



Metabolic responses of channel catfish (*Ictalurus punctatus*) exposed to phenol and post-exposure recovery

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ABSTRACT

Metabolic adjustments were studied in channel catfish *Ictalurus punctatus* exposed to 1.5 mg L⁻¹ of phenol (10% LC50) for four days and recovered for seven days. Lower triacylglycerol (TGA) stores and increased muscle fat free acids (FFA) suggest fat catabolism in muscle. Remarkable liver FFA decrease (-31%) suggests liver fat catabolism as well. Increased muscular ammonia levels and ASAT (aspartate aminotransferase) and decreased plasma aminoacids suggest higher muscular amino acid uptake. Constant levels of glucose and increased liver glycogen stores, associated with lower amino acids in plasma, indicate gluconeogenesis from amino acids. This is supported by higher hepatic ALAT and ASAT. Higher hepatic LDH followed by lower plasma lactate may indicate that plasma lactate was also used as gluconeogenic substrate. Biochemical alterations were exacerbated during the post-exposure recovery period. Reduction in muscle and plasma protein content indicate proteolysis. A higher rate of liver fat catabolism was resulted from a remarkable decrease in hepatic TGA (-58%). Catabolic preference for lipids was observed in order to supply such elevated energy demand. This study is the first insight about the metabolic profile of *I. punctatus* to cope with phenol plus its ability to recover, bringing attention to the biological consequences of environmental contamination.

Key words: metabolic adjustments, enzymes, lipid stores, intermediary metabolism, recovery, xenobiotic.

INTRODUCTION

Phenols and their derivatives are aromatic, organic compounds present in a wide variety of biotic and abiotic factors, and under numerous circumstances, they can be considered pollutants. Domestic and industrial effluents are the major source of such pollutants in aquatic environments, causing damages at several levels of biological organization

(Moens et al. 2007). Despite the maximum limit for the concentration of phenol in treated effluents (0.5 mg L⁻¹) and freshwater (0.003 - 1 mg L⁻¹) be restrictive in Brazil (Brasil 2005, Cetesb 2014a), phenol contamination in water basins is often from either industrial wastewaters or accidental phenol discharges. Total phenols concentrations are above the allowed limit in some Brazilian freshwater and coastal water points (Cetesb 2014b). Moreover, phenols concentrations ranging from 0.37 to 3.92 mg L⁻¹ are reported in the Brazilian rivers (Possa

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et al. 2008, Watthier et al. 2008, Porto and Ethur 2009). When toxic substances reach the fish, it can be absorbed through the skin, gills, intestine and mucous membranes. Into bloodstream, it spreads out to body compartments and causes several physiological disturbances as hematological and metabolic disorders (Hori et al. 2006, Avilez et al. 2008, Moraes et al. 2015). Depending on the environmental concentration, phenol can lead to death to fishes (Gupta et al. 1982, Saha et al. 1999, Moraes et al. 2015). Multiple mechanism of action of phenol or some phenolic compounds have been reported, including: drug antagonists (Roche and Bogé 2000), induction of genotoxicity (Bolognesi et al. 2006), carcinogenesis and mutagenesis (Tsutsui et al. 1997, Yin et al. 2006), endocrine disruption (Kumar and Mukherjee 1988), and metabolic disruption (Hori et al. 2006).

The evaluation of metabolic parameters has currently been used as biomarkers of environmental contamination by phenol (Hori et al. 2006, Abdel-Hameid 2007, Avilez et al. 2008, Varadarajan et al. 2014), pesticides (Venkateswara Rao 2006, Aguiar et al. 2004, Sancho et al. 2009, Venturini et al. 2015) and metals (Pretto et al. 2014). Evaluation of metabolic profile can help to elucidate how metabolic disruption caused by xenobiotics may affect fish physiology and behavioral state. (Oost et al. 2003, Scott and Sloman 2004, Schlenk et al. 2008). The metabolism of protein in fishes can be altered by phenol. Adjustments in the activities of alanine (ALAT) and aspartate (ASAT) aminotransferases and changes in tissular protein concentration are reported in fish exposed to phenol (Barse et al. 2006, Dangé 1986b, Gupta et al. 1983, Hori et al. 2006, Sannadurgappa et al. 2007). Phenolic compounds affect the lactate dehydrogenase (LDH) activity, altering the carbohydrate metabolism through the inter-conversion of lactate-pyruvate (Barse et al. 2006, Dangé 1986a, Gupta et al. 1983, Hori et al. 2006, Sannadurgappa et al. 2007). Adjustments in the lipid metabolism are also

