30th ESCPB Congress

Unraveling complexity: from molecules to ecosystems

BOOK OF ABSTRACTS

Barcelona, 4th to 7th of September 2016

INSTITUT DE DIAGNOSI AMBIENTAL I ESTUDIS DE L'AIGUA

Universitat de Barcelona

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PRESENTATION

The **local organizing committee** and **Cosmocaixa** would like to welcome you to the 30th Congress of the New European Society for Comparative Physiology and Biochemistry (ESCPB 2016).

ESCPB was founded in 1978 and soon became one the most important scientific platforms for promoting and supporting comparative physiology and biochemistry in Europe. The society was renewed in 2007 to incorporate recent developments and novel technologies in environmental and life sciences, gaining a wider perspective and potential for innovation. Since its foundation, 29 ESCPB congresses have been organized in locations across Europe (Ravenna, Innsbruck, Bilbao, Glasgow). ESCPB is open to scientists interested in comparative aspects of physiology and biochemistry, with a special emphasis on integration of environmental aspects as modulating factors of physiological, cellular, biochemical and molecular processes.

We have prepared an attractive program aimed to encourage the interaction between basic and applied research science and encouraging the participation of young researchers. We hope you enjoy the meeting and your stay in Barcelona!

Cinta Porte, Carlos Barata, Joaquim Gutiérrez

Local Organizing Committee

(www.escpb.eu)

CONGRESS VENUE

Cosmocaixa

C/ Isaac Newton, 26. Barcelona

PROGRAM

4th September	Room Auditorium		Room Agora	
17:00-17:15	**OPENING CEREMONY**			
17:15-18:15	IS-01: VANCE TRUDEAU	p.8		
18:30-19:30	WELCOME COCKTAIL RECEPTION (HALL)			
5th September				
08:30-08:50	O-1	p.14	O-15	p.28
08:50-09:10	O-2	p.15	O-16	p. 29
09:10-09:30	O-3	p.16	O-17	p.30
09:30-09:50	O-4	p.17	O-18	p.31
10:00-10:30	COFFEE BREAK AND **PS-I**: MO01 to MO44 (HALL)	p.98-141		
10:30-11:15	IS-02: ILARIA CORSI	p.9		
11:20-11:40	O-5	p.18	O-19	p.32
11:40-12:00	O-6	p.19	O-20	p.33
12:00-12:20	O-7	p.20	O-21	p.34
12:20-12:40	O-8	p.21	O-22	p.35
12:40-14:00	LUNCH AND **PS-I**: MO01 to MO44 (HALL)	p.98-141		
14:00-14:20	O-9	p.22	O-23	p.36
14:20-14:40	O-10	p.23	O-24	p.37
14:40.15:00	O-11	p.24	O-25	p.38
15:00-15:20	O-12	p.25	O-26	p.39
15:20-15:40	O-13	p.26	O-27	p.40
15:40-16:00	O-14	p.27	O-28	p.41
16:00-18:00	COFFEE AND **PS-I**: MO01to MO44 (HALL)	p.98-141		
18:00	VISIT TO PLANETARIUM			

PROGRAM

6th September	Room Auditorium		Room Agora	
08:30-08:50	O-29	p.42	O-43	p.56
08:50-09:10	O-30	p.43	O-44	p.57
09:10-09:30	O-31	p.44	O-45	p.58
09:30-09:50	O-32	p.45	O-46	p.59
10:00-10:30	COFFEE BREAK AND **PS-I**: MO01 to MO44 (HALL)			p.98-141
10:30:11:15	IS-03: DANIEL ZALKO	p.10		
11:20-11:40	O-33	p.46	O-47	p.60
11:40-12:00	O-34	p.47	O-48	p.61
12:00-12:20	O-35	p.48	O-49	p.62
12:20-12:40	O-36	p.49	O-50	p.63
12:40-14:00	LUNCH AND **PS-II**: TU01 to TU41 (HALL)			p.142-182
14:00-14:20	O-37	p.50	O-51	p.64
14:20-14:40	O-38	p.51	O-52	p.65
14:40.15:00	O-39	p.52	O-53	p.66
15:00-15:20	O-40	p.53	O-54	p.67
15:20-15:40	O-41	p.54	O-55	p.68
15:40-16:00	O-42	p.55	O-56	p.69
16:00-16:20			O-57	p.70
6:20-18:00	COFFEE AND **PS-II**: TU01to TU41 (HALL)			p.142-182
20:30	CONGRESS DINNER			

PROGRAM

7th September	Room Auditorium		Room Agora	
08:30-08:50	O-58	p.71	O-71	p.84
08:50-09:10	O-59	p.72	O-72	p.85
09:10-09:30	O-60	p.73	O-73	p.86
09:30-09:50	O-61	p.74	O-74	p.87
10:00-10:30	COFFEE BREAK AND **PS II**: TU01 to TU41 (HALL)			p.142-182
10:30:11:15	IS-04: KRISTIN SCHIRMER	p.11		
11:20-11:40	O-62	p.75	O-75	p.88
11:40-12:00	O-63	p.76	O-76	p.89
12:00-12:20	O-64	p.77	O-77	p.90
12:20-12:40	O-65	p.78	O-78	p.91
12:40-14:00	LUNCH AND **PS- II**: TU01 to TU41 (HALL)			p.142-182
14:00-14:20	O-66	p.79	O-79	p.92
14:20-14:40	O-67	p.80	O-80	p.93
14:40.15:00	O-68	p.81	O-81	p.94
15:00-15:20	O-69	p.82	O-82	p.95
15:20-15:40	O-70	p.83	O-83	p.96
15:40-16:25	IS-05: S. (SACHI) KAUSHIK	p.12		
16:25-17:00	**CLOSING CEREMONY**			
17:00	FAREWELL DRINK			

ENCODE NAME

IS → Invited speaker
O → Oral
PS-I → Poster session I
PS-II → Poster session II

CONGRESS SESSIONS

ENVIRONMENTAL PROBLEMS OF NANOMATERIALS

BIOMARKERS

ENVIRONMENTAL PROBLEMS OF MICROPLASTICS

ENDOCRINE DISRUPTORS

THE –OMIC TECHNOLOGIES

COMPARATIVE PHYSIOLOGY AND BIOMARKERS

ANIMAL REPLACEMENT

OCEANS AND HUMAN HEALTH: ACIDIFICATION

LIPID HOMEOSTASIS AND OBESOGENS

COMPARATIVE PHYSIOLOGY AND AQUACULTURE

COMPARATIVE PHYSIOLOGY AND BIOREMEDIATION

Invited speakers

IS-01 *Opening Ceremony*

INTEGRATIVE NEUROENDOCRINE CONTROL OF FISH REPRODUCTION: MULTIPLICITY AND APPLICATION

Vance L. Trudeau[1], Kimberly Mitchell[1], Marilyn Vera Chang[1], Dillon Da Fonte[1], Lei Xing[1], Wei Hu[2]

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The multiplicity (>20) of stimulatory neuropeptides and neurotransmitters is in marked contrast to the limited number of inhibitory factors known to control teleost reproduction. Preoptic and hypothalamic neuronal networks synthesizing neuropeptides, catecholamine and amino acid neurotransmitters send projections to the anterior pituitary where luteinizing hormone (LH), follicle stimulating hormone, growth hormone (GH) and prolactin are both directly and indirectly regulated. The neurohypophysial peptides isotocin and vasotocin are also involved in reproduction. Emerging data on kisspeptins, gonadotropin-inhibitory hormones and neurokinin B in tetrapod models has led comparative endocrinologists to explore their potential roles in fish. Negative and positive steroid feedback at the levels of the brain and pituitary remains poorly understood. Radial glial cells are stem-like neuronal precursors that expression aromatase B. Strong evidence for neuroendocrine roles for neurosteroids synthesized by RGCs remains elusive, despite the observation that teleosts are champion neuroestrogen producers. The discovery of intrapituitary paracrine control by traditional hormones (LH, GH) and novel peptides (e.g., secretoneurin) raises fundamental questions about the integration of signaling pathways. While many aspects of the control of reproduction are conserved in evolution, recent functional and anatomical studies, including genetic manipulations (TALEN or CRISPR) in teleost models reveal major differences that must be understood. Receptor signal transduction studies for some teleost neurohomones are well-advanced; however, the integration of the multiplicity of stimulatory and inhibitory signals by neurons and pituitary cells is unclear. Critically missing is a unifying model incorporating endocrine, neuroendocrine, paracrine and autocrine mechanisms. A simplified view recognizing these aspects will be presented. This may serve as the framework for a systems biology approach to the complexity of teleost reproductive control. The application of such basic knowledge may lead to new approaches for the management of cultured or endangered species, or the development of novel methods for testing the effects of environmental pollutants on neuroendocrine systems.

IS-02 *Environmental problems of nanomaterials*

ECOSAFETY ASSESSMENT AND DESIGN OF NANOMATERIALS ENTERING THE MARINE ENVIRONMENT

Ilaria Corsi

Dept. of Physical, Earth and Environmental Sciences, University of Siena, Italy

Engineered nanomaterials (ENMs) are nowadays used extensively in a variety of emerging technologies and commercial products, including biomedicine, pharmaceuticals and personal care, renewable energies, and electronic devices. As these materials are used, disposed of, and degraded, they can release ENMs into the environment. Fate and transport models indicate that ENMs entering soil and waterways will eventually reach the marine environment as nanowaste that can cause human injuries as well as ecological impact with significant socioeconomic consequences. Moreover, some accidental releases and exposures cannot be excluded since a suite of new marine nanotechnologies, including antifouling paints and pollution remediation systems, are also being developed with great uncertainty about their ecosafety and sustainability for the marine environment. An increasing number of short-term, well-controlled laboratory studies have tested ENM's toxicity on marine organisms and showed a wide variety of potential biological injuries. Whether ENMs cause similar injuries in the dynamic natural marine environment is uncertain because the fate, transport, and behavior of many ENMs in natural seawater, and thus their biological risks, remain poorly understood. The chemistry of ENMs plays a crucial role as their bioavailability, bioaccumulation, and toxicity are difficult to predict in seawater because the materials undergo complex interactions/transformations when exposed to elevated ionic concentrations and in the presence of natural organic matter. Therefore, developing tools to predict, estimate and compare short and long-term effects and risks of ENMs entering the marine environment presents many challenges. State-of-the-art approaches used to measure the exposure and toxicity of ENMs in marine ecosystems will be discussed as well as the influence of key environmental conditions and other multiple environmental stressors including interaction with existing contaminants.

IS-03 *The –omic technologies*

THE COMPLEX FATE AND EFFECTS OF BISPHENOLS: FROM EXPOSURE ISSUES TO METABOLOMICS-BASED SYSTEMS BIOLOGY STUDIES

Daniel Zalko

Toxalim, Université de Toulouse, INRA, Toulouse, France

Bisphenols form a large and diverse family of chemicals. They are mainly used for manufacturing bisphenol polymers, namely epoxy resins (paints, lacquers, food can internal lacquers) and polycarbonates (compact discs, plastic ware) which properties and industrial use vary according to the chemical structure the bisphenol monomer they are based on. Human exposure to bisphenols remains insufficiently documented but has unequivocally been demonstrated for bisphenol A (BPA). Evidence for human exposure also exists for BPA substitutes (BPF, BPS…) as well as halogenated bisphenols, which are mainly used as fire retardants. BPA, one of the largest selling chemicals worldwide, is a well-known xeno-estrogen and a model endocrine disruptor. Its adverse effects have been demonstrated including at very low doses of exposure, when exposure occurs during critical stages of development (perinatal period). Over the last decade, it gradually became obvious that bisphenols, as well as other xeno-estrogens, not only can target the reproductive system, but could also durably modulate general metabolism and play a role in the onset of obesity and/or type II diabetes. BPA is even suspected to exert neuro-developmental effects through metabolic disruption. The metabolic fate, but also the toxicology of bisphenols, largely depends on their structure. For instance, halogenated analogues of BPA are poor estrogen receptor activators, but conversely are excellent ligands for other key nuclear receptors involved in metabolic homeostasis. Moreover, some metabolites of bisphenols are the object of scientific controversies regarding their pharmacological activity and potential effects. Novel metabolomics approaches are increasingly used in toxicology, with the aim to characterize and model the mechanisms of effects of toxicants, including the complex effects of endocrine disruptors. Together with a thorough understanding of bisphenols fate, they provide new keys for a better characterization of bisphenols targets and ability to disrupt general homeostasis.

IS-04 *Animal replacement*

USING FISH CELLS IN CULTURE TO PREDICT THE IMPACT OF CHEMICALS TO FISH

Kristin Schirmer*[1,2,3]

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[2]ETH Zürich, Institute of Biogeochemistry and Pollutant Dynamics, CH 8092 Zürich
[3]EPF Lausanne, School of Architecture, Civil and Environmental Engineering, CH 1015 Lausanne
*(kristin.schirmer@eawag.ch)

Fish cell-based systems hold great potential for deciphering the molecular mode of action of chemicals and, provided the right choice of *in vitro* model and exposure set-up, may supplement or even substitute fish toxicity tests. Conceptually, if the cell-based responses reflect the initial stage for an adverse outcome seen at the organism level, it must be possible to develop an array of cellular systems suitable to identify mechanisms of action and adverse outcomes. I will present three examples of recent advances in support of this concept: (1) We have developed a rainbow trout (*Oncorhynchus mykiss*) gill cell line assay that is capable to predict fish acute toxicity: in fact, we found that, overall, the cell line responds like a fish in quantitative terms to acute chemical exposure. (2) We investigate the biotransformation of chemicals in gill, intestinal and liver cells of fish and input the kinetic information into physiologically-based toxicokinetic models to predict bioconcentration in fish. (3) We recently demonstrated that it is possible to predict the impact of chemicals on fish growth *(in vivo)* based on fish cell population growth *(in vitro)*. Like for bioconcentration prediction, we used a mechanistic model to scale between the *in vitro* and the *in vivo* outcome. These examples show that, when fish cell culture systems are purposely combined, systematically linked to chemical concentrations, and outcomes scaled to fish via computational approaches, they provide foundation for the development of a "virtual fish".

IS-05 *Comparative physiology and aquaculture*

THE ROLE OF SOLID NUTRITIONAL SCIENCE FOR SUSTAINABLE AQUACULTURE DEVELOPMENT

Sadasivam (Sachi) Kaushik

ERA Chair, EcoAqua Institute, Universidad Las Palmas de Gran Canaria, Spain

Aquaculture is now the major source of seafood for humans around the world, exhibiting continued growth. The major responsibility of the actors involved in aquaculture is to ensure that that the growth in fish production is managed in a sustainable manner. Given the economic importance of nutrient supply through feeds, there is a clear need for demonstrating and further improving the efficiency of resource (nutrient, energy, water, land) utilisation to make aquaculture sustainable. In this context, basic nutrition research has a strong role to play. There is concern as regards the reliance of fish and shrimp farming on feeds containing high levels of fish meal (FM) and fish oil (FO) derived from capture fisheries. Research in the area of replacement of FM and FO as dietary sources of essential amino acids, fatty acids and micronutrients has made much progress in the recent years and is reflected by the significant reductions in the inclusion of these ingredients in the commercial feeds. Ensuring the nutritional value of farmed fish as food for man, reducing the environmental footprint of aquaculture and enhancing the resource utilisation efficiencies are some of the challenges. To apply precision-farming approach in aquaculture, we need to fully understand the high protein / amino acid requirements of aquatic animals analyse and exploit the differences in fatty acid bioconversion capacities and improve micronutrient supply and utilisation especially since feed composition is the fastest changing environmental factor. We need to properly analyse genotype nutrition interactions with dedicated studies to separate the effects of specific nutrients from those due to ingredients themselves by using robust non-lethal biomarkers to assess physiological consequences. We should recognise the importance of complementary analyses linking responses at different levels: from animal trait ontology to gene ontology. These are some of the main challenges for sustainable aquaculture development.


effects
zebrafish
fish
Mytilus
galloprovincialis
environmental
nanoparticles
species
expression
physiological
marine
exposure
model
responses
monitoring
early
activity
mussel
response
toxicity
freshwater
biochemical
approach
metabolism
biomarkers
embryos
stages
water
concentrations
different
gene
disruption
reproductive
Daphnia
magna
sea
lipid
development
using
larval
salmon
two
acidification
Atlantic
life
Mediterranean
liver
analysis
mussels
horse
protein
impact
vitro
stress
oyster
urchin
quality
Salmo
change
conditions
molecular
organisms
biological
earthworms
Biomphalaria
Reduced
Graphene
drugs
alterations
Carbon
temperature
glabrata
trout
Dicentrarchus
Cytotoxicity
microplastics
immunotoxicity
performance
bivalve
Japanese
Oncorhynchus
oxidative
retardants
behaviour
developmental
Cd
tool
crab
labrax
human
osmoregulation
age
genes
receptor
contaminants
Comparative
comparison
flame
Trojan
action
Endocrine
oxygen
Sea
Chemical
assessment
ecotoxicological
exposed
estrogen
investigation
larvae
potential
River
identification
reproduction
stage
seawater
mykiss
European
mechanisms
philippinarum
assay
radiation
Oxide
silver
copper
Medaka
mixtures
Lysosomal
Molecular
metal
immune

Orals

O-1 *Environmental problems of nanomaterials*

INTERACTIONS OF CATIONIC POLYSTYRENE NANOPARTICLES WITH MARINE BIVALVE HEMOCYTES IN A PHYSIOLOGICAL ENVIRONMENT: ROLE OF SOLUBLE HEMOLYMPH PROTEINS

Laura Canesi[1], Caterina Ciacci[2], Rita Fabbri[1], Teresa Balbi[1], Annalisa Salis[3], Gianluca Damonte[3], Katia Cortese[4], Marco Monopoli[5], Kenneth Dawson[5], Ilaria Corsi[6]

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[2]Dept.of Biomolecular Sciences -DIBS, University of Urbino, Italy
[3]Centre of Excellence for Biomedical Research, University of Genoa, Italy
[4]Department of Experimental Medicine-DIMES, University of Genoa, Italy
[5]Centre for BioNanoInteractions, University College Dublin, Ireland
[6]Dept. of Physical, Earth and Environmental Sciences, University of Siena, Italy

In the marine bivalve *Mytilus galloprovincialis,* the immune system represents a sensitive target for different types of nanoparticles (NPs) both *in vitro* and *in vivo*. In environmental conditions, both NP intrinsic properties and those of the receiving medium will affect particle behaviour and consequent bioavailability/uptake/toxicity. However, the evaluation of the biological effects of NPs requires additional understanding of how, once within the organism, NPs interact at the molecular level with cells in a physiological environment. In mammalian systems, different NPs associate with serum soluble components, organized into a ‘‘protein corona’’, which affects particle interactions and effects with target cells. However, no information is available so far on the interactions of NPs with biological fluids of aquatic organisms. In this work, the influence of hemolymph serum (HS) on the *in vitro* effects of amino modified polystyrene NPs (PS-NH_2) on *Mytilus* hemocytes was investigated. Hemocytes were incubated with PS-NH_2 suspensions in HS (1, 5 and 50 µg/ml). Cell functional parameters (lysosomal membrane stability, oxyradical production, phagocytosis) were evaluated, and morphological changes were investigated by TEM. The activation state of the signalling components p38 MAPK and PKC was determined. The results show that in the presence of HS, PS-NH_2 increased lysosomal destabilization, oxyradical production and cellular damage with respect to artificial sea water (ASW). The effects were mediated by disregulation of p38 MAPK signalling. PS-NH_2–protein complexes in HS were isolated by centrifugation and SDS gel electrophoresis. The results of nano-HPLC-ESI-MS/MS identified the Putative C1q domain containing protein of *M. galloprovincialis* (MgC1q6) as the main component of the PS-NH_2 protein corona in *Mytilus* hemolymph. The results underline the importance of the physiological exposure medium in *in vitro* testing with marine invertebrate cells. These data will contribute to a better understanding of the *in vivo* effects of NPs in aquatic invertebrates.

O-2 *Environmental problems of nanomaterials*

CHANGES IN PROTEIN EXPRESSION IN MUSSELS *MYTILUS GALLOPROVINCIALIS* DIETARILY EXPOSED TO PVP/PEI COATED SILVER NANOPARTICLES AT DIFFERENT SEASONS

Nerea Duroudier[1], Câtia Cardoso[2], Maria J. Bebianno[2], Eider Bilbao[1], Miren P. Cajaraville[1]

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[2]CIMA, Marine and Environmental Research Center, University of Algarve, Campus de Gambelas, 8000-135 Faro, Portugal.

The rapidly growing benefits and applications of silver nanoparticles (Ag NPs) have increased concerns about their potential input into aquatic ecosystems and associated environmental hazards. Toxicity of Ag NPs to waterborne exposed aquatic organisms has been widely studied. However, potential toxic effects of Ag NPs ingested through the food web and depending on the gametogenic developmental stage are still unexplored. The aim of this work was to assess differences in protein expression profiles in the digestive gland of female mussels after dietary exposure to Ag NPs both in autumn and in spring. Mussels *Mytilus galloprovincialis* were fed daily with microalgae *Isochrysis galbana* previously exposed for 24 hours to 10 µg/L of PVP/PEI coated 5 nm Ag NPs. The aggregation and dissolution behavior of NPs was studied in seawater. After 21 days of exposure, differentially expressed protein profiles between control and exposed mussels were discriminated using two-dimensional electrophoresis. In autumn, 104 significantly different spots were detected (Mann-Whitney U-rank test, $p < 0.05$), while in spring the number of spots increased to 142. Among them, 46 and 26 spots were specific for control mussels in autumn and in spring, respectively, while 36 spots were specific for exposed mussels in autumn and 83 in spring. Based on the Bivalvia database, 27 out of 60 differentially expressed spots were identified by MALDI-TOF. In autumn, proteins related to cytoskeleton (*paramyosin* and *vinculin*) and proteins involved in metabolic processes (*glyceraldehyde-3-phosphate dehydrogenase* and *chitinase-like protein-3*) were differentially expressed after Ag NPs exposure. In spring *glyceraldehyde-3-phosphate dehydrogenase* and *chitinase-like protein-3* were downregulated and proteins related to stress response (*superoxide dismutase* and *putative C1q domain containing protein MgC1q52*) were upregulated. Overall, season and Ag NPs exposure dependent protein expression profiles were determined.

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O-3 *Environmental problems of nanomaterials*

MOLECULAR RESPONSES OF FRESHWATER MUSSELS TO NANO-ZNO DEPENDING ON THE *IN SITU* EXPOSURE HISTORY

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Electrical power production affects freshwater ecosystems due to chemical and thermal pollution and may sensitize organisms to emerging stressors such as metal nanoparticles. We studied stress responses to nano-ZnO in indigenous mussels *Unio tumidus* from a pristine area (I-group) and from the cooling reservoirs of power plants (PP) including a nuclear PP (N-) and two thermal PPs (B-, D-groups). Mussels were exposed for 14 days to elevated temperatures (T, 25^{o}C), nano-scale zinc oxide (n-ZnO, 3.1 μM), Zn^{2+} (3.1 μM), or n-ZnO at 25 ^{o}C (n-ZnO+T). Control groups from each site were held at 18^{o}C for 14 days. Mussels from the PPs had higher background levels of stress biomarkers and were more sensitive to chemical pollutants than their counterparts from a pristine habitat, whereas mussels from I-site were most vulnerable to warming. Exposures to Zn and n-ZnO induce upregulation of MT levels by ~ 30% in most groups. However, warm exposure suppressed MT levels in I- and N- treated groups, and abolished MT upregulation in ZnO-exposed I-group. The changes of caspase-3 and cathepsin D activities reveal high sensitivity to all impacts with opposite direction of changing in I- and B- *versus* N- and D-groups. Mussels from DPP showed higher level of DNA fragmentation and oxidative injury. Our data show that long-term acclimation and/or adaptation of mussels to elevated temperatures in the N- and B-cooling ponds results in increased thermotolerance. However, the mussels from the highly polluted DPP had the lowest tolerance to all tested stressors. Our findings indicate the limited capacity of cellular mechanisms to protect against multiple environmental insults and demonstrate that combination of multiple stressors (each relatively benign by itself) can overwhelm the cellular protective systems.

O-4 *Environmental problems of nanomaterials*

CYTOTOXICITY OF COPPER OXIDE NANOPARTICLES ON HAEMOCYTES OF THE MARINE BIVALVE *RUDITAPES PHILIPPINARUM*: AN *IN VITRO* APPROACH

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Copper oxide nanoparticles (CuO NPs) are increasingly investigated, developed and manufactured for a wide and promising array of commercial and industrial products. Their increased production use and disposal will eventually lead to their increased release into the aquatic environment and potentially affect non-target organisms within. Although CuO NPs have been identified to be potentially cytotoxic, little is known about their behaviour in the marine environment and its potential influence on their cytotoxicity. We studied aggregation and dissolution kinetics of two commercially available bare copper oxide nanoparticles with nominal similar mean sizes (~ 40 nm), but distinct synthesis processes (wet chemistry and combustion synthesis) in various environmental and experimental relevant conditions. In addition cytotoxicity and DNA damage, as well as gene expression of oxidative stress, inflammatory response, DNA damage repair and cell death mediator markers were studied in *Ruditapes philippinarum* haemocytes after 24 h *in vitro* exposure and compared with sub-micron CuO (~ 500 nm) and water soluble Cu treated cells. Our results indicate that aggregating behaviour largely influences the toxicity of CuO NPs by influencing their susceptibility to ion leaching from the particle/aggregate surface. Gene expression analysis identified highly similar modes of action for all tested particulate and ionic Cu forms, further substantiating that nano and bulk CuO toxicity might largely be driven by ionic Cu. In addition our work highlights various differences in the behaviour of CuO NPs in environmental and culture conditions that need consideration when extrapolating results from *in vitro* to natural (or environmental) conditions.

O-5 *Environmental problems of microplastics*

A META-ANALYSIS OF THE ECOTOXICOLOGICAL EFFECTS OF PHARMACEUTICALS ON AQUATIC ORGANISMS

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Pharmaceutical compounds are considered emerging pollutants of priority concern due to their ubiquity in the aquatic environment and to their potential to elicit biological effects even at low concentrations. Given their increased use and continuous release of pharmaceutical residues to surrounding waters, they are also considered persistent or pseudo-persistent contaminants. Over the past two decades an increasing number of studies have assessed the toxicity of several pharmaceutical compounds using different biological endpoints in various taxa, and reported varying effects. Therefore, a systematic quantitative assessment is of the utmost importance to improve current understanding of the ecological risks of pharmaceuticals to nontarget organisms in the aquatic environment. To unravel patterns in biomarker responses across different taxa and aquatic environments a meta-analysis was performed on reported effects of exposure to pharmaceutical compounds (according to therapeutic class). Studies were selected based on a set of objective criteria considering organisms' exposure to pharmaceutical residues under controlled conditions. Several endpoints were considered, namely biochemical, developmental (e.g. growth), reproductive and behavioral responses, and lethality, for studies reporting effects on fish, crustaceans and molluscs. The value and sensitivity of different biomarkers and endpoints was evaluated according to pharmaceutical class and taxa. The meta-analysis provided a key framework to compare effects among multiple taxa and aquatic environments. Overall the implications of current findings for environmental monitoring and ecological risks of pharmaceuticals in aquatic ecosystems are discussed.

O-6 *Environmental problems of microplastics*

ECOTOXICOLOGICAL POTENTIAL OF PHARMACEUTICALS IN MARINE ENVIRONMENT

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Pharmaceutical residues in aquatic ecosystems represent a growing concern for possible deleterious effects on non target organisms. Despite their ubiquitous occurrence in natural environments, information on the biological consequences, bioavailabilty and distribution pathways are still limited, especially in coastal areas. To unravel the complexity of this environmental issue, the ecotoxicological potential of five Non-Steroidal Anti Inflammatory-Drugs (NSAIDs) was investigated using the mussel *Mytilus galloprovincialis* as typical bioindicator organism. Mussels were exposed to various environmentally realistic concentrations of the different NSAIDs, both in short and long term conditions. Analyses of drugs bioaccumulation were integrated with early molecular responses measured as changes in transcriptomic profile, and with functional analyses at cellular level through a large panel of ecotoxicological biomarkers: these included alterations of the immune system, variations of oxidative stress biomarkers and appearance of genotoxic damage. The presence of pharmaceuticals was also investigated in natural mussels harvested for human consumption along the central Adriatic coast. Obtained results demonstrated the capability of mussels to accumulate tested compounds and the onset of biological alterations, with some differences depending on the type of molecule or the exposure dose. Analyses of transcriptomic profile indicated the modulation of cell cycle, lipid and arachidonic acid metabolisms in mussels exposed to lower doses: at higher exposure conditions, such alterations progressed at cellular level, with variations of immune system responses, oxidative stress and early onset of genotoxic effects. Measurable concentrations of various pharmaceuticals in wild mussels further highlighted the importance of this environmental risk, and the suitability of *M. galloprovincialis* as sentinel organism for assessing the ecotoxicological potential of pharmaceutical drugs in marine environment.

O-7 *Environmental problems of microplastics*

TROPHIC TRANSFER OF MICROPLASTICS IN FISH LARVAE OF TWO SPECIES WITH DIFFERENT MATURATION RHYTHMS

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Microplastics (MPs) are small plastic particles in the µm to mm range. MPs in the environment, in particular aquatic environment, have dramatically increased over the last decades. There are some evidences of toxicity of MPs for aquatic fauna which varies according to e.g. chemical composition, shape, size. An additional level of variation in toxicity comes from the fact that MPs can serve as vector for a wide range of chemicals either intrinsic or adsorbed during weathering. The goal of the work presented here is to evaluate the transfer of MPs and chemicals vectorised by MPs in the food chain. For this purpose, model spheric fluorescent MPs from two size classes (1-5 or 10-20 µm in diameter) were fed to artemia nauplii which in turn were fed to fish larvae. Larvae of two species were used, zebrafish and marine medaka as models for fresh- and marine water fish respectively. A trophic transfer of MPs has been observed for both species starting at 8 days post-fertilisation (dpf; corresponding to 5 days after hatching) for zebrafish and 17 dpf (corresponding to 7 days after hatching) for medaka. A higher MPs load in artemia nauplii was observed for 1-5µm MPs class compared to the 10-20 µm MPs class and this was logically also observed in fish larvae. Clearance analysis revealed that two days after the end of feeding with MPs no more fluorescence could be observed in larvae. These preliminary results revealed a difference in fluorescence uptake in larvae depending on the species which may rely on differential maturity stage. Behavioural tests were used as a proxy of physiological status and revealed differences in locomotor activity which may be indicative of physiological distress.

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O-8 *Environmental problems of microplastics*

PRELIMINARY ECOTOXICOLOGICAL INVESTIGATION ON MICROPLASTICS IMPACT IN WHALE SHARKS FROM THE GULF OF CALIFORNIA (MEXICO) USING SKIN BIOPSIES

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The impacts of microplastics on large marine vertebrates are largely unknown. The whale shark (WS) (*Rhincodon typus*) is a vulnerable (IUCN) species that may be exposed to microplastic ingestion as a result of their filter-feeding activity. In this work, we perform the first ecotoxicological investigation, using skin biopsies, on whale sharks sampled in the Gulf of California. The increasing human activity in WS grounds gives rise to chemical pollution from urban waste waters, vessels, agriculture and waste including marine litter. In order to evaluate the potential impact of microplastic and related anthropogenic contaminants on this species, 13 skin biopsy samples were collected in January 2014 from 12 males and 1 female WS in La Paz Bay. PCBs (twenty-one *ortho* PCB congeners), DDTs, PBDEs (fourteen congeners from tri- to deca-substituted) and HCB were analyzed by GC-qMS. Cytochrome P450 1A (CYP1A1) was analyzed in skin biopsies, using western-blotting (WB) techniques. A preliminary investigation on microplastic density and plastic additives in superficial zooplankton/microplastic samples collected in the Gulf of California was also carried out. The average abundance pattern for the target contaminants in skin biopsies was PCBs>DDTs>PBDEs>HCB. Mean concentration values of 8.42 ng/g w.w. were found for PCBs, 1.31 ng/g w.w. for DDTs, 0.29 ng/g w.w. for PBDEs and 0.19 ng/g w.w for HCB. CYP1A1 was also detected for the first time (by WB techniques), in WS skin samples. First data on the average density of microplastics in the superficial zooplankton/microplastic samples collected from the Gulf of California showed values ranging from 0.00 items/m^3 to 0.14 items/m^3; furthermore, concentrations of mono-(2-ethylhexyl) phthalate (MEHP), used as a tracer of plastic additives, ranged from 13.08 ng/g to 13.69 ng/g. Further ecotoxicological investigation on whale shark skin biopsies will be carried out for a worldwide ecotoxicological risk assessment of this vulnerable species including microplastic impacts.

O-9 *Endocrine disruptors*

IS 4-NONYLPHENOL CAPABLE TO DISRUPT OSMOREGULATION IN THE TELEOST EURYHALINE FISH *DICENTRARCHUS LABRAX*?

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4-nonylphenol (4-NP) is a surfactant degradation product capable of mimicking the action of the natural hormone 17β-estradiol. In seawater the concentrations of 4-NP can reach maximum concentrations of several µg/L in polluted areas. Estuaries and lagoons are the most contaminated coastal areas. Euryhaline fish species such as the European sea bass *D. labrax* are thus potential target for 4-NP pollution in the wild. As a xenoestrogen, 4-NP can potentially modulate various estrogen-regulated functions including osmoregulation. The aim of our work was to investigate the effect of 4-NP on the osmoregulatory function in sexually immature sea bass. Juvenile sea bass were exposed during 2 weeks at two nominal concentrations of 4-NP (5 $\mu g.L^{-1}$ and 25 $\mu g.L^{-1}$) or at the solvant as a control (0.0005% methanol). The biological functioning of osmoregulation was evaluated at different levels: blood osmotic pressure, expression and activity of major proteins involved in ion transport in gills and its endocrine control by the GH/IGF axis. The expression of estrogen receptors ERβ1 and ERβ2 was also measured in gills. Our results highlighted that osmoregulation was significantly disrupted after 2 weeks exposure to 4-NP. The osmotic pressure in blood was significantly increased. In gills, the expression of the genes encoding the main ionic transporters involved in the regulation of ionic balance in seawater (NKAα1b, CFTR and NKCC1) was significantly decreased, as well as the activity of the Na^+/K^+ ATPase pump. Regarding the endocrine control of osmoregulation, our first results suggest that the GH/IGF1 axis was also significantly disturbed. Additionally, a significant modification of estrogen receptors ERβ1 and ERβ2 expression was measured in gills, suggesting a possible modification of signal transduction by the estrogen receptor. Altogether, these results showed that 4-NP can disrupt osmoregulation in juvenile sea bass at environmentally realistic concentrations. The involved pathways are currently being further explored.

O-10 *Endocrine disruptors*

IN VITRO ESTROGEN DISRUPTION OF CURRENTLY-USED FLAME RETARDANTS

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Flame retardants (FRs) are ubiquitously-used chemicals that are added to nearly all manufactured materials. Accordingly, FRs have been detected in human serum and urine, soil, aquatic environments, and biota. Additionally, there has been a steady increase in diseases resulting from endocrine-disruption with an aligned increase in use of chemicals. Given the persistence, potential bioaccumulation, limited toxicological understanding, and vast use of FRs, there is a need to investigate potential endocrine-disruptive activity associated with these compounds in an effort for better risk assessment. We therefore used the MCF-7 flow-cytometric proliferation assay in an effort to establish potential estrogen-disrupting effects of twelve currently-used flame retardants. The assay is an adaption of the classical E-Screen assay which relies on the proliferation of estrogen receptor α-positive MCF-7 cells in response to xenoestrogens. The assay allows for the rapid screening of potential xenoestrogens while also maintaining comparable performance to other *in vitro* estrogen assays. Five FRs showed statistically significant estrogenic activity while seven FRs harboured anti-estrogenic activity when co-treating with 17β-estradiol. However, potencies were many orders of magnitude lower than those for control compounds (17β-estradiol and the estrogen receptor antagonist fulvestrant for estrogen and anti-estrogen activity respectively). Interestingly, some compounds showed both estrogenic and anti-estrogenic effects, indicating both receptor-dependant and –independent mechanisms attributed to some of these compounds, in line with other studies. Multiple currently-used flame retardants may therefore act as xenoestrogens and anti-estrogens, or alter estrogen homeostasis, which could affect endocrine function.

O-11 *Endocrine disruptors*

RESPONSIVENESS TO A MODEL XENOESTROGEN OF TURBOT, SEABASS AND MULLET IN COMPARISON TO ESTABLISHED FISH MODEL SPECIES (ZEBRAFISH AND TROUT)

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Xenoestrogenic responses in fish vary according to the environment (freshwater vs marine), temperature or species sensitivity. Thus, extrapolation of data between species inhabiting different habitats and/or showing different behavioural habits within a given ecosystem could result in erroneous effect estimations. The aim of the present work was to study the sensitivity to the model xenoestrogen 17α-ethinylestradiol (EE2), of three marine species (turbot, European sea bass and thicklip grey mullet) and compare the xenoestrogenic early response to the response of aquatic model freshwater species (zebrafish and rainbow trout). For this purpose, well-known biomarkers of xenoestrogenicity, such as vitellogenin (*vtg*), choriogenin (*chg*) and brain aromatase *cyp19a1b* transcription levels were quantified by qPCR. Juvenile fish were exposed for 10 days to 5 ng/L, 25 ng/L and 50 ng/L of EE2 and samples of liver and brain collected after 2 and 10 days of exposure. Results showed increased liversomatic index (LSI) in EE2 exposed fish that might be related to alterations in the metabolic activity of the liver. Accordingly, up-regulation of *vtg* and *chg* was determined in studied species. No such a clear response was obtained for *cyp19a1b* in brain. Overall, freshwater species were more responsive to EE2 at short exposure times, 2 days, while after 10 days marine species turbot and thicklip grey mullet, resulted more sensitive. Transformation of nominal EE2 exposure concentrations to ng/g fish load demonstrated that turbot and thicklip grey mullet were the most sensitive of all tested species. Results showed that the response to EE2 was species specific and highlights the importance of characterizing the sensitivity to EDCs of experimental fish species in order to avoid erroneous extrapolations in data interpretation.

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O-12 *Endocrine disruptors*

EFFECTS OF 17 A-ETHINYLESTRADIOL AT DIFFERENT WATER TEMPERATURES ON ZEBRAFISH SEX DIFFERENTIATION AND GONAD DEVELOPMENT

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Zebrafish (*Danio rerio*) sex determination is described to have a polygenic genetic basis, which can be secondarily influenced by environmental factors, such as temperature and exposure to endocrine disrupting chemicals (EDCs). Considering current climatic changes scenarios, it is relevant to study how EDC effects change depending on temperature. Therefore, zebrafish were raised at three distinct water temperatures (23, 28 or 33±0.5 °C) and were exposed to 4 ng/L of EE2, from 2 hours to 60 days post-fertilization. A quantitative (stereological) assessment of zebrafish gonads was performed, at 35 and 60 dpf, to identify alterations on gonadal development and differentiation. The results showed that at 23 ºC there was a general growth delay, as well as, of gonad differentiation and maturation; while at 33 ºC opposite effects were observed. Sex ratio was skewed toward males when zebrafish were maintained at the highest temperature. EE_2 exposure promoted gonad maturation in both genders, independently of the temperature. However, at 33 ºC, the exposure to EE_2 induced a delay in the male gonad development, with some individuals still showing differentiating gonads at 60 dpf. In summary, the results indicate that zebrafish sex determination is sensitive to environmental conditions and that EDCs effects may be temperature dependent. Further studies combining temperature and environmental contaminants are necessary to predict the potential implications of global warming on fish populations.

