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Uptake and toxicity of waterborne uranium and cadmium to Atlantic salmon (*Salmo salar* L.) depends on life stage and combined exposure☆

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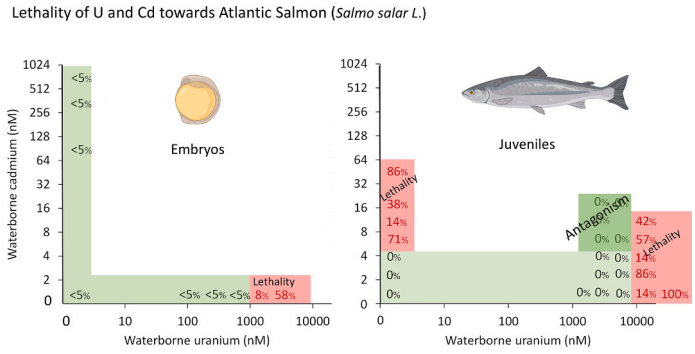
HIGHLIGHTS

- Combined exposures to reduce uncertainties in cumulative hazard assessments of multiple stressors
- Toxicity of radionuclides and trace metals to fish depends on life stages.
- Fish embryo stage is more sensitive to uranium than juvenile stage, while opposite for cadmium.
- Uranium blocking uptake of cadmium in fish results in antagonistic mixture effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Controlled laboratory experiments were performed to investigate the toxicity of depleted uranium (U) and the frequent co-contaminant cadmium (Cd), individually and in combination, on two life stages (embryos and juveniles) of Atlantic salmon (*Salmo salar* L.).

U and Cd accumulation in both eggs and juveniles were positively correlated with waterborne concentrations and toxic effects. The embryo stage was more sensitive to U (LC_{50}: 3.17 μM U), while the juveniles were more sensitive to Cd (LC_{50}: 19.6 nM Cd). Continuous uptake of U in eggs during the 90-day exposure period was observed to our knowledge for the first time, and could explain why U was more toxic to fish embryos than Cd, taken up only during the early days. The acute toxic mechanisms of U and Cd were different as the U gill and liver concentration correlated with increased blood glucose and reduced plasma Cl concentrations, while no such responses were observed during Cd exposure, although negative invers correlation of Cd uptake in liver to survival rate (LC_{50}: 4.8 nmol g^{-1} liver) was also observed for U (LC_{50}: 8.66 nmol g^{-1} liver).

Combined exposure to juveniles showed an antagonistic interaction between the two elements, where U reduced systematic uptake of Cd in liver and thereby increased the survival of lethal Cd concentrations. Using

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independent action as reference model, antagonistic effects on both the toxicokinetic and the toxicodynamic level were confirmed.

Results demonstrated the importance of integrating life stage dependent toxicokinetic and toxicodynamic effects into risk assessment associated with multiple stressor scenarios.

1. Introduction

Following releases from natural geological sources (e.g., alum and black shales), the nuclear weapon and fuel cycles as well as from anthropogenic activities associated with non-nuclear industries, organisms in affected ecosystems will be exposed to a series of chemical stressors, especially a variety of radionuclides such as uranium (U) and trace metals such as cadmium (Cd) (UNSCEAR Report, 2000).

Elevated concentrations of U in surface fresh waters can reach up to 14 mg L^{-1}, mostly due to localized sources such as NORM bedrocks and U mining activities (Salbu et al., 2013; Smedley and Kinniburgh, 2023), as radionuclides as well as a series of metals can be mobilized. Due to weathering processes, mixtures of radionuclides such as U and metals such as Cd can be transferred to downstream environments potentially causing multiple stressor situations for biota (Salbu et al., 2013). Thus, U often coexist in the environment with a number of co-contaminants, such as Cd, albeit the latter being present at much lower concentration levels in for instance mine influenced surface waters (Nichols and Scholz, 1989). Thus, organisms in affected areas will not only be exposed to one stressor at a time but could be exposed simultaneously to a cocktail of stressors. Then, the toxicity of multiple stressors should be expressed as the cumulative effects of U in combination with other stressors such as Cd.

The speciation (i.e., physico-chemical form) of trace elements affects its mobility and bioavailability. In freshwater, both U and Cd can be present in different physico-chemical forms e.g., varying with respect to size (molecular masses) and charge properties (Salbu, 2006; Smedley and Kinniburgh, 2023). While U and Cd present as low molecular mass (LMM) species are believed to be mobile and bioavailable, U and/or Cd colloids are assumed to be mobile, and particles containing U and/or Cd will to a large degree be retained in soil and sediments (Salbu, 2006). Thus, the bioavailability and uptake of U and Cd by organisms depends strongly on speciation. In addition, different environmental factors and processes can also influence the uptake in aquatic organisms (Cheng et al., 2010; Wood et al., 2012).

In fresh waters, LMM U species such as the UO_2^{2+} and $UO_2(OH)^+$ ions are assumed to be the predominant bioavailable form of uranium (Cheng et al., 2010; Lofts et al., 2015). The distribution of U species in water will, however, change depending on water characteristics such as pH and concentration of complexing agents such as humic and fulvic material (Nehete et al., 2017; Smedley and Kinniburgh, 2023). Similarly, the speciation and bioavailability of Cd are also reported to change with water characteristics (Wood et al., 2012; Liu et al., 2022). Particularly the waterborne concentration of calcium (Ca) and humic substances is assumed to affect the toxicity of Cd towards fish (Wood et al., 2012; Richards et al., 1999).

Due to the long half-lives of the naturally occurring uranium isotopes (^{238}U (99.27 %), ^{235}U (0.72 %) and ^{234}U (0.0057 %)), the radiotoxicity of both naturally occurring U and depleted U (which contains smaller proportion of ^{234}U and ^{235}U) is low, while the metal toxicity of U is of greater significance (Craft et al., 2004). The toxicity of uranium has been reported for several aquatic organisms including fish (Cheng et al., 2010; Lourenço et al., 2017; Li et al., 2024b). For comparison, Cd is generally assumed to be more toxic than U (Wood et al., 2012; Shankar et al., 2021). Available literature indicates that waterborne uranium can sorb to the external surface of fish egg chorion and then be transferred to the developing embryo (Bourrachot et al., 2008). Uranium can also sorb to fish gills and then be subject to transport across the gill membrane to blood (Barillet et al., 2010), absorption through the diet and transfer to internal tissues (Li et al., 2024b; Croteau et al., 2005). Uranium is accumulated in the kidney, liver and bone of fish (Kraemer and Evans, 2012; Salbu et al., 2013). Similarly, waterborne Cd is also known to be taken up by fish eggs and gills of fish, and can accumulate in kidney and liver of fish (Wood et al., 2012; Lee et al., 2024). Cd taken up through the gills enters the cells through divalent metal transporters and the epithelial Ca channel of the gills (Niyogi and Wood, 2004). Similar uptake mechanisms could be relevant for U, as significant deposition of U in bones of fish (Kraemer and Evans, 2012) support that U can replace Ca in biological systems. Thus, competition between U and Cd with respect to gill deposition, transfer to blood as well as further translocation may occur. The balance between uptake and excretion of U and Cd as well as the kinetics of these processes determine the biodistribution and the total bioaccumulation within fish.

Several studies have investigated the toxic effects of U in fish and other aquatic organisms. It is well known that U induces free radicals, producing reactive oxygen species (ROS), and induce oxidative stress in organisms. Uranium has been shown to cause several toxic effects such as DNA damage, altered mitochondria metabolism and ion transport, neurotoxicity, reproductive toxicity and suppression of the immune system (Bourrachot et al., 2008; Song et al., 2014; Shankar et al., 2021). Several of these effects are related to chronic exposure, while interferences with membrane associated proteins and failure of the functions of enzymes/proteins can also result in physiological changes and acute ion regulatory or respiratory failure (Annamalai and Arunachalam, 2017).

Cd exposure of fish cause a plethora of toxic effects. Cd is well known neurotoxic agent, e.g., increasing the neurotransmitter release of acetylcholine and disrupting neurotransmitter signaling proteins (Wood et al., 2012; Liu et al., 2022). Furthermore, it may generate production of ROS and oxidative stress, cause suppression of the immune system and induce DNA damage (Lee et al., 2024). Cd may bind to functional groups of enzymes or be enclosed in protein structures, replacing Ca. Acute Cd exposure to fish is also associated with physiological changes and tissue damage, as well as interferences with the ability of fish to regulate the balance of water and ions in their body, particularly affecting the Ca levels, leading to hypocalcemia (da Silva and Martinez, 2014).

For assessment purposes potential impact from U has typically been based on total concentrations in water, representing a conservative approach, assuming that all U is present as bioavailable forms. Furthermore, the cumulative effects of U in combination with other stressors are generally assumed to be additive. Cumulative effects are commonly predicted using concentration addition (CA) and independent action (IA) models, widely accepted for metal mixture toxicity assessments (Jonker et al., 2005). Both models assume that the toxicity of the mixture components is additive but have different approaches for determining contributions from the individual contaminants. While IA calculates the toxicity of a mixture as the product of the individual contaminant's toxicity, assuming that they have dissimilar modes of action (Bliss, 1939), CA assumes that contaminants within a mixture have a similar mode of action (Loewe, 1927). In both cases, when observed cumulative effects exceed that expected for additivity, then synergism is assumed. Conversely if cumulative effects are less than expected from additivity, then antagonism is assumed.

The accumulation and toxic effects of U depend also on the exposed species of fish (Nichols and Scholz, 1989; Kraemer and Evans, 2012; Salbu et al., 2013) and may also differ between different life stages of fish. The fish embryo has often been reported to be more sensitive to

metals than the juvenile stages. According to Bourrachot et al. (2008) and Wood et al. (2012) fish embryos seem to be more sensitive to U and Cd exposure than more developed. Identification of interacting effects and their underlaying mechanism is therefore essential for better characterization of the risk at sites polluted with both radionuclides and metals, such as U and Cd. As water composition impacts the speciation, bioavailability and thereby the bioaccumulation of U and Cd, experiments with similar water composition under identical environmental conditions are required when comparing the sensitivity between life stages and between toxic compounds present in exposure mixtures.

