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# Changes in nutritional parameters in diploid and triploid African catfish *Clarias gariepinus* following chlorpyrifos exposure

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ABSTRACT: There is a paucity of information about the influence of ploidy status on physiological parameters in organisms following contaminant stress. Also, little is known about the changes in nutritional values of fishes in response to the exposure to contaminants. Full-sibling juvenile *Clarias gariepinus* were exposed to 3 levels (mean measured 8.9, 17.5, or 28.0 µg $l^{-1}$) of chlorpyrifos for 21 d. The levels of 3 fatty acids (FAs) in the muscle were significantly different between unexposed diploid and triploid fish. In the chlorpyrifos-exposed fish, the levels of most amino acids (AAs), some FAs, protein, and moisture content were affected in the muscle of diploids, while the levels of some FAs and ash content were changed in the muscle of triploid fish. This work represents the first study of changes in FAs and proximate composition in triploid animals in response to environmental stressors. Fewer changes of nutritional values in triploid *C. gariepinus* under chlorpyrifos exposure may indicate higher adaptability of triploids than diploids towards environmental stressors. Significant changes in the muscle protein content and AA and FA compositions in diploid *Clarias gariepinus* in response to chlorpyrifos exposure suggest their application as sensitive indicators of aquatic environmental pollution.

KEY WORDS: Triploid · Diploid · Organophosphate pesticide · Nutritional values

## INTRODUCTION

Fish are ubiquitous in most aquatic environments, representing an important component of the food chain, and are a rich source of amino acids (AAs), fatty acids (FAs), and protein to humans (Tidwell & Allan 2001). AAs are the building blocks of proteins, which play a vital role in regulating key metabolic pathways necessary for health, growth, development, reproduction, and homeostasis of organisms (Wu 2009). FAs are a major source of energy and are the chief constituents of cell membranes. Polyunsaturated fatty acids (PUFAs) serve as the precursors to potent lipid mediator signalling molecules, namely eicosanoids, that play various roles in therapeutic benefits for human health such as blood pressure regulation and immune responses (Dunbar et al. 2014). Lipids and proteins are the main organic components in fishes, playing major roles in the fish's life history and physiology (Tocher 2003).

Extensive application of pesticides in modern agriculture has resulted in their widespread distribution

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in the environment (Yadav et al. 2015). The release of pesticides into aquatic ecosystems via spray drift, volatilization, wind erosion of soil, and accidental spillage may exert severe adverse effects on non-target organisms (Gill & Garg 2014). Chlorpyrifos (O,O-diethyl O-3, 5, 6-trichloropyridin-2-pyridinyl phosphorothioate) is a broad-spectrum organophosphate (OP) insecticide that has been proved to be toxic to non-target animals including fishes (Barron et al. 1991). The factors that affect the environmental concentrations of chlorpyrifos in water bodies vary according to physical parameters, namely size of the water body, properties and quality of the soil, and application concentrations. Temporal factors including rainfall events, frequency of application, and season may also contribute to the environmental concentration of chlorpyrifos. Concentrations of chlorpyrifos in small water bodies adjacent to croplands have been reported to range from 73 to over 700 µg $l^{-1}$ (Moore et al. 2002, USEPA 2002, Mazanti et al. 2003).

Biochemical parameters such as protein and lipid concentrations in tissue are regarded as sensitive indicators in fish exposed to OP pesticides (Almeida et al. 2010). However, limited information is available regarding the effects of environmental contaminants on levels of nutritional parameters such as AAs and FAs (Zuraini et al. 2006). For example, changes in FA compositions were employed to evaluate stress responses to trace elements and polycyclic aromatic hydrocarbons in Mediterranean mussels *Mytilus galloprovincialis* (Signa et al. 2015). In another study, a 28 d exposure to sublethal chlorpyrifos concentrations decreased total protein, free AA, and ammonia contents in the gill, kidney, liver, and muscle of walking catfish *Clarias batrachus* (Narra et al. 2011).

Cells of triploid organisms have 3 sets of chromosomes, compared to 2 in diploids (Piferrer et al. 2009). The unbalanced chromosomal number in triploid fish cells may hinder meiotic division and the production of gametes (Gold & Avise 1976). The channelled energy from reproduction into somatic growth may cause the better growth performance of triploid fish as compared to their diploid counterparts (e.g. Fast et al. 1995, Karami et al. 2016a). Triploidy has been shown to be naturally occurring in several fish species (e.g. *Carassius auratus*; Murayama et al. 1986, Xiao et al. 2011). Despite the presence of triploid fish in aquatic environments and their promising application in the aquaculture industry (e.g. salmonids, Mayer 2015), reports on the impacts of contaminants on the physiological responses of polyploid fish are limited to our recent studies (e.g. Karami et al. 2015b, 2016a,b,c,d).

African catfish *C. gariepinus* is a major source of animal protein for humans and animals in tropical and subtropical regions (Adewolu et al. 2008) and is farmed in and around agricultural lands (Marimuthu et al. 2013).This species is especially suited for aquaculture due to its high fecundity and its resistance to poor oxygen content due to auxiliary respiratory organs (Toko et al. 2007).

The primary objectives of this study were to compare AA, FA, and proximate compositions in the skeletal white muscle of unexposed diploid and triploid *C. gariepinus* and to investigate the effect of chlorpyrifos exposure on these nutritional values in diploid and triploid *C. gariepinus*. The chlorpyrifos concentrations used in the study (nominal concentrations) were 50, 100, and 150 µg $l^{-1}$, levels that are within the range observed in small water bodies or what has been used in recent ecotoxicological studies (Nwani et al. 2013, Jin et al. 2015).

## MATERIALS AND METHODS

### Triploidy induction

Broodstock were obtained from local farmers in Selangor, Malaysia, and raised for 12 mo in 2000 l fiberglass tanks. Triploid *Clarias gariepinus* were produced as described by Karami et al. (2010). Briefly, mature male and female *C. gariepinus* were injected with Ovaprim® 10 h before breeding. Eggs and milt were gently mixed with a bird feather for 3 min and then divided into 2 batches. Triploids were produced from the first group by cold-shocking of the fertilized eggs at 5°C for 40 min. The second batch received no treatment and, therefore, was considered as diploid. Each batch of eggs was transferred to a 2000 l fiberglass tank filled with 500 l UV-sterilized water. To confirm successful initial triploidy induction, the chromosomal spreads of 20 larvae from the cold shock treated groups were prepared according to the method of Karami et al. (2015a). Larvae were initially fed ad libitum with freshly hatched *Artemia* nauplii 3 times a day for 1 wk. Then, fish were fed at a rate of 5 to 10% of body weight with fish powder (Cargill, crude protein: 38 to 40%, crude fat: 3%, crude fiber: 6%, moisture content: 13%) for 2 wk and then with commercial pellets (Star Feed, crude protein: 45%, crude fat: 6%, crude fiber: 4%, moisture content: 12%) for 11 wk prior to the start of the experiment. Total lengths and weights of fishes were measured after 14 wk of growth.

