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REPORT ON THE EFFECTS OF TOTAL DISSOLVED SOLIDS ON ARCTIC GRAYLING AND DOLLY VARDEN FERTILIZATION SUCCESS

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1. INTRODUCTION

This report describes a study conducted to assess the effects of total dissolved solids (TDS) on fertilization success of the Arctic grayling (*Thymallus arcticus*) and Dolly Varden (*Salvelinus malma*). The study consisted of a series of laboratory experiments to develop dose-response relationships for TDS exposed embryos.

1.1. Background

Teck Cominco's Red Dog Mine (RDM) is located north of Kotzebue, Alaska. The RDM is a lead-zinc mine with onsite milling operations. In the milling process, RDM generates tailings that are deposited in an impoundment. All mine drainage water and mining impacted water are also collected in the tailings 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, as measured as total dissolved solids (TDS). The Red Dog Mine 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. Subsequently, the treatment plant effluent, which is ultimately discharged to Red Dog Creek contains high levels of calcium sulfate TDS. The total dissolved solids concentration in the whole effluent is approximately 3,300 mg/L (ranging between 2,400 and 3,900 mg/L). The average ionic composition of this effluent is summarized in Table 1.

RDM has been granted a site-specific water quality standard for TDS of 1,500 mg/L in the Mainstem Red Dog Creek during periods when salmonids are not spawning. During spawning periods, the limit has been set at 500 mg/L TDS. The 500 mg/L TDS limit during periods of salmonid spawning is based on current State of Alaska Water Quality Regulations. A series of toxicity studies conducted by Stekoll et al., has recently raised questions regarding the validity of the State Water Quality Standards for TDS. The results and uncertainties associated with these studies have been previously summarized by Brix et al. (2004).

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 (based on presence of young of the year juveniles), 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 studies described in this report were to develop sufficient data to provide a better understanding of the potential effects of TDS on local salmonid fertilization success.

Table 1. Ionic Composition of RDM Effluent

Ion	mg/L	mM[1]
Ca^{2+}	655	16.4
K^{+}	39	1.0
Mg^{2+}	40	1.6
Na^{+}	59	2.6
Cl^{-}	24	0.7
HCO_3^{-}	34	0.6
SO_4^{2-}	1838	19.2

[1] mM = millimolar

2. STUDY DESIGN

2.1. General

Studies on Arctic grayling (May-June) and Dolly Varden (September) were conducted in 2004. As discussed below, relatively high variability in the Arctic grayling results, prompted additional studies on this species in May 2005. Results from the two years of Arctic grayling experiments are assessed in an integrated manner in this report.

The Stekoll et al. (2003a; 2003b) studies evaluated a number of different endpoints. They considered fertilization success, embryo development, 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 fertilization success was the most sensitive endpoint of those evaluated. Based on these results and discussions with the regulatory agencies, our experiments focused on the fertilization endpoint and this report does not consider the potential effects of TDS on other endpoints.

2.2. Fish Sampling

Adult fish were collected with the assistance of ADNR and ADF&G personnel. The movement of fish upstream 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 (Station 12) using a fyke net. Sufficient fish were collected over a 2 d period (May 28-29) in 2004 to conduct a total of 4 toxicity tests (Table 2). After this period, additional females collected from Station 12 were either partially or completely spawned out, making them unsuitable for use in additional toxicity testing. In 2005, Arctic grayling collected from Bons Pond on May 28-May 31, and from the North Fork June 1-June 3. A total of 7 toxicity tests were initiated with gametes from these animals (Table 2).

Dolly Varden were collected from Ikalukrok Creek approximately 3.25 miles downstream of Station 160 and on the Wulik River approximately 26.5 miles upstream of the confluence with Ikalukrok Creek. All fish were collected by beach seine from September 12 through 16. 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 8 toxicity tests were conducted on Dolly Varden over the course of the study period (Table 2).

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 temperature 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 for pooled milt samples used to conduct the toxicity tests were within a factor of 2 of each other for all four tests conducted in 2004 (Table 3). Sperm density was not determined in 2005.

Table 2. Summary of Adult Arctic Grayling and Dolly Varden Captures Used in Toxicity Testing

Test	Collection Date	Location	Collection Method	# Adults
AG1	5/28/04	Bons Pond	Hook and Line	4M/3F
AG2	5/29/04	Station 12	Fyke Net	3M/1F
AG3	5/29/04	Station 12	Fyke Net	5M/2F[1]
AG4	5/29/04	Station 12	Fyke Net	2M/2F
AG5	5/28/05	Bons Pond	Hook and Line	12M/2F
AG6	5/29/05	Bons Pond	Hook and Line	12M/5F
AG7	5/30/05	Bons Pond	Hook and Line	14M/4F
AG8	5/31/05	Bons Pond	Hook and Line	13M/7F
AG9	6/1/05	Station 12	Fyke Net	2M/5F
AG10	6/2/05	Station 12	Fyke Net	3M/3F
AG11	6/3/05	Station 12	Fyke Net	3M/7F
DV1	9/12/04	Wulik R.	Beach Seine	1M/3F
DV2	9/14/04	Wulik R.	Beach Seine	1M[2]/1F
DV3	9/15/04	Ikalukrok Cr.	Beach Seine	1M/1F
DV4	9/15/04	Ikalukrok Cr.	Beach Seine	1M/1F[3]
DV5	9/15/05	Ikalukrok Cr.	Beach Seine	1M/1F
DV6	9/16/04	Wulik R.	Beach Seine	3M/1F
DV7	9/16/04	Ikalukrok Cr.	Beach Seine	1M/1F
DV8	9/16/04	Ikalukrok Cr.	Beach Seine	1M/7F