It has been hypothesized that the toxicity of U in Atlantic salmon is not influenced by life stage of fish, and that the cumulative effects of U and Cd in exposed Atlantic salmon are additive. The objectives of the current study were therefore to provide toxicokinetic and toxicodynamic information on single and combined U and Cd exposure of fish to improve cumulative risk assessments by 1) identifying toxicity of U at different life stages of fish using identical water quality with similar U speciation and 2) comparing toxicity of U with toxicity of Cd, and 3) identifying interactions between U and Cd as well as combined cumulative effects.

2. Methods

Transfer and toxicity of waterborne uranium (U) and cadmium (Cd) were assessed separately to Atlantic salmon embryos and juveniles according to OECD guideline 210 (OECD, 2013) for fish early-life stage toxicity test, and OECD guideline 203 (OECD, 1992) for fish acute toxicity test, respectively. In addition, binary mixtures to juveniles were also studied using US EPA very soft water, at pH 6.7 ± 0.4 for 96 h. Atlantic salmon was selected as a representative of fish species sensitive to metal exposure (Poléo et al., 1997) with high commercial value. The exposures were conducted in accordance with the Norwegian Welfare Act and research animal legislation. The experiments were approved in advance by the Norwegian Animal Research Authority (NARA ID: 4615 and 8474).

2.1. Preparation of the exposure water

Synthetic US EPA very soft water (U.S. Environmental Protection Agency, 2002) was used as the reference water for both juveniles and embryos. The reference water was selected to represent typical Norwegian river and lake waters low in ionic strength. The reverse osmosis water was produced in 1000 L tanks and mixed with salts and aerated using circulation and aquaria pumps to equilibrate the dissolved CO_2 with the atmosphere at least two days prior to use.

The U and Cd concentrations in the exposure water were selected based on literature and results from pilot tests with juveniles, the concentration range for eggs and juveniles overlapped, but was somewhat different to include both predicted no effects and >50 % lethal concentrations. To obtain the different water based U concentration, U was added from stock solution (223 mM) prepared by dissolving $UO_2(NO_3)$ $2H_2O$ in Type 2 water 24 h prior to use. Cd was added from stock solutions (31.1 μM Cd) prepared by dissolving $CdCl_2$ in Type 2 water. U and Cd was added to the very soft EPA water 3 days (72 h) prior to exposure of eggs or juveniles of Atlantic salmon, and during this period the solutions (16 L for eggs; 180 L for juveniles) were aerated using aquaria pumps to mix and equilibrate the exposure solution before the start of exposure. During the egg experiment water was replaced when U or Cd concentrations deviated more than 10 % compared to nominal concentrations, while exposure solutions were not replaced during the 96 h exposure period for juveniles.

2.2. Characterization of the exposure water

Water quality parameters such as pH, dissolved oxygen, conductivity, and temperature were continuously logged during the exposures. In addition, water samples were collected regularly in 50 mL Sarstedt tubes during the experiments (6 times during the egg experiments and 2 times during the juvenile experiment). Major cations (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}), U and Cd were determined in 2 % HNO_3 acidified water samples using ICP-MS (8800 ICP-MS Triple Quad, Agilent Technologies). Major anions were determined in non-acidified samples (separate tubes) using Iachat IC5000 ion chromatography. Organic carbon was determined using a total organic analyzer (Shimadzu TOC cpn, Kyoto, Japan). Water samples were filtered (0.45 μm syringe filter Millexwater®-AA, MF Millipore membrane, Millipore Ireland) prior to analysis.

To obtain information about U and Cd species in exposure waters, size fractionation was performed. Membrane filtration (0.45 μm) and utrafiltration (Pall hollow fiber, cutoff of 10 kDa) were used to exclude particles and colloids, respectively. The U and Cd concentrations of the following fractions were obtained:

- total: concentration of U and Cd in unfiltered water;
- particulate: derived by difference, concentration of U and Cd in unfiltered water subtracting concentration of U and Cd in 0.45 μm filtered water;
- colloids: derived by difference, concentration of U and Cd in 0.45 μm filtered water subtracting concentration of U and Cd in ultrafiltered water;
- LMM: concentration of U and Cd in ultrafiltered water (cutoff 10 kDa).

Visual MINTEQ (3.1) was used to predict the % distribution of Cd or U species in the exposure water taken into account measured composition of the exposure water.

2.3. Exposure of Atlantic salmon embryos

Dry stripped eggs from three different Atlantic salmon females (biological replicates) were dry fertilized by using milt from one male, before transfer to exposure units. Each exposure units containing 500 eggs from one female, placed on plastic grids in a flow-through box (155 mm × 106 mm × 45 mm, 739 cm^3). Three exposure units, one for each female, receiving water from a common 16 L reservoir of water, one reservoir for each treatment. The water from the reservoir was pumped to each exposure unit by a small aquarium pump and the overflow of the exposure unit was returned to the reservoir in a recycling mode. The exposure was conducted under dark conditions following the OECD guidelines 210 (OECD 201) in a temperature and light controlled room. One-cell stage fertilized embryos (n = 500 per replicate) were exposed for 88 days at 6.3 °C (550 day degrees) to 5 different concentrations of U and 3 different concentrations of Cd, and in addition to only very soft EPA water as control. The design allowed the water in the reservoir to be replaced without disturbing the eggs, as the water remained in the exposure unit when stopping the pump and changing the water in the reservoir. The replaced water in the reservoir was recirculated through the exposure unit when the pump started until the next water change.

2.3.1. Determination of U and Cd uptake in fish eggs

To obtain information about transfer of waterborne U and Cd during exposure, eggs were collected from each treatment at 4 different timepoints in addition to hatched alevins. Collected eggs were either sampled whole or inside content were collected by a syringe. The content inside egg was sucked out using a needle connected to the syringe. The whole egg or the inside content without chorion and hatched alevins were weighed and acid digested (HNO_3 and ultraclave) before dilution to 10 % HNO_3 and determination of U and Cd using ICP-MS. In addition, a Zeiss XRadia Micro XCT-400 (Carl Zeiss, Oberkochen, Germany; 40 kV, 200 μA) was used to conduct x-ray absorption tomography analysis of an U exposed egg (3.6 μM U) as well as a control egg before hatching to characterize the distribution of high-density material as a proxy for U biodistribution. Immediately after sampling, eggs were fixed in 2 %

paraformaldehyde (PFA), rinsed twice in 1 X Phosphate-Buffered Saline (PBS) with 0.1 % Tween-20, and step wise dehydrated in 20 %, 30 %, 50 % and 70 % ethanol. While kept in Eppendorf tubes and immersed in 70 % ethanol, the samples were rotated 360°along their central axis, and 2D tomographic projections based on X-ray attenuation were collected. Projections were rendered volumetrically (voxel-size 12 μm) then exported for 3D visualization using CT Analyzer software such as Sky-Scan (Forsman et al., 2025). To maximize the intensity projection of the internal parts of the fish eggs, the chorion was removed from the 3D reconstruction model.

2.3.2. Determination of effects on exposed fish embryos

Lethality and morbidity were recorded daily during the entire experimental period. Unfertilized embryos were removed from the exposure units and not included for further analysis. Survival rate was calculated for each day as total number of fertilized eggs subtracted dead embryos (white or opaque) divided by the number of fertilized eggs in the exposure unit at that day, whereas cumulative survival was calculated based on the last day of exposure as total number of survived embryos divided by the number of fertilized eggs at the start of exposure subtracted sampled eggs.

For all exposure units, time of hatching was recorded by daily counting the total number of alevins. Fraction of hatched eggs was calculated as a percentage of the initial number of fertilized embryos per experimental unit, while cumulative hatching was calculated based on the last day of exposure.

2.4. Exposure of Atlantic salmon juveniles

Juvenile (parr) of Atlantic salmon with length 10.7 ± 0.9 cm, weight 10.4 ± 3.1 g from the Fish Laboratory of Norwegian University of Life Sciences (NUMB, Ås, Norway) was acclimated for 7 days (1000 L tank) to the very soft EPA water (9 ± 0.5 °C) before exposure. The fish were fed daily (1 % of total fish weight) during the first four days of acclimation period. After acclimation 7 fish were transferred to each exposure tanks for steady state 96 h exposure. Each tank was black lined with transparent plastic bags and covered with a loose lid on top. Each tank represented one exposure unit and contained about 180 L water as required according to the biomass of fish following OECD guidelines 203 (L/g fish). The exposure water was continually aeriated using aquarium air pump. Seven fish were exposed to water with different concentrations of U and Cd, separately or in binary mixtures. Results from the exposure of U and Cd separately were used to select the exposure concentration in the binary mixtures. Partial factorial design was used with seven nominal U concentrations (0, 4.2, 8.4, 9.9, 11.3, 12.6 and 14.7 μM) and ten nominal Cd concentrations (0, 0.9, 1.8, 3.6, 5.3, 7.1, 8.9, 12.5, 17.8 and 35.5 nM) to ensure responses from 0 to 100 % effects. Tanks were placed in temperature and light controlled room.

2.4.1. Determination of U and Cd uptake in juveniles (parr)

After 96 h of exposure fish from all exposure units were dissected to collect different tissues and organs according to the EMERGE protocol (Rosseland et al., 2001). The second gill arch from the right side and the liver of each fish were collected after killing the fish by a blow to the head. The gill and liver samples were freeze-dried, weighed, digested (ultrapure HNO_3 and ultraclave), and analyzed with respect to Cd and U using ICP-MS. Gills and liver were also sampled before the exposure was initiated from acclimated fish.

