Effects of cadmium on the behaviour of *Cnesterodon decemmaculatus*

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Abstract: Recognising biomarkers for assessing early ecotoxicity effects using test species exposed to reference toxicants is important, hence the aim of this work was to study changes in some behavioural parameters of *Cnesterodon decemmaculatus*. Fish were under stress conditions induced by exposure to a subtoxic concentration of cadmium (Cd) in the laboratory. Acute bioassays (96 h) were performed under laboratory conditions, with medium renewal every 48 h. Animals were randomly assigned into two groups of 10 individuals each: Cd-exposed (0.50 mg Cd. L^{-1}) and control (moderate tap water). Both groups were video-recorded every day for 15 min at the same time of day. Each individual was evaluated for: a) aggressive behaviour; b) sexual behaviour; and c) swimming alterations. Fish exposed showed irregular swimming, hyperactivity periods, fewer copulation attempts and same aggressive behaviour as compared with the control group. Our results suggest that these behavioural alterations may serve as early and reliable biomarkers.

Keywords: *Cnesterodon decemmaculatus*; cadmium; aggressive behaviour; sexual behaviour; swimming behaviour; bioassay; video recorder; behavioural biomarkers.

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1 Introduction

A biomarker is defined as an observable and/or measurable change at the molecular, biochemical, cellular, physiological or behavioural level that reveals present or past exposure of an individual to a pollutant. Behavioural biomarkers in vertebrates are gaining increasing interest in aquatic ecotoxicological studies. Individuals exposed to toxicants may exhibit behaviour alterations (i.e., effect biomarkers) having potential impacts at higher levels of organisation such as the population and community levels. Fish are a good model for the study of behavioural toxicology due to their ecological relevance and because their behaviours can be easily observed and quantified. They can be found at different levels of the trophic chain and have a high morphological, physiological and ecological diversity (Dell'Omo, 2002).

Cnesterodon decemmaculatus (Poeciliidae, Cyprinodontiformes) is a South American native species widely distributed throughout the Neotropical Region including the La Plata basin where it reaches high densities in lotic and lentic water bodies of the pampean region. This species can be found from pristine to heavily degraded habitats. It is a small viviparous, micro-omnivorous, benthic-pelagic, non-migratory fish, which is easy to handle and acclimate to laboratory conditions (Ferrari et al., 2017). Due to intrinsic characteristics, such as its small size, wide range of tolerance, easy breeding in laboratory conditions it is commonly employed in bioassays to study heavy metals (Mastrangelo and Ferrari, 2013; Baudou, 2019), pesticides (Menendez-Helman et al., 2015) and contaminated river waters (Ossana et al., 2019).

Cadmium is a well-studied, non-ferrous metal of unknown physiological role which has been classified as a priority pollutant in ecosystems. The limits for surface water argentine guidelines for protection of aquatic life are 01–0.4 µg/L. In Argentina was found in 2 µg/L in Reconquista river (Rigacci et al., 2013) and 0.02 mg/L in Pilcomayo river (Casares, 2012). Alam et al. (2011) found Cd concentrations between 0–70 µg/L in Bangladesh wetlands.

In aquatic environments, anthropogenic activity increases Cd concentrations and in fish Cd may be accumulated in multiple target organs, (e.g., kidneys, liver and gills) due to its long biological half-life (Rani et al., 2014). Cadmium has toxic effects in fish, causing abnormal swimming activity in a concentration of 0.3–0.6 mg/L (Sloman et al., 2003; Eissa, 2009), alterations in gill morphology observed in 300 µg/L (Eissa, 2009), alterations in the energetic balance in concentration of 0.5 mg/L (Baudou, 2019), increase in antioxidant enzymes activity in Nile tilapia exposed to 0.75 mg/L (Almeida et al., 2009), and genotoxic effects in erythrocytes evaluated by micronuclei test and comet assay in a concentration of 2.0 mg/L (Ossana et al., 2019), among others. For *C. decemmaculatus* ecotoxicological studies, cadmium was proposed as reference toxicant, both for lethal and sub-lethal response (Mastrangelo and Ferrari, 2013). In fish, cadmium is taken up directly from the environment but may also be ingested with contaminated food. It is distributed in most tissues and accumulated in gills, liver, kidney, hepatopancreas. Cd in the cell is often bound to cytoplasmatic proteins (detoxifying mechanism), elimination occurs via the kidney (Rani et al., 2014).

