Chemosphere xxx (xxxx) xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Copper and cadmium impair sperm performance, fertilization and hatching of oocytes from Amazonian fish *Colossoma macropomum*

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HIGHLIGHTS

- *Colossoma macropomum* spermatozoids are affected by copper and cadmium exposure.
- The motility rate of spermatoazoids from *Colossoma macropomum* decrease in the presence of copper and cadmium.
- High concentration of cadmium and copper cause a reduction of fertilization rate in *Colossoma macropomum*.
- Cadmium and copper cause a reduction of hatching rate of *Colossoma macropomum* eggs.
- Antioxidant enzymes of spermatozoids of *Colossoma macropomum* were affected by copper and cadmium.

ARTICLE INFO

Article history:
Received 28 August 2020
Received in revised form
8 November 2020
Accepted 10 November 2020
Available online xxx

Handling Editor: Willie Peijnenburg

Keywords:
Fish sperm
Transition metals
Gametes
Tambaqui
Reproduction

ABSTRACT

The contamination of aquatic environments by transition metals can have a direct influence on the reproductive process of several organisms in the aquatic biota. This study aimed to evaluate the effect of cadmium and copper on the sperm of tambaqui (*Colossoma macropomum*). Male (n = 4) and female (n = 4) specimens of *C. macropomum* were induced to spermiation and ovulation, with sperm being activated in the following media: 0; 0.6; 1.2 and 1.8 mg/L of cadmium ($CdCl_2$) and 0; 0.4; 0.8 and 1.2 mg/L of copper ($CuCl_2$). Sperm quality was assessed through time (s) and motility rate (%), superoxide dismutase (SOD) and glutathione S-transferase (GST) activities, lipoperoxidation levels (LPO), and morphological characteristics. In parallel, the effects of these metals on the rate of fertilization and hatching of the oocytes were evaluated. The duration and motility rate of sperm were longer in the control treatment, 85.67 ± 11.01 s; 90 ± 0.01%, and progressively decreased to 44.67 ± 4.16 s and 60 ± 5%, respectively, in concentrations of 1.8 mg/L (44.67 ± 4.16 s; 60 ± 5%) of $CdCl_2$ and to 65.67 ± 3.30 s; 70 ± 5%, respectively, in concentrations of 0.8 mg/L of $CuCl_2$. We observed an increase in the activity of the SOD enzyme in sperm cells exposed to 1.2 mg/L of $CdCl_2$. The LPO levels were increased significantly in sperm cells exposed to 1.2 and 1.8 mg/L of $CdCl_2$ and 0.8 mg/L of $CuCl_2$. Fertilization and hatching were severely impaired in the presence of Cd and Cu. These data indicate that environments contaminated with cadmium and copper harm the gametes of *C. macropomum*.

1. Introduction

Transition metals are continuously released into the aquatic environment through anthropic activities (Kollár et al., 2018) and have a toxic, persistent and bio-accumulative effect on organs and tissues of aquatic organisms (Liang et al., 2016). In aquaculture, fish are exposed to these toxic elements during the production process. Excessive use of feed, pesticides, fertilizers, medicines and release of effluents, which include inorganic components such as cadmium and copper that can accumulate in the sediment, are the major problems for cropping systems (Mendiguchía et al., 2006; Oga et al.,

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https://doi.org/10.1016/j.chemosphere.2020.128957
0045-6535/

2008; Leira et al., 2017).

Contamination by transition metals in aquatic environments can directly affect the reproductive potential of fish by disturbing different mechanisms: endocrine disruption and/or impaired embryonic development, hatching and larval growth, among others (Kime, 1995; Dey et al., 2009). There are also direct effects on sperm as motility disturbances, speed reduction, impairment of fertilization capacity, and decrease of DNA integrity (Dietrich et al., 2010; Hatef et al., 2013).

Currently, in vitro tests are widely used in aquatic toxicology due to their practical and ethical advantages over in vivo tests (Kollár et al., 2018). Mainly primary fish cells (Maunder et al., 2017), such as sperm are used. The spem cells have characteristics that make them a suitable toxicological model for in vitro experiments, including ease collection by a non-invasive method and measurable parameters, such as motility, morphology, speed, metabolism, among others, that can respond to effects of toxic substances (Olsen et al., 2005; Gazo et al., 2015).

Exposure of fish sperm to different metals, such as cadmium and copper, affects the quality of these cells, decreasing the mobility time and increasing morphological damage, oxidative damage and lipid peroxidation (Dietrich et al., 2010; Bombardelli et al., 2016), compromising the fertilization process. In the present study, sperm from tambaqui, *Colossoma macropomum* (Cuvier, 1816), an omnivorous species of the family Serrassalmidae, widely used in studies of environmental contamination in the Amazon, were evaluated (Araujo-Lima and Goulding, 1998; Sadauskas-Henrique et al., 2017). This species is exploited in artisanal fisheries in the Amazon region, contributing to the regional economy. As it easily adapts to breeding conditions, presenting rapid growth worldwide (Mendonça et al., 2009), it is under a significant pressure. *Colossoma macropomum* has been used as a model species in ecotoxicological studies, with emphasis on physiology and bioaccumulation of metals in the gills and liver (Matsuo et al., 2005; Matsuo and Val, 2007). As far as we know, there are no studies related to the in vitro effect of copper and cadmium on *C. macropomum* sperm.

