# Acute and chronic toxicity of nitrate to brook trout (Salvelinus fontinalis). [DNR-140] [2000?]

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Project # 140

# Acute and Chronic Toxicity of Nitrate to Brook Trout Salvelinus Fontinalis

Summary

Title: Acute and chronic toxicity of nitrate to brook trout Salvelinus fontinalis

Project ID: 3230 DNR project number

Investigators: Principal Investigator, Ronald Crunkilton, Associate Professor of Water Resources, University of Wisconsin, Stevens Point, WI , Project Assistant, Todd Johnson, M.S. candidate University of Wisconsin, Stevens Point, WI ,

Period of Contract: July 1, 1998- June 30, 2000

Background/Need: Nitrates in surface waters of the Little Plover River, a cold groundwater fed stream located in central Wisconsin, have increased from about 2 mg/L $NO_3$-N in the 1960s to over 8 mg/L $NO_3$-N by 1997. In groundwater upwelling zones on the Little Plover River, nitrate concentrations can exceed 28 mg/L $NO_3$-N. Nitrate may be a potential problem for the successful recruitment of cold water species such as brook trout, because they prefer to spawn in groundwater upwelling zones in streams and lakes. Water from upwelling zones flush fines from the redd, provide oxygen, and remove metabolic wastes produced by the embryos. Typical incubation times range from 32 to 165 days depending on temperature. During incubation, the embryo is buried in the substrate, where it may be exposed to high concentrations of nitrate for an extended period, until emergence or swim-up.

Objectives: The purpose of this study was to determine the acute and chronic toxicity of nitrate to feral and domestic brook trout (Salvelinus fontinalis) embryos and larvae, in moderately hard and soft water and to predict the potential for nitrate toxicity to brook trout in Wisconsin streams.

Methods: The acute and chronic toxicity of nitrate to brook trout embryos and fry was measured in short-term (96-h) and long-term (96-d) exposures. The endpoints measured were mortality and growth. Two domestic strains and one feral strain were tested.

Results and Discussion: Exposure to nitrate at environmentally realistic concentrations significantly increased mortality in brook trout embryos and reduced growth or biomass in long-term exposures. The lowest observable effect concentration was 6.25 mg/L $NO_3$-N for both mortality and growth of feral brook trout. The lowest observable effect concentration was 12.5 mg/L $NO_3$-N for mortality in domestic brook trout embryos and 100 mg/L $NO_3$-N for biomass reduction. Mortality was greater in soft water compared to hard water and the effect was independent of nitrate concentration. The 96-hour $LC_{50}$ values for the domestic and feral brook trout were 2151.4 and 2645.3 mg/L $NO_3$-N respectively.

Conclusions: Concentrations of nitrate in groundwater are a concern because they influence surface water and because trout actively seek out groundwater upwelling zones

for spawning. The Little Plover River derives more than 40% of it base flow from groundwater. Concentrations of nitrate in groundwater feeding central Wisconsin streams such as the Little Plover River and the Plover River range from 2.8 to 37.8 mg/L $NO_3$-N. In some areas of upwelling in the Little Plover River, nitrate concentrations average 15.5 mg/L and specific sites of upwelling have reached 28 mg/L $NO_3$-N. Projected nitrate concentrations for areas of surface water for the Plover River, WI are estimated to reach 28.1 mg/L $NO_3$-N under current land practices by 2005. Trends in the Whiting, WI (Central Wisconsin sand plains near Stevens Point) well-field and Little Plover River support the notion that nitrate concentration will rise to over 8 mg/L in the surface water.

Concentrations of nitrate in the surface waters of the mid-west U. S. are a concern because they fall within ranges that are harmful to aquatic organisms. Fifty-three rivers sampled by the USGS in 1994 and 1995 in the 9 mid-western states (Illinois, Indiana, Iowa, Kansas, Minnesota, Missouri, Nebraska, Ohio and Wisconsin) indicated. Eighty-eight percent had concentrations that exceeded 2mg/L $NO_3$-N, while 43 % had concentrations exceeding 6 mg/L NO3-N. Kincheloe et al. (1979) suggested 2 mg/L $NO_3$-N as a guideline for protection of developing salmonid embryos in soft water. Effect levels reported for domestic and feral brook trout in this study support the need for a similar guideline.

Under current conditions, there is the potential to have increased embryo mortality in brook trout in coldwater streams, especially in areas with high agricultural uses. To fully understand how nitrate affects aquatic organisms, in-situ tests should be preformed to validate laboratory studies. Additional research is needed to expand our knowledge of ancillary effects seen in the current experiment, such as effects on behavior, nitrate avoidance and effects on different species of coldwater organisms.

Related Publications: none yet submitted

Key Words: nitrate, brook trout, groundwater, toxicity

Funding: Groundwater Consortium

Final Report: A final report containing more detailed information on this project is available for loan at the Water Resources Center, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.

Abstract

Increasing concentration of nitrate in groundwater is becoming an environmental health concern. Nitrate levels in pore spaces of groundwater upwelling zones of the Little Plover River, a cold groundwater fed stream located in central Wisconsin, frequently exceed 30 mg/L $NO_3$-N (nitrate-nitrogen). This is a concern because brook trout, *Salvelinus fontinalis*, spawn within these zones exposing developing embryos to high concentrations of nitrate for extended periods of time. The effect of nitrate and water hardness was examined in developing embryos and fry of two domestic and one feral strain of brook trout. The lowest observable effect concentration for feral brook trout was 6.25 mg/L $NO_3$-N for both mortality in developing embryos (96-d test) and reduced growth in fry (14-d test) in hard water. The lowest observable effect concentration was 12.5 mg/L $NO_3$-N for mortality (96-d test) in domestic brook trout embryos and 100 mg/L $NO_3$-N for biomass reductions (14-d test) in fry. Mortality of embryos was greater in soft water compared to hard water and the effect was independent of nitrate concentration. The 96-hour $LC_{50}$ values for the domestic and feral brook trout fry were 2151.4 and 2645.3 mg/L $NO_3$-N respectively. Pre-exposure of developing embryos to nitrate slightly decreased acute toxicity in 96 hour tests indicating the potential for brook trout to develop some tolerance to subsequent high-level exposures. Under current conditions in central Wisconsin, there is the potential to have increased embryo mortality and reduced growth in brook trout in coldwater streams, especially in areas with high agricultural uses.

Acknowledgment

I would like to thank the Wisconsin Department of Natural Resources for funding this project. I would also like to thank the Wisconsin Cooperative Fishery Research Unit for the use of vehicles and equipment use on this project. Thank you, I'm grateful for the experience that you have provided by contributing to this project

I need to thank the staffs of the fish hatcheries that contributed resources and knowledge to help make this project work. Steve Fajfer, Randy Larson, Lee Haass and Peter Jensen, thank you for the lessons and the experience culturing brown and rainbow trout. I can not express how much help it was to have your assistance in getting started. Jeffrey Tabat, Mike Aquino, John Komassa, Don Dodge, and Gregory Fischer, thank you for providing the eggs needed for this project, and providing valuable advise. With out your help and expertise this project would have never gotten as far as we did, thank you.

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## Introduction

Nitrate has long been considered nontoxic to juvenile and adult fish (Westin, 1974), in part because other forms of nitrogen, such as nitrite and ammonia, are considered to be more toxic (Lewis and Morris, 1986). Because of these perceptions, nitrate was virtually ignored as a toxicant to aquatic organisms for a number of years. Nitrate is toxic in warm-blooded animals where it is converted to nitrite in the gut and reacts to form methemoglobin, which is incapable of carrying oxygen in the blood (Shuval and Gruener, 1972). Early developmental stages and young are particularly susceptible because they lack methemoglobin reductase, an enzyme responsible for restoring the oxygen carrying capacity of the hemoglobin (Shuval and Gruener, 1972; Bodansky, 1951).

More recently nitrate has been identified as a possible source of toxicity to early life stages of aquatic organisms. The 120-hour $LC_{50}$ values (lethal concentrations that kill 50 percent of the population) for early instars of the caddisflies *Hydropsyche occidentalis* and *Cheumatopsyche pettite* ranged from 65.5 to 106.5 ppm nitrate-nitrogen ($NO_3$-N) (Camargo and Ward, 1992). The 96-hour $LC_{50}$ value for amphibian embryos (common frogs and toads) ranged from 13.21 to 39.3 mg/L $NO_3$-N (Hecnar, 1995). The $LC_{50}$ value for Oregon spotted frog and northwestern salamander (*Rana pretioso* and *Ambystoma gracile*) during the tadpole stage was 16.45 and 23.39 mg/L $NO_3$-N, respectively (Marco et al., 1999). Other effects on cold-blooded organisms include increased egg mortality in steelhead trout (*Oncorhynchus mykiss*) at 1.1 ppm $NO_3$-N, rainbow trout (*O. mykiss*) at 2.3 ppm $NO_3$-N and cutthroat trout (*Salvelinus clarki*) at 4.5 ppm $NO_3$-N (Kincheloe et al., 1979). These levels of nitrate are commonly found in surface water drainages of the upper Midwest (Mechenich and Kraft, 1997; Stark, 1997; Peters et al., 1998; Mason et al., 1990).

Nitrogen enters watersheds from a variety of sources, including domestic and industrial sewage, animal feedlots, and fertilizer application (van Eerdt and Fong, 1998; Peters et al., 1998; Gellenbeck, 1994). The major source of nitrogen contamination of surface and groundwater is from agricultural application of nitrogen-based fertilizer (Toth and Fox, 1998; Guimera, 1998; Peters et al., 1998). Concentrations above 3 mg/L $NO_3$-N are considered elevated due to human activities (Madison and Brunett, 1984). Nitrates in surface waters of the Little Plover River, a cold groundwater fed stream located in central Wisconsin, have increased from about 2 mg/L $NO_3$-N in the 1960s to over 8 mg/L $NO_3$-N by 1997 (Mechenich and Kraft, 1997). In groundwater upwelling zones on the Little Plover River, nitrate concentrations can exceed 28 mg/L $NO_3$-N (Browne et al., In Review).

Nitrate may be a potential problem for the successful recruitment of cold water species such as brook trout, because they prefer to spawn in groundwater upwelling zones in streams and lakes. Water from upwelling zones flush fines from the redd, provide oxygen, and remove metabolic wastes produced by the embryos (Peters, 1965). Typical incubation times range from 32 to 165 days depending on temperature (Embody, 1934). During incubation, the embryo is buried in the substrate, where it may be exposed to high concentrations of nitrate for an extended period, until emergence or swim-up stage.

Brook trout occupy streams with a wide range of water hardness, which can alter toxicity of certain ions. Hardness is known to effect toxicity of heavy metals to a variety of aquatic organisms including fish (Erickson et al., 1996) and daphnia (Penttinen et al.,

1998) by effecting ion absorption (Bradley and Sprague, 1985). Calcium concentrations alter permeability of the chorion of fish embryos to some ions (Potts and Rudy, 1969). Water hardness may effect the uptake of nitrate in brook trout embryos, hence a potential for difference in toxicity.

The purpose of this study was to determine the acute and chronic toxicity of nitrate to feral and domestic brook trout (*Salvelinus fontinalis*) embryos and larvae, in moderately hard and soft water and to predict the potential for nitrate toxicity to brook trout in Wisconsin streams.

## Methods

### *Brook trout strains*

Two domestic and one feral strain of brook trout were tested. One domestic strain of brook trout was obtained from the St. Croix State hatchery, St. Croix, WI. The St. Croix domestic strain originated from the Pink Banks domestic strain in New Hampshire and has been held captive for over 30 years at the St. Croix hatchery. The second domestic strain, the Lake Superior coaster brook trout, was provided by the Red Cliff tribal fish hatchery, Red Cliff, WI. Coaster brook trout spawn in-stream, but live at least part of their life cycle in a lake (Becker, 1983). This strain of brook trout has been propagated within the hatchery for 10 years. Feral trout are captured wild trout, transported and held at a hatchery for a short time for spawning and then released. Feral brook trout were collected in late September from Melancthron Creek, Richland County in southeastern Wisconsin and held for spawning in November at the Nevin State hatchery, Fitchburg, WI.

