



SETAC Latin America 11th Biennial Meeting

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THE ROLE OF SCIENCE IN ENVIRONMENTAL DECISION-MAKING



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11th Biennial Meeting
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Abstract Book

Buenos Aires 2015

Abstract Book

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This book comprises the abstracts of the presentations for the platform and poster sessions of the Society of Environmental Toxicology and Chemistry (SETAC) Latin America 11th Biennial Meeting, conducted at the “Usina del Arte” in Buenos Aires, 7–10 September 2015. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order.

In each abstract, the presenting author’s name is underlined. The author index cross-references the corresponding abstract numbers. Affiliation, session, and keyword indices are also included.

the North shore there was a substantial number of larger plastic nurdles. In the second stage, a standardised procedure was established for extraction and quantification of MPs in two marine mussels, *Mytilus edulis* (intertidal) and *Modiolus modiolus* (subtidal) collected from the East and West coasts of Scotland. Previously used methods for extraction and quantification of MPs from marine organisms (e.g. use of strong acids or bases) are likely to damage MPs. We developed a new method based on enzymatic digestion that is relatively easy, reproducible, and provides good recovery rates of MPs from spiked tissue samples. Based on this method, processing samples of mussels collected from various field sites on the East and West coast of Scotland is ongoing and we anticipate providing information on the level of MPs contamination in mussels according their site of collection and habitat. A field campaign is underway that involves transplantation of *M. edulis* into purpose built cages and use of passive water samplers to quantify MPs presence and abundance at specific sites on the Scottish coast.

PT32. Polychlorinated Biphenyls (PCBs) in plastic pellets in beaches close to Port of Santos.

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Plastic pellets, or nubs, are small plastic granules (< 5mm) that serve as raw material for production of many manufactured plastic products. They can be composed of different polymers and present plastic additives in their composition; moreover, hydrophobic contaminants that can be adsorbed onto the pellets. Plastic pellets end up into the marine environments due to losses during the processes of production, transport and manufacturing, and in the last few years, microplastic pollution in coastal environments has become of main concern. The present study describes the spatial distribution of pellets on sandy beaches along the central portion of the São Paulo coast (Southern Brazil), a region that is under influence of the Port of Santos, and analyzes the variability in the concentration of Polychlorinated Biphenyls (PCBs). We sampled pellets in eight beaches, using a 1/4m² quadrant with 3 replications in 3 points, at the superficial sandy layer. Afterwards, pellets were classified by color and some were separated for chemical analysis. A total of 4,277 pellets were collected. A decreasing gradient of pellets concentration along with the increasing the distance from the Port of Santos ($r(\text{Pearson}) = -0.8083$). White (43%) and clear yellow (20%) pellets were predominant, representing a potential threat to the marine animals, in special the marine birds, which selectively tend to ingest pellets with these colors. The PCBs were found in all samples, in high concentrations (1471.36 to 3891.84 ng/g-pellet). Some pellets from our study presented the highest PCB concentrations in the world (>3544.75 ng/g-pellet). All the other sites presented pellets with PCBs concentrations ≥ 1471.36 ng/g-pellet, 12 times higher than the maximum value permitted by Brazilian standards for sediments on industrial areas and 49 times higher than the sea water. The pellets from the farthest beach (Itaúgaré), supposedly to be the control area, presented the highest PCB concentrations in this study, indicating that contaminated pellets area not be restricted to harbor zones. Our results show the influence of the Port of Santos as a source of contaminated pellets for the adjacent marine environments. The concentrations of PCBs in plastic pellets are surprisingly high in the studied area, and probably represent a relevant threat to the marine biota.

