Int. J. Lim. 2024, 60, 24
© M. Mäkinen et al., Published by EDP Sciences, 2024
https://doi.org/10.1051/limn/2024023

Available online at:
www.limnology-journal.org

Special issue - Biology and Management of Coregonid Fishes - 2023
Guest editors: Orlane Anneville, Chloé Goulon, Juha Karjalainen, Jean Guillard, Jared T. Myers and Jason Stockwell

RESEARCH ARTICLE OPEN ACCESS

Differences in sulfate sensitivity of early development between brackish and freshwater coregonines

Mikko Mäkinen*, Xiaoxuan Hu and Juha Karjalainen

Department of Biological and Environmental Science, University of Jyväskylä, Survontie 9C, PO Box 35, FI 40014, Finland

Received: 19 December 2023; Accepted: 30 September 2024

Abstract – Sulfate is found naturally in the aquatic environments, but due to various anthropogenic activities the sulfate concentrations in surface waters have increased globally. High levels of sulfate can cause adverse effects on aquatic organisms. In this study we explored the effects of sulfate on the reproduction of two coregonine species, whitefish (*Coregonus lavaretus* (L.)) and vendace (*Coregonus albula* (L.)), in Baltic Sea brackish water and soft boreal freshwater. The chronic toxicity tests lasted from fertilization to hatching of the larvae, endpoints being embryonic and larval survival, and size of newly hatched larvae. The chronic 196–214-day tests were conducted in different sodium sulfate (Na_2SO_4) solutions at water temperatures simulating natural conditions during the egg incubation from autumn to spring. The separate fertilization tests were carried out to measure fertilization success (%). The fertilization and early embryonic phase were found to be the most sensitive periods for sulfate toxicity. The survival in late embryonic phase, hatching and 5-day larval phase was high (>80%). In the acute fertilization tests with brackish water populations, the LC50-values were between 2554 and 2575 mg/L and with freshwater populations between 1107 and 1230 mg/L of sulfate. In the chronic experiments from fertilization to hatching the LC10-values for brackish water populations were between 1800 and 1820 mg/L and for freshwater populations between 335 and 624 mg/L of sulfate. The tolerance for sulfate in freshwater coregonines was significantly lower than brackish water coregonines, but it was in similar range as to what has been observed in other freshwater species.

Keywords: Fish / embryo / fertilization / lethal concentration / salinization

1 Introduction

Sulfate (SO_4^{2-}) is found naturally in aquatic environments. Due to anthropogenic activities, concentration of sulfate in waterways can increase to levels which can be harmful to aquatic organisms. Point sources of sulfate are primarily industrial processes that utilize sulfuric acid as part of their production cycle, drainage from mining areas and municipal wastewaters (Elphick *et al.*, 2011; Nordstrom *et al.*, 2015; Ekholm *et al.*, 2020). Major diffuse sources are run offs from forestry areas, fertilization runoffs and tilling of soil in agricultural areas, especially from fields located on acid sulfate soil (Elphick *et al.*, 2011; Ekholm *et al.*, 2020).

In boreal freshwaters, background sulfate levels range from 3 to 30 mg/L, but in areas heavily affected by anthropogenic activities sulfate levels can reach values as high as over 2000 mg/L (Ekholm *et al.*, 2020). In the coastal regions of the northern Baltic Sea the sulfate levels range from 100 to 500 mg/L (Lehtoranta *et al.*, 2008; Zak *et al.*, 2021). Increased sulfate load into surface waters can lead to salinization and stratification, modify the cycle of nutrients and cause eutrophication and anoxic conditions in hypolimnion (Cañedo-Argüelles *et al.*, 2019; Wiltse *et al.*, 2020; Kaushal *et al.*, 2021; Zak *et al.*, 2021). In addition, sulfate affects aquatic organisms by inducing osmotic stress which may lead to increased consumption of energy to maintain homeostasis (Griffith, 2017; Cañedo-Argüelles *et al.*, 2019) or by inducing specific ion toxicity (Davies and Hall, 2007). The toxicity mechanism of sulfate is also linked to water hardness, as low water hardness increases the toxic effects of sulfate (Soucek and Kennedy, 2005; Davies and Hall, 2007; Elphick *et al.*, 2011; Soucek *et al.*, 2011).

In this study, we aim to further contribute to the knowledge of the effects of sulfate on aquatic organisms in soft boreal waters. We continued the work from our previous research (Karjalainen *et al.*, 2021, 2023) by examining the fertilization and early development of two different coregonine (Coregoninae) species, whitefish (*Coregonus lavaretus* (L.)) and vendace (*Coregonus albula* (L.)), from both brackish and freshwater environments. In this study in addition to anadromous whitefish which we have studied earlier

*Corresponding author: mikko.v.j.makinen@jyu.fi

Table 1. Sulfate toxicity tests carried out in soft waters (RW = River Kokemäenjoki water, LW = Lake Konnevesi water) and in brackish water (BW = Kokkola brackish water). Chronic toxicity tests lasted from fertilization to the moment when the hatched larvae reached age of 5 days except from fertilization of *Salmo trutta* from fertilization to estimated emergence moment (see Karjalainen *et al*., 2023). Results of five toxicity tests were published first time in this study and rest of them were published previously in Karjalainen *et al.* (2023).

Test species and spawning habitat	Test type and water	Endpoint	Source
Coregonus lavaretus, brackish water	Acute, BW	Fertilization rate	This study
Coregonus albula, brackish water	Acute, BW	Fertilization rate	This study
Coregonus lavaretus, anadromous	Acute, RW	Fertilization rate	Karjalainen *et al.*, 2023
Coregonus lavaretus, fresh water	Acute, LW	Fertilization rate	Karjalainen *et al.*, 2023
Coregonus albula, fresh water	Acute, LW	Fertilization rate	Karjalainen *et al.*, 2023
Salmo trutta, fresh water	Acute, LW	Fertilization rate	Karjalainen *et al.*, 2023
Rutilus rutilus, fresh water	Acute, LW	Fertilization rate	Karjalainen *et al.*, 2023
Coregonus lavaretus, brackish water	Acute, BW	Survival	This study
Coregonus lavaretus, anadromous	Acute, RW	Survival	Karjalainen *et al.*, 2023
Coregonus lavaretus, freshwater	Acute, LW	Survival	This study
Coregonus albula, freshwater	Acute, LW	Survival	This study
Salmo trutta, freshwater	Acute, LW	Survival	Karjalainen *et al.*, 2023
Rutilus rutilus, freshwater	Acute, LW	Survival	Karjalainen *et al.*, 2023

(Karjalainen *et al*., 2021), new toxicity tests of sea-spawning whitefish and vendace in brackish water and lake-spawning whitefish and vendace in freshwater were carried out. Vendace is mainly an inland species, but it is found in Gulf of Bothnia and Gulf of Finland regions of the Baltic Sea where it spawns in rivers, estuaries or less saline coastal habitats (HELCOM, 2013; Lehtonen *et al.*, 2023). In Baltic Sea the vendace is commercially caught for its roe which is a commercially important product (Lehtonen *et al.*, 2023). In Finland, vendace generated the largest portion of the revenue for the commercial inland fisheries and together with pikeperch (*Sander lucioperca* L.), it is one of the most important target species of commercial fishers in recent years (Luke, 2023).