2.4.2. Determination of effects on exposure juveniles

The effect endpoint was mortality or survival, but to understand underlying mechanisms blood samples were collected from the caudal vessels using heparinized syringes and analyzed on site using an I-stat blood/gas analyzer (Abbott, Chicago, USA) with EC8+ cartridge. Lethality was recorded daily during the entire experiment. Survival rate was calculated for each exposure as total number of fish subtracted dead fish divided by the total number of fish in the exposure unit. Blood samples were collected at the end of the experiment.

2.4.3. Determination of binary interaction effects on exposed juveniles

In order to describe the joint effects, additive effects were predicted using IA and CA according to Junker et al., 2005 using observed effects from single exposure of U and Cd. Antagonism was defined as observed effects below the predicted additive effects, while synergism was defined as observed effects higher than predicted additive effects.

2.5. ICP-MS analysis

The concentration of U and Cd in water and digested fish tissues were determined using ICP-MS (Agilent 8800). Measurements of certified reference materials (CRM) indicated good accuracy (IAEA-414: 85.9 ± 0.9 μg U kg^{-1} compared to CRM 95 % confidence interval value 86 to 101 μg U kg^{-1} and DOLT-4: 23.2 ± 2.8 mg Cd kg^{-1} compared to CRM value 24.2 ± 0.8 μg Cd kg^{-1}).

2.6. Statistical analysis

Statistical analyses were performed using SigmaPlot version 14.0 (Systat Software, San Jose, Ca) and JMP Pro v17 (SAS institute, Cary, NC, USA). Generalized Linear Model (GLM) and Analysis of Variance (ANOVA) were adopted for comparison between different exposure groups. Significance was considered when p-value < 0.05. Time series analysis of mortality and hatching was performed by using Probit 4P model as four parameter logistic regression model, and inverse prediction adopted for identification of inflection points such as Lethal Concentration (LC) and Effective Concentration (EC) estimates of U or Cd for 10 and 50 % of salmon populations (LC_{10}, LC_{50} and EC_{10} and EC_{50}). Comparisons between treatments were analyzed using one-way ANOVA (Holm-Sidak method for multiple comparisons). Linear regressions analyses were performed to determine the relationship between waterborne U or Cd and concentration in eggs or tissues of juveniles when suitable and expresses as R^2 values. Data are given as arithmetic means with their respective standard deviation (S.D.) unless otherwise stated. For ethical reasons the number of fish used in the experiments was kept to a minimum, which means that the juvenile data analysis was based on pseudo-replicates.

3. Results and discussion

3.1. Characteristics of exposure water

The water qualities were saturated with oxygen (100 %) and generally characterized with pH between 6.7 and 7.0, conductivity of 51.5 ± 4.9 μS cm^{-1}, low concentration of organic carbon for the eggs (0.04–0.12 mM) and juveniles (<0.05 mM), as well as concentrations of major cations similar to nominal concentration for “Very soft” EPA water (Table S1). The concentration of Cl was, however, higher due to the use of HCl (1 M) for pH adjustments, when required. The water composition was similar in all U and Cd experiments where embryos and juveniles were exposed. However, the temperature was on average lower in the experiments with embryos (6.5 °C) than with juveniles (9 °C).

3.1.1. U and cd fractions in exposure water with fish egg

The concentration of waterborne U in the exposure units with eggs ranged between 150 nM to 3.6 μM, with maximum 25–35 % variation in concentration in each exposure unit during the experimental period of 92 days. Uranium was mainly present as LMM species (43–96 %), while a minor fraction was present as particulate and colloidal U (Table 1). The proportion of particulate and colloidal U was highest at the lowest waterborne U concentration. Using Visual MINETEQ, less than 3.5 % was predicted to be preset as UO_2^{2+} and UO_2OH^+ in the exposure water

Table 1
Waterborne concentration of U and Cd fractions in exposure solutions.

Groups	U						Cd					
	Total	Particulate	Colloidal	LMM	LMM	UO_2^{2+}, UO_2OH^+	Total	Particulate	Colloidal	LMM	LMM	Cd^{2+}
	(nM)	(nM)	(nM)	(nM)	%	%[1]	(nM)	(nM)	(nM)	(nM)	%	%[1]
Eggs												
Control	<0.3	<0.3	<0.3	<0.3			<0.3					
150 nM U	150 ± 70	17 ± 25	69 ± 98	65 ± 21	43	3.5	<0.3					
450 nM U	421 ± 145	24 ± 34	45 ± 63	350 ± 139	84	2.2	<0.3					
900 nM U	871 ± 280	40 ± 57	49 ± 70	779 ± 258	90	1.6	<0.3					
1800 nM U	1814 ± 482	30 ± 42	51 ± 71	1735 ± 189	96	1.1	<0.3					
3600 nM U	3347 ± 976	87 ± 123	259 ± 366	3005 ± 1164	90	0.8	<0.3					
80 nM Cd	<0.3						82 ± 16	4 ± 4	10 ± 3	71 ± 2	86	93
300 nM Cd	<0.3						298 ± 187	13 ± 2	29 ± 1	202 ± 4	68	93
800 nM Cd	<0.3						780 ± 203	57 ± 14	70 ± 88	653 ± 197	83	93
Juveniles												
Control	<0.3						<0.3					
8 μM U	8703	433	90	8181	94	0.3	<0.3					
9.2 μM U	10,362	348	<0.3	10,014	97	0.3	<0.3					
10.5 μM U	11,363	260	<0.3	11,103	98	0.3	<0.3					
12 μM U	13,101	723	71	12,307	94	0.3	<0.3					
15 μM U	14,920	876	538	13,506	91	0.3	<0.3					
3.0 nM Cd	<0.3						3 ± 1	<1	2	2	45	96
10 nM Cd	<0.3						10.0	<1	6	3	35	95
36 nM Cd	<0.3						36.0	7	21	8	22	96

[1] % of Cd^{2+} and UO_2^{2+}, UO_2OH^+ derived by the Visual MINTEQ.

(Table 1), while the main fraction was present as less toxic U-carbonate species. The concentration of waterborne Cd in exposure units ranged from 82 to 780 nM, with 21–45 % variation in concentration within each exposure unit during the experimental period. Independent of the waterborne concentration, Cd was mainly present as LMM species (68–86 %), while a minor fraction was present as particulate and colloidal Cd (Table 1). Using Visual MINETEQ, about 90 % of Cd was predicted to be preset as Cd^{2+} in the exposure water (Table 1), while 6 % was probably associated with organic material. The concentration of both U and Cd was observed to decrease by time in the exposure units with embryos, and the water in the reservoirs was therefore renewed every 2 weeks to keep the concentrations of U and Cd relative stable during the experimental period. The decrease in concentration of waterborne U and Cd over time was attributed to sorption of U and Cd to eggs and to surfaces of the exposure unit. Egg-shell membranes include several functional groups that provide the potential for sorption of metal species (Jezierska et al., 2009).

3.1.2. U and Cd fractions in juvenile exposure water

The concentration of waterborne U in exposure units with juveniles varied between 2.0 μM and 16.5 μM in separate tanks and was in line with the nominal concentration with less than 10 % decrease during the

Table 2
Uranium and Cd concentration per dry weight in Atlantic salmon eggs at 520 day degree of exposure and in alevin after hatching. Concentration ratios (CR) calculated by total concentration in whole egg divided by total concentration in water.

Groups	U				Cd			
	Whole egg	Internal content	CR whole egg	Alevin	Whole egg	Internal content	CR whole egg	Alevin
	(nmol g^{-1})	%		(nmol g^{-1})	(nmol g^{-1})	%		(nmol g^{-1})
Control	<0.01	–	–	0.02 ± 0.01	<0.01			<0.01
150 nM U	27 ± 1	5–13	0.75	0.31 ± 0.02				
450 nM U	43 ± 1	7–9	0.43	0.57 ± 0.13				
900 nM U	73 ± 10	5–7	0.35	1.02 ± 0.21				
1800 nM U	154 ± 10	9	0.36	1.00 ± 0.24				
3600 nM U	688 ± 61	9	0.86	–				
80 nM Cd	<0.3				10 ± 1	22–29	0.126	0.91 ± 0.24
300 nM Cd	<0.3				20 ± 1	56–60	0.067	0.52 ± 0.16
800 nM Cd	<0.3				54 ± 3	21–58	0.068	1.58 ± 0.50

96 h of exposure. Uranium in the juvenile experiments was mainly present as LMM species (91–98 %) (Table 2). The concentration of waterborne Cd in exposure units varied between 0.6 nM and 36 nM in line with nominal concentrations. Cd was mainly present as colloidal (49–64 %) and LMM species (22–45 %), while a minor fraction was present as particles (Table 2).

Eggs were exposed to even lower concentrations and juveniles to even higher concentrations of U, while the opposite was the case for Cd. The concentration of U was generally higher than the Cd concentration, but overlapping for the eggs. The fraction of colloidal U species was lower than for Cd, but highest for the lowest concentration tested (150 nM). It is well known that both Cd and U interact with humic and fulvic substances in organic-rich surface water (Richards et al., 1999; Smedley and Kinniburgh, 2023; Nehete et al., 2017). Although the concentration of dissolved organic carbon in the synthetic water used was less than 0.12 mM about 4 % of Cd was predicted to be associated with organic carbon (Visual MINETEQ) and probably sufficient to affect the bioavailability of Cd, but not significantly for U.

3.2. Uptake of U and Cd in eggs

The U concentration associated with eggs increased with increasing concentration of waterborne U and with duration of the exposure (Fig. 1). Thus, the concentration of U associated with eggs increased between each time of sampling and was highest at the end of exposure (after 520 day degree). Waterborne U ranging within 150 nM–1800 μM was positively correlated with the U concentration in whole eggs (R^2 = 0.99). The overall U concentration ratio (CR) at the end of the exposure (520 day degree) was 0.34 (Table 2), while higher when eggs were exposed to even higher U concentrations (i.e., 3.6 μM).