PT33. The potential for young citizen scientist projects: a case study of Chilean schoolchildren collecting data on marine litter and microplastics

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Citizen science is scientific research conducted by the collaboration of citizens and scientist, in order to address a wealth of scientific questions. Schoolchildren rarely participate in these projects, which is surprising considering that many citizen science projects would ideally complement modern school curricula, ranging from science, to math, reading and arts. The accumulation of debris in the marine ecosystem is a problem throughout the entire world, causing ecological impacts to marine wildlife and biodiversity. Projects that study the sources, amounts, impacts and solution of the marine debris issue have a long standing tradition in North America and Europe, but just a few projects have been made at other places of the world, such as South America and the SE Pacific. In Chile, the program Científicos de la Basura (Scientific of Marine Debris) is a citizen science project supported by schoolchildren who investigate the problem of marine litter along the Chilean coast. Schoolchildren received specially designed education materials, carefully tested instructions and sampling kits.

Wherever possible they were accompanied by trainers, who supported the teacher in supervising the sampling process. More than 3000 students and 300 teachers from all over Chile (Continental Chile from Arica to Punta Arenas, Eastern Island and Juan Fernandez Islands) have participated. These activities revealed that in Chilean beaches the average of macrodebris is 1.8 items per m², mainly plastics, cigarettes butts and glass. The microplastics abundance is 30 items per m² at the continental coast, and 800 items per m² at Eastern Island. Also, that a vast percentage of Chileans admits to have littered at beaches and considered that environmental education is the better solution for the marine debris problem. The experience of Científicos de la Basura shows that schoolchildren are enthusiastic researchers and expressed interest in participating in future environmental projects. We suggest that involving schoolchildren in citizen science projects will not only enhance the spatial and temporal scale of data collection of marine debris and microplastics, but also support school curricula, public understanding of the scientific process, and environmental management decisions.

PT34. Accumulation of PAHs and heavy metals by marine microplastics in the north of Scotland

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Microplastics (pieces of plastic measuring < 5 mm) are a widespread and often ubiquitous component of the natural environment. The relentless use and release of lightweight and durable plastics during the last century has resulted in greater environmental densities of primary and secondary microplastics, which are now a common component of marine litter. However, there is still little understanding regarding the distribution of microplastics in remote and rural regions, and their impact on biological and ecological systems. Microplastics pose a potential threat to a number of species as they can accumulate organic pollutants and heavy metals and then be consumed. We examine this potential threat by examining sites adjacent to the Pentland Firth, a sea strait between the Scottish mainland and the Orkney Islands, renowned for its very strong tides, and high flow of water, and is a designated site for imminent marine renewable devices. The Pentland Firth channels a significant proportion of water and therefore represents a major channel by which microplastics from the Atlantic may be distributed to Scandinavia and north west Europe. Consequently, we address two aspects which are: 1) examine marine environments adjacent to a high flow marine system to determine the presence, and quantify the abundance of microplastics and 2) establish and quantify the ability of plastics in the marine environment to adsorb polycyclic aromatic hydrocarbons (PAHs) and heavy metals at sites around the coast of Caithness, Sutherland, and Orkney Islands in the far north of Scotland.

Detection of Endocrine Disruptors and Other Micropollutants through Effects on Aquatic Organisms

PT35. Avances en estudios de perturbación endocrina reproductiva en peces nativos de la Orinoquia Colombiana

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Las actividades antropogénicas han conducido a un deterioro progresivo de los ecosistemas, siendo los cuerpos de agua vulnerables a la contaminación ambiental. Diversas alteraciones sobre los organismos vivos son ocasionadas por dichos contaminantes, dentro de los cuales los efectos sobre el sistema endocrino en humanos y animales ha generado gran interés debido a su efecto deletéreo sobre las poblaciones terrestres y acuáticas. Las sustancias que generan alteraciones sobre el sistema endocrino se han denominado compuestos de perturbación endocrina (EDCs). En Colombia son casi nulos los estudios de perturbación endocrina en especies nativas, por tanto uno de los intereses del grupo de investigación BioTox de la Universidad de los Llanos se fundamenta en estudiar el impacto de los EDCs sobre organismos acuáticos. Para este fin se han llevado a cabo estudios en dos especies nativas de peces *Aequidens metae* y *Astyanax gr. bimaculatus* como modelos para estudios de perturbación endocrina en la Orinoquia Colombiana. Se han conducido exposiciones durante 21 días a etinilestradiol (EE2) tanto en hembras como en machos, evaluando variables de respuesta estrogénica. De igual modo, *cyp19a1a* and *cyp19a1b* en *A. metae* se clonó en el vector P-GEM-T Easy Vector (Promega) y transformado dentro de *E.*

