Session 1: Microbial genomics and metagenomics

P01 | Changes in the Caulerpa microbiome due to abiotic stresses

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Morphologically complex seaweeds such as siphonous green algae are among the most notorious invasive species in many parts of the world. Their ecological success has repeatedly been linked to their association with endo- as well as epiphytic bacteria. Indeed, recent studies based on 16S rDNA barcoding revealed rich associated bacterial communities. However, little is known about their functional diversity as well as the principles underlying their assembly. To address how bacteria contribute to the ecological success of siphonous green algae and whether the competitive potential of invasive species may be at least partly shaped by associated microbes, we will apply a metagenomic approach to analyse the diversity of epi- and endophytic bacterial communities associated with native and invasive species of Caulerpa in the presence of abiotic stresses. In this study, natural populations of two Caulerpa species (C. cylindracea and C. prolifera) found along the Turkish coastline of the Izmir region were sampled. Addtionally, in situ experiments combined with lab experiments were conducted in which abiotic conditions were altered to assess the role of environmental factors in bacterial recruitment and microbiome stability. Characterization of bacterial communities involved Illumina-based 16S rDNA amplicon sequencing. To provide more comprehensive insight into the functional diversity of bacterial communities, metatranscriptomics will be done. In addition we will develop and apply DNA-stable isotope probing to label bacteria and hence provide detailed phylogenetic and functional information about the microorganisms responsible for the metabolism of a particular substrate.

P02 | An association between a neogregarine parasite and the microbial community of bumblebees

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Bumblebees are important pollinators in temperate and cold regions of the world. They pollinate a range of wild and agricultural flowering plants, and contribute to the plant diversity and human food supply. Hereby bumblebees provide valuable ecosystem services. The characterized gut microbial communities of bumblebees and honeybees have a highly specialized but species-poor community. These gut bacterial communities revealed related functions to nutrition, pathogen defense and immune response, showing their important role in the host's health status. To better understand these host-microbe interactions, the dependency of the environmental context needs to be considered, especially the presence of parasitic species is an important factor herein. Both the gut and fat body microbial communities of foragers (Bombus terrestris) originating from two locations were analyzed, using the 16S ribosomal amplicon sequencing with the Illumina technology. Also the relation with the neogregarine parasite Apicystis bombi was explored. A. bombi infection disturbs the microbial association network, as positive correlations between the Operational Taxonomic Units (OTUs) residing in the gut and fat body increase from 0.18% to 0.69%. Also the OTU identified as Arsenophonus sp., which has a location dependent interaction with A. bombi, is a possible candidate to influence the bee health in collaboration with A. bombi.

P03 | Temporal dynamics of bacterial colonization of plastic debris in the North Sea

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Previously we have demonstrated that bacteria can colonize marine plastic debris. We hypothesized that three major factors may influence the bacterial colonization of plastic: changes in environmental conditions (e.g. salinity, temperature), differences in biofilm formation stages and plastic-related factors (shape, colour, polymer type). Additionally, it has been proposed that bacteria could use plastic debris as a transport vector or that within the plastic colonisers, bacteria are present that are able to degrade these polymers.

Whereas bacterial colonization has been shown to occur within weeks, little is known about the temporal dynamics of marine bacterial communities on plastic debris and the influence of environment or plastic type. Therefore, we exposed two polyethylene materials (sheet and dolly rope) to the marine environment for six months on two locations: the harbour of Ostend and near the offshore wind farm, the "Thornton bank". Once a month, plastics were sampled and the temporal dynamics of the bacterial communities was analysed using 16S V3-V4 rDNA amplicon sequencing.

For both locations and plastic types, Bacteroidetes and Proteobacteria dominate the bacterial communities. Temporal shifts in community composition were observed, especially during the first months. The bacterial community composition of plastic materials sampled at the harbour of Ostend are substantially different from those at the Thornton bank, indicating a major influence of the environmental parameters. Furthermore, differences in bacterial community composition and richness were found between the two plastic items, independent from the sampling location, suggesting that the shape and/or colour of the plastic influences the bacterial colonization. Further on, we investigated if a core microbiome could be found on plastic items across all time points. This could indicate the presence of bacterial genera that can easily colonise marine plastic debris and are therefore omnipresent.

P04 | The microbiome of sympatric cryptic nematode species reflects resource differentiation which alters by ecological interactions

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Differences in resource use or in tolerances to abiotic conditions are often invoked as potential mechanisms underlying the sympatric distribution of cryptic species. Additionally, the microbiome can provide physiological adaptations of the host to environmental conditions. We determined the intra- and interspecific variability of the microbiomes of three cryptic nematode species of the Litoditis marina species complex that co-occur, but show differences in abiotic tolerances. Roche 454 pyrosequencing of the microbial 16S rRNA gene revealed distinct bacterial communities characterized by a substantial diversity (85 – 513 OTUs) and many rare OTUs. The core microbiome of each species contained only very few OTUs (2 – 6), and four OTUs were identified as potentially generating tolerance to abiotic conditions. A controlled experiment in which nematodes from three cryptic species (Pm1, Pm3 and Pm4) were fed with either an E. coli suspension or a bacterial mix was performed and the 16S rRNA gene was sequenced using the MiSeq technology. OTU richness ranged between 1118 – 7864. This experiment confirmed the existence of species-specific microbiomes, a

core microbiome with few OTUs, and high interindividual variability. The microbiome of starved nematodes was clearly different from that of nematodes offered food. The offered food source affected the bacterial community and illustrated different feeding behavior between the cryptic species, with Pm3 exhibiting a higher degree of selective feeding than Pm1. A second experiment in which the three species were incubated together illustrated that Pm1 and Pm4 feed on the same bacteria, while Pm3 had a very distinct microbiome. Similar resource use may explain the largely allopatric distribution of Pm1 and Pm4 in the field. Morphologically similar species belonging to the same feeding guild (bacterivores) can thus have substantial differences in their associated microbiomes and feeding strategy, which in turn may have important ramifications for biodiversity – ecosystem functioning relationships.

Session 2: Evolutionary and ecological omics

P05 | Earthworm adaption to volcanic stressors

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The volcanic islands of the Azores in the mid-Atlantic are home to a variety of earthworm species. Separated from largescale outside genetic interference, these populations have colonised a variety of hostile environments. From high altitude peaks rising 2000m from the Atlantic to steaming calderas the worms provide a fantastic opportunity to investigate adaptation to stress. This study investigates the localised micro-evolutionary changes that occur due to the impact of multiple stressor considering both the phylogeography distribution of earthworm communities in addition to providing a foundation to identify functional loci under selection across these extreme environments. The effect of altitude was investigated through sampling a transect running up the main volcano on the island of Pico, the main stressors include: low temperature, low soil depth and quality and low levels of vegetation. In contrast adaptation to an active volcanic field was investigated in the active fumerals on the neighbouring island of Treceira where stresses include: high CO2, high soil temperature, low soil depth and high chemical toxicity due to the high bioavailability of metal ions. Worms were collected in a single day at 12 sampling sites, across a North-western transect of Pico's main volcanic peak, ranging from an altitude of 1167m to 1622m. Sampling sites included a range of soil depth and vegetation cover. Worms were also collected in a single day at 5 sampling sites, in and around the steaming fumerals of Treceira. Although altitude did not differ greatly, (534-615m), soil depth and vegetation cover provided a variety of habitation environments. Site 2 in Treceira was located very close to active fumerals and whilst soil was still present, no worms were found. Worms from Pico and Terceira were grouped by sites into species by observation and transported back to Cardiff for DNA extraction and genotyping. Three species of worm were identified during collection; Aporrectodea caliginosa which was found at the majority of sites sampled, Lumbricus terrestris and Amynthas corticis which were less universally distributed. Mitochondrial genotyping revealed the haplotype distribution allowing analysis of relationship between communities to be determined. This fuelled a wider analysis of the colonisation dynamics of earthworms onto the Azorean Archipelago in addition to the impact of the two multi-stressor environments under investigation.