*Spawning*

Trout were artificially spawned using the dry method (Piper et al., 1982). Trout were separated by ripeness and sex, paired by availability of gender and then anesthetized. Eggs from one or two females were gently expressed into a dry pan. Milt from a single male was similarly expressed over the eggs, mixed with a feather, and allowed 5 minutes for fertilization to take place. Fertilized eggs were then volumetrically distributed in equal amounts to nitrate treatments of 100, 50, 25, 12.5 and 6.25 mg/L nitrate-nitrogen ($NO_3$-N) and a control. After distribution, a new set of trout were spawned and fertilized eggs were distributed in the same manner as described above until each nitrate treatment had approximately 1000 embryos. This required six to eight sets of trout. Fertilized embryos were rinsed and water hardened for one hour within the nitrate concentration used for each treatment. The embryos were transported to the University of Wisconsin, Stevens Point, during the shock resistant stage, within 8 hours after water hardening. The embryos for each treatment were then volumetrically measured to deliver approximately 100 embryos to each of eight replicate chambers within each treatment concentration.

*Exposure system*

Embryos were exposed in temperature controlled flow-through tanks. Each treatment consisted of a compartmentalized 54-L tank that contained eight replicate egg baskets. Egg baskets consisted of 7.62 cm diameter PVC pipe, 16 cm high, with a fine polyethylene screen (33.6 x 33.6 strands per cm) affixed to the bottom and were partially submerged in the tank compartments. Egg baskets were hung from a rocker arm powered by a 12 V DC Dayton gear motor (Dayton Electric Inc. Chicago, Ill.) turning at 0.45 revolutions per minute.

A model Q fluid-metering pump (Fluid Metering Inc. Oyster Bay, NY) delivered a concentrated stock solution of reagent grade sodium nitrate ($NaNO_3$, Fisher Scientific, lot # 960785) to a proportional flow-through diluter. The concentrated stock solution was diluted with soft or hard water to the specified nitrate treatment concentration and delivered in 2-L increments. Turnover of exposure water was once every 20 hours.

*Exposure water*

Hard water treatments for the flow-through tests consisted of municipal well water filtered through two 27 cm-diameter by 107 cm-vertical activated carbon filters, each containing 23 kg of carbon to remove chlorine.

Soft water, was prepared by adding 81.74 g sodium bicarbonate ~~nitrate~~ ($NaHCO_3$, Fisher Scientific, lot # 987580) 51.09 g calcium sulfate dihydrate ($CaSO_4 \cdot 2H_20$, ACROS lot # B0100958) 51.09 g magnesium sulfate anhydrous ($MgSO_4$, Fisher Scientific, lot # 986620) and 3.41 g potassium chloride (KCl, Fisher Scientific, lot # 946305A) to 1700L of distilled water (APHA, 1992). Batches were stored in a 122 cm-diameter 183 cm vertical 2000L polyethylene tank (Norwesco, St. Bornifacius, MN). A ¾ horsepower Jacuzzi Magnum pump (Jacuzzi, Little Rock, AK) was used to vigorously mix and aerate the water for at least 24 hours before use. A Teel pump (model 3P715B) and a Teel epoxy-lined pressure tank (model 3P557C, Dayton Electric MFG. Co. Chicago, Ill.) pumped water through an activated carbon filter to the flow-through system.

*Embryo Mortality- 90 day*

Mortality was assessed by counting and removing dead embryos. To minimize handling loss, dead embryos were not removed from test containers until eye pigmentation developed (eyed stage), about 30 days. Embryo testing lasted until yolk sac absorption and larvae started to actively search for food. This took approximately 90 days.

*Chronic exposure-growth and biomass*

Surviving larvae from the eight replicates in each treatment concentration were pooled after approximately 90 days, then 10 larvae were randomly distributed back into each of the eight replicate chambers of the same treatment concentration. Fry were fed excess brine shrimp (0.20 mL twice daily at 6-hour intervals). One hour after the second feeding uneaten brine shrimp was removed. Tests were terminated at day 14 and larvae were sacrificed using 70% isopropyl alcohol and rinsed in distilled water. Larvae were placed in pre-weighed aluminum drying pans and dried to a constant weight (less than 1% difference in weighing) at 105°C. Weight measurements were taken to 0.00001 g on an analytical balance (Mettler AE240- Greifensee, Switzerland) and recorded. Growth and biomass were the two endpoints measured. Growth is defined as total dry weight of each replicate divided by the number of larvae surviving in the replicate at the end of the test. This endpoint did not include the effect of mortality (< 16% for any treatment or the control) that occurred during the 14- day test. Biomass is defined as the total dry weight of larvae from each replicate at the end of the test divided by 10, the number of larvae at the start of the test. Thus, this endpoint included the effect of mortality or biomass lost during the 14-day test.

*Water chemistry for embryo mortality and chronic exposures*

Water chemistry measurements for DO were taken with a dissolved oxygen meter (YSI model 58 - Yellow Springs, OH), pH was taken with a pH meter (Orion model 920 A - Boston, MA), hardness was determined by EDTA titration (APHA, 1992). Nitrate grab samples were analyzed by the Environmental Task Force Laboratory (Lachat Instruments, 1992).

Water for feral brook trout tests was characterized by temperature (8.5 to 9.0 $^0$C), DO (8.62 to 11.75 mg/L $O_2$), total hardness (180 to 216 mg/L $CaCO_3$), and pH (7.28 to 8.24). Soft water for the Lake Superior domestic brook trout tests was characterized by temperature (7.9 to 12.0 $^0$C), DO (7.54 to 12.14 mg/L $O_2$), total hardness (40 to 60 mg/L $CaCO_3$), and pH (7.00 to 8.22). Hard water for the Lake Superior domestic brook trout tests was characterized by temperature (7.8 to 8.0 $^0$C), DO (7.41 to 12.00 mg/L $O_2$), total hardness (192 to 256 mg/L $CaCO_3$), and pH (7.28 to 8.22). These measurements were preformed daily. Embryos were kept in darkness until hatched after which embryos were put on a regime of 12-hour light 12-hour dark photoperiod. Nitrate concentrations were measured biweekly. Mean measured nitrate concentrations were within 20% of nominal nitrate concentrations. A complete characterization of all other chemical constituents measured during the test can be seen in Appendix A.

*Acute toxicity testing*

The 96-hour $LC_{50}$ of nitrate to brook trout fry with and without prior exposure to nitrate was determined. The St. Croix domestic strain and the feral strain of brook trout from Melancthron Creek were used. Both strains were tested at 96 days post-fertilization in hard water. The St. Croix domestic larvae were exposed to the nitrate concentrations of 25 and 100 mg/L $NO_3$-N until hatched (39 days) then moved to a clean water source (carbon filtered municipal tap water) for 57 days before starting tests. The feral larvae were continuously exposed in 25 and 100 mg/L $NO_3$-N nitrate treatments up to the start of the test. Control organisms were hatched and incubated in moderately hard water without added nitrate.

Larvae were exposed to a geometric series of five concentrations of reagent grade $NaNO_3$ (Fisher Scientific, lot # 960785) ranging from 1771 to 3000 mg/L $NO_3$-N in a 96-hour renewal test. Five larvae were placed in each of four replicates for each concentration. Two-L glass jars filled with 1500-mL test solution served as testing chambers. Test solutions were renewed daily with a stock solution prepared at the start of the test and stored at 4° C.

Tests were conducted in an environmental chamber with a photoperiod of 12 hours light to 12 hours dark. Test solutions were characterized by total hardness (160 to 172 mg/L as $CaCO_3$), pH (7.60 to 8.27), and DO (8.32 to 10.90 mg/L $O_2$), temperature (10°C $\pm$ 1°). Water chemistries were taken daily.

Dilution and control water was reconstituted moderately hard water prepared according to standard methods (APHA, 1992). Reconstituted water was from municipal well water that was softened, deionized and pumped through a high purity water processor (Barnstead Nanopure model - D4741, Dubuque, Iowa) with one carbon, two ion exchange and one organic extraction filter in sequence resulting in type 1 reagent grade water.

*Statistical analysis*

Mortality data for the feral brook trout tested in hard water was analyzed using a one-way analysis of variance (one-way ANOVA) model with the Fisher's Least-Significant-Difference post hoc test to determine the no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) of nitrate. Mortality data for the Lake Superior domestic brook trout in hard and soft water was analyzed using a two-way analysis of variance (two-way ANOVA) model, to examine the effects of nitrate concentration and water hardness. The Fisher's Least-Significant-Difference post hoc test was used to determine the no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC). The percent embryo mortality values for all tests were arcsine square root transformed prior to statistical analysis, normally distributed (Chi-square test for normality) and had equal variances (Hartley's test for homogeneity of variance).

The endpoints of the 14-day chronic exposures were measurements of growth and biomass. Growth and biomass in the feral brook trout were analyzed with a one-way ANOVA with the Fisher's Least-Significant-Difference post hoc test to determine the no observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC). Endpoints for the Lake Superior domestic brook trout were analyzed with one-way ANOVA and the Fisher's Least-Significant-Difference post hoc test to determine the no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC). One-way ANOVA was used because there was significant interaction between concentration and treatment in the two-way ANOVA ($p= 0.037591$). Data for the chronic tests were normally distributed (Chi-square test for normality) and had equal variances (Hartley's test for homogeneity of variance).

The $LC_{50}$ values for acute exposures of the feral brook trout from Melancthron Creek and St. Croix domestic brook trout larvae were determined by the Spearman-Karber procedure (West and Gully, 1996).

## Results

### *Embryo mortality*

Exposure to nitrate significantly increased ($p < 0.05$) mortality in feral brook trout embryos from Melancthron Creek (Table 1). This brook trout strain was tested in hard water only. The LOEC was 6.25mg/L $NO_3$-N, and the NOEC could not be statistically determined, but was below 6.25 mg/L $NO_3$-N (Figure 1). The average increase in the mortality at the LOEC was about 4% greater than the control. There was also a significant increase ($p<0.004$) in embryo mortality of Lake Superior domestic trout exposed to nitrate in hard and soft water (Table 2, Fig. 2a). Because the interaction between nitrate concentration and water hardness was unimportant in the two-way ANOVA, the response slopes for hard and soft water were assumed to be equal. Thus, the post hoc separation procedure identified 12.5 and 6.25 mg/L $NO_3$-N as the common

LOEC and NOEC values respectively (Fig. 2b). The average increase in mortality at the LOEC was about 5% greater than the control.

*Chronic effects-growth and biomass in feral strain*

Exposure to nitrate reduced growth ($p < 0.002$; average dry weight of surviving larvae) in feral brook trout larvae at day 14 (Table 3). This stain was tested in hard water only. The LOEC was 6.25 mg/L $NO_3$-N, and NOEC could not be determined, but was below 6.25 mg/L $NO_3$-N (Figure 3). The average weight at the LOEC was about 7% less than the control. Biomass (total dry weight of larvae divided by the number of larvae at the start of test) was not significantly different because higher mortality (16%) occurred in the control than in any of the nitrate treatments.

*Chronic effects-growth and biomass domestic strain*

Growth of the Lake Superior domestic strain was not significantly different in soft or hard water. Biomass for the Lake Superior domestic strain water was significant ($p<0.05$) in soft, but not hard water (Table 4). The LOEC was 100 mg/L $NO_3$-N, with a NOEC at 50 mg/L $NO_3$-N (Figure 4). The average weight at the LOEC was about 20% less than the control. Larvae appeared to be more lethargic during feeding at the concentrations of 25, 50 and 100 mg/L $NO_3$-N than the control, 6.25 and 12.5 mg/L $NO_3$-N concentrations, but this apparent effect was not quantified.

*Acute effects*

Ninety-six-hour $LC_{50}$ values for St. Croix domestic and Melancthron Creek feral brook trout larvae (96-days old) were 2151.4 and 2645.3 mg/L $NO_3$-N respectively. When the developing embryos were exposed to 25 and 100 mg/L $NO_3$-N prior to testing the $LC_{50}$ values generally increased (Table 5).

**Discussion**

The effect range for mortality in brook trout embryos reported here is similar to results reported for several other species of cold-water fish. Kincheloe et al. (1979) examined mortality from incubation to hatch and then 30 days past yolk sac absorption for several species of salmonids and reported statistically significant increases in mortality at the $p<0.05$ value. Coho salmon (*Oncorhynchus kisutch*) showed no effect in concentrations up to 4.5 mg/L $NO_3$-N, while Chinook salmon (*Oncorhynchus tshawtscha*) had an increase in total mortality at 4.5 mg/L $NO_3$-N. The non-anadromous rainbow trout and cutthroat trout had an increased total mortality at 2.3 mg/L $NO_3$-N. The anadromous rainbow trout had an increased total mortality at 1.1 mg/L $NO_3$-N. These effect concentrations are similar to the range reported in the present study for brook trout.

