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Agrichemicals chronically inhibit the cortisol response to stress in fish

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HIGHLIGHTS

- We studied the stress response of *Rhamdia quelen* following exposure to agrichemicals.
- Acute exposure of fingerling-aged fish to agrichemicals chronic inhibits stress response.
- The stress axis of fish exposed to MPBI and to TBF were fully recovered after a 180 d.
- The acute exposure to the tested agrichemicals impairs fish growth and survival.

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ABSTRACT

We studied the stress response of *Rhamdia quelen* fingerlings at 45, 90, 135 and 180 d following acute exposure to agrichemicals. Herein, we report the novel observation that acute exposure of fingerling-aged fish to a methyl parathion-based insecticide (MPBI) and to a tebuconazole-based fungicide (TBF) induced chronic inhibition of the stress response. In contrast, fish exposed to an atrazine–simazine-based herbicide (ASBH) recovered the stress response on day 45, and fish exposed to a glyphosate-based herbicide (GBH) did not present stress response inhibition. Additionally, fish exposed to MPBI, GBH and ASBH showed lower survival rates and attained lower final weights. In the case of TBF, the presence of the stressful stimulus more strongly influenced the changes in the performance parameters than did the agrichemical exposure itself. An impairment of the cortisol response may seriously hamper the adaptive response and the ability to promote the necessary metabolic and ionic adjustments to respond to environmental stress.

1. Introduction

Exposing fish to various stressful situations, such as environmental changes, prey-predator interactions and, in the case of aquaculture, several different management procedures (such as the transfer of fish to a different tank or biometrics measurements), triggers a cascade of adaptive alterations. These events have been classified as a stress response (Barton and Iwama, 1991; Wendelaar Bonga, 1997) and are coordinated by the hypothalamus–pituitary-interrenal (HPI) axis (Barton, 2002). The end product of activation of the HPI axis is the glucocorticoid cortisol, which is a regulator of the necessary metabolic and ionic adjustments for coping with stress (Mommsen et al., 1999). Thus, an impairment of the cortisol response may seriously hamper the overall adaptive response and the ability to maintain metabolic and osmoionic homeostasis.

Both chronic and acute exposures to environmental contaminants might disrupt the stress axis and consequently affect stress reactivity in fish (Hontela et al., 1997; Girard et al., 1998; Norris et al., 1999; Pacheco and Santos, 2001; Dorval et al., 2005; Gravel and Vijayan, 2007; Hori et al., 2008), including in jundiá (Cericato et al., 2008, 2009). However, the long-term effects on fish lifespan and stress

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reactivity after acute exposure to agrichemicals during an early life stage have not been reported in the literature. This type of exposure is plausible because most natural and constructed water bodies are located near agricultural areas or have been filled with water that ran through cultivated soil. Significant amounts of the products used in crop production, such as herbicides, pesticides and fungicides, could reach these water bodies and affect non-target organisms (van der Oost et al., 2003).

To address this potential exposure scenario, we posed three questions. First, does an impairment of the cortisol stress response occur following exposure to the test agrichemicals? Second, can the fish restore their ability to trigger the response? Third, could this initial exposure affect survival rates and performance parameters? To answer these questions, fingerlings were acutely exposed to agrichemicals and then monitored for 6 months to assess the long-term effects on both the cortisol response to new stressors and growth performance parameters. This exposure and recovery paradigm was evaluated with fish fingerlings that were stocked in aquaculture ponds at the time of agrichemical application to nearby agricultural fields.

2. Material and methods

2.1. Ethical note

This study was approved by the Ethics Commission for Animal Use (CEUA) of Universidade de Passo Fundo, UPF, Passo Fundo, RS, Brazil (Protocol#3/2011-CEUA, July 2009) and met the guidelines of the Brazilian College for Animal Experimentation (COBEA; http://www.cobea.org.br).

Stress response evaluation based on only peak cortisol measurements (Koakoski et al., 2012; Barcellos et al., 2012a) is derived from established animal welfare science. Several previous studies have used this methodology to evaluate the stress response in *Rhamdia quelen* (Cericato et al., 2008, 2009). Thus, we selected this single point evaluation methodology to prevent the use and sacrifice of more experimental fish than necessary to draw conclusions regarding agrichemical effects on cortisol profiles.

2.2. Location and study subjects

Experiments were conducted from September 2011 to March 2012 at the facilities of the Universidade de Passo Fundo in Rio Grande do Sul, Brazil. We used 90-day-old, mixed-sex *R. quelen* (Heptapteridae, Teleostei) juveniles from Jundiá municipality with an average weight of 11.2 ± 0.32 g (mean ± SEM, n = 1440, 360 exposed to agrichemicals). The fish were kept in a 6200-L plastic tank prior to being transferred into experimental tanks under a natural photoperiod. The fish were fed twice a day, at 10:00 and 16:00 h, with commercial extruded food provided at 5% of body weight (42% crude protein, 3400 kcal kg^{-1} digestive energy, DE).