coli JM109 para el posterior secuenciamiento del gen de estas aromatasas. Los resultados evidenciaron diferencias en las variables de respuesta de las dos especies. En *Astyanax* gr. *bimaculatus* se observó un efecto deletéreo de EE2 en los índices gonado (IGS) y hepatosomáticos (IHS); sin embargo, en *A. metae* dicho efecto no se evidenció claramente. Los análisis histológicos han revelado alteraciones a nivel testicular y ovárico en los peces expuestos a las mayores concentraciones de EE2. A nivel molecular, se secuenció e identificó satisfactoriamente fragmentos del gen de *cyp19a1a* and *cyp19a1b* en *A. metae*. Se observó que *A. metae* y *A. gr. bimaculatus* no responden a EE2 de la misma manera. Los resultados obtenidos a la fecha se constituyen en los primeros realizados en peces nativos colombianos evaluando los efectos de sustancias xenoestrogénicas. Igualmente, estos estudios son pioneros ya que se ha logrado identificar el gen de la aromatasa en *A. metae* como biomarcador de perturbación endocrina.

PT36. Toxicity of E2 and EE2: cell damages in zebrafish male gonads and biological recover

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This study aimed to evaluate the toxicity of 17 β -estradiol (E2) and 17 α -ethinylestradiol (EE2) hormones on zebrafish adult males (*Danio rerio*) exposed to chronic tests in laboratory using concentrations present in nature (30 ng.L⁻¹, nominal concentration). The application of two treatments (powdered activated carbon- PAC, and aquatic humic substances - AHS) to remove these hormones from water were also evaluated through biological recovery tests performed using the same organisms, and histological analyzes of male gonads were used as toxicological biomarker. Twenty six fishes were exposed to each experimental condition (control, E2 and EE2) during 21 days. At 21th test day, randomly selected organisms (n=3) were euthanized for gonads removal. The remaining fishes then followed to biological recovery tests for more 7 days with test solutions treated with PAC (0.5g.L⁻¹, n=12) or AHS (20 mg L⁻¹, n=11) for two hours in shaker and, at 28th test day, the same number of organisms (n=3) were selected for gonads extraction. The gonads were submitted to fixation (Bouin liquid over 18 hours), washing (running water over 12 hours), dehydration (with 70% alcohol), and were included in paraffin. Serial sections (4 μ m) were obtained with microtome and stained with hematoxylin-eosin (HE). Qualitative analysis was conducted through a light microscopy and classified (OECD, 2010). The results were compared, before and after the period of biological recovery. Histological analysis revealed widespread cellular damage in gonadal tissue after chronic exposure to EE2, as the increased cell volume, degeneration, and sperm cell death. Furthermore, these changes were maintained irreversible after 7 days of biological recovery with both treatments. Males of control and E2 group apparently showed no visible histological changes based on analyzes conducted at the end of each assay. Biological injuries observed at the cellular level in this research highlights the cells as the primary target of environmental contaminants remained altered despite the environmental improvement. Therefore, our results warn about the impact of female sex hormones found at low concentrations and long-term exposure to aquatic organisms, and its implications for the preservation of the species in their natural environments. More investigations to find out about the potential reversibility of such injuries and an acceptable concentration for the exposed organism should be performed.