In recent years, sexual activity has been used as a non-invasive and sensitive indicator of toxicity, with alterations in reproductive behaviour including a decrease in the intensity of sexual display and in the frequency of copulation attempts and copulation successes (Wang et al., 2014).

It is well known that pollutants result in alterations in fish social behaviour. Sloman et al. (2002), who studied social dominance behaviour in rainbow trout exposed to copper (30 µg/L), observed that metal accumulation in tissues influenced their social rank. In fish, the olfactory organ is one of the first targets of pollutants, leading to impaired chemoreception and associated changes in aggression levels. Cadmium is taken up in the olfactory rosette and then moved along the olfactory nerve to finally accumulate in the olfactory bulb, thus affecting responses to natural pheromones. Non-visual sensory systems such as olfaction are undoubtedly important to successful migration (Sloman et al., 2003).

The goal of this work was to assess the effect of acute exposure to a sublethal cadmium concentration on some behavioural parameters of *Cnesterodon decemmaculatus*.

2 Methods and materials

We used fish from our stock, adults of *Cnesterodon decemmaculat*us. They were reared in aquaria filled with dechlorinated tap water and controlled temperature (21°C) and photoperiod (16 hL/8 hD). Then were transferred to glass aquaria to conducted the bioassay. A total of 40 animals were used, measuring 24.6 ± 0.6 mm in length and 108.4 ± 9.6 mg in weight.

Fish used in the experiments have been cared for according to the University Bioethics Committee (Universidad Nacional de Luján, DISP SE ACAD LUJ-0001438-18) criteria.

Hardness, dissolved oxygen (oxymeter Hach), pH (pHmeter Mettler), hardness (Aquamerck test kit, sensitivity 1 mg/L $CaCO_3$) and conductivity (conductivity meter Hach) were recorded daily in each aquarium.

Cadmium solutions were prepared from a stock solution containing 1000 mg Cd/L (prepared from $CdCl_2.2.5H_2O$, J. T. Baker, Phillipsburg, NJ, USA, in double distilled water). The effective concentration of Cd in the solutions assayed was measured by atomic absorption spectroscopy (Perkin Elmer, AAnalyst 200 model, quantification limit: 0.048 ± 0.003 mg/L) equipped with hollow cathode lamps (□ = 228.8 nm). The calibration curve was in the range of 0.1 to 1.0 mg Cd/L ($y = 0.167x – 0.016$; $r^2 = 0.997$).

Bioassays were carried out in duplicate and included two steps: a 15-d acclimation period to the experimental conditions mentioned above and a 96-h exposure to $CdCl_2$. The aquaria media were renewed every 48 h over the duration of the bioassay. During the acclimation period, 10 fish were kept in each of four glass aquaria (14 x 14 x 20 cm) containing moderately hard water (MHW) with the following composition: $NaHCO_3$ 96 mg/L; $CaSO_4.{}_2H_2O$ 60 mg/L; $MgSO_4$ 60 mg/L; KCl 4 mg/L; pH, 7.4–7.8; hardness 80–100 mg CO_3Ca/L. Aquaria were placed in an incubation chamber supplied with continuous aeration, at constant temperature (21 ± 1°C) and photoperiod (16 h L/8 h D), and these conditions were maintained until the end of the assays. After the completion of the acclimation period, fish were separated into two groups, one of which was exposed to 0.5 mg/L of $CdCl_2$ (nominal concentration) in MHW (exposed group-EG) and the other remained in MHW (control group-CG). At initial time, 10 fish (six females and four males) were used per replicate. Hardness, dissolved oxygen (DO), pH, Cd concentration and conductivity were recorded daily in each aquarium.