P06 | Transcriptional Rewiring: Synthetic and Evolutionary Processes Governing Plant Environmental Responses

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Plant defence responses are modulated by substantial transcriptional reprogramming, up to 40% of the genome can be differentially expressed following pathogen challenge. High temporal resolution differential expression analysis and subsequent network inference has revealed a large transcriptional networks with a complex hierarchies. Overall this indicates that transcriptional factors play an important role in the defence response. The complexity, diversity and redundancy in these defence response networks further suggests interesting evolutionary principles may govern and give rise to these highly structured and plastic networks. Previously we have shown that a synthetic technique known as genetic rewiring, where the open reading frames of selected transcription factors are fused to different promoters altering the natural expression of the regulator, can be used to synthetically evolve novel phenotypic diversity. As a transcription factor can potentially regulate thousands of target genes alteration of its natural expression has the potential to generate radically new phenotypes through cascading regulatory events. We have shown through this approach, in yeast, that heterologous protein expression can be significantly enhanced using this rewiring approach. Furthermore, network analysis reveals that rewired open reading frames and promoters possess characteristic topological network features that can serve as predictive measures for future rewiring endeavours. As such in conjunction with a presentation of our work on rewiring the yeast transcriptome I will briefly discuss how we are using a combined systems and synthetic biology approach to first construct large scale transcriptional networks responding to pathogen challenge in plants. Secondly, I will show how through a process of topological network analysis how we are now selecting promoters and open reading frames which we will use to rewire the plant defence transcriptome. This novel transciptome diversity will be used to help reveal key regulatory points and potential weaknesses in these networks as well as highlighting potential solutions to help improve tolerance to plant pathogens.

Session 3: (Eco)toxicological omics

P07 | Toxicogenomics of the flame retardant tris (2-butoxyethyl) phosphate (TBEP) in HepG2 cells

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Tris (2-butoxyethyl) phosphate (TBEP) is a ubiquitously-used flame retardant and plasticizer in consumer goods. TBEP has been shown to diffuse out of materials and has therefore been detected in house dust, drinking water, and even in human mothers' milk and placenta. Worryingly, TBEP has been shown to elicit neurotoxic, developmental, and estrogenic effects. Further toxicological data is largely limited to monitoring classical endpoints such as measuring lethal dose values. Insight into the molecular modes-of-actions underlying toxic phenotypes is therefore scarce. Few existing omics studies have shown TBEP to effect metabolism, apoptosis, organ development which results in decreased heart rate and body length in zebrafish larvae, while affecting protein metabolism, biosynthesis, and energy metabolism in daphnia. Given the lack of unbiased omics data, and the lack of potential toxicological effects in human, we used RNA-seq to investigate potential effects of TBEP in the human hepatoma cell line, HepG2, at two concentrations. Of the 19,000 transcripts detected as expressed, treatment with 125µM TBEP resulted in the detection of 4,060 differentially-expressed genes (DEGs) (FDR≤0.05), while treatment with 25µM TBEP induced 1,055 DEGs, a majority of which were shared with the 125µM treatment. Over-representation analysis of the high-dose DEGs indicated enrichment for Gene Onthology (GO) terms involved in response to stimuli, wound healing, carbohydrate metabolism, angiogenesis, cell proliferation, cell migration, and cellular development

and differentiation. Gene set enrichment analysis showed that such GO term enrichment was accompanied by the upregulation of ribosomal genes, along with genes involved in oxidative phosphorylation, and DNA replication. This indicates that TBEP may result in a diverse set of toxicological effects by altering cellular protein and energy metabolism. Such effects may include regulation of angiogenesis, carbohydrate metabolism, cellular chemotaxis and migration, wound healing, and cell proliferation, indicating the ability for TBEP to induce systemic stress. In addition, effects on organ development, in particular vascular development, and response to estrogen stimuli were also observed in agreement with previous studies.

P08 | Protein differential expression and biochemical effects of pesticide exposure in the midge Chironomus riparius

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One of the ultimate goals in pest management is to discover efficient pesticides for specific pests while maintaining adverse outcomes on non-target species to a minimum. Some non-target insects play a crucial role in freshwater systems and, due to runoff or leaching events from nearby agricultural fields are often subjected to significant concentrations of pesticides. The ecotoxicological effects of pesticide exposure seen on higher levels of organization result from impacts at molecular levels and therefore assessing earlier sub-organismal endpoints on key species may provide insights on the long-term consequences for natural populations. Here, larvae of the nonbiting midge Chironomus riparius were exposed to environmentally relevant concentrations of 3 pesticides with distinct modes of action: fipronil, indoxacarb and spinosad, and their effects evaluated in terms of life history responses using standard laboratory tests as well as at cellular and molecular levels using biochemical biomarkers and proteomics. Besides impairment of developmental and/or emergence rates caused by chronic exposure to all compounds, acute exposure to fipronil induced alterations in glutathione S-transferase (GST), acetilcholinesterase and catalase enzyme activities, along with changes in cellular respiration and metabolism. Spinosad caused changes in the electron transport system and glutathione peroxidase (GPx) activities and induced lipid peroxidation. Indoxacarb caused changes in GPx and GST activities. Acute exposures (48h) were also performed for proteomic analysis. Proteins were iTRAQ labeled, separated and analyzed by LC-MS/MS. Data analysis revealed changes in expression of globin proteins for all pesticides, suggesting globin expression as a potential biomarker for pesticide-induced stress in C. riparius. For fipronil and indoxacarb, motility related proteins (actin and myosin) expression was altered, which could explain effects observed at organism level: lack of movement and changes in burrowing behavior. Regarding spinosad, ATP synthase expression changes were observed, which is in accordance to changes in the electron transport system detected at biochemical level. Protein profile changes can be used as early warning biomarkers of pesticide exposure. Proteomic approaches have the potential to provide a better mechanistic interpretation of the toxic response of organisms and thus to aid in the assessment of the ecological effects of environmental contamination.

P09 | Coupling Transcriptomics with in vitro Multi-Cellular Co-Culture Assays Provides Systemic Toxicity Screening Representative of in vivo Effects

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The need for non-animal-based toxicity screening and evaluation methods that can accurately identify in vivo effects are in high demand as the regulatory community continues to pursue minimized animal use and animal replacement. Unfortunately, many cell-based in vitro screening methods lose the systems context necessary to holistically understand toxicity. As a means to remedy this shortcoming, we have employed the Integrated discrete Multiple Organ Co-Culture (IdMOC) system in conjunction with global-transcriptomics expression assessment to provide a systems context of interactive organ and molecular pathway responses. We present a specific case study evaluating toxicological responses in IdMOC exposures containing human cell cultures representative of 5 organ systems exposed in a common media compared to mono-culture exposures, each exposed to high-nitrogen (high-N) munitions of military concern. This approach was applied to evaluate the toxicity and toxicogenomic responses of a well-studied legacy munition, 2,4,6-trinitrotoluene (TNT), which has robust toxicological description in the literature and a novel insensitive munition, 2,4-dinitroanisole (DNAN), which has very sparsely described toxicity. Cytotoxicity was assessed for each chemical using cell viability characterizations and toxicogenomics assessed using microarray-based global transcript expression analysis. The toxicity of DNAN, based on the lethal concentrations 50 (LC50s), was significantly lower for than TNT for three of the five cell lines: liver, vascular endothelium, and heart. The observed pathway enrichment within kidney cells exposed to TNT and DNAN through the IdMOC system reflected the literature-based in vivo toxicity of parent and active toxicants generated from the metabolism of each chemical, with common pathways among chemicals related to p53 signaling, biological oxidative stress and the metabolism of xenobiotics by cytochrome P450. Enrichment of pathways unique to DNAN and TNT exposures were also observed where multiple pathway-level responses suggest that the effects of DNAN exposure may reflect the toxicity of a primary metabolite, dinitrophenol. Overall, the IdMOC results provided a higher number and more robust enrichment of pathways involved in known toxicity mechanisms versus monocultures thus demonstrating the utility of the system for accurate chemical hazard assessment.

