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Effect of Sub-Lethal Concentrations of Manganese on Sperm Motility of the Caspian lamprey (*Cspimyzon wagneri*)

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ABSTRACT The pollution of aquatic ecosystems may affect natural reproduction of fish populations by decreasing the fertilization efficiency. Among the various sources of pollution, heavy metals are important group, being found in both freshwater and marine environment. The present study investigated changes of sperm characteristics of *Caspimyzon wagneri* when exposed to Manganese (Mn). Spermatozoids were exposed to 0 (control), 0.01, 0.1, 1, 10, 100 and 1000 mg l^{-1} of Mn, duration of sperm motility and percent of motile sperms were measured using a light microscope and digital camera as a semi-quantitative method. Total duration of spermatozoids motility decreased with increase of Mn concentration in all treatments. This study indicated that Mn could seriously affect the reproductive success of the Caspian lamprey in a polluted environment through decreasing the duration of spermatozoid motility and possibly fertilization and hatching rates. Hence, contact to this metal could decrease the survival rate of this endangered species, especially during the reproduction.

Key words: *Fish, Motility indices, Pollution, Reproduction*

1 INTRODUCTION

Contamination of aquatic ecosystem influences the natural reproduction of fishes by decreasing the quality of sperm and ovule and thus fertilization efficiency (Kime *et al*., 1996). Among contaminants, the Endocrine Disrupting Chemicals (EDCs) can affect the homeostasis, reproduction and early development of aquatic organisms due to disturbing the synthesis, secretion, transport and metabolism of hormones (Kandarakis *et al*., 2009). Mobility ratio, mobility behavior patterns, duration of mobility, structural and ultra-structural characteristics of spermatozoid are the most important factors in study of fish sperm (Billard and Cosson, 1992; Cosson *et al*., 1999; Abascal *et al*., 2007; Dietrich *et al*., 2010). Studies on spermatozoid mobility and structure are limited to commercially important fishes and the species that have conservational importance (Ciereszko *et al*., 2000).

Caspimyzon wagneri is a native species of the Caspian Sea, which exists in the north, south and western basin of the Caspian Sea (Coad, 2014). This species reproduces from April to June, at 15 to 23 °C and migrates to rivers for spawning (Satari *et al*., 2002). In recent years, the stock of *C. wagnery* have been

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dramatically decreased due to dam construction, habitat destruction and pollution of rivers (Close *et al*., 2002). The present investigation was carried out to evaluate effects of different levels of Mn on sperm motility parameters over the reproduction season that may be used as an indicator of reproductive success. This study may help providing useful information for conservational managers.

2 MATERIALS AND METHODS

Fifteen male *C. wagneri* were caught using a hand-net from the down-stream of the Shirud River (36° 51' 20" N, 50° 47' 57" E) which were migrating to the spawning ground located in the upstream of the River in spring 2012. Immediately after sampling and drying with a clean towel, milting were performed by pressing the abdominal area (Linhart *et al*., 1995). One mL sperm was obtained from each specimen and stored in separate tubes. Then, sperms of all 15 specimens were mixed together to eliminate effects of quality of spawners on results of the experiment. The stock of sperm was kept in ice flask during the sampling and the experiment was started immediately in the field (Shirud River, Mazandaran Province).

Concentrations of 0.01, 0.1, 1, 10, 100 and 1000 mg l^{-1} Mn were prepared using $MnCl_2$ (Merck®; Fadakar Masouleh *et al*., 2011). Total duration of motile sperm and percentage of motile sperm were studied using a light microscope (Leica, Swaziland) and a digital camera with 14 megapixels (Canon 210, Japan) as a semi-quantitative method (Figure 1) (Alavi *et al*., 2004). To measure the duration of motility, one µL sperm plus 50 µL distilled water (for control), and various concentrations of Mn were added to the sperms on a glass slide. The experiment was performed with five treatments, four replicate for $MnCl_2$ and four replicates for the control group.

