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Ploidy-, gender-, and dose-dependent alteration of selected biomarkers in *Clarias gariepinus* treated with benzo[*a*]pyrene

Ali Karami[1,*], *Swee J. Teh*[2], *Mohamad Pauzi Zakaria*[3], *Simon C. Courtenay*[4]

1. *Laboratory of Aquatic Toxicology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Selangor, Malaysia. E-mail: alikaramiv@gmail.com*
2. *School of Veterinary Medicine, Department of Anatomy, Physiology, and Cell Biology, University of California, Davis, CA 95616, USA*
3. *Centre of Excellence for Environmental Forensics, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400, UPM, Selangor, Malaysia*
4. *Department of Environment and Resource Studies, Canadian Water Network, Canadian Rivers Institute, University of Waterloo, Canada*

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ABSTRACT

Naturally-occurring and artificially-induced polyploids have been documented in various fish species but to date no comparison has been reported of the impacts of ploidy on fish biomarker responses to organic pollutants. This study describes effects of ploidy, gender, and dose on biliary fluorescent aromatic compound (FAC) concentrations, hepatic ethoxyresorufin-O-deethylase (EROD) and glutathione S-transferase (GST) activities in one of the most commonly cultured warm-water species, the African catfish *Clarias gariepinus*. Recently matured male and female diploid and triploid fish were intraperitoneally (i.p.) injected with 0, 5 or 25 mg/kg benzo[*a*]pyrene (BaP) and liver and gallbladder were sampled 48 hr later. No significant differences were found between ploidies in bile concentrations of 7,8 dihydrodiolbenzo[*a*]pyrene (7,8D BaP), 1-hydroxybenzo[*a*]pyrene (1-OH BaP) or 3-hydroxybenzo[*a*]pyrene (3-OH BaP). However, concentrations of the biliary FACs did differ between males and females at different dose of injection with generally higher concentrations in females at the low dose of BaP and higher concentrations in males at the higher BaP concentration. Hepatic EROD activity did not exhibit gender-dependent difference, whereas it was significantly higher in triploids than diploids. GST activities were not significantly influenced by any of the tested factors. This work advanced our understanding of the role of ploidy, gender, and dose in biotransformation of pollutants in fish.

Introduction

Fish are the most diversified and wide spread vertebrates on the planet (Nelson, 2006) increasing the chance of their exposure to a wide range of pollutants discharged into aquatic environments. Some studies have highlighted the influence of confounding factors (*e.g.*, age, gender, length, water temperature, and nutritional status) on fish physiological responses towards water pollutants (Široká and Drastichova, 2004; Napierska and Podolska, 2005). However, reports on the influence of ploidy on fish responses to pollutants are scarce.

Polyploidy occurs naturally in several orders of fish including the Perciformes and the Cypriniformes (Comber and Smith, 2004). Triploid cells possess 50% more genes as compared to the diploids. Number and expression of genes and their interactions define organisms' responses to

* *Corresponding author*. E-mail address: alikaramiv@gmail.com (Ali Karami).

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environmental conditions (Ching et al., 2009). Triploid fish have fewer but bigger cells than their diploid counterparts (Benfey, 1999). This phenomenon reduces the ratio of cell surface area to volume and, therefore, could affect cellular performance (*e.g.*, enzyme abundance, recovery after stress, diffusion) (Hyndman et al., 2003). Some studies have reported inconsistent trends of responses of diploid and triploid fish towards stressors. Juvenile triploid silver catfish (*Rhamdia quelen*) exhibited greater sensitivity than diploids to ammonia during the first 48 hr of exposure but comparable sensitivity after 96 hr (Weiss and Zaniboni-Filho, 2009). Atkins and Benfey (2008) reported higher metabolic rates in triploid than diploid Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) at low temperatures but lower metabolic rates at high temperatures. Orrego et al. (2008) reported ethoxyresorufin-O-deethylase (EROD) induction and elevated plasma vitellogenin levels in triploid rainbow trout (*Oncorhynchus mykiss*) exposed to pulp mill effluent but no comparison was made with diploid fish. Despite the enormous importance of triploid fish for the aquaculture industry, no study has yet investigated the effects of organic pollutants on any triploid fish species.

The role of gender on toxicity of polycyclic aromatic hydrocarbons (PAHs) in aquatic organisms is well documented (Fossi et al., 2002). In sexually mature fish, females often exhibit lower levels of detoxifying enzymes than males and may therefore have lower levels of biliary fluorescent aromatic compounds (FACs) compared to males (Vuorinen et al., 2006). In Turbot (*Scophthalmus maximus* L.), sexually mature females showed lower cytochrome P4503A (CYP3A) levels than males (Arukwe and Goksøyr, 1997). Similarly, Winzer et al. (2002a) reported decreased supply of hepatocytic NADPH, reduced and/or delayed NADPH-dependent activity of CYP450 and a lower capacity of reduced glutathione (GSH) in female than male European flounder (*Platichthys flesus*) exposed to BaP.