[1] One of the two females used in Test #3 was also the single female that supplied eggs for Test #2.

[2] The male used in this test was collected on 9/12/04.

[3] The female used in this test was half spent at time of collection.

Table 3. Sperm Cell Density in Pooled Milt Samples for Arctic Grayling in 2004

Test	Sperm Density (cells ml^{-1})
1	1.35×10^{10}
2	1.10×10^{10}
3	6.63×10^{9}
4	9.38×10^{9}

In contrast to this, Dolly Varden milt generally had low or no activity in all males sampled. Only the milt used in DV3 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 more variable than for Arctic grayling, varying in density by a factor of 5.5.

Table 4. Sperm Cell Density in Milt Samples for Dolly Varden

Test	Sperm Density (cells ml^{-1})
1	4.98×10^{9}
2	1.28×10^{10}
3	1.37×10^{10}
4	1.37×10^{10}
5	1.37×10^{10}
6	7.58×10^{9}
7	4.70×10^{9}
8	2.50×10^{9}

2.3. TDS Toxicity Tests

The general experimental design (e.g., test chambers, replicates, etc.) was similar for all tests as described in Section 2.3.2.

2.3.1. Test Facilities

All testing was conducted on-site at Teck Cominco's Red Dog Mine in a building separate from the mine/mill facilities. Deionized water was provided by the TCAK analytical laboratory. Tests were maintained in a refrigerator unit with double glass sliding doors to maintain the appropriate test

temperature. Test chambers were gently aerated using polyethylene tubing (PE%50) and aquaria air pumps.

2.3.2. Test Methods

The original intent was to closely follow the methods used by Stekoll et al. (2003a). However, conditions on site necessitated several deviations from the methods described by Stekoll et al. Specifically, modifications to the fertilization process were made in response to the low volume of milt available from Arctic grayling. Milt volume ranged from approximately 0.1 to 0.7 mL per male. The total volume of milt available for any test (after pooling) ranged from 0.3 to 1.2 mL. The workplan specified that fertilization would be accomplished in 1000 mL polypropylene containers each containing 30 eggs. To each container, 50 mL of test solution was to be added followed by the addition of 0.2 mL of milt and an addtional 100 mL of test solution to facilitate mixing of milt and eggs. Assuming 3 replicates of 6 treatments, at least 3.6 mL of milt would be required to conduct a test.

Given the low volume of milt available, the following modified protocol was used to accomplish fertilization. Approximately 30-50 eggs were placed in a 30 mL polypropylene cups along with 20 µL (0.02 mL) 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. The resulting solution is equivalent to adding 0.6 mL of milt to 150 mL of test solution (as compared with 0.2 mL of milt to 150 mL of solution in the original workplan). Eggs were allowed to fertilize for two minutes 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. Dolly Varden males supplied considerably more milt than Arctic grayling, ranging between ~10 mL and 40 mL per male. However, for the sake of comparability to the Arctic grayling tests, we opted to continue with the modified fertilization protocol described above for the Dolly Varden testing.

For each test conducted in 2004, target TDS concentrations of 125, 250, 500, 750, 1000, and 2000 mg/L were evaluated. The 125 mg/L (nominal) 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 TDS

treatment was omitted due to the limited number of eggs available for testing. For the 2005 Arctic grayling tests, it was decided that the objective should be to test water simulating a mixture of the ionic compositions of effluent from Outfall 001 and Station 12. As a result, target concentrations were 0, 6.25, 12.5, 25, 50 and 100% simulation 001 effluent using simulation Station 12 water for dilution.

All salts used to make the test waters were either technical or reagent grade (purchased from 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 our 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[1] after which the embryos were fixed in Stockard solution for later inspection. In contrast, fertilization was easily discernable at 24 hours 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.

2.3.3. Composition of Test Water

In 2004, we reasoned that because salmonids will not be exposed to whole effluent, but rather a mixture of effluent and receiving water, the most realistic exposure regime would mimic the ionic composition of these mixed waters. Further, the water quality during the period of time when salmon are spawning should be specifically considered. For Arctic grayling, Station 10[2] represents the most upstream condition to which embryos might be exposed, while Station 160 best represents the conditions to which Dolly Varden might be exposed. The ionic composition of the test waters was intended to mimic the composition at Station 10 in the May-June time period when Arctic grayling spawn (Table 3) and Station 160 in the August-September time period when Dolly Varden spawn (Table 4).

