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The effects of total dissolved solids on egg fertilization and water hardening in two salmonids—Arctic Grayling (*Thymallus arcticus*) and Dolly Varden (*Salvelinus malma*)

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ABSTRACT

Previous studies have indicated that salmonid fertilization success may be very sensitive to elevated concentrations of total dissolved solids (TDS) with effects at concentrations as low as 250 mg l^{-1} being reported. However, interpretation of these studies is complicated by poor control performance and variable concentration response relationships. To address this, a series of experiments were performed to evaluate TDS effects on Arctic Grayling (*Thymallus arcticus*) and Dolly Varden (*Salvelinus malma*) fertilization success and identify possible mechanisms for previously observed test variability and any observed effects of TDS. Results indicate that some of the experiments reported here were likely confounded by extended milt holding times prior to experiment initiation. Milt holding times >6 h were shown to significantly reduce control fertilization and corresponding concentration response relationships were variable. When milt holding time was minimized during fertilization experiments, consistent control performance with >90% control fertilization was achieved and consistent concentration response relationships were observed for both species examined. Experiments performed under these conditions indicate that Arctic Grayling and Dolly Varden fertilization success is not sensitive to elevated TDS with EC20s (concentration causing 20% effect) of >2782 and >1817 mg l^{-1} (the highest concentrations tested), respectively. However, TDS was shown to significantly affect embryo water absorption during the water hardening phase immediately following fertilization. The lowest observable effect concentrations (LOECs) for this endpoint were 1402 and 964 mg l^{-1} for Arctic Grayling and Dolly Varden, respectively. The effect of reduced embryo turgidity, due to impaired water absorption, on resistance to mechanical damage under real world conditions needs further investigation in order to understand the implications of this observed effect.

1. Introduction

Teck Cominco's Red Dog Mine (RDM) is located north of Kotzebue, Alaska. The RDM is a lead–zinc mine with on-site milling operations. In the milling process, RDM generates tailings that, together with mine drainage and otherwise impacted waters are deposited in an impoundment. Mining activities accelerate the naturally occurring oxidation of sulfide minerals such as pyrite (FeS_2) and sphalerite (ZnS), which results in the mine drainage water containing high levels of dissolved metal sulfates. Typical of many hard rock mining operations, the RDM utilizes a lime treatment plant for the removal of heavy metal contamination in the tailings impoundment water. The treatment plant removes the dissolved metals from solution and replaces them with calcium resulting in a high total dissolved solids (TDS) concentration in the whole effluent (comprised primarily of $CaSO_4$) of approximately 3300 mg l^{-1} (ranging between 2400 and 3900 mg l^{-1}). TDS is typically defined as the sum of major cations (Ca^{2+}, Na^+, Mg^{2+}, K^+) and anions (SO_4^{2-}, Cl^-, HCO_3^-) present in water.

The effluent is discharged to Red Dog Creek, a first order tributary of the Ikalukrok River, which is part of the larger Wulik River drainage. This drainage supports large populations of several salmonids including Dolly Varden (*Salvelinus malma*) and Arctic Grayling (*Thymallus arcticus*) which spawn in the upper drainage, including Red Dog Creek and the upper Ikalukrok River. As a result, the elevated TDS concentrations in the RDM discharge are of potential concern with respect to the protection of these fish.

The effects of elevated TDS, or the specific ions comprising TDS, on freshwater aquatic organisms have largely been limited to acute

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lethal testing of several standard test organisms (e.g., *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas*). Data from these studies have allowed development of statistical models investigating the sublethal toxicity of TDS to aquatic biota (Mount et al., 1997; Tietge et al., 1997). Specific to fish, these studies have investigated the effects of elevated TDS on egg fertilization, water hardening, and early survival.

Early studies on the effects of high TDS (13 mM $CaSO_4$; 1597 mg l^{-1} TDS) showed that egg fertilization was significantly impacted and that Ca^{2+} was the primary cause of observed effects (Ketola et al., 1988). More recently and specific to the RDM, Chapman et al. (2000) investigated the effects of elevated TDS on rainbow trout (*Oncorhynchus mykiss*). They simulated the ionic composition of the RDM effluent and a second mining effluent and evaluated the effects of increasing TDS concentrations on *O. mykiss* embryo viability as well as fry survival and growth during a 7-d exposure. No significant effects were observed for either simulated effluent at any concentration tested (up to 2000 mg l^{-1} TDS) for any of the endpoints.