Whitefish populations are found from both inland lakes as well as from the Baltic Sea. In the Finnish coastal regions two ecotypes of whitefish are present, a river-spawning whitefish and a sea-spawning whitefish (Lehtonen, 1981). The aim of this study was to compare the new and previously published sulfate toxicity test results of several whitefish and vendace populations to examine the differences in sulfate sensitivity of early development between brackish and freshwater coregonines. The endpoint variables in the toxicity tests in our study were: 1) fertilization success, 2) survival in early embryonic phase, 3) survival in late embryonic phase, 4) survival in hatching and as yolk sac larvae and 5) the size of the hatched larvae.

2 Materials and methods

2.1 Test species

The toxicity tests were carried out using whitefish and vendace from both Baltic brackish water and inland freshwater (Tab. 1). Vendace and whitefish both spawn in late autumn and eggs incubate until spring. The incubation time can last up to 6 months. We followed the incubation period from fertilization to hatching of brackish water whitefish for 196 days and anadromous whitefish for 165 days and freshwater whitefish and vendace for 214 days and 203 days, respectively. The parental fish of whitefish and vendace were caught by professional fishers on 26th of October 2022 from Baltic Sea in Larsmo archipelago (N63° 72' 5418", E22° 62' 8513"). Eggs and sperm of whitefish were stripped in harbour and stored in separate chilled containers. Eggs from vendace were stripped in site, but males were transported in ice to the university where the sperm was stripped. In the whitefish test, we used gametes of 7 females and 7 males and in vendace experiment, we used 6 females and 9 males (Supplementary material Tab. 1). Anadromous sea-migratory whitefish gametes from 5 females and 5 males were obtained from River Kokemäenjoki (N61° 33' 8384", E22° 11' 7176") (Karjalainen *et al.*, 2023) (Supplementary material Tab. 1). Freshwater whitefish and vendace were obtained from Lake Konnevesi (N62° 61' 7519", E26° 35' 1125") on 2nd of November 2022, whitefish by gill nets and vendace by seine net. The fish were transported in chilled containers to the university where the eggs and sperm were stripped. In freshwater whitefish test, we used gametes from 3 females and 6 males and in vendace test we used 9 females and 12 males (Supplementary material Tab. 1). Number of parental fish varied due to the ripeness to spawn of the fish caught. The toxicity test results of Karjalainen *et al.* (2023) of roach (*Rutilus rutilus* L.) and brown trout (*Salmo trutta* L.) were compared to whitefish and vendace toxicity test results.

2.2 Test setup and chemicals

Both the acute and chronic toxicity tests were carried out: the acute short-term fertilization test and the chronic toxicity test from fertilization to hatching including the short 5-day incubation of hatched larvae. In the acute fertilization test, 50–100 eggs from each sulfate treatment were sampled to determine fertilization success (%). In the chronic incubation test each treatment had 36 eggs which were followed throughout the early development. The tests were applied in same manner for each test species and population and were analysed separately.

Table 2. The nominal and measured sulfate (mg/L SO_4) concentrations (mean ± SD), amount of added Na_2SO_4 into experimental treatments and the pH (mean ± SD) in brackish water control and experimental treatments (number of samples taken, $n = 5$), in Lake Konnevesi control and experimental treatments ($n = 4$) and in River Kokemäenjoki control and experimental treatments ($n = 5$) (Karjalainen *et al.*, 2023).

	Added Na_2SO_4	Nominal SO_4	Measured SO_4	pH
Brackish water				
		Kokkola control	240.0 ± 7.1	7.6 ± 0.3
	384 mg/L	500 mg/L	496.0 ± 13.6	7.5 ± 0.1
	828 mg/L	800 mg/L	810.0 ± 16.7	7.5 ± 0.1
	1567 mg/L	1300 mg/L	1280.0 ± 40.0	7.5 ± 0.1
	2307 mg/L	1800 mg/L	1780.0 ± 40.0	7.5 ± 0.1
	2898 mg/L	2200 mg/L	2180.0 ± 40.0	7.4 ± 0.0
	3490 mg/L	2600 mg/L	2600.0 ± 70.7	7.5 ± 0.1
	4081 mg/L	3000 mg/L	3050.0 ± 50.0	7.4 ± 0.0
	4820 mg/L	3500 mg/L	3475.0 ± 43.3	7.4 ± 0.1
	6299 mg/L	4500 mg/L	4500.0 ± 0.0	7.4 ± 0.0
Lake water				
		L. Konnevesi control	5.0 ± 0.5	7.3 ± 0.2
	436 mg/L	300 mg/L	307.5 ± 8.3	7.1 ± 0.1
	880 mg/L	600 mg/L	615.0 ± 11.2	7.0 ± 0.1
	1323 mg/L	900 mg/L	920.0 ± 18.7	7.0 ± 0.0
	1767 mg/L	1200 mg/L	1175.0 ± 43.3	7.0 ± 0.0
	2358 mg/L	1600 mg/L	1600.0 ± 0.0	6.9 ± 0.0
	2654 mg/L	1800 mg/L	1800.0 ± 0.0	6.9 ± 0.0
	2950 mg/L	2000 mg/L	2000.0 ± 0.0	6.9 ± 0.0
	3541 mg/L	2400 mg/L	2500.0 ± 0.0	6.7 ± 0.0
	4281 mg/L	2900 mg/L	2900.0 ± 81.6	6.8 ± 0.0
	5168 mg/L	3500 mg/L	3600.0 ± 0.0	6.8 ± 0.0
River water				
		R. Kokemäenjoki control	12.2 ± 2.8	7.3 ± 0.1
	426 mg/L	300 mg/L	304.0 ± 8.9	7.2 ± 0.2
	869 mg/L	600 mg/L	598.0 ± 22.8	7.1 ± 0.2
	1313 mg/L	900 mg/L	892.0 ± 29.5	7.2 ± 0.2
	1757 mg/L	1200 mg/L	1200.0 ± 0.0	7.1 ± 0.2
	2200 mg/L	1500 mg/L	1500.0 ± 0.0	7.1 ± 0.1
	2644 mg/L	1800 mg/L	1800.0 ± 0.0	7.1 ± 0.3
	3531 mg/L	2400 mg/L	2380.0 ± 44.7	7.1 ± 0.2

Experimental treatment solutions were made by dissolving sodium sulfate (Na_2SO_4, Merck, purity ≥ 99%) into filtered natural water (Millipore Pellicon & Durapore GVPP, 0.22 µL). In brackish water (BW) experiments we used natural water taken from the Baltic Sea coastal area in Kokkola (N63° 92' 5371", E23° 03' 8227", background sulfate 240 mg/L) (Tab. 2). For freshwater coregonine toxicity tests natural water was taken from Lake Konnevesi (LW) (N62° 61' 7519", E26° 35' 1125", background sulfate 5.0 mg/L) (Tab. 2) and for anadromous whitefish test from River Kokemäenjoki (RW) (N61° 33' 8384", E22° 11' 7176", background sulfate 12.2 mg/L) (Tab. 2) (Karjalainen *et al.*, 2023). The water used as a base for treatment solutions for fertilization and throughout the incubation was similar water from which the parental fish originated from. These natural waters taken from the different locations were also the control treatments in the toxicity tests. Stock solutions were prepared from the waters by dissolving the Na_2SO_4 into filtered natural water to achieve the highest concentration. Other treatment solutions were prepared by diluting the stock solutions with natural water to achieve the target concentrations in each experiment. Number of treatments in BW experiment including control were 10, in LW experiment 11 and in RW experiment 8 (Tab. 2). Water hardness for natural waters were calculated from the measured Ca^{2+} and Mg^{2+} concentrations. The hardness values for natural waters were 5.188 ± 0.147 mmol/L (mean ± SD) (518.8 ± 14.7 mg/L as $CaCO_3$) in BW, 0.139 ± 0.004 mmol/L (13.9 ± 0.4 mg/L) in LW and 0.284 ± 0.026 mmol/L (28.4 ± 2.6 mg/L) in RW. Thus, BW water was hard (> 300 mg/L), while LW and RW waters were soft (<30 mg/L).

