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Research Article

Effects of Cd, Cu and Zn on sperm motility indicators of the Caspian lamprey, *Caspiomyzon wagneri*

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Abstract

The present study investigated the effects of Cd, Cu and Zn on the sperm motility of *Caspiomyzon wagneri*. The sperm of the specimens were exposed to 0.01, 0.1, 1, 10, 100 and 1000 mg L^{-1} of the heavy metals, and the duration of sperm motility and percentage of motile sperms were measured using a compound microscope and digital camera. Based on the results, sperm motility parameters decreased with increasing the concentrations of heavy metals. A concentration of 1000 mg L^{-1} of heavy metals stopped completely the motile sperms. The present study indicated that heavy metals have detrimental effects on sperm motility and possibly decrease the fertilization and hatching rates of *C. wagneri* in the spawning ground, and thus a serious threat to the survival of this rare and native species.

Keywords: Pollution, Reproduction, Spawning, Mobility indices, Heavy metals.

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Introduction

Pollution of water resources threatens the survival of fishes (Kime *et al*., 1996). Among the various sources of pollution, heavy metals are important group, being found both in freshwater and marine environments (Sumpter, 2005). Heavy metals are natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities (Sprocati *et al*., 2006). All these sources of pollution influence the physiochemical characteristics of the water, sediment and biological components, and thus quality and quantity of fish stocks (Singh *et al*., 2006).

Examples of heavy metals include Cadmium (Cd), Zinc (Zn) and Copper (Cu). Cadmium is the fifth most toxic metal for vertebrates and the fourth for vascular plants (Bradl, 2005). Cadmium salts with strong acids bases are easily soluble in water, but sulfides, carbonates, hydroxides, and fluoride have low solubility (Bradl, 2005). In aquatic ecosystems, cadmium accumulates in river mussels, shrimps, crabs and fishes. The biological half-life of cadmium in the organisms' soft tissue and bone is 10 to 30 years (Kumar and Singh, 2010). Zinc inhibits the activity of the thyroid gland (Calesnick and Dinan, 1988). Zinc sulfate increases concentration of T4, decreases the concentrations of TSH and T3. Decrease of T3 levels in fish exposed to zinc metal may be due to impairment in the conversion of T4 to T3 (Calesnick and Dinan, 1988). Copper, in small amounts, is considered as an essential element in the metabolism of organisms, but in concentrations greater than 20 μg g^{-1}, can have significant toxic effects (Bradl, 2005). Fish and aquatic organisms compared to mammals are 10 to 100 times more sensitive to copper toxicity.

The obvious sign of highly polluted water, dead fish, is readily apparent, but the sublethal pollution might result only in unhealthy fish. Very low-level of pollutions may have no apparent impact on the fish itself, which would show no obvious sign of illness, but it may decrease the fecundity of fish populations, leading to a long-term decline and eventual extinction of these important natural resources (Krishnani *et al*., 2003; Burger and Gochfeld, 2005). Such low-level pollution could have an impact on reproduction, either indirectly via accumulation in the reproductive organs, or directly on the free gametes (sperm or ovum) which are released into the water. It has already been demonstrated that exposure to heavy metals affects the spermatogenesis, and decreases spermatogenic cells and motility of spermatozoa in fishes (Vos *et al*., 2000). Studying the impact of pollutants on natural reproduction of ichthyofauna is essential for conservational programs.

Caspiomyzon wagneri is native lamprey of the Caspian Sea and its

catchment area (Esmaeili *et al*., 2018) and have evolutionary importance (Satari *et al*., 2002). It is distributed over 75% of the southern Caspian Sea basin with a moderate frequency. In recent years, the stocks the Caspian lamprey has remarkably declined because of construction of dams, habitat destruction and pollution of rivers, (Close *et al*., 2002; Nasrolah Pourmoghadam *et al*., 2015). Most migratory rivers of the *C. wagneri* are located in the southern Caspian Sea basin in the vicinity of urban areas and agricultural lands. Heavy metals concentrations in the Shirud River, which is one of the main spawning rivers for the Caspian lamprey, indicate a moderate to severe pollution (Kharat Sadeghi and Karbasi, 2006). Therefore, this study was conducted to investigate the effect of different levels of heavy metals i.e. Cd, Cu and Zn on sperm motility of *C. wagneri* as an indicator of spawning success. By date, no study has investigated the effects of heavy metals on sperm quality of the Caspian lamprey.