Based on this study and a review of the extant literature, the State of Alaska granted RDM a site-specific water quality standard for TDS of 1500 mg l^{-1} in the mainstem Red Dog Creek during periods when salmonids are not spawning. During spawning periods, the limit was set at 500 mg l^{-1} TDS. The 500 mg l^{-1} TDS limit during periods of salmonid spawning is based on current State of Alaska Water Quality Regulations. However, a series of unpublished (see Weber-Scannell and Duffy (2007) for summary review) toxicity studies conducted by Stekoll et al. (2003a,b), has recently raised questions regarding the validity of the State Water Quality Standards for TDS.

Stekoll et al. (2003a) conducted several types of studies evaluating various endpoints including fertilization, early embryonic development, and long-term embryonic development. The first studies evaluated fertilization and development in 96-h assays with coho salmon (*Oncorhynchus kisutch*). By exposing eggs or embryos to elevated TDS before, after or during both fertilization and development the researchers determined which life stage was most sensitive. Results from these experiments clearly showed that eggs exposed to elevated TDS during fertilization were most sensitive.

In another set of experiments, Stekoll et al. (2003b) further evaluated TDS toxicity to site-specific populations of Arctic Grayling, Dolly Varden and chum salmon. The TDS composition was a simulation of RDM effluent. In the Arctic Grayling assay, a significant difference in fertilization was observed between the controls and the lowest concentration tested of 500 mg l^{-1}. However, there was no effect at the next two higher concentrations. In the chum salmon assay, Stekoll et al. reported there was strong evidence for fertilization effects at 250 mg l^{-1}; however, fertilization was also low in the controls. Finally, in the Dolly Varden assay, the authors could make no conclusions on the effects of TDS to this species due to low fertilization rates in all treatments including the control.

Overall, the results for all three species were inconclusive due to interrupted concentration-response relationships or poor fertilization rates in the controls. However, they raise the possibility of effects on salmonid spawning at TDS concentrations below the site-specific criterion issued for mainstem Red Dog and Ikalukrok Creeks of 500 mg l^{-1} TDS currently applied to RDM effluent during salmonid spawning periods. Given the intra- and inter-species variability observed in the Stekoll et al. studies, and the fact that some of the effects thresholds estimated approach background TDS concentrations where salmonids successfully spawn, further research is needed on the effects of TDS on salmonid embryo fertilization. Ideally, additional studies would be conducted to determine effect thresholds with greater precision than has been achieved to date.

The objectives of the present studies were to provide a better understanding of the potential effects of TDS on salmonid fertilization success using the two species known to spawn near in the Red Dog and upper Ikalukrok creeks, *T. arcticus* and *S. malma*. Further, if significant effects were observed, we sought to gain an improved understanding of the mechanism(s) by which elevated TDS may affect the early life stages of salmonids. While the current study is site-specific, discharge of high TDS hard rock mining effluent into streams where salmonids spawn is relatively common in western North and South America giving broader applicability to the results and implications of this study.

2. Methods and materials

2.1. General

Studies on Arctic Grayling were conducting in May–June 2004 during their spawning season while Dolly Varden experiments were performed in September 2004 during their corresponding spawning season. As discussed below, relatively high variability in the Arctic Grayling results prompted additional studies on this species in May 2005.

The Stekoll et al. studies evaluated the relative sensitivity of several different endpoints/life stages. The first endpoint, termed the fertilization endpoint, included fertilization, water hardening and embryo development through 50% epiboly. Other endpoints included embryo development from 50% epiboly up to hatching, hatching success, and larval growth and survival. Although there were significant uncertainties regarding what concentration of TDS caused effects, these studies were relatively conclusive in demonstrating that the fertilization endpoint (fertilization, water hardening and development through 50% epiboly) was the most sensitive of those evaluated. Based on these results our experiments focused on this endpoint as well. However, in addition to the standardized tests that generally copied the Stekoll et al. methodology, we also conducted experiments to evaluate the effects of elevated TDS on water absorption and net ion flux during the water hardening phase of development, as well as experiments evaluating TDS effects and milt storage time on sperm longevity. The objective of these experiments was to potentially elucidate physiological mechanisms by which any observed toxicity might be manifested.

2.2. Fish collection

The movement of adult fish upstream into the upper Ikalukrok and Red Dog creeks was closely monitored to facilitate collection as soon as they reached spawning beds, when gametes are at their optimum quality. Adult Arctic Grayling were collected from Bons Pond using hook and line and from North Fork Red Dog Creek using a fyke net. Sufficient fish were collected over a 2-d period in 2004 to conduct a total of 4 toxicity tests. After this period, additional females collected from Red Dog creek were either partially or completely spawned out, making them unsuitable for use in additional toxicity testing. In 2005, Arctic Grayling were collected from the same two locations allowing 4 additional toxicity tests to be performed with gametes from these animals.