Uranium in the egg interior constituted 5–13 % of the egg total U concentration. The internal U fraction was positively correlated (R^2 = 0.99) with the whole egg U concentrations (Table 3), showing that U was predominantly associated with the chorion and the eggshell surface as corroborated via micro-CT (Fig. 2A–B). These findings supports that sorption of metal species onto egg-shell membranes may be efficient (Jezierska et al., 2009). A significant proportion of U was however also translocated across the chorion (Table 2). Micro-CT imaging indeed indicated the presence of U in direct contact with the embryo, while only a minor fraction appeared to be associated with perivitelline fluid (Fig. 2C–D). The concentration of U in the interior of eggs increased with time. To our knowledge, this is the first time U transfer into fish eggs has been shown to be increased continually during the whole period from fertilization to hatching, being associated with the embryo. Our results are in line with Bourrachot et al. (2008), who also observed that U mainly accumulated on the surface of the chorion of fish eggs (zebrafish), but was also able to penetrate the chorion, albeit at a substantially lower fraction (0.002 %) than observed in this study. Uranium was also associated with the alevin after hatching (Table 2), underpinning the uptake and interaction of U with the embryo inside the egg.

The Cd concentration associated with eggs increased with increasing concentration of waterborne Cd, but only during the first part of the exposure period (Fig. 1). The increase was rapid during the first days of exposure and occurred at a lower rate during the following days, from 6 to 60 day degree. Then, the Cd concentration associated with egg did not significantly increase thereafter, neither at 200 day degree nor at 520 day degree. There was a positive correlation between Cd concentrations in water and Cd concentrations in whole egg (R^2 = 0.98) with a concentration ratio of 0.07 observed at the end of the exposure (520 day degree). The rapid increase in Cd uptake shortly after the exposure start is supported by earlier finding showing a dose dependent uptake until saturation. Rombough and Garside (1982) observed that saturation was reached after 24 h which was subsequently maintained until hatching while in the current study the saturation was reached later (10 days).

The concentration of Cd in the interior of eggs was strongly correlated with the total Cd associated with the egg (R^2 = 0.98). About 60 % of the total Cd concentrations associated with the egg were observed internally. The fact that Cd was associated with the alevin after hatching (Table 2), underpins the observed uptake and interaction of Cd with the embryo. High retention of Cd to the chorion of fish eggs (both *Salmo salar* and *Oriz latipes*) has also previously been reported (Michibata, 1981). However, these authors identified only a fraction (5–6.6 %) of cadmium in the embryo or yolk sac, indicating a significantly lower transfer of Cd than observed in this study. Differences in Cd uptake could be explained by differences in exposure situations; in earlier reported studies the exposure was initiated at the blastula stage, while in the present experiment exposure was initiated immediately after fertilization. Upon fertilization, the major chorion proteins are expected to polymerize to insoluble high molecular mass proteins, thereby increasing the chorion toughness (Iwamatsu et al., 1995). The chorion is therefore less protected against metal penetration during the swelling stage compared to the post swelling stage (Jezierska et al., 2009). This supported also the fact that the uptake of waterborne Cd in eggs was rapid initially and more limited after the first days of exposure. This is also supported by González-Doncel et al. (2003).

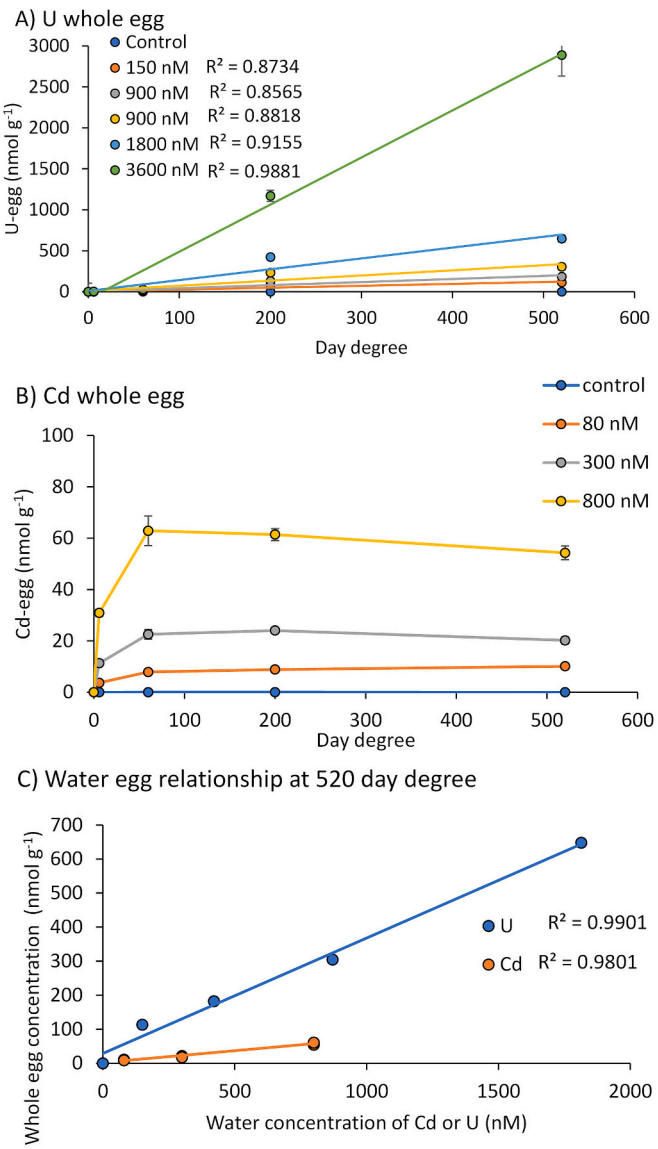

Fig. 1. Relationships between concentration of A) U and B) Cd in whole egg and time of exposure present as day degree at different waterborne concentration of U and Cd. C) relationship between water concentration and whole egg concentration of U and Cd after 520 day degree. N = 3.

Table 3
Uranium and Cd concentration in gills and liver per dry weight after 96 h of exposure of Atlantic salmon juveniles. Concentration ratios (CR) in gills calculated by total concentration in gills divided by total concentration in water.

Groups	U			Cd		
	Gill	Liver	CR gill	Gill	Liver	CR gill
	(nmol g^{-1})	(nmol g^{-1})		(nmol g^{-1})	(nmol g^{-1})	
Control	0 ± 0	0 ± 0	–	<0.005	<0.0007	
2 μM U	47 ± 12	0.3 ± 0.1	0.02 ± 0			
4 μM U	97 ± 33	0.5 ± 0.2	0.02 ± 0.01			
8 μM U	303 ± 102	1.7 ± 1.1	0.04 ± 0.01			
9.2 μM U	604 ± 113	2.9 ± 0.4	0.07 ± 0.01			
10.5 μM U	615 ± 189	na	0.06 ± 0			
12 μM U	836 ± 900	6.4 ± 1.7	0.07 ± 0.14			
15 μM U	na					
17 μM U	na					
0.6 nM Cd				0.009 ± 0.002	<0.0007	0.016 ± 0
1.2 nM Cd				0.014 ± 0.006	<0.0007	0.013 ± 0
3.0 nM Cd				0.023 ± 0.004	0.001 ± 0.001	0.008 ± 0.004
8.0 nM Cd				0.022 ± 0.015	0.003 ± 0.002	0.003 ± 0.002
10 nM Cd				0.031 ± 0.002	0.003 ± 0.002	0.003 ± 0.002
17 nM Cd				0.016 ± 0.007	0.005 ± 0.001	0.001 ± 0
36 nM Cd				0.022 ± 0	0.009 ± 0	0.001 ± 0