observed in *Oreochromis mossambicus* exposed to phenol (Sannadurgappa et al. 2007).

Biological effects of phenol are relevant, particularly when several derivatives of it should present similar effects. This is particularly relevant to fish, which is able to uptake and retain different xenobiotic dissolved in water via active or passive processes (Fernández-Vega et al. 2015). Channel catfish *Ictalurus punctatus* is a freshwater fish responsive to phenol contamination (Moraes et al. 2015). At the present days, it is distributed in several countries, including Brazil (FAO 2015), and it is easily maintained in the lab conditions. Besides, *I. punctatus* have large amount of fat, which is subject to phenols bioaccumulation, as reported to the catfish *Clarias gariepinus*, and *Carassius carassius* exposed to phenol and effluents, respectively (Ibrahem 2012, Zheng et al. 2015).

Even at low levels, environmental contamination may result in no perceptible, immediate or acute consequences. Sublethal concentrations (as 10% of LC50) can be explored through biochemical biomarkers to predict impairment at the high levels of organization. It is assumed that risks can be mitigated when contaminants are applied to aquatic environments at concentrations 10 - 50% of LC50 (Touart 1995). In addition, studies on the post-exposure recovery period (Aguiar et al. 2004, Begum 2004, Sancho et al. 2009, Pretto et al. 2014, Venturini et al. 2015) can elucidate the ability of fish to reestablish the homeostasis, when the stressor is discontinued.

Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity (Begum 2004). The biochemical responses of organisms are the most sensitive and relatively early events of pollutant damages and can be accessed into few hours; besides, biochemical alterations could be part of mitigating effects from toxicant presence (Pickering and Pottinger 1995, Begum 2004, Schlenk et al. 2008). Among

the many biochemical responses, those observed at metabolic level are particularly relevant since energy is partially and insidiously shifted to attend the detoxification processes. No data are available concerning the effects of phenol on metabolic activity of *I. punctatus*. Therefore, the evaluation of intermediary metabolism of *I. punctatus* exposed to phenol plus its ability to recover was the first insight into the metabolic adaptability of the species to cope with this kind of stressor, allowing us to infer on the magnitude of the metabolic expenditures and losses.

MATERIALS AND METHODS

CHEMICALS

Phenol was purchased from Sigma-Aldrich and it had previously been dried into a vacuum desiccator at room temperature in the dark. All chemicals and reagents were purchased from Sigma-Aldrich, Merck or Mallinckrodt.

FISH MAINTENANCE AND EXPERIMENTAL DESIGN

The fish *I. punctatus* were obtained from a local commercial fish farm and, acclimated for 15 days in a water flow-through system of 2000 L fiber tanks at following: temperature 25-27 °C; pH 7.0-7.2; $[\text{NH}_3\text{-NH}_4^+]$ 0.4-0.6 mg L⁻¹; and dissolved oxygen 5.0-6.0 mg L⁻¹. The fish samples (37-48 g; 15.3-17.9 cm) were fed twice a day with commercial pellets until satiety. After acclimation, 48 fish were transferred to the experimental system wherein they equally distributed into eight 250L-tanks (n=6 fish/tank) with the same water quality of a new flow-through system and fed the same regimen. The fish were kept undisturbed in this new condition for seven days and the feeding was discontinued 24 hours before starting the phenol exposure to prevent fecal debris deposition over the assay. The experiment was carried out in duplicates in a semi-static system. Afterward, the water flow was ceased and phenol was added to four tanks to final