Dolly Varden were collected from upper Ikalukrok Creek and on the Wulik River approximately 43 km upstream of the confluence with Ikalukrok Creek. All fish were collected by beach seine in September 2004. Adult fish not used the day of capture were held at the collection site in hoop nets (males and females kept in separate nets). A total of 7 toxicity tests were conducted on Dolly Varden over the course of the study period.

Collected fish were spawned in the field with gametes from individual males and females collected separately into 50 ml polypropylene test tubes and placed on ice for transport back to the laboratory. Once in the laboratory, both eggs and milt were stored in an environmental chamber maintained at the test tem-

perature used for each of the species. Milt was carefully inspected for quality prior to experimentation. When excessive blood or feces were present, the milt was discarded. Once in the laboratory, milt quality was further evaluated by placing a small subsample on a microscope slide, adding a drop of freshwater and observing motility (Environment Canada, 1998). For the Arctic Grayling testing, only highly active milt was used in testing and the sperm density ($0.66–1.35 \times 10^{10}$ sperm ml^{-1}) for pooled milt samples used to conduct the toxicity tests were within a factor of 2 of each other for all tests conducted.

In contrast to this, Dolly Varden milt generally had low or no activity in all males sampled. Milt used in only one of the Dolly Varden experiments had activity that approached what was typically observed for Arctic Grayling. Low sperm motility has previously been observed for Dolly Varden (F. Decicco, personal communication) that still produced high fertilization rates. Given this, we did not use sperm motility as a screening tool for milt quality in the Dolly Varden testing. Sperm density for the Dolly Varden used in toxicity testing was also more variable than for Arctic Grayling, varying in density by a factor of 5.5 ($0.25–1.37 \times 10^{10}$ sperm ml^{-1}).

2.3. Toxicity tests

The general experimental design was similar for all tests. All testing was conducted on-site at Teck Cominco's RDM in a building separate from the mine/mill facilities. Approximately 30–50 eggs were placed in 30 ml polypropylene cups along with 20 μl of milt. Care was taken to ensure milt and eggs did not contact each other (i.e., no dry fertilization was allowed). To this, 5 ml of the test solution was added in a manner that rapidly mixed the milt and eggs together. Eggs were allowed to fertilize for 2 min after which they were rinsed twice with 10 ml of fresh solution and then transferred to 1 l beakers. Each test beaker contained 500 ml of test solution and was placed on gentle aeration (~100 bubbles/min.) in a temperature controlled environmental chamber at 6 °C for the Arctic Grayling and 5 °C for the Dolly Varden.

For each test conducted in 2004, nominal TDS concentrations of 125, 250, 500, 750, 1000, and 2000 mg l^{-1} were evaluated. The 125 mg l^{-1} treatment served as the control group for all toxicity tests. The exception to this was the first Arctic Grayling test (AG1) in which the 2000 mg l^{-1} TDS treatment was omitted due to the limited number of eggs available for testing. In 2005, higher nominal TDS concentrations of 150, 300, 500, 750, 1500, and 3000 mg l^{-1} TDS were tested based on 2004 results where no significant effects were observed at the highest TDS concentration tested in tests with acceptable control performance (see Section 3).

All salts used to make the test waters were either technical or reagent grade (Sigma Chemicals, St. Louis, MO). Temperature, dissolved oxygen and pH were measured at test initiation and termination. Samples were collected from each treatment for measurement of ionic composition. Each treatment was tested in either triplicate or quadruplicate depending on the amount gametes available.

Because of the difficulty in discerning fertilization in Arctic Grayling embryos at 24 h, the exposure period was extended to 72 h after which the embryos were fixed in Stockard solution (5% formaldehyde, 4% glacial acetic acid, 6% glycerin) for later inspection. In contrast, fertilization was easily discernable at 24 h for Dolly Varden embryos, and all tests were terminated at this time. Embryos for both species were scored as fertilized/unfertilized at the University of Miami using a dissecting microscope.

2.4. Characterization of water uptake in embryos

To characterize the potential effects of elevated TDS on water hardening of the newly fertilized embryos, a separate experiment was performed in which embryo mass was determined as a function of TDS exposure concentration. It was assumed that changes in embryo mass would primarily be a function of water absorption during initial development. In the experiment with Arctic Grayling, embryos were exposed to measured TDS concentrations of 145, 784, 1402, and 1381 mg l^{-1} for up to 840 min after fertilization. At 0, 5, 10, 20, 40, 60, 90, 120 and 840 min after fertilization, 10 embryos were sampled from each treatment and individual embryo mass was determined to the nearest 0.1 mg. The Dolly Varden experiment used the same design as described above, but measured TDS concentrations were 250, 585, 964, and 1789 mg l^{-1} in this experiment.