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[1] Terminating the exposures at 72 h was determined to be acceptable based on a blind scoring trial conducted in concert with ADNR scientists on site at the time of testing.

[2] Arctic grayling will spawn further upstream in the North Fork of Red Dog Creek, but Station 10 is the most upstream station prior to the confluence of the mainstem and North Fork of Red Dog Creek.

Because the ionic composition of Station 10 is very comparable to the effluent, the ionic strength of the Arctic grayling test media was varied by simply diluting a 2000 mg l^{-1} TDS test solution to achieve the specified TDS concentrations of 125, 250, 500, 750, 1000 and 2000 mg l^{-1}. In contrast, the ionic composition at Station 160 differs significantly from the effluent. This is not surprising given it is farther downstream and mixes with additional receiving water. Specifically, the relative contributions of Ca^{2+} and SO_4^{2-} to overall ionic strength are substantially lower, while that of HCO_3^- is substantially higher. The result is that Ca^{2+}, SO_4^{2-}, and HCO_3^- are present at approximately equimolar concentrations at Station 160, whereas Ca^{2+} and SO_4^{2-} occur at concentrations 6 and 30 times those of HCO_3^- at Station 10 and in the whole effluent, respectively. Our approach aimed at taking these differences into account as best possible.

The substantially different ionic composition at Station 160 has important implications for how TDS effects on Dolly Varden should be evaluated. It would not be appropriate to simply vary the ionic strength of Station 160 water by diluting a 2000 mg l^{-1} TDS test solution to achieve varying TDS concentrations. This would result in environmentally unrealistic HCO_3^- concentrations at higher TDS levels and would cause problems with precipitation of $CaCO_3$. Instead, varying TDS concentrations for the Station 160 water was achieved using only $CaSO_4$, as these are the predominant ions varying as a function of TDS concentration and the primary source of TDS in the effluent. All other parameters were maintained at the mean Station 160 concentrations shown in Table 4.

After discussions with USEPA and other regulatory agencies, it was concluded that a different approach would be used with respect to tests waters. Specifically, tests would be conducted with waters simulating the ionic composition of Outfall 001 (as best can be achieved given the limits of $CaSO_4$ solubility) and Station 12, effectively testing 0, 6.25, 12.5, 25, 50 and 100% simulated effluent. The ionic composition of Station 12 in the May-June time period was used for these experiments (Table 5).

Table 3. Ionic Composition at Station 10 (n=13)

Ion	mg l^{-1}	mM[\1]
Ca^{2+}	145	3.6
K^{+}	6.3	0.2
Mg^{2+}	12	0.5
Na^{+}	10	0.5
Cl^{-}	6.8	0.2
HCO_3^{-}	48[\2]	0.6
SO_4^{2-}	377	3.9

[\1] mM = millimolar

[\2] No data available, value derived by balancing charges

Table 4. Ionic Composition at Station 160 (n=10)

Ion	mg l^{-1}	mM[\1]
Ca^{2+}	67	1.6
K^{+}	1.8	0.05
Mg^{2+}	14	0.6
Na^{+}	4.5	0.2
Cl^{-}	2.6	0.07
HCO_3^{-}	88[\2]	1.5
SO_4^{2-}	152	1.6

[\1] mM = millimolar

[\2] No data available, value derived by balancing charges

Table 5. Ionic Composition at Station 12 (n=33)

Ion	mg l^{-1}	mM[\1]
Ca^{2+}	26.1	0.65
K^{+}	2.1	0.05
Mg^{2+}	3.5	0.14
Na^{+}	1.2	0.05
Cl^{-}	0.8	0.02
HCO_3^{-}	62[\2]	1.02
SO_4^{2-}	30.5	0.32

[\1] mM = millimolar

[\2] No data available, value derived by balancing charges

2.3.4. Analytical Chemistry

Cations and anions were measured using atomic absorption (Varian SpectrAA 220FS) and ion chromatography (DIONEX DX-120), with the exception of bicarbonate, which was measured by

double titration in the Arctic grayling testing and using a total CO2 analyzer (Corning 965) in the Dolly Varden tests. These two methods for 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 was determined as the sum of measured ion concentrations.

2.3.5. 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) was 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 (e.g., linear interpolation, probit analysis, as appropriate to the data). Data analysis was performed on measured TDS concentrations (not nominal).

3. RESULTS

3.1. WATER QUALITY

Test temperature was maintained at 6 °C 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) in all tests. The individual ion concentrations for each of the test treatments are summarized in Table 6 for Arctic grayling (2004), Table 7 for Arctic grayling (2005) and Table 8 for Dolly Varden.

