



Abstracts from Fourteenth International Symposium on Pollutant Responses in Marine Organisms (PRIMO 14) – Methodological approaches in environmental research

The flux of stuff: Developing an inexpensive and accurate method to track dispersion of environmental contaminants in aquatic ecosystems

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Abstract

For decades, aquatic toxicologists and environmental scientists have been frustrated in accurately measuring, across a range of scales, the dispersion of contaminants, effluent plumes, pollution gradients, suspended particulates, sediments, biological propagules, natural and manmade tracers. Of particular concern for aquatic toxicologist is the necessity to establish contaminant exposure levels at a given field site from water-borne effluent of interest, whether it be pulp mill and other industrial effluents, municipal sewage, mine acid drainage, produced waters from offshore oil and gas exploration, etc. Conventional technologies include instrumented drifters, current meters, various dyes and chemical tracers, and a plethora of numerical models. These technologies suffer prohibitive expense, compromised time and space resolution, and the paucity of numerical-model validation. We present concepts, rationale and field-trial results of an inexpensive magnetically-attractive particle and magnetic particle-collector system (patent pending) for measuring dispersion. The system is based on specially designed magnetically-attractive non-toxic particles that incorporate user-designed specific gravity, size and shape (to mimic study propagules) and markings (for multiple release purposes) used to measure dispersion via the autonomous magnetic particle-collector array. The system has the rare ability to time-integrate particle dispersion at scales of hours to months and meters to thousands of kilometers² without using power or electronics. In addition to applications in aquatic toxicology mapping effluent plumes, sewer systems, etc., this technology can be used to examine erosion and sedimentation in watersheds, larval dispersion, alien species invasion, population/species connectivity, aquaculture settings, and each with the added advantage of dispersion model development and validation.

Dioxin-induced protein-protein interactions between the aryl hydrocarbon receptor and the aryl hydrocarbon receptor nuclear translocator from Atlantic salmon (*Salmo salar*)

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Abstract

Dioxins are a group of planar, aromatic compounds with great affinity for the aryl hydrocarbon receptor (AhR), a ligand-activated cytosolic receptor found in all vertebrates. The dioxin-activated AhR heterodimerize with the aryl hydrocarbon receptor nuclear translocator (ARNT), and this complex induces transcription of specific genes, e.g. cytochrome P450 1A (CYP1A) through binding to specific DNA elements. The goal of this study has been to better understand the function of this system in Atlantic salmon (*Salmo salar*), since fish in general and salmonids in particular are highly sensitive to dioxin toxicity. Salmon ARNT cDNA was cloned and transfected into an S2 insect cell system for stable expression, and purified from cell lysates using magnetic beads. Salmon AhR cDNA was cloned and expressed as a GFP-fusion protein in COS-7 cells. In addition, salmon liver cytosol was used as a source for AhR. AhR-ARNT interactions were studied in the presence or absence of ligand (e.g. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD, or beta-naphthoflavone, BNF) in both *in vitro* and in transfected cell systems. Confocal imaging was used to determine the location of AhR-ARNT complexes in COS-7 cells incubated with different concentrations and types of ligand. Salmon ARNT immobilized on magnetic beads was used in pull-down assays to investigate AhR-ARNT complex formation upon ligand exposure. Salmon ARNT was stably expressed in S2 cells, inducible through the metallothionein promoter, and could be harvested through an inserted His-tag or a CYP1A epitope with the use of peptide-specific monoclonal antibodies. SDS-PAGE and western blotting was used to reveal the nature of the complexes formed after ligand activation. *Support:* Norwegian Research Council and Biosense Laboratory.