Table 1
2004 Arctic Grayling test media ionic composition (mM).

Parameter	Nominal TDS concentration (mg l^{-1})					
	125	250	500	750	1000	2000
Ca^{2+}	0.90	1.82	3.24	3.67	4.34	8.33
K^{+}	0.03	0.07	0.15	0.20	0.28	0.51
Mg^{2+}	0.10	0.20	0.38	0.62	0.74	1.60
Na^{+}	0.11	0.23	0.48	0.87	0.87	1.65
Cl^{-}	0.03	0.06	0.19	0.28	0.38	0.70
HCO_3^{-}	0.13	0.25	0.48	0.69	0.85	1.82
SO_4^{2-}	0.83	1.57	3.26	5.01	6.63	8.53
pH	7.1	7.3	7.7	7.8	7.6	7.8
TDS (mg l^{-1})	132	254	503	719	921	1381
Hardness (mg l^{-1})	100	202	362	429	509	1002

2.5. Analytical chemistry

Cations and anions were measured using atomic absorption (Varian SpectrAA 220FS) and ion chromatography (DIONEX DX-120), respectively, with the exception of bicarbonate, which was measured by double endpoint titration in the Arctic Grayling testing and using a total CO_2 analyzer (Corning 965) in the Dolly Varden tests. These two methods for determining bicarbonate concentrations have been cross validated to reveal excellent agreement (Grosell et al., 1999). Hardness was determined from measured Ca^{2+} and Mg^{2+} concentrations. Total dissolved solids were determined as the sum of measured ion concentrations.

2.6. Data analysis

The no observable effect concentration (NOEC), lowest observable effect concentration (LOEC), chronic value (geometric mean of the NOEC and LOEC) and concentration causing a 20% and 50% effect (EC20 and EC50) were determined in each test. The NOEC and LOEC were determined using analysis of variance after appropriate transformations and checks for normality and homogeneity of variance. The EC20 and EC50 (and their 95% confidence limits) were estimated using linear regression techniques (linear interpolation or probit analysis, as appropriate to the data). Data analysis was performed on measured TDS concentrations (not nominal) using ToxCalc Version 5.0 (Tidepool Scientific, McKinleyville, CA, USA).

3. Results

3.1. Water quality

Test temperature was maintained at 6 and 5 °C throughout all of the studies conducted, for the Arctic Grayling and Dolly Varden tests, respectively. Dissolved oxygen was maintained near saturation (~12 mg l^{-1}) in all tests. The individual measured ion concentrations for each of the test treatments are summarized in Tables 1 and 2 for Arctic Grayling (2004 and 2005), and Table 3 for Dolly Varden. In general, measured ion concentrations closely

Table 2
2005 Arctic Grayling test media ionic composition (mM).

Parameter	Nominal TDS Concentration (mg l^{-1})					
	150	300	500	750	1500	3000
Ca^{2+}	0.62	1.20	1.87	3.27	6.06	13.77
K^{+}	0.04	0.08	0.12	0.19	0.36	0.66
Mg^{2+}	0.15	0.21	0.28	0.40	0.66	1.19
Na^{+}	0.05	0.28	0.57	1.09	2.09	4.18
Cl^{-}	0.08	0.12	0.18	0.31	0.48	0.93
HCO_3^{-}	0.15	0.31	0.41	0.69	1.15	2.05
SO_4^{2-}	1.06	2.17	3.48	5.81	10.36	20.02
pH	6.8	6.8	6.9	7.1	7.3	7.6
TDS (mg l^{-1})	145	294	465	784	1402	2782
Hardness (mg l^{-1})	77	141	214	367	671	1497

Table 3
Dolly Varden test media ionic composition (mM).

Parameter	Nominal TDS concentration (mg l^{-1})					
	125	250	500	750	1000	2000
Ca^{2+}	0.70	1.30	2.52	3.74	4.72	9.81
K^{+}	0.05	0.05	0.05	0.05	0.05	0.05
Mg^{2+}	0.58	0.58	0.58	0.58	0.58	0.58
Na^{+}	0.96	1.04	1.04	1.04	1.04	1.09
Cl^{-}	0.05	0.05	0.05	0.06	0.06	0.05
HCO_3^{-}	1.05	1.11	1.23	1.16	1.20	1.23
SO_4^{2-}	1.36	2.10	3.73	5.19	6.80	13.25
pH	7.7	7.7	7.7	7.7	7.7	7.7
TDS (mg l^{-1})	263	363	576	761	957	1784
Hardness (mg l^{-1})	127	187	310	432	529	1036

approximated nominal values but were slightly higher at low TDS concentrations due to low levels of TDS in the on-site deionized water.