Sulfate concentrations were monitored by taking samples throughout the experiments (Tab. 2). The uncertainty of the sulfate analysis was 10%. The analysis was provided by an independent accredited laboratory and done according to SFS-EN ISO 10304–1 (2009) standard. Water temperature simulated the natural temperature rhythm and was monitored throughout the experiments with automatic temperature loggers. The pH (SevenEasy, Mettler-Toledo) (Table 2) and oxygen (Microx 4 trace, PreSens Precision Sensing GmbH) levels were monitored throughout the experiments. The mean oxygen concentration was 11.5 ± 0.1 mg/L (± SD, $n = 18$).

2.3 Fertilization

Approximately 100 eggs per each female were put in to a 500 mL glass jar and 3–5 μL of sperm from each male was distributed evenly among all eggs from the females. 25 mL of water was added into the jar to activate the gametes. The jar was gently stirred and left to sit for 40 seconds after which the eggs were rinsed with fresh water to remove the excess sperm. Once the water in the jar was clear it was filled up to 200 mL and provided with aeriation. This was repeated for each different treatment concentration (Tab. 2). The fertilization success rate (%) was determined after 48–96 hours post fertilization. Around 50 to 100 randomly selected eggs from each treatment were observed under a microscope to see if the cell divisions had started to develop. The fertilization temperature was 5 °C for brackish water whitefish and vendace and 6 °C for freshwater whitefish and vendace. The fertilization and its success were regarded as an acute exposure experiment.

2.4 Embryonic period and hatching

After 24-h period eggs were moved to 6-well plates with movable inserts (VWR 6-well plate type 734–2717 6) with cut out Pasteur pipette. The wells were prefilled with 10 mL of treatment solution corresponding with the jar from which the eggs were moved. Randomly selected eggs were individually placed into the inserts within the wells. The plates were covered with lids to prevent evaporation during the experiments. Each treatment (Tab. 2) had total of 36 eggs distributed into 6 plates and divided on two separate aluminium trays. Trays were placed on a shelf in the cold room. Placement of the trays was randomized. Temperature of the cold room was adjusted throughout the experiment to follow the simulated natural temperature cycle. The temperature at the fertilization was 5 °C for brackish water whitefish and vendace and 6 °C for freshwater whitefish and vendace. The average temperature during the incubation period was 2 °C for brackish water whitefish and for freshwater whitefish and vendace. The average temperature during the hatching and larval period w was 4 °C for brackish water whitefish and 5 °C for freshwater whitefish and vendace. The temperature was monitored with two separate temperature loggers placed in to 6-well plates filled with tap water.

The water in the toxicity tests was changed weekly. Inserts containing the eggs were gently moved from the old plates to new ones which were prefilled with the corresponding sulfate treatment. The trays housing the plates were also rotated during each water change to even out the possible temperature fluctuations in the room. The observation of embryonic period was divided into early and late periods. Once the eggs reached the eyed stage dead eggs were recorded and removed from the setup, thus ending the early embryonic period. Late embryonic period ended into hatching.

After the first hatching was observed plates were inspected on daily basis for new hatchings. The date of each hatched larvae was recorded, and larvae was followed for 5 days counting the day of hatching as the first day. After the 5-day period larvae were removed from the well and preserved in 95% ethanol for further analysis. The larvae were collected into concentration specific tubes, respectively. The incubation period from fertilization to hatching and to 5-day-old larvae was regarded as chronic toxicity test.

2.5 Total length and mass measurements

Total length, fresh and dry mass, and dry matter (%, dry mass divided by fresh mass) were measured from the preserved larvae. Before measuring, larva was taken from ethanol and put into a petri dish filled with water to soak for 15 min to restore the size of tissues (Karjalainen, 1992). When taken out the larva was wiped gently with a moist paper towel to get rid of the excess moisture and length was measured immediately. After length measurement, a larva was placed in a pre-weighed aluminium cup and the fresh mass was weighted with a microscale. The larvae were dried at 60 °C for 24 h after which the cups were reweighed to determine the dry mass for the calculation of dry matter.

2.6 Statistical analysis

The effects of sulfate on the fertilization rate (%) and the embryonic survival were analysed by using Kruskall–Wallis *H* test and pairwise comparisons by Bonferroni test between the control and added sulfate treatments. The effects on the larval length and fresh mass were analysed with one-way ANOVA and the effects on dry matter ratio were analysed with Kruskall–Wallis *H* test, pairwise comparisons were done for both by Bonferroni test. These analyses were done in IBM SPSS (v28.0.1.1) statistics software. The concentration-response analysis was done by using 'drc' package (Ritz *et al.*, 2015) in R (R Core team, 2022) to analyse the toxicity data. The lethal concentration LC10 (the concentration, which was lethal to 10% of tested individuals) with the 95% confidence intervals was calculated for chronic exposure (toxicity test from fertilization to 5-day-old larvae) and the lethal concentration LC50 (the concentration, which was lethal to 50% of tested individuals) with the 95% confidence intervals was estimated for acute exposure (fertilization test) (Supplementary material Tab. 3). The statistical analyses were made for each toxicity test separately. The LC values of the separate toxicity tests were compared to evaluate the differences in sulfate sensitivity of different whitefish and vendace populations originated in brackish and freshwater environments.

3 Results

3.1 Sulfate concentration affects fertilization and early embryo development

Sulfate concentration significantly affected fertilization rate of whitefish and vendace in all brackish and freshwater experiments (Fig. 1, Supplementary material Tab. 2). Sulfate disturbed early cell division and embryo development, and majority of the mortality in all experiments occurred in the early embryonic phase from fertilization to the eyed stage (Fig. 2, Supplementary material Tab. 2). In the late embryonic phase from the eyed stage to 5-day-old newly hatched larvae, sulfate did not significantly affect the survival of the developing embryos or living hatched larvae (Fig. 3, Supplementary material Tab. 2). The survival of vendace in

