) and neurotoxicity (AChE) biomarkers. Early molecular effects were examined by quantifying the relative expression of genes related to cell proliferation, apoptosis, cellular stress and energy metabolism through RT-qPCR analysis. A lipidomic study characterized variations in the lipidome in response to the different exposure conditions. The study provides insights into the biochemical and molecular responses in blue mussels to simultaneous waterborne and foodborne PFAS exposure, contributing to our understanding of the risks associated with these contaminants.

Key words
Mytilus, PFAS, dietary exposure, biomarker, multi-omics

Type of presentation
Platform

Session
"New Approach Methodologies (NAMs) to assess pollutant toxicity"

Ecological factors driving the evolution of mammalian estrogen receptor alpha (ERα)

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Estrogen receptor alpha (ERα) plays a critical role in the reproduction of animals. However, the evolutionary dynamics influencing interspecies differences in ERα sequences remain unclear. This study aimed to evaluate ecological factors driving interspecies differences in mammalian ERα by analyzing representative sequences of 88 mammalian species and 63 ecological factors using two-way cluster analysis (TWCA), together with *in silico* and *in vitro* approaches. Human, killer whale, Baikal seal, mouse, cat, and dog ERαs were exposed to 17β-estradiol (E_2), genistein (GEN), and diethylstilbestrol (DES) in *in vitro* reporter gene assays. TWCA revealed that marsupials, monotremes, rodents, primates, and cetaceans formed unique clusters, underscoring ecological factors such as main diet, habitat, and reproductive strategies contributing to ERα sequence variations. Notably, omnivores exhibited larger ligand binding pockets of ERα than carnivores and herbivores, likely due to exposure to diverse dietary chemicals. EC_{50} values in *in vitro* assays showed minimal variations in E_2 response among ERαs, whereas there were larger variations in response to GEN and DES. A strong association emerged between a mutation from Asn (corresponding to N349 in humans) to Ser in the ligand binding domain of cetaceans and the aquatic habitat, wherein Ser349 formed a hydrogen bond with Tyr538 of helix-12, suggesting its impact on ligand binding and co-activator recruitment. Overall, these results offer valuable insights into the impact of ecological factors on the ERα evolution and its chemical sensitivity.

Keywords
Estrogen receptor alpha, evolution, ecological factors, two-way cluster analysis

Type of presentation
Poster

Session
1. New approach methodologies (NAMs) to assess pollutant toxicity
2. Biomonitoring and integrative assessment approaches
3. EDCs & Neuroendocrine effects

Ecotoxicological Effects of Plastic-Associated Chemicals from Consumer Products on Atlantic Halibut (*Hippoglossus hippoglossus*) Hepatocytes

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The increasing prevalence of microplastics in marine environments is well-known, yet the risks posed by plastic-associated chemicals remain underexplored. This study, aiming to enhance the knowledge in this area, focused on the toxic impacts of these chemicals on a marine fish species significant to the Norwegian ecosystem. Utilizing *in vitro* methods under New Approach Methodologies (NAMs), this study examined primary liver cells from the Atlantic Halibut (*Hippoglossus hippoglossus*). The toxicity of leachates from five consumer products—shoe soles, balloons, "end-of-life" tyre granulates, washing gloves, and a polyethylene terephthalate (PET) bottle—was evaluated. These items were chosen for their varied toxicity levels in marine organisms at different developmental stages and trophic levels, including bacteria, algae, copepods, bivalves, sea urchins, and fish. Hepatocytes were exposed to these leachates for 48 and 96 hours, after which effects on cell viability, endocrine disruption, oxidative stress, and detoxification were measured. Advanced chemical analysis techniques, including Gas-chromatography-Mass-spectrometry and Inductively Coupled Plasma Mass Spectrometry, identified multiple plastic-associated additive chemicals in each product. The findings of this study contribute with vital information on the toxic action of chemical additives present in consumer products to fish liver cells, further supporting hazard and risk assessment efforts within the broader MicroLEACH project. The MicroLEACH project is funded by the Research Council of Norway (project No. 295174).

Key words
In vitro, NAMs, Marine fish, Plastic additives.

Type of presentation
Poster presentation.

Session
New Approach methodologies (NAMs) to assess pollutant toxicity.

Evaluating Cadmium-Induced Toxicity in Marine Mussels (*Mytilus edulis*) by Raman Spectroscopy and Chemometrics

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Raman spectroscopy is widely used in biology due to its non-invasive analytical capabilities. Several studies have been conducted for medical application, such as the development of cellular markers of carcinogenesis and detection of pathogenic bacteria. In the environmental field, this technique has been used for the detection of microplastic/nanoparticles in organisms. However, only few studies have focused on assessing toxic effects using Raman spectroscopy, reporting its ability to discriminate copper-tolerant yeast isolated from contaminated soil and monitor arsenic toxicity on bacteria. The European Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) both aim to achieve and maintain a good general water status by 2027. For this, an assessment of the environmental status is necessary, considering chemical pollution and their effects on living organisms. In this context we propose using Raman spectroscopy as a diagnostic tool for evaluating the toxic impact on marine invertebrates.

To test this hypothesis, mussels (*Mytilus edulis*) were exposed to two concentrations of cadmium (50 and 500 µg/L), alongside control groups. Then, the gills and the mantle were analyzed using Raman micro-spectroscopy. Results showed that it is possible to discriminate the mantle of the exposed mussels from the controls, as well as differentiate the two concentrations of exposure.

Our results show that Raman spectrometry, coupled with chemometric tools, holds promise and could be used to define a toxic fingerprint for marine invertebrates in environmental risk assessment.

Key words

Raman Spectroscopy, Toxicity, Marine Mussel, Chemometrics

Type of presentation

Platform

Session

1- New Approach Methodologies (NAMS) To Assess Pollutant Toxicity
2- Biomonitoring and Development of Integrative Assessment Approaches

In vivo assessment and *in silico* prediction of estrogenic potency of endocrine disrupting chemicals using zebrafish

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The present study aimed to evaluate the estrogenic potency of a wide array of estrogenic endocrine-disrupting chemicals (E-EDCs) via *in vivo* and *in silico* approaches using zebrafish (*Danio rerio*). Zebrafish embryos were exposed to E-EDCs to investigate the effect on the estrogen-responsive cytochrome P450 19A1b (*CYP19A1b*) gene expression. Tested chemicals include bisphenol A and its analogs (BPs), equine estrogens (EEs), and zearalenone and its metabolites (ZENs), as well as 17β-estradiol (E2). We also performed *in silico* screening of estrogenic potency by constructing 3D homology models of ligand binding domains (LBDs) of three zebrafish estrogen receptor (zfER) subtypes and subsequently simulating interactions between each of E-EDCs and zfER subtype LBDs. Exposure of embryos to E-EDCs resulted in the induction of *CYP19A1b* expression in a concentration-dependent manner by many tested chemicals. Based on the half maximal effective concentration (EC_{50}), the estrogenic potencies were basically ranked as EEs (0.8-79 nM) > ZENs (24-163 nM) > BPs (290-35,000 nM). *In silico* docking simulations revealed that three amino acids (e.g., Glu321, Leu355, and His492 in zfERα LBD) were important for hydrogen bonding with those E-EDCs. The interaction energy of E-EDCs to each of zfER subtypes showed positive correlations with EC_{50} for *in vivo CYP19A1b* induction by those compounds. The *in silico* simulations of interactions between ligands and zfER subtypes may help predict *in vivo* estrogenic potencies of untested chemicals.

Key words
Endocrine disrupting chemicals; Estrogen receptors; Docking simulation; *CYP19A1b*; Zebrafish

Type of presentation
Poster

Session
New Approach Methodologies (NAMs) to assess pollutant toxicity, EDCs & Neuroendcrine Effects

New approach methodologies (NAMs) to assess developmental toxicity in early chicken embryos

[1,2]Kazuki Kanda, [1]Kaori Chigusa, [1]Hisato Iwata

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Developing vertebrate embryos are known to be sensitive to exposure to environmental contaminants. Despite the need to assess these chemical hazards during the developmental stages of birds, the OECD Test Guidelines, the internationally accepted tests for acute exposure to post-hatched birds, are costly and time-consuming. Over the last decade, the *in ovo* exposure tests have been widely used as an alternative approach to assess embryonic toxicity in birds. However, due to the difficulty of *in situ* observation, *in ovo* exposure studies can only examine limited phenotypic effects (e.g., tarsus length and liver mass) by sacrificing embryos and few adverse cardiovascular endpoints have been investigated. Tahara and Obara (J. Poult. Sci., 51, 307–312, 2014) succeeded in hatching chicken embryos in artificial culture vessels. Chicken embryos in this shell-less (*ex ovo*) incubation system are able to visualize embryogenesis *in situ*. *Ex ovo* studies have not been applied to developmental toxicology despite their great potential to serve as new approach methodologies (NAMs). Thus, we have developed a novel method for *ex ovo* developmental toxicity testing based on the shell-less incubation system established by Tahara and Obara (2014) and measured a variety of phenotypic endpoints in early chicken embryos treated with organophosphate flame retardants (OPFRs) (Kanda et al., Ecotox. Environ. Safe., 207, 111263, 2021; Chigusa et al., Ecotox. Environ. Safe., 264, 115445, 2023). In this presentation, we will review our recent studies on the hazard assessment of OPFRs using the *ex ovo* incubation system.

Keywords
Chicken embryo, Organophosphate flame retardants, Shell-less (*ex ovo*) incubation system

Type of presentation
Poster

Session
1. New approach methodologies (NAMs) to assess pollutant toxicity
2. Biomonitoring and integrative assessment approaches

Planaria: a great organisms to evaluate rivers quality

[1]Caroline VIGNET., [1]Jean-Michel Malgouyres,[2] Jan Heuschele., [2]Khuong Van Dinh.,

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Currently, single pollutants and mixtures increase in the environment, particularly in aquatic environments. Environmental monitoring can be challenging due to the simultaneous presence of many pollutants in the environment. We aim to develop tools to understand such cocktail effects on endemic aquatic organisms: planaria. These small freshwater invertebrates, considered as bioindicator and present in rivers all over the world, have a simplified central and peripheral nervous system compared to higher vertebrates but with many common components such as neurotransmitters.

We collected planarian from previously undescribed locations and evaluated the behavioral responses of these planarians from different locations. In order to get a locomotion base, we first analyzed the behavior of the planaria in the original river water and also exposed them to copper in order to determine the ability of organisms from different places to cope with anthropogenic stressors. Copper is used as a biocide in both agri- and aquaculture. Behavior is one of the first responses to environmental change. We, therefore, analyzed the behavior using traditional movement analysis and labeled particular behavioral responses called stereotypes, such as the C-shape. These stereotypes are known to be linked to specific neurological reactions.

Then, the catalog can be used to train an AI-based image recognition algorithm to automatize and speed up the assessment of the state of an environment using the behavioral responses of planaria. It can also aid in understanding the effect of certain chemicals on species.

Key words
Planaria, copper, invertebrate, behavior, machine learning

Type of presentation
Platform presentation

Session

1) **New approach methodologies (NAMS) to assess pollutant toxicity**
2) **Mixture effects of pollutants**
3) **Acclimation and adaptation to chemical stress**

Regulation of redox and bioenergetics dynamics in early bivalve development: a molecular and biochemical perspective on shellfish reef health and sustainability

[1]Rafael Trevisan, [1]Ika Paul-Pont, [1]Caroline Fabioux, [1]Arnaud Huvet, [1]Jeremy Le Luyer

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Bivalve early life is especially vulnerable to natural and anthropogenic disturbances. It is also crucial for the long-term viability of bivalve populations and shellfish reefs, key contributors to coastal biodiversity. As miners in the 19th century used canaries as early signs of environmental hazards, we suggest using regulatory systems of bivalve development to predict environmental threats to shellfish reefs. Building on research from other species, this "canary in the mine model" implies that bioenergetic systems promote metabolic flexibility and redox state affects cellular proliferation, differentiation, and death during bivalve early development. Environmental factors alter these systems in adult bivalves, yet a tight control may limit flexibility during early life. Indeed, *in silico* analysis of the Pacific oyster early-life transcriptomes showed that antioxidant expression is minimal during cleavage, but Nrf2 and GSH synthesis activate during embryogenesis, and overall antioxidant amplification occurs with larval development. Expression of mitochondrial bioenergetic machinery is also absent during cleavage but starts throughout embryogenesis and increases as larval development progresses. Functional redox and bioenergetics assays are of concern to validate these molecular patterns. Evaluating the success or failure of managing such events in the light of environmental stresses allows for modeling bivalve early development outcomes and its shellfish reef consequences in a context of global change and growing anthropogenic pressures.

Key words

Oyster; redox biology; energy metabolism; ontogeny; coastal ecosystems.

Type of presentation

Poster

Session

Please propose one to three sessions in their order of preference.

t04: New approach methodologies (NAMs) to assess pollutant toxicity

t09: AOP, System Biology approaches and other conceptual modeling tools

t10: Biomonitoring and development of integrative assessment approach

Subcellular responses of *Mytilus galloprovincialis* to fluoroquinolone antibiotics

[1]Joanna Giannessi., [1]Lucia De Marchi, [1]Valentina Meucci, [1]Luigi Intorre, [1]Gianfranca Monni, [2]Mariella Baratti, [1,3]Carlo Pretti

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This study investigated the subcellular responses of the Mediterranean mussel, *Mytilus galloprovincialis*, to environmentally relevant concentrations of various fluoroquinolone antibiotics (FQs). The evaluation was performed on the digestive gland (DG) and gills (G) through a multi-biomarker approach employing *in vitro* assays. The research explored potential interactions between FQs and enzymes linked to antioxidant defenses, metabolism, and neurotransmission, along with assessing their impact on membrane lipids, proteins, and DNA integrity (Fig. 1).

Results indicate tissue-specific responses to each FQ, with an overall reduction in antioxidant defenses, suggesting potential alterations in enzyme structure and functionality in both DG and G. In the G, a significant increase in GST activity was observed, suggesting the activation of detoxification mechanisms. Additionally, inhibition in CbE was observed after exposure to MARB and DI. While DG showed no cellular damage, G exposed to ENR, MARB, and DI exhibited altered carbonylated protein content, indicating oxidative potential. All tested FQs strongly inhibited AChE in DG and compromised DNA integrity in both tissues, suggesting potential neurotoxic and genotoxic effects on non-target organisms like mussels. These findings offer insights into aquatic organisms' responses to these emerging pollutants, aiding in designing future *in vivo* experiments focused on understanding their ecotoxicity, in addition to the well-known effects related to environmental antibiotic resistance.

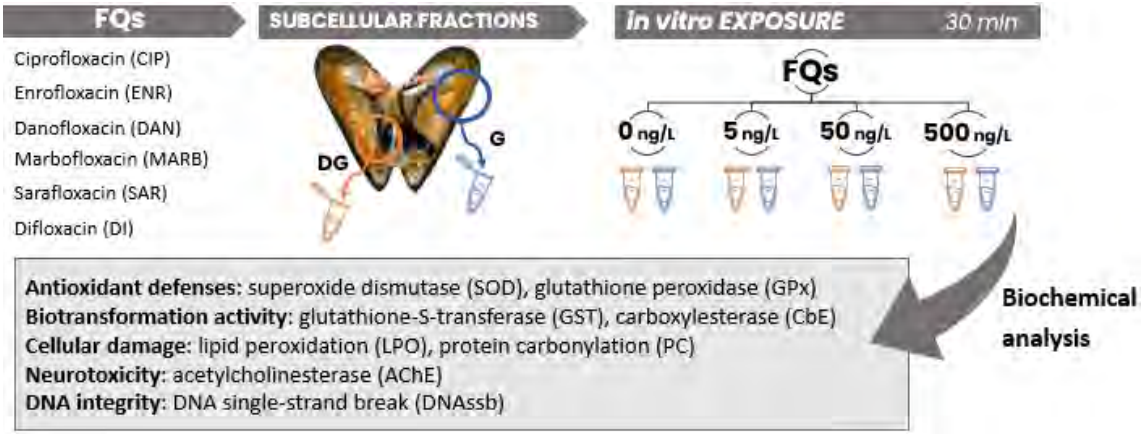

Figure 1: Schematic representation of experimental setup. Tested FQs, concentrations, and biomarker assays were reported.

Key words: Fluoroquinolones, *Mytilus galloprovincialis*, subcellular fractions, biomarkers

Type of presentation: Poster

Session: Monday, May 27th

SCIENTIFIC PROGRAM OF PRIMO 22

Mixture effects of pollutants

(Oral talks)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

Gender differentiating effects of a mixture of emerging contaminants in the Mediterranean mussel *Mytilus galloprovincialis.*

J.M. Gonçalves[1], M. Benedetti[2,3], G. d'Errico[2], F. Regoli[2,3] and M.J. Bebianno[1]

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Mussel gametes are released into the water column, where fertilization takes place, being subjected to the surrounding contaminants. Emerging contaminants, such as nanoplastics and pharmaceuticals, have been gaining vast attention; however, gender-differentiating effects on the reproductive organs of mussels remain scarce. In this instance, the gonads of *Mytilus galloprovincialis* were exposed to polystyrene nanoparticles (50 nm; 10 µg/L), the cytotoxic drug 5-fluorouracil (10 ng/L), and a combination of the two for 21 days for a multi-biomarker assessment and 28 days for the ingestion of nanoplastics, with gender differentiation. The effects on oxidative stress and damage were assessed, and synergistic and antagonistic interactions between the contaminants within the mixture were calculated. A weight-of-evidence model was also used to evaluate the hazardous level of biomarker results in relation to nanoplastic and mixture-exposed mussels. The ingestion of nanoplastics, being females the most compromised, was shown to be gender and time-specific. A synergistic interaction between the cytotoxic drug and the nanoplastics was computed, suggesting that the mixture is more dangerous than the individual contaminants. Additionally, the weight-of-evidence model confirms that female mussels are mostly compromised at longer exposure times compared to males. Thus, these exposure conditions can possibly impair robust oocyte formation during gametogenesis, jeopardising reproductive success and consequently the viability of future generations, negatively impacting the whole ecosystem.

Keywords
Mytilus galloprovincialis; gender ; nanoplastics; 5-Fluorouracil; mixture

Type of presentation
Oral

Session
Mixture effects of pollutants

Mixture effects of pharmaceuticals carbamazepine, diclofenac and venlafaxine on *M. galloprovincialis* probed by metabolomics and proteogenomics combined approach

[1]Courant F., [1]Dumas T., [2,3]Boccard J., [1]Ramirez G., [4]Armengaud J., [1]Escande A., [1,5]Mathieu O., [1]Fenet H. and [1]Gomez E.

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Exposure to single molecules under laboratory conditions has led to a better understanding of the mechanisms of action (MeOAs) and effects of pharmaceutical active compounds (PhACs) on non-target organisms. However, not taking the co-occurrence of contaminants in the environment into account may lead to underestimation of their impacts.

In this study, mussels collected from a lagoon around Montpellier were exposed (or not) to a mixture of 3 PhACs at low (0,1 µg/L) and high (10 µg/L) concentrations. After 7 days of exposure, the digestive glands were collected to carry metabolomic and proteomic analyzes, on the same samples, considered as paired samples. Our multi-omics approach and data fusion strategy highlighted direct links between metabolites and proteins and revealed toxicological pathways activated by the mixture. The response was mainly characterised by energy metabolism disruption, fatty acid degradation, protein synthesis and degradation, and the induction of endoplasmic reticulum stress and oxidative stress. The known MeOAs and molecular signatures of each PhAC (investigated earlier) were taken into consideration to gain insight into the mixture effects, thereby revealing an additive effect.

The integration of multi-omics data made it possible to more robustly document the molecular understanding of the mixture effects on non-model organisms. Multi-omics approaches offer a comprehensive overview of molecular responses, that can be precursors of physiopathological effects, triggered by exposure to contaminant mixtures, even at environmental concentrations.

Key words
multi-omics; pharmaceutical active compounds; bivalve mollusks; data fusion; adverse effects

Type of presentation
Platform

Sessions
1/Mixture effects of pollutants
2/New approaches methodologies (NAMs) to assess pollutant toxicity
3/Biotransformation pathways and mode of action (MOA) of chemical pollutants

Interactive biological responses modulate the toxicity of pharmaceuticals mixtures.

Mezzelani, M., Panni, M., d'Errico, G., Di Carlo, M., Nardi, A., Benedetti, M., Gorbi, S., Regoli, F.

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The constant exposure to chemical mixtures represents one of the major challenges affecting marine ecosystems. Among the large variety of contaminants of emerging concern, pharmaceuticals are increasingly documented in seawater, sediments, and marine wildlife. However, the influence of additive, synergic or antagonistic effects in modulating pharmaceutical 's mixtures toxicity remains still unclear. This study aimed at a multidisciplinary characterization of adverse effects caused on different model species by various pharmaceuticals (Ibuprofen, Paroxetine, Venlafaxine, Metformin, Gemfibrozil, Ramipril) dosed both alone and as mixtures. Analyses on bioavailability in *Mytilus galloprovincialis* were integrated with a panel of biochemical and cellular biomarker. Ecotoxicological effects were also evaluated through a battery of bioassay with different endpoints and species (bacterial bioluminescence, algal growth, oyster embryotoxicity). Interactive and competing mechanisms among tested drugs modulated bioaccumulation and biological responses in mussels, with both additive and antagonistic interactions depending on investigated mixture and metabolic pathways. Ecotoxicological bioassays highlighted different sensitivity of tested species toward selected drugs and mixtures, with major effects on the oyster embryotoxicity and algal growth. Using a quantitative Weight of Evidence model, the risk of pharmaceutical mixtures was summarized, highlighting the added value of such multidisciplinary approach for an effect-based risk assessment of chemical mixtures in marine ecosystem.

Key words (up to 5)
Pharmaceutical mixtures, biological responses, interactive effects, multidisciplinary approach, environmental risk assessment.

Type of presentation
Platform.

Session (list 3 in order of preference)

1) Mixture effects of pollutants
2) Biomonitoring and development of integrative assessment approaches
3) Biotransformation pathways and mode of action (MOA) of chemical pollutants

Mixture Effects of Per- and Polyfluoroalkyl Substances on Embryonic and Larval Sheepshead Minnows (*Cyprinodon variegatus*)

[1]Philip Tanabe, [1]Peter B. Key, [1]Katy W. Chung, [1]Emily C. Pisarski, [2]Jessica L. Reiner, [3]Alix E. Rodowa, [4]Jason T. Magnuson, [1]Marie E. DeLorenzo

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants originating from many everyday products. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are two PFAS that are particularly pervasive in aquatic environments. Both chemicals are toxic to fish and have complex and largely uncharacterized mixture effects. However, limited information is available on saltwater species. In this study, embryonic and larval sheepshead minnows (*Cyprinodon variegatus*) were exposed to several PFAS mixtures to assess lethal and sublethal effects. PFOS alone was acutely toxic to larvae, with a 96 h LC_{50} of 1.97 mg/L (1.64–2.16). PFOS + PFOA resulted in a larval LC_{50} of 3.10 (2.62–3.79) mg/L, suggesting an antagonistic effect. These observations were supported by significant reductions in malondialdehyde (105% ± 3.25) and increases in reduced glutathione concentrations (43.8% ± 1.78) in PFOS + PFOA exposures compared to PFOS-only treatments, indicating reduced oxidative stress. While PFOA reduced PFOS-induced mortality (97.0% ± 3.03), perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA) did not. PFOS alone did not affect expression of peroxisome proliferator-activated receptor alpha (pparα) but significantly upregulated apolipoprotein A4 (apoa4) (112.4% ± 17.8), a downstream product of pparα, while none of the other PFAS affected apoa4 expression. These findings suggest that there are antagonistic interactions between PFOA and PFOS that may reduce mixture toxicity in larval sheepshead minnows through reduced oxidative stress.

Key words: PFAS; PFOS; PFOA; mixtures; mechanism of toxicity; PPARα; oxidative stress

Type of Presentation: Oral

Sessions in order of preference:

1. Mixture effects of Pollutants
2. Biotransformation pathways and mode of action (MoA) of chemical pollutants
3. AOP, Systems Biology approaches and other conceptual modeling tools

Impaired metabolism of a common isothiazolinone biocide in the presence of a the CYP inhibitor clotrimazole in a fish liver cell line (PLHC-1)

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Isothiazolinones (ITs) are widely and increasingly used as antimicrobial biocides. In 2015, EU lowered the limit values for methylated ITs due to increased cases of allergic dermatitis. Unfortunately, this has led to a shift to the use of more lipophilic IT derivatives that are allowed at 20 times higher levels. However, these lipophilic ITs are less investigated and suggested to pose an ecotoxicological risk. We studied metabolism of the lipophilic benzisothiazolinone (BIT) and dichloro-octyl-isothiazolinone (DCOIT) biocides which both are increasingly used in numerous consumer products. Fish liver cells (PLHC-1 cell line) were exposed to 0.5-10 µM BIT and/or DCOIT and cell culture media were sampled after 1 to 72h. Concentrations of BIT and DCOIT were analyzed in the media using HPLC-UV. There was a time-dependent decrease of BIT concentrations in the cell culture media, whereas levels of BIT remained stable over time in the cell-free media. The concentration of BIT in the media was lowered in the presence of DCOIT. A more polar BIT-metabolite was detected in the cell culture media, but not in cell-free media. Levels of the BIT-metabolite increased with time parallel with decreased levels of BIT, indicating hepatic metabolism of BIT. Addition of the antimycotic drug and broad CYP enzyme inhibitor clotrimazole reduced the amount of metabolite and stabilized the concentrations of BIT in the media. The specific CYP1A inhibitor α-naphthoflavone had no effect on formation of the BIT-metabolite. This suggests that BIT is metabolized in these cells, presumably by CYP enzymes though not by CYP1A isozymes.

