



Effects of metals on sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey fish (*Odontesthes bonariensis*)

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Accepted: 19 June 2020 / Published online: 3 July 2020
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Abstract

Some species of fish have been used as bioindicators of aquatic environmental pollution all over the world. Pejerrey (*Odontesthes bonariensis*) was selected for the current study due to its sensitivity to pollutants and because is one of the emblematic fish species that inhabits shallow lakes of the Pampa region (Argentina). Recently, in Chascomús lake were recorded concentrations of Cd, Cr, Cu and Zn with values above the Argentine National Guidelines for the Protection of the Aquatic life. Regarding this, the aim of the present study was to investigate the effects of environmental concentrations of these metals on the sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey. Also, the same endpoints were analyzed with concentrations ten times higher to simulate a polluted worst-case scenario. The results showed that the presence of some metals in aquatic environments reduced pejerrey sperm motility (in ~50%) and velocity (in ~30%). These results were obtained using a computer assisted sperm analyzer enforcing the application of this analysis as a tool or bioindicator of aquatic pollution. In addition, fertilization rate was diminished (in ~40%) for all treatments. Besides, the hatching rate, and embryo and larval survival were drastically affected being zero for the highest metal concentrations assessed. All together these results, showed that even lower metal concentrations can negatively affect different reproductive parameters of one of the most emblematic fish species of the Argentinean water bodies.

Keywords Atherinopsidae · CASA · Embryo–larval assays · Metals · Pollution

Introduction

Some metals, like copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), are essentials for the normal cellular metabolism, but in higher concentrations, they could become toxic substances (Farkas et al. 2000; Handy 2003). However, other metals like arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb), are nonessentials and produce adverse effects on aquatic organisms (Velma et al. 2009; Jabeen et al. 2012). Metals have the singularity of being non-biodegradable and to accumulate in different substrates like water, sediments or in organic tissues (Linnik

and Zubenko 2000; Uysal et al. 2008). Due to these characteristics, there is an ecological and global public health concern regarding environmental pollution by metals (Tchounwou et al. 2012). Furthermore, these pollutants are present in almost all water bodies around the world and their concentrations are increasing (Karadede and Ahmet Oymak 2004; Naigaga et al. 2011) linked to the expansion of agriculture, industries and urbanization (Jorgensen 2012).

A recent study reported metal concentrations above the national guideline's levels in Chascomús lake (35°38' S 58° 0' W), a typical water body within the Pampa region of Argentina. The maximum concentrations were: 0.23 µg/L for Cd, 4.28 µg/L for Cr, 22.09 µg/L for Cu and 210.76 µg/L for Zn (Gárriz et al. 2019). There are also other previous studies that reported similar concentrations for this lake and for nearby water bodies (Schenone et al. 2014; Avigliano et al. 2015). The source of these metals could be the discharges of some metallurgical and textile industrial plants located near the lake (Gárriz et al. 2019). Nevertheless, much higher values were determined in some extreme

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polluted lakes in other countries such as China, India and Pakistan (Cheung et al. 2003; Malik et al. 2010; Nawaz et al. 2010).

Water bodies from the Pampa region (Argentina) are shallow lakes with an average depth of about 3 meter and are characterized by a high degree of natural eutrophication, which is often increased by different anthropic activities (Diovisalvi et al. 2015). In this sense, the *Encadenadas de Chascomús* are an important group of chained shallow lakes where Chascomús lake is the largest one. A characteristic of this lake is a high concentration of suspended particulate matter and low transparency, that consequently allows the accumulation of different pollutants, including metals (Carrquiriborde and Ronco 2002; Schenone et al. 2014; Avigliano et al. 2015; Diovisalvi et al. 2015). Particularly, in Chascomús shallow lake inhabits a large population of pejerrey fish, *Odontesthes bonariensis* (Colautti et al. 2015), an important native fish species from the Pampa region of Argentina, due to the sport fishing and its flesh quality (Somoza et al. 2008; Berasain et al. 2015). Moreover, this fish species is considered an excellent model for aquatic ecotoxicology studies due to its sensitivity to different pollutants (Carrquiriborde and Ronco 2002, 2008; Gárriz et al. 2015, 2017; Menéndez-Helman et al. 2015).

Some species of fish have been used as bioindicators of aquatic environment pollution all over the world (Nawaz et al. 2010; Corredor-Santamaría et al. 2019) mainly because they are the predominant vertebrate group in this type of environments and due to their position within the food web (Nawaz et al. 2010; Ambreen et al. 2015). The presence of high concentrations of metals inside these organisms can alter their homeostasis through the availability or transport of other essential elements such as sodium (Na), potassium (K) or calcium (Ca; Farkas et al. 2000). Another aspect that can be altered by these pollutants is the reproduction, because fish gametes are directly exposed to pollutants during external fertilization and consequently can also affect the embryo and/or larval viability or development (Jeziarska et al. 2009). Furthermore, there is evidence that showed that in vitro fertilization with the presence of Cd, Cu or Zn reduced the total amount of fertilized eggs and hatching success of different fish species (Flik et al. 2002; Bulut et al. 2008).

Regarding the study of reproductive parameters affected by pollutants, the analysis of sperm motility has the potential to be used as an easy, fast, and sensitive bioindicator to determine the effects of pollutants in aquatic environments (Kime 1995; Dietrich et al. 2010). Nowadays, with the development of computer assisted sperm analyzer (CASA) for fish species, the analysis of sperm quality is one of the most objective endpoints used to determine the effects of pollutant in fish reproduction (Hashimoto et al. 2008; Dietrich et al. 2010; Acosta et al. 2016). Among this,

it has been reported that the presence of Cd in the activation solution of *Danio rerio* sperm reduced its motility and velocity (Acosta et al. 2016). Furthermore, the survival rate and growth of juveniles of *Oncorhynchus tshawytscha* exposed to Cr and of *Oncorhynchus mykiss* exposed to Cd were negatively affected (Hansen et al. 2002; Farag et al. 2006). Moreover, some metals can generate osmotic disturbances or increases of ROS (reactive oxygen species) at cellular level, and alterations of enzyme synthesis and activity (Jeziarska et al. 2009; Dietrich et al. 2010).

