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Negative impact of a cadmium concentration considered environmentally safe in Brazil on the cardiac performance of bullfrog tadpoles

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ABSTRACT

A drastic amphibian decline has been observed worldwide, which can be attributed (among other factors) to exposure to pollutants. Considering that cadmium corresponds to the most rapidly increasing trace metal in the environment, the aim of this work was to evaluate whether the exposure (2 and 16 days) of bullfrog tadpoles to this trace metal, at the concentration currently considered environmentally safe (at 1 ppb) in class 1 and 2 waters by the Brazilian Environmental Council, can affect the cardiac performance of these animals. The acute exposure (2 days) of tadpoles to cadmium resulted in a marked bradycardic response, which was correlated with an incomplete cardiac relaxation, without any compensation by improved cardiac twitch force (Fc) or contraction velocity (TPT), nor even by cardiac hypertrophy. Indeed, after 16 days of exposure, the cardiac function of tadpoles became even more depressed due to a marked decrease in Fc, a prolongation of TPT, and also incomplete relaxation (*i.e.* increases in the ventricle resting tension), without changes in ventricle relative mass. Altogether, the cardiodepressive effects of cadmium (especially after more prolonged exposure periods) impose negative alterations on a tadpole's development and also impede adequate homeostatic adjustments to respond appropriately to the exposure to cadmium with increase in energetic demand to counteract the deleterious effects of the xenobiotic. These disturbances can impair tadpoles' growth, development and reproduction. It is a fact that allows us to strongly suggest that cadmium concentrations, which are currently considered environmentally safe in Brazil, should be revised.

1. Introduction

Amphibians correspond to the vertebrates that present the highest diversity of modes of life, reproductive behavior, and life cycles (Duellman, 1989). The peculiarities of their physiological processes and high susceptibility to pollutants due to their permeable skin make them eligible as adequate indicators of environmental health (Relyea, 2003). Additionally, their dual (aquatic and terrestrial) life cycle and migration to different breeding sites increase the probability of exposure to environmental contaminants (Murphy et al., 2000).

Xenobiotics – alone or in association with habitat loss, over harvesting, ultraviolet-B radiation, global warming, and/or diseases – have been considered one of the main factors underlying the decline in amphibian populations, especially over the last three decades (Collins and Storfer, 2003). Despite this, toxicological research on amphibians has been rather scarce compared to research on other vertebrates (Venturino et al., 2003).

Some studies demonstrated that amphibians are lethally impacted by exposure to petrol and its derivates (Mahaney, 1994), acidification (Rowe et al., 1996; Sadinski and Dunson, 1998), pesticides (Johansson et al., 2006; Gurushankara et al., 2007), and several trace metals (Freda, 1991). Murphy et al. (2000) also describe sublethal effects of amphibians' exposure to these pollutants, such as reductions in body length and mass, intersex gonads, limb malformations, and suppression of immune system. Noteworthy in this aspect is that studies examining toxicity using endpoints that can be the consequence of numerous etiologies are barely instructive when compared to those that focus on potential underlying mechanisms, the latter providing a basis for understanding how exposure to chemicals is likely to influence larval growth, behavior, and development (Mann et al., 2009). In this context, the study of physiology becomes a useful tool to ecotoxicologists.

The trace metals can bioaccumulate, resulting in morphological and/or physiological alterations (Rowe et al., 1996, 1998). Despite being naturally found in the environment at very low concentrations,

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anthropogenic actions are also responsible for cadmium accumulation. In the decade of 1990, cadmium corresponded to the trace metal that presented the most rapidly increasing concentrations in the environment (Alloway et al., 1990). Among the sources of the increasing environmental contamination by cadmium, it is worthwhile to mention other metal exploitation and refining (*e.g.* zinc and lead) (Boon and Soltanpour, 1992), residues of industries of taint, batteries (Potsch, 1967), fuels, and lubricants (Olsen, 1972), as well as the utilization of agricultural correctives, fertilizers (Amaral-Sobrinho et al., 1992), and fungicides (Lagerwerff, 1972). Considering there is no data from then until now concerning the actual increments of the concentration of this metal in the environment, one could expect it remained increasing due its continuous and crescent employment worldwide in the above mentioned activities from then until now, what can make us infer that now cadmium represents an even higher concern to ecotoxicologists.

Cadmium is potentially toxic to humans, as well as to plants and other animals (Pierangeli et al., 2005). This trace metal accumulates in vertebrates by biomagnification over the trophic chain. This process can last several years, considering that cadmium elimination occurs very slowly (Vogiatzis and Loumbourdis, 1998). Flament et al. (2003) point out that amphibians play a key role in the cadmium biomagnification process, even when exposed to an extremely low concentration of this metal.

Several studies focused on the effects of amphibians' exposure to cadmium. Among the effects, we highlight the following: a delay in metamorphosis, reduced growth (Fort et al., 2000; Flament et al., 2003), and theratogenesis of skin, eye, and digestive system (Sunderman et al., 1991). Of particular interest in the present context is the fact that ventricular myocytes, *Pelophylax ridibundus* tadpoles, present marked histogenesis alterations in response to increasing cadmium concentrations (Rudenko and Iamshchikov, 2001), resulting in tissue differentiation disorders, suppression of proliferative capacity, and death of cardiomyocytes. Cardiac deformities due to exposure to cadmium have also been observed by Sunderman et al. (1991) with *Xenopus laevis* tadpoles.