### Triploidy confirmation

Individual fish from the shocked group were labelled with plastic T-bar anchor tags followed by blood sampling. The ploidy status of each fish from the shocked group was confirmed by determining nuclear DNA content of erythrocytes using flow cytometry (LSR Fortessa, BD Biosciences) (Karami et al. 2016a).

### Experimental design

Prior to exposure, 14 wk old diploid and triploid fish were acclimatized in 84 l glass aquaria filled with UV-treated water (1 fish per aquarium, 5 fish per replicate per treatment) for 1 wk. Stock solutions of chlorpyrifos (analytical standard grade, Sigma-Aldrich) were prepared in HPLC-grade acetone (Fisher Scientific) every 3 d and kept refrigerated in dark bottles. Following OECD guidelines (OECD 1994, 2011), a solvent (acetone <0.001% V/V) control group was applied in our experiment to assess the toxicity of acetone. Diploids (mean ± SD weight: 140 ± 19 g, total length: 27.9 ± 1.4 cm; n = 25) and triploids (weight: 184 ± 31 g, total length: 29.7 ± 3.2 cm; n = 25) were exposed to 1 of 3 nominal concentrations of chlorpyrifos (50, 100, or 150 µg $l^{-1}$) for 21 d. Besides a solvent control group, a negative control (chlorpyrifos-free water) group was used for diploid and triploid fishes. To minimize the stress on the fish posed by the water change, every day, 70% of the test solution was refreshed in the morning and 30% in the evening (10 h later) (Karami et al. 2016a). During the exposure period, fish were fed ad libitum once daily with commercial pellets (Star Feed). Adequate aeration was provided using airstone blowers. Water quality parameters were measured every 3 d throughout the experiment, mean ± SD (n = 42): temperature 28.0 ± 0.97°C, pH 6.81 ± 0.34, dissolved oxygen 6.59 ± 0.78 mg $l^{-1}$, total hardness 58.1 ± 4.4 mg $CaCO_3$, alkalinity 37 ± 6.5 mg $CaCO_3$, and salinity <1 mg $l^{-1}$. At the end of the experiment, fish were sacrificed by an overdose of clove oil. The skeletal muscles (tissue samples) were taken from the same section of each organism for analysis of each parameter to minimize experimental variability.

### Measured chlorpyrifos concentrations

Every 3 d, water samples were collected in 500 ml amber glass bottles (Schott) from each treatment 30 min before and after water changes in the morning and also 30 min before water change in the evening. Water samples were analysed for chlorpyrifos according to the procedure of Mamun et al. (2009) with some modifications as described by Karami et al. (2016a). Chlorpyrifos concentrations were analysed by gas chromatography (GC, Agilent 6890N) equipped with an electron capture detector and an HP-5 capillary column (30 m × 0.25 mm inside diameter × 0.25 µm film thickness). The carrier gas was nitrogen at a flow rate of 1 ml $min^{-1}$. The extraction recovery was calculated by spiking the distilled water with chlorpyrifos at concentrations of 0.1, 0.01, and 0.001 µg $ml^{-1}$. Average recoveries (SD) (n = 3) for 0.1, 0.01, and 0.001 µg $ml^{-1}$ spiked samples were 110 (3.1), 99.2 (4.8), and 88.3% (2.6), respectively.

### AA analysis

The AA levels of muscle samples were determined with an AccQ-Fluor™ Reagent Kit (Waters) using the AccQ-Tag™ AA analysis method. Muscle tissues (0.2 g) were added to 5 ml of 6 M HCl (R & M Chemicals) in a high temperature resistant bottle and incubated in an oven at 105°C for 24 h (AOAC 1984). The solution was diluted to 100 ml with distilled water and filtered through a syringe filter (PVDF, 0.2 µm, Jet Biofil). Then, 40 µl of the internal standard L-aminobutyric acid (AABA, Sigma) was added to 960 µl of treated sample. For derivatization, 10 µl of sample was mixed with 70 µl of AccQ-Fluor borate buffer reagent and 20 µl of AccQ-Fluor reagent. Prior to injection into HPLC, the derivatized compound was heated on a digital block heater at 55°C for 10 min. AA composition was analysed using HPLC (Waters Alliance System e2695) equipped with fluorescence detection (Waters 2475 multi-λ fluorescence detector) set at 250 nm excitation and 395 nm emission. AAs were separated on an AccQ-Tag AA analysis column (150 × 3.9 mm, 4 µm, RP18, Waters). The column was operated at 31°C. The eluent system consisted of the aqueous solution of AccQ-Tag™ eluent reagent (Waters) and 60% acetonitrile (Fisher Scientific) at a flow rate of 1 ml $min^{-1}$.

### FA analysis

Total FA of muscle tissue was extracted according to the method of Rajion et al. (1985) as described by Ebrahimi et al. (2012). Briefly, 7 ml of chloroform–methanol (2:1 V/V, R & M Chemicals) was mixed

with 1 g of minced muscle and shaken by vortex. The mixture was centrifuged at 3000 rpm for 5 min, and the lower layer was dried by nitrogen gas after transferring to the methylation tube. The extracted FAs were transmethylated to their FA methyl esters (FAME) using 0.66 M KOH and 14% methanolic boron trifluoride (Sigma) (AOAC 1999). The FAME was separated and quantified by gas liquid chromatography using an Agilent 7890A GC system equipped with a 100 m × 0.25 mm inside diameter (0.20 µm film thickness) Supelco SP-2560 capillary column and a flame ionization detector. Helium was used as the carrier gas. The column temperature program was set at 100°C, held for 2 min, heated to 170°C at 10°C $min^{-1}$, held for 2 min, heated again to 220°C at 7.5°C $min^{-1}$, and subsequently held for 20 min. The injector and detector temperatures were kept at 250 and 300°C, respectively. A reference standard (mix C4-C24 methyl esters, Sigma-Aldrich), conjugated linoleic acid standard mixture (O-5507, Sigma-Aldrich) and conjugated linolenic acid standard mixture (47792, Supelco) were used to measure recoveries and correction factors for the evaluation of individual FA levels.