From each video recording the following behavioural parameters were analysed in the treated and control groups during the experimental period:

- swimming alterations (number of hyperactivity and loss of balance episodes)
- sexual behaviour (number of copulation attempts)
- aggressive behaviour (number of biting, fighting, chasing and tail lashing observed).

Fish were filmed daily with a digital video camera for 15-min. Behavioural assessment was performed by the Multifocal or Sweeping method, in which different observers focus on each individual of the studied groups. The first two minutes of the film were excluded to avoid potential bias in fish responses induced by the operator.

Statistical differences in aggressive behaviour, swimming alterations and sexual behaviour between the Cd-exposed and control groups were tested using the Wilcoxon-Mann-Whitney test at the $p<0.05$ significance level. Statistical analysis was carried out with Infostat software v.26.01.

3 Results and discussion

During the experimental period, no mortality was recorded in fish either from the control or the exposed groups.

Table 1 shows the values of water quality parameters in the aquaria of the control and exposed groups during the experimental period. Except for Cd^{2+} concentrations, there were no significant differences ($p > 0.05$) in the measured parameters between groups. The levels of dissolved oxygen were suitable for fish, resulting in no additional stress.

Table 1 Physicochemical parameters of the assay media. Mean ± SD. ND = not detected

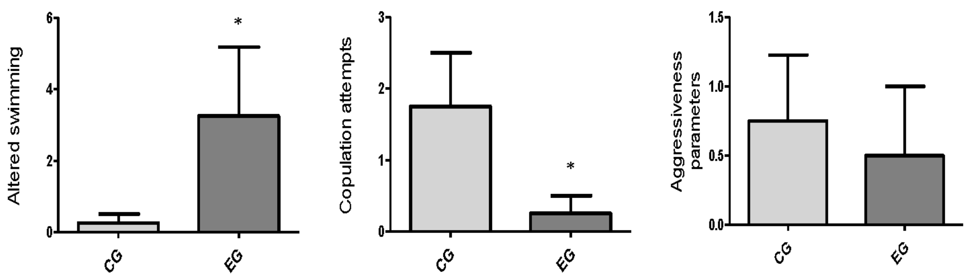
Parameters	*Control*	*0.5 mg Cd/L*
pH	7.36 ± 0.4 ($n = 8$)	7.44 ± 0.22 ($n = 7$)
DO ($mg.L^{-1}$)	7.62 ± 0.4 ($n = 6$)	7.52 ± 0.8 ($n = 8$)
Conductivity ($\mu S.cm^{-1}$)	340 ± 21 ($n = 8$)	325 ± 31 ($n = 8$)
Hardness (mg $CaCO_3.L^{-1}$)	9 ± 7 ($n = 9$)	92.6 ±1($n = 4$)
Cadmium ($mg.L^{-1}$)		
Nominal (0.50) – real:	ND	0.46 ± 0.03 ($n = 4$)

Cadmium-exposed fish (EG) showed a significant increase ($p < 0.038$) in the episodes of abnormal swimming (e.g., hyperactivity, loss of balance and jerky movements) (Figure 1(a)), a significant decrease ($p < 0.044$) in copulation attempts (Figure 1(b)) and same levels in aggression with respect the control group animals (CG) (e.g., biting, fighting, chasing and tail lashing) (Figure 1(c)).

Animal behaviour results from the complex interaction between an organism and its environment. Therefore, it can be used as an indicator of the individual's health status. Exposure to pollutants can induce alterations in a variety of behaviours, associated with sexuality and reproduction, activity, motivation, communication, aggression, dominance and cognitive abilities such as learning, among others (Sloman et al, 2002). Changes in habitat selection behaviour also occurs when contaminants lead organisms to avoid contaminated areas making them migrate to other regions, leading to an imbalance in the food chain (Araújo et al., 2018).

