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BRIEF COMMUNICATION

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Ultra-acute exposure to cadmium does not impair whitefish sperm motility

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Abstract

Cadmium (Cd) exposure can impair the traits of aquatic animals associated with reproduction. In natural lakes Cd is typically detected at concentrations below 0.001 mg l^{-1}. The authors investigated the impact of ultra-acute Cd exposure on sperm motility in European whitefish (*Coregonus lavaretus*). They activated sperm with water containing various nominal concentrations of Cd and recorded sperm motility parameters. Only the highest Cd concentration (500 mg l^{-1}) was associated with decreased sperm swimming velocity and increases in both the percentage of static cells and curvature of the sperm swimming trajectory. The results indicate that environmentally realistic concentrations of Cd during the sperm motility activation are not critically harmful to male *C. lavaretus* fertilization potential.

KEYWORDS
aquatic organisms, cadmium, sperm motility, whitefish

Heavy metals are naturally occurring elements found throughout the Earth crust, but anthropogenic activities such as mining, smelting operations and industrial production often lead to unnatural environmental contamination (*e.g.*, Du *et al*., 2020). Aquatic animals are particularly vulnerable to detrimental effects of these pollutants (Coward *et al*., 2002). Heavy metals can be easily absorbed and concentrate in internal organs of aquatic animals and affect relevant biochemical, physiological and behavioural processes (Golovanova, 2008; Solgi & Galangashi, 2018). Further, in externally fertilizing species, gametes may become directly exposed to heavy metals present in the surrounding water body.

Along with many other heavy metals, cadmium (Cd) is naturally occurring in the environment but non-essential for organisms. Cd disperses readily into the environment through the air especially from many industrial and agricultural sources and has thus been a major cause for concern for many decades (Järup & Akesson, 2009). Environmental concentrations in unpolluted natural waters are usually below 0.001 mg l^{-1} but can be up to 0.6 mg l^{-1} in polluted waters (Du *et al*., 2020; Duncan *et al*., 2018) and up to 3 mg l^{-1} in highly polluted waters (Jaeckel *et al*., 2005). The concentrations of up to 5 mg kg^{-1} have been detected in lake sediments (OSPAR Commission, 2002; Parviainen *et al*., 2012). The release of Cd from the sediment may further increase in acidic conditions (Zhang *et al*., 2018), and possibly also at low oxygen levels (Li *et al*., 2013), both phenomena being increasingly prevalent due to human activities and climate change (*e.g.*, forest draining, surface run-off and eutrophication). Cd accumulation has been associated with reduced fertilization success in aquatic animals, and it can affect embryonic development (Acosta *et al*., 2016; Au *et al*., 2001; Witeska *et al*., 1995).

Sperm is known to act as a sensitive bioindicator of environmental pollution (Rurangwa *et al*., 1998). Several studies across different

taxa, from invertebrates to mammals, have revealed that heavy metal exposures are associated with a number of alterations in sperm physiology (*e.g.*, Huang *et al.*, 2001; Young & Nelson, 1974). For example, Cd concentration of 5, 50 and 100 mg l^{-1} reduced sperm motility in rats (*Rattus norvegicus domestica*). This reduction was presumably caused by the cadmium-induced alterations in calcium channels of the sperm flagella (Benoff *et al.*, 2008). In rabbits (*Oryctolagus cuniculus*), exposure to relatively low concentrations of Cd (0.6–1.0 μg $CdCl_2$ ml^{-1}) weakened sperm motility and altered sperm morphology and plasma membrane integrity (Roychoudhury *et al.*, 2010). Acosta *et al.* (2016) demonstrated in zebrafish (*Danio rerio*) that exposure of sperm to various concentrations of Cd (0.5–10 μg Cd l^{-1}) for 10 min detrimentally influenced sperm motility and longevity, thus impairing male fertility.