Early developmental stages of other species of cold-blooded vertebrates have been reported to be sensitive to low levels of nitrate in acute tests. The 96-hour $LC_{50}$ for American toad (*Bufo americanus*) tadpoles was 13.6 and 39.3 mg/L $NO_3$-N in two populations. The 96-hour $LC_{50}$ of tadpoles for western chorus frog (*Pseudacris triseriata*), northern leopard frog tadpoles (*Rana pipiens*), and green frog tadpoles (*Rana *clamitans melanota*) was 17.0, 22.6 and 32.4 mg/L $NO_3$-N respectively (Hecnar, 1995). The 15 day $LC_{50}$ value for the Oregon spotted frog tadpole was 16.45 mg/L $NO_3$-N and the northwestern salamander larvae had a value of 23.39 mg/L $NO_3$-N tadpoles (Marco et al., 1999).

Certain species of aquatic insects have also been reported to be adversely affected by low concentrations of nitrate. The 120-hour $LC_{50}$ of the caddisflies was higher than the LOEC range for brook trout, but safe concentrations, 352-day $LC_{0.01}$, for the early instars of caddisflies (*C. pettiti* and *H. occidentalis*) were 1.4 and 2.4 ppm $NO_3$-N respectively (Camargo and Ward, 1995). The 120-hour $LC_{50}$ values for early instars of two caddisflies (*C. pettiti* and *H. occidentalis*) ranged from 65.5 to 106.5 ppm $NO_3$-N (Camargo and Ward, 1992).

Low water hardness decreased survival of brook trout embryos. We are unaware of any literature on the effect of hardness on nitrate toxicity. Hardness effects are well documented for other toxicants such as metals. Increased hardness from 30 to 60 mg/L $CaCO_3$ significantly (non-overlapping $LC_{50}$ confidence interval) reduced toxicity of $AgNO_3$ (silver nitrate) to rainbow trout after 96-hour acute exposure (Karen et al., 1999). Increased hardness from 31 to 387 mg/L $CaCO_3$ decreased the acute 96-hour rainbow trout $LC_{50}$ from 0.17 to 5.16 mg/L total Zn at pH 7 (Bradley and Sprague, 1985). A greater concentration of calcium outside of the cell may reduce uptake of potentially toxic ions that are similarly shaped or charged by reducing membrane permeability (Potts and Rudy, 1969). The slopes of the dose response curves for nitrate were similar in both hard and soft water (i.e. the interaction term in the two-way ANOVA was not significant) indicating there were no synergistic effects between these factor levels. The difference in survival was uniform across all nitrate concentrations including the control suggesting that soft water alone was responsible for the increased toxicity.

Growth was reduced in brook trout exposed to nitrate for 14 days in this study. Research on other species has also shown effects on measures of growth. Ten-day LOAEL (lowest observable adverse effect level) for reduce weight and length for pacific tree frog and African clawed frog (*Pseudacris regilla* and *Xenopus laevis*) at early hind-limb bud stage ranged from <30.1 to126.3 mg/L $NO_3$-N (Schuytema and Nebeker, 1999). At 9.0 and 23.0 mg/L $NO_3$-N decreased growth and increased mortality occurred in the tree frog (*Littoria caerrulea*) tadpoles (Baker and Waights, 1994). At 10 mg/L $NO_3$-N significantly fewer chorus frog tadpoles were metamorphosing to adults (Hecnar, 1995). Reduced growth was shown in rainbow trout after a 14-day flow-through exposure of 50 mg/L $NO_3$-N (Bance and Johnson, 1997).

In this study, brook trout exhibited a lower toxicity to short term nitrate exposure than other fishes. The 96-h $LC_{50}$ values for Chinook salmon (*Oncorhynchus tshawytscha*, rainbow trout, channel catfish (*Ictalurus punctatus*), fathead minnows (*Pimephales promelas*) and guadalupe bass (*Micropterus treculi*) all range from 1250 to 1400 mg/L $NO_3$-N, about half the value we found in brook trout (Scott and Crunkilton, in-press; Tomasso and Carmichael, 1986; Colt and Tchobanoglous, 1976; Westin 1974).

The acute toxicity of nitrate can vary between strains or populations. Feral brook trout had an overall higher tolerance to nitrate than the St. Croix domestic strain of brook trout. Two populations of American toads (*Bufo americanus*) tadpoles from Ojibway pond (Essex county, Ontario) had a 96-hour $LC_{50}$ value of 13.6 mg/L $NO_3$-N while American toads from Harrow pond (Essex county, Ontario) had a value of 39.3 mg/L

$NO_3$-N (Hecnar 1995). Differences in toxicity to embryos of anadromous and non-anadromous rainbow trout have also been reported as previously described (Kincheloe et al., 1979).

There also seems to be a tolerance developed by early exposure to nitrate. The acute toxicity values show an increasing tolerance with early exposure at concentrations of 25 and 100 mg/L $NO_3$-N. No references were found on the development of tolerance to nitrate, although it has been reported for other toxicants. The 48-hour $LC_{50}$ for rainbow trout larvae exposed to cadmium (Cd) was < 0.1 mg/L Cd, while for rainbow trout larvae pretreated for 7-days at 0.001 and 0.01 mg/L Cd had 48-hour $LC_{50}$ values of 0.11 an 1.5 mg/L Cd respectively (Pascoe and Beattie, 1979). In another study, the $LC_{50}$ of arsenic for rainbow trout increased from 13.2 mg/L to 19.7 mg/L after 21 days of pre-exposure at 0.22 mg/L (Dixon and Sprague, 1981).

Concentrations of nitrate in groundwater are a concern because they influence surface water and because trout actively seek out groundwater upwelling zones for spawning. The Little Plover River derives more than 40% of it base flow from groundwater. Concentrations of nitrate in groundwater feeding central Wisconsin streams such as the Little Plover River and the Plover River range from 2.8 to 37.8 mg/L $NO_3$-N (Albertson, 1998; Mechenich and Kraft, 1997). In some areas of upwelling in the Little Plover River, nitrate concentrations average 15.5 mg/L and specific sites of upwelling have reached 28 mg/L $NO_3$-N (Browne et al., In Review). Projected nitrate concentrations for areas of surface water for the Plover River, WI are estimated to reach 28.1 mg/L $NO_3$-N under current land practices for the year 2005 (Mechenich and Kraft, 1997). Trends in the Whiting, WI (Central Wisconsin sand plains near Stevens Point) well-field and Little Plover River support the notion that nitrate concentration will rise to over 8 mg/L in the surface water (Mechenich and Kraft, 1997).

Surface waters in the mid-west U. S. are a concern because current nitrate levels are within ranges that are harmful to aquatic organisms. Fifty-three rivers sampled by the USGS in 1994 and 1995 in the 9 mid-western states (Illinois, Indiana, Iowa, Kansas, Minnesota, Missouri, Nebraska, Ohio and Wisconsin) had 6 rivers (11 %) with concentrations below 2 mg/L $NO_3$-N. Eighty-eight percent had concentrations that exceeded 2mg/L $NO_3$-N, while 43 % had concentrations exceeding 6 mg/L NO3-N. Eleven percent had nitrate concentrations that exceeded 10 mg/L $NO_3$-N, and 9 % had levels greater than 12 mg/L $NO_3$-N (Scribner et al., 1998). Kincheloe et al. (1979) suggested 2 mg/L $NO_3$-N as a guideline for protection of developing salmonid embryos in soft water. Effect levels reported for domestic and feral brook trout in this study support the need for a similar guideline.

Under current conditions, there is the potential to have increased embryo mortality and reduced growth in brook trout in coldwater streams, especially in areas with high agricultural uses. The increase in mortality or reduction in growth at statistical effect levels was small less than 10%. The relevance of this magnitude of mortality to population of wild brook trout is unknown. To more fully understand how nitrate affects aquatic organisms, in-situ tests should be preformed to verify these laboratory studies. Additional research is also needed to expand knowledge of effects seen in the current experiment, such as effects on behavior, nitrate avoidance and effects on different species of coldwater organisms.

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Table 1. Mortality in feral brook trout (Melancthron Creek) embryos analyzed with one-way ANOVA and Fisher's Least-Significant post hoc test to determine differences from the control. Embryos were exposed to nitrate concentrations for 95 days post fertilization. Values are arcsine (square root (X)) transformed percent mortality.

1a. One-way ANOVA

| Source | Sum of squares (transformed) | df | Mean-square | F-ratio | P-value |
|---|---|---|---|---|---|
| Concentration | 0.000441 | 5 | 0.000088 | 3.312347 | 0.046034 |
| Error | 0.001119 | 42 | 0.000027 | | |

1b. Fishers least-significant-difference post hoc test

| Concentration | % mean mortality (Back transformed) | P-value |
|---|---|---|
| Control | 0.051280 | 1.000000 |
| 6.25 | 0.129032 | 0.007490 |
| 12.5 | 0.093023 | 0.030294 |
| 25 | 0.122449 | 0.005017 |
| 50 | 0.096774 | 0.079486 |
| 100 | 0.080645 | 0.257955 |

Table 2. Mortality of Lake Superior strain domestic brook trout embryos in hard and soft water analyzed with two-way ANOVA and Fisher's Least-Significant post hoc test to determine differences within treatments. Embryos were exposure to nitrate and hardness treatments for 88 days post fertilization. Values are arcsine (square root (X)) transformed percent mortality data.

2a. Two-way ANOVA

| Source | Sum of squares (transformed) | df | Mean-square | F-ratio | P |
|---|---|---|---|---|---|
| Water hardness | 0.109361 | 1 | 0.109361 | 24.850871 | 0.000003 |
| Nitrate Conc. | 0.083498 | 5 | 0.016700 | 3.794784 | 0.003792 |
| Interaction | 0.049194 | 5 | 0.009839 | 2.235750 | 0.058126 |
| Error | 0.369657 | 84 | 0.004401 | | |

2b. Fishers least-significant-difference post hoc test

| Concentration | % mean mortality (back transformed) | P-value |
|---|---|---|
| Control | 0.721305 | 1.000000 |
| 6.25 | 0.735811 | 0.488709 |
| 12.5 | 0.769220 | 0.021297 |
| 25 | 0.751709 | 0.153360 |
| 50 | 0.730544 | 0.659942 |
| 100 | 0.795124 | 0.003663 |

Table 3. Growth of feral brook trout larvae (Melancthron Creek) analyzed with one-way ANOVA and Fisher's Least-Significant post hoc test to determine differences from the control. Units are average dry weight (grams) per surviving larvae at end of 14-day exposure.

3a. One-way ANOVA

| Source | Sum of squares | df | Mean-square | F-ratio | P |
|---|---|---|---|---|---|
| Concentration | 0.000022 | 5 | 0.000004 | 4.798607 | 0.001471 |
| Error | 0.000039 | 42 | 0.000001 | | |

3b. Fishers least-significant-difference post hoc test

| Concentration | Growth | P-value |
|---|---|---|
| Control | 0.014178 | 1.000000 |
| 6.25 | 0.013138 | 0.036848 |
| 12.5 | 0.013054 | 0.024605 |
| 25 | 0.013488 | 0.159576 |
| 50 | 0.012182 | 0.000164 |
| 100 | 0.012299 | 0.000344 |

Table 4. Biomass of Lake Superior domestic brook trout larvae analyzed with one-way ANOVA and Fisher's Least-Significant post hoc test to determine differences from the control. Units are average dry weight (grams) of ten larvae after 14-day exposure.

