Aquatic Toxicology 174 (2016) 10–21

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Endocrine-disruptor molecular responses, occurrence of intersex and gonado-histopathological changes in tilapia species from a tropical freshwater dam (Awba Dam) in Ibadan, Nigeria

Aina O. Adeogun[a], Kolawole Onibonoje[a], Oju R. Ibor[a], Roseline A. Omiwole[a], Azubuike V. Chukwuka[a], Alex O. Ugwumba[a], Adiaha A.A. Ugwumba[a], Augustine Arukwe[b,*]

[a] Department of Zoology, University of Ibadan, Ibadan, Nigeria
[b] Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 3 February 2016
Accepted 5 February 2016
Available online 6 February 2016

Keywords:
Intersex
Endocrine disruption
Fish
Environmental health
Developing country

ABSTRACT

In the present study, the occurrence of endocrine disruptive responses in Tilapia species from Awba Dam has been investigated, and compared to a reference site (Modete Dam). The Awba Dam is a recipient of effluents from University of Ibadan (Nigeria) and several other anthropogenic sources. A total of 132 Tilapia species (*Sarotherodon malenotheron* (n = 57 and 32, males and females, respectively) and *Tilapia guineensis* (n = 23 and 20, males and females, respectively)) were collected from June to September 2014. At the reference site, samples of adult male and female *S. melanotheron* (48 males and 47 females) and *T. guineensis* (84 males and 27 females) were collected. Gonads were morphologically and histologically examined and gonadosomatic index (GSI) was calculated. Hepatic mRNA transcriptions of vitellogenin (Vtg) and zona radiata protein (Zrp) genes were analyzed using validated RT-qPCR. Significant increase in Vtg and Zrp transcripts were observed in male tilapias from Awba Dam, compared to males from the reference site. In addition, male tilapias from Awba Dam produced significantly higher Vtg and Zrp mRNA, compared to females in June and July. However, at the natural peak spawning period in August and September, females produced, significantly higher Vtg and Zrp mRNA, compared to males. Fish gonads revealed varying incidence of intersex with a striking presence of two (2) pairs of testes and a pair of ovary in *S. melanotheron* from Awba Dam. The entire fish population examined at Awba Dam showed a high prevalence of intersex (34.8%), involving phenotypic males and females of both species. Analysis of sediment contaminant levels revealed that As, Cd, Pb, Hg and Ni (heavy metals), monobutyltin cation, 4-iso-nonyphenol and PCB congeners (138, 153 and 180) were significantly higher in Awba Dam, compared to the reference site. Principal component analysis (PCA) showed that fish variables were positively correlated with sediment contaminant burden at Awba Dam, indicating that the observed endocrine disruptive responses are associated with contaminant concentrations. Overall, the occurrence of intersex and elevated expressions of Vtg and Zrp in male fish, suggest that the measured contaminants were eliciting severe endocrine disruptive effects in Awba Dam biota, which is an important source of domestic water supply and fisheries for the University of Ibadan community.

1. Introduction

The human, wildlife and biota health consequences of environmental pollution represent an issue of serious concern in developing countries, both for sustainable management and protection of aquatic resources and fisheries (Robles-Molina et al., 2014). Particularly for Nigeria, coastal and inland waters, such as dams, lakes, rivers and streams represent one of the most vulnerable aquatic environments, due to the high contaminant inputs through effluents, resulting from extensive urbanization, waste disposal, industrial and agricultural activities and the absence of established monitoring protocols for sustainable environmental health management (Eruola et al., 2011; Onyeike et al., 2002). The aquatic ecosystem is a significant sink for a wide variety of

* Corresponding author.
E-mail address: arukwe@bio.ntnu.no (A. Arukwe).

http://dx.doi.org/10.1016/j.aquatox.2016.02.002

Table 1
Biometric data of *Sarotherodon melanotheron* and *Tilapia guineensis*, sampled at awba Dam and a reference site (Modete Dam).