Sperm motility parameters are reliable predictors of fertilization success (Kime *et al.*, 2001; Browne et al. 2015), serving as valuable attributes in ecotoxicology; apart from being practical and cost-effective monitoring tools, they could be applied more often as optimal alternatives to full organism models in bioassay monitoring studies (Fabbrocini *et al.*, 2013). Computer-assisted sperm analysis (CASA) is often used for aquatic animals like fishes to predict sperm quality and fertility potential (Egeland *et al.*, 2015; Fauvel *et al.*, 2010). It provides quick quantitative measures compared to a traditional microscopic estimation, and thus is a relevant tool to study the effects of environmental stressors on fish sperm (Kime *et al.*, 2001; Browne et al. 2015).

CASA technique was used in this study to assess the impact of ultra-acute Cd exposure on sperm motility parameters using the European whitefish (*Coregonus lavaretus*) as a model species. This species has an important ecological and commercial value, and previous works indicate that it shows notable sensitivity to aquatic toxicants (Cooley *et al.*, 2002; Kashulin *et al.*, 2008). Importantly, the *C. lavaretus* possesses external fertilization, and its spawning can occur close to contaminated sediments in the wild. Thus, experimental ultra-acute exposure of sperm to certain concentrations of Cd may mimic the actual spawning event in the wild, where released sperm gets into direct contact with polluted water during activation. To authors' knowledge, this study is the first to test the sperm motility of a coregonid under a short-term Cd exposure during the sperm activation.

The *C. lavaretus* males were obtained from a hatchery-reared migratory River Koitajoki population maintained at the Saimaa Fisheries Research and Aquaculture Station of the Natural Resources Institute Finland (Luke), Enonkoski, Finland. On 15 November 2018, 15 mature males (mean length 455 ± 21 S.D. mm; mean body weight 1378 ± 272 SD g) were haphazardly sampled from the broodstock. The fish were sedated using sodium bicarbonate-buffered tricaine methanesulphonate (MS-222; 100 mg l^{-1}) before milt stripping. The stripped milts were kept in oxygen-filled plastic zipper bags on ice until the sperm motility analyses, which were performed during the same day at the laboratory of University of Eastern Finland, Joensuu.

Cadmium chloride ($CdCl_2$) was used as a source of Cd, and the used nominal concentrations were prepared by a careful dilution procedure based on commercial Cd (MERCK, 2001.0250). Sperm motility was measured using CASA (Integrated Semen Analysis System, ISAS v1: Proiser, Valencia, Spain) with B/W CCD camera (capture rate 60 frames s^{-1}) attached to negative phase contrast microscope (100× magnification). Sperm motility analyses were performed after vortexing the milt samples for 5 s and then by adding 0.1 μl of milt to Leja 2-chamber (chamber height 20 μm, volume 6 μl) microscope slides (Leja, Nieuw-Vennep, The Netherlands). Then, the sperm were activated with 3 μl of 4°C natural water containing the following nominal concentrations of Cd: 0 (control), 0.1, 2, 10, 100, and 500 mg l^{-1} (six independent treatments). Sperm were allowed to stay in aforementioned nominal Cd concentrations for 10 s prior to motility measurements.

Five sperm motility parameters (straight line velocity, VSL; linearity of sperm swimming tracks, LIN; straightness of sperm swimming trajectory, STR; curvilinear velocity, VCL; proportion of static sperm cells, %STATIC) were measured from 10 to 11 s after the activation (*i.e.*, sperm motility was recorded for 1 s). As a reliable QA/QC procedure for the CASA methodology (*e.g.*, Kekäläinen *et al.*, 2018), the average of two replicate measurements per Cd concentration was used as an observation for each male.

The effect of nominal Cd concentration (within-subject factor) on sperm motility parameters was analysed using repeated-measures ANOVA. For each individual, the averages from two activations were used as observations. Pair-wise differences were studied by Sidak *post hoc* tests. All statistical analyses were performed using IBM SPSS Statistics 25.0, after checking for data normality and homogeneity of group variances.

