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Cortisol response to acute stress in jundiá *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals

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ABSTRACT

Exposure to agrichemicals can have deleterious effects on fish, such as disruption of the hypothalamus–pituitary–inter-renal axis (HPI) that could impair the ability of fish to respond to stressors. In this study, fingerlings of the teleost jundiá (*Rhamdia quelen*) were used to investigate the effects of the commonly used agrichemicals on the fish response to stress. Five common agrichemicals were tested: the fungicide — tebuconazole, the insecticide — methyl-parathion, and the herbicides — atrazine, atrazine+simazine, and glyphosate. Control fishes were not exposed to agrichemicals and standard stressors. In treatments 2–4, the fishes were exposed to sub-lethal concentrations (16.6%, 33.3%, and 50% of the LC_{50}) of each agrichemical for 96 h, and at the end of this period, were subjected to an acute stress-handling stimulus by chasing them with a pen net. In treatments 5–7 (16.6%, 33.3%, and 50% of the LC_{50}), the fishes were exposed to the same concentrations of the agrichemicals without stress stimulus. Treatment 8 consisted of jundiás not exposed to agrichemicals, but was subjected to an acute stress-handling stimulus. Jundiás exposed to methyl-parathion, atrazine+simazine, and glyphosate presented a decreased capacity in exhibiting an adequate response to cope with stress and in maintaining the homeostasis, with cortisol level lower than that in the control fish ($P<0.01$). In conclusion, the results of this study clearly demonstrate that the acute exposure to sub-lethal concentrations of methyl-parathion, atrazine+simazine, and glyphosate exert a deleterious effect on the cortisol response to an additional acute stressor in the jundiá fingerlings.

1. Introduction

In southern Brazil, aquaculture is still considered as an activity complementary to agriculture, and thus, many ponds used for fish culture are located closer to or inside the agricultural areas, or are filled by water springs that run through the cultivated soil. Because of pest-management practices, large amounts of agrichemicals are used on crops and, as a result, small amounts of these products may reach the ponds used for fish culture (Van der Oost et al., 2003). In southern Brazil, the herbicides glyphosate, atrazine, and Herbimix™ (a combination of simazine+atrazine) are widely used. However, these herbicides are considered as aquatic contaminants (Oulmi et al., 1995). The fungicide tebuconazole is used in plant cultures or as wood preservative (Lebokowska et al., 2003), and the pesticide methyl-parathion is used in fish-culture ponds to kill the aquatic larvae of the predatory insects (Szarek et al., 2000).

The active ingredient of Folicur200CE, tebuconazole, rapidly degrades with short persistence in the environment, and is not bioaccumulative (http://www.milenia.com.br). However, methyl-parathion (Folidol 600) is a “less-persistent” organophosphate insecticide, which is moderately soluble in water and acutely toxic to fishes (Walton et al., 1997). Atrazine and simazine are little affected by the natural degradation processes, resulting in almost permanent contamination of the surface and ground waters (as reviewed by Saglio and Trijasse (1998)). However, glyphosate formulations are rapidly dissipated from the surface waters, and undergo biodegradation by the soil microflora to form α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and CO_2 (reviewed by Glusczak et al., 2007).

Several field studies have focused on the prolonged exposures to agrichemicals, especially in wildlife, which adversely affect the hypothalamus–pituitary–inter-renal axis (HPI) (reviewed by Hontela, 1998). However, the possible effects on HPI axis caused by an acute exposure to these chemicals are not well described. Pacheco and Santos (1996, 2001) verified that fish acutely exposed to xenobiotics

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lost their capacity to elevate cortisol in response to additional stressors. Studies using experimental designs to access the HPI-endocrine disruption, caused by acute exposure to agrichemicals were not carried out for *Rhamdia quelen* or other fish that are suitable for production in the southern South America.

The HPI axis coordinates the stress response in fish, with adverse effects widely known (Wendelaar Bonga, 1997), including in jundiás (Barcellos et al., 2003, 2004). However, works focusing on the adaptive role of the stress response are rare for *R. quelen*. The end product of the HPI axis, the glucocorticoid cortisol, plays a key role in the metabolic and ionic adjustments necessary for coping with stress (Mommsen et al., 1999). Consequently, any adverse effect on the functioning of the HPI axis would compromise the ability of the animal to mount an adequate response to stressors (Hontela, 1998).

Jundiá, *R. quelen*, is an endemic species of southern South America, and is capable of growing in any region with a temperate or subtropical climate. Because of their characteristics, the jundiá has received great attention from the Brazilian researchers. Aspects of its reproductive physiology (Barcellos et al., 2001b, 2002), larviculture (Townsend et al., 2003), stress response (Barcellos et al., 2004, 2006a,b), toxicology (Soso et al., 2007; Kreutz et al., 2008), general physiology (Bello et al., 2000), and transportation (Golombieski et al., 2003) have already been studied. Thus, *R. quelen* were the preferred models for our study, owing to their commercial importance, especially their well-characterized stress response.