### Proximate composition analysis

Proximate composition (protein, lipid, moisture, and ash) of muscle tissue was determined according to the procedures of AOAC (2000). Protein content was assessed by the Kjeldahl method (protein = total nitrogen × 6.25). Lipid was extracted with petroleum ether (boiling point, 60 to 80°C, R & M Chemicals) using a Soxhlet extractor. Moisture content was quantified by drying the sample in an oven at 105°C for 24 h until obtaining a constant weight. Ash content was assessed by incinerating a muscle sample at 550°C in a Thermolyne 62700 muffle furnace.

### Statistical analysis

Data were assessed for normality with the Shapiro-Wilks test and for homogeneity of variance with Levene's test. Data were log transformed where necessary. The AA, FA, and proximate compositions of the fish belonging to the solvent and negative control groups were compared using Student's *t*-test (OECD 2011). Data from the 2 groups were pooled if no significant differences were observed (OECD 2011). AA and FA levels and protein, ash, lipid, and moisture contents of diploid and triploid fish from the control groups were compared using Student's *t*-test. Within each ploidy, alterations in the AA and FA levels and protein, lipid, moisture, and ash contents across chlorpyrifos concentrations were analysed using 1-way ANOVA, with post hoc comparisons using Tukey's test if a significant difference ($p < 0.05$) was found. All statistical analyses were performed using IBM SPSS Statistics (v.22).

## RESULTS

### Measured chlorpyrifos concentrations

The measured chlorpyrifos concentrations in 50, 100, and 150 µg $l^{-1}$ treatments were (mean ± SD) 8.9 ± 2.7, 17.5 ± 5.8, and 28.0 ± 6.3 µg $l^{-1}$, respectively. In the negative and solvent control groups, the chlorpyrifos concentration was below the detection limit. The detection limit was 0.002 µg $l^{-1}$ for the tested chlorpyrifos.

### Effect of chlorpyrifos on proximate composition

Moisture, lipid, protein, and ash contents in *Clarias gariepinus* muscle were not significantly different between solvent and negative control groups in diploid or triploid fish (Student's *t*-test, $p > 0.05$). The muscle tissues of diploids in the 28 µg $l^{-1}$ chlorpyrifos treatments had significantly ($p < 0.05$) lower protein (ANOVA, $F_{3,21} = 15.2$; Fig. 1A) content (1.10%). Moisture content (ANOVA, $F_{3,21} = 3.98$; Fig.1B) was greater (1.02%) in the 28 µg $l^{-1}$ chlorpyrifos treatment compared to the 8.9 µg $l^{-1}$ chlorpyrifos treatment in diploid fish. Also, ash content (ANOVA, $F_{3,21} = 4.57$; Fig. 1C) was greater (2.24%) in the 17.5 µg $l^{-1}$ chlorpyrifos treatment compared to the 8.9 µg $l^{-1}$ chlorpyrifos treatment in triploid fish.

### Effect of chlorpyrifos on AA levels

Individual AA levels or their sum (total AAs) were not significantly different between solvent and negative control groups in diploid and triploid fish (Student's *t*-test, $p > 0.05$), so they were pooled together as total AAs for subsequent analyses. There were no significant differences in AA levels between unexposed diploid and triploid *C. gariepinus* (Student's *t*-test, $p > 0.05$).

The muscles of diploids in the 28 µg $l^{-1}$ chlorpyrifos treatments had significantly ($p < 0.05$) decreased lev-

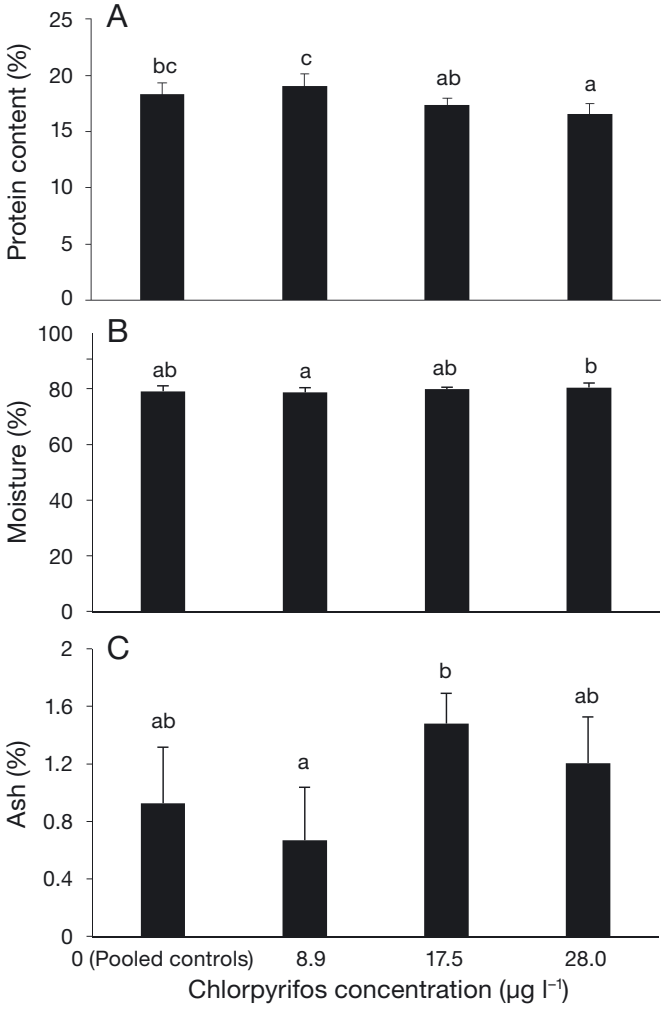


Fig. 1. Mean + SD of (A) protein and (B) moisture contents in the muscle of diploid and (C) ash content in the muscle of triploid *Clarias gariepinus* across different chlorpyrifos treatments. Bars with different letters are significantly different ($p < 0.05$, Tukey's multiple range tests); n = 5 for all treatments except for pooled controls (0 µg $l^{-1}$), where n = 10

