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Effect of road salt and copper on fertilization and early developmental stages of Atlantic salmon (Salmo salar)

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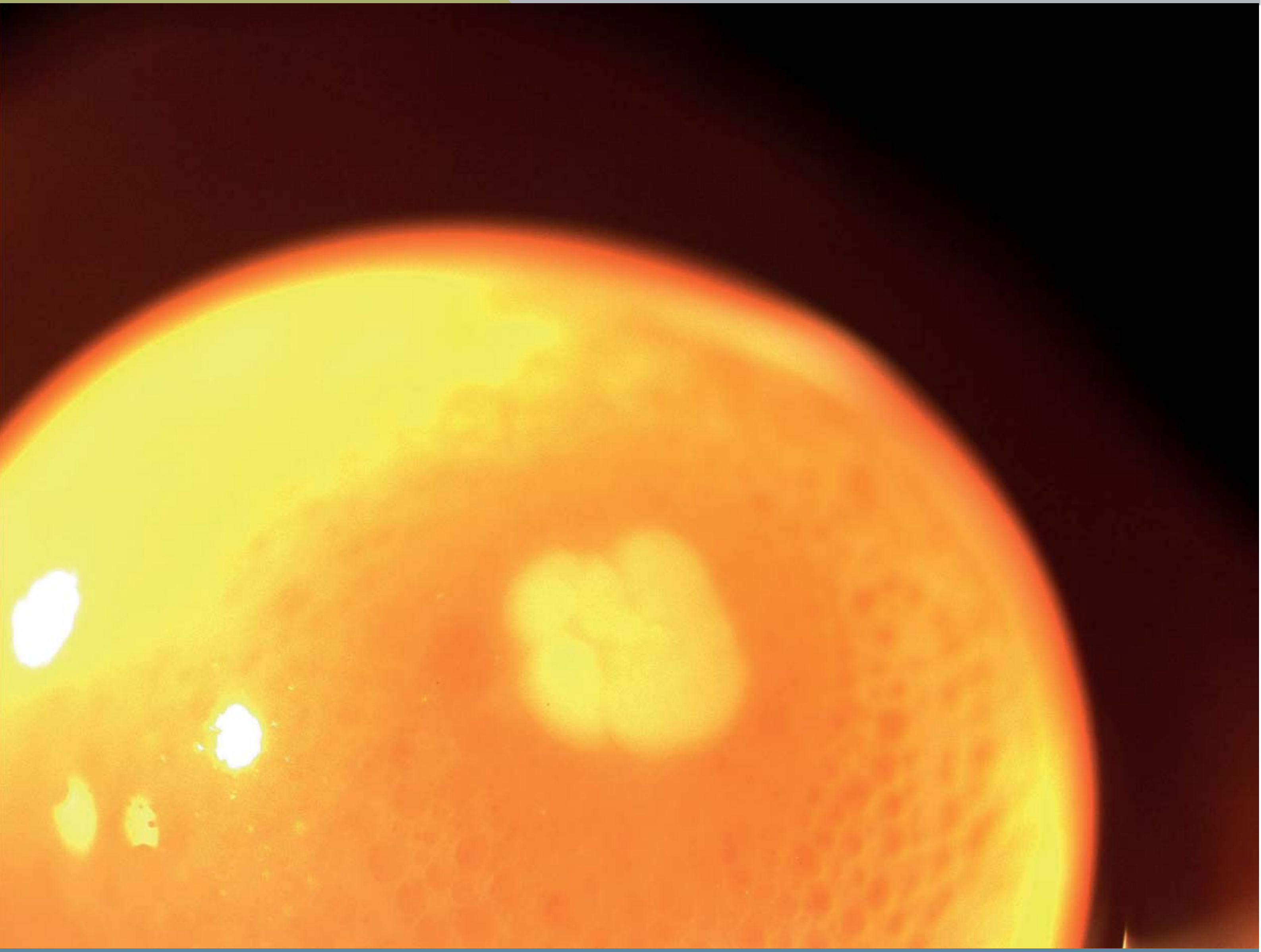

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VD rapport

Tittel
Effekter av vegsalt og kobber under befruktning og tidlige utviklingsstadier hos Atlantisk laks (Salmo salar)

Forfatter
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Sammendrag

Rapporten oppsummerer effektene av vegsalt og blandingen vegsalt og kobber (Cu) på tidlige utviklingsstadier hos Atlantisk laks (Salmo salar L.), fra befruktning til klekking og "swim-up". Forsøket ble gjennomført ved Fiskelaboratoriet, UMB. Resultatene indikerte subletale (ikke-dødelige) effekter under befruktning ved høye vegsaltkonsentrasjoner (≥5000 mg/L), mens senere livsstadier var mindre følsom. I tillegg ble det observert deformiteter som følge av eksponeringen med vegsalt og Cu i kombinasjon. Det synes derfor som om sensitiviteten øker hos laks i tidlige livsstadier når de eksponeres for vegsalt i kombinasjon med andre forurensninger i avrenningsvann fra veg.

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VD report

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Key words
Road salt toxicity, Early life history stage, Atlantic salmon, Cu toxicity

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This report summarizes the effects of road salt as well as of mixtures of road salt and cupper (Cu) on early developmental stages of Atlantic salmon (Salmo salar L.), from fertilization to hatching and swim-up. The experiments have been performed at the Fish Laboratory, UMB. The results indicated sub-lethal effects from road salt at high salt concentrations (≥5000 mg/L) during fertilization, while early developing stages until hatching were less susceptible. In addition, deformities were observed when multiple stressors exposures (road salt in mixture with Cu) were applied. Hence, the sensitivity of early stages of salmon seems to increase when exposed to road salt mixed with interacting stressors in road runoff.

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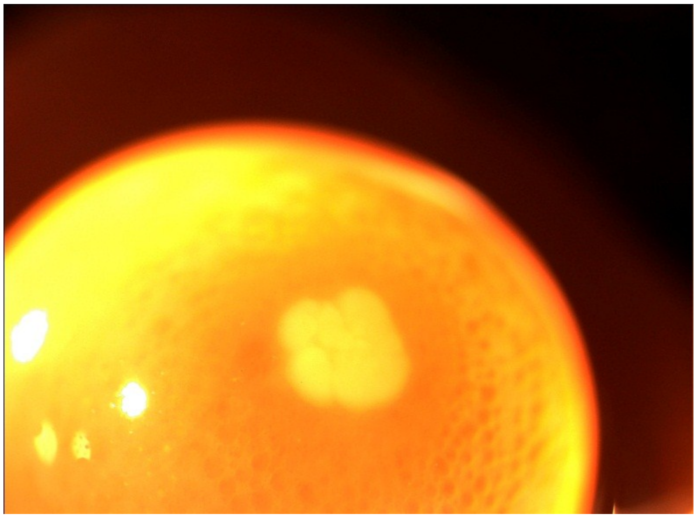

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Key word, English
1. Road salt toxicity
2. Early life history stage
3. Atlantic salmon
4. Cu toxicity

Preface

UMB has in collaboration with the Norwegian Public Roads Administration (NPRA) performed an experiment with Atlantic salmon (*Salmo salar L.*) to obtain information about the environmental effects of road salt on aquatic ecosystems. The sensitivity of road salt to the early development stages of Atlantic salmon, from fertilization until hatching and swim-up, exposed to road salt was tested in controlled experiments for 6 months (November 2010 until May 2011). The susceptibility of road salt on fertilization stage was compared to other developmental stages until hatching. In addition, the effects of multiple stressors (salt mixed with Cu) were also studied during fertilization and development stages.