Considering the complex interactions between pollutants and aquatic biological systems, our objective was to analyze sperm quality, fertilization and hatching rate of *C. macropomum* oocytes exposed to different concentrations of cadmium and copper. Furthermore, we hypothesize that exposure to copper and cadmium affects specific characteristics of *C. macropomum* sperm and hinders fertilize ability.

2. Material and methods

All procedures and experimental manipulations used in this study were carried out following the Brazilian Guidelines for Animal Care and were approved by the Ethics Committee for the Use of Animals of the National Institute for Research in the Amazon- INPA, under the protocol number 004/2018.

2.1. *Semen* and oocyte *collection*

Males (n = 4; 58.7 ± 0.25 cm; 3.9 ± 0.43 Kg) and females (n = 4; 60.85 ± 1.28 cm; 5.37 ± 0.64 Kg) of *C. macropomum* were randomly selected from the Aquaculture Technology, Training and Production Center - CTTPA (Balbina, Presidente Figueiredo, Amazonas - 1° 55′54.4 "S; 59° 24′39.1″ W). The animals were acclimated for 6 h in 500L masonry tanks, with continuous aeration and flow thru well water (pH 6.3, 6.7 mg O_2 L^{-1} and 29.5 °C). Then, the animals were induced to produce sperm and oocytes by intraperitoneal application of crude carp pituitary extract (CPE). The induction period for males and females occurred in the interval of 2 h ensuring the collection of gametes for the immediate fertilization process. Doses of 0.5 mg of CPE/kg and 1.0 mg of CPE/kg for males, and 1.0 mg of CPE/kg and 2.5 mg of CPE/kg for females, with a 12 h interval between each dose were injected in the experimental animals. The sperm was collected 6 h after injection of second hormone dose, and oocytes were collected 8 h after injection of second hormone dose. The first batches of oocytes and sperm were discarded to avoid contamination with water, blood, feces, or urine. Approximately 4 mL of sperm from each specimen were collected in graduated Falcon tubes (15 mL), immediately diluted in a 1:10 ratio (50 μL of semen: 450 μL of diluter) in Beltsville Thawing Solution (BTS-MINITUB®) (Pestrana et al., 2018) and refrigerated at 4 °C for immediate motility analysis under an optical microscope (Leica DM500; 40x). The oocytes were collected in a Petri dishes. Oocytes and sperm from each fish were mixed and stored separately, according to Sanches et al. (2011). For enzymatic analysis, sperm were immediately frozen in liquid nitrogen after exposure to metal, and for morphological analysis, fixed in formaldehyde-citrate and transported to the Laboratory of Ecophysiology and Molecular Evolution of the Brazilian National Research Institute of the Amazon - INPA, for later analyzes.

2.2. Means of activation

The effect of copper and cadmium on sperm motility, the following nominal concentrations of copper (0; 0.4; 0.8 and 1.2 mg/L) (Vetec Ltda) and cadmium (0; 0.6; 1, 2 and 1.8 mg/L) (BDH Ltda) were diluted in the activation solution (distilled water - 0 mOsm/kg), at 28 °C for all analyzes. Copper and cadmium solutions were prepared with copper chloride ($CuCl_2$) and cadmium chloride ($CdCl_2$). Samples of water from each nominal concentration were collected for real quantification of copper and cadmium, which were analyzed by atomic absorption spectroscopy, flame mode (PerkinElmer model 3100: PerkinElmer Inc, USA). The measured concentrations were 0; 0.38; 0.79 and 1.22 mg/L for copper and 0; 0.63; 1.19 and 1.77 mg/L for cadmium. The sperm were activated in the 2:20 ratio (v:v). After activation, the spermatozoa were analyzed using an optical microscope (Leica DM 500) (400x) by one observer to avoid subjective bias of the analysis. Motility time was measured with a stopwatch in seconds (s), from the start of the movement until 100% of sperm became motionless. To identify the percentage of mobile cells, a scale from 0 to 100% was used, according to Cosson et al. (2008), where: 1 = 0–5%; 2 = 5–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100%. For each treatment, mobile cells were measured in triplicate using a pooled sperm of all males.

2.3. Evaluation of antioxidant enzymes and lipoperoxidation

For the antioxidant enzymes analysis, 200 μL of semen were activated from each fish, in triplicates for each treatment. After the end of motility, 800 μL of the homogenization buffer (pH 7.6) containing (in mM) Tris base 20, EDTA 1, dithiothreitol 1, sucrose 50 and KCl 150, were added. The sperm were centrifuged at 9000 g for 10 min at 4 °C and the supernatant were used to analyze the activities of superoxide dismutase (SOD) and glutathione S-transferase (GST) and determination of levels of lipid peroxidation (LPO). SOD activity was quantified by inhibiting the reduction rate of cytochrome *c* using the 550 nm xanthine/xanthine oxidase system (Flohi and Tting, 1984), and its activity is represented as $U.min^{-1}.mg$ of $protein^{-1}$. To measure GST activity, changes in absorbance at 340 nm and using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate were recorded, as suggested by Keen et al. (1976). GST activity was calculated as conjugated nmol of $CDNB.min^{-1}.mg$ of $protein^{-1}$, using a 9.6 mM cm^{-1} M extinction coefficient. The levels of lipid peroxidation (LPO) were quantified by the FOX method (Jiang et al.,

1991), based on the oxidation of Fe^{+2} to Fe^{+3} by hydroperoxides in acidic medium at 560 nm. The total protein concentration of the sperm was determined spectrophotometrically at 595 nm, according to the colorimetric assay described by Bradford (1976), using bovine serum albumin (BSA) as a standard. A SpectraMax M2 spectrophotometer (Molecular Devices Inc., Sunnyvale, CA, USA) was used in all above determinations.