3.2. Toxicity test results

A total of 8 toxicity tests were successfully conducted with Arctic Grayling and 7 with Dolly Varden embryos (Table 4). All 8 of the Arctic Grayling tests resulted in >80% control fertilization while 5 of 7 Dolly Varden tests resulted in control fertilization >80%. The two Dolly Varden tests that did not achieve acceptable control fertilization are discussed further below.

Results from all tests are summarized in Table 4 and Figs. 1 and 2. Considerable inter-test variability was observed in the 2004 Arctic Grayling experiments with 2 tests exhibiting high control fertilization (≥97%) and no significant effect of TDS up to the highest concentration tested (Fig. 1a). In contrast, the other two tests, one of which had lower (83%) control fertilization, exhibited a U-shaped concentration response similar to that observed in some of the Stekoll et al. experiments. Less variability was observed in the 2004 Dolly Varden studies with 5 of the 7 tests exhibiting high control fertilization (≥90%) and no significant effect of TDS up to the highest concentration tested (Fig. 2). However, for 2 tests, an inverse

Table 4
Toxicity testing results with Arctic Grayling and Dolly Varden (mg l^{-1} TDS).

Test	NOEC	LOEC	EC20	EC50
AG1	921	>921	>921	>921
AG2	1381	>1381	>1381	>1381
AG3	254	503	748	>1381
AG4	132	254	202	>1381
AG5	2782	>2782	>2782	>2782
AG6	2782	>2782	>2782	>2782
AG7	2782	>2782	>2782	>2782
AG8	2782	>2782	>2782	>2782
DV1	1817	>1817	>1817	>1817
DV2	1789	>1789	>1789	>1789
DV3	1704	>1704	>1704	>1704
DV4[a]	1762	>1762	>1762	>1762
DV5	1777	>1777	>1777	>1777
DV6	1796	>1796	>1796	>1796
DV7[a]	1808	>1808	>1808	>1808

[a] Inverse dose response relationship observed in this test.

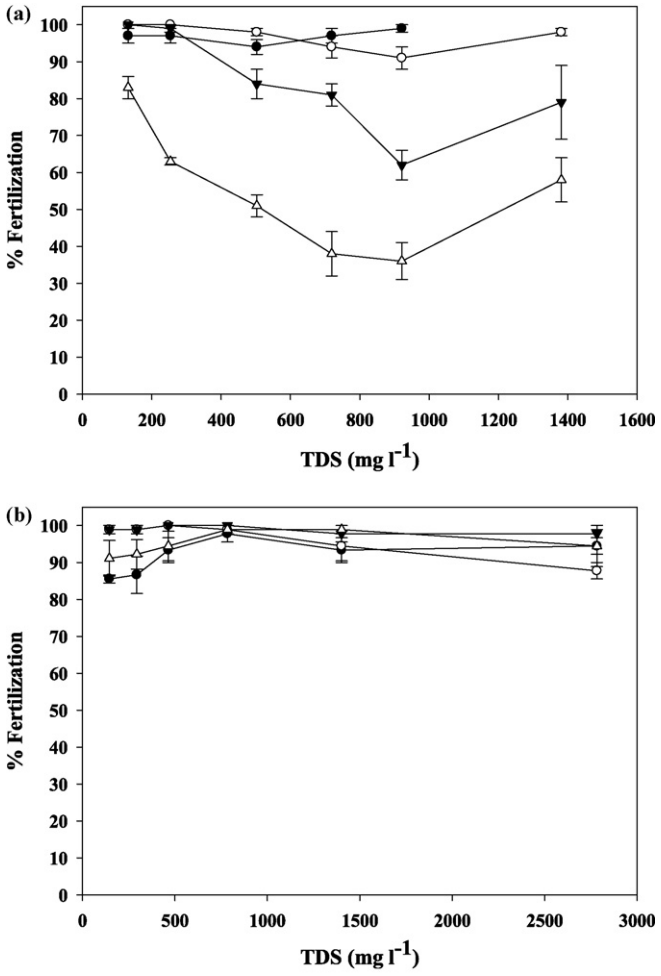

Fig. 1. (a) Arctic Grayling embryo fertilization as a function of TDS concentration. Results of 4 individual tests performed in 2004. (b) Arctic Grayling embryo fertilization as a function of TDS concentration. Results of 4 individual tests performed in 2005.