The effect of nominal Cd concentration was statistically significant on VSL, LIN and proportion of static sperm cells (Table 1a; Figure 1). Pair-wise comparisons for these parameters revealed, however, that Cd treatment affected sperm parameters mainly at the highest (500 mg l^{-1}) nominal concentration with decreases in VSL and LIN and an increase in %STATIC relative to lower concentrations and controls (see Sidak *post hoc* tests in Table 1b).

This study demonstrated that ultra-acute exposure of *C. lavaretus* sperm to environmentally realistic nominal concentrations of Cd (0–2 mg l^{-1}; Parviainen *et al.*, 2012) during sperm activation did not affect any of the measured motility parameters. A clear detrimental effect for sperm motility was found only in the highest, hundreds-fold concentration of Cd (500 mg l^{-1}), though not all motility traits were affected even in that level of treatment. These results indicate that functionality of *C. lavaretus* spermatozoa tolerates extremely high Cd concentrations in a short-term exposure during sperm activation.

Various effects of different Cd concentrations on fish sperm have been reported earlier. According to Hayati *et al.* (2017), *e.g.*, ultra-acute Cd exposure reduced sperm motility of carp (*Cyprinus carpio* L.) at concentrations of 100 mg l^{-1} or higher. Contrary to the finding of this study, toxic effects of Cd on sperm motility were observed at much lower concentrations in some species. In bocachico (*Prochilodus magdalenae)*, ultra-acute Cd exposure caused lowered sperm motility already at concentrations of 0.25 and 25 mg l^{-1} (Sierra-Marquez *et al.*, 2019). Acosta *et al.* (2016) observed that a short-term (10 min)

TABLE 1 The effect of different cadmium concentrations (0.1, 2.0, 10, 100 and 500 mg l^{-1}) on different sperm traits (a) and the Sidak *post hoc* comparison between concentrations (b)

(a)

Parameter	df_1/df_2	*F*	*P*
VCL	5/10	2.139	0.143
VSL	5/10	4.989	**0.015**
LIN	5/10	16.076	**<0.001**
STR	5/10	2.558	0.097
% STATIC CELLS	5/10	3.821	**0.034**

(b)

		VSL	LIN	%STATIC
Treatments (mg l^{-1})		*P*	*P*	*P*
0	0.1	0.083	**0.016**	0.987
	2	0.974	1.000	1.000
	10	0.253	0.965	1.000
	100	0.932	0.811	0.954
	500	0.062	**0.009**	**0.049**
0.1	2	1.000	0.567	0.990
	10	1.000	0.617	1.000
	100	0.874	0.269	0.159
	500	**0.002**	**0.001**	**0.004**
2	10	0.991	0.997	1.000
	100	1.000	1.000	0.918
	500	**0.070**	0.083	**0.037**
10	100	0.445	1.000	0.596
	500	**0.026**	**0.035**	**0.037**
100	500	**0.050**	**0.006**	0.131

Note. The *P*-values indicating statistically significant difference are highlighted. LIN, linearity of sperm swimming tracks; %STATIC, proportion of static sperm cells; STR, straightness of sperm swimming trajectory; VCL, curvilinear velocity; VSL, straight line velocity. Bold values indicates $P < 0.050$.