Key words
mixture effects, benzisothiazolinone, dichloro-octyl-isothiazolinone, biotransformation, cytochrome P450

Type of presentation
Platform

Session

1. Mixture Effects of Pollutants
2. Biotransformation Pathways and Mode of Action (MoA) of Chemical Pollutants

An *in vitro* approach to assess the cellular effects of several therapeutic classes of pharmaceuticals as single compounds and mixtures

[1]Panni, M., [1]Mezzelani, M., [2]Vitale, M., [1]d'Errico, G., [1]Gorbi, S., [1]Regoli, F., and [2]Pampanin, D.M.

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Pharmaceuticals are contaminants of emerging concern due to their biological activity and ubiquitous detection in environmental matrices. Wild organisms are continuously exposed to cocktails of these substances, that can interact producing unexpected effects in terms of toxicity or metabolic pathway changes. Since these interactions can lead to changes at the cellular level, *in vitro* assays are regarded as a suitable and quick screening method in their toxicity assessment. The aim of this work was to characterize the ecotoxicological potential of environmental concentrations (1-10 μg/L) of 15 pharmaceuticals and their mixtures using fish hepatic (PLHC-1) and gill (RTgill-W1) cell lines, measuring cell viability, production of reactive oxygen species and changes in mitochondrial membrane potential. Obtained results revealed a major sensitivity of PLHC-1 cell line, revealing the key role of liver tissue in the drugs-metabolization processes. Non-Steroidal Anti-Inflammatory drugs (NSAIDs) were shown as the less reactive therapeutic class. Paroxetine, Naproxen and Atenolol were the most toxic molecules for psychiatric drugs, NSAIDs and cardiovascular drugs, respectively. Single pharmaceuticals showed greater effects than their mixtures, in which the toxicity was not related to the exposure dose or the number of combined molecules, indicating the presence of some antagonistic mechanisms in the tested conditions. The findings provide novel insights into pharmaceuticals mechanisms of toxicity, which can be used to improve future environmental risk assessments in marine ecosystems. *(1600/1600 characters including spaces).*

Key words
Pharmaceutical contaminants, *in vitro* integrated approach, mechanisms of toxicity, oxidative stress, cytotoxicity.

Type of presentation
Platform

Session
1. New approach methodologies (NAMS) to assess pollutants toxicity.
2. Biotransformation pathways and mode of action (MOA) of chemical pollutants

SCIENTIFIC PROGRAM OF PRIMO 22

Mixture effects of pollutants

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Biochemical and cellular alterations of a mixture of glyphosate and aminomethylphosphonic acid in mussels *Mytilus galloprovincialis*

Vivani, V., Nardi, A., Mezzelani, M., d'Errico, G., Pittura, L., Benedetti, M., Gorbi, S., Regoli, F.

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Glyphosate (GLY) is among the most widely used non-selective systemic herbicides globally, detected in all environmental matrices along with its main breakdown product aminomethylphosphonic acid (AMPA). Given the wide use and long persistence, these molecules have raised concern for environmental and human health. In this study, the biological effects of environmentally realistic concentrations of GLY (0.5 μg/L) and AMPA (0.5 μg/L), alone and in mixture, were evaluated in mussels *Mytilus galloprovincialis*. After 28 days of exposure and 14 days of recovery phase, organisms were collected to analyze a wide panel of putative biochemical and cellular targets of tested compounds, including neuro-immune system alterations, antioxidant defenses, oxidative damages, lipid and xenobiotics metabolism. At the end of the exposure, both GLY and AMPA caused alterations of the immune parameters, as well as of xenobiotic biotransformation system and lipid metabolism, with slightly harsher outcomes caused by AMPA compared to GLY, and slight interactive effects in organisms co-exposed to the two compounds. After the recovery period, certain alterations persisted, and the delayed onset of cholinergic alterations and oxidative stress was observed, suggesting both a limited capability to recover from the exposure and non-synchronous biological responsiveness to GLY, AMPA and their mixture. Overall, results provided evidence of the effects and mechanisms of interaction of environmentally realistic concentrations of GLY and AMPA, with implications for their environmental risk assessment.

Key words
Herbicides, Glyphosate, Aminomethylphosphonic acid, Mussels, Biomarkers

Type of presentation
Poster

Session

1. Mixture effects of pollutants
2. Biotransformation pathways and mode of action (MOA) of chemical pollutants

Ecotoxicological impacts of metals in single and co-exposure on mussels: Comparison of observable and predicted results

[1]Rosa Freitas, [2]Tiago Morais, [2]Pedro Moleiro, [1]Carla Leite, [1]Francesca Coppola, [3]João Pinto, [3]Bruno Henriques, [1]Amadeu M.V.M. Soares, [3]Eduarda Pereira

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Used in high-tech and everyday products, mercury (Hg), cobalt (Co), and nickel (Ni) are known to be persistent and potentially toxic elements that pose a serious threat to the most vulnerable ecosystems. Despite being on the Priority Hazardous Substances List, existing studies have only assessed the individual toxicity of Co, Ni and Hg in aquatic organisms, with a focus on the latter, ignoring potential synergistic effects that may occur in real-world contamination scenarios. The present study evaluated the responses of the mussel *Mytilus galloprovincialis*, recognized as a good bioindicator of pollution, after exposure to Hg (25 µg/L), Co (200 µg/L) and Ni (200 µg/L) individually, and to the mixture of the three metals at the same concentration. The exposure lasted 28 days at 17 ± 1 °C, after which metal accumulation and a set of biomarkers related to organisms' metabolic capacity and oxidative status were measured. The results showed that the mussels could accumulate metals in both single- and co-exposure conditions (bioconcentration factors between 115 and 808) and that exposure to metals induced the activation of antioxidant enzymes. Although Hg concentration in organisms in the mixture decreased significantly compared to single exposure (9.4 ± 0.8 vs 21 ± 0.7 mg/kg), the negative effects increased in the mixture of the three elements, resulting in depletion of energy reserves, activation of antioxidants and detoxification enzymes, and cellular damage, with a hormesis response pattern. This study underscores the importance of risk assessment studies that include the effects of the combination of pollutants and demonstrates the limitations of applying models to predict metal mixture toxicity, especially when a hormesis response is given by the organisms.

Key words
Mixture of metals, Bivalves, Metabolism, Oxidative stress, Independent action model.

Type of presentation
poster

Session
Mixture effects of pollutants.

Effects of environmentally relevant concentrations of diclofenac, ciprofloxacin and their binary mixture on *Mytilus edulis* sperm function and fertilization

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Active pharmaceutical ingredients have become increasingly significant environmental contaminants, representing a new challenge for non-target aquatic organisms, and laboratory studies are necessary to fill knowledge gaps. The present research aimed to investigate the effects of diclofenac (DIC), a non-steroidal anti-inflammatory compound, ciprofloxacin (CIP), a broad-spectrum antimicrobial, and their binary mixture at environmentally relevant concentrations (5, 50, 500, 1000 and 10000 ng/L) on the sperm function and fertilization success of *Mytilus edulis*.

To better understand the potential negative effects both *in vitro* and *in vivo* experiments were conducted. Male gametes from *in vitro* and *in vivo* exposures were analyzed for cell viability, intact mitochondrial membrane potential (MMP) and production of reactive oxygen species (ROS). Fertilization assays were performed in mussels exposed *in vivo* for 10 days, and fertilization success, mortality and spawning were recorded.

In vitro exposure to both single compounds (500, 1000 and 10000 ng/L) and binary mixture (500 ng/L) significantly decreased mussel sperm viability and MMP intactness. However, the percentage of ROS remained unchanged. The 10 days *in vivo* exposure resulted in a significant increase in sperm ROS production, accompanied by a decrease in viability and MMP, ultimately decreasing the fertilization success. Results show that CIP and DIC individually, and as a mixture, can impact male gamete functionality and fertilization in marine invertebrates at concentration levels common for their habitat.

Key words

Diclofenac, ciprofloxacin, spermatozoa, fecundity, binary mixture, invertebrates.

Type of presentation

Platform

Session

1. Mixture effects of pollutants
2. EDCs and neuroendocrine effects
3. Chemical exposome and non-target screening approaches

Glutathione-S-transferase determination in *Mysidopsis juniae* exposed of crude oil

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The study assessed the toxicity of petroleum on the marine microcrustacean *Mysidopsis juniae*, as well as the potential use of this species as a test organism in detecting the effects of acute exposure to petroleum hydrocarbons. The mysids were exposed for 96 hours to six different concentrations (i.e., 50, 25, 12.5, 6.25, 3.125, and 1.563%) of the Water-soluble Fraction (WSF) of crude oil - obtained following a 1:9 ratio (oil: natural seawater) (ABNT NBR15469, 2021). Two experiments with the same experimental design (6 concentrations and 3 replicates per concentration) were conducted simultaneously. In the first experiment, 10 juvenile organisms per replicate (aged between 4 and 5 days) were exposed to evaluate mortality, and in the second, 80 adults per replicate (aged 20 days) were exposed to determine protein activity and evaluate the activity of glutathione S-transferase (GST) using the method described by Habig and Jakoby (1981). Samples of the test water were analyzed for the quantification of PAH and THP in solution. The LC50 found in the juvenile assay was 32.99% (28.53 – 38.14%). GST activity levels increased with the increasing concentration of petroleum in the water, indicating an apparent correlation. The results of the chemical analyses of the water reflected the variations in concentration used.

Key words
Mysidopsis juniae; hydrocarbon; GST; Oil.

Type of presentation
Poster.

Session
Mixture effects of pollutants; Chemical exposome and non-target screening approaches.

SCIENTIFIC PROGRAM OF PRIMO 22

Particles, fibres, plastics and their additives

(Oral talks)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

The Mediterranean mussel *Mytilus galloprovincialis* as a potential bioindicator of microplastic pollution

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Microplastics are abundant and ubiquitous, representing a threat to all marine species. Biases in current methods of microplastics quantification in aquatic environments lead to a lack of reliable data on small microplastics (<25µm) concentrations. Considering the awareness that monitoring microplastic pollution is essential to predict ecological and sanitary risks, new measuring tools as bioindicators are needed.

Mussels are already used as bioindicators for other pollutants and, since they are non-specific suspension feeders filtering large volumes of water, they appear to be good candidates as microplastics bioindicators. For this purpose, we investigated the potential of microplastics bioaccumulation concomitantly with the maintenance of a good health status of the Mediterranean mussel *Mytilus galloprovincialis* exposed to polyethylene microplastics.

An acute 48h-exposure to various increasing concentrations of microplastics (between 100 and 2000 MPs/L) reveals that the number of particles accumulated in mussels is proportional to the water concentration, suggesting that mussels allow a direct assessment of environmental microplastic pollution. A chronical exposure during 100 days of mussels to 200 particles/L did not show any effects on condition index, total lipid content and shell growth, whereas at a concentration of 2000 particles/L the shell growth was reduced and the valve activity modified.

These results support the choice of mussels for monitoring environmental marine microplastic pollution but their use as bioindicator might be limited at higher concentrations.

Key words

Plastic, pollution, biomonitoring, sentinel organisms

Type of presentation

Oral

Session

Particles, fibres, plastics and their additives

Aquaculture environment interactions

Biomonitoring and development of integrative assessment approaches

Analysis of rubber-derived contaminants in surface water by liquid chromatography hybrid linear ion-trap-Orbitrap high-resolution mass spectrometry

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Rubber-derived organic contaminants are of emerging environmental concern because of their toxicity for aquatic organisms. Their occurrence and fate in the aquatic environment are largely unknown, in part due to the limited analytical methods available. The objective of this study is to develop a method to analyze eight paraphenylenediamine (PPDs), five PPD-Quinone, and seven amine and urea-type rubber additives in the dissolved phase and suspended particulate matter (SPM) of riverine and estuarine surface waters. This method extracted the dissolved phase using solid phase extraction (SPE) by hydrophilic-lipophilic balance cartridges. SPM was extracted using ultrasonic-assisted extraction followed by SPE cleanup. The separation and quantification were achieved by high-performance liquid chromatography (HPLC) reversed-phase C18 coupled with positive electrospray ionization (+ESI) linear ion trap (LTQ) Orbitrap high-resolution mass spectrometry detection. Linearity for all compounds throughout the 12-point calibration range was >0.99. The instrument detection limit was from 0.1 to 12 ng/mL. The absolute recovery range was 70-90 % in environmental water. The surface water samples collected from the Rimouski River and the St. Lawrence River and Estuary (Canada) were used to determine the limits of detection and quantification and validate the method. To our knowledge, this is the first research to use an LTQ-Orbitrap to simultaneously analyze these 20 target contaminants in the dissolved phase or the SPM of riverine and estuarine surface waters.

Key words

Tire additives; PPDs; PPD-Qs; surface water

Type of presentation

Platform

Session

1- Particles, fibres, plastics and their additives
2- New tools to track pollutant sources and transfers

Microplastics and Plasticizers in Estonian Wastewater Treatment Plants

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Wastewater treatment plants (WWTPs) are important pathways of microplastics (MPs) and plastic additives incl plasticizers (PLZs), into the environment. Until recently, phthalates were dominant PLZs but due to increasingly restricted use, alternative PLZs have emerged as contaminants. To date, the extent of MPs and PLZs pollution load from Estonian WWTPs is not known. To address this knowledge gap, six WWTPs in Estonia were selected for MPs analysis in each wastewater treatment stage and PLZs in the sludge. Composite 24 h samples were collected in raw influent, before biological step, and effluent, using an automated sampler with a double-layered sieve system. Upon Fenton-H_2O_2 digestion of organic matter, ≥ 300 µm MPs were quantified in stereomicroscope and categorized based on size, shape and color. Polymer types were identified using µFTIR. For PLZs, raw and dewatered sludge was sampled and upon lyophilization and sieving, analyzed for PLZs by GC-MS. MPs were detected in all the studied samples, with the highest concentrations in the raw influent and the lowest in the effluent. Fibers, fragments, films were recorded in the influent whereas in the effluent, fibers dominated. Fragments and films were mainly composed of PP and PE, while fibers had more diverse compositions incl PP, PET, and PAN. Selected PLZs (DEHT, DPHP, DINCH) showed comparable levels to those of restricted DEHP. This research establishes baseline levels for MPs and novel PLZs in Estonian WWTPs that is essential for future regulatory and research endeavors.

Keywords: plastic pollution, additives, phthalates, wastewater sludge, effluent

Abbreviations: DEHT - bis(2-ethylhexyl)terephthalate; DEHP - Bis(2-ethylhexyl)phthalate; DINCH - 1,2-cyclohexanedicarboxylic acid disonyl ester; DPHP - di(2-propylheptyl)phthalate; GC-MS - PAN – polyacrylonitrile; PE - polyethylene; PET - polyethylene terephthalate; PP - polypropylene (PP)

Type of presentation
Oral.

Session
Particles, Fibers, Plastics and their Additives

Acknowledgements: This research was funded by Estonian Research Council grant PRG1427. The wastewater treatment plants leaders' willingness to cooperate is highly appreciated. The sampling for plasticizers was performed by Dr. J. Reinik. The authors wish to thank R. Nagorka and J. Koschorreck from German Environment Agency for advising on plasticizer GC-MS protocol optimization. A. Y. Ayankunle is employed by AS Emajõe Veevärk and acknowledges the administrative and technical support of the staff and colleagues from AS Emajõe Veevärk.

The neglected impact of sedimentable metallurgical atmospheric particulate matter on coastal zones: Bioaccumulation and effects on mangrove crabs

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Micro and nanoparticles of metals are emitted into the atmosphere through metallurgical activities. When these activities are located in coastal zones, estuarine and marine ecosystems can be contaminated by settleable atmospheric particulate matter (SePM). On the Brazilian Southeast Coast (Tubarão Metallurgical Complex – Espírito Santo), this pollutant contains fractions formed by an agglomeration of metallic nanoparticles, which present non-essential metals and rare earth elements. Our study assumes the hypothesis that coastal organisms experience bioaccumulation and effects as a result of exposure to metallurgical SePM. To verify this hypothesis, mangrove crabs (*Ucides cordatus*) were exposed to environmentally relevant concentrations of SePM for 30 days. Several tissues were evaluated for bioaccumulation of metals and sublethal effects (oxidative stress, cyto, geno and neurotoxicity). It was observed bioaccumulation of Ce, Y, La, Al, Ni, Zr, and Nb in the gills. The hepatopancreas accumulated Ce, Y, La, Cd, Ti, and Zr, while the muscle accumulated Sr, Ag, and Ba. Oxidative stress was observed in the gills. Genotoxicity was observed in the hemolymph, while neurotoxicity was observed after 15 and 30 days in the hemolymph and muscle, respectively. The stability of the lysosomal membrane pointed out severe stress after 30 days of exposure. This study sheds light on the consequences of marine pollution due to atmospheric particulate matter originating from coastal metallurgical activities, a topic still underexplored by environmental assessments and regulations.

Key words
Metallic particles, rare earth elements, sublethal effects, tropical zones, *Ucides cordatus*.

Type of presentation
Platform

Session
Particles, Fibers, Plastics, and their Additives.

Investigating the impact of nanoplastic pollution in commercially important fish under laboratory conditions

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To investigate the impact of nanoplastics (NPs) on marine species, particularly those of economic interest, is of utmost importance to determine the repercussions that pollution by these emergent contaminants may have on the aquaculture sector.
Automated analysers offer the opportunity to extract data from samples in a more cost-effective and efficient manner, but these must be validated first. The present study focused on validating automated systems for both haematological analyses and biochemical profiling of plasma in fish, and to further investigate the effects of a chronic exposure to polystyrene nanoplastics (PSNPs) in commercially important fish. To this end, automated haematological and plasma biochemical analysers were validated with rainbow trout (*Oncorhynchus mykiss*) and goldfish (*Carassius auratus*), respectively used as model organisms. Subsequently, juvenile gilthead seabream (*Sparus aurata*) were challenged with a 28-day waterborne exposure to PSNPs under three distinct experimental conditions: control (0mg/L); low concentration (100µg/L) and high concentration (1000µg/L). The samples collected included blood for haematology and plasma biochemistry, organs for histopathological analyses, and video recordings for behavioural analyses, amongst others. PSNPS did not appear to have significant effects on the histology nor on the haematological profile, but slight behavioural changes were observed. Further research is needed to fully understand the effects of NPs on *S. aurata* health and on the respective aquaculture sector.

Key words
Polystyrene nanoplastics, aquaculture, automated methods validation.

Type of presentation
Platform (Oral presentation)

Session
Particles, fibres, plastics, and their additives

Sympathy for the Plastic: Leachates Fueling *Oxyrrhis marina* Growth

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Marine protozooplankton (e.g., heterotrophic dinoflagellates, ciliates) are key components of aquatic food webs as major phytoplankton grazers and trophic upgraders, but their responses to plastic pollution remain underexplored. The potential toxicity of leachates from conventional and bioplastics to marine life has raised concerns, with evidence linking microplastic toxicity to additives. This study investigated the effects of leachates from two types of micronized plastics, a biodegradable commercial plastic bag and beach-collected microplastics, on the marine heterotrophic dinoflagellate *Oxyrrhis marina*. Aliquots of an *O. marina* culture were exposed to different leachate concentrations (equivalent to 0.03-1 g L^{-1}) from micronized plastics (< 250 μm) and to 0.1 μm filtered seawater (control). *O. marina* was exposed to the leachates for 72 hours in the dark and in the absence of food. Following exposure, a significant increase in the concentration of *O. marina* cells was observed with increasing leachate concentration, reaching up to three times higher than the control. The average concentration of leached dissolved organic carbon (DOC) was 391 μM for weathered plastics and 2799 μM for bioplastics, 2 and 3 orders of magnitude, respectively, higher than in the control. The observed growth stimulation in *O. marina* showed a correlation with the concentration of leached DOC. The mechanisms underlying this previously undescribed effect will be discussed, with a focus on the role of DOC leached from microplastics, bacterial stimulation, and their interactions with *O. marina* and its trophic modes. This discovery underscores the critical need for further research on the effects of plastic leachates on protozoans and the microbial loop to better evaluate the global implications of plastic pollution on the functioning of marine ecosystems.

Key words
Microplastics leachate; Plastic alternatives; Heterotrophic dinoflagellate; Plastic additives

Type of presentation
Platform presentation

Session
PARTICLES, FIBRES, PLASTICS AND THEIR ADDITIVES

Currents of Contamination: Navigating the Microbial Seascape of Kuroshio's Microplastic Plastisphere

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Abstract

The surge in oceanic plastic waste is expanding the habitat for microorganisms, with taxonomically distinct microbial communities known as the 'Plastisphere' thriving on plastic materials. This study investigates the microbial populations associated with floating microplastics in the vulnerable Kuroshio circulation of the North-eastern Pacific Ocean, highlighting the intricate relationships between plastics and marine microorganisms. High-throughput sequencing (Oxford Nanopore sequencing) of 16S rRNA was employed to analyze microbiome profiles of microplastic assemblages sampled from the Kuroshio current. Distinct microbial communities exclusive to plastic surfaces were identified, emphasizing the unique nature of the plastisphere. Alpha diversity analysis revealed a significant difference in diversity between surface seawater and plastisphere communities across all stations. Notably, alpha diversity indices were consistently higher on microplastics compared to surface seawater communities ($p < 0.05$). Using the FAPROTAX database, prokaryotic metabolic functions based on 16S rRNA gene data were analysed. The results showed that chemoheterotrophy and aerobic chemoheterotrophy dominate in wood and seawater, while phototrophy-related functions prevail in plastics. STAMP analysis further uncovered distinct metabolic profiles between seawater, microplastics, and wood, underscoring the influence of substrate type on bacterial functions. This comprehensive study provides insights into the dynamic microbial communities across diverse environments, particularly in the context of plastic pollution. The findings contribute to our understanding of ecosystem dynamics and have implications for effective management strategies in marine ecosystems impacted by plastic waste.

Keywords
Plastisphere, Microplastic, Kuroshio current, Nanopore sequencing.

Organophosphate ester plastic additives and microbial communities in marine sediments: a complex relationship

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Organophosphate esters (OPEs) flame retardants and plasticizers are among the most used organic plastic additives at global scale. These chemicals can effectively accumulate in marine sediments. However, the way OPEs can interact with naturally occurring microbial communities in marine sediments is poorly understood. These interactions can determine their environmental persistency as well as their potential impacts on microbial communities. An integrated chemical-microbiological approach was applied to study this issue in an impacted sediment (NW Mediterranean). Sediments were spiked with an OPE mix to achieve environmental relevant concentrations and incubated in dark at 13°C during one month. Two conditions (i.e. abiotic, biotic) were tested. OPE were quantified by GC/MS after ultrasound extraction and clean-up, and total sedimentary DNA was extracted in each treatment to quantify the number of bacterial 16S rRNA genes (proxy of bacterial abundance) and to characterize the structure and composition of the bacterial community (16S metabarcoding MiSeq Illumina). A significant decrease of half-lives was verified for most OPEs under biotic conditions, highlighting the relevant role of microbial assemblages enhancing OPE degradation. In addition, OPE contamination induced a decrease on the diversity of the bacterial community in the coastal sediment, noticeable after 14 days of incubation. It is likely that on one side the contamination had favored the growth of some bacterial groups maybe involved in the biodegradation of these compounds but, on the other side, had also impacted some sensitive bacteria.

Key words
Contaminants of emerging concern, flame retardants, degradation, biodiversity, toxicity

Type of presentation
Platform

Session
(1) Particles, fibres, plastics and their additives
(2) Microorganisms as target and vector for chemical pollutants

Unraveling the Impacts of Microplastics and their Additives in Marine Ecosystems

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Microplastics (MP) and their chemical additives are complex yet poorly understood hazards to marine ecosystems. Most toxicity assessments of MP rely on pristine reference materials that don't represent environmental particles from everyday consumer products, leading to gaps in understanding their impact. In this study, we unravel the role of MP as a pollutant and a carrier of chemical additives and thus distinguish long-term effects derived from MP versus those from additives in marine species. Fifty plastic products with different polymer and additive chemical content were screened using non-target chemical analysis and baseline toxicity tests (Bacterial Luminescence and Algal Growth). Results showed varied toxicity, with higher chemical content correlating with higher toxicity, particularly in elastomer-based products. Comprehensive toxicity testing of leachates from 5 products with high (car tire rubber (CTR), balloons, washing gloves, shoe soles) or low (PET bottle) baseline toxicity revealed significant impacts on organisms across different life stages and trophic levels, namely microalgae, copepods, oysters, mussels, sea urchins, polychaetes and fish. Investigations using cod larvae and adult mussels showed that the additive chemicals in CTR, rather than the particles alone, drove significant adverse effects on survival, hatching success, neurotoxicity and oxidative stress. This research emphasizes the critical need for mechanistic assessments to distinguish toxicity drivers between MP particles and leachates, especially at different levels of biological organization.

Acknowledgments
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Key words
Microplastics, consumer products, chemical additives, marine organisms.

Type of presentation
Platform presentation.

Session
Particles, fibres, plastics and their additives.

Phototransformation of Microplastics Derived from Sunscreen and Their Mixture Effect with ZnO Nanoparticles

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The environmental impact of sunscreen is a growing concern, yet the combined effects of its components on marine animals are poorly understood. As two main particulate ingredients, ZnO nanoparticles (*n*ZnO) and microplastics (MPs) were isolated from commercial sunscreens based on the "like dissolves like" principle. MPs were further extracted by acidic digestion of *n*ZnO and characterized as spherical particles of approximately 5 µm with irregular layered sheets on surface. Although primary MPs (PMPs) were stable in the presence of simulated sunlight and water after 12 h of exposure, *n*ZnO promoted the photooxidation by producing hydroxyl radicals, promoting surface oxidation of PMPs with a 2.5-fold increase in the carbonyl index. The produced secondary MPs (SMPs) were more soluble in water and fragmented to irregular shapes with sharp edges. The mixture effects of sunscreen extracted *n*ZnO and MPs on the development of barnacle larvae were investigated, focusing on the different roles played by PMPs and SMPs. Co-exposure to PMPs had no significant effect on larval development, whereas SMPs mitigated the impact of *n*ZnO by restricting its in vivo transformation to ionic Zn. Accumulated SMPs reduced gut dissolution of *n*ZnO by up to 40%, lowering gut acidity by 85% and buffering the in vivo dissolution of *n*ZnO. A rough-surfaced Si-fragment in SMPs was considered to decrease gut acidity. Our study explored the activation of MPs by *n*ZnO derived from commercial sunscreens and highlighted the combined effects of sunscreen components on marine organisms.