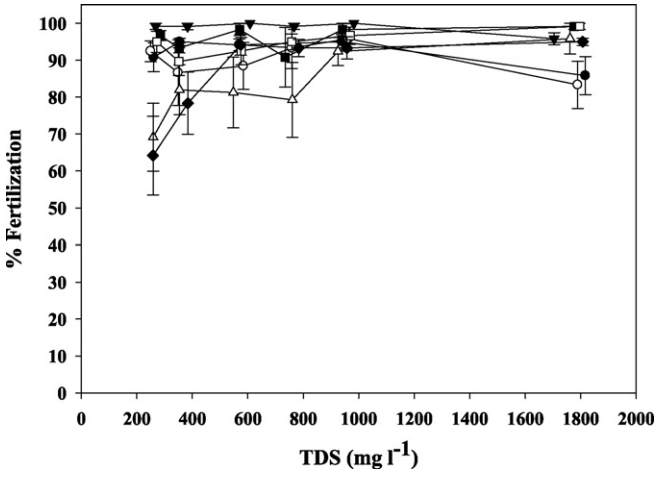

Fig. 2. Dolly Varden embryo fertilization as a function of TDS concentration. Results of 7 individual tests performed in 2004.

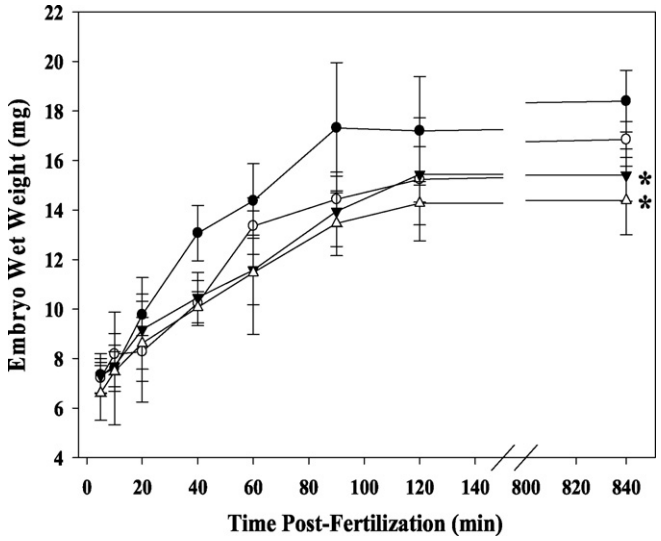

Fig. 3. Arctic Grayling embryo water absorption as a function of TDS concentration ($n = 10$ for each sampling point). (●) 145 mg l^{-1}, (○)784 mg l^{-1}, (▼) 1402 mg l^{-1}, and (△) 2782 mg l^{-1} TDS. *Significantly different from control ($p < 0.05$). Statistical analysis of only the last time point is shown.

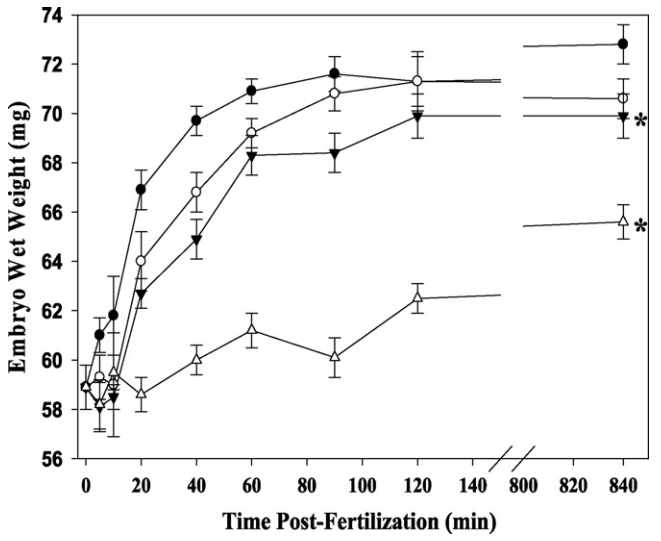

Fig. 4. Dolly Varden embryo water absorption as a function of TDS concentration ($n = 10$ for each sampling point). (●) 250 mg l^{-1}, (○) 585 mg l^{-1}, (▼) 964 mg l^{-1}, and (△) 1789 mg l^{-1} TDS. *Significantly different from control ($p < 0.05$). Statistical analysis of only the last time point is shown.

concentration response relationship was observed with relatively low control fertilization (64% and 69%), but fertilization comparable to the other 5 tests at higher TDS concentrations. All 4 of the Arctic Grayling experiments performed in 2005 were similar, with high control fertilization and no effects of TDS observed up to the highest concentration tested (Fig. 1b).