O-13 *Endocrine disruptors*

MOLECULAR COMPLEXITY IN REPRODUCTIVE BEHAVIOUR IN THE GUPPY (*POECILIA RETICULATA*): LINKS BETWEEN BRAIN TRANSCRIPTOME, BEHAVIOUR AND EFFECTS OF ETHYNYLESTRADIOL

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The guppy (*Poecilia reticulata*) provides an ideal model in behavioural ecotoxicology. Females are selective of males based on elaborate courtship behaviours. We have previously shown that exposure to EE2 alters reproductive behavior in several fish species. Here we conducted a study, which investigated the effects of EE2 exposure on male and female mate choice behaviour, and combined the behavioural data with an NGS-based investigation of the transcriptome of whole brain of those same individual fish. Fish were acclimated in sex-separated tanks for two weeks and then exposed to EE2 (measured = 5 ng/L) for 21 days. We found that EE2-exposed males spent more time associating with exposed females when they could use visual cues. In contrast when they were given only chemical cues, EE2-exposed males visited control females more often than when provided with a cue from EE2-treated females. Interestingly, control females spent significantly more time associating with control males and repeated that behaviour more often than EE2-exposed females. After these experiments the fish were sacrificed and total RNA isolated from whole brain. Samples from individual fish were then sent for sequencing on an Illumina HiSeq2000 for 100 bp paired-end reads at a commercial centre. Samples consisted of individual males and females and they were grouped based on behavioural data (fish associating or not associating with the other sex) (n = 3 for each group, total n = 24). All reads were used for transcriptome assembly using Trinity prior to identification of differentially expressed transcripts (RSEM and edgeR) by a series of comparisons to dissect out sex-, behavior- and treatment-specific genes. The results of this ongoing analysis will be described but we have already identified genes associated with behaviour. Further, we will describe possible roles of transcripts from LINE1 and other Transposable Elements in modulating behaviour and response to treatment.

O-14 *Endocrine disruptors*

OESTROGEN RECEPTOR DISTRIBUTION ON A PRIMARY IMMUNE ORGAN, THE THYMUS OF EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

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Recently the European Sea bass has received special attention due to a huge stock decline in Western Europe. One of the reasons might be the presence of specific pollutants, the Oestrogenic Endocrine Disrupting Chemicals (EEDC) highly present in its nursery, the Seine Estuary (France). It is well known that those chemicals disturb the reproduction but they have also an immune-modulatory role, affecting the development and function of the immune system of teleost. Oestrogens are known to modulate thymic activity and production of self-tolerant T-cells in mammals and are therefore suspected to act similarly in teleost fish. Indeed we could demonstrate previously that oestrogens impact the thymus of *D. labrax* during key steps of its ontogenesis. We demonstrated that Sea bass thymus express transcripts for (*i*) the well-documented nuclear Oestrogen Receptors ERα, ERβ1, ERβ2 but (*ii*) we could also identify a G Protein-coupled membrane Oestrogen Receptor (GPER and GPER-like), which suggests that alternative oestrogen-mediated signalling also exists. ERs and GPER distribution was identified by histological staining and immunochemistry within the various thymic cellular subtypes. The ER-isoforms and GPER/GPER-like were found to be expressed in the capsular zone, in the medulla, in myoid cells and in Hassal's corpuscule-like, the former one being described for the first time in Sea bass. In mammals those structures are crucial for T-cell maturation and functionality. We observed that the distribution of ERs and GPERs in the various structures of the Sea bass thymic microenvironment shares similarity with mammals. We can thus hypothesise that (1) the complex oestrogenic effects on the thymus are evolutionary conserved between teleost and mammals and that therefore (2) EEDCs are likely to affect thymic development and function in teleosts. Therefore those pollutants might impair the immunocompetence of Sea bass as observed for mammals.

O-15 *Biomarkers*

A COMPARATIVE APPROACH OF DEVELOPMENTAL IMMUNOTOXICITY: *ORYZIAS MELASTIGMA* AS MARINE FISH MODEL FOR IMMUNOTOXICOLOGY

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Research in mammals has highlighted a strong connection between toxicants and significant alterations of immune system development at critical developmental stages. The resulted developmental immunotoxicity (DIT) may manifest as impairments in immune organ integrity and/or immune cell differentiation, rendering the organism more vulnerable to pathogens and/or may induce persistent changes affecting the adult immunity. The marine medaka, *Oryzias melastigma*, is an emerging model for immunotoxicology, however, essential knowledge on developmental critical windows for DIT has not yet been established. This study employed a comparative approach, using DIT for mammals as reference, to investigate the critical windows (CWs) for marine medaka at the molecular, cellular, organ and whole organism levels. The appearance of first phagocytes at stage 22 marks the onset of the innate immune defense in the embryo. The assessments of the *recombinant activation gene 1* and the *T-cell receptors β* and *γ* gene expression and immune organs histopathology allowed us to identify the beginning of lymphocyte colonization in the head kidney and thymus shortly before hatching (stage 39-41). The differentiation of T-lymphocytes started from 5-6 dph together with the onset of the thymus compartmentalization. The host resistance to bacterial challenge (*Edwardsiella tarda*) was determined and immune competence of larvae was found increased with advanced development. A comparison between the marine *O. melastigma* and the freshwater medaka (*O. latipes*) revealed a disparity in T-lymphocyte maturation pattern, possibly indicative for a difference in DIT between marine and freshwater teleost. The developmental pattern observed in *O. melastigma* is highly comparable to the immune system ontogenesis of commercial important species like rainbow trout and sea bass. Biomarkers relevant for DIT in teleost will be discussed. Overall, the results suggest similar CWs for DIT in vertebrates from teleost to mammals, substantiating the application of *O. melastigma* to assess the risk of immunotoxicants in marine environments.

O-16 *Biomarkers*

LYSOSOMAL BIOMARKERS IN JUVENILE *SOLEA* SP. FOR EARLY WARNING ASSESSMENT OF MARINE ECOSYSTEM HEALTH

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Flatfish, amongst which the sole (*Solea* sp.) is common in the Bay of Biscay, are recognised as sentinel species in pollution monitoring programmes. Previous research confirmed the sensitivity of sole to the effects of pollution using mostly biochemical and histopathological endpoints. Though lysosomal responses are considered general health biomarkers in a variety of sentinel species, little is known about lysosomal responses to environmental stressors in sole. Thus, the present study aimed at determining the suitability of lysosomal biomarkers (LP, Vv_{LYS}, Sv_{LYS}, S/V_{LYS}, Nv_{LYS}, Vv_{NL}) measured in juvenile sole liver on the basis of histochemical methods. Standard procedures were successfully adapted to juvenile sole using both wild and farmed individuals. Lysosomal changes were first assessed in samples taken from laboratory experiments where animals were exposed to different stress conditions. Lysosomal biomarkers in sole were sensitive enough to differentiate degrees of responses and exposure times between experimental groups. They were also contrasted against histopathological effects and bioaccumulation of pollutants in the tissues. Overall, similar trends were recorded in lysosomal parameters and histopathological effects, which were in agreement with pollutant tissue levels. Consequently, the present study confirmed the suitability of lysosomal biomarkers in juvenile sole liver for early warning assessment of marine ecosystem health in response to different types of pollution and environmental stress.

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O-17 *Biomarkers*

EXPLORING THE RELATIONSHIP BETWEEN CARBOXYLESTERASE ACTIVITY AND TROPHIC HABITS OF ABUNDANT AND THREATENED CHONDRICHTHYANS IN THE WESTERN MEDITERRANEAN

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Esterase activity is a good biomarker to detect exposure to organophosphates (OP) and other xenobiotic contaminants in the environment. Although these enzymes are present in all organisms, not many studies have been conducted in Chondrichthyans. In the present study, we examined the activities of these enzymes in 23 rare, endangered and common elasmobranch species inhabiting the Western Mediterranean Sea. In particular, we determined carboxylesterase (CbE) and butyrylcholinesterase (BChE) activities in the liver of 11 sharks, 11 rays and 1 chimaera. Moreover, by using intrinsic trophic markers (stable isotopic analyses and isotopic mixing models) we related the activity of these enzymes with the trophic habits at both individual and population level. Hepatic BChE activity was very low for all species, mostly under 1 nmol/min/mg prot. CbE activity showed a strong intraspecific variability, without any relationship with the body measures (body length and body mass) of the individuals. Within elasmobranch groups, we found differences in CbE activities between sharks and rays and between species belonging to the same subfamily. Also, we found a negative relationship between CbE activity and trophic habits (δ^{13}C and δ^{15}N measured in the muscle of the same individuals, with p-values of 0.0017 and 0.0008, respectively). Within the ecosystem, most of the Chondrichthyans are located in high trophic positions. This makes them likely to accumulate contaminants and they could be used as good sentinels to assess contaminant exposures; and esterase activities could be a potentially good biomarker of choice.

O-18 *Biomarkers*

FISH BILE PROTEOME AS AN ENVIRONMENTAL MONITORING TOOL

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Examining fish bile for PAH metabolites has become an established method of analysis for oil exposure. In this analysis, the metabolites are detected and quantified. Despite the fact that analytical biochemistry has made large improvements, the methods used have predominantly remained the same. Following up the iBILE concept (published in 2014), the use of the bile proteome (i.e. the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions) is proposed to determine the presence and source of contamination in fish species. Armed with the right knowhow and instrumentation, the study of the fish bile proteome can reveal the conditions of the environment the fish has been living in. In studies performed in our research group, the bile proteome of different fish species exposed to oil or PAHs in laboratory (cod and haddock) and field (tusk, haddock, ling, saithe and red fish) has been determined using LC-MS/MS analysis. The analysis was conducted on a linear ion trap-Orbitrap mass spectrometer. The raw files were analysed by Proteome Discoverer 2.0 using PMI-Byonic 1.0 (Protein Metrics Inc.). The laboratory exposure results represent a solid base in the use of bile proteome as multi-biological markers of exposure. In particular, new sensitive indicators, in the form of expressed proteins affected by exposure to PAH (i.e. PAH-protein adducts) were included in the analysis, increasing the power of the investigation. The list of identified bile proteins provides unique information about the presence, the site and the type of modification/s (i.e. PAH-protein adducts). In the field survey, the bile proteome analysis was able to differentiate between sites (reference vs exposed), confirming the potential of this approach to evaluate the effect of contamination in fish

O-19 *Biomarkers*

IMPORTANCE OF MAINTENANCE OF PHYSIOLOGICAL PERFORMANCE IN THE INTERDIDAL SCALLOP *MIMACHLAMYS VARIA*: MULTI-BIOMARKERS APPROACH

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Studies in bivalves have documented a number of physiological changes that are associated with alternating periods of immersions and aerial emergence that include compensatory changes in metabolic rate, oxygen consumption and heart rates. The present study aimed to determine metabolic cycles by metabolome comprehensive approach and biochemical alterations in the intertidal mollusk *Mimachlamys varia.* In this context we sampled variegated scallops at Britany Atlantic coast site, characterized by low contamination levels, to study the short-term responses of this species to physiological stress associated with low tide aerial exposure. We used biomarkers (superoxide dismutase, lipid peroxidation levels and citrate synthase) as indicators of oxidative stress, mitochondrial respiration and general metabolism. Moreover, this study aims to understand relations between enzymatic activities and tide effect on oxidative capacities. High-resolution analysis of metabolic cycles in the intertidal scallops revealed the presence of alternating phases of aerobic and anaerobic system. Thus, we created a simulated intertidal environment in which scallops were acclimated to alternating high and low tide in aquaria and samples were taken every 2 hours during 48 hours (early, middle, end high tide and early, middle and end low tide). Results of our study were confirmed by a modulation of reaction biochemical substrates, which are implicated in physiological response and cellular metabolism in *Mimachlamys varia*. Indeed, the increases in antioxidant enzyme activities suggest that cell try to compensate the cell damage caused by oxidative stress due to low tides. Metabolomics approaches help to identify more sensitive metabolites implicated in aerobic and anaerobic pathways, changes that are probably due to level of stress imposed by a low tide. The new findings in scallops *M. varia* helped identify new quantifications levels of biochemical and metabolic control in intertidal adaptation. Multidisciplinary approaches are effective tools for assessing environmental influence (contaminated, uncontaminated areas) on heath status of bivalve.

O-20 *Biomarkers*

AN ECOPHYSIOLOGICAL APPROACH TO MONITORING THE EFFECTS OF WASTEWATER DISCHARGE USING A TROPICAL MANGROVE CRAB

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The crab *Neosarmatium meinerti* is largely present in the mangrove system of Mayotte (Comoros archipelago), where it is potentially threatened by uncontrolled wastewater discharges. Such streams can affect their physiology due to their content in xenobiotics, but also due to their low osmotic pressure. These wastewater discharges have just recently started to be treated through small sewage stations, but this situation calls anyhow for the need to evaluate their effectiveness. Here, we present our results using *N. meinertii* to assess the impact of such wastewaters, using an ecophysiological approach. After conducting a quantitative analysis of the chemical profile of the effluent, salinity levels at the surface of the sediment at low tide and crab burrow density were quantified in the field and mapped around the discharge areas and nearby undisturbed sites. Animals were also collected from clean sites for caging experimentation in the field (in both disturbed and undisturbed areas), while others and acclimated to laboratory conditions for further *in-vitro* experimentation. For both laboratory and field experimentation, a complete evaluation of the redox metabolism (antioxidant content, oxidative damage and reactive oxygen and nitrogen species formation assessments) was carried out on gills. Furthermore, laboratory tests were carried out on the osmoregulatory mechanisms and energetic requirements associated to exposure to seawater and diluted seawater (clean and polluted, the later directly collected from the effluent). Our results provide a comprehensive overview on the effects of salinity changes and effluent exposure on the energetic and redox metabolisms of this decapod crab. Our results will help to understand the effects of these effluents on the intertidal communities, contributing to adequately propose methods for minimizing their impact on mangrove ecosystems.

O-21 *Biomarkers*

CRITICAL CONCENTRATIONS OF ENVIRONMENTAL VARIABLES AND ACCUMULATED POLLUTANTS IN RELATION TO ECOLOGICAL WATER QUALITY

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In the present study two different approaches were followed to assess the protectiveness of environmental quality standards (EQS) for the ecological quality of surface waters. In the first approach we investigated whether concentrations of some environmental variables including metals, chloride and sulfate are related to ecological quality as assessed by a macroinvertebrate-based biotic index, the MMIF (Multimetric Index of Flanders). This way it was evaluated whether the current EQS for these variables was protective enough to reach a good ecological status. Large datasets from total and dissolved metal concentrations and other variables in Flemish (Belgium) fresh water systems and the associated macroinvertebrate-based biotic index MMIF (Multimetric Macroinvertebrate Index Flanders) were used to estimate critical environmental concentrations for good ecological water quality, as imposed by the European Water Framework Directive (2000). Measurements of metals in the environment only reflect the momentary pollution status and do not take into account differences in bioavailability, affected by abiotic factors such as pH, water hardness, temperature and biotic factors such it as feeding habits. As a consequence, current water or sediment quality criteria for micro pollutants are not necessarily adequate and well related to effects on aquatic communities observed in the real world. Direct measurement of pollutants in biota could tackle these problems. Therefore in a second approach we also related accumulated metal levels to effects at the community level again by combining datasets of accumulated metals in larvae of the non-biting midge (*Chironomus* sp. gr. *thummi*) with the MMIF. For both approaches quantile regression analysis was used. We were able to evaluate the existing EQS of the measured metals, chloride and sulfate and to derive for a set of metals save body burdens in midge larvae that are protective of ecological quality.

O-22 *Biomarkers*

MULTIPLEXED QUANTITATION OF PROTEIN BIOMARKERS IN THE INVERTEBRATE SPECIES *GAMMARUS FOSSARUM*: NEW PERSPECTIVES FOR MOLECULAR ENVIRONMENTAL MONITORING

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Modulations of molecular biomarkers can be related to an exposure to chemical compounds and provide early warning indicators of possible hazard on the ecosystem. However, routine use of these tools in biomonitoring faces several drawbacks, especially in invertebrates: lack of robust species-specific quantification methods and needs of numerous biomarker-specific protocols. This leads to very expensive biomonitoring strategies in time, cost and biological samples. Recently, "proteogenomics" emerged as a relevant strategy for the discovery of proteins in non-model organisms. With this approach, our consortium created a database consisting of 1873 experimentally validated proteins of the amphipod crustacean *Gammarus fossarum*, a sentinel species used for freshwater biomonitoring. The objective of the present study was to setup an innovative approach that allowed a fast, specific and simultaneous quantification of several proteins of interest in this invertebrate species. We applied a mass spectrometry multiplexed quantitation methodology (Selected Reaction Monitoring) to study 55 protein biomarker candidates. Identification of specific proteotypic peptides, in agreement with putative physiological roles of the associated proteins, and assessment of their interest as biomarkers in *G. fossarum* were achieved by studying their exact quantitative changes through male and female reproductive cycles and after exposure to stresses/contaminations (food privation, exposure to model chemical compounds in the laboratory and *in situ* caging).The levels of 21 biomarkers of interest (sex-specific proteins and/or with key physiological functions) were successfully monitored simultaneously in biological samples during the physiological processes and their sensitivity to toxic contamination was demonstrated both in laboratory and during field monitoring. For example, the laboratory contamination with environmental concentrations of cadmium modulated the concentrations of some biomarkers as the Na^+K^+ATPase (cellular pump), catalase (oxidative defense) and GST (detoxification) proteins. This breakthrough methodology in ecotoxicology is a valid alternative to currently used protocols.

O-23 *Biomarkers*

GENDER AS POTENTIAL VARIABILITY FACTOR IN CELL AND TISSUE-LEVEL BIOMARKERS OF MUSSEL DIGESTIVE GLAND

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Mussels are widely used sentinel species for marine ecosystem health assessment based on biomarkers. Standard procedures are available for the determination of biomarkers but there is a need to optimize them by reducing the influence of natural variability and the effects of confounding factors. In the present study, it was investigated how gender may affect cell and tissue level biomarkers in digestive gland. For this purpose, mussels (*Mytilus galloprovincialis*) were collected from a reference site (Plentzia) and from a chronically polluted site (Arriluze) in the Basque Coast in March 2013 and January, April, August and November 2014. Thirty mussels were collected per sample and a battery of biomarkers was analysed: lysosomal structural changes (Vv_{LYS}, Sv_{LYS}, S/V_{LYS}, Nv_{LYS}), lysosomal membrane stability (LP), intralysosomal neutral lipid accumulation (Vv_{NL}), cell-type composition (volume density of basophilic cells, Vv_{BAS}) and structural changes in digestive gland (mean luminal radius/mean epithelial thickness, MLR/MET, and connective tissue to digestive tissue ratio, CTD). Gamete development stages were determined. Whilst considering males and females both together lysosomal biomarkers differed between sampling sites, once separated by gender, significant differences were only found in Vv_{NL} at certain gamete developmental stages. Regarding, Vv_{BAS}, MLR/MET and CTD, there were differences between genders depending on the season, especially in Plentzia. Overall, gender seems to be a confounding factor of minor relevance for lysosomal and tissue-level biomarkers in mussels, except for specific seasons and therefore seasonality is seemingly more crucial when designing and performing biological effects monitoring programmes based on biomarkers.

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O-24 *Biomarkers*

APPLICATION OF MICRONUCLEUS ASSAY FOR GENOTOXICITY MONITORING IN FRESHWATER CLADOCERANS

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Assessment of DNA damage is of primary concern when determining the pollution-related stress in biota. The present study intends to determine a new application of micronucleus assay to microcrustaceans (Cladorerans). To assess the sensitivity of MN assay in Daphnids, an adapted methodology for micronucleus (MN) assay was applied to *Daphnia magna* collected from a clean site and acclimated in the lab for 24 h. Cladocerans were experimentally exposed (in triplicate) to different concentrations of metals (Cu, Zn, Cd) and deltamthrin for 48 h. Whole organisms were crushed, incubated at 37 °C in a solution of Dispase I (Neutral protease, Sigma, France) and centrifuged. The pellets were spread on microscope slides, air dried, then fixed in methanol: acetic acid (3:1) for 1 min and stained with 0.4% Giemsa. Slides were examined under 100× magnification using an optical microscope to MN counting. Results showed a dose dependent micronucleus induction, the highest value was found to be 11.5 ‰. An *in-situ* application of the MN assay was then performed. Identified Daphnid species have been collected from different freshwater environments in Tunisia. For each collection site, micronucleus assay was performed, in triplicates, using a pool of 10 specimens of the same Daphnid species. The micronucleus frequencies varied between 0.67 ‰ and 7.30 ‰. This work can be considered as a preliminary study to use the Daphnid model to set up a reliable test for hazard and risk assessment for aquatic genotoxicity.

O-25 *Biomarkers*

EFFECT OF MUSSEL AGE ON THE RESPONSES OF PHYSIOLOGICAL AND BIOCHEMICAL BIOMARKERS TO FLUORANTHENE EXPOSURE

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Mussels are commonly used to monitor the quality of coastal waters by the measurement of both, pollutant concentrations and biological changes. For that, mussels of a standard size are periodically collected from the sampling sites included in a monitored area. Nonetheless, several endogenous and exogenous variables may influence pollution biomarkers apart from chemical exposure, acting as "confounding factors" and hampering the environmental assessment. Among them, mussel age has been considered as a variable which might influence pollutant accumulation and its biological responses. Large-scale monitoring programs are characterized by covering a high variability of habitats, which prompts differences in growth of the mussel populations therein. Thus, mussel size might not necessarily be related to mussel age, coexisting in the same sampling survey populations of different ages and the same size. The aim of this study was to evaluate the effect of mussel age in mussels of the same size, on the toxicity of the polycyclic aromatic hydrocarbon fluoranthene (FLU), measured by means of physiological (the scope for growth –SFG-) and biochemical (antioxidant enzymes –CAT, SOD, GR, GPx-, glutathione-S-transferase –GST- and lipid peroxidation –LPO-) biomarkers. For that purpose, mussels of two different ages and the same size (45 mm), collected at the same area and sampled at the same time, were exposed to 30 µg L^{-1} of FLU for 21 days. Some physiological measurements as respiration rates were clearly age-dependent, but the overall SFG was similar in both mussels. On the contrary, biochemical biomarkers studied were not only affected by mussel age, but also by the effect of FLU exposure that, at the same time, was dependent on mussel age. These results evidence the importance of endogenous factors, such as the age, in the biological responses to pollutants, which highlight the need of considering this parameter in toxicological studies and in biomonitoring programs.

O-26 *Biomarkers*

INTERACTIVE EFFECTS OF NUTRITION, REPRODUCTIVE STATE AND POLLUTION ON MOLECULAR STRESS RESPONSES IN MUSSELS, *MYTILUS GALLOPROVINCIALIS*

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Understanding the effects of physiological variability (such as variation in nutritive or reproductive state) on molecular responses to stressors is important for understanding of the stress physiology and development of effective stress biomarkers in marine organisms. We determined the effects of nutritive state (due to long-term exposure to 3 different food rations), reproductive stage and pollution [exposure to 0, 30 and 60 µg of a polycyclic aromatic hydrocarbon, fluoranthene (FLU)] on mRNA expression of key genes involved in stress protection and detoxification pathways in mussels *Mytilus galloprovincialis*. Nutritive and reproductive state strongly modulated molecular stress responses of mussels. Control mussel showed a significant decrease of mRNA expression of several genes (including CYP450, GST-S2 and MT20) with decreasing nutrition. Exposure to FLU led to upregulation of metallothioneins (MT10, MT20), molecular chaperones (HSP22, HSP70-3 and HSP70-4) and Phase II detoxification (GST-S2) genes, depending on the FLU concentration. Exposure to high FLU concentration led to downregulation of P-glycoprotein (PgP) mRNA. Reproducing mussels showed elevated mRNA expression of gametogenesis-related genes (Vitel, VCLysine) as well as stress- and detoxification-related genes (PgP, GST-α, and MT20). These findings show that expression of stress- and detoxification-related genes is a good indicator of pollution exposure in mussels, but effective biomarker-based environmental assessment must take into account the shift in the baseline mRNA expression and modulation of stress responses caused by the physiological condition of mussels.

O-27 *Biomarkers*

METABOLISM AND SEQUESTRATION OF ZINC IN THE LAND SLUG *ARION VULGARIS*

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Zinc (Zn^{2+}) is a transition metal being a co-factor and an essential metal ion in the active centre of more than a hundred enzymes. Despite its essential nature, excess Zn^{2+} may interfere with the absorption and/or transport of other essential trace elements such as iron (Fe) and copper (Cu). A pivotal role in metal detoxification and trace element metabolism is attributed to metallothioneins (MTs). These low-molecular mass proteins contain a high proportion of Cys residues, by which they specifically bind transition metal ions such as Cu^{+}, Cd^{2+} and Zn^{2+}. In metal-exposed and control individuals of the terrestrial slug *Arion vulgaris*, Zn^{2+} is predominantly accumulated in the midgut gland. In contrast to most other animals, however, no significant proportion of Zn^{2+} in *Arion vulgaris* is bound to MTs, in spite of the presence of metal-specific MT isoforms in the slug's midgut gland. Instead, most of the Zn^{2+} is allocated to high molecular weight proteins (> 100 000 Da) and a low molecular weight compound with an estimated apparent molecular mass of ~ 1 000 Da, clearly distinct from salt fractions. Preliminary results indicate that this compound may contain sulphur and/or an aromatic core. Conclusive analyses are in progress. Moreover, histochemical detection of Zn^{2+} on freeze sections of midgut gland revealed that a large proportion of the metal is present in the cytoplasm of calcium cells. In addition, some minor Zn^{2+} deposits are visible on the surface of calcium granules. It is concluded that the pathways of Zn^{2+} metabolism in *Arion vulgaris* are distinct from those of other transition metals such as Cd^{2+} and Cu^{2+}/Cu^{+}.

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O-28 *Biomarkers*

EPIGENETIC ALTERATIONS 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 overall health status of the earthworms was monitored at the cellular (DNA damage, oxidative stress, lipid damage) and organismic level (fitness, reproductive success). 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 exposure. During the exposure, earthworm fitness and cellular mechanisms remained unaffected by Cd. However, on the epigenetic level, already after 1 month of exposure, Cd induced significant increase in DNA methylation, which remained constant 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 impacts on the cellular and organismic level can be observed.

O-29 *Environmental problems of nanomaterials*

NANOPARTICLES AS TECHNOLOGICAL INNOVATIONS: STAIRWAY TO HEAVEN OR SYMPATHY FOR THE DEVIL?

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Nanometric revolution is underway, promising technical innovations in numerous domains (such as industry, cosmetic, construction, medicine…). However, the significant arise of nanomaterials application leads consequently to a boost of environmental discharges and especially in aquatic systems representing an important receptacle of pollutants. Therefore, we need knowledge about transfer and toxicity of nanomaterials to preserve aquatic ecosystems sustainability. Trophic transfer is referred as an important pathway of nanoparticles contamination in aquatic systems being reportedly the main exposure route to organisms. Among their great variety of nature and characteristics, gold nanoparticles (AuNPs, amine-PEG coating, diameter 10 nm) have been chosen as model contaminant due to their high stability in solution. A subchronic exposure (21-days) to AuNPs-spiked food has been performed on an aquatic predator, the European eels *Anguilla anguilla*. Two contamination levels have been tested: the first (NP1, 1 mgAu kg^{-1}) corresponds to a concentration obtained in a previous assay for an herbivorous fish, and the second (NP10, 10 mgAu kg^{-1}) is 10-fold higher. Eels exposed to the highest AuNPs level presented an irregular and avoidance feeding behavior compared to controls and NP1 eels. Blood plasma analyses highlighted significant modifications for chloride, aspartate transaminase and urea rates in NP10 eels, probably related to the fasting effects. In reference to previous studies, RNA-sequencing approach was applied in order to assess the change in gene expression at whole transcriptomic level in liver and brain tissues in NP1 exposed eels.

O-30 *Environmental problems of nanomaterials*

SALINITY-DEPENDENT TOXICITY OF WATER-DISPERSIBLE, SINGLE-WALLED CARBON NANOTUBES TO JAPANESE MEDAKA EGGS

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To investigate the effects of salinity on the behavior and embryonic fish toxicity of functionalized, single-walled carbon nanotubes (SWCNTs), medaka eggs were exposed to non-functionalized single-walled carbon nanotubes (N-SWCNTs), water-dispersible, cationic, plastic-polymer-coated, single-walled carbon nanotubes (W-SWCNTs), or hydrophobic polyethylene glycol–functionalized, single-walled carbon nanotubes (PEG-SWCNTs) at different salinities, from freshwater to seawater. As reference nanomaterials we tested dispersible chitin nanofiber (CNF), chitosan-chitin nanofiber (CCNF), and chitin nanocrystal (CNC, i.e. shortened CNF). Under freshwater conditions, with exposure to 10 mg/l W-SWCNTs, the yolk sacks of 57.8% of eggs burst, and the remaining embryos had reduced heart rate, eye diameter, and hatching rate. Hatched larvae had severe defects of the spinal cord, membranous fin, and tail formation. These toxic effects increased with increasing salinity. Survival rates declined with increasing salinity and reached 0.0% in seawater. In scanning electron microscope images, W-SWCNTs, CNF, CCNF, and CNC were adsorbed densely over the egg chorion surface; however, because of chitin's biologically harmless properties, only W-SWCNTs had toxic effects on the medaka eggs. No toxicity was observed from N-SWCNT and PEG-SWCNT exposure. We demonstrated that water dispersibility, surface chemistry, biomedical properties, and salinity were important factors in assessing the aquatic toxicity of nanomaterials.

O-31 *Environmental problems of nanomaterials*

LOW CONCENTRATIONS OF SILVER NANOPARTICLES IMPAIR ZEBRAFISH REPRODUCTION AND EMBRYO DEVELOPMENT

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The cellular and molecular mechanisms of toxicity of nanomaterials (NMs), and especially of silver nanoparticles (Ag NPs), have become an issue of extensive investigation. A lot of data have been published regarding embryo toxicity of these NMs, but much less information is available regarding the effect of NMs on physiological functions, such as growth or reproduction. In this work, we address the hazard posed by PVP/PEI coated Ag NPs of 5 nm for zebrafish reproduction and for further embryo development compared to the direct exposure of embryos to the NMs. For this, 1) selected breeding zebrafish were exposed for 3 weeks to 100 ng Ag/L (environmentally relevant concentration) or to 10 µg Ag/L of Ag NPs; reproductive parameters were recorded and resulting embryos were allowed to develop up to five days in clean water, and 2) embryos from unexposed parents were exposed for 5 days to 0.01-10 mg Ag/L and development was monitored. Chemical analysis of exposed adults showed a significant higher content of silver in fish exposed to 10 µg Ag/L than in those exposed to 100 ng Ag/L, which showed values similar to the controls. However, even at the environmentally relevant silver concentration, a significant reduction in the number of spawned and viable eggs was registered in exposed fish by the second week of treatment. Direct exposure of embryos to these Ag NPs resulted highly toxic (LC50 at 120 h = 50 µg Ag/L) causing 100% mortality during the first 24 h of exposure at 0.1 mg Ag/L. These results show that Ag NPs at an environmentally relevant concentration are able to affect population level parameters in zebrafish.

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O-32 *Environmental problems of nanomaterials*

HUNTING NANOPARTICLES IN MARINE FISH: A TROJAN HORSE *IN VIVO* STUDY USING TITANIUM DIOXIDE NANOPARTICLES IN COMBINATION WITH DIOXIN AND CRUDE OIL IN JUVENILES OF EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*

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The aim of the present study was to investigate the effect of titanium dioxide nanoparticles (nano-TiO2) towards 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and water accommodated fraction of crude oil (WAF) in juveniles of European sea bass *Dicentrarchus labrax*. A Trojan horse *in vivo* study was performed using nano-TiO_2 (1 mg/L and 10 mg/L) alone and in combination with 2,3,7,8-TCDD (46 pg/L) and WAF (0.068 g/L) for 7d and 48h respectively. Genes expression profile of phase 0, I, II and III of liver biotransformation as *abcb1, abcc1-c2-g2, Ahrr,* c*yp1a, gsta*, g*r, erβ2,* engulfment and motility domain-containing protein 2 (*elmod2*) were investigated. Activities of 7-ethoxyresorufin-*O*-deethylase (EROD) and glutathione-*S*-transferase (GST) enzymes in liver and PAH metabolites in bile were also measured. Nano-TiO_2 (10 mg/L) resulted of a micrometric size of 1300nm and 1500nm in artificial and natural sea water respectively (Z-Average 972±35.37 and 1152±27.62 nm, PdI 0.295±0.028 and 0.267±0.024, ζ-potential -7.19±1.24 and 7.28±2.20 mV). Nano-TiO_2 did not caused any significant alteration in gene expression profile except for a*bcg2, abcc1* and *abcc2* genes which resulted down-regulated compared to controls in both experiments. Both 2,3,7,8-TCDD and WAF up-regulated *cyp1a, gsta* and *elmod2* genes as expected. Co-exposure to 2,3,7,8-TCDD caused a further significant down-regulation of *abcb1* and *abcc2* and of *ahrr, erβ2,* while *cyp1a*, *gsta* and *elmod2* were not affected compared to single 2,3,7,8-TCDD. Co-exposure to WAF caused a significant reduction of *cyp1a, ahrr, abcg2* and *abcc2* genes expression compared to single WAF. EROD was also reduced in co-exposure with 2,3,7,8-TCDD and WAF. Pyrene metabolites increase significantly in WAF and a further significant increase in co-exposure was observed. Nano-TiO_2 is unlikely to interfere with 2,3,7,8-TCDD-dependent biotransformation while a clear effect towards WAF of crude oil can be hypothesized probably related to its photocatalytic activity towards PAHs able to reduce their bioavailability and toxicity.

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THE USE OF TRANSGENIC DAPHNIA MAGNA LINES TO UNRAVEL THE MECHANISMS OF ACTION OF PROZAC AND OTHER PSYCHIATRIC DRUGS

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New ecotoxicological approaches are currently focused on a mechanistic understanding of how toxic chemicals affect organisms. This is the core of the adverse outcome pathway framework-AOPs. This framework requires establishing a causal link between key molecular events and adverse outcomes. This is not easy as most empirical evidence for non vertebrate model species is inconclusive. Here we present our current work on thestudy of the mechanisms of action by which selective serotonin reuptake inhibitors (SSRI) affect the life-history performance in Daphnia. We first used toxicological studies to establish adverse outcomes of regulatory relevance. The use of transcriptomic and immunological studies aid us to identify potential altered molecular signalling pathways. Finally the use of tryptophan hydroxylase (TPH) targeted gene knock-down using CRISPR/Cas enable us to obtain stable clones lacking of serotonin. Results indicated that the SSRRI fluoxetine, which is the pharmacologically active chemical of Prozac, increased serotoninergic activity in the brain of Daphnia magna disrupting the correct perception of food. Individuals exposed to fluoxetine at low food levels had greater serotoninergic activity in the brain and behave as those cultured at high food (matured earlier and produced more offspring). Daphnia TPH knock-down clones lacking of serotonin were viable and at high food behave like the original clone at low food (growth less, reproduced latter and had less offspring). Thus than TPH knock-down D. magna clones had a reverse phenotype than those exposed to fluoxetine. Our next step is performing experiments with fluoxetine and other neuroactive compounds using TPH knock-down clones to further study the involvement of the serotonin signalling pathway.

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IDENTIFICATION OF ESTROGEN AND PHYTOESTROGEN-RESPONSIVE GENES IN FISH SCALES

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Estrogens are essential regulators of numerous physiological processes including calcium regulation in the vertebrates. Teleost fish scales are a reservoir of readily available calcium and are estrogen (E_2) responsive due to the presence of both membrane and nuclear estrogen receptors. RNA-seq was used to detail the short (1 day) and long term (5 days) molecular responses of sea bass scales to E_2 or a phytoestrogen that is frequently present in aquaculture feeds, genistein (Gen, 5 µg/g body weight). Thirty libraries (five biological replicates per treatment) were sequenced by Illumina RNA-seq producing 1,103 million of good quality paired-end reads. These were assembled into 35,214 contigs with an average length of 2,825 bp, 91% of which were annotated. Pairwise comparisons of treatment versus control identified 262 genes with a significantly regulated (more than 2 fold) expression in the E_2 and/or Gen treatments. The global changes in gene expression in scales mainly consisted of short term regulation by Gen and up-regulation of most genes by Gen or E_2 after 5 days. E_2 and Gen had a similar action on fish scales, although 69 genes were specifically regulated by E_2 and 107 by Gen and compound-specific enrichment occurred in some cellular pathways. Gene expression of ten responsive genes was verified by quantitative PCR with additional biological replicates, confirming a significant ($p < 0.001$) correlation between RNA-seq and qPCR. To our knowledge this is the first genome-wide transcriptome analysis of fish scales. It provides an important resource to understand E_2 mechanisms of action on fish mineralized tissues and the possible impact of phytoestrogens and other estrogenic-disrupting compounds. New biomarkers for E_2 and EDC have been identified with potential for application in aquaculture or environmental pollution control.

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REDOX PROTEOMIC ANALYSIS OF *MYTILUS EDULIS* GILL: COMPARATIVE EFFECTS OF MODEL PRO-OXIDANTS BISPHENOL A AND DICLOFENAC

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The pro-oxidant bisphenol A (BPA) is a common environmental pollutant which can induce oxidative stress in the tissues of aquatic organisms. The blue mussel, *Mytilus edulis*, were exposed to sub-lethal doses of bisphenol A (BPA; 50 µg/L and 500 µg/L) for 7 days and a week of recovery time. Effects on the gills were investigated, followed by measurement of enzymatic activities and protein thiol status. Oxidation of protein thiols was detected by tagging thiols with 5-iodoacetamido fluorescein (IAF). The changes were studied by using one-dimensional (1DE) and two-dimensional electrophoresis (2DE). Protein 2DE profiles were compared with Progenesis SameSpots software both for IAF fluorescence and protein abundance for both datasets with a dataset previously obtained in response to diclofenac exposure. Results revealed that five matched proteins (Arganine kinase, b-tubulin, calreticulin, heavy metal-binding protein-HIP and GAPDH) were affected (increased or decreased in abundance) by these two pro-oxidants. One protein was affected by both diclofenac and BPA that is arginine kinase. Analysis of blue mussels' proteome facilitates detection of subtle changes at the level of individual proteins in response to environmental stressors. These findings support redox proteomics as a strategy for detecting common responses to model pro-oxidants in environmental toxicology.

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TROJAN HORSE STRATEGY TO INTERFERE *CLOCK* GENE EXPRESSION IN THE OYSTER *CRASSOSTREA GIGAS*

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Using a non-invasive RNA interference (RNAi) approach, we are studying the role of biological rhythms in the bioaccumulation process in the oyster *Crassostrea gigas*. We present here a study showing that a disruption of the circadian clock could modify the accumulation of toxin in *C. gigas* during a harmful algae bloom. Biological rhythms are generated by an endogenous clock, which organize temporal structure of organisms from metabolism to behavior. The *clock* gene, a central gene of the circadian clock, was targeted for interference by feeding bacteria-alga complex. *Escherichia coli* strain HT115, engineered to express dsRNA *clock* gene, was adsorbed on unicellular alga (*Heterocapsa triquetra*). We evaluated the feasibility, at gills level, of this Trojan strategy on: (1) the *clock* gene silencing, (2) the consequences on the canonical genes of the circadian clock (*cycle*, *period*, *timeless 1*, *cryptochrome 1*, *cryptochrome 2*, *rev-erb* and *ror*) and (3) the bioaccumulation of harmful alga toxins (saxitoxins) produced by the dinoflagellate *Alexandrium minutum*. The results show a 40% of inhibition of *clock* gene expression in 62.5% of the oysters. Moreover, the consequences of *clock* interference lead to a repression of clock canonical genes. Finally, we show that an efficient disruption of molecular clock induces a modification of saxitoxin bioaccumulation after an *A. minutum* bloom exposure mimicking environmental concentration. Those results highlight that the circadian rhythm plays a role in the contamination process in *C. gigas*. Furthermore, our results bring out that it is feasible to silence genes by feeding bacteria expressing dsRNA - alga complex in the oyster *C. gigas*. It is the first time such complex is used as "Trojan horse" in bivalves. This non-invasive method of RNAi is a promising tool for elucidating the function of a gene in filter-feeder bivalves.