There are studies that evaluate the negative effects of metals particularly in pejerrey fish, between them: the setup of LC50 for Cd (0.013 mg/L), Cu (0.218 mg/L) and Cr (8.2 mg/L) for pejerrey juveniles (Carrquiriborde and Ronco 2002); the analysis of genotoxic effects of these metals by comet assay in pejerrey gill and liver (Gasulla et al. 2016). Also, it has been reported their bioaccumulation rates both in laboratory (Carrquiriborde and Ronco 2008) and also in wild fish sampled from Adela and Barrancas lakes belonging to the *Encadenadas de Chascomús* system (Avigliano et al. 2015). There is a more recent study that evaluated the effects of Cd, Cr, Cu and Zn in the endocrine-reproductive axis of sexual mature pejerrey males (Gárriz et al. 2019), however, none of these studies analyze the global effects of metals in the sperm, fertilization and/or in early stages of pejerrey development. According to this, the aim of the present study was to investigate for the first time the effects of certain metals (Cd, Cr, Cu and Zn) on the sperm quality, fertilization and hatching rates, and, embryo and larval survival of pejerrey, *O. bonariensis*, using environmental concentrations of Cd, Cr, Cu and Zn measured in Chascomús lake and higher concentrations to simulate a polluted worst-case scenario.

Materials and methods

Metal solutions

The metals treatments tested were: 0.25 µg/L Cd, 4 µg/L Cr, 22 µg/L Cu, 211 µg/L of Zn and ten times more of each metal concentration (2.5 µg/L Cd, 40 µg/L Cr, 220 µg/L Cu and 2110 µg/L Zn). These concentrations were established based on the ones reported for Chascomús shallow lake (Gárriz et al. 2019) above the Argentinian guideline's levels allowed for the protection of the aquatic biota (Argentinian national law N° 24,051; Decret 831/93; Anex II). Also, ten times of each metal concentration were assayed in order to simulate a worst-case scenario. Stock solutions (100 mg/L) for each tested metal (Cd, Cr, Cu and Zn) were prepared by a dilution with distilled water (pH 7.01) and they were preserved in the refrigerator (4 °C) for no more than 15 days. The metals salts used were cadmium chloride

(CdCl₂; ACS Reagent Sigma-Aldrich, USA), copper sulfate (CuSO₄; Mallinckrodt Chemicals, USA), potassium dichromate (K₂Cr₂O₇; ACS Reagent Anedra, Argentina) and zinc sulfate-7-hydrated (ZnSO₄ 7H₂O; Sigma-Aldrich, USA). To reach each final metal concentration, another dilution of each metal solution stock with ground water (pH 7.97; osmolarity 141 mOsm/L; alkalinity 564 mg CaCO₃/L; hardness 198 mg CaCO₃/L; nitrites 100 µg/L; nitrates 19 mg/L) was performed. The trace metal concentrations in the ground water was non detectable.

Sperm quality analysis

Sperm samples were obtained during spawning season (spring) from 5 sexually mature pejerrey males of 5-years reared since hatching in the aquaculture facilities of the INTECH (Instituto Tecnológico de Chascomús) following the procedures described in Miranda et al. (2006) and Chalde et al. (2016) using underground water in an open flow water system and were feeding twice a day with commercial dry feed (Shulet®) at a rate of 1% body mass. This age class was chosen because in a previous study it was demonstrated that at this age pejerrey fish had the best gamete quality (Chalde et al. 2016).

The fish were anesthetized by immersion in 100 ppm benzocaine solution and sperm samples from each male, from the treated and control groups, were collected with a syringe by abdominal massage, drawn into a capped tube (0.06 mL) and kept on ice. To activate the sperm, 1 µL of each sperm sample was diluted in 1799 µL of ground water or the corresponded metal solution (dilution rate 1:1800) in a 50 ml falcon tube. This dilution rate was established in order to observe a range of 100–150 spermatozooids per frame, require to have a proper CASA analysis. Immediately after, 10 µL of the activated sperm solution was placed in a Neubauer chamber to record one single video. The software used was IMAGEJ (National Institute of Health, USA, <http://rsb.info.nih.gov/ij/>) compiled with the CASA application (University of California and Howard Hughes Medical Institute, USA); following the methodology described by Gárriz et al. (2015). The sequences of each video analyzed were the seconds 10, 20 and 30 post-activation (pa). The parameters evaluated were: Motility (%), VCL (Curvilinear velocity), VAP (Average path velocity), VSL (Straight line velocity) and LIN (Linearity); using the CASA system already set for pejerrey species (Gárriz et al. 2015; Chalde et al. 2016).

In vitro fertilization assay

The eggs, were collected by stripping from 3 sexual mature pejerrey females of 5-years kept since hatching in the aquaculture facilities of INTECH and, there were separated

in 27 different samples containing each of them approximately 100 eggs (24 treatments and 3 controls). Each sample was fertilized in vitro with sperm activated with each metal solution. The sperm used belonged to a pool of 5 sexually mature pejerrey males of 5 years that was activated with the following metal solutions: 0.25 µg/L Cd, 4 µg/L Cr, 22 µg/L Cu, 211 µg/L Zn; and ten times each metal concentration (2.5 µg/L Cd, 40 µg/L Cr, 220 µg/L Cu and 2110 µg/L Zn). For control groups, the sperm was activated only with ground water. Later, each egg sample was hydrated with 15 ml of each treatment solution and then incubated at 24 °C for 24 h. At that moment, the fertilization rate (%) was assessed following procedures described in Gárriz et al. (2015) and Chalde et al. (2016).