2.4. Sperm morphology

Morphological analysis aims to assess the integrity of sperm structures such as the head, intermediate part and tail after sperm activation. These changes can be caused by several environmental, physiological and genetic factors, in addition to external stressors. When the sperm cells are activated, the activation channels are opened, causing it to move. During this process there is an exchange of ions from the environment with the internal ions of the cell, changing the movement mechanisms, impairing swimming. At the end of motility, fixative solutions, such as formaldehyde-citrate, are used to keep their sperm characteristics unchanged (Maria et al., 2017). To estimate the rate of sperm morphological differences, after activation in the respective treatments, 10 μL of sperm were immediately fixed in 990 μL formaldehyde-citrate (1:90). Then, 15 μL of semen were added to a slide, stained with 0.5 μL Rose Bengal (1:30) and smeared over the slide (Maria et al., 2017). The procedure was performed in triplicate for each treatment. The slides were dried and examined under a microscope (400x). In each slide, 100 sperm were analyzed, and the number of anomalous sperm cells was recorded concerning the total number of cells evaluated (%). The cells were grouped as presenting absence of pathology, mild pathologies, and severe pathologies. Bent and loose tail, loose head, proximal and distal cytoplasmic gout were considered mild pathologies. Broken, curled, degenerated, abaxial, and bifurcated tail, degenerated head, microcephaly, and macrocephaly were considered severe pathologies (Streit et al., 2006).

2.5. Fertilization

The fertilization test was carried out in vitro in a completely randomized design with 8 treatments (0; 0.4; 0.8 and 1.2 mg/L $CuCl_2$ and 0; 0.6; 1.2 and 1.8 mg/L $CdCl_2$), with individual recipients, in triplicate, in total 21 recipients were used. To perform fertilization, 40 μL of pooled semen of all males was added to 0.5 g of pool oocytes off all females (approximately 700 oocytes), then homogenized in 200 ml in the respective treatment solutions diluted in distilled water (0 mOsm/kg) for activate gametes. Castro et al. (2020) showed in detail the effect of different means of activation (pH, temperatures, dissolved oxygen) on sperm of this species. Immediately after activation, the gametes for each treatment were kept in a smooth motion for 60 s. Subsequently, the eggs were washed three times in distilled water and incubated in the respective experimental solution. Fertilization rates were determined 6 h after activation, by counting 130 eggs from each experimental unit. Cells with well-formed core after this period were considered fertilized. The fertilized cells were kept in incubation for another 10 h for hatching. Subsequently, cells that passed the gastrula stage, with internal movement in the cell and with a formed larva, were counted as a hatch(Leite et al., 2013).

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean. To test the effect of different concentrations of copper and cadmium on time of motility and motility rate, on antioxidant enzymes, on fertilization and on morphological damage, an analysis of variance (ANOVA *one way*) was performed with a significance level of $p < 0.05$. When differences between the means were verified, Tukey's post-hoc test was applied. Data that did not meet the assumption of homogeneity of variances were log transformed. All statistical analyzes were performed using the R-3.5.2 software (R Core Team, 2018).

3. Results

3.1. Sperm motility

Sperm exposed to different copper concentrations showed no differences for duration and motility rate (Fig. 1A and B) ($p > 0.05$). Exposed to cadmium, duration and motility rate of *C. macropomum* spermatozoa was longer in the control treatment (85.67 ± 11.01s; 90 ± 0.01%), showing a progressive decrease ($p < 0.05$) with the increase of cadmium concentrations, showing the shortest duration and motility rate in the treatment of 1.8 mg/L (44.67 ± 4.16 s; 60 ± 5%) (Fig. 2A and B).

3.2. Biochemical analyzes

There were no differences in GST activity in any of the treatments ($p > 0.05$). Exposure to copper did not cause changes in the activity of the SOD enzyme at any of the concentrations ($p > 0.05$) (Fig. 3A) but caused an increase of LPO levels in exposed sperm to 0.8 mg/L (Fig. 4A) ($p < 0.05$). In contrast, *C. macropomum* sperm activated at a concentration of 1.2 mg/L of $CdCl_2$ showed an increased activity of SOD (Fig. 3B) and lipid peroxidation in 1.8 mg/L $CdCl_2$ (Fig. 4B) ($p < 0.05$).

3.3. Oocyte fertilization and hatching rate

The fertilization rate of *C. macropomum* oocytes decreased significantly ($p < 0.05$) in all exposures to copper and cadmium (Fig. 5A and B). The concentrations of 1.2 mg/L $CdCl_2$ and 0.8 mg/L $CuCl_2$ resulted in no fertilized oocytes. Regarding hatching, all treatments showed a significant decrease compared to control ($p < 0.05$) (Fig. 5A and B).