concentration of 1.5 mg L⁻¹ (10% of 96 hour LC50 for *I. punctatus*, Moraes et al. 2015). The four tanks remaining were kept with clean water and assigned as control. Over the exposure period, water was renewed each 24 hours, the phenol concentration was readjusted, and the water quality was preserved. This procedure was done in nearly 20 minutes and the whole semi-static exposure lasted four days. At the fourth day, four tanks (two exposures and two controls) were randomly pointed and the fish were netted to sample tissues and blood. In the other four tanks the water was kindly renewed to keep the fish undisturbed and the tanks returned to the same initial flow-through system for seven more days (post-exposure recovery). Ended this period, the fish were equally netted for blood and tissue sampling.

BLOOD AND TISSUE SAMPLING

At the ending of each experimental spans (four days of exposure or seven days of recovery), the fish were dived into 40 mg L⁻¹ eugenol solution for one minute (Inoue et al. 2003), blood was withdrawn from the caudal vein with heparinized syringes and then the fish were immediately killed by transecting the spinal cord. Liver and white muscle samples were quickly excised over a chilly glass plate, rinsed with cold saline solution and quickly plunged in liquid nitrogen. Total blood was centrifuged for three minutes at 13,400 x g and the pellets were discarded. Plasma and tissues were kept at -80 °C until analyses.

ENZYME ASSAYS

White muscle and liver samples were homogenized at 4 °C in a buffer containing 50% 10 mM phosphate buffer pH 7.0 plus 50% glycerol at a ratio 1:10 (w/w). For enzyme extraction, a rotative high-speed homogenizer was used for tissue disruption. To cool the sample down to 4 °C tubes were place in ice bath. The tissue homogenates were firstly centrifuged at 600 × g for three minutes at 4 °C

and secondly the supernatants were centrifuged at $6,000 \times g$ for eight minutes at 4°C . The protein concentrations were determined in the supernatants which were used as crude enzyme source. White muscle and liver ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) were assayed kinetically (Bergmeyer et al. 1978); LDH (lactate dehydrogenase) and MDH (malate dehydrogenase) were used as auxiliary enzyme for ALAT and ASAT assays, respectively. The final concentration of the reaction mixture was 500 mM alanine (ALAT) or 220 mM aspartate (ASAT), 10 mM 2-ketoglutaric acid and liver/muscle homogenate adjusted to 0.04/0.40 mg of protein to a final volume of 1.0 mL with 100 mM Tris buffer, pH 7.5. All the reagents were previously diluted into 100 mM Tris buffer, pH 7.5. The reaction was followed by NADH^+H^+ extinction, read at 340nm and expressed in mIU per mg of protein. One IU corresponded to 1.0 μmol of NADH oxidized per minute ($\epsilon_0 = 6.22 \text{ mM}^{-1} \cdot \text{cm}^{-1}$). Enzyme activity of LDH (lactate dehydrogenase) was assayed according to Hochachka et al. (1978). The tissue homogenates were prepared as described above. The LDH reaction was carried out in a medium containing 5.0mM pyruvic acid, 0.1mM NADH, 42.5 mM TRIS buffer pH 7.5, and liver/muscle homogenate adjusted to 0.02/0.10 mg of protein to a final volume of 1.0 mL. The reaction was followed by NADH^+H^+ extinction, read at 340nm and expressed in mIU per mg of protein. One IU corresponded to 1.0 μmol of NADH hydrolyzed per minute ($\epsilon_0 = 6.22 \text{ mM}^{-1} \cdot \text{cm}^{-1}$). Liver and white muscle activities of ACP (acid phosphatase) and ALP (alkaline phosphatase) were assayed by endpoint method read at 405nm (Bergmeyer 1986). The reaction was based in hydrolyses of 120 mM p-nitrophenyl phosphate, in 50mM sodium citrate buffer pH 5.0 (ACP) or 50mM glycine buffer pH 8.5 plus 10mM MgCl_2 (ALP) and liver/muscle homogenate adjusted to 0.04/0.40 mg of protein to a final volume of 1.0 mL. The reaction was stopped by 2M NaOH addition and the p-nitrophenolate

was optically determined at 405nm and expressed in mIU per mg of protein. One IU corresponded to 1.0 μmol of hydrolyzed p-nitrophenyl phosphate hydrolyzed per minute ($\epsilon_0 = 18,200 \text{ mM}^{-1} \cdot \text{cm}^{-1}$)