P10 | Transcriptional responses and prediction of metabolic changes in marine medaka fish (Oryzias javanicus) to acute exposure to endocrine disrupting chemicals

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The differentially expressed genes in the liver tissue of the marine medaka fish, Oryzias javanicus, were profiled using an oligo-microarray (EnviHaz-Fish Array) which contains oligonucleotide probes for 22,796 unigenes of O. javanicus, after the fish were exposed to five kinds of endocrine disrupting chemicals (EDCs), bisphenol A (BPA), 17β-estradiol (E2), nonylphenol (NP), perfluorooctanesulfonate (PFOS), and triclosan (TCS) for 12, 24, 48, or 72 h to predict the metabolic and physiological changes with exposure time. The transcriptomic changes were highly dynamic in the EDC-exposed fish groups by the exposure time. The differentially expressed genes were used to predict the changes that occurred in the metabolic pathways and processes in response to EDCs exposure.

P11 | Developing an enhanced experimental workflow for maximising the use of 'omics data within the Adverse Outcome Pathway framework

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Developments in 'omics technologies have opened new possibilities for assessing the molecular and biochemical responses of organisms to chemicals. Feature selection tools are increasingly being used to discover responses within the 'omics datasets that may be predictive of adverse outcomes. These molecular responses can help to define the "key events" (KEs) underpinning the toxicity pathway within an Adverse Outcome Pathway (AOP) framework. KEs are deviations from a healthy state, through a stress response, that may predict an adverse outcome and therefore indicative of a chemical's potential health hazards. Here we investigate the potential of multi-omics technologies to discover KEs of specific acting chemicals and baseline narcotics. The first phase of this work has been the development of a proposed work-flow of how to utilise the non-targeted, information rich, data attainable from omics investigations; incorporating this into an enhanced AOP driven risk assessment approach. The second phase has been to start to develop comparative multi-omics approaches as part of an integrated (weight of evidence) approach in combination with available in silico or in vitro data to support the categorisation of chemicals by their KEs based upon their molecular and biochemical responses in Daphnia and algae. We aim to achieve this by elucidating the relevant KEs of specifically acting chemicals and baseline narcotics and measuring the strength of the association with the subsequent adverse outcome. The use of organisms that span two trophic levels could enable qualitative assessment of the predictive capability of the KE(s) both within and across species, improving toxicity predictions for untested species and in setting exposure thresholds in environmental risk assessment. To identify target homology and further enhance cross species applicability, molecular target sequence analysis tools; such as the US-EPA developed SeqAPASS (Sequence Alignment to Predict Across-Species Susceptibility), will be utilised. Sampling at high temporal resolution (spanning the acute and chronic experimental stages) for the 'omics assays will provide reproducible toxicological effects measurable by gene expression and metabolite profiling. By detecting the point of departure from a healthy state, we aim to use acute exposure scenarios to predict chronic outcomes, ultimately streamlining the experimental design to capture just the KEs making the work flow rapid and cost-effective.

P12 | Metabolomic Response of Gilt-headed Sea Bream Following Sub-chronic Exposure to Amitriptyline at Environmentally Relevant Concentrations

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Amitriptyline is a serotonin-norepinephrine reuptake inhibitor, and one of the most commonly prescribed antidepressants globally. The extensive use of amitriptyline has led to its ubiquitous occurrence in the aquatic environment, with concentrations up to several hundred ng/L reported in wastewater treatment plant effluents and surface waters. Despite considerable knowledge of the mode-of-action for this substance in humans, it remains unclear how non-target organisms, such as fish, respond to amitriptyline exposure. In the present work, we investigated metabolomic perturbations in juvenile gilt-headed sea bream (Sparus aurata) exposed over 7 days via the water to an environmentally relevant concentration of amitriptyline (200 ng/L). Liver and brain were collected prior to dosing and on exposure days 2, 4, and 7 from control (n=10) and exposed (n=10) animals. Samples were flash frozen and then stored at -80°C prior to analysis. Following extraction with

MeOH:chloroform (80:20), metabolite analysis was performed using a combined target/non-target approach: 38 metabolites (amino acids, biogenic amines, TCA metabolites, thyroid hormones) were quantified by UHPLC-MS/MS while an additional 167 polar lipids were determined by flow injection-MS/MS with isotopic overlap deconvolution. Non-target analysis was performed by UHPLC-Orbitrap MS in positive and negative modes with both C18 and HILIC separation. Metabolites were identified using Compound Discoverer (Thermo) interfaced to MZmine. QA/QC measures included the use of isotopically labelled internal standards, NIST reference material, pooled fish tissue, and procedural blanks. Multivariate statistical analysis was carried out on metabolites detectable in >50% of samples using MetaboAnalyst. Metabolites driving group separation were further investigated using the Kyoto Encyclopedia for Genese and Genomes (KEGG) in order to determine affected pathways. Overall these data demonstrate the use of metabolomics as a sensitive and specific approach for probing molecular-level effects of emerging contaminants. Acknowledgements: This work was financially supported by the Ministry of Economy and Competitiveness through the project CTM2014-56628-C3-1-R and H. Ziarrusta is grateful to the Spanish Ministry for her pre-doctoral fellowship.

P13 | Development and validation of a multi-matrix Targeted/Non-targeted Metabolomics Method

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Metabolomics offers an unprecedented opportunity to probe molecular-level effects of environmental contaminants at low dose. In order to facilitate widespread adoption and incorporation of metabolomics into regulatory hazard assessment for environmental contaminants, sensitive and specific methods which produce robust, quality-controlled data are required. Here we present the development and validation of a combined targeted/non-targeted approach which offers the opportunity to quantify known metabolites while facilitating identification of novel biomarkers. The multi-platform method is based on: a) ultra-high performance hydrophilic interaction liquid chromatography-tandem mass spectrometry (UHP-HILIC-MS/MS) for quantitative analysis of 18 amino acids, 8 biogenic amines, 5 neurotransmitters, and 4 nucleobases; b) flow-injection (FI)-MS/MS for semi-quantitative determination of 167 sphingomyelins, gylcerophospholipids and carnitines; and c) a non-target approach for determination of novel metabolites using UHP-HILIC-Orbitrap MS and UHP-reversed phase-Orbitrap MS. Method optimization involved testing a number of chromatographic stationary and mobile phases, solvent extraction efficiency tests, evaluation of carry-over, blank contamination checks as well as a lipid isotopic deconvolution & quantification assessment. An investigation into different extraction solvents in brain and liver tissues from gilthead bream revealed that 4:1 MeOH:$CHCl_3$ was the most effective in terms of extraction efficiency, with typically >80% extracted in a single extraction. Carry over was reduced to <4% using a three-step needle cleaning program and by extending cleaning steps between runs. Lipid isotopic deconvolution was performed using an in-house R-script which was validated using a series of spiking experiments. Application of the method to replicates of NIST SRM1950 revealed identification of a total of 340 metabolites, 156 of which were determined quantitatively. For 11 targets for which reference concentrations were available, measured concentrations were within 12% of known values, and precision was <4.7%. A comparison of the results of non-target analysis using HILIC or reversed phase chromatography revealed HILIC under positive mode to be the most effective technique for maximizing metabolite coverage.

P14 | Are microRNAs involved in regulating the oxidative stress response in Oryza sativa to gamma radiation?

