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In situ combined chemical and biological assessment of estrogenic pollution in a water recycling system

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ABSTRACT

Estrogenic pollution and its control in aquatic systems have drawn substantial attention around the world. The chemical and biological assessment approaches currently utilized in the laboratory or field cannot give an integrated assessment of the pollution when used separately. In this study, *in situ* chemical and biological methods were combined to detect pollution in a water recycling system. Data for the water quality index (WQI) demonstrated that the water treatment resulted in the decline of pollution from upstream to downstream. Wild male Nile tilapia, *Oreochromis niloticus*, was sampled in June and September. The concentrations of four common endocrine disrupting chemicals (EDCs) were determined in the tilapia liver by chromatographic analysis methods. The level of 17β-estradiol (E2) declined from upstream to downstream in both months. In contrast, the levels of bisphenol A (BPA), di-(2-ethylhcxyl) phthalate (DEHP), and perfluorooctane sulfonate (PFOS) did not display this declining tendency. The highest relative expression of vitellogenin 1 (VTG1) was observed in tilapia from upstream, then the level significantly decreased along the water system. The relative expression levels of CYP1A1 in the water system were also significantly higher than that of the control. However, no declining trend could be observed along the water system. The change of VTG1 expression corresponded well with that of E2 levels in the tilapia liver. Overall, our study assessed the pollution by endocrine disruptors using chemical and biological data with good correspondence. This study also demonstrated the effectiveness of the water recycling system in eliminating estrogen pollution in municipal sewage.

Introduction

Endocrine disrupting chemicals (EDCs) have received increasing attention because of their adverse influence on organisms. The aquatic environment is the ultimate destination for most environmental contaminants derived from industrial, agricultural and domestic wastewater. Studies have indicated that EDCs are prevalent in aquatic environments and induce endocrine dysfunction in aquatic organisms (Bertin et al., 2011; Oehlmann et al., 2000). EDCs are known to act by interfering with normal hormone biosynthesis (Patrick et al., 2014).

Vitellogenin 1 (VTG1) is synthesized in the liver and induced by estrogens. The induction of VTG1 synthesis in male fish by environmental estrogens has been proposed to be an effective and sensitive biomarker of estrogenicity (Goksøyr, 2006; Selcer and Verbanic, 2014). High levels of hepatic VTG1 were observed in Japanese medaka exposed to estrogenic EDCs (Yamaguchi et al., 2005). EDCs induced the increase of plasma VTG1 in a dose-related

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manner, and estrogenic chemicals shared the identical mechanism for inducing VTG1 synthesis (Chang et al., 2011). The cytochrome P450 (CYP450) gene signaling pathway plays an important role in the defense against environmental stress (Kim et al., 2015). CYP1A1, one of the CYP1 family isoforms, is the most relevant form described so far (Costa et al., 2012; Koenig et al., 2013; Solé et al., 2012). The level of CYP1A1 expression is inducible by exposure to certain EDCs (Zhu and Lee, 2005).

Oreochromis niloticus is a good biological model for toxicological studies. This fish has high growth rates, is efficient in acclimatizing to various diets and is resistant to diseases (Eshel et al., 2012; Fırat et al., 2011). *O. niloticus* is generally found in estuaries around the world and responds rapidly to environmental change. The biochemical parameters of *O. niloticus* are sensitive for detecting potential negative effects (Almeida et al., 2002).

Although laboratory studies have indicated negative influences on fish exposed to estrogen compounds, laboratory investigations of EDCs tend to use high chemical dose levels. The exposure cycle is relatively short. In general, chemical pollutants are present as a complicated environmental mixture and at relatively low concentrations in water ecosystems (Michel et al., 2013). Whether chronic exposures under environmentally relevant levels elicit adverse effects on wild fish populations (Kidd et al., 2007) in the natural environment has not been determined. Currently, measuring only the chemical characteristics does not allow one to judge the health of an aquatic ecosystem. Combined chemical and biological evaluations could provide a more systematic picture of water pollution (Oberholster et al., 2008). However, few previous studies combine chemical and biological data.