O-37 *The –omic technologies*

PEPTIDOMICS OF THE ZEBRAFISH: IDENTIFICATION OF NEUROPEPTIDES BY LC-MS

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(Neuro)peptides are small messenger molecules that are derived from larger, inactive precursor proteins by the highly controlled action of processing enzymes. These biologically active peptides can be found in all metazoan species where they orchestrate a wide variety of physiological processes. Obviously, detailed knowledge on the actual peptide sequences, including the potential existence of truncated versions or presence of post-translation modifications is of high importance when studying respective signaling cascades. A peptidomics approach therefore aims to identify and characterize the endogenously present peptide complement of a defined tissue or organism using liquid chromatography and mass spectrometry (LC-MS). While the zebrafish *Danio rerio* is considered as an important aquatic model, either in the domain of ecotoxicology or rather as general vertebrate model in a medical context, very little is known about their peptidergic signaling cascades. We therefore set out to biochemically characterize endogenously present (neuro)peptides from the zebrafish. The brain region of adult zebrafishes were carefully dissected and (neuro)peptides were extracted using a specific extraction protocol. Resulting peptide samples were analyzed using a nanoLC instrument that is directly coupled with an LTQ-Orbitrap mass spectrometer to yield biochemical identifications of about 100 peptides including several shortened forms (aminoterminally or carboxyterminally truncated) of the presumed biologically active peptides. These peptide variants may result from further *in vivo* processing in the vesicles or might be the result of extracellular (in vivo) peptide processing by specific peptidases. Alternatively, they can also occur from in vitro degradation during sample processing. Obtained sequence data from these first zebrafish peptidomics experiments are likely to pave the way for further functional studies concerning peptidergic signaling in fish.

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NMR-BASED METABOLOMICS REVEALS CUO NP-INDUCED INTERFERENCES IN LARVAL MORPHOGENESIS, NEUROTRANSMISSION AND SKELETOGENESIS OF SEA URCHIN EMBRYOS

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In the last decades, a great deal of concern has been raised over the extensive use of nanoparticles (NPs) in a variety of fields and their potential toxicity to the environment and human health. In this context, the embryotoxicity of copper oxide (CuO) NPs was assessed in an intertidal species commonly present in the Mediterranean, the black sea urchin *Arbacia lixula* and, for the first time to our knowledge, a nuclear magnetic resonance (NMR)-based metabolomic approach was applied on this species. Fertilized eggs were exposed to five doses of CuO NPs ranging from 0.009 to 2.9 μM, until the pluteus larva stage. Developmental delay and morphological abnormalities were found, as well as interferences with the normal neurotransmission pathways. In detail, evidence of serotoninergic and cholinergic systems affection was revealed by dose-dependent decreased levels of *N*-acetyl serotonin and choline, respectively, measured by metabolomics. These results were in compliance with an immunohistochemically observed reduction in serotonin (5-HT) and inhibition of AChE enzymatic activity. The metabolic profile also highlighted a significant CuO NP-dependent increase of anhydroglucose and glycine, which are respectively, components of matrix glycoproteins and/or proteins involved in the biomineralization process, suggesting perturbation in the skeletogenesis of sea urchin embryos, accordingly to the observed skeletal defects in spicule patterning. However, the expression of skeletogenic genes, i.e. msp130 and sm30, did not differ among groups, allowing hypothesizing altered PMC migration. Noteworthy, other unknown metabolites were detected from the NMR spectra, and their concentrations found to be reflective of the CuO NP exposure levels. Overall, these findings demonstrate the toxic potential of CuO NPs to interfere with larval morphogenesis, neurotransmission and skeletogenesis of sea urchin embryos. Moreover, the integrated use of embryotoxicity tests and metabolomics represent a highly sensitive and effective tool for assessing the effects of NPs on aquatic biota health.

O-39 *The –omic technologies*

INTEGRATIVE -OMICS ANALYSES REVEAL PERTURBATION OF LIPID METABOLIC PATHWAYS IN THE LIVER OF ATLANTIC COD (*GADUS MORHUA*) EXPOSED TO THE PERSISTENT ORGANIC POLLUTANT PCB 153

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Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), are increasingly recognized as contributing factors in the incidence of metabolic syndrome. PCB 153 has strong tendencies to bioaccumulate and biomagnify up the food-chain, and is one of the most abundant PCB congeners detected in biological samples. In order to study mechanisms of toxicity, particularly any effects on the energy metabolism, we exposed Atlantic cod (*Gadus morhua*) to increasing concentrations of PCB 153 and examined the effects on the hepatic gene expression and protein synthesis. Integrative analysis of the transcriptomics and proteomics data using various bioinformatics tools revealed significant effects of PCB 153 on many cellular processes and pathways, of which lipid metabolism associated pathways were predominant. Particularly, a highly coordinated up-regulation of genes and enzymes in the *de novo* fatty acid synthesis pathway was observed, suggesting perturbations of the lipid metabolism. Parallel to increased levels of lipogenic enzymes, some enzymes in the gluconeogenisis and fatty acid beta-oxidation pathways were down-regulated, indicating overall lipogenic effects. Furthermore, in accordance with the prediction of increased lipid-synthesis in the cod liver, elevated levels of triglycerides were confirmed in plasma samples obtained from the same fish. Thus, our integrative analysis of transcriptomics, proteomics, and metabolomics offers new insights into the toxicity mechanisms of this environmental contaminant. Our future projects are aimed at using systems toxicology approaches to integrate transcriptomics, proteomics, metabolomics, toxicopathological studies and mathematical modeling to elucidate pathways of toxicity of environmental chemicals, including mixtures, in Atlantic cod.

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O-40 *The –omic technologies*

GENOME AND POPULATION RESPONSE TO ENVIRONMENTAL CHANGE

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Differences in inter-individual response are described by variation in environments and genomes that combine to give rise to phenotypic variation observed in populations. Environmental genomics provides tools for understanding these features that offer links to phenotypes, which are in-line with proposed changes in regulatory toxicology. In this talk we draw from recent environmental genomic studies to explore how environmental stress contributes to genome variability, influences the fate of genetic variation in populations, and over micro-evolutionary time scales determines the fate of phenotypes. These studies contribute to and make use of maturing genomic tool kits for the killifish, *Fundulus heteroclitus*, and the water flea, *Daphnia pulicaria*. Using these animal models we explore how functional variation in gene expression and gene regulatory networks contributes to phenotypic plasticity, and influence homeostasis in response to environmental change, and how environment-induced alterations in the magnitude and distribution of gene copy number (CNV) in natural populations contributes to adaptations to extreme environments. We will discuss the importance of understanding genome variation and the evolutionary forces that shape it in light of environmental stress.

O-41 *The –omic technologies*

CHEMICAL WARFARE IN THE MARINE ENVIRONMENT: GENOMIC SURVEY OF THE ATLANTIC COD (*GADUS MORHUA*) DEFENSOME REVEALS THE ABSENCE OF AN IMPORTANT XENOSENSOR

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The complete set of gene families and pathways that together function to detoxify and eliminate harmful compounds, including both xenobiotic and endobiotic chemicals, are denoted as the chemical defensome. Cytochrome P450s (CYPs) constitute a large superfamily of enzymes involved in the initial phase of biotransformation of numerous compounds in the whole range of living organisms. Similar to a number of other proteins involved in the chemical defensome, the transcription of CYPs are tightly regulated by ligand-activated transcription factors, including nuclear receptors and the aryl hydrocarbon receptor (AHR). By searched the newly sequenced Atlantic cod (*Gadus morhua*) genome, we recently mined the full suite of CYP genes, termed the "CYPome". However, when exposing precision-cut cod liver slices to well-known ligands of the nuclear receptor promiscuous xenobiotic receptor (PXR, NR1I2), the transcription cod *cyp3a* genes remained unaffected. The object of the following study was thus to identify the complete set of chemical defensome genes in Atlantic cod, including the transcription factors. Interestingly, hidden Markov model (HMM) searches and BLAST searches were unable to identify a cod ortholog to the PXR. Analysis of the genomic region surrounding the neighboring gene *MAATS1* confirmed the absence of a cod *pxr* gene. Subsequent mapping of the upstream nucleotide sequence of the cod *cyp3a* genes revealed a high number of response elements corresponding to aryl hydrocarbon receptor (Ahr)-binding motifs, suggesting a different transcriptional regulation of *pxr* target genes in this teleost compared to other vertebrate species.

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O-42 *The –omic technologies*

GAMMA RADIATION EFFECTS IN *DAPHNIA MAGNA*: OXIDATIVE STRESS AND DIFFERENTIAL GENE EXPRESSION

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As the aquatic environment is unavoidably exposed to ionizing radiation from both natural and anthropogenic sources, the characterization of ecological and health risks linked to radiation are of immediate need. For the crustacean *Daphnia magna* several studies have mainly focused on effects of chronic ionizing radiation on growth and reproduction, leaving a significant gap in responses at the individual, biochemical and molecular level especially after acute exposure. The main biological effects of gamma radiation can be attributed to formation of reactive oxygen species and consequent oxidative damage to lipids, proteins and DNA. In this context, the aim of the present study was to understand the effects and modes of action (MoA) of gamma radiation (0.4, 1, 4, 10, 40 and 100 mGy/h) in *D. magna* after short-term exposure (24 h). Several individual, physiological and molecular endpoints were addressed, namely ROS formation, lipid peroxidation, DNA damage and global transcriptional changes. Results for ROS formation, lipid peroxidation and DNA damage showed significant increase after 24h exposure, especially at higher doses, showing the capacity of gamma radiation to induce oxidative stress. The gene expression patterns obtained showed dose-dependent transcriptional alterations in *D. magna* after 24h exposure. A total of 95 and 821 genes were confirmed as differentially expressed (DEGs) at 40 mGy/h and 100 mGy/h, respectively, 81 of which commonly regulated by both dose rates. No significant DEGs were found for the remaining dose rates. Of these responding genes, several were associated with known gene ontology processes such as energy production, growth and development, apoptosis, ion transport and general stress response (including oxidative stress). Overall, the results obtained revealed multiple MoA of gamma radiation in *D. magna* after short-term exposure, which can be used to develop a more effective set of tools to assess future impacts of radiation in the environment.

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O-43 *Biomarkers*

A COLOUR PREFERENCE TECHNIQUE TO EVALUATE THE TOXICITY OF ACRYLAMIDE IN ZEBRAFISH

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The zebrafish has become the most common vertebrate model in environmental research. Here we present an improved colour preference-based technique to evaluate the toxicity of acrylamide in zebrafish. A digital camera was used to record the locomotor behaviour of individual zebrafish swimming in a water tank consisting of two compartments separated by an opaque perforated wall through which the fish could pass. The overall level of the ambient lighting could be varied to study the effects of illumination on swimming behaviour, while the colour of the lighting in each compartment could be altered independently (producing distinct but connected environments of white, red or blue) to allow association of the zebrafish's swimming behaviour with its colour preference. The functionality of the photoreceptors was evaluated based on the ability of the zebrafish to sense the different colours and to swim between the compartments. The zebrafish tracking was carried out using our algorithm developed with MATLAB. We found that zebrafish preferred blue illumination to white and white illumination to red. Acute treatment with acrylamide (2 mM for 36 hours) resulted in a marked reduction in all indices of locomotion and a concomitant loss of colour-preferential swimming behaviour. Chronic treatment with acrylamide (0.25 mM for 2 weeks) resulted in a shift in velocity distributions to higher values when fish were tested in uniform lighting conditions (identical colour in each compartment). When different colour combinations were used the chronically-treated fish displayed a similar colour preference to that of untreated fish but were less likely to move from a red to a white compartment when placed initially in the former, indicating a possible change in their perception of the colour red. Histopathological examination of acrylamide-treated zebrafish eyes showed that acrylamide exposure had caused retinal damage. The colour preference tracking technique has applications in the assessment of environmental toxicity.

O-44 *Biomarkers*

IMMUNOTOXIC IMPACT OF B[A]P AND PAH MIXTURES ON JAPANESE MEDAKA LARVAE: DEVELOPMENT OF AN IMMUNE CHALLENGE ASSAY

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Nowadays, the number of pollutants released into the aquatic environment is increasing and represent a threat for aquatic ecosystems. The immune system is one of the targets of certain pollutants such as PAHs. Immuno-suppression by pollutants could increase individual susceptibility to infectious diseases. Since in natural environment, fish and other aquatic organisms are co-exposed to numerous chemicals and pathogens, analysis of the immunotoxic effects of pollutants is particularly relevant. Development of an immune challenge assay on fish early life stage could be particularly useful because of the high sensitivity of this stage to both pollutants and pathogens. Besides being a potent chemical carcinogen, benzo[a]pyrene (BaP) has also been shown to suppress the immune response of mammals and fish, including Japanese medaka. This model pollutant was used to validate a virus challenge protocol on Japanese medaka larvae. Following exposure to BaP for 9 days at the embryonic stage or for 24 h for hatchlings, larva immunity status was assessed by waterborne exposure to the betanodavirus RGNNV. Larva mortality and swimming behavior were recorded for 14 days after virus exposure. Genotoxic effects and EROD activity induction were also observed in PAH mixture exposed larvae. Preliminary results showed impact of chemical and virus alone on mortality and biometry. Swimming activity of larvae was not affected by BaP treatment. In contrast, larvae contaminated with betanodavirus had very low swimming activity after light stimulation and larvae exposed to BaP and virus had no activity at all. These preliminary results confirm the immunotoxic potency of BaP and highlighted the sensitivity and usefulness of the immune challenge assay on Japanese medaka larvae. Other experiments are being carried out with environmental mixtures of PAHs.

O-45 *Biomarkers*

MULTI-STEP INVESTIGATION OF COCAINE AND ITS MAIN METABOLITES TOXICITY ON ZEBRAFISH EMBRYOS

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The increase in global consumption of illicit drugs caused both social and medical problems, but recently it raised the awareness of a potentially new environmental problem. In fact, several monitoring studies showed that after human consumption, illicit drugs and their metabolites end up in surface waters, where they are commonly detected the high ng/L to low µg/L range worldwide. Considering their widespread distribution and their high biological activity, their presence in freshwater ecosystems may lead to a potential risk for aquatic organisms. However, at present the information on their harmful effects to non-target organisms is inadequate. To fill this gap of knowledge, the aim of the present study was to investigate the embryotoxicity of environmentally relevant concentrations of cocaine (COC) and its main metabolites, the benzoylecgonine (BE) and the ecgonine methyl ester (EME), in the zebrafish (*Danio rerio*). We exposed zebrafish embryos for four days (up to 96 hours post fertilization) to the same four concentrations of COC, BE and EME (0.04, 0.4; 4 and 40 nM, respectively) and we assessed their toxicity by a multi-step approach, based on biomarker, transcriptomic and proteomic analyses. The results obtained by our investigation showed that also environmentally relevant concentrations of all the tested illicit drugs can induce significant cyto-genotoxic effects, modulate the expression of some specific genes and alter the protein pattern of treated zebrafish embryos with respect to controls. Our findings point out the potential embryotoxicity of these emerging aquatic pollutants towards non-target organisms, also at concentration commonly found in surface waters, and suggest the needing to further in-depth analysis the delineate their real hazard for aquatic communities.

O-46 *Biomarkers*

ENVIRONMENTAL CONCENTRATIONS OF GLYPHOSATE CAN INDUCE SUBLETHAL EFFECTS IN EARLY LIFE STAGES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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One of the most commonly used pesticides worldwide is the glyphosate-based herbicides, whose molecules, usually transported by agricultural runoff, are frequently detected in surface water at elevated concentrations that can even reach up to the order of 10^1 mg/L. The aim of this work was to study the possible sublethal effects that glyphosate-based herbicides could cause in the early developmental stages of the salmonid rainbow trout (*Oncorhynchus mykiss*). For 21 days, eyed-stage embryos were exposed up to the larval stage to sublethal concentrations of 0.1 and 1 mg/L of glyphosate, using the commercial formulation Roundup® GTMAX. Water samples were analyzed by analytical chemistry to ensure the studied concentrations. Several toxicity endpoints were recorded such as survival, hatching success, larval biometry and malformations. Swimming activity was also conducted at the end of the experience using the video tracking system Daniovision (version 10.0 of Noldus). Additional to that, a comet assay was performed on larvae in order to evaluate genotoxic effects. Both concentrations did not affect embryo and larval survival, and there was no significance increase of morphological abnormalities. However, a slight significant decrease was observed on head size for larvae exposed to 1 mg/L of glyphosate, and a reduction of head/length ratio was also observed for both concentrations. The locomotion assay highlighted a significant increase of mobility between larvae exposed to 0.1 mg/L of glyphosate and control groups. Also it was observed that larvae exposed to this condition swam faster than larvae exposed to 1 mg/L of glyphosate, but no difference was remarked with control groups. On the other hand, no significant increase of DNA damage was observed at both tested concentrations. These results revealed that glyphosate-based herbicides have the capacity to induce adverse effects in early life stages of rainbow trout at concentrations that occur in aquatic environments.

O-47 *Comparative physiology and biomarkers*

COPING STRATEGIES IN EARTHWORMS TO LOW LEVELS OF CADMIUM

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Earthworms are ecologically important soil dwelling organisms which are affected by anthropogenic pollution through agriculture and industry. Cd is one of the main soil pollutants with a high carcinogenic and toxicogenic potential. *Lumbricus terrestris*, the common earthworm, has therefore been used in lab exposures to low doses of environmentally relevant Cd concentrations over three months with a subsequent recovery period in control soil followed by an exposure to high levels of Cd. Quantitative RealTime PCR, comet assay, and enzyme activity assays have been applied to determine metallothionein (MT) and phytochelatin synthase (PCS) levels as well as DNA damage and oxidative stress in earthworms. MTs are probably able to act as the exclusive mechanism to detoxify low doses of Cd over several months and to prevent the organism from Cd-induced damages and oxidative stress. Control earthworms exposed to high Cd concentrations revealed an increase of MT gene expression levels. Low dose Cd-exposed earthworms which face a high Cd concentration are able to induce MT expression to a much higher degree. The transcriptional regulation mechanism of MTs is therefore suggested to be responsive to the exposure history. Therefore, it seems that highly flexible coping strategies have evolved in earthworms facing environmental stress like Cd pollution.

O-48 *Comparative physiology and biomarkers*

BIOLOGICAL RESPONSES TO CADMIUM EXPOSURE IN *ONCORHYNCHUS MYKISS* ERYTHROCYTES

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Nucleated trout erythrocytes were used in this study as an "*in vitro* model" to investigate Cadmium uptake and toxicity. Cadmium does not have a biological role in living systems, on the other hand is known to be citotoxic, although mechanisms beyond its toxicity are not clear. Being a transition metal these processes could be associated with an increased Reactive Oxygen Species (ROS) production. The aim of this work was to relate Cadmium uptake with its ability to promote ROS formation and induce oxidative stress in trout erythrocytes (*Oncorhynchus mykiss*). Blood was withdrawn by a heparinized syringe from trout's caudal vein. Cadmium uptake analysis was performed by atomic absorption spectrometry on pelleted erythrocytes. The presence of a trascriptionally active nucleus and mitochondria allowed the functional evaluation of different Cadmium concentrations on cellular viability, intracellular ROS production and mitochondrial membrane potential (MMP) using fluorescent probes associated with flow-cytometric determinations. The effect of Cadmium on the hemoglobin stability was evaluated spectrophotometrically. The data showed a positive correlation between cellular accumulated cadmium and time of exposure (2 and 4 hours) as well as Cd concentrations (1, 10 and 25 ppm). At cellular level 30 min exposure at different cadmium concentrations significantly decreased viability, promoted ROS formation level and mitochondrial membrane depolarisation. Hemolyzate incubation with Cadmium at 37 °C increased the rate of hemoglobin autoxidation and precipitation highlighting a potential toxicity mechanism in this cellular model involving met-hemoglobin formation. In fact, the conversion of oxyhemoglobin in met-Hb is associated with superoxide production and thereby of products such as H_2O_2 or hydroxyl radical. Moreover, the autoxidation of oxygenated hemoglobin can lead to the formation of precipitation products. These results indicate that cadmium exposure in nucleated erythorcyte produces cellular toxicity mediated by different triggers of oxidative stress such as mitochondrial depolarization and hemoglobin oxidation.

O-49 *Comparative physiology and biomarkers*

CADMIUM AS ENDOCRINE DISRUPTIVE CHEMICAL IN THE FRESHWATER GASTROPOD *BIOMPHALARIA GLABRATA*

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Cadmium (Cd) is a transition metal known to exert toxic effects on animals and humans. In metal-polluted areas, Cd can be accumulated by plants and thereby enter the food chain. Direct exposure of animals to Cd may occur in aquatic habitats, where Cd^{2+} is solved in water. At low concentrations, Cd^{2+} is suspected to act as an endocrine disrupting chemical, interfering with the endocrine balance of many species, especially during reproduction. The gastropod *Biomphalaria glabrata* is a model organism and freshwater species easy to culture. Individuals of *B. glabrata* were exposed to Cd at a low (2 µg/L) and a high exposure level (10 µg/L). Cd accumulation was measured in four different tissues, including the midgut gland, the ovotestis, the foot and the albumen gland. In a Quantitative Real Time PCR (qPCR) approach, the effects of Cd on the expression of two genes involved in reproduction, ovipostatin and yolk ferritin, were detected in the ovotestis of *B. glabrata*, in order to verify the metal's endocrine disrupting potential. The results are discussed in view of applying this qPCR approach as a tool for detecting the endocrine disrupting potential of Cd in aquatic gastropods from natural habitats, with the aim of establishing the two genes as endocrine biomarkers in aquatic gastropods from polluted freshwater habitats.

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O-50 *Comparative physiology and biomarkers*

COMPARATIVE SUB-LETHAL EFFECTS OF COPPER IN DEEP-SEA AND SHALLOW-WATER SHRIMPS

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Deep-sea organisms are physiologically adapted to strive in an environment void of sunlight and with high pressures. Some are also adapted to live in a metal rich environment with warmer temperatures such as at hydrothermal vents. However, anthropogenic pressures may reach these ecosystems with the exploitation of deep-sea minerals. Massive sulphide deposits are one potential target for deep-sea mining and with the exploitation activities mineral plumes with high concentrations of toxic metals may be released. The aim of this study is to compare the effects of copper in deep-sea hydrothermal vent shrimps (*Rimicaris exoculata* and *Mirocaris fortunata*) with their shallow-water counterparts (*Palaemon elegans*, *P. serratus* and *P. varians*). The accumulation of copper in different tissues (gills, hepatopancreas and muscle) and a battery of biomarkers was analyzed: metal exposure (metallothioneins), oxidative stress (catalase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase) and oxidative damage (lipid peroxidation). The effects of copper in the different species will help to understand the potential effects of deep-sea mining on the deep-sea species and the comparison with their shallow-water relatives will enable to compare adaptations to different environments and to evaluate the potential use of shallow-water species as surrogates to evaluate the ecotoxicological impacts of deep-sea mining.

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O-51 *Comparative physiology and biomarkers*

SALINITY INFLUENCES THE BIOCHEMICAL RESPONSE OF THE PORTUGUESE OYSTER TO ARSENIC

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Low-lying marine ecosystems such as estuaries are expected to become increasingly impacted by climate change. Sea-level rise and increasing frequency of flooding and drought events pose major threats to these ecosystems, by causing shifts in salinity, one of the most important physico-chemical parameters influencing species survival, distribution and physiology. Estuaries are also important sinks for contaminants, namely Arsenic (As) an ubiquitous toxic compound worldwide. The increasing rate of occurrence and persistence of climatic events causing salinity shifts, in combination with contamination, may further challenge organisms' response to environmental stress. Hence, we studied the effects of different salinity levels (10, 20, 30 and 40) on the response of the Portuguese oyster *Crassostrea angulata* to As exposure (4 mg.L-1). Biochemical analysis were performed to assess osmotic regulation (CA), metabolism (ETS), enzymatic (SOD, CAT and GSTs) and non-enzymatic (GSH/GSSG and LPO) markers of oxidative stress. Results showed significantly higher metabolic activities in oysters maintained in low salinity (10), coupled with higher As accumulation, as well as higher SOD and CAT activities, when compared to higher salinities. GSTs activity and LPO levels were higher in oysters exposed to As at salinities 20, 30 and 40, when compared to the same conditions without As. We concluded that the response of C. angulata to As is influenced by salinity. At the lowest salinity (10) oysters accumulated higher As concentrations, here attributed to higher metabolic rate involved in physiological osmoregulation, also stimulating antioxidant enzymes activity (SOD and CAT) and thus preventing increased LPO. On the contrary, at salinities 30 and 40 with As, antioxidant SOD and CAT were inhibited, enabling for LPO generation. Given our results, the effects of As on the oysters antioxidant capacity appears to be more deleterious under higher salinities (20, 30 and 40), comparing to salinity 10.

O-52 *Comparative physiology and biomarkers*

SHEDDING LIGHT ON THE NON-METAL-SPECIFIC CADMIUM/COPPER-METALLOTHIONEIN IN THE ROMAN SNAIL *HELIX POMATIA*

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Metallothioneins (MTs) are multifunctional proteins involved in many biological tasks such as metal homeostasis and detoxification, stress response, cell differentiation and development. In helicid snails, three different MT isoforms can be distinguished: a Cadmium-selective, predominantly Cd^{2+} or Zn^{2+} binding variant (CdMT) and a Copper-selective isoform (CuMT), predominantly binding Cu^{+}. These two MT isoforms show a distinct tissue-specific expression pattern and maintain differential, metal-specific functions. The CdMT, expressed most in hepatopancreatic and digestive tissues, is mainly involved in Cd^{2+} detoxification and stress response whereas the CuMT, only expressed in a certain cell type called rhogocyte, plays a crucial role in metal homeostasis and hemocyanin synthesis. A third, non-metal-specific isoform, the Cd/CuMT, was first found in the garden snail, *Canatreus aspersus*, and subsequently partially characterized from the Roman snail at the mRNA level. In *Cantareus aspersus*, this intermediate isoform shows high transcription rates in embryonal snails indicating a possible role in embryonal development. In *Helix pomatia*, however, no expression data on the Cd/CuMT gene are so far available. The aim of this study was, therefore, to identify the complete Cd/CuMT mRNA sequence of *Helix pomatia*. In addition, the transcriptional activity of all three MT genes was quantified in unexposed and metal-exposed (Cd, Cu) embryonal Roman snails. The highest MT mRNA gene expression in 7 and 20 days old unexposed embryos was detected for the Cd/CuMT gene. Furthermore, the Cd/CuMT gene of 20-day-old embryos showed a distinct response to Cu, but not Cd exposure. In addition, a significant upregulation of the CdMT gene due to Cd exposure was observed in 7 and 20 days old embryos. In contrast, the CuMT was not expressed in 7-day-old embryos. Instead, a low expression level of this gene was detected in 20-day-old embryos, irrespective of their previous treatment with and without metals.

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O-53 *Comparative physiology and biomarkers*

GENE STRUCTURE, PROTEIN SEQUENCE AND FUNCTION OF A NOVEL MULTI-DOMAIN METALLOTHIONEIN FROM THE FRESHWATER SNAIL *BIOMPHALARIA GLABRATA*

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Metallothioneines (MTs) are low-molecular mass proteins capable of binding metal ions to the sulfur atoms of their cysteine residues. They are involved in the regulation of metal homeostasis, oxidative stress protection, and detoxification. The large family of gastropod MTs has achieved through evolution an unprecedented degree of diversity, with unique features and adaptations such as multiplicate and truncated domain structures, as well as metal-selective binding features. Intriguing MT genes with metal-specific isoforms have already been described from the clade of Stylommatophora. We show here that the MT gene of *Biomphalaria glabrata* (from the clade of Hygrophila) is one of the largest MT genes identified so far, suggesting a three-domain partition of the encoded protein. Using a bioinformatic approach involving gene sequencing, structural and *in silico* analysis of transcription factor binding sites (TFBs), we found that the MT gene shows differing adaptations regarding the composition and features when compared to the intensively studied MT gene of *Helix pomatia*. It exhibits a regulatory promotor region containing three Metal Responsive Elements (MREs), a high number of Heat Shock Elements (HSEs) and additional TFBs with putative involvement in environmental stress response, immune competence, and regulation of gene expression. Exposure experiments to metals and non-metallic stressors of Biomphalaria snails suggest, indeed, that this protein may play a major role in coping with environmental stress. Its plasticity for adaptation may even be increased by the presence of several allelic variants with modifications in the primary protein structure.

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O-54 *Comparative physiology and biomarkers*

REDUCED SURVIVAL AND DISRUPTION OF FEMALE REPRODUCTIVE OUT IN TWO COPEPOD SPECIES (*ACARTIA CLAUSI* AND *A. TONSA*) EXPOSED TO 17A-ETHINYLESTRADIOL

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Estuaries are heavily impacted by pollutants from different sources such as urban sewage, industrial waste and agriculture runoff. Endocrine disrupting chemicals (EDCs) have been identified as very concerning pollutants to estuarine wildlife, but little is known on their impact into microscopic biota such as zooplankton. In this work, we aimed to investigate the effects of a model EDC, such as 17α-ethinylestradiol (EE2), on two copepod species inhabiting Basque Country (South East Bay of Biscay) estuaries: *Acartia clausi* (autochthonous neritic species) and *Acartia tonsa* (non-indigenous brackish species). Female copepods were collected at population maximum time (spring for *A. clausi* and summer for *A. tonsa*) and exposed individually to 5 ng/L, 5 µg/L and 500 µg/L EE2, from environmental concentrations in sewage effluents to toxicological concentrations. After 24 h of exposure, studied endpoints included individual survival, egg laying (fecundity) and egg hatching. Female survival was only reduced at the highest EE2 dose in both copepod species, being *A. tonsa* more sensitive than *A. clausi*. Fecundity was reduced in both species at EE2 medium and high doses. At the highest EE2 dose fecundity was reduced to a half. No differences with the control samples were detected in egg hatching success, but marked trends were calculated for *A. clausi*. In *A.clausi* control and low EE2 exposure groups 70% egg hatchability was observed but in medium and high EE2 exposed groups this was reduced to 50%. In *A. tonsa* the hatching percentages were high (> 80%) for all experimental groups. In conclusion, EE2 reduced female copepod survival and disrupted reproductive output, but only at high non-environmentally relevant concentrations. Further work is required to assess the sensitivity of selected copepod species to EDCs present in the aquatic environment.

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O-55 *Comparative physiology and biomarkers*

PARENTAL EXPOSURE TO GAMMA RADIATION CAUSES INCREASED ROS FORMATION, LIPID PEROXIDATION AND DNA DAMAGE IN ZEBRAFISH EMBRYOS

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The effects of exposure during two sensitive life stages to ^{60}Co γ-radiation (nominal[a] dose rates 10 and 40 mGy/h) on demographics and oxidative stress parameters were assessed in two generations (F0 and F1) of zebrafish. Significant reduction in embryo production was observed in F0, with sterility observed 1 year post irradiation to 40mGy/h (for 27 days). F1 embryos from F0 irradiated at 40mGy/h showed 100% mortality occurring at the gastrulation stage corresponding to 8 hours post fertilization. Control and F0 exposed to 10 mGy/h (27 days) were used to create 4 ZF lines: control, an E line irradiated (10 mGy/h) only in F1 embryogenesis (3 days), a G line irradiated only during F0 gametogenesis (27 days) and a GE line irradiated during F0 gametogenesis and F1 embryogenesis. Demographic scoring showed that GE and E embryos hatched earlier than G and controls (48-72 hpf). A significantly higher mortality of 3.1, 4.5 and 7.2% occurred in embryos from E, G and GE lines compared to controls, respectively. A higher number of deformities compared to controls was seen in G and GE, but not E embryos. ROS production was highest in the GE and E, while higher DNA damage and lipid peroxidation were observed in G embryos compared to controls and other lines. Overall, this study showed adverse reproductive and developmental effects of γ-radiation, which may be induced by the observed increase in ROS formation and DNA damage. The results also revealed heritable effects in offspring of γ-irradiated parents, highlighting the necessity for multigenerational studies to assess the environmental impact of this type of radiation.

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O-56 *Comparative physiology and biomarkers*

INTRACELLULAR PROTEASE ACTIVITY IN THE EARLY DEVELOPMENT OF ATLANTIC SALMON (*SALMO SALAR* L.)

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Among the main pathways of protein degradation the lysosomal-autophagic and calpain are considered principal in fish; besides calpain contribute to initial disintegration of native structures and lysosomal cathepsins subsequently hydrolyze resultant fragments to short peptides and amino acids. The dynamics of intracellular protease activity was studied in Atlantic salmon *Salmo salar* L. unfertilized eggs, embryos and young fish of 0+ to 3+ ages. The activity of μ- and m-calpains, cathepsins B and D, and total exopeptidase activity was accessed. Activation of most studied enzymes was detected on late blastula/early gastrula and eye pigmentation stages with peak of peptidase/cathepsin activity in eggs just before hatching. Among young fish, maximal protease activities were obtained in underyearlings (0+). Besides, on salmon parrs from the Varzuga river (the White Sea basin) leaving spawning redd and separately settling river side of the mainstream or tributaries, it was shown that the difference in ecological conditions in biotopes promotes the differentiation of salmon underyearlings on two phenotypic subgroups distinguishing by metabolic specificities, including proteolytic ones. Relatively high activities of intracellular proteases indicative for intensive protein turnover were observed in underyearlings inhabiting tributaries. It was suggested that revealed underyearling differentiation may result in more significant divergence in the course of salmon development and, as a consequence, two salmon subgroups would enter smoltification in the different age.

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O-57 *Comparative physiology and biomarkers*

DECIPHERING BEHAVIOURAL DISRUPTION IN ZEBRAFISH EXPOSED TO PCBS AND PBDES ENVIRONMENTAL MIXTURES AND IN UNEXPOSED OFFSPRING OVER SEVERAL GENERATIONS

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Harmful effects of Persistent Organic Pollutants (POPs), in particular behavioural disruptions, have been shown in several species in directly exposed animals. This has recently led to an extensive use of behaviour as readout in ecotoxicology and neurotoxicity assessment. In the frame of Fish'N'POPs project, our aim was to improve knowledge on behavioural and physiological effects of POPs dietary exposure with a mixture of PCBs and PBDEs at concentrations mimicking environmental conditions. In this study, zebrafish were exposed to spiked-diet starting at the first meal and lasting all their life (F0 fish). We investigated behaviour in F0 as well as in unexposed offspring first (F1) and second (F2) generation. We measured different behavioural traits at early and late stages such as anxiety, boldness, exploration and sociability. First results showed behavioural disruptions in these unexposed F1 and F2. Offspring from exposed parents appeared more anxious, less social and less explorer than offspring of control parents. To investigate underlying molecular mechanisms driving those effects in F1 and F2 generation not directly exposed to POPs, we analysed DNA methylase and *cfos* genes expression which could provide mechanical clues for the observed disruptions. In nature, behavioural disruptions observed could impede access to food, exploration of new territories or reproduction and hence contribute to exposed population decrease and the existence of effects over several generations enhances this threat.

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O-58 *Animal replacement*

CHEMICAL ACTIVATION OF THE PREGNANE X RECEPTOR SHOWS SPECIES SPECIFICITY: CASES FOR FLAME RETARDANTS AND AZOLES

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In vertebrates, the nuclear receptor pregnane X receptor (PXR) is one of the main transcription activators for cellular metabolic detoxification. However, the processes involved are known to vary among species, and between individuals, tissues and even cell-types. Understanding the metabolic capabilities in different fish is important for assessing their susceptibility to chemicals. Here, using reporter gene assays, we assessed for differences in PXR activation in carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*), for two classes of chemicals, azoles and polybrominated diethylethers that are persistent in fresh water environments and toxic to fish. A Dual Luciferase assay was employed in which COS7 cells were transiently transfected with the PXR-LBD/pBind construct. Serial dilutions of azole and polybromodiphenyl ethers (10^{-5}M-10^{-12}M) were tested in 3 consecutive cell passages and 5β-pregnan-3,20-dione (an established PXR agonist) was included to probe the responsiveness of the bioassay. 5β-pregnan-3,20-dione activated carp-PXR, but not brown trout-PXR. Similar differences in activation were observed for azoles. Clotrimazole (CLO), miconazole and propiconazole activated carp-PXR in a dose-dependent manner, but only CLO activated brown trout-PXR. Bromodiphenyl ether 47 (BDE47), BDE100, and tetrabromobisphenol A activated brown trout-PXR in a dose-dependent manners and were more effective in doing so than carp-PXR. In conclusion, the activation of PXR appears to show differences between these two fish species. In brown trout detoxification for azoles may operate *via* other nuclear receptors, since only CLO of the tested azoles activated btPXR. In contrast, the polybrominated diethylethers activated both cPXR and btPXR with btPXR generally appearing the most sensitive. These findings highlight the need for better understanding the metabolic pathways of chemical detoxification in different fish species for their environmental protection.

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O-59 *Animal replacement*

PRECISION-CUT LIVER SLICES (PCLS) FROM MARINE ORGANISMS AS AN ALTERNATIVE BIOLOGICAL MODEL FOR ENVIRONMENTAL CONTAMINANTS AND BIOMOLECULES RESEARCH

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In the last decade, precision-cut liver slices (PCLS) have been developed as an *in vitro* model to reduce the number of organisms necessary for *in vivo* experiments. Unlike cell cultures, the PCLS maintain the original three-dimensional organization of the tissue and, although their use is widely diffused in mammalian studies, the application to other model species is still limited, especially for marine organisms. In this study, 250 µm thick slices were successfully prepared from fresh livers of the white sea bream (*Diplodus sargus*) through a vibratome. The obtained PCLS, cultured in Leibovitz (L-15) medium at 18 °C, maintained the cellular viability up to 72 h, and the analyses of specific enzymatic systems (antioxidant, peroxisomal and biotransformation activities) further demonstrated that such tissue slices preserve their cellular functionality at least for 48 h. To evaluate the suitability of *D. sargus* PCLS for ecotoxicological studies, the transcriptional modulation of several genes was measured in response to polycyclic aromatic hydrocarbons and biomolecules of natural origin. The results showed that 24 h exposure to benzo(a)pyrene increased the transcription of the cytochrome P450 *CYP1A1*, while the peroxisome proliferator activated receptors *PPARα* and β–oxidation genes were up-regulated after the exposure to the biomolecule caulerpin, the main metabolite from the invasive alga *Caulerpa racemosa*. Overall, our results highlighted a good responsiveness of PCLS and their efficacy as alternative experimental model in the study of molecular mechanisms of response to environmental contaminants. The use of fish PCLS may represent an efficient support to the environmental research allowing to reduce the need of fish for *in vivo* experimental activities.

O-60 *Animal replacement*

CYTOTOXICITY AND ENDOCRINE DISRUPTING POTENTIAL OF BADGE AND ITS DERIVATIVES IN HUMAN PLACENTAL CELLS

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BADGE (bisphenol A diglycidyl ether) is an epoxy resin monomer used as a coating in cans and food containers. It is a synthesis product of bisphenol A (BPA) that can migrate into food and generate different derivatives by hydrolysis of epoxy groups. BADGE is suspected to disrupt the endocrine system and to induce adipogenic differentiation in mammalian models, but no information is available for its derivatives. Thus, this work investigates the comparative toxicity, endocrine disruption potential and ability to alter cellular lipids of BADGE, BADGE·H_2O and BADGE·2HCl in human placental choriocarcinoma JEG-3 cells. After 24 h exposure, BADGE·2HCl (EC_{50}: 32-39 μM) and BADGE (EC_{50}: 38-43 μM) showed the highest cytotoxicity, followed by BADGE·H_2O (EC_{50}: 81-101 μM). All three compounds showed higher cytotoxicity than its precursor, BPA (EC_{50}: 138-218 μM). Regarding endocrine disrupting potential, only BADGE·H_2O was able to significantly inhibit P450 aromatase (IC_{50} 49 ± 5 μM) in JEG-3 cells at a range of concentrations similar to those observed for BPA (IC_{50} 71 ± 7 μM). Additionally, placental cell lipids namely, phosphatidylcholines (PC), PC-plasmalogen and triacylglycerols (TAGs), analyzed by UPLC-ToF-MS, were no significant altered after 24 h exposure to BADGE (0.5, 5, 20 μM), despite a tendency towards decreased levels of PCs and PC-plasmalogens and increased concentration of TAGs in those cells exposed to the highest concentration. Finally, the analysis of BADGE and its derivatives in culture medium by HPLC-ESI(+)-QqQ evidenced a good bioavailability of BADGE·2HCl, but a very low stability of BADGE and BADGE·H_2O. This is of particular concern since the observed effects for BADGE might occur at concentrations up to 20-fold lower than nominal concentrations. This study (a) evidences the higher cytotoxicity and ability to inhibit aromatase activity of BADGE and its derivatives in comparison to BPA, and (b) highlights the need for an accurate determination of experimental concentration of chemicals to improve the sensitivity and accuracy of in-vitro tests.

