

health risks, since can be carcinogenic, mutagenic and/or teratogenic. Upon entering the cell, its metabolism occurs through the activation of biotransformation reactions. In order to evaluate the molecular responses and biotransformation enzymes in Oysters *Crassostrea brasiliana* were exposed to two concentrations of pyrene (50 and 100 mg. L<sup>-1</sup>) and fluorine (100 and 200 mg. L<sup>-1</sup>), for 12 and 96 h. Half-life of these compounds in water were quantified by fluorescence and chemical analyses were carried out to check the concentration of the PAHs in the soft tissue of oysters. Transcription levels of genes of biotransformation of phase I (*CYP1-like*; *CYP2-like*; *CYP2A1* and *CYP356A1-like*) and phase II (*GST W-like*; *Gstm-like* and *SULT-like*) and EROD activity, GST and GSTm were evaluated in the gills. Both PAHs were bioaccumulated by oysters. The half-life of the pyrene in water was (100 mg.L<sup>-1</sup> = 2 h and 12 min), lower than that of fluorine (100 mg.L<sup>-1</sup> = 5h and 54 min). This may be related to greater lipophilicity of pyrene, facilitating your entry in the intracellular medium through the plasma membrane. After exposure to fluorine, there was only an increase in the level of gene transcripts in *CYP2A1* (200 mg.L<sup>-1</sup>, 96 h). In oysters exposed to pyrene, there were increased levels of transcripts of *CYP2A1* (24 and 96 h); *GST W-like* (24 and 96 h) and *SULT-like* (24h) in 50 mg.L<sup>-1</sup> and in all genes assessed in 100 mg.L<sup>-1</sup> 24h exposed group. In addition there was an increase of EROD activity and GSTm (96 h), suggesting a significant participation of enzymes and genes related to metabolism of biotransformation of phases I and II of the pyrene. The results contribute in the search for biomarkers of contamination by PAHs in *C. brasiliana* and show a possible participation of these genes and enzymes in the metabolism of biotransformation of pyrene. In addition, suggest the participation of the *CYP2A1* gene in the PAHs Biotransformation in gills of *C. brasiliana*.

#### 95 The transcriptome of the brown mussel *Perna perna* when exposed to anthracene

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The brown mussel *Perna perna* (Linnaeus, 1758) helps the monitoring of chemical compounds in marine ecosystems. However its molecular mechanisms of detoxification and stress response remain unclear. Elucidating these mechanisms is crucial to understand the toxic effects of chemical pollutants and to develop biomarkers to assess marine ecosystems. In this study, *P. perna* individuals were exposed to anthracene (ANT) and its mRNA complement was sampled sequenced with Illumina technology. Chemical analysis of the soft tissue identified ANT concentrations 268 - 715 fold higher in the exposed group compared to controls, demonstrating that the exposure procedure was successfully accomplished. Transcriptome sequencing of *P. perna* generated 273.152.390 paired reads that were assembled in 231.728 contigs of average length 720 bp and N50 1083 bp, which 66.541 contigs (28,7%) could be annotated using GenBank genes, Pfam domains, Gene Ontology (GO) terms and KEGG pathways. The terms "oxidation-reduction process" and "binding" were the most abundant terms in biological process and molecular functions GO categories, respectively. In KEGG pathways, "Signal transduction" in "Environmental Information Processing" was the pathway with the most number of predicted proteins assigned. It was possible to identify transcripts similar to genes related with biotransformation reactions of phases I, II and III, including CYPs and GSTs. Transcripts similar to CYPs and GSTs isoforms were highly expressed in the group exposed to ANT, however no CYP, GST, or even other genes related with biotransformation reactions were classified as differentially expressed. On the other hand, several hypothetical genes were differentially expressed, which suggests that *P. perna* uses unknown mechanisms of biotransformation to deal with ANT stress contamination. Immune related-genes were both up and down-regulated, as was also observed for *Perna viridis* exposed to benzo(a)pyrene, suggesting that ANT promotes alteration in the immune response of *P. perna*. A qPCR validation is being carried out to verify results here described.