Key words
Secondary microplastic; sunscreen; mixture effect; zinc oxide; phototransformation.

Type of presentation
Platform.

Session
Particles, fibres, plastics and their additives

Mixture effects of pollutants

Is replacing Bisphenol A with Bisphenol analogues an environmentally friendly decision?

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Bisphenol A (BPA) is one of the most used plasticizers in the industry. Increased regulations have been placed on its production and usage due to human health and environmental concerns. This has led to the production of several bisphenol alternatives (BPs) as a replacement for BPA. Even though BPA is still the major bisphenol found in the environment, BPs are also frequently detected. Data available in literature suggests that BPA alternatives can have similar toxicity to BPA, as for example endocrine disrupting effects, cytotoxicity and genotoxicity.
The Partnership for the Assessment of Risks from Chemicals (PARC) was established in 2022 to develop next-generation chemical risk assessment to help protect human health and the environment. Our aim under PARC was to generate data to fill gaps on poorly characterised BPA alternatives on aquatic organisms. Bisphenol AP (BPAP), Bisphenol Z (Bis-Z) and Bisphenol AF (BPAF) were used in our study. 10-days bioassays with the sediment dwelling *Arenicola marina* were run to obtain behavioural and mortality data. In addition, energetic and oxidative stress biomarkers were investigated to understand sublethal impacts of selected BPs.
Our initial results suggest that acute toxicity of the selected BPA alternatives is similar to that of BPA itself. The results help filing a gap on data for environmental toxicity of BPA alternatives in the marine environment and, in particular, in a model benthic organism key in supporting marine ecosystem functioning.

Key words
Bisphenol A, Bisphenol alternatives, sediment toxicity, Arenicola marina, environment

Type of presentation
Platform presentation

Session
PARTICLES, FIBRES, PLASTICS AND THEIR ADDITIVES

Tyre-driven ecotoxicity: differentiating the effects from particles and leachates to *Mytilus edulis*

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The chemical composition of tyre wear particles and end-of-life tyres (ELT), originating from the additives incorporated during manufacturing, poses significant risks to aquatic biota, as these chemicals can leach into the environment. Despite research on the effects of tyre particles and individual chemical additives on aquatic species, a crucial gap remains regarding their toxicity drivers and pathways. This study aims to address this gap by differentiating the impacts of "pristine" ELT particles (TP, <300µm), leachates (L) and particles after 14 days of leaching (TPL, <300µm) on mussels *Mytilus edulis* after 36 days of exposure at a concentration of 0.1 g/L. Toxicity was assessed by a multi-biomarker approach consisting of condition index, lysosomal membrane stability, lipid peroxidation, acetylcholinesterase activity and antioxidant enzymes. Mussels were also examined for particle and chemical bioaccumulation. L exposure induced neurotoxic effects after 7 and 36 days, the same pattern was observed for TP at days 28 and 36 of exposure. Lipid peroxidation was detected at day 7 after TP exposure, and both TP and TPL caused lysosomal membrane disfunction at days 7 and 28, respectively. Overall, preliminary results indicate that the chemicals are the main toxicity drivers, however, there also seems to be a synergistic effect of chemicals/leachates and the particles. These findings also highlight the complex tyre-related pollution dynamic, emphasizing the need for a thorough understanding of the toxicity pathways of chemicals and particles towards marine species.

Key words
Car tyre particles, car tyre leachates, chemical mixtures, rubber additives, biomarkers

Type of presentation
Platform presentation.

Session
Particles, fibers, plastic and their additives.

Distinct toxicity profiles caused by conventional and biodegradable fishing nets' leachates on the marine bacteria *Aliivibrio fischeri* and the fish *Oryzias latipes*

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Fishing nets (FNs) are a significant source of macroplastic pollution, but their contribution to pollution by micro and nanoplastics (MNPs) and associated additives is poorly understood. To fill this gap, we studied a co-polyethylene-polypropylene trawl net and two trammel nets, one made of polyamide 6 and another of biodegradable polybutylene succinate polybutyrate co-adipate-co-terephthalate (PBS-PBAT). Artificial aging (AA) based on dry UVA&B irradiation (0-6-12-25-60 days) and agitation was carried out to characterize the degradation kinetics and to study the toxicity of FNs leachates. SEM, ATR-FTIR, and MPs quantification (5-1000 µm) were used to assess degradation, and GC/MS and SIM methods to quantify the leached compounds. The ecotoxicity of the leachates was investigated using the marine bacteria *A. fischeri* and the Japanese Medaka's larvae *O. latipes* with heart rate and behavior endpoints. AA caused a slight degradation but a non-negligible production of MPs. FN leachates contained hazardous additives known as neurotoxic and endocrine disruptors. However, only the PBS-PBAT leachates exert significant toxicity to *A. fischeri*. In contrast, exposure to *O. latipes* caused a UV irradiation time-, FNs' leachates- and endpoint-dependent sublethal toxicity. Results demonstrate that leachates produced by the aging of FNs can lead to variable ecotoxicity according to FNs composition, UV irradiation time, and exposed organisms. Although PBS-PBAT seems promising to mitigate the impact of derelict and end-of-life FNs, the substantial toxicity of their leachates is highlighted.

Key words
Fishing gears, micro and nanoplastics, additives, degradation, toxicity.

Type of presentation
Platform.

Session
1. Particles, fibers, plastics, and their additives
2. Mixture effects of pollutants

Are commercial synthetic biopolymers more toxic than conventional plastics? A plankton community mesocosm experiment

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Synthetic biopolymers—in a broad sense called "bioplastics" —have emerged as sustainable alternatives to conventional petroleum-derived plastics. However, little is still known about the potential impacts of bioplastics on marine food webs. In this study, we investigated the effects of two commercial biopolymers, polylactic acid (PLA) and poly hydroxybutyrate-co-hydroxyvalerate (PHBv), and a conventional plastic, polypropylene (PP), on the structure and dynamics of a Baltic Sea planktonic community. Specifically, we used a mesocosm experimental approach ($2m^3$ tanks of 5m depth) where a planktonic community was exposed to 200 mg L^{-1} of the different micronized (< 250 µm) materials for 3 weeks. The abundance and composition of phytoplankton and zooplankton and the chlorophyll (Chl) concentration were determined in the different mesocosm treatments (control, PP, PLA, PHBv) twice a week throughout the experiment. Both synthetic biopolymers were more toxic than PP to the studied plankton community and their toxicity increased with the exposure time. The total microphytoplankton abundance was up to 2.1. and 3.2 times lower after exposure to PLA and PHBv than to PP respectively. Among phytoplankton, diatoms were the most sensitive group to the studied synthetic biopolymers. The Chl concentration in PLA and PHBv treatments was reduced by half compared to the PP treatment. Zooplankton abundance was up to 1.5 times lower in the treatment with synthetic biopolymers than in the one with PP by the end of the exposure. Overall, in most cases, PP caused a slight effect on plankton compared to the synthetic biopolymers, and PHBv was generally more toxic than PLA. Harmful chemicals were identified in leachates from PLA and PHBv (e.g., 2,4-di tert-butyl-6-methylphenol in the PHBv mesocosms), indicating that functional additives introduced during the manufacturing of these synthetic polymers had an impact on the observed toxicity. Although more research is needed to understand the ecological impacts of bioplastic pollution, the toxicity of the studied bioplastics raises concerns about the chemical safety of these alternative materials and calls for more research to find less toxic plastic additives to allow environmentally safer replacements for conventional plastics.

Keywords
Synthetic biopolymers, microplastics, mesocosm, plankton community, additives

Type of presentation
Platform.

Session
Particles, Fibres, Plastics, and their additives

Alternative “bio” plastics do not improve chemical safety compared to conventional ones

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A comparative ecotoxicological profile of the same plastic items made of conventional non biodegradable plastics vs alternative polymers, frequently commercially advertised with the prefix “bio” and claiming a lower ecological impact, has been conducted using aquatic marine models. Plastic knifes of two different colors, vegetables nets and small trash sacs were purchased in local markets. For each object, a brand using conventional (PE, PS) polymer and an alternative (“bio”, compostable, etc) polymer was purchased. Plastics were grinded in an ultracentrifuge mill, analysed by ATR-FTIR to identify main polymeric matrix, extracted and analysed by GC-MS for additives, and tested for ecotoxicity with the sea-urchin embryo test according to standard methods.

Alternative materials consistently showed a slightly higher aquatic ecotoxicity than conventional ones. Chemical analyses identified a longer list of intentionally or unintentionally added chemicals in the PLA knifes than in the PS ones. Chemicals identified in the “bio” materials and not in the homologous conventional ones include dibutylphthalate (in the knifes), heptadecanenitrile (in the net), o-toluidine and behenylchloride (in the trash sac).

	Conventional				Alternative			
Object	Pink knife	Green knife	net	Trash sac	Pink knife	Green knife	net	Trash sac
Sample Code	ID-051	ID-053	ID-096	ID-087	ID-052	ID-054	ID-079	ID-069
Description	PS	PS	PE	PE	PLA	PLA	PBAT	PBAT
SET TU	<1	<1	<1	<1	1.14	1.46	2.06	2.67
Chemicals identified by GC-MS	N=12	N=13	In progress	N=23	N=19	N=22	N=13	N=13

Therefore, alternative plastics may contribute to reduce the environmental persistence of plastic objects but they clearly do not improve their ecotoxicological impact or chemical safety.

Key words
Biodegradable plastic, compostable plastic, polyethylene, ecotoxicology, chemical additives.

Type of presentation
Platform.

Session
t05: Particles, fibers, plastics and their additives.

Impact of leachates from compostable and non-degradable plastic bags on the transcriptome of the marine fish model *Cyprinodon variegatus*

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New commercial plastic products, as compostable bags, reach the environment due to improper waste disposal. Plastic additives, that can leach into water, and the new properties of these potentially biodegradable materials may lead to unknown toxic effects. To evaluate this, males of the marine fish *Cyprinodon variegatus* were exposed to 1 $g \cdot L^{-1}$ leachates of a compostable bag and a non-degradable polyethylene bag (LDPE). Exposure to two reference chemicals, the estrogenic endocrine disruption (EED) ethinylestradiol (EE2, 100 ng L^{-1}), and the antiandrogenic plastic additive Di(2-ethylhexyl) phthalate (DEHP, 5 µg L^{-1}), were also performed. RNA sequencing (RNAseq) was conducted on fish liver tissue.

RNAseq quantified 3148 transcripts, 987 of which were affected by compostable bag leachate and 480 by LDPE leachate. The compostable bag affected genes related to nervous system, immune system, lipid biosynthesis, and epithelium. LDPE induced changes in nervous system and development. These results differed from EED since EE2 alterations were related to the estrogen receptor activation and protein synthesis. DEHP affected 607 transcripts related to apoptosis, amino acid transport, development, energy metabolism and immune system. DEHP was detected in the tested compostable and LDPE bags at concentrations of 28.4 µg g^{-1} and 16.1 µg g^{-1} respectively.

In conclusion, compostable bag affect twice as many transcripts as LDPE, both affecting genes related to the nervous system. DEHP was present in both bags, and its exposure induced extensive alterations in *C. variegatus* transcriptome.

Key words
Compostable bag

Phthalate

Cyprinodon variegatus

Transcriptomic

Marine fish model

Type of presentation
Platform

Session
-t05: Particles, fibres, plastics and their additives

-t10: Biotransformation pathways and mode of action (MoA) of chemical pollutants

-t09: AOP, System Biology approaches and other conceptual modeling tools

Hemolymph microbial composition of the *Mytilus galloprovincialis* after exposure to microfibers

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Plastic microfibers (MF) of polyethylene terephthalate (PET) belonging to the polyester family are among dominant synthetic fibers in the ocean, and their size and shape make them readily available for filtering marine bivalves. In the present work, MF were obtained by cryo-milling of a pink fleece cover that mimic textile washing. *In vivo* exposure of *Mytilus galloprovincialis* were performed for 96h using two MF concentrations 10 and 100 µg/L, equivalent to 150 and 1500 MF/mussel/L, respectively, to investigate their possible effects on hemolymph immune defenses and microbial composition.

The results showed that mussels were affected by MF, showing induction of immune/inflammatory processes together with accumulation in different tissues. In hemolymph, MF trigger immune response with induction of cytotoxic mechanisms (released by hemocytes), mainly at lower concentrations. These effects were accompanied by shifts in the bacterial composition in hemolymph with respect to the unexposed group. Stronger effects were also observed at lower MF concentration, with decreases in abundances in some genera (e.g. *Vibrio*) and increases in others (e.g. *Sulfitobacter*). Interestingly, half of the top 20 most abundant genera were part of the Rhodobacteraceae family and were increased upon MF exposure.

Overall, the effects of PET MF on mussel hemolymph were more pronounced at lower MF concentrations and seemed to be linked to the degree of the immune response induced in the host rather than to the degree of exposure to MF.

Key words
Mussel, microfibers, immune parameters, microbiota.

Type of presentation
platform

Session
t05: Particles, fibres, plastics and their additives

Distribution and effects of model palladium doped nanoplastics in mussels *Mytilus galloprovincialis*

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While it is anticipated that the concentration of nanoplastics (NPs, <1 µm) in the marine environment will continue to increase, both the analytical tools for quantification and data on their impacts are scarce. This work aimed to expand knowledge on potential bioaccumulation and effects of NPs on mussels *Mytilus galloprovincialis*. Mussels were dietarily exposed to model palladium doped-polystyrene NPs (Pd-PS NPs) for 7 d at $1x10^9$ NPs/mL. Pd levels in water samples (t0, t1, t7) and mussel feces (t1, t3, t7), hemolymph (t7) and soft tissues (t7) were analyzed by ICP-MS. Hemocyte responses, digestive gland (DG) and gonad histopathology and condition index were measured at t7. After 1 d exposure less than 2% of the NPs remained in the water. After 7 d, only between 0.001 and 0.004% of the NPs passed into the hemolymph. Consequently, no differences in hemocyte responses were observed. Approximately 0.5% of the NPs were found in soft tissues. This could be linked to a higher prevalence of digestive tubule atrophy in exposed mussels. No effects were observed at the whole organism. Nearly half of the NPs introduced in the tanks were found in the feces. In conclusion, mussels were able to uptake and then eliminate a large amount of NPs through the feces after 7 d exposure. Whether longer exposures could cause a significant accumulation of NPs and associated biological effects remains to be studied. *Work funded by EC CAS NANOPLASTICS project, Spanish MICINN FIERA project and the Basque Gov (grant to consolidated group and postdoc grant to NGS). DMM was funded through the Swiss NSF.

Key words
Palladium doped polystyrene nanoplastics, dietary exposure, mussels, distribution, hemocytic and histopathological responses.

Type of presentation
Platform presentation

Session
Particles, Fibres, Plastics and their additives

Bioenergetic implications of plastic pollution in a warming ocean: a potential hazard to coastal oyster reefs in the 2100s

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Coastal marine biodiversity is expected to suffer from rising temperatures and plastic waste. Both can disrupt animal bioenergetic systems, threatening their physiological adaptations to coastal habitats. Here we investigated the temperature-dependent metabolic toxicity of nanoplastics in oysters *Crassostrea gigas*. We exposed hemocytes and embryos to 50 nm plain (PS-NPs) and amino-modified (NH2-PS-NPs) polystyrene nanoparticles to 0.1 to 10 mg/L for 24 h under 16-28°C (cells) or 24-30°C (larvae) (2°C steps). Both models indicated that higher temperatures significantly increased cellular metabolism, mitochondrial content, and ROS production, all signs of bioenergetic stress. In cells, PS-NP did not cause mortality or metabolic impairment at any temperature. In contrast, NH2-PS-NPs exacerbated cell mortality with rising temperatures by partially blocking metabolic responses to temperature and increasing oxidative stress. In developing larvae, both PS-NPs and NH2-PS-NPs increased mortality and deformity with temperature. NH2-PS-NPs was one order of magnitude more lethal but did not cause metabolic alterations in D-larvae. However, PS-NPs restricted metabolic responses to temperature and larval growth. Our findings show that plastic pollution limits the bioenergetics responses of early-life stages of bivalves to stress. While more research using environmental nano or microplastics is required, our working hypothesis is that plastic pollution poses a threat to shellfish reefs through energy balance and makes it more challenging for bivalves to adapt to climate change.

Key words
Nanoplastics; bivalves; climate change; mitochondria; ecophysiology.

Type of presentation
Platform.

Session
Please propose one to three sessions in their order of preference.

1 - t05: Particles, fibres, plastics and their additives

2 - t01: Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

Impact and interactions of macro- and microplastic plastispheres with the microbiome of cold-water corals

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Plastic pollution has been identified as a major threat to marine life and ecosystems, but their biological impacts are still largely unknown. Coral reefs, one of the most biodiverse ecosystems on earth which provide essential ecological and economical services, are now recognized to be impacted by plastic pollution from the surface to the deep. Here, we investigated the impact of colonized macro- and microplastics on the microbiome of the most emblematic cold-water coral, *Lophelia pertusa* to help better understand the early biological pathways that may lead to coral physiological alterations. Both plastics induced early (7 days) microbial shifts for *L. pertusa*, that were exacerbated at two months, with specific effects between plastic sizes. We observed a proliferation of opportunistic bacteria, with some may having pathogenic properties. These proliferations can be induced by physical, chemical, and/or biological impacts of both plastispheres (i.e., bacteria associated to plastics).

Our results suggest that shift in coral microbiome was directly due to general dysbiosis from stress inducing proliferation of opportunists allowing specific bacterial transfer between plastisphere and the coral microbiome. These microbial observations highlighted for the first time the impact of the plastisphere on coral microbiome, that lead to biological alterations. Considering the widespray distribution of macroplastics in the ocean that are continuously fragmented into microplastics, our conclusions suggest that plastics could seriously endangered the cold-water corals reefs.

Key words
Cold-water corals, plastisphere, microbiome, dysbiosis, interactions.

Type of presentation
Oral presentation, platform.

Session
t05: Particles, fibres, plastics and their additives.

Filling the gap: improving knowledge of the impact of marine debris on Mediterranean seabirds by combining ingestion of marine debris with loads of phthalates

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Although the Mediterranean has been identified as one of the hotspots for marine litter pollution, the impact on Mediterranean seabirds is underestimated. The analysis of the GIT content of birds has been adopted to measure trends and regional variations in marine debris some monitoring activity. Within the Plastic Busters project, it was developed an approach that goes beyond the mere analysis of the amount of ingested and include analysis of phthalates in different tissue. The aim of this study is to properly assess the impact of marine litter on carcasses of *Calonectris diomedea* (1), *Puffinus yelkouan* (34), *Puffinus mauretanicus* (1) and *Gulosus aristotelis* (2) from Tyrrhenian and Ligurian Sea (Italy). The different sections of the GIT were examined separately according to standardized protocol. A total of 925 pieces of debris were isolated and characterized from 35 of the 38 (92%) birds analysed, with man-made polymers being the predominant category of debris. Polyethylene and polypropylene microplastics in the form of fragments or films were identified as the most common type of debris found in all species. Accumulation patterns and phthalate burdens vary between different species and tissues. Bis(2-ethylhexyl) phthalate (DEHP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP) and di-n-octyl phthalate (DNOP) are the most abundant compounds. The results confirm the importance of seabirds as valuable indicators of marine debris in monitoring programmes and highlight the urgent need for an in-depth assessment of this impact in the Mediterranean basin.

Key words
Seabirds, marine debris, microplastics, platicizer.

Type of presentation
Oral

Session
t05: Particles, fibres, plastics and their additives.

In the clam *Ruditapes decussatus*, are combinations of micro/nanoplastic exposures more harmful than their separate counterparts?

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The increasing amount of micro and nano-sized plastic particles entering the ocean is a cause for concern due to their high level of toxicity, potential interactions, and repercussions. The clam *Ruditapes decussatus* was exposed to polystyrene nanoparticles (50 nm, 10 µg/L), polyethylene microparticles (4-6 µm, 10 µg/L), and their mixtures both *in vitro* (24 h) and *in vivo* (10 d). Using a multi-biomarker approach; clam haemolymph was used to analyse cytotoxicity (*in vitro*), and the gills and digestive glands were utilised to evaluate genotoxicity, neurotoxicity, oxidative stress, and damage, and ingestion. Furthermore, the characterization of these plastic particles was assessed both separately and in combination. The mixture produced larger aggregates than the individual plastic particles. Nanoplastics led to higher levels of cytotoxicity. Nonetheless, there were no indications of genotoxicity in any treatment. Regarding neurotoxicity, a time-dependent rise was noted, and oxidative stress was found to be tissue- and time-dependent. Conversely, the absence of oxidative damage suggests that the antioxidant defence system was successful in preventing the effects of the production of any reactive oxygen species brought on by the exposure. Substantial ingestion rates were seen in all treatments and tissues, suggesting that *R. decussatus* use more than only size to discriminate between various particle types. The digestive glands and the 10th day were most influential on the overall hazardous assessment, and antagonistic interaction between the two plastic particles was calculated.

Keywords: Nanoplastics; microplastics; mixture; *Ruditapes decussatus*

Type of presentation: Oral

Session: particles, fibres, plastics, and their additives; mixture effects of pollutants

POLYETHYLENE MICROPLASTIC-INDUCED HISTOPATHOLOGICAL LESIONS, OXIDATIVE STRESS, AND STRESS RESPONSE GENES OF HYDROTHERMAL VENT CRAB, *Xenograpsus testudinatus*

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ABSTRACT

Hydrothermal vent (HV) crabs inhabit sulfur-rich shallow hydrothermal vents. This present study aimed to investigate the impact of polyethylene microplastic (MPs) on the HV crab organs using histopathological examination, enzymatic activities, and gene expression. Prior investigations have yet to be undertaken to assess the ecotoxicological effects of MPs on HV crabs. Fluorescent green polyethylene microspheres (FGPE) were administered via feeding and immersion (0, 0.3, 0.6, and 1%) to the male (n=168), *Xenograpsus testudinatus*. Histopathology of various organs (gills, hepatopancreas, midgut, and muscles), oxidative stress markers (SOD, GPx, MDA, and CAT), and stress response genes (SOD, GPx, HSP, and CAT) were analyzed after 7 days of the experiment. FTIR was used to confirm the MPs in the HV crabs' fecal matter, hence capable of ingesting MPs. Results demonstrated that FGPE at 1% had severe damage and atrophy in the hepatopancreas and gills of the HV crab in both feeding and immersion. However, FGPE at 0.3 and 0.6% showed mild changes in the tissues of the HV crab. Further, the oxidative stress response of HV crab of SOD, MDA, GPX, and CAT increased ($p<0.05$) at 1% of FGPE. Moreover, FGPE at 1% significantly ($p<0.05$) affects the performance of SOD, GPx, HSP, and CAT genes. Ingesting MPs showed changes in the physiology of the HV crab. Hepatopancreas as energy and toxin storage exhibited extreme changes in histology, enzymatic activities, and stress response genes. This study sheds light on oxidative defense and relative gene expressions in response to ingesting MPs of the unique species inhabiting shallow hydrothermal vents.

Keywords: vent crab, polyethylene microplastic, histology, oxidative stress, RNA,

Distinctive molecular events induced by polystyrene micro- and nano-plastics in zebrafish liver cells

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The presence of micro- and nano-plastics (MNPs) in ecosystems and organisms has raised significant concerns due to their potential ecological and physiological impacts. Despite this, the mechanisms underlying their toxicity remain poorly understood. This study utilized zebrafish liver (ZFL) cells as an in vitro model to investigate the effects of MNPs and the associated key molecular events. After a 24-hour exposure, microplastics (MPs, 1.06 μm) lead to minimal effects on cellular viability, but triggered the production of reactive oxygen species (ROS). This was accompanied by a decrease in specific phospholipids and storage lipids, especially at the highest concentration (135 μg/mL). In contrast, exposure to nanoplastics (NPs, 52 nm) resulted in more significant effects, including reduced cell viability, increased ROS production, and notable changes in the cellular lipid composition, characterized by elevated cholesterol esters, triacylglycerols, and ceramides. Moreover, exposure to MNPs altered the expression of genes related to lipid metabolism, oxidative stress, and energy metabolism pathways in a size- and dose-dependent manner. MPs (2.7-135 μg/mL) primarily downregulated genes involved in lipid and energy metabolism (fasn, scd, mtp, acc, g6pd, agpat4), while NPs (2.6-130 μg/mL) mainly altered genes associated with oxidative stress (nrf2, sod2, cat). This research highlights distinct biological and molecular responses to polystyrene MPs and NPs and emphasizes the importance of considering particle size when assessing the environmental and health implications of MNPs, calling for further research to explore the consequences of long-term exposure.

Key words
Micro and nano plastic, lipidomic, gene expression, size and dose-dependent, zebrafish liver cell

Type of presentation
Oral presentation

Session
PARTICLES, FIBRES, PLASTICS AND THEIR ADDITIVES

SCIENTIFIC PROGRAM OF PRIMO 22

Particles, fibres, plastics and their additives

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Biological effects of petroleum and bio-based microplastics in fish *Gasterosteus aculeatus* and clam *Macoma balthica*

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The use of new biobased polymers as a sustainable alternative to conventional ones, is raising some concern related to virtually unknown effects of these compounds once released in the marine environment.
In this respect, laboratory experiments were carried out to investigate the onset of biological alterations caused by exposure to petroleum-based (polypropylene) and biobased polymers (polylactic acid and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) in the fish *Gasterosteus aculeatus* and in the clam *Macoma balthica,* chosen as bioindicators of marine Baltic ecosystems. Organisms were exposed to micronized plastic particles (<250 µm; 0.2g/L) and, after 7 and 21 days of exposure, a wide panel of biological alterations were investigated at biochemical and cellular level, including cholinergic system, oxidative and lipid metabolism.
Significant variations of all investigated parameters such as total oxyradical scavenging capacity, activities of single antioxidants, acetylcholinesterase and AcylCoA were observed in *G. aculeatus* after one week of exposure to biobased polymers, while no effect was measured in clams. After 21 days only biobased polymers caused a higher level of total oxyradical scavenging capacity toward peroxyl and hydroxyl radicals in fish, and a significant inhibition of acetylcholinesterase and lower total oxyradical scavenging capacity toward hydroxyl radicals in clams. In conclusion, preliminary results highlight a potentially higher toxic effect of biobased polymers compared to traditional ones.