For the 2004 Arctic grayling experiments, the general ratio of ions closely approximated nominal concentrations in the treatments ≤750 mg/L TDS (nominal). Calcium and sulfate concentrations were significantly less than nominal in the two highest treatments. In 2005, significantly higher TDS concentrations were achieved in the Arctic grayling experiments. Although there were minor difference in the ionic compositions of the waters from 2004 to 2005, it seems unlikely that this caused the significantly higher TDS concentrations achieved in 2005. For the Dolly Varden, measured test concentrations approximated nominal concentrations for all treatments, except for the 125 mg l^{-1} and 250 mg l^{-1} treatments, which had higher TDS concentrations (263 and 363 mg l^{-1}, respectively) than targeted.

Table 6. 2004 Arctic Grayling Test Media Ionic Composition (mg l^{-1})

	Nominal TDS Concentration					
Parameter	**125 mg l^{-1}**	**250 mg l^{-1}**	**500 mg l^{-1}**	**750 mg l^{-1}**	**1000 mg l^{-1}**	**2000 mg l^{-1}**
Ca^{2+}	36	73	130	147	174	337
K^{+}	1.3	2.7	5.7	7.7	11	20
Mg^{2+}	2.5	4.8	9.2	15	18	39
Na^{+}	2.5	5.2	11	20	20	38
Cl^{-}	1.2	2.3	4.6	6.7	9.3	17
HCO_3^{-}	8.1	15	29	42	52	111
SO_4^{2-}	80	151	313	481	637	819
pH	7.1	7.3	7.7	7.8	7.6	7.8
TDS	132	254	503	719	921	1381
Hardness	100	202	362	429	509	1002

Table 7. 2005 Arctic Grayling Test Media Ionic Composition (mg l^{-1})[1]

	Simulated Effluent Concentration with Simulated Station 12 as the Dilution Water					
Parameter	**0%**	**6.25%**	**12.5%**	**25%**	**50%**	**100%**
Ca^{2+}	25 ± 1	48 ± 0	75 ± 1	131 ± 2	243 ± 7	552 ± 58
K^+	1.7 ± 0.1	3.3 ± 0.1	4.8 ± 0.1	7.6 ± 0.1	14 ± 0	26 ± 1
Mg^{2+}	3.7 ± 0.1	5.0 ± 0.1	6.7 ± 0.1	9.7 ± 0.1	16 ± 0	29 ± 0
Na^+	1.2 ± 0.1	6.5 ± 0.2	13 ± 0	25 ± 1	48 ± 1	96 ± 4
Cl^-	3.0 ± 0.9	4.4 ± 0.2	6.3 ± 0.3	11 ± 1	17 ± 0	33 ± 1
HCO_3^-	9.3 ± 0.2	19 ± 1	25 ± 1	42 ± 3	70 ± 3	125 ± 5
SO_4^{2-}	102 ± 1	208 ± 0	334 ± 8	558 ± 3	995 ± 7	1922 ± 46
pH	6.75 ± 0.03	6.81 ± 0.01	6.90 ± 0.03	7.05 ± 0.03	7.33 ± 0.03	7.61 ± 0.03
TDS	145 ± 3	294 ± 1	465 ± 9	784 ± 4	1402 ± 14	2782 ± 75
Hardness	77 ± 2	141 ± 0	214 ± 2	367 ± 5	671 ± 17	1497 ± 145

[1] All values presented as mean ± s.d. of three measurements.

Table 8. Dolly Varden Test Media Ionic Composition (mg l^{-1})[1]

	Nominal TDS Concentration					
Parameter	**125 mg l^{-1}**	**250 mg l^{-1}**	**500 mg l^{-1}**	**750 mg l^{-1}**	**1000 mg l^{-1}**	**2000 mg l^{-1}**
Ca^{2+}	28 ± 1	52 ± 1	101 ± 4	150 ± 2	189 ± 5	393 ± 9
K^+	2.1 ± 0.5	2.1 ± 0.3	2.1 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	2.1 ± 0.2
Mg^{2+}	14 ± 0	14 ± 0	14 ± 0	14 ± 0	14 ± 1	14 ± 0
Na^+	22 ± 5	24 ± 1	24 ± 1	24 ± 1	24 ± 1	25 ± 2
Cl^-	1.8± 0.2	1.8 ± 0.2	1.8 ± 0.2	2.2 ± 0.6	2.3 ± 0.5	1.9 ± 0.2
HCO_3^-	64 ± 7	68 ± 4	75 ± 4	71 ± 5	73 ± 4	75 ± 5
SO_4^{2-}	131 ± 12	202 ± 13	358 ± 14	499 ± 12	653 ± 16	1273 ± 43
pH	7.7 ± 0.0	7.7 ± 0.0	7.7 ± 0.0	7.7 ± 0.0	7.7 ± 0.0	7.7 ± 0.0
TDS	263 ± 12	363 ± 15	576 ± 17	761 ± 16	957 ± 20	1784 ± 37
Hardness	127 ± 3	187 ± 5	310 ± 10	432 ± 6	529 ± 14	1036 ± 23

[1] All values presented as mean ± s.d. of eight measurements.