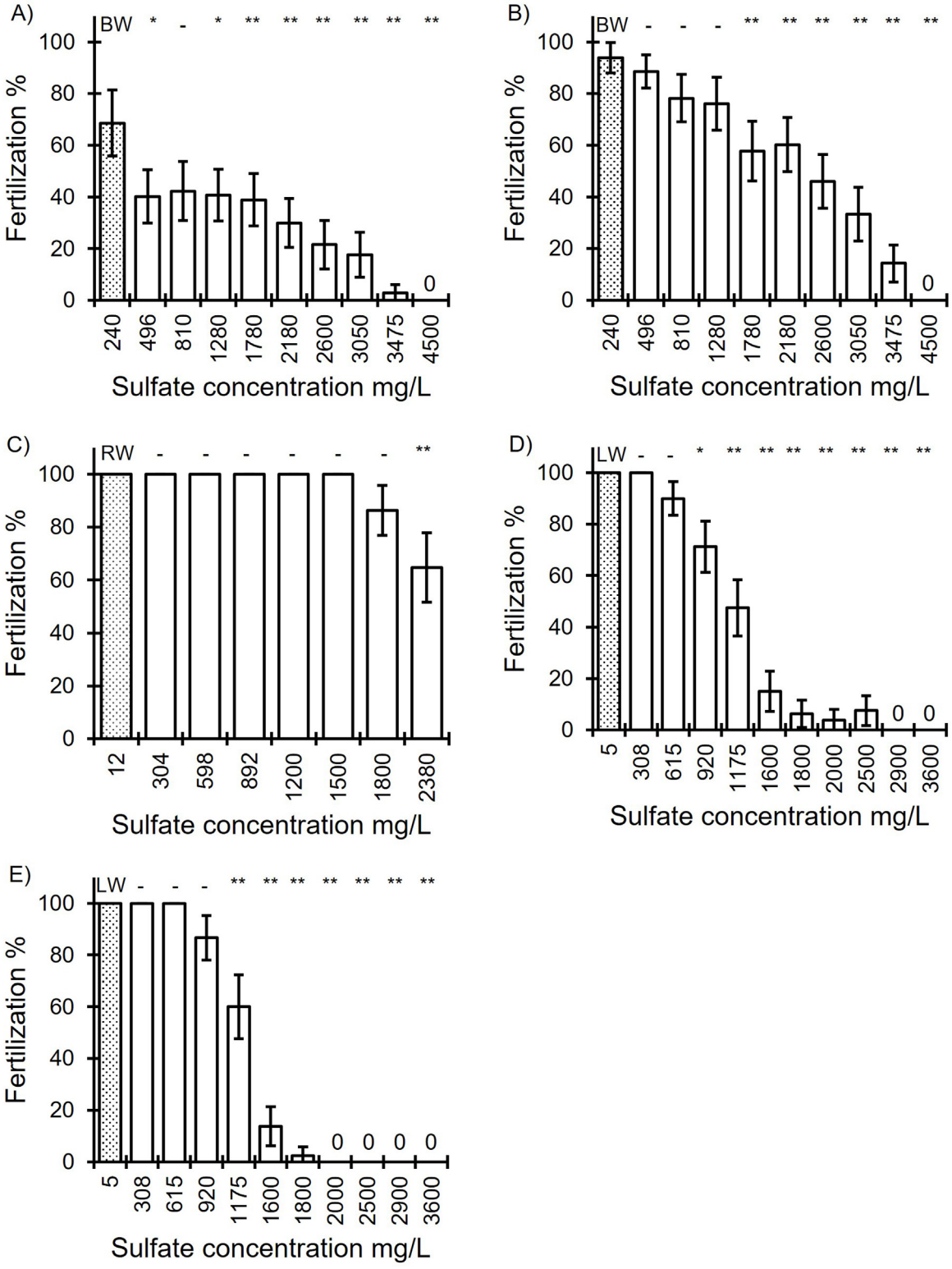

Fig. 1. Proportion of successfully fertilized eggs in brackish water vendace (A) (%, n = 51–104 eggs per treatment), brackish water whitefish (B) (%, n = 51–104 eggs per treatment), anadromous sea-migratory whitefish (C) (%, n = 50–51 eggs per treatment), freshwater vendace (D) (%, n = 55–80 eggs) and freshwater whitefish (E) (%, n = 55–80 eggs). Experiments done in different sulfate (SO_4^{2-}) treatments and in control treatments (BW = brackish water, RW = R. Kokemäenjoki water, LW = L. Konnevesi water) with background sulfate concentrations. The significance levels are above the bars (Kruskall-Wallis): − = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$. Vertical lines on bars represent the 95% confidence limits.

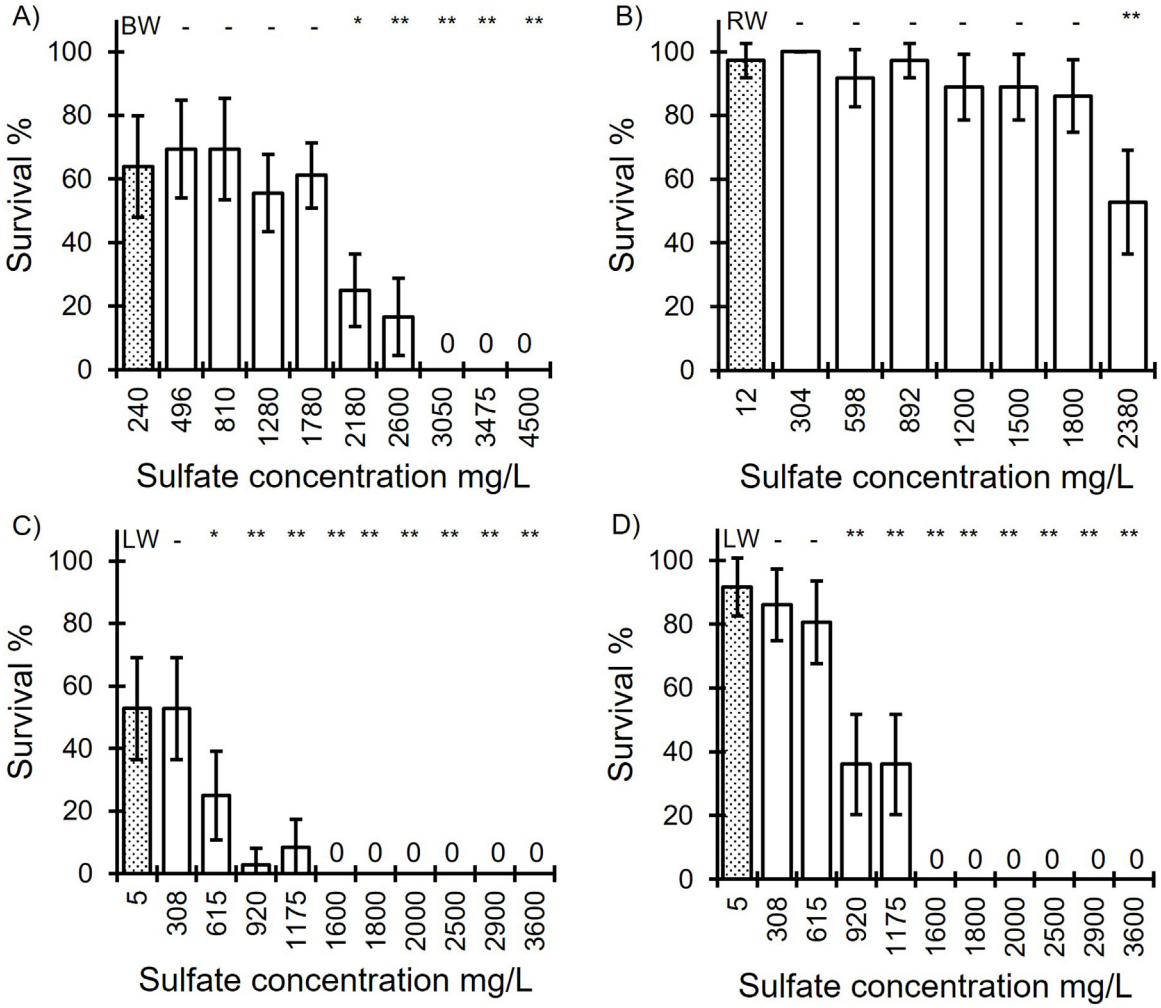

Fig. 2. Survival (%) of brackish water whitefish (A) (34 days from the fertilization, n = 36), anadromous sea-migratory whitefish (B) (25 days from the fertilization, n = 36), freshwater vendace (C) (34 days from the fertilization, n = 36) and freshwater whitefish (D) (34 days from the fertilization, n = 36) in the early embryonic phase from fertilization to eyed stage in different sulfate (SO_4^{2-}) concentrations with control treatments (BW = brackish water, RW = R. Kokemäenjoki water, LW = L. Konnevesi water) and their background SO_4^{2-}concentrations. The significance levels are above the bars (Kruskall-Wallis): – = P > 0.05, * = P < 0.05, ** = P < 0.01. Vertical lines on bars represent the 95% confidence limits.

the LW experiment at sulfate concentration of 920 mg/L was low (0%, Fig. 3C) but also the individuals after the sulfate exposure by the eyed stage was low, with only one alive embryo.