3.3. Water uptake in embryos

In Arctic Grayling, water absorption in control embryos appeared to reach steady-state 90 min after fertilization, with no significant change in wet weight from 90 min until the end of the exposure at 840 min (Fig. 3). At elevated TDS concentrations, steady-state water absorption appeared to be slightly delayed requiring between 90 and 120 min. After the full 840 min exposure, embryo wet weight was significantly ($p < 0.05$) lower in the 1402 and 2782 mg l^{-1} TDS treatments compared to the control. Although we did not perform a quantitative analysis, qualitatively, embryos from these higher TDS treatments exhibited an obvious reduction in turgidity.

Similar to Arctic Grayling, water absorption in Dolly Varden control embryos appeared to reach steady-state approximately 90 min after fertilization (Fig. 4). Steady-state water absorption was not significantly delayed at 585 or 964 mg l^{-1} TDS, but was significantly delayed in the 1789 mg l^{-1} TDS treatment with a significant ($p < 0.05$) increase in embryo wet weight between the 120 and 840 min sampling points. Additionally, again similar to Arctic Grayling, embryo wet weights were significantly lower in the two highest TDS treatments at the end of the 840 min exposure period, suggesting impairment of water absorption at elevated TDS concentrations. A reduction in embryo turgidity, although not quantified, was not as obvious in Dolly Varden embryos.

4. Discussion

4.1. Toxicity test results

The objective of this study was to resolve some of the uncertainties associated with previous studies conducted by Stekoll et al. on Arctic Grayling and other salmonids. Stekoll et al. (2003b) had previously reported an NOEC of 250 mg l^{-1} and LOEC of 500 mg l^{-1} TDS when testing Arctic Grayling embryos using methods similar to those reported here. However, mean control fertilization (~68%) was below what is normally considered acceptable in embryo studies (ASTM, 1998). Additionally, although statistically significant effects on fertilization were observed at 500 mg l^{-1} TDS, they did not observe statistically significant effects at 750 and 1250 mg l^{-1} TDS, creating uncertainty as to where the true effect level occurred.

In comparison, in 2004 all of the Arctic Grayling tests conducted in the present study achieved >80% control fertilization. However, results from the 2004 studies did not resolve the uncertainty associated with previous studies as two of the tests conducted indicated no significant effect of TDS on fertilization success up to the highest concentration tested while the other two tests did indicate effects with one test having effects below the 500 mg l^{-1} TDS water quality standard. Further, for the two tests where effects were observed, a reduced effect was observed in the highest TDS concentration tested, repeating the unusual concentration-response previously observed by Stekoll et al. (2003b).

The observed variability in the 2004 tests may be the result of natural differences in the quality and sensitivity of Arctic Grayling embryos to TDS. The increased sensitivity to TDS in the second two tests from 2004 generally corresponded to reduced control fertilization suggesting the embryos were less robust than in the first two tests. The increased sensitivity in the second two tests also corresponded with an increase in ambient temperature from which the adults were collected. Finally, the increased sensitivity also corresponded with the end of the spawning window for the Arctic Grayling. One or more of these factors may have contributed to the observed variability. It is also possible that the variability in the 2004 tests is the result of an artifact in the test method, a possibility discussed further below.

The Dolly Varden studies were much more conclusive than the 2004 Arctic Grayling studies. None of the experiments on Dolly Varden indicate a significant effect on fertilization success even up to the highest concentration tested. However, two of the Dolly Varden tests did exhibit an inverse concentration response relationship with the lowest fertilization success in the controls and highest fertilization success in the highest TDS treatment. We hypothesize this inverse concentration response relationship may be a result of extended milt holding time. When milt holding time is plotted

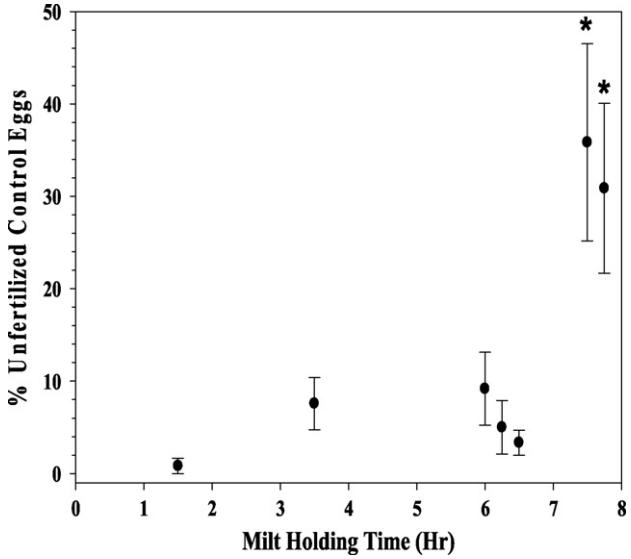

Fig. 5. Effect of milt holding time on Dolly Varden control fertilization. *Statistically different from control ($p < 0.05$).

against control fertilization, there appears to be a clear effect on milt quality when the holding time exceeds 6 h (Fig. 5).