O-61 *Animal replacement*

ZEBRAFISH PROVIDES A PREDICTIVE MODEL FOR IDENTIFICATION OF DRUGS PROVIDING NEUROPROTECTION IN HUMAN SEVERE ACUTE ORGANOPHOSPHORUS POISONING

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Acute organophosphorus poisoning (OPP) has become a worldwide clinical and public health problem, with an estimation of around 3 million cases and 300,000 deaths annually. Along with acetilcholinesterase (AChE) inhibition, neurodegeneration and brain damage is one of the hallmarks of severe OPP. Recently, we have generated a zebrafish severe model for human OPP using the prototypic organophosphorus (OP) compound, chlorpyrifos-oxon (CPO). This model was characterized by a compacted head, with areas of opacification indicating necrosis of the brain. Further investigation revealed mechanistic similarities in the pathophysiological processes behind human severe OPP as in AChE inhibition, activation of the NMDA-receptor, inflammatory and immune responses and calcium dysregulation. The purpose of this study was to assess the suitability of the developed chemical zebrafish model for severe OPP to be used in the identification of new molecules providing neuroprotection in human severe OPP. Considering this, we tested prophylactic and treatment properties of standard human OPP treatment drugs (atropine and pralidoxime), reversible AChE inhibitors (huperzine-A, galantamine, physiostigmine and pyridostigmine) and molecules modulating pathways involved in human OPP, such as NMDA-receptor antagonists (MK-801, memantine), dual-functional NMDA-receptor and acetylcholine receptor (AChR) antagonists (caramiphen, benactyzine) and anti-inflammatory drugs (dexamethasone, ibuprofen). The effect on the 24 h survival and the prevalence of “abnormal” heads was determined for all the compounds. Moreover, effectiveness of the countermeasures was further confirmed by histopathological analysis and by the quantification of gene expression levels of four selected genes (*opn1mw1*, *il-12*, *hspb11*, *pth1a*) potentially involved in the severe OPP pathogenesis. Our results demonstrate that the zebrafish model for severe OPP provides reasonable accurate evaluations of the neuroprotective effect offered by these drugs that are well characterized in mammalian models.

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O-62 *Lipid homeostasis and obesogens*

PRIMARY ADIPOCYTE CULTURE AS A COMPLEMENTARY TOOL FOR UNDERSTANDING FISH LIPID METABOLISM

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In the recent years, white adipose tissue (WAT) has been recognized as a multi-functional tissue that regulates different physiological processes, such as appetite, glucose and lipid metabolism and inflammation. Besides its classical role in the regulation of energy balance and lipid storage, WAT is a highly active endocrine organ responsible for the secretion of a variety of adipokines, which exert an impact on whole-body metabolism and homeostasis. Although this field is extensively studied in mammals, the physiological and metabolic role of bWAT in cultured fish is not clearly elucidated. Moreover, the rapid growth of aquaculture and the subsequent increase of fish feed production result in a high demand of fish meal and fish oil. Therefore, development of new fish diets in order to improve product quality, control of adiposity and sustainable practices are essential to strengthen the fish farming industry. In this framework, our research group established and characterized primary adipocyte cells cultures from gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) in order to study adipose tissue dynamics and adipogenesis in fish. Results have shown that our model can be successfully used as an *in vitro* approach for testing compounds ranging from dietary ingredients with antioxidant properties to endocrine disruptors with a remarkable adipogenic potential. Specifically, we have confirmed *in vitro* the anti-adipogenic effect of two vegetal compounds, Caffeic acid and Hydroxytyrosol as well as the estrogenic effect of Genistein, another vegetal ingredient currently used in fish feeds. In summary, *in vitro* studies using adipocyte cells, can serve not only to examine the different pathways implicated in lipid metabolism and adipogenesis, but also can be used as an adequate screening mechanism to characterize different compounds and make suggestions for further *in vivo* testing.

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O-63 *Lipid homeostasis and obesogens*

IN VITRO MODELS FOR STUDYING HEPATIC LIPID HOMEOSTASIS

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The liver is able to store triacylglycerols (TAGs) and other lipids in cytosolic lipid droplets (LDs) that are dynamic organelles consisting of a core of neutral lipids surrounded by phospholipids and proteins of the PAT family. Excess lipid accumulation leads to hepatic steatosis, a multifactorial disorder which can be elicited by dietary, genetic and environmental factors. In order to study the direct effects of pro- or anti- steatotic compounds, minimizing the use of animal models, we developed *in vitro* models of liver steatosis. Primary cultures of rat hepatocytes and hepatoma FaO cells were incubated with a mixture of free fatty acids-FFAs (oleate/palmitate) leading to a mild steatosis condition in the absence of cytotoxicity. Parallel studies were performed on *in vivo* rat models of steatosis. In different models, steatosis was associated with increased number/size of LDs, modulation of mRNA expression or activity of genes coding for the main PAT proteins, peroxisome proliferator-activated receptors (PPARs), and lipolytic enzymes. In both animal and *in vitro* models of steatosis we tested the effects of thyroid hormones (THs), as major modulators of hepatic lipid metabolism. Our results showed that THs directly promoted a marked reduction in TAG content and LD diameter in hepatocytes mainly by stimulating mitochondrial oxidative metabolism of FFAs, rather than TAG secretion. In FaO cells, two xenobiotics such as Bisphenol A, a well known xenoestrogen, and Tetrabromobisphenol A, that shares structural similarities with THs, induced pro- and anti-steatotic effects, respectively. Moreover, in FaO cells, steatosis was promoted by exposure to ethanol, alone and in combination with exogenous FFAs: the results showed cumulative effects in impairment of FFA oxidation and stimulation of lipogenic pathways. We can conclude that our *in vitro* models represent a valuable tool to study the regulation of hepatic lipid homeostasis by endogenous and exogenous compounds.

O-64 *Lipid homeostasis and obesogens*

LIPID AND GLUCOSE METABOLISM IN GOLDFISH (*CARASSIUS AURATUS*) LIVER IS MODIFIED BY OLEOYLETHANOLAMIDE TREATMENT

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Oleoylethanolamide (OEA) is an acylethanolamide mainly synthesized in the gastrointestinal tract, which binds to the peroxisome proliferator-activated receptor α (PPARα) to regulate a wide variety of biological processes, such as food intake, locomotor activity or energy metabolism. These regulatory effects on food intake and locomotor activity were studied in our laboratory, but those related to energy metabolism have not been yet assessed in fish. Therefore, we evaluated the effects of acute (6 h) and chronic (11 days) treatments with OEA (5 μg g^{-1} body weight) on the levels of metabolites and enzyme activities related to lipid and glucose metabolism in liver of goldfish (*Carassius auratus*). We also determined the mRNA abundance of *pparα* and the clock gene *bmal1a* in both experiments. Regarding hepatic lipid metabolism, OEA decreased lipolytic capacity after both acute and chronic treatments, while lipogenic capacity increased after acute and decreased after chronic OEA treatment. These results are different than those observed in mammalian adipose tissue, but not so clearly in liver, and might be attributed to the absence of changes in the expression of *pparα* and/or the increase in the expression of *bmal1a* after chronic OEA treatment. Concerning glucose metabolism, a clear decrease in the hepatic capacity to use glucose was observed in OEA-treated fish. Altogether, these results suggest that OEA plays an important role in the regulation of liver energy metabolism in fish, which could be related to the metabolic changes associated with circadian rhythms and feeding regulation.

O-65 *Lipid homeostasis and obesogens*

MECHANISMS OF ACTION OF OBESOGENS IN DAPHNIA MAGNA

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Accumulation of storage lipids in the crustacean *Daphnia magna* can be altered by a number of exogenous and endogenous compounds, like 20- hydroxyecdysone (natural ligand of the ecdysone receptor, EcR), methyl farnesoate, pyrirproxyfen (agonists of the methyl farnesoate receptor, MfR) and tributyltin (agonist of the retinoid X acid receptor, RXR). This effect, analogous to the obesogenic disruption in mammals, alters Daphnia's growth and reproductive investment. Here we propose that storage lipid accumulation in droplets is regulated in Daphnia by the interaction between the nuclear receptor heterodimer EcR:RXR and MfR. The model was tested by determining changes in storage lipid accumulation and on gene transcription in animals exposed to different effectors of RXR, EcR and MfR signaling pathways, either individually or in combination. RXR, EcR and MfR agonists increased storage lipid accumulation, whereas fenarimol (an inhibitor of ecdysteroid synthesis) decreased it. Joint effects of mixtures with fenarimol and ecdysone were antagonistic, mixtures of juvenoids showed additive effects as predicted by the concentration addition concept, and combinations of tributyltin with juvenoids resulted in greater than additive effects. Exposure to these combinations resulted in de-regulation of ecdysone- and farnesoid-regulated genes, accordingly with the observed changes in lipid accumulation. The results indicate that the EcR:RXR complex behave as a non-permissive heterodimer, requiring binding of ecdysone to activate transcription, and that an excess of ecdysone disrupt the whole process, probably by triggering negative feedback mechanisms.

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O-66 *Comparative physiology and aquaculture*

DOES EXPOSURE TO VARIATIONS OF WATER TEMPERATURE AND OXYGENATION AT LARVAL STAGE HAVE LONG-TERM IMPACT ON METABOLISM OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)?

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European sea bass (*Dicentrarchus labrax*) is a highly valued fish that is likely to migrate from offshore areas to the coast during the last stages of larval development. Once arrived in the estuarine and coastal waters, larvae can be exposed to environmental constraints related to global change including fluctuating temperatures and dissolved oxygen levels. While the immediate effects of thermal and oxygenation conditions have been extensively investigated in marine fish larvae, the long term implications of early exposure to these constraints are poorly documented. In particular, it would be of great interest to determine whether early exposure to environmental constraint leads to fish individual phenotypes, with specific metabolisms, able to better cope with these constraints at later life stages. The objective of the present study was to evaluate the long-lasting impact of an experimental exposure to the combination of two oxygen concentrations (40% and 100% air saturation) and temperatures (15 and 20 °C) between 28 and 50 days post-hatching on metabolic parameters of European sea bass. After a common period of seven months on optimal condition (17 °C, normoxia), fish were again exposed to environmental constraints similar to the initial one. Effects of the early exposure were evaluated in terms of growth rate and metabolic parameters in the liver. The capacity for anaerobic energy production was investigated by assessing glycogen and glucose metabolites while lipid content was evaluated as energy supply for aerobic metabolism. The functioning of several biological pathways was investigated at the molecular level with a particular attention to the specific role of different metabolites. The results of this study provide precious knowledge about the capacity of marine species to buffer effects of climate change, particularly the understanding of key trait limits of plasticity.

O-67 *Comparative physiology and aquaculture*

A PHYSIOLOGICAL AND DEVELOPMENTAL ANALYSIS OF METAL MIXTURE TOXICITY IN ADULT AND EARLY-LIFE STAGES OF THE ZEBRAFISH

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A number of metals are key to life while others do not appear to fulfil essential functions and both become toxic at elevated concentrations. Although the effects of metals on biological systems are well documented on the macroscopic scale, our understanding of the molecular, biochemical and physiological mechanisms of metal toxicity is still limited. Especially the effects of metal mixtures remain very poorly documented and understood. Within this context we are performing experiments with early life stages and adult zebrafish to investigate the effects of single and combined exposures to Cu and Cd. In adults, effects on survival and behavioural responses were followed up to 28 days and the body burdens of both metals and the major cations were analysed. In zebrafish embryo acute toxicity tests survival and a number of sub-lethal endpoints, including swim bladder inflation, heart rate, mobility and malformations were recorded up to 120 hours post fertilization. In general, both zebrafish embryos and adults were much more sensitive to Cu than to Cd in single metal exposures on the basis of the observed mortalities. Exposure to Cu and Cd together showed interactions pointing to strong synergistic effects. The metal uptake measured in the adult fish showed a clear dose-response relationship, however neither the metal uptake rates nor body burdens in individual or mixture exposures explain the observed effects. Analysis of the major body cations showed that a loss of cations, in particular sodium has a large impact on the observed mortality in adults. The results obtained so far with early life stages show a high sensitivity of the development of the posterior swim bladder chamber to the exposure to Cu and Cd compared to other endpoints. The obtained information will be used as a starting point to construct adverse outcome pathways for metal and metal mixture toxicity.

O-68 *Comparative physiology and aquaculture*

OXIDATIVE RESPONSE AND ADAPTATION MECHANISMS TO EXTREME CONDITIONS AND TOWARD CLIMATE CHANGE: COMPARISON BETWEEN TWO ANTARCTIC SPECIES

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The high-latitude marine ecosystems are ranked to be among the most sensitive regions to climate change since highly stenothermal and specially adapted organisms might be seriously affected by global warming and ocean acidification. These processes can be further exacerbated by the presence of specific local features such as high cadmium bioavailability in upwelling area. Despite the importance to understand vulnerability of Antarctic organisms to climate change, synergistic effects of such multiple stressors have never been investigated. In this study, the delicate balance between oxidative metabolism and lipid homeostasis were compared in the scallop *Adamussium colbecki* and the emerald rockcod *Trematomus bernacchii*, chosen as keys sentinel species, among to Antarctic invertebrates and vertebrates. Organisms were exposed for 2 weeks to cadmium (Cd-40 µgL-1) and a combinations of two levels of pH (8.0 and 7.6) and temperature (- 1 and + 1 °C). Several parameters were analyzed both in digestive glands/livers and gills of exposed organisms, including cadmium bioaccumulation, metallothioneins levels, antioxidant system measured as individual defenses (catalase, glutathione, glutathione reductase, glutathione peroxydases, glutathione S-transferases) and total oxyradicals scavenging capacity (TOSC) toward hydroxyl and peroxyl radicals, lipid peroxidation, peroxisomal proliferation (AcylCoA oxidase) and genotoxic damage (% of DNA fragmentation and micronuclei frequencies). Results indicated reciprocal interactions between multiple stressors, with a different sensitivity among tissues and species. Despite that, a variable cadmium bioaccumulation was observed, limited variations occurred for metallothioneins. A certain modulation of single antioxidants and TOSC in all treated groups reflected an unbalance in oxyradical metabolism and an increased cellular damage, generally greater in scallops than in fishes.

O-69 *Comparative physiology and aquaculture*

METABOLIC ENZYMES ACTIVITIES AND GROWTH-RELATED GENES EXPRESSION IN ATLANTIC SALMON (*SALMO SALAR* L.): AGE AND SMOLTIFICATION ASPECTS

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The study was conducted to characterize the energy metabolism level and processes of muscle growth during development of Atlantic salmon (*Salmo salar*) inhabiting river Indera (Kola Peninsula, Russia). The activities of aerobic and anaerobic enzymes (cytochrome *c* oxidase and lactate dehydrogenase) and carbohydrate metabolism enzymes (glucose-6-phosphate dehydrogenase, glycerol-3-phosphate dehydrogenase and aldolase) in muscles and liver and expression levels of genes of myosin heavy chain (*MyHC*), myostatin (*mstn1*), myogenic regulatory factors (MRFs – *MyoD1*, *Myf5*, *myogenin*) in white muscles were studied in salmon parr of ages 0+, 1+, 2+, 3+ and smolts of ages 2+, 3+. Multidirectional changes in activities of enzymes of aerobic and anaerobic energy metabolism with age were shown in white muscles of parr. The changes in expression levels of MRFs, MyHC and myostatin indicating the extent of hyperplasia, hypertrophy, and restriction of muscle growth at different ages of parr were revealed. Particularly, the expression of genes *MyoD1*, *myogenin*, *MyHC* and *mstn1* peaked in yearling parr (1+). The differences were revealed in parameters studied between parr and smolts, which are directly associated with the peculiarities of metabolism caused by smoltification. Leveles of aerobic and anaerobic metabolism were higher in white muscles of smolts than in parr. The activities of carbohydrate metabolism enzymes were decreased in liver of smolts. The expression level of *MyoD1* and *myogenin* were lower in smolts in comparison to parr.

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O-70 *Comparative physiology and aquaculture*

MODULATION OF PITUITARY ACTIVITY AND THYROIDAL AXIS OF GILTHEAD SEA BREAM THROUGHOUT THERMAL FLUCTUATIONS: EFFECTS OF DIETARY ENERGY.

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Pituitary gland acts as an intermediary organ for physiological signals exchanged between the hypothalamus and peripheral organs. It is well known that gilthead sea bream (*Sparus aurata*) exposed to falling temperatures arrest growth due to cold-associated fasting and metabolic depression, decreasing fish-farm production. However, there is little knowledge about the role played by the multiple endocrine axes during the wintering period and on subsequent recovery. The aim of the present work was to explore the pituitary functionality of sea bream, focusing on the thyroidal axis, during induced thermal fluctuation and under two dietary regimes. Immature fish (around 145 g, in triplicate tanks) were fed two isoproteic diets (47%) differing in lipid content: 14% (D14 diet) and 18% (D18 diet) of crude fat. Experimental period (115 days) was comprised by 3 thermal periods: "Pre-cold" period at 22 ºC for 35 days; "Cold" period cooling down in 5 days to 14 ºC and maintaining for 45 days and "Recovery" period divided as "Early Recovery" rising up to 22ºC in 7 days and "Late Recovery" at 22 ºC for 23 days. Changes in pituitary gene expression for TSH, GH and POMC as well as for both thyroid-receptors (TRα and TRβ) and glucocorticoid receptor (GR) were evaluated by qPCR. Results revealed that at 22 ºC fish fed high dietary energy (D18) showed higher expression for all genes studied, except for TRα. Plasma levels of thyroxine (T_4), but not triiotyronine (T_3), were also diet-dependent. Surprisingly and contrary to global depression, overall pituitary gene expression was up-regulated by the end of the cold period. These data were also related to plasma indicators such as glycaemia, lactemia or lipemia throughout the experimental period. The role of pituitary gland regulating energy uptake and metabolism under thermal fluctuations is discussed for the first time in this species.

O-71 *Oceans and human health: acidification*

HEDISTE DIVERSICOLOR UNDER PREDICTED SEAWATER ACIDIFICATION SCENARIOS: BIOCHEMICAL ALTERATIONS

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Seawater pH is among the environmental factors controlling the performance of marine organisms, including non-calcifying organisms such as polychaetes, often the most abundant group of organisms in estuarine systems. Thus, the present study evaluated the impacts of seawater acidification in the polychaete *Hediste diversicolor*. For this organisms were exposed to different pH levels (7.9, 7.6 and 7.3) during 28 days and several biochemical markers were measured. The results obtained demonstrated that pH decrease negatively affected osmotic regulation and polychaetes metabolism, with individuals under low pH (7.6 and 7.3) presenting higher carbonic anhydrase activity (CA), lower energy reserves (protein and glycogen content) and higher metabolic rate (measured as Electron transport system activity (ETS)). The increase on CA activity was associated to the organism osmoregulation capacity while the increase on ETS and decrease on energy reserves was associated to the polychaetes capacity to activate defence mechanisms (e.g. antioxidant defences and maintenance of pH homeostasis). In fact, despite having observed higher lipid peroxidation (LPO) at pH 7.6 in polychaetes at the lowest pH tested (7.3) LPO levels were similar to values recorded in individuals under control pH (7.9). Such findings may result from higher antioxidant enzyme activity at the lowest tested pH, which prevented organisms from higher oxidative stress levels. Overall, our study demonstrated how polychaetes may respond to near-future ocean acidification conditions, exhibiting capacity to develop biochemical strategies which will prevent organisms from lethal injuries. Such defense strategies will contribute for polychaetes population maintenance and survival under predicted ocean acidification scenarios.

O-72 *Oceans and human health: acidification*

SEASONAL-DEPENDENT EFFECTS OF TEMPERATURE AND PH/pCO_2 ON CADMIUM ACCUMULATION AND BIOLOGICAL EFFECTS IN THE MEDITERRANEAN MUSSEL *MYTILUS GALLOPROVINCIALIS*

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Rising ocean temperature and ocean acidification represent a serious threat for marine ecosystems and have been recently addressed as potential factors modulating accumulation and toxicity of environmental pollutants in marine organisms. The impacts of these interactions could be highly influenced by strong seasonal fluctuations that characterize temperate areas, where physiology and responsiveness of organisms to stressors can greatly vary. In this study, the interactive effects of acidification and warming on cadmium exposure were evaluated in mussels *Mytilus galloprovincialis* sampled in different seasons. Mussels were exposed in a full 2 x 2 x 2 experimental design to two levels of temperature (control and $\Delta T = + 5$ °C), two levels of pH (control and $\Delta pH = 0.8$) and two doses of cadmium (0 and 20 µg/L), both in summer and winter. After one month of exposure, digestive gland, gills and haemolymph were analyzed to investigate cadmium bioaccumulation and a wide panel of biomarkers, including metallothioneins, antioxidant defenses, oxidative stress products, immune system parameters and onset of genotoxic damage. Our findings indicate that (1) pH/pCO_2 and temperature are key factors in modulating organisms responsiveness to cadmium and oxidative insult; (2) seasonality affects the influence of pH/pCO_2 and temperature on cadmium accumulation in mussels tissues and modulate the capability of organisms to counteract multiple stressors; (3) ocean acidification and warming represent additional risks for marine organisms experiencing exposure to pollutants.

O-73 *Oceans and human health: acidification*

EARLY LIFE STAGES OF THE SEA URCHIN *PARACENTROTUS LIVIDUS* COPING WITH SEAWATER ACIDIFICATION AND ENVIRONMENTAL CONTAMINANTS

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Sea water acidification poses increasing concern for broadcast spawning species which release gametes in the water column and have planktonic larvae with calcareous structures. Indeed, the functionality of gametes and their interactions, as well as the normal larval growth may be negatively affected by reduced pH. Susceptibility to other environmental stressors, such as pollutants, may be also altered under acidified conditions, resulting in more detrimental effects. To verify this hypothesis, combined exposures to CO_2-driven acidification and environmentally relevant concentrations (0.5 µg/L) of three contaminants (caffeine, diclofenac and PFOS, both singularly and in mixture) were carried out to asses motility of sperms and their ability to fertilize eggs in the sea urchin *Paracentrotus lividus*. Larvae from different families were also exposed to reduced pH and contaminants for 48 hours after fertilization in order to highlight different patterns of response due to the transmission of phenotypes from parents differently resilient to the tested stressors. Our results show a significant reduction in sperm motility, as well as in the percentage of fertilized eggs when sperms were pre-exposed to reduced pH (pH 7.7) compared to controls (pH 8.1). Conversely, at both pH values tested, no significant effect due to the contaminants was found. Larval growth was significantly affected by reduced pH, contaminants and pH/ contaminants interactions, with higher reduction in body length and increased percentage of morphological abnormalities when the two stressors were used in combination. The magnitude of the detrimental effects observed in larvae was different among different families suggesting the transmission of more or less performing phenotypes from parents to offspring.

O-74 *Oceans and human health: acidification*

SEAWATER ACIDIFICATION AND SALINITY EFFECTS ON PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE OF INTRODUCED CLAM *R. PHILIPPINARUM*

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Climate change is predicted to occur in the next years, including an increase on the frequency and intensity of extreme weather events (namely rainy and drought periods) and ocean acidification, which can have negative impacts to aquatic species. Due to this, there is an urgent need to increase the knowledge on the effects of these environmental factors (pH decrease and salinity shifts), especially when acting in combination, namely for marine bivalves. Thus, the objective of this study was to evaluate the effects of the combined impact of seawater acidification and salinity changes on the physiological and biochemical performance of the clam *R. philippinarum*. For this, clams were chronically exposed for 28 days to a combination of different salinities (14, 28 and 35) and pH levels (7.8 and 7.3). The results revealed that when combining pH 7.8 and salinity 14, clams physiological status and biochemical performance were negatively affected, resulting in an increase of cellular oxidative stress. On the contrary, clams exposed to pH 7.8 and salinity 28 and 35 were able to maintain their physiological status and biochemical performance. In addition, our findings showed that clams under low pH and different salinities were able to maintain their physiological and biochemical performance independently on the salinity tested. Our results further demonstrated that all salinities tested resulted in similar physiological and biochemical responses in clams under both tested pH levels. Also, clams exposed to low pH (salinities 14, 28 and 35) and control conditions (pH 7.8 and salinity 28) tended to present a similar response pattern, which may indicate that pH may have a lower impact on clams than salinity. Overall, the present study points out that the predicted increase of CO_2 in seawater and consequent seawater acidification will have a lower impact on physiological and biochemical performance of clams than salinity shifts.

O-75 *Environmental problems of nanomaterials*

TROJAN HORSE MECHANISM BY CARBON NANOPOWDER TOWARDS B(A)P AFFECTS UPTAKE AND TOXICITY FOR ZEBRAFISH EMBRYOS

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This study investigates the potential role of Carbon nanopowder (CNPW) as carrier for Benzo(α)pyrene B(α)P in zebrafish embryos. To this aim CNPW was contaminated with B(α)P, and GC-MS/MS analysis revealed a significant sorption of the hydrocarbon on CNPW surface. Embryos were exposed to CNPW (50mg/L), B(α)P (0.2-6-20 µg/L) alone and to the CNPW doped with the three B(α)P concentrations, from 24 hours post fertilization to 96 hpf. The uptake of CNPW and B(α)P and their tissue and intra-cellular localization were investigated by immunofluorescence and transmission electron microscopy. Results identified gills and gut as uptake tissues for CNPW, showing also the ability to translocate into the embryos, B(α)P alone accumulated mostly in the yolk sac and gut. In co-exposure, significant accumulation of B(α)P was evident in the gut, but not in the yolk. To evaluate the toxic effects due to CNPW interaction with B(α)P, biomarkers of cyto/genotoxicity and oxidative stress were applied. Any significant genotoxicity was induced by CNPW and B(α)P alone, while in co-exposure an increase of cytotoxicity, and necrotic and apoptotic cells was observed. The co-exposure determined antagonistic effects on the activity of SOD, Catalase and GST respect to single exposure. The application of transcriptomics and proteomics allowed the identification of molecular events involved in the responses to pollutants alone and in co-exposure. B(α)P alone determined an up-regulation of *cyp1a*, but the gene was not affected by co-exposure. A significant down-regulation of *sod2* was observed upon exposure to CNPW alone, but not in co-exposure. Proteomic analysis showed distinct patterns of protein modification induced by the three different exposure conditions, highlighting the alteration of key signaling pathways involved in ocular disorders (B(α)P), cytoskeleton assembly (CNPW), embryo development and patterning of the nervous system (CNPW + B(α)P).

O-76 *Environmental problems of nanomaterials*

SILVER NANOCOLLOIDS DISRUPT MEDAKA IMMUNE SYSTEM AND RESISTANCE AGAINST A COMMON PATHOGEN *EDWARDSIELLA TARDA*

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In this study we estimated immunotoxicity of silver nanocolloid (SNCs), which is one of silver nanomaterials, using medaka. To investigate exposure effects of SNCs on medaka immune responses, medaka embryos (developmental stages 11, 21, and 30) were exposed to 0.05 mg/L of SNCs until hatching. Post-hatched larvae (stage 40) were also exposed for 7 days. Adult (5 mouth old) were exposed to 0.05 mg/L of SNCs for 24 hours. Exposed eggs, larvae, or adult were subjected to qRT-PCR analyses of immune relative genes, such as nuclear factor kappa B (NFκB) p105 and NFκB p100, and tumor necrosis factor α (TNFα). In case of exposure from stage 11, *NFκB p105* was suppressed and other two genes (*NFκB p100* and *TNFα*) were enhanced compared with control. In exposure from stage 21, the three gene expressions were all enhanced. From stage 30, stage 40, and adult, the three gene expressions were all suppressed. Eggs exposed from stage 21 were subjected to whole mount *in situ* hybridization on day 3. Enhanced *NFκB p105*, *NFκB p100*, and *TNFα* genes were detected at pectoral fins and head; at a liver and intestinal tract; and at ducts of Cuvier and a heart, respectively. To see exposure effects of SNCs on medaka tolerance to pathogenic bacteria, medaka embryos, larvae and adult were treated following four conditions: (i) control, (ii) SNC exposure, (iii) pathogenic bacteria (*Edwardsiella tarda*) infection, (iv) SNC exposure and *E. tarda* infection; after which survival ratios were counted for 10 days. Under non-infected conditions, all medaka survived despite SNCs exposure. Regarding the embryos and larvae under *E. tarda* infected condition, there were no significant differences in survival ratios regardless of SNCs exposure. However, regarding adults under *E. tarda* infected condition, survival ratios were reduced to 20% by SNCs exposure.

O-77 *Environmental problems of nanomaterials*

BIOREACTIVITY OF GRAPHENE OXIDE AND REDUCED GRAPHENE OXIDE WITH HUMAN ALVEOLAR TYPE-I-LIKE EPITHELIAL CELLS

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Graphene and its derivatives, such as graphene oxide (GO) and reduced graphene oxide (rGO), have attracted great interest due to their potential applications in electronics, energy, materials and biomedicine. GO nanoplatelets are chemically reduced in order to reestablished electrical conductivity. This rGO shows different properties and behavior than GO and both have been increasingly used in many industrial products, increasing the risk of human exposure. In the form of nanoplatelets these nanomaterials could pose unusual risks to the respiratory system after inhalation exposure. Here we used highly relevant human alveolar type-I-like epithelial cells (TT1 cells) to assess the risks of GO and rGO on human health. TT1 cells were initially exposed to a wide range of concentrations of both types of nanoplatelets (with and without polyvinylpyrrolidone (PVP) as stabilising agent) to assess cell viability and then exposed to sublethal concentrations to evaluate their effects on the reactive oxygen species (ROS) production, inflammatory mediator release and cell membrane integrity. GO and rGO showed relatively low cytotoxicity to TT1 cells, being rGO more toxic than GO. PVP did not cytotoxic but increased bioavailability of nanoplatelets. ROS production and inflammatory mediator release were significantly increased after exposure to both GO and rGO. At the same time, GO and rGO caused a significant decrease in cell membrane integrity. Chemical reduction of GO increased its bioreactivity possibly due to the restoration of its surface electronic structure. In conclusion, GO and rGO are not highly cytotoxic for TT1 cells but they trigger important cellular mechanisms leading to toxic responses. Also, the use of human pulmonary cells offers a sensitive approach to detect differences in cellular reactivity of nanomaterials related to differential physico-chemical properties.

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O-78 *Environmental problems of nanomaterials*

COMBINED EFFECTS OF BENZO(A)PYRENE AND MULTI-WALLED CARBON NANOTUBES IN *MYTILUS GALLOPROVINCIALIS* TISSUES: A TRANSCRIPTOMIC AND IMMUNOHISTOCHEMICAL STUDY

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Despite the growing concern over the potential biological impact of polyaromatic hydrocarbons such as benzo(a)pyrene (B[a]P) in aquatic ecosystems , little is known about their interactions with non conventional emergent contaminants like multi-walled carbon nanotubes (MWNT). In this work, the interactive effects of B[a]P and MWNT at cellular/ tissue levels were investigated in mussel tissues (gills and digestive gland) using an integrated approach transcriptomics/immunohistochemistry. Mussels were exposed to B[a]P (5, 50 and 100 µg.L^{-1}) and MWNT (1 mg.L^{-1}), alone and in combination, for 72 h. Tissue B[a]P accumulation was evaluated by immunofluorescence with an anti- PAHs antibody showing an increasing trend in mussels exposed to BaP only. However, in animals exposed to MWNT along with the B[a]P gradient, no interactive accumulation was reported. Transcriptomic analysis using the new low-density targeted microarray identified several processes associated to DNA metabolism, cytoskeleton, oxydatif stress and heat shock response. In trend with the chemical data, no synergic effect was reported in term of gene expression neither in term of number of gene neither in term of gene expression trend in mussels exposed to B[a]P alone and to MWNT along with the B[a]P gradient. Transcription of selected genes was verified by qPCR. Moreover, expression of tubulin, as an example of target protein of interest identified by gene transcription data, was confirmed in tissue sections by immunolabelling. These represent the first data on transcriptional responses of marine invertebrates to exposure not only to B[a]P as a model ubiquitous polycyclic aromatic hydrocarbon and known genotoxic agent but also to emergent contaminant like MWNT. Finally, our data gives clues about the non occurrence of the Trojan Horse effect of MWNT at the tested concentrations.

O-79 *Comparative physiology and bioremediation*

INVESTIGATING APPEARANCE AND REGULATION OF THE MXR PHENOTYPE IN EARLY LARVAL STAGES FROM THE MEDITERRANEAN MUSSEL (*MYTILUS GALLOPROVINCIALIS*)

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MXR (*Multixenobiotic resistance*) efflux transporters constitute a general and broad-spectrum physiological defense system allowing marine bivalves to cope with environmental challenges. There is, however, less information on the type and role that different MXR transporters may have in larval stages, which represent the most sensitive stages of the bivalves to environmental stress. In this study, regulation of MXR-related transporters was investigated in early larval stages of the Mediterranean mussel (*Mytilus galloprovincialis*) attempting to elucidate their potential involvement in embryo protection and development. *In vitro* fertilization experiments using gametes from naturally-spawning broodstocks have been performed to follow embryo development from fertilized eggs (30 min post fertilization), first oocyte divisions (120 min post fertilization), trochophora stage (24 h post fertilization), and D-shape veliger stage (48 h post fertilization). QPCR analyses indicated that *ABCB* and *ABCC* transcripts encoding the main MXR-related transporters P-glycoproteins (P-gp) and Multidrug resistance proteins (Mrp) were expressed soon after 30 min from oocyte fertilization, with *ABCC* being more expressed than *ABCB*. Both transcripts were dramatically up-regulated in trochophorae and D-veligers. MXR efflux activity assessed using the fluorescent substrate rhodamine 123 (2.5 μM) and selective P-gp or Mrp inhibitors (verapamil and MK571, respectively) showed that the P-gp mediated efflux can be detected only in veliger, while a significant Mrp mediated efflux can be detected soon after 120 min post fertilization and remained almost unchanged in trochophora and veliger stages. Together with these basal transporter activity and expression patterns, MXR modulation by putative transcriptional regulators as well as substrates lead to hypothesize that while P-gp aid xenobiotic efflux performing a prominent protective role, Mrp could be a dual-functioning transporter also in mussel development as in mammals, performing both protective and physiological functions.

O-80 *Comparative physiology and bioremediation*

BIOCHEMICAL CHARACTERIZATION AND QUANTITATIVE GENE EXPRESSION ANALYSIS OF OXIDATIVE STRESS MARKERS IN *MYTILUS GALLOPROVINCIALIS* EARLY LIFE STAGE EXPOSED TO METAL CONTAMINATION AND HEAT STRESS

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The present study aims to evaluate metal accumulation, transcriptional and biochemical levels markers of oxidative stress responses in *Mytilus galloprovincialis* D-shaped larvae exposed to combine heat stress and metal contamination. For this purpose, we investigated the response of a large panel of oxidative stress markers such as catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and accumulation of malonedialdehyde (MDA)) and metallothionein (MT) as well as gene expression and metal accumulation in mussels larvae exposed to a sub-lethal concentration of Cu (9.54 µg/l), Ag (2.55 µg/l) and the mixture of the two metals (Cu (6.67 µg/l) + Ag(1.47µg/l)) along with a temperature gradient (18, 20 and 22 °C) for 48 h. Results suggest that co-exposure to metals and moderate temperature (20 °C) increase the antioxidant enzyme activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) and caused an increase of MDA and metallothionein accumulation. Meanwhile an exposure to 22 °C decreased the antioxidant enzyme activities. Sod, cat, gst and mt-10 gene expression levels showed an important increase in larvae exposed to copper silver or to the mix compared to the control condition at 18 °C. The same pattern but with higher induction levels was recorded in animals co-exposed to metals at 20 °C. At 22 °C, a significant decrease in mRNA abundance of cat, gst and sod and a significant up-regulation of mts targets (mt10 and mt20) was observed. Cu and Ag were differentially accumulated in larvae according to the exposure temperature. This study provides for the first time, the importance of the early protective response of oxidative stress related-gene expression and regulation in mussel's early life stages in multi stressors situations.

O-81 *Comparative physiology and bioremediation*

REACTIVE OXYGEN SPECIES: THEIR INFLUENCE ON OSMOREGULATION AND REDOX METABOLISM IN A DECAPOD CRAB

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The Mediterranean crab *Carcinus aestuarii* is a hyperosmoregulator in diluted seawater (dSW). As other Carcinids, it possesses two types of gills: while anterior gills are mainly for respiration, upon dSW exposure, posterior gills serve osmoregulatory purposes and increase their antioxidant defense to successfully combat free radical-induced stress. In this study, the effects of pro- and anti-oxidant exposures were analyzed in anterior and posterior gills of dSW-exposed crabs. Animals were collected from the field and allowed to acclimate to laboratory conditions in seawater (SW). They were then injected with an anti-oxidant (N-acetylcysteine (NAC), 150 $mg \cdot kg^{-1}$), a pro-oxidant (10 µl of 0.88 M $H_2O_2 \cdot g^{-1}$) or PBS. After 1 h of first injection, half of the animals were transferred to dSW. Injections were then carried out in 12-hour intervals. All injected solutions were adjusted to the osmotic pressure of crab hemolymph at SW or dSW to avoid osmotic alterations. After aproximatley 48 h of acclimation, all animals were sacrificed and gills dissected, flash frozen and stored at -80 ºC until analysis. For crabs exposed to dSW, H_2O_2 induced an increase in catalase (CAT) and superoxide dismutase (SOD) activities in both gill types, although these differences were higher for anterior gills (1.5 and 3.2-fold increase for CAT and SOD, respectively). NAC caused CAT to decrease, but had no effects on SOD. Furthermore, the osmoregulatory capacity of posterior gills was not significantly altered by either H_2O_2 or NAC treatments but these had a remarkable effect on pump expression in anterior gills: pro-oxidant treatment caused a 1.3-fold increase of Na^{2+}/K^{+}-ATPase gene expression (and activity measurements) while NAC raised $Na^{2+}/K^{+}/2Cl^{-}$ expression levels to posterior gill levels. Here, a functional model is proposed explaining how reactive oxygen species are involved in the physiological behavior of crab gills, which have two different physiological functions and strategies during hyper-osmoregulation in dSW.