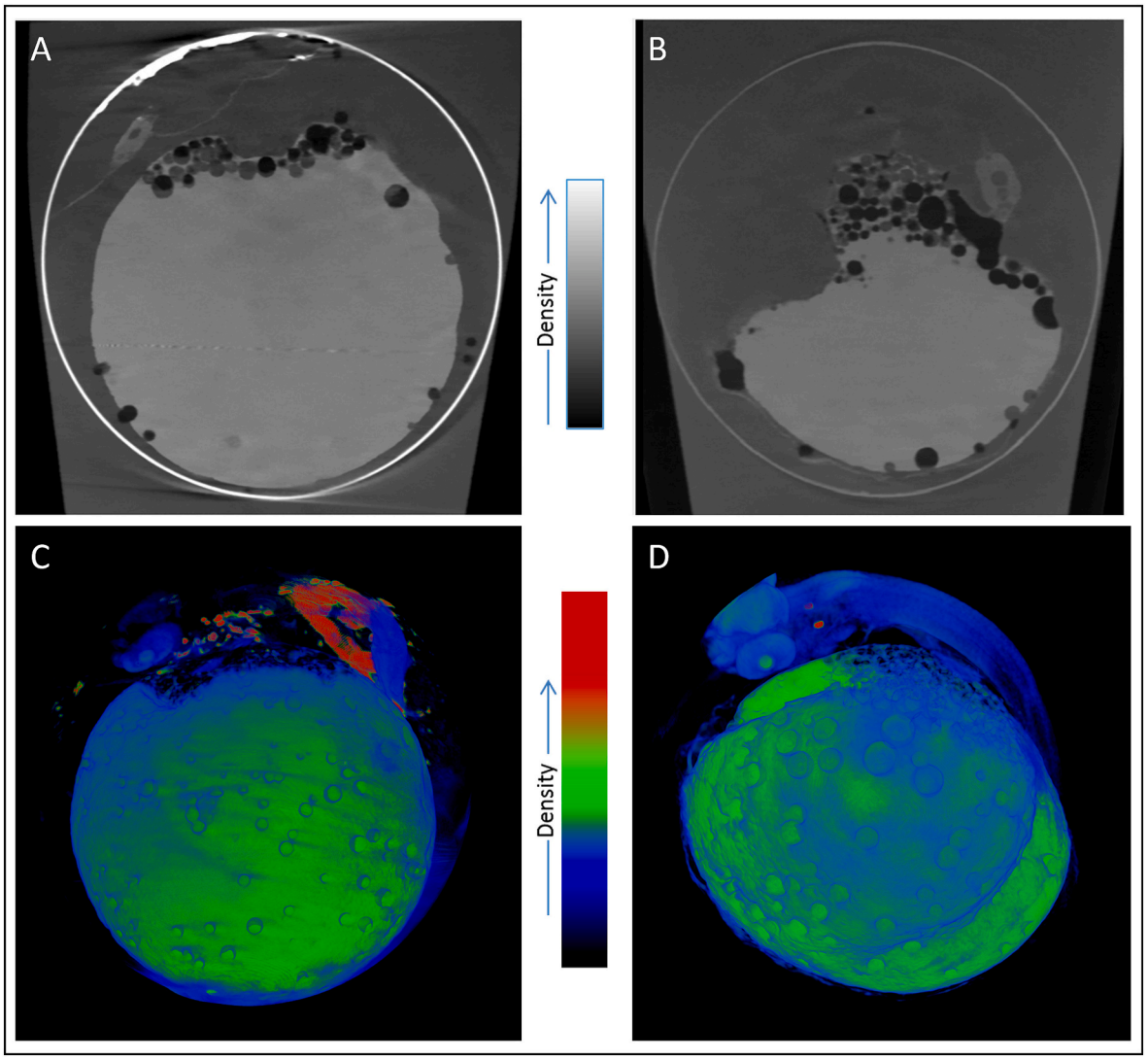

Fig. 2. Micro CT scan of eggs before hatching A) 2D slice of U exposed egg (3.6 μM) with chorion B) 2D slice of control egg with chorion, C) 3D reconstruction of U exposed egg (3.6 μM) without chorion D) 3D reconstruction of control egg without chorion. The distributions of dense material indicate U biodistribution in the U exposed egg, while in the control only the auditory vesicles feature signals corresponding to relatively high density material.

3.2.1. Comparison of U and Cd uptake in fish eggs

The sorption and uptake of U occurred during the entire period of exposure and was a factor of 4.9 higher than for Cd, although the estimated fraction of bioavailable U species (UO_2^{2+} and $UO_2(OH)^+$) were small (few %), while was dominating for Cd. Cd was sorbed to the chorion and was only able to penetrate the chorion initially, during the swelling stage. The prolonged uptake of U after the swelling stage of fish eggs at the level observed in these experiments has not been reported earlier for metals (Jezierska et al., 2009). It could be speculated if U is interacting with chorion proteins, limiting the polymerization or formation of new proteins that do not increase chorion toughness as normally. If U impairs the toughness of the chorion of fish eggs, then uptake of other metals could be expected in mixed exposure with U. However, increased uptake of other metals to fish eggs in mixed exposure with U has not been reported (Lourenço et al., 2017). At 10 day degree the CR was significantly higher for Cd than for U, while the situation was opposite at the end of the exposure period.

3.3. Effects of U and Cd on developing fish embryo

Results showed that the exposure to the highest U concentrations tested was lethal as only 94 % and 9 % of the eggs survived until hatching during exposures to 1.8 and 3.6 μM of U, respectively. LC_{50} was predicted to be 2.15 μM of waterborne U. The exposure to U induced also premature hatching of eggs (Fig. 3A), with 50 % of the eggs being hatched after 492 day degree when exposed to 900 nM of U and even earlier when exposed to higher concentrations of U (at 460 day degree when exposed to 1.8 μM of U) compared to 518 day degree for the control eggs. As the U concentration in the water decreased between each water replacement, the observed LC values may be somewhat underestimated. Bourrachot et al. (2008) have also found effects of U on hatching of fish eggs (zebra fish) at similar concentrations (1.05 μM) as in our experiments, albeit in the form of delayed hatching and opposed to premature hatching. Delayed hatching of zebra fish eggs after exposure to U has also been reported by Lourenço et al. (2017). It is unclear why eggs of salmon responded to U exposure by premature hatching in contrast to eggs of zebra fish. Exposure to U could potentially shorten the entire embryonic development time. However, chemical interaction between U and chorion proteins leading to faster degradation of the chorion during long time exposure cannot be excluded. Before hatching, the embryos develop so called hatching glands that produce chorionase, the enzyme necessary for egg chorion disintegration during hatching (Jezierska et al., 2009). It is a tentative assumption that U could trigger the chorionase protein leading to increased activity and degrade the structure of the chorion, although earlier reports have mainly implied that metals can decrease the activity of these enzymes keeping the chorion more intact (Norrgren and Degerman, 1993).

Results showed that the exposure to Cd at concentrations tested did not cause any significant effects on the survival of the eggs (Fig. 3B). About 50 % of the eggs were hatched earlier, at 501–511 day degree compared to 518 day degree for the control. This is supported by earlier findings reporting that the LC_{50} values of Atlantic salmon (*Salmo salar*) embryos lie between 2.7 and 7.1 μM (300–800 μg L^{-1}, Rombough and Garside, 1982) at water concentrations higher than tested in this study. No consistent effects on hatching time associated with Cd exposure are reported, as some reported premature hatching after exposure to Cd, while other published results showed delayed hatching of fish eggs (*Cyprinus carpio*) incubated in water with Cd (Jezierska et al., 2009). Variable levels of waterborne concentration of Cd have been reported to cause developmental failure in fish eggs, in *Salmo trutta* at 16 and 30 nM Cd, in common carp at 90–445 nM Cd, in *Salmo salar* LC_{50} was reported to range between 2.6 and 7.1 μM, in *Melanotaenia fluviatilis* at 29 μM and in *Oryzias latipes* at 178 μM (Rombough and Garside, 1982; González-Doncel et al., 2003; Jezierska et al., 2009). The large differences in chemical speciation and bioavailability of Cd in different water qualities (González-Doncel et al., 2003) could explain some of the differences, but also different sensitivity between fish species should be taken into account (Niyogi and Wood, 2004). In the current experiment, Cd was present mainly as Cd^{2+} ions and thus assumed bioavailable.

3.3.1. Comparison of U and Cd effects in fish embryo

None of the elements tested caused any impact on egg survival at waterborne concentrations lower than 800–900 nM. Only U was tested at higher concentrations, which resulted in reduced survival. Literature indicated that Cd is more toxic than U. However, the present revealed that this is not the case for the embryo stage of Atlantic salmon. Calculated CRs were significantly higher for U than for Cd (Table 2), demonstrating that U was more bioavailable with respect to eggs than Cd, although Cd was mainly present as species assumed to be bioavailable, while the bioavailable fraction for U was limited. High U sorption and uptake in eggs resulted in mortality when the whole egg U concentration was 154 ± 10 mmol g^{-1} or higher. At lower whole egg concentration (73 ± 10 mmol U g^{-1}, 54 ± 10 mmol Cd g^{-1}) no mortality was observed for any of the elements. Earlier hatching of embryos was observed when exposed to U (0.9 μM) than exposed to Cd (0.8 μM), although concentration of U and Cd in hatched alevins was in similar range (Table 2). Thus, results indicated that U is more toxic to embryos than Cd.

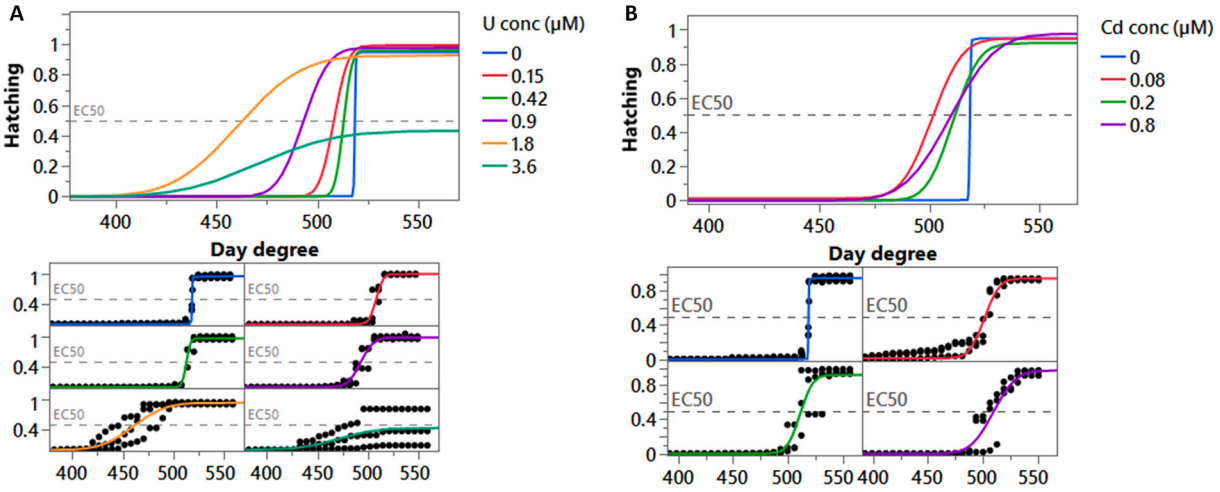

Fig. 3. Cumulative hatching of eggs A) at different U exposures and B) at different Cd exposures. Each line represents an average of 3 separate units with eggs. Dotted line represents 50 % hatching.