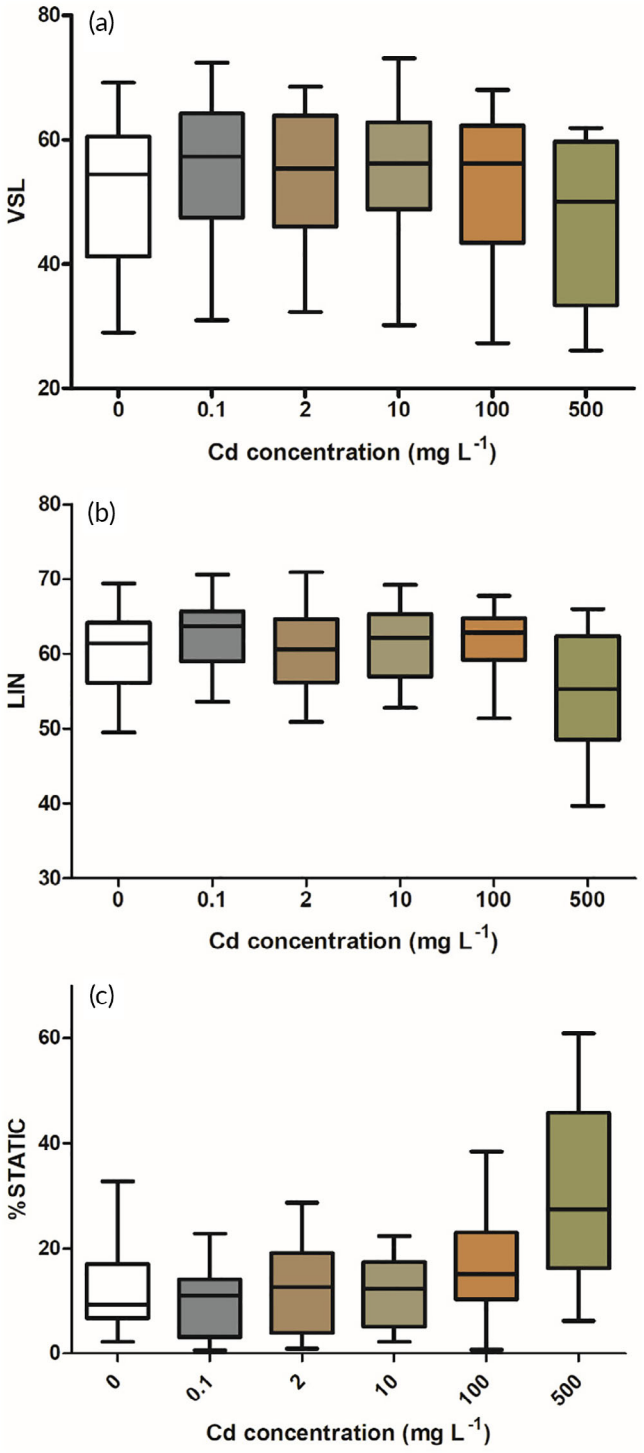

FIGURE 1 Effect of cadmium concentrations on (a) sperm straight line velocity (VSL: μm s^{-1}), (b) linearity of sperm swimming (LIN: %) and (c) proportion of sperm static cells (%STATIC) in different concentrations of cadmium: 0.1, 2.0, 10, 100 and 500 mg l^{-1}. Whiskers in boxplots indicate 95% C.I., and the lines within each box represent median

Cd exposure induced highly toxic effect on sperm cells of *D. rerio* already in a concentration range of 5×10^{-3} to 10×10^{-3} mg l^{-1} by reducing membrane integrity, whereas sperm motility of sterlet (*Acipenser ruthenus*) was not affected by 2 h Cd exposure at a concentration of 0.05 mg l^{-1} (Li *et al*., 2010). The present and previous studies thus indicate that fish sperm sensitivity to Cd may be a species-dependent attribute. Plasma membrane of sperm is very likely to be intensively affected by toxicants (Harayashiki *et al*., 2013). In rainbow trout (*Oncorhynchus mykiss*), seminal plasma has been suggested to protect sperm against toxicants (Dietrich *et al*., 2010). Similar protective mechanisms against Cd may occur also in *C. lavaretus*, but these mechanisms are yet unknown and remain to be explored.

In externally fertilizing fish that spawn in shallow areas, such as the studied *C. lavaretus* population, the released gametes may be exposed to contaminants of sediments during spawning act (Amundsen *et al*., 2011; Haakana & Huuskonen, 2012). The present results suggest, however, that in ultra-acute exposure the performance of *C. lavaretus* sperm is tolerant to considerably high nominal

concentrations of Cd, and thus no naturally occurring concentrations should compromise the fertilization potential of males. Further studies are needed to test whether the negative effects of Cd manifest themselves only after longer exposure time or occur later during embryonic period.

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