For the entire duration of the experiment from the fertilization to the end of larval period, a statistically significant difference in survival was found between treatments in BW (H = 108.35, DF = 9, $P < 0.001$), RW (H = 50.11, DF = 7, $P < 0.001$) and LW whitefish (H = 255.94, DF = 10, $P < 0.001$) and LW vendace (H = 146.86, DF = 10, $P < 0.001$) (Supplementary material Tab. 2, Supplementary material Fig. 1).

3.2 Sensitivity of freshwater fish and brackish water fish

The sulfate affected the fertilization of freshwater whitefish and vendace in lower concentrations than the fertilization of anadromous whitefish or brackish water whitefish and vendace (Fig. 1, Supplementary material Tab. 3). The acute LC50 (fertilization rate) of freshwater fishes varied from 1107 to 1353 mg/L while the LC50 of anadromous whitefish and sea-spawning whitefish and vendace were over 2500 mg/L. Similarly, the chronic LC10 of freshwater fishes for the entire incubation experiment (from fertilization to 5-day-old larvae) was clearly lower (335–1139 mg/L) than the LC10 of Baltic Sea whitefish and vendace (Fig. 4, Supplementary material Tab. 3).

3.3 Size of newly hatched larvae

Sulfate significantly affected length and fresh mass of the 5-day-old larvae in anadromous whitefish and freshwater vendace and whitefish (Fig. 5, Supplementary material Tab. 4). Length of the larvae in the concentration of 1175 mg/L differed statistically from the control treatment in both freshwater species, but in terms of fresh mass none of the sulfate

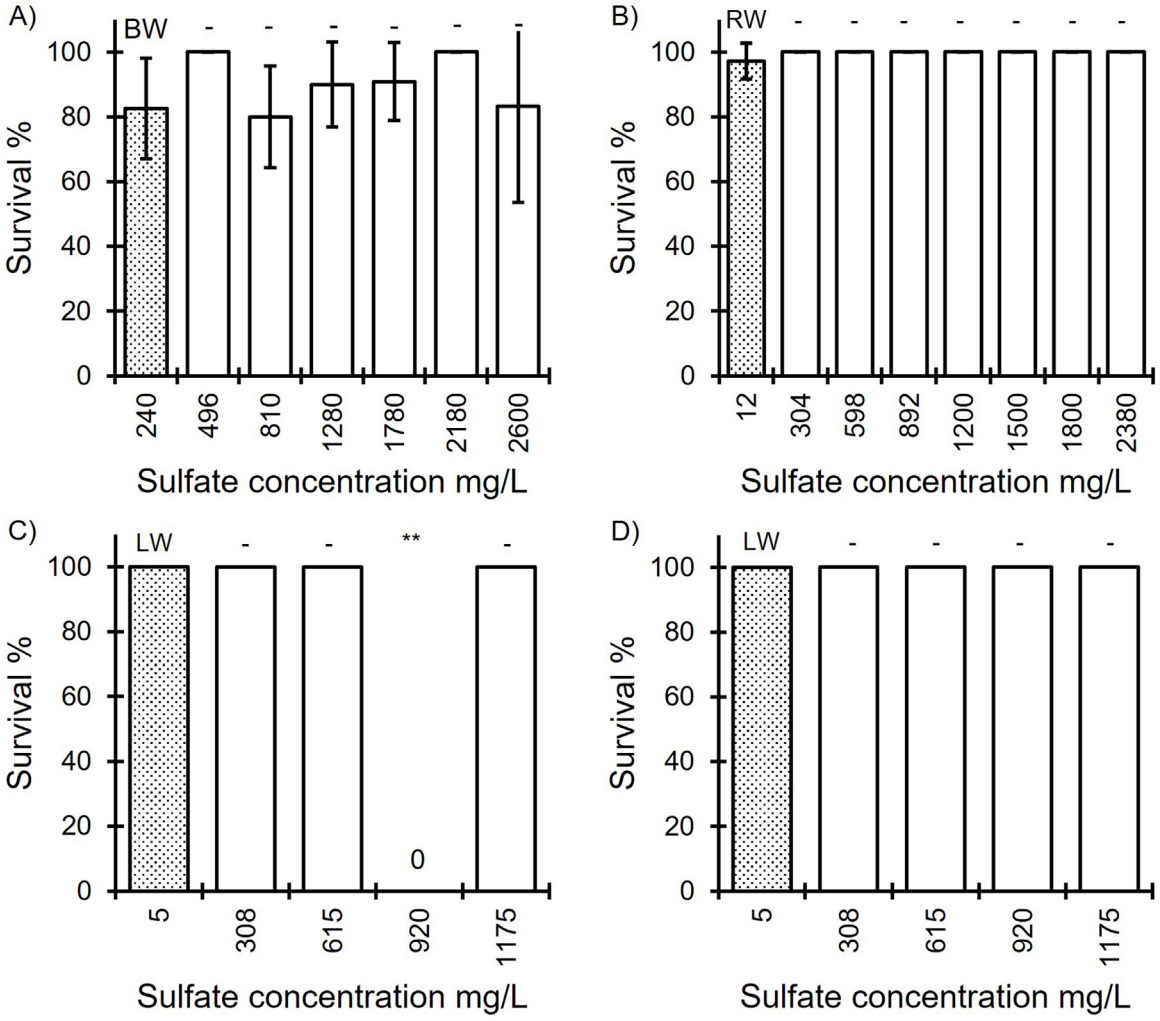

Fig. 3. Survival (%) of brackish water whitefish (A) (169 days from the end of early embryonic period, n = 36), anadromous sea-migratory whitefish (B) (140 days from the end of early embryonic period, n = 36), freshwater vendace (C) (175 days from the end of early embryonic period, n = 36) and freshwater whitefish (D) (186 days from the end of early embryonic period, n = 36) during the late embryonic phase, hatching and larval period in different sulfate (SO_4^{2-}) concentrations with control treatments (BW = brackish water, RW = R. Kokemäenjoki water, LW = L. Konnevesi water) and their background SO_4^{2-}concentrations. The significance levels are above the bars (Kruskall-Wallis): − = P > 0.05, * = P < 0.05, ** = P < 0.01. Vertical lines on bars represent the 95% confidence limits.

concentrations differed statistically from control treatment (Fig. 5). In anadromous whitefish experiment a significant difference was found in length and fresh mass measurements of the 5-day-old larvae, but not in dry matter (Fig. 5, Supplementary material Tab. 4).