3.4. Sperm cell morphology

Mild and severe morphological changes were related to the increase in copper and cadmium concentrations used in the activation of *C. macropomum* sperm (Figs. 6–8). The highest number of normal sperm cells was observed in the absence of copper and cadmium. The concentrations of 1.2 mg/L $CuCl_2$ and 1.8 mg/L of $CdCl_2$ caused the highest incidence of cells with severe changes compared to the control (0 mg/L) ($p < 0.05$).

4. Discussion

4.1. Effect of copper

Several studies have already shown the negative effects of copper on sperm cells of fish (Zebral et al., 2019; Kowalska-Góralska et al., 2019; Vergilio et al., 2015). However, these studies are limited to a few tropical species and conditions. Our analyzes reveal a negative effect of copper on the sperm quality of *C. macropomum*, evidenced by the decrease in duration and motility rate at higher concentrations (0.8 and 1.2 mg/L $CuCl_2$) compared to the control. The results found in this study corroborate previous studies by Lahnsteiner et al. (2004) that, evaluating the effect of copper on the sperm cells of *Clarias gariepinus* (African catfish), found a decrease

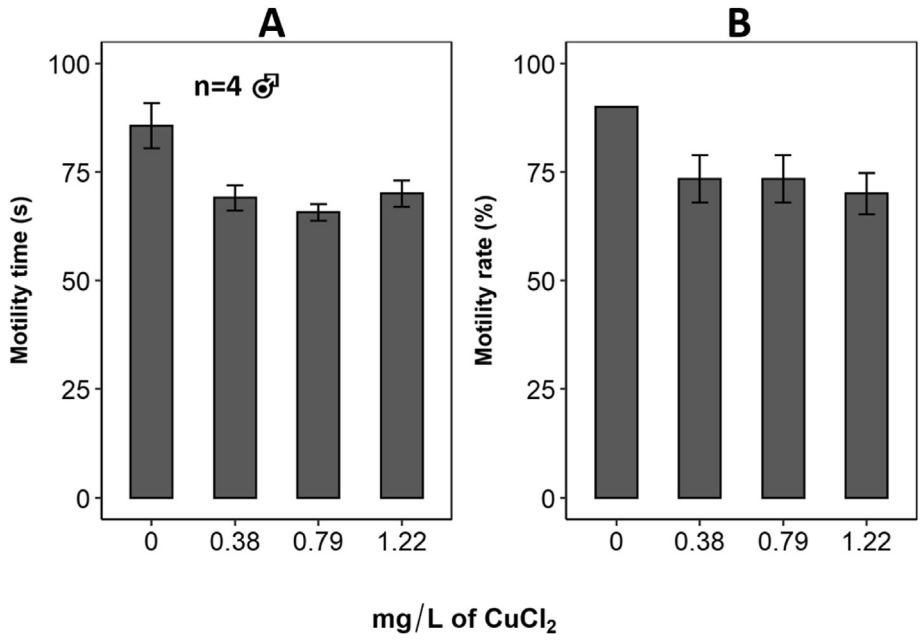

Fig. 1. In vitro effects of copper on motility duration (A) and rate (B) of spermatozoa of *Colossoma macropomum*. Data are presented as mean ± SEM.

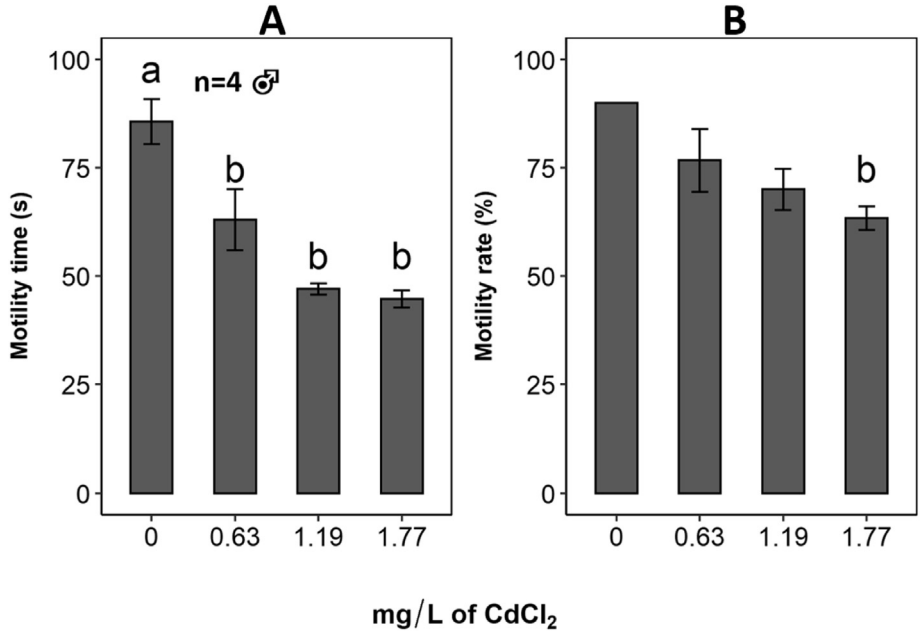

Fig. 2. In vitro effects of cadmium on motility time (A) and rate (B) of spermatozoa of *Colossoma macropomum*. Data are presented as mean ± SEM. Lower case letters indicate a difference in motility duration in relation to the control ($p < 0.05$); Capital letters indicate the difference in motility rate compared to control (0 mg/L) ($p < 0,05$).