The duration of sperm motility was measured using a digital stopwatch and calculated as the time when 95-99% of sperms had no movement (Linhart *et al*., 1995; Cosson *et al*., 1999; Alavi *et al*., 2004). The percentage of motile sperms was measured according to Liley *et al*. (2002) using a hemocytometer slide and the time when 80%, 50% and 20% of sperms retained their mobility, was recorded. In addition, the proportion of the motile to immobile sperms was calculated.

Normality and homogeneity of variance were examined using the Kolmogorov-Smirnov and the Levene tests, respectively. A one-Way ANOVA was used to examine a significant difference between effects of various concentrations of Mn on sperm characteristics. When there was a significant effect of Mn concentration on sperm characteristics, a Duncan multiple range test was used to compare the means of different treatments. The Pearson correlation analysis was performed to examine the correlation between different concentrations of Mn and the sperm motility index. All analyses were performed using SPSS 20.

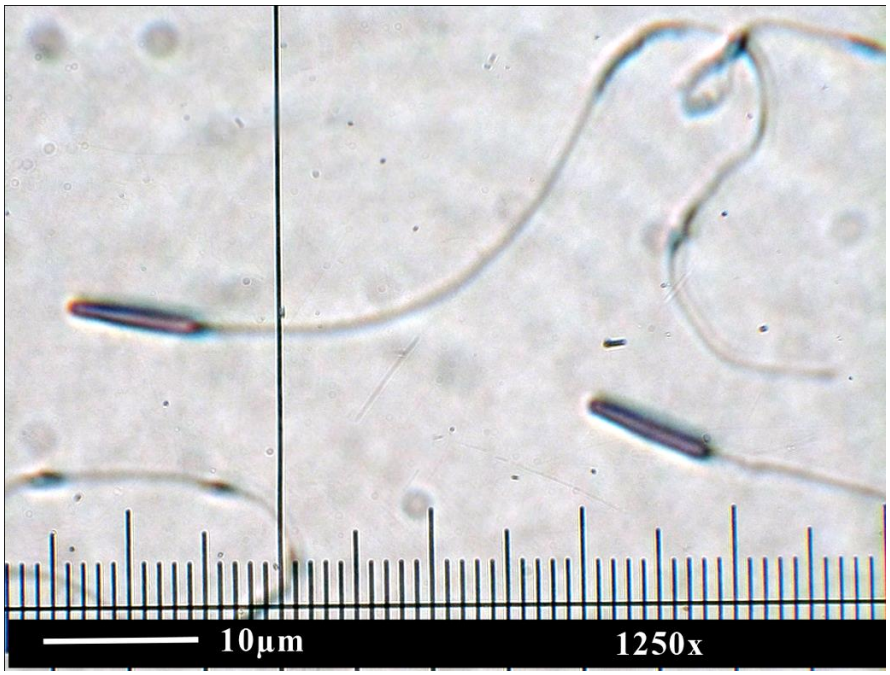

Figure 1 Natural shape of *C. wagneri* sperm under the light microscope (1250X)

3 RESULTS

The Kolmogorove-Smirnov and Levene tests indicated that the data had normal distribution and there was no significant difference in variance of the data across the treatments. There was no significant difference between sperms that exposed to 0 (control) and 0.01 mg l^{-1} Mn at the time when 80% of the sperms motile. Similarly, no significant difference was detected between sperm motility in those exposed to 1, 10 and 100 mg l^{-1} Mn ($P>0.05$). However, there were significant differences between treatments at the time when 50% and 80% of the sperms were motile.

A Pearson correlation between different Mn concentrations and motility duration of spermatozoids found a significant negative correlation when 80% ($r = -0.781^{**}$), 50% ($r = -0.776^{**}$) and 20% ($r = -0.747^{**}$) of spermatozoids were motile.

The long term mobility was recorded as the time when 95-100% of spermatozoids stopped. With increase of Mn concentration, the total duration of spermatozoids motility decreased in different treatments. Mn decreased the duration of sperm mobility at a nearly constant rate. At the concentration of 1000 mg l^{-1} < 20% of the sperms had a 10-second motility, and a greater motility was seen as a pendulum and not progressive, with all sperms being stopped after this time (Figure 2 and Table 1).