4a. One-way ANOVA

| Source | Sum of squares | df | Mean-square | F-ratio | P |
|---|---|---|---|---|---|
| Concentration | 0.000046 | 5 | 0.000009 | 2.487437 | 0.047175 |
| Error | 0.000147 | 40 | 0.000004 | | |

4b. Fishers least-significant-difference post hoc test

| Concentration | mean weight | P-value |
|---|---|---|
| Control | 0.013085 | 1.000000 |
| 6.25 | 0.013315 | 0.811241 |
| 12.5 | 0.013521 | 0.662354 |
| 25 | 0.012911 | 0.856371 |
| 50 | 0.012079 | 0.299704 |
| 100 | 0.010505 | 0.012860 |

Table 5. Ninety-six-hour acute $LC_{50}$ values and 95 % confidence intervals, from Spearman-Karber procedure, for St. Croix domestic brook trout and feral brook trout (Melancthron Creek) with and without pre-exposure to nitrate.

| Pre-exposure concentration during incubation mg/L $NO_3$-N | St. Croix Domestic mg/L $NO_3$-N | Feral brook trout mg/L $NO_3$-N |
|---|---|---|
| Control | 2151.4 (2063.2 – 2239.6) | 2645.3 (2551.2 – 2739.5) |
| 25 | 2254.0 (2093.4 – 2414.6) | 2631.5 (2537.0 – 2725.9) |
| 100 | 2569.4 (2435.0 – 2701.8) | 2758.8 (2686.2 – 2831.4) |

Figure 1. Average mortality of feral brook trout embryos from Melancthron Creek exposed to nitrate concentrations for 96 days post-fertilization. LOEC is determined by the first value to be statistically different from the control at the $p < 0.05$ level using Fishers least-significant-difference post hoc test. Vertical bars are ± one standard error of the mean.

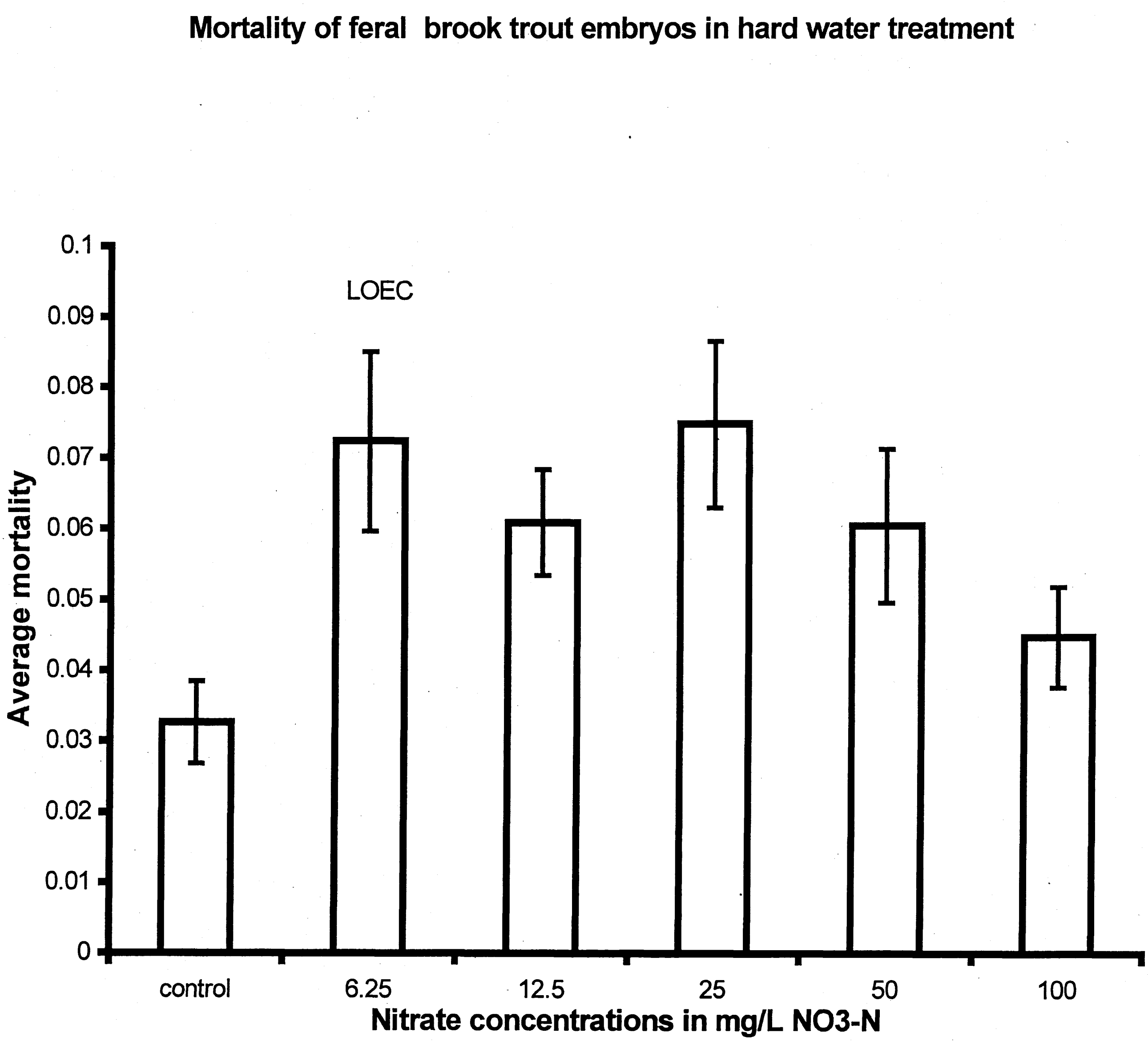
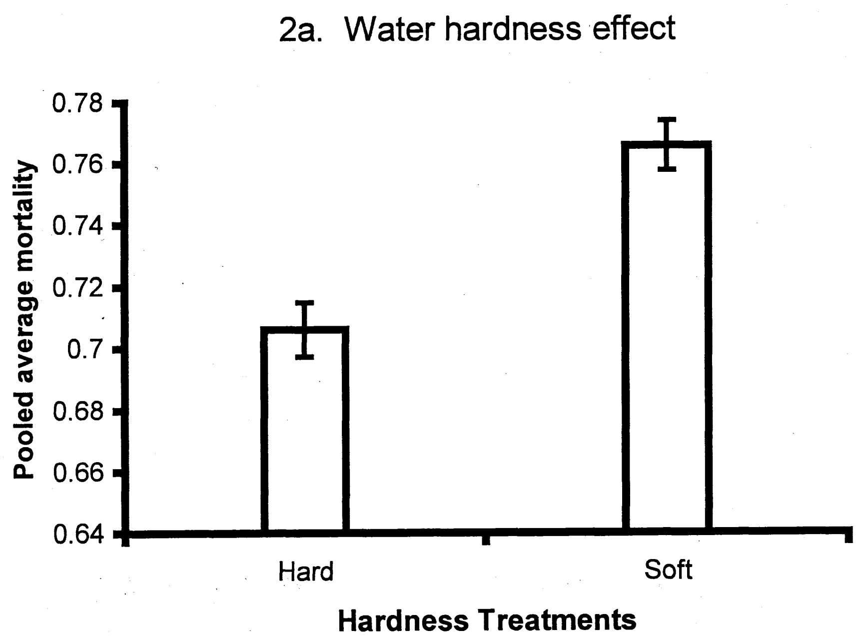


Figure 2. Effect of water hardness and nitrate concentration on embryo mortality in Lake Superior brook trout exposed for 96 days. Pooled values are from two-way ANOVA model. Vertical bars are ± one standard error of the mean.



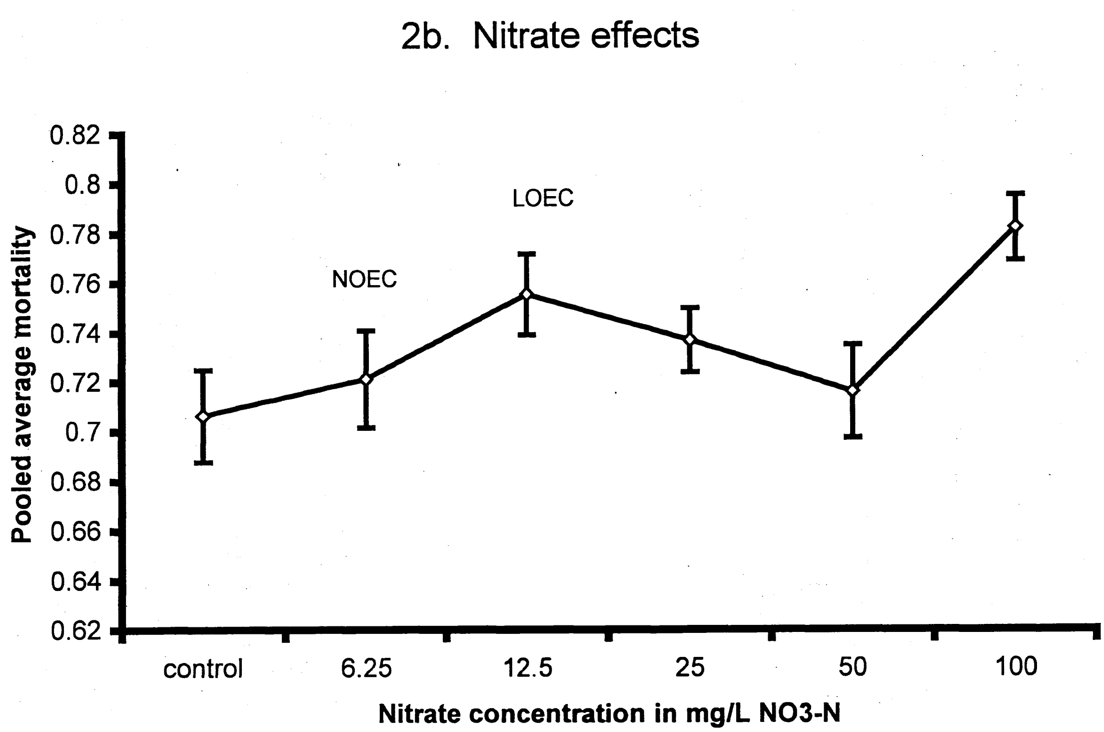



Figure 3. Average growth of feral brook trout larvae from Melancthron Creek exposed to nitrate concentrations for 14 days. LOEC is determined by the first value to be statistically different from the control at the $p<0.05$ level using Fishers least-significant-difference post hoc test. Vertical bars are $\pm$ one standard error of the mean.
Average dry weight of fish per replicate

