Ecotoxicology and Environmental Safety 124 (2016) 82–90

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of waterborne exposure to 17β-estradiol and 4-*tert*-octylphenol on early life stages of the South American cichlid fish *Cichlasoma dimerus*

Fernando J. Meijide [a,b,*], Graciela Rey Vázquez [a,b], Yanina G. Piazza [a], Paola A. Babay [c], Raúl F. Itria [d], Fabiana L. Lo Nostro [a,b]

[a] Laboratorio Ecotoxicología Acuática, DBBE, FCEN, Universidad de Buenos Aires, Ciudad Universitaria, Intendente Güiraldes 2160, 1428 CABA, Argentina
[b] IBBEA, CONICET-UBA, CABA, Argentina
[c] Gerencia Química, Centro Atómico Constituyentes, CNEA, Avenida General Paz 1499, 1650 Buenos Aires, Argentina
[d] INTI, Avenida General Paz 5445, 1650 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form
24 September 2015
Accepted 5 October 2015

Keywords:
Teleosts
Environmental estrogens
Gonad development
Sex differentiation
Testicular oocytes
Acute toxicity

ABSTRACT

Estrogenic chemicals are often detected in the aquatic environment and can negatively affect animal development and reproduction. In teleost fishes, the hormonal regulation during a critical period of larval development has a strong influence on gonadal sex differentiation; thus this process may be affected by the exposure to environmental estrogens. In this study, we first assessed the lethal acute toxicity of the natural estrogen 17β-estradiol (E_2) and the weaker estrogen mimics 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP) on larval stages of the South American cichlid fish *Cichlasoma dimerus*. In a further experiment, we analyzed the effects of chronic waterborne exposure to E_2 and OP on gonad development and sex differentiation. Exposure to high concentrations of E_2 had a pronounced feminizing effect directing sex differentiation towards ovarian development, while testis development was inhibited at a lower, environmentally relevant concentration. Among OP-exposed fish, 15–38.5% of the males exhibited testicular oocytes (TOs), a commonly reported biomarker of estrogenic exposure. However, since TOs were also recorded in control males and the proportion of males with TOs was not significantly higher in OP treatments, their occurrence could not be attributed to OP exposure. In addition, TOs did not seem to impair male gonad development and functionality since normal spermatogenesis was observed in testes of OP-treated fish. These results indicate that E_2 occurring in the South American aquatic environment may affect male reproductive development and pose a risk for wild *C. dimerus*, especially under prolonged exposure, while the effects of weaker xenoestrogens such as OP would be negligible for gonad development in this species. As illustrated by this study, the natural occurrence of TOs indicates that conclusions concerning the causes of this phenomenon must be drawn with care.

1. Introduction

Chemicals capable of affecting the endocrine function of aquatic organisms are often detected in waters that receive effluents from municipal and industrial wastewater treatment plants or untreated wastewaters. One class of such endocrine disruptors is represented by estrogenic chemicals such as the natural estrogen 17β-estradiol (E_2), the synthetic estrogen 17α-ethynylestradiol (EE_2) used in birth-control pills, or weaker estrogen mimics such as nonylphenol (NP) and octylphenol (OP) (see White et al., 1994; Desbrow et al., 1998; Giesy and Snyder, 1998; Sumpter and Jobling, 2013). Like EE_2, E_2 reaches the aquatic environment via human excretion or through improper disposal of unused pharmaceuticals containing it as active ingredient. Also effluents from feedlots are a significant source of E_2 input into the aquatic systems in rural areas with intensive cattle breeding (Ying et al., 2002b; Soto et al., 2004). Alkylphenol ethoxylates (APEs) are nonionic surfactants used as detergents, emulsifiers, wetting and dispersing agents in products for agricultural, industrial, commercial and domestic applications. Although the use of APEs has been restricted in the European Union (Soares et al., 2008; Sumpter and Jobling, 2013), they are still widely employed in other regions, including South America, where no action has been taken to reduce or eliminate their usage. As two of the main breakdown products of APEs, both NP and OP are ubiquitous in the aquatic

* Corresponding author at: Laboratorio Ecotoxicología Acuática, DBBE, FCEN, Universidad de Buenos Aires, Ciudad Universitaria, Intendente Güiraldes 2160, C1428EGA CABA, Argentina. Fax: +54 11 45763384.
E-mail address: fmeiji@bg.fcen.uba.ar (F.J. Meijide).

http://dx.doi.org/10.1016/j.ecoenv.2015.10.004
0147-6513/

environment. NP is the preponderant alkylphenol (AP), constituting 80% of APs found in surface waters and sediments (Ying et al., 2002c). In comparison, OP accounts for about 15% of the commercial AP input (Bennett and Metcalfe, 1998). Due to its lower usage, OP has been less analyzed than NP in ecotoxicological studies. The reports about the relative estrogenic potency of both APs are inconclusive; while OP probed to be more estrogenic than NP in rainbow trout vitellogenin production and human breast cancer cell growth assays (Jobling and Sumpter, 1993; White et al., 1994), NP presented a higher estrogenicity factor in a recombinant yeast estrogen receptor (ER) assay (Céspedes et al., 2004). In addition, while OP probed to be more potent than NP when competing with E_2 for binding to the trout hepatic ER (White et al., 1994), both APs had a similar affinity for the ER when tested for competitive binding with E_2 to the rat uterine ER (Laws et al., 2000).