Key words
Microplastics, biobased polymers, biological alterations, biomarkers

Type of presentation
poster

Session
Particles, fibres, plastics, and their additives

Comparative assessment of the toxicity of leachates from beach-collected cigarette butts and microplastics on marine plankton.

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Cigarette butts (CBs) and microplastics (MPs) are globally the most common types of litter found on urban beaches. While MPs have been intensively studied in recent years, the potential effects of CBs on the marine environment have received less attention. In this study, we determined and compared the acute toxicity of leachates from beach-collected cigarette butts and microplastics on marine plankton. CBs and MPs were collected from beaches in Bahia del Confital on the Island of Gran Canaria. We used a similar protocol for obtaining leachates from micronized plastics and similar toxicity tests for both types of debris. Specifically, we assessed the toxicity of leachates (0.0625-1 g L^{-1}) on the microalgae *Rhodomonas salina*, nauplii of the marine copepods *Acartia tonsa* and *Amonardia normanni,* and embryos of the sea urchin *Arbaxia lixula*. Leachates of the field collected MPs showed no toxicity at the used exposure concentration whereas exposure to CB leachates caused negative effects to all the studied organisms. Nauplii of *A. tonsa* were the most sensitive to CB leachates with a median lethal concentration (LC_{50}) of 0.14 g L^{-1} followed by the microalgae *R. salina* with a median effective concentration (EC_{50}) of 0.20 g L^{-1}. The low toxicity of oceanic MPs, collected on a beach of Canary Islands, could be related to the loss of their additives. The chemical composition of materials and their leachates will be discussed. Overall, our results show that leachates from CBs are acutely toxic to key organisms on marine food webs, which calls for more research on the fate and impacts of CBs on coastal ecosystems and mitigation measures.

Key words
Microplastics, cigarette butts, leachates, toxicity, plankton

Type of presentation
Poster

Session
Particles, fibers, plastics, and their additives.

Comparison of the Environmental Toxicity of Micro, Nano, and Leachate Fractions of Three Rubber Materials to *Danio rerio* and *Daphnia magna*

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Rubber materials enter aquatic environments via sources such as playground mulch, athletic fields, and roadway surfaces. Plastics break down into micro (<5 mm) and nano (<1 µm) sized plastics. Rubber particles are complex and variable depending on the type, source, and age of rubber. Zebrafish *(Danio rerio)* embryos and *Daphnia magna* were exposed to nano- (<1 µm) or micron-sized (1 – 20 µm) particles, or leachate from recycled rubber (RR), crumb rubber (CR), and cryo-milled tire tread (CMTT). We hypothesized *Daphnia* and zebrafish exposure responses vary based on the origin and fraction of tire rubber due to distinct physical and chemical characteristics. Embryos and daphnids were evaluated for lethal and sublethal effects during 120-hour and 48-hour exposures, respectively. Nano-scale RR, CR, and CMTT particles elicited a hatch delay in zebrafish embryos having similar EC_{50} values. Leachate fractions of RR, CR, and CMTT particles elicited a hatch delay with EC_{50} values of 77%, 103%, and 151%, respectively. Micro-scale particles did not elicit any significant effects in zebrafish. Nano-scale particles of all rubber materials significantly increased hatch delay compared to leachate and micro-scale particles, suggesting an adverse nano-particle effect unexplained by chemical leaching. *Daphnia* RR micro and nano exposures resulted in mortality, with LC_{50} values of 9.8×10^5 micro-particles/mL and 5.0×10^8 nano-particles/mL. Leachate exposures did not elicit significant *Daphnia* mortality. The effects of tire-derived exposures observed pose a risk to aquatic organism survival.

Key words
Ecotoxicology, nano-particles, microplastics, rubber

Type of presentation
Poster

Session
Particles, fibres, plastics and their additives

Cytogenoxicity of settleable atmospheric particulate matter on Pacific White Shrimp *Litopenaeus vannamei*

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This study assumes the hypothesis that micro and nanoparticles of metals from metallurgical atmospheric emissions can trigger sublethal effects on Pacific White Shrimp *Litopenaeus vannamei*. Our aim was to analyze cytotoxicity (Neutral red retention assay) and genotoxicity (DNA strand break) in shrimps exposed to environmentally relevant concentrations (0.001, 0.1, and 1.0 g/L) of settleable atmospheric particulate matter (SePM) for different times (T2, T4, T7, T15 and T30 days), and in several tissues (gills, hemolymph, intestine and hepatopancreas). Two-way ANOVA (time x concentration) was employed to analyze the data set and Dunett's method to compare control vs. treatments. The results revealed time- and dose-dependent responses. Within the first 4 days, only the highest concentration showed a significant difference. From the seventh to the fifteenth day of exposure, the concentration of 0.1 g/L exhibited significant effects. In the longest exposure period (30 days), all concentrations triggered cytogenetic effects on the Pacific White Shrimp *Litopenaeus vannamei*. These findings shed light on the toxicological potential of SePM on estuarine organisms exposed to short and long periods to environmentally relevant concentrations.

Key words
Metallic particles, sublethal effects, tropical estuaries, shrimp, toxicological potential

Type of presentation
Poster

Session
Particles, Fibers, Plastics, and their Additives.

Effects of a Multi-Polymer Microplastic Mixture on *Mytilus edulis*

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Due to their ubiquity and small size, microplastics (MP) are easily taken up by aquatic organisms, causing a wide range of adverse biological effects. Research so far has focused primarily on the impacts on organisms in response to pristine test materials that are often single polymer made and spherical. However, MP found in the environment are mainly composed of a mixture of different polymers, sizes and shapes, which raises questions about their combined impacts in marine organisms. This study aims to investigate the accumulation and toxicity of a MP mixture of polypropylene, polyamide and polyethylene (<200 µm) at three concentrations (0.1, 1 and 10 mg/L) in the mussel *Mytilus edulis* over a three-week period. A battery of cellular, biochemical, and physiological biomarkers was subsequently determined, including micronuclei formation, DNA damage, acetylcholinesterase activity, antioxidant enzymes activity, lipid peroxidation, cellular energy allocation, histochemistry and digestive enzymes. The occurrence of the MP mix in mussel tissues was also investigated. Analyses are ongoing, but it is expected that the results obtained will contribute to understanding the interactions between different MP polymers and their potential toxic effects in organisms. Overall, this study will help to establish a baseline hazard knowledge for a MP mixture of environmentally relevant polymers and support future hazard and risk assessment of MPs in Norwegian ecosystems. This work was supported by the MicroOPT Project funded by the Norwegian Retailers Association – Handelens Miljøfond.

Key words
Microplastic mixture, mussels, multi-biomarker approach.

Type of presentation
Poster presentation.

Session
Particles, fibres, plastics and their additives.

Evaluation of the potential mitochondrial toxicity of polystyrene nanoparticles to marine bivalves: an *in vitro* study with hemocytes of *Crassostrea gigas*

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Plastic particles are contaminating our marine habitats on a worldwide scale. Characterizing their effects on marine life is critical for predicting possible threats to coastal species. This study investigates the mitochondrial toxicity of aluminum-doped polystyrene nanoplastics (Al-NanoPS) using a marine cellular *in vitro* method with *Crassostrea gigas* hemocytes. The LC50 of Al-NanoPS after 24 hours of exposure was 269.0 ± 65.9 mg/L (neutral red assay) and 119.9 ± 72.3 mg/L (resazurin assay), indicating a stronger influence on metabolic activity rather than lysosomal stability. Further metabolic and mitochondrial techniques investigated this hypothesis. We analyzed whether co-exposure to mitochondrial toxicants such as sodium azide and FCCP would increase the metabolic toxicity of Al-NanoPS, which was not proven. However, when galactose was added to the medium instead of glucose, Al-NanoPS toxicity increased. Galactose strongly favors aerobic respiration, implying that mitochondria disruption play a significant role in Al-NanoPS toxicity. Finally, the toxicity of Al-NanoPS was associated with increased ROS production. This could be linked to mitochondrial dysfunction, which remains to be assessed. Our findings imply that nanoplastics can disrupt mitochondrial function and affect cellular bioenergetics. Further TEM examinations of mitochondrial structure and Al-Nano-PS localization might provide more insights into the possible interactions between nanoplastics and mitochondria, an issue that has received little attention in marine bivalves.

Keywords: Nanoplastics, metabolism, cells, bivalves, ecotoxicity.

Type of presentation
Poster.

Session
t05: Particles, fibres, plastics and their additives

Fate of an innovative antifouling coating in seawater and ecotoxicity to the common mussel *Mytilus edulis*

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This study analyzed the potential toxicity of the biomimetic polyamide-based antifouling coating Finsulate for the common mussel Mytilus edulis and the release of its fibers into seawater. The mussels were exposed to Finsulate alone, to Finsulate combined with a resin epoxy or regular antifouling paint for 21 days under controlled laboratory conditions. No significant mortality was observed in the control, Finsulate, and Finsulate + epoxy conditions, while all mussels died in the antifouling paint conditions within 6 days of exposure. Gills and digestive glands from control, unexposed, Finsulate- or Finsulate + epoxy-exposed mussels were analyzed for enzymatic biomarkers of oxidative stress (CAT, GST, SOD) and neurotoxicity (AChE). Exposure to Finsulate and Finsulate + epoxy did not induce oxidative stress or neurotoxicity compared to untreated control mussel tissues. The Finsulate antifouling coating was analyzed by SEM to assess the release of its fibers into seawater. The Finsulate fibers used for the 21-day exposure were not altered compared to the new Finsulate fibers. This study showed that the Finsulate antifouling coating did not induce any toxic effects on the biomarkers tested in *Mytilus edulis*, and that its fibers were not altered under laboratory conditions during an exposure period of 21 days.

However, to assess whether Finsulate is likely to be a new source of microplastics in the oceans due to alteration of coating fibers caused by shipping-induced hydrodynamics, further analyzes should be carried out, exposing *Mytilus edulis* with Finsulate used.

Key words
Antifouling, *Mytilus edulis*, Biomarker, Oxidative stress, Neurotoxicity.

Type of presentation
Poster presentation.

Session
t05: Particles, fibres, plastics and their additives

t03: New tools to track pollutant sources and transfers

Functionalized polystyrene micro- and nano-plastics modulate the lipidome of zebrafish liver cells

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While micro- and nano-plastics (MNPs) have emerged as a significant environmental concern, our understanding of their toxicity in aquatic organisms remains limited. Once in the environment, MNPs undergo physical and chemical alterations. Thus, in an attempt to emulate the diversity of particle surface properties resulting from environmental weathering conditions, zebrafish liver (ZFL) cells were exposed to functionalized and plain micro- (MP, MP-COOH, MP-OH, MP-NH2) and nano-plastics (NP, NP-COOH) at an equivalent concentration of 27 μg/mL (4.55 x 1010 particles/mL). Effects on cell viability and changes in the expression of genes involved in lipid metabolism were investigated together with the modulation of the lipidome after 24 h exposure. Exposure to NP led to a slight decrease in cell viability and an increase in the production of reactive oxygen species, together with a significant increase of ether-linked phosphatidylcholines (PC-Ps/PC-Os), mainly involved in cell signaling and acting as precursors of inflammatory factors. Regarding MPs, exposure to MP-NH_2 caused a down-regulation of microsomal triglyceride transfer protein gene (*mtp*), involved in the transport of neutral lipids and cholesterol metabolism, and a significant accumulation of cholesterol ester in exposed cells. This study underscores the importance of considering both the size and functional group of MNPs when assessing their toxicity. Further research is needed to thoroughly characterize and evaluate the toxicological and cellular responses of authentic environmental MNP samples.

Key words
Functionalized, microplastics, nanoplastics, lipidomics, in vitro

Type of presentation
Poster

Session

Particles, fibres, plastics and their additives

Impact of Chemical Leachates from Car Tire Rubber on Marine Microalgae: Toxic Mechanisms and Ecosystem Implications

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Car tire rubber (CTR) particles contribute significantly to the presence of micronized particles in the environment, raising concerns about the release of plastic additives into marine ecosystems. These plastic additives are highly toxic to aquatic organisms, posing a serious threat to microalgae, at the basis of the aquatic food web. This study investigated the toxic effects of chemicals released from CTR after 7 and 14 days of leaching, on four microalgal species: *Skeletonema pseudocostatum*, *Rhodomonas baltica*, *Isochrysis galbana*, and *Tetraselmis suecica*. General toxicity of CTR leachates was first assessed using flow cytometry and PAM fluorometry, focusing on growth rate, cell size, complexity, natural pigments content, and photosystem II performance. The 14-day leachate exhibited the highest toxicity across all species, with *S. pseudocostatum* displaying the greatest sensitivity (EC_{50}=3.26 mg/mL). Subsequently, the sub-lethal effects of CTR leachates were investigated, for which metabolic activity, cell viability, cytoplasmic and mitochondrial membrane potentials, reactive oxygen species (ROS) formation, lipid peroxidation, neutral lipids, cellulose, and DNA contents were determined. This study uncovered the specific toxic mechanisms of CTR leachates, with notable impacts on ROS formation, oxidative stress, and cellulose content. This comprehensive analysis provides new insights into the toxicity mechanisms of CTR leachates on microalgae, highlighting the importance of understanding the potential impacts of plastic-associated chemicals on the marine ecosystem.

Acknowledgments

This research was supported by the MicroLEACH project (#295174), funded by the Norwegian Research Council.

Key words

Car tire rubber, leachates, microalgae, toxic mechanisms.

Type of presentation

Poster presentation.

Session

Particles, fibres, plastics and their additives.

Investigation of interactions between environmentally relevant microplastics and pathogenic bacteria in zebrafish larvae: integrating histology and scanning electron microscopy

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The present study investigated the effects of environmentally relevant microplastics (MPs) on the intestinal integrity of zebrafish larvae with and without concurrent bacterial challenge with *Aeromonas hydrophila*. Zebrafish larvae (3 days post-fertilization (dpf)) were exposed to MPs-PET fragments or fibers for 5 days. Half of the larvae underwent a concurrent bacterial challenge with *Aeromonas hydrophila* starting at 5 dpf. Whole larvae were collected at 8 dpf for histological processing and for scanning electron microscopy. The effect of MPs alone and combined with *Aeromonas hydrophila* on the intestinal integrity were investigated and both contaminants were marked, allowing to locate them in the gut and to visualize their interaction. SEM images allowed to investigate the physical interactions between *Aeromonas hydrophila* and MPs. Overall, this study provides insight into the interactions between MPs and pathogenic bacteria in the gut of aquatic vertebrates at early life stages and the consequences of such an exposure on the intestine at a tissue and cellular level.

Key words
Zebrafish larvae, Microplastics, Bacteria, Histology, Scanning electron microscopy

Type of presentation
Poster

Session
Mixture effects of pollutants

Microplastics identification and characterization in *Mullus Barbatus* fishes sampled from the Eastern of Tunisia

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Abstract

The prevalence of microplastics in aquatic environments has raised concerns about their availability and risks to aquatic biota. Since fish is an important source of animal protein for human beings, the occurrence and potential impacts of microplastics in fishes deserve particular attention. In this context, within the framework of the COMMON project, a sampling campaign was carried out in December 2020. Microplastics (MP_S) were identified in *Mullus Barbatus* collected from from Teboulba fishing area (governorate of Monastir, Eastern of Tunisia). Gastrointestinal tracts of fishes (n = 24 per species) were examined to analyse microplastics following digestion protocol, microscopic observations and characterization of COMMON project. All analyzed specimen are contaminated with MP_S, a total of 417 microplastic items were found in the intestines of *Mullus Barbatus*, all identified particle sizes are ranging from 0.26 to 162.26 µm. Among various types and colors of microplastics : fragment (56.83%) and Black (42.82%) were dominant.

Key words

Microplastics, Marine Pollution, Fishes, Polymer, stereomicroscope.

Type of presentation

Platform.

Session

1- Particles, Fibres, Plastics And Their Additives
2- Aquaculture Environment Interactions
3- Mixture Effects of Pollutants

Mineral UV filter and their effects on coral: comparative toxicity of two different-sized zinc oxide nanoparticles on *Pocillopora damicornis*

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The rising use of sunscreens and cosmetics containing UV filters has increased their presence in marine ecosystems. UV filters include a broad range of organic and mineral compounds with varying behaviors and properties in the aquatic environment. A recent report from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) highlighted that 45% of the tested organic UV filters were associated with proven toxic risks to coral reefs. Mineral UV filters such as zinc oxide (ZnO) or titanium dioxide (TiO_2) are considered more environmentally friendly. ZnO and TiO_2 are found in cosmetic products as nanoparticles (NPs) and are increasingly used as alternatives organic UV filters. Although prior studies have focused on the impact of ZnO NPs on aquatic photosynthetic organisms, their potential toxicity to corals remains understudied. This work compared the effects of two ZnO particles sizes (60 and 250 nm) on the symbiotic tropical coral *Pocillopora damicornis* through untargeted metabolomic analyses. Corals were exposed to environmentally relevant concentrations (5-50-300-1000 µg/L) for 7 days, and their metabolome was characterized using UHPLC-HRMS/MS analysis. Both sizes demonstrated a negative impact on the coral's symbiotic algae, with a major shift in lipid and pigment composition at higher concentrations. The impact on symbiotic algae occurred from 300µg/L for the smallest particles, emphasizing the role of NP physical properties in toxicity. This research contributes to a better understanding of how sunscreens may adversely affect coral reefs.

Key words: Coral bleaching, metabolomics, mineral sunscreen, nanoparticles, *Symbiodiniaceae*

Type of presentation
Platform

Session
Chemical exposome and non-target screening approaches

Nile Red staining for detecting microplastics in the invasive Atlantic blue crab

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Microplastics (MPs) have been found in many marine species, and they have potential health and economic implications for fisheries and the humans that rely on them. For monitoring purposes, staining techniques have been proposed as alternative or complementary methods for quicker, more economical and routine analysis of MNPs in biological samples. This work tested the suitability of using Nile Red to identify and quantify MPs in hepatopancreas and hemolymph samples of the Atlantic blue crab *Callinectes sapidus*. To this end, crabs were exposed for 14 days to microplastic mixtures through the diet under controlled laboratory conditions. Contaminated seafood batches were prepared using seafood as matrix and subsequently spiked with MPs, homogenised, blended and moulded to obtain food pellets. MPs comprised different size of polyethylene microparticles (from 4 to 100 µm), and polyester and bulked textile microfibers. After the exposure period, crabs were sacrificed and hepatopancreas and hemolymph samples analysed. Hepatopancreas samples were digested with 10% KOH for 72 hours at 40 °C, then filtered and dyed with Nile Red solution to perform fluorescent microscope quantification of MPs using blue excitation. The number of MPs quantified in both hepatopancreas and hemolymph samples were higher in crabs exposed to MPs than in control ones, the minimum size particle recovered being ≥9-10 µm. Furthermore, hemolymph cell viability was lower in microplastic-exposed crabs as compared to control ones. The results of this study show that Nile Red staining could be used in a relatively simple and standardized manner to quantify MP concentrations in *C. sapidus* and that this species can be used as a biomonitor for MP in invaded Mediterranean coastal ecosystems.

Keywords
Callinectes sapidus; microplastic; Nile red; biomonitoring;

Type of presentation
Poster

Session

t05: Particles, fibres, plastics and their additives

t10: Biomonitoring and development of integrative assessment approach

Plastmorne: Exposure to microplastics associated with 17α ethinylestradiol causes biochemical disturbances in different tissues of *Centropomus undecimalis*.

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This study hypothesizes that microplastics (MPs) spiked with 17α ethinylestradiol (EE2) can cause disturbed biochemical pathways in tropical fish *Centropomus undecimalis* after short periods of exposure. It was verified through chemical analyses of the transference of EE2 from MPs to water and organisms, in addition to the sublethal effects related to biotransformation and conjugation enzymes, antioxidant defenses, citogenotoxicity, and neurotoxicity. Specimens were exposed for 7 days to virgin microplastic (MP) and MPs spiked with the hormone EE2 (MPE). Biomarkers were analyzed at different times (T0, T3, and T7 days) and tissues (gills, liver, muscle, intestine, and brain). The presence of EE2 was verified in the water and the organisms after the exposure periods. Biomarker results showed changes associated with the MPE group in the gills and liver after a short period of (T3). At the end of the experiment (T7), an Integrated Biomarkers Responses index pointed out that all tissues suffer biochemical disturbances, and the main effects were lipid peroxidation in gills, liver, and intestine, as such as DNA damage in gills linked to the treatments MP and MPE. Once present in the water column, MPs with their additives or adsorbed contaminants such as hormones, can come into contact with fish via breathing, being retained in the gills, and through ingestion of particles causing responses in different digestive organs. It could be stated that virgin polyethylene microplastics or those associated with 17α ethinylestradiol represent a threat to tropical estuarine ecosystems.

Keywords
Plastic pollution, polyethylene, spiked microplastic, estrogen vector, common snook fish.

Type of presentation
Poster

Session
Particles, Fibers, Plastics, and their Additives.

Simple detection of polystyrene nanoparticles and effects in freshwater mussels; method development and *in situ* application to urban pollution.

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Abstract
The ubiquity of plastics in environments worldwide is raising concerns about their toxicity to organisms. The purpose of this study was to investigate simple means to determine the exposure and effects of nanoplastics (NPs) in the freshwater mussels *Elliptio complanata*. NP tissue levels were determined using a plasmonic nanogold sensor probe and effects were determined using the refractive index (RI) and thiol-reaction rates (TRR) in protein-dense tissue extracts. This method was adapted to quantitatively measure the concentration of NPs in tissues using a salting out extraction in the presence of ACN. Concentrated solutions of albumin were first spiked with NPs to evaluate changes in RI and TRR to determine crowding effects. The data revealed that NPs readily decreased the RI and TRR in albumin *in vitro*. These 3 simple assays were then applied on freshwater mussels caged for 3 months at various sites in a largely populated area. Mussels downstream of the city center and found at the street runoff discharge sites were highly contaminated by NPs and the RI and TRR were also reduced. In conclusion, simple and readily accessible assays to assess the NP contamination based on a visual nanogold sensor technology, and the effects of these plastics are proposed for freshwater mussels.

Keywords: Polystyrene nanoplastics, nanogold sensor probe, refractive index, thiol reaction rates, Elliptio complanata, municipal effluents.

Toxicity assessment of partially biobased waterborne polyurethane nanoparticle suspensions in zooplankton and zebrafish embryos*

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Waterborne polyurethanes (WBPUUs), a type of PU obtained in a mainly organic solvent-free synthesis process, produced from renewably sourced precursors show properties comparable to fossil-based PUs. In spite of their wide range of applications, there is no information on the environmental hazard posed by WBPU nanoparticles (NPs) produced during the synthesis. Here, the toxicity of two latexes of partially bio-based WBPUU NPs synthesized using a polyol derived from castor oil with and without 3% cellulose nanocrystals (CNC) as reinforcement, as well as CNC alone, were tested in zebrafish (*Danio rerio*) embryos, rotifers *Brachionus plicatilis* and brine shrimps *Artemia salina.* WBPUU suspensions up to 100 mg/L did not cause acute toxicity, malformations, hatching delay, or cell death in zebrafish embryos. In rotifers, exposure to 200 mg/L or to ≥100 mg/L for 24 or 48 h, respectively, caused significatly higher mortality rate, but no acute effects were seen in artemia. CNC alone at 3 mg/L also provoked significant mortality in rotifers. WBPUU at ≥12.5 and 25 mg/L, but not WBPUUCNC, caused a decrease in the ingestion of both rotifers and artemia, respectively. Additionally, CNC alone at ≥0.375 mg/L decreased the ingestion rate in artemia. Both WBPUU suspensions strongly aggregated in contact with saltwater. In summary, tested partially bio-based WBPUU NP suspensions did not cause acute toxicity in zebrafish embryos nor in artemia, but rotifers seem to be more sensitive to these materials. Moreover, there is some evidence of sublethal toxicity at high exposure concentrations in artemia. Further studies are being developed to investigate the cellular and biochemical mechanisms of toxicity. *Funded by the Spanish MICIN project ENSURE2 (TED2021-131147B-I00, MCIN/AEI/10.13039/501100011033/NextGenerationEU/PRTR), Basque Government grant to consolidated research groups (IT1743-22 & IT1690-22) and UPV/EHU predoctoral grant to GAS.

Key words
Partially biobased polymers, waterborne polyurethane nanoparticles, environmental hazard

Type of presentation
Poster

Session
1. Particles, fibres, plastics and their additives

Toxicity of Two New Emerging Nanomaterials in the Mussel *Mytilus edulis*

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The fast expansion and use of nanotechnology has continuously led to the development of new emerging nanomaterials (NMs) with unique properties but diverse applications. Layered nanostructures, such as defect-rich molybdenum disulfide (MoS_2) and layered double hydroxide nanosheets (LDH), have shown significant potential in environmental technology and remediation due to their specific physicochemical properties. However, their potential toxic effects towards organisms, as well as their overall impact on aquatic ecosystems are still poorly understood. Thus, the present study investigated the toxicity of MoS_2 and Mg-Al-LDH NMs (0.1 and 1 mg/L) to the mussel *Mytilus edulis* over a 3-week period. A biomarker battery was deployed, including lysosomal membrane stability (LMS), micronuclei formation, DNA damage, acetylcholinesterase activity, antioxidant enzymatic activities, lipid peroxidation, cellular energy allocation and metallothionein concentrations. Behavior of the NMs in exposure media was evaluated using Transmission Electron Microscopy and Dynamic Light Scattering and their bioaccumulation in mussel tissues was also quantified. Analyses are ongoing, but the obtained results seem to indicate a significant effect in mussels that is concentration and time dependent, with Mg-Al-LDH NMs demonstrating a clearer toxic response than MoS_2 NMs, as evidenced by the results obtained from LMS. Overall, this study provides valuable information on how new emerging NMs can become a potential risk for the aquatic environment and organisms living therein.