Embryo and larval survival analysis

Advanced stage embryos (with ocular pigments; Chalde et al. 2011) and recently hatched larvae were selected from INTECH stock. Both embryos ($n = 270$) and larvae ($n = 270$) were divided in groups of 10 individuals and they were placed in plastic containers (50 mL total volume). The metals treatments were the same tested for the sperm quality analysis and the fertilization assay, plus a control group with only ground water; each treatment was done by triplicate. The total volume of each container was 30 mL and each solution was renewed daily at the same time, for a total of 10 days. All the treatments were kept in a growth chamber (GC 300, JEIO TECH, Korea) with controlled photoperiod (12L:12D) and temperature (22 °C).

Embryo and larval survival were recorded daily, and dead organisms were removed. For the embryos the cumulative survival rate (%) and hatching rate (%) were analyzed; and for the larvae the cumulative survival rate (%) and the point of no return (PNR = days at which 50% of starved larvae died) were assessed.

Statistical analysis

All the data are shown as mean ± SEM. The normal distribution of the data was analyzed by the Shapiro–Wilk test and the Brown-Forsythe test was performed to check the homogeneity of variance. The values of the sperm quality parameters were averaged from two recorded video sequences to obtain a single value per parameter per fish ($n = 5$). For the analysis of the fertilization rate (%), each female represents a replica ($n = 3$). To evaluate the differences between metals treatments and controls in all the analysis an ANOVA (One-way Analysis of Variance) was performed for normally distributed variables, followed by post hoc Tukey's HSD test ($P < 0.05$). All statistical analyses were done running the software SPSS (version 20) and GraphPad Prism 7.0.

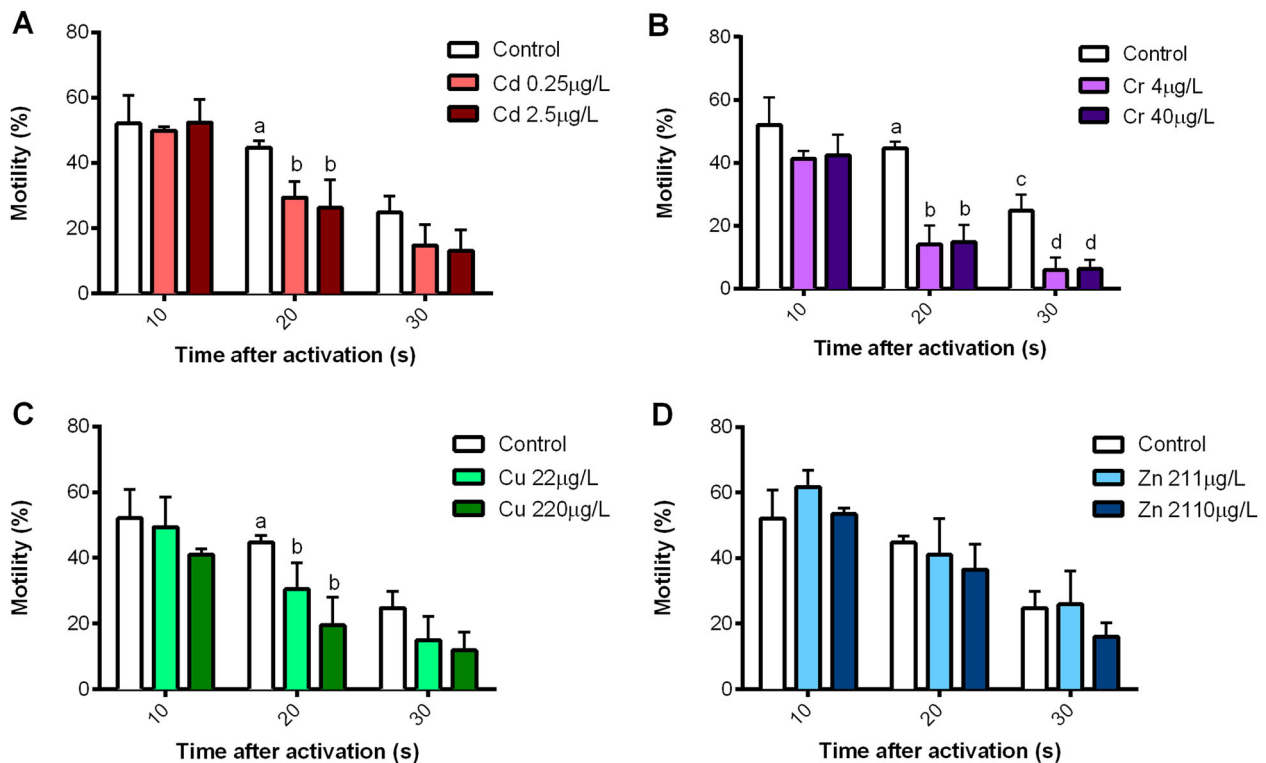


Fig. 1 Effects on the pejerrey (*O. bonariensis*) sperm motility (%) after the activation with different metals solutions with Cd (a), Cr (b), Cu (c) and Zn (d). Values are means \pm SEM ($n = 5$) and the statistically

significant differences between treatments and control ($P \leq 0.05$) are shown with lower case letters

Results

Analysis of sperm quality

The motility % was significantly decreased in the Cd, Cr and Cu treatments at 20 s pa compared with the control group (Fig. 1a–c). When the sperm was activated with Cr solution this difference was also observed at 30 s pa (Fig. 1b). The Zn treatment did not show any significant difference compared with the control (Fig. 1d). Regarding the sperm velocities, the VSL was statistically reduced at 10 s pa in the higher concentrations of Cd, Cr and Cu compared with both the control and the environmental concentration of each metal (Fig. 2a–c). The Zn treatment was not statistically different compared with the control (Fig. 2d). For VAP, there was a significant decrease at 10 s pa in the higher concentration of Cd compared with its environmental concentration and with the control (Fig. 3a). For Cu, VAP parameter was significantly lower in the higher concentration compared with its environmental concentration at 10 s pa (Fig. 3c). In addition, VCL and LIN were analyzed but there were not differences between metals treatments and control (data not shown).