els of aspartic acid (1.09 times) (ANOVA, $F_{4,20} = 9.10$), serine (1.11 times) (ANOVA, $F_{4,20} = 8.08$), glutamic acid (1.10 times) (ANOVA, $F_{4,20} = 10.18$), histidine (1.18 times) (ANOVA, $F_{4,20} = 5.34$), alanine (1.08 times) (ANOVA, $F_{4,20} = 4.81$), tyrosine (1.12 times) (ANOVA, $F_{4,20} = 9.59$), valine (1.12 times) (ANOVA, $F_{4,20} = 6.27$), lysine (1.11 times) (ANOVA, $F_{4,20} = 8.21$), isoleucine (1.12 times) (ANOVA, $F_{4,20} = 6.31$), leucine (1.10 times) (ANOVA, $F_{4,20} = 8.00$), phenylalanine (1.12 times) (ANOVA, $F_{4,20} = 6.98$), and total AAs (1.10 times) (ANOVA, $F_{4,20} = 8.47$) compared to the control group. In triploids, only the levels of cysteine were significantly lower (1.37 times) in the 17.5 µg $l^{-1}$ treatment compared to the 8.9 µg $l^{-1}$ treatment (ANOVA, $F_{4,20} = 3.79$). The AAs for which significant changes in AA levels were observed across different chlorpyrifos exposures are shown in Table 1.

### Effect of chlorpyrifos on FA levels

In both ploidies, the level of palmitic acid (C16:0) was significantly different between negative and solvent controls (Student's *t*-test, $p < 0.05$). In triploids, acetone treatment resulted in significant differences in the level of total PUFAs compared to the negative control group. The levels of arachidonic acid (C20:4n-6), total saturated FAs (SFAs), and total unsaturated FAs (UFAs) were significantly different between the control and solvent controls in diploid fish (Student's *t*-test, $p < 0.05$). The levels of most of the FAs were not significantly different between the control diploid and triploid fish except for myristic acid (C14:0) (Student's *t*-test, $t_{18} = -2.32$), which was 1.11 times higher in triploids, and docosahexaenoic acid (C22:6n-3) (Student's *t*-test, $t_{18} = 2.44$) and total omega-3 FAs (Student's *t*-test, $t_{18} = 2.35$), which were 1.23 and 1.16 times greater in diploids than triploids, respectively.

In diploids, exposure to 17.5 µg $l^{-1}$ chlorpyrifos resulted in significantly ($p < 0.05$) higher levels of C16:0 (1.02 times) (ANOVA, $F_{4,20} = 4.19$) and total SFAs (1.03 times) (ANOVA, $F_{4,20} = 4.62$) but lower total UFAs (1.01 times) (ANOVA, $F_{4,20} = 4.62$) compared to the control group. In triploids, the levels of stearic acid (C18:0) (ANOVA, $F_{4,21} = 4.44$) and docosapentaenoic acid (C22:5n-3) (ANOVA, $F_{4,21} = 3.44$) were significantly greater (1.14 and 1.72 times, respectively) in the 8.9 µg $l^{-1}$ chlorpyrifos treatments, and the levels of C22:6n-3 (ANOVA, $F_{4,21} = 3.29$) and total omega-3 fatty acids (ANOVA, $F_{4,21} = 3.26$) were significantly greater (1.41 and 1.33 times, respectively) in the 17.5 µg $l^{-1}$ chlorpyrifos treatments compared to the control group. Also, the level of C16:0 (ANOVA, $F_{4,20} = 6.77$) was significantly lower (1.11 times) in the 17.5 µg $l^{-1}$ chlorpyrifos treatment compared to the control group. The level of total SFAs was significantly lower (1.08 times) (ANOVA, $F_{4,21} = 4.84$) in the 17.5 µg $l^{-1}$ chlorpyrifos treatment compared to the 8.9 µg $l^{-1}$ chlorpyrifos treatment. In contrast, the level of total UFAs was significantly greater (1.05 times) (ANOVA, $F_{4,21} = 4.84$) in the 17.5 µg $l^{-1}$ chlorpyrifos treatment compared to the 8.9 µg $l^{-1}$ chlorpyrifos treatment. The muscle FA levels in diploid and triploid fish in response to different chlorpyrifos exposures are summarized in Table 2.

Table 1. Amino acid composition (mg $g^{-1}$ wet weight) in the skeletal muscle of diploid and triploid *Clarias gariepinus*. Data are mean ± SD; n = 5 for all treatments except for 0 µg $l^{-1}$ (pooled controls), where n = 10. Values in the same line with different letters indicate differences among groups ($p < 0.05$, Tukey's multiple range tests). *Significant ($p < 0.05$) differences compared with control value