4 Discussion

In this study the fertilization and the early embryonic phase of coregonines were the most sensitive periods to sulfate exposure in the terms of survival. In previous studies Karjalainen *et al.* (2021) reported that the fertilization and the early period of embryonic development of a migratory anadromous whitefish were found to be sensitive to the sulfate exposure. Meays and Nordin (2013) noted in their study that the sensitive phase for sulfate in development of rainbow trout (*Oncorhynchus mykiss* (Walbaum)) embryo was from eyed stage to alevin, but they also mentioned that pre-eyed stage could be even more sensitive endpoint. Our results showed that the pre-eyed stage and fertilization were indeed the most sensitive periods in early development of fish. The mechanism of sulfate toxicity is either through osmotic stress or specific ion toxicity (Davies and Hall, 2007; Griffith, 2017; Cañedo-Argüelles *et al.*, 2019). Sulfate can disturb the osmotic homeostasis of the cell and create imbalance in water regulation within, thus causing toxic effects to the organism (Davies and Hall, 2007). Compared with organisms developed in saline environments, organisms developed in hypo-osmotic conditions of freshwater environments are more threatened by increases of salinity and are more susceptible to the adverse effects of osmotic stress and specific ion toxicity (Cañedo-Argüelles *et al.*, 2019). The osmoregulatory adaptation to saline environments (Hart *et al.*, 1991; Koel and Peterka, 1995; Fyhn *et al.*, 1999) can explain the difference found in our study

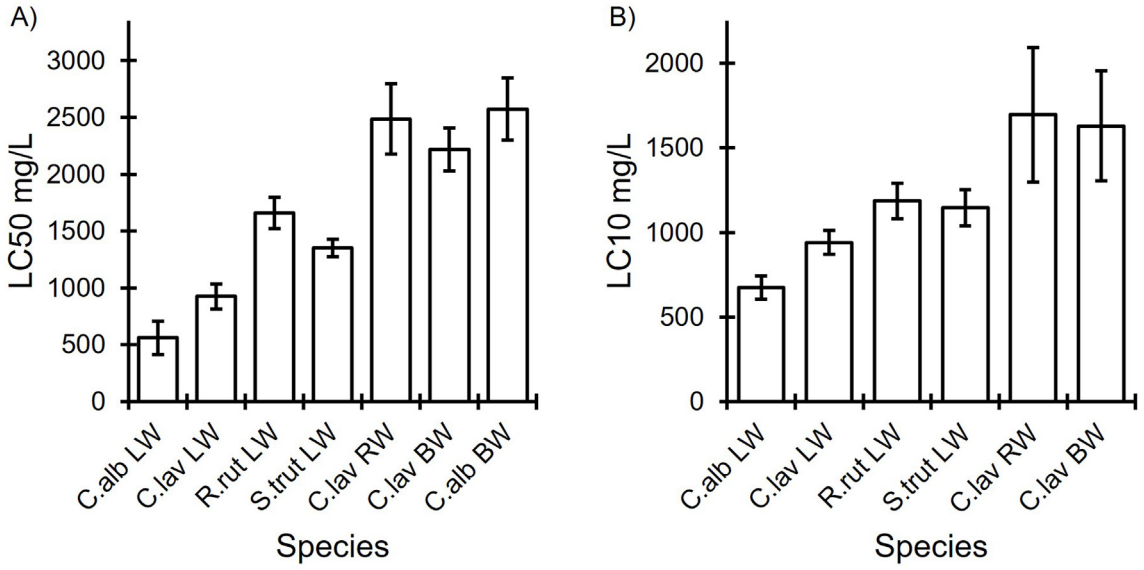

Fig. 4. The LC50 values for fertilization (A) and LC10 values for entire incubation period (B) in different sulfate (SO_4^{2-}) concentrations with results from earlier experiments (Karjalainen *et al.*, 2021; Karjalainen *et al.*, 2023). The species in the chart are vendace (C.alb), whitefish (C.lav), roach (R.rut) and brown trout (S.trut) with different backgrounds (LW = lake water, RW = river water, BW = brackish water). Vertical lines on bars represent the 95% confidence limits.

between the brackish water and freshwater forms. The anadromous sea-migratory whitefish and both brackish water vendace and whitefish exhibited high tolerance (*i.e.*, having high LC values) against sulfate exposure in the acute fertilization tests as well as in the chronic incubation experiments. The tolerance of the freshwater forms was lower, and the LCs were at the level of common roach and brown trout reported in Karjalainen *et al.* (2023) (Supplementary material Tab. 3).

High sulfate concentrations affected the hatching length of freshwater vendace and whitefish. The length of 5-day old larvae was shorter in high sulfate concentrations. The decrease in size and mass in relation to the increase of sulfate concentration might be the result of increased osmotic stress which may hinder growth and development (Cañedo-Argüelles *et al.*, 2019). On the other hand, the length of the 5-day-old larvae increased in high sulfate concentrations in the anadromous whitefish experiment, reflecting again higher sulfate tolerance of sea-migratory whitefish. In all, the differences in hatching size of larvae between the sulfate concentrations were small, which indicated that the embryonic growth of vendace and whitefish after eyed stage was not very sensitive to sulfate. Karjalainen *et al.* (2021) reported that size differences in larvae hatched from a 31-day egg incubation sulfate-exposed experiment eventually evened out during the following sulfate-free depuration process. Further research is required to find out how extended sulfate exposure affects the growth of whitefish larvae.

The fertilization success and survival in embryonic development in our control groups of natural freshwater or brackish water were at the same level as reported in earlier studies (Karjalainen *et al.*, 2015; Karjalainen *et al.*, 2021; Stewart *et al.*, 2021). In present study, whitefish had >94% fertilization success, and 53–92% survival during the embryonic and larval period. For comparison, in Karjalainen *et al.* (2015) whitefish had >85% embryonic survival in freshwater experiment and in Stewart *et al.* (2021) the survival was 51%. In our previous study (Karjalainen *et al.*, 2021), the fertilization rate for whitefish was >88% and the embryonic survival 58–85%. In the present sulfate experiments, vendace fertilization success was >69% in control treatments and survival in embryonic period was 53%. In Karjalainen *et al.* (2015) the embryonic survival of vendace was much lower (35–50%) while in Stewart *et al.* (2021) the embryonic survival of whitefish in Konnevesi control water was >80%. Altogether, the fertilization success and the survival of embryonic development in coregonines have been very variable in the different experimental studies and it is well-known that the reproduction success of whitefish can be affected by male-female pairings and their genetic compatibility (Wedekind *et al.*, 2001, 2008; Wedekind and Müller, 2004). Thus, random selection of females and males in crossing may lead to variable fertilization success and embryonic survival, even in the cases where the eggs and milt of several individuals have been mixed. According to Wedekind *et al.* (2001), mate selection and pair spawning behaviours of whitefish play an important role in selecting compatible genes for future offspring. Furthermore, male-female interactions influence early embryonic survival (Wedekind and Müller, 2004; Wedekind *et al.*, 2008) but in the late embryonic stage, survival seemed to be affected by male traits such as size of the breeding tubercles which correlated with the survival (Wedekind *et al.*, 2008). The reproductive behaviour of vendace has been studied much less. Vendace has pair spawning but no breeding tubercles (Karjalainen and Marjomäki, 2017). It is possible that they have also mate selection, though mechanisms and selection cues are unknown.