3.4. Uptake of U and Cd in juveniles

The U concentrations in gills and liver increased exponentially with waterborne U concentrations (Fig. 4A and B) up to 836 ± 900 nmol g^{-1} for the gills (R^2 = 0.93) and to 6.4 ± 1.7 nmol g^{-1} for the liver (R^2 = 0.78). The concentration ratio (CR) was about 0.02 for gills: water (96 h) in the range 2–4 μM waterborne U (Table 3). At higher waterborne U concentrations (9.2–12 μM U) the CR of U was 0.07 or higher, showing higher transfer of U to the gills with increasing concentration of waterborne U. There was a linear correlation between U concentration in gills and in liver (R^2 = 0.96, P = 0.001), with CR value of 0.006 (Fig. 4C). Results indicated that U in fish gills was further transferred

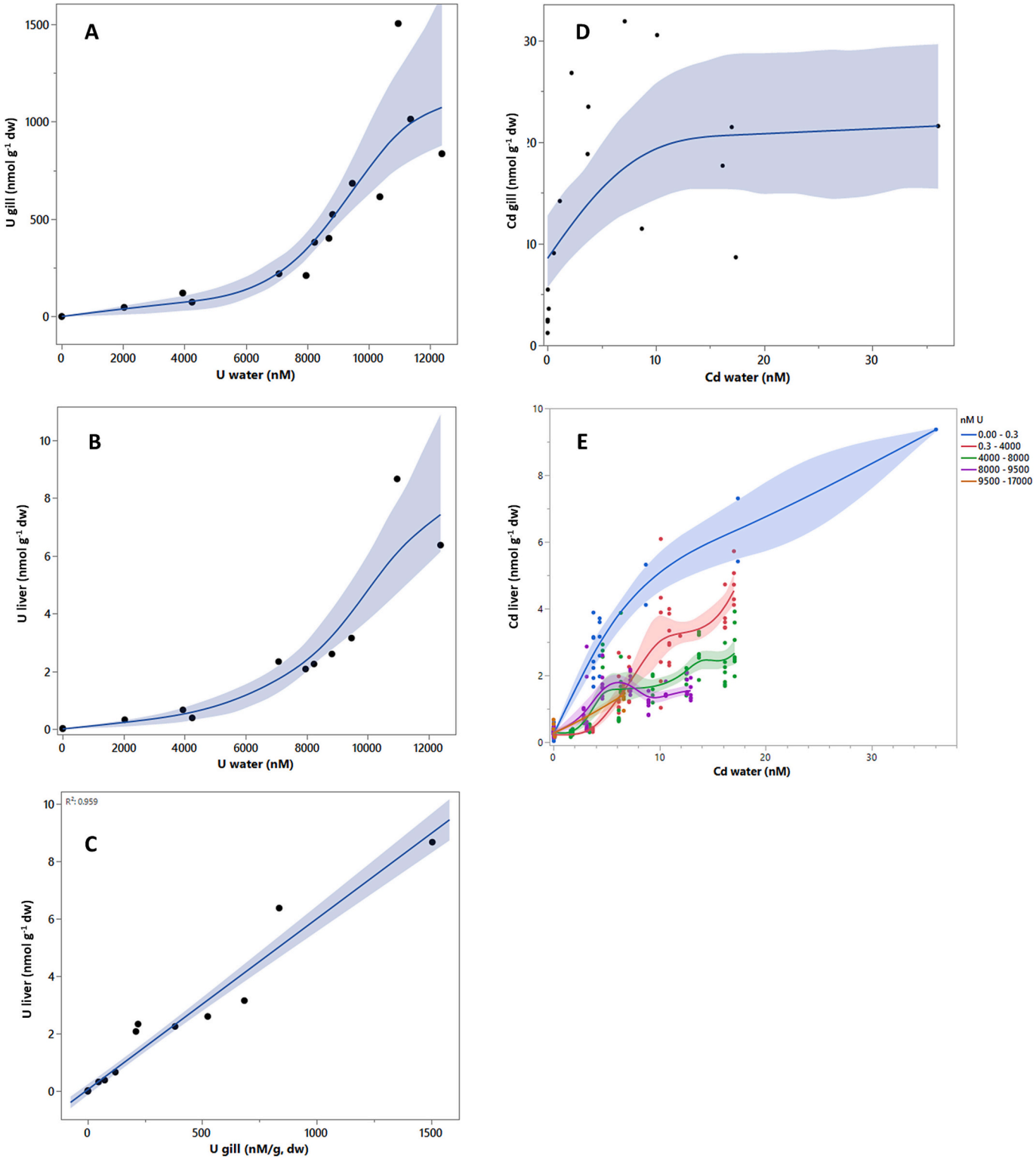

Fig. 4. Relationships between waterborne concentration and concentration in tissue of exposed Atlantic salmon juveniles, A) U in water and U in gills, B) U in water and U in liver, C) U in gills and U in liver D) Cd in water and Cd in gills and D) Cd in water and Cd in liver at different concentration of U in water. Shaded area shows 95 % confidence interval.

into the blood and liver, as waterborne uptake from intestines is not expected as freshwater fish do not drink water (Heath, 2018).

The Cd concentration in gills increased with increasing concentration of waterborne Cd up to a maximum at about 10 nM, while there was no significant increase at higher waterborne concentrations (Fig. 4D). The concentration of Cd in liver continued to increase also at waterborne Cd concentrations higher than 10 nM (Fig. 4E). The CR was about 0.016 for gill: water (96 h) at the lowest Cd concentration tested and was 0.003 at the point of saturation (8–10 nM Cd). Results indicated that Cd in fish gills was further translocated into the blood and to liver as waterborne uptake in intestines is not expected for freshwater fish (Heath, 2018). However, Cd saturation in gills was observed before saturation in liver, most likely due to the delay in Cd transfer from gills to the circulatory system.

3.4.1. *Comparison of U and Cd uptake in juveniles*

Results demonstrated that both waterborne U and Cd were taken up in juveniles, and that the accumulation of waterborne U in gills was higher than for Cd (CR 0.02–0.07 compared to 0.001–0.02). However, the transfer from gills to liver was higher for Cd than for U. Whereas

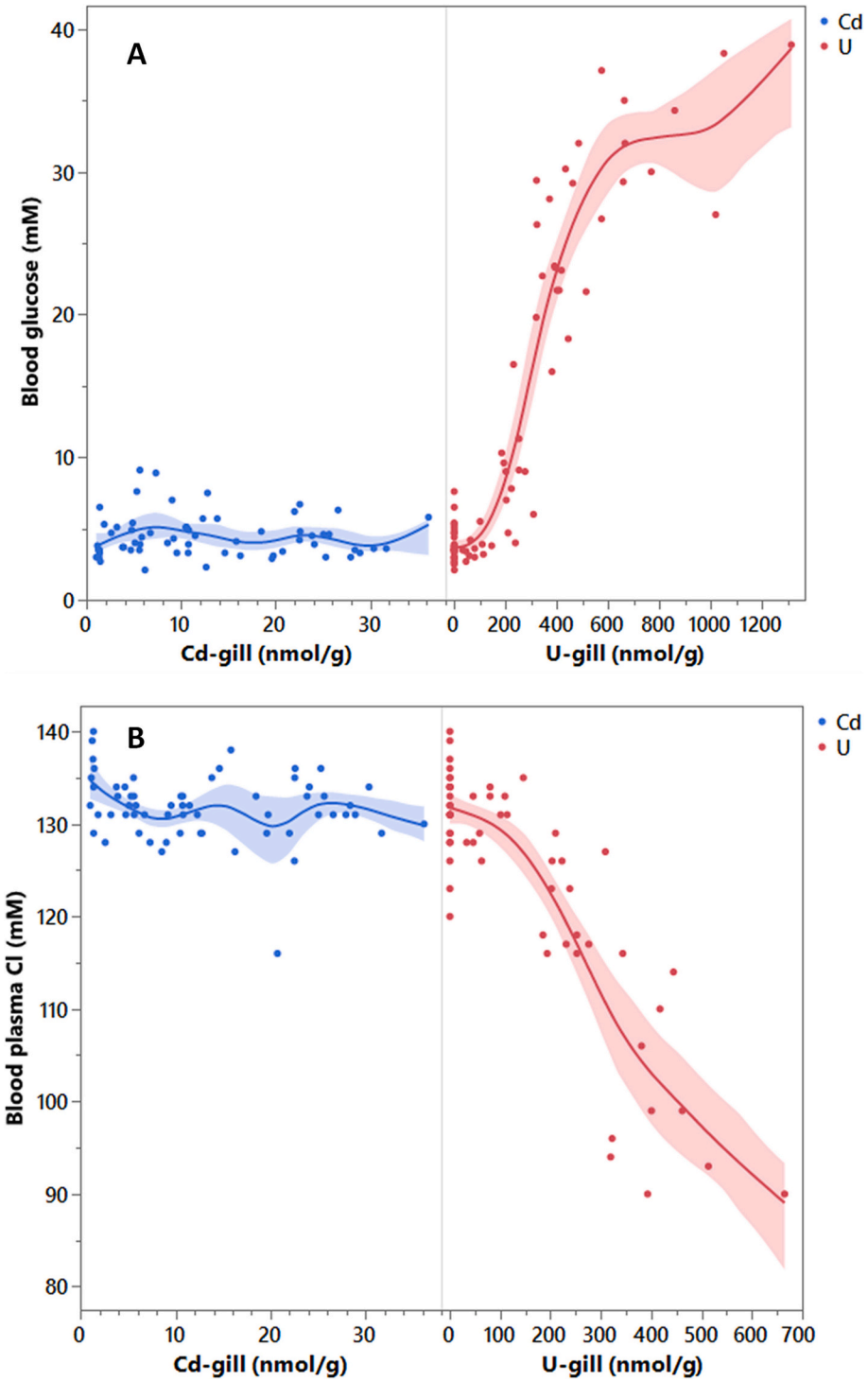

Fig. 5. Relationship between gill concentration of U or Cd with A) blood glucose and B) plasma Cl in exposed Atlantic salmon juveniles.

accumulation of U in gills increased with increasing exposures, the Cd uptake tended to reach a maximum at 20 nmol g^{-1} gill and then plateaued. Waterborne metals can accumulate to different extents in gills, and the order of accumulation depends on several factors such as type of metals and speciation. However, it is surprising that the accumulation of U is higher than Cd in the gills. Higher uptake of Cd compared to U in liver is expected as the bioavailable fraction of Cd was predicted to be significantly higher than for U. The transfer of Cd from the gills to the blood is assumed to occur through Ca channels and by Ca-ATPase enzymes (Niyogi and Wood, 2004). Uranium is also assumed to be transferred from gills to the blood by Ca channels, as demonstrated for plant root transport (Sarthou et al., 2022). However, the uptake of U has also been demonstrated to occur by endocytosis (Huang et al., 2023).