3.2. BIOLOGICAL RESULTS

As shown in Table 2, a total of 11 toxicity tests were conducted with Arctic grayling embryos and 8 toxicity tests with Dolly Varden embryos. All 4 Arctic grayling tests conducted in 2004 and 4 of the 7 tests conducted in 2005 resulted in >80% control fertilization. The first three tests conducted in 2005 all exhibited very low control fertilization (0, 18 and 7%, respectively). This low fertilization is attributed to collection of slightly "green" eggs very early in the spawning season. Because control fertilization was so low in these tests, eggs in higher treatments were not scored and these data were excluded from our analysis. For the Dolly Varden, 5 of 8 Dolly Varden tests

resulted in control fertilization >80%. The three Dolly Varden tests that did not achieve acceptable control fertilization are discussed further below.

Results from all tests are summarized in Table 9 and Figures 1 through 5. Tests with similar dose-response relationships are grouped together in Figures 1 through 5. Considerable inter-test variability was observed for both species, but within treatment variability for each test was low. Further the inter-test variability could be grouped into several distinct dose-response patterns. For six of the Arctic grayling tests (AG1, 2, 8, 9, 10 and 11) and five of the Dolly Varden tests (DV1, 2, 3, 6 and 7), no significant effect of TDS on fertilization success was observed (Figures 1, 3, and 4). For the other two Artic grayling tests (AG3[3] and 4), a decrease in fertilization success with increasing TDS concentrations up to 921 mg l^{-1} followed by a slight increase in fertilization success at the highest TDS concentration tested was observed (Figure 2). The remaining Dolly Varden tests fell into one of two distinct groups: In one group of two Dolly Varden tests (DV5 and 8), an inverse dose response relationship was observed with control fertilization ranging from 64-69% and then increasing with increasing TDS up to 95-96% in the highest treatment (Figure 5). Finally, in the remaining Dolly Varden test (DV4), low fertilization (14%) was observed in the control with a generally flat dose response relationship observed with increasing TDS concentrations (Figure 6).

[3] In AG3, a significant number of eggs did not contain viable embryos. When viewed under a dissecting microscope, these eggs appeared dark brown and contained no discernable embryo, unlike unfertilized eggs which contained an embryo, but showed no signs of development. AG3 used eggs from two females and it is likely that eggs from one of the females were largely inviable. For purposes of scoring this test, eggs without viable embryos were excluded from data treatment.

Table 9. Toxicity Testing Results with Arctic Grayling and Dolly Varden (mg l^{-1} TDS)

Test	NOEC	LOEC	Chronic Value	EC20	EC50
AG1	921	>921	>921	>921	>921
AG2	1381	>1381	>1381	>1381	>1381
AG3	254	503	357	748	>1381
AG4	132	254	183	202	>1381
AG8[\3]	2782	>2782	>2782	>2782	>2782
AG9	2782	>2782	>2782	>2782	>2782
AG10	2782	>2782	>2782	>2782	>2782
AG11	2782	>2782	>2782	>2782	>2782
DV1	1817	>1817	>1817	>1817	>1817
DV2	1789	>1789	>1789	>1789	>1789
DV3	1704	>1704	>1704	>1704	>1704
DV4	N/A[\1]	N/A	N/A	N/A	N/A
DV5	1762[\2]	>1762	>1762	>1762	>1762
DV6	1777	>1777	>1777	>1777	>1777
DV7	1796	>1796	>1796	>1796	>1796
DV8	1808[\2]	>1808	>1808	>1808	>1808

[\1] Invalid test due to low control fertilization
[\2] Inverse dose response relationship observed in this test
[\3] Results for AG5-7 are not reported due to unacceptable control fertilization