Thus, the aim of this work was to verify if acute exposures to sub-lethal concentrations of the selected agrichemicals may affect the cortisol response to an acute stressor in *R. quelen* fingerlings.

2. Materials and methods

The experiments were conducted in March and April 2007, at the facilities of the Universidade de Passo Fundo, Rio Grande do Sul, Brazil. We used 120-day old, mixed-sex jundiá *R. quelen* (Heptapteridae, Teleostei) fingerlings, weighing 15.7±3.3 g (S.E.M., $n=120$). The fingerlings were kept in a 6200-L plastic tank prior to transferring into experimental tanks under natural photoperiod, and were fed twice a day (10:00 and 16:00 h) with commercial extruded food at 5% of body weight (42% crude protein, 3400 kcal kg^{-1} DE).

Water temperature (26±1 °C) and dissolved oxygen concentrations (5.6–7.5 mg L^{-1}) were measured with a YSI model 550A oxygen meter (Yellow Spring Instruments, USA). The pH values (6.6–7.0) (Bernauer pH meter), total ammonia-*N* (<0.5 mg L^{-1}) (colorimetric test), total alkalinity (60 mg L^{-1} $CaCO_3$), and hardness (65 mg L^{-1} $CaCO_3$) were also measured (colorimetric tests).

2.1. Experimental design and treatments

Five experiments were conducted, each with one specific agrichemical, tested with three sub-lethal concentrations (Table 1). The agrichemicals tested were tebuconazole (Folicur200CE™), methyl-parathion (Folidol600™), atrazine+simazine (Herbimix™), atrazine (Siptram500™), and glyphosate (Roundup™).

All the experiments consisted of 8 treatments with 4 replicates (total 32 tanks), containing 95-L chlorine free, well-aerated tap water and 10 fingerlings. Treatment 1 comprised the control (C) group, in which the fingerlings were kept in water without any agrichemicals and no stress was applied; in treatments 2, 3, and 4, the fingerlings were kept in water containing three sub-lethal concentrations of the agrichemical, corresponding to 16.6%, 33.3%, and 50% of the lethal concentrations for acute exposure ($LC_{50-96\ h}$) (Kreutz et al., 2008), and after 96 h were subjected to an acute stress-handling stimulus (chasing them with a pen net for 60 s). In treatments 5, 6, and 7, the fingerlings were kept in water contaminated with the same sub-lethal concentrations for 96 h, but without any application of stress. In treatment 8, considered as the stressed (S) group, the fingerlings were kept in water

Table 1
Commercial and chemical name, manufacturer, usage, $LC_{50-96\ h}$ and the concentrations of agrichemical used in the experiments

	Agrichemical	Commercial name***/ manufacturer	Chemical name	Use	$LC_{50-96\ h}$* (mg L^{-1})	Treatments							
						Control	Agrichemical**+stress			Agrichemical only			Stress only
							16.6%	33.3%	50%	16.6%	33.3%	50%	
						T1	T2	T3	T4	T5	T6	T7	T8
1	Tebuconazole	Folicur™ Bayer SA	2-[2-(4-chlorophenyl)ethyl]-3, 3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)buta *n*-2-ol	Fungicide	5.3	–	0.88	1.76	2.65	0.88	1.76	2.65	–
2	Methyl-parathion	Folidol600™ Bayer SA	*O*-*O*-dimethyl *O*-4-nitrophenyl thiophosphate	Insecticide	4.8	–	0.80	1.60	2.40	0.80	1.60	2.40	–
3	Atrazine+Simazine	Herbimix™ Milenia SA	6-chloro-*N*,*N*′-diethyl-1,3,5-triazine-2,4-diamine+ 2-chloro-4-ethylamine-6-isopropylamino-*S*-triazine	Herbicide	10.5	–	1.74	3.50	5.25	1.74	3.50	5.25	–
4	Atrazine	Siptram500™ SipCam Agro	6-chloro-*N*,*N*′-diethyl-1,3,5-triazine-2,4-diamine	Herbicide	10.2	–	1.69	3.40	5.10	1.69	3.40	5.10	–
5	Glyphosate	Roundup™	*N*-phosphonomethylglycine	Herbicide	7.3	–	1.2	2.43	3.65	1.2	2.43	3.65	–

Each experiment was conducted separately and consisted of 8 treatments as indicated.
*(Kreutz et al., 2008); **Refers to percentage of the $LC_{50-96\ h}$. ***Commercial names might be trademark protected by law. All products were purchased at local stores.