in the percentage of mobility at higher concentrations of copper. Those authors however, found no significant differences in motility time. In *Salvelinus fontinalis* (brook trout), Kutluyer et al. (2018) reported a decrease of 10 s in motility duration of sperm exposed to 1 mg/L of copper, similar to what was observed in the present study. This decrease of motility duration caused by copper seems to be related to the amount of ROS in the sperm cell, decreasing its viability (Kutluyer et al., 2018) and, in addition, to inhibiting the glucose metabolism that influences the decrease of sperm cell movement (Maidin et al., 2014). In fact, we found a significant increase in LPO levels in the treatment of 0.8 mg/L of copper compared to the control group, a direct marker of the occurrence of o0xidative stress (Pandey et al., 2001). Note that the shortest motility duration was observed for this copper exposure, compared to cells exposed to other copper concentrations.

Analysis of fertilization and hatching rates suggest that copper can negatively influence the population of *C. macropomum*, as in vitro experiments showed a drastic decrease of both parameters, with the hatching rate reaching 0% or close to it at all copper concentrations. Copper can alter the selective permeability of the membrane, leading to disturbances in the exchange of cations between the perivitelline liquid and water (Stouthart et al., 1996). Our

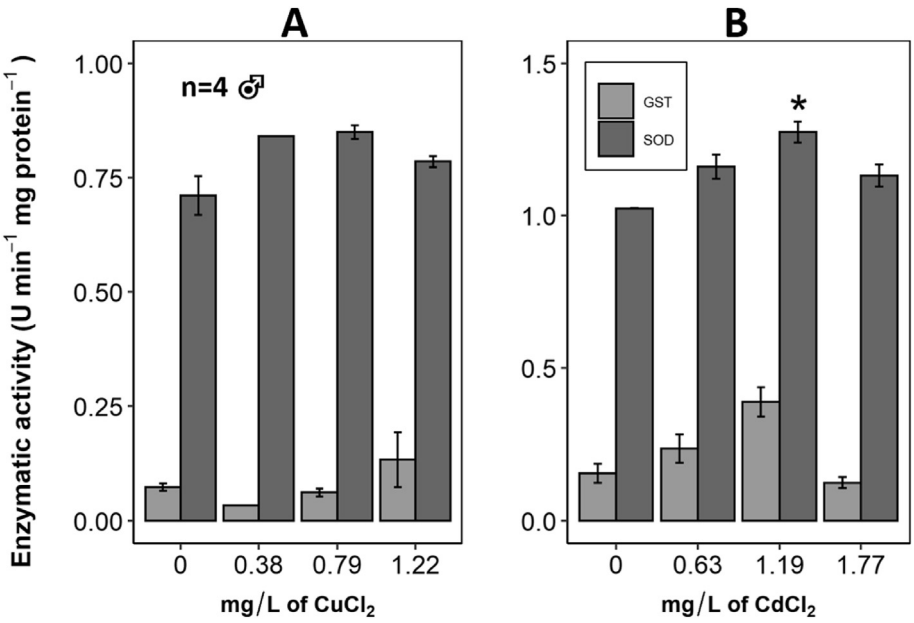

Fig. 3. In vitro effects of copper (A) and cadmium (B) on the activity of the enzymes SOD (superoxide dismutase) and GST (GlutationeS-Tranferase) of spermatozoa of *Colossoma macropomum*. Data are presented as mean ± SEM. Asterisks (*) indicate significant difference from control (0 mg/L) ($p < 0.05$).

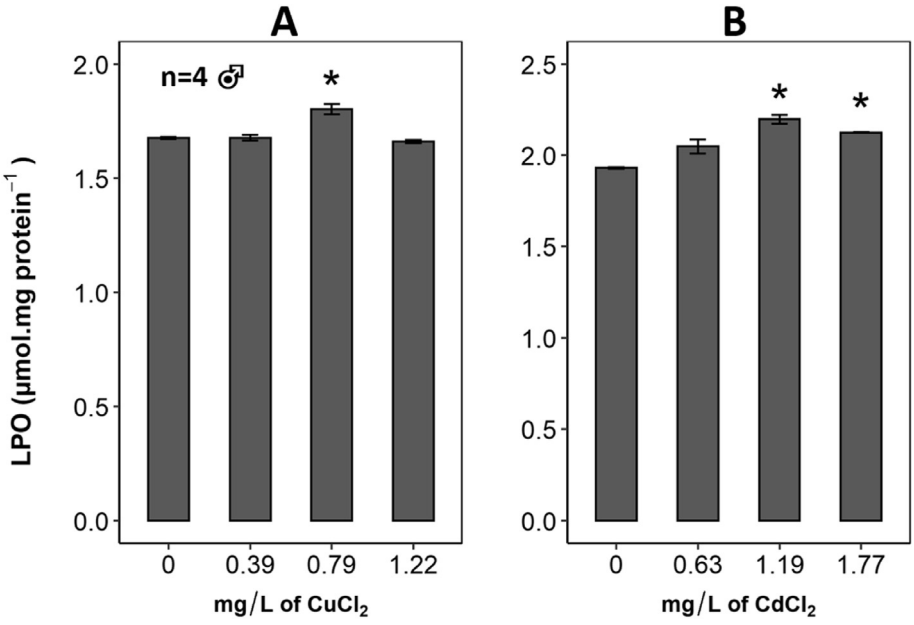

Fig. 4. In vitro effects of different concentrations of copper (A) and cadmium (B) on lipoperoxidation levels of *Colossoma macropomum* spermatozoa. Data are presented as mean ± SEM. Asterisks (*) indicate significant difference from control (0 mg/L) ($p < 0.05$).