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It is difficult to extrapolate effects of chronic low dose radiation observed at cellular level to those observed in higher levels of organisation. In this study, the effect of radiation in rice (Oryza sativa cv. Nipponbare) was analysed by irradiating 7-day old seedlings with 4 gamma dose rates: 20, 40, 90 and 350 mGy/h for 2 weeks followed by a 2-week recovery period. Growth measurements revealed a decrease in shoot length and fresh weight especially at the highest dose rate. In recovery plants, controls had the highest shoot length and fresh weight compared to exposed plants whereas plants exposed to the highest dose rate had the lowest fresh weight. Anti-oxidative stress enzymes were measured after dividing the plants into two groups. The first consisted of pooled plants (leaves of different plants but same physiological age bulked together) while the second consisted of non-pooled plants (leaves from the same plant but different physiological age bulked together). In non-pooled plants, highest catalase activity was observed in shoots exposed to highest dose rate. Ascorbate, guajacol and syringaldazine peroxidase enzyme activities was high in exposed plants compared to controls. In contrast, overall superoxide dismutase activity was lowest in plants exposed to highest dose rate. No catalase activity was observed in roots of all plants whereas peroxidase activity was found to be high in roots of plants exposed to lowest and highest dose rates. These results are the first indication of oxidative stress in gamma radiation exposed rice plants. At the moment, expression profile of rice microRNAs involved in regulating stress is being analysed. Four genes were selected for analysis in the pooled, non-pooled leaves and roots of exposed and recovered plants to determine whether their expression depends on dose rate and physiological age. The miRNAs selected from literature are: osa-miR169b, osa-miR397b which were shown to be up-regulated after seedlings were treated with methyl viologen whereas osa-miR1425 and osa-miR827 were up-regulated when they were treated with hydrogen peroxide. Future work will further investigate the activity of anti-oxidative stress enzymes and antioxidants in pooled and recovery plants as well as test the expression profile of more miRNA genes that have been shown to be involved in oxidative stress response. The possible link between effects observed on growth, leaf age and the changes on cellular and molecular level will be discussed.

P15 | RNAseq analysis reveals that chronic gamma radiation affects the developmental biology during embryogenesis in Atlantic salmon.

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Ionizing radiation such as gamma radiation, cause harmful effects by one principal process, the excitation of atoms or molecules including DNA, RNA, proteins and lipids. Here we report RNAseq analysis of eyed egg embryos of Atlantic salmon exposed to chronic gamma radiation from fertilization. Specific toxicological and/or biological processes were determined after mapping the salmon DEGs to mammalian orthologs and subjection to protein-protein network and pathway analysis. Results showed a dose dependent change in number of DEGs. Gene ontology analysis showed that a number of genotoxic insult related pathways were upregulated. DEG's included central genes that govern important aspects of the developmental program during embryogenesis. Specifically, this involved both stem cell differentiation, neuron, mussel, brain and eye development. Downregulated haem/erythrocyte metabolic pathways and O2/CO2 homeostasis genes characterized pathological changes in haematological parameters. Histopathological analysis of

exposed embryos corroborated the involvement of the proposed pathways and molecular mechanisms responsible for harmful effects of chronic gamma radiation on the development of Atlantic salmon embryos.

P16 | Transcriptional and epigenetic responses to bisphenol A in breeding zebrafish

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Bisphenol A (BPA) is a commercially important chemical used in the production of epoxy resins and polycarbonate plastics, with wide uses including in food packaging and other plastics. As a consequence, BPA is ubiquitous in the environment leading to exposure of humans and wildlife. Evidence suggests that this chemical can affect reproduction via oestrogenic and anti-androgenic signalling pathways, and exposure to BPA has been associated with pathologies in a range of species, including reproductive disruption, cardiovascular disease and behavioural abnormalities. Recent studies also suggest that BPA may affect epigenetic signalling pathways, and there is a significant research need to document these effects both in wildlife and humans. We aimed to investigate the epigenetic and reproductive effects of BPA in a fish model, in order to link its mechanisms of toxicity to adverse effects on the reproductive health of the populations studied. Breeding groups of zebrafish (Danio rerio) were exposed to 0.01, 0.1 and 1mg BPA/L for 15 days, and reproduction was quantified over time. We observed a significant increase in egg production, together with a reduced rate of fertilisation in fish exposed to the highest concentration tested (1mg BPA/L). Exposure to BPA was associated with significant alterations in the transcription of genes involved in reproductive function and epigenetic processes in both liver and gonadal tissue. Of note, reduced expression of DNA methyltransferase 1 (dnmt1) was observed at all concentrations tested, including environmentally-relevant concentrations (0.01mg BPA/L), together with a significant reduction in global DNA methylation in testes and ovaries following exposure to 1mg BPA/L. These findings demonstrate that BPA disrupts reproductive processes in zebrafish, likely via oestrogenic mechanisms, and that environmentally-relevant concentrations of BPA are associated with altered transcription of key enzymes involved in DNA methylation maintenance. A further study is underway to investigate how previous exposure to BPA affects the response of fish upon re-exposure and whether there is an epigenetic basis for these effects. We hypothesise that exposure to BPA will alter the transcriptional response after re-exposure, possibly due to stable epigenetic alterations in DNA methylation. Our findings to date provide evidence of reproductive and epigenetic effects of BPA in a model vertebrate, and advocate for its reduction in the environment.

P17 | Predictive potential of molecular biomarkers of endocrine disruption in the freshwater gastropod Lymnaea stagnalis: transcriptomic and proteomic responses vs reproductive output

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The freshwater gastropod Lymnaea stagnalis is one of the test species of recently adopted OECD Technical Guidelines for reprotoxicity testing in molluscs. This in vivo test appears as a Mollusc Partial Life-Cycle Assay at Level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (EDs). For such an apical test, endpoints can be sensitive to more than one mechanism and measured effects may be due to non-ED mechanisms. Therefore, screening methods are needed to establish unequivocally endocrine disruption in L. stagnalis. Molecular-based screening methods for endocrine disruption are currently developed in this freshwater gastropod. Transcriptomic and proteomic approaches have been implemented in order to identify response patterns specific of snail exposure to selected endocrine active compounds (tributyltin, vinclozolin, chlordecone). In parallel, these chemicals have been tested using Yeast Estrogenic and Androgenic Screening (YES and YAS) assays, and also for their potential adverse reproductive effects (28-day fecundity assay). Cross-comparisons between the results of YES and YAS assays, transcriptome and proteome response pattern analyses and in vivo reprotoxicity tests revealed very limited consistency. First, endocrine disruptive activity was not supported by YES and YAS assays at the concentrations used in in vivo tests, whatever the compound. In L. stagnalis, tributyltin (TBT) and chlordecone (CLD) were the most effective compounds. At the transcriptional level, TBT and CLD exposure triggered dose-dependent responses, in terms of number of differentially expressed transcripts. However, transcriptomic functional annotation and enrichment tests did not identify disruption of any endocrine system available in GO and KEGG databases. Proteomic analysis showed that four proteins related to reproduction (ovipostatin, schistosomin, ovulation hormone, and yolk ferritin) were affected by one or several compounds. However, results were not consistent with effects observed on fecundity. Interestingly, ethinyl-estradiol (EE2), used as a putative positive control in this study, had no effect on any level of expression or endpoint. Collectively, results indicate that, in absence of a reference genome, effects on L. stagnalis reproduction cannot be explained by or predicted from in vitro or de novo omics assays.