PT37. Molluscs and endocrine disruption - progress in the development of biomarkers

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With around 85,000 registered mollusc species, the Mollusca phylum is the second largest animal phylum. Despite their wide terrestrial, marine- and fresh water distribution and importance to almost all ecosystems as well as human economy (both as food source and as agricultural pests), much knowledge is still lacking in relation to their endocrinology including the endocrine control of reproduction. The main reasons for this lack of knowledge have been a general focus on vertebrate endocrinology and that molluscan endocrinology seems much less preserved between classes and species than is the case for vertebrates. It has been known for decades that many mollusc species can be affected at population level by the antifouling agent TBT (tributyltin) via endocrine mode(s) of action.

However, increasing attention has been given to effects of endocrine disrupting chemicals (EDs) on molluscs over the latest years, including initiation of development of OECD test guidelines (TG) to assess the effect of EDs in molluscs. One of the main challenges with the development of standardized tests to detect ED effects in molluscs is the lack of validated endocrine specific endpoints/biomarkers. Attempts have been made to copy biomarkers developed to detect chemicals affecting the vertebrate steroid hormone system to molluscs. One example is the estrogen dependent vertebrate yolk protein precursor vitellogenin used as a biomarker for estrogenic exposure in fish for decades and included in several OECD fish TGs: It has been proposed that yolk proteins in molluscs have the same estrogenic dependence and they have been used as biomarkers for estrogenic exposure in molluscs. This is controversial because the direct link between mollusc yolk proteins and vertebrate steroid hormones has not been verified. Further, the yolk protein quantifications have been performed with non-validated indirect alkali-labile phosphate (ALP) methods that have been outdated in vertebrate yolk protein quantification for years. The present works investigates and discuss the possible applicability of yolk proteins in molluscs as biomarkers for exposure to endocrine disrupting chemicals and especially the specific use of yolk proteins as biomarker for estrogenic exposure in molluscs. We have developed antibodies against yolk protein from three species (two bivalves and one gastropod) and present results of exposure to both vertebrate- and invertebrate types of hormones and EDs.

PT38. Cytochrome P450 induction and antiestrogenic effects caused by trace levels of contaminants in farmed rainbow trout

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Fish can be exposed to trace levels of contaminants that induce detoxification processes and endocrine disruption. These effects can be so subtle that no external damage is observed in fish. However, essential functions as growth, sexual development or reproduction can be compromised. Exposure to these contaminants can be evidenced by measuring the induction of detoxification related cytochromes. For instance, cytochrome P4501A (CYP1A) is induced by polycyclic aromatic hydrocarbons and other related chemicals. On its hand, CYP3A is activated by a wide variety of substances including a number of pharmaceuticals. The main objective of this work was to determine if farmed rainbow trout (*Oncorhynchus mykiss*) were really affected by trace levels of contaminants and to characterize the damage provoked on animals. For that, three different approaches were used simultaneously: biomarker measurement, chemical analysis, and active biomonitoring (ABM) transferring the animals to clean waters. Regular samplings were performed all along three years in several fish farms and the induction of CYP1A and CYP3A was studied at the transcriptional and enzyme (by measuring CYP1A and CYP3A dependent EROD and BFCOD activities, respectively) levels. Strong inductions of CYPs, showing actual exposure to contaminants, were observed at different dates in two fish farms. Extracts from sediments taken from the water entry channels of fish farms also activated EROD activity in a rainbow trout cell line maintained in vitro, corroborating the presence of contaminants in these sediments. In ABM experiments, CYP1A and CYP3A inductions disappeared after some days of fish being in clean waters, confirming the presence of chemicals in waters. In sediments, some anthropogenic chemicals were detected. However, they were not able to induce enzyme activities in the in vitro experiments, indicating that other substances, different from those detected by chemical analysis were responsible of the observed effects. These results suggest a real exposure of fish to very low concentrations of mixtures of pollutants whose interactions is possibly provoking the reported inductions. Acknowledgements: this work was financially supported by INIA project RTA2012-00053-00-00

PT39. Altered endocrine signalling in freshwater fish and amphibian model organisms in response to crude oil exposure

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