O-82 *Comparative physiology and bioremediation*

THE FEEDING PHYSIOLOGY OF TWO COMMERCIAL BIVALVES IN THE INDIAN RIVER LAGOON (FL, USA) AND THEIR ABILITY TO DEPLETE BROWN TIDES

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The Indian River Lagoon, located on the Atlantic coast of Florida (USA), is one of the most diverse estuaries in the country gathering both temperate and subtropical species. The enormous biodiversity of the Lagoon has been recently threatened by multiple, massive, phytoplankton blooms since fall 2011. As a consequence, the sea grass coverage of the Lagoon was severely reduced and the fauna and flora suffered negative effects. Therefore, to elucidate potential top down control of blooms by filter-feeding organisms, we studied the feeding physiology of two native bivalves, oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*). The feeding behavior of the bivalves was studied *in situ* using filter-feeding, flow-through devices under normal water conditions. Results revealed that oysters had higher clearance rates than clams (2.33±0.20 L h^{-1} and 0.57±0.11 L h^{-1} respectively; $p<0.001$). A few months later, a brown tide caused by the phytoplankton species *Aureoumbra lagunensis* developed in the Lagoon with concentrations up to $3x10^6$ cells ml^{-1}. The same experiment under these new, changing conditions revealed that oysters only cleared 0.07±0.03 L h^{-1}, whereas a clearance rate for clams was not quantified as they did not seem to filter. To understand if oysters and clams could bio-remediate pre-algal bloom conditions we exposed the bivalves to a series of brown tide dilutions, from 10^4 to 10^6 cells ml^{-1} with increases every 50,000 cells ml^{-1}. Results showed that both species significantly reduced their clearance rates when algal concentration increased ($p<0.01$). Oysters decreased their clearance rates from 2.16±0.30 L h^{-1} to 0.16±0.03 L h^{-1} and clams from 0.41±0.12 L h^{-1} to 0.07±0.03 L h^{-1}. In summary, oysters seemed good candidates to deplete brown tide at low concentrations, but neither oysters nor clams seem to be good candidates to bio-remediate *A. lagunensis* blooms.

O-83 *Comparative physiology and bioremediation*

ANALYZING THE POTENTIAL OF THE SEA CUCUMBER *PARASTICHOPUS REGALIS* FOR BIOREMEDIATION OF AQUACULTURE WASTES

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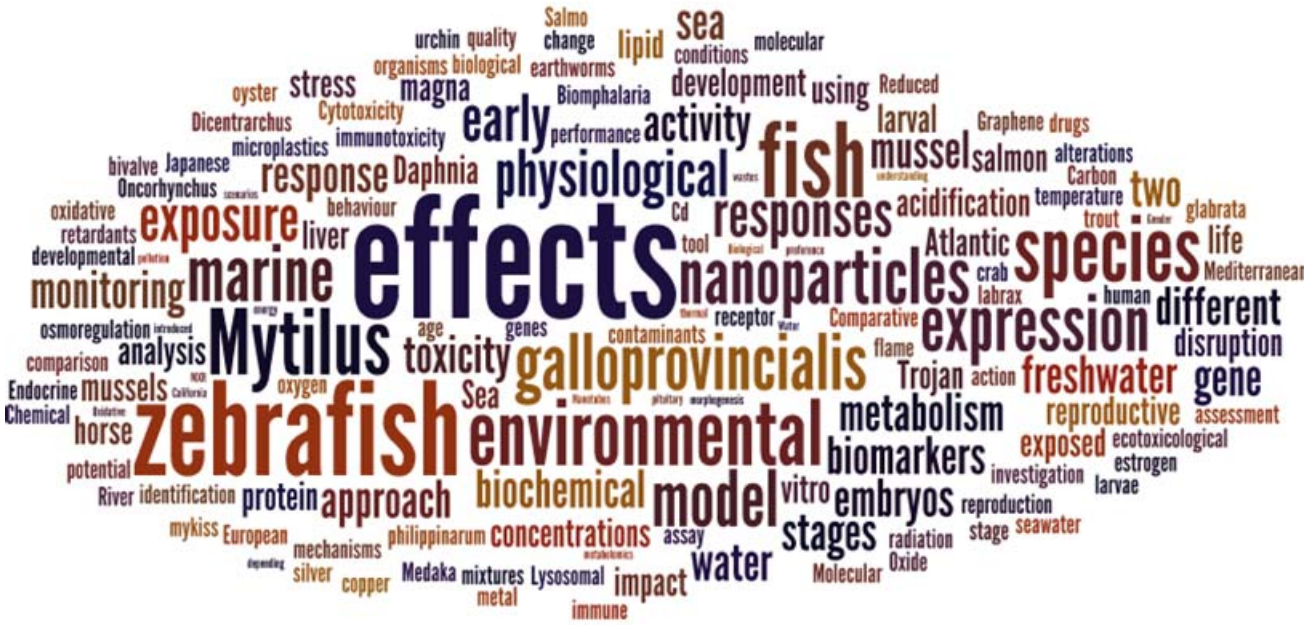
Aquaculture enhances food availability and provides economic and social benefits to coastal communities but there are concerns about the ability of the environment to sustain aquaculture expansion. Farming operations release particulate organic matter (unconsumed feed and fish feces) and inorganic nutrient excretions. The organic wastes settle onto the seabed and produce enriched sediments, which organic matter can be potentially consumed by bottom feeders. In recent years, integrated multi-trophic aquaculture (IMTA) has been proposed as a sustainable approach to mitigate the ecological effects of monoculture systems. Several studies have demonstrated the functional role of sea cucumbers such as *Apostichopus japonicus, Australostichopus mollis* and *Parastichopus californicus* to ameliorate the adverse effects of organic matter enrichment in coastal ecosystems. *Parastichopus regalis* is a holoturian of the family Stichopodidae which five muscle strips have high commercial value in the Spanish Mediterranean Sea. In a recent project (ref. AGL2011-25382) we have studied several aspects of its biology (feeding, reproduction, habitat requirements and behavior). Experimental estimation of feeding parameters (gut transit time, feeding rate and food assimilation efficiency) was performed. The main results obtained showed that *P. regalis* exhibited a positive selectivity for organic particles. The organic matter assimilation efficiency of the digestive tract was 26%. The estimated gut residence time ranged between 4 and 15 hours. Feeding rate estimates ranged between 0.01 and 1.78 g h^{-1}. In light of these results, the sediment processing rate of *P. regalis* will be compared with published data on other sea cucumbers to evaluate if this species is an elective candidate for bioremediation in Mediterranean aquaculture farms.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Posters

MO01 *Regulation and homeostasis from molecules to populations and beyond*

INTRACEREBROVENTRICULAR CERAMIDE TREATMENT AFFECTS HINDBRAIN AND HYPOTHALAMIC FATTY ACID-SENSING AND CONTROL OF FOOD INTAKE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Ceramides is a family of lipid composed of a sphingosine linked to a fatty acid. In mammals, ceramide metabolism plays important roles in different physiological functions, and its synthesis is controlled by hormonal (ghrelin and leptin) signals. Ceramides are involved in the control of feeding and body weight at hypothalamic level. We have demonstrated in fish the existence in hypothalamus of rainbow trout of fatty acid sensing systems modulated by ghrelin and involved in the control of food intake. We hypothesise that ceramides in rainbow trout are involved in the control of food intake modulated by central fatty acid sensing systems. We administered ICV to 100g rainbow trout 1µl of DMSO-Saline alone (control) or containing 2.5 µg of C6:0 ceramide. Ceramide treatment activated fatty acid sensing systems, increased anorexigenic potential and inhibited food intake. Particularly, we observed significant decreased production of the orexigenic factors AgRP (hypothalamus and hindbrain) and NPY (hindbrain); and a significant increase in the production of the anorexigenic factors POMC (hindbrain) and CART (hypothalamus and hindbrain), whose overall balance would be an increased anorexigenic potential in agreement with decreased food intake. These responses are exclusive of hypothalamus and hindbrain, since midbrain was unaffected. We therefore provide, for the first time in fish, evidence for a specific role of ceramides in hypothalamus and hindbrain fatty acid sensing systems and the control of food intake.

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MO02 *Regulation and homeostasis from molecules to populations and beyond*

EFFECTS OF DIFFERENT SALINITIES ON THE OSMOREGULATORY CAPACITY OF MEDITERRANEAN STICKLEBACKS

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Anthropogenic pressures and climate change put southern populations of three-spined sticklebacks (*Gasterosteus aculeatus* L.) at risk. This is especially relevant for the mesohaline and freshwater populations living along the northern Mediterranean coast. Therefore, a possible conflict for habitat and resources between these populations is anticipated. To initiate this study, individuals of a mesohaline population from the Camargue region (Rhone delta) were sampled and acclimated to laboratory conditions for at least two weeks in freshwater (FW; 5‰), brackish water (BW; 15‰), and seawater (SW; 30‰) . To explore their hydromineral regulatory mechanisms, blood osmotic pressure and gill Na^+/K^+-ATPase (NKA) gene expression of the α1a and α1b isoforms were determined. Furthermore, the NKA protein expression in the gill ionocytes and the remodelling of these specialised cells were investigated through NKA immunolabelling. Blood osmolalities of FW-, BW- and SW-fish were significantly different (291±5.8, 311±6.0 and 326±6.1 mosm/kg, respectively). In SW and FW conditions, branchial NKA α1a and α1b expressions were also different with less NKA α1b in FW than in SW. Ionocytes in FW-fish gills appeared located along the lamellae and at their base, whereas, in SW-fish, these cells are restricted to gill filaments. Moreover, ionocytes are elongated in FW-fish but possess a round shape in SW-fish. Finally, scanning and transmission electron microscopy revealed three different types of apical structures for these ionocytes: honeycomb-like structure and dome shape in FW, or deeply encrypted in SW. These results indicate that the morphological changes of the ionocytes and the different expressions of NKA isoforms are salinity-dependent. This is in agreement with previous studies on other euryhaline Teleosts. This remodelling must be directly linked to the different physiological homeostatic status reached by the fish. It also highlights that this Mediterranean mesohaline stickleback populations can rapidly acclimate to different salinity conditions and can easily migrate to freshwater.

MO03 *Regulation and homeostasis from molecules to populations and beyond*

EFFECTS OF DIETARY ELECTROLYTE BALANCE ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND ENERGY USE IN MEAGRE (*ARGYROSOMUS REGIUS*)

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The gastro-intestinal tract (GIT) is not only the site for digestion and nutrient absorption, but is involved in maintaining ionic and water balance in fish. The effect that dietary ionic and water disruptions have on digestion and nutrient assimilation itself remains unexplored in marine fish. The objective of the present study was to investigate the effect that dietary electrolyte balance (DEB) has on growth, nutrient digestibility, and energy use in meagre (*Argyrosomus regius*). For this purpose, fish (104±2 g) were fed to satiation for 75 days a rich-base (DEB 700) or a more acidic (DEB 200) diet, and changes in growth performance, metabolic rates and nutrient digestibility were assessed. Meagre fed the DEB 200 diet had better specific growth rate than fish fed the DEB 700 diet (0.8±0.0 and 0.5±0.0 %.day^{-1}, respectively), although absolute feed intake was not significantly different between both groups. Therefore, meagre fed the DEB 700 diet were less efficient in terms of gained mass per unit of feed consumed. Results also revealed lower standard metabolic rate in fish fed the DEB 700 diet than in fish fed the DEB 200 diet (64±5 and 99±31 mgO_2.Kg^{-1}.h^{-1}, respectively). Two hours after feeding, the pH of stomach chyme in fish fed the DEB 700 diet was more alkaline and blood pH was more acidic than in fish fed the DEB 200 diet (3.9±0.6 and 2.9±0.6 in chyme, 6.9±0.2 and 7.3±0.1 in blood, respectively). Meagre fed the DEB 700 diet also showed higher amylase activity in intestine but lower plasma glucose and TAG levels ($P<0.05$). Results suggest that a high DEB diet triggers mechanisms in order to re-establish acid-base homeostasis at the expense of an increased energetic cost. In addition, DEB may also reduce digestion efficiency and nutrient assimilation by altering chyme properties, which impacts growth and may have implications for aquaculture.

MO04 *Regulation and homeostasis from molecules to populations and beyond*

INTERACTIONS BETWEEN CORTICOTROPIN-RELEASING FACTOR (CRF) AND THE CIRCADIAN SYSTEM AT THE HYPOTHALAMUS-PITUITARY-INTERRENAL GLAND AXIS IN GOLDFISH

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Glucocorticoids act as regulatory signals of the circadian system in mammals and teleosts, modulating clock genes expression in several central and peripheral oscillators. In goldfish (*Carassius auratus)* the existence of glucocorticoid-sensitive clocks in the Hypothalamus-Pituitary-Interrenal Gland (HPI) has been suggested. The objective of the present work was to investigate if the hypothalamic CRF (corticotropin-releasing factor), involved in the HPI activation, could also be an input for the goldfish circadian system. First, the effect of an acute intracerebroventricular (ICV) injection of CRF (15 ng/g body weight, bw) on the HPI oscillators was studied. The expression of clock genes (in the hypothalamus, pituitary and interrenal gland) and plasma cortisol levels were analyzed at 2 and 8 h post-injection. The *gper1a* expression is induced by CRF in all studied tissues at both sampling times, while cortisol increased only at 8 h post-injection. To test if cortisol was involved in the CRF-induced *per1a* increment, goldfish were intraperitoneally injected with the glucocorticoids inhibitor metyrapone alone (1 μg/g bw), CRF alone (ICV, 10 ng/g bw) or metyrapone plus CRF. Clock genes expression was analyzed in the HPI at 2 h post-injection. The CRF-induction of *gper1a* was not reverted by metyrapone in all studied tissues, suggesting that this effect of CRF is not mediated by cortisol. Finally, if glucocorticoids are able to synchronize daily locomotor activity rhythms in goldfish was studied. The effect of a chronic cortisol treatment (1 and 3 μg/g bw) on locomotor activity synchronization was studied in fish under dim constant light and random schedule fed. Under these free running conditions, a daily cortisol injection for 15 days was unable to synchronize daily locomotor activity rhythms in goldfish. These data suggest that the stress hormone CRF (by independent glucocorticoid mechanisms) might play a functional role in the maintenance of the temporal homeostasis in teleosts.

MO05 *Regulation and homeostasis from molecules to populations and beyond*

ANALYSIS OF GUT MICROBIOME AND BIOCHEMICAL PARAMETERS IN OBESE PATIENTS, PRE- AND POST- BARIATRIC SURGERY

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The aims of this study were to evaluate the composition of gut faecal microbiome and host biochemical parameters (glucose, bile acids, short-chain fatty acids, hormones etc) of obese patients before and after bariatric surgery. Faecal and blood samples were taken 4 weeks prior to surgery and then 13 weeks post-operation (n=6). Bacterial identity was established by Illumina MiSeq sequencing of the variable region (V3V5) of the 16S rRNA gene. Sequence data were analysed with the Qiime tool to establish phylum, genus and species. Seven phyla were identified, with Firmicutes dominant and followed with Actinobacteria, Bacteroidetes, and Proteobacteria, and a lower abundance of Euryarchaeota, Fusobacteria and Verrucomicrobia. The abundance of organisms in the phylum Firmicutes and Proteobacteria were decreased, while Bacteroidetes was increased at 13 week post-operation. Significant decreases in weight, changes in microbiome and in biochemical profile (decreased glucose, triglyceride and cholesterol) suggest a complex interaction between host homeostatic metabolic control and the microbiome.

MO06 *Environmental problems of nanomaterials, microplastics and emerging compounds*

EVALUATION OF CHRONIC TOXICITY INDUCED BY THE ANTIDEPRESSANTS FLUOXETINE AND CITALOPRAM IN ZEBRA MUSSEL (*DREISSENA POLYMORPHA*)

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Antidepressants are among the most used pharmaceuticals and their concentration detected in freshwaters worldwide is in the order of ng/L. This issue is mostly associated to the inability of traditional wastewater treatment plants to remove these compounds from wastes, where they are spilled with urine and feces. However, despite the presence of pharmaceuticals in the aquatic environments is well documented, very few studies have been conducted to evaluate their potential adverse effects on aquatic organisms. Therefore, the aim of our study was the evaluation of sub-lethal toxicity induced by two common antidepressants, Fluoxetine (FLX) and Citalopram (CT), on the well-known biological model *Dreissena polymorpha*. Several mussels were exposed at these antidepressants for 14 days in semi-static conditions at the environmental concentration of 500 ng/L each one. In addition, considering that in the aquatic ecosystem these molecules are found as complex mixtures, we co-exposed the bivalves at FLX and CT at the same concentrations. The antidepressants added in the exposure tanks were quantified through liquid chromatography-mass spectrometry (LC-MS). Chronic toxicity was then evaluated by a multi-biomarker suite. The cellular stress was assayed by monitoring the activity of antioxidant/detoxifying enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and by the efflux activity of an ABC transporter, namely the P-glycoprotein (P-gp). The oxidative damage was assessed through lipid peroxidation (LPO) and protein carbonylation content (PCC). Finally, the genetic damage was evaluated on bivalve hemocytes by the Single Cell Gel Electrophoresis assay (SCGE), DNA diffusion assay and micronucleus test.

MO07 *Environmental problems of nanomaterials, microplastics and emerging compounds*

THE ROLE OF CARBON NANOPOWDER AS CARRIER FOR BENZO(A)PYRENE IN ZEBRAFISH: A PROTEOMIC INVESTIGATION

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During the last decades, considerable efforts have been made to investigate the toxicity of carbon nanoparticles (CNPs) since they are reactive oxygen species generator, can mediate secondary genotoxicity and they aggravate pulmonary and systemic cardiovascular disorders. Although the increasing interest in CNPs' toxicity, some aspects associated to their particular behaviour are still unexplored. One of the most recent and controversial features of the nanoparticles' effect is the contribution of the environmental contaminants adsorbed on CNPs to their toxicity because they are powerful adsorbent for organic compounds, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins and pesticides. Our Project aims to investigated the role played by carbon nanopowder (CNPW) as possible carrier of benzo(a)pyrene (BaP), one of the most frequent and dangerous atmospheric contaminants. We selected the embryos of *Danio rerio* (zebrafish) as well-known model system both for ecotoxicology and some human disease studies, exposed until 96 hpf (hours post-fertilization) to CNPW (50 mg/L) and BaP (20 μg/L) administered alone and to a co-exposure of CNPW preliminary doped with BaP at the same concentration. The proteomics analysis showed a proteins' modulation more than double for the co-exposure (16 proteins changed) in comparison to CNPW (8 proteins) and BaP (6 proteins), suggesting a possible role of carbon nanopowder as carrier for this hydrocarbon. In details, the proteins' identification highlighted the different cellular targets due to contaminants: the BaP seemed to act mainly on the eye, as indicated by the over-expression of some structural proteins, as the crystallins, while the down-expression of some other proteins due to CNPW showed as specific targets the cytoskeleton and some elements involved in the nervous system maintenance. The co-exposure highlighted the same effects of CNPW, combined with the modulation of other proteins mainly interested in the nervous system development and patterning.

MO08 *Environmental problems of nanomaterials, microplastics and emerging compounds*

IS *HEDISTE DIVERSICOLOR* A GOOD BIOINDICATOR OF PHARMACEUTICAL POLLUTION? RESULTS FROM SINGLE AND COMBINED EXPOSURE TO CARBAMAZEPINE AND CAFFEINE

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Several stressors have been identified as key and/or emerging drivers of environmental change that could significantly influence marine near-shore ecosystems. Among these stressors, pharmaceutical contaminants are often detected in aquatic environments, although information concerning their toxicity impacts on inhabiting species is scarce, especially when acting in combination. Furthermore, almost no information is available on the impacts of pharmaceuticals in polychaetes, often the most abundant group of organisms in benthic communities and including various species commonly used as bioindicators of environmental conditions. Therefore, the present study aimed to assess the biochemical alterations induced in the polychaete *Hediste diversicolor* by the antiepileptic drugs carbamazepine and the stimulant caffeine, acting alone and in combination. The results obtained clearly revealed that, after a 28 days exposure period, both drugs induced oxidative stress in *H. diversicolor*, shown by the increase on lipid peroxidation (LPO) levels and decrease on total glutathione and reduced to oxidized glutathione ratio (GSH/GSSG) with the increase of exposure concentrations. Furthermore, the present findings demonstrated that polychaetes biotransformation capacity as well as antioxidant defense mechanisms were not sufficiently efficient to fight against the excess of reactive oxygen species (ROS) leading to LPO when organisms were exposed to both drugs. Our results also demonstrated that polychaetes tended to decrease the activity of the Electron transport system (ETS) when exposed to drugs, avoiding energy expenditure which may prevent them from greater damages. The present study further revealed that the impacts induced by the combination of both drugs were similar to those obtained at the highest drugs concentrations acting alone.

MO09 *Environmental problems of nanomaterials, microplastics and emerging compounds*

ARTEMIA GENUS MAY BE AFFECTED BY THE WIDESPREAD USE OF METHYLPARABEN: THE EXAMPLE OF *A. FRANCISCANA*

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Nowadays parabens are used as preservatives in cosmetics, toiletries, pharmaceutical drugs and foodstuffs. Because of its widespread use, the occurrence of parabens in aquatic environments, including marine and hypersaline environments, are frequent. Methyl- and propylparaben are the most commonly used in cosmetics. To date there are very few studies on the toxicity of methylparaben (MeP) on aquatic invertebrates, and in the case of Artemia, no data exists. In this study, the toxicity of an emerging pollutant, such as MeP is analyzed in the sexual species *Artemia franciscana*, due its presence in coastal areas and marine saltworks in the Mediterranean region. For this reason, we tested MeP acute toxicity (LC_{50}-24h) in nauplii, and their chronic effect (9 days) was evaluated by measuring survival and growth under two sublethal concentrations (0.05 and 0.1 $mg \cdot L^{-1}$). Also, the effect on several key enzymes involved in: antioxidant defences (catalase (CAT), and gluthathion-S-transferase (GST)), neural activity (cholinesterase (ChE)) and xenobiotic biotransformation (carboxylesterase (CbE), was assessed after 48h under sublethal exposure. *A. franciscana* nauplii are resistant to MeP (LC_{50}-24h = 131.4 $mg \cdot L^{-1}$). MeP significantly affects survival and growth in chronic exposure to sublethal concentrations tested. In addition, MeP causes significant alterations in CAT activity after 48h exposure to the highest MeP concentration tested. However, no significant effect on ChE, CbE and GST activities was found. These results indicate that *A. franciscana* is resistant to MeP, although chronic exposures (up to 9 days) to sublethal concentrations affect survival and growth. Furthermore, the inhibition of CAT activity points out the oxidative stress effect of MeP in Artemia. We discuss the potential implications of our results on Artemia genus biodiversity and aquaculture development.

MO10 *Environmental problems of nanomaterials, microplastics and emerging compounds*

THE EMERGING USE OF KETAMINE AND ITS IMPLICATIONS IN EARLY VERTEBRATE DEVELOPMENT

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According to the United Nations Office on Drugs and Crime 2014 World Drug Report, ketamine and PCP-type substances recreational abuse correspond to about 4% of new psycho-active substances (NPS)" worldwide derived from their hallucinogenic effects. In humans and other organisms, ketamine is not completely metabolized and about 10% can be excreted by faeces and urine. Environmental contamination is an ecotoxicological concern, as ketamine has been reported to persist through conventional wastewater treatment plants (WWTPs), being detected in surface and seawaters. Furthermore, there is limited data on the toxic consequences and ecotoxicity of ketamine to aquatic organisms. Therefore, the aim of this study was to establish the 24-hour lethal concentration (LC50) of ketamine to zebrafish (*Danio rerio*) embryos and to evaluate its impact on development. The lethal median concentration (LC_{50}) in embryos was conducted according to OECD standard protocol (OECD 236). Based on the LC_{50} calculation, early blastula embryos (~2.0 hours post-fertilization) were statically exposed for 24 h to freshly ketamine concentrations (50, 70 and 90 mg L^{-1}), along with controls containing no ketamine. Developmental analysis was based on observable lethal and sublethal morphological parameters. Defects in cartilage (alcian blue) and bone (calcein) elements were assessed. To quantify similarities and differences between head shapes of zebrafish embryos, geometric morphometrics was used. The results showed that endpoints such as mortality, edema, heart rate, malformation rate, and growth rate were significant related with ketamine exposures. Cartilage and bone malformations in the head of zebrafish were also observed and corroborated by the geometric morphometrics analysis. This study provides new evidence on the ketamine teratogenic potential that will contribute to a better understanding of the environmental toxicity and aquatic risks of ketamine.

MO11 *Environmental problems of nanomaterials, microplastics and emerging compounds*

INGESTION OF MESOPOROUS SILICA NANOPARTICLES AND OXIDATIVE STRESS ENZYMES IN THE GERMAN COCKROACH *BLATTELLA GERMANICA* (LINNAEUS 1767)

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Nanotechnology has been recently increasing its presence in a wide range of science fields, developing technologies and consumer products, and therefore, the complex ways in which nanoparticles and nanomaterials could negatively affect the living beings and the environment must be assessed. In the case of silica nanoparticles, they have been suggested for the formulation of novel nanopesticides. However, their environmental fate and potential effects on non-target organisms remain still unknown. In this sense, oxidative stress is considered an emerging, general mechanism underlying nanoparticles toxicity and the activation of its pathways has been previously reported in experiments where NPs were ingested. In our work, we have used the model insect *Blattella germanica* (German cockroach), as it presents a world-wide distribution and is considered an important pest species. Furthermore, the German cockroach has been proposed as a useful bioindicator of indoor pollution and a good candidate for nanoparticles toxicity studies. Newly emerged adults (20 females and 20 males, parental generation) were daily administered with mesoporous silica nanoparticles (SiO_2NPs) through the diet. Couples were maintained in separated containers until fertilization was assured. After ootheca hatching, females were separated in new containers; this process was repeated until formation of the third ootheca. Nymphs hatched from each ootheca (F1) continued with daily ingestion of SiO_2NP-dosed food until reaching the adult stage, then they were frozen for enzymatic activity determinations. The oxidative stress state of treated insects was studied through the measurement of some antioxidant enzymatic defenses present in insects (Glutathione-*S*-transferase, Glutathione Reductase and Catalase) and the lipid peroxidation (TBARS) that may have occurred and could affect membrane stability.

MO12 *Environmental problems of nanomaterials, microplastics and emerging compounds*

DOES SIMVASTATIN ADMINISTRATION DISRUPT PLASMA METABOLITE HOMEOSTASIS AND BIOTRANSFORMATION ACTIVITIES IN *SOLEA SENEGALENSIS*?

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Statins, among them simvastatin (SV), are a diverse class of pharmaceuticals designed to lower human plasma cholesterol levels (LDL), the cholesterol with the strongest links to vascular diseases, and thus, reducing the risk of heart attack. These lipid regulators are among the most prescribed human pharmaceuticals in western European countries and are present in the aquatic environment at increasing concentrations because of their ability to pass waste-water treatment plants. Despite of its widespread use, many of its toxicokinetics and toxicodynamics aspects in non-target organisms remain unknown. SV is administered as lactone prodrug and generally has higher toxicity than the biologically active acid form of statins. This study aimed to identify the main metabolic responses after an intraperitoneal injection (IP; 10 mg/kg) of SV on a set of physiological markers in juveniles of *Solea senegalensis*. Blood, muscle and liver samples were collected at 20, 26 and 44 hours after IP injection. None of the plasma metabolites (cholesterol, triglycerides, glucose, lactate, ammonia, osmolality), liver enzymatic activities (EROD, BFCOD, CbE, CAT, GST) nor AChE activity and lipid peroxidation levels in muscle were responsive to SV. However, positive correlations were found between plasma cholesterol and triglyceride levels, liver BFCOD and GST activities, and between lactate levels and CAT activity. Additional research is in progress to unveil other more specific effects of SV in *S. senegalensis.*

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MO13 *Environmental problems of nanomaterials, microplastics and emerging compounds*

TOXICITY OF TIO2 NANOPARTICLES AND BULK IN FRESHWATER AND MARINE MICROALGAE, UNDER VISIBLE LIGHT AND UV-A RADIATION

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Titanium dioxide nanoparticles (TiO_2 NPs) has become a part of our daily life in the form of drug delivery systems, therapeutics and biosensors, cosmetics, production of paints, coatings, plastics, skin care products, foods, water remediation devices and pharmaceuticals, and the predicted environmental concentrations are the highest in aquatic ecosystems. Although TiO_2 has got a limited reactivity, under UV-A radiation can increase adverse effects on organisms, due to its photocatalytic properties; and the occurrence at nanoscale level change its physicochemical properties and toxicity. Phytoplankton is a key trophic level in aquatic ecosystems, and the toxicity provoked by these nanoparticles can affect structure and function of the ecosystems. Two microalgae species, from freshwater (*Chlamydomonas reinhardtii*) and seawater (*Phaeodactylum tricornutum*) have been selected for testing toxicity of TiO_2 NPs and TiO_2 bulk form. Due to their photo-catalytic properties, UV-A effect was checked, also. TiO_2 NPs and bulk TiO_2 showed a relation between the size of agglomerates (homoagglomerates) and time in freshwater and saltwater but not in ultrapure water. Under both, UV-A and no UV-A treatments, NPs triggered higher cytotoxic responses than conventional bulk material. TiO_2 NPs produced higher increasing in ROS production, damage to membrane and quantum yield. The marine microalgae species (*P. tricornutum*) showed to be more sensitive than the selected freshwater species and higher Ti internalization was reported. Exopolymeric substances (EPS) in the culture media are produced in presence of TiO_2 NPs and bulk from microalgae, pointing out a possible defense mechanism, enhancing homoagglomeration and settling processes and reducing bioavailability. In order to assess environmental risk assessment in realistic conditions, UV radiation should be considered as key element in toxicity assay guidelines.

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MO14 *Environmental problems of nanomaterials, microplastics and emerging compounds*

ECOTOXICOLOGY STUDY OF DILTIAZEM AND DOXEPINE IN *VIBRIO FISCHERI, DAPHNIA MAGNA, SELENASTRUM CAPRICORNUTUM* AND *DANIO RERIO* BIOASSAYS

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In the past few years, human health has benefited from consumption of drugs. However, nowadays, drugs are becoming a serious environmental and health problem. There are not enough studies addressing what happens with these substances in the environment and how they affect nature. Main problems are related to the stability, persistence and the action of drugs in many organisms. The aim of this study is to conduct an ecotoxicological evaluation of two drugs (Diltiazem and Doxepine) across four aquatic bioassays (*Vibrio fischeri*, *Daphnia magna*, *Selenastrum capricornutum*, and *Danio rerio).* These bio-indicators correspond to different trophic levels in order to provide sufficient information for performing an Assessment of Environmental Risk (ERA). A relationship between the chemical structure of the studied compounds and their ecotoxicity was determined using several physicochemical properties such as the partition coefficient, the critical micelle concentration and solubility. Futhermore, the relationship of the ecotoxicity and hydrophobicity was also investigated; the log EC_{50} values of chemicals studied were plotted against their log *P* values. According to the results obtained, these compounds can be categorized as "Moderately Toxic" and "Practically Harmless" in accordance with Passion and Smith's classification.

MO15 *Environmental problems of nanomaterials, microplastics and emerging compounds*

COMPARISON OF ECOTOXICITY OF THREE FAMILIES OF BIOMASS DERIVED COMPOUNDS BY MEANS OF FISH EMBRYO TOXICITY TEST

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Solvents are used in many processes and reactions of the chemical industry. The use of new environmental-friendly solvents has increased in the last decades although their final impact on the environment has not been widely studied. Some of these solvents are those derived from biomass which have been received much attention as renewable organic resources and have demonstrated a wide variety of applications. Most of the ecotoxicological studies have been focused in the analysis of acute toxicity of these compounds in invertebrate models such as algae, bacteria or crustacean. To complete the available information and to compare it with toxicity in vertebrate, embryos of zebrafish have been exposed to different solvents. Ecotoxicity of several compounds divided in three important families of solvents from biomass; lactic acid, levulinic acid and furfural derived chemicals has been analysed. Zebrafish embryo bioassay based on OECD 236 (Fish Embryo Toxicity Test, 2013) protocol has been used to observe signs of lethality of embryos after 24 and 48 hours of exposure to the chemicals and to determine LC_{50} at 48 hours. Ecotoxicity of compounds has been compared and differences among families have been evaluated.

MO16 *Environmental problems of nanomaterials, microplastics and emerging compounds*

PRESENCE, DISTRIBUTION AND CHARACTERIZATION OF MICROPLASTICS IN MEDITERRANEAN ORGANISMS

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In this work, the presence, distribution and characterization of microplastics (MPs) were assessed in several species from different Mediterranean areas, including Central-North Adriatic sea and Northern Tyrrhenian Sea. A recently validated protocol was applied to extract MPs from gastrointestinal tracts of fish and soft tissue of invertebrates; after the extraction, particles were further characterized in term of size, shape and polymer typology through microscopy and FT-IR analyses. In Adriatic organisms, MP items were observed in 45% of analyzed specimens. Invertebrates typically exhibited a lower frequency of MPs in soft tissues compared to the stomach of fish. MPs were mostly represented by fragments and lines, while polyethylene, polystyrene and nylon were the predominant polymers.In the Tyrrhenian Sea, several species were collected at the Giglio Island during the parbukling project of the Costa Concordia Wreck. Benthic fish were collected in proximity of the stern of the wreck and in a control site during the summer 2014, while mussels were translocated at two depth (-5 and -40m) and in three different season (winter and spring 2013, summer 2014) at different distance from the wreck. Results showed that fish were highly susceptible to MPs ingestion: extracted MPs were mostly represented by fragments and lines, suggesting a possible relationship with human activities related to the wreck removal. Transplanted invertebrates typically exhibited a lower frequency of MPs in soft tissues and the translocation period did not allow to highlight significant differences between sites at different distance from the wreck. On the other hand, a higher ingestion of MPs was observed in surface- compared to bottom-transplanted specimens, and in summer compared to winter and spring experiments. These studies provide new insights on the presence, distribution and typology of MPs in Mediterranean organisms, representing an important baseline assessment on the levels of these emerging pollutants.

MO17 *Environmental problems of nanomaterials, microplastics and emerging compounds*

TRANSCRIPTOMIC AND CELLULAR EFFECTS OF DIETARY EXPOSURE OF ZEBRAFISH TO SILVER NANOPARTICLES

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Waterborne exposure of fish to silver nanoparticles (Ag NPs) is known to cause deleterious effects, but much less is known about the effects of nanomaterials transferred through the diet. In this study we have daily exposed larvae of brine shrimps (*Artemia*) to 100 ng/L (Low Dose, LD) or 100 µg/L (High Dose, HD) of PVP/PEI-coated Ag NPs of 5 nm and these larvae were used to feed adult zebrafish for 21 days, resulting in a nominal exposure concentration of 0.17025 ng Ag/fish/day and 2.1817 ng Ag/fish/day, respectively. Silver content (ICP-MS) increased in whole zebrafish fed with the HD and autometallography reflected a time- and dose-dependent increase of metal content, both in the intestine and in the liver. After the microarray (44 K) analysis in fish fed with the HD, only one transcript resulted significantly regulated in the liver after 3 days of exposure and 261 transcripts after 21 days. In the intestine, no significantly regulated transcripts were detected after 3 days and only one transcript was significantly altered after 21 days. Among significantly regulated genes, *vtg*, *apoptosis inducing factor*, *gst*, *vegf*, *er*, *pparα*, *mt2* and *dna-j* were selected for microarray validation. Hepatic lysosomal membrane stability was significantly reduced after 3 and 21 days treatment in both exposed groups, indicating impairment of general fish health. In the intestine, the proportion of goblet cells did not change in any of the treatment groups. In conclusion, metal accumulation detected by autometallography did not provoke significant changes in the intestinal transcriptome, while liver transcriptome was more responsive after 21 days of exposure. These results point out to liver as the main target organ for Ag NPs toxicity in zebrafish after dietary exposure.

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MO18 *Environmental problems of nanomaterials, microplastics and emerging compounds*

ACUTE OXIDATIVE STRESS RESPONSE TO SILICA NANOPARTICLES ON *BLATTELLA GERMANICA*

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The environmental release of engineered nanoparticles is a matter of growing concern nowadays, receiving considerable attention of society and academic circles. The increased use of silica nanoparticles in emerging applications may result to obtain them in substantial concentrations which may lead to potential impacts in ecosystems. Little is known about the real exposure and their toxicity to organisms so that a greater research is of utmost importance and undeniably necessary. Some studies suggest that oxidative stress is one of the main paths responsible for toxicity due to nanoparticles. Moreover, the employment of biological models, such as insect models, is an accurate tool to evaluate the possible effects of nanoparticles that could carry environmental hazard. In this study we have assessed the oxidative stress response induced by acute exposure to silica nanoparticles to newly emerged adults of *Blattella germanica* cockroaches which is a species well studied and of economic importance. Two different ways of delivering the nanoparticles to the insects were chosen and compared between them; the first consisted of nourishing the animals with food containing silica nanoparticles and the second one was indirectly by means of tarsal contact. Animal survival rate was followed throughout seven days and the total amount of silica within the animal body was measured. Several oxidative stress biomarkers were studied in the exposed cockroaches, among them we can count ethoxyresorufin-O-deethylase (EROD), glutathione S-transferase, glutathione reductase and catalase activities as well as lipid peroxidation via thiobarbituric acid reactive substances (TBARS) assay.

MO19 *Environmental problems of nanomaterials, microplastics and emerging compounds*

TOXICITY OF UNCOATED VERSUS COATED TIO_2, CEO_2 AND AG NANOPARTICLES IN *DAPHNIA MAGNA* AND RAINBOW TROUT

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Different coatings are being used to improve the functionality of nanoparticles (NPs) by modifying their physicochemical and toxicological properties. However, this supposes that a high number of assays should be conducted for all possible coating-NPs combinations. For this reason, one of the objectives of the Project GUIDEnano is to establish relationships between different types of coatings and toxicity in an attempt to reduce *in vivo* assays. In this study, we investigated how two hydrophilic coatings, citrate (CIT) and polyethylene-glycol (PEG), and two hydrophobic coatings, dodecylphosphonic acid and oleylamine, modulate the toxicity of the corresponding uncoated TiO_2, CeO_2 and Ag NPs. All NPs were provided by PlasmaChem GmbH (Germany). The acute toxicity of these NPs has been studied in *Daphnia magna* and rainbow trout (*Onchorrynchus mykiss*) following the OECD guidelines 202 and 203, respectively. Pristine NPs and NPs in the exposure medium were characterized by TEM, DLS and ICP-MS. The uncoated NPs produced toxicity to *D. magna*. AgNPs were the more toxic followed by CeO_2NPs and TiO_2NPs. Interestingly, the coating of the metal oxide NPs prevented the toxic effects on this organism. However, in the case of the coated AgNPs an increase of the toxic effects was observed, indicating differences in coating-core interaction between the metal oxide and the metal nanoparticles used in this work. The toxicity in fish didn't follow the same pattern. Any of the uncoated TiO_2 and CeO_2 NPs were toxic at the limit dose (100µg/mL). Similarly, the corresponding hydrophilic coatings did not lead to an increase of the toxicity of these NPs. The hydrophobic NPs couldn't be tested for technical reasons. The results with TiO_2 and CeO_2 NPs indicated a different taxa susceptibility to a same uncoated NP but a same sensitivity to the CIT and PEG coated NPs.

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MO20 *Environmental problems of nanomaterials, microplastics and emerging compounds*

ISOLATION AND IDENTIFICATION OF CELLULOSE-DEGRADATION BACTERIA FROM CATTLE SLURRY

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Cellulases are the third most widely used group of enzymes obtained from microbial sources. Despite the large number of microorganisms available to degrade cellulose, few bacterial sources can be produced in sufficient quantities to completely hydrolyze cellulose. The present study was undertaken to investigate and identify cellulolytic bacteria found in agriculture waste in a state of anaerobic digestion. For the purposes of the study, bacterial isolates were obtained from three samples of cattle slurry: (1) fresh, prior to treatment; (2) after one month of anaerobic digestion; (3) after six months of anaerobic digestion. We employed the Sanger Sequencing Method of detection for plasmid isolation from bacterial isolates, to identify bacteria up to the genus level, using the 16 S rRNA PCR technique. Of all the isolates, the majority of the bacteria identified belonged in order of prevalence to: (1) the genera *Bacteroides*, *Clostridium*, *Enterobacter*, *Lactobacillus* and *Bifidobacterium*; (2) the genera *Bacteroides*, *Bifidobacterium*, *Klebsiella*, *Enterococcus* and S*taphylococcus*; (3) the genera *Clostridium*, *Lactobacillus*, *Bifidobacterium*, *Staphylococcus* and *Campylabacter*. The study showed the bacterial community's composition differed across all three samples, and depended on the times at which the samples were taken prior or after treatment. Furthermore, future work is indicated to assess the anaerobic digestion potential of these bacteria isolates by measuring produced methane gas. This can be done after investigating and identifying bacterial isolates up to the species level, using Pyrosequencing.