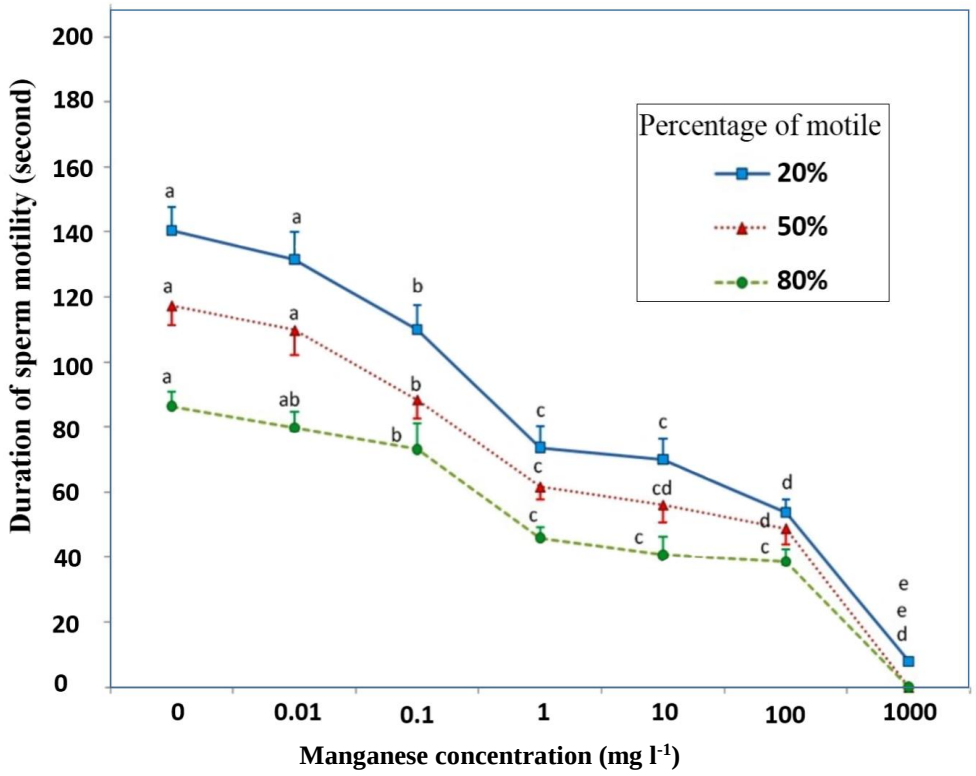

Figure 2 Motile spermatozoids percentage in different concentrations of Manganese

Table 1 Mean duration time of spermatozoids motility in connection with different concentrations of Manganese (mean± SD)

Concentration (mg l^{-1})	**0**	**0.01**	**0.1**	**1**	**10**	**100**	**1000**
Motility duration time (s)	184.8±10.1[a]	142.2±9.6[a]	128.0±15.3	90.1±18.4[b]	81.0±11.6[b]	58.7±3.3[b]	10.2±1.6

Values with similar letter do not have significant difference (P>0.05)

4 DISCUSSION

Sperm motility parameters (duration of sperm motility and mobility) are the most important criteria that have been used to assess the quality of spermatozoa (Cosson *et al*., 1999). In this respect, if spermatozoids show a high motility percentage and duration, they have a high quality and then increase the fertilization rate (Fauvel *et al.*, 2010). There is limited information about biological characteristics of sperms in lampreys, especially motility parameters. In particular, there is not much information on the motility and structure of the sperm in *C. wagneri*. The present study showed that the sperm of *C. wagnery* was activated by water and only 20% of sperms lost their mobility after 85 seconds. After two minutes, approximately half of the sperms stopped. After 182 seconds, all sperms were immotile. However, Kobayashi (1993) revealed that after 5 min, 40% of *Lampetra japonica*'s sperms were able to remain motile. Also, in the marine

lamprey duration of the sperm motility had been reported as 7 minutes (Jaana and Yamamoto, 1981). Therefore, the duration of sperm motility in *C. wagnery* is less than those species. Changes in environmental parameters such as salinity, pH, Ca^{2+}, Mg^{2+} and Na^{+} may be the reasons that have decreased motility duration of sperms in *C. wagnery* compared to other marine species of the same family (Baynes *et al*., 1981; Billard and Cosson, 1992; Cosson *et al*., 1999).