P18 | 1H-NMR metabolomic study of mussels exposed to a controlled mixture of hydrophobic organic microcontaminants

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Mussels have been extensively used as sentinel organisms to monitor polluted aquatic environments. Since they are not ubiquitous in many locations or they cannot withstand in highly polluted aquatic environments, passive sampling strategies are gaining also interest to be used with the same purpose. In this work, we have used a continuous flow system that allows the continuous exposure of Mytilus galloprovincialis mussels and passive sampler devices (PMDS Stir bar) to a controlled mixture of 8 hydrophobic organic microcontaminants at 20 ng/L for 14 days. The 3rd, 7th and 14th day 40 mussels were taken from the tanks for a set of bioanalysis including the 1H-NMR metabolomic analysis of hemolymph, gonad and foot tissues. All the tissue samples were proceed according to standard procedures leading to the hydrophilic and the hydrophobic fractions. The NMR measurements were collected in a 500 MHz NMR spectrometer and the data was treated with the PLS Toolbox (Matlab). An OPLS-DA model was built for each kind of samples and the three models showed a close class distribution, the first component is able to distinguish the control and the 3rd day data sets from those obtained in the 7th and the 14th day. From each model the VIPs (Very Important Projections) were studied and the annotation of the peaks was proceeded. From the identified peaks and the VIP intensities a heatmap was build and the biochemical interpretation of the results was carried out combining these results together with the bioaccumulation rates and the

histopathological analysis. The most remarkable fact of this experiment was the spontaneous spawning of the exposed mussels the 3rd day surely due to the subjected stress for the exposure to contaminants cocktail. This fact was also observed in two previous experiments at higher concentrations. However, metabolomics results confirm the class clustering based on the biochemical profiles in the studied tissues and hemolymph. In fact, enhanced effects were observed in the gonads, especially in the ketogenesis pathway (hydroxybutyrate, acetate), the TCA cycle (α-ketoglutarate, pyruvate) and some aminoacids and intermediates (leucine, glycine, serine, methylmalonate). Additionally, remarkable changes in creatine, creatinine, homarine and betaine as well as the depletion of glucose, glutamate or phosphocholines were also observed.

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P19 | Transcriptomic alterations in zebrafish larvae exposed to obesogens

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Obesogens are an important group of Endocrine Disrupting Chemicals (EDCs), able to cause adverse effects in exposed organisms both at the short and at the long terms. Moreover, some of their long-term effects can be transferred from generation to generation and they are assumed to be at least partially driven by the epigenetic regulatory machinery. For example, exposures to Tributyltin (TBT), a well-studied obesogen, can increase the adipocyte lipid droplets in zebrafish or increase adipose depots in mice up to the third generation. Our study aims to identify transcriptomic alterations (as characteristic biomarkers) of TBT exposure in zebrafish larvae. Based on previous publications, we selected genes involved in different pathways as possible biomarkers of obesogenicity. This include genes related to 1) lipid metabolism: lipoprotein lipase (lpl), acyl-coenzyme A oxidase 1 (acox1), cAMP response element binding protein 3-like-3 (creb3l3), peroxisome proliferator-activated receptor gamma (ppar-γ), and fatty acid binding protein 11a (fabp11a); 2) methyltransferases: DNA (cytosine-5-)-methyltransferase 1 (dnmt1), glycine N-methyltransferase (gnmt). We studied the mRNA expression profiles of these genes through the first 21 days of development to identify putative sensitive windows to TBT exposures. Then we performed acute TBT exposures (from 0 to 100 nM) in zebrafish larvae during different developmental windows and studied the alterations in mRNA levels produced by the TBT exposure. Results of this study will exert a positive influence in the study of the effects of TBT, increasing the knowledge of regulatory mechanisms and modes of action of obesogens at a global scale. In addition, we aim (in the future) to identify epigenetic and lipidomic footprints, characteristics of that exposure, and to integrate them with the transcriptomic footprint.

Session 4: Epigenetics in ecology and (eco)toxicology: science and technology

P20 | Reprogramming of the epigenome in the self-fertilizing mangrove rivulus, Kryptolebias marmoratus, and the effects of environmental stressors

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The mangrove rivulus, Kryptolebias marmoratus, is a cyprinodontiform fish native from the mangrove ecosystems of Florida, the Bahamas, and Central America. It presents a great capacity to withstand the constantly fluctuating environmental conditions that define mangrove ecosystems, and is characterized by a high level of phenotypic plasticity. Natural populations are androdioecious (hermaphrodites coexist with a low proportion of males) and hermaphrodites display the unique ability for a vertebrate of self-fertilization. Selfing has resulted in populations composed of distinct isogenic strains with a variety of identifiable phenotypes. Using rivulus to study phenotypic plasticity permits the construction of "true" reaction norms by reducing genetic noise within lineages. Epigenetic modifications such as DNA methylation have important regulatory functions controlling gene expression. They potentially represent molecular mechanisms that can generate phenotypic plasticity under natural conditions in the absence of genetic variability. In the present study, the dynamic of global DNA methylation during development, in juveniles and in diverse adult tissues, was investigated using LUminometric Methylation Assay (LUMA). A significant higher level of DNA methylation was observed in male testes (87.2%) compared to hermaphrodite ovotestes (79.6%). After fertilization, a decrease in DNA methylation occurred from 27.8% in fertilized eggs to 15.8% in gastrula, immediately followed by an increase and re-establishment of the adult pattern by the stage 26 (liver formation) (70.0%). In addition, characterization of genes coding for DNA-methyltransferase enzymes (DNMT1, DNMT3A and DNMT3B) suggests evolutionary conservation of this gene family. Together these results provide evidence of a long apparent zygotic transition and an original reprogramming pattern of DNA methylation among vertebrates. The effects of environmental contaminants (EE2, Cu and oil-spill pollutants) during this reprogramming period are being investigated with the objective to determine impacts on DNA methylation and gene expression. We hypothesize that DNA methylation, and more generally epigenetic mechanisms, may have a crucial role in adaptive evolution of rivulus in populations with very little genetic diversity. Overall DNA methylation reprogramming during development might be a sensitive period during which exposure to environmental challenges can have significant impacts on adult phenotype.

P21 | Impacts of fine particle mine tailings on early life stages of cod

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Mineral industries produce many millions of tons of mine tailings waste that are partly deposited in coastal marine environments. In Norway, submarine tailings disposal has been common practice and is planned for some future mining activities. Mine tailing slurry can include a fraction of fine particles smaller than 100 µm, and can have elevated levels of heavy metals from incomplete recovery of ore minerals. Potential prolonged suspension of these small particles in the water column may bring them into contact with locally spawned pelagic fish eggs, including commercially valuable Atlantic cod (Gadus morhua). Cod eggs were exposed to environmentally-relevant concentrations (1-10 mg/l) of suspended mine tailings particles in flow-through aquaria, and mortality and development was assessed over 21 days (2 hours post fertilization - 5 days post hatching). Physical characterization of the particles will evaluate particle concentration (Beckman Multsizer Analyzer), particle size profile (laser diffraction particle size analyzer), shape and elemental composition (scanning electron microscopy and energy-dispersive spectroscopy), and chemical analyses (inductively-coupled plasma optical emission spectroscopy) of the water and larvae will measure metal concentration. Molecular analyses are ongoing and include quantitative reverse transcription PCR assessment of a panel of genes involved in general stress response (e.g. heat shock proteins and metallothioneins),

genotoxicity (e.g. gadd45), and epigenetic markers (e.g. DNA methyltransferases). Experimental research into ecotoxicological implications of fine-fraction mine waste is limited and the long-term heritable impacts are unknown. Assessment of epigenetic and genotoxic biomarkers can clarify transgenerational and population-level impacts of industrial waste in the environment and will provide valuable information to guide best practice management of future mine tailings deposits.

P22 | Effects on DNA Methylation Pattern in the Freshwater Snail Physa acuta (Gastropoda, Pulmonata) and Chironomus riparius (Dipteran) after the exposure to Vinclozolin.