	Sarotherodon melanotheron				*Tilapia guineensis*			
	Awba Dam		Reference site		Awba Dam		Reference site	
	Male	Female	Male	Female	Male	Female	Male	Female
June								
Total length	21.8 ± 3.6	25.8 ± 6.4*	16.5 ± 0.8	17.8 ± 2.7	22.6 ± 3.6	22.6 ± 2.5	21.2 ± 4.6	21.9 ± 1.3
Body weight	244 ± 16.2*	426.9 ± 66.5*	94.0 ± 52	123.2 ± 51.3	273.6 ± 112.2*	295.6 ± 39.8*	207.2 ± 28.8	152.5 ± 21.1
Gonad weight	0.8 ± 0.2*	19.9 ± 5.9*	0.1 ± 0	4 ± 2.2	0.6 ± 0.2*	4 ± 1.7*	0.3 ± 0.1	1.1 ± 0.1
GSI	0.6 ± 0.2*	4 ± 1.3*	0.2 ± 0	2.4 ± 1	0.3 ± 0*	2.6 ± 2*	0.2 ± 0	0.9 ± 0.2
–								
July								
Total length	28 ± 2.5*	27.4 ± 0.6*	21.7 ± 1.8	21.5 ± 0.5	31.3 ± 1.2*	27.3 ± 0.6*	22.5 ± 1.1	19.5 ± 0.5
Body weight	425.7 ± 87*	370.3 ± 88.2	210.8 ± 55.6	205.8 ± 27.1	503.7 ± 79.4*	384.7 ± 24.8*	225.3 ± 26.8	132.3 ± 10.1
Gonad weight	0.9 ± 0.2*	7.8 ± 2.2*	0.1 ± 0.0	0.9 ± 0.2	0.7 ± 0.1*	7.8 ± 1*	0.2 ± 0.1	0.2 ± 0
GSI	0.5 ± 0.3*	2.2 ± 0.6*	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0	2.2 ± 0.6*	0.1 ± 0	0.2 ± 0.1
–								
August								
Total length	29.6 ± 1.5*	28.9 ± 1.7*	21.9 ± 2.0	22.5 ± 1.2	28.2 ± 0.1*	27.7 ± 0.6*	24.0 ± 0.9	19.7 ± 0.4
Bodyweight	445.0 ± 58.8*	409.7 ± 33.8*	214.8 ± 16.7	212.5 ± 15.5	425.1 ± 16.3*	413.1 ± 18*	260.7 ± 24.3	139.9 ± 14.1
Gonad weight	3.9 ± 1.6*	14.5 ± 3.2*	0.1 ± 0	1 ± 0.3	5.2 ± 2.2*	14.5 ± 2.4*	0.4 ± 0.1	1 ± 0.2
GSI	0.8 ± 0.2	3.4 ± 0.7*	0.1 ± 0	0.7 ± 0.1	1.3 ± 0.6*	3.2 ± 0.9*	0.1 ± 0	0.7 ± 0
–								
September								
Total length	29.7 ± 1*	29.6 ± 0.5*	22 ± 1.8	21.4 ± 0.3	28.2 ± 0.1*	32.2 ± 3.6*	24.8 ± 0.9	20 ± 1.7
Bodyweight	**440.1** ± **36***	439.3 ± 35.7*	219 ± 16.8	206.4 ± 7.9	425.5 ± 16.3*	**428.4** ± **84.5***	260.7 ± 79.8	185.6 ± 40.7
Gonad weight	3.8 ± 1.6*	**13.9** ± **2.8***	0.2 ± 0.1	3 ± 0.3	1.6 ± 0.5*	**12.3** ± **3.2***	0.4 ± 0.1	0.9 ± 0.3
GSI	0.8 ± 0.2*	**3** ± **0.4***	0.1 ± 0	1.4 ± 0.7	1.3 ± 0.6*	**2.9** ± **0.8***	0.2 ± 0.1	0.6 ± 0.1

GSI = Gonadosomatic index.
Values are given as mean ± standard deviation (stdev).
* Significant difference ($p < 0.05$) within same sex (male or female) and species at Awba Dam, compared to the reference site.

industrial chemicals, pharmaceuticals and personal care products, pesticides and surfactants that are ubiquitous contaminants in these ecosystems (Kolpin et al., 2013). Exposure to environmental chemicals or their mixtures may produce complex biological responses that may have profound consequences on aquatic organisms, including effects on hormonal responses, physiology and reproductive process (Brian et al., 2005; Beresford et al., 2011). However, the relative importance of the influence of complex chemical mixtures on biological systems is not well understood or quantified mechanistically (Balaguer et al., 1996; Carvalho et al., 2014).

