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*Abstract*

# Evaluation of Effluent Water Effects on *Cyprinodon variegatus* Larvae for the Ecotoxicological Assessment of Endocrine Disruptors Acting on Estrogenic and Androgenic Pathways †

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## Abstract

**Introduction:** The discharge of treated wastewater into coastal and marine environments represents a continuous source of pollutants, including pharmaceuticals and plastic additives with endocrine-disrupting (ED) potential. These compounds are of increasing concern for the European Union due to their capacity to interfere with hormonal systems and their inclusion in current environmental monitoring priorities. ED compounds may induce sublethal effects in aquatic organisms, particularly in vertebrates, where endocrine pathways are highly conserved. In this context, the use of *Cyprinodon variegatus*, a euryhaline fish species, provides a suitable model to assess potential risks in marine ecosystems. Despite advances in wastewater treatment technologies, the persistence of biologically active substances in treated effluents remains a concern. **Objective:** This study aims to evaluate whether treated effluent water still contains compounds with endocrine-disrupting activity and to assess their potential effects on marine organisms. **Methodology:** Larvae of *C. variegatus* from a laboratory stock maintained at ECIMAT (University of Vigo), one of the few available stocks of this species in Europe, were exposed for 48 h to environmentally relevant dilutions (1:10, 1:30, and 1:100) of wastewater treatment plant effluent collected after UV disinfection as the final treatment step. Pools of 10 larvae were used for each condition. Sublethal effects were assessed through gene expression analysis using quantitative PCR (qPCR), targeting biomarkers involved in endocrine regulation. Two housekeeping genes (*tbp* and *hprt*) were used for normalization. Estrogenic responses were evaluated through *vtgab* and *zp2*, while androgenic responses were assessed using *17hsd* and *11hsd*. **Results:** Preliminary results indicate significant alterations in estrogen-related gene expression, particularly in vitellogenin (*vtgab*) and zona pellucida (*zp2*), highlighting the activation of estrogenic pathways and supporting the presence of endocrine-disrupting activity in treated effluent water. **Conclusions:** This study highlights the relevance of assessing endocrine disrupting activity in treated effluents and supports the use of molecular biomarkers as sensitive tools for evaluating their potential impact on marine ecosystems, contributing to the improvement of wastewater monitoring and management strategies.

**Keywords:** endocrine disruptors; wastewater effluent; *Cyprinodon variegatus*; qPCR; marine ecotoxicology

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