Materials and methods

Fifteen male specimens of *C. wagneri* were caught using electrofishing device from the upstream of the Shirud River (36°51'20"N, 50°47'57"E). Immediately after sampling and drying with a clean towel, sperm was collected by pressing the abdominal area. In order to eliminate the impact of quality of spawners, sperms of all individuals were mixed.

$CuSO_4$, $ZnCl_2$ and $CdCl_2$ in various concentrations (0.01, 0.1, 1, 10, 100 and 1000 mg L^{-1}) were prepared (Fadakar Masouleh *et al*., 2011). A semi-quantitative method was used to measure sperm motility and percentage of motile sperm (Alavi *et al*., 2004) via a light microscope (Leica, Swaziland) and a digital camera with a resolution of 14 megapixels (Canon, IXUS 210, Japan). To find the duration of motility, one µL of semen was poured on a microscope slide. At the same time, 49 µL of distilled water (for control) and various concentrations of heavy metals were added to the slide. Time was recorded using a digital stopwatch. The duration of spermatozoa motility was considered the time when 95-99% of sperms stopped moving (Linhart *et al*., 1995; Cosson *et al*., 1999). The percentage of motile sperm was measured using a hemocytometer slide according to Liley *et al.* (2002), i.e. the time when 80%, 50% and 20% of sperm in various treatments had retained their mobility were recorded. The experiment was performed with 18 treatments and four replications for heavy metals, and five replications for control group.

A one-way ANOVA was used to analyze the effects of various heavy metals on sperm characteristics. Normality of data and homogeneity ofvariance were examined using the Kolmogorov-Smirnov and Levene's tests, respectively, followed by a Duncan's multiple range tests, when there was a significant effect of a heavy

metal on the sperm characteristics. In all analyses, the type-I error was 0.05. Pearson correlation analysis was performed to examine correlation between different concentrations of heavy metals and sperm motility index. All analyses were performed using SPSS 15.

Results

In the control group, 20, 50 and 80% of sperms were motile for 140.40±7.19, 117.40±6.18 and 86.40±4.30 s (mean±SD), respectively. Over time, the percentage of motile sperm decreased to > 95%, i.e. 184.80±10.13 s after the start of the experiment.

Effects of cadmium

With increase of Cd concentration, sperm motility decreased (Fig. 1). There was a sharp decrease in the sperm mobility at 0.01 mg L^{-1}, compared to the control treatment. From 0.01 to 10 mg L^{-1} Cd, duration of sperm motility decreased slowly, so that there was no significant difference between 0.01 and 0.1, 0.1 and 1, 1 and 10 mg L^{-1} Cd on the time when 80 and 20% of sperms were motile. No significant difference was detected between 0.01 and 0.1 mg L^{-1} Cd on the time when 50% of the sperms were motile. There was a significant difference among 10, 100 and 1000 mg L^{-1} at the time when 80, 50 and 20% of the sperms were motile. Pearson correlation analysis, between various concentrations of Cd, and the duration of sperm motility, showed a significant negative correlation at 80% (r=-0.497, p<0.01), 50% (r=-0.494, p<0.01), and 20% (r=-0.507, p<0.01).

