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# Alterations in juvenile diploid and triploid African catfish skin gelatin yield and amino acid composition: Effects of chlorpyrifos and butachlor exposures☆

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ABSTRACT

Skin is a major by-product of the fisheries and aquaculture industries and is a valuable source of gelatin. This study examined the effect of triploidization on gelatin yield and proximate composition of the skin of African catfish (*Clarias gariepinus*). We further investigated the effects of two commonly used pesticides, chlorpyrifos (CPF) and butachlor (BUC), on the skin gelatin yield and amino acid composition in juvenile full-sibling diploid and triploid African catfish. In two separate experiments, diploid and triploid *C. gariepinus* were exposed for 21 days to graded CPF [mean measured: 10, 16, or 31 μg/L] or BUC concentrations [Mean measured: 22, 44, or 60 μg/L]. No differences in skin gelatin yield, amino acid or proximate compositions were observed between diploid and triploid control groups. None of the pesticide treatments affected the measured parameters in diploid fish. In triploids, however, gelatin yield was affected by CPF treatments while amino acid composition remained unchanged. Butachlor treatments did not alter any of the measured variables in triploid fish. To our knowledge, this study is the first to investigate changes in the skin gelatin yield and amino acid composition in any animal as a response to polyploidization and/or contaminant exposure.



## 1. Introduction

Gelatin is a high molecular weight protein compound produced by the partial denaturation or hydrolysis of collagen (Gómez-Guillén et al., 2011). It has been used extensively by different food and health industries (Jellouli et al., 2011; Karim and Bhat, 2009). Skin and bone of animals particularly pig and cattle are the main sources of commercial gelatin production (Mariod and Adam, 2013). Fish have not been used as a major source of gelatin in the past but that is changing. Fish processing by-products constitute the major portion of the catch (Wasswa et al., 2007) and because of religious barriers and/or safety considerations about the consumption of bovine and porcine gelatins, fish skin has become a promising source for gelatin production (Chiou et al., 2008; Kittiphattanabawon et al., 2016).

Despite the widespread distribution of pesticides in aquatic environments (Masiá et al., 2013), very little is known about the effects of contaminants on nutritional parameters in fish. Narra et al. (2011) showed a reduction in total free amino acids in the gill, kidney, liver, and muscle of walking catfish (*Clarias batrachus*) after a 28-day sub-lethal exposure to the commonly used pesticide chlorpyrifos (CPF). In another study, chlorantraniliprole decreased free amino acid contents in the kidney, liver, gill, and muscle of Indian carp (*Labeo rohita*) (Bantu et al., 2013). Chlorpyrifos, an organophosphate (OP) insecticide, and butachlor (BUC), an organochlorine (OC) herbicide, are widely employed in paddy fields to control insects and weeds, respectively (Zhang et al., 2012; Zhu et al., 2014). Toxicities of CPF and BUC to aquatic organisms have been reported by earlier studies (Baorong et al., 2015; Deb and Das, 2013). African catfish (*Clarias gariepinus*) is an important source of protein in tropical and subtropical countries, which is cultivated in

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and around paddy fields (Marimuthu et al., 2013). Hence, the presence of different classes of pesticides in natural and man-made aquatic ecosystems could adversely influence the health of this species.

Biochemical parameters are regarded as sensitive and robust biomarkers of OP and OC pesticide exposure and effect in fish (Karami-Mohajeri and Abdollahi, 2010; Kavitha and Rao, 2008). However, the potential of gelatin metabolism in fish as a biomarker response has never been investigated before. Mayer et al. (1977) introduced collagen metabolism as a sensitive indicator of the chronic effects of four organic chemicals in rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*). The present study is the first, to our knowledge, to elucidate the impacts of contaminants on gelatin yield or amino acid composition in fish skin.

Triploid organisms possess 50% more genes relative to their diploid counterparts. The presence of an extra set of chromosomes in triploid organisms can result in an increase in the cell size and a decrease in cell number (Benfey, 1999). These fundamental changes in cellular and tissue structure may in turn result in physiological differences between diploid and triploid organisms. Triploid fish have been used in the aquaculture industry due to their sterility and higher growth rate (Burke et al., 2010; Ruiz-Verdugo et al., 2000), superior flesh quality (Bjørnevik et al., 2004; Poontawee et al., 2007) and higher disease resistance (Nichols, 2009) than diploid fish. The majority of studies on the impacts of polyploidization on nutritional values has focussed on plants. For example, tetraploid kaffir potato (*Plectranthus esculentus*) were shown to have higher starch content than their diploid counterparts (Hannweg et al., 2016). In contrast, very little is known about the effects of polyploidization on nutritional values in animals but there is reason to think that differences from diploids may exist. Fillets of triploid *O. mykiss* contained 3% higher crude fat and 6% lower moisture than diploids, while the protein content remained unchanged (Poontawee et al., 2007).

Despite the promising use of triploid fish in the aquaculture industry, studies on their physiological responses following exposure to contaminants are scarce. Information on biomarker responses in polyploid animals following exposure to contaminants is limited to our three recent studies in triploid *C. gariepinus* (Karami et al., 2016b, 2015; Karami and Courtenay, 2015).