The mean water temperature in all of the tanks was maintained at 24 ± 2 °C, and the dissolved oxygen concentrations varied from 5.6 to 7.2 mg L^{-1} (both measured using a YSI model 550A oxygen meter; Yellow Springs Instruments, USA). The pH values ranged from 6.2 to 7.4 (measured using a Bernauer pH meter). The total ammonia–nitrogen concentration was less than 0.5 mg L^{-1} in each of the tanks (measured using a colorimetric test), the total alkalinity was 60 mg L^{-1} of $CaCO_3$, and the hardness was 65 mg L^{-1} of $CaCO_3$ (both measured using colorimetric tests).

2.3. Agrichemicals tested

Four experiments were conducted, each with one specific agrichemical used to expose the fish. The agrichemicals used were a methyl-parathion-based insecticide (MPBI, Folisuper600™, 600 g L^{-1} of O,O-dimethyl O-4-nitrophenyl phosphorothioate), a tebuconazole-based fungicide (TBF, Folicur200CE™, 200 g L^{-1} of RS-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol), a glyphosate-based herbicide (GBH, Roundup Original™, 360 g L^{-1} of N-phosphonomethylglycine) and an atrazine–simazine-based herbicide (ASBH, Herbimix™, 450 g L^{-1} of 6-chloro-*N2*-ethyl-*N4*-isopropyl-1,3,5-triazine-2,4-diamine + 450 g L^{-1} of 6-chloro-*N2,N4*-diethyl-1,3,5-triazine-2,4-diamine). The exposure concentration used for each agrichemical was based on previously reported results (Cericato et al., 2008, 2009; Kreutz et al., 2008; Ferreira et al., 2010), and corresponded to 16.6% of the calculated lethal concentration for 50% of animals (LC_{50}) at 96 h (MPBI = 0.80 mg L^{-1}; TBF = 0.88 mg L^{-1}; GBH = 1.21 mg L^{-1} and ASBH = 1.74 mg L^{-1}). All chemicals were obtained from commercial sources.

2.4. Study design

In each experiment, fish were divided into four treatment groups with three replicates per group, i.e., a total of 12 tanks. Each tank contained 900 L of chlorine-free, well-aerated tap water and 30 fish. Treatment 1 was the control (C) group, in which the fish were kept in water without agrichemical exposure and were not subjected to stress. In treatment 2, the stressed (St) group, the fish were kept in water without agrichemical exposure but were subjected to an acute stress stimulus after 96 h. In treatment 3, the fish were kept in water containing a sub-lethal concentration of the specific agrichemical for 96 h. Finally, in treatment 4, the fish were kept in water contaminated with the same sub-lethal concentration of an agrichemical for 96 h and were subjected to an acute stress stimulus, i.e., being chased with a pen net for 60 s (Barcellos et al., 2004). The elapsed time between the stress application and sampling for all fish was 30 min because previous results indicated that cortisol peaks at this time in fingerling *R. quelen* (Koakoski et al., 2012; Barcellos et al., 2012a).

After this initial sampling, the fish in all treatment groups were maintained in water for a 180-d recuperation period, in the absence of exposure. During this period, fish from treatment groups 2 and 4 were subjected to a stress test on days 45, 90, 135 and 180, and all of the fish were sampled at these times. A schematic representation of the experimental design is depicted in Fig. 1.

All experiments were conducted using a static-test design during the exposure period. Because cortisol is a glucocorticoid that might be influenced by starvation (Barcellos et al., 2010), the fish were fed daily during the 96 h exposure (24, 48, and 72 h after the beginning of exposure) at a rate of 0.75% of their biomass. During the 180-d recuperation period, the fish were fed twice daily at 5% of their biomass. Food residues and feces were not removed during the exposure period to prevent any stress caused by introducing a cleaning siphon. During the recuperation period, a water change rate of 100% per day (in open circulation system) helped keep the tanks clean, as the tanks had a conical bottom. To ensure optimal conditions for viability, water quality parameters were accessed daily to verify whether parameters ranged within normal concentrations.

The fish were closely observed to identify potential declines in individual and/or group health over the entire exposure period. Abnormal swimming behavior, skin darkening, anorexia and body lesions were observed to detect fish at a moribund stage. Fish in this situation were immediately captured, anesthetized with buffered (NaH_2CO_3) MS222 (300 mg L^{-1}) and euthanized by spinal section.