In vitro fertilization % and hatching rate

The fertilization rates (%) for Cd, Cr and Cu treatments were statistically lower compared with the control group (Fig. 4a–c). The Zn treatment did not show significant differences compared with the control group (Fig. 4d). Moreover, the hatching % was significantly lower in all metal treatments compared with the control group (Fig. 4e–h), reaching even 0% for the highest tested concentrations of Cu and Zn (Fig. 4g, h).

Embryo and larval survival, and PNR

The cumulative embryo survival in all metal treatments dropped in a statistically significant way compared with the control group and, it was even more affected in the higher concentrations of each metal (Fig. 5). The cumulative embryo survival was significantly reduced since day 6 for both concentrations of Cd and Cr (Fig. 5a, b) and since day 4 for both Cu treatments (Fig. 5c). In the case of Zn, both treatments showed a decrease in the embryo survival, being the highest concentration the most harmful with a significant mortality (80%) since day 2 (Fig. 5d). The cumulative larval survival (%) was reduced in all metal

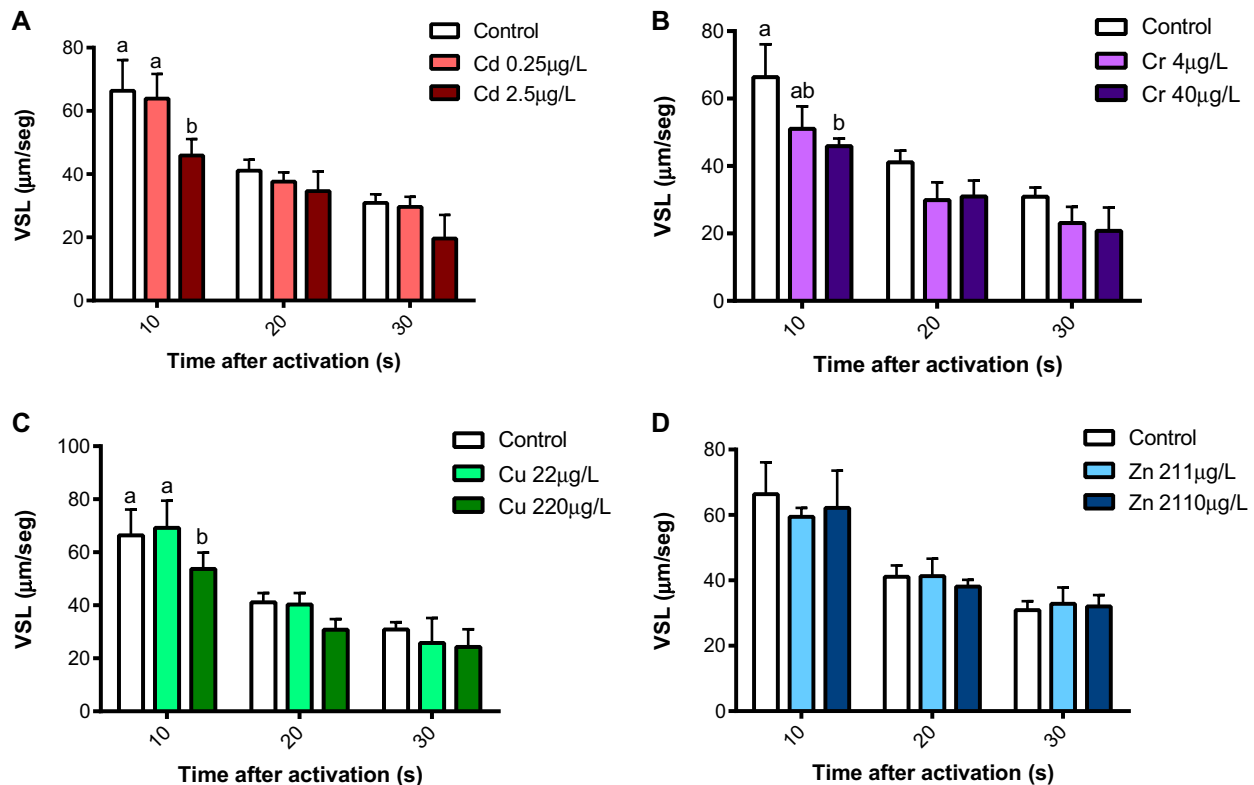


Fig. 2 Effects on the VSL (straight line velocity; $\mu\text{m}/\text{seg}$) of *O. bonariensis* sperm after the activation with different metals solutions with Cd (a), Cr (b), Cu (c) and Zn (d). Values are means \pm SEM ($n = 5$)

and the statistically significant differences between treatments and control ($P \leq 0.05$) are shown with lower case letters

treatments compared with the control group being statistically different for 0.25 $\mu\text{g}/\text{L}$ Cd treatment (at day 4 and 5; Fig. 6a) and in both Cu treatments (since day 2; Fig. 6c). In the case of Zn, the larval survival showed the most pronounced drop reaching 0% at day 2 for both concentrations tested (Fig. 6d). The PNR was significantly decreased in the larvae exposed to 220 $\mu\text{g}/\text{L}$ of Cr (2.33 ± 0.67 days) and in both Zn treatments (211 and 2110 $\mu\text{g}/\text{L}$; 1.67 ± 0.33 and 1 ± 0.0 days, respectively) compared with the control group (5.67 ± 0.33 days). For the rest of the metal treatments the PNR was: 3.67 ± 0.33 days for 0.25 $\mu\text{g}/\text{L}$ Cd; 5.33 ± 0.67 days for 2.5 $\mu\text{g}/\text{L}$ Cd; 6 ± 0.0 days for 4 $\mu\text{g}/\text{L}$ Cr; 5.67 ± 0.33 for 40 $\mu\text{g}/\text{L}$ Cr and 2.67 ± 0.33 for 22 $\mu\text{g}/\text{L}$ Cu.

In addition, two of the larvae exposed to 40 $\mu\text{g}/\text{L}$ of Cr showed a morphological alteration showing a “C-shape body” (Fig. 7).