The present work is performed within the scope of the joint UMB and NPRA project "Effects of road salt on early life history stages of Atlantic salmon (*Salmo salar L.*)". Grants from NPRA in addition to support from UMB are appreciated. We are also grateful to AquaGen for their contribution of eggs and sperm from Atlantic salmon and to the Fish laboratory at UMB for their assistance during the experimental work.

Ås, 30 August 2011

Hans-Christian Teien

Hans-Christian Teien

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1 Introduction

Atlantic salmon (*Salmon salar L*) is highly sensitive towards environmental pollution, especially during the specific life history stages. The early developmental stages are sensitive, and during the time from fertilization until hatching and swim up stage the fish cannot move away from polluted areas with potential episodic marginal water quality. Survival and normal development is highly dependent upon good water quality at the location of the eggs in tributaries and rivers.

The widespread use of road salt (mainly sodium chloride (NaCl)) for snow and ice removal has resulted in environmental impacts in many areas (e.g. Amrhein et al, 1992). It has been reported that aquatic ecosystem located close to roads receive episodic high concentration of salt and that lakes nearby roads have higher concentrations of salt than lakes located further away from the roads (Bækken and Haugen, 2006). High concentration of salt in runoff is especially linked to episodic events with high precipitation or snowmelt.

In addition, road salt as well as other deicing agents such as calcium magnesium acetate (CMA) may also have a mobilizing effect on several heavy metals found in roadside soils (Backstrom et al, 2004). For example, Amrhein and Strong (1990) and Amrhein et al., 1993 showed in laboratory studies that NaCl releases metals (Cr, Pb, Ni, Fe and Cu) associated to organic matter and colloids and that the release was most prominent when adsorbed sodium was high and the ionic strength was low.

In Norwegian fresh waters the background concentrations of Cu is normally less than 1-2 µg/L (Lydersen et al., 2002). However, increased concentration of Cu has been reported in salt impacted lakes located close to roads compared to salt un-impacted lakes located further away (Bækken and Haugen, 2006). Although copper (Cu) is an essential metal for nearly all organisms, including fish (Owens, 1981; Maage et al, 1989), elevated concentrations may cause toxic effects (McGeer et al., 2000; Niyogi and Wood, 2004).

In fish, Cu seriously interferes with branchial ion transport (Lauren and McDonald, 1987; Wilson and Taylor, 1993) plasma ion concentrations (Stagg and Shuttleworth, 1982), hematologic parameters, and enzyme activities (Lauren and McDonald, 1987; Jackim et al, 1970; Lorz and McPherson, 1976). In addition, Cu may cause immunosuppression (Anderson and Dixon, 1989), vertebral deformities (Birge and Black, 1979; Bengtsson and Larsson, 1986) and neurological disorders. Furthermore, aquatic freshwater organisms are sensitive to high concentration of salt. Salt could also interact on the toxicity of Cu (Anderson et al. 1995). According to Anderson et al (1995) a number of studies have investigated the relationship between salinity and toxicity using heavy metals (Cd, Zn, Ni, Pb, Cr, Cu, Hg). For example, it is recommended that the Cu concentration is less than 3 µg/L in water used in Atlantic salmon hatcheries (Rosseland, 1999).

To reduce the environmental impact of road salt, the strategy of its usage is aimed to be improved The knowledge of the sensitivity of the early developmental stages of Atlantic salmon and the combined exposure of salt and Cu are both limited. To improve the present knowledge, the objectives of the current study were the following:

1. Identify possible negative effect (toxicity) of road salt road salt (the term “road salt” refers to NaCl throughout the text) to early developmental stages of Atlantic salmon, from fertilization to swim-up.
2. Identify possible negative effect (toxicity) of multiple stressors; mixtures of road salt and Cu to early developmental stages of Atlantic salmon, from fertilization to swim-up.
3. Compare the susceptibility of the fertilization stage to the salt and Cu mixture exposure to other developmental stages.

2 Methods

The experiment was designed to obtain information about toxic levels of road salt on the fertilization and early developmental stages of Atlantic salmon and information on effects when road salt was mixed with Cu in the exposure water. Four types of exposure regimes were included:

1. exposure of road salt during fertilization and not thereafter
2. exposure of road salt and Cu during fertilization and not thereafter
3. exposure of road salt episodically after fertilization and not during fertilization
4. exposure of road salt and Cu episodically after fertilization and not during fertilization

The experiment was conducted from 23 of November 2010 until May 2011 in the Fish Laboratory at UMB in a temperature controlled system.

Dry stripped Atlantic salmon eggs and sperms from Aquagen were transported in plastic bags on ice in a box of polystyrene. Before dry fertilization (Fig. 1), the viability of two milt batches was tested under the microscope by adding a drop of water to a tiny drop of milt. The most viable group was selected. All equipments were washed, cleaned with freshwater and dried before mixing eggs and milt. This procedure was followed between each fertilization batch. After fertilization, eggs were transferred to the specific water qualities for swelling in corresponding fridges according to Källqvist et al. (2003).

Figure 1. Dry fertilization of eggs (Photo by Sondre Meland*)*

2.1 *Experimental setup*

The reference water was obtained from the Lake Maridalsvann, which is the drinking water reservoir of the City of Oslo. The water had a temperature of 6.3-6.6 °C and was kept in two tanks (2 x 600 L) from where water was distributed to different exposure boxes placed in the fridge.

To utilize realistic concentrations of Cu in the salt mixtures, a pilot study was performed where collected road dust was extracted using 10000 mg NaCl/L. Results showed that a Cu concentration of 26 µg/L could be achieved under realistic conditions.

One of the tanks was spiked with Cu giving a concentration 10 µg Cu/L, compared to <3 µg Cu/L in Lake Maridalsvatn, respectively. The stock solution for Cu (100 mg/L) was made from Cu chloride ($CuCl_2$).Water was continuously pumped by peristaltic pumps from the big tanks to the small exposure boxes with a flow rate of approximately 40-60 mL/min (Fig. 2). The water returned to the big tanks by gravitation. The eggs were placed in the exposure boxes making replicates of each treatment in both fridges according to Haugen et al. (2006).