The results showed that at 0.001 mg l^{-1} of Mn, when 80, 50 and 20% of sperms were motile, there was no significant difference between those treatment and the control group, and the decreasing process occurred slowly at higher concentrations. Thus, the energy reserves of sperms in lower concentrations of Mn are able to overcome the inhibitory effects of Mn. Abdullah *et al* (2007) reported a 50% mortality in *Labeo rohita* after 30-day exposure of to 64 mg l^{-1} of Mn.

In one and 1000 mg l^{-1} Mn, a sharp decrease in mobility duration was found. Therefore, the mobility duration at one mg l^{-1} varied from 128 to 90 seconds, and at 1000 mg l^{-1} from 58 seconds up to a maximum of 10 seconds. At 1000 mg l^{-1}, the maximum sperm duration of mobility was nearly 10 seconds but was not progressive. Kharat-sadeghi and Karbasi (2006) reported that the concentration of Mn in the Shirud River (northern Iran), one of the important rivers for *C. wagnery*, to be > 500 mg l^{-1} which may play an effective role in decreasing the duration of sperm motility in *C. wagnery*. Manganese is one of the essential elements for livings, however, in high concentrations shows negative and destructive effects (Sindayigaya *et al*., 1994).

5 CONCLUSION

The present study indicated that heavy metals can seriously affect the sperm quality and thus the reproductive success of *C. wagneri* through the duration of the sperm motility. There are several anthropogenic pollution sources based around the Caspian Sea entering noticeable amount of pollution into the sea. Hence, any contact with this metal, especially during the reproduction season, can affect the recruitment of this native species. Anadromous species are inevitably being hit by river and stream pollution that may have detrimental effects on their recruitments. Heavy metals are not the only threat to the stocks of anadroms but pesticide as the agriculture is a major activity, especially, in the Southern Caspian Sea. Therefore, studying the synergistic effects of heavy metals and other chemicals including pesticides are recommended.

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اثر غلظت‌های تحت کشنده منگنز بر تحرک اسپرم مارماهی دهان‌گرد خزری (*Cspimyzon wagneri*)

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چکیده آلودگی اکوسیستم‌های آبی می‌تواند بر تولیدمثل طبیعی جمعیت ماهی‌ها به‌واسطه کاهش کارایی لقاح آن‌ها تاثیر بگذارد. از بین آلاینده‌های متنوع، فلزات سنگین یک گروه مهم می‌باشند که در اکوسیستم‌های آب‌شیرین و دریایی یافت می‌شوند. مطالعه حاضر با هدف بررسی تغییر ویژگی‌های اسپرم مارماهی دهان‌گرد خزری در مواجهه با منگنز (Mn) به اجرا درآمد. برای این منظور اسپرم این ماهی در معرض غلظت‌های ۰ (شاهد)، ۰/۰۱، ۰/۱، ۱، ۱۰، ۱۰، ۱۰۰ و ۱۰۰۰ میلی‌گرم در لیتر منگنز قرار گرفت و طول دوره تحرک اسپرم و درصد اسپرم‌های متحرک با استفاده از یک میکروسکوپ نوری و دوربین دیجیتال براساس روش نیمه-کمی مورد سنجش قرار گرفت. براساس نتایج، در تمامی تیمارها مدت کل تحرک اسپرم‌ها با افزایش غلظت منگنز کاهش یافت. نتایج این تحقیق نشان داد که منگنز می‌تواند بر موفقیت تولید مثل مارماهی دهان‌گرد خزری در محیط‌های آلوده از طریق کاهش دوره تحرک اسپرم و احتمالاً میزان لقاح و تخم‌گشایی تاثیر بگذارد. از این‌رو این فلز می‌تواند میزان بازماندگی این گونه در معرض خطر را به‌ویژه در طول دوره تولیدمثل آن را کاهش دهد.

کلمات کلیدی: آلودگی، تولیدمثل، شاخص‌های تحرک، ماهی