3.5. Effects of U and Cd in juveniles

Results showed that the blood glucose in fish increased, and blood plasma Cl and Na decreased following exposure to U (Fig. 5A, B), at concentrations higher than 200 nmol U g^{-1} gill. There was a positive correlation between gill U concentration and glucose ($R^2 = 0.81$) and negative correlation with plasma Cl ($R^2 = 0.74$). Results indicated that concentrations higher than 9.5 μM U in water (at pH 6.7), higher than 400 nmol U g^{-1} gill or higher than 3 nmol U g^{-1} liver were associated with reduced survival of fish (Fig. 6). LC_{50} was reached at 1.15 μM of waterborne U, at 836 nmol U g^{-1} gill and at 8.66 nM U g^{-1} liver. Above 400 nmol g^{-1} gill U, the blood glucose was higher than 15 mM and plasma Cl lower than 115 mM. Reported lethal U concentration for fish vary widely (Wood et al., 2012), most likely as a result of varying exposure conditions including differences in water quality that influence U speciation as well as different sensitivities among the exposed fish. Labrot et al. (1999) reported U accumulation in gills and LC_{50} at 725 nM U for fish (*Brachydanio rerio*). Blood glucose of 3–5 mM has been used as a criterion for no effect in Atlantic salmon (Kroglund et al., 2007), and increased level is known to induce secondary stress response in fish due to additional energy costs maintaining homeostasis during metal exposure (Wendelaar Bonga, 1997). The positive correlation between U in gills or liver with blood glucose in the current study shows that U interfered with metabolism and cellular processes. Barillet et al. (2010) reported gill damage and hyperplasia of chloride gill cells after exposure to waterborne U (*Danio rerio*). Physiologically, chloride cells are responsible for the maintenance of the ion balance and hyperplasia of chloride cells could be the reason for reduced plasma Cl due to failure in osmoregulation. As decreased plasma Cl has also been reported as a physiological compensation for respiratory acidosis (higher HCO_3 in blood) (Grøttum and Sigholt, 1996), respiratory failure cannot be excluded as a contributing effect in addition to osmoregulatory failure,

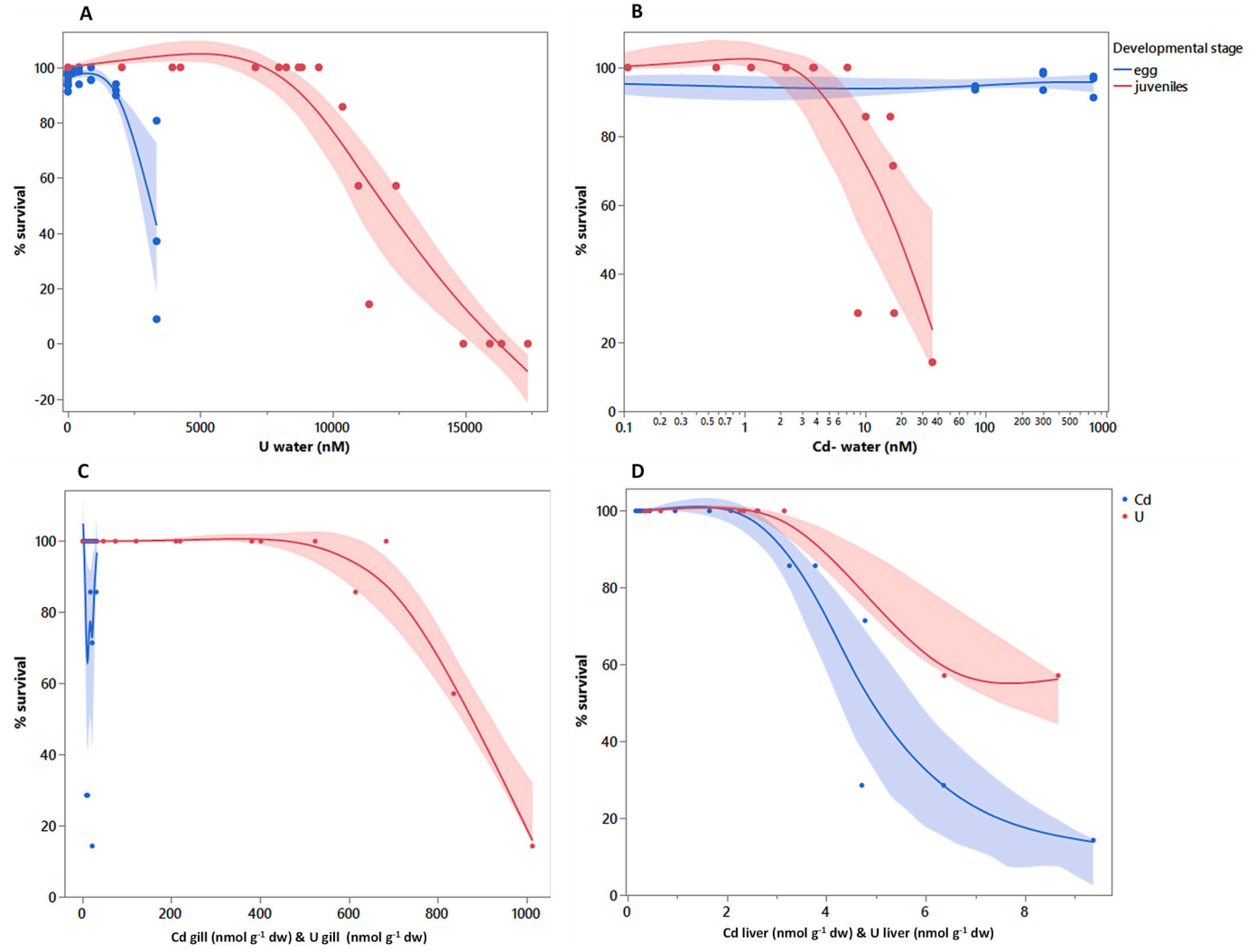

Fig. 6. Relation between A) waterborne U and survival of eggs and juveniles, B) waterborne Cd and survival of eggs and juveniles, C) gill concentration of U or Cd and survival of juveniles of Atlantic salmon and D) liver concentration of U or Cd and survival of juveniles. Each egg point represents % survival of 250 eggs and each juvenile point represent % survival of 7 fish of Atlantic salmon (*Salmon salar* L.).

both associated with increased blood glucose after exposure to metals (Kroglund et al., 2007). It is also possible in the present study could induce inflammation and hyperplasia in gill tissue, enlarging the distance between blood and water and thereby causing respiratory failure. Annamalai and Arunachalam (2017) reported hematological effects in fish after acute exposure to U, which also affected oxygen carry capacity.

Exposure to Cd reduced the survival of the juveniles at waterborne concentration at 8.7 nM or higher, with LC_{50} value 19.5 nM (Fig. 6). Cd concentration in gills increased to a maximum plateau that coincided with reduced survival. There was a good correlation between Cd in liver of juveniles and survival, with LC_{50} at 4.8 nmol g^{-1} of Cd in liver. No change in blood glucose or plasma Cl due to exposure to Cd was observed (Fig. 5). In literature, acute LC_{50} values for freshwater fish range from 4.4 nM to more than 500 μM (Wood et al., 2012), thus it is rather difficult to compare toxicity values. However, the LC_{50} values in our experiments are among the lowest reported values in literature (Liu et al., 2022). This is not unexpected as the low concentrations of DOC and Ca in the tested water were expected to result in very limited protection against Cd toxicity (Wood et al., 2012). Indeed, more than 90 % of the Cd species were presumably present as Cd^{2+} in the exposure water. Disruption of ion homeostasis, particularly Ca regulation in gills is reported to be one of the primary acute effects of Cd in fish (Niyogi and Wood, 2004). Neither plasma Ca nor Ca-ATPase activity were analyzed in exposed fish, but the absence of effects on Cl regulation in fish was in agreement with earlier findings (McGeer et al., 2000). No change in blood glucose could indicate that the Cd exposure did not cause additional energy costs or more likely that Cd could disrupt the glycogen metabolism and alter glucose levels in the blood. Cd could impact the signaling pathways involved in glycogen breakdown producing glucose (Lin et al., 2011), which also would increase glycolysis (Li et al., 2024a). Previous studies have demonstrated increased blood glucose in the blood of fish after Cd exposure (Hontela et al., 1996), while other not (Hosseini and Hoseini, 2012).

3.5.1. Comparison of U and Cd toxicity in juveniles

Comparing observed effects, Cd was significantly more toxic to juveniles than U in the water tested. The waterborne concentration of U was more than 600 times higher than the Cd concentration when mortality was observed, while the U gill concentration was a factor of 20–70 higher and the U liver concentration was in the same range as for Cd. Furthermore, results indicate different acute toxic mode of action due to U and Cd exposure, as increased blood glucose and reduced plasma ions were only observed when exposed to U. Although Na imbalance only was observed after U exposure and not after Cd exposure, disruption of Na homeostasis could also occur after Cd exposure in fish (Niyogi and Wood, 2004). It is well known that both U and Cd can lead to oxidative stress, DNA damage and similar downstream effects following chronic exposure (Song et al., 2014; Shankar et al., 2021; Lee et al., 2024). Similar toxic modes of action cannot therefore be excluded.