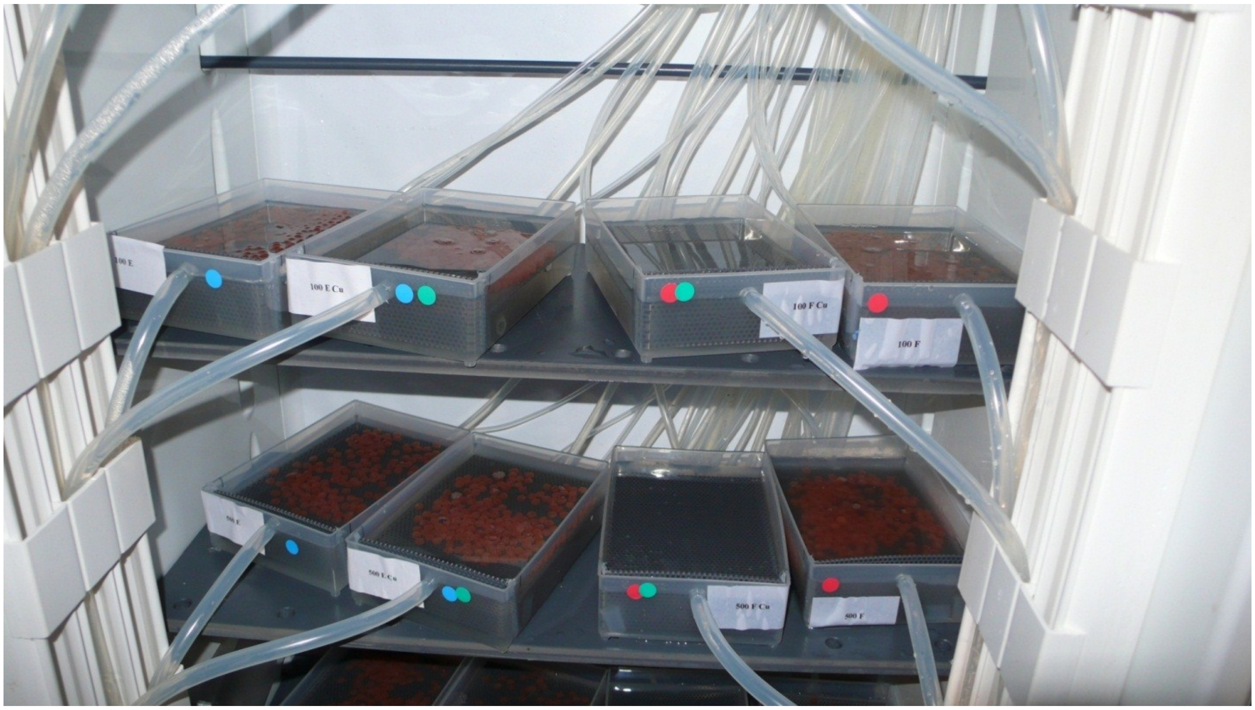


Figure 2. The experimental setup. Each treatment had 2 replicate boxes with 220 eggs (Photo by Merethe Kleiven)

2.2 Road salt and Cu exposures

To obtain information of toxic level of road salt, the eggs of Atlantic salmon were exposed to the following five different water qualities**:**

1: Reference water Maridalsvann
2: Reference water Maridalsvann with 50 mg road salt / L.
3: Reference water Maridalsvann with 100 mg road salt / L.
4: Reference water Maridalsvann with 500 mg road salt / L.
5: Reference water Maridalsvann with 5000 mg road salt / L.
6: Reference water Maridalsvann with 10000 mg road salt / L.

To obtain information about the interaction of road salt on Cu toxicity, eggs of Atlantic salmon were exposed to following five different water qualities:

7: Reference water Maridalsvann added Cu, totally having 10µg Cu /L.
8. Reference water Maridalsvann having 10 µg Cu /l with 50 mg road salt / L.
9: Reference water Maridalsvann having 10 µg Cu /l with 100 mg road salt / L.
10: Reference water Maridalsvann having 10 µg Cu /l with 500 mg road salt / L.
11: Reference water Maridalsvann having 10 µg Cu /l with 5000 mg road salt / L.
12: Reference water Maridalsvann having 10 µg Cu /l with 10000 mg road salt / L.

To obtain further information about the Cu toxicity, Atlantic salmon eggs was also exposed to 20 and 30 µg Cu/L in additional boxes with 500 mg NaCl/L during and after fertilization. Only one replicate was, however, included for this additional exposure.

During episodic treatments, the water was pumped from separate 30 L tanks into each box containing eggs, and recirculated to the tank with the same water quality. This episodic treatment occurred for 24 hrs every week. Between episodes each box received reference water form big tanks (600 L).

2.2.1 Exposure types and duration

220 eggs were placed in each of the boxes with episodic treatments while 240 eggs were placed in the boxes having treatment only during the fertilization. This difference in number was due to sampling of eggs taken out to check fertilization success and increase in swelling after fertilization due to different treatments during fertilization. The eggs were given two types of exposure treatments with all water qualities mentioned in chapter (2.2):

1. During the first 24 hours after dry fertilization. After 24 hours, all eggs were given normal reference water supply until end of the experiment.

2. Weekly 24 hours exposure (Episodic treatment). During the fertilization and pre- and post exposures, eggs were given reference water with and without Cu.

2.3 Water quality parameters

To control the system water was weekly checked for the concentration of salt (measured as conductivity), total Cu, temperature, pH, organic material (TOC) and oxygen concentration. In addition information of different physico-chemical forms of Cu, including particules, colloids, positively charged Cu species and low molecular mass Cu were also obtained. The conductivity, pH, temperature and oxygen of water in both fridges were also logged continuously with an automatic electronic logger (Campell CR200). Water samples were acidified with 2 % ultrapure nitric acid (HNO_3) before major cations and Cu was determined using by using Inductive Coupled Plasma Emission Spectroscopy (ICP-OES, Perkin Elmer, Optima 5300 DV). In addition Iachat IC5000 Ion Chromatograph was used to determine major anions and the Total organic analyzer (Shimadzu TOC cpn) to determine TOC.

2.3.1 Fractionation of water

Different Cu species, formed by different transformation processes (Fig. 3), were separated using various fractionation techniques. As natural waters are dynamic system, therefore, in situ fractionation was preferred over laboratory based methods, where storage effects may occur. To obtain information about the size and charge of Cu species, the separation according to size and charge are performed in a combined system (Salbu and Oughton 1995; Teien et al.2004). The concentration of Cu, in different fractions, was determined by ICP-OES.

2.3.1.1 Size fractionation

Size fractionation was performed three times during the experimental period by filtration and ultrafiltration utilizing membrane filters (0.45µm) and hollow fibers. Ultrafiltration was performed using the Amicon H1P1-20 hollow fiber with a nominal molecular cut-off level of 10kDa.

2.3.1.2 Charge fractionation

Charge fractionation was based on ion-exchange chromatography using the cation exchange Chelex-100. The resin material was placed in columns and the metal concentration, retained in the resin, was calculated by the difference between metal concentration in the inlet sample and outlet sample. The flow rate through the column was 30 ml/min check and at least 60 ml of the sample was pumped through the resin for conditioning purposes.

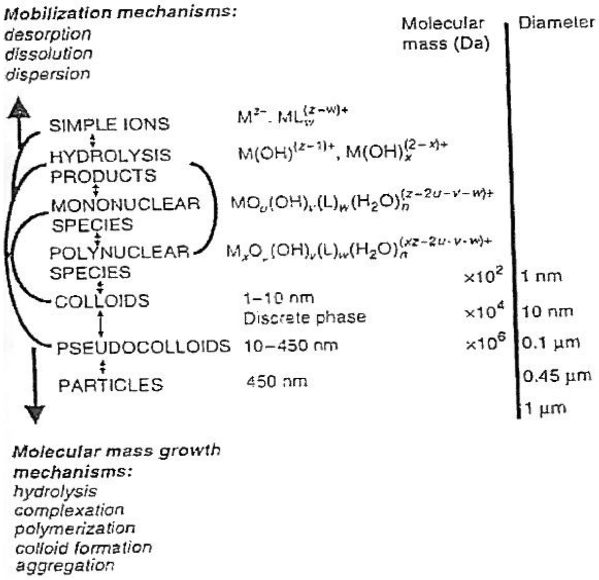


Figure 3. Transformation processes changing the distribution of metal species (after Salbu, 2000).