Frequently, the concentrations of estrogens recorded in the aquatic environment (ng/L to μg/L range) are above the thresholds for which adverse reproductive effects have been reported for teleost fishes, raising concern about possible negative impacts on wild populations. Laboratory studies have shown that exposure to environmentally relevant concentrations of these chemicals can cause reproductive disorders of various types, including abnormal gonad development, altered secondary sex characteristics, changes in reproductive behavior, altered hormone levels and reduced reproductive success (Jobling et al., 1996; Gray and Metcalfe, 1997; Panter et al., 1998; Gray et al., 1999a; Kinnberg et al., 2000; Harris et al., 2001; Knörr and Braunbeck, 2002; Kang et al., 2002, 2003; Seki et al., 2002, 2003; Balch et al., 2004; Nash et al., 2004; Fenske et al., 2005; Balch and Metcalfe, 2006; Salierno and Kane, 2009; Guyón et al., 2012; Maltais and Roy, 2014; Roggio et al., 2014). Field assessments have demonstrated that estrogenic exposure can lead to reduction of the reproductive performance (Harris et al., 2011) and even to collapse (Kidd et al., 2007) of wild fish populations. In South America, records on environmental levels of estrogenic chemicals are scarce. However, the generally defective treatment of effluents and the lack of control of effluent discharges suggest that the concentration of these compounds in the aquatic systems might exceed the levels causing endocrine disruption in fishes. In addition, although the reproductive effects of environmental estrogen exposure have been analyzed in a number of teleosts, little is known about the sensitivity of native South American species to this class of pollutants.

The process of sex differentiation in teleosts is highly dependent on the hormonal regulation during a critical period of larval development. The administration of exogenous sex steroids can strongly influence the course of sex differentiation, suggesting that they play a critical role in this process (Devlin and Nagahama, 2002). The lability of sex-determination systems in this group of fishes makes them particularly sensitive to the influence of environmental estrogens. Few studies have addressed the effects of waterborne exposure to these chemicals on gonadal morphology and histology during the period of sex differentiation. A commonly reported sign of feminization is the presence of previtellogenic oocytes within the testicular tissue of exposed males. However, the natural occurrence of testicular oocytes is largely unknown for most fish species, and therefore conclusions concerning the causes of this phenomenon must be drawn with care. This concern is especially valid regarding the ambisexual nature of teleosts that results in a sexual plasticity that is not yet completely understood (Hecker et al., 2006).

The acará, *Cichlasoma dimerus*, is a neotropical cichlid quite common in shallow waters of the Paraguay river and most of the Paraná river basins (Kullander, 1983). This species is representative of the teleost fish fauna in the La Plata River Basin and results relevant to the riverine ecosystems of Argentina. It adapts easily to laboratory conditions and displays an elaborate social and reproductive behavior, which includes biparental breeding activities (Pandolfi et al., 2009). In addition, *C. dimerus* has been extensively used in our laboratory for ecotoxicological research (Moncaut et al., 2003; Rey Vázquez et al., 2009; Da Cuña et al., 2011, 2013; Genovese et al., 2011, 2012, 2014; Piazza et al., 2011; Rey Vázquez and Lo Nostro, 2014), and it is considered an appropriate native species for xenobiotic toxicity assays by the Argentinean Institute of Standardization and Certification (IRAM 29112, 2008). In a previous study, we showed that sublethal OP exposure induced vitellogenin synthesis and disrupted testis morphology in adult *C. dimerus* (Rey Vázquez et al., 2009). The aims of this study were to assess the lethal acute toxicity of E_2, OP and NP on larval stages of *C. dimerus* and to evaluate the effects of sublethal concentrations of E_2 and OP on gonad development and sex differentiation. As far as we are aware, this is the first study reporting the effects of waterborne exposure to environmental estrogens on early life stages of a South American freshwater fish.

2. Materials and methods

2.1. Animals

Adult specimens of *C. dimerus* were captured by local fishermen from the natural environment in Esteros del Riachuelo, Corrientes, Argentina (27° 25′ S, 58° 15′ W), an area with minimal human influence. Fish were held in 100 L aquaria where they were allowed to acclimate to laboratory conditions for two months prior to the onset of experimentation. Pairs formed in community tanks were isolated in 45 L aquaria and maintained at 26 ± 2 °C and a 12:12 h photoperiod. Aquaria were well aerated and provided with a layer of gravel and smooth stones for egg deposition on the bottom. Fish were fed once a day with pelleted commercial food (Tetra food sticks). Larvae used in the experiments were obtained from natural spawns of 6 pairs. On the 10th day posthatch (PH), each lot of offspring was isolated in a bare 20 L aquarium and reared until they reached the larval stage used in each experiment. Fry were initially fed with freshly hatched nauplii of *Artemia sp.* and later fed finely ground, dried flake food. Guidelines on the care and use of fish in research and testing from the Canadian Council on Animal Care (2005) were followed.

2.2. Tests conditions and chemicals

Experiments were performed at 26 ± 1 °C and a 12:12 h photoperiod, using filtered tap water (pH 7.8, conductivity 250 μS/cm, total alkalinity 44.1 mg/L, O_2 8 mg/L). The test substances, 17β-estradiol (E_2), 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP) (> 97% pure) were obtained from Sigma (St. Louis, MO). Stock solutions of each chemical were prepared every week by dissolving them in 100% ethanol and stored in the darkness at 4 °C. During each water renewal, the necessary volume of stock solution was added to the aquarium water in order to achieve the desired final concentrations (solvent = 0.01% *per* treatment).

2.3. Lethal acute toxicity

Since NP is the preponderant alkylphenol in the aquatic environment (Ying et al., 2002c), the lethal toxicity of this compound was assessed in addition to that of OP and E_2. Toxicity of the three substances was assessed in larvae at 10, 18 and 26 days PH. For each larval stage, a 96 h exposure was conducted under semi-static conditions with daily renewal of water and test chemical solutions. Exposure concentrations were determined by a preliminary range-finding test for each chemical. In the definitive