Our experiments with brackish and freshwater coregonines from Kokkola region, Lake Konnevesi and River Kokemäenjoki, lasting from fertilization to hatching and larval stage, showed that the success of fertilization and the survival during

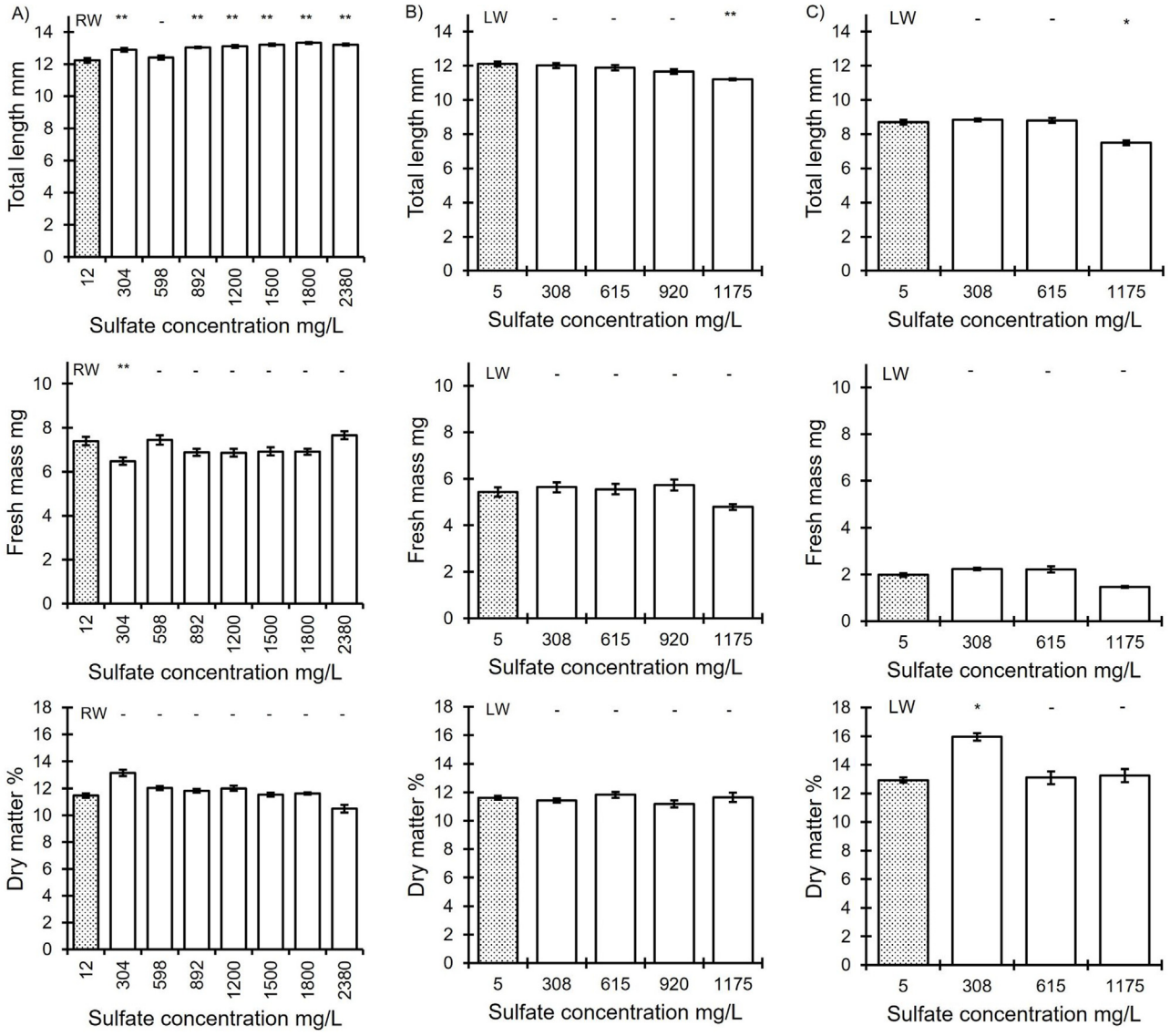

Fig. 5. Total length (mm), fresh mass (mg) and dry matter ratio (%) of the 5-day old anadromous sea-migratory whitefish (A), freshwater whitefish (B) and freshwater vendace (C) larvae in different sulfate (SO_4^{2-}) concentrations and in control treatments (RW = R. Kokemäenjoki water, LW = L. Konnevesi water) with background SO_4^{2-}concentration. The significance levels are above the bars (one-way Anova for length and mass, Kruskall-Wallis for dry matter): − = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$. Vertical lines on bars represent standard error.

the early embryonic phase were the most sensitive periods to sulfate toxicity. The observation that freshwater coregonines in the toxicity tests started to exhibit negative effects in relatively low concentrations of sulfate warrants further research to assess toxicity effects of sulfate on other freshwater species from lakes and rivers. The mortality rate was low during late embryonic and larval period of the experiments, which suggests the utility of shortening the length of chronic experiments for coregonines. Since both diffuse and point loads of sulfate are expected to increase in the future, toxicological testing of fish provides important knowledge when developing water quality guidelines in the boreal regions.

Acknowledgments

We like to thank professional fishers in Pietarsaari and Konnevesi and Kimmo Puosi from Pro Agria/Satakunnan Kalatalouskeskus for providing the fish for the experiments; Emma Pajunen, Nina Honkanen and Mervi Koistinen from University of Jyväskylä for technical assistance in producing the test solutions and KVVY for laboratory analyses.

Conflicts of 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.

Author contribution statement

MM: study conception and design, data collection, data analysis and interpretation, manuscript writing XH: data analysis and interpretation, reviewing JK: study conception and design, supervision, data collection, reviewing.

Animal care

Sampling and handling of parental fish, embryos and larvae have been done according to the current permits and guidelines for experimental animals.

Supplementary material

Summary of parental fish used in fertilization and incubation experiments (Supplementary material Table 1). Statistical tests and results for incubation period in this study (Supplementary material Table 2). Summary of tests carried out in this and our previous studies with different species and their EC/LC values (Supplementary material Table 3). Statistical tests and results for larval measurements in this study (Supplementary material Table 4A). The survival during the entire test period for species in this study (Supplementary material Fig. 1).

Supplementary material Fig. 1: Survival (%) of brackish water whitefish (A) (203 days from the fertilization, n = 36), anadromous sea-migratory whitefish (B) (165 days from the fertilization, n = 36) and freshwater vendace (C) (209 days from the fertilization, n = 36) and whitefish (D) (220 days from the fertilization, n = 36) during the experiment from fertilization to 5-day larvae in different sulfate (SO_4^{2-}) concentrations with control treatments (BW = brackish water, RW = R. Kokemäenjoki water, LW = L. Konnevesi water) and their background sulfate concentrations. The significance levels are above the bars: – = P > 0.05, * = P < 0.05, ** = P < 0.01. Vertical lines on bars represent the 95% confidence limits.

Supplementary material Table 1: The wet mass (g, mean ± SD), total length (cm, mean ± SD) and number of parental fish used in fertilization and incubation tests.

Supplementary material Table 2: Summary of statistical test results for fertilization and incubation periods. Tests were made with Kruskall-Wallis H test (K-W).