The combined size and the charge fractionation system was applied. The following physico-chemical forms were identified.

- Total Cu, determined based on unfiltered sample

- Particulate Cu species obtained by following calculation: Cu concentration in unfiltered samples subtracted Cu concentration in 0.45 µm filtered samples
- Colloidal or high molecular mass (HMM) Cu species obtained by following calculation: Cu concentration in 0.45µm filtered samples subtracted Cu concentration in ultrafiltrated samples (≤10kDa).

- Low molecular mass (LMM) Cu species (≤10kDa). Determined in ultrafiltered samples.

- Positively charged Cu species: passing 0.45µm membrane filter and retained in Chelex 100 resin.

- LMM positively charged Cu species: passing 10 kDa ultrafilter and retained in chelex 100 resin

2.4 Biological endpoints

2.4.1 Fertilization

To check the influence of the salt and Cu mixtures on fertilization success, 10 eggs were randomly collected after 24 hours of fertilization, from each of the replicates of all the treatments which were exposed during fertilization. The collected eggs were examined under a binocular microscope after being treated in a fixation liquid containing 14 g NaCl/L and 35 % vinegar. Eggs were classified as *not fertilized*, if lacking a white mass of cells in the centre.

2.4.2 Degree of swelling

To obtain information about the degree of swelling, the mean diameter of 20 eggs were measured with a ruler pre-fertilization, and compared to 20 randomly collected eggs of each treatment after 24 hours of post-fertilization.

2.4.3 Eyed eggs

Time was noted and the number of eyed egg in each boxes were counted and the percentage was calculated (according to the total number of egg is the box). The percentage of eyed eggs was linked to the number of day-degrees (sum of mean temperature during the period from fertilization to eyed egg).

2.4.4 Egg mortality

Dead eggs were picked and counted on daily basis and checked afterwards, under the microscope, according to classification of fertilized or not fertilized egg. Eggs were considered dead when parts of the content turned opaque and white.

2.4.5 Hatching success

Time and degree days at hatching were noted and the number of hatched and/or not hatched egg in each boxes were counted daily after hatching. Fully as well as incompletely hatched larvae were included (Stouthart et al, 1996).

2.4.6 Deformities

The percentage of larval deformation was determined after microscopic examination. Both alevins (after hatching) and embryos within non-hatched eggs were included.

3 Results and discussion

3.1 Water chemistry

The temperature during the whole experimental period ranged from 6.1-7.0℃, while pH was within 6.9-7.2 in all water qualities. During the total experimental period (exposure to reference water only or 24 hours to episodic treatments weekly) no significant differences in pH between treatments or temperature were observed. The concentration of oxygen in all water qualities was almost 12 mg/L (Table 1), indicating that the water in flow through boxes and circulating tanks had similar normoxic concentration. The conductivity in water increased linearly ($R^2 = 0.98$, $P<0.0001$) with increased concentration of salt, on average from 54 µS/cm to 13258 µS/cm for reference water and 10000 mg road salt/L, respectively. The concentration of TOC was 4.6±0.6 mg/L.

Table 1 Physical–chemical variables and chemical compositions in different treatments (n=19)

Water quality	Temp. (℃)	Conductivity (µS/cm)	pH	O_2 (mg/L)
Reference water	6.6±2.2	53.9±9.5	7±0.6	12.2±0.7
50 mg road salt/L	6.1±2.7	141±11	7.2±0.4	11.9±1.0
100 mg road salt /L	6.3±2.5	208±35	6.9±0.4	12.0±0.8
500 mg road salt /L	6.4±2.5	878±187	6.9±0.4	11.9±0.8
5000 mg road salt /L	6.3±2.7	8313±2891	6.9±0.2	11.8±1.1
10000 mg road salt /L	6.8±2.9	13258±3843	7.0±0.1	11.9±1.0
Reference water added with 10µg Cu/L	6.3±1.3	47.6±8.5	7±0.5	12.3±0.6
50 mg road salt /L added with 10 µg Cu/L	6.7±2.8	180±139	7.0±2.3	12.0±0.9
100 mg road salt /L added with 10 µg Cu/L	6.6±2.7	219±34	7.0±0.3	11.9±1.0
500 mg road salt /L added with 10 µg Cu/L	7.0±2.7	902±128	6.9±0.4	11.9±0.9
5000 mg road salt /L added with 10 µg Cu/L	7.0±3.0	6883±2372	7.0±0.1	11.9±1.0
10000 mg road salt /L added with 10 µg Cu/L	7.0±3	15079±3966	7.0±0.2	11.9±1.0
500 mg road salt /L added with 20 µg Cu/L	6.4±2.8	1865±1934	6.7±1.6	11.9±0.9
500 mg road salt /L added with 30 µg Cu/L	6.5±2.7	923±64	7.0±0.3	11.9±0.9

The concentration of total Cu in the reference water was low < 3 µg/L. In water with added Cu, the concentrations were 9.5, 24 and 28 µg Cu/L (Table 2). Results indicated that the concentration of colloidal Cu was reduced and that the concentration of particulate Cu increased with increasing concentration of road salt, probably due to aggregation. Results indicated also that the concentration of positively charged Cu species increased with increasing salt concentration, being higher at 10000 mg road salt /L than at lower salt concentration. This showed that increasing salt concentrations change the speciation and increase the bioavailability of Cu. This confirms the results from the pilot study as well as from the findings of Backstrom et al, (2004) that road salts mobilizes more reactive form of Cu.

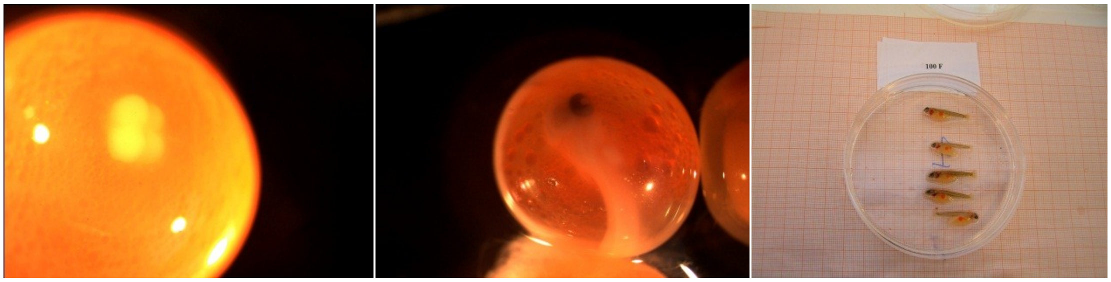
Table 2 Concentration of different Cu-fractions in treatments having different concentration of salt and Cu (n=19)