Esterase activity as endpoint in benthic marine microalgae assays: A comparison with standard growth inhibition and chlorophyll fluorescence

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Abstract

This study assessed the response of *Cylindrotheca closterium* (Ehremberg) Lewin and Reimann to copper, comparing esterase activity as endpoint with standard growth-inhibition bioassays related to biomass (cellular density evolution as endpoint) and chlorophyll fluorescence (excitation at 485 nm, emission at 680 nm) as biomass biomarker. Microalgae populations were exposed to selected copper concentrations for 72 hours in flasks or multi-well plates (in the latter, volumes of 200 and 300 µL were compared). Four bioassays were performed for each technique. Each bioassay was also performed in quadruplicate. The effective concentration which inhibits diatom population growth in flasks to 50% in 72 h (EC50%72 h) for copper was 7.35 (±0.42) and 7.79 (±1.45) µg L⁻¹ when esterase activity was the endpoint (for 200 µL and 300 µL aliquots, respectively). This parameter was 29.57 (±0.29) and 27.82 (±0.74) µg L⁻¹ for bioassays completely carried out in microplates, taking chlorophyll fluorescence as endpoint (200 and 300 µL volume, respectively). Cellular counting by microscopy (standard bioassay on flasks) resulted in an EC50% of 10.08 (±0.162) µg L⁻¹. Chlorophyll fluorescence measurement of aliquots from these flasks resulted in EC50%72 h values of 12.85 (±6.17) and 10.16 (±5.89) mg L⁻¹ (for 200 and 300 µL aliquots, respectively). Differences between flask and microplate exposure bioassays can be explained by the differential volume/surface ratio of the vessels (and thus different metal adsorption capacity of the vessel walls). Differences between direct cellular counts and microplate measurements for the same flasks can be due to different chlorophyll content of exposed cells. *This work has been funded by the project MECASEC (CTM2006-01473/MAR), from the National Spanish Plan for Scientific and Technique Research. First author has been granted by the CAPES foundation (Coordination of Improvement for High Level Personnel from the Science and Education Ministry of Brazil).*

Field testing of a fish bioconcentration model used in assessing the risks posed by pharmaceutical residues in aquatic environments

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Abstract

A theoretical model has been proposed by Pfizer to assess the probability of biological effects of pharmaceutical residues in fish. In the model, bioconcentration in fish is calculated using a quantity structure-property relationship for hydrophobic organic pollutants. We have field tested the model by exposing rainbow trout to pharmaceutical residues in sewage effluents at three sites. Measurements of the pharmaceuticals in the effluent and fish blood

plasma was conducted via GC/MS. Measured and modelled plasma values agreed within several orders of magnitude indicating the potential usefulness of the model but that further testing and refinement is necessary. Strategies to do this will be outlined.

Metallothionein quantification in the polyp and zooxanthellae of the coral *Porites astreoides*: A new spectrophotometric technique

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Abstract

A novel procedure based on the typical spectral characteristics of metallothionein (MT) was developed to determine its concentration in cnidaria. This method was applied to obtain the MT concentration in the polyp and zooxanthellae (compartments) of the coral *Porites astreoides*, exposed to 0 (control), 0.01 and 0.10 mg/l of mercury (Hg), during 72 h. A calibration curve was prepared with standard MT-I from rabbit liver, after evaluating the apoMT-I and HgMT-I conditions, using the maximum absorbance of Hg bound at 260 nm. The calibration curve obtained with standard MT-I was adjusted to a linear regression ($r=0.9788$) and the detection limit was 0.2 ngMT/100 µl. The heat-treated cytosolic fraction of both compartments was treated in the same conditions to determine the MT content. Additionally, the thiol groups were determined using Ellman reaction. In zooxanthellae, the MT concentrations increased at 0.10 mgHg/l of exposure and were correlated with bound-proteins thiols ($r=0.943$, $p=0.000$). In the polyp, the mercury exposure did not produce any changes in the thiols and MT concentrations. An electrophoretic SDS-PAGE - fluorimetric assay of the heat-stable proteins was made with the fluorescent reagent thiolite monobromobimane to observe proteins rich in -SH groups. The fluorimetric assay of the heat-stable proteins showed a distinctive band of 6.5 kDa rich in -SH groups, in both coral compartments. This is the first report of MT quantification in the scleractinian coral *P. astreoides* using a novel spectrophotometric technique.