METABOLITES

Metabolites were quantified in liver and white muscle acidic homogenates prepared in 20% trichloroacetic acid (TCA) or neutral homogenates made in distilled water. Plasma was only diluted in 20% TCA or water before using. Homogenates were performed in a rotative high-speed tissue disruptor with three strokes of 20 seconds under ice bath. The homogenates were centrifuged for three minutes at $21,000 \times g$ and the supernatant extracts were used. Ammonia (Gentzkow and Masen 1942), lactate (Harrower and Brown 1972) and pyruvate (Lu 1939) were photometrically quantified in acid extracts. Free amino acids (Copley 1941), glucose (Bergmeyer 1986), free fatty acids (Novák 1965) and triacylglycerol (Stein and Myers 1995) were photometrically determined in extracts at neutral pH. Hepatic and muscular glycogen content was determined by the method of Bidinotto et al. (1997). The tissues were solubilized with 6.0N KOH in a boiling water bath for five minutes, and the glycogen was precipitated by ethanol and K_2SO_4 saturated solution. After centrifugation at $2000 \times g$ for three minutes, the pellets were resuspended in distilled water and the glycogen was quantified as glucosyl-glucose by phenol-sulfuric acid (Dubois et al. 1956).

PROTEIN

The homogenate and plasma protein concentrations were determined with Bradford reagent against bovine albumin solution as standard (Kruger 1994).

STATISTICAL APPROACH

The biochemical parameters from each group are presented as mean \pm SE. Groups (including

controls) were tested for homogeneity concerning the respective replicates. The data were analyzed concerning the groups normality by Kolmogorov-Smirnov test and the differences among means were evaluated by Student's *t*-test at the level of $P < 0.05$. Each treatment (exposed to phenol and post-exposure recovery) was evaluated against the respective control group.

ETHICS

The conditions and experimental procedures of this study were approved by Ethic Committee for Animal Research of Federal University of São Carlos (CEEA n° 039).

RESULTS

I. punctatus exposed to phenol did not exhibit any evident signs of poisoning. The metabolic profile of *I. punctatus* depicted relevant tissular and plasmatic adjustments (Table I). All differences described in this section were statistical significant. The liver glycogen stores enhanced after exposure, however, in white muscle it was not mobilized. The exposure to phenol caused a sinking of plasmatic

concentrations of amino acids, protein, free fatty acids (FFA), triacylglycerol (TAG) and lactate. After the post-exposure recovery period, plasma lactate concentration increased, while protein concentration decreased. The levels of glucose and TAG were fallen in the muscle after exposure, and pyruvate and protein just after the post-exposure recovery period. However, the levels of muscle ammonia and FFA were heightened, and this one was kept high even after post-exposure recovery period. The relevant changes in the liver were observed after the post-exposure recovery period. Except by decrease of the liver FFA levels after exposure, those of glucose and ammonia were increased after post-exposure recovery while the TAG level was dropped. The activities of the enzymes assayed depicted changes either after the exposure or the post-exposure recovery period (Table II). In the liver, the enzymatic activity of ALAT, ASAT, LDH and ALP were increased; ACP activity increased only after post-exposure recovery period. In white muscle, ACP activity was reduced after exposure. The activity of the ALAT was increased in white muscle after post-exposure recovery.

TABLE I
Concentration of metabolites in the *Ictalurus punctatus* exposed to sublethal concentration of phenol for 4 days (10% LC50) and post-exposure recovery for 7 days.