MO21 *Environmental problems of nanomaterials, microplastics and emerging compounds*

ENDOCYTIC UPTAKE AND INTRACELLULAR FATE OF TITANIUM DIOXIDE NANOPARTICLES IN RAINBOW TROUT LIVER CELLS

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Titanium dioxide (TiO2) nanoparticles (NPs) belong to the most widely used nanomaterials. Concomitantly they are increasingly released into surface waters, where they can be taken up by aquatic organisms. Previous studies measured increases in the amount of Ti in rainbow trout liver after exposure to TiO2 NPs via the diet. It is still unknown how TiO2 NPs accumulate in fish liver and whether or not they can be eliminated again. The objective of this study was to determine if and through which mechanism TiO2 NPs are taken up by fish liver parenchymal cells using the rainbow trout liver cell line RTL-W1 as in vitro experimental model. RTL-W1 cells were exposed for 15 min, 30 min, 1 h, 2.5 h, 4 h and 24 h to100 µg/ml TiO2 NPs. The exposure conditions were thoroughly characterized by measuring morphology, size distribution and stability of TiO2 NPs in cell culture medium using transmission electron microscopy and dynamic light scattering analysis. TiO2 NP uptake and intracellular fate was studied by means of transmission electron microscopy. TiO2 NPs agglomerates/aggregates were identified in smooth, flask-shaped plasma membrane invaginations at the apical side of the cell. The NPs were taken up into endocytic vesicles and routed to other intracellular compartments including endosomes, multivesicular bodies and multilamellar, autophagosome-like vacuoles. The amount of TiO2 NP entrapped within intracellular compartments increased with exposure time. The results suggest that RTL-W1 internalize TiO2 NPs via a clathrin-independent mechanism and accumulate them in specialized compartments for isolation, degradation and/or excretion.

MO22 *Comparative physiological adaptations in a changing environment*

INTER- AND INTRA-POPULATIONAL VARIATIONS OF CD TOXICITY ON OSMOREGULATORY MECHANISMS IN *GAMMARUS FOSSARUM*

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In order to explore the importance of life history and sensitivity of organisms in ecotoxicological studies, we investigated the impact of Cd exposure in three natural populations of *Gammarus fossarum* from different rivers of southeastern France. The first population lives in a Cd-contaminated river from a geochemical background, while the others inhabit Cd-free sites with one river situated in a weakly anthropized context (agriculture) and the last one in a pristine environment (reference population). Osmoregulation, a relevant biomarker to evaluate crustacean health, was used as an endpoint of potential local physiological adaptation to Cd. Specimens from each population were experimentally exposed to Cd for 7 days and hemolymph osmolality (HO) was then individually measured. To avoid any modification of HO unrelated to Cd exposure, each population was exposed in water sampled from its river of origin, which led to adjust Cd concentrations towards physicochemical parameters to provide a common level of exposure of 9 µg Cd^{2+}/L for the three populations. In each population, high inter-individual variations in HO values were noted, resulting in their separation into unimpacted and slightly or highly impacted (with lower HO) animals. In gills of impacted organisms, deep histopathological alterations and a protein overexpression of Na^+/K^+-ATPase and V-H^+-ATPase were observed through histology and immunolocalization, while non-impacted animals showed profiles comparable to controls. Moreover, the population living in the non-contaminated site was the least impacted by Cd exposure in the laboratory. The changes found in individual and population responses thus revealed no obvious adaptive phenomena, but they may be due to differences in fitness between populations in relation to the features of their respective environments.

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MO23 *Comparative physiological adaptations in a changing environment*

COMPARISONS OF THE INTEGRATED RESPONSES BETWEEN THE NONLETHAL AND LETHAL HYPOTHERMAL STRESSES OF MILKFISH (*CHANOS CHANOS*): A PROTEOMIC STUDY

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The milkfish is a marine species and is one of the major aquaculture fish in Taiwan. The high mortality of milkfish in cold snap of winter, however, usually causes huge economic loss. To understand the critical influences and compensatory responses of milkfish induced by hypothermal stress, this study compared the protein profiles of milkfish livers and gills among the control (28°C), nonlethal (18°C) and lethal (16°C) hypothermal groups, using the two-dimensional electrophoresis proteomics approach. The protein spots of livers were classified into three categories according to their cellular functions: (1) anti-oxidative stress, (2) apoptotic pathway, and (3) cytoskeleton. Meanwhile, five functional categories were sorted in the gills: (1) cytoskeleton, (2) immunoresponse, (3) protein quality control, (4) energy production, and (5) intracellular homeostasis. Based on the functional information of identified proteins, this study assumed that different responses and influences were stimulated by varied hypothermal stresses. Upon the nonlethal hypothermal stress, the milkfish encountered oxidative stress in the liver and inflammation responses in the gill since the identified proteins were involved in the roles of anti-oxidative stress and anti-inflammation. Upon the lethal hypothermal stress, however, the identified proteins induced by nonlethal hypothermia were associated with apoptosis in the liver and regulation of intracellular homeostasis in the gill. The present study provided proteomic evidences to illustrate differences between multi-physiological responses to nonlethal and lethal hypothermal stresses in livers and gills of the milkfish.

MO24 *Comparative physiological adaptations in a changing environment*

TOLERANCE OF NATIVE AND INTRODUCED *RUDITAPES* SPECIES TO COMBINED EFFECTS OF OCEAN ACIDIFICATION AND ARSENIC

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Organisms in marine systems are exposed to a combination of multiple stressors, that creat a range of associated environmental and ecotoxicological risks. Examples of stressors include alterations in the range and variability of physical and chemical conditions related to climate change (e.g. extremes in temperature, alterations in pH and dissolved oxygen regimes) and the magnitude and duration of exposure to chemical pollutants (including metals and metalloids) arising from human activities. These environmental stressors have been identified as key and/or emerging drivers that could significantly influence the marine near-shore ecosystems and inhabiting organisms, namely bivalves. The clams *Ruditapes decussatus* (European clam) and *R. philippinarum* (endemic species from the Indo-Pacific region) have been used as sentinel organisms to assess the impact of pollutants in different marine ecosystems, being good candidates to provide relevant information on environmental changes. Thus, the aim of this study was to compare physiological and biochemical responses of sympatric clam species, *R. decussatus* and *R. philippinarum*, when exposed to combined effects of pH (7.8 and 7.3) and As concentrations (0, 4 and 17 mg L^{-1}). Preliminary results suggested that As concentrations clearly influenced the physiological and biochemical performance of both clams species. In general, our findings revealed that for each As concentration no differences were found among pH conditions (7.3 and 7.8), but at each pH level differences were found among As concentrations. Overall, our findings demonstrated that As contamination induced biochemical and physiological alterations in clams, independently on the seawater pH values. Also, our results point out that clams under predicted seawater pH values may develop mechanisms that allow them cope with such changes.

MO25 *Comparative physiological adaptations in a changing environment*

MOLECULAR CHARACTERIZATION AND EXPRESSION OF NA^+/K^+-ATPASE A1 ISOFORMS IN THE EUROPEAN SEA-BASS OSMOREGULATORY TISSUES FOLLOWING SALINITY TRANSFER

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The Na^+/K^+-ATPase (NKA), considered as the main pump involved in active ion transport, presents in the European sea-bass (*Dicentrarchus labrax*) two isoforms of the alpha 1 subunit (NKA α1a and α1b). Analysis of amino acid (aa) sequences of both isoforms revealed a high degree of conservation across teleosts. NKA α1a and α1b isoform aa sequences are compared through phylogeny and regarding key functional motifs between salmonids and other acanthomorph species. The expression pattern of *α1a* and *α1b* genes were measured in the gill, kidney and posterior intestine of fish in seawater (SW) and transferred to fresh water (FW) at different exposure times. *α1a* seems to be more expressed than *α1b* whatever the condition and the tissue analyzed. In FW and SW conditions after long-term salinity acclimation (2.5 years), the levels of *α1a* mRNA transcripts present in the kidney were highest followed by the posterior intestine and the gill. Compared to SW conditions, expression of *α1a* was significantly increased or decreased respectively in gill and posterior intestine. Branchial *α1b* in contrast was significantly decreased in FW acclimated fish. Short-term FW acclimation seems to rapidly increase *α1a* transcript levels in the kidney unlike in gill tissues where different gene expression levels are detected only after long-term acclimation.

MO26 *Comparative physiological adaptations in a changing environment*

TEMPORAL VARIATION OF CORTISOL BIOSYNTHETIC PATHWAY AFTER STRESS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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In fish, the stress response involves the activation of the hypothalamus-sympathetic-cromaffin (HPC), and the hypothalamus-pituitary-interrenal cells (HPI) axes, leading to increased plasma catecholamines and cortisol levels. Serotonergic and dopaminergic systems have been reported to play a main role in initiating and maintaining such response. As a consequence, cortisol synthesis and release increase. After stress exposure, the physiological response tends to dissipate, with cortisol levels, among other parameters, turning back to normal. Specifically for cortisol, little is known about time needed for the hormone to stabilize to normal non-stress levels. To address that question, a cohort of rainbow trout (*Oncorhynchus mykiss*) was subjected to stress by high stocking density for up to 7 or 10 days and then sacrificed, whereas another cohort was stressed for 7 days and afterwards un-stressed and sacrificed for the following 2-h, 6-h, 24-h and 72-h. Individual samples of blood (for cortisol assessment) and head kidney (for the assessment of parameters related to cortisol synthesis: *StAR*, *3βHSD*, *P450ssc* and *11βH*) were collected. Our results show increased plasma cortisol levels and mRNA abundance of *StAR*, *3βHSD*, *P450ssc* and *11βH* in stressed trout at both 7 and 10 days. The hormone levels decreased to those comparable to control group at 6-h after stress and afterwards. The expression of *StAR*, *P450ssc* and *11βH* recovered normal levels at 6-h after stress, whereas only 2-h were needed for *3βHSD*. Thus, the physiological response to chronic stress in rainbow trout, characterized by the activation of both HPC and HPI axes, and elevated plasma cortisol levels, among other parameters, dissipates in a relatively short time period (within 6-h) after exposure to stress, which is in support of the role played by the hormone in mediating the upstream initiated physiological response to stress in trout.

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MO27 *Comparative physiological adaptations in a changing environment*

BRAIN MONOAMINERGIC NEUROTRANSMITTERS IN CHRONIC STRESS AND DURING STRESS RECOVERY IN RAINBOW TROUT

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The activation of brain dopaminergic and serotonergic neurons plays an important role in initiating the stress response in fish. As a consequence cortisol synthesis and release increases shortly after stress, remaining elevated for several days until it dissipates progressively or stress disappears. However, little is known about the role of brain monoamine neurotransmitters during chronic stress and indeed no studies focused after stress, when cortisol decays to basal non-stress levels. To address such concern, a cohort of rainbow trout (*Oncorhynchus mykiss*) was subjected to stress by high stocking density and sacrificed after 7 and 10 days, whereas a second cohort was stressed for 7, then unstressed and sacrificed at 2h, 6h, 24h and 72-h post-stress. A third cohort of un-stressed control fish was also sacrificed at 7 and 10 days. Food intake was daily evaluated for each cohort all over the experiment. Plasma levels of cortisol, glucose and lactate were assayed, but also the serotoninergic and dopaminergic activities in several brain regions. Fish stressed for 7 and 10 days reduced food intake, increased plasma cortisol levels, and enhanced dopaminergic and serotonergic activities in telencephalon, hypothalamus, optic tectum and hindbrain. Monoaminergic activities decreased to basal values from 2 to 6 hours after stress, which parallels decreased cortisol levels. These results suggest that brain monoamines participate in maintaining, but also in ending the neuroendocrine response to stress.

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MO28 *Comparative physiological adaptations in a changing environment*

LIFE HISTORY TRAITS AFFECT TRANSCRIPTIONAL RESPONSES TO THERMAL STRESS IN THE REEF-BUILDING CORAL *POCILLOPORA VERRUCOSA*

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Global climate changes are impacting coral reefs worldwide, with approximately 19% of reefs being permanently degraded, and 15% showing symptoms of imminent collapse. This alarming level of reef degradation is mainly due to an increase in frequency and intensity of natural and anthropogenic disturbances. Recent evidence has called into question whether corals may acclimatize or adapt to climate changes, and some groups of corals showed inherent physiological tolerance to environmental stressors. This study reports the first investigations on molecular mechanisms underlying physiological plasticity in the reef-building coral *Pocillopora verrucosa*. Corals were sampled at three locations and two depths per location (3 m and 12 m) in Bangka Island waters (North Sulawesi, Indonesia). Differences in expression levels of mRNAs encoding ATP synthase (*ATPs*), NADH dehydrogenase (*NDH*), and a 70-kDa heat shock protein (*HSP70*) evaluated under field conditions suggested an adaptation to local environmental conditions in corals collected at 3 m and at the different sites. Corals collected at 12 m showed a less pronounced site-to-site separation suggesting more homogenous environmental conditions. After a 14-day acclimatization to common and fixed temperature conditions in the laboratory (28°C; 10h:14h light:dark daily cycles), corals were subjected for 7 days to an altered thermal regime, where temperature was elevated at 31°C during the light phase and returned to 28°C during the dark phase. Results pointed out that corals collected at 12 m were more sensitive to the effects of thermal stress, so that reacted by significantly over-expressing the selected transcripts. Being continuously exposed to fluctuating environments, 3-m collected corals are more resilient to the stress stimulus, showing unaffected mRNA expressions. These results highlighted that expression levels of transcripts involved in coral stress response are modulated by natural seasonal temperature changes. Specimens living in more variable habitats exhibited higher basal *HSP70* levels, possibly enhancing physiological tolerance towards environmental stressors.

MO29 *Comparative physiological adaptations in a changing environment*

THE EFFECT OF SEASONALITY ON GLYCERONEOGENESIS IN THE HEPATOPANCREAS OF *NEOHELICE GRANULATA* SUBJECTED TO DIFFERENT DIETS AND STARVATION

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The aim of this study was to investigate the influence of seasonal and nutritional variations on the glyceroneogenesis in the hepatopancreas of *Neohelice granulata*. Male (n=80) *N. granulata* were collected in the Tramandaí Lagoon (29º58'S 50º08'W), Brazil, in winter and summer. The animals were kept in aquaria (20‰, 25ºC, natural photoperiod) and fed *ad libitum* for 15 days with an HP diet (beef) or an HC diet (boiled rice). Both the HP and HC crabs were then subjected to starvation for 5 weeks in each case (HCS and HPS groups, respectively). After this, the animals were anaesthetized on ice for 15'. PEPCK (EC 4.1.1.32) activity was analysed based on the exchange reaction between $H^{14}CO_3^-$ and oxaloacetate. The PEPCK activity was expressed as μmol $H^{14}CO_3^-$ incorporated into oxaloacetate.mg^{-1} protein.min^{-1}. To the glyceroneogenesis analysis, hepatopancreas were incubated (1 hour, 25ºC) in crab physiologic solution with 1 mM (0.2μCi) of pyruvic acid [2-^{14}C] plus 5.0 mM of pyruvate. The ^{14}C-glycerol content was quantified in a β counter. The homogeneity of data was analyzed by Levene test, followed by 2-way ANOVA and Tukey tests (significant when $p<0.05$). The concentration of ^{14}C-glycerol was higher in summer than in winter ($p<0.05$). In summer, starvation increased ($p<0.05$) the synthesis of ^{14}C-glycerol in the HPS group. PEPCK activity was higher in summer than in winter in the HP, HC and HPS groups ($p<0.05$), while in the HCS crabs it was higher in winter. Our findings demonstrate for the first time the presence of glyceroneogenesis in an adult invertebrate and reveal a marked sazonal difference in this pathway. Moreover, the nutritional status modified the glyceroneogenesis in hepatopancreas of *N. granulata*, similarly to what occurs in liver and adipose tissue of vertebrates, suggesting that the function of this pathway was maintained throughout evolution.

MO30 *Comparative physiological adaptations in a changing environment*

SEASONAL VARIATIONS IN THE METABOLIC PARAMETERS OF *CALLINECTES SAPIDUS* CRABS IN SOUTHERN BRAZIL

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The aim of this study was to obtain information about the seasonal morphometric and metabolic parameters of *C. sapidus* crabs in southern Brazil, in order to help promote the development of sustainable management and stock maintenance of the species. Male, female and young crabs (n=92) were seasonally collected from 2013 to 2015 in the Lagoa de Tramandaí estuary (-29.97, -50.15). The crabs were kept in aquaria (natural photoperiod, 20‰, 25±2°C), and fed on squid for 14 days. Levels of glucose, triglycerides, cholesterol and total proteins in the hemolymph were determined using commercial enzymatic assays (Labtest, Brazil). Levels of glycogen in the tissues were also assessed. Homogeneity was analyzed by Levene test, and the data were submitted to 2-way ANOVA or Kruskal-Wallis tests. The number of adult males collected was higher than the number of adult females in all seasons, although overall numbers were reduced in winter. Hemolymph glucose levels differed only between gender ($p<005$): they were higher in adult female than young crabs in summer, and higher in adult male than young crabs in spring. Adult female triglyceride levels were higher than in adult males in summer ($p<0.05$), and the cholesterol levels of adult males and young crabs were higher in autumn than in spring ($p<0.05$). Levels of protein were higher in autumn than in winter ($p<0.05$). Glycogen levels in the mandibular muscle were lower in females than in males in summer ($p<0.05$). Even though the crabs were kept in similar laboratory conditions, the metabolic parameters presented seasonal differences, suggesting that laboratory acclimatization does not cancel out natural seasonal variations in the metabolism of blue crabs. This indicates that, in future metabolic studies, the season in which the crabs were collected must always be taken into consideration.

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MO31 *Comparative physiological adaptations in a changing environment*

IMMUNOHISTOCHEMICAL STUDY FOR LOCALIZATION OF GLUCOSENSING MARKERS IN BRAIN OF RAINBOW TROUT

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In mammals, glucosensing markers reside in brain areas known to play an important role in the control of food intake. The most important and best characterized glucosensing mechanism is that dependent on glucokinase (GK). However, the existence of alternative glucosensing mechanisms based on LXR, SGLT-1, sweet taste receptor, and mitochondrial activity has been suggested. In fish, we previously obtained evidence in rainbow trout for the distribution of GK immunoreactive cells in brain areas such as in the preoptic area and in several hypothalamic areas. In this study, we aimed to evaluate the possible presence of glucosensing markers independent on GK in the same areas to support our previous studies demonstrating the response of parameters related to these mechanisms to changes in the levels of glucose. Fish were anesthetized, their brains fixed with 4% paraformaldehyde or 10% formalin solution and subsequently embedded in paraffin and sectioned at 7-10 µm thickness, deparaffinized and rehydrated. The sections were treated with 3% H_2O_2 to block the endogenous peroxidase activity and overnight incubated with rabbit polyclonal antibodies (anti-SGLT-1, anti-GPCR TAS1R3 and anti-LXR alpha) at room temperature. After several washes in PBS, the sections were incubated subsequently in biotinylated ant-rabbit IgG and in ABC-kit reagent. Finally, the sections were developed using 0.003% 3,3´-diaminobenzidine and 0.01% H_2O_2, and same sections were counterstained with hematoxylin. The immunochemical results obtained support our previous physiological studies demonstrating the presence of glucosensing markers based on SGLT-1, LXR and sweet taste receptor in the preoptic area and several hypothalamic nuclei of rainbow trout, and also in another areas where the glucosensing mechanism are functional.

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MO32 *Comparative physiological adaptations in a changing environment*

BIOCHEMICAL DEFENSES OF APPLE SNAIL EGGS. UNDERSTANDING THE SUCCESS OF AN UNUSUAL REPRODUCTIVE STRATEGY

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Despite of being aquatic organisms, apple snails deposit brightly colored eggs above the water surface that are ignored by most predators. Egg proteins (perivitellines) provide nutrients and defenses that play a critical role in this peculiar reproductive strategy. To understand the evolution of perivitellines that facilitate the switch from underwater to aerial egg deposition we studied from a comparative point of view the structure-function relationship of PmPV1, the most abundant perivitelline of *Pomacea maculata*. Their subunit sequences, proteolysis resistance, structural stability related to its capacity to withstand the gastrointestinal environment of a potential predator and its agglutinating activity were determined. N-terminal sequences of PmPV1 subunits allowed us to detect 4 sequences (196-203 translated residues) in the albumen gland transcriptome. Phosphorylation and glycosilation sites were predicted in all subunits. Phylogenetic analysis between PmPV1 and the ortholog PcOvo reveal a high similarity among subunits. Low similarities among PmPV1 subunits sequences indicate that gene duplication may have occurred before speciation. *In silico*, *in vitro* and *in vivo* gastrointestinal proteolysis assays, and fluorescence and absorption spectroscopy assays indicate that PmPV1 withstands the gastrointestinal environment of a potential predator: is highly resistant to protease digestion and displays high structural stability between pH 2.0–12.0. Moreover, after intragastric administration of PmPV1 to mice, it was recovered unchanged in feces, supporting an antinutritive defensive function. PmPV1 showed no protease inhibitor activity, indicating that its structural rigidity itself is responsible for its antinutritive property. PmPV1 is apparently closely related to PcOvo from *Pomacea canaliculata* as they share several similar structural and functional properties. However, no hemagglutinating activity was observed in PmPV1 whereas the ortholog PsSC from *Pomacea scalaris* displays a strong lectin activity, a property not present neither in PcOvo. Altogether, these results provide evidence that these protective carotenoproteins/perivitellins have undergone a rapid evolution in closely related species.

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CLONING AND CHARACTERIZATION OF GHRELIN O-ACYLTRANSFERASE (GOAT) IN GOLDFISH (*CARASSIUS AURATUS*)

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The brain-gut peptide hormone ghrelin is the only known protein posttranslationally modified by an acylation with a fatty acid. This modification is crucial for most of ghrelin's physiological effects and is catalyzed by the recently discovered enzyme ghrelin O-acyltransferase (GOAT). Despite the importance of ghrelin acylation, many aspects of GOAT remain unknown to date, especially in fish. Therefore, the main aims of this study were to clone the GOAT cDNA sequence and to characterize its pattern of expression in different tissues of the goldfish (*Carassius auratus*). First, the full-length DNA sequence was obtained using RT-PCR and rapid amplification of cDNA ends. This resulted in the identification of two highly homologous cDNAs of 1491 and 1413 bp, respectively, named *goat-V1* and *goat-V2*. Deduced protein sequences (393 and 367 amino acids, respectively) contain 11 and 9 transmembrane regions, respectively, and both contain the two conserved key residues proposed to be involved in catalysis: asparagine and histidine. The RT-qPCR assay of gene expression reveals that both forms of *goat* show a similar tissue distribution, with the highest expression in gonads and the gastrointestinal tract. Immunostaining of intestinal sections reveals also the presence of GOAT immunoreactive cells in the gastrointestinal mucosa, some of which colocalize with ghrelin. Finally, we characterized possible daily variations of *goat* expression in goldfish maintained under a 12h light:12h darkness photoperiod and different feeding schedules, and observed a rhythmical oscillation of mRNA transcript levels in hypothalamus, pituitary and intestinal bulb of goldfish fed during the photophase, but not during the scotophase, in all cases with the acrophase occurring at nighttime. Thus, it seems that rhythmicity of *goat* expression is conditioned by both *zeitgebers*, the photoperiod and feeding time. Together, these findings offer novel information about GOAT in goldfish, extending the knowledge of the ghrelinergic system in fish.

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TOXICITY EFFECTS OF ORAL P,P'-DICHLORODIPHENYLDICHLOROETHYLENE (DDE) EXPOSURE IN RAT LIVER

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Environmental modulators of liver chronic diseases can include nutrition, lifestyle, and exposure to toxicants such as POPs. We investigated morphological, functional and molecular changes in liver affected by both dietary fat and exposure to a low dose of DDE, the bioaccumulative metabolite of DDT. Male Wistar rats were exposed to DDE (10 mg/kg) or vehicle (corn oil) via gavage every days for 4 weeks; concurrently, animals were placed on either a low fat (10% lard, N and N+DDE rats) or high fat (45% lard, D and D+DDE rats) diet. DDE-treated rats exhibited a higher liver weight compared to untreated rats. This hepatomegaly, due to swelled, vacuolated hepatocytes, was not associated to an increase in lipid accumulation nor to body weight gain; conversely, DDE-treated rats showed a lower hepatic lipid content, probably due to the higher mitochondrial fatty acids oxidation observed in these rats. We determined the expression of glucose-regulated protein (GRP) 78, as ER-stress marker, superoxide dismutase (SOD), as ROS marker, and metallothionein (MT), as cellular stress marker. Western blot analysis demonstrated higher GRP78 expression and ROS production in D rats vs. N rats; a further increase was observed in DDE-treated rats. Interestingly, analysis of MT expression showed reverse results: the major amount of MT transcripts and proteins was found in N rats; both high-fat diet and DDE-treatment led to a decrease in MT expression. In addition, immunohistochemical analysis showed a nuclear translocation of the MT in these hepatocytes. In conclusion, we can affirm that chronic low dose of DDE did not induce obesity development in standard fed rats, neither a further increase in body weight gain in high-fat fed rats. The intake of DDE caused mitochondrial dysfunction and lipids peroxidation; the increase of substrate oxidation may be useful to cope with increased energetic demand associated with hepatic detoxification mechanism. Possibly, the MT nuclear translocation protects DNA from oxidative damage.

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EXPOSURE TO LOW DOSES OF P,P'-DDE EXERTS TESTICULAR TOXICITY IN MALE RATS

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The 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (DDE), the major metabolite of DDT, is a persistent organic pollutant and a male reproductive toxicant. Up today, mechanism of DDE-mediated reproductive toxicity remains unclear; in addition, previous studies had been performed on DDE-exposed rats via intraperitoneal injections.We investigated the effects of chronic exposure to a low dose of DDE (10 mg/kg b.w. by gavage) on rat testis, in presence or absence of a high fat diet. The simultaneous fat intake facilitates DDE assimilation and increases cell stress. For the study, morphological, physiological and molecular analyses were performed. Four groups of rats (n=8) were treated for 4 weeks: 1) standard diet (10% fat J/J, N rats); 2) standard diet plus DDE (N+DDE); 3) high-fat diet (45% fat J/J, D rats); 4) high-fat diet plus DDE (D+DDE). Light microscopy analysis demonstrated in both DDE-treated groups hypertrophy of Leydig cells and disruption of the arrangement of spermatogenic layers. The seminiferous tubules contained few spermatogenic cells; spermatogonia showed cytoplasmic vacuolization with pyknotic nuclei and sperms were scattered in the lumen of the tubules. Spermatids retention, degenerating germ cells and occlusions of the efferent ductules were also evident. Interestingly, slight alterations in tubule cells differentiation and testicular degeneration were observed also in D rats. Studies on mitochondrial efficiency showed that respiratory rates decreased in both DDE-treated groups in isolated mitochondria, thus demonstrating that testis mitochondrial functions were damaged by chronic DDE exposure. Gene expression analysis demonstrated that no significant differences in metallothionein (MT) expression were observed between the 2 diets; an appreciable decrease in MT transcripts was found only in N+DDE rats. No differences were recorded between D and D+DDE rats. Data were confirmed by MT immunolocalization. In conclusion, results demonstrate that DDE exerts testicular toxicity by acting at multiple levels interfering with tissue structure and function even when present at very low concentrations.

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RESPONSE PLASTICITY TO CADMIUM STRESS IN THE MARINE PERIWINKLE (*LITTORINA LITTOREA*)

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The plasticity of animals for adaptation to environmental stressors is paradigmatically evidenced in species of the mollusk clade of Gastropods. The metabolic processing of non-essential trace elements, for example, can be handled in gastropods by activating manifold – often coexisting – response strategies at the molecular, biochemical and cellular levels. The aim of the present work was to determine the onset of biological effects and response mechanisms induced in the marine periwinkle (*Littorina littorea*) by Cadmium (Cd) stress at increasing sub-lethal concentrations (controls, 0,25 and 1 mg Cd/l) through an exposure period of 21 days. Cd was increasingly accumulated in the midgut gland, foot and mantle of exposed individuals, depending on exposure time and the level of metal concentrations applied. Cd-related effects at the organism level (growth, mortality) along with cellular effects in midgut gland were accompanied by molecular transcription of the MT gene and MT protein expression patterns. In particular, intra-lysosomal metal accumulation and alterations of lysosomal structure were observed in midgut gland cells of metal-exposed winkles. Moreover, changes in the relative proportion of digestive and basophilic cells of the midgut gland were detected. Accumulation of metals in lysosomes occurred at very short exposure times (< 1d) for the highest Cd concentration applied, whereas changes of cellular composition at the tissue level were seen at longer exposure periods, showing that the relative proportion of basophilic cells increased with a concomitant decrease in the number of digestive cells. At the same time, the upregulation of the MT gene and of expressed MT protein was quantified. Our results show that the response of *Littorina littorea* towards Cd stress occurs simultaneously at different levels of biological organization. This may improve the overall resistance capacity of this species towards harsh environmental conditions.

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MO37 *Comparative physiological adaptations in a changing environment*

ENERGY AND CARBOHYDRATE METABOLISM STATUS OF THE WHITE SEA HERRING *CLUPEA PALLASII MARISALBI* BERG: ECOLOGICAL, AGE- AND SEX-RELATED ASPECTS

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The activity of key energy and carbohydrate metabolism enzymes (CO, LDH, MDH, aldolase, 1-GPDH) were measured in muscle, liver, gill and kidney of males and females White Sea herring (age 1+, 2+, 3+) from Onega, Dvina and Kandalaksha Bays. These bays are characterized by specific trophic, ecological and hydrological conditions. For herring groups there were shown variations in aerobic and anaerobic ATP synthesis, the carbohydrate utilization in energy metabolism and in the glycerophosphate synthesis. The determined differences in enzyme activity were more expressed in first year fish. The intensity and direction of the key metabolic reactions provide the maintenance of energy homeostasis, the choice of strategies for effective use of nutrients, regulation of the structural and storage substances synthesis, and define an adaptation strategy of each herring groups to hydrological and feeding conditions of bays. The metabolism of females from all investigated bays characterized by more high activity of CO and 1-GPDH that indicating a higher level of aerobic ATP synthesis and increasing of glycerophosphate component synthesis for structural and storage lipids. Detected features of female are demonstrated regulation mechanisms for metabolic supply of generative processes in adaption to the specific conditions of White Sea bays.

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COMPARING NATIVE AND EXOTIC OYSTER SPECIES FROM BRAZIL UNDER INCREASING SEAWATER TEMPERATURE

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Global warming is affecting aquatic ecosystems worldwide, particularly in low lying areas such as estuaries and mangroves. Since differences in species ecophysiology and tolerance limits to changing temperature may dictate competitive advantages for habitat occupation, species performance under a changing environment is therefore important to understand. In addition to environmental alterations, the introduction of exotic species may threaten native species. In aquatic ecosystems oysters are important socio economic resources, playing numerous ecosystem functions in the areas they inhabit. Hence, the objective of this study was to evaluate the biochemical responses of two oyster species currently harvested in Brazil, the mangrove oyster *Crassostrea brasiliana* (native) and the pacific oyster *Crassostrea gigas* (introduced), under different water temperatures. Juvenile and adult oysters from both species were exposed to temperatures 24, 28 and 32ºC during 28 day. Biochemical parameters analyzed included enzymatic (Catalase and Superoxide dismutase activity) and non-enzymatic (Lipid peroxidation levels, Glutathione content) markers of oxidative stress; metabolic rate (Electron transport system activity); and energy reserves (Glycogen content). Results showed different response patterns by both species to different exposure temperatures, with *C. gigas* presenting overall higher sensitivity, including high mortality rates in juveniles exposed to 32ºC. In *C. gigas* oysters, biochemical alterations induced by increasing temperature included decreasing lipid peroxidation (LPO) levels and higher superoxide dismutase (SOD) activity in juveniles, and increasing oxidized glutathione content and decreasing energy reserves (glycogen) in adults. As for the native species, *C. brasiliana* adults showed little or no variation on biochemical markers studied among tested temperatures, whereas juveniles showed increasing SOD activity and LPO levels towards the lowest tested temperature. Despite a less apparent fitness of the introduced species *C. gigas* to temperatures closer to those naturally experienced by the native species, concerns should still be risen towards the native species (*C. brasiliana*) management and protection.

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IN-SITU IMPACT OF UNDERWATER NOISE POLLUTION ON THE BEHAVIOR OF A BIVALVE MOLLUSC, THE OYSTER *CRASSOSTREA GIGAS*

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Underwater noise has become a major problem in the marine environment. High intensity seismic survey, pile driving and/or military sonar activities can induce dramatic disturbances. The impact of less intense anthropogenic noise, such as vessel noise, is more difficult to assess. We studied the sensitivity of oysters *Crassostrea gigas* to large vessel noise. Experiments were performed under laboratory conditions and in the Santander port. Our aim was first to characterize the sense of hearing in *C. gigas* by performing a study of short-term behavioral responses. Second, we studied during a few weeks the behavior of 2 groups of oysters in the Santander port in presence of nearby large vessels (distance ≈ 400 m). We used HFNI Valvometry that is based on the measurement of voltage variations produced from the electromagnetic field between two 56 mg coils of wire glued on their valves (project MolluSCAN eye: http://molluscan-eye.epoc.u-bordeaux1.fr/). One group was native from the Santander port, the other one came from a nearby bay without shipping. Our data show that *Crassostrea* can hear vessel noise in the frequency range 20-600 Hz. Their maximum sensitivity is in the range 10-100 Hz. At 100 Hz the minimal power required to elicit a transient valve closure is 129 dB re 1 µPa@1m. Above 140 dB, all oyster shut their valve simultaneously. The response vanished with time. In the field, at ≈ 400 m from the vessels, we did not record any simultaneous valve closing. The maximum intensity of the noise at oyster level was 126 dB. It is concluded that oysters can hear ship noise. This noise, at high enough intensity, has the potential to induce a transient valve closure. To conclude, we show that vessel noise is heard by oysters, possibly recognized as a negative thing (they shut their valves) and that acclimation occurs.

MO40 *How to address biological complexity: the –omics technologies*

CHANGES ON TRANSCRIPTIONAL LEVELS OF FOUR COMMON REFERENCE GENES IN FISH OVARY: ARE REFERENCE GENES SUITABLE FOR QPCR NORMALIZATION DURING OOGENESIS?

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Transcriptomics has become a common tool for the study of molecular mechanisms controlling gametogenesis and reproduction in fish. This approach has relayed on the assessment of target genes by normalizing against a single or a combination of reference genes. However, the validity of this procedure should be questioned since the transcriptional stability of well known reference genes during gametogenesis is controversial, as they fluctuate following endogenous and external stimuli. Furthermore, during gametogenesis a total transformation of the gonad tissue composition and physiology occurs thus, the assumption of a non-variable transcript is inaccurate. In this study we tested the variability of commonly used four reference genes (β-actin, elongation factor α, glyceraldehyde-3-phosphate dehydrogenase and 18S rRNA) by qPCR, during the oogenic cycle in thicklip grey mullet (*Chelon labrosus*). Total cDNA concentration was also calculated and used as reference. In addition, target genes associated with gonad steroidogenesis such as *star*, *cyp19a1a* and *cyp11b* were also studied and normalized using cDNA concentration or transcription levels ofsingle reference genes or combined genes (Geometric mean method) and analyzed by the Genorm and Normfinder tools. Results showed that *b-actin* and *elongation factor* showed a CV lower than 5% during oogenesis and thus are suitable reference genes in the ovary of mullets. Higher variability was observed for *gapdh* and *18S rRNA*. Noteworthy, *18S rRNA* transcription levels in developing and mature oocytes exceeded by far any other tested transcript levels in oocytes and should be discarded as reference gene in fish oogenesis transcriptional studies. Methods based on the combination of different gene transcription levels were the best normalizing procedures and therefore they are recommended for future studies aiming to assess transcriptomic changes during oogenesis in fish.

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APPLICATION OF DEUTERATEDANILINE AND P-(TRIMETHYLAMINO) ANILINE AS TAGGING AGENTS IN THE METABOLOMICS ANALYSIS OF SUGARS BY LC-MS

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Analysis of sugars in metabolomics studies is challenging because of the presence of various isomers in α- and β-pyranose and furanose forms respectively, which are in equilibrium with each other. The presence of several isomers leads to poor peak shapes in LC-MS andthe sugars cannot be distinguished simply based ontheir MS/MS or accurate masses. Thus it is necessary to achieve their chromatographic resolution for accurate identification. Reductive aminationat low pH offers the best approach for chromatographic separation since it causes all the four conformations of each of each sugar to form only one final product. This approach uses a weak base and a reducing agent, which is stable at the low pH required to keep the sugars in their ring open aldehydic forms, to convert the sugars into amines. The current study is evaluating the application of two aniline-basedtagging agentsD_5-aniline and p-(trimethylamino)anilinefor the derivatisation of hexoses, pentoses, disaccharides and glycoproteins in different biological fluids including urine, plasma and tissue extracts. The analysis employs ZIC-HILIC columns with 0.01% formic acid in water (A) and 0.01% formic acid in acetonitrile (B) as mobile phases with isocratic conditions at 80%, 90% and 95% of B. The derivatives of common hexoses such as glucose, galactose, fructose and mannose, and those of common pentosesincluding xylose, ribose, 2-deoxy-D-ribose, and arabinose were resolved with greatly improved peak shapes. The yield of the tagging reaction was high and D_5-aniline has so far proved to be the more effective at achieving separation of the isomeric derivatives. The method has been successfully applied in the analysis of the sugars in urine samples. Later studies will evaluatedeuteratedp-(trimethylamino)anilineand appropriate ^{13}C-labbeled internal standards to quantify these metabolites in urine, plasma and body tissues and to link them to different metabolic disease states.

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OESTROGENIC EFFECT OF 17B-ESTRADIOL ON PITUITARY GLAND PROTEOME OF EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

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In vertebrates, the neuroendocrine network that regulates the gametogenic and reproductive cycle implies steroidogenic feedback systems, directed from the gonads to the hypothalamus and pituitary, which control the synthesis and secretion of the pituitary gonadotropins. Thus, the pituitary constitutes a potential target for Estrogenic Endocrine Disrupting Chemicals (EEDCs). Moreover, non-hormonal proteins interfere in synthesis, cleavage, post-translational modifications and release of follicle-stimulating and luteinising hormone. Little is known, however, on how oestrogens regulate these non-hormonal anterior pituitary proteins. Recently the European Sea bass, *Dicentrachus labrax*, has received special attention because of a huge stock declines in Western European seas. As Sea bass populations are potentially subject to exposure to EEDCs in highly contaminated estuaries, such as that of the Seine in Normandy, France, we applied a proteomic approach to investigate the effect of 17β-oestradiol (E2) on the pituitary gland of female sea bass in order to better understand the cellular mechanisms underlying gonadotropin synthesis and secretion by exogenous oestrogens. We collected pituitary glands of adult sea bass exposed over one week to E2 or solvent control. Proteins were extracted and analysed with Off-Gel isoelectric focusing coupled to nano-LC-MS/MS. Relative quantification of alterations in the pituitary gland proteome following E2-exposure was carried out with isobaric tags (iTRAQ). Preliminary results identified 1172 pituitary proteins distributed in 18 cellular functions.