Figure 1. Arctic Grayling Test #1 and #2 Results

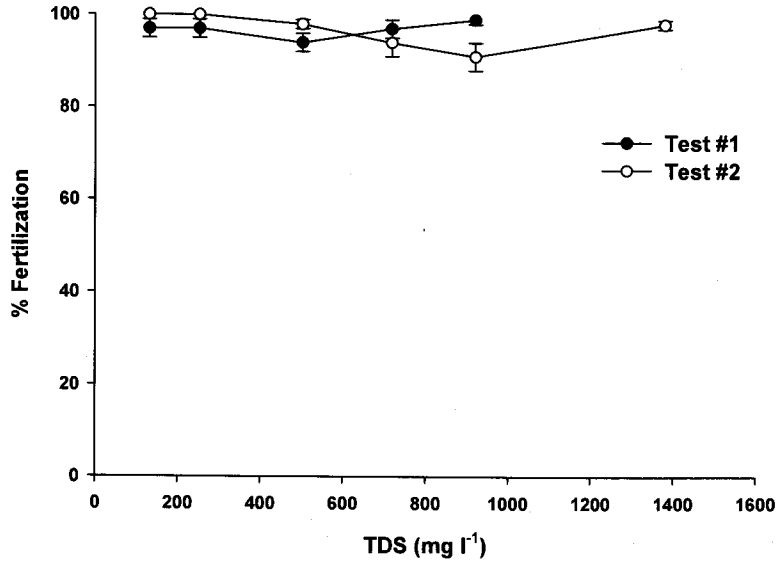


Figure 2. Arctic Grayling Test #3 and #4 Results

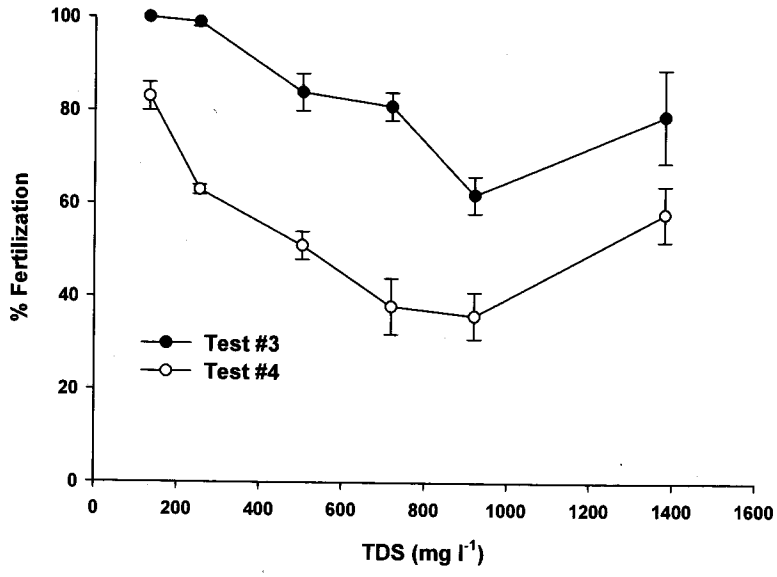


Figure 3. Arctic Grayling Test #8, 9, 10, 11 Results

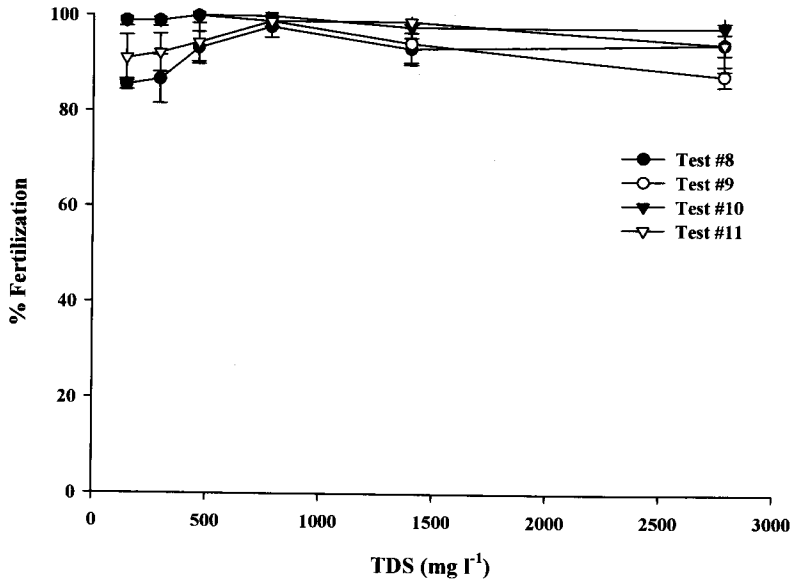


Figure 4. Dolly Varden Tests #1, 2, 3, 6, and 7 Results

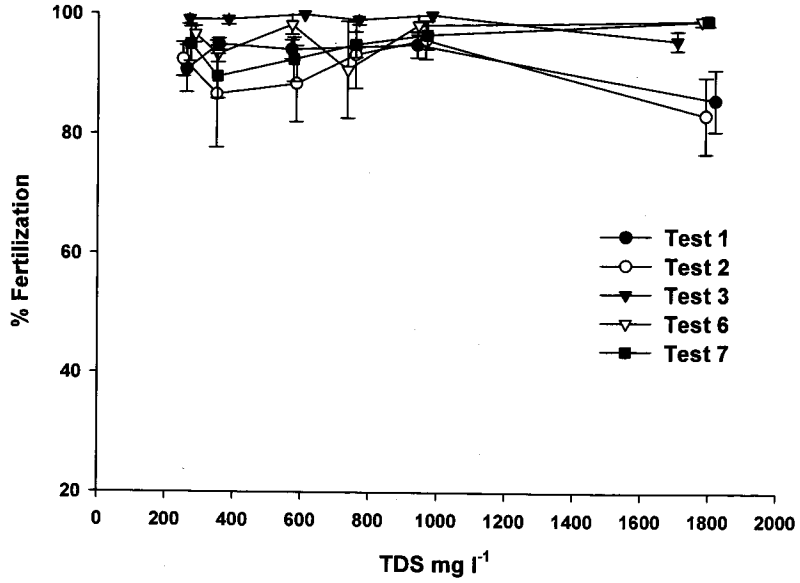


Figure 5. Dolly Varden Test #5 and #8 Results

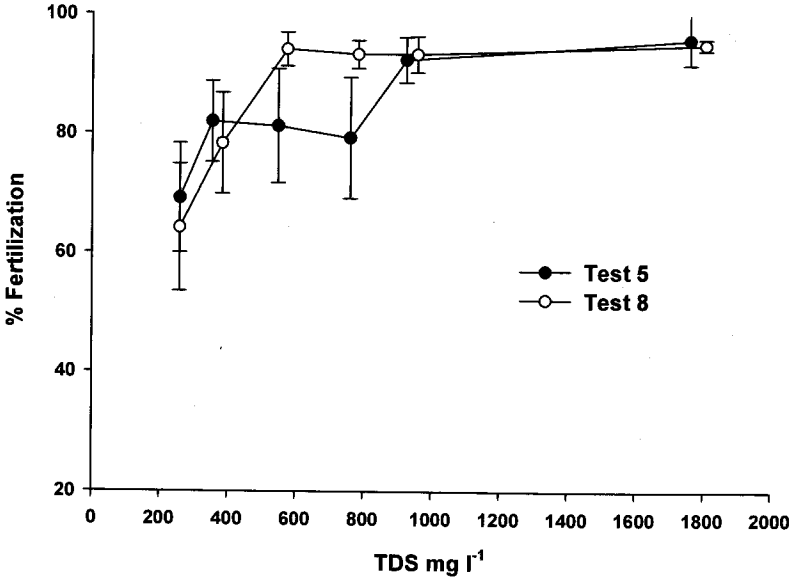
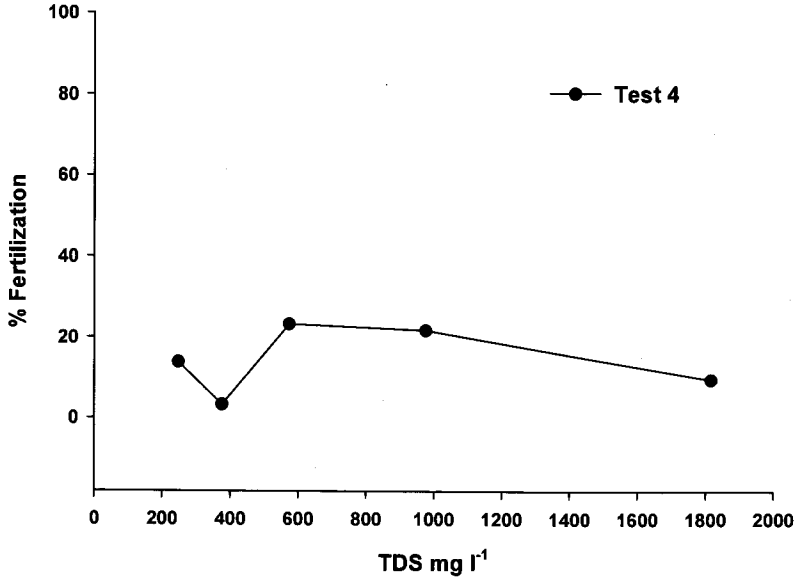


Figure 6. Dolly Varden Test #4 Results (partially spent female)



4. DISCUSSION

4.1. 2004 Arctic Grayling Experiments

The objective of this study was to resolve some of the uncertainties associated with the previous studies conducted by Stekoll et al. on Arctic grayling and other salmonids. Stekoll et al. had previously reported an NOEC of 250 mg/L TDS and LOEC of 500 mg/L TDS when testing Arctic grayling embryos using methods similar to those reported here. However, mean control fertilization was below (~68%) what is normally considered acceptable in embryo studies (ASTM 1992). Additionally, although statistically significant effects on fertilization were observed at 500 mg/L TDS, they did not observe statistically significant effects at 750 and 1250 mg/L 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. Results from the 2004 studies were did not resolve the uncertainty associated with previous studies. While two of the tests conducted indicated no significant effect of TDS on fertilization success up to the highest concentration tested, the other two tests did indicate effects, and in the case of AG4 effects at TDS concentrations below the standard of 500 mg l^{-1}. Further, for the two tests where effects were observed, a reduced effect was observed in the highest TDS concentration tested, repeating the unusual dose-response previously observed by Stekoll et al. (2003).

The observed variability in the 2004 tests may be the result of natural variability in the viability and sensitivity of Arctic grayling embryos to TDS. The increased sensitivity to TDS in the second two tests 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.