In addition to the previous findings, cadmium is employed in several physiological experiments as a pharmacological tool to analyze the cardiac function of isolated preparations, due to its property of selectively blockading sarcolemmal Ca^{2+} influx pathways (*i.e.*, L-channels and the Na^{+}/Ca^{2+} exchanger), not only in myocytes from mammals (Lee and Tsien, 1984; Lansman et al., 1986; Mitra and Morad, 1985; Kirby et al., 1993; Vornanen, 1996; Ásgrímsson et al., 1995; Adachi-Akahane et al., 1997; Hobai et al., 1997), but also in amphibian myocytes (Bonvallet and Rougier, 1989; Shepherd et al., 1991; Alvarez and Vassort, 1992; Badaoui and Leoty, 1994). Despite these studies clearly indicating that exposure to cadmium chloride results in negative cardiac inotropy, ecotoxicological studies that evaluate the possible effect of exposure to sublethal concentrations of cadmium chloride in premetamorphic amphibians are lacking.

In this sense, particularly regarding to the relevance of the employment of cardiac biomarkers as sensitive endpoints, it is worthy to mention a study performed by Thomaz et al. (2008) with the juvenile fish *Oreochromis niloticus* exposed (96 h) to a sublethal concentration of trichlorfon that demonstrated that among the endpoints analyzed (oxidative stress biomarkers, *in vitro* contraction force and heart frequency), that the cardiac function was the most sensitive biomarker. This was not the only study employing the cardiac function as a biomarker. Other authors have already proved this endpoint is very sensitive and useful when we intend to notice the negative impact of a sublethal concentration of environmental stressors over an organism before it will cause mortality or other multifactorial deleterious effects that could ultimately result in a wide decline in the population level in mammals (Calore et al., 2007), fish (*e.g.*, Martins et al., 2013; Andrade Waldemarin et al., 2012), but also in amphibians (*e.g.*, Cos et al., 2004; Costa et al., 2008).

In this context, the goal of this work was to test whether different exposures (2 and 16 days) of bullfrog tadpoles (25 Gosner stage), *Lithobates catesbeianus* (Shaw, 1802), to the sublethal cadmium chloride concentration of 1 ppb – which is considered environmentally safe by the Brazilian Environmental Council (CONAMA, resolution # 357/2005) to class 1 and 2 waters – can potentially have a negative effect on cardiac function. Classes 1 and 2 waters are considered the inland waters that correspond to the habitat of the anura biota due to the quality required by CONAMA's #354 Resolution (2005) to their main uses.

The relevance of selecting this endpoint as the biomarker of exposure to cadmium chloride is that this trace metal is usually found in water in concentrations that are rarely high enough to directly cause mortality, but rather disturb some mechanism (such as cardiac performance) that impairs the maintenance of body homeostasis and ends up indirectly jeopardizing anuran populations. Indeed, while most studies focused on the negative impacts xenobiotics cause on growth, development, and behavior of anuran tadpoles, this study focused on cardiac performance, which is a much more specific and sensitive biomarker.

2. Materials and methods

2.1. Animal care

Newly hatched *L. catesbeianus* (Shaw, 1802) tadpoles were obtained from a breeding colony at Ibaté, in southeast Brazil. Tadpoles were housed in 50 L aquaria equipped with a continuous supply (1.2 L/h) of well-aerated and dechlorinated water at a constant temperature (25 ± 1 °C) under natural photoperiod (~12 h light/dark cycle) until they reached Gosner (1960) developmental stage 25 (~ 1 week), at which time cadmium chloride was applied. Animals were fed *ad libitum* every two days with cooked lettuce leaves, which were withheld 48 h before exposure. This feeding pattern was maintained for the animals exposed during 16 days, while animals exposed during 2 days were not fed. All leftover food and feces remaining in the aquaria were removed 12 h later (at the time of the water renewal, in the case of the 16 days tests).

2.2. Design of ecotoxicological experiments

The water was monitored daily to ensure that the physical and chemical parameters were kept at acceptable levels (pH 7.1–7.3; hardness as $CaCO_3$ 28–34 mg L^{-1}; dissolved oxygen 6.8–7.5 mg L^{-1}), similar to most Brazilian inland waters. Ammonia concentrations in the water was monitored every two days (K-1510; CHEMets) and remained below 2 mg L^{-1}. All physical-chemical parameters feel within acceptable ASTM (2000) guidelines.

Tadpoles, with body mass between 2.85 and 9.41 g (3.68 ± 2.57 g – mean ± SE), were randomly divided into two triplicated experimental groups: control (02CT; $n=6$, and 16CT; $n=10$) and cadmium chloride-exposed (Sigma-Aldrich Chemical Co) at the sublethal concentration of 1 μg L^{-1} for 2 days (02CD; $n=6$) in a static-system, and for 16 days (16CD; $n=10$) in a semi-static system (renewed every 2 days). This concentration was chosen based on the highest concentration considered environmentally safe by CONAMA (Resolution # 357/2005) for classes 1 and 2 waters in Brazil.

All experimental groups were placed in 12 L glass aquaria filled with dechlorinated well-aerated water (> 6.0 mg O_2/L), with a controlled temperature (25 ± 1 °C) on a 12:12 h light:dark cycle. Aquaria sides were dark-covered to prevent external disturbance. All procedures followed ASTM (2000) guidelines, and the experiments were previously approved by the University Ethics Committee (Protocols # 3573921/2008-3), which follows Brazilian regulating laws.

From the first day after exposure until the last one, the activity level of bullfrog tadpoles of all experimental groups was monitored twice a day according to the methodology proposed by Fraker and Smith (2004) for *Lithobates pipiens* tadpoles. Briefly, "activity" was defined as a constant movement through aquaria, while "inactivity" meant that the animal keeps stationary on the bottom or passively floats.

2.3. Determination of the "in loco" heart rate

For the determination of the heart rate, four animals of each treatment were randomly taken from each of the three replicate aquarium of each treatment