results differ from the findings by Shaw and Brown (1971), who found no differences in the fertilization rate for *Oncorhynchus mykiss* (rainbow trout) exposed to 1 mg/L of copper and those described by Billard and Roubaud (1985) showing favorable effects on fertilization for *Salmo gairdneri* exposed to copper (0.5 and 5 mg/L). However, our data corroborate the findings of Anderson and Middaugh (1991) in experiments carried out with *Atherinop saffinis*, in which they found a significant decrease in the fertilization rate under exposure to 0.18 mg/L of copper. Other studies have shown that the hatching rate of fish exposed to different copper concentrations is delayed or decreased (Witeck et al., 2011; Bombardelli et al., 2016). Thus, the exposure to copper cannot yet be generalized as detrimental or beneficial and should be considered according to the species and, possibly, according to the environmental characteristics.

The increase in $CuCl_2$ concentrations caused a decrease in the number of normal cells. A study by Ebrahimi (2004) observed extensive morphological changes that hinder the swimming of sperm cells, in carp and trout sperm under the influence of 10 ppm of copper. Also, the study reveals that organisms exposed in vivo to high levels of copper during the reproductive period, produce sperm with a higher percentage of vacuoles in the region of the flagellum when compared to sperm from control animals (Ackerman et al., 1999). During the activation process, the

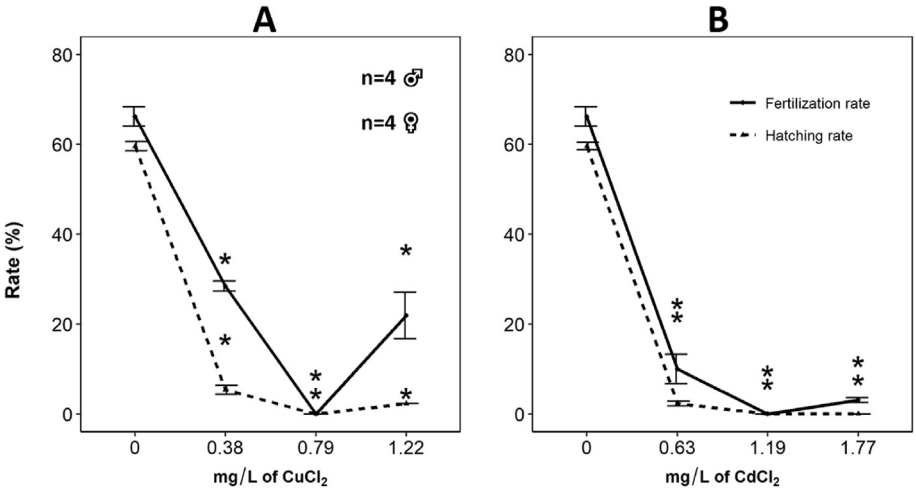

Fig. 5. In vitro effects of copper (A) and cadmium (B) on the fertilization and hatching rate of *Colossoma macropomum*. Asterisks (*) indicate a significant difference from control (0 mg/L) ($p < 0.05$).

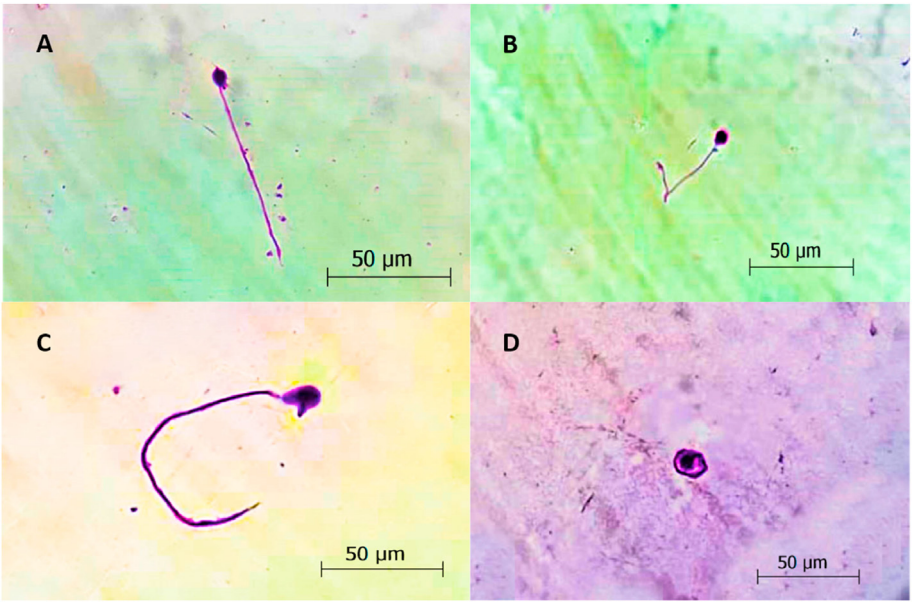

Fig. 6. Morphological changes in sperm and *Colossoma macropomum* exposed to different concentrations of copper and cadmium. A) normal sperm; B) sperm with a light tail; C) sperm with a folded tail (mild); and D) sperm with the tail wrapped around the head (severe).

transition metals can move through the activation channels and, when reaching the sperm cytoplasm, they bind to various proteins and enzymes, affecting the symmetry of sperm movements and causing morphological changes (Dietrich et al., 2010). The analyzed spermatozoa of *C. macropomum*, demonstrated a series of physical and biochemical disturbances, corroborating the proposed hypothesis, in which the presence of copper in the activation medium decreased the quality of the spermatozoa, hindering in vitro fertilization. Additional studies are needed to understand the toxicity of this metal to sperm and ova of tropical fish and global warm challenges.