| Amino acid | Ploidy | Chlorpyrifos concentration (µg $l^{-1}$) 0 | 8.9 | 17.5 | 28.0 |
|---|---|---|---|---|---|
| Aspartic acid | Diploid* | 1.81 ± 0.07$^{b}$ | 1.82 ± 0.03$^{b}$ | 1.73 ± 0.04$^{ab}$ | 1.65 ± 0.04$^{a}$ |
| | Triploid | 1.71 ± 0.08 | 1.77 ± 0.08 | 1.76 ± 0.07 | 1.80 ± 0.09 |
| Serine | Diploid* | 0.75 ± 0.04$^{b}$ | 0.74 ± 0.005$^{b}$ | 0.72 ± 0.006$^{ab}$ | 0.67 ± 0.01$^{a}$ |
| | Triploid | 0.73 ± 0.02 | 0.762 ± 0.03 | 0.72 ± 0.01 | 0.73 ± 0.01 |
| Glutamic acid | Diploid* | 2.71 ± 0.1$^{b}$ | 2.67 ± 0.008$^{b}$ | 2.59 ± 0.03$^{ab}$ | 2.46 ± 0.07$^{a}$ |
| | Triploid | 2.63 ± 0.15 | 2.721 ± 0.1 | 2.58 ± 0.02 | 2.67 ± 0.06 |
| Glycine | Diploid | 0.74 ± 0.07 | 0.78 ± 0.01 | 0.70 ± 0.02 | 0.67 ± 0.02 |
| | Triploid | 0.78 ± 0.07 | 0.712 ± 0.04 | 0.84 ± 0.02 | 0.80 ± 0.07 |
| Histidine | Diploid* | 0.44 ± 0.04$^{b}$ | 0.45 ± 0.01$^{b}$ | 0.41 ± 0.007$^{b}$ | 0.37 ± 0.01$^{a}$ |
| | Triploid | 0.42 ± 0.04 | 0.44 ± 0.06 | 0.414 ± 0.01 | 0.44 ± 0.03 |
| Arginine | Diploid | 1.09 ± 0.09 | 1.11 ± 0.05 | 1.1 ± 0.01 | 1.02 ± 0.01 |
| | Triploid | 0.98 ± 0.08 | 1.103 ± 0.05 | 0.97 ± 0.09 | 1.11 ± 0.07 |
| Threonine | Diploid | 0.85 ± 0.05 | 0.843 ± 0.009 | 0.82 ± 0.01 | 0.78 ± 0.03 |
| | Triploid | 0.83 ± 0.05 | 0.79 ± 0.03 | 0.81 ± 0.01 | 0.83 ± 0.01 |
| Alanine | Diploid* | 1.01 ± 0.04$^{b}$ | 1.00 ± 0.01$^{ab}$ | 0.97 ± 0.03$^{ab}$ | 0.93 ± 0.02$^{a}$ |
| | Triploid | 0.98 ± 0.03 | 1.01 ± 0.4 | 0.97 ± 0.01 | 1.01 ± 0.03 |
| Proline | Diploid | 0.57 ± 0.02 | 0.59 ± 0.01 | 0.57 ± 0.01 | 0.54 ± 0.01 |
| | Triploid | 0.58 ± 0.01 | 0.56 ± 0.02 | 0.56 ± 0.01 | 0.59 ± 0.02 |
| Cysteine | Diploid | 0.11 ± 0.01 | 0.115 ± 0.01 | 0.11 ± 0.003 | 0.10 ± 0.01 |
| | Triploid | 0.09 ± 0.01$^{ab}$ | 0.11 ± 0.008$^{b}$ | 0.08 ± 0.02$^{a}$ | 0.1 ± 0.02$^{ab}$ |
| Tyrosine | Diploid* | 0.63 ± 0.03$^{b}$ | 0.62 ± 0.01$^{ab}$ | 0.60 ± 0.01$^{ab}$ | 0.56 ± 0.02$^{a}$ |
| | Triploid | 0.60 ± 0.04 | 0.617 ± 0.02 | 0.60 ± 0.01 | 0.61 ± 0.01 |
| Valine | Diploid* | 0.8 ± 0.05$^{b}$ | 0.79 ± 0.008$^{ab}$ | 0.76 ± 0.01$^{ab}$ | 0.71 ± 0.03$^{a}$ |
| | Triploid | 0.78 ± 0.03 | 0.79 ± 0.03 | 0.78 ± 0.02 | 0.78 ± 0.01 |
| Methionine | Diploid | 0.47 ± 0.06 | 0.54 ± 0.02 | 0.46 ± 0.005 | 0.43 ± 0.05 |
| | Triploid | 0.43 ± 0.07 | 0.47 ± 0.03 | 0.43 ± 0.08 | 0.49 ± 0.04 |
| Lysine | Diploid* | 1.71 ± 0.09$^{b}$ | 1.68 ± 0.01$^{b}$ | 1.62 ± 0.02$^{ab}$ | 1.53 ± 0.04$^{a}$ |
| | Triploid | 1.67 ± 0.1 | 1.74 ± 0.08 | 1.64 ± 0.03 | 1.68 ± 0.05 |
| Isoleucine | Diploid* | 0.72 ± 0.04$^{b}$ | 0.71 ± 0.009$^{b}$ | 0.68 ± 0.009$^{ab}$ | 0.64 ± 0.02$^{a}$ |
| | Triploid | 0.70 ± 0.04 | 0.70 ± 0.03 | 0.70 ± 0.01 | 0.71 ± 0.01 |
| Leucine | Diploid* | 1.42 ± 0.06$^{b}$ | 1.41 ± 0.02$^{b}$ | 1.37 ± 0.01$^{ab}$ | 1.29 ± 0.05$^{a}$ |
| | Triploid | 1.38 ± 0.08 | 1.366 ± 0.06 | 1.36 ± 0.02 | 1.41 ± 0.03 |
| Phenyl-alanine | Diploid* | 0.74 ± 0.03$^{b}$ | 0.73 ± 0.01$^{b}$ | 0.71 ± 0.01$^{ab}$ | 0.66 ± 0.02$^{a}$ |
| | Triploid | 0.72 ± 0.03 | 0.72 ± 0.03 | 0.718 ± 0.02 | 0.74 ± 0.02 |
| Total amino acids | Diploid* | 15.6 ± 0.73$^{b}$ | 15.5 ± 0.13$^{b}$ | 14.9 ± 0.18$^{ab}$ | 14.1 ± 0.48$^{a}$ |
| | Triploid | 16.09 ± 1.02 | 16.72 ± 0.75 | 15.86 ± 0.25 | 16.57 ± 0.40 |

### Effect of ploidy on growth

Triploid fish were 31% heavier and 6% longer than diploid fish at the end of the 14 wk rearing period (Fig. 2).

## DISCUSSION

The unbalanced chromosome number in triploid fish led to considerable differences in AA composition, omega-3 FAs, and protein of diploid and triploid *Clarias gariepinus* white muscle tissue after exposure to an OP pesticide. Changes in protein, AA, and FA compositions in animals can be protective strategies under environmental stressors (Silva et al. 2017).

Lack of significant differences in the proximate composition of white muscle between diploid and triploid control groups was consistent with the comparable whole-body proximate composition observed for diploid and triploid *Oncorhynchus mykiss* (Oliva-Teles & Kaushik 1990). In line with the results of the present study, diploid and triploid *C. gariepinus* had a similar proximate composition in skin gelatin (Karami et al. 2016b). Despite the same protein content per gram of muscle in diploid and triploid *C. gariepinus*, triploid fish were heavier in weight and of a longer body length than diploid fish, and this should guarantee a higher total protein yield in the aquaculture industry.