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Epigenetics is known to be involved in several biological processes with the participation of different mechanisms like DNA methylation, histone modifications, and ncRNA interaction. DNA methylation has been the most extensively studied epigenetic mechanism and has been shown to play a role in the regulation of gene expression and splicing during normal cell metabolism and in response to environmental factors such as chemical stress. Vinclozolin (Vz) is a fungicide with anti-androgen activity in vertebrates. Transgenerational effects in mammals through epigenetic mechanism after Vz exposures has been also observed. The likelihood of Vz being released into surface waters, together with its known endocrine disruption effect, justifies studies with aquatic organisms. This work analyzes DNA methylation alterations on two aquatic invertebrates; Physa acuta, a freshwater Gastropoda, and Chrionomus riparius, an insect, after exposure to Vz. Two sets of restriction enzymes were used: MspI/HpaII and MboI/Sau3A, which allow the detection of 5-methyl cytosine sites and N6-methylated adenine respectively. Digested DNAs were used in PCR to obtain the DNA methylation patterns of Physa acuta embryos and Chironomus riparius fourth instar larvae. Additionally, an inhibitor of DNA methylation (5-azacytidine) and a direct-acting alkylating agent that can transfer its methyl group to DNA (N-methyl-N-nitrosourea) were used as model compounds in experiments with Physa acuta. The results showed for the first time that Physa acuta embryos display DNA methylation because we observed differences between MspI and HpaII profiles. Finally, although no clear indication of CpG methylation can be observed in C. riparius, as expected since is proposed that dipteran has few or none CpG methylated, some variations are observed in methylated adenine suggesting that Vz could be altering the DNA in this group of insects in other nucleotides. This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Spain), grant CTM-2015-64913-R from the Ciencias y Tecnologías Medioambientales program. M.A. is the receiver of a predoctoral contract Ministry of Economy and Finance (BES-2013-064041).

P23 | Changes in DNA methylation in response to cyanobacteria in the model crustacean Daphnia

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Despite the recent developments in molecular technologies, little attention has been given in environmental toxicology to epigenetic modifications and their potential consequences for environmental risk assessment. We focused on the crustacean Daphnia whose well-known ecology and phenotypic plasticity offer unique opportunities to enhance our understanding of epigenetic mechanisms and their importance in environmental challenges. We characterized the methylation pattern in Daphnia in response to a rich diet and a diet contaminated with the toxic cyanobacterium Microcystis aeruginosa, a common harmful algal bloom known to have detrimental effects on higher organisms. We used bisulfite sequencing to identify methylated cytosines in genomic features. Methylation was primarily targeted to exonic regions while introns and intergenic regions were generally devoid of methylation. We observed that despite the low methylation level in Daphnia, some genes are preferably methylated with methylation levels higher than 50% while others have no methylation at all. Roughly 300 genes were differentially methylated between the two treatments. This geneset included ribosomal proteins and RNA polymerases as well as histones and methyl-binding proteins suggesting a functional role for DNA methylation in gene regulation. Overall, the results suggest that DNA methylation may play a significant role in environmental stress responses in Daphnia.

P24 | Effects of salinity during the development of mangrove rivulus (Kryptolebias marmoratus): anchoring behavioural traits to DNA methylation and protein expression profiles in adult brain

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Personality traits are known to be individually consistent across time and situations. Among them, boldness and aggressiveness strongly influence social relationships, reproductive success, food access and survival and, consequently, fitness. Mangrove rivulus, Kryptolebias marmoratus (cyprinodontiform), is one of two self-fertilizing hermaphroditic vertebrate species able to produce isogenic lineages. Variability of these personality traits in rivulus exists even in the absence of genetic diversity. We hypothesized that epigenetics can explain this behavioural plasticity when organisms are exposed to an environmental stress during development, and thus delayed behavioural responses can be observed in adults. Even if recent researches suggest that DNA methylation status in brain could influence behaviour by regulating gene expression, this mechanism remains largely uncharacterized and need to be expanded to personality traits. For this purpose, rivulus larvae were exposed to either 5 ppt or 25 ppt salinity from 0 till 60 days post hatching (dph). At 150, 170 and 190 dph shelter and model tests were performed to measure adult boldness and aggressiveness respectively. Genome-wide patterns of DNA methylation and hydroxymethylation at single-nucleotide resolution will be investigated in adult brain using Reduced Representative Bisulfite Sequencing (RRBS) and oxidative bisulfite sequencing. In parallel, the cellular phenotype will be assessed at the protein expression level using a label-free quantitative proteomic workflow. Preliminary results indicated that rivulus brain global DNA methylation at CpG sites, assessed by Luminometric Methylation Assay (LUMA) reached 75,9%, which seems lower than values reported in other fish species (around 85%). On the other hand, after two weeks exposure to different salinities, larvae do not show any significant change in global DNA methylation (79,2 % at 25 ppt and 77,7 % at 5 ppt). Understanding the molecular mechanisms underlying changes in personality traits in the absence of genetic diversity in rivulus is an appealing question in evolutionary physiology. Linking information from the cellular and whole-organism phenotypes will allow a better understanding of the sequence of key molecular events leading to behavioral modifications. Finaly, comparing this phenotype anchoring among lineages will help to deepen our knowledge about the genetic underpinnings of phenotypic plasticity.

P25 | Use of LUminometric Methylation Assay (LUMA) to measure global DNA methylation in ecological organisms

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The LUminometric Methylation Assay (LUMA) is a convenient method for measuring global DNA methylation. LUMA depends on digestion of DNA with methyl-sensitive and methyl-insensitive restriction enzymes, followed by pyrosequencing. Until recently, LUMA has been principally used for biomedical research. Here, we apply LUMA to a wide range of vertebrate species, compare the resulting data to measures obtained by other techniques, and investigate issues related to sample quality. LUMA was used to assess DNA methylation in 12 species representing five animal classes: amphibians (African and Western clawed frog), reptiles (green anole lizard), fish (yellow perch, goldfish, lake trout), mammals (American mink, polar bear, short-beaked common dolphin, Atlantic white-sided dolphin) and birds (chicken, Japanese quail). We saw a pattern of high DNA methylation in fish (84–87%), and intermediate levels in mammals (68–72%) and birds (52–71%). This pattern corresponds well with previous measures of DNA methylation generated by HPLC. We then assessed the effect of tissue storage conditions on DNA methylation values. This is an important consideration for ecological species because samples are not always ideally preserved and LUMA is sensitive to poor DNA quality. We found that good quality LUMA data could be obtained from chicken liver and brain tissues stored at 21 °C for at least 2 and 12 h, respectively. Longer storage times introduced nonspecific peaks to pyrograms which were associated with reduced DNA methylation. Repeatedly, freezing and thawing the tissues did not affect LUMA data. Current work is focused on assessing whether different DNA isolation methods affect LUMA pyrogram quality, and evaluating the effectiveness of LUMA for invertebrate samples. Our data represent the first CpG methylation values to be reported for several species of vertebrates and provide a basis for studying patterns of epigenetic inheritance in an ecological context.

P26 | Epigenetic signatures as sensitive tool in soil ecotoxicology: alterations and relation to biomarker response in heavy metal stressed earthworms

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Emerging evidence suggests that soil heavy metal pollution can alter epigenetic marks in soil fauna, with DNA methylation as one of the underlying mechanisms - a process which can be persistent and even heritable. The aim of this study was to assess the consequences of chronic heavy metal exposure on genome wide DNA methylation in the earthworm Lumbricus terrestris. Earthworms were exposed to low (10 mg/kg) environmentally relevant concentration of cadmium (Cd) for a period of 3 months. Through the exposure period, the biomarker response and health status of the earthworms was monitored at the cellular (DNA damage, oxidative stress, lipid damage) and organismic level (fitness, reproductive success) and their detoxification capacity was assessed by monitoring the expression of metallothionein (MT) gene. Genome wide DNA methylation status was assessed by methylation sensitive amplification polymorphism (MSAP) at the beginning of the exposure and after 1 and 3 months of exposure. During the exposure, earthworm fitness remained unaffected by Cd. Cellular stress was evident in the first 2 weeks of the exposure but afterwards it returned to the control level which corresponded well with the MT expression results. On the epigenetic level, already after 1 month of exposure, Cd induced significant increase in DNA methylation, which remained increased until the end of the exposure. The results of this study demonstrate that low levels of environmental pollution can lead to the occurrence of subtle epigenetic modifications, even when no or very low impact on the cellular and organismic level can

be observed. These findings reinforce the application of epigenetic tools as predictors of toxicological response and suggest their use in environmental toxicology assessment.

