



LABORATORIO TECNOLÓGICO DEL URUGUAY

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

The Low Uruguay River is a bi-national watercourse shared by Uruguay and Argentina. It holds a rich aquatic biodiversity and provides resources to various human activities, including drinking water abstraction.

Therefore, it is important to assess if industrial sources, such as bleached pulp mill effluents, domestic sources like untreated municipal wastewaters and/or non-point source agricultural pesticides used in soy-fields, could affect the river water quality [1].

In this framework we studied the potential estrogenic effect of pulp mill and domestic wastewater discharges into Uruguay river on the male gonads of the Fathead minnow, and we compared it to the effect of treatment with 17 β -estradiol (E2).

Methods

Location:

- Surface water receiving municipal wastewater from Fray Bentos stream (33° 07' 16" S, 58° 19' 02" W) (fig 1).
- Final pulp mill effluent.

Test Organisms:

Fathead Minnow *Pimephales promelas* (fig. 2) cultured at Laboratorio Tecnológico del Uruguay according to EPA protocols [2].

Experimental design:

Age: 7 months old
3 control + 3 treated groups
Each group = 2 males + 4 females
Exposed to 10L for 21 days
Semistatic flow with renewal of the testing solution every 48h.

Tested solutions were:

- surface water receiving municipal wastewater
- Pulp mill effluent
- 150 ng L⁻¹ of the estrogen 17 β -estradiol (E2)
- 450 ng L⁻¹ of the estrogen 17 β -estradiol (E2).

Fish Sampling:

On the 21 day fish were sacrificed with a cervical incision. Testis were fixed in Bouin and analysed by histological techniques.

Histological analysis:

The sections were examined for abnormal findings such an increase in spermatogonias, and signs of testis degeneration as cell apoptosis or vacuolization.

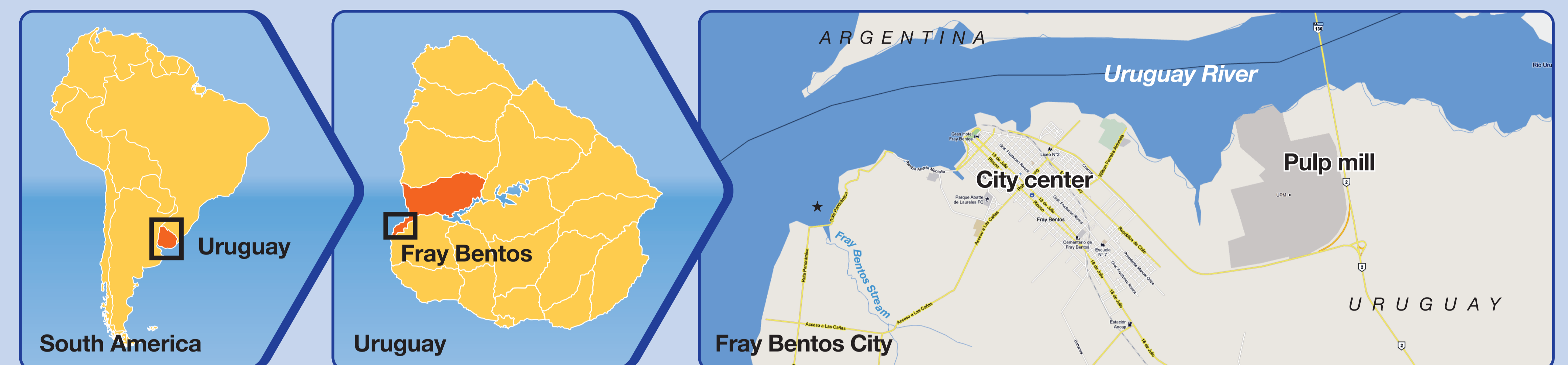


Fig. 1. Location of the Pulp mill and the Fray Bentos stream on the Fray Bentos city, Río Negro, Uruguay

Results and Discussion

Controlling the sensitivity of the experiment

The gene expression of vitellogenin was evaluated in liver of control and treated groups. Only E2 (450 ng L⁻¹) induced a significant increase in testis and ovaries vitellogenin expression (Keel, Parodi & Miguez *Pers comm.*).

Morphological evaluation of testis

- The testis were all at spermatogenic stages, but for the different experiments the control fish were at different degree of spermatogenic development.
- Testes contained complete spermatogenic cell stages such as spermatogonia, spermatocytes, spermatids and spermatozoa.
- 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. 3).

Discussion

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. 2. Male (above) and Female (below) individuals of Fathead minnow cultured at the Water & Chemical products in Laboratorio Tecnológico del Uruguay.