Water quality	Cu_{tot} (µg/L)	Particulate (µg/L)	Colloidal (µg/L)	0.45µm Cu positively charged (µg/L)	LMM (µg/L)	LMM Cu positively charged (µg/L)
Reference water	<3	<3	<3	<3	<3	<3
50 mg road salt/L	<3	<3	<3	<3	<3	<3
100 mg road salt /L	<3	<3	<3	<3	<3	<3
500 mg road salt /L	<3	<3	<3	<3	<3	<3
5000 mg road salt /L	<3	<3	<3	<3	<3	<3
10000 mg road salt /L	<3	<3	<3	<3	<3	<3
Reference water added with 10µg Cu/L	9.4±3.7	<3	5.8	<3	3.9	3.2
50 mg road salt /L added with 10 µg Cu/L	7.5±3.4	<3	3.8	<3	3.8	<3
100 mg road salt /L added with 10 µg Cu/L	10.7±1.1	<3	3.7	<3	3.7	<3
500 mg road salt /L added with 10 µg Cu/L	8.6±1.4	<3	<3	<3	3.6	<3
5000 mg road salt /L added with 10 µg Cu/L	11.5±1.3	4.4±2.3	<3	<3	6.9	3.3
10000 mg road salt /L added with 10 µg Cu/L	11.2±2	6.6±5	<3	3.4±2	3.7	3.7
500 mg road salt /L added with 20 µg Cu/L	24±6.4	3.9±3.7	8	7.9±7.4	5.8	6.6
500 mg road salt /L added with 30 µg Cu/L	28±12	<3	<3	11±6.5	17.7	8.4

3.2 Biological responses

3.2.1 Fertilization success

The fertilization success was 90-100 % in all groups. Neither salt nor Cu exposure during fertilization had any effect on the overall fertilization success. Some developmental stages after fertilization are present in Fig. 4.

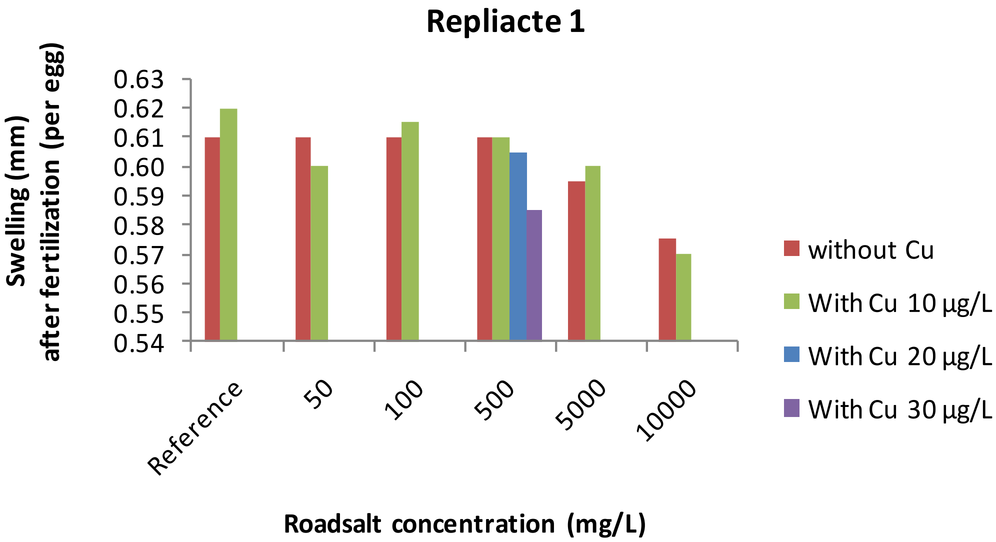


4 celled-stage Eyed egg Alevin

Figure 4. Development stages from 4-celled stage, through eyed egg to alevins

3.2.2 Degree of swelling

The results showed that high salt doses (≥ 5000 mg/L), regardless of Cu inhibited the swelling (Fig. 5). In addition, inhibition of swelling was also observed at 500 mg road salt/L in mixtures with elevated Cu concentrations (>20µg/L).



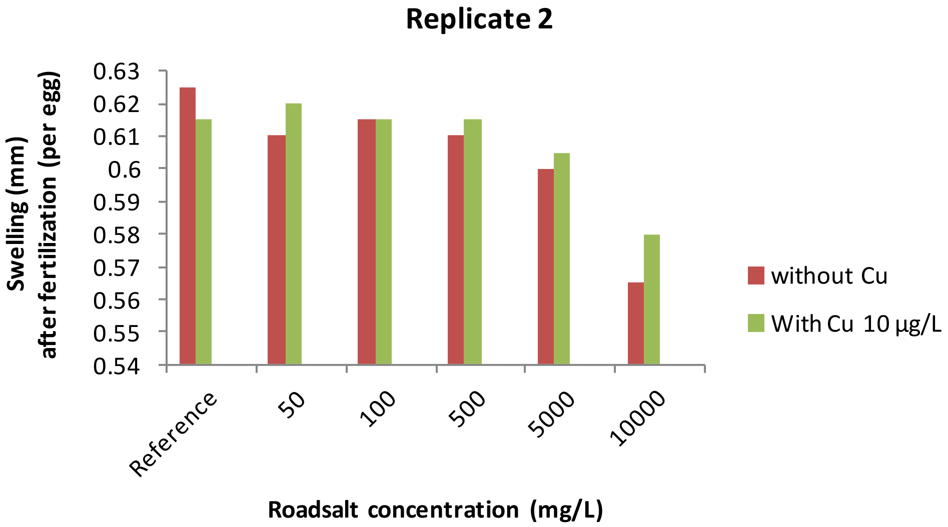


Figure 5. Increase in swelling (mm) in treatments having different concentration of road salt and Cu (Replicate 1 and 2).

3.2.3 Egg mortality

High mortality of egg was observed in the groups exposed to road salt during the fertilization, irrespective of Cu concentration (Fig. 7). More than 85 % egg mortality was found at 10000 mg road salt/L exposure and about 20 % egg mortality was found at 5000 mg road salt/L exposure. Compared to the reference water no significant egg mortality was observed at lower salt concentrations. For eggs episodically exposed to road salt after fertilization, no mortality was observed. This indicates that high concentrations of road salts are lethal for eggs of Atlantic salmon during fertilization, but not thereafter, when exposed to 24 hours weekly episodes. This reflects that exposure to episodic events is not that serious, and probably less serious than exposure to constant salt treatment after fertilization. Fig. 6 shows the time after exposure during fertilization when egg mortality was observed.

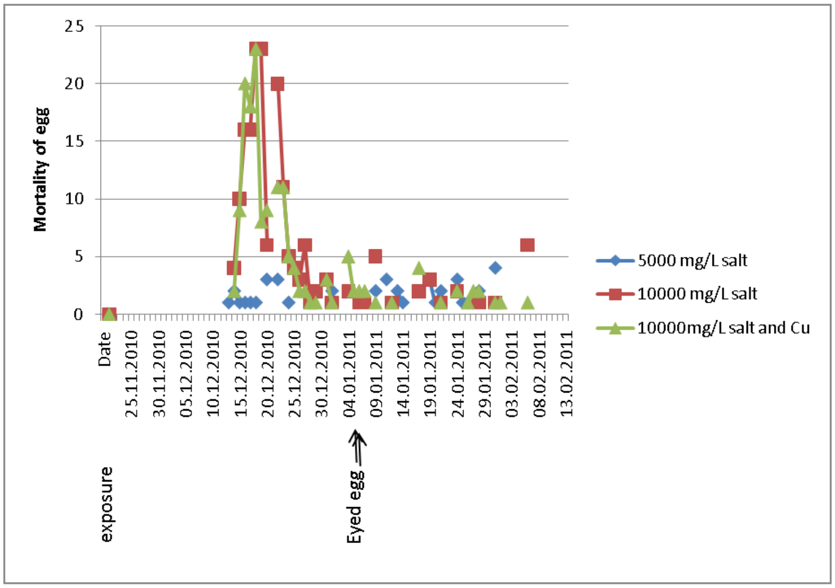


Figure 6. Time of egg mortality observed after exposure during fertilization.