Methods for biomonitoring of DDT in the Amazon environment

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Abstract

Until the end of the 90s, the defense against mosquitoes was indoor spraying of the organochlorine insecticide DDT. In spite of the huge efforts made in the past, the overall morbidity from malaria in the Brazilian Amazon is still very high. This work documents the 10-year effort in sampling and analyzing of DDT and its metabolites in hundreds of biotic (mainly fish and human breast milk) as well as abiotic samples (fluvial sediments, forest and urban soils). The con-

ventional approach using Soxhlet extraction as well as alternative extraction devices tested, including new techniques for clean-up of samples using SEP-PAK cartridges, are described. There is clear evidence that the indoor use of DDT resulted in unexpected environmental contamination. The levels in fish seem to be decreasing but the presence of DDT and its main metabolites in breast milk is very high in some of the riverside villages of the Madeira river basin. *This work was partially supported by CNPq-Prosul, CNPq-PPG7 and Fogarty International Center of the US-NIH (Grant No.: 1D43TW00640 of the Mount Sinai School of Medicine).*

PAH metabolites in urine of crab *Ucides cordatus* by HPLC/fluorescence detection

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Abstract

Mangroves are tropical ecosystems of great ecological and economical importance and are often impacted by human activities. Crabs *Ucides cordatus* are potential bioindicators of pollution in these ecosystems, because of their large distribution (Florida, USA to Santa Catarina State, Brazil), robustness and human consumption. In this study, a rapid screening method for polycyclic aromatic hydrocarbons was developed for crab urine for assessing exposure to PAHs in the aquatic environment. Also, the biotransformation of pyrene and phenanthrene was investigated by exposure of crabs to these compounds in the laboratory. The PAH metabolites were qualitatively analyzed by HPLC (LC-10AD Shimadzu) using fluorescence detection (RF-10AXL Shimadzu). The analyses were carried out in reverse phase with a solvent gradient from 1:8.5 (methanol:water) to 100% of methanol in 15 min; this condition was kept over 20 min with constant flow of 1 ml min⁻¹; the wave lengths in the fluorescence detector were adjusted at $\lambda_{ex}=341$ and $\lambda_{em}=383$ nm for pyrene, and for phenanthrene at $\lambda_{ex}=233$ and $\lambda_{em}=383$ nm. The present study proved that *U. cordatus* exposed to pyrene and phenanthrene assimilates and metabolizes these PAHs, excreting the products in the urine. These metabolites are detected using HPLC/F and they are correlated with the concentrations of PAHs in the environment. This technique presented several

advantages as it is sensitive (DL = 0.01 $\mu\text{g mL}^{-1}$), non-destructive, rapid and of low cost.

Sex determination methods in marine mussels

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Abstract

Ecotoxicology and environmental monitoring strategies involving sentinel organisms are increasingly utilising omic technologies. Previous studies have shown that in order to effectively interpret information acquired from such techniques, knowledge of the organism's genotype and phenotypic factors such as gender are essential. Marine mussels are one such organism extensively involved in biomonitoring. Their increasing use in ecotoxicogenomics necessitates the development of a technique that can 'phenotypically anchor' the gender in *Mytilus* spp. which can be used all year round. This study examines four different methods of sex determination for *Mytilus edulis*, *Mytilus galloprovincialis* and a viable hybrid species. Methods include histology (the 'gold standard'), a lipid-based technique (J. Exp. Mar. Biol. Ecol. 107(1): p.39–44, 1987), a genetic method involving RT-PCR, and NMR-based metabolomics. Evaluation of each technique was initially conducted using sexually ripe mussels sampled from April, May and June which were distinguishable as male or female by histology. Next, to test that each method could be used throughout the year, spent *M. edulis* from September that could not be distinguished by histology were investigated. The results showed that for sexually ripe animals, histology and RT-PCR were the most accurate techniques followed closely by metabolomics. However, spent animals that could not be discriminated by histology were able to be classified using RT-PCR and metabolomics. In addition, RT-PCR, metabolomics and the lipid-based method appeared to detect animals of 'intermediate' sex that were undetected by histology. Our findings therefore highlight the application of both RT-PCR and metabolomics as novel, robust and sensitive techniques for the year round sexual discrimination of marine mussels. *This work was funded by the Natural Environment Research Council (NERC) and supported by Cefas.*