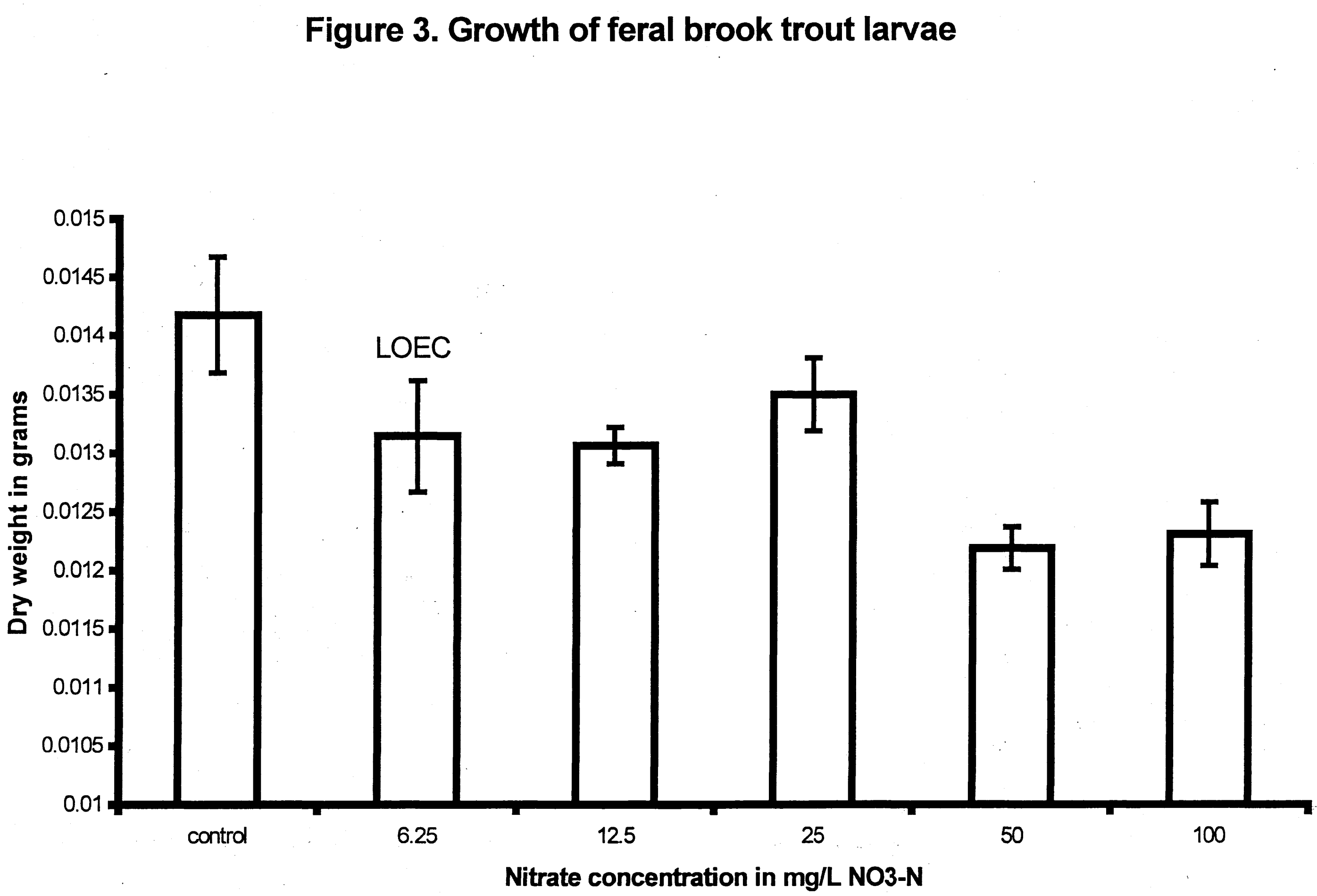
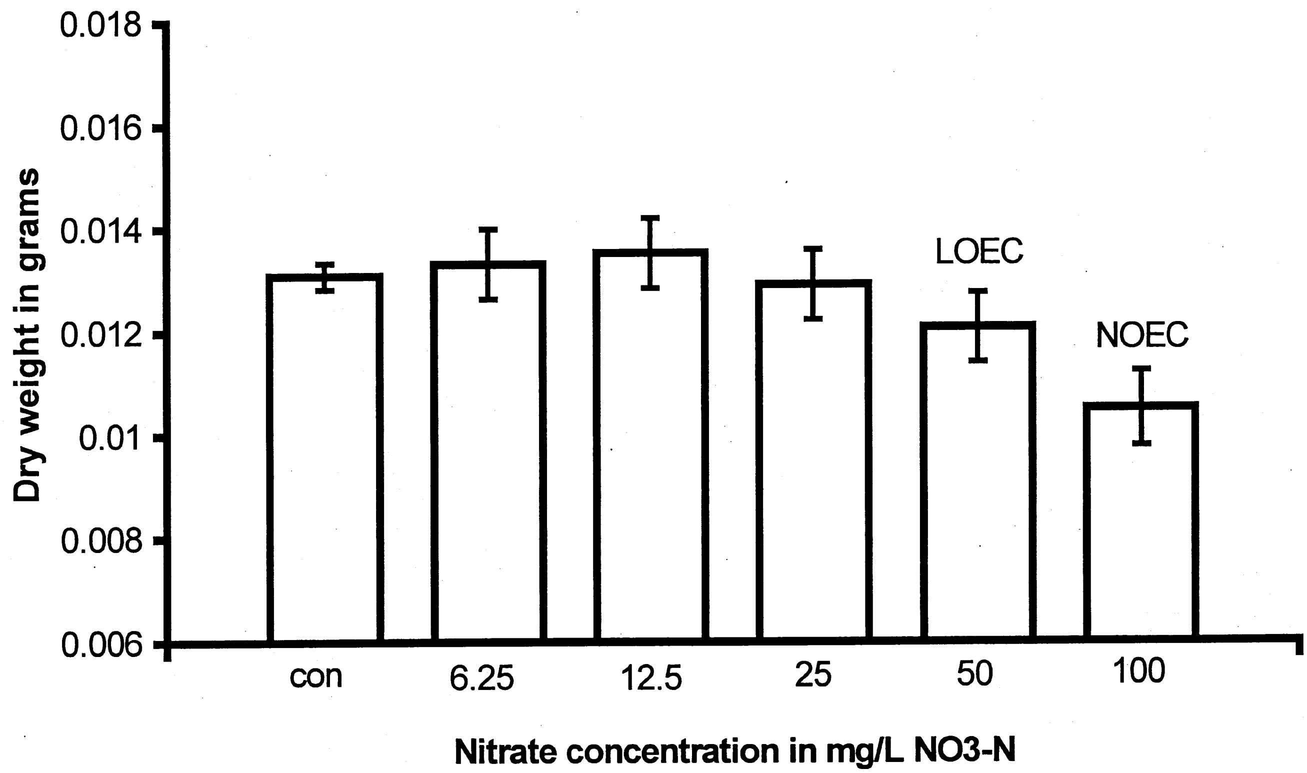


Figure 4. Average biomass of Lake Superior domestic brook trout exposed to nitrate concentrations for 14 days. LOEC is determined by the first value to be statistically different from the control at the $p<0.05$ level using Fishers least-significant-difference post hoc test. Vertical bars are $\pm$ one standard error of the mean. Average dry weight per replicate.

Figure 4. Biomass of Lake Superior domestic brook trout larvae soft water treatment



# Appendix A

## Nominal and measured nitrate-nitrogen for exposures

Appendix A1. Nominal and measured concentration of nitrate-nitrogen for flow-through test with feral embryos and larvae from Melancthron Creek. Measured values are reported in mg/L $NO_3$-N

| Concentration | Measured | n (# of samples) |
|---|---|---|
| Control | 2.5 - 3.0 | 4 |
| 6.25 | 8.5 – 9.5 | 4 |
| 12.5 | 14.8 – 15.8 | 4 |
| 25 | 25.2 – 27.1 | 4 |
| 50 | 46.5 – 49.9 | 4 |
| 100 | 102.0 – 104.0 | 4 |

Appendix A2. Nominal and measured concentration of nitrate-nitrogen for flow through-test with Lake Superior domestic brook trout embryos and larvae in hard water. Measured values are reported in mg/L $NO_3$-N

| Nominal | Measured | n (# of samples) |
|---|---|---|
| Control | 3.1 – 3.5 | 5 |
| 6.25 | 9.4 – 12.1 | 6 |
| 12.5 | 14.7 - 18.3 | 6 |
| 25 | 23.0 – 39.9 | 6 |
| 50 | 55.6 – 67.0 | 6 |
| 100 | 79.0 - 130 | 6 |

Appendix A3. Nominal and measured concentration of nitrate-nitrogen for flow-through test with Lake Superior domestic brook trout embryos and larvae in soft water. Measured values are reported in mg/L $NO_3$-N

| Nominal | Measured | n (# of samples) |
|---|---|---|
| Control | 0.03 – 0.2 | 6 |
| 6.25 | 3.5 – 7.3 | 5 |
| 12.5 | 9.7 – 13.9 | 6 |
| 25 | 15.4 –26.4 | 6 |
| 50 | 36.5 – 52.0 | 6 |
| 100 | 64.0 – 112.0 | 6 |