Polycyclic aromatic hydrocarbons are a ubiquitous group of organic pollutants in aquatic environments (Wang et al., 2014). Fish possess phase I enzymes of mixed-function monooxygenase that convert PAHs into more hydrophilic components such as phenols, dihydrodiols and quinines which can then be eliminated from the body (Liu et al., 2014). Hence, quantifying PAHs in fish tissue may underestimate the exposure level (Beyer et al., 2010). Following conjugation with some molecules (*e.g.*, glutathione) through phase II enzymes (*e.g.*, glutathione S-transferase (GST)), biotransformed PAHs are stored in the gall bladder before excretion from the body (Varanasi et al., 1989). Benzo[*a*]pyrene is a member of the PAHs family and is a well-known oxidative stress inducer (Winzer et al., 2002b; Wang et al., 2014). Biliary FACs are reliable and sensitive biomarkers of recent PAH exposure in fish (van Schanke et al., 2001). The three main BaP FACs reported in fish are 7,8-dihydrodiolbenzo[*a*]pyrene (7,8D BaP) and the two phenolic compounds, 1-hydroxybenzo[*a*]pyrene (1-OH BaP) and 3-hydroxybenzo[*a*]pyrene (3-OH BaP) and were therefore used as the selected biliary FACs in this study (van Schanke et al., 2000; Telli-Karakoç et al., 2002; Karami et al., 2012b).

The main objective of this study was to evaluate the role of gender and ploidy in *Clarias gariepinus* on concentrations of glucuronides and sulfate conjugated biliary FACs, EROD and GST activities at different doses of BaP following intraperitoneal (i.p.) injection. Injection of substances into the peritoneal cavity is commonly used for exposing small laboratory animals to target compounds (Turner et al., 2011).

African catfish (*C. gariepinus*) is among the leading food fishes in tropical and subtropical countries (Adewolu et al., 2008). To our knowledge no study to date has investigated the role of ploidy on fish biomarkers in combination with gender and/or dose of organic pollutants.

1. Materials and methods

1.1. Chemicals

Colchicine, BaP, 1-chloro-2,4-dinitrobenzene (CDNB), dithiothreitol (DTT), GSH, 7-ethoxyresorufin (7-ER) and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical (USA); β-glucuronidase/arylsulfatase (30/60 U/mL, from *Helix pomatia*) and Giemsa from Merck (Germany); 7,8-D BaP, 1-OH BaP, and 3-OH BaP were supplied by the Mid-west Research Institute (USA); methanol and acetone (HPLC grade) were purchased from JT Baker (USA); 0.2 μm syringe filter was obtained from Whatman (UK); and distilled water (HPLC grade) was produced in the laboratory.

1.2. Broodstock

Immature *C. gariepinus* were purchased from local farmers in Selangor, Malaysia and raised for 4 months until reaching maturity in 1000-L fibreglass tanks under a 12-hr light and dark cycle (12:12). Throughout the study, fish were fed *ad-libitum* with commercial pellets (Cargill, Malaysia). The mean (±SE) water temperature and dissolved oxygen were 27.2°C (±0.1) and 6.9 mg/L (±0.09), respectively.

1.3. Fish breeding and triploidy induction

Male and female broodstocks were injected with 0.25 mL/kg and 0.5 mL/kg body weight of Ovaprim®, respectively, 10 hr prior to breeding. Eggs and milt were mixed together and then divided into two batches 3 min later. The second batch was cold-shocked 3 min after fertilization at 5°C for 40 min to induce triploidy as described by Richter et al. (1986). Shocked and unshocked eggs were incubated separately in fibreglass tanks filled with 200 L of UV-sterilized water. Primary triploidy induction success rate was evaluated through chromosomal preparation of 2 day post-hatch larvae according to Karami et al. (2010). Chromosomal spreads with 56 and 84 chromosomes were considered as diploid ($2n = 56$) and triploid ($3n = 84$), respectively (Miskolczi et al., 2005).

Fish from both ploidy groups were fed three times a day *ad-libitum* and reared for 5 months in 1000-L fibreglass tanks prior to the start of the experiment. Recently matured fish from diploid and triploid groups were sexed according to the size, shape and colour of genital papilla and size of the belly. Individuals were labelled by plastic T-bar anchor tags. Triploidy was confirmed through evaluating size and shape