Metabolite	Tissue	Exposure		Recovery	
		C-4	P-4	C-7	P-7
Glycogen ^(μ)	L	235.8 ± 12	274.4 ± 11*	53.4 ± 5.4	67.6 ± 6.2
	Wm	8.4 ± 0.4	7.8 ± 0.05	14.0 ± 0.3	14.4 ± 0.2
Glucose ^(μ)	L	62.4 ± 1.3	66.2 ± 1.2	54.9 ± 1.2	59.1 ± 1.2*
	Wm	1.1 ± 0.05	0.9 ± 0.02*	1.2 ± 0.05	1.2 ± 0.03
	P	2.3 ± 0.05	2.2 ± 0.1	1.5 ± 0.09	1.8 ± 0.2
Amino acids ^(μ)	L	36.8 ± 1.0	35.9 ± 0.6	44.0 ± 1.0	42.5 ± 1.3
	Wm	8.8 ± 0.1	8.7 ± 0.3	14.2 ± 0.2	15.1 ± 0.5
	P	5.3 ± 0.1	4.8 ± 0.05*	4.6 ± 0.1	4.5 ± 0.1
Ammonia ^(μ)	L	47.1 ± 2.5	50.9 ± 3.0	57.8 ± 2.1	78.5 ± 2.0*
	Wm	19.5 ± 0.6	21.0 ± 0.3*	17.2 ± 0.2	17.1 ± 0.3
	P	1.4 ± 0.03	1.4 ± 0.03	2.5 ± 0.03	2.7 ± 0.1
FFA ^(μ)	L	9.9 ± 0.6	6.8 ± 0.5*	27.0 ± 2.6	21.5 ± 2.2
	Wm	0.4 ± 0.01	0.5 ± 0.02*	0.7 ± 0.01	0.9 ± 0.01*
	P	0.8 ± 0.02	0.6 ± 0.01*	--	--

TABLE I (continuation)

Metabolite	Tissue	Exposure		Recovery	
		C-4	P-4	C-7	P-7
TAG ^(mg)	L	26.6 ± 0.08	25.0 ± 0.1	13.8 ± 1.1	7.3 ± 0.8*
	Wm	2.4 ± 0.1	2.0 ± 0.02*	1.1 ± 0.02	1.1 ± 0.02
	P	2.5 ± 0.08	2.1 ± 0.05*	2.3 ± 0.05	2.3 ± 0.03
Pyruvate ^(μ)	L	0.7 ± 0.03	0.7 ± 0.03	0.9 ± 0.03	0.9 ± 0.03
	Wm	0.7 ± 0.03	0.7 ± 0.03	0.7 ± 0.03	0.6 ± 0.01*
	P	0.30 ± 0.01	0.30 ± 0.005	0.24 ± 0.01	0.25 ± 0.005
Lactate ^(μ)	L	10.8 ± 0.5	10.3 ± 0.5	7.1 ± 0.6	6.1 ± 0.2
	Wm	22.5 ± 0.8	23.6 ± 0.6	22.9 ± 0.4	21.3 ± 0.6
	P	3.4 ± 0.2	2.8 ± 0.1*	3.0 ± 0.2	3.8 ± 0.1*
Protein ^(mg)	L	65.5 ± 1.5	63.9 ± 1.6	65.5 ± 1.7	59.6 ± 2.6
	Wm	19.7 ± 0.5	18.4 ± 0.7	15.6 ± 0.2	14.5 ± 0.3 *
	P	31.53 ± 0.9	27.89 ± 0.5 *	20.40 ± 1.1	15.16 ± 0.9*

Metabolites concentrations are expressed in μmol (^{μ}) or mg (^(mg)) per g of tissue or per mL of plasma. C-4 control of exposure for 4 days; P-4 exposed for 4 days; C-7 control of recovery; P-7 recovered for 7days; L = liver; Wm = white muscle; P = plasma; FFA = free-fatty acids; TAG = triacylglycerol; Values are followed by mean \pm SE (*) difference between the exposed group and the corresponding control is significant at $P < 0.05$ ($n=12$).

TABLE II
Enzyme activities of aminotransferases, phosphatases and dehydrogenase in *Ictalurus punctatus* exposed to sublethal concentration of phenol for 4 days (10% of LC50) and post-exposure recovery for 7 days.