Salmonid sperm is typically viable for several days when held at 5–6 °C (Scott and Baynes, 1980). However, it has also been shown that it is critical that sperm be well oxygenated during holding as cellular respiration is occurring to some extent in unactivated sperm. Consistent with the Stekoll et al. studies, milt was not actively aerated during holding, rather sperm were maintained in 50 ml polypropylene test tubes that were loosely capped to reduce evaporation and provide good air exchange. It is possible that these holding conditions did not provide sufficient oxygen for the sperm and the threshold for viability was being reached at approximately 7 h. Several previous studies have shown that sperm rapidly lose viability (within 3–5 h) if sufficient oxygen supply is not available (Smith and Quistorff, 1943; Henderson and Dewar, 1959). Interestingly, it has also been shown that exposure to high concentrations (10 mM) of Ca^{2+} and Mg^{2+} can counteract the effects of low oxygen supply, though the mechanism for this effect is unknown (Pautard, 1962). Given this, it is possible that the elevated Ca^{2+} in the higher TDS concentrations counteracted the oxygen depletion effect, providing a mechanism for the observed inverse concentration response relationship observed in several experiments during this study.

Based on the results of the Dolly Varden experiments, we hypothesized that the variable results observed in the 2004 Arctic Grayling experiments may also have been caused by excessive holding time for the milt. Exact records were not taken regarding milt holding time in the 2004 Arctic Grayling experiments, but in general holding time was >4 h and exceeded 8 h in at least one test. To address this issue, holding times for milt in the 2005 experiments were <3 h for all studies and <2 h for 3 of the experiments. With the exception of reducing the holding time, milt in the 2005 studies was treated exactly the same as in the 2004 studies. Similar to the Dolly Varden experiments, the 2005 Arctic Grayling experiments provided very consistent data indicating no effects on fertilization success up to the highest TDS concentration tested (2782 mg l^{-1}).

4.2. Effect of TDS on embryo water absorption

Because we were assessing fertilization success on embryos that have reached the epiboly stage of development, we hypothesized that any observed effects on fertilization success might actually be an effect on early embryo development. In particular, given the results of previous studies, we hypothesized that elevated TDS might interfere with water hardening. While the fertilization success experiments show rather conclusively that elevated TDS is not affecting Arctic Grayling and Dolly Varden fertilization, significant effects on water absorption were observed (Figs. 3 and 4).

There were interesting differences between the two species under control conditions. The small (6 mg wet weight) Arctic Grayling eggs underwent a nearly 3-fold increase in mass during water hardening while the comparatively larger (59 mg wet weight) Dolly Varden egg mass only increased by ~25%. Despite these inherent differences in water absorption, the two species appeared to be roughly similar in sensitivity with respect to the effect of TDS on water absorption.

The long-term impact of observed reductions in water absorption during the water hardening phase is unclear. In addition to the short-term fertilization assays, Stekoll et al. (2003a) performed longer exposures evaluating hatching success and early larval development. These life stages were less sensitive than fertilization suggesting that the long-term impacts of reduced water absorption may not be significant. However, these experiments obviously do not simulate real world conditions where embryo turgidity and corresponding resistance to mechanical damage are likely to be much more important. Given the data in this study indicate that fertilization success is not very sensitive to elevated TDS for Arctic Grayling and Dolly Varden, effects on water hardening appear to be the most sensitive endpoint evaluated to date and further research on this endpoint is needed.

5. Conclusions

Results from the experiments presented in this paper suggest that previous studies (and some of our own experiments) showing variable but relatively high sensitivity of salmonid fertilization success in high TDS waters may have been confounded by variation in milt holding time. When milt holding time is minimized, more consistent results are achieved indicating that TDS does not have a significant impact on Arctic Grayling and Dolly Varden fertilization up to the highest concentrations evaluated (2782 and 1817 mg l^{-1}, respectively). However, elevated TDS did significantly affect embryo water absorption at concentrations as low as 964 mg l^{-1} TDS (NOEC of 585 mg l^{-1}) and the ecological implications of this effect on embryo survival under real world conditions is worth further investigation. Given these results, the current site-specific water quality standard of 500 mg l^{-1} TDS during salmonid spawning periods and 1500 mg l^{-1} during other periods appears adequate for protection of salmonid reproduction. However, the 500 mg l^{-1} TDS standard during salmonid spawning periods should not be increased based on results from the fertilization study given the observed sensitivity of embryo water absorption to elevated TDS.

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