Session 5: Transgenerational and epigenetic effects of chemicals

P27 | Does prior exposure to heavy metals protect future generations of plants to metal stress?

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Many anthropogenic activities have contributed to a release of pollutants, including heavy metals, into the environment. As plants are sessile organisms, they cannot avoid exposure to harmful environmental conditions. It is therefore essential for plants that they are able to defend themselves by responding and adapting to harmful environments. The main objective of this study is to determine if long-term exposure of plants to low concentrations of uranium (U) in a previous generation induces altered tolerance or sensitivity towards different abiotic stressors. Therefore, two different seed-types were used: control seeds (that have never been exposed before to heavy metal stress) and seeds that were exposed in their previous generation to 5 µM U (i.e. U-seeds). Next, 18-day-old Arabidopsis thaliana plants were exposed to 5 or 10 µM Cd or to 25 or 50 µM U during 3 days. Exposing A. thaliana plants to U or Cd resulted in a reduced growth of both roots and shoots. In addition, an increased lipid peroxidation was observed, although no significant differences were present between the control seeds and the U-seeds. Also for different enzymes of the antioxidative defence system and for the concentration of oxidized and reduced glutathione, differences were observed for the different metal treatments but not between the different seed-types. However, at gene expression level, differences between control seeds and U-seeds were observed. As such, a significant increase in the transcript levels of different DNA-repair related genes was observed in the roots for U-seeds after exposure to 10 µM Cd, 25 µM U or 50 µM U. This possibly indicates that plants from the U-seeds have an increased capacity to repair DNA damage that can occur after heavy metal exposure. In addition, differences in transcript levels of genes related to DNA methylation were observed, with an increased expression in MET1 and DRM2 after exposure to 10 µM Cd, 25 µM U and 50 µM U in the U-seeds as compared to the control seeds. An increased expression of CMT3 was also observed in the U-seeds after exposure to 25 µM U and 50 µM U. Those changes can induce differences in the DNA methylation state of the U-plants which in turn can influence differences in gene expression. This can possibly induce an enhanced stress tolerance in plants that have been exposed in their previous generation to a low U concentration. To further complement the current analyses, global methylation levels will be analysed.

P28 | Implications of DNA methylation in the response of early-life stage zebrafish after TCS exposure

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DNA methylation is a dynamic epigenetic mark that contributes to gene regulation and genome maintenance. It appears mostly on cytosine residues within a CpG context. There are increasing evidences that toxicant exposure can alter methylation profiles, particularly during embryogenesis when DNA methylation patterns are established. In some extent these modifications can possibly last

in adulthood and might be inherited through successive generations. In the present study, we analysed the effects of triclosan (TCS) at 50 and 100 µg/L on DNA methylation during early zebrafish (Danio rerio) embryogenesis during 7 days post fertilisation (7dpf). We used Reduced Representative Bisulfite Sequencing (RRBS), which allows genome-wide investigation of methylation patterns at single-nucleotide resolution. Significant (cut-off q-value of < 0.01 after FDR adjustment and with percent methylation difference ≥ 15%) differentially methylated fragments (DMFs) between conditions were identified using DMAP software. We also investigated the correlation between DMFs within intragenic regions and their corresponding gene expression levels using high throughput quantitative PCR (Fluidigm Biomark-HDTM system). A total of 171 DMFs were identified, mostly between fish exposed to 50 µg/L and 100 µg/L (58% of the DMFs). The main biological pathways involved were the metabolic, the cellular, the biological regulation and the developmental processes. We also found that 18 DMFs were implicated in nucleic acid binding transcription factor activity. Almost 50% of the DMFs resided within CpG island shore (defined as 2 Kb from either side of a CpG island core) while 48% of the DMFs were outside any CpG feature. These results highlighted the relevance of "non-CpG island core" regions for genes regulation after stress exposure. Among all DMFs, we identified 51 fragments overlapping with intronic regions, 18 with exonic regions and only 4 fragments resided in a gene promoter region. 43 corresponding genes were selected for gene expression analysis. These data will provide a deep understanding of the links between gene regulation and the modifications of the methylation landscape at specific CpG island and genomic features. Overall this study emphasizes the epigenetic effects of an exposure to TCS during early embryogenesis and the necessity to take into account the possible long term and transgenerational impacts of pollutant exposure during developmental stages.

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P29 | Multigenerational exposure to silver ions and silver nanoparticles reveals heightened sensitivity and epigenetic memory in Caenorhabditis elegans

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The assessment of the environmental impact of engineered nanoparticles (ENPs) generally relies on the use of short term toxicity tests despite multigenerational exposures being an increasingly common scenario in natural environments. Current exposure patterns, in case of e.g. single or repeat pulses, may also include the potential to recovery from contamination. In this study we examine the impact of exposure of the nematode Caenorhabditis elegans to Ag ENPs and AgNO3 over ten generations. Continuous exposures were conducted at the respective previously determined reproductive EC30 (Ag ENP = 1.5 mg Ag/l and AgNO3 = 0.1 mg Ag/l) and effects of a range of concentrations on reproduction, growth and lifespan were measured at the parent, F2, F5, F8 and F10 generation. We further assessed the potential for recovery in populations that were initially exposed for six generations but subsequently placed in a clean environment for four generations before re-exposure. By combining these lines of investigation we were able to address both multigenerational and transgenerational effects. Toxicity of AgNO3, which was included to mimic complete dissolution of the Ag ENPs, was found to be greater than in Ag ENPs. However, continuous exposure to both silver forms caused a pronounced sensitization (ca. 10 fold) in reproduction within

two generations. This increased sensitivity was sustained until F10. Additionally, subtle effects on size and lifespan were observed. In the recovery populations the sensitization to both silver forms persisted despite removal of the exposure over four generations. Responses in all measured endpoints were most closely related to those of the last exposed ancestral generation (F5) rather than unexposed populations indicating a clear transgenerational effect. Various mechanisms may be postulated to have caused the observed sensitization. Given that the reproductive output of the control populations remained unchanged effects of culturing alone or artificial selection toward sensitive individuals are unlikely. Considering that sensitization was sustained across multiple unexposed generations with levels corresponding to the last exposed ancestors mechanisms are likely organised through the epigenome. This suggests that multigenerational and transgeneration effects of chemicals may have important consequences for the sensitivity of species that will be important considerations for risk assessment.

