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Comparative study of the estrogenic responses of mirror carp (*Cyprinus carpio*) exposed to treated municipal sewage effluent (Lisbon) during two periods in different seasons

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Abstract

To assess the estrogenic potency of the treated domestic sewage effluent from a sewage treatment plant (STP) located in Lisbon (Chelas), 60 mirror carp (*Cyprinus carpio*) were exposed to different concentrations of the sewage effluent (0%, 25%, 50% and 100%) for two periods of 28 days in two different seasons (winter/spring). Vitellogenin induction in males was used as a biomarker of exposure to xenoestrogens. At the end of the experiment, blood samples were taken for vitellogenin analysis and the fish were sacrificed and dissected. Gonad samples were taken for histological evaluation of the sewage effects. The results showed an increase in vitellogenin induction in exposed fish, both males and females, depending on the different dilutions of the sewage effluent. In comparison with controls, the gonadosomatic index decreased significantly ($P<0.05$) in fish exposed to 100% treated effluent. Although statistically not significant, the hepatosomatic index (HSI) was high in all exposed fish. Histological abnormalities in fish gonads were evaluated and related to the different percentages of sewage effluent. Seasonal variations found in estrogenic responses were attributed to weather influences on sewage dilution.

Keywords: Endocrine disruption; Vitellogenin; *Cyprinus carpio*; Sewage effluent; Seasonal variations

1. Introduction

The number of anthropogenic chemicals released into the aquatic environment is increasing year by year and is the cause of great concern, particularly with regard to xenoestrogens (Allen et al., 1999; Harris et al., 2000), which have bioactivity similar to endogenous steroid hormones and are known to affect the development, sexual maturation and reproduction of vertebrates (Ackermann et al., 2002). Some of these xenoestrogens, also called EDCs (Endocrine Disruptor Compounds), are structurally similar to the

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endogenous steroid hormone 17 β-estradiol, while others may not be structurally related to naturally occurring steroids. Thus, many non-steroidal compounds, such as flavonoids, lignins, sterols and fungal metabolites, and synthetic chemicals can interact with sex hormone receptors or can modulate their metabolism and biosynthesis (Combes, 2000).

In the current scientific literature, treated and non-treated sewage effluents have been identified as the main sources of EDC discharges into the aquatic environment (Sumpter, 1995; Tyler and Routledge, 1998; Solé et al., 2001, 2002). Indeed, STPs (Sewage Treatment Plants) receiving domestic and industrial sewage release a complex mixture of chemicals (natural and synthetic), some of which are difficult to biodegrade in the treatment process (Sumpter, 1997, 1998; Petrovic et al., 2002; Solé et al., 2003).

According to previously published literature, estrone, 17 β-estradiol (E2), synthetic estrogen 17 α-ethinylestradiol (EE2) and the degradation products of alkylphenol polyethoxylates (APEOs) are major causes of estrogenic effects from STP effluents (Harries et al., 1997; Desbrow et al., 1998; Sonnenschein and Soto, 1998; Petrovic et al., 2002). APEOs are a group of non-ionic surfactants that are microbially degraded into more lipohilic products with estrogenic potential (Hawrelak et al., 1999), such as nonylphenol or octylphenol, substances that may act as endocrine disruptors at the estrogen receptor level by mimicking the activity of E2. There is also evidence that alkylphenols can disrupt endocrine-mediated events by inhibiting enzymes involved in the metabolism of sex steroids (Harris et al., 2000).

Moreover, synthetic estrogens, such as EE2, are even more potent than natural estradiol (Allen et al., 1999; Van den Belt et al., 2004). These chemicals can reach the aquatic environment in biologically active amounts and bioaccumulate to significant concentrations in aquatic organisms (Sumpter, 1995; Arukwe et al., 2000).

The first reports in U.K. rivers concerning intersex fish (presence of oocytes within the testes) were related with the proximity to STPs (Purdom et al., 1994). Subsequent studies identified EDCs in lagoons and rivers close to STPs in the U.K, where intersex fish were often found (Allen et al., 1999). Other studies identified and quantified chemicals with estrogenic activity in STPs (Spengler et al., 2001). Studies carried out by Harries et al. (1999) showed estrogenic potency from STPs in rainbow trout (*Oncorhynchus mykiss*) and Solé et al. (2002) reported high levels of vitellogenin (Vtg) in male carp (*Cyprinus carpio*) collected downstream of a sewage treatment plant in Spain. In the U.S.A, Folmar et al. (2001) demonstrated that STPs release estrogenic effluents capable of inducing vitellogenin production and altering normal serum sex steroids in a commercially valuable fish, the walleye (*Stizostedion vitreum*).

The use of biomarkers of potential endocrine disruption in fish is important for the detection and monitoring of the potentially adverse effects of environmental contaminants on aquatic organisms. Usually Vtg is used as a monitoring tool to assess estrogenic effects in studies involving EDCs (Allen et al., 1999). Vtg is an estrogen-inducible phosphoprotein that is normally synthesized by the liver of female oviparous vertebrates during oogenesis. It is the precursor of egg yolk proteins but both males and juveniles can synthesise Vtg if estrogens or xenoestrogens are present. Accordingly, this protein can be used as a biomarker for estrogenic exposure in male and juvenile fish (Folmar et al., 1996).

Desbrow et al. (1998) isolated and identified the major estrogenic chemicals present in sewage effluents discharged into U.K. rivers. Synthetic estrogen EE2, natural hormone E2 and estrone were present in all effluents assessed, which suggests that these chemical compounds may be responsible for the induction of Vtg synthesis in male fish near effluent discharges from STPs.

Few studies have focused on seasonal variations in sewage effluent estrogenicity or on how these changes can be influenced by variations in STPs efficiency or by the volume/dilution of the influents (Rodgers-Gray et al., 2000). Nevertheless, there is little information available and a strong need to understand which factor(s) or mechanisms contribute to seasonal variations and how this can influence estrogenic responses in fish.

The main objective of the present work is to evaluate the estrogenic responses of mirror carp (*C. carpio*) exposed to treated sewage effluent from an STP located in Lisbon (Portugal), in two periods in different seasons (winter and spring). To achieve the proposed objectives Vtg was used as a biomarker of