Enzyme	Tissue	Exposure		Recovery	
		C-4	P-4	C-7	P-7
ALAT	L	180 ± 12	250 ± 2.6*	210 ± 14.7	210 ± 26.5
	Wm	10.0 ± 1.1	10.0 ± 0.6	7.0 ± 0.3	10.0 ± 0.9*
ASAT	L	350 ± 32	440 ± 17*	490 ± 20	510 ± 27
	Wm	96 ± 7.1	121 ± 5.8*	120 ± 7.3	120 ± 7.5
ACP	L	65 ± 0.6	63 ± 0.1	63 ± 0.05	78 ± 0.6*
	Wm	14 ± 0.6	12 ± 0.6*	15 ± 0.6	15 ± 0.8
ALP	L	9.5 ± 0.4	11.5 ± 0.02*	12.3 ± 0.3	13.0 ± 0.03
	Wm	1.9 ± 0.01	2.2 ± 0.02	2.6 ± 0.03	3.0 ± 0.01
LDH	L	320 ± 30	420 ± 30 *	460 ± 35	480 ± 32
	Wm	2550 ± 264	2670 ± 260	3770 ± 205	3640 ± 176

The specific activities of: ALAT (alanine aminotransferase), ASAT (aspartate aminotransferase), ACP (acid phosphatase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) are expressed in mIU (mg of protein)⁻¹. L = liver; Wm = white muscle. C-4 control of exposure for 4 days; P-4 exposed for 4 days; C-7 control of recovery; P-7 recovered for 7days. The values are followed by mean \pm SE; (*) difference between the exposed group and the corresponding control is significant at $P < 0.05$ ($n=12$).

DISCUSSION

The intermediary metabolism of *I. punctatus* was altered due to phenol exposure. A switch from anabolic to catabolic processes is commonly observed in stressed fish. It involves major changes in intermediary metabolism and may have important consequences for somatic growth (Pickering and Pottinger 1995). The energy demanded to cope with poisoning was clearly observed in white muscle of *I. punctatus*. Increase in the glycolytic activity in white muscle of *I. punctatus* could be inferred by the presence of less glucose. This seems to be a general response of fish exposed to phenol (Hori et al. 2006, Abdel-Hameid 2007), and such decrease was to attend energetic expenditures caused by phenol. In this particular, a glycogen synthesis, to justify the glucose decrease, was not carried out since no glycogen increase was observed in the white muscle. It is expected over the muscular fermentative processes an increase of lactate concentration and LDH activity. Then, invariable activity of LDH enzyme and steady concentrations of lactate suggest that glucose oxidation has been the metabolic preference of *I. punctatus*. Although glycogen can provide the immediate energy requirements of fish under stress (Fernández-Vega et al. 2015), it was observed the maintenance of the glycogen bulks in the muscle of *I. punctatus*, as also reported to *O. mossambicus* (Dangé 1986a) and *B. amazonicus* (Hori et al. 2006) exposed to phenol. This metabolic scenario points out an exogenous source of glucose to the white muscle of *I. punctatus*. Therefore, the metabolic preference of white muscle in *I. punctatus* seemed to be oxidative, in which glucose was certainly fundamental to the mitochondrial metabolic pathways, and lipid catabolism.

High level of fat catabolism have decreased the triacylglycerol stores (17%) followed by an increase of FFA in white muscle of *I. punctatus*. Lipids are the major energy reserves in fish and

their mobilization suggest high energy demands state (Fernández-Vega et al. 2015). Catabolism of such amount of lipid in *I. punctatus* reflects a higher demand for ATP as a consequence of elevated stress and detoxification process. Lipid catabolism is also observed in *O. mossambicus* exposed to phenol for 30 days (Sannadurgappa et al. 2007). However, not only glucose and lipids were required to cope with phenol poisoning. The increase in the white muscle ammonia in *I. punctatus* was a consequence of an increase in the amino acids catabolism. The activity increase of white muscle ASAT corroborates such assumption since this enzyme provides oxaloacetate to the oxidative metabolism. In spite of the amino acids steady-state, which is usual among tissues (Houlihan et al. 1995), the plasma amino acids decreased in *I. punctatus*. Decrease of plasma amino acids and increase of ammonia in the muscle indicate amino acids catabolism in the muscle. Alterations on muscle protein metabolism is clearly reported in catfish *Rhamdia quelen* exposed to cadmium for 7 days, whereas protein concentration decreased, and amino acids and ammonia concentrations increased (Pretto et al. 2014).