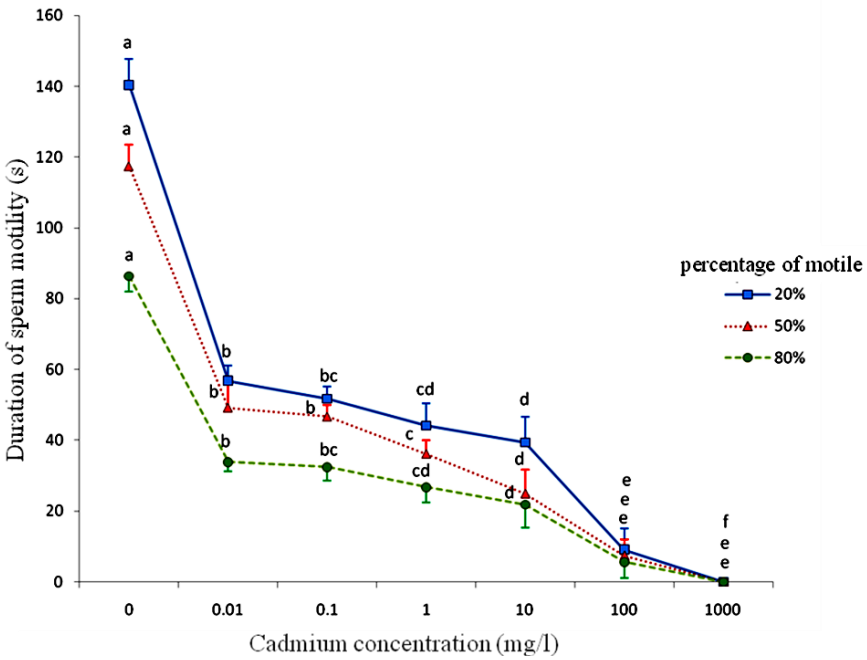

Figure 1: The effects of different cadmium concentrations on percentage of motile sperms in *Caspiomyzon wagneri.*

Effects of copper

The duration of sperm motility changed with increase of Cu concentration over all measured percentages (Fig 2.). There was a sharp decrease in duration of sperm motility with increase of Cu concentration from 0 to 0.01 mg L^{-1} (Fig. 2). The duration of sperm motility had a low fluctuation between 0.1 and 10 mg L^{-1} Cu and decreased onwards. No significant difference was detected at a time when 80% of sperms were motile among 0.01, 0.1, and 1 mg L^{-1}. However, there were significant differences between 10, 100 and 1000 mg L^{-1}. At a time when 50% of sperms were motile, significant differences between the concentrations became more obvious. When 20% of sperms were motile, it showed the highest significant level than the lower percentages. So that the concentrations of 0, 0.01, 1, 10, 100 and 1000 mg L^{-1} have significant differences with each other in terms of mobility time. Pearson correlation analysis showed a significant negative correlation in motile sperms at level 1% at 80% (r = -0.515, p<0.01), 50% (r=-0.555, p<0.01), and 20% (r=-0.587, p<0.01).

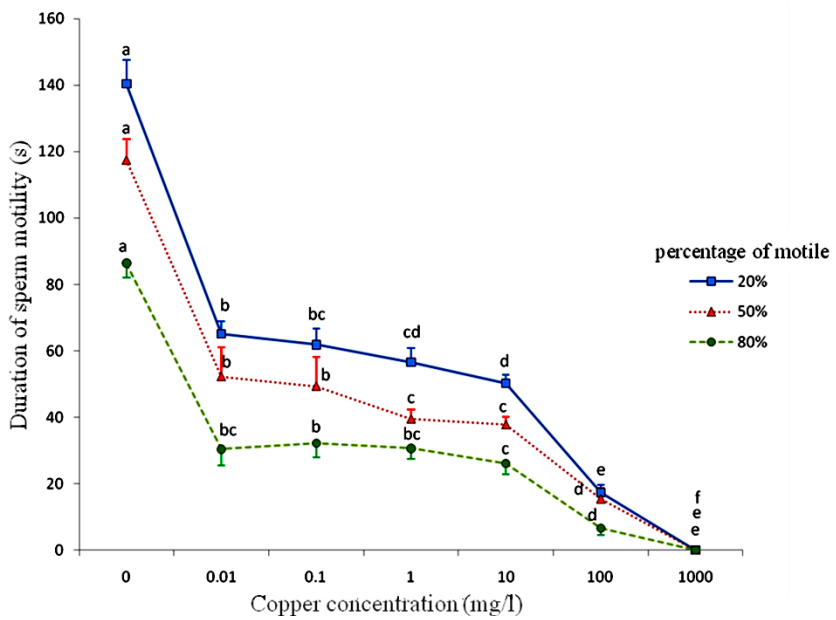

Figure 2: The effects of different copper concentrations on percentage of motile sperms in *Caspiomyzon wagneri.*