#### 96 Cytogenotoxic effects of a cocaine byproduct (crack cocaine) to marine mussel *Perna perna*.

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The cocaine and its byproducts have grown into epidemics in metropolitan regions, becoming a social and environmental problem in Brazil. Their occurrences have been reported in domestic effluents, rivers and coastal waters. Our study aims to evaluate the cytogenotoxicity of the crack cocaine in the marine mussel *Perna perna*, considered a sentinel organism in programs of environmental monitoring. Adult organisms (n=160) were exposed to two controls (marine water and DMSO 0.001% v/v) and to three concentrations of crack cocaine: 0.5 µg.L<sup>-1</sup> (environmental concentration), 5 µg.L<sup>-1</sup> and 50 µg.L<sup>-1</sup>, for seven days. Gills and digestive glands were collected after 48, 96 and 168 hours of exposure. Biochemical responses related to the xenobiotics metabolism were determined through biomarkers, including Phase I and II, antioxidant defenses and subcellular effects. After 96h of exposure, the lysosomal membrane stability decreased to all crack cocaine concentrations (p< 0.05). In gills, GST activity raised after 48h of exposure to 5 and 50 µg.L<sup>-1</sup>, and after 168h this activity was significantly higher in organisms exposed to 5 and 50 µg.L<sup>-1</sup> when compared to controls (p< 0.05). In digestive glands, GST activity decreased after 96h of exposure to 50 µg.L<sup>-1</sup> when compared to controls (p< 0.05). GPX activity increased in gills after 48h of exposure to 5 µg.L<sup>-1</sup> when compared to controls (p< 0.05). It was also observed significant increase of lipid peroxidation and DNA damage in gills after 96h exposed to 5 µg.L<sup>-1</sup>. In digestive glands, it was detected a significant increase in DNA damage after 96h in organisms exposed to 5 µg.L<sup>-1</sup> when compared to DMSO control (p< 0.05). The results bring evidences that exposure to crack cocaine is able to generate oxidative stress and cytogenotoxic effects in gills of *Perna perna*, which proved to be a reliable model to assess environmental risk of cocaine in marine ecosystems.

#### 97 Lysosomal stability in oysters *Crassostrea* sp. from three different populations from the coast of São Paulo, Brazil

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Coastal zones continuously receive inputs of contaminants, which greatly affect its quality. Biological effects resulting from environmental pollution are complex and have not been properly estimated by chemical analysis and toxicity tests. A reliable form to assess the environmental quality consists of analyzing organisms exposed to constant, complex and diffuse sources of contamination. The appropriate use of biomarkers in sentinel organisms may provide an estimate of the potential risk associated with contamination. This study aims to evaluate if different levels of potential contamination affect differently the stress responses in oysters from three sites along the coast of São Paulo state. The physiological condition of oysters *Crassostrea* sp. from Cananéia (reference site), Santos and Bertioga was studied by analyzing the lysosomal membrane stability in haemocytes, measured using the neutral red retention time (NRRT) assay. Adult organisms were collected in spring (August, September, and October/2016) and autumn (April and May/2017). During the spring, the times of retention of the neutral red dye were similar in oysters from different sites and the reference area (p > 0.05). Interestingly, an uncommon red tide occurred along the coast of the state mainly affecting Cananéia, which may have influenced the reduction of the NRRT for oysters from this region. Such biological events (algal blooms) are more likely to occur at this time of year due to the typical climatic conditions, and may have the potential to even the adverse effects of pollution on distinct bivalve populations subject to different levels of contamination. During the autumn campaign, organisms from both contaminated sites (Santos and Bertioga) showed significant reduction in the mean NRRT relative to the reference site (p < 0.05). A previous study in the same region (Catharino *et al.*, 2015) observed that in both seasons the oysters from Cananéia had a higher NRRT than the oysters from the other two sites. Overall, the NRRT were lower in the present study. These results may be due to the exposure of the organisms to pollutants, since lysosomes are organelles that absorb a wide variety of organic and inorganic substances. However, further investigations are required and being carried out to confirm this hypothesis.

#### Reliable analytical data in environmental studies: sample treatment and analytical determination issues

#### 98 Innovative methods for the determination of PPCPs in drinking water treatment sludge

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