Supplementary material Table 3: Summary of the sulfate tests carried out in soft waters (RW = River Kokemäenjoki water, LW = Lake Konnevesi water) and in brackish water (BW: Kokkola brackish water). LC10 and LC50 values for sulfate concentrations (mg/L SO_4^{2-}) (95% confidences limits in parentheses) (* = published previously in Karjalainen *et al.* 2023).

Supplementary material Table 4: Summary of statistical test results for larval measurements between the treatments. Tests were made with one-way Anova (Anova) and Kruskall-Wallis H tests (K-W).

The Supplementary Material is available at https://www.limnology-journal.org/10.1051/limn/2024023/olm.

References

Cañedo-Argüelles M, Kefford B, Schäfer R. 2019. Salt in freshwaters: causes, effects and prospects – introduction to the theme issue. *Phil Trans R Soc B* 374: 20180002.

Davies TD, Hall KJ. 2007. Importance of calcium in modifying the acute toxicity of sodium sulfate to Hyalella azteca and Daphnia magna. *Environ Toxicol Chem* 26: 1243.

Ekholm P, Lehtoranta J, Taka M, Sallantaus T, Riihimäki J. 2020. Diffuse sources dominate the sulfate load into Finnish surface waters. *Sci Tot Env*iron 748: 141297.

Elphick JR, Davies M, Gilron G, Canaria EC, Lo B, Bailey HC. 2011. An aquatic toxicological evaluation of sulfate: the case for considering hardness as a modifying factor in setting water quality guidelines. *Environ Toxicol Chem* 30: 247–253.

Fyhn HJ, Finn RN, Reith M, Norberg B. 1999. Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater. *Sarsia* 84: 451–456.

Griffith MB. 2017. Toxicological perspective on the osmoregulation and ionoregulation physiology of major ions by freshwater animals: teleost fish, crustacea, aquatic insects, and mollusca. *Environ Toxicol Chem* 36: 576–600.

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* 210: 105–144.

HELCOM. 2013. Red List Fish and Lamprey Species. *Helsinki Commission*. www.helcom.fi.

Karjalainen J. 1992. Effects of different preservation methods on total length and weight of larval vendace (Coregonus *albula* (L.)). *Nord J Freshw Res* 67: 88–90.

Karjalainen J, Hu X, Mäkinen M, Karjalainen A, Järvistö J, Järvenpää K, Sepponen M, Leppänen MT. 2023. Sulfate sensitivity of aquatic organism in soft freshwaters explored by toxicity tests and species sensitivity distribution. *Ecotoxicol Environ Saf* 258: 114984.

Karjalainen J, Keskinen T, Pulkkanen M, Marjomäki TJ. 2015. Climate change alters the egg development dynamics in cold-water adapted coregonids. *Environ Biol Fish* 98: 979–991.

Karjalainen J, Marjomäki TJ. 2017. Communal pair spawning behaviour of vendace (*Coregonus albula*) in the dark. *Ecol Freshw Fish* 27: 542–548.

Karjalainen J, Mäkinen M, Karjalainen AK. 2021. Sulfate toxicity to early life stages of European whitefish (*Coregonus lavaretus*) in soft freshwater. *Ecotoxicol Environ Saf* 208: 111763.

Kaushal SS, Likens GE, Pace ML, Reimer JE, Maas CM, Galella JG, Utz RM, Duan S, Kryger JR, Yaculak AM, Boger WL, Bailey NW, Haq S, Wood KL, Wessel BM, Park CE, Collison DC, Aisin BYI, Gedeon TM, Chaudhary SK, Widmer J, Blackwood CR, Bolster CM, Devilbiss ML, Garrison DL, Halevi S, Kese GQ, Quach EK, Rogelio CMP, Tan ML, Wald HJS, Woglo SA. 2021. Freshwater salinization syndrome: from emerging global problem to managing risks. *Biogeochemistry* 154: 255–292.

Koel TM, Peterka JJ. 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. *Can J Fish Aquat Sci* 52: 464–469.

Lehtoranta J, Ekholm P, Pitkänen H. 2008. Eutrophication-driven sediment microbial processes can explain the regional variation in phosphorus concentrations between Baltic Sea sub-basins. *J Mar Sys* 74: 495–504.

Lehtonen H. 1981. Biology and stock assessments of Coregonids at the Baltic coast of Finland. *Finn Fish Res* 3: 31–83.

Lehtonen TK, Gilljam D, Veneranta L, Keskinen T, Bergenius Nord M. 2023. The ecology and fishery of the vendace (*Coregonus albula*) in the Baltic Sea. *J Fish Biol* 1–13.

Luke. 2023. Commercial inland fishery 2022. Natural Resources Institute Finland, Published 20.10.2023. https://www.luke.fi.

Meays C, Nordin R. 2013. Ministry of Environment, Providence of British Columbia Ambient Water Quality Guidelines For Sulphate. Water Protection & Sustainability Branch, Environmental Sustainability and Strategic Policy Division, *BC Ministry of Environment*, Updated Technical Appendix, April 2013.

Nordstrom DK, Blowes DW, Ptacek CJ. 2015. Hydrogeochemistry and microbiology of mine drainage: an update. *Appl Geochem* 57: 3–16.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Available from https://www.R-project.org.

Ritz C, Baty F, Streibig JC, Gerhard D. 2015. Dose-response analysis using R. *PLoS One* 10: e0146021.

SFS-EN ISO 10304–1. 2009. Water quality. *Determination of dissolved anions by liquid chromatography of ions. Part 1: Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulfate (ISO 10304-1:2007)*. SFS-EN ISO 10304–1.

Soucek DJ, Kennedy AJ. 2005. Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Ecotoxicol Environ Saf* 24: 1204–1210.

Soucek DJ, Linton TK, Tarr CD, Dickinson A, Wickramanayake N, Delos CG, Cruz LA. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. *Environ Toxicol Chem* 30: 930–938.

Stewart TR, Mäkinen M, Goulon C, Guillard J, Marjomäki TJ, Lasne E, Karjalainen J, Stockwell JD. 2021. Influence of warming temperatures on coregonine embryogenesis within and among species. *Hydrobiologia* 848: 4363–4385.

Wiltse B, Yerger EC, Laxson CL. 2020. A reduction in spring mixing due to road salt runoff entering Mirror Lake (Lake Placid, NY). *Lake Reser Manag* 36: 109–121.

Zak D, Hupfer M, Cabezas A, Jurasinski G, Audet J, Kleeberg A, McInnes R, Kristiansen SM, Petersen RJ, Liu H, Goldhammer T. 2021. Sulphate in freshwater ecosystems: a review of sources, biogeochemical cycles, ecotoxicological effects and bioremediation. *Earth Sci Rev* 212: 103446.

Wedekind C, Evanno G, Urbach D, Jacob A, Müller R. 2008. 'Good-genes' and 'compatible-genes' effects in an Alpine whitefish and the information content of breeding tubercles over the course of the spawning season. *Genetica* 134: 21–30.

Wedekind C, Müller R. 2004. Parental characteristics versus egg survival: towards an improved genetic management in the supportive breeding of lake whitefish. *Ann Zool Fennici* 41: 105–115.

Wedekind C, Müller R, Spicher H. 2001. Potential genetic benefits of mate selection in whitefish. *J Evol Biol* 14: 980–986.

Cite this article as: Mäkinen M, Hu X, Karjalainen J. 2024. Differences in sulfate sensitivity of early development between brackish and freshwater coregonines. *Int. J. Lim.* 60: 24.