Discussion

The results of the current study demonstrated that Cd, Cr and Cu negatively affect the sperm quality of pejerrey fish, mainly, reducing the sperm motility and velocity (VSL and VAP). These results were demonstrated using the CASA system for pejerrey fish, enforcing the use of this analysis as

a tool or bioindicator of aquatic pollution, as it was suggested in previous studies for other fish species (Dietrich et al. 2010; Acosta et al. 2016). Furthermore, the present study showed that the hatching rate and the survival of pejerrey embryo and larvae were significantly reduced with all the metals tested (Cd, Cr, Cu and Zn).

There is wealth of data about lethal and sublethal effects of metals in different fish species (Witeska et al. 2014; Jezierska et al. 2009; Lizardo-Daudt and Kennedy 2008) but their mechanism of action is still elusive. Environmental pollutants, like metals, could damage the plasma membrane and the axoneme of sperm leading to the loss of fertility abilities (Hatef et al. 2013). In addition, there are some studies that reported osmotic imbalance or alteration of the synthesis and/or activity of certain enzymes due to metal expositions (Jezierska et al. 2009). The Cd cation (Cd^{++}) in particular, can go through the Ca channels of the gill surface into the organism getting in competition with Ca (due to the similarity in size) and reducing the Ca-ATPase activity (McGeer et al. 2011). Also, Zn is another metal that competes with Ca in the gill surface and can inhibit the Ca channels (Cousins et al. 2006). Therefore, the presence of Cd and/or Zn in the aquatic environment lead to a lower absorption of Ca causing hypocalcemia in fish and affecting the whole integrity and growth of these organisms (De La

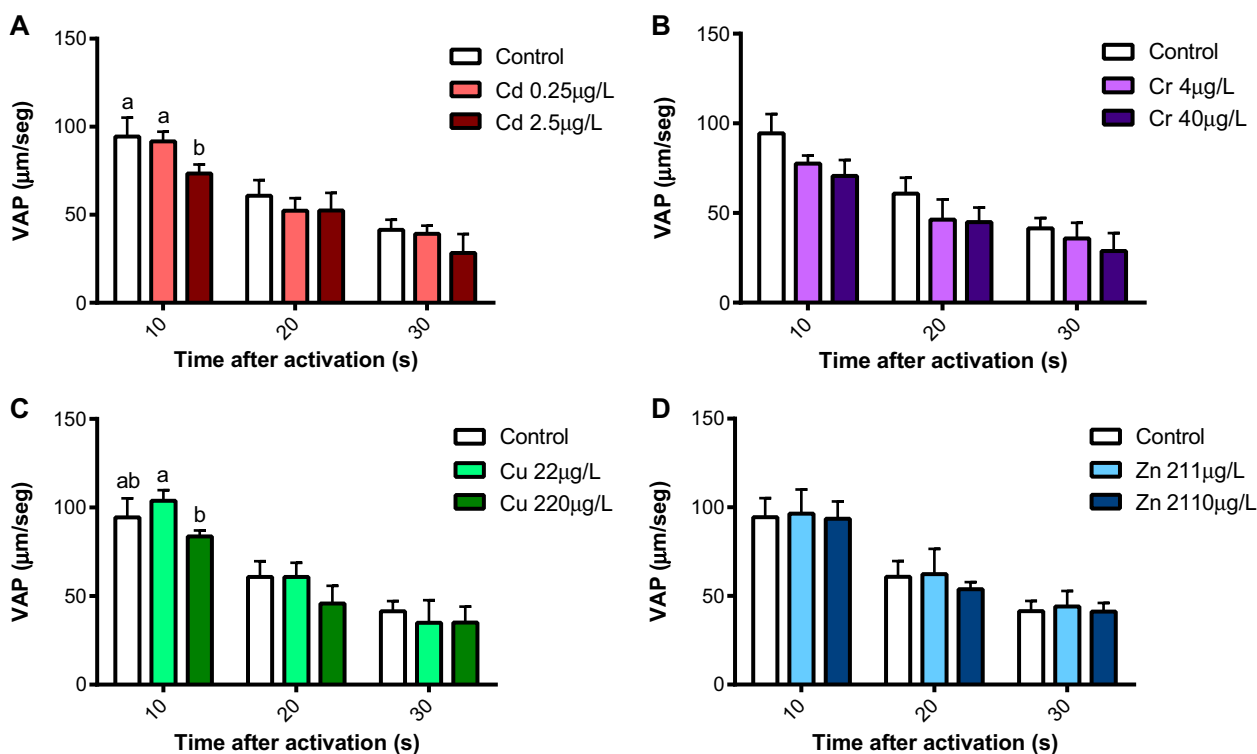


Fig. 3 Effects on the VAP (average path velocity; $\mu\text{m}/\text{seg}$) of *O. bonariensis* sperm after the activation with different metals solutions with Cd (a), Cr (b), Cu (c) and Zn (d). Values are means \pm SEM

($n = 5$) and the statistically significant differences between treatments and control ($P \leq 0.05$) are shown with lower case letters

Torre et al. 2000). It is well known, that in many fish species, Ca^{2+} influx and K^{+} or Na^{+} efflux through specific ionic channels change the membrane potential and eventually lead to an increase in cAMP concentration inside the cell, which constitutes the initiation signal for sperm motility activation (Alavi and Cosson 2006). According to these observations, it is possible to assume that some parameters of sperm quality could be reduced after the activation with heavy metals solution.

In the case of Cr, it could be absorbed by sulfate and phosphate transporters into the organism (Kotás and Stasicka 2000). Likewise, Cu could interfere with the transporting of Na into the organism (Alsop and Wood 2011), and both metals can alter the Na/K-ATPase activity and cause osmoregulation failure (Grosell et al. 2004).