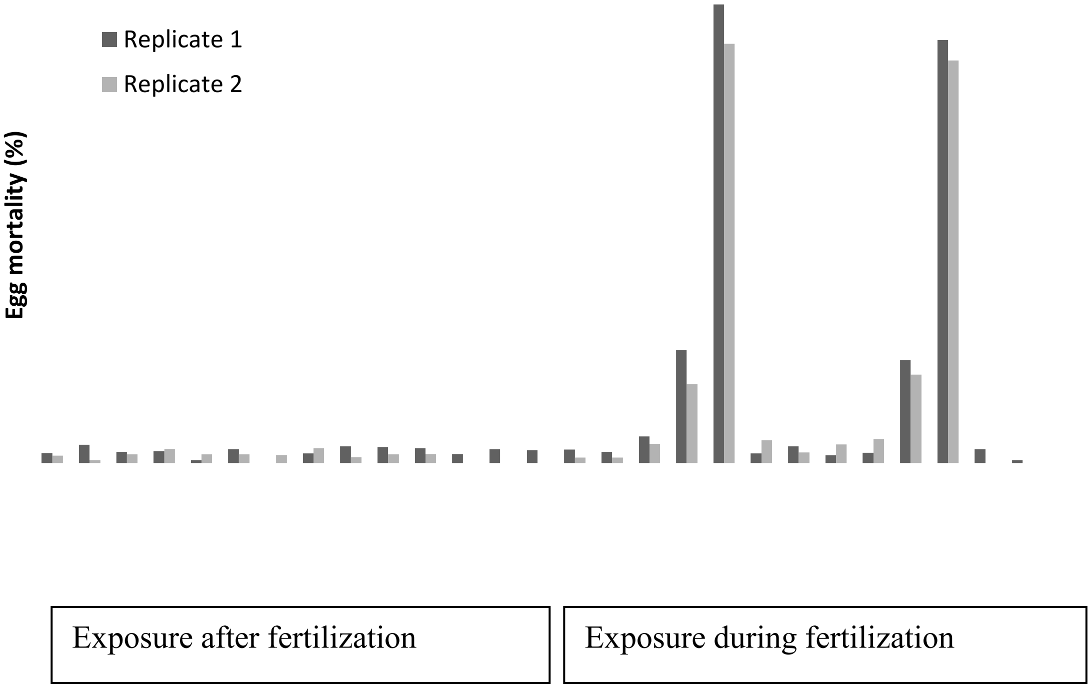


Figure 7. The percentage egg mortality in salt treatments during fertilization (F) and episodic (E) after fertilization, with and without Cu.

3.2.4 Hatching time

Eggs from all treatments reached the eyed-egg stage at the same date, 4th of January, 2011 after 255 degree days. Hatching started from 8th of February 2011 until 1st of March 2011, based on the average temperature, corresponding to 395 to 621 degree days. Hatching started and finished almost at the same time in all treatments for eggs exposed during fertilization. This showed that the exposure to salt during and after fertilization did not have any delaying effect on the development. In addition, the eggs exposed to different concentration of road salt and Cu during fertilization did not show any effect on hatching (Fig. 8 and 10).

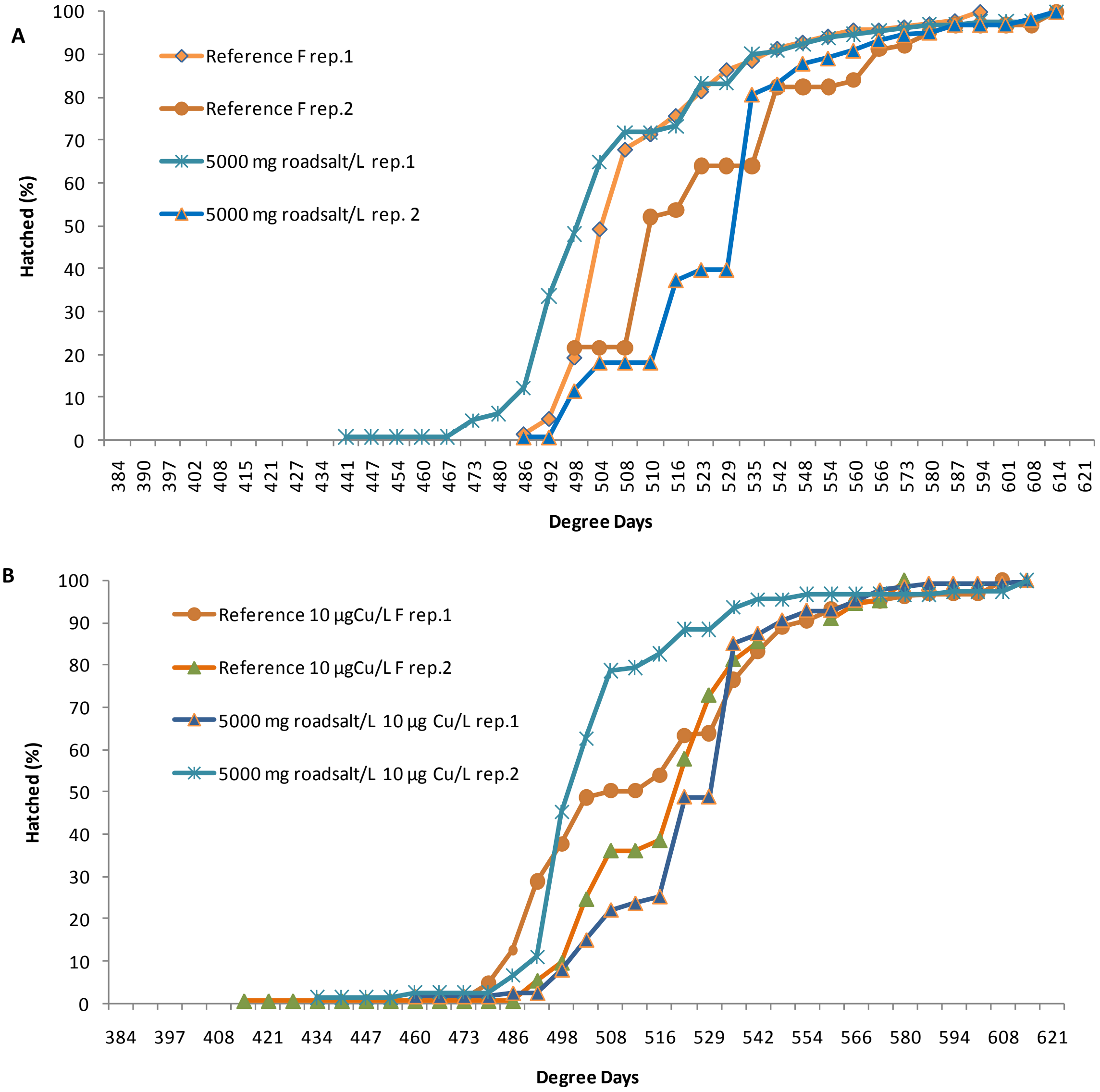


Figure 8. Hatching groups being exposed to road salt during the fertilization without (a) and with (b) Cu (Replicate 1 and 2)

Delayed time of hatching was however observed for eggs exposed to different concentrations of Cu after fertilization (Fig. 9 and 11). The time of hatching was more delayed with increasing the Cu concentrations in water (Fig. 11). This showed that Cu had significant negative effect on hatching, when combined with episodic salt treatments after fertilization.