# Appendix B

Daily pH measurements for exposures

Appendix B1. The pH measurements made in one compartment of exposure treatments for feral brook trout from Melancthron Creek.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 12-Nov-98 | 7.90 | 7.83 | 7.72 | 7.89 | 7.83 | 7.78 |
| 13-Nov-98 | 8.12 | 8.12 | 8.09 | 8.04 | 8.02 | 8.00 |
| 14-Nov-98 | 7.99 | 8.00 | 7.98 | 7.95 | 7.88 | 7.99 |
| 15-Nov-98 | 7.82 | 7.72 | 7.81 | 7.90 | 7.91 | 7.90 |
| 16-Nov-98 | 8.01 | 8.24 | 8.20 | 8.14 | 8.21 | 8.02 |
| 17-Nov-98 | 7.77 | 7.82 | 7.88 | 7.92 | 7.96 | 7.94 |
| 18-Nov-98 | 8.00 | 8.07 | 8.06 | 7.98 | 7.99 | 7.94 |
| 19-Nov-98 | 7.89 | 7.90 | 7.97 | 7.99 | 8.14 | 8.09 |
| 20-Nov-98 | 8.01 | 8.07 | 8.10 | 8.05 | 8.05 | 8.01 |
| 21-Nov-98 | 8.14 | 8.01 | 8.07 | 8.16 | 8.19 | 8.03 |
| 22-Nov-98 | 8.09 | 8.02 | 8.03 | 8.15 | 8.17 | 8.01 |
| 23-Nov-98 | 7.86 | 8.08 | 8.09 | 8.02 | 7.98 | 7.97 |
| 24-Nov-98 | 7.96 | 8.00 | 8.11 | 8.03 | 8.02 | 7.98 |
| 25-Nov-98 | 7.97 | 8.03 | 8.15 | 8.08 | 8.09 | 8.04 |
| 26-Nov-98 | 7.99 | 8.02 | 8.04 | 7.97 | 8.01 | 7.94 |
| 27-Nov-98 | 8.04 | 8.04 | 8.08 | 8.07 | 8.01 | 7.96 |
| 28-Nov-98 | 8.01 | 7.87 | 8.00 | 8.11 | 8.03 | 7.99 |
| 29-Nov-98 | 7.79 | 7.89 | 7.91 | 7.91 | 7.95 | 7.97 |
| 30-Nov-98 | 8.02 | 8.14 | 8.12 | 8.05 | 8.02 | 8.00 |
| 01-Dec-98 | 7.91 | 8.05 | 8.06 | 8.03 | 8.00 | 7.95 |
| 02-Dec-98 | 8.02 | 8.08 | 8.10 | 8.01 | 8.00 | 7.96 |
| 03-Dec-98 | 8.03 | 7.86 | 8.10 | 8.04 | 8.01 | 7.98 |
| 04-Dec-98 | 7.95 | 8.03 | 7.94 | 8.05 | 8.01 | 7.98 |
| 05-Dec-98 | 7.97 | 8.04 | 8.07 | 8.03 | 7.98 | 7.96 |
| 06-Dec-98 | 7.97 | 8.06 | 8.06 | 7.98 | 7.99 | 7.97 |
| 07-Dec-98 | 7.93 | 7.73 | 8.06 | 7.98 | 7.95 | 7.89 |
| 08-Dec-98 | 7.74 | 8.02 | 7.80 | 7.74 | 7.74 | 7.81 |
| 09-Dec-98 | 7.88 | 8.06 | 8.04 | 8.05 | 7.94 | 7.89 |
| 10-Dec-98 | 8.03 | 7.88 | 8.05 | 7.86 | 7.95 | 7.91 |
| 11-Dec-98 | 7.78 | 8.09 | 7.92 | 7.89 | 7.90 | 7.97 |
| 12-Dec-98 | 8.04 | 8.08 | 8.07 | 7.99 | 7.99 | 7.96 |
| 13-Dec-98 | 8.01 | 8.02 | 8.07 | 7.99 | 7.99 | 7.97 |
| 14-Dec-98 | 8.01 | 8.02 | 8.10 | 7.98 | 7.90 | 7.93 |
| 15-Dec-98 | 8.03 | 8.03 | 8.05 | 7.98 | 7.97 | 7.93 |
| 16-Dec-98 | 7.97 | 8.00 | 8.00 | 7.92 | 7.91 | 7.88 |
| 17-Dec-98 | 7.73 | 7.83 | 7.84 | 7.83 | 7.85 | 7.93 |
| 18-Dec-98 | 7.93 | 7.95 | 7.95 | 7.87 | 7.86 | 7.85 |
| 19-Dec-98 | 8.05 | 8.08 | 8.07 | 7.97 | 7.92 | 7.91 |
| 20-Dec-98 | 8.06 | 8.19 | 8.15 | 8.05 | 8.03 | 8.02 |
| 21-Dec-98 | 7.95 | 8.02 | 8.02 | 7.89 | 8.05 | 8.05 |
| 22-Dec-98 | 8.05 | 8.02 | 8.04 | 8.13 | 8.02 | 8.00 |
| 23-Dec-98 | 7.90 | 7.96 | 8.02 | 8.07 | 8.08 | 8.14 |
| 24-Dec-98 | 7.84 | 7.92 | 7.92 | 7.86 | 7.85 | 7.88 |
| 25-Dec-98 | 7.91 | 7.94 | 7.93 | 7.90 | 7.97 | 8.01 |
| 26-Dec-98 | 7.98 | 8.00 | 8.02 | 7.97 | 7.96 | 8.03 |
| 27-Dec-98 | 7.74 | 7.87 | 7.91 | 7.89 | 7.93 | 7.99 |
| 28-Dec-98 | 7.88 | 7.96 | 7.97 | 7.89 | 7.96 | 7.98 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 29-Dec-98 | 7.65 | 7.78 | 7.82 | 7.74 | 7.75 | 7.90 |
| 30-Dec-98 | 7.95 | 7.95 | 7.94 | 7.90 | 7.88 | 7.94 |
| 31-Dec-98 | 7.94 | 7.96 | 7.98 | 7.87 | 7.87 | 7.89 |
| 01-Jan-99 | 7.94 | 7.86 | 7.88 | 7.83 | 7.80 | 7.89 |
| 02-Jan-99 | 7.78 | 7.94 | 7.95 | 7.89 | 7.85 | 7.86 |
| 03-Jan-99 | 7.86 | 7.90 | 7.92 | 7.84 | 7.80 | 7.86 |
| 04-Jan-99 | 7.87 | 8.02 | 8.01 | 7.91 | 7.89 | 7.93 |
| 05-Jan-99 | 7.90 | 7.97 | 8.01 | 8.02 | 8.04 | 8.04 |
| 06-Jan-99 | 7.83 | 7.89 | 7.95 | 7.95 | 7.99 | 8.09 |
| 07-Jan-99 | 7.45 | 7.56 | 7.64 | 7.64 | 7.68 | 7.74 |
| 08-Jan-99 | 7.98 | 7.99 | 7.97 | 7.94 | 7.94 | 7.90 |
| 09-Jan-99 | 7.93 | 7.97 | 7.95 | 7.91 | 7.90 | 7.75 |
| 10-Jan-99 | 7.95 | 8.02 | 7.98 | 7.93 | 7.92 | 7.92 |
| 11-Jan-99 | 8.01 | 8.00 | 8.02 | 7.88 | 7.98 | 7.95 |
| 12-Jan-99 | 7.96 | 8.04 | 8.05 | 7.90 | 7.96 | 7.95 |
| 13-Jan-99 | 8.10 | 8.06 | 8.08 | 7.96 | 8.01 | 8.01 |
| 14-Jan-99 | 7.80 | 7.82 | 7.85 | 7.82 | 7.89 | 7.89 |
| 15-Jan-99 | 7.98 | 8.00 | 8.00 | 7.94 | 7.97 | 8.00 |
| 16-Jan-99 | 8.01 | 8.01 | 8.01 | 7.91 | 7.90 | 7.89 |
| 17-Jan-99 | 7.98 | 7.96 | 7.92 | 7.93 | 7.93 | 7.95 |
| 18-Jan-99 | 7.81 | 7.80 | 7.84 | 7.86 | 7.89 | 7.85 |
| 19-Jan-99 | 7.96 | 7.98 | 7.94 | 7.82 | 8.09 | 7.87 |
| 20-Jan-99 | 8.00 | 8.00 | 7.95 | 7.84 | 7.86 | 7.87 |
| 21-Jan-99 | 8.08 | 8.05 | 8.02 | 7.95 | 7.92 | 7.90 |
| 22-Jan-99 | 8.03 | 8.00 | 7.97 | 7.92 | 7.29 | 7.90 |
| 23-Jan-99 | 8.09 | 8.00 | 7.97 | 7.92 | 7.92 | 8.06 |
| 24-Jan-99 | 8.08 | 8.04 | 7.99 | 7.94 | 7.95 | 7.98 |
| 25-Jan-99 | 8.04 | 8.00 | 7.99 | 7.93 | 7.94 | 7.94 |
| 26-Jan-99 | 8.02 | 8.09 | 8.02 | 7.99 | 7.99 | 8.01 |
| 27-Jan-99 | 7.90 | 7.98 | 7.95 | 7.85 | 7.85 | 7.86 |
| 28-Jan-99 | 8.03 | 8.00 | 7.88 | 7.92 | 7.91 | 7.98 |
| 29-Jan-99 | 7.83 | 7.95 | 7.98 | 7.89 | 7.89 | 7.84 |
| 30-Jan-99 | 8.18 | 8.18 | 8.17 | 7.99 | 8.06 | 8.04 |
| 31-Jan-99 | 8.13 | 8.12 | 8.10 | 8.01 | 8.03 | 7.99 |
| 01-Feb-99 | 8.05 | 8.04 | 7.99 | 7.96 | 7.98 | 8.03 |
| 02-Feb-99 | 8.07 | 8.04 | 8.03 | 8.01 | 7.98 | 7.99 |
| 03-Feb-99 | 8.01 | 8.00 | 8.02 | 7.98 | 7.95 | 7.92 |
| 04-Feb-99 | 8.06 | 8.03 | 8.05 | 8.00 | 7.97 | 7.95 |
| 05-Feb-99 | 8.00 | 7.99 | 8.04 | 7.99 | 7.97 | 8.03 |
| 06-Feb-99 | 7.87 | 7.91 | 7.95 | 8.00 | 7.93 | 7.96 |
| 07-Feb-99 | 7.79 | 7.87 | 7.88 | 7.84 | 7.87 | 7.87 |
| 08-Feb-99 | 7.80 | 7.99 | 8.04 | 8.00 | 7.97 | 7.94 |
| 09-Feb-99 | 8.13 | 8.08 | 8.07 | 8.03 | 8.03 | 8.00 |
| 10-Feb-99 | 7.76 | 7.72 | 7.77 | 7.80 | 7.79 | 7.89 |
| 11-Feb-99 | 7.87 | 7.80 | 7.90 | 7.81 | 7.89 | 7.91 |
| 12-Feb-99 | 7.77 | 7.69 | 7.75 | 7.72 | 7.77 | 7.80 |

Appendix B2. The pH measurement made in one compartment of exposure treatments for Lake Superior domestic brook trout in hard water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 7.72 | 7.76 | 7.76 | 7.78 | 7.80 | 7.80 |
| 01-Dec-99 | 7.87 | 7.80 | 7.81 | 7.79 | 7.81 | 7.81 |
| 02-Dec-99 | 7.76 | 7.71 | 7.69 | 7.83 | 7.90 | 7.95 |
| 03-Dec-99 | 7.85 | 7.94 | 7.94 | 7.94 | 7.93 | 7.92 |
| 04-Dec-99 | 7.63 | 7.64 | 7.63 | 7.62 | 7.62 | 7.60 |
| 05-Dec-99 | 7.95 | 7.90 | 7.91 | 7.92 | 7.90 | 7.94 |
| 06-Dec-99 | 7.95 | 7.90 | 7.91 | 7.92 | 7.90 | 7.94 |
| 07-Dec-99 | 7.68 | 7.72 | 7.72 | 7.74 | 7.72 | 7.64 |
| 08-Dec-99 | 7.75 | 7.67 | 7.67 | 7.73 | 7.70 | 7.70 |
| 09-Dec-99 | 8.03 | 7.96 | 7.92 | 7.90 | 7.87 | 7.85 |
| 10-Dec-99 | 7.65 | 7.59 | 7.58 | 7.56 | 7.57 | 7.61 |
| 11-Dec-99 | 7.76 | 7.70 | 7.66 | 7.65 | 7.62 | 7.62 |
| 12-Dec-99 | 7.47 | 7.46 | 7.49 | 7.48 | 7.46 | 7.44 |
| 13-Dec-99 | 7.35 | 7.32 | 7.40 | 7.42 | 7.40 | 7.41 |
| 14-Dec-99 | 7.56 | 7.51 | 7.49 | 7.50 | 7.44 | 7.44 |
| 15-Dec-99 | 7.41 | 7.43 | 7.46 | 7.48 | 7.52 | 7.56 |
| 16-Dec-99 | 7.90 | 7.82 | 7.81 | 7.80 | 7.80 | 7.77 |
| 17-Dec-99 | 7.49 | 7.46 | 7.45 | 7.44 | 7.41 | 7.38 |
| 18-Dec-99 | 7.41 | 7.37 | 7.36 | 7.34 | 7.32 | 7.28 |
| 19-Dec-99 | 7.42 | 7.37 | 7.37 | 7.35 | 7.33 | 7.30 |
| 20-Dec-99 | 7.66 | 7.61 | 7.60 | 7.56 | 7.51 | 7.48 |
| 22-Dec-99 | 7.66 | 7.62 | 7.67 | 7.64 | 7.62 | 7.60 |
| 23-Dec-99 | 7.46 | 7.42 | 7.41 | 7.33 | 7.35 | 7.31 |
| 24-Dec-99 | 7.52 | 7.47 | 7.48 | 7.44 | 7.42 | 7.39 |
| 26-Dec-99 | 7.77 | 7.72 | 7.68 | 7.60 | 7.58 | 7.54 |
| 29-Dec-99 | 7.86 | 7.96 | 7.63 | 7.59 | 7.60 | 7.60 |
| 30-Dec-99 | 7.80 | 8.01 | 7.94 | 7.88 | 7.83 | 7.82 |
| 31-Dec-99 | 7.57 | 7.62 | 7.59 | 7.53 | 7.49 | 7.52 |
| 01-Jan-00 | 7.41 | 7.39 | 7.36 | 7.30 | 7.30 | 7.31 |
| 02-Jan-00 | 7.41 | 7.72 | 7.70 | 7.66 | 7.63 | 7.60 |
| 03-Jan-00 | 7.65 | 7.64 | 7.69 | 7.67 | 7.66 | 7.67 |
| 04-Jan-00 | 8.07 | 8.00 | 8.00 | 7.92 | 7.87 | 7.84 |
| 05-Jan-00 | 7.78 | 7.78 | 7.76 | 7.71 | 7.67 | 7.64 |
| 06-Jan-00 | 8.03 | 7.98 | 7.94 | 7.87 | 7.79 | 7.76 |
| 07-Jan-00 | 7.84 | 7.78 | 7.74 | 7.68 | 7.60 | 7.55 |
| 08-Jan-00 | 7.49 | 7.61 | 7.66 | 7.58 | 7.53 | 7.50 |
| 09-Jan-00 | 7.68 | 7.67 | 7.65 | 7.58 | 7.54 | 7.48 |
| 11-Jan-00 | 8.07 | 7.99 | 7.97 | 7.90 | 7.83 | 7.78 |
| 12-Jan-00 | 7.56 | 7.54 | 7.52 | 7.51 | 7.44 | 7.42 |
| 13-Jan-00 | 7.97 | 7.89 | 7.89 | 7.80 | 7.82 | 7.80 |
| 14-Jan-00 | 8.01 | 8.01 | 8.12 | 8.08 | 8.04 | 8.00 |
| 15-Jan-00 | 7.67 | 7.73 | 7.74 | 7.70 | 7.65 | 7.76 |
| 16-Jan-00 | 7.80 | 7.77 | 7.73 | 7.68 | 7.62 | 7.69 |
| 17-Jan-00 | 7.77 | 7.75 | 7.71 | 7.66 | 7.76 | 7.62 |
| 18-Jan-00 | 7.50 | 7.49 | 7.47 | 7.48 | 7.42 | 7.37 |
| 19-Jan-00 | 7.56 | 7.51 | 7.57 | 7.45 | 7.52 | 7.57 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 20-Jan-00 | 7.50 | 7.48 | 7.46 | 7.40 | 7.36 | 7.31 |
| 21-Jan-00 | 7.58 | 7.64 | 7.69 | 7.65 | 7.59 | 7.55 |
| 22-Jan-00 | 7.82 | 7.74 | 7.80 | 7.89 | 7.72 | 7.83 |
| 23-Jan-00 | 7.60 | 7.62 | 7.60 | 7.53 | 7.45 | 7.38 |
| 24-Jan-00 | 7.70 | 7.66 | 7.64 | 7.55 | 7.49 | 7.43 |
| 25-Jan-00 | 7.66 | 7.61 | 7.60 | 7.53 | 7.54 | 7.58 |
| 26-Jan-00 | 7.96 | 7.85 | 7.79 | 7.70 | 7.62 | 7.62 |
| 27-Jan-00 | 7.41 | 7.53 | 7.54 | 7.56 | 7.58 | 7.60 |
| 28-Jan-00 | 7.74 | 7.76 | 7.74 | 7.81 | 7.80 | 7.83 |
| 29-Jan-00 | 7.72 | 7.75 | 7.77 | 7.78 | 7.77 | 7.79 |
| 30-Jan-00 | 7.70 | 7.72 | 7.72 | 7.73 | 7.86 | 7.89 |
| 31-Jan-00 | 7.80 | 7.84 | 7.85 | 7.87 | 7.85 | 7.87 |
| 02-Feb-00 | 7.52 | 7.56 | 7.61 | 7.63 | 7.63 | 7.66 |
| 03-Feb-00 | 7.42 | 7.44 | 7.48 | 7.51 | 7.53 | 7.57 |
| 04-Feb-00 | 7.50 | 7.50 | 7.53 | 7.57 | 7.57 | 7.62 |
| 05-Feb-00 | 7.77 | 7.30 | 7.33 | 7.34 | 7.36 | 7.44 |
| 06-Feb-00 | 7.82 | 7.82 | 7.83 | 7.83 | 7.86 | 7.84 |
| 07-Feb-00 | 7.78 | 7.80 | 7.80 | 7.80 | 7.78 | 7.83 |
| 08-Feb-00 | 7.32 | 7.34 | 7.38 | 7.38 | 7.36 | 7.39 |
| 09-Feb-00 | 7.75 | 7.80 | 7.83 | 7.84 | 7.84 | 7.83 |
| 10-Feb-00 | 8.11 | 8.10 | 8.07 | 8.08 | 8.05 | 8.06 |
| 11-Feb-00 | 7.42 | 7.38 | 7.39 | 7.44 | 7.54 | 7.66 |
| 12-Feb-00 | 7.33 | 7.33 | 7.32 | 7.38 | 7.36 | 7.45 |
| 13-Feb-00 | 7.54 | 7.53 | 7.51 | 7.51 | 7.48 | 7.51 |
| 14-Feb-00 | 7.97 | 7.95 | 7.91 | 7.97 | 7.95 | 7.95 |
| 15-Feb-00 | 7.36 | 7.46 | 7.46 | 7.50 | 7.59 | 7.61 |
| 16-Feb-00 | 7.66 | 7.69 | 7.70 | 7.72 | 7.83 | 7.98 |
| 18-Feb-00 | 7.78 | 7.78 | 7.77 | 7.78 | 7.75 | 7.87 |
| 19-Feb-00 | 7.70 | 7.70 | 7.71 | 7.72 | 7.70 | 7.71 |
| 20-Feb-00 | 8.02 | 7.99 | 7.97 | 7.97 | 7.92 | 7.97 |
| 21-Feb-00 | 7.55 | 7.61 | 7.63 | 7.87 | 7.83 | 7.81 |
| 22-Feb-00 | 7.82 | 7.85 | 7.83 | 7.83 | 7.81 | 7.84 |
| 23-Feb-00 | 7.87 | 7.85 | 7.83 | 7.83 | 7.81 | 7.84 |
| 24-Feb-00 | 7.86 | 7.88 | 7.87 | 7.87 | 7.84 | 7.84 |
| 25-Feb-00 | 7.87 | 7.91 | 7.91 | 7.92 | 7.91 | 7.88 |
| 26-Feb-00 | 7.56 | 7.62 | 7.59 | 7.61 | 7.64 | 7.75 |
| 27-Feb-00 | 7.50 | 7.54 | 7.58 | 7.57 | 7.58 | 7.65 |
| 28-Feb-00 | 7.48 | 7.52 | 7.50 | 7.52 | 7.55 | 7.59 |
| 29-Feb-00 | 7.93 | 7.94 | 7.92 | 7.90 | 7.93 | 7.89 |
| 01-Mar-00 | 7.38 | 7.57 | 7.62 | 7.69 | 7.77 | 7.73 |
| 02-Mar-00 | 7.74 | 7.75 | 7.78 | 7.75 | 7.76 | 7.72 |
| 03-Mar-00 | 7.40 | 7.44 | 7.44 | 7.45 | 7.53 | 7.53 |
| 04-Mar-00 | 7.50 | 7.55 | 7.63 | 7.63 | 7.69 | 7.71 |
| 05-Mar-00 | 7.68 | 7.71 | 7.70 | 7.70 | 7.74 | 7.73 |
| 06-Mar-00 | 7.43 | 7.50 | 7.51 | 7.53 | 7.58 | 7.57 |
| 07-Mar-00 | 7.50 | 7.52 | 7.55 | 7.55 | 7.57 | 7.62 |
| 08-Mar-00 | 7.82 | 7.83 | 7.82 | 7.79 | 7.82 | 7.81 |