The first hypothesis ($H_{01}$) tested in the present study was that there is no significant difference in gelatin yield, amino acid content and proximate composition (i.e., moisture, lipid, ash and protein content of extracted gelatin) between diploid and triploid *C. gariepinus*. Secondly, we hypothesized ($H_{02}$) that CPF and BUC exposures have no impacts on gelatin yield and amino acid composition in diploid and triploid *C. gariepinus*. Therefore, the first aim of this study was to investigate the effects of ploidy on skin gelatin yield, amino acid and proximate compositions in *C. gariepinus*. Secondly, we examined the effects of waterborne CPF or BUC exposures on skin gelatin yield and amino acid composition in diploid and triploid *C. gariepinus*.

## 2. Material and methods

### 2.1. Chemicals

Chlorpyrifos (purity 99.8%), BUC (Pestanal® analytical standard grade), phosphate buffer saline (PBS), propidium iodide, RNase and amino acid standards were purchased from Sigma Chemical (USA). AccQ.Fluor reagent kit and AccQ.Tag eluent were purchased from Waters Corporation (Milford, Massachusetts, USA). Sodium hydroxide, hydrochloric acid, methanol, acetic acid, sodium sulfate, sodium chloride were obtained from R&M chemicals (Essex, UK). Coomassie brilliant, Tris-HCl buffers and protein marker were purchased from Bio Rad (USA). Dichloromethane (DCM), HPLC-grade ethanol and acetone, and kjeldahl tablets were obtained from Fisher Scientific (UK); and sodium dodecly sulphate (SDS) and tetramethylethylenediamine (TEMED) were supplied by Merck (Germany).

### 2.2. Experimental design

A flow diagram of the experimental design is presented in Fig. 1.

#### 2.2.1. Fish breeding and triploidy induction

Brood stock fish were injected with 0.5 mL/kg (♀) and 0.25 mL/kg (♂) body weight of Ovaprim® 10 h before breeding. Eggs and milt were gently mixed for 3 min and then divided into two batches. The first batch (diploids) proceeded with incubation in a 2000 L fiberglass tank filled with 500 L UV-sterilized water. The second batch (triploids) was subjected to a cold shock at 5 °C for 40 min (Karami et al., 2010; Richter et al., 1987) and incubated in a separate fiberglass tank. Initially, larvae were fed *ad libitum* with newly hatched *Artemia* nauplii, 3–5 times a day for one week. Then, fish were fed at a rate of 5–10% of body weight three times a day with fish powder (Cargill, crude protein: 38–40%, crude fat: 3%, crude fiber: 6%, Moisture content: 13%) during the early stage and later fed with commercial pellets (Star Feed, crude protein: 45%, crude fat: 6%, crude fiber: 4%, Moisture content: 12%).

#### 2.2.2. Triploidy confirmation

Ploidy of individuals from the shocked group (presumed to be triploid) was confirmed by flow cytometric measurement of nuclear DNA content of erythrocytes using the method adopted by Darzynkiewicz et al. (1997) with some modifications as described by Karami et al. (2016b). Specimens were implanted with plastic T-bar anchor tags in their dorsal musculature. Blood (2 μL) was sampled and diluted in cold PBS, then with chilled HPLC grade ethanol, and stored at 4 °C for 48 h. The mixture was centrifuged and the pellet was washed twice with cold PBS and re-suspended in PBS, after which propidium iodide and RNase were added. Finally, mean fluorescence intensity (Gmean) was measured using BD LSR Fortessa and FACSDiva software (BD Biosciences).

#### 2.2.3. Exposure to the pesticides

*2.2.3.1. CPF exposure.* Fourteen week old diploid and triploid fish were divided among 324 L glass aquaria filled with UV treated water (5 fish per aquarium, one aquarium per treatment) and acclimatized for one week prior to the exposure. Stock solutions were prepared every three days by dissolving CPF in HPLC-grade acetone and kept refrigerated in dark. Diploids (mean weight ± SD: 144.63 ± 20.42 g, mean total length: 28.30 ± 1.71 cm; n = 25) and triploids (mean weight ± SD: 189.32 ± 40.00 g, mean total length: 30.11 ± 2.39 cm; n = 25) were exposed one of three nominal concentrations of CPF (50, 100, and 150 μg/L) for 21 day. Aquarium water was removed daily, 70% in the morning and 30% in the evening and replaced with freshly-spiked water each day. Three negative control groups (three aquaria, 15 fish, 5 fish per aquarium) and one solvent (acetone: <0.001% v/v) control group were used for each ploidy. Average ± SD water temperature was 27.08 ± 0.73 °C, pH 6.69 ± 0.24, dissolved oxygen 6.44 ± 0.61 mg/L, total hardness 60.65 ± 5.98 mg $CaCO_3$, alkalinity 39.85 ± 6.67 mg $CaCO_3$, and salinity <1 mg/L.

*2.2.3.2. BUC exposure.* Fifteen week old diploid and triploid fish were distributed among 324 L glass aquaria (5 fish per aquarium, one aquarium per treatment). Stock solutions of BUC in HPLC-grade