Effect of zinc

The duration of sperm motility changed similarly with increasing of Zn concentration over all measured percentages (Fig. 3), i.e. the duration of sperm motility decreased gently when exposed to 0-10 mg L^{-1}, and decreased sharply at higher concentrations. At a time when 80% of the sperm were motile, no significant difference was found between concentrations of 0.01 and 0.1 mg L^{-1} and also 1 and 10 mg L^{-1}. At a time when 50% of the sperm were motile, there was no significant

difference between concentrations of 0, 0.01 and 0.1 and 1 mg L^{-1}. At a time when 20% of the sperm were motile, no significant difference was detected between concentrations of 0.1, 1 and 1 and 10 mg L^{-1}. However, there were significant differences among concentrations of 10, 100 and 1000 mg L^{-1} in all proportions of mobility (Fig. 3). Pearson correlation analysis between various concentrations of Zn and the duration of sperm motility showed a significant negative correlation in motile sperms at level 1% at 80% (r=-0.810, p<0.01), 50% (r=0.825, p<0.01), and 20% (r=-0.816, p<0.01).

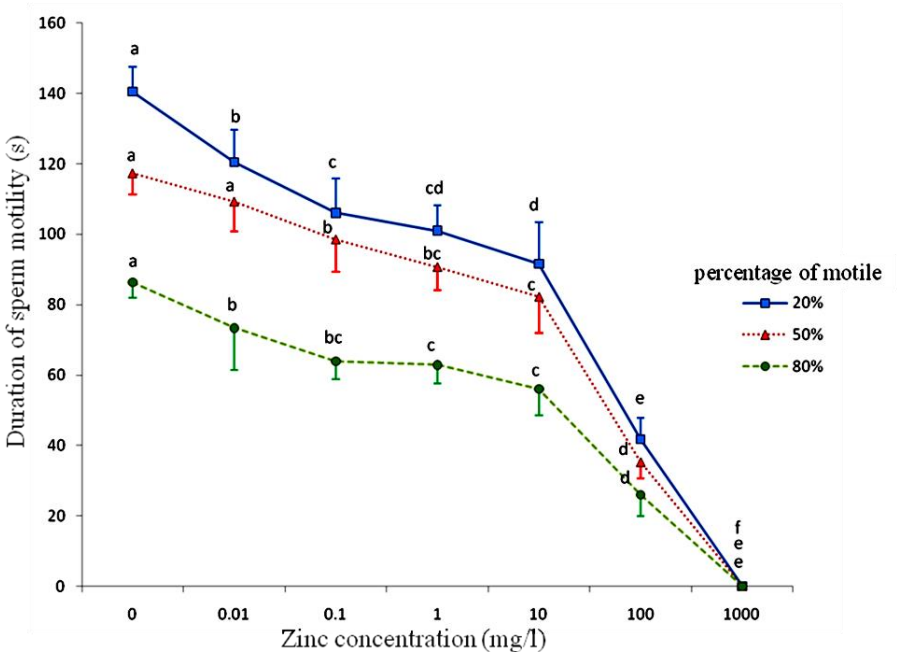

Figure 3: The effects of different zinc concentrations on percentage of motile sperms in *Caspiomyzon wagneri.*

Duration of sperm motility

The total duration of sperm motility was decreased in different treatments with increasing concentrations of Cd, Cu and Zn (Table 1). The trend of decrease was dissimilar in different metals. At concentration of 1000 mg L^{-1} of Zn, two to three percent of the sperm had a pendulum motion, and the rest were clots. At concentration of 1000 mg L^{-1} of Cu and Cd, no movement was observed, and all sperm were clots. Comparison between heavy metals in the reduction of sperm motility reflected more impact of Cu and Cd than Zn. Overall, cadmium was more effective in the reduction of motility duration compared with the other metals in decreasing the duration of motility. Pearson correlation analysis showed a significant negative correlation at level of 1% between total duration of motility of sperm and different concentrations of heavy metals of Cd (r=-0.481, p<0.01), Cu (r=-0.565, p<0.01), and Zn (r=-0.734, p<0.01).

Table 1: The average duration (second) of the sperm motility under the contact of different concentrations of heavy metals.