The objective of this study was to assess estrogenic pollution through combined chemical and biological methods in a field survey of wild *O. niloticus* from upstream to downstream locations in a water system. The levels of four commonly used endocrine disruptors were measured in the tilapia liver. Meanwhile, the Messenger ribonucleic acid (mRNA) levels of both VTG1 and CYP1A1 were also measured. The correspondence of both data was analyzed to validate the effectiveness of the method. Through this study, we established an effective method to assess estrogenic pollution.

1. Materials and methods

1.1. Standards and reagents

All reagents were of high performance liquid chromatography (HPLC) grade. The standard chemicals were purchased as follows: di-(2-ethylhcxyl) phthalate (DEHP) (Supelco, Bellefonte, PA, USA), 17β-estradiol (E2) and perfluorooctane sulfonate (PFOS) (Sigma, St. Louis, MO, USA), and bisphenol A (BPA) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). $^{13}C_{12}$BPA (Cambridge Isotope Laboratories Ins, USA) and $^{13}C_4$PFOS (Wellington Laboratories Inc., Guelph, ON, Canada) were added as internal standards.

1.2. Sample collection in the water recycle system

Wild *O. niloticus* were collected using a fish net at eight sampling sites (S1–S3, upstream; S4–S6, midstream; S7–S8, downstream) in the water recycling system during June and September 2014 (Fig. 1). The water recycling system is located in Xiamen, China near the Xinglin Wan Reservoir. The sewage treatment unit is located upstream and includes an activated carbon filter, sand filters and plant absorption area. In the unit, effluent is first sent to a primary sedimentation tank and subsequently flows to the secondary sedimentation tank. Sewage treatment and recycling processes take place after the precipitation. Two small dams are present between the up- and middle-streams and middle- and down-streams. Each section has a plant adsorption area in the water reuse system. The tilapia were captured separately in each section. At each sampling point, dozens of fish were captured, and sex identification was subsequently performed according to the aquaculture industry standard of the People's Republic of China (SC/T 1105–2007). The male fish were used for the experiments. The weight and length of each fish were recorded, and the liver was then dissected and weighed. The hepatosomatic index (Huang et al., 2008) was calculated using the following equation: HSI = liver weight (g) / total fish weight (g) × 100%.

1.3. Measuring the water quality index (WQI)

The WQI was measured by a HACH Hydrolab multi-parameter probe (Hach Company, USA). The water was obtained from three sites (upstream, midstream and downstream). Each index was determined six times. The index included eight parameters: pH, temperature (T), oxidation reduction potential (ORP), turbidity (TUR), specific conductance (SPC), salinity (SAL), luminescent dissolved oxygen (LDO) and chlorophyll (CHL) content.

1.4. Determining DEHP and E2 by HPLC

To quantify DEHP and E2 in tilapia liver, the samples were extracted by solid phase extraction (SPE) with Oasis HLB cartridges (60 mg, 3 cc, Waters, Milford, MA, USA). The process was as follows: 3 mL of ethyl acetate was added to the liver samples, which were then immersed in an ultrasonic bath for 25 min. Next, 1 mL of Milli-Q water was added. The homogenates

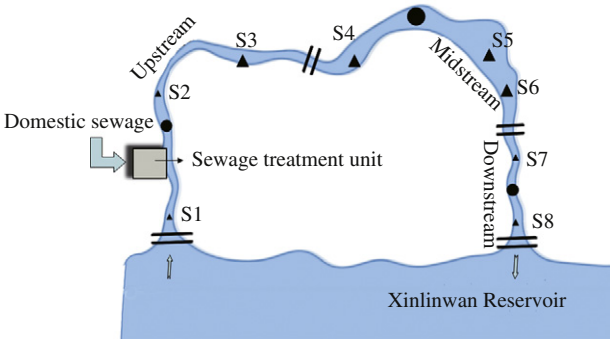

Fig. 1 – Sampling sites for wild *O. niloticus* in the water reuse system in Xiamen, China. S1–S8 (black triangle) were the sampling sites, S1–S3 was the upstream area, S4–S6 was the midstream area, S7–S8 was the downstream area, and the square was the sewage treatment unit, the black rounded areas represented plant purification areas.