The exposure to phenol was likely effective in expending metabolic energy to detoxification processes in the liver. The increase of liver glycogen in fish exposed to phenol demands glucose input. Considering that fish were kept starved the only source of glucose backbones should be amino acids. In *I. punctatus*, constant levels of liver glucose and decrease of plasma amino acids are suggestive that gluconeogenesis has occurred from free amino acids supplied by peripheral tissues. This assumption is strengthened by the increase of hepatic ALAT and ASAT. Such increases of ALAT and ASAT are usual biomarkers in fish exposed to xenobiotics (Gupta et al. 1983, Dangé 1986b, Begum 2004, Sancho et al. 2009). Enhanced activity of aminotransferases can be providing the oxaloacetate, pyruvate, α -ketoglutarate and glutaric

acid due to increase of energy demand imposed by stressing conditions (Begum 2004). The oxaloacetic acid and α -ketoglutarate might have been channeled into the Krebs's cycle, as proposed to *C. batrachus* exposed to carbofuran, which presents ALAT and ASAT induction in the liver (Begum 2004). The increase of hepatic LDH of *I. punctatus* would also support gluconeogenesis from lactate in the liver cells, as reported in *Brycon amazonicus* and *Oreochromis aureus* exposed to phenol (Abdel-Hameid 2007, Hori et al 2006). Increased hepatic LDH and ALAT activity are also reported to *O. mossambicus* exposed to 10% LC50 of phenol (Varadarajan et al. 2014). The rise in muscular ALAT activity of *Cyprinus carpio* exposed to 4-*tert*-butylphenol suggests amino acid catabolism to support the hepatic gluconeogenesis (Barse et al. 2006). Therefore, glucose synthesized *de novo* in the liver of *I. punctatus* should be responsible for maintaining the observed normoglycemia, and this profile was enhanced in the post-exposure recovery period. Normoglycemia accompanied by hepatic gluconeogenesis is also reported in silver catfish *Rhamdia quelen* exposed to cadmium for 7 days (Preto et al. 2014). Significant addition of metabolic energy was required to *I. punctatus* carried out phenol detoxification processes, which resorted to lipid catabolism in the liver to supply such demand. This explains the remarkable decrease (31%) of the liver content of FFA.

In the course of the post-exposure recovery period, the biochemical alterations were exacerbated. The teleost *Piaractus mesopotamicus* exposed to 10% LC50 of trichlorfon also presented metabolic alterations over the post-exposure recovery period (Venturini et al. 2015). In *I. punctatus*, reduction in the muscle protein content was observed, indicating that proteolysis was necessary in the recovery period. *R. quelen* recovered for 7 days to cadmium exposure also presented muscle proteolysis (Preto et al. 2014). Decrease of pyruvate concentration and increase of ALAT activity in the white muscle

are suggestive that alanine was synthesized and likely exported to the liver, performing the alanine cycle. The increased glucose concentration in the liver of *I. punctatus* indicates that rate of liver gluconeogenesis was maintained, and this fact was provided by amino acids backbones. Increased liver ammonia of *I. punctatus* indicates catabolism of amino acids, as also reported to the catfishes *Clarias batrachus* and *R. quelen* after post-exposure recovery of carbofuran and cadmium, respectively (Begum 2004, Preto et al. 2014). High rates of fat catabolism were active in liver of *I. punctatus* and it is resulted from the remarkable decrease (58%) of liver TGA. Thus, depletion of energy stores, such as muscular proteolysis and hepatic lipolysis, was observed at the end of the recovery post-exposure period in the *I. punctatus*, indicating metabolic high cost of compensatory responses of stressed fish, as early proposed to *Anguilla anguilla* exposed to propanil (Fernández-Vega et al. 2015).