In the aquatic environment, the effects of endocrine disrupting chemicals (EDCs) on reproduction have been reported in several invertebrate and vertebrate species (Degen and Bolt, 2000; Witorsch, 2002). For example, altered steroid hormone levels and abnormal male and female gonads were observed in juvenile alligators exposed to organochlorine contaminants in Lake Apopka (Florida) (Guillette et al., 1994; Gunderson et al., 2001). Several exogenous compounds are found to produce estrogenic effects in fish and these include phytoestrogens (Pelissero et al., 1991), synthetic estrogens such as 17α-ethynylestradiol (EE2) used in birth control pills (Fent et al., 2006) and several other synthetic substances such as alkyl phenols (nonylphenol; NP), insecticides, phthalates and hydroxylated (OH)-metabolites of polychlorinated biphenyls (PCBs) (Braathen et al., 2009). The causative agent of estrogenic effects on fish in rivers and estuaries appear to be the natural endogenous steroids 17β-estradiol (E2) and estriol, EE2 as well as alkyl phenols derived from polyethoxylated phenols (Arukwe et al., 1997; Labadie and Budzinski, 2006). In heavily contaminated rivers in the United Kingdom, there have been reports of male roach with ovotestis (the presence of male and female gonadal cells), abnormal glands and duct, morphological changes of secondary sexual structures and overt reproductive effects (Elango et al., 2006; Jobling et al., 1996; Matthiessen et al., 2002).

In developing molecular and cellular responses for evaluating the presence of EDCs in the environment, the detection of vitellogenin (Vtg) and zona radiata proteins (Zrp) in males and juveniles of oviparous species have been used as sensitive early warning signals (Arukwe et al., 2000; Rey et al., 2006; Soverchia et al., 2005). Vtg and Zrp are produced in the liver under stimulation by estradiol-17β (E2), secreted and transported through the blood to the ovary where they accumulate into maturing oocytes (Arukwe and Goksoyr, 2003). In the ovary, Vtg provides nutrition, while Zrp form the eggshell that provides protection against mechanical disturbances for the developing embryo during the early fragile period (Arukwe and Goksoyr, 2003; Tyler and Sumpter, 1996). The molecular basis for Vtg and Zrp mRNA expression are estrogen receptor (ER) mediated responses that are E2 or E2 mimics (EDCs) dependent interactions with the ER, probably involving several ER isoforms (Arukwe and Goksoyr, 2003). Ligand-activated ER complex exerts a diverse array of biological effects that include the transcriptional induction of ER-controlled genes after binding to the estrogen-responsive elements (EREs) upstream of ER-controlled genes (Wahli et al., 1981). Under normal physiological condition, very little (if any at all) Vtg or Zrp can be detected in male and juvenile fish, presumably because of low estrogen concentrations (Arukwe and Goksoyr, 2003). But, it is known that these proteins are synthesized by the liver cells (both in vivo and in vitro) of male and juvenile fish treated with E2 or EDCs (Arukwe et al., 2000; Rey et al., 2006; Soverchia et al., 2005) and has been extensively used as biomarkers of exposure and effect for the presence of EDCs in the environment (Adeogun et al., 2016; Jasinska et al., 2015; Bizarro et al., 2014; Mdegela et al., 2010; Palermo et al., 2008).

Fish form an important protein source for human nutrition, and contaminated fishes are of potential risk to human health (Aravindakshan et al., 2004; Berntssen et al., 2010), and have been used as an important and sensitive indicator species of contamination in the aquatic environment (Aravindakshan et al., 2004; Berntssen et al., 2010). The release of contaminants into the aquatic environment represents potential environmental, wildlife and human health consequences of societal concern, both for sustainable management and protection of aquatic resources and