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LIPIDOME PROFILING OF PLHC-1 CELLS UNDER DIFFERENT GROWTH CONDITIONS

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Ultrahigh resolution mass spectrometry and accurate mass measurements was applied for lipid profiling of PLHC-1 cells in order to obtain basal information for further use of the fish cell line as an in-vitro model for the screening of pollutants that are likely to alter lipid metabolism in fish. Cells were routinely cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 5% FBS and then transferred for 24 h to (a) MEM-5% FBS as control, (b) MEM with 1% FBS or with no FBS; (c) Leibovitz's L15-ex medium suplemented with 0.08% galactose and 0.05% pyruvate, and (d) Dulbecco's phosphate buffered saline (DPBS) with 0.2% glucose. Flow injection was used to introduce samples into the mass spectrometer, allowing the analysis of more than 200 individual lipid species including phosphatidylcholines (PC), phosphatidylinositols (PI), phosphatidylethanolamines (PE), PE- and PC-plasmalogens, phosphatidylserines (PS) and diacyl- and triacylglycerols (DAGs, TAG), among others. The lipid profile of PLHC-1 cells under standard growing conditions (MEM-5% FBS) was enriched in PC (68%), followed by PI (6%), PC-plasmalogens (4%), TAGs (8%) and DAGs (4%). When FBS was absent or restricted to 1%, cell growth was reduced up to 21% and a relative depletion of TAGs was observed. In contrast, cells cultured in DPBS-glucose and L-15ex medium showed a significant reduction of membrane lipids (PC and PI) together with a relative increase in TAGs that represented up to 16-32% of total lipids. Interestingly, cell growth was reduced up to 56% after 24 h incubation in these mediums in comparison to MEM-5% FBS. The obtained results evidenced the ability of PLHC-1 cells to synthesize fatty acids (14:0, 16:0, 18:0) from glucose and pyruvate and to store them in specific TAGs 46:1, 48:1, 50:1, 52:1. This work provides background data on the modulation of PLHC-1 cells lipidome under different culture conditions.

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DIFFERENTIAL PROTEIN EXPRESSION PROFILE IN MUSSELS *MYTILUS GALLOPROVINCIALIS* EXPOSED TO CDTE QUANTUM DOTS AND DISSOLVED CADMIUM

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Cadmium (Cd) is a well-known toxic metal to marine organisms, but its effects at nanoscale (Cd-based quantum dots - QDs) are less known. In the nanoecotoxicological context, proteomics approaches applied on marine invertebrates are suitable tools for description of the mode of action (MoA), identification of novel or unbiased biomarkers and safety assessment of nanomaterials in aquatic environment. In this work, a proteomic approach was performed to assess differential protein expression signatures (PESs) in the digestive gland of the marine mussel *Mytilus galloprovincialis* exposed to CdTe QDs and their dissolved counterpart. Mussels were exposed to CdTe QDs (size: 6 ± 1 nm; 10 µg Cd L^{-1}) and dissolved Cd (Cd nitrate; 10 µg Cd L^{-1}) for 14 days and the PESs were analyzed by two-dimensional electrophoresis (2D-PAGE) associated to mass spectrometry and bioinformatics, along with the determination of Cd concentration in the mussel tissue. Results showed that both Cd forms induce changes in the protein expression, indicating nano-specific effects. PESs show significant differences (≥ 2 folds) in 304 protein spots between mussels exposed to both Cd forms and unexposed ones, wherein 32 and 123 proteins were specific to QDs and dissolved Cd, respectively, and 28 proteins were observed only after the exposure to both Cd form. Results indicated that the MoA and toxicity of QDs is not only due to QDs dissolution and release of ionic Cd (Cd^{2+}), but also related to its nano-specific properties. To our knowledge, this is the first proteomics study in bivalves exposed to Cd-based QDs.

TU01 *Biomarkers: present and future perspectives*

MUSSEL BIOMONITORING DURING DREDGING OPERATIONS IN THE LA SPEZIA HARBOUR, ITALY: PRELIMINARY OBSERVATIONS

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Dredging operations in harbours are recurrent to maintain accessibility and navigational depths. The main environmental risks of these operations is the remobilization of contaminants from sediments, increasing their bioavailability, as well as increases in water turbidity, that could affect respiration and filtration rates in marine biota. However, regulatory policies regarding the contamination risk of dredging chiefly apply to the disposal of dredged materials rather than the direct impact of the procedure itself. The gulf of La Spezia (Ligurian Sea, Italy) is in an intensive rearing area for aquacultured bivalves (mainly mussels and oysters). In order to assess the possible impact of dredging operations in the La Spezia harbor on mussel aquaculture, a biomonitoring plan has been developed utilizing the sentinel organism *Mytilus galloprovincialis*. A biomarker approach was applied in order in order to assess the health status of mussels grown at 3 different sites of the aquaculture plant in the La Spezia gulf in comparison with a reference site. Mussels were sampled monthly and biomarker responses were evaluated according to the two tiered approach. In Tier 1, lysosomal membrane stability and stress on stress response were evaluated as general biomarkers at the cellular and whole organism level. In Tier 2, biomarkers related to exposure to specific classes of contaminants (i.e. metallothionein and Glutathione transferase activity for heavy metals and organic contaminants, respectively), as well as immune biomarkers (phagocytosis) and antioxidant biomarkers (catalase activity) were evaluated in hemocytes and tissues (gills and digestive gland). The results do not indicate a significant impact of dredging operations on mussels' health. Moreover, biomarker data are in accordance with heavy metal and PAH loads and presence of potential pathogens in mussel tissues.

TU02 *Biomarkers: present and future perspectives*

EXPRESSION OF STRESS-INDUCED HEPATIC GENES IN *ALBURNUS ALBURNUS* (L.) FROM THE RIVER DANUBE – CHALLENGES IN ASSESSMENT OF CHEMICAL STRESS

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In the frame of FP 7 funded SOLUTION project, along with the Joint Danube Survey 3 (JDS3), liver samples of bleak (*Alburnus alburnus* (L.), Cyprinidae) were collected. The choice of the species was directed by its cosmopolitan distribution. Study objectives were: a) to link potential *in situ* effects on resident biota with pollution profiles along the river; b) to check suitability of selected species for monitoring chemical – induced *in situ* effects. The liver samples from ten JDS3 sampling sites were used for quantitative real-time PCR analysis (qRT-PCR) of selected stress-induced genes. Since *A. alburnus* is poorly defined genetically, 21 *Danio rerio* (Cyprinidae) specific primer pairs, standing for stress-induced genes, were tested using a cDNA template from *A. alburnus*. The expression of four target genes could be analyzed: extra-cellular signal regulated protein kinase 2 (*erk2)*, glutathione peroxidase 1 (*gpx1*), nuclear factor erythroid 2-related factor 2A (*nrf2a*) and membrane integral glutathione S-transferase 3a (*mgst3a*). No statistical difference was observed among the groups for any of the investigated genes, so based on these results, no sampling site could be distinguished as more- or less-polluted. Comparison with other biomarker data and results of chemical analysis will clarify if the expression of stress-induced hepatic genes in *A. alburnus* is insufficiently responsive biomarker, or similar responses at different sites are due to the high self-purification potential of the River Danube. This is the first analysis of the stress-induced hepatic genes in *A. alburnus*, so the results also represent a contribution to understanding of their basal expression.

TU03 *Biomarkers: present and future perspectives*

THE USE OF *SOLEA SOLEA* AS SENTINEL IN THE EBRE RIVER MARINE PLATFORM

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In order to assess if extractive works from a toxic load situated in an industrial plant about 90 km upstream of the Ebre river could be reflected in the health status of the fish living at the continental platform in the river mouth, 150 specimens of *Solea solea* were sampled. The samplings were carried out during spring in 3 consecutive years (2013, 2014 and 2015) nearby the river mouth (l=40.65° N, L=0.84° E) at 12 m depth. Infection parasite level (prevalence and abundance), enzymatic data and histopathological alterations were obtained from fish and used as health indicators. The biomarkers selected are part of the energy metabolism system and pollution indicators of toxic exposures, neurotoxicity, xenobiotic metabolism and oxidative stress. In addition, analyses of heavy metals were also obtained from both fish and sediments. An in vitro approach was also attempted to see the capacity of common emerging contaminants to interact with the liver microsomal detoxification system of this species. A total of 9 metazoan parasite taxa were found: two digeneans, three cestodes, one acantocephala, two nematodes and one copepod. The most prevalent and abundant parasite was the acantocephalan *Pseudorhadinorhynchus* sp. The most prevalent histopathological alterations were unspecific inflammatory foci in liver and cysts of unknown etiology (CUEs) in liver, spleen and gills. Higher levels of the cestode Tetraphyllidea gen. sp. and CUEs were observed in 2015 related with higher levels of copper in fish. An enhancement in most detoxification parameters and an elevation of the tissue damage was also observed in 2015 due to an oxidative stress situation. Also five, out of the ten chemicals tested in vitro, interacted with the CYP3A4 (BFCOD) enzymatic activity while the lipid regulators simvastatin and fenofibrate inhibited CbE activity as it occurs in higher vertebrates.

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TU04 *Biomarkers: present and future perspectives*

EFFECT OF *IN SITU* EXPOSURE HISTORY ON THE MOLECULAR RESPONSES OF FRESHWATER BIVALVE *ANODONTA ANATINA* (UNIONIDAE) TO IONIZING RADIATION

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Freshwater ecosystems are exposed to multiple stressors from anthropogenic activities including warming and pollution that can affect survival and performance of resident organisms. We investigated responses of unionid mollusks *Anodonta anatina* to ionising radiation depending on their different *in situ* history. Male specimens from pristine (F-), agricultural (A-) sites and a cooling reservoir of a nuclear power plant (N-site) were exposed for 14 days to 2 mGy X-ray radiation over the body (R-groups) or to control conditions (C-groups), and oxidative stress, geno- cyto- and neurotoxicity, and apoptosis markers were used to assess cellular injury and stress. Control group from the cooling reservoir (CN) had higher levels of caspase-3 activity in the digestive gland, higher metallothionein concentrations and nuclear lesions in the gills and low levels of superoxide dismutase (SOD) and glutathione in the gills compared to other control groups (CF and CA). Irradiation induced cellular damage in mussels from all three sites including increased level of nuclear lesions in hemocytes, depletion of caspase-3, a decrease of SOD and catalase activities, an increase of the lipid peroxidation and oxidized glutathione levels, as well as down-regulation of cholinesterase indicating neurotoxicity. The up-regulation of ethoxiresorufin-*O*-deethylase activity in the digestive gland and vitellogenin-like protein level in gonads were also found in most radiation-exposed groups. The RA-group showed the greatest magnitude of radiation-induced stress responses compared to the other two groups. Overall, unionid mollusks, particularly those from a chronically polluted agricultural site, were highly sensitive to low-dose radiation indicating insufficient stress protection and cellular repair activities.

TU05 *Biomarkers: present and future perspectives*

DEVELOPMENT OF BIOMARKER GENES FOR ENDOCRINE DISRUPTION ASSESSMENT IN THE NON-MODEL CRUSTACEAN SPECIES *GAMMARUS FOSSARUM*

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Despite the importance of invertebrates in aquatic systems, we lack specific biomarkers to assess the effects of endocrine disruption (ED) on these animals. This can be attributed to a poor knowledge of their endocrine systems and to the absence of genomic data for non-model species. The development of relevant tools for hazard assessment of ED is currently a challenge for crustacean species used in aquatic ecotoxicology. Our research teams have conducted a "proteogenomic" approach to generate a large transcriptomic and proteomic dataset for the amphipod crustacean *Gammarus fossarum*, a sentinel species used widely in aquatic ecotoxicology in Europe. Based on these "omic" resources, the aim of this study was to identify, in *G. fossarum*, some key players involved in the endocrine regulation of crustaceans/amphipods. For this, we first established a list of candidate genes known to play an essential role in hormonal systems of crustaceans and insects: nuclear hormone receptors, other regulatory genes, enzymes of the hormone metabolism and hormone-regulated genes. Using sequence similarity and phylogenetic analyses, we identified similar sequences of three candidate genes in our transcriptomic *G. fossarum* database: the nuclear receptors RXR and E75, and the regulator broad-complex. The three genes were cloned in order to obtain reliable nucleotide sequences and primers for the subsequent expression studies. Their validation as biomarkers was then performed by studying gene expression during the male and female reproductive cycles, and after laboratory exposure to model ED chemical compounds.

TU06 *Biomarkers: present and future perspectives*

CALL TO BUILD A COLLABORATIVE NETWORK FOR THE PROFILING OF FISH GONADAL RIBOSOMAL RNA

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Fish constitute the most diverse group within vertebrates involving a wide array of specialized developmental/physiological traits reflected in an ample spectrum of sex determination and differentiation mechanisms. Our group has described that an electrophoresis of total gonadal RNA allows identification of high levels of 5S rRNA present in oocytes. It diagnostically distinguishes fish ovaries, testis and intersex testis in a wide array of teleost species. 5S rRNA is the prevailing transcript in previtellogenic oocytes. As oogenesis progresses accumulation of 18S and 28S rRNA begins. Thus, a 5S/18S rRNA index has been developed that allows identifying sex and ovarian developmental stage. It also works as a quantitative method to rank intersex severity in fish exposed to xenoestrogens. The objective hereby is to create a collaborative network to extend this quantitative and objective molecular approach as a robust method to identify fish sex and female reproductive stage across different laboratory set-ups, fish species and environmental scenarios. In a Cloud computing based survey (GoogleDrive user: userfishoo, Password: UserFishO) researchers can share Agilent 2100 Bioanalyzer files used to assess their fish gonad/eggs RNA quality. File submission will not take longer than 10 minutes. Requested information will be: RNA quality files (2100 Bioanalyzer original XAD files), RNA extraction method, fish species and individual characteristics when available. Our group will calculate the 5S/18S rRNA index and correlate values to the characteristics detailed for each individual. Data coming from intersex fish individuals will be mostly welcome. It will allow establishing threshold values for intersex severity indexes in different species. This metadata will offer new research and collaboration opportunities for the study of fish sex differentiation and will open new avenues for developments and application in fish fecundity analysis.

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TU07 *Biomarkers: present and future perspectives*

A COMPREHENSIVE EVALUATION OF THE ENVIRONMENTAL HEALTH OF A HIGHLY PRESSURED COASTAL LAGOON (RAVENNA, ITALY): PHYSIOLOGICAL RESPONSES IN MARINE MUSSELS AS A BIOMONITORING TOOL

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A physiological approach was employed to evaluate the environmental quality of the Pialassa Piombone (Ravenna, Italy), a highly industrialized coastal lagoon recently subjected to dredging activities aimed at the enlargement of its harbour area. Research purposes were pursued through the analysis of a wide set of biomarkers of environmental stress in specimens of the Mediterranean mussel *Mytilus galloprovincialis* exposed *in situ* for 28 days in 7 different sites within the lagoon. Assessments encompassed structural and functional lysosomal parameters, lipid peroxidation endpoints, antioxidant responses and biomarkers of exposure to specific pollutants, such as heavy metals, neuro- and genotoxic substances. In addition, the concentration of different classes of organic (PAH, pesticide and pharmaceuticals) and inorganic (heavy metals) compounds was measured in mussel tissues to reveal their occurrence and bioavailability within the lagoon. Exposed mussels displayed a generalized destabilization of the haemocytes lysosomal membrane, which is typically associated to the onset of a stress syndrome. As well, histological alterations of the digestive gland lysosomal compartment were appreciated independently from the site of exposure, including the increase of the lysosomal volume and the intra-lysosomal accumulation of lipofuscin and neutral lipids. The activity of the antioxidant enzymes catalase and glutathione S-transferase resulted generally altered following the *in situ* exposure, suggesting the occurrence of conditions potentially detrimental for the homeostasis of exposed animals. A clear relation was observed between tissue concentrations of Al, Co, Cr, Cu, Fe, Ti and Hg and levels of metallothionein measured within the digestive gland of mussels exposed within the same sites and a similar concordance was noticeable between PAH levels and primary DNA damages. As a whole, information acquired in this study confirmed the suitability of combining physiological and chemical analysis on model organisms to provide a more reliable and predictive assessment of the environmental quality of a coastal lagoon.

TU08 *Biomarkers: present and future perspectives*

USE OF BIOMARKERS IN SUSTAINABLE AGRICULTURE: INVESTIGATING TOXICOLOGICAL IMPACT OF OLIVE MILL WASTE BEFORE AND AFTER BIOREMEDIATION PROCESS

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The use of biomarkers to support sustainable agriculture has been until now poorly investigated, while it can represent a strong tool to highlight practices with a low environmental impact. Olive oil extraction process produces a large quantity of olive mill waste (OMW) that can be re-used in agriculture in amounts defined by law. Farmers spread it on the bare soil before the planting or seeding or on perennial olive trees fields. The disposal of these byproducts represents one of the most serious problems that olive mill factories face. OMWs have a low pH and high salinity and contain high amounts of polyphenols, potentially dangerous for the environment; in fact they seem to be responsible for the phytotoxic and antimicrobial effect of these byproducts. It is therefore mandatory to develop procedures able to produce OMW with lower environmental impact. In this study we applied a biomarker approach on aquatic and terrestrial bioindicators to evaluate the efficiency of a bioremediation treatment of OMWs. Specimens of *Eisenia fetida* and *Gambusia affinis* were exposed under laboratory condition at three concentrations of olive humid husks and olive mill wastewater before and after a bioremediation treatment based on bioaugmentation with fungi strains able to metabolize polyphenols. A set of biomarkers was applied to investigate eventual neurotoxic affects, oxidative stress, and genotoxicity of treated and untreated OMWs. We found genotoxic (comet assay), and oxidative stress (lipid peroxidation) in organisms exposed to OMWs before the bioremediation treatment. A significant reduction of the toxicological effects was found in bioindicators exposed to bioremediated OMW. The biomarkers approach contributed to evaluate the environmental sustainability of the new products for their use in agriculture.

TU09 *Biomarkers: present and future perspectives*

TOWARDS A TISSUE-ARRAY TECHNOLOGY FOR THE ASSESSMENT OF CELL AND TISSUE-LEVEL BIOMARKERS IN MARINE POLLUTION BIOMONITORING PROGRAMMES

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The application of biomarkers in marine pollution monitoring programmes is recognized by international intergovernmental institutions. Although detailed conventional procedures (Standard Operating Procedures (SOPs) or consensus) are available for several biomarkers, the development of improved protocols and novel procedures to speed up measurements and to reduce their cost are major challenges at present. Amongst the currently available high-throughput technologies, the Tissue-Array (TA) responds to most of the restraints encountered in conventional procedures; it can provide quick and cost-effective approaches (frozen and paraffin embedded samples) in homogeneous conditions (e.g., quantity and intensity of staining) and uses a large number of samples. In a preceding study, TA technology was successfully applied in samples of various marine sentinel species (bivalve and fish species) for gender determination screening and several general effect biomarkers commonly used in biomonitoring programmes (histological, immunohistochemical and histochemical endpoints). Thus, the present study was designed to investigate the suitability of TA technology for determining lysosomal biomarkers (LP, Vv_{LYS}, Sv_{LYS}, S/V_{LYS}, Nv_{LYS}, Vv_{NL}) in fish liver in comparison with conventional methods. Samples were taken from previous laboratory experiments where sole (*Solea senegalensis*) were subjected to different stress conditions. Comparable trends in lysosomal biomarkers were detected using both TA and conventional methods, with differences between experimental groups being identified in both cases. These results confirm the suitability of TAs for the assessment of lysosomal biomarkers which are considered core parameters for the biological effects assessment in marine pollution monitoring programmes.

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TU10 *Biomarkers: present and future perspectives*

IMPROVED METHOD FOR HIGH-THROUGHPUT TISSUE-LEVEL BIOMARKERS IN MUSSEL DIGESTIVE GLAND THROUGH SPECIFIC LABELING OF BASOPHILIC CELLS

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For biological effects monitoring using mussels as sentinels, ICES proposed cell-type composition (Vv_{BAS}) and structural changes of digestive alveoli in digestive gland (mean luminal radius/mean epithelial thickness, MLR/MET, and connective tissue to digestive tissue ratio, CTD) as tissue-level biomarkers (quantitative histopathology). Usually, stereology is applied on hematoxylin-eosin (H&E) stained digestive gland paraffin sections in order to quantify these parameters. However, the identification of basophilic cells is sometimes unclear and to a large extent it depends on the experience of the operator. For instance, beyond certain pathological situations basophilic cells and digestive cell nuclei may be hardly distinguishable and basophilic cells may loss their basophilia. Thus, the goal of this study was to develop a specific staining (not based on their basophilic properties) to label basophilic cells in the epithelium of the digestive gland alveoli of mussels (*Mytilus galloprovincialis*). Different staining approaches were applied including, amongst others, Masson Trichrome, Azan Trichrome, Mallory Trichrome, toluidine-eosin (T&E) and H&E. The combination of toluidine-eosin (T&E) was selected due to the optimum results afforded. H&E and T&E staining approaches were compared in field and laboratory samples. In the field study, both stains produced the same high quality results although in the laboratory experiment, the identification of basophilic cells was better in the case of T&E stained sections. Besides, the background noise of nuclei was largely reduced, and therefore it was concluded that measuring Vv_{BAS} after T&E staining was easier, faster and more reliable than after H&E staining. Moreover, T&E staining also enables measuring other tissue-level biomarkers, such as MLR/MET or CTD. Consequently, T&E stain is proposed to be applied to determine tissue-level biomarkers in sentinel mussels.

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TU11 *Biomarkers: present and future perspectives*

MOLECULAR, BIOCHEMICAL AND CELLULAR RESPONSES TO ASSESS TOXICITY OF MECHANICALLY AND CHEMICALLY DISPERSED CRUDE OIL IN *MYTILUS GALLOPROVINCIALIS*

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Oil spills can seriously affect the marine environment causing toxicity through both physical smothering and carcinogenic effects of their constituents' particularly polycyclic aromatic hydrocarbons (PAHs). The severity of impact typically depends on the concentration and typology of spilled oil, environmental characteristics of the area and associated biota. The use of dispersants is often presented as a rapid technique to reduce the oil slick, by promoting its oil dispersion in the water column; however, the use of such compounds is highly controversial and debated within scientific community, for potential synergistic effects of chemical dispersants with oil, and the possible increase in PAHs bioavailability and toxicity. The aim of this study was to fill our knowledge gaps investigating early molecular and cellular effects caused by oil and dispersant co-exposure, and evaluating the recovery of these effects in the Mediterranean mussel, *Mytilus galloprovincialis,* selected as bioindicator organism. Organisms were exposed to dispersant, mechanically dispersed oil and chemically dispersed oil, and analysed parameters included bioaccumulation of PAH, and a wide array of biological analyses, to elucidate modulation, of biotransformation pathways, oxidative stress, lipid metabolism, and onset genotoxic damage at molecular, biochemical and cellular levels. Preliminary results showed a different bioavailability of PAHs in various treatments, and changes of biological parameters at molecular and biological levels.

TU12 *Biomarkers: present and future perspectives*

VITELLOGENIN EXPRESSION AND SYNTHESIS IN TISSUES OF MALE LIZARD ENVIRONMENTALLY OR EXPERIMENTALLY EXPOSED TO ESTROGENIC COMPOUNDS

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In the environment, many substances mimicking the action of endogenous estrogens interfere with the reproductive physiology of animals. Endocrine Disruptor Chemicals (EDC) have multiple origins. Pesticides used in intensive agriculture contain large amounts of chemicals with estrogenic properties, such as nonylphenol (NP). However, animal feeding operations are important sources of estrogen metabolites, introduced into the environment through manure application in organic farming. The most validated biomarker of estrogenic exposure is the expression in male liver of the vitellogenin (VTG), an estrogen-dependent glycolipophosphoprotein naturally expressed only in oviparous females' liver during the reproductive season. We investigated the presence of VTG transcript and protein in male lizards *Podarcis sicula* caught in areas devoted to organic farming, where the manure is the unique fertilizer used (environmentally-exposed sample), and in males fed with NP-polluted food or intraperitoneally treated with estradiol-17β (E2) (experimentally-exposed samples). In all exposed samples, we found appreciable amounts of VTG in livers; this result confirms the estrogenic nature of NP; data highlight also the estrogenic activity of manure. Interestingly, our findings demonstrate the expression and synthesis of VTG in testis and epididymis of all exposed lizards. To data, the extra-hepatic expression of VTG is reported only for aquatic vertebrates. Our study demonstrates the ability of testis and epididymis of this oviparous lacertid to synthesize VTG following estrogenic exposure. The testicular VTG could have a dual origin: could be synthesized from the transcripts present in the testicular cells, but the hepatic VTG, synthesized under estrogenic stimulation and released into the bloodstream, could be taken up by receptor mediated endocytosis, as naturally occurs in the ovary. The failure to detect transcripts for VTG receptors suggests that the testicular VTG derives entirely from the biosynthetic process locally activated by estrogen metabolites, NP or E2. Considering that exposure to estrogens impairs spermatogenesis, soil contamination by steroids and EDC could represent a serious risk for lizard reproductive success.

TU13 *Biomarkers: present and future perspectives*

AGE-RELATED CHANGES OF THE EXPRESSION OF METALLOTHIONEIN ISOFORMS IN RAT BRAIN

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Metallothioneins (MTs) are multigenic proteins with antioxidant, anti-inflammatory and metal-binding properties. In mammals, the MT1 and MT2 isoforms, ubiquitously present in tissues, are inducible by metals, inflammatory and stress stimuli; MT3 exists mainly in the central nervous system and its functional role and regulation are controversial. In mouse, MTs down-regulation result in a progressive neurodegeneration, leading to early aging, morbidity, and mortality. The neurodegenerative changes are attenuated in MTs over-expressing mice, suggesting the neuroprotective role of MTs in aging. These studies, however, lacks to identify the MT isoforms functionally involved. In this study, by using Real-Time PCR analysis, we examined and compared the expression of MT1/2 and MT3 genes during rat brain aging. In particular, we evaluated the MT1/2 and MT3 expression profiles in cerebral cortex and hippocampus of adolescent (2 months), adult (4 and 8 months), and aged (16 months) rats. First of all, real-time PCR confirmed that MT3 was very abundant in brain (cortex > hippocampus); however, appreciable amounts of MT1/2 transcripts were found, as well as some MT3 transcripts were detected in rat liver. Interestingly, in adult rats, both MT1/2 and MT3 transcripts were below the amounts measured in cortical and hippocampal areas of 2-months young rats. On the contrary, a huge increase in the expression of the MT3 isoform was observed in 16-months aged rats; in particular, the level of MT3 mRNA increased of 16-folds compared to the 2-months rats and of 17-folds compared to both 4- and 8-months adult rats. As regarding the MT1/2 expression in brain areas of aged rats, the amount of these transcripts slightly increased reaching the level found in 2-months rats. In conclusion, the results demonstrate an age-related expression of MT3, whereas the MT1/2 isoforms seem to play a marginal role in the brain cells aging.

TU14 *Biomarkers: present and future perspectives*

TEMPORAL AND SPATIAL VARIABILITY IN THE SCOPE FOR GROWTH (SFG) BIOMARKER IN *MYTILUS GALLOPROVINCIALIS* FROM THE SPANISH MEDITERRANEAN COAST

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The scope for growth (SFG) measured in mussels to assess the quality status of marine ecosystems has been considered an environmental monitoring technique since it is sensitive, non-specific, comprehensive and ecologically relevant. It has been described that this measurement is highly dependent on endogenous factors such as the animal condition. Moreover, mussels are highly seasonal animals. Their physiology depends, among other factors, on the annual reproductive cycle, which is regulated by two main environmental factors: temperature and food availability. Mussel condition depends on both: food availability and sexual maturity which are strongly linked. Integrated monitoring of pollution carried out by the IEO along the Spanish Mediterranean coast (Spanish Marine Pollution Monitoring Program, SMP) has evidenced important spatial differences in condition between mussel populations. Thus, there is a need to study the natural variability of biological responses used as pollution biomarkers at different seasons and in different habitats to establish an adequate link between chemical pollution and biological responses. This study aims to assess the natural variability of SFG in the mussel, *Mytilus galloprovincialis,* in 5 different sites of the Mediterranean coast selected from the SMP Program, which differ in both natural ecology and anthropogenic pressure. Mussel biological characterization from a biochemical, histological and anatomical point of view was performed. Physiological biomarkers were clearly influenced by the annual reproductive cycle, but in a particular way for each site, so that inter-site differences in SFG were dependent on the sampling time. It seems that temporal biomarker variability at each site was higher than inter-site variability at each sampling season. Therefore, SFG assay should be applied with great care in pollution studies due to the strong interaction between temporal and spatial variability.

TU15 *Biomarkers: present and future perspectives*

PHENOLOXIDASE ACTIVITY IN WILD MUSSELS (*MYTILUS GALLOPROVINCIALIS*) FROM THE SPANISH COAST: RELATIONSHIP BETWEEN SEASONALITY AND ENVIRONMENTAL QUALITY

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The implementation of the European Union's Marine Strategy Framework Directive (2008/56/EC) requires the knowledge of the biological responses of organisms to pollution. The interaction of factors such as environmental quality (presence of pollutants), mussel nutrition and mussel reproductive state may lead to the misinterpretation of the biomonitoring data. The wild mussel (*Mytilus galloprovincialis*) is the most common sentinel organism used for marine pollution assessment. In the Spanish coasts, the IEO is responsible of the Spanish Marine Pollution Monitoring Program (SMP) which comprises both, chemical analysis of pollutants in environmental compartments (water, sediment and biota) and determination of the effects of pollutants on biological systems (biomarkers). The aim of this study was to evaluate the phenoloxidase activity (PO) in wild mussel haemolymph from the Atlantic, Cantabrian and Mediterranean coasts of Spain along an annual cycle. Mussels were selected according to their pollution levels and trophic conditions. Mussel haemolymph was obtained and PO activity was analysed by spectrophotometry. The PO activity in haemolymph in this species is considered as a good pollution biomarker and that is capable of detecting differences in organisms proceeding from areas with different environmental qualities. Spatial and temporal variability was related to mussel biochemical, histological and anatomical characteristics. The results obtained evidenced seasonal variations of PO in relation to the level of pollution of the sampling sites and among sampling seasons.

TU16 *Biomarkers: present and future perspectives*

SWIMMING ACTIVITY OF D-LARVAE OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* EXPOSED TO COPPER AND S-METOLACHLOR

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France is the fourth world producer of oysters and *Crassostrea gigas* is the major cultivated species in the Arcachon bay. However, in recent years, the increased frequency of problems in oyster farming can be indicative of a change in the environmental quality of the lagoon. This work was focused on the impact of copper and S-metolachlor, two major pollutants of the Arcachon bay, on the swimming activity of *C. gigas* D-larvae. Previous studies have highlighted the embryotoxic and genotoxic effects of Cu and S-metolachlor on this species but impact on swimming behavior has not been studied yet. Swimming activity was measured using a plug in developed for the ImageJ software. Three parameters were measured (average and maximum speeds, trajectories) following a 24h-exposure to different concentrations of Cu and S-metolachlor. Larvae from adult oysters from a commercial hatchery were used to establish and validate the software (NIS Element D). Then, the swimming behavior of larvae from native oysters from the Arcachon bay was analyzed. Oyster embryos obtained by *in vitro* fecundation were exposed to 1 $\mu g.L^{-1}$ (environmental concentration) and 10 $\mu g.L^{-1}$ of Cu or 10 $ng.L^{-1}$ (environmental concentration), 100 and 1000 $ng.L^{-1}$ of S-Metolachlor. After 24h-exposure, swimming behavior of D-larvae was video tracked for 2 min at 24 °C using an inverted microscope equipped with a digital camera (Nikon Eclipse). Preliminary results in control condition, showed higher average speed and maximum speed of D-larvae from the Arcachon bay in comparison to larvae from hatchery's oysters. Rectilinear trajectories have emerged as the normal behavior of larvae. An increase of circular trajectories was observed from 10 $\mu g.L^{-1}$ of Cu or 10 $ng.L^{-1}$ of S-metolachlor. This study demonstrated for the first time that environmental concentration of S-metolachlor is sufficient to significantly modified swimming behavior of Pacific oyster D-larvae.

TU17 *Biomarkers: present and future perspectives*

COMPARATIVE ANALYSIS OF BIOMARKER RESPONSES TO ENVIRONMENTAL CONTAMINATION IN ESTUARIES: A MULTI-TAXA APPROACH

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Estuaries sustain valuable ecologic and economic resources, yet they are often subjected to various pressures derived by human activities in and around these water bodies. In this study, various biomarker responses were determined in six fish and invertebrate species (Dicentrarchus labrax, Pomatoschistus microps, Scrobicularia plana, Hediste diversicolor, Crangon crangon and Carcinus maenas), collected in two estuaries of the Portuguese coast (Ria de Aveiro and Tejo). Four estuarine sites were selected, based on previous knowledge and characterization of anthropogenic stressors from multiple sources in both estuaries (e.g. industrial, agricultural and shipping activities) and respective levels of environmental contamination. Biomarker responses analyzed across all species included the activities of superoxide dismutase (SOD), catalase (CAT), ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST), as well as lipid peroxidation (LPO) and DNA damage (DNAd). For each species, appropriate tissues were selected for biomarker analysis. Spatial variability in species responses was observed, as well as inter-specific differences in biomarker responses, which was linked to the varying degree of site contamination and species-specific behaviors and habitat use patterns. Overall, this approach provided important insights into the variability patterns of species responses to contaminants exposure in estuaries, and highlighted the difficulties associated with establishing ecological risk assessments based on multi-biomarker and multi-taxa.

TU18 *Animal replacement for an efficient environment and human health assessment*

COMPARATIVE TOXICITY, OXIDATIVE STRESS AND ENDOCRINE DISRUPTION POTENTIAL OF PLASTICIZERS IN HUMAN PLACENTAL CELLS

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Plasticizers are suspected to be toxic and/or to modulate or disrupt the endocrine system of humans and to cross the placental barrier, being embryonic and fetal development a particularly vulnerable period. This work investigates the comparative toxicity, ability to interfere with the synthesis of steroids and to generate reactive oxygen species (ROS) of a selected number of plasticizers, including bisphenol A (BPA), nonyl- (NP) and octylphenol (OP), and several phthalates (benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di(2-ethylhexyl)phthalate (DEHP) and dimethyl phthalate (DMP)) in the human placenta choriocarcinoma cell line JEG-3. Moreover, the bioavailability of chemicals in culture medium was investigated in order to relate the observed effects to experimental rather than nominal concentrations. After 24 h exposure, OP and NP showed the highest cytotoxicity (EC_{50}: 36-40 μM) followed by BPA (138-219 μM), whereas no significant toxicity was observed for phthalates. OP, NP and BPA significantly induced the generation of ROS. The apparent insensitivity of JEG-3 cells to phthalates was attributed to their very low bioavailability in culture medium. Notwithstanding, BBP and DBP significantly decreased P450 aromatase activity in JEG-3 cells at experimental concentrations in the range of 5 to 40 μM, respectively. These concentrations are well below the IC50s calculated based on nominal doses (104-166 μM). On the other hand, NP and OP (20 μM) increased aromatase activity in human placental cells, while exposure to BPA inhibited the activity (IC_{50}: 71 μM). Overall, this study (a) evidences the differential toxicity and ability to modulate aromatase activity of some of the compounds nowadays used as plasticizers, and (b) highlights the need of an accurate determination of the bioavailability of the chemicals to improve the sensitivity of in-vitro tests. The observed effects on placental P450 aromatase are of particular concern, since this activity is responsible for estrogen production and the sexual steroid balance necessary for normal embryonic and fetal development in pregnant women.

TU19 *Animal replacement for an efficient environment and human health assessment*

A HUMAN TROPHOBLAST CELL LINE AS A MODEL FOR EVALUATING POSSIBLE EFFECTS OF 217-GSM MOBILE PHONE SIGNALS ON THE PLACENTATION PROCESS

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Human exposure to radio-frequency electromagnetic fields (RF-EMF) has increased dramatically over the last few decades, mainly because of the massive development of wireless communication devices including cell phones, base stations, WiFi and Bluetooth technologies. Along with this increase, considerable concerns have been expressed about possible risks to human health. A relevant number of investigations were performed to ascertain whether RF-EMF interact with cells to induce adverse biological effects. Considering the need to deepen knowledge about the exposure of gestational tissue to RF-EMF, we decided to investigate their effects on HTR-8/SVneo cells. These cells, derived from extravillous trophoblast cells (EVT), represent a suitable model for the *in vitro* study of molecular mechanisms at the basis of placentation. The proliferation, migration and invasiveness of EVT are finely regulated and alteration of such integrated processes may also lead to early pregnancy failure. HTR-8/SVneo cells sensitivity to external stimuli make them an attractive model for investigating possible detrimental effects of RF-EMF on cell physiology and more specifically gestation processes. We presently exposed cells to 1.8 GHz continuous wave signal or to two different GSM modulation schemes, GSM-217Hz and GSM-Talk, for different periods (1, 4, 16 and 24 h) at a specific adsorption rate of 2 W/kg. After that, we assessed possible effects on Mytogen Activated Protein Kinases (MAPK) pathways by Western blotting. In particular we analysed ERK1/2 pathway, physiologically activated by growth factors, and JNK1/2 and p38MAPK pathways, addressed to as stress activated protein kinases. Our data showed that oxidative stress caused by H_2O_2 exposure (positive control) triggers the activation of MAPK signaling pathways, whereas the phosphorylation status of these proteins was not affected by the exposure to any of the three different 1.8 GHz signals.

TU20 *Animal replacement for an efficient environment and human health assessment*

AN *IN VITRO* BIOASSAY TO SCREEN FOR ENDOCRINE DISRUPTING COMPOUNDS USING FISH SCALES

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A wide range of natural and anthropogenic compounds are accumulating in the aquatic environment, many of which can interact with and disrupt the endocrine system. Estrogenic endocrine disruptors (EDCs) are a particular problem with impact on humans, ecosystems and wildlife and are particularly relevant in aquatic organisms like fish that may experience life-long exposures. The effects of EDCs in fish have mainly been assessed using reproductive endpoints and *in vivo* animal experiments. We propose that using other potential endpoints, such as the effect of estrogens on mineralized tissue, would allow development of a simple non invasive assay using scales. Fish scales are mineralized tissues that express both membrane and nuclear estrogen receptors, and are targets for natural estrogens and EDCs. The *in vitro* bioassay optimized in this work includes sampling of fish scales, incubation in culture media containing the tested compounds and measurement of enzymatic activities related to calcium turnover (TRAP, tartrate-resistant acid phosphatase and ALP, alkaline phosphatase). Several variables were optimized including culture media, compounds concentrations and incubation conditions (e.g. temperature, time), using both sea bass (*Dicentrarchus labrax*) and tilapia (*Oreochromis mossambicus*) scales. Significant effects of E_2 and EDCs were detected, including both rapid (30 minutes) or slow (1day) changes in scale TRAP or ALP activities, but the responses were of low magnitude and varied with the individual, age, time of year, species and culture conditions. The *in vitro* fish scale assay is a promising non-invasive screening tool for E_2 and EDCs effects, complying with the 3Rs of animal welfare. However, current technical limitations are its limited sensitivity for some parameters eg. TRAP/ALP activity and alternative, sensitive, robust and easy to measure endpoints are under investigation.

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TU21 *Animal replacement for an efficient environment and human health assessment*

USING PRECISION-CUT LIVER SLICES (PCLS) AND LUCIFERASE REPORTER GENE ASSAYS FOR CHARACTERIZING THE ARYL HYDROCARBON RECEPTOR 2 (AHR2) PATHWAY IN ATLANTIC COD (*GADUS MORHUA*)

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The aryl hydrocarbon receptor (AHR) is a transcription factor that regulates the expression of important enzymes involved in the biotransformation of xenobiotics, including the cytochrome P450 protein CYP1A. The main group of pollutants involved in AHR activation is polycyclic aromatic hydrocarbons (PAHs). However, also other chemicals, such as certain pesticides and dioxin-like polychlorinated biphenyls (PCBs), act as AHR agonists. Aquatic organisms, such as Atlantic cod (*Gadus morhua*), are potentially exposed to such pollutants. Atlantic cod is important to fisheries industry, and is also commonly used as a monitoring species in marine waters. Thus, increased knowledge of AHR mediated xenobiotic responses in Atlantic cod is important, and can potentially be used in extrapolation of biological effects across species. *In vivo* exposures are commonly used in the majority of aquatic toxicological studies. However, *ex vivo* and *in vitro* techniques, such as precision-cut liver slices (PCLS) and reporter gene assays allow more efficient and high throughput analyses of an increased number of compounds. In the present work, we have cloned and characterized the AHR2 receptor from Atlantic cod, and established a luciferase reporter gene assay for studying AHR2-ligand activation. Furthermore, these analyses have been complemented with exposure of PCLS to the same compounds, allowing the study of AHR2 activation and gene regulation within intact liver tissue. A variance of compounds, including 6-formylindolo(3,2-b) carbazole (FICZ), benzo(a)pyrene (BaP), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the dioxin-like 3,3',4,4',5-pentachlorobiphenyl (PCB 126), among others, were used with both the reporter gene assay and the PCLS exposure. Transcriptomics levels of AHR2 target genes, such as *cyp1a1*, were assessed by quantitative real time PCR (Q-PCR).