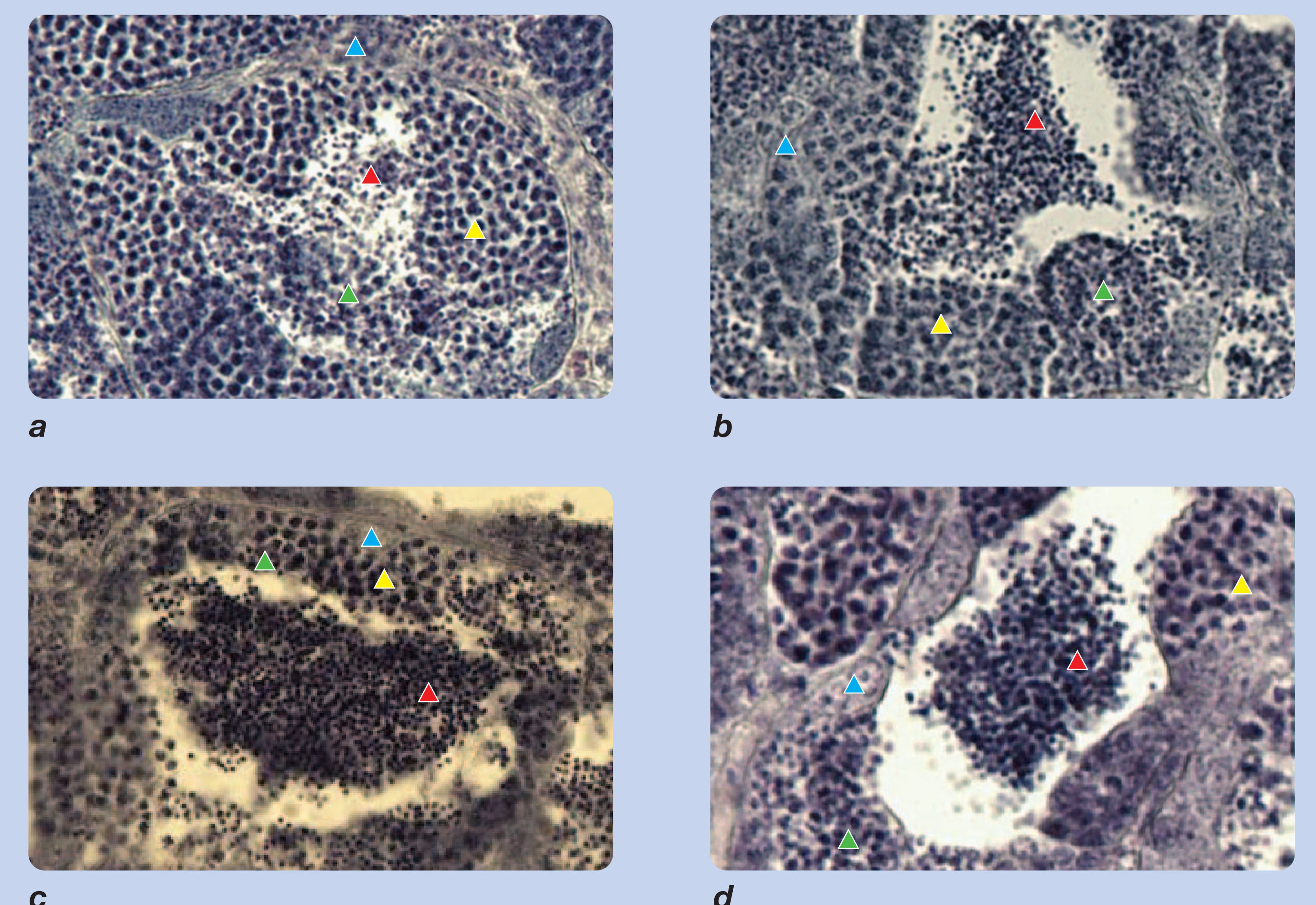


Conclusions

We conclude that neither pulp mill nor surface water receiving domestic wastewater altered the testis morphology of Fathead Minnow in the experimental conditions using a qualitative methodology.

We are now studying the potencial effects of these stressors at molecular level (i.e. brain aromatase, key gonadal genes sensitive to estrogens).

Fig. 3. Histological sections of testes in the different experiments: (3a) Urban Effluent Control group (3b) Urban Effluent treated group. (3c) E2 (150 ng L⁻¹) experience Control group, (3d) E2 (150 ng L⁻¹) treated group. Triangle Red: spermatozoa; Yellow: Spermatocytes; Green: Spermatids; Blue: Spermatogonia



References

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