Appendix B3. The pH measurement made in one compartment of exposure treatments for Lake Superior domestic brook trout in soft water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 7.40 | 7.37 | 7.36 | 7.34 | 7.31 | 7.34 |
| 01-Dec-99 | 8.20 | 8.15 | 8.05 | 8.00 | 7.92 | 8.00 |
| 02-Dec-99 | 7.12 | 7.15 | 7.16 | 7.14 | 7.13 | 7.14 |
| 03-Dec-99 | 7.24 | 7.29 | 7.27 | 7.26 | 7.23 | 7.26 |
| 04-Dec-99 | 7.16 | 7.18 | 7.15 | 7.15 | 7.13 | 7.15 |
| 05-Dec-99 | 7.15 | 7.18 | 7.20 | 7.19 | 7.16 | 7.19 |
| 06-Dec-99 | 7.15 | 7.18 | 7.20 | 7.19 | 7.16 | 7.19 |
| 07-Dec-99 | 7.34 | 7.35 | 7.34 | 7.32 | 7.27 | 7.32 |
| 08-Dec-99 | 7.15 | 7.16 | 7.19 | 7.17 | 7.14 | 7.17 |
| 09-Dec-99 | 7.22 | 7.21 | 7.18 | 7.19 | 7.14 | 7.19 |
| 10-Dec-99 | 7.13 | 7.12 | 7.15 | 7.11 | 7.07 | 7.11 |
| 11-Dec-99 | 7.06 | 7.09 | 7.13 | 7.11 | 7.08 | 7.11 |
| 12-Dec-99 | 7.05 | 7.05 | 7.07 | 7.05 | 7.05 | 7.05 |
| 13-Dec-99 | 7.09 | 7.10 | 7.10 | 7.09 | 7.07 | 7.09 |
| 14-Dec-99 | 7.17 | 7.22 | 7.17 | 7.13 | 7.08 | 7.13 |
| 15-Dec-99 | 7.30 | 7.26 | 7.26 | 7.27 | 7.23 | 7.27 |
| 16-Dec-99 | 7.12 | 7.10 | 7.13 | 7.13 | 7.11 | 7.13 |
| 17-Dec-99 | 7.12 | 7.11 | 7.10 | 7.09 | 7.05 | 7.09 |
| 18-Dec-99 | 7.14 | 7.12 | 7.12 | 7.08 | 7.05 | 7.08 |
| 19-Dec-99 | 7.15 | 7.14 | 7.16 | 7.10 | 7.05 | 7.10 |
| 20-Dec-99 | 7.20 | 7.14 | 7.15 | 7.11 | 7.04 | 7.11 |
| 22-Dec-99 | 7.18 | 7.08 | 7.13 | 7.12 | 7.09 | 7.12 |
| 23-Dec-99 | 7.14 | 7.16 | 7.12 | 7.10 | 7.03 | 7.10 |
| 24-Dec-99 | 7.17 | 7.14 | 7.14 | 7.13 | 7.08 | 7.13 |
| 26-Dec-99 | 7.17 | 7.15 | 7.14 | 7.17 | 7.09 | 7.12 |
| 29-Dec-99 | 7.18 | 7.21 | 7.20 | 7.15 | 7.12 | 7.15 |
| 30-Dec-99 | 7.21 | 7.17 | 7.14 | 7.11 | 7.04 | 7.11 |
| 31-Dec-99 | 7.25 | 7.23 | 7.17 | 7.12 | 7.06 | 7.12 |
| 01-Jan-00 | 7.32 | 7.25 | 7.28 | 7.17 | 7.09 | 7.17 |
| 02-Jan-00 | 7.31 | 7.32 | 7.34 | 7.27 | 7.19 | 7.27 |
| 03-Jan-00 | 7.26 | 7.25 | 7.24 | 7.23 | 7.19 | 7.23 |
| 04-Jan-00 | 7.21 | 7.19 | 7.17 | 7.15 | 7.11 | 7.15 |
| 05-Jan-00 | 7.50 | 7.51 | 7.49 | 7.45 | 7.39 | 7.45 |
| 06-Jan-00 | 7.24 | 7.20 | 7.19 | 7.17 | 7.12 | 7.17 |
| 07-Jan-00 | 7.32 | 7.29 | 7.26 | 7.19 | 7.14 | 7.19 |
| 08-Jan-00 | 7.84 | 7.78 | 7.74 | 7.68 | 7.60 | 7.68 |
| 09-Jan-00 | 7.26 | 7.26 | 7.27 | 7.25 | 7.16 | 7.25 |
| 11-Jan-00 | 7.35 | 7.37 | 7.38 | 7.30 | 7.24 | 7.30 |
| 12-Jan-00 | 7.34 | 7.32 | 7.30 | 7.22 | 7.17 | 7.22 |
| 13-Jan-00 | 7.42 | 7.46 | 7.43 | 7.39 | 7.31 | 7.39 |
| 14-Jan-00 | 7.01 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| 15-Jan-00 | 7.40 | 7.37 | 7.35 | 7.32 | 7.25 | 7.32 |
| 16-Jan-00 | 7.23 | 7.20 | 7.17 | 7.11 | 7.06 | 7.11 |
| 17-Jan-00 | 8.00 | 8.00 | 7.89 | 7.83 | 7.70 | 7.83 |
| 18-Jan-00 | 7.26 | 7.24 | 7.18 | 7.14 | 7.06 | 7.14 |
| 19-Jan-00 | 7.31 | 7.22 | 7.16 | 7.11 | 7.02 | 7.11 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 20-Jan-00 | 7.76 | 7.25 | 7.17 | 7.11 | 7.01 | 7.11 |
| 21-Jan-00 | 7.30 | 7.22 | 7.17 | 7.14 | 7.11 | 7.14 |
| 22-Jan-00 | 7.35 | 7.27 | 7.21 | 7.14 | 7.06 | 7.14 |
| 23-Jan-00 | 7.24 | 7.30 | 7.24 | 7.27 | 7.06 | 7.27 |
| 24-Jan-00 | 7.30 | 7.25 | 7.17 | 7.15 | 7.06 | 7.15 |
| 25-Jan-00 | 7.28 | 7.18 | 7.12 | 7.06 | 7.02 | 7.06 |
| 26-Jan-00 | 7.51 | 7.36 | 7.28 | 7.22 | 7.12 | 7.22 |
| 27-Jan-00 | 7.02 | 7.03 | 7.03 | 7.03 | 7.02 | 7.03 |
| 28-Jan-00 | 7.53 | 7.54 | 7.52 | 7.51 | 7.49 | 7.51 |
| 29-Jan-00 | 7.96 | 7.93 | 7.92 | 7.90 | 7.89 | 7.90 |
| 30-Jan-00 | 7.24 | 7.20 | 7.18 | 7.14 | 7.14 | 7.14 |
| 31-Jan-00 | 7.24 | 7.27 | 7.27 | 7.29 | 7.36 | 7.29 |
| 02-Feb-00 | 7.06 | 7.07 | 7.07 | 7.08 | 7.06 | 7.08 |
| 03-Feb-00 | 7.55 | 7.55 | 7.54 | 7.53 | 7.51 | 7.53 |
| 04-Feb-00 | 7.86 | 7.83 | 7.82 | 7.79 | 7.75 | 7.79 |
| 05-Feb-00 | 7.75 | 7.74 | 7.71 | 7.69 | 7.63 | 7.69 |
| 06-Feb-00 | 8.07 | 8.06 | 8.04 | 7.98 | 7.94 | 7.98 |
| 07-Feb-00 | 7.98 | 7.96 | 7.93 | 7.93 | 7.89 | 7.93 |
| 08-Feb-00 | 7.71 | 7.66 | 7.64 | 7.60 | 7.57 | 7.60 |
| 09-Feb-00 | 8.07 | 8.06 | 8.03 | 7.83 | 7.82 | 7.83 |
| 10-Feb-00 | 7.39 | 7.40 | 7.40 | 7.41 | 7.41 | 7.41 |
| 11-Feb-00 | 7.89 | 7.87 | 7.83 | 7.81 | 7.72 | 7.81 |
| 12-Feb-00 | 7.72 | 7.70 | 7.67 | 7.65 | 7.61 | 7.65 |
| 13-Feb-00 | 7.78 | 7.71 | 7.56 | 7.55 | 7.51 | 7.55 |
| 14-Feb-00 | 7.28 | 7.31 | 7.33 | 7.32 | 7.30 | 7.32 |
| 15-Feb-00 | 7.03 | 7.05 | 7.05 | 7.07 | 7.05 | 7.07 |
| 16-Feb-00 | 7.20 | 7.21 | 7.23 | 7.22 | 7.19 | 7.22 |
| 18-Feb-00 | 7.22 | 7.24 | 7.22 | 7.27 | 7.21 | 7.22 |
| 19-Feb-00 | 7.48 | 7.48 | 7.47 | 7.46 | 7.43 | 7.46 |
| 20-Feb-00 | 8.22 | 8.19 | 8.17 | 8.15 | 8.14 | 8.15 |
| 21-Feb-00 | 7.30 | 7.30 | 7.31 | 7.34 | 7.33 | 7.34 |
| 23-Feb-00 | 7.07 | 7.08 | 7.11 | 7.09 | 7.07 | 7.06 |
| 24-Feb-00 | 7.16 | 7.22 | 7.24 | 7.22 | 7.22 | 7.17 |
| 25-Feb-00 | 7.16 | 7.21 | 7.18 | 7.15 | 7.14 | 7.23 |
| 26-Feb-00 | 7.13 | 7.11 | 7.16 | 7.14 | 7.12 | 7.08 |
| 27-Feb-00 | 7.18 | 7.16 | 7.17 | 7.16 | 7.13 | 7.09 |
| 28-Feb-00 | 7.11 | 7.13 | 7.14 | 7.12 | 7.11 | 7.07 |
| 29-Feb-00 | 7.21 | 7.25 | 7.25 | 7.22 | 7.20 | 7.20 |
| 01-Mar-00 | 7.06 | 7.04 | 7.04 | 7.04 | 7.03 | 7.00 |
| 02-Mar-00 | 7.55 | 7.37 | 7.33 | 7.36 | 7.34 | 7.27 |
| 03-Mar-00 | 7.27 | 7.19 | 7.16 | 7.15 | 7.12 | 7.08 |
| 04-Mar-00 | 7.60 | 7.61 | 7.60 | 7.58 | 7.54 | 7.49 |
| 05-Mar-00 | 7.10 | 7.08 | 7.07 | 7.15 | 7.12 | 7.15 |
| 06-Mar-00 | 7.15 | 7.15 | 7.18 | 7.13 | 7.13 | 7.09 |
| 07-Mar-00 | 7.10 | 7.11 | 7.12 | 7.10 | 7.09 | 7.05 |
| 08-Mar-00 | 7.27 | 7.28 | 7.27 | 7.26 | 7.24 | 7.19 |

