



Apresentações Internas do CIIMAR

Data: 12/12/2005

Horário: 14:00 – 15:00

Local: Auditório CIIMAR, Rua dos Bragas 289

Coordenador: Dr. Rodrigo Ozorio

Programa:

14:00 - 14:15 Estrogens counteract the masculinizing effect of tributyltin in zebrafish

Miguel Santos – Toxicologia Ambiental

14:20 - 14:35 Seasonal variations in the morphology of brown trout (*Salmo trutta f. fario*) kidney peroxisomes

Dolores Resende– Laboratorio de Estudios Celulares e Moleculares

14:40 - 14:55 The Asiatic clam *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries: genetic vs. morphometry.

Ronaldo Sousa - Laboratório de Ecotoxicologia

Resumos:

Estrogens counteract the masculinizing effect of tributyltin in zebrafish

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Recently, it has been demonstrated that the biocide tributyltin (TBT) can interfere with fish sex differentiation, leading to a bias of sex toward males. On the contrary, it is well known that

estrogenic compounds can induce fish feminization. Yet, the combined effects of mixtures of androgenic and estrogenic compounds on fish sex differentiation have never been investigated before, even though in the environment animals are frequently exposed to both groups of xenobiotics. Therefore, in order to investigate if exposure to estrogenic compounds can block the masculinizing effect of TBT, 5 days post-fertilization zebrafish (*Danio rerio*) larvae were exposed for a four month period to TBT and to the synthetic estrogen - ethinylestradiol (EE2). The fish were fed a diet containing TBT at nominal concentrations of 25 and 100 ngTBT/g, and two groups of animals were also dosed with TBT plus EE2 at nominal water concentration of 3.5 ng/l, using a flow-through design. As expected, fish exposed to TBT showed a bias of sex toward males (62.5% males in control tanks and 86 and 82% in TBT 25 and TBT 100 ngTBT/g, respectively). Co-exposure to EE2 completely blocked the masculinizing effect of TBT, with 7% males in the TBT 25 ng/g + EE2 treatment and 0% in the EE2 alone and in the TBT 100 ng/g + EE2 exposed groups. These results clearly indicate that EE2, at environmentally relevant concentrations, can block the TBT masculinizing effects in zebrafish, which suggests that in the aquatic environment the presence of estrogens may neutralize the fish masculinizing effect of TBT. Our findings highlight the need of testing the combined effects of contaminants, as single exposure studies may not be sufficient to predict the effects of mixtures of xenobiotics with antagonistic properties.

Seasonal variations in the morphology of brown trout (*Salmo trutta f. fario*) kidney peroxisomes

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Peroxisomes are cytoplasmic organelles present in most eucaryotic cells, which are particularly abundant in hepatocytes and kidney proximal tubules. These organelles have been intensively investigated in mammals and yeasts, but the literature about fish peroxisomes is still scarce. However, morphological and biochemical changes were previously detected in brown trout liver peroxisomes during the annual reproductive cycle. In females of this species, peroxisomal volume

decreases significantly during vitellogenesis being accompanied by a reduction of peroxisomal enzymatic activities, suggesting a regulation by estrogens [1, 2].

In order to investigate kidney peroxisomal variations, a stereological study was carried out on three-years-old brown trout. Five males and five females were collected in February (end of spawning), May (early vitellogenesis), September (vitellogenesis) and December (pre-spawning). Trunk kidney was fixed and processed for catalase cytochemistry (Fig.1). Classical stereological methods were used at ultrastructural level. Data were examined by two-way ANOVA and Newman-Keuls post-hoc test.

According to the results presented in Table 1, in female kidney proximal tubules there are no significant seasonal changes in the percentage of cell volume occupied by peroxisomes (V_V). Although in females some seasonal variations in the mean peroxisome volume and the relative number of these organelles were detected, these changes occur in opposite directions and compensate each other causing an absence of significant variation in V_V . In males no significant variations were found in segment I of proximal tubules for all measured parameters. However, in segment II of males the V_V was significantly higher in May and September. Significant V_V differences between genders were only detected in segment II of proximal tubules, with higher values for females in February and December.

The morphological variation pattern of kidney peroxisomes during the year is different from the previously shown in the liver of this species [1], suggesting that such variations in renal peroxisomes are not correlated with sex steroids levels. The seasonal changes of peroxisomal enzymatic activities are also very different in both organs [2, 3]. These morphological and functional differences are probably related to the distinct functions of each organ and consequently a peroxisome involvement in diverse metabolic pathways. The physiological significance of the present data will be further investigated.

[1] Rocha, E. *et al.* (1999). *J. Submicrosc. Cytol., Pathol.*, **31**(1): 91-105.

[2] Rocha, E. *et al.* (2001). *J. Morphol.* **248**: 277.

[3] Resende, A.D. *et al.* (2005). *Ann. N. Y. Acad. Sci.* **1040**: 444-447

The Asiatic clam *Corbicula fluminea* (Müller, 1774) in two Portuguese estuaries: genetic vs. morphometry

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Species of the *Corbicula* genus are important nonindigenous invasive species (NIS) in aquatic ecosystems with potential ecological, environmental and economical impacts. The identification of different species inside the *Corbicula* genus is difficult due to their marked variation in shell morphology, colour, sculpture and reproductive biology. The Minho and Lima estuaries were, recently, colonized by individuals belonging to this genus. The populations of the two estuaries show different invasive behaviour and have significant morphological differences in the shape and colour of the shell. Therefore, the main objective of this study was to investigate if *Corbicula* individuals from Minho and Lima estuaries are different populations of the species *Corbicula fluminea* or if two different species exist in these estuaries. To attain this objective, a molecular genetic study based on restriction fragment length polymorphism (RFLP) analysis of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) was performed. The genetic analysis indicates that both populations belong to the species *Corbicula fluminea* (Müller, 1774).