In the present study, it was observed that the sperm motility (%) decreased when certain concentrations of metals (0.25 and 2.5 $\mu\text{g}/\text{L}$ of Cd; 4 and 40 $\mu\text{g}/\text{L}$ of Cr and 220 $\mu\text{g}/\text{L}$ of Cu) were added in the activation solution and also, the sperm velocity decreased with the highest concentrations of Cd, Cr and Cu. According to these findings, a diminish in the VSL was reported in *Clarias gariepinus*, *Salmo trutta*, *Leuciscus cephalus* and *Lota lota* with the presence of Cd, Pb, Hg or Zn in the sperm activation solution (Lahnsteiner et al. 2004). These adverse effects might affect consequently the fertilization success

(Lahnsteiner et al. 2004), due that metals can penetrate the sperm cells disrupting directly the cellular membrane function or, they can bind to proteins or enzymes in the flagellum changing its metabolism or movement (Dietrich et al. 2010). Some of these alterations could be occurring in pejerrey because in all the metal treatments tested a low fertility rate was also observed. A similar result was reported for *Oryzias javanicus* with a Cd solution assay (Ismail and Yusof 2011); but more research is needed to reveal the complete mechanism of action of metals in this step of reproduction.

Fish embryos are particular sensitive to the presence of pollutants in the aquatic environment and there are many studies that demonstrated the reduction in survival and hatching rates in several fish species exposed to different metals: *Cyprinus carpio* (Cu; Flik et al. 2002), *Pagrus major* (Cd, Cao et al. 2009), and *Melanotaenia fluviatilis* (Cd and Zn; Williams and Holdway 2000), between others. However, the toxicity may depend on the stage of development of the exposed embryo, because there is more harm as earlier as the exposure to the metal is (Cao et al. 2009; Jezierska et al. 2009). In the present study, the pejerrey embryos exposed to metals were in an advanced stage of development and the survival was significantly reduced. In this sense, it can be predicted that in early stages, the consequences of the metal exposure could be worst.

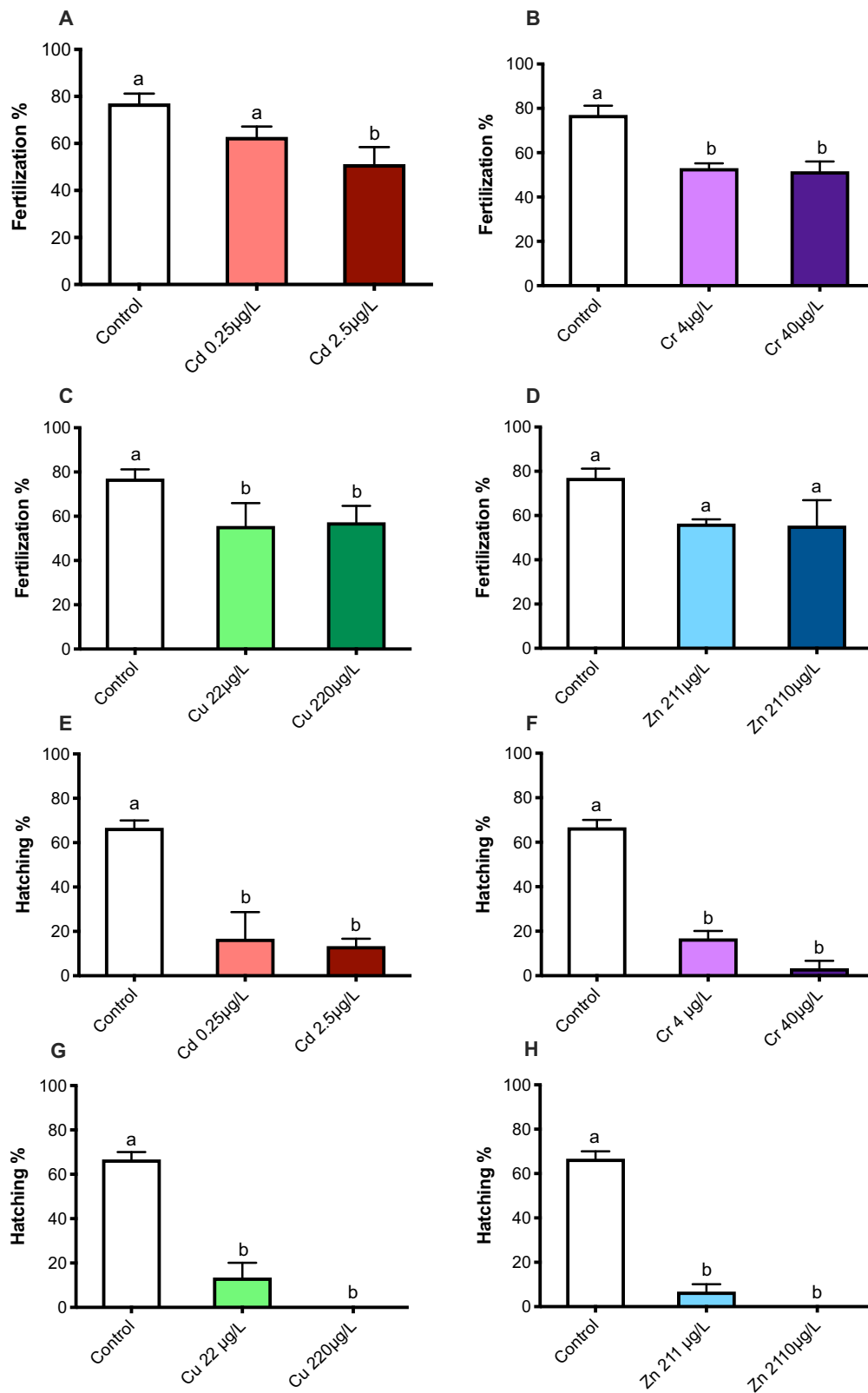


Fig. 4 Fertilization rates (%) in pejerrey fish (*O. bonariensis*) after different metals exposures with Cd (a), Cr (b), Cu (c) and Zn (d). Hatching rates (%) after in vitro fertilization with solution of Cd (e), Cr

(f), Cu (g) and Zn (h). Values are means \pm SEM ($n = 3$) and the statistically significant differences between treatments and control ($P < 0.05$) are shown with lower case letters