TU22 *Animal replacement for an efficient environment and human health assessment*

CYTOTOXICITY OF NEW GLYCEROL-DERIVATIVE SOLVENTS IN HUMAN EPIDERMAL KERATINOCYTES, (HEKN)

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Glycerol is the main by-product in industrial biodiesel synthesis, amounting to *ca*. 10% wt. of the total outcome. Due to the increasing interest in this fuel, the availability of glycerol is growing. As a consequence current glycerol supply exceeds the demand as a raw material, causing a surplus in the market. Hence, new applications of this by-product have been proposed, as its use as a source of alternative solvents. However, the complete toxicological characterization of these new solvents is necessary to ensure safety. The present work forms part of a broader project regarding the toxicological and the ecotoxicological characterization of several glycerol-derivative solvents. The aim of the present study was to evaluate the toxicity in human cells of these compounds and to determine if it is related with their lipophilicity, as we shown in previous works with other biological models. To this end, we show their cytotoxic effects in human epidermal keratinocytes, neonatal (HEKn) after an exposure of 24 hours to determine the LD_{50}. Prestoblue Assay (Invitrogen, Darmstadt, Germany) was used to analyze HEKn cell viability and proliferation ability. The obtained results have revealed significant differences according to compound lipophilicity. In line with our previous studies, the toxicity of the compounds increases with its lipophilicity. This may be due to its greater ability to pass through biological membranes reaching higher rates of bioaccumulation.

TU23 *Tissue hypoxia and oxidative stress*

EVALUATING THE THERAPEUTIC POTENTIAL OF GYPENOSIDE IN A ZEBRAFISH RETINAL DEGENERATION MODEL

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Retinal degeneration is a common clinical manifestation in various ciliopathies and is characterized by death of photoreceptor cells. Leber congenial amaurosis (LCA) is one type of inherited retinal degenerations and causes severe vision loss and often blindness. Zebrafish has been increasingly used as a model to study a variety of human diseases. We have characterized a zebrafish mutant line that carries a nonsense mutation in the *RPGRIP1* gene that is mutated in LCA patients. The *RPGRIP1* mutant zebrafish exhibited absence of rod photoreceptor segments and early retinal degeneration. It has been suggested that oxidative stress plays a major role in the death of photoreceptors. Gypenoside (GP) is an extract derived from a Chinese plant and has antioxidant properties. Prior to using GP to treat the RPGRIP1 zebrafish model, the toxicity of the drug was evaluated from 6 hours post fertilization (hpf) to 120hpf in zebrafish embryos that were treated with GP at different concentrations. Morphological abnormalities, hatching, oedema, and heart rate were assessed at 24, 48, 72, 96 and 120hpf in treated and control embryos. There was a significant decrease in heart rate and in hatching of zebrafish embryos treated with GP at 50 µg/ml but there was no significant change with GP at 25 µg/ml. The results indicated an appropriate non-toxic concentration of the drug that could be used as a potential treatment for zebrafish retinal degeneration. Consequently, RPGRIP1 mutant embryos were treated with GP at 25µg/ml and compared with an untreated control group. Histological, immunofluorescence and gene expression analysis showed that treatment resulted in a decrease in the expression of Caspase 3 leading to decreased cell death and an increase in the expression of antioxidant genes such as SOD1 and NQ-1. Our results suggest that GP has a therapeutic potential for treating RPGRIP1 mutated LCA patients.

TU24 *Tissue hypoxia and oxidative stress*

PROTECTIVE EFFECTS OF CARNOSIC ACID AGAINST ACRYLAMIDE-INDUCED TOXICITY IN ZEBRAFISH EMBRYOS

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Acrylamide (ACR) is a substance that is neurotoxic in humans. It is formed in starchy foods cooked at high temperatures above 120°C as a result of interaction between the amino acid asparagine and monosaccharides. Carnosic acid (CA) accounts for over 90% of the antioxidant properties of rosemary extract, and purified carnosic acid is a powerful inhibitor of lipid peroxidation in microsomal and liposomal systems. This study investigated the protective effects of carnosic acid against acrylamide-induced toxicity in zebrafish embryos. Zebrafish embryos at 6 hours post fertilization (hpf) were divided into five groups: Group 1 was untreated (control); Group 2 was treated with ACR (1mM); Group 3 was treated with ACR (1mM) and CA (10µM); Group 4 was treated with ACR (2mM), while Group 5 was treated with ACR (2mM) and CA (10µM). Embryos treated with ACR alone (Groups 2 and 4) showed a significant decrease in heart rate and hatching compared with embryos treated with combined ACR and CA (Groups 3 and 5). Axial malformation and oedema were observed in embryos treated with ACR alone. There was a significantly increased expression of SOD1, SOD2, CAT, NQO-1 and GCLM genes in zebrafish embryos treated with ACR and CA compared to that of embryos treated with ACR alone. There was a significant decrease in the expression of Caspase 3 gene in embryos treated with combined ACR and CA compared to that in embryos treated with ACR alone. In addition, the photoreceptor degeneration that was observed in ACR-treated embryos was reduced when the ACR treatment was combined with CA. Our study results suggest that carnosic acid has protective effects against ACR-induced toxicity in zebrafish embryos.

TU25 *Tissue hypoxia and oxidative stress*

OXIDATIVE STRESS INDUCED BY HYPOXIA CAN BE REVERSED BY HYPOTHERMIA IN RAT LIVER

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It is known that hypoxia exposure induces oxidative stress and therefore tissue damage. Recently, hypothermia has been proposed to prevent hypoxic damage because of its protective effect against reactive oxygen and nitrogen species (RONS) formation. The present work aims to study the scope of hypothermia in preventing hypoxia-induced oxidative damage in rat liver. Several hepatic markers of oxidative stress were analysed in three groups of animals (n = 8): Control group was kept in Normothermia (CN) while breathing room air, and two groups were under extreme hypoxia (breathing 10% O2), one of this in Normothermia (37ºC body temperature, HN group) and the other in Hypothermia (22ºC, HH group). The procedures were based on oxidative stress techniques: Immunoblot analysis of 4-hydroxy-nonenal (4-HNE) protein adducts, nuclear factor NF-κB, hypoxia inducing factor (HIF1α) and inducible nitric oxide synthase (iNOS) were performed in liver cell lysates and normalized to β-actin. Lipid peroxidation was determined using thiobarbituric acid reactive substances assay, and quantified as the end reactive product malondialdehyde (MDA). Nitric oxide (NO) assessment was performed by quantifying the final concentrations of its products nitrite plus nitrate. We found an increased expression of HIF1α due to hypoxia in HN and HH groups, when compared to CN. Hepatic levels of nitric oxide and the expression of iNOS were higher in HN group than HH and CN groups ($p < 0.05$). Formation of 4-HNE adducts showed higher values in HN versus CN and HH groups ($p < 0.05$), which agreed with the levels found of the lipid peroxidation end-product MDA. Our results indicate that hypoxia induced RONS, leading to lipid peroxidation in the liver, although both can be reduced by hypothermia. Our experimental findings suggest that hypothermia plays a protective role against lipid peroxidation in vivo and provide new insights into the therapeutic use of hypothermia.

TU26 *Tissue hypoxia and oxidative stress*

REDOX METABOLISM IN TWO COMMERCIALLY IMPORTANT MARINE TELEOST SPECIES EXPOSED TO DIFFERENT SALINITY AND TEMPERATURE REGIMES

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European sea-bass *Dicentrarchus labrax* and milkfish *Chanos chanos* are eurytherm and euryhaline species that have to cope with fluctuating physico-chemical parameters in their habitats. The temperate species (*D. labrax*) is generally farmed at temperatures close to 18°C and seawater (SW, 34ppt). In the wild, *D. labrax* migrates in spring to lagoons for growth where temperatures can rise sharply, notably during the summer season and salinities may also fluctuate. The subtropical species (*C. chanos*), more generally raised at 28°C, inhabits estuaries or river mouths. In the winter season, temperatures can decrease drastically and lead to high mortality rates. This comparative study analyzes individual and combined effects of salinity and temperature on the redox metabolism of these two species. Animals were maintained for 4 weeks in cold (18°C) or warm (24-28°C) conditions in SW and fresh water. After exposure time, plasma, liver and gill tissues were used to quantify reactive oxygen species formation, antioxidant enzymes (superoxide dismutase and catalase at the protein and gene expression levels) and oxidative damage (caspase activities), respectively. Through the analysis of oxidative stress biomarkers, this study shows interesting aspects regarding adaptive strategies in a temperate and tropical species facing redox imbalance as well as its relation with the energetic requirements to achieve ion homeostasis.

TU27 *Tissue hypoxia and oxidative stress*

EFFECTS OF DIETARY SEAWEED SUPPLEMENTATION ON HYPOXIA TOLERANCE AND OXIDATIVE STRESS RESPONSE IN GILTHEAD SEA BREAM (*SPARUS AURATA*)

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Intensive aquaculture practices involve rearing fish at high densities in enclosed spaces, circumstances in which they may experience hypoxic events. Seaweeds (SW) and their derived compounds when included into fish diets may increase antioxidant defenses, improving welfare. The gilthead sea bream (*Sparus aurata*) has a growing importance in the European aquaculture sector. Thus, our aim was to evaluate the effect that dietary SW supplementation has on the metabolic profile and antioxidant capacity of sea bream juveniles subjected to an acute hypoxic event and ulterior recovery to normoxia. For this purpose, fish (105±2 g) were distributed into 24 tanks (60 L, 7 fish/tank) and fed for 34 days a commercial diet supplemented with *Gracilaria sp.* (+GR, 5%) or *Ulva sp.* (+UL, 5%), or without SW supplementation (Control). Fish were subjected to acute hypoxia (4 tanks/diet, 24h, DO 1.3mg/L) in a controlled system and sampled 24h after returning to normoxia (Hypoxia/Recovery). Another group remained in normoxia during the whole trial (4 tanks/diet, DO 8.6mg/L). Hypoxia produces an increase in the O_2 carrying capacity in the fish, displayed by an increase in HCT values in all dietary groups, although the increase was most noticeable in the MC[Hb] of +GR and +UL treatments. At transcriptional level, a PCR-array of 29 selected genes in liver and heart showed differentially expressed markers related to oxidative stress, which improve oxidative capacity during normoxic and/or hypoxia/recovery conditions preferentially in those fish fed +GR diet. Such results are in accordance with the higher survival rates of fish fed +GR diet than of those fed a control diet (61±4% and 29±6%, respectively, P=0.017). Our results suggest that compounds with antioxidant properties in +GR diet may have a protective role during hypoxia. The nature of these compounds and possible mechanisms implied in the response are currently being investigated.

TU28 *Tissue hypoxia and oxidative stress*

CELLULAR SIGNALING IN EPO-STIMULATED NEUROBLASTOMA CELLS (SH-SY5Y)

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The study of cell signaling triggered by neuroprotective cytokines is reaching high interest in neurodegenerative diseases. The hormone erythropoietin (EPO) has a well-described role in the homeostatic response against hypoxia. In addition to erythroid progenitors, EPO receptor has been found in neurons and preliminary studies indicate that it plays a role in neuroprotection against brain hypoxia. The neuroprotective action of the neuronal EPO receptor would be conducted by triggering PLC/$InsP_3$ and PI3K/AKT signaling pathways. It has been described that an increase in cellular calcium level by the PLC/$InsP_3$ pathway could have a neuroprotective action. The inactivation of the proapoptotic factor FoxO3A by the PI3K/AKT system has also been associated with neuroprotection. On the other hand, the AKT kinase may indirectly increase the cGMP levels (activation of the cGMP/PKG pathway). The main purpose of this study is to explore these possibilities using SH-SY5Y cultured cells that express EPO receptor. Confluent cultures were stimulated with EPO and cells were extracted at different times. In the resulting lysates was evaluated total and phosphorylated FOXO3A, PI3K and AKT (Western Blot); cAMP, ATP, ADP, GTP and soluble phosphorylated inositols (HPLC analysis) and cGMP (Immunoassay technique). The results obtained indicate an increase in the $InsP_2$ and $InsP_3$ levels at the first minute of EPO stimulation, which suggests an activation of the PLC/$InsP_3$ pathway. It was also observed an increase in the PI3K and FoxO3A phosphorylation after 3 minutes of stimulation. However, the AKT phosphorylation remained constant during the whole stimulation. These results suggest that PI3K may contribute to the activation of the $InsP_3$ pathway and that the FoxO3A might be activated through another kinase, like ERK, instead of AKT. The absence of changes in cGMP agrees with the unaltered phosphorylated AKT levels.

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TU29 *Tissue hypoxia and oxidative stress*

ANAEROBIC TRAINING IN HYPOXIA INCREASE THE RATING OF EFFORT PERCEPTION IN ATHLETES

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This study compared subjective effort perception with objective physiological measures during high-intensive intermittent exercise performed in normoxia, moderate hypoxia (FiO2: 16.5%) and severe hypoxia (FiO2: 13.5%). Sixteen physically active subjects performed an equal training session on three different days. Training consisted of 6 "all-out" series of continuous jumps lasting for 15 seconds each. Twenty-four hours before and after training, HRV parameters (R-R, RMSDD, pNN50 and TP, LF, HF and LF/HF ratio) were measured. Average power output during the jumps was similar in all three conditions (~3200W). Greater hypoxemia was observed in hypoxia as compared to normoxia. Likewise, a significantly higher value in perceived effort was observed after hypoxia training as compared to normoxia training ($p<0.05$). Whereas blood lactate concentrations immediately after training were not different between normoxia and hypoxia, creatine kinase increased in moderate ($p=0.02$) and severe ($p<0.01$) hypoxia compared to normoxia 24h after the training. Perceived fatigue was also significantly elevated 24h after hypoxic exercise only. Heart rate variability 24h after exercise showed a tendency to sympathetic predominance when exercising in severe hypoxia as compared to moderate hypoxia and normoxia. A single session of anaerobic exercise can be executed at the same intensity in moderate/severe hypoxia as in normoxia. While several studies have shown that hypoxia and aerobic exercise modify HRV parameters, our study, involving only anaerobic metabolic pathways, showed a negligible perturbation of HRV after 24 hours of recovery in normoxia. This type of hypoxic training may be considered as a potential method to improve the ability of tolerating discomfort and consequently also exercise performance.

TU30 *Tissue hypoxia and oxidative stress*

INTERMITTENT HYPOXIA INCREASES MITOCHONDRIAL DYNAMICS AND BIOGENESIS AFTER ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE IN TRAINED RATS

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Mitochondria play a key role in (and are modulated by) exercise physiology, response to hypoxia and to cellular damage. Consequently, these phenomena could play an important role in muscle damage and recovery through mitochondrial regulation. To analyze the mitochondrial biogenesis and dynamics in eccentric-exercise induced muscle damage (EEIMD) and its recovery with intermittent hypobaric hypoxia (IHH), alone or combined with light exercise. Muscle injury was induced by downhill running to forty-eight Sprague-Dawley trained rats. They were divided into three groups: (1) Ctrl (passive recovery); (2) HYP (exposed to IHH: 4-hour session per day, at 4,000 m); and (3) EHYP (IHH + light aerobic exercise). Each group was analyzed 1, 3, 7 and 14 days after the muscle damage. The following proteins were determined by Western Blotting: PGC-1α, TFAM, TOM20 (biogenesis markers), Mfn1, OPA-1 and DRP-1 (mitochondrial dynamics markers) and Sirt3. All results were normalised to the Ctrl group. Seven days after the damage induction, both HYP and EHYP groups showed significant increased levels of PGC-1α (161 and 159%, respectively) and TOM20 (156 and 147%), while only EHYP had a significant increase of TFAM. At t14 only EHYP rats had increased levels of PGC-1α (128%), but both HYP and EHYP groups showed increased TFAM (153 and 155%) and TOM20 (145 and 144%). Sirt3 was significantly increased in HYP t03 (200%) and in the EHYP group at t07 (127%). Regarding to mitochondrial fusion proteins, HYP and EHYP rats had increased levels of OPA-1 at t07 (171% both) and t14 (136 and 128%), while Mfn1 was only increased at t14 in EHYP (158%). On the other hand, DRP-1 appeared elevated in HYP t07 (133%) and EHYP t14 (162%) groups. These differences had a p-value <0.05. After EEIMD, IHH exposure increased mitochondrial biogenesis and dynamics, as well as Sirt3 protein. When combined with light aerobic exercise, these increments were more consistent and took place in a later stage of the recovery. These results suggest that these protocols can improve the muscle damage recovery.

TU31 *Tissue hypoxia and oxidative stress*

EXERCISE TRAINING IMPOSES STRONGER IMPACT ON THE DIFFERENTIAL GENE EXPRESSION OF *PER1* AND *ARNT* THAN OF INTERMITTENT HYPOBARIC HYPOXIA EXPOSURE

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The protein coding genes *per1* and *arnt* are both associated with hypoxia via the HIF-1alpha Pathway. Evidence suggest the protein encoded by *per1* is upregulated during hypoxia modulated by Hif1a both under hypoxic and normoxic conditions, whilst one of the function of the protein encoded by *arnt* is a co-factor for the Hif1a regulation itself. With the aim to understand the combining effects of exercise and hypoxia, 42 Sprague-Dawley male rats went through a 4-weeks exercise training period ending in a specific downhill eccentric exercise training (likely to induce some muscle damage). Thereafter, 4 times over 2 weeks, the m. soleus muscle was sampled for differential gene expression, from rats with passive recovery, 4-hour intermittent hypobaric hypoxia sessions alone, and the hypoxia sessions followed by light exercise session. Our results indicate that after the relatively extensive exercise training period the necessity of some components of the HIF-1alpha pathway is reduced, reflected in a down regulation of *per1*. The intermittent hypoxia alone only reduces this down regulation slightly. The addition of light exercise with the intermittent hypoxia is reflected in even less down regulation as it is more likely to provoke or maintain a slight muscle damage. The differential expression of *arnt* has similar pattern tendency as *per1* regarding the down regulation, still, the role of exercise-related muscle damage seems more emphasised than the exercise per se. The exercise training, in the beginning, does not leave any significant mark on the expression, still, the light exercise with intermittent hypoxia is reflected in upregulation.

TU32 *Endocrine disruptors and obesogens in the aquatic environment*

EFFECTS OF VINCLOZOLIN IN THE AQUATIC GASTROPOD *PHYSA ACUTA*

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Vinclozolin (Vz) is a fungicide used in agriculture that can reach aquatic ecosystems and affect the organisms living there. Its effects have been intensively studied in vertebrates, where it acts as an antiandrogen, but there is a lack of information about its mechanistic effects on invertebrates. *Physa acuta* is a hermaphrodite snail that lives in freshwater rivers, streams, lakes, ponds, and swamps. This gastropod appears in anthropogenic reservoirs, occurring in warm water discharges from power stations and in some rivers. It can survive well under temporary harsh conditions (extreme temperature and water pollution) and eats dead plant and animal matter and other detritus. In this work, we have analyzed the response of this organism to the presence of Vinclozolin. Using comet assay and retrotranscription coupled with Real-Time PCR animals exposed to 20 and 200 μg/L of Vz were analyzed to evaluate the acute genotoxics effects and the acute alterations in gene expression that produces this compound. Comet assay results showed that this compound can produce a dose dependent DNA damage. On the other hand, the expression of three genes was analyzed. Two of them, Estrogen Related Receptor (*ERR*) and Retinoid X Receptor (*RXR*), are related with the hormonal system. The third one, the Heat Shock Protein 70 (*hsp70*), is the main heat shock protein which is known responsive to several pollutants in other organisms. The Real-Time PCR results showed that Vz can reduce the mRNA levels of *ERR* and *RXR* while no effects are observed in *hsp70* expression at 24h and concentrations used. All together these results suggest that *Physa acuta* is a suitable organism to analyze the effects of pollutants and show that Vinclozolin, a model anti-androgen in vertebrates, alters the expression of hormonal related genes at low concentrations.

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TU33 *Endocrine disruptors and obesogens in the aquatic environment*

IMPACT OF FATTY ACIDS ON PREADIPOCYTE DIFFERENTIATION AND LIPID ACCUMULATION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Adipose tissue is an essential organ involved in energy homeostasis and secretion of endocrine factors. Lipid vacuoles in adipocytes are mainly composed of fatty acids that are mostly supplied by the diet. They play important roles as bioactive compounds and as a source of energy. Adipose tissue is also the main reservoir of lipophilic pollutants, which may disrupt its functions. The impacts of fatty acids and pollutants have to be investigated jointly to better understand their interactions on adipose tissue. We used an *in vitro* approach with primary cultures of trout adipocytes to study this question. Firstly, we assessed the impact of oleic (C18:1 n-9) and eicosapentaenoic (C20:5 n-3) acids on cell differentiation and lipid accumulation. Precursor cells were isolated from trout visceral adipose tissue and grown until reaching confluence. Their differentiation was then induced with a cocktail containing insulin (1,7 µM), dexamethasone (0,25 µM), TZD (10 µM) and a lipid mixture (85 µM). After 48h, the differentiation cocktail was removed (except for the lipid mixture) and oleic or eicosapentaenoic acid was added to the culture medium at 600 µM. No cytotoxic effect was observed (LDH assay). Control cells received only the lipid mixture. First results show a higher accumulation of neutral lipids in cells enriched with oleic acid after 10 days as compared to control cells. Neutral lipids were mainly composed of oleic acid in enriched cells (80% from total fatty acids) as compared to control cells (30%). Interestingly, fatty acid profile of the phospholipids was modified in a lesser extent (25% of oleic acid in control cells and only 45% in enriched cells). Next steps will be to compare the effects of eicosapentaenoic acid on lipid accumulation and fatty acid profile as well as investigate the effects of both fatty acids on gene expression throughout the differentiation process.

TU34 *Endocrine disruptors and obesogens in the aquatic environment*

LIPID SYNTHESIS IN DIGESTIVE GLAND MICROSOMAL FRACTION OF *MYTILUS GALLOPROVINCIALIS* AND ITS MODULATION BY TRIBUTYLTIN

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The endoplasmic reticulum is involved in the metabolism of lipids and lipid-soluble compounds in eukaryotic cells, but it is also the site of synthesis for several important lipids, including cholesterol, triacylglycerols and phospholipids. This work aims at (a) assessing lipid synthesis in digestive gland microsomal fractions incubated with either 100 µM palmitoyl-CoA or 1 mM ATP & 2 mM CoA, and (b) investigating whether the organotin compound, tributyltin (TBT) interferes with this process. The use of ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-MS) allowed the detection and identification of a total of 110 lipid species, including phosphatidylcholines (PC), lyso-PC, di- and triacylglycerols (DAGs, TAGs) and cholesterol esters. When microsomal fractions were incubated with palmitoyl-CoA, an increase in specific lipids, namely TAGs 48:0 (3.5-fold) and 48:1 (1.8-fold), PC 32:0 (5-fold) and DAG 32:1 (3.5-fold), was observed, suggesting the incorporation of palmitic acid (16:0) into these molecules. However, the incubation of microsomal fractions with ATP & CoA allowed the activation of endogenous fatty acids (16:0, 18:0, 18:1, 20:5, 22:6) and their incorporated into several PCs (i.e. 36:1, 36:5, 38:7) (1.6-fold increase), DAGs (i.e. 36:5, 36:6) (4-fold) and TAGs (1.5-fold). Interestingly, the presence of 100 µM TBT in the incubation mixture lead to a significant inhibition of the incorporation of activated fatty acids into TAGs and a concomitant increase in DAGs, suggesting the specific inhibition of acyl-CoA:diacylglycerol acyltransferase by TBT. In contrast, TBT did not alter the incorporation of endogenous fatty acids into PCs. This work evidences the potential of digestive gland microsomal fractions as *in-vitro* models for the screening of environmental contaminants suspected to disrupt lipid metabolism in molluscs.

TU35 *Endocrine disruptors and obesogens in the aquatic environment*

ENDOCRINE AND CELLULAR STRESS EFFECTS OF ZINC OXIDE NANOPARTICLES AND NIFEDIPINE IN MARSH FROGS *PELOPHYLAX RIDIBUNDUS*

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Freshwater organisms including amphibians experience increasing exposures to emerging pollutants such as nanoparticles and pharmaceuticals, which can affect their fitness and performance. We studied the effects of two common pollutants extensively used in industry, pharmaceutical and personal care products, nano-zinc oxide (nZnO) and a Ca-channel blocker nifedipine (Nfd), on endocrine status and cellular stress markers in the marsh frog *Pelophylax ridibundus*. Males were exposed for 14 days to nZnO (3.1 μM), Zn^{2+} (3.1 μM, as a positive control for nZnO exposures), Nfd (10 μM), and combination of nZnO and Nfd (nZnO+Nfd). Exposure to nZnO and Zn^{2+} led to increase of Zn, Zn-bound metallothioneins (Zn-MTs) in the liver and vitellogenin in the serum, whereas exposures to Nfd and nZnO+Nfd had the opposite effect. Deiodinase activity in the liver was up-regulated by all Zn-containing exposures and serum thyrotropin level was increased by exposure to Zn, Nfd, or nZnO+Nfd. All exposures caused increase in DNA fragmentation, lipofuscin accumulation as well as caspase-3 and CYP450-dependent activities reflecting genotoxicity and oxidative damage in the liver. All exposures except for nZnO caused prominent oxidative stress response indicated by up-regulation of superoxide dismutase, increase in the glutathione and MT levels, and stimulated total and lysosomal activities of cathepsin D. This indicates that biodegradation of nZnO in the frog organism and correspondent Zn-depending endocrine effects are modulated by Nfd and demonstrates disruption of hormonal pathways and cellular damage caused by environmentally relevant exposures to nZnO and Nfd which may have implications for survival and reproduction of amphibian populations from polluted areas.

TU36 *Endocrine disruptors and obesogens in the aquatic environment*

CHARACTERIZATION OF MOLECULAR BIOMARKERS OF ZEBRAFISH 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. 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.The purpose of our study was to identify and characterize molecular biomarkers of exposure to obesogens in zebrafish, using TBT as a model compound. Thereby, we exposed zebrafish during 48 h to a range of 0-50 nM TBT at different windows of development. In addition, a chronic exposure from 5 to 30 dpf was included. Our study aims to: 1) Establish a relationship between TBT exposure and zebrafish total lipid contents; 2) Characterize the lipid profile of the zebrafish exposed to TBT; 3) Identify genetic biomarkers of 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 will identify molecular biomarkers and whole genome molecular footprint characteristics that could be applied to the study of other possible obesogenic compounds. Finally, as some obesogenic trans-generational effects are assumed to be at least partially driven by the epigenetic regulatory machinery, we propose to use zebrafish to study and to identify epigenetic biomarkers in animals exposed to different EDCs.

TU37 *Towards a sustainable aquaculture*

B_2-ADRENOCEPTOR AGONISTS EFFECTS IN GILTHEAD SEA BREAM CULTURED MYOCYTES

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In mammals, activation of β_2-adrenergic receptors in skeletal muscle increases protein synthesis and decreases degradation, leading to muscle hypertrophy. Once ligand-receptor binding occurs, G-protein dissociates into two parts, Gα and Gβγ. Gα causes the production of cAMP, which in turn activates protein kinase A (PKA). PKA triggers different processes related to growth; specifically inducing myocyte proliferation, differentiation and the gene expression of some myogenic regulatory factors (Pax, MyoD and Myf5). Gβγ activates the PI3K/AKT/target of rapamycin (TOR) signaling pathway, a key regulator of protein synthesis, cell proliferation and survival. The aim of this work was to define the signal transduction molecules dependent on the activation of β_2-adrenoceptors in gilthead sea bream skeletal muscle. A myocyte primary culture was used as a model system. Four days cultured cells were incubated with 1 μM noradrenaline or the β_2-agonists, formoterol or salmeterol. First, the activated pathways were evaluated by measuring key signaling molecules by means of HPLC or Western blot. Next, the effects on cell proliferation were analyzed by proliferating cell nuclear antigen (PCNA) immunocytochemistry and, the quantitative expression of the most important genes involved in protein synthesis, degradation, and myogenesis was also determined by qPCR. Preliminary results indicated that the signaling pathways activated through the Gα and Gβγ subunits (cAMP levels and TOR phosphorylation, respectively) are very similar between fish and mammals, being the gilthead sea bream myocytes quite sensitive to β_2-adrenergic stimulation. Furthermore, PCNA data showed that β_2-agonists stimulate significantly myocytes proliferation with respect to control cells, presenting the 3 ligands similar activation levels. Myogenic factors and signaling molecules gene expression will be also discussed. Altogether these data will help to understand the effects of β_2-adrenergic activation on fish skeletal muscle growth regulation.

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TU38 *Towards a sustainable aquaculture*

CHARACTERIZATION OF ADIPOGENESIS IN GILTHEAD SEA BREAM MESENCHYMAL STEM CELLS FROM DIFFERENT ORIGIN

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In fish, excessive fat accumulation can have negative effects affecting both, fish health and welfare and aquaculture production. Thus, to better understand the process of adipogenesis is of great importance. For this purpose, we recently established two *in vitro* models of primary mesenchymal stem cells (MSCs) from adipose tissue and vertebra of gilthead sea bream (*Sparus aurata*) with adipogenic potential. The main objective of this study was to determine the gene expression profile of primary cultured MSCs derived from both tissues during induced adipogenesis. Both, preadipocytes and bone-derived cells were capable of differentiating into adipocyte-like cells and to accumulate lipids in their cytoplasm after incubation with an adipogenic medium for 4 days. In preadipocytes, some lipid metabolism-related genes were down-regulated after 4 days, such as lipoprotein lipase (LPL), hormone sensitive lipase (HSL), peroxisome proliferator-activated receptor gamma (PPARγ), alpha (PPARα) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH2); other genes decreased later, like GAPDH1 (day 8), and fatty acid synthase (FAS) and PPARβ (day 12). On the contrary, an increase of glucose-6-phosphate dehydrogenase (G6PDH) expression was observed during preadipocyte differentiation, while liver X receptor alpha (LXRα) remained stable. Protein expression of GAPDH and PPARγ in preadipocytes was also detected, with GAPDH increasing its expression during adipogenesis. Regarding the bone-derived cells, down-regulation of some lipid metabolic genes at day 10 (FAS and G6PDH) or 15 (HSL, PPARβ and LXRα) was observed. Overall, the intracellular accumulation of lipids occurred during adipocyte maturation appeared to down-regulate most genes controlling lipid metabolism in this species. Cell lineage-specific regulation may explain the transcriptional differences detected between both MSCs cultures; an issue to be explored in future experiments. The knowledge acquired on the genes that participate in fish adipogenesis and their regulation, can help to modulate lipid accretion in fish species for aquaculture sustainability.

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TU39 *Towards a sustainable aquaculture*

GROWTH OF JUVENILE GILTHEAD SEA BREAM (*SPARUS AURATA*) PROMOTED BY LONG-ACTING RECOMBINANT BOVINE GROWTH HORMONE (GH): SEARCHING FOR OPTIMAL GROWTH CAPACITY

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Vertebrate growth implies complex interactions of genes, nutrient supply and environmental conditions under the control of growth hormone (GH). Exogenous GH increases growth rate in several fish species. To know the potential of growth improvement of gilthead sea bream under culture, juveniles of 16 g body weight were injected with prolonged-release recombinant bovine growth hormone (rbGH, Posilac®) at 6 mg/g. Fish were fed at visual satiety three times per day and sampled after 9 weeks. The GH-group grew more than controls (+24%; specific growth rate of 2.33 *vs* 1.98 of controls), with similar muscle-somatic index. Feed efficiency of GH-group increased (from 0.58 to 0.78), because feed intake was not modified. Although mesenteric fat content did not differ significantly, liver size decreased as well as its glycogen and lipid depots. In white muscle, lipid content also decreased but muscle protein content maintained whereas RNA levels increased, suggesting a higher capacity of protein synthesis. The isotopic values of muscle tissue components ($\delta^{13}C$ for lipids and glycogen; $\delta^{15}N$ for proteins) revealed higher recycling of dietary non-protein energy fuels (lipids and glycogen), and an extremely lower recycling of dietary proteins. Post-feeding plasma profile (at 10 minutes, 3 hours and 24 hours) of essentials amino acids (AAs) did not differ between groups, but non-essential AAs were higher throughout the day in GH-group. After 9 weeks of rbGH treatment, the intermediary metabolism of gilthead sea bream remodelled because several enzyme activities of cellular processes changed: higher citrate synthase and glutathione reductase, and lower alanine aminotransferase activities were found in liver of GH-group. All the variables measured corroborate that GH acted saving dietary proteins for muscle growth and promoting the use of lipids and carbohydrates as energy fuels. All these effects occurred with a similar ration through higher nutrients assimilation.

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TU40 *Towards a sustainable aquaculture*

EFFECTS OF DIETARY FISH OIL REPLACEMENT BY VEGETABLE OILS ON OXIDATIVE STRESS STATUS IN GILTHEAD SEA BREAM

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The increase of world aquaculture production and its consequent raw material demand has required the research for novel fish meal (FM) and fish oil (FO) alternatives. Vegetable meal and vegetable oil (VO) are interesting choices; however, their use may cause nutritional imbalances and negative physiological effects on lipid storage and oxidative stress. This constitutes an important basis for various liver disorders redounding the susceptibility to pathologies, lowering product growth and quality. The aim of this experimental trial was to study the effect of partial dietary FO substitution by different VO, each one with a different fatty acid (FA) profile as: palm oil (PO), rich in saturated fatty acids (SFA); rapeseed oil (RO), rich in monounsaturated fatty acids (MUFA); soybean oil (SO), rich in n-6 unsaturated fatty acids (UFA) and linseed oil (LO), rich in n-3 UFA; on oxidative stress status in liver and adipose tissue in gilthead sea bream. The results showed significant effects on redox status caused by distinct lipid accumulation and peroxidation due to dietary FA profile, which could be attributed to the ease of UFA to be oxidized and a more efficient storage for SFA in mesenteric adipose tissue. Gene expression analyses of oxidative stress-related genes did not show differences among diets; nevertheless, there was a positive correlation between superoxide dismutase relative expression and UFA/SFA ratio. In the liver, oxidative stress genes expression and antioxidant enzymes activities were also affected by dietary FA composition. Currently we are performing additional analysis of plasma parameters, oxidative stress, lipid metabolism and growth-related genes expression in other tissues to better understand the effects of these VO based diets in gilthead sea bream. The study of lipid metabolism and physiological status could help us in formulation and optimization of fish feeds.

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TU41 *Towards a sustainable aquaculture*

CHARACTERIZATION OF THE LIPID PROFILE OF THE COLD-WATER SCALLOP, *CHLAMYS ISLANDICA*, A MODEL ORGANISM OF THE ARCTIC AND SUBARCTIC SEAS

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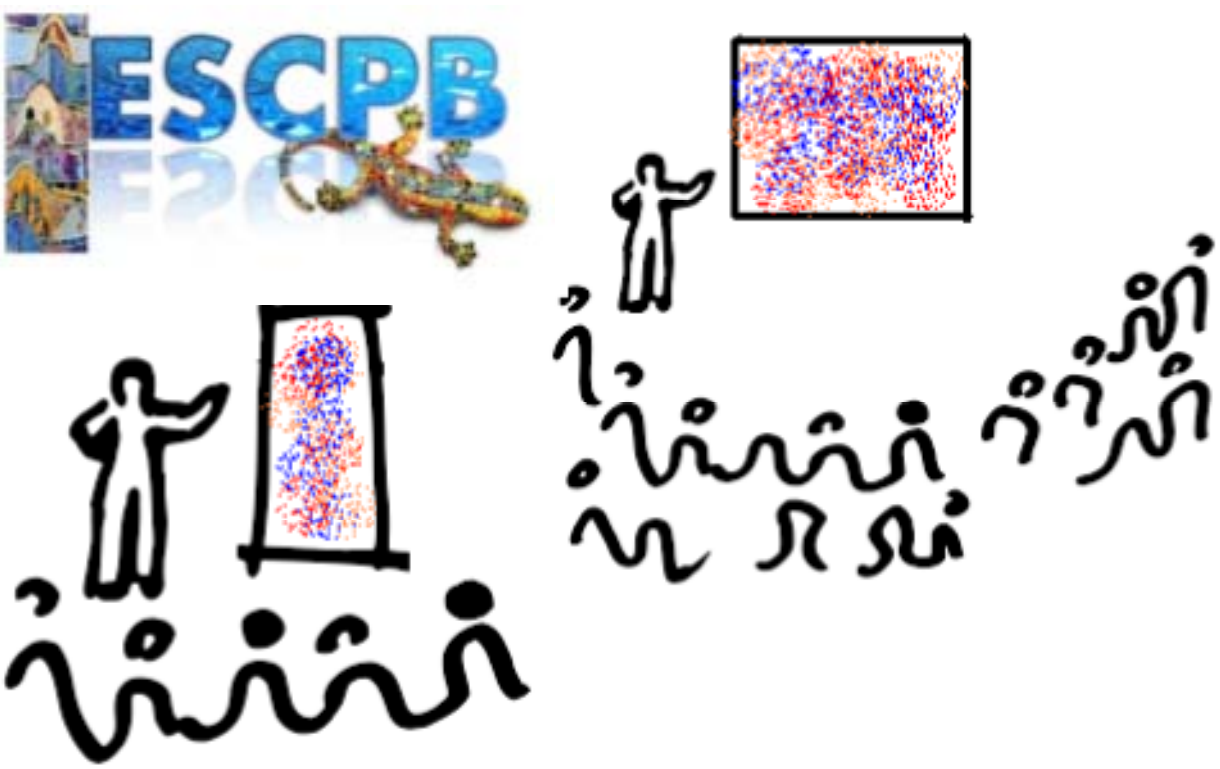
The Icelandic scallop, *Chlamys islandica*, is an abundant species inhabiting the hard seafloor bottom of the Subarctic. Although many studies have characterized the lipid profile and investigated seasonal variation of lipids in both, vertebrates and invertebrates from temperate areas, information regarding cold-water inhabitants is still poor. Therefore, this study aimed at analysing the lipid composition of the digestive gland of the Icelandic scallop collected in the Tromsø area in spring, summer and autumn. About 60 animals were analyzed using ultra-high performance liquid chromatography coupled with time of flight high-resolution mass spectrometry, UPLC-ToF/HRMS, which allowed the identification of 166 lipids by exact mass. The effect of seasonal variation was determined in six different lipid families, including phospholipids (PL) constituents of cell membranes such as phosphatidylcholines (PCs) or lyso-phosphatidylcholines (Lyso-PCs), and neutral lipids used as energy storage, such as diacylglicerols (DAGs) or triacylglicerols (TAGs). The highest accumulation of lipids in the digestive gland of scallops was observed in summer. The relative abundance of membrane lipids did not show significant changes over the sampling period, while the amount of neutral lipids (DAGs, TAGs) showed a strong seasonal variation, the highest accumulation detected in summer and autumn. The digestive gland of scallops was enriched in PCs containing 4 to 7 unsaturations, which agrees with the fact that cold-water marine invertebrates possess altered cell membranes enabling them to survive at low temperatures. Interestingly, also TAGs and DAGs were formed with fatty acids with a high degree of unsaturations. This study provides base line data for further study of the effects of pollutants on the lipidome of the Icelandic scallop, often used as a sentinel species in biomonitoring programs.



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