HISTOMORPHOLOGICAL EVALUATION OF *Pimephales promelas* MALE GONADS AFTER EXPOSURE TO PULP MILL AND DOMESTIC DISCHARGES INTO THE URUGUAY RIVER (FRAY BENTOS-URUGUAY)

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**Introduction:**

Fish are subject to a broad variety of stressors because their homeostatic mechanisms are highly dependent on prevailing conditions in their immediately surroundings [1]. Moreover, they are suitable models for evaluating endocrine-active compounds in the water column. Fathead minnow (*Pimephales promelas*) Pisces: Cypriniforms has been used as a sentinel organism for ecotoxicity since the sixties for USEPA, Environment Canada and OECD. An increased number of widely used urban, industrial and agricultural chemicals are being found to cause endocrine disruption. In fishes, xenoestrogens can induce female proteins in males, and in some cases, the development of testis-ova [2]. Histomorphological alterations of the testis in Fathead minnow have been previously described for 17β-estradiol [3, 4], 4-nonylphenol and nonylphenol ethoxylate [5]. We studied here the potential estrogenic effect of pulp mill and domestic wastewater discharges located on the Uruguay River on the male gonads of the Fathead minnow, and we compared it to the effect of treatment with 17β-estradiol (E2).

**Methods:**

**Samples** - surface water receiving municipal wastewater was obtained from Fray Bentos stream in March 2010. A sample of final pulp mill effluent was collected by sampling technicians from Laboratorio Tecnológico del Uruguay.

Test Organisms: Fathead Minnow *Pimephales promelas* were cultured at Water & Chemicals department in the Laboratorio Tecnológico del Uruguay according to EPA protocols [6].

**Experimental design** - Three control and three treated groups, containing two males and four females (7 months old) were exposed in 10L aquaria (n=36) with spawning substrates for 21 days on a semi-static flow with renewal of the testing solution every 48h. Tested solutions were: surface water receiving municipal wastewater, a pulp mill effluent and 150 ng L⁻¹ of the estrogen 17β-estradiol (E2). In order to confirm the sensitivity of the experiments the expression of vitellogenin was evaluated in liver of both males and females in control and treated groups. Only E2 induced a significant increase in testis and ovaries vitellogenin expression (Keel, Parodi & Miguez).

**Fish Sampling** - On the 21 day fish were weighed and measured and then sacrificed with a cervical incision. Testis were dissected, weighed and fixed in Bouin’s fixative for 24 h and treated by classic histological procedures using Hematoxylin and Eosin (H&E).

**Testis Histology** - The sections were examined for abnormal findings such as increase in spermatogonia, and signs of testis degeneration as cell apoptosis or vacuolization.

**Results and Discussion:**

Males were mature and testes contain complete spermatogenic cell stages such as spermatogonia, spermatocytes, spermatids and spermatozoa. For the different experiments, control fish were not all at the same maturation stage. However, none of the treatments delayed or advanced the spermatogenesis when compared with controls. No evidences of histopathological alterations were found for the domestic and pulp mill effluent neither for the E2 concentration applied (Fig. 1). Previous studies on the histopathological effects in gonads of *P. promelas* males exposed to high concentrations (2780 ng L⁻¹) of E2 had shown moderate alterations such as Sertoli cells hyperplasia and hypertrophied with loss of germinal cells, presence of degenerated spermatozoa and occasionally germ cell syncytia [3], vacuolated cells and apoptotic body cells [4]. Vacuolated cells and apoptotic body cells could be attributed to E2 [4], but this alterations were not found in the present study.

Fig.1. Histological sections of testes in the control (left) and E2 (150 ng L⁻³) treated fish (right).
Conclusion:
We conclude that neither pulp mill nor surface water receiving domestic wastewater altered the testis morphology of Fathead Minnow in the experimental conditions used.

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