Acknowledgments
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Key words
New emerging nanomaterials, mussels, biomarker battery.

Type of presentation
Poster presentation.

Session
Particles, fibres, plastics and their additives.

Toxicity to fish liver cell line of leachates and extracts from micro and nanoplastics produced from plastic items collected at different beaches of the Bay of Biscay

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Despite increasing micro and nanoplastic (MNPs) research, limited information on toxicity of realistic environmental MNPs exists. This study compares MNPs' toxicity from beach-collected objects using different solvents to produce leachates (lch) and extracts (ext) in the rainbow trout liver cell line (RTL-W1). Items were picked on 3 beaches of the Spanish Basque Country (BC) (Gorrondatxe (G), Murgita (M) and Zumaia (Z)), in winter (J) and spring (A) 2023, and 1 beach in the French BC (Tarnos) in A 2021. Polymer composition was identified by FTIR and each item was ground to <250 µm. MNPs were mixed with original polymer proportions by weight, at each beach and season. Lch and ext were made by adding filtered distilled water, DMSO or methanol (10 g/L) to MNPs and agitating them (24h, 190 rpm, 18°C, darkness). Liquid phase was recovered by filtration (0.8 µm). In methanol ext, DMSO was added postfiltration (1:1) and methanol was evaporated with N_2. RTL-W1 cells were exposed to lch (100-0.01%) and ext (1-0.01%) for 24 h for cytotoxicity and to 20% and 1% lch and 1% ext for 4h to assess ROS production. All lch caused cytotoxicity at ≥40% and almost all ext at ≤1%. Exceptions were GJ and MJ in DMSO ext and GJ in methanol ext. ROS production increased in cells exposed to 20% lch and to almost all ext at 1% (except ZJ and ZA), but different responses were seen in cells exposed to DMSO and methanol ext. These results highlight the complexity of analyzing toxicity of environmental MNPs due to differences in polymer types and proportions, additives, sorbed pollutants and aging degree.
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Key words
Environmental micro and nanoplastics, Bay of Biscay, aging, leachates and extracts, *in vitro* toxicity

Type of presentation
Poster presentation

Session
Particles, Fibres, Plastics and their additives

Toxicological evaluation of biodegradable and conventional plastics: Impact of photodegradation and composting on environmental safety

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In recent years, biodegradable/compostable plastic (BPs) have been promoted as eco-friendly alternatives to traditional plastics for addressing environmental concerns. However, the potential toxic effects resulting from leachable chemicals in these plastics have been relatively overlooked. This study evaluates the toxicity of methanolic extracts of BPs compare to conventional plastics (both virgin and recycled), and explores the potential impact of plastic photodegradation and composting on toxic responses, employing a battery of in vitro assays conducted in PLHC-1 cells. The results reveal a noteworthy decrease in cell viability (<70%) in PLHC-1 cells after a 24-hour exposure to extracts from compostable plastics, but not those of conventional plastics. Toxicity was enhanced by photodegradation and partial composting of BPs. Extracts of conventional plastics, and particularly those of recycled plastics, induced 7-ethoxyresorufin-O-deethylase (EROD) activity and micronucleus formation in exposed cells, indicating the presence of significant amounts of CYP1A inducers and genotoxic compounds in the extracts, which was enhanced by photodegradation. These findings underscore (a) the importance of investigating the impact of degradation mechanisms, such as sunlight exposure and composting, on the toxicity of plastics, and (b) the need to investigate the composition of newly formulated compostable plastics, as they could potentially exhibit a greater risk of chemical toxicity when compared to traditional ones.

Key words
Plastics, compostable, extracts, toxicity, in vitro

Type of presentation
Poster

Session
PARTICLES, FIBRES, PLASTICS AND THEIR ADDITIVES

Transcriptomic Alteration of *Mytilus galloprovincialis* Exposed to Virgin and Marine Incubated Microparticles Made of Biodegradable and Conventional Polymers

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Biodegradable polymers are considered a potential solution to the problem of plastic litter but their use in marine applications has raised concern, making it necessary to investigate their ecotoxicity. This study aims to evaluate the ecotoxicological effects of microparticles (MPs) of different biodegradables polymers (Polycaprolactone, Mater-Bi, cellulose) and conventional polymers (Polyethylene) on *Mytilus galloprovincialis*.

Selected polymers were grinded to MPs (100 to 300 µm) and incubated at sea for 60 days. Fifty mussels per group (5 replicate tanks), were exposed to virgin and marine incubated MPs (0.1 mg/l) for 21 days. Total RNA was isolated from mussels' hepatopancreas and sequenced in 150 bp paired-end mode. Differential expression and gene set enrichment analysis were carried out on the de novo reconstructed transcriptome.

Marine Incubated polycaprolactone affect the highest number of genes, followed by virgin polycaprolactone. Other biodegradable polymers and polyethylene cause weaker transcriptional response. All MPs regardless of polymeric composition, affect key biological processes involved in innate immunity and fatty acid biosynthesis. Furthermore, the preponderant effect is attributable to the MPs' marine incubation, underlining that a key factor of mussel's biological response is the particles' modifications occurring in the environment.

This study contributes to the ecotoxicological characterization of conventional and biodegradable polymers, suggesting that some bio-based biodegradable polymers may constitute a promising alternative to conventional plastics.

Key words
Microplastics, bivalves, transcriptome, biodegradable plastics, marine incubation

Type of presentation
Platform presentation

Session
Proposed sessions: PARTICLES, FIBRES, PLASTICS AND THEIR ADDITIVES

Uncoupling between microplastic inorganic and organic pollutants and toxicity in nine European rivers

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Keywords: plastic pellets, chemical pollution, toxicity

Session: t05: Particles, fibres, plastics and their additives

The contamination of freshwater and marine ecosystems with plastic is a global ecological problem of increasing scientific concern. Estimation of plastic debris entering the oceans has been recently reevaluated to about 500 kilotons per year. These plastics can adsorb from aquatic environments persistent organic pollutants or toxic metals, which can impact at the end aquatic biodiversity. Here, we characterised along nine European river-to-sea continuum the contaminant sorption and their toxicity levels, after sorption on two different plastics. After 1 month, we observed for several rivers high contents of pharmaceuticals, pesticides, antimicrobials or Per- and Polyfluoroalkyl Substances (PFAS). Interestingly, a high variability was observed across and along the nine European rivers, suggesting the different influences of anthropogenic activities.

We observed that both plastics can impact sea urchin and oyster reproductions, with a high variability across and along the nine rivers. Interestingly for one plastic, a toxicity gradient of contaminant sorption followed the salinity gradient for the Seine and Thames rivers. Thus, our study highlighted that along rivers, plastic adsorb several pollutants from aquatic environment leading to increase toxicity for aquatic ecosystems.

SCIENTIFIC PROGRAM OF PRIMO 22

Pollution by renewable marine energy technologies

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Effects of chemical elements resulting from the degradation of aluminum-based galvanic anode on 4 microalgae species and on the larval stage of the Pacific oyster *Crassostrea gigas*

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In the context of the deployment of Offshore Renewable Energy (ORE) farms in France, the potential impact of cathodic protections (CP) was highlighted as an environmental concern. Based on this issue, the ECOCAP project aims to perform a conclusive chemical risk assessment for elements released by CP in the marine water column. This study describes the methodology implemented at BOREA laboratory (University of Caen Normandy) to assess the ecotoxicological impacts of the dissolution of elements released by cathodic protection systems using an aluminium-based galvanic anode (GACP) and/or an impressed current (ICCP). Experiments under controlled conditions were conducted on 4 species of microalgae and on the larval stage of the Pacific oyster. Element concentrations, microalgal growth inhibition and the percentage of abnormal larvae were recorded to define baseline toxicity values (EC_{10}, EC_{20} and EC_{50}) from dose-response curves. The different organisms tested showed a large variation in sensitivity to aluminium produced by GACP. Under representative environmental concentrations of Al, two microalgae were affected, while no effect was observed on the embryo-larval development of oyster larvae in response to elements released from GACP and ICCP systems. Only Al concentration 100 times greater than the environmental level induced significantly abnormal larvae. This work enhances our knowledge on the impact of CP on the marine ecosystem and could therefore provide useful information for the development of offshore wind technologies by improving their environmental integration.

Key words
Galvanic anode, impressed current, microalgae, oyster, biological effects

Type of presentation
Platform (oral presentation)

Session
Pollution by renewable marine energy technologies

Assessment of the ecotoxicological effects of chlorination and ozonation for biofouling control in a tropical OTEC pilot plant

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Ocean thermal energy conversion (**OTEC**) is a renewable energy technology that produces electricity thanks to the temperature difference between warm surface waters and deep cold waters. Because biofouling can significantly hamper the efficiency of OTEC plants, we investigated the effectiveness in biofouling control of alternative oxidant ozone (O_3) and commonly being used chlorine. This was done during long run experiments at Ifremer pilot plant at La Martinique (French Antilles) with the complementary aim to assess the environmental impact of such a technology.

The pilot plant is equipped with heat exchanger models and wide range of instrumentation (temperature probes, flowmeters). Two concentrations of ozone 0.25 and 1 ppm/hr per day in intermittent mode and a concentration of chlorination at 1 ppm trigged by biofilm sensor were evaluated. Moreover, direct monitoring of the biofouling was successfully carried out by the biofilm sensor (Alvim system) to assess chlorination and ozonation efficiency. In addition, the ecotoxicological effects of some of the identified halogenated chlorination and ozonation by products generated during pilot plant operation was investigated. Ecotoxicological tests were conducted for individual halogenated by-products, on several marine algae species, *Tisochrysis lutea, Skeletonema marinoi*, *Tetraselmis suecicca, Emiliania huxleyi* (inhibition growth assay), the Pacific oyster, *Crassostrea gigas* (embryo-larval bioassay, ISO 17244:2015), the freshwater zebra fish, *Danio rerio* (OECD 236 - Fish Embryo acute Toxicity, FET), and the marine medaka, *Oryzias melastigma* (protocol adapted using 3 standards: OECD 210 - Fish, Early-life Stage Toxicity Test, OECD 212 - Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages and OECD 236 - Fish Embryo Acute Toxicity (FET) Test). Behavior defects were also monitored in fish using the photomotor response test. Moreover, tests were also conducted with effluents collected at the outlet of the test bench.

The lower concentration of ozonation and chlorination showed a very efficient biofouling control over a a full year in tropical conditions. Results of this pilot scale study will be helpful in the selection of appropriate oxidant for OTEC system employed in the worldwide. The chemical risk for pelagic species living in marine water column was assessed using existing PNEC $_{\text{marine water}}$ (Predicted No-Effect Concentration) and when possible, using additional ecotoxicological results obtained in this study to refined PNEC for some of the identified by-products.

Key words: OTEC, Ozonation by products, Ecotoxicological effects, Chemical risk assessment.

Type of presentation

Oral

SCIENTIFIC PROGRAM OF PRIMO 22

Pollution by renewable marine energy technologies

(Posters)

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NANTES FRANCE

Assessment of the toxicity of aluminum from anti-corrosion devices to fish using marine medaka

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In the context of the deployment of Offshore Renewable Energy (ORE) farms in France, the potential impact of cathodic protections (CP) was highlighted as an environmental concern. Based on this issue, the ECOCAP project aims to perform a conclusive chemical risk assessment for elements released by CP in the marine water column. One of such CP is galvanic anode cathodic protection system (GACP) which relies on the degradation of an anode mainly composed of aluminum (Al ~95%) and zinc (~5%), released in the environment during functioning. The purpose of this study was to evaluate toxicity of GACP to fish.

A GACP system was settled in a tank filled with 43 L marine water and an electric current (I = 0.1 A) was applied for 24 h. The solution was further decanted for 24 h to eliminate the particle phase and the final stock solution was used to prepare dilutions for toxicity assessment. For comparison, we produced in parallel an equivalent $AlCl_3$, 6 H_2O solution by dissolving 4.5 g of salt into 43 L of seawater. Embryo-larval (OECD 212) and chronic exposure over 4 months were performed as previously described using marine medaka (*Oryzias melastigma*).

Acute exposure resulted in no toxicity at concentration up to 1 mg L^{-1} of total Al. Chronic exposure resulted in no modification of growth or reproduction while an increase in anxiety was observed at the highest tested concentration (~400 µg L^{-1} of total Al).

In our system, we conclude that Al is not acutely toxic, while effects on behavior would require further studies to evaluate additional behavioral traits and understand underlying mechanisms.

This project was financially supported by French National Agency for Research “Investissement d'Avenir” under the agreement number ANR-10-IEED-0006-34.

Key words
Toxicity, aluminum, medaka, acute, chronic.

Type of presentation
Poster

Session
Pollution by renewable marine energy technologies

Chemical risk assessment in the context of Offshore Renewable Energy: the case of cathodic protections

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In the context of the deployment of Offshore Renewable Energy (ORE) farms in France, the potential impact of cathodic protections (CP) was highlighted as an environmental concern. Based on this issue, the ECOCAP project aims to perform a conclusive chemical risk assessment for elements released by CP in the marine water column. To date, two methodologies of CP are currently used in ORE, Galvanic Anode (GACP) and Impressed Current (ICCP). The risk assessment process described in this study follows the REACH technical guidance described by the ECHA (European Chemicals Agency). This methodology is based on a 4-steps procedure. First, an inventory of all compounds potentially released into the environment is performed by combining bibliographic review and experimental data acquisitions. Secondly, a Predicted Environmental Concentration (PEC) in marine water is estimated for each element potentially released in the dissolved fraction. This step is based on two complementary approaches: a 3D hydro-numerical modelling of CP released element dispersal and *in situ* measurements. The 3rd step of the risk assessment is dedicated to the refinement of threshold of hazard through the estimation of Predicted No Effect Concentrations (PNEC) for water column using literature review and ecotoxicological experimentations (both acute and chronic) on model marine species representative of several trophic levels. Finally, thanks to the PEC and PNEC definitions, the 4th and final step of the procedure is performed by characterizing the risk for the release of CP elements in the water column.

Key words
Offshore Renewables energies; Cathodic protections; Ecotoxicity; Chemical Risk assessment

Type of presentation
Poster

Session
Pollution by renewable marine energy technologies

Effect of magnetic fields related to submarine power cables on the expression of selected genes in larval rainbow trout *Oncorhynchus mykiss*

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The introduction of artificial magnetic fields into the environment through submarine cables poses a potential threat to ecosystems associated with the operational phase of offshore wind farms and other marine renewable energy devices. The vulnerability of fish, particularly early life stages of those reproducing on the bottom, arises as they may directly encounter cables on the seabed. While some negative effects of magnetic fields on fish have been documented, certain critical processes remain unexplored, such as ion transport, inflammatory responses, apoptosis, and neuronal development. **This study aimed** to investigate the impact of an electromagnetic field (EMF) at levels typically observed near submarine cables (1 mT, 50 Hz) on the expression of genes encoding selected proteins. Fish were exposed to EMF for 52 days, starting from fertilized embryos to mobile yolk sac larvae. The experimental setup featured an EMF generator employing Helmholtz coils. **Results** indicated no statistically significant differences in the expression of most tested genes between EMF-exposed and non-treated larvae. However, the gene encoding interleukin-6 exhibited an alteration. In trout larvae from the EMF group, IL-6 gene expression was 5.8 times higher compared to the control group ($p=0.0004$). **Conclusions** drawn from the study suggest that prolonged exposure to EMF likely impacts immune responses by stimulating the production of proinflammatory cytokines during the early life stages of *O. mykiss* fish.

Impact assessment of metals released by aluminum-based galvanic anode on the physiology of the abalone *Haliotis tuberculata* in controlled conditions

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To protect metal structures immersed in the seawater from corrosion, the galvanic anode cathodic protection system (GACP) is often applied. However, this association leads to continuous oxidation of the galvanic anode and therefore to a release of a metal cocktail in the forms of ions or oxy-hydroxides. Therefore, the main objective of our study was to investigate the toxicity of elements released from the dissolution of an aluminium-based galvanic anode (~95% Al, ~5% Zn, <0.1% for In, Cu, Cd, Mn, Fe) on a grazing gastropod, the abalone *Haliotis tuberculata*. Gastropods were exposed for 16 weeks (12 weeks of exposure and 4 weeks of decontamination phase) to 6 conditions including a control, 4 concentrations based on total aluminium level (86, 425, 1096 and 3549 µg L^{-1}) and a trophic control, corresponding to abalones placed in non-contaminated natural seawater but fed with contaminated algae. The effects of metals on growth, glycogen levels, Brix index of hemolymph, MDA levels in the digestive gland and gills, hemocyte phagocytic activity, ROS production, lysosomal system and the progress of gametogenesis were investigated throughout the entire exposure allowing the realization of kinetics. The results revealed that the aluminium-based anode did not seem to have an effect on the health status of the individuals for environmentally realistic concentrations. However, in extreme conditions strong effects were reported on the growth, immune system and reproduction of abalone.

Keywords: galvanic anode, aluminum, zinc, *Haliotis tuberculata*, biological effects

Type of presentation

Poster

Session

Pollution by renewable marine energy technology

The effect of electromagnetic fields on the embryos and early larvae of Atlantic cod and haddock

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Controlled Source Electromagnetic (CSEM) surveying is widely used to explore subsea hydrocarbon reservoirs. While the signal propagates through the water column, it could affect marine organisms, particularly if they are electro-or magneto-sensitive. We examined the effect of the electromagnetic fields (EMF) generated by such surveys on Atlantic haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*). Cod and haddock embryos were exposed at three developmental stages to EMF intensities representing three different distances from the EMF source Embryo-larval survival, hatching, morphological development, and cardiac function until the yolk sac stage were monitored. None of the treatments caused mortality or delayed hatching in either species. EMF exposure induced slight tachycardia in haddock (1.2-fold compared to control) which also exhibited reduced yolk reserves. This effect was significant at the lowest EMF intensity (equivalent to a distance of 1000 m from the EMF source) and increased with exposure time and intensity; however, we cannot say whether these effects are reversible. No developmental effects were recorded in cod. Haddock larvae seem to be more vulnerable to EMF than cod. An EMF exposure was conducted on male parent cod, revealing no impact on the sperm quality of exposed males and there was no effect on fertilization rate or the subsequent development of cod embryos. Our findings highlight the nuanced effect of CSEM-generated EMF on development of Atlantic haddock and cod.

Key words
CSEM, morphology, offspring, larval stages, heart rate

Type of presentation
Poster

Session
t06: Pollution by renewable marine energy technologies

SCIENTIFIC PROGRAM OF PRIMO 22

Aquaculture Environment interactions

(Oral talks)

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Oyster transcriptomic responses to septic effluent

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The Atlantic oyster *Crassostrea virginica* a popular and important aquaculture species in the Eastern US. These bivalves are sessile filter feeders and thus reflect changes in the contaminant status of the environment. *Crassostrea* species are found in temperate and tropical waters in both open waters and brackish estuaries where runoff is at its highest. As they accumulate contaminants, bivalves may exhibit sublethal changes, evident in gene expression changes that arise in response to chemical insult, and which may be useful as contamination bioindicators. The effects of on-site sewage disposal via septic systems on coastal environments has been much less studied than direct sewer effluent. Twenty-five percent of the households in the United States, and nearly fifty percent of the households in the Northeastern US dispose of sewage using on-site systems. We exposed *C. virginica* to different concentrations of septage and performed RNA-seq analyses to search for biomarkers of septic effluent. We also analyzed oysters held in a saline coastal pond impacted by septic effluent, comparing pond-exposed oysters with a coastal site not impacted by septic effluent. Out of more than 37000 mapped transcripts we found more than 4000 differentially regulated genes. While some transcripts exhibited expected patterns, most transcripts are not sufficiently annotated to provide pathway analysis. Clear differences between laboratory-exposed and field-exposed oysters challenge the idea that robust biomarkers of dilute pollutant exposure can be determined from limited sampling. Funding: WHOI.

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Key words
In Calibri 11, 5 key words at maximum.

Type of presentation
Poster

Session
1. t11: Aquaculture Environment interactions

2. t08: Mixture effects of pollutants

Toxicity of antifoulant coatings used in Norwegian aquaculture on two scavenging amphipods, *Orchomenella obtusa* and *Tmetonyx cicada*

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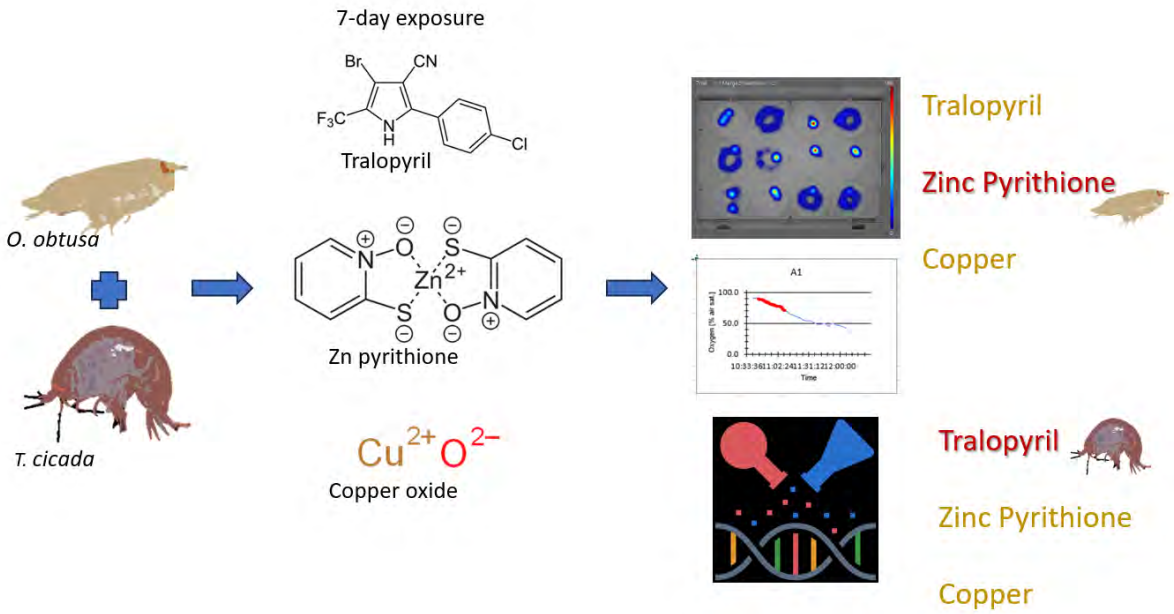
Offshore aquaculture farms suffer from biofouling, biological growth, on nets. Traditionally, copper-based antifoulants have been used to reduce this growth. However, copper from these antifoulants can leach into the environment and net cleaning methods have also been found to disperse particles into the local environment. Novel biocides have started to be put in use to replace copper, but not all have been assessed for impacts on non-target crustaceans. Scavenging amphipods found in the vicinity of aquaculture farms (<50 m) are attracted to salmon feed, which makes them a potential first responder species to aquaculture pollution.

This study aims to establish if these local deep water amphipod species, *T. cicada or O. obtusa*, can be used as an animal model for environmental monitoring. Amphipods were assessed for mortality, swimming speed, as well as respiration rate for sublethal doses of three antifoulants: copper oxide, zinc pyrithione, and tralopyril. Behavior was assessed with the DanioVision system. Metabolic oxygen consumption was assessed using a the Loligo® respiration system.

Species specific effects were seen in dosage for each chemical, with *T. cicada* having higher resilience to copper oxide and zinc pyrithione exposures, but much higher sensitivity to tralopyril. Some behavioral impacts were seen at sublethal concentrations of zinc pyrithione, and effects on respiration were seen at sublethal levels of tralopyril. This study aims to be a first step to better understand the potential impact of these chemicals on the benthic community near aquaculture farms.



Key words
Aquaculture, biofouling, amphipod, ecotoxicology, antifoulants
Type of presentation
Oral presentation or poster
Session
Aquaculture Environment Interactions
Biomonitoring and development of integrative assessment approaches

Developmental toxicity of an antifoulant and two sea lice chemotherapeutants used in aquaculture on the green sea urchin (*Strongylocentrotus droebachiensis*)

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Aquaculture can cause different environmental impacts due to the discharge of nutrients, the use of antifoulants and the application of veterinary medicinal products (VMPs) used to treat fish for parasites. Copper based antifoulants and chemotherapeutant treatments with avermectins (e.g. emamectin benzoate) and fluorobenzoyl ureas (e.g. teflubenzuron) are used in the salmonid industry and are discharged to the marine environment through their use. The effects of these compounds have mostly been studied in crustaceans. In this study, we examined the impact of emamectin benzoate, teflubenzuron and copper (II) sulfate at environmentally relevant concentrations (5 to 90 µg/L) on the embryonic and larval development of the green sea urchin (*Strongylocentrotus droebachiensis*), an ecosystem engineer and common species in Norway. The embryogenic development was assessed at blastula, gastrula, prism and pluteus larval stages, where the severity of the malformations was analyzed using a toxicity classification index. Larval growth was also assessed via morphometric measurements (body length, somatic rods, post-oral arm, anterolateral arm, and larval width). Preliminary results show that embryos exposed to copper caused a reduced or total absence of pluteus larvae (50 and 90 µg/L) and body rod and skeletal malformations at the highest concentrations of the VMPs.