4.2. Effect of cadmium

Transition metals that accumulate in fish tissue and cells can decrease the quality of gametes, especially sperm motility (Govind and Madhuri, 2014). This is directly related to the decline in fish reproduction as motility is associated with the fertilization capacity of sperm (Hayati et al., 2019). The increase in $CdCl_2$ concentrations in the activation medium causes a decrease of sperm quality of *C. macropomum*. These data corroborate, in part, the report of Chyb et al. (2001) that, analysing the effect of different concentrations of cadmium (10, 50, 100, 200, 500, 1000 and 2000 ppm) on *Cyprinus carpio* sperm (common carp), observed a reduction in motility rate. However, the authors found no statistical differences regarding motility duration. The reduction in motility duration found here for *C. macropomum* may be related to the change that cadmium causes in the specific calcium biding sites, affecting the voltage-activated channel (VAC), one of the mechanisms of calcium entry into the cell (Büsselberg, 1995). The entry of calcium into the cell is important to start sperm motility, as clearly shown by Tanimoto and Morisawa (1988) that studying *Salmo gairdneri*, reported that in the absence of calcium, sperm cells are not activated. The Amazonian waters, in particular, have relatively low calcium levels

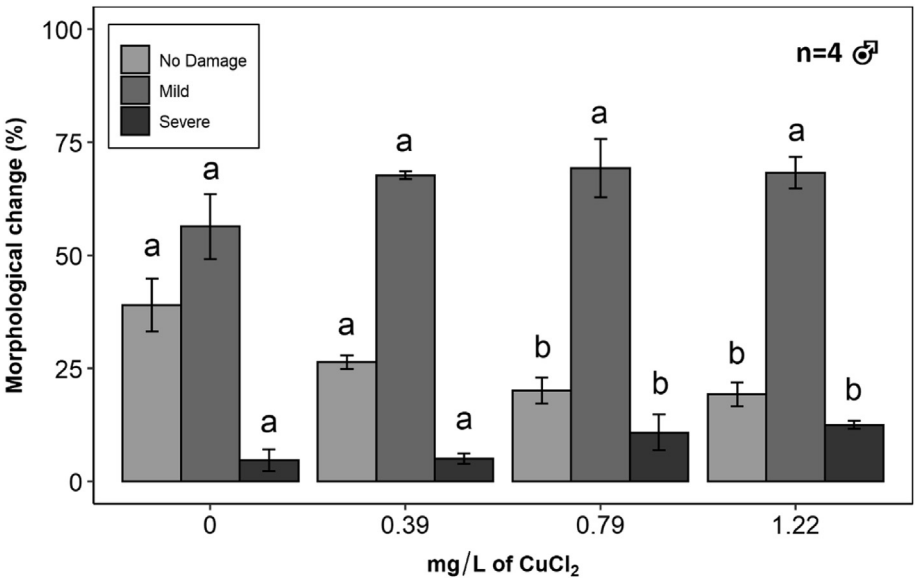

Fig. 7. In vitro effect of copper on morphological changes (see text for details) of sperm cells of *Colossoma macropomum*. Lower case letters indicate significant differences in lesions between treatments ($p < 0.05$).

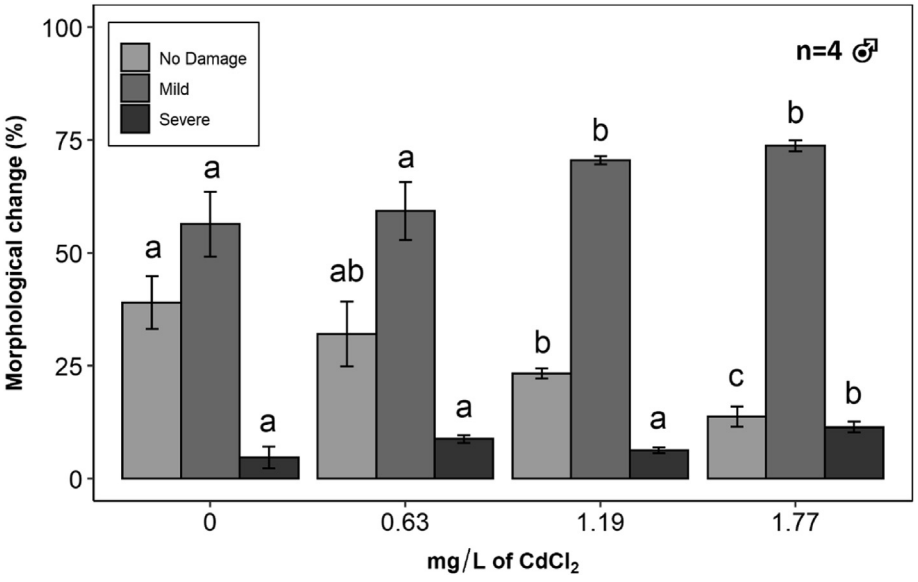

Fig. 8. In vitro effects of cadmium on morphological changes (see text for details) of sperm cells of *Colossoma macropomum*. Lower case letters indicate differences in lesions between treatments ($p < 0.05$).