Proteolytic activities can be resultant from catabolic processes induced by higher metabolic demand and cleaning of dead cells or components of the poisoned cells. Consequently, increase of hydrolytic enzymes from lysosomes, such as ACP, or bounded to cell membrane, such as ALP, can be observed in plasma. Structural damages followed by leaking of cell components reduce the content of enzymes in poisoned cells. Hepatocytes of rainbow trout *Oncorhynchus mykiss* exposed to alkyphenols for 96 hours lost the cell membrane integrity (Tollefsen et al. 2007). The increase of plasmatic ALP and ACP reported in the African catfish *Clarias gariepinus* exposed to the phenyl derivative λ -cyhalothrin and in the *O. mossambicus* exposed to phenol is attributed to a leakage of hepatic of enzymes into the blood (Okechukwu and Auta 2007, Varadarajan et al. 2014). The profile of phosphatases activity observed in *I. punctatus* was likely due a leakage of muscle ACP in consequence of intoxication. The levels of hepatic ALP were raised likely to provide phosphate radicals to

metabolic processes of detoxification, as already reported to *C. carpio* exposed to *tert*-butylphenol (Barse et al. 2006).

In summary, the results indicate that *I. punctatus* is adversely affected by sublethal concentration of phenol. The increased rates of carbohydrates, proteins and fat oxidation were to attend the metabolic demand, resultant from stress and detoxification processes. This fact is usually accepted in other fishes exposed to a number of xenobiotics (Scott and Sloman 2004, Fernández-Vega et al. 2015). Since a long time, the preference for other fuels rather than carbohydrates is widely accepted in fishes (Thillart and Raaij 1995). *I. punctatus* presented a catabolic preference for lipids to cope with phenol exposure in the liver. Proteins were hydrolyzed in white muscle as a secondary metabolic source of energy. The energy level expended by *I. punctatus* exposed to sublethal concentration of phenol was significant enough to bring attention to the resultant biological consequences. Even after post-exposure recovery period, metabolic alterations were maintained, indicating that compensation or adaptation was not achieved by *I. punctatus*. The injuries caused by sublethal concentration of phenol could have ecological implications. The metabolic losses due to phenol contamination can be reflected in impairments on growth, disease resistance and reproduction.

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RESUMO

Os ajustes metabólicos foram estudados em bagre do canal *Ictalurus punctatus* exposto a 1.5 mg L⁻¹ de fenol (10% da CL50) por quatro dias e após recuperação por sete dias. Redução da concentração de triacilglicerol e aumento de ácidos graxos livres no músculo são indicativos de catabolismo de lipídeos. Redução de ácidos graxos livres (-31%) no fígado também indica catabolismo de lipídeos. Aumento da concentração de amônia e da atividade da aspartato aminotransferase (ASAT) no músculo, e a redução na concentração plasmática de aminoácidos sugerem aumento na captação muscular de aminoácidos. Níveis constantes de glicose e o aumento da reserva hepática de glicogênio, associados a baixos níveis de aminoácidos no plasma, indicam gliconeogênese a partir de aminoácidos. Este fato é respaldado pelo aumento de ALAT e ASAT. A indução da LDH hepática acompanhada da redução de lactato plasmático podem indicar que o lactato plasmático foi também utilizado como substrato para a gliconeogênese. As alterações bioquímicas foram exacerbadas após o período de recuperação. Reduções no conteúdo de proteína muscular e plasmática indicam proteólise. O aumento do catabolismo lipídico no fígado foi resultado de uma expressiva redução da concentração hepática de TGA (-58%). Uma preferência catabólica por lipídeos foi observada na tentativa de suprir a elevada demanda energética. Este estudo é o primeiro a abordar o perfil metabólico de *I. punctatus* frente à exposição ao fenol e sua capacidade de recuperação, trazendo a atenção para as consequências biológicas da contaminação ambiental.

Palavras-chave: ajustes metabólicos, enzimas, reservas de lipídeos, metabolismo intermediário, recuperação, xenobiótico.

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