Heavy metals	0 ppm	0.01 ppm	0.1 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Cadmium	184.8±10.1	65.6±6.8	59.5±5.0	53.5±9.5	49.6±7.4	12.6±6.3	0
Copper	184.8±10.1	84.4±7.2	78.6±10.1	69.6±3.9	57.4±2.3	22.8±3.2	0
Zinc	184.8±10.1	127.9±7.6	116.1±11.8	107.5±8.3	98.0±10.6	45.5±6.5	0

Discussion

There is limited information about sperm characteristics such as motility parameters in lampreys particularly in case of *C. wagneri*. The results of the present study showed that 20 percent of the sperms in *C. wagneri* lost their activity after about 85 seconds, half of them after 2 min and after 182 seconds all of the sperm were stopped. Kobayashi (1993) reported that 40% of sperms of *Lampetra japonica* had motility after 5 minutes. Also, in the marine lamprey, duration of sperm motility has also been reported up to 7 min (Jaana and Yamamoto, 1981). Thus, that duration of sperm motility of *C. wagneri* is less than the other lamprey species. Different sperm characteristics, including pH, concentrations of calcium, magnesium and sodium may explain the short duration of sperm motility in *C. wagneri* than the other members of its family (Baynes *et al*., 1981; Billard and Cosson, 1992).

The results of the effect of Cd on sperm motility parameters of *C. wagneri* showed a sharp decline in the percentage of motile sperm and their motility time. Ebrahimi (2005) showed that in African catfish (*Clarias gariepinus*) exposure of sperm to 1 mg L^{-1} of Cd for 24 hrs can stop sperms motility and at 50 mg L^{-1}, all sperm stopped. Moreover, the total stop of sperms by Cd at a concentration of 100 mg L^{-1} have been reported in *Oncorhynchus mykiss*, *Acipencer ruthenus* and *A. baeri* (Dietricha *et al*., 2010). Sperm exposed to Cd in this experiment had a speed higher than the control group, but their motility duration was lower and sperms with high speed stopped in a small interval. This could be due to the rapid consumption of energy reserves mitochondrial affected by cadmium (Kime *et al*., 2001).

At the present study, the duration of motility in all percentages of the treatments containing Cu decreased compared to the control and at the concentration of 1000 mg L^{-1}, all sperm as a flocculent mass was observed, and no motility was found. Abascal *et al.* (2007) reported that Cu only at concentrations greater than 100 mg L^{-1} can affect sperm motility in *Dicentrarchus labrax*. However, in another experiment conducted by Lahsteiner *et al.* (2004), it is reported that the concentration of effective Cu in reducing sperm motility of *C. gariepinus*, *Salmo trutta fario*, *Lota lota* and *Squqlius cephalus* 10 seconds after activation is at least 5 mg L^{-1}. Our results suggest that energy reserves in

spermatozoa of *C. wagneri* was not able to overcome detrimental effects of pollutants in this concentration and led to a significant decrease in percentage of motile sperm (Kime *et al*., 2001)

The results showed a decrease in the time and percentage of motile sperm affected by the different concentrations of Zn. However the duration of mobility affected by Zn was different from those of Cd and Cu. In a study conducted by Chyb *et al*. (2000) by the CASA system, it is reported that the concentrations of 10 and 50 mg L^{-1} of Zn did not show significant effects on sperm motility parameters of *Cyprinus carpio*. On the other hand, at concentrations of 100 and 200 mg L^{-1}, significant decrease in sperm motility parameters was observed. However, complete stopping of sperm motility in *C. carpio* affected by concentrations of 500, 1000 and 2000 mg L^{-1} was reported. Ebrahimi *et al*. (1996) showed that Zn may play a role in the removal of Ca from the sperm, because the calcium channels are target place of zinc metal, and calcium removal will stop sperm motility (Busselberg, 1995). While, Zn is an essential element in some reproductive processes (Mills, 1988), but the results showed that Zn at high concentrations can have a negative impact on sperm motility.

In conclusion, the heavy metals of Cd, Cu and Zn have strong effects on the reproductive success of *C. wagneri* in polluted environments by reducing the mobility time of spermatozoa. Thus, exposure to any contamination at all stages of life, particularly in the breeding season, even at low concentrations might also affect the reproductive success of this species, and therefore it is necessary to reduce the possible risksand increase the survival of this species.

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