AAs play a significant role in metabolism and immunity against diseases in organisms (Wu 2013). Similar AA composition in the diploid and triploid control groups is in agreement with our recent study that demonstrated AA composition did not change in the

Table 2. Fatty acid composition (% total fatty acids) in the muscle of diploid and triploid *Clarias gariepinus* across different chlorpyrifos concentrations. Data are mean ± SD; n = 5. Values in the same line with different letters indicate differences among groups ($p < 0.05$, Tukey's multiple range tests). (D) indicates dropped (negative control group dropped according to OECD [2011] guidelines); (P) indicates pooled (negative and solvent controls pooled according to OECD [2011] guidelines). *Significant ($p < 0.05$) differences compared with control value. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; UFAs: unsaturated fatty acids

| Fatty acid | Ploidy | Chlorpyrifos concentration (µg $l^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 0 (control group) | | 8.9 | 17.5 | 28.0 |
| | | Negative | Solvent | | | |
| C14:0 | Diploid | 1.76 ± 0.2 | 1.61 ± 0.1 | 2.30 ± 0.2 | 1.93 ± 0.2 | 1.88 ± 0.2 |
| | Triploid | 2.04 ± 0.2 | 1.73 ± 0.3 | 2.55 ± 0.5 | 1.74 ± 0.3 | 2.55 ± 0.6 |
| C16:0 | Diploid* | 27.2 ± 0.8 (D) | 28.6 ± 0.2$^{a}$ | 27.7 ± 0.6$^{ab}$ | 29.4 ± 1.4$^{b}$ | 28.5 ± 0.5$^{ab}$ |
| | Triploid* | 27.8 ± 0.5 (D) | 29.6 ± 1.4$^{b}$ | 28.6 ± 1.02$^{b}$ | 26.6 ± 0.5$^{a}$ | 29.1 ± 1.2$^{b}$ |
| C18:0 | Diploid | 10.0 ± 0.4 | 9.86 ± 0.3 | 10.0 ± 0.6 | 10.2 ± 1.0 | 9.95 ± 0.9 |
| | Triploid* | 9.74 ± 0.5$^{a}$ (P) | 9.07 ± 0.4$^{a}$ (P) | 10.8 ± 0.9$^{b}$ | 10.3 ± 0.8$^{ab}$ | 9.89 ± 0.5$^{ab}$ |
| Total SFAs | Diploid* | 39.0 ± 0.9 (D) | 40.0 ± 0.4$^{a}$ | 40.0 ± 0.2$^{ab}$ | 41.5 ± 1.3$^{b}$ | 40.4 ± 0.7$^{ab}$ |
| | Triploid | 39.6 ± 0.4$^{ab}$ (P) | 40.4 ± 0.6 $^{ab}$ (P) | 41.9 ± 2.4$^{b}$ | 38.7 ± 0.9$^{a}$ | 41.5 ± 2.1$^{b}$ |
| C16:1 | Diploid | 3.80 ± 0.3 | 3.94 ± 0.2 | 4.20 ± 0.5 | 3.94 ± 0.6 | 3.76 ± 0.6 |
| | Triploid | 4.07 ± 0.4 | 4.37 ± 0.7 | 3.94 ± 0.7 | 3.32 ± 0.6 | 4.1 ± 0.5 |
| C18:1n-9 | Diploid | 24.2 ± 1.3 | 25.1 ± 1.5 | 23.4 ± 3.2 | 25.3 ± 2.8 | 23.6 ± 3.9 |
| | Triploid | 25.6 ± 1.7 | 26.7 ± 1.8 | 25.2 ± 2.1 | 23.6 ± 4.1 | 25.1 ± 2.6 |
| Total MUFAs | Diploid | 28.0 ± 1.6 | 29.1 ± 1.7 | 27.6 ± 3.8 | 29.3 ± 3.4 | 27.4 ± 4.5 |
| | Triploid | 29.1 ± 2.1 | 31.1 ± 2.4 | 29.2 ± 2.7 | 26.95 ± 4.7 | 29.2 ± 3.2 |
| C18:3n-3 | Diploid | 0.95 ± 0.1 | 1.02 ± 0.1 | 1.12 ± 0.1 | 1.07 ± 0.3 | 0.83 ± 0.08 |
| | Triploid | 1.02 ± 0.08 | 1.00 ± 0.2 | 1.01 ± 0.1 | 0.88 ± 0.2 | 1.18 ± 0.1 |
| C20:5n-3 | Diploid | 2.95 ± 0.2 | 2.58 ± 0.3 | 2.93 ± 0.3 | 2.45 ± 0.5 | 2.94 ± 0.6 |
| | Triploid | 2.90 ± 0.2 | 2.64 ± 0.4 | 2.67 ± 0.6 | 3.35 ± 0.4 | 2.65 ± 0.4 |
| C22:5n-3 | Diploid | 1.08 ± 0.09 | 1.01 ± 0.1 | 1.06 ± 0.2 | 1.31 ± 0.5 | 1.02 ± 0.1 |
| | Triploid* | 0.96 ± 0.1$^{a}$ (P) | 0.96 ± 0.2$^{a}$ (P) | 1.66 ± 0.5$^{b}$ | 1.24 ± 0.3$^{ab}$ | 1.01 ± 0.2$^{ab}$ |
| C22:6n-3 | Diploid | 15.8 ± 2.2 | 13.7 ± 1.3 | 15.0 ± 3.4 | 11.0 ± 3.1 | 14.2 ± 2.5 |
| | Triploid* | 13.5 ± 1.7$^{a}$ (P) | 10.5 ± 2.8$^{a}$ (P) | 12.2 ± 2.4$^{a}$ | 17.0 ± 3.9$^{b}$ | 12.9 ± 3.3$^{ab}$ |
| Total omega-3 fatty acids | Diploid | 20.8 ± 2.4 | 18.3 ± 1.6 | 20.2 ± 3.8 | 15.8 ± 3.7 | 19.0 ± 3.5 |
| | Triploid* | 18.4 ± 1.9$^{a}$ (P) | 15.2 ± 2.5$^{a}$ (P) | 17.6 ± 3.1$^{ab}$ | 22.4 ± 4.5$^{b}$ | 17.7 ± 3.9$^{ab}$ |
| C18:2n-6 | Diploid | 9.68 ± 0.8 | 10.2 ± 0.7 | 10.0 ± 0.4 | 9.95 ± 0.5 | 10.4 ± 0.3 |
| | Triploid | 10.2 ± 0.8 | 9.86 ± 0.4 | 9.35 ± 0.8 | 9.41 ± 0.6 | 9.67 ± 0.6 |
| C20:4n-6 | Diploid | 2.48 ± 0.1 | 2.01 ± 0.1 | 2.21 ± 0.3 | 1.95 ± 0.3 | 2.36 ± 0.4 |
| | Triploid | 2.20 ± 0.2 | 2.02 ± 0.5 | 1.95 ± 0.3 | 2.51 ± 0.5 | 1.91 ± 0.5 |
| Total omega-6 fatty acids | Diploid | 12.2 ± 0.8 | 12.2 ± 0.8 | 12.2 ± 0.1 | 12.0 ± 0.4 | 13.1 ± 0.8 |
| | Triploid | 12.4 ± 0.7 | 11.9 ± 0.7 | 11.3 ± 1.2 | 11.9 ± 0.8 | 11.6 ± 0.7 |
| Total PUFAs | Diploid | 33.0 ± 2.2 | 30.5 ± 2.1 | 32.4 ± 3.8 | 28.4 ± 4.3 | 32.2 ± 4.2 |
| | Triploid | 30.7 ± 1.8 | 27.0 ± 2.3 | 28.9 ± 4.0 | 34.4 ± 4.9 | 29.3 ± 4.4 |
| Total UFAs | Diploid* | 61.0 ± 0.9 (D) | 59.6 ± 0.4$^{b}$ | 60.0 ± 0.2$^{ab}$ | 58.5 ± 1.3$^{a}$ | 59.6 ± 0.7$^{ab}$ |
| | Triploid | 60.4 ± 0.4$^{ab}$ (P) | 59.6 ± 0.6$^{ab}$ (P) | 58.1 ± 2.3$^{a}$ | 61.3 ± 0.9$^{b}$ | 58.5 ± 2.1$^{a}$ |
| Omega-3/ omega-6 | Diploid | 0.59 ± 0.09 | 0.66 ± 0.05 | 0.62 ± 0.1 | 0.81 ± 0.2 | 0.69 ± 0.07 |
| | Triploid | 0.68 ± 0.09 | 0.80 ± 0.1 | 0.65 ± 0.08 | 0.54 ± 0.09 | 0.67 ± 0.1z |