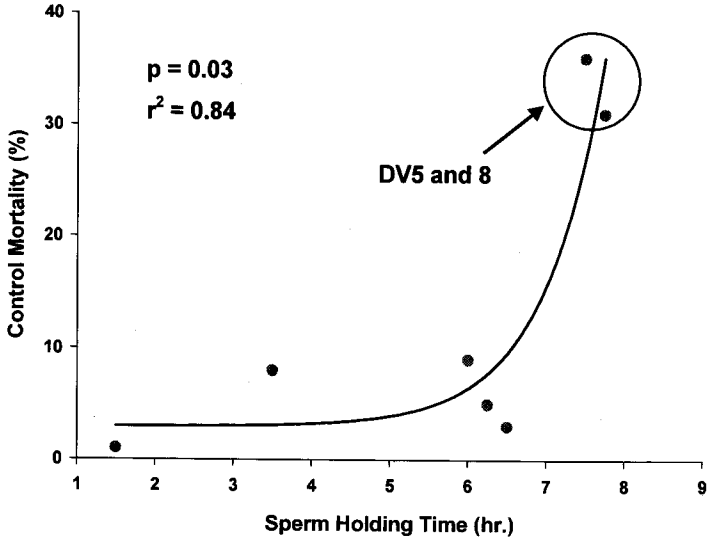
It is also possible that the variability in the 2004 tests is the result of an artifact in the test method. This possibility is discussed further below.

4.2. 2004 Dolly Varden Experiments

The Dolly Varden studies were much more conclusive than the 2004 Arctic grayling studies. None of the studies suggest a significant effect on fertilization success is occurring. One test (DV4) exhibited very poor control performance and a generally flat dose-response relationship. This test used eggs from a female that had already partially spawned her eggs and milt from the same male used in DV3 which had high fertilization success and indicted no effects from TDS on fertilization success. These data may suggest that eggs from partially spent females have generally low viability.

Two other Dolly Varden tests (DV5 and DV8) exhibited inverse dose-response relationships with the lowest fertilization success in the controls and highest fertilization success in the highest TDS treatment. At this time, we cannot provide an explanation for these results, although possible reasons are discussed below. However, as shown in Figure 6, there does appear to be a relationship between the length of time milt is held after collection before use in testing and control performance. The results from DV5 and DV8 would also suggest that the reduced viability of milt as a result of extended holding is ameliorated by elevated TDS, or components thereof.

Figure 6. Dolly varden control fertilization as a function of milt holding time



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 even though they are not activated, cellular metabolism (respiration) is still occurring. Several studies have shown that sperm rapidly lose viability (within 3-5 hours) if sufficient oxygen supply is not available (Smith and Quistorff 1943; Henderson and Dewar 1959). 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).

In the current studies, Dolly Varden sperm were maintained in 50 ml polypropylene test tubes that were loosely capped to reduce evaporation and contamination potential. 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 hours (Figure 6). If this were the case, the elevated Ca^{2+} concentrations in the higher TDS concentrations would potentially have counteracted this effect, resulting in the higher fertilization observed in these treatments for tests DV5 and DV8.

4.3. 2005 Arctic grayling Experiments

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}). The early control failures were clearly the result of attempts to use "green" eggs.

Based on the results of the Dolly Varden experiments, we hypothesized that the variable results observed in the 2004 Arctic grayling experiments may have been caused by excessive holding time for the milt. Exact records were not taken regarding milt holding time, but in general holding time was >4 hours and exceeded 8 hours in at least one test. Holding times for milt in the 2005 experiments were <3 hours for all studies and <2 hours 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. While this does not provide definitive evidence that milt holding time was the sole factor contributing to the observed variability in 2004, considering the Dolly Varden and 2005 Arctic grayling studies in concert is supportive of this hypothesis.

4.4. Regulatory Implications

Given the available data, standard methods for assessing toxicity data for the purpose of setting water quality criteria/standards indicate that the geometric mean of available toxicity values for a given species be used (Stephan et al. 1985; Erickson and Stephan 1988) to derive a species mean value (SMV). Using current practices, the SMV could be derived using the EC20 (USEPA 1999a; 1999b; 2001). We suggest this may be the most appropriate treatment of the data from this study. Use of this approach would result in a SMV of 1357 mg l^{-1} TDS for Arctic grayling and >1779 mg l^{-1} TDS for Dolly Varden.

Its is worth considering that the weight of evidence now strongly suggests that TDS is having no significant effect on Arctic grayling fertilization success and that the EC20 of 202 mg l^{-1} observed in a single experiment is perhaps erroneous. According to USEPA guidelines if a single outlier value exists for a given species, best professional judgement can be used to discard some or all of the data (Stephan et al. 1985). Exerting such professional judgement and excluding the low value for Arctic grayling would result in a SMV of 1782 mg l^{-1}, consistent with the SMV for Dolly Varden.

5. CONCLUSIONS AND RECOMMENDATIONS

Given the available data, SMVs of 1357 and 1779 mg l^{-1} TDS were estimated for Arctic grayling and Dolly Varden. The high variability observed in the 2004 Arctic grayling studies, may have been the result of extended milt holding times, although no definitive evidence demonstrating this is available. Given all of the results obtained on the effects of TDS on fertilization success (the only endpoint considered in this report), it appears that the current site-specific limit of 1500 mg l^{-1} TDS or a value near it, is appropriate for the protection of this endpoint in salmonids.

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