Key words
Antifoulants, veterinary medicinal products, embryotoxicity

Assessing the impact of chemical pollution on the Pacific oyster (*Crassostrea gigas*): effects on telomere dynamics following an *in situ* transplantation

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French coastal waters are contaminated by numerous pesticides, particularly within shellfish farming areas. Those contaminants are known to impact physiological parameters and genome integrity of non-target marine species. Telomeres play a crucial role in maintaining genome stability by protecting the coding sequence of DNA at the end of chromosomes. Oxidative stress induced by pesticides may inhibit telomerase activity or prematurely reduce the length of telomeres (TL), therefore affecting their dynamics. Investigating the impact of pesticides on telomere dynamics in *C. gigas*, a valuable aquatic species, is therefore of great interest in ecotoxicology. It may result in the development of new biomarkers of pollutant effects and exposure and novel biomarker of longevity.
Consequently, we first developed (i) a RT-qPCR protocol to measure telomerase gene expression and (ii) a qPCR protocol for measuring relative TL. Methodological developments and validation were established on frozen oyster tissues at different ages. These protocols were then applied to a 1-year field study in the Bourgneuf bay (France), to evaluate impacts of chemical pollution on oyster telomere dynamics. A control sub-sample was kept at the experimental hatchery in control seawater (UV treated and active carbon filtered) for the duration of the transplantation. TLs and gene expression were measured every 3 months along with genotoxicity (comet assay) and physiological (growth and survival) endpoints. POCIS have also been deployed in the field and the hatchery to characterize pesticide contamination of water and hence oyster exposure. Results will be presented during the conference.

Key words
oyster, telomeres dynamics, chemical contaminants, (RT-)qPCR, genotoxicity

Type of presentation
Oral presentation

Sessions
1. t10: Biomonitoring and development of integrative assessment approaches
2. t11: Aquaculture Environment interactions

Curcumin as a natural compound supporting oyster stress tolerance and aquaculture sustainability in a changing planet

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The Pacific oyster *Crassostrea gigas* is one of the world's most cultivated bivalve. Several countries have recently experienced mass mortalities in their cultivation because of pathogens and global changes, such as marine pollution. The development of therapeutics to minimize mass mortalities is of major importance for economic gain in the aquaculture sector. Curcumin (CUR) is a natural plant compound with strong antioxidant and anti-inflammatory properties as evidenced in vertebrate models. We were pioneers in demonstrating that CUR induces antioxidant amplification and stress tolerance in adult *C. gigas*. In this study, we expanded investigations on the effects of CUR *in vivo*, using oyster larvae and juveniles, and *in vitro*, using immune cells (hemocytes). We detected higher antioxidant responses in all tested models. This was directly correlated to a higher ROS detoxification capacity in hemocytes and larvae and a higher tolerance of oyster larvae to toxic challenges using a pro-oxidant and the antifouling compound DCOIT. In juveniles, we also demonstrated that despite its beneficial antioxidant effects, CUR pre-treatment did not increase oyster survival against bacterial infections (three different *Vibrio* species). We'll next test for its protective effect against a viral disease (OsHV-1). Overall, our results demonstrate a conserved role of CUR in antioxidant amplification and stress tolerance in oysters. While its role in disease resistance remains elusive, we highlight CUR as a promising natural compound supporting blue economy and sustainable aquaculture.

Keywords

Bivalve, antioxidant, immunology, antifouling, *Vibrio*.

Type of presentation
Platform.

Session
t11: Aquaculture Environment interactions

Comparison of uptake and toxicity of two antifoulants on blue mussels: copper and tralopyril

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Norwegian aquaculture has used large amounts of copper (Cu_2O) for antifouling on net pens over many years, although since 2019 the use has decreased by 75 % and copper has mainly been replaced by tralopyril also called Econea. Tralopyril has a short half life in sea water but little is known of stability and toxicity of its degradation products, except that high concentrations are measured in blue mussels growing on tralopyril treated net pens.
We have performed dose response studies after short term exposures (1 hour and 24 hours) followed by 2 days of recovery. Parameters measured are uptake of copper, tralopyril and one of its degradation products, oxygen consumption, histological changes in digestive gland and gills and gene expression in digestive gland and gills by RNAseq.

The results demonstrated half life of tralopyril to 9 hours in sea water at 12°C, up concentration of its de-brominated form and uptake of copper and tralopyril in mussel tissues depending on exposure. Tralopyril seemed more potent on oxygen consumption, gill histology and gene expression of gill cells, while copper showed strongest dose response regarding differential gene expression in digestive cells. KEGG pathways in digestive gland and gills most affected by both exposures were NOD-like receptor signaling pathway, phagosome, apoptosis and ubiquitin mediated proteolysis.

Key words
Biomarkers; toxicity; coper; tralopyril; RNAseq

Type of presentation
Platform

Session
t07: Biotransformation pathways and mode of action (MoA) of chemical pathways.

T11: Aquaculture Environment interactions

Ecological risks assessment of sulfur and heavy metals in sediments in a historic mariculture environment, North Yellow Sea

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Abstract: The environment behaviors of sulfur and heavy metals in sediments is closely related to sediment aging in mariculture area. In this study, the distributions and ecological risks of reduced inorganic sulfur (RIS) and heavy metals were investigated, along with the relationships between different occurrences of RIS and heavy metals. The results indicated that the adequate organic matter in mariculture sediments significantly enhanced the accumulation of acid volatile sulfur (AVS) compared to the control area. In shellfish farming area, biological sedimentation contributed to accumulation of AVS. The chromium (II)-reducible sulfur (CRS) was the main component of RIS in mariculture area. The environmental risks of heavy metals in mariculture area presented low levels. Principal component analysis (PCA) showed that distribution of Cu closely related to mariculture activities compared to other heavy metals. For ecological risks of heavy metals, the ratio of ∑(acid-soluble fraction (F1)+ reducible fraction (F2)+oxidizable fraction (F3))/AVS was the appropriate index rather than conventional simultaneous extraction of heavy metals (SEM)/AVS, because SEM/AVS would overestimate the toxicity of heavy metals. AVS/RIS ratios significantly positively correlated with Pb (F2/(F1+F2+F3+ residual fraction (F4)), F2/∑F), Pb (F3/∑F), and Zn (F3/∑F), while significantly negatively correlated with Pb (F4/∑F) and Cu (F1/∑F). These results indicated that the accumulation of AVS during the mariculture process was conducive to the formation of F2 and F3 of Pb, and F3 of Zn, conversely to the formation for F4 of Pb and F1 of Cu, because it was opposite to the accumulation of CRS.

Key words:

Sediment; Reduced inorganic sulfur; Heavy metals; Ecological risks; Mariculture

Type of presentation
Platform.

Session
Aquaculture environment interactions.

SCIENTIFIC PROGRAM OF PRIMO 22

Aquaculture Environment interactions

(Posters)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Enhanced resilience of marine fish to extreme environments by nano-ZnO exposure

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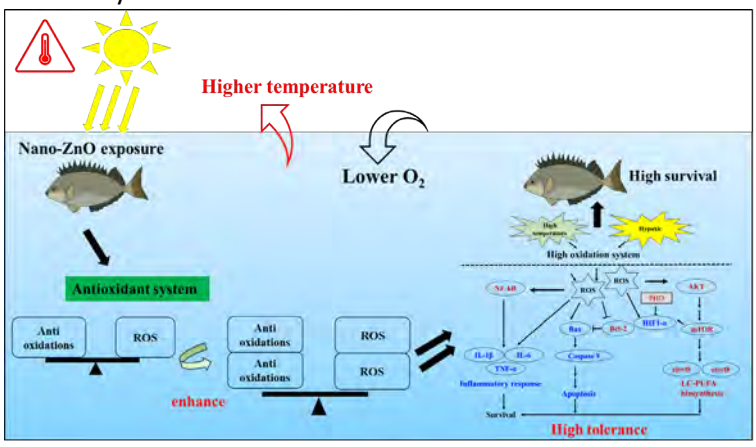
Marine hydrosphere is in grave danger if extreme weather conditions such as high temperatures and heat waves become the norm. Nano Zn oxides (*n*ZnO) functional characteristics might provide a "booster substance" that helps marine species to endure environmental stress. In the present study, rabbitfish *Siganus fuscessens* were exposed to different diets with $ZnSO_4$ and *n*ZnO at the optimum requirement levels (Zn content of 62.5 μg/g) and were then challenged with low dissolved oxygen concentration and higher temperature. We demonstrated that dietary *n*ZnO promoted the anti-oxidation defense system by improving the activities of antioxidant enzymes, i.e., catalase (CAT), superoxide dismutase (SOD) and the reduced glutathione/oxidized glutathione (GSH/GSSG) ratio without tissue damage. Compound stressors reduced the median survival duration of rabbitfish from 4.25 h to 2.5 h (dietary Zn^{2+} exposure) and 9 h to 5.75 h (dietary *n*ZnO exposure) compared to single environmental stress. *n*ZnO downregulated the HIF gene expression and increased the prolyl hydroxylase (PHD) activity in the liver. After 10 h of extreme environmental exposure, gene expression results indicated that *n*ZnO inhibited the NF-κB/IL-1β/IL-6 pathway and TNF-α/ Bcl-2/ Bax/ Caspase 9 pathway and activated the akt/mTOR/elovl5 and elovl8 pathway of rabbitfish to maintain the resilience of fish at extreme environement. These results strongly suggested that *n*ZnO may improve the anti-oxidation ability of fish to resist extreme environments.



Key words

*n*ZnO, anti-oxidative capacity, heat stress, hypoxia, lipid metabolism

Type of presentation

Oral

Session

Aquaculture environment interactions, Biotransformation pathways and model of action (MoA) of chemical pollutants, Acclimation and adaptation to chemical stress.

Mussel biofiltration of noxious chemical in an experimental aquaculture system

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Aquaculture stands as one of the most rapidly expanding sectors in food production globally, contributing slightly over half of the world's fish for human consumption (FAO, 2016). However, concerns regarding the environmental impact of aquaculture operations and the potential for sustainability persist. Coastal areas designated for aquaculture often experience compromised water quality due to human activities, such as wastewater drainage and discharge of aquaculture residues, resulting in ecosystem damage. In light of these concerns, the integration of effective remediation systems with aquaculture becomes imperative.

Previous studies have highlighted the role of mussels in reducing chlorophyll, phosphorus (P), and nitrogen (N) levels in salmon farming (Soto and Mena, 1999). Additionally, recent research has demonstrated their efficacy as biofilters for microplastics (Cole et al., 2023) and metals (Magni et al., 2015). This study aims to further explore the potential of mussels as biofilters in fish farming systems.

In initial exposures with mussels at a density of 1 individual per liter, results indicate varying removal efficiencies for different metals: lead exhibited a removal efficiency of 50% in less concentrated exposures (initial concentration 100 ppb) and 40% in more concentrated ones (initial concentration 1000 ppb); nickel displayed a removal efficiency of 40% in less concentrated exposures (initial concentration 10 ppb) but showed no significant removal in highly concentrated ones (initial concentration 100 ppb); cadmium demonstrated removal efficiencies of 30% and 10% in less and more concentrated exposures, respectively (initial concentration 10 ppb and 100 ppb). These initial experiments were conducted in the absence of algae.

Future efforts will explore potential variations in removal efficiency in the presence of various algae concentration in seawater and different mussel density and will involve testing in relevant environmental settings. This research is part of the Italian project Remote, Intelligent & Sustainable Aquaculture System for Fish – fish RISE, aimed at advancing aquaculture practices toward greater sustainability and environmental stewardship.

This work was performed within the framework of the “Blue growth”, FISHRISE Project No. ARS01_01053, Mininstry of Research and Education (MUR-Italy).

Key words
Mussel, biofiltration, chemicals, aquaculture

Type of presentation
poster

Session
First or second

Nanoplastics are accumulated in the gut and blood and induce a decrease in plasma cortisol levels

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Nanoplastics (NPs) are plastic particles of less than 1 µm in size formed in the environment by the degradation larger plastic waste. These particles can cross biological barriers, such as the gastrointestinal and brain blood barriers, which could make them the most dangerous among plastic debris. Studies on the effects of NPs have increased in recent years, with findings ranging from metabolism disruption to developmental and behavioural problems. However, very little information exists on their endocrine disrupting potential. The main objective of the present work was to evaluate if polystyrene-NPs (PS-NPs) are recognized as a stressor by the hypothalamus-pituitary-interrenal (HPI) axis of *Oncorhynchus mykiss* by measuring plasma cortisol levels. For this purpose, adult rainbow trout (50.2 g mean weight) were orally intubated with PS-NPs (44 nm, 100 µg/L, 1 mL per fish). After 96 h fish were sacrificed, and blood, liver and gut were sampled. Cortisol, intermediary and lipid metabolism (glucose, cholesterol, and triglycerides) biomarkers were determined in plasma after PS-NPs exposure. Additionally, quantification of PS-NPs levels was carried out in blood, gut, and liver of fish. Histology was done in the gut to assess damage. The results of the present study revealed that PS-NPs were detected and quantified in both blood and gut of exposed fish, but not in liver. Plasma cortisol levels showed a significant decrease in PS-NPs exposed fish when compared to control group. In fish, cortisol is involved in the stress response as well as in many aspects of the endocrine-mediated immune response and therefore the observed decrease could suggest an impairment of the HPI axis. Triglycerides levels in plasma were increased in the exposed individuals, which could point to altered lipid metabolism, potentially affecting the energetic status of rainbow trout. No histological alterations were found in gut.

Key words
Fish, nanoplastics, stress response, bioaccumulation

The possible hazard of ichthyotoxic microalgal blooms to French aquaculture: metabolic toxicity as a critical criterion determining the mechanism of action of *Karlodinium* toxins on *Crassostrea gigas*.

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Recently, harmful algal blooms (HABs) have become a major threat to coastal areas. For instance, *Karlodinium* HABs can produce ichthyotoxins with lytic activities, harming marine life and aquaculture. *Karlodinium* species are present in France, however, the toxicity of French strains upon shellfish production is unknown. We used hemocytes from oysters *Crassostrea gigas* to compare the toxicity of *Karlodinium* intra and extracellular extracts from six French strains and four ichthyotoxic strains from Spain, the US, and Australia. Strains KVEN CBC7, IFR-CC-2044, and MAR1F7 (*K. veneficum*, France), K-0668 (*K. armiger*, Spain), and CCMP2936 (*K. veneficum*, US) presented intra and extracellular extracts with lytic activity. They also revealed mitochondrial overactivity, including enhanced mitochondrial ROS and NADH production and higher mitochondrial membrane potential. The extracts from the French strains toxic to hemocytes were also toxic to oyster spermatozoa and embryos but not oocytes, suggesting that higher-metabolizing cells may be under greater stress. Cellular assays also indicated that the strains KGE-01C (*K. gentienii*, France), KVEN DIA2C2 (*K. veneficum*, France), and CCMP1975 (*K. veneficum*, US) induced mitochondrial stress but not cellular permeability. We propose for the first time that besides their predicted lytic action, *Karlodinium* compounds promote mitochondrial overactivity, inability to regulate ROS and ATP generation, and developmental toxicity in bivalves. These findings highlight the potential threats of *Karlodinium* HABs to French shellfish aquaculture.

Key words
Toxic microalgae; bivalves; mitochondria; development; toxicity screening.

Type of presentation
Platform.

Session
t11: Aquaculture Environment interactions

t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

SCIENTIFIC PROGRAM OF PRIMO 22

Acclimation and adaptation to chemical stress

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Unraveling Multigenerational Impacts: Persistent and Sex-Specific Behavioral Alterations in Atlantic Killifish Exposed to BDE-99

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Pollutants can have long-term impacts on health and wellbeing. These are poorly understood but may be important for risk assessment. Using Atlantic killifish (*Fundulus* heteroclitus), we investigated long-term and multigenerational effects of BDE-99, a ubiquitous polybrominated diphenyl ether (PBDE) flame retardant linked to adverse impacts on development and neurobehavior. Two exposure regimes were compared: maternal transfer and direct exposure. For maternal transfer, adult fish were exposed to BDE-99 through diet and spawned to produce parentally exposed (F1) offspring. Concurrently, with embryos from unexposed parents, aqueous exposures were conducted from 1 to 6 days post-fertilization (dpf). Parentally exposed F1 and directly exposed F0 embryos were raised under clean conditions and spawned to produce F2 and F1 generations, respectively. Photomotor response and anxiety-like behavior were assessed at various life stages across generations and exposure regimes. Parentally exposed F1 fish from BDE-99 lineages exhibited larval hypoactivity and reduced juvenile anxiety-like behavior, but these effects were not propagated to F2. In both parentally exposed and directly exposed embryos, behavioral alterations persisted into adulthood, with effects differing by sex. RNA-seq analyses of juvenile and adult brain tissues aim to elucidate molecular mechanisms associated with observed behavioral outcomes. Examining multigenerational consequences of BDE-99 will enrich our understanding of long-term implications and risks associated with these ubiquitous pollutants.

Key words
Multigenerational, PBDEs, Atlantic killifish, Behavior

Type of presentation
Oral preferred, but poster okay.

Session
1. Acclimation and Adaptation to Chemical Stress
2. EDCs & Neuroendocrine Effects

Investigating the adaptive mechanisms to ocean acidification in two calcifying mollusks from the CO_2 vent systems of Ischia (Italy)

Camilla Della Torre[1,2*], Silvia Giorgia Signorini[1,2], Fabio Crocetta[2], Lorenzo Federico[3], Lara Nigro[1], Ilaria D'Aniello[4], Marco Munari[2,4]

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Based on laboratory studies, ocean acidification (OA) has several detrimental effects across many taxa. However, different investigations performed in CO_2 vent systems demonstrated the ability of some species, including calcifying organisms, to survive even in the most acidified areas. Nonetheless, the molecular and physiological mechanisms underpinning tolerance to OA remain largely unknown.
In this study, specimens of *Mytilus galloprovincialis* (early recruited) and *Patella caerulea* (long-term resident) were collected along the pH gradient of the Castello Aragonese vent (8.1 - 7.7 - 7.4) and from a nearby control site in order to investigate the biochemical, molecular, and physiological mechanisms that allow survival and may be involved in acclimation/adaptation.
Biomarkers related to oxidative stress, energy metabolism and neurotoxicity, and physiological traits were assessed, together with an untargeted metabolomics analysis. Both species displayed slight oxidative stress conditions. *Mytilus galloprovincialis* exhibited a significant downregulation of aminoacids, nucleosides, lipids, carbohydrates, and several osmolites, and a significant decrease of glycogen content in specimens from the extreme acidified site compared to those from the ambient pH. Conversely, *P. caerulea* mainly showed an upregulation of metabolites, with a significant increase of carnitine and its metabolites.
Our study contributes to understand the mechanisms that promote tolerance to OA in marine invertebrates, and to predict the vulnerability of organisms in changing oceans.

Key words
Ocean acidification, oxidative stress, metabolomics, Mediterranean sea, Mollusca

Type of presentation
platform

Session
Acclimatation and adaptation to chemical stress

Assessing transgenerational effects of environmentally relevant concentrations of Bisphenol S in *Danio rerio*

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Bisphenol S (BPS), a substitute for Bisphenol A, has been detected in surface waters in the range of ng/L to low µg/L, due to its wide industrial applications, such as in polycarbonate plastics. The main objective of this study is to assess BPS risk in aquatic environments using a transgenerational exposure (F0-F3) to environmentally relevant levels (0.4, 2 and 10 µg/L BPS), with *Danio rerio* as the model organism. We characterized physiological (morphometric, reproductive and behavioural effects), biochemical (oxidative stress and neurotoxicity biomarkers) and molecular (transcriptomic analyses and epigenetic mechanisms - RNA-seq and DNA methylation, respectively) responses to disclose cause-effects relationships and contribute to the understanding of the underlying mechanisms behind the adverse physiological responses. Another aim was to identify whether the transmission of altered phenotypes is by paternal, maternal or both lines, by crossing exposed females with unexposed males and vice-versa (F0-F3). The present results showed several generational, intergenerational, and transgenerational effects at different levels of biological organization, after parental exposure to environmental relevant levels of BPS. Therefore, with this conceptual approach, we expect to contribute to the establishment of adverse outcome pathways for BPS.

This study is supported by TRANSEPIC (2022.02922.PTDC, doi: 10.54499/2022.02925.CEECIND/CP1728/CT0004), by MCIN/AEI/10.13039/501100011033 (PID2020-117686RB-C32) and Xunta de Galicia (ED431C 2021/06).

Key words
Contaminants of emerging concern; Bisphenol S; *Danio rerio*; Risk assessment; Transgenerational effects.

Type of presentation
Platform.

Session
T13: Acclimation and adaptation to chemical stress

T07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

T14: EDCs & Neuroendocrine Effects

Parental early exposure to environmentally realistic pesticide mixture drives offspring phenotype in a larger extent than direct exposure in the Pacific oyster.

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Many marine organisms exhibit external fertilization, thus expose early life stages to environmental stressors like pesticides. Among them, the Pacific oyster is an emblematic lophotrochozoan model in marine ecotoxicology. In the last decades, numerous studies highlighted the epigenetic outcomes of early exposures to xenobiotics on DNA methylation and their related delayed effects in several species, notably *C. gigas*. Because DNA methylation is an epigenetic mark implicated in organism development, and is meiotically heritable, it raises the question of the multigenerational consequences of the pesticide-induced alterations. Therefore, we performed a multigenerational exposure to an environmentally relevant mixture of 18 pesticides (nominal sum concentration: 2.85 µg·L−1) during embryo−larval stages (0−48 hpf) of a second generation (F1) for which parents where already exposed or not in F0. We performed DNA methylation, gene expression and SNP analyses, as well as, phenotypic analyses among the whole life-cycle. The study revealed that parental (F0) exposure to the pesticide mixture has a greater influence on the offspring (F1) phenotype than the direct exposure. Multigenerational effects were revealed at phenotypic endpoints, i.e. individuals descending from exposed parents were less able to metamorphose under epinephrine stimulation. At the molecular level, RNA-seq and Methyl-seq data analyses performed in gastrula embryos and metamorphosis-competent pediveliger (MCP) larvae revealed a clear F0 treatment-dependent discrimination. F1:F0 treatments interaction patterns were observed in genes related to shell formation and immunity (i.e. *Calm* and *Myd88*), suggesting adaptive mechanisms. Those results suggest that low environmental pesticide contamination can alter organisms beyond the individual scale level and have long-term adaptive implications that we should consider in the environmental risk assessment framework.

Key words: epigenetics, gene expression, molluscs, multigenerational, contaminant cocktail

Type of presentation: platform

Session: Acclimation and adaptation to chemical stress or mixture effects of pollutants

An epigenetic memory at *CYP1A* protects against PAH cancer

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Polycyclic aromatic hydrocarbons (PAH) are toxic combustion byproducts, some of which activate the aryl hydrocarbon receptor (AHR). Some PAHs are metabolically activated by AHR-inducible cytochrome P450 1 (CYP1) enzymes to form carcinogenic DNA adducts. We have characterized an epigenetic memory at the *cytochrome P450 1A* (*CYP1A*) gene in wild Atlantic killifish (*Fundulus heteroclitus*) that have adapted to chronic, extreme PAH pollution. *CYP1A* is normally highly induced by the AHR in response to PAH. In PAH-tolerant fish, *CYP1A* induction is blunted, which protects them from cancer, but this phenotype is partially reversible. Here, we show that the *CYP1A* promoter-enhancer contains an environmentally responsive bivalent domain (BD) comprising both activating and repressive histone post-translational modifications. Altered BD regulation corresponds with blunted *CYP1A* induction in tolerant fish. In addition, repeated PAH hits trigger a secondary memory in tolerant adult fish in which *CYP1A* is inefficiently shut off after each hit, leading to progressive loss of epigenetic control and upward drift of *CYP1A* expression. We also evaluated DNA methylation (DNAm) responses to PAHs. AHR pathway genes contained more DNAm changes than expected by chance in both populations. Sensitive fish had a higher active mutation rate at CpG sites and more differentially methylated CpGs in response to PAH than tolerant fish, indicating that loss of CpG sites and epigenetic plasticity is a cost of PAH tolerance. These data support a strong role for altered epigenetic regulation in PAH tolerance.

Key words
Polycyclic aromatic hydrocarbons, epigenetics, chromatin

Type of presentation
Platform or Poster

Session
Acclimation and adaptation to chemical stress

How do environmental concentrations of metformin impact zebrafish metabolism, growth, reproduction and epigenetic machinery?

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Metformin (MET) is an antidiabetic drug used worldwide for the treatment of type-2 diabetes mellitus. Therefore, in the past few years, it has been detected in surface waters at increasing concentrations, from ng/L to low µg/L, raising concerns regarding its potential risk to non-target organisms. Given that the long-term effects of MET are still poorly understood, the present study aimed at investigating its generational (F0) and intergenerational (F1) effects at concentrations ranging from 390 to 14423ng/L in *Danio rerio*. By integrating several apical endpoints (survival, growth, and reproduction), with molecular (qRT-PCR and RNA-seq) and biochemical (mitochondrial complex I activity and lipid content) analyses, it was possible to obtain further insights into MET's underlying mode of action.

Multiple biological functions were found to be affected by MET long-term exposure, including metabolism, growth, reproduction and differential expression of epigenetic genes. Moreover, compelling evidences from our findings suggest that MET acts as an endocrine-disrupting chemical and has the ability to disrupt zebrafish epigenetic machinery, potentially affecting future generations.

Acknowledgements: This study was developed under the project TRANSEPIC ([Reference: 2022.02922.PTDC, doi:10.54499/2022.02925.CEECIND/CP1728/CT0004], financed by the Portuguese Foundation for Science and Technology (FCT). RM, RR and JBQ acknoweldge funding provided by MCIN/AEI/10.13039/501100011033 (PID2020-117686RB-C32) and Xunta de Galicia (ED431C 2021/06).

Key words
Danio rerio, Metformin, Long-term Exposure, Reproduction, Epigenetic Modifications

Type of presentation
Platform.