P30 | Behavioral and transcriptional effects of bisphenol A in zebrafish embryos

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Several studies have shown that the epigenome of fish can be affected by environmental contaminants. The endocrine disruptor bisphenol A is one of the best-studied contaminant proven to induce epigenetic alterations in animals. In this study we used behavioral and singe-gene transcriptional endpoints to search for low-dose effects of bisphenol A (BPA) on zebrafish embryos, to be followed up by evaluation of whole-genome DNA methylation (WGBS). Zebrafish embryos were exposed to sublethal concentrations of BPA from 2 to 26 hours post fertilization (hpf) (24 h exposure during critical development period). Treatments were 0, 0.0001, 0.001, 0.01, 0.1, 1, 10, 30 µM of BPA (dissolved in DMSO). Higher concentrations were not used as they resulted in high levels of embryo deformity. At 72 hpf, embryos showing no signs of deformities from each treatment replicate were transferred to individual wells of a 96 well plate in a randomized design, and analyzed for locomotor activity at 3, 4 and 5 dpf in response to changing light conditions, using an automated video tracking system (Zebralab). The results showed no differences ($p>0.05$) in hatching or survival rates between controls and BPA treated. A small but statistically significant ($p<0.05$) increase occurred in the rates of pericardial edema in embryos from the highest BPA-treated group (30 µM) at 24 hpf. Over the examined concentration ranges hyperactivity was demonstrated with exposure to 0.001 µM BPA in comparison to embryos exposed to lower or higher BPA concentrations. The locomotor response of larvae to BPA demonstrate that a) early developmental exposure of BPA can affect larval locomotor activity over large concentration differences in exposure, b) light conditions (dark:light variation) is a factor in response to BPA exposure and c) BPA can induce hyper or hypoactivity depending on concentration, light conditions and larval age. In addition, the amount of space to move also appears to be a factor in the larval response to BPA. Dose-response effects were determined for several genes with RT-qPCR. Compared to the control, two DNA methylation genes (dnmt1, cbs) showed significant effects at 0.01 µM BPA ($p<0.0001$). Significant effects were also seen for estrogenic markers (ar, esr2). In conclusion, this study shows that BPA can affect zebrafish embryos at very low concentrations, and suggests that this system represents an interesting model for further epigenetic research.

P31 | Cross-generational epigenetic effects of diet restriction in Daphnia magna

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The condition of mothers, as determined by the environment in which they live, has strong effects on offspring development, birthweight, infection, and later chronic disease. We aim to advance understanding of how epigenetic inheritance modulates these traits in offspring using the crustacean Daphnia magna. This species is ideal for demonstrating epigenetic inheritance: (a) multi-generation adaptive maternal effects on performance traits have been demonstrated; (b) mothers can reproduce clonally which removes paternal and genetic variation effects from experiments; (c) there is a sequenced genome and extensive metabolic pathway annotation. Food stressed Daphnia mothers produce fewer but larger offspring. These offspring are more resistant to starvation and parasites, but have smaller clutch sizes than the offspring of unstressed mothers. As a result, the two offspring types have different life-history trajectories; they are prepared for different challenges. Here, we varied mothers' nutritional states and measured epigenetic changes in the offspring (F1) and great granddaughter (F3) generations. We predicted that changes in epigenetic modifications will be strongest in the F1 and degrade with each generation. However, to demonstrate a truly transgenerational effect some differing modifications must be maintained to the F3 generation. We are now analysing changes in three epigenetic mechanisms due to nutritional regime: genome methylation (via bisulphite sequencing), histone modification patterns (via whole genome chromatin immunoprecipitation (ChIP-seq), and small RNA expression differences (via RNAseq). We will then identify which mechanism is most important in D. magna and whether they correlate with one another in a coordinated manner. We will link these patterns to offspring, performance and global gene expression differences.

P32 | Metabolomic, transcriptomic, and epigenetic effects of BPA exposure during the early zebrafish development

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Increasing evidence demonstrates that environmental insults occurring during the early development may have life-long effects, indicating that epigenetic mechanisms could be involved. However, epigenetic approaches in ecotoxicology are still lacking. Due to its similarities in epigenetic regulatory machinery to mammals, zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining metabolomic, transcriptomic and epigenomic tools. Our long-term target is the fusion of multiple omic techniques to identify models to predict phenotypic traits and outcomes. The main objectives of our project are: 1) recognize target genes regulated by EDC exposures at the transcriptomic and epigenomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate metabolomic, transcriptomic and epigenetic platforms to identify whole genome molecular footprints characteristic of EDCs exposures. In the present study, we exposed zebrafish larvae to BPA from 0 to 5 dpfs, and analyzed changes in gene expression patterns by RNA-sequencing (RNA-seq) and in the metabolome by untargeted LC-MS. From the commonly altered pathways, we selected a set of promoters to study DNA methylation profiles. We intend to determine the role of epigenetic mechanisms in life-long lasting effects of BPA and other endocrine disruptors. This study will increase the knowledge of regulatory mechanisms and modes of action of EDCs at a global scale, including epigenetic effects. We anticipate that studies on ecotoxicoepigenetics will assist in early detection and risk assessment of environmental emerging contaminants in the near future.

P33 | Impacts of Environmental chemicals on epigenetic modulation in Ecotoxicity model species

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Epigenetics, phenotypic characters without modification of gene sequence, possess reversibility as well as the heritable transgenerational transfer of epigenetic marks which argues for it inclusion as sensible biomonitoring programs. Environmental epigenetics principally deals with the cause-effect relationships between the altered adaptive responses of biological organisms and the specific environmental factors. Indeed, several epigenetic biomarkers (DNA methylation, histone modification and non-coding RNA) have been successfully applied not only in traditional model organisms but also in human epidemiology. The epigenetic analysis with its' dynamic and extraordinary potentiality, has now also been a reality in ecologically and environmentally relevant organisms. In our current ongoing projects, we found that bisphenol-A (BPA) caused hypermethylation in various ecological model species, such as, daphnia, chironomus, zebrafish (adult as well as embryo). In addition, a typical alteration in global DNA methylation was evident in transgenerational adaptation, from F0 to F3/F4, of daphnia (exposed to BPA and organic contaminated stream water) as well as in chironomus (exposed to organic contaminated sediment). Besides global DNA methylation, the changes in histone methylation were also observed in BPA exposed chironomus and crude oil exposed C.elegans which was possibly related to their decrease in reproductive potentiality. Taken together, our results strongly support that epigenetic marks not only could function as sensible tools for pollution biomonitoring, but also could serve as indicator of trangerational transferability of the phenotypic effects in unexposed subsequent generations.

Session 6: Epigenetics in risk assessment: academia, industry and regulator perspective

P34 | Cadmium hepatotoxicity: an epigenetic gastropod-based approach

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Background. Cadmium (Cd) affects our bodies at multiple levels, including the epigenome.W Methylation of DNA at the 5 position of cytosine (5-methylcytosine, 5mC) can influence gene expression and DNA metabolism. Depending on concentration and exposure duration, Cd can induce both global hyper- and hypomethylation in human cells/tissues. Although gastropods serve as pertinent bioindicators for environmental cadmium, there is no information linking Cd exposure with changes in global 5mC levels in these terrestrial invertebrates. Objective. We evaluated global 5mC levels in DNA of the hepatopancreas of land snails with the aim to develop this epigenetic mark as a potential biomarker of chronic low-level Cd exposure and hepatotocixity. Materials and Methods. Adult Cantareus aspersus specimens were reared for 112 days under controlled laboratory conditions and fed Cd-enriched diets, i.e., 0, 0.02, 0.05, 0.2, 1, 10, and 100 parts per million (ppm). Hepatopancreas samples were collected at 0, 14, 28, 56, and 112 days. We measured Cd levels in this organ using Flame Atomic Absorption Spectrometry and the percentage of 5-mC in samples using an ELISA-based colorimetric assay. Snail death rates were used as a lethality endpoint. Statistical analysis was used to estimate the level over which Cd accumulation may alter the snail DNA

methylome. Results and Discussions. This is the first study to document the presence of 5mC in C. aspersus and to reveal the potential epigenetic effect of cadmium exposure on mollusks. Our results showed that cadmium levels in snail hepatopancreas increased with time and concetrations in food ($p < 0.05$). Detectable levels of 5mC were observed in hepatopancreas samples. Variable methylation levels (analysis in progress) were found in response to cadmium exposure. Conclusions. Our findings indicate that global 5mC levels are sensitive and respond to chronic dietary low-level cadmium exposure in the hepatopancreas. Acknowledgements. The present work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, Research Council, project number PN-II-RU-TE- 2014-4-0776 (awarded to DVN).