skin gelatin of diploid and triploid *C. gariepinus* (Karami et al. 2016b). In contrast, the muscle of diploid and triploid tench *Tinca tinca* showed differences in free AA composition, indicating species-specific differences (Buchtová et al. 2005). In the present study, the most dominant AAs in both ploidies control groups were glutamic acid and aspartic acid, representing about 2.7 and 1.8 mg $g^{-1}$ (wet weight), respectively. These AAs are proven to have a fundamental role in the wound healing process in traumatized rats

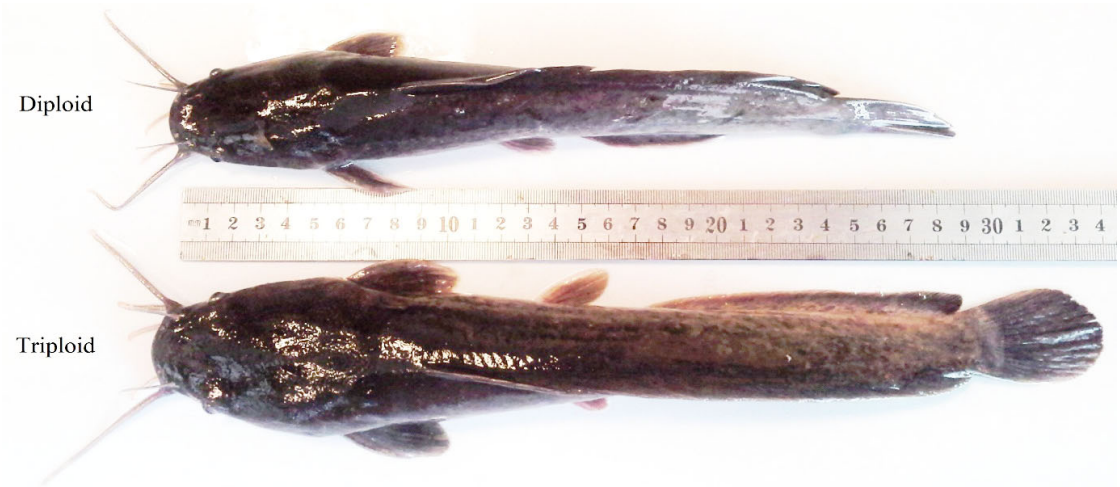


Fig. 2. Differences in length between full-sibling 14 wk old diploid and triploid *Clarias gariepinus* used in this study

(Chyun & Griminger 1984). Anticancer activity of glutamic acid (Dutta et al. 2013) and the impacts of aspartic acid in enhancing athletic performance (Meehan 2016) may suggest *C. gariepinus* as a rich source of nutrients for human health.

Unexposed diploid fish might show superior nutritional values compared to triploids due to a higher level of C22:6n-3 and total omega-3 FAs in their skeletal muscle. Contrary to our results, triploid *Salmo salar* had a significantly higher percentage of C22:6n-3 compared to diploids (Taylor et al. 2013), while diploid and triploid salmon (*O. masou*) had a comparable FA composition in the muscle (Wang et al. 2015). These results highlight the effect of ploidy and species-specific differences in FA metabolism.

The increase in the number of genes in triploid organisms compared to diploids may change nutritional composition such as fat content, AA, and FA composition (Buchtová et al. 2005, Taylor et al. 2013). No significant differences in AAs, most of the FAs, and proximate composition indicated that gene dosage had minimal impacts on nutritional values in *C. gariepinus.* In polyploid organisms, duplicated genes may interact through recombination, gene conversion, epigenetic silencing, or other mechanisms (Wendel 2000). The observed lack of differences in the levels of AAs and most FAs or protein, lipid, moisture, and ash contents in *C. gariepinus* may be attributed to the silencing of one of the 3 alleles which modify gene expression in triploid fish. Woods & Buth (1984) showed a high level of gene copy silencing in tetraploid goldfish *Carassius auratus*.