Session
t13: Acclimation and adaptation to chemical stress

t07: Biotransformation pathways and mode of action (MoA) of chemical pollutants

t14: EDCs & Neuroendocrine Effects

Amelioration of Transgenerational Liver Disease by an Epigenetic Modifier Treatment

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Abstract

Epigenetic inheritance of environmentally and lifestyle-induced phenotypes has been demonstrated in many model organisms in a laboratory setting, suggesting that current generations could be harboring ancestral exposure-induced molecular memories, or future generations will be carrying such memories due to exposure at the current generation. Ancestral exposure-related transgenerational traits may be detrimental at the population level if occurring in a natural population. It is essential to develop strategies to correct transgenerational abnormalities before they are transferred to offspring via germline transmission or to block the heritable pathways leading to the abnormal health phenotype before they induce the disease. As a proof of concept, using medaka fish as an aquatic model organism of human and ecosystem health, we investigated whether the environmentally induced transgenerational non-alcoholic fatty liver disease can be reversed to normal by epigenetic modifiers targeting epigenetic hotspots of liver differentiation and NAFLD development. Ancestral bisphenol A (BPA) exposure from the day of fertilization through 15 days after fertilization at F0 generation led to transgenerational NAFLD leading to nonalcoholic steatohepatitis (NASH) at F2 generation, which persisted for five generations. The intergenerational transmission of the disease trait was mediated by both male and female germlines. The severity of the disease was sexually dimorphic- with females affected more than males. Past F2 generation, the BPA-exposure-initiated transgenerational NAFLD started after puberty, developed in adulthood, and progressed toward NASH as the fish aged. We performed transcriptomic, metabolomic, and methylome analyses to understand the mechanisms involved. An integrated multi-omics analysis revealed impairments in metabolic pathways involved in proline, tryptophan, and bile acids. A larger percentage of BPA-induced transgenerational differentially methylated regions (DMRs) present in the NAFLD liver at the F2 generation were removed in the F4 generation, but the phenotype in the F4 generation persisted with comparatively reduced severity. Treatment of the F4 generation BPA-lineage fish with vitamin C resulted in a) correction of the majority of BPA-induced DMRs, irrespective of their methylation status, b) restoration of the DNA methylation signatures in the BPA-lineage livers comparable to that in control livers, and c) blockade of development and progression of NAFLD in adulthood in F4 generation fish leading to restoration of normal liver health from transgenerational injury caused by BPA exposure. The present results suggest that low-concentration epigenetic erasures may be used to correct BPA-induced transgenerational liver injury. The potential of other epigenetic modifiers in reversing BPA-induced intra-, inter-, and transgenerational DMRs and correcting liver and other injuries is under investigation.

Acknowledgment: This study was supported by funds from the National Institutes of Health of the United States to RKB.

SCIENTIFIC PROGRAM OF PRIMO 22

Acclimation and adaptation to chemical stress

(Posters)

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NANTES FRANCE

Development of a chronic toxicity bioassay in the larvae of the shrimp *Palaemon serratus*

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In marine decapods, most bioassays focus on acute toxicity in adult stages. Data on chronic toxicity are limited, particularly for the early life stages, which are a critical phase for organisms as they are particularly sensitive to pollutants and play a key role in population dynamics. In this context, the present study aims to develop a bioassay on *Palaemon serratus* larvae. This shrimp is native to the western Atlantic coast of Europe and presents an important ecological and socio-economic interest. Based on previous work, we consider the entire development, from hatching to larval metamorphosis, with regular and precise observations of the development. In this way, we monitored three times a week larval stage succession, feeding rates, extra larval stage events and survival. We also determined developmental duration, juvenile energy reserves, and metamorphosis success. The experimental design was conceived to incorporate a large number of chemical/contaminant concentrations, and a large number of replicates, to provide a basis for further statistical works on design optimization. This study will then allow us to study variability between populations and within populations. Larvae from several females were grouped (n=50 per group) and then exposed to a gradient of 8 concentrations. This experimental set-up was tested in the context of exposure to a model contaminant, the juvenoid insecticide Fenoxycarb (*i.e.* an inhibitor of arthropod metamorphosis).

Keywords
Bioassay - Crustacean – Early life stage – Ecotoxicology - Larval stage

Type of presentation
Poster

Session
t13: Acclimation and adaptation to chemical stress
t04: New approach methodologies (NAMs) to assess pollutant toxicity

Does direct detection of mitochondrial DNA damage protect against mitochondrial DNA mutagenesis?

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Key words
Mitochondrial DNA damage, mitochondrial DNA mutagenesis, mitophagy

Type of presentation
No preference between poster or platform

Session
t13: Acclimation and adaptation to chemical stress

Abstract

The mitochondrial genome (mtDNA) mutates more rapidly than the nuclear genome both in somatic tissues with age, and over evolutionary time, in all species examined. mtDNA is more susceptible to damage from exogenous stressors than nDNA and lacks some DNA repair pathways. Further, there is little evidence that cells can directly sense mtDNA damage. Nonetheless, there is very limited evidence for environmental genotoxins driving mtDNA mutagenesis, in contrast to nuclear DNA mutagenesis, Using *Caenorhabditis elegans*, cell culture, and *in vitro* biochemical techniques including protein-binding microarrays and atomic force microscopy, we have been testing whether cells can detect irreparable mtDNA damage, whether such damage causes mutagenesis, whether damaged mtDNAs are digested by mitophagy or exported from the cell, and whether these non-repair mechanisms for reducing the cellular burden of mtDNA damage protect against mutagenesis. We found that *C. elegans* is remarkably resistant to mtDNA mutagenesis after exposure to aflatoxin B_1 and cadmium, even with mutations in the mitophagy genes *dct-1* and *pink-1*. We also found that the mitochondrial protein TFAM binds differently to mtDNA after ultraviolet C radiation-induced damage, that cells respond to such damage by increasing mtDNA replication and transcription, and that *C. elegans* responds to such damage by exporting mtDNA and inducing mitophagy. These results inform a current model that we are testing, in which TFAM sequesters irreparably damaged mtDNA, permitting its selective degradation, thus preventing mutagenesis.

MANILA project: testing innovative strategies to mitigate shellfish vulnerability to climate change and pollutants.

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Sessile filter-feeders as bivalves are increasingly subjected to multiple co-occurring stressors deriving from marine heatwaves events and chemical pollution, which may promote biodiversity loss and socio-economic impacts on aquaculture, as the case of Manila clam, *Ruditapes philippinarum* in Adriatic Sea: indeed, several mass mortality events related to the persistence of harsh stressful environmental conditions caused a dramatic decrease of clams' stock availability in recent years. The project MANILA intends to provide novel strategies to ameliorate Manila clam performance under multiple stressful conditions, using innovative tools as thermal and chemical priming and microbiota manipulation, with the final aim to explore biodiversity conservation and sustainable aquaculture management strategies. Priming refers to a process where exposure to mild/sub-lethal stress or stimuli triggers a heightened response that enhances the organism's ability to withstand subsequent, more severe stressors. In this study, a wide panel of key molecular, biological and cellular processes are investigated in chemically- and thermally-primed Manila clams, to explore the efficiency of these techniques to ameliorate the performance of this species under multiple stressors. Overall, MANILA project will provide a new point of view on actions/interactions of biological processes involved in responsiveness and adaptation to environmental changes, opening new scenarios for research in ecology, ecotoxicology, biology, and for sustainable bivalve aquaculture management.

Key words
priming, heatwaves, *Ruditapes philippinarum*, biological effects, multiple stressors.

Type of presentation
Poster

Session
t13: Acclimation and adaptation to chemical stress

Variability of responses to multiple chemical stressors in invasive mosquitofish

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Rapid adaptation to pollution in wildlife is still debated. Recent studies document fish adaptation to dioxin like pollutants, but a limited number of empirical studies in realistic environmental conditions are available so far. In addition, chemical pollution in aquatic environment is frequently characterized by a cocktail of chemicals including several historical and emerging compounds at sub-lethal concentrations, which could complicate the evolutionary outcome of pollution exposure in the wild. In this study, we evaluated the phenotypic divergence of wild populations of invasive mosquitofish (*Gambusia holbrooki*) exposed to gradients of multiple chemical stressors in French rivers. In addition, we tested the inter-populational divergence in resistance to an experimental exposure to a cocktail of non-persistent pollutants to test for potential local adaptation to pollution. We hypothesized that increased tolerance to pollutants in fish living in polluted streams would be associated with: (i) no or moderate detrimental effects of chemical stressors on fish health, due to increased detoxication metabolism and/or higher non-enzymatic antioxidant defenses and (ii) a higher resistance to an experimental exposure to a realistic cocktail of pollutants. First, we performed an extensive field study in 12 populations and compared the phenotype of F0 fish living along gradients of chronic pollution. Second, we experimentally tested the survival of their F1 offspring to an experimental cocktail of non-persistent pollutants (pesticides-pharmaceutics). Results show that F0 fish from highly polluted sites had an increase biotransformation activity and a decrease non-enzymatic antioxidant capacity associated with limited oxidative damages. In addition, their F1 offspring had a higher survival when experimentally exposed to a realistic cocktail of pollutants, revealing a higher resistance of juveniles coming from the most exposed populations. Further studies are now necessary to investigate the underlying genetic and epigenetic mechanisms explaining this phenotypic divergence.

Key words
phenotypic divergence, tolerance, cocktail of pollutants, mosquitofish, acclimation

Type of presentation
Poster

Session
Acclimation and adaptation to chemical stress

SCIENTIFIC PROGRAM OF PRIMO 22

Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

(Oral talks)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

The influence of temperature on the effects of lead and lithium *in Mytilus galloprovincialis* through biochemical, cell and tissue levels: Comparison between mono and multi-element exposures

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Lead (Pb) and lithium (Li) are metals which have been detected in the environment and, at high concentrations, can induce toxic effects that disturb the growth, metabolism or reproduction of organisms along the entire trophic chain. The impacts of these metals have scarcely been investigated using marine bivalves, especially when acting as a mixture. The present study aimed to investigate the influence of temperature on the ecotoxicological effects caused by Pb and Li, acting alone and as a mixture, on the mussel species *Mytilus galloprovincialis* after 28 days of exposure. The impacts were evaluated under actual (17 °C) and projected (+4 °C) warming conditions, to understand the influence of temperature rise on the effects of the metals (both acting alone or as a mixture). The results obtained showed that the increased temperature did not influence the accumulation of metals. However, the biomarkers evaluated showed greater responses in mussels that are exposed to metals under increased temperature (21 °C). The IBR index showed that there is a comparable toxic effect of Li and Pb separately, while exposure to a mixture of both pollutants causes a significantly higher stress response. Overall, the results obtained revealed that temperature may cause extra stress on the mussels and exposure to the metal mixture caused the greatest impacts compared to each metal acting alone.

Key words
Bivalves; Multiple stressors; Warming; Metals; Integrated biomarker response.

Type of presentation
Platform.

Session
Impact of Climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems.

Innovative ecologically realistic study of multiple stressors, marine heatwaves and microplastics, on biological response of *Crassostrea gigas*

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Abstract : Microplastics (MPs) and rising marine seawater temperatures are one of the major environmental problems threatening the survival of marine organisms and biodiversity. However, interactions between such multiple stressors are almost unexplored. In this study, we have assessed the combined effect of marine heatwaves (MHWs) and polyethylene-type MPs on pacific oyster (*Crassostrea gigas*), one of the most globalized mollusc species for aquaculture. We realized an experimentation in mesocosm preceded by a 7-day acclimatization period and a 14-day laboratory exposure with two environmental concentrations of MPs (0 and 0.01 mg.L-1) and two temperatures (15°C and 22°C). The aim of this study was to evaluate how *C. gigas* response to these multiple stressors. We studied the responsiveness of various biological parameters including laccase (LAC), superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity as well as the malondialdehyde (MDA) concentration in the digestive gland of *C. gigas*. Our results show that MPs concentration and temperature significantly influenced *C. gigas* survival rate, both alone and in interaction. It also emerged that combined effects of MPs and MHWs can interfere significantly with the stress marker studied. In conclusion, these data give us knowledge about the combine effects of MPs pollution and MHWs on *C. gigas* and highlight the potential ecological risk posed by these two factors on organisms in the marine environment.

Rather resilient then stressed: cellular response of ragworms (*Hediste diversicolor*) to high environmental ammonia and low oxygen conditions

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The Baltic Sea faces deoxygenation and nutrient pollution due to agricultural and industrial run-off, as well as potential eutrophication due to recent coastal habitat restoration such as coastal realignment or rewetting. Ammonia is one of the most potent nutrient pollutants, being directly toxic to biota and having indirectly the ability to promote deoxygenation via microalgae blooms. High ammonia and low oxygen conditions affect the physiology of marine biota via oxidative stress, altered metabolic rate, cell damage and energy deficiency, impairing their survival and ecological performance.
We investigated in an experimental set-up the single and combined effects of high environmental ammonia and low oxygen conditions on the bioturbator *Hediste diversicolor*. After 21 days of exposure, we examined cellular damage, anti-oxidative capacity, and survival.
Our results show that the ragworm can cope with environmentally realistic ammonia and low oxygen concentrations. The cellular damage was not pronounced and probably a result of effective defense mechanisms against reactive oxygen species stress. However, this species showed reduced survival due to very high environmental ammonia in combination with low oxygen conditions. The tolerance of *H. diversicolor* to ammonia and oxygen deficiency might reflect adaptation to life in organic-rich sediments that often experience natural deoxygenation. Therefore, this polychaete is a successful colonizer of eutrophicated habitats and could be an important pioneer species for the natural succession and transformation of restored coastal areas.

Key words
Ecophysiology, eutrophication, deoxygenation, polychaetes

Type of presentation
Talk

Session
Mixture Effects of Pollutants

Interactive Effects of Climate and Chemical Contaminants in Estuarine Systems

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The cumulative and interactive stressors of chemical contaminants and environmental factors are especially relevant in estuaries where tidal fluctuations cause wide variability in water quality parameters. Toxicity testing for environmental regulation typically adheres to a standard set of abiotic parameters, which may not be predictive under variable climate conditions. This presentation summarizes results of laboratory toxicity testing with multiple classes of chemicals (oil, oil spill dispersants, pesticides, PFAS) under a range of testing conditions (temperature, salinity, dissolved oxygen, pH, UV light). The species selected for testing represent common fish and invertebrates of Southeastern U.S. tidal systems. While these assessments revealed that the nature of the interaction between climate variables and chemical pollution will depend on estuarine species, life stage and contaminant class, some general trends were observed. Most species tested were more sensitive to selected pesticides, oil, and oil dispersants under elevated temperatures and under lower salinity conditions. PFOS was more toxic to some species under elevated temperatures and lower salinity. Hypoxia and hypercapnia exacerbated toxicity of the pyrethroid insecticide resmethrin to larval hard clams. UV light increased the toxicity of crude oil in all species tested. These findings suggest that expanding toxicity testing to include a wider range of parameters will improve environmental risk assessment of chemical contaminants and management capabilities under changing climate conditions.

Key Words: Climate, Toxicity, Estuarine, Fish, Invertebrates

Type of presentation: Platform

Session: Impact of Climate Change on the Ecodynamics of Legacy and Emerging Pollutants in Marine Ecosystems

Single and combined effects of increased temperature and methylmercury on different stages of the marine rotifer Brachionus plicatilis

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Rapid, anthropogenic activity–induced global warming is a severe problem that not only raises water temperatures but also shifts aquatic environments by increasing the bioavailability of heavy metals (HMs), with potentially complicated effects on aquatic organisms, including small aquatic invertebrates. For this paper, we investigated the combined effects of temperature (23 and 28 °C) and methylmercury (MeHg) by measuring physiological changes, bioaccumulation, oxidative stress, antioxidants, and the mitogen-activated protein kinase signaling pathway in the marine rotifer *Brachionus plicatilis*. High temperature and MeHg adversely affected the survival rate, lifespan, and population of rotifers, and bioaccumulation, oxidative stress, and biochemical reactions depended on the developmental stage, with neonates showing higher susceptibility than adults. These findings demonstrate that increased temperature enhances potentially toxic effects from MeHg, and susceptibility differs with the developmental stage. This study provides a comprehensive understanding of the combined effects of elevated temperature and MeHg on rotifers.

Key words
Global warming, Methylmercury, Oxidative stress, MAPK signaling pathway, *Brachionus plicatilis*

Type of presentation
platform

Session
Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

The multistressor effect of ocean acidification, microplastic and lanthanum on sea urchin *Arbacia lixula*

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Ocean acidification (OA), microplastics (MP) and lanthanum (La) are of importance due to their increasing trends and effects on marine species. This study investigates the single and combined effects of those stressors (low pH: pH_T: 7,45, polyethylene MP: 26 µg L^{-1} and La: 9 µg L^{-1}) on the physiology, histology and transcriptomics of sea urchin *Arbacia lixula*. The stressors did not lead to mortality in sea urchins. The physiological and histological effects of OA, MP and La on the sea urchins were evaluated by considering respiration rate, the quantity and viability of coelomocytes, and histopathology. The respiration rate was not impacted by the stressors. Although the total coelomocytes were slightly affected by exposure to the stressors, coelomocyte viability was the most affected. The coelomocyte viability was suppressed most in the triple stressor group (OA-MP-La). According to the histological examination results, crypt lengths were shorter and epithelial layers were thinner in the OA-La, MP, MP-La and OA groups compared to the control group. These results indicated an atrophy in the crypt structure. Following de novo transcriptome assembly, annotation, and differential expression analysis, various genes were found to respond to environmental stressors, unlike the control group. Functional enrichment analysis showed that these genes were functionally clustered in signal transduction, ion transport, apoptosis, heat stress, glycerolipid metabolism, and glutathione metabolism.

Key words
Ocean acidification, microplastic, lanthanum, sea urchin.

Type of presentation
Oral presentation

Session
1. Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems.
2. Mixture effects of pollutants.

The impacts of carbamazepine and temperature on the diurnal and nocturnal behaviour of yellow perch larvae

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The yellow perch (*Perca flavescens*) is a freshwater perciform that breeds in cool water. Recently, perch populations have declined in the Great Lakes. Climate change may pose a risk to fish species; expected increases are ~3°C over the next 2 decades. Rearing temperature can lead to substantial changes in behavioural performance at post-hatch stages in the yellow perch. Perch larvae exhibit increased movements at night, prompting us to measure both diurnal and nocturnal behaviour. The anticonvulsant carbamazepine is one of the most frequently detected pharmaceuticals in freshwater and induces behavioural responses in zebrafish. We tested the hypothesis that rearing temperature would alter the behavioural response of larvae to carbamazepine. Yellow perch were reared at 12°C, 15°C, and 18°C until hatch. Following hatch, fish were slowly raised over 7 days to a common garden temperature (18°C). We assessed fish behaviour at 1, 5 and 10 day post hatch (DPH); timepoints that are the onset of movement, transition to light-preference, and a critical stage for recruitment. Perch were exposed to either 0, 0.5, or 10 µg/L carbamazepine and immediately assessed for behaviour during peak activity periods. Carbamazepine induced hyperactivity in 1DPH larvae, but only at night, suggesting a time-based sensitivity to contaminants. At 5 DPH, only fish reared in 15 °C were susceptible to exposure with more active animals. This work highlights the need to consider environmental temperature and climate change in toxicity studies.

Key words
Yellow perch, carbamazepine, climate change, behaviour

Type of presentation
Platform

Session
Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

Impact of pollutant mixtures and climate change on *Paracentrotus lividus* embryos and larvae: biological and biochemical markers of toxicity

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This work assesses the impact of a combination of stressors and pollutants commonly found in the ocean on the model species *Paracentrotus lividus*. We studied how chlorpyrifos (CPF) and microplastics (MP), alone or in combination, impact the fertilisation, the development and biochemical biomarkers (GST, GRx, AChE) of larvae under conditions of Ocean Acidification (OA) and Ocean Warming (OW). Results showed that CPF has a marked effect on larvae growth, but less on the fertilisation rate. When embryos are exposed to both MP and CPF, the effect on fertilisation and growth is higher than when CPF is added alone. Temperature has more influence when embryos or larvae are exposed to a combination of stressors. GST activity was induced by thermal stress and low concentrations of CPF (Effective Concentration at 10%; EC10), but inhibited by OA. At high CPF concentration (EC50), GST activity was inhibited, contradicting the induction observed at EC10, suggesting a complex relationship between environmental stressors and detoxification capacity. At EC10, all treatments subjected to thermal stress showed higher levels of GRx activity. AChE activity increases with the increase of temperature at EC10 of CPF, and this could be associated with a status of oxidative stress. In this work we demonstrated that global climate change conditions increase the sensitivity of embryos to MP and CPF. Global change conditions could have a severe impact at different organizational levels on marine life, increasing the negative effect of pollutant mixtures commonly present in the marine environment.

Key words
Biomarkers; Microplastics; Ocean Acidification; Ocean Warming; Pollutant Mixtures.

Type of presentation
Platform.

Session
t01: Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems.

t08: Mixture effects of pollutants.

Pharmaceutical pollution under a changing climate: Unravelling the ecotoxicological effects of carbamazepine in marine mussels *Mytilus galloprovincialis*

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Pharmaceutical pollution is considered one of the most pressing challenges of the Anthropocene with direct and indirect consequences for marine and coastal ecosystems. Yet, climate change exacerbates the interplay of multiple stressors through increased seawater temperature and salinity fluctuations, leading to unknown impacts on the marine biota. Therefore, there is an emerging need to understand the detrimental biological effects of these forthcoming interactions. The antiepileptic carbamazepine (CBZ) has been detected in different marine matrices with significant biological responses to concentrations up to 1 µg/L. Thus, the present study aimed to assess the effects of CBZ combined with a rise in seawater temperature and salinity. Mediterranean mussels *Mytilus galloprovincialis* were exposed to CBZ (5 µg/L) in a current environmental (temperature 17 °C and 35 salinity) and climate change (23 °C and 40) conditions' over 28 days. Thus, a multiple biomarker approach was explored, including energy and biotransformation metabolism, antioxidant activity, neurotoxicity, oxidative damage, and genotoxicity. Results revealed that climate change stressors increase the ecotoxicological effects of CBZ in marine mussels. Given the importance of *M. galloprovincialis* in ecosystem functioning and its economic relevance, the increasing concentrations of pharmaceuticals in environmental compartments, and the potential exacerbation of threats posed by climate change, additional long-term studies are recommended to unravel the ecological consequences of predicted scenarios.

Key words
Pharmaceuticals; Multiple stressors; Biochemical biomarkers; Genotoxicity; Mussels.

Type of presentation
Platform: oral presentation

Session
T01: Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

SCIENTIFIC PROGRAM OF PRIMO 22

Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

(Posters)

PRIMO22

26-29 MAY 2024

NANTES FRANCE

Combined effects of temperature and copper exposure to shrimp *Palaemon serratus* in the context of global change

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The shrimp *Palaemon serratus* is a high commercially value species fished by professional and recreative fishermen, and living among algae and on the rocky shores in Normandy, France. However, this species is affected by anthropic activities, and for two decades the populations show high fluctuations and tend to decrease, justifying the European Project GeDuBouq (2020-23). In the frame of this project, we investigated the effects of copper on shrimps brought to the lab and maintained at 3 temperatures: 15 vs 22 and 24°C. For 21 days, 8 shrimps were exposed to 3 concentrations plus control (10, 100 and 1000 µg L^{-1} of Cu; 12 conditions), and this experiment was duplicated (January and October 2022). Mortality, molting and behaviour (activity and localisation in aquaria, reaction to a stimulus and to food) were daily or every other day checked whereas other biomarkers were studied at T0 and after 21d: amount of glycogen in muscle, malondialdehyde (MDA) and proteins in the digestive gland and hemocyte phagocytic activity.

The results showed that a concentration of 1000 µg L^{-1} of Cu was lethal, and that a high temperature accelerated mortality and the molting rate. Moreover, a high temperature significantly increased the shrimp activity and their ability to quickly react to stimulus and food while their behavioural responses were more variable according to copper concentrations. Temperature had very slight effects on the biochemical biomarkers; by contrast, copper led, for example, to a lower amount of MDA in the digestive gland and also a stimulation of the phagocytic activity.

Key words

Palaemon serratus, copper, temperature, behaviour, biomarkers

Type of presentation

Poster

Session

Acclimatation and adaptation to chemical stress

Effects of ocean acidification and warming on Mediterranean gorgonians: physiological, transcriptomic, and epigenetic responses

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The current rate of climate change may outweigh marine metazoans' ability to adapt to future conditions. Acidification and marine heat waves threaten the Mediterranean gorgonian corals. Phenotypic plasticity is critical for slow-growing gorgonians as adaptation through natural selection might not be fast enough to cope with rapid environmental changes. Epigenetic mechanisms such as DNAm (DNA methylation) could offer gorgonians greater ability to buffer the impacts of environmental changes by fine-tuning gene expression in response to warming and acidification. The aim of this study is to assess the physiological responses of the gorgonians to warming, acidification and their combination over two weeks to understand the underlying transcriptome and epigenetic mechanisms of phenotypic plasticity.

In controlled lab conditions, gorgonian corals (*Eunicella singularis* and *Paramuricea clavata*) were exposed to low pH (-0.35 units) and high temperature (+ 4℃), either alone or in combination. Low pH, high temperature and their combination did not lead to necrosis or mortality. Oxygen consumption rate and polyp activity in *E. singularis* significantly affected by the interaction between pH and temperature. High temperature induced up/down regulation in transcripts (De novo transcriptome assembly) involves intracellular protein transport, protein cargo traffic, protein export, protease and isomerase activity. There was no significant effect of low pH, warming and their interactions on global DNA methylation in *E. singularis*. Oxygen consumption rate in *P. clavata* was not significantly affected by pH, temp and their interaction. We suggest that acidification and warming alter the physiological and transcriptome responses of gorgonians.