The inhibition of mechanosensory systems is one way through which cadmium exposure alters animal behaviour. In fish, olfactory impairment affects food search, the selection of an appropriate mate and the ability to follow migratory routes or to evaluate the risk of predation (Lürling and Scheffer, 2007). Exposure to cadmium also alters behaviours involving swimming, intraspecific interactions, predator/prey interactions, escape behaviour and avoidance responses (Scott et al., 2003; Sloman et al., 2003; Eissa, 2009).

Figure 1 (a) episodes of abnormal swimming; (b) copulation attempts and (c) aggressiveness parameters recorded in *Cnesterodon decemmaculatus* individuals from the control (CG) and the Cd-exposed (EG) groups. Mean ± SD. Asterisk indicates significant difference ($p < 0.05$) from the CG



In our study, *C. decemmaculatus* individuals acutely exposed to cadmium mainly displayed hyperactivity. Alterations in swimming behaviour characterised by hypo-and hyperactivity responses resulting from sub-lethal exposure to 0.30–0.60 mg Cd/L were previously observed in our laboratory in *Cyprinus carpio*, *Australoheros facetum* and *Astyanax fasciatus* (Eissa, 2009). Individuals of *Poecilia reticulata* acutely exposed to $CdCl_2$ (22.5–36.0 mg/L) exhibited various behavioural disorders such as imbalanced swimming, capsizing, attaching to the surface, difficulty in breathing and gathering around the ventilation filter, slowness in motion and sinking down to the bottom (Yilmaz et al., 2004). Baudou (2019) observed in *C. decemmaculatus* exposed to comparable Cd (0.445 ± 0.043 mg/L) concentrations a decreased food intake and increased metabolic rate.

In the present study, acute exposure of *C. decemmaculatus* to Cd resulted in fewer copulation attempts. Rainbow trout exposed to 2.27 ± 0.05 µg/L Cd showed severely impaired social interactions (Sloman et al., 2003), while concentrations as low as 1 µg/L Cd decreased the reproductive capacity of fathead minnows (Wang et al., 2014). In fish Cd is a well-known endocrine disruptor due to its neuroendocrine toxicity (García-Santos et al., 2013), affecting multiple important pathways involved in reproduction and development in *Oreochromis niloticus* exposed to 25 mg/L of $CdCl_2$.

We observed that the Cd did not induced changes in the aggressive behaviour, in the studied individuals of *C decemmaculatus*. Other authors such as Almeida et al. (2009) observed a reduction in aggressiveness in Nile tilapia exposed to 0.75 mg/L $CdCl_2$ for 15 days. These behavioural responses would be due to the disruptive effect of cadmium on the olfactory system, which plays a key role in foraging and social interactions. Olfactory impairment is due to accumulation of Cd in the olfactory rosette, altering the fish's ability to respond to natural pheromones (Scott et al., 2003). The use of behavioural biomarkers in environmental monitoring studies requires the establishment of baseline levels to characterise their intrinsic natural variability (Baudou, 2019). This allows to distinguish between natural variability (noise) and the adverse effect induced by pollutants (signal).

In brief, short-term exposure of *C. decemmaculatus* to a sub-lethal concentration of Cd (0.50 mg/L) induced alterations in behavioural parameters involving locomotion (i.e., increase in abnormal swimming and hyperactivity) and reproduction (i.e., fewer copulation attempts). It is worthy to mention that these responses were observed from the beginning of the experiment. The study of these parameters can be linked with apical endpoints such as: foraging, predator avoidance, reproduction, social structures and survival.

The knowledge that certain particular behavioural abnormalities are indicative of exposure to specific chemicals and their study would greatly improve the predictive capabilities of behavioural endpoints in environmental monitoring for risk assessment. The level, frequency and duration of exposure (dose) relative to the inherent sensitivity of the organism and its metabolic (detoxification) capabilities largely determine the magnitude of the resultant changes in behaviour. The initial response of fish to the exposure of contaminants is often behavioural, such as in altered habitat selection, abnormal swimming, feeding, social and reproductive behaviour. This, in turn, influences the survival, reproductive success and distribution of the individuals; and ultimately in the environmental dynamics of the population and biodiversity.

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