(Holland et al., 2017), which in synergy with metals, probably can hinder the activation of sperm from different fish species.

Exposure to high concentrations of $CdCl_2$ also caused an increase in SOD activity in *C. macropomum* sperm cells, suggesting the formation of free radicals that can decrease sperm quality. Superoxide dismutase is considered the first line of defense against the increase in reactive oxygen species and the increase in the activity of this enzyme suggests a response against environmental stress, such as the presence of the metal analyzed here (Li et al., 2010). An increase in SOD activity at a concentration of 1.2 mg/L of cadmium was observed and, subsequently, a decrease with an increase in concentration to 1.8 mg/L, suggesting that, possibly, the presence of cadmium causes an increase in stress activating another control pathway, such as catalase (CAT). Regarding GST, we found no significant difference in any treatment. According to Pandey et al. (2001), the formation of reactive oxygen species results, in general, in the peroxidation of unsaturated lipids, causing an increase in LPO. This stress was evidenced in treatments with a concentration of 1.2 mg/L and 1.8 mg/L of cadmium that caused a significant increase in the levels of LPO in *C. macropomum*.

The fertilization and hatching rates observed in the present study showed a significant decrease in all cadmium exposure levels. This study corroborates the report of Witeska (1995) on *Cyprinus carpio* (common carp) showing a decrease of hatching rate from 86% to 88% in the control group to 8%–35% in the presence of cadmium. According to Dumorné et al. (2018), the decrease in sperm motility duration limits their arrival at the oocyte surface, which may explain the reduction in the fertilization and hatching rate observed here for *C. macropomum*. Cadmium also promoted a reduction in motility duration in all concentrations further

affecting fertilization. Another possible explanation for the marked decrease in the rate of fertilization in cadmium exposed cells is that the presence of this contaminant can obstruct the micropyle, preventing the entry of sperm into the oocyte, and thus the fertilization process (Kime, 1995).

In addition, we observed an increase in morphological damage in sperm cells of *C. macropomum* exposed to increased $CdCl_2$ concentrations. Morphological damage in fish sperm influences their ability to move, reducing fertilization success (Look and Kime, 2003), which was observed in the present study. Changes in sperm structures decrease the motility and the induction of the acrosomal reaction, which are considered the main steps of fish fertilization (Meeker et al., 2008). Sperm exposed in vitro to transition metals may show changes in head length, median piece size, and flagellum length, reducing cell motility (Lüpold et al., 2009). These damages were observed in the sperm cells after exposure to cadmium and explain reduction in motility duration. Au et al. (2001) reported that the plasma membrane of *Anthocidaris crassispina* sperm (sea urchins) exposed in vivo to 0.01 mg/L of cadmium has become more convoluted, possibly due to the binding of cadmium to the plasma membrane of sperm, interfering with the functioning of calcium channels during acrosomal reactions.

Cadmiun caused a significant increase of sperm cell damage in *C. macropomum*, reducing their motility duration and fertilization rates. These results corroborate previous studies (Acosta et al., 2016; Rocha et al., 2018). Similar to copper, cadmium caused a decrease of sperm quality and, consequently, the rate of in vitro fertilization and hatching. Although more studies are needed to clarify the mechanisms of action of copper and cadmium, it is clear that these metals affect the fertilization rates of *C. macropomum*. Therefore, it is necessary to limit the presence of these metals in the natural environment where the reproduction of the studied species occurs and in the farms of *C. macropomum*, particularly in the water used in the reproduction processes.

5. Conclusion

In the present study, we showed that copper and cadmium affected the quality of *C. macropomum* sperm, decreasing the motility duration of these cells, increasing the activity of antioxidant enzymes and causing lipid peroxidation. The rate of fertilization and hatching of the oocytes were significantly influenced by these metals, suggesting that reproduction in contaminated environments may be compromised.

Credit Author Statement

Gustavo Lemes Pinto, Methodology, Formal analysis, Investigation, Writing - original draft. Jonatas da Silva Castro, Conceptualization, Methodology, Investigation, Writing - review & editing. Adalberto Luis Val, Validation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We wish to thank the Center for Technology, Training, and Production in Aquaculture (CTTPA) of the state of Amazonas-AM for providing the tambaqui breeders for experimentation. This study was funded by the Brazilian National Research Council (CNPq, 465540/2014-7) and the Amazonas State Research Foundation (FAPEAM, 062.1187/2017) and the Coordination for the Improvement of Higher Education Personnel (CAPES) (code 001), which supports INCT/ADAPTA II. ALV (303930/2014-5) is recipient of CNPq research fellowships. JSC is recipient of post-grad fellowships from CAPES (finance code 001); GLP is the recipient of a research fellowship through the Aristides Pacheco Leão Program (PAPL) from the Brazilian Academy of Science (ABC) through Arisyíd. We would also like to thank the collaborators at the Balbina fish farm.

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