Key words
Gorgonian coral, acidification, warming, physiology

Type of presentation
Platform (Oral)

Session
Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

Multigenerational effects of elevated temperature on host-microbiota interactions in the marine water flea Diaphanosoma celebensis exposed to micro- and nanoplastics

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Rising ocean temperatures are driving unprecedented changes in global marine ecosystems. Meanwhile, there is growing concern about microplastic and nanoplastic (MNP) contamination, which can endanger marine organisms. Increasing ocean warming (OW) and plastic pollution inevitably cause marine organisms to interact with MNPs, but relevant studies remain sparse. Here, we investigated the interplay between ocean warming and MNP in the marine water flea *Diaphanosoma celebensis*. We found that combined exposure to MNPs and OW induced reproductive failure in the F2 generation. In particular, the combined effects of OW and MNPs on the F2 generation were associated with key genes related to reproduction and stress response. Moreover, populations of predatory bacteria were significantly larger under OW and MNP conditions during F2 generations, suggesting a potential link between altered microbiota and host fitness. These results were supported by a host transcriptome and microbiota interaction analysis. This research sheds light on the complex interplay between environmental stressors, their multigenerational effects on marine organisms, and the function of the microbiome.

Key words
Nanoplastics, Ocean warming, Combined effects, Marine water flea, Microbiome

Type of presentation
poster

Session
Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

Synergistic adverse effects of microfibers and freshwater acidification on host-microbiota interactions in the water flea Daphnia magna

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Microfibers are the most common type of microplastics in freshwater environments. Anthropogenic climate stressors, such as freshwater acidification (FA), can interact with plastic pollution to disrupt freshwater ecosystems. However, the underlying mechanisms responsible for the interactive effects of microfibers and FA on aquatic organisms remain poorly understood. In this study, we investigated individual *Daphnia magna*–microbiota interactions affected by interactions between microfibers and FA (MFA). We found that the accumulated amount of microfibers in pH-treatment groups was significantly higher than in the control groups, resulting in negative consequences on reproduction, growth, and sex ratio. We also observed that MFA interactions induced immunity- and reproduction-related biological processes. In particular, the abundance of pathogenic bacteria increased only in MFA groups, indicating that MFA interactions can cause intestinal damage. Our integrated analysis of microbiomes and host transcriptomes revealed that synergistic adverse effects of MFAs are closely related to changes in microbial communities, suggesting that *D. magna* fitness and the microbial community are causally linked. These finding may help elucidate the toxicity mechanisms governing the responses of *D. magna* to microfibers and acidification interactions, and to host-microbiome-environment interactions.

Key words
Microfiber, Freshwater acidification, *Daphnia magna*, Microbiome, Host-microbiome interactions

Type of presentation
poster

Session
Impact of climate change on the ecodynamics of legacy and emerging pollutants in marine ecosystems

The effects of ocean warming on the biochemical and transcriptional responses of Mediterranean mussels exposed to the fluoroquinolone antibiotic enrofloxacin

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This study aimed to evaluate cellular responses in the mussel *Mytilus galloprovincialis* exposed to environmentally relevant concentrations of the fluoroquinolone antibiotic enrofloxacin (ENR) under ambient and predicted warming conditions (Fig. 1). Responses were assessed in mussels' gills after a 14-day exposure period (EXP) followed by a 14-day recovery period (REC) through a multi-biomarker approach and gene expression analysis.

Detectable ENR concentrations were measured in mussels' whole tissue only after EXP at 500 ng/L, with no differences observed across temperatures. Results indicated that uncontaminated mussels exposed to 25°C exhibited biochemical impairments, including reduced metabolic capacity, elevated energy reserve utilization, and increased detoxification mechanisms. Similar patterns were observed in ENR-exposed mussels at both 20 and 25°C, together with a decreased antioxidant activity. After REC, mussels restored their metabolic capacity, energy reserves, and antioxidant defenses, gradually returning to control conditions, except for GST, which remained elevated. RT-PCR revealed variations in gene expression due to ENR exposure and temperature conditions. The selected genes of interest (GOIs) displayed upregulated (i.e. Catalase, SOD, GST, Isocitrate dehydrogenase) and downregulated (i.e. ABCB, Caspase, P53, CYP4y1, PKPYR) expression, although statistical significance was achieved only in some cases. Overall, these findings indicated that both stressors, acting alone or in combination, may represent a risk for marine bivalves like mussels.

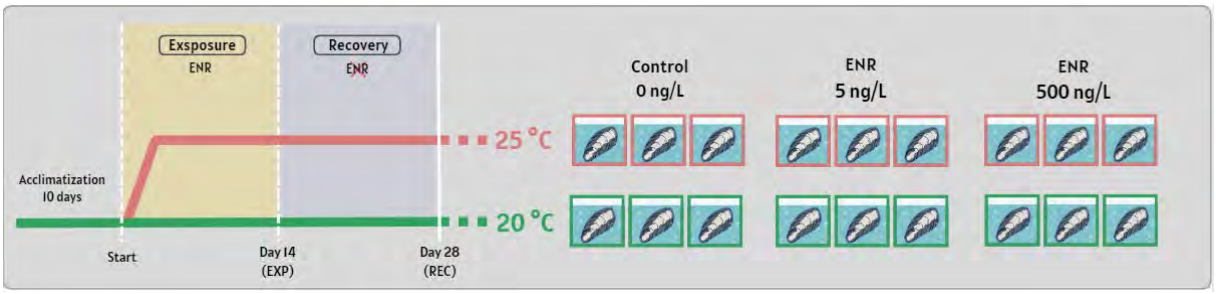

Figure 1: Representation of experimental setup. Tested temperature (20 vs 25 °C), ENR concentrations (5 and 500 ng/L), and experimental periods (EXP and REC) are reported. The aquaria represent the replication for each condition (N=3).

Key words: enrofloxacin, ocean warming, mussels, gene expression, biomarkers

Type of presentation: Poster

Session: Monday, May 27th

THE EVERTEA FOUNDATION IN 2023: A PRIVILAGED PARTNER SUPPORTING RESEARCH AND RESEARCHERS IN ECOTOXICOLOGY AND ENVIRONMENTAL TOXICOLOGY

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For 10 years, the evertéa Foundation[1] has been an integral part of the French ecotoxicology and environmental toxicology research network. Acting as a major partner for research teams and networks throughout Europe, the Foundation also plays a part in transferring knowledge to the general public, particularly on issues relating to environmental pollution, climate change and the concept of "One Health".

Support for research takes the form, for example, of funding projects under an annual Call for Proposals. To date, the evertéa Foundation has contributed to the funding of 23 research projects, in both ecotoxicology and toxicology, covering a wide range of topics, from the study of the "Effects of pollutants on human health" to the "Influence of climate change on ecotoxicology and associated field methodologies".

Support for research is also expressed through the organization of workshops (on Adverse Outcome Pathways, for example) and the co-organization of conferences, while maintaining our desire to forge links between different disciplines, from ecotoxicology to human toxicology, via ecology.

On the basis of our experience and recent dynamics, we are now looking forward in working with new research teams and create bridges between ecotoxicology and other related disciplinary fields, in order to continue to promote and communicate health-environment research. Similarly, we are open to become a partner in your research projects, whether in terms of coordination, human resources or communication.

[1] https://fondationevertea.org/

Key words
Ecotoxicology, human and environmental health, climate change, partnership, One Health.

Type of presentation
Poster.

Session
t01, t09.

SCIENTIFIC PROGRAM OF PRIMO 22

AOP, System Biology approaches and other conceptual modeling tools

(Oral talks)

PRIMO22

26-29 MAY 2024
NANTES FRANCE

Adverse effects on eye development in larval zebrafish exposed to tricyclic antidepressants.

[1]Marwin Jafari, [1]Jason T. Magnuson, [2]Katharina Brotzmann, [3]Sebastian Eilebrecht, [3]Fabian Essfeld, [1]Daniela M. Pampanin

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To assess the effects on development and function of the zebrafish eye caused by tricyclic antidepressant (amitriptyline, AMI, and nortriptyline, NOR) exposures, endpoints at different levels of biological organization were assessed. At 120 h post fertilization (hpf), zebrafish larvae were evaluated for the retinal pigment epithelium (RPE) thickness via histology and for the eyes' ability to trace movement via optokinetic response assay (OKR). Transcriptomic analysis was carried out at 48, 72, and 96 hpf to evaluate biological processes (BPs) affected and to better understand the mechanism of effect.

A significant increase of the RPE thickness in larvae exposed to 300 µg/L of AMI (15.8%) and NOR (15.3%) was observed. OKR showed a significant decrease in frequency of eye movements in larvae exposed to 3 and 300 µg/L of AMI (17.21 and 3.83 sac/min) and 300 µg/L of NOR (5.75 sac/min) compared to the control (22.65 sac/min).

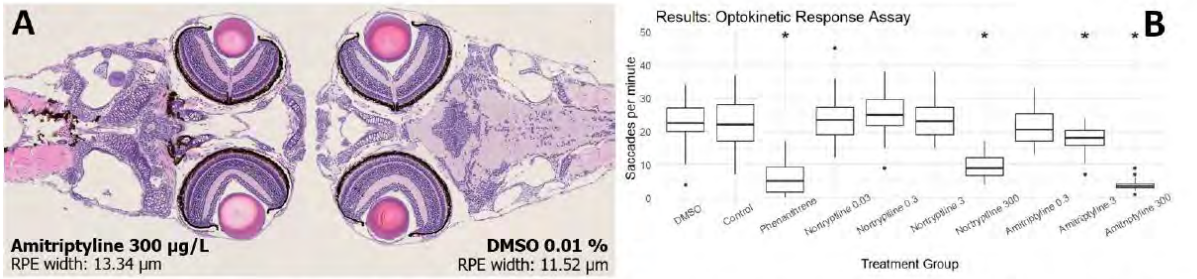

Figure 1: (A)Comparison between eye structure of larva exposed to 300 µg/L of AMI and 0.01% DMSO. (B) Boxplot depicting the results for the optokinetic response assay in saccades/minute. (*=p value < 0.05, Welch ANOVA)

mRNA sequencing found 3014 differentially expressed genes across treatments. Enrichment Analysis showed eye development to be among affected BPs, along with muscle development and neural signaling.

Thyroid disruption (TD) is known to result in adverse eye function by affecting the RPE layer, and to affect muscle development. Genes involved in thyroid signaling were differentially expressed, supporting the hypothesis that TD plays a role in the observed effects.

These results provide evidence that eye development and function are affected by tricyclic antidepressant exposure, and transcriptome data and histology gave valuable information about the underlying mechanism.

Key words
Zebrafish, Transcriptomics, Histology, Pharmaceuticals

Type of presentation
Platform

Session
AOP, SYSTEM BIOLOGY APPROACHES AND OTHER CONCEPTUAL MODELING TOOLS; EDCS & NEUROENDOCRINE EFFECTS

DRomics, a workflow to exploit and make sense of dose-response (multi-)omics data

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Omics technologies has opened new possibilities to assess environmental risks and to understand the mode(s) of action of pollutants. Coupled to dose-response experimental designs, they allow a non-targeted assessment of organism responses at the molecular level along an exposure gradient. However, describing the dose-response relationships on such high-throughput data is no easy task. DRomics is a workflow we developed for this purpose, and made available both as an R package and through interactive web applications.

Concretely the DRomics workflow selects items (e.g. contigs, metabolites) that are significantly deregulated along the dose/concentration gradient, and for each of them characterizes its response using parametric modeling and estimates a sensitivity threshold as a benchmark dose (BMD). Recently we added in the package different functions to help interpreting the outputs of this workflow, in the light of their biological annotation obtained from databases such as KEGG or Gene Ontology (GO). Those functions were especially designed to help the comparison of responses obtained at different experimental levels: different measurement levels (e.g. multi-omics), different exposure histories, time points, etc.

We propose to give an overview of the methodological choices we made in DRomics to rationalize and optimize a strategy to analyze dose-response omics data and to illustrate the use of DRomics on different case studies. We will also discuss the challenges and open questions we face while working on such problematics.

Key words
modeling, transcriptomics, metabolomics, proteomics, multi-omics, environmental risk assessment

Type of presentation
Platform

Session
AOP, system biology approaches and other conceptual modeling tools

Biotransformation pathways and mode of action (MOA) of chemical pollutants

Bioenergetic versus redox stress in neurotoxicity in *Caenorhabditis elegans* exposed to mechanistically distinct mitotoxicants

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Key words
Mitochondrial toxicity, Caenorhabditis elegans, neurotoxicity, neurodegeneration

Type of presentation
No preference between poster or platform

Session
t04: New approach methodologies (NAMs) to assess pollutant toxicity, t09: AOP, System Biology approaches and other conceptual modeling tools

Abstract

Mitochondrial dysfunction is associated with neurotoxicity, in particular dopaminergic cell death. 5-15% of pollutants likely affect mitochondrial function as their primary mechanism of action. However, there are many different mechanisms of mitocondrial toxicity, and it is not clear which drive neuronal cell death. Furthermore, the reason for the unusual sensitivity of neurons and especially dopaminergic neurons to mitochondrial toxicity is unclear. We hypothesized that exposures resulting in redox stress, but not exposures causing bioenergetic deficiency only, would cause dopaminergic cell death. We have been exposing *Caenorhabditis elegans* to mitochondrial toxicants acting by different mechanisms, assessing redox and bioenergetic changes, measuring morphological degeneration and loss of neurons, and testing rescues. We have tested inhibitors of electron transport chain complexes, mitochondrial uncouplers, redox cyclers, and mitochondrial DNA damage. We have also tested how high sugar diets, exercise, and microbiome changes that alter mitochondrial function affect neuronal toxicity. To date, all exposures that cause a significant increase in oxidation state cause neuronal loss. Dopaminergic neurons have been the most sensitive. Exposures that cause energetic depletion only cause neuronal cell death if they also caused intracellular oxidation. We propose that mechanistic neurotoxicity analyses with *C. elegans* are a useful New Approach Methodology to generate data informing mechanism-specific mitochondrial toxicity-initiated Adverse Outcome Pathways.

Paternal sperm epigenome as an indicator of modified offspring brain development

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DNA methylation has been shown potential to serve as molecular marker for intergenerational inheritance of modified offspring development extending the developmental onset of health and disease (DOHaD) hypothesis towards the pre-conceptional parental environment. The ubiquitous environmental pollutant Benzo[a]pyrene (BaP) reduces DNA methyltransferase activity in vertebrate models and affects offspring methylation profiles. Sperm harbors a repertoire of methylation markers that are associated with different physiological functions, especially neurobehavioral activity. Sperm-borne differential DNA methylation in genes that regulate the development and function of the CNS in offspring has been demonstrated in fish and mammals. Comparing sperm bisulfite sequencing data from adult male and gene expression, acetylcholinesterase activity and swimming behavior in larval offspring upon parental BaP exposure for 21 days (1µg/L) revealed modified methylation in pathways associated with neuronal development and function (netrin signaling; synaptogenesis pathway). Rho guanine nucleotide exchange factor 7, neuroligin 2 and adenylate cyclase 8 were found hypomethylated in the offspring sperm and exhibited reduced gene expression in the larvae, and thus, are possibly responsible for an increased anxiety behavior identified in larval and adult offspring. The data presented here provide a potential connection between parental BaP exposure, neurodevelopmental impacts, and the risk of behavioral and psychiatric disorders in vertebrates.

Key words
Polycyclic aromatic hydrocarbons, neurotoxicity, DNA methylation, inheritance.

Type of presentation
\Platform

Session
AOP, System Biology approaches and other conceptual modeling tools

EDCs & Neuroendocrine effects

Developmental immunotoxicity of PFAS in Marine medaka (*Oryzias melastigma*)

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Production and use of legacy per- and polyfluoroalkyl substances (PFAS) is heavily regulated due to their immunotoxicy. Still, it remains unclear if replacement PFAS pose a similar risk. To evaluate potential immunotoxic effects of PFAS during critical windows of immune system development, marine medaka (*Oryzias melastigma*) were exposed to PFAS levels equivalent to prenatal/postnatal human blood concentrations. Legacy PFAS (PFOS, PFOA) and replacements (PFHxS, PFBS, PFHxA, GenX) were assessed for immunotoxic impacts using a bacterial challenge and transcriptomics. Exposure windows encompassed lymphocyte progenitor cell migration and thymus colonization (7-11 days post fertilization (dpf)) and the establishment of immune competence in the thymus (12-19 days post hatching (dph)). PFHxA exposure at 7-11dpf reduced immune competence, while exposure during 12-19dph revealed diminished immune competence in response to PFHxS, PFBS, PFOA, and GenX. The transcriptome indicated changes in expression profiles of PFAS-exposed larvae (12-19 dph). Similar numbers of genes were differentially regulated for PFOA (919) and its replacement GenX (964). Comparatively, fewer genes were differently expressed after exposure to PFOS (492) and its replacement PFHxS (666). However, the shortest PFOS replacement, PFBS, resulted in the highest deregulation with 1697 differentially expressed genes. The data demonstrate variability in mechanisms of PFAS-induced immunotoxicity. Thus, specific critical windows may be more susceptible to certain PFAS which is important to consider for risk assessment.

Key words
fish, immune, development, environmental pollution

Type of presentation
Platform.

Session
AOP, system biology approaches and other conceptual modeling tools

Expanding endocrine AOPs from laboratory fish models: a case for developing an AOP for AR agonism for annual spawners.

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The Adverse Outcome Pathways (AOPs) available in the AOP wiki are dominated by human (mammalian) pathways whilst the environment is vastly under-represented, especially considering the biodiversity it supports. This is also true for fish, a key phylogenetic group of tremendous ecological and economic importance. The current AOPs relevant to sex steroid receptors, are based on laboratory fish (i.e. zebrafish), which once they reach sexual maturation, they continue to spawn until the end of their life. In contrast, most fish species have an annual spawning cycle, in preparation for which the endogenous levels of steroids reach levels orders of magnitude higher than those presented by continuous spawners. We hypothesize that the hypothalamic-pituitary-gonadal (HPG) axis is a positive feedback loop accommodate this steroid flux, not a negative loop as required for repeat-spawning. We will describe the experimental studies we have initiated using the three-spined stickleback as a model, which focus on the effects of AR agonists on the reproductive physiology of both sexes. The data aim to build the mechanistic and functional information needed to develop an AOP for AR agonists in annually spawning fish, expanding the taxonomic domain of AOP#23, which is limited to repeat-spawning fish. Development and validation of New Approach Methodologies (NAMs) will be targeted at fish embryonic stages, allowing future replacement in animal testing. Our work will contribute to PARC, by developing further the endocrine AOPs and environmental NAMs.

Key words
Androgen, Reproduction, Development, Stickleback, NAMs.

Type of presentation
Platform

Session
AOP, System Biology approaches and other conceptual modeling tools

EDCs & Neuroendocrine Effects.

SCIENTIFIC PROGRAM OF PRIMO 22

AOP, System Biology approaches and other conceptual modeling tools

(Posters)

PRIMO22

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NANTES FRANCE

From dose-response modelling of transcriptomic data to biological interpretation: application to embryonic exposure of zebrafish to di-n-butyl phthalate

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Transcriptomic dose-response (DR) data has recently emerged, enabling the exploration of mechanistic exposure-effect relationships of xenobiotics using DR modeling. However, unraveling the intricate signals along the dose gradient represents a real challenge, with their interpretation proving equally demanding.

To characterize the relationships between exposure and effect, we adopted the DRomics workflow, computing transcript benchmark doses (BMDs) and depicting response trends. To address the complexity of transcriptomic DR data and its biological interpretation, we propose a reproducible workflow that harmonizes DR modelling metrics with bioinformatic techniques such as Markov Clustering and Over-Representation Analysis (ORA), leveraging complementary databases like STRING, KEGG, Wikipathways and AnimalTFDB. This approach aims to provide arguments from biological and functional databases to prioritize the exploration of responsive transcript groups, thereby improving the relevance and objectivity of biological interpretation.

Utilizing zebrafish embryos as an in vivo model, we investigate the dose-dependent transcriptional effects of di-n-butyl phthalate (DBP). Comparing our workflow with more conventional methods for biological interpretation, we demonstrate its ability to distinguish more sensitive groups and reveal biological pathways that would otherwise have been overlooked. Our analysis effectively uncovers disrupted biological pathways, aligning with prior findings on phthalate esters while introducing an unprecedented sensitivity dimension to these pathways.

Key words
di-n-butyl phthalate, RNA-seq, dose-response framework, functional enrichment, clustering

Type of presentation
Platform

Session
AOP, system biology approaches and other conceptual modeling tools

Biotransformation pathways and mode of action (MOA) of chemical pollutants

Investigating effects and mechanistic pathways of perfluoroalkyl substance (PFAS) toxicity in developing mummichog (Atlantic killifish) using multi-omics

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Per- and polyfluoroalkyl substances (PFAS) are widespread, persistent chemicals associated with adverse health effects. However, mechanistic pathways underlying toxicity and hazards to wildlife, particularly estuarine and marine fish, are not fully characterized. We investigated developmental toxicity and multi-omic response after perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) exposures in mummichog (*Fundulus heteroclitus*). An ecologically important estuarine fish with a well-annotated genome, mummichogs are amenable to morphology, behavior, and 'omics assessments. Embryos were nominally exposed to PFHxS or PFOS (1, 10, and 100 µM) from 1 to 6 days post-fertilization (dpf). RNA-seq was conducted at 3 or 4 and at 10 dpf, metabolomics at 10 dpf, and developmental endpoints (e.g., morphology, heart rate, growth, light/dark behavior) evaluated 10-30 dpf. 'Omics analyses revealed similar and distinct responses. At 3 or 4 dpf, differentially expressed genes (DEGs) increased with concentration for both chemicals. At 10 dpf, 1 µM exposures induced the most DEGs, and PFOS elicited more than PFHxS. Metabolomic changes were also greater after PFOS exposure relative to PFHxS but greatest after 10 µM for both. Despite molecular changes, neither PFAS induced mortality or morphological effects, though additional data analysis is underway. Multi-omic data integration, alongside apical organismal effects, will contribute to understanding PFAS toxicity, elucidate underlying mechanistic pathways, and inform predictions of population-level impacts for marine species.

Key words
developmental toxicity, PFAS, transcriptomics, metabolomics

Type of presentation
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Session
AOP, systems biology approaches and other conceptual modeling tools

Physiologically Based Toxicokinetic Models: Chemical Exposure Simulations Applied to Novel Fish Species

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To model a complete Source To Outcome Pathway (STOP) for arctic species, it is essential to parameterize the potential environmental concentrations and the pathways that link outside environment concentrations to internal target site concentrations so that an Adverse Outcome Pathway (AOP) can be modeled starting with the Molecular Initiating Event based on target site concentrations. To come to this Aggregate Exposure Pathway model, physiologically based toxicokinetic (PBTK) models can be applied to predict bioconcentration and internal distribution of various chemicals in different species. PBTK models consist of a variety of basic equations that describe the absorption, distribution, metabolism, and excretion (ADME) of a compound in an organism. These equations are linked in a series of ordinary differential equations and parameterized by the physiology (e.g. blood flow to organs, tissue volumes, cardiac output, etc.) of the species being investigated. Hybrid striped bass are an important game fish in North America and are commonly stocked in ponds and lakes for fishing and human consumption. Despite this, there are no PBTK models for this species. This work aims to use newly generated physiological parameter values for tissue specific blood flows and weights for hybrid striped bass into an existing PBTK model structure as a predictive tool in the event of harmful chemical exposure in hybrid striped bass habitats. This work is preliminary to a larger undertaking by the EXPECT project in association with the Norwegian Institute for Water Research.

Susceptibility of PFAS-exposure during critical windows for RAG+ T-lymphocyte maturation

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T-lymphocyte maturation is a critical developmental phase for an intact immune response and the generation of immune competence in vertebrates. The *recombination activating gene 1* (*RAG1*) is a crucial lymphocyte marker responsible for T-cell receptor and immunoglobulin diversity, which are essential for an adaptive immune response. Dysfunction of *RAG1* has been associated with increased oxidative stress, defect lymphocyte maturation and immune disorders in vertebrates. Notwithstanding in vivo assessment of T-lymphocyte maturation during the embryonic stage are scarce and difficult to obtain in mammals. Observation of the *RAG1:gfp* transgenic medaka (*Oryzias latipes*) line via fluorescence microscopy revealed critical windows of T-lymphocyte maturation during progenitor migration and thymus colonization between 2 to 5 days post fertilization. As previously reported, anterior to thymus and posterior to thymus *RAG1*-positive progenitor cell migration was observed. Impairment of this critical window through endocrine disrupting chemicals, like flame retardants (PFAS), demonstrated a modified progenitor migration and delayed onset of *RAG1* expression. Differences in the response are reported between legacy and replacement PFAS, indicating that PFAS exposure during development may severely affect the T-lymphocyte maturation and functionality of the immune system.

Key words
Developmental Toxicity, Immunotoxicity, flame retardants, DOHaD
Type of presentation
Poster

Session
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The Mysid Shrimp as a Model for Endocrine Disruption Screening: Identification of Transcriptomic Biomarkers

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The mysid shrimp (*Americamysis bahia*) is a commonly-employed model organism for the routine assessment of effluents discharged into marine environments. Though this species is primarily used to evaluate acute and chronic toxicity, the results of previous studies provide information indicating its potential as an invertebrate model for endocrine disruption screening. The number of endpoints available for detecting ecdysteroid signaling disruption in this species is limited; therefore, the aim of this work was to assess the impacts of ponasterone A (a model ecdysteroid), 17β-estradiol, and trenbolone on mysid growth and global gene expression profiles in an effort to identify biomarkers indicative of endocrine disruption. To accomplish this, newly-hatched mysids were exposed to five concentrations of each chemical for 14 d. Growth was assessed in all groups at 7 and 14 d, while transcriptomic profiles were determined for a subset of groups on day 7. Ponasterone A exposure significantly increased length on day 7, but did not alter growth parameters after 14 d of exposure. Transcriptomic analysis identified a total of 253 downregulated and 172 upregulated genes between control mysids and those exposed to the highest concentration of ponasterone A. The nature of these differentially-expressed genes will be discussed, along with the concentration-dependent transcriptional responses to 17β-estradiol and trenbolone. Overall, the results of this study provide key insights into the molecular responses of mysid shrimp to endocrine disruptors and aid in biomarker identification.

Key words
Endocrine Disruption, Invertebrate, Gene Expression

Type of presentation
Poster

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Chemical exposome and non-target screening approaches

Biomonitoring and development of integrative assessment approach