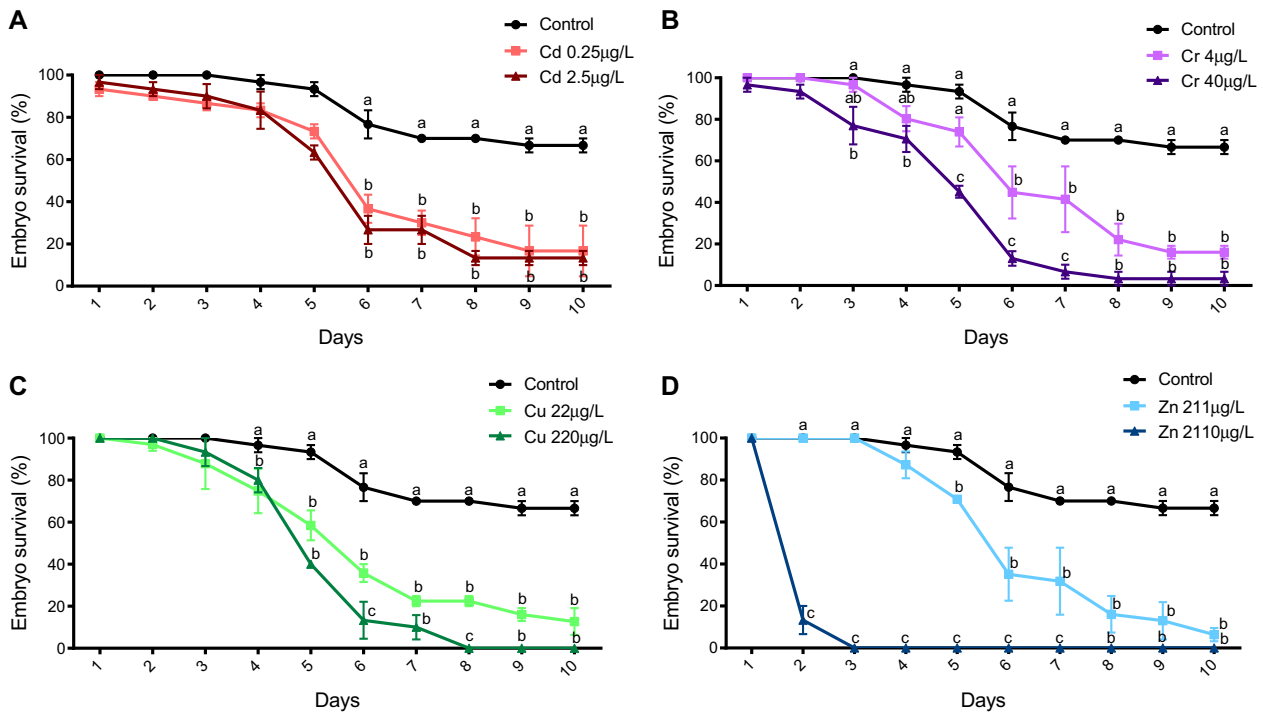


Fig. 5 Cumulative embryo survival (%) of pejerrey fish (*O. bonariensis*) exposed to Cd (a), Cr (b), Cu (c) and Zn (d). Values are means ± SEM ($n = 3$) and the statistically significant differences between treatments and control ($P \leq 0.05$) are shown with lower case letters

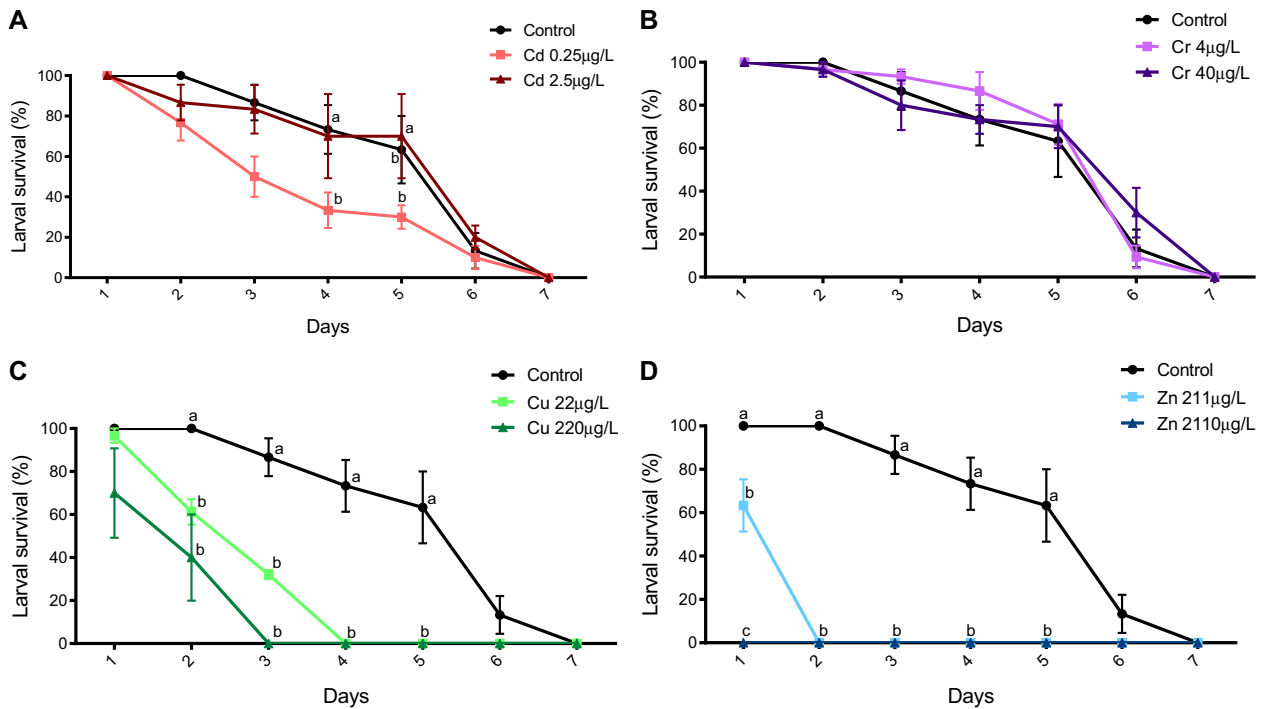


Fig. 6 Cumulative larval survival (%) of pejerrey fish (*O. bonariensis*) exposed to Cd (a), Cr (b), Cu (c) and Zn (d). Values are means ± SEM ($n = 3$) and the statistically significant differences between treatments and control ($P \leq 0.05$) are shown with lower case letters