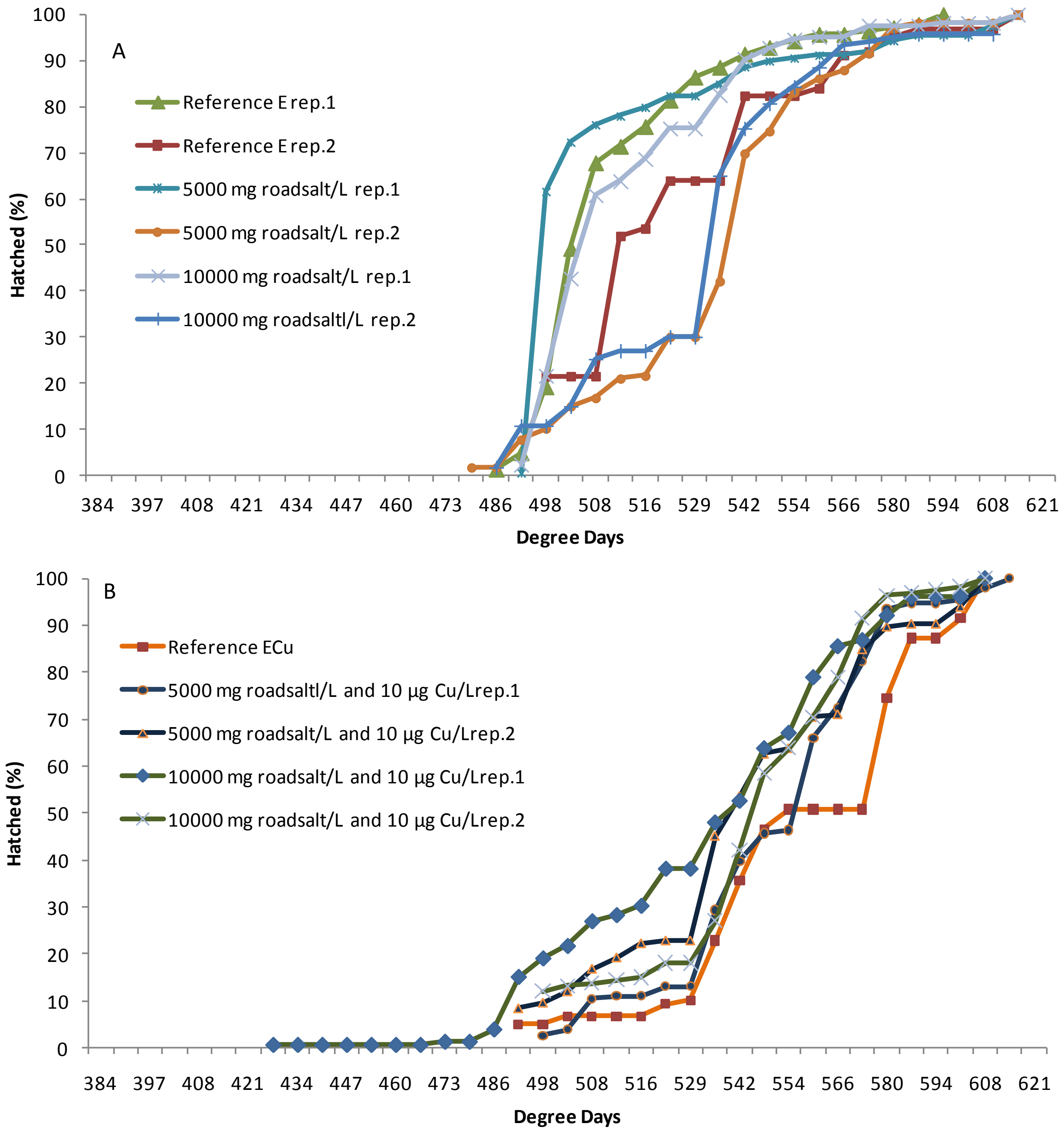


Figure 9. Hatching in groups being fertilized in reference water and the exposed to episodic road salt treatments, with and without Cu (Replicate (rep.) 1 and 2).

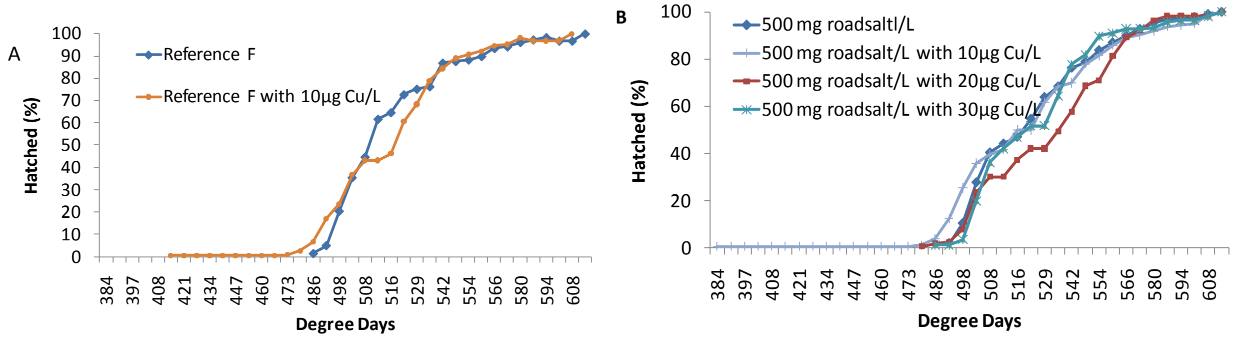


Figure 10. Hatching in groups being exposed to Cu during the fertilization

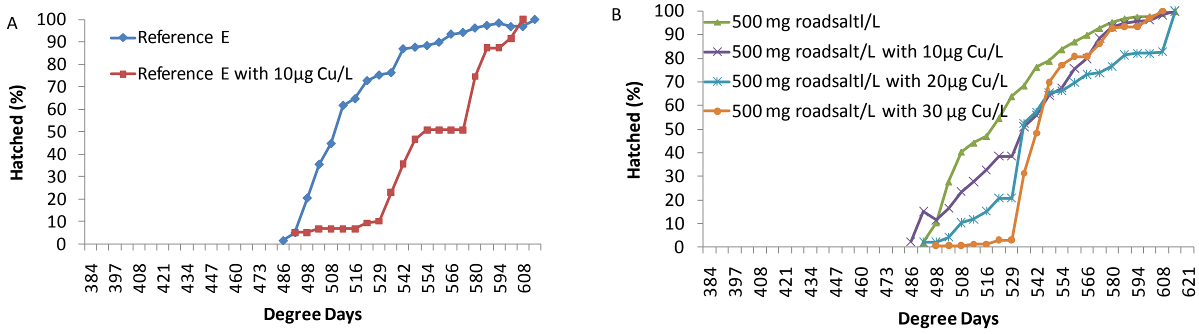


Figure 11. Hatching in groups being fertilized in reference water and exposed to Cu after fertilization (a) and episodic exposure to salt and increased Cu concentrations (b).