Comparing toxic effects of U and Cd at different life stages in Atlantic salmon, results showed that the embryo stage was more sensitive to U than juvenile stage, as reduced survival was observed at lower waterborne concentrations for eggs (LC_{50} = 3.17 μM) than for juveniles (LC_{50} = 11.5 μM). However, for Cd the juvenile stage was more sensitive than the embryo stage, as reduced survival was observed at significant lower waterborne Cd concentrations for juveniles (LC_{50} = 19.6 nM) than for eggs (LC_{50} > 800 nM). Thus, juveniles were more sensitive to Cd than egg stage, while the egg stage was more sensitive to U than juvenile stage. Results indicated that changes in bioavailability of the elements between eggs and juveniles could partly explain the difference in sensitivity as increased sorption and uptake of U, compared to Cd, was observed at the egg stage.

3.6. Joint effect of U and Cd in juveniles

Exposure of Atlantic salmon juveniles to lethal U concentration in mixture with waterborne Cd resulted in similar or reduced survival. However, when exposing juveniles to waterborne Cd at lethal concentration in combination with U, the survival of the juveniles increased. Independent action (IA) model described 61 % of the variation in the data and showed that there was a significant antagonistic effect in the mixture where Cd dominates the effects (Fig. 7A). Using the concentration addition (CA) model there was also a significant antagonistic effect in the same mixtures. Although both models show similar results and the uptake pathways for U and Cd are assumed to be similar in fish, the IA model was selected to be most relevant as measured physiological effects were different for U and Cd. Results showed that waterborne U reduced the toxicity of waterborne Cd, while the opposite was not observed. Antagonistic effects between U and Cd are in line with what others have reported for other organisms (Margerit et al., 2015).

Comparing uptake of waterborne U in gills and liver of fish in mixture exposures with single waterborne U exposures, results showed that Cd did not change the bioaccumulation of U in the gills nor uptake in the liver of the juveniles. Also, the exposure to high concentration of Cd combined with U did not change the uptake of Cd in gills as the maximum plateau was the same. However, the bioaccumulation of Cd in gills at low waterborne concentration of Cd and in liver was reduced when fish was exposed to waterborne Cd in combination with waterborne U (Fig. 4E). Thus, results indicate that U blocked the transfer of Cd from water to gill and thereby transfer of Cd from gills to blood and bioaccumulation in livers. Reduced internal uptake of Cd (e.g., in liver) and subsequent lower toxic effects of Cd in combination with waterborne U could be attributed to antagonistic effects. Pelgrom et al. (1994) observed also reduced uptake of Cd in fish (*Oreochromis mossambicus*) following exposures of binary metal mixtures with Cu, while no reduced Cu burden in co-exposure with Cd. It is believed that both U and Cd are taken up in cells by Ca channels (da Silva and Martinez, 2014) and that Ca^{2+} can compete with Cd^{2+} for uptake in fish gills (Niyogi and Wood, 2004). Thus, competing effects between uranyl ion and Cd^{2+} ion should also contribute to reduced uptake of Cd in fish. The fact that U did not affect the maximum concentration of Cd in the gill was surprising and indicated that U did not influence the number of binding sites at the gill surface, but rather competed with Cd for the avaible sites. This is supported by the observation of lower gill Cd concentration in presence of U when the maximum Cd concentration was not reached, and that Cd has a higher affinity for gills compared with U (Niyogi and Wood, 2004; Lofts et al., 2015). Obviously, complex interaction mechanisms on the toxicokinetic level are involved between U and Cd species in water.

When the concentrations of U and Cd in liver tissue of the juveniles were taken into account, the IA additive model (Fig. 7B, C) also showed that the interaction between Cd and U resulted in less than additively, i.e., antagonistic interaction between Cd and U. The IA reference model described 64 % of the variation in the dataset. Thus, results indicated that antagonistic effects occurred both on the toxicokinetic level causing lower uptake of Cd in presence of U, but also on the toxicodynamic level taking the tissue concentrations of both U and Cd into account.

4. Conclusion

The results demonstrate differences in bioavailability and toxicity of waterborne U and Cd in very soft EPA water (pH 6.7–7.0) to two different live stages of Atlantic salmon exposed under controlled experimental conditions. Uranium was mainly present in the LMM fraction in water and was taken up in both eggs and juveniles, causing reduced survival in eggs and juveniles. Results are the first to show that U inside chorion of fish eggs increased with exposure time from fertilization until hatching. Observed LC_{50} for waterborne U were 3.17 μM for eggs but significant higher for juveniles (11.5 μM). Based on tissue concentrations LC_{50} values were 1900 nmol U g^{-1} whole egg concentration, 836 nmol U g^{-1} gill concentration and 8.66 nmol U g^{-1} liver concentration. Results demonstrated that the embryo stage was more sensitive to waterborne U than the juvenile stage with a significant

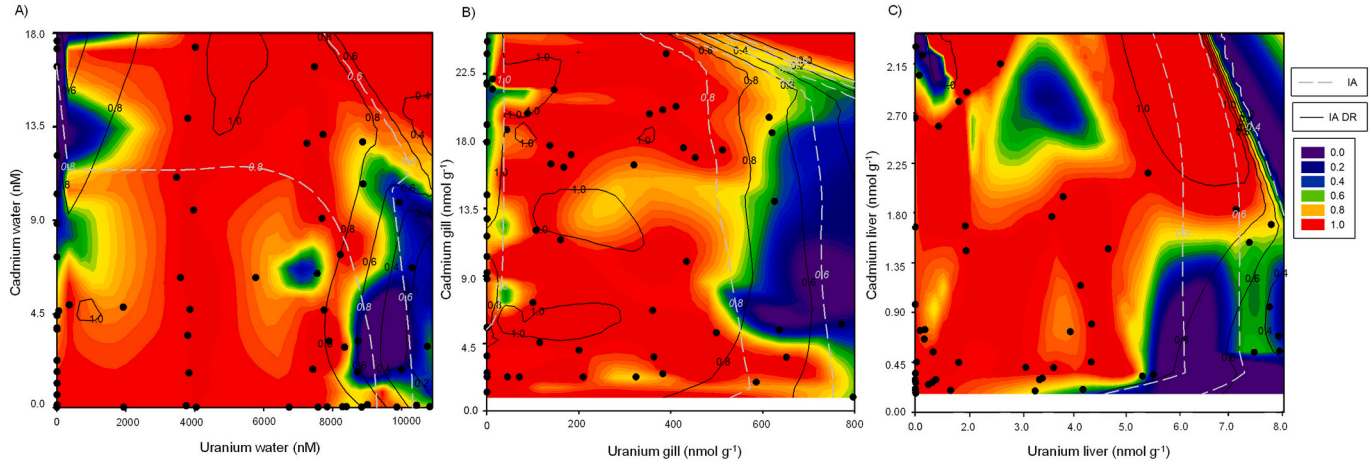

Fig. 7. Survival of Atlantic salmon juveniles exposed to U and Cd in mixtures A) based on waterborne concentrations (mM), B) based on concentrations in gills (nmol g^{-1}) and C) based on concentration in liver (nmol g^{-1}) of exposed juveniles. The color represents the observed data (red 100 % survival and purple 0 % survival). The gray isobols represent the modeled survival using independent action (IA) as reference model. The solid black isobols describe the most significant deviation model (values indicate % survival). Each black point represents tests performed. The IA models based on water concentration, gill concentration and liver concentration of U and Cd describe 67 %, 11 % and 64 %, respectively of the variance of survival and the deviation models 73 %, 26 % and 71 %.

positive correlation between tissue concentrations and effects for both life stages.

Waterborne Cd in the tested concentration range up to 800 nM was mainly present in the LMM fraction, but also featured a significant colloidal fraction, was taken up in both eggs and juveniles, causing reduced survival in juveniles (LC_{50} = 19.6 nM waterborne U and 4.8 nmol Cd g^{-1} liver) while not in fish eggs. Results showed that the embryo stage was less sensitive to waterborne Cd than the juvenile stage. The uptake in eggs was, however, limited to the swelling stage. Limited uptake thereafter was assumed to be the main reason for limited effects of Cd on the embryo stage.

Cadmium was significant more toxic to juveniles than U, while Cd was less toxic than U for the embryo stage. Only U was observed to cause mortality in eggs, but at higher concentrations than tested with Cd. The acute toxic mode of action of U and of Cd in juveniles was observed to be different as changes in blood glucose and blood plasma Cl were only observed for U exposed juveniles.

Using independent action as reference model, antagonistic effects on both toxicokinetic and toxicodynamic level were observed for exposures to U and Cd as binary mixtures, partly due to reduced uptake of Cd in presence of U. Results highlighted that interactions on the toxicokinetic level can reduce joint effects, but demonstrated also that joint effects can occur at the toxicodynamic level of U and Cd. Results also demonstrated the importance of linking water concentration and speciation, bioaccumulation and adverse effects in organisms in order to understand factors and processes resulting in life stage dependent toxicokinetic and toxicodynamic during multiple stressor situations.

CRediT authorship contribution statement

Hans-Christian Teien: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Dag Anders Brede:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Yetneberk Ayalew Kassaye:** Methodology, Investigation, Formal analysis. **Erica Maremonti:** Writing – review & editing, Software, Formal analysis. **Claus Svendsen:** Methodology, Formal analysis, Conceptualization. **Ole Christian Lind:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Brit Salbu:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180733.

Data availability

Data will be made available on request.

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