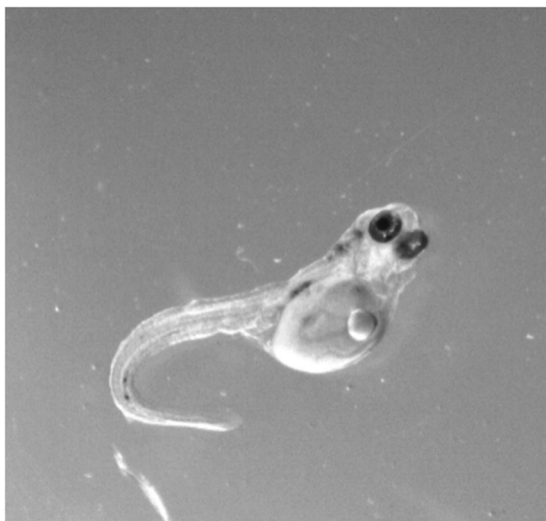


Fig. 7 Metal induced pejerrey (*O. bonariensis*) larval deformations in a C-shape body after being exposed to 40 $\mu\text{g/L}$ of Cr

In addition, focusing on the effects of Cd in pejerrey, it was peculiar that the highest concentration tested (25 $\mu\text{g/L}$) was less harmful for the larval survival than the lower concentration (0.25 $\mu\text{g/L}$), having a non-strict concentration-dependent manner.

Regarding the embryo and larval survival, Zn was the most hazardous metal due that the 100% of the embryo mortality was reached just after 1 day of exposure with the highest concentration (2110 $\mu\text{g/L}$). However, Zn was the least harmful metal in the sperm quality and hatching % assessment. The effects of this particular metal in reproduction and development is controversial. On one hand, it was demonstrated that Zn is an essential trace element to maintain the germ cells regular functions, the spermatogenesis process and to regulate the sperm motility (Yamaguchi et al. 2009). But on the other hand, embryos of *M. fluviatilis* (Williams and Holdway 2000) and of *P. major* (Huang et al. 2010) exposed to Zn decreased the hatching rate and increased the presence of morphological abnormalities in exposed larvae. More studies are needed to clarify the ambiguous role of Zn in reproduction and embryo–larval survival. Moreover, premature hatching was reported in *O. mykiss* embryos exposed to sublethal concentrations of Cd (Lizardo-Daudt and Kennedy 2008) and a decrease in the hatching rate of *C. carpio*, *Orzias latipes* and *M. fluviatilis* exposed to similar concentration of Cd used in the current study (Jeziarska et al. 2009; Gonzalez-Doncel et al. 2003; Williams and Holdway 2000). Likewise, embryos of *D. rerio* exposed to 11 to 1000 $\mu\text{g/L}$ of Cu showed a rise in mortality, inhibition of the hatching success and different alterations in the lateral line system development (Johnson et al. 2007; Linbo et al. 2009). Also, it was reported a reduction of the yolk sac size, indicating

that these embryos are consuming more nutrients, linked with a rise in the metabolism (Jeziarska et al. 2009). May be these metals are inactivating the coriolisine enzyme activity, altering the osmoregulation balance or even affecting the embryos muscular movements during the hatching process (Calta 2001).

The larval survival is also reduced by metal exposures as it was reported for *Silurus soldatovi* exposed to Cd (Zhang et al. 2012), and for *Ide Leuciscus idus* exposed to Cd and Cu (Witeska et al. 2014). Even more, the larval growth rate of *O. mykiss* could be negatively affected by Cd exposure in a concentration-dependent manner (Lizardo-Daudt and Kennedy 2008) and in *Oreochromis mossambicus* and *C. gariepinus* exposed to Cu (Nguyen and Janssen 2002; Chen et al. 2012). In addition, several morphological alterations in larvae are associated to Cd, Cu or Pb exposures in different fish species (Williams and Holdway 2000; Cao et al. 2009; Jeziarska et al. 2009). In this study, Cr was associated with less mortality effect in pejerrey larvae. However, some larvae exposed to 40 $\mu\text{g/L}$ of Cr developed a C-shape body. Similar malformations, were also observed in *C. carpio* (Jeziarska et al. 2009), *P. major* (Cao et al. 2009; Huang et al. 2010) and *Leuciscus idus* (Witeska et al. 2014) larvae, but exposed to Cd or Cu. Probably, these malformation were caused by the toxic potential of these metals, being the skeleton the main target (Jeziarska et al. 2009; Cao et al. 2009).

Conclusion

It is important to note that the number of samples analyzed could have been higher, especially in the first part of the work (sperm quality and in vitro fertilization assays) to make the statistical analysis more robust. However, this was impossible because the INTECH was closed temporally by COVID-19 from the beginning of March 2020 to the present. Considering this limitation, this study demonstrated for the first time that the environmental concentrations of metals such as Cd, Cu, Cr and Zn recorded in Pampas shallow lakes, negatively affected pejerrey sperm quality, altering consequently, the fertilization process and decreasing the embryo and larval survival. These findings added to the adverse reproductive responses previously reported on the same species to heavy metals pollution, should be an alarm signal for decision-making on the protection of pejerrey wild population.

Acknowledgements The authors thank Javier Herdman, Rocio Pala-dea, Pamela del Fresno and Martin Estermann for their technical assistance during the experiments.

Funding This study was funded by a grant to LAM National Agency of Promotion of Science and Technology (ANPCyT): PICT 2017-2506.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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