Chemical pesticides used in agriculture impact the activity of biologically active molecules such as proteins, lipids, and carbohydrates (Singh & Sharma 1998). Protein plays a key role in metabolic pathways (Suryavanshi et al. 2009) and in improving the health, growth, development, and disease resistance of organisms (Xu et al. 2016). Exposure to 28 µg $l^{-1}$ chlorpyrifos significantly reduced protein content in the skeletal muscle of diploid *Clarias gariepinus* compared to the control group. Consistent with the present study, a decrease in protein content was observed in different tissues of *C. batrachus* following exposure to sublethal chlorpyrifos concentrations for 28 d (Narra et al. 2011). Possible explanations for the reduced protein content are the destruction or necrosis of cells, resulting in protein synthesis impairment (Bradbury et al. 1987), and the enhanced proteolytic activity in the muscle under pesticide exposure. In contrast, lipid composition showed no alteration throughout the study across each ploidy, which is consistent with our recent study where phenanthrene had no impacts on the serum lipid profile in *C. gariepinus* (Karami et al. 2016d). Changes in the muscle moisture content in diploid fish following chlorpyrifos treatments are in agreement with the alterations in moisture content in monocrotophos-exposed juvenile Indian carp *Labeo rohita* (Ramani et al. 2002). Alterations in moisture and ash contents have been suggested to be due to the reduction of food consumption and food conversion efficiency under stress (Nair & Sherief 1998).

The decrease in levels of aspartic acid, serine, glutamic acid, histidine, alanine, tyrosine, valine, lysine, isoleucine, leucine, phenylalanine, and total AAs in diploid *C. gariepinus* exposed to 28 µg $l^{-1}$ chlorpyrifos may be attributed to the utilization of AAs for energy provision to cope with the stress (Shobha Rani & Janaiah 1991). Similarly, a reduction in free AA levels was observed in the muscle of *C. batrachus* after 28 d of exposure to 825 and 1650 µg $l^{-1}$ chlorpyrifos (Narra et al. 2011). Fewer changes in AA levels in chlorpyrifos-exposed triploids may indicate the

impact of ploidy on the adaptive mechanisms of fish following exposure to environmental stressors. In diploids, lower protein content and AAs at the high concentration of chlorpyrifos may highlight an interruption in AA synthesis followed by impairment in protein synthesis (Narra et al. 2011). Furthermore, these results may demonstrate the poor nutritional value of the farmed or wild diploid fish from areas with high chlorpyrifos concentrations. However, the extra set of chromosomes in triploid cells may neutralize the adverse impacts of the chlorpyrifos on AA synthesis and protein content of the muscle.

PUFAs, especially omega-3, are important for aquaculture, as they influence the nutritional value of the fillet (Manor et al. 2012). According to the findings of this study, either the 8.9 or the 17.5 µg $l^{-1}$ chlorpyrifos exposure caused an increase in levels of C18:0, C22:5n-3, C22:6n-3, and total omega-3 fatty acids and decreased the levels of C16:0 and total SFAs in triploid *C. gariepinus*. However, the chlorpyrifos exposures did not affect the level of omega-3 FAs in the muscle of diploid fish. Stearic acid (C18:0) is an SFA but is less efficiently absorbed by the body and, therefore, has limited bioavailability (Clarke et al. 1977, Kritchevsky 1994). In contrast, the association between the high C16:0 intake and various diseases has been suggested earlier (see review by Fattore & Fanelli 2013). Long-chain highly unsaturated FAs including C20:5n-3, C22:6n-3, and C20:4n-6 are needed for normal growth and development in fish (Sargent et al. 1999). They are also a part of membrane phospholipids and are the precursors of biologically active eicosanoids (Sargent et al. 2002). In addition, C20:5n-3 and C22:6n-3 play a vital role in humans, including promoting cardiovascular health (Calder & Yaqoob 2009). In contrast to triploids, diploid fish showed an increase in C16:0 and total SFA levels and a decrease in total UFA levels. In freshwater rosy barb *Puntius conchonius*, the sublethal concentration of phosphamidon, an OP insecticide, increased the level of free FAs in the liver and skeletal muscles (Gill et al. 1990). A few studies have shown the effects of pesticides on FA metabolism in fish species under controlled conditions due to alterations in the enzymes responsible for metabolism such as catalase, allantoinase, uricase, FA synthase (multienzyme complex characterized in fish species), and acetylCoA carboxylase (see review by Olivares-Rubio & Vega-López 2016).

No alterations in protein content, along with little change in the AA levels or composition in triploid fish, could indicate the better adaptability of triploid fish to environmental stressors. Earlier studies have found protein–DNA interactions within organisms (Luscombe & Thornton 2002, Stormo 2013). Also, OP pesticides are known to induce DNA damage to fish species (Ali et al. 2008). Therefore, extra DNA contents in triploid fish (Wendel & Doyle 1998) might have resulted in alteration of gene expression and, consequently, fewer responses of triploids to chlorpyrifos exposure compared to their diploid counterparts.

We showed in this study that diploid and triploid *C. gariepinus* respond differently to the stress induced by chlorpyrifos exposure. Similarly, the increased level of C22:6n-3 and C20:5n-3 in freshwater clams *Dipolodon chilensis* was suggested as an adaptive mechanism to enhance their resistance to sewage discharges (Rocchetta et al. 2014). Further studies on other biomarkers are required to elucidate better the impacts of environmental stressors on polyploid fish.

This study showed that ploidy status had a small but significant influence on nutritional values in unexposed *C. gariepinus*. Chlorpyrifos at the concentrations applied in this study (up to 28 µg $l^{-1}$) was observed to induce potentially harmful effects to diploid *C. gariepinus*. Significant changes in the muscle protein content and AA and FA compositions in diploid fishes in response to chlorpyrifos exposure suggest their application as sensitive indicators of aquatic environmental pollution. Dissimilar patterns of change in the levels of particular AAs and FAs, and in proximate composition between diploid and triploid fish following chlorpyrifos exposure highlight different mechanisms used by the fish from different genetic types to cope with the stress. In addition, no change in muscle protein content, little change in AA levels, and increased UFA levels in chlorpyrifos-exposed triploid *C. gariepinus* may highlight their higher resistance to environmental stressors as compared to the diploid fish. Moreover, higher growth of triploid *C. gariepinus* compared to diploids and their sterility (Karami & Courtenay 2015), which limits their breeding with wild populations, could support application of triploid *C. gariepinus* as an alternative to their diploid counterparts for the aquaculture industry.

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