3.2.5 Deformities

Four types of deformities were observed in alevins after hatching (Table 3):

1. Coiled tail.
2. Twin bodies.
3 Twin heads
4.Spinal cord deformation.

Different treatments induced different types of deformities and the number of deformed alevins was also different (Fig. 12). Some of the treatments did not produce any deformities at all, showing no effect on the alevins.

Table 3 Deformities (%) of alevins after exposure to different levels of salt without and with Cu during and after fertilization.

	Exposure episodic after fertilization						Exposure during fertilization				
Road salt (mg/L)	100	500	5000	100	500	5000	50	100	50	100	5000
Cu (µg/L)	<2	<2	<2	10	10	10	<2	<2	10	10	10
Deformities types											
Coiled tail	0.4	0.4	0.4	0.4	0	0	0.4	0.4	0.4	0.4	4
Twins (two bodies)	0	0	0	0	0	0	0	0	0	0	1
Twins (two heads)	0	0	0	0.4	0	0	0	0	0	0	1
Spinal cord deformation	0.4	0	0.4	0	0.4	0.4	0.4	0.4	0.4	0.4	3

No deformities were found in the reference water, either with or without Cu (Table 3). Highest percentage deformities were found in 5000 mg road salt/L with 10 µg Cu/L. High road salt concentrations without Cu, or only Cu exposure even at high concentrations during the fertilization, gave low percentage deformities. The results indicated interacting effects of salt and Cu.

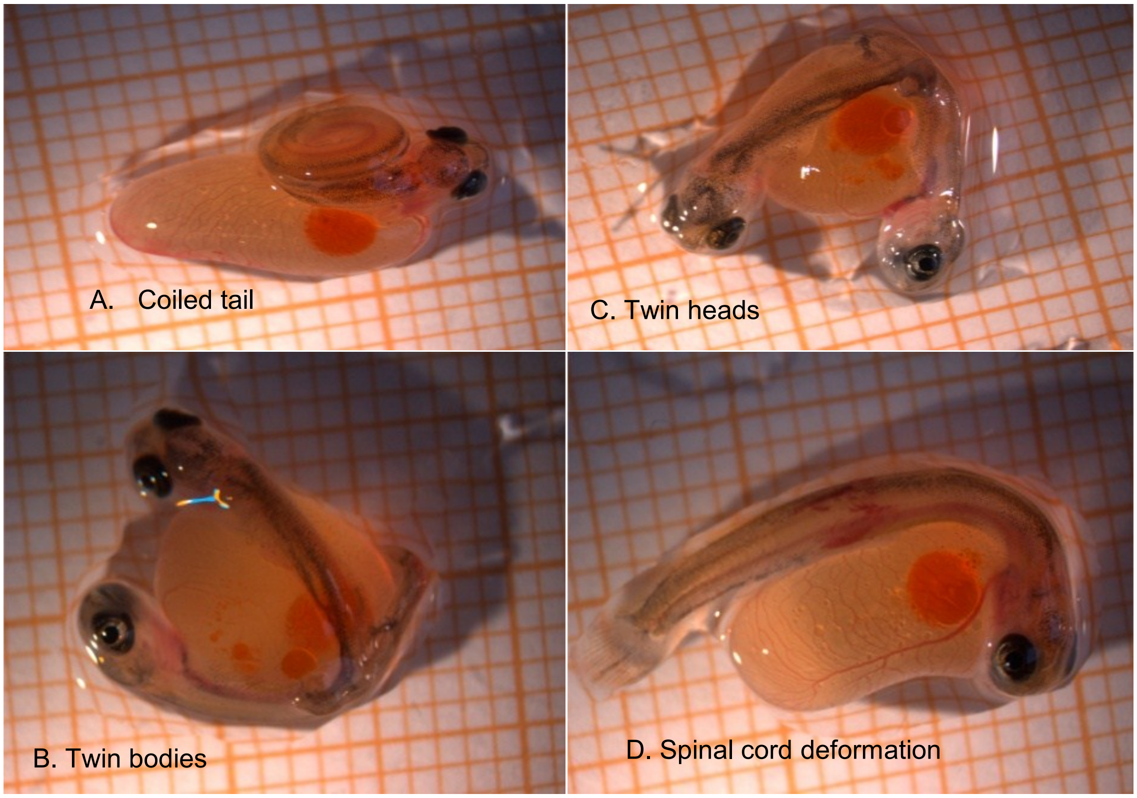


Figure 12. Different types of deformities observed in Atlantic salmon alevins due to exposure to road salt and Cu (Table 3)

4 Conclusion

The results indicated negative and lethal effects on fertilization and the early development stages of Atlantic salmon exposed to high road salt concentrations (≥5000 mg/L). Different susceptibility was, however, observed for the fertilization stage compared to the embryonic post-fertilization stage. The results can be summarized as:

- High mortality due to salt exposure during fertilization indicated high sensitivity towards salt at this stage.
- When eggs were exposed to different road salt concentrations in weekly episodes post fertilization, no effects were seen on mortality.
- Exposure to salt in combination with Cu during fertilization resulted in other detrimental effects, as an increased incident of deformities was observed due to salt and co-exposure of Cu compared to only salt exposure or only Cu exposure. This is observed at 5000 mg road salt/L and with 9.5 µg Cu/L. Effects at lower concentrations could not be omitted.

The results indicated, therefore, multiple stressor effects of road salt in combination with other stressors (Cu) in runoff from roads. Road salt could cause toxic effects itself towards aquatic organisms in freshwater, but also increase the mobilisation and possibly the bioavailability of other elements, like metals such as Cu, causing toxic effects and interacting effects.

In an environmental context, the spawning period seems to be the most sensitive period for Atlantic salmon at the earliest life history stage. In most parts of Norway, this will be in October-November and in a few areas also in December to early January. Depending on the snow condition, this probably means that the most significant exposures to combination of stressors from road salt and multiple elements from road pollution will be on the embryonic post-fertilization stage through hatching.

Concentrations of 5000 mg road salt/L will hardly ever occur in nature, and in addition, the episode needs to be at the exact time as spawning and fertilization. So the overall conclusion is that there is a relatively low risk of causing any detrimental effect on early life stages of Atlantic salmon. However, the endpoints used in this experiment is rather rough and we cannot exclude that there may be other effects at a lower biological complexity level (e.g. genetic, molecular, cellular) at lower road salt concentrations. A series of analyses, like gene analyses of eggs exposed to different salt/Cu combinations as well as the use of tracers to study fluxes of elements over the egg membrane will strengthen our knowledge of the effects of salt and their combined stressors. This will be evaluated in an follow-up project involving gene expression analysis.

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