# Appendix C

Daily DO measurements for exposures
mg/L $O_2$

Appendix C1. DO measurements (mg/L $O_2$) for nitrate concentrations in tests of feral brook trout from Melancthron Creek.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 12-Nov-98 | 9.91 | 9.55 | 9.22 | 9.55 | 9.65 | 9.80 |
| 13-Nov-98 | 10.50 | 10.36 | 10.44 | 10.35 | 10.21 | 10.33 |
| 14-Nov-98 | 10.42 | 10.40 | 10.36 | 10.10 | 10.39 | 10.13 |
| 15-Nov-98 | 10.99 | 10.82 | 10.51 | 10.69 | 10.49 | 10.62 |
| 16-Nov-98 | 11.31 | 11.03 | 10.81 | 10.38 | 10.41 | 10.60 |
| 17-Nov-98 | 10.12 | 10.09 | 10.23 | 10.01 | 10.12 | 10.24 |
| 18-Nov-98 | 10.62 | 10.61 | 10.16 | 10.48 | 10.26 | 10.46 |
| 19-Nov-98 | 11.13 | 10.53 | 10.72 | 10.34 | 10.31 | 10.46 |
| 20-Nov-98 | 11.27 | 11.01 | 10.90 | 10.48 | 10.39 | 10.53 |
| 21-Nov-98 | 9.90 | 9.98 | 9.95 | 10.24 | 10.28 | 10.38 |
| 22-Nov-98 | 9.84 | 9.90 | 9.75 | 10.24 | 10.25 | 10.46 |
| 23-Nov-98 | 11.34 | 11.09 | 10.91 | 10.44 | 10.35 | 10.48 |
| 24-Nov-98 | 10.61 | 10.68 | 10.69 | 10.35 | 10.45 | 10.60 |
| 25-Nov-98 | 11.17 | 10.91 | 10.77 | 10.40 | 10.34 | 10.45 |
| 26-Nov-98 | 11.24 | 10.83 | 10.71 | 10.44 | 10.44 | 10.50 |
| 27-Nov-98 | 10.65 | 10.86 | 10.90 | 10.58 | 10.60 | 10.74 |
| 28-Nov-98 | 11.21 | 11.12 | 10.62 | 10.57 | 10.72 | 10.65 |
| 29-Nov-98 | 10.70 | 10.78 | 10.72 | 10.29 | 10.28 | 10.36 |
| 30-Nov-98 | 10.74 | 10.78 | 10.70 | 10.24 | 10.16 | 10.42 |
| 01-Dec-98 | 10.82 | 10.63 | 10.57 | 10.24 | 10.28 | 10.35 |
| 02-Dec-98 | 11.37 | 11.00 | 10.83 | 10.35 | 10.23 | 10.40 |
| 03-Dec-98 | 11.21 | 11.06 | 11.75 | 10.28 | 10.18 | 10.37 |
| 04-Dec-98 | 11.15 | 10.98 | 10.81 | 10.36 | 10.30 | 10.54 |
| 05-Dec-98 | 10.50 | 10.53 | 10.51 | 10.14 | 10.07 | 10.30 |
| 06-Dec-98 | 11.02 | 10.84 | 10.75 | 10.33 | 10.17 | 10.51 |
| 07-Dec-98 | 10.30 | 10.49 | 10.38 | 10.20 | 9.96 | 10.39 |
| 08-Dec-98 | 10.56 | 10.48 | 10.40 | 9.96 | 9.82 | 10.13 |
| 09-Dec-98 | 10.86 | 10.48 | 10.32 | 10.36 | 9.87 | 10.09 |
| 10-Dec-98 | 9.92 | 10.22 | 10.36 | 9.99 | 9.94 | 10.26 |
| 11-Dec-98 | 10.42 | 10.48 | 10.30 | 9.99 | 9.87 | 10.90 |
| 12-Dec-98 | 10.70 | 10.50 | 10.35 | 9.77 | 9.88 | 10.04 |
| 13-Dec-98 | 10.72 | 10.61 | 10.27 | 9.93 | 9.74 | 9.94 |
| 14-Dec-98 | 10.01 | 10.04 | 10.12 | 10.43 | 10.40 | 10.32 |
| 15-Dec-98 | 10.92 | 10.89 | 10.73 | 10.19 | 10.24 | 10.50 |
| 16-Dec-98 | 11.01 | 10.87 | 10.67 | 10.20 | 10.12 | 10.43 |
| 17-Dec-98 | 10.53 | 10.61 | 10.40 | 10.06 | 10.02 | 10.33 |
| 18-Dec-98 | 10.35 | 10.17 | 10.18 | 9.78 | 9.81 | 10.04 |
| 19-Dec-98 | 10.37 | 10.43 | 10.42 | 10.05 | 9.93 | 10.45 |
| 20-Dec-98 | 10.87 | 10.82 | 10.66 | 10.27 | 10.19 | 10.60 |
| 21-Dec-98 | 11.25 | 11.03 | 10.81 | 10.25 | 10.19 | 10.57 |
| 22-Dec-98 | 10.57 | 10.65 | 10.42 | 10.68 | 10.46 | 10.73 |
| 23-Dec-98 | 10.19 | 10.25 | 10.21 | 9.84 | 9.78 | 10.24 |
| 24-Dec-98 | 10.62 | 10.42 | 10.15 | 9.74 | 9.77 | 9.97 |
| 25-Dec-98 | 10.51 | 10.34 | 10.01 | 9.60 | 9.48 | 9.92 |
| 26-Dec-98 | 10.28 | 10.40 | 10.21 | 9.70 | 9.67 | 10.20 |
| 27-Dec-98 | 10.92 | 11.06 | 10.96 | 10.75 | 10.14 | 10.64 |
| 28-Dec-98 | 10.84 | 11.03 | 10.48 | 10.20 | 10.08 | 10.14 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 29-Dec-98 | 10.20 | 9.92 | 9.72 | 9.45 | 9.20 | 9.78 |
| 30-Dec-98 | 10.20 | 10.27 | 10.25 | 9.76 | 9.78 | 10.17 |
| 31-Dec-98 | 10.15 | 10.30 | 10.25 | 9.75 | 9.74 | 10.25 |
| 01-Jan-99 | 9.81 | 9.96 | 9.97 | 9.50 | 9.40 | 9.95 |
| 02-Jan-99 | 10.38 | 10.45 | 10.25 | 9.84 | 9.63 | 10.27 |
| 03-Jan-99 | 9.96 | 9.87 | 9.78 | 9.42 | 9.19 | 9.86 |
| 04-Jan-99 | 9.80 | 9.91 | 9.80 | 9.22 | 9.23 | 9.84 |
| 05-Jan-99 | 9.66 | 9.82 | 9.99 | 9.80 | 9.66 | 10.18 |
| 06-Jan-99 | 10.24 | 9.97 | 10.00 | 9.66 | 9.63 | 9.96 |
| 07-Jan-99 | 9.95 | 10.04 | 10.03 | 9.76 | 9.85 | 10.10 |
| 08-Jan-99 | 9.97 | 10.01 | 9.90 | 9.75 | 9.73 | 10.03 |
| 09-Jan-99 | 9.50 | 9.88 | 9.98 | 9.51 | 9.71 | 8.67 |
| 10-Jan-99 | 10.06 | 10.07 | 9.87 | 9.74 | 9.69 | 9.87 |
| 11-Jan-99 | 10.18 | 10.71 | 10.44 | 10.25 | 10.14 | 10.26 |
| 12-Jan-99 | 9.56 | 10.07 | 10.15 | 9.77 | 9.91 | 10.20 |
| 13-Jan-99 | 9.68 | 10.00 | 9.99 | 9.70 | 9.77 | 9.97 |
| 14-Jan-99 | 9.54 | 9.77 | 9.76 | 8.82 | 9.45 | 9.27 |
| 15-Jan-99 | 10.10 | 10.14 | 10.02 | 9.79 | 9.81 | 9.98 |
| 16-Jan-99 | 10.03 | 10.04 | 9.90 | 10.06 | 10.20 | 10.11 |
| 17-Jan-99 | 9.32 | 9.48 | 9.49 | 9.77 | 9.49 | 10.34 |
| 18-Jan-99 | 8.78 | 9.10 | 9.31 | 9.21 | 9.07 | 9.27 |
| 19-Jan-99 | 9.77 | 9.65 | 9.52 | 9.57 | 9.46 | 9.27 |
| 20-Jan-99 | 8.99 | 8.96 | 8.90 | 9.14 | 8.80 | 8.62 |
| 21-Jan-99 | 10.01 | 9.72 | 9.50 | 9.83 | 9.28 | 9.86 |
| 22-Jan-99 | 9.70 | 9.68 | 9.63 | 9.50 | 9.25 | 10.23 |
| 23-Jan-99 | 9.90 | 9.73 | 9.42 | 9.26 | 9.45 | 10.03 |
| 24-Jan-99 | 9.74 | 9.88 | 9.58 | 9.12 | 9.56 | 10.30 |
| 25-Jan-99 | 9.74 | 9.92 | 9.56 | 9.32 | 9.61 | 10.12 |
| 26-Jan-99 | 9.68 | 10.00 | 9.65 | 9.26 | 9.77 | 10.17 |
| 27-Jan-99 | 9.43 | 9.75 | 9.39 | 9.42 | 9.40 | 10.04 |
| 28-Jan-99 | 9.58 | 9.77 | 9.52 | 9.27 | 9.33 | 9.91 |
| 29-Jan-99 | 9.15 | 9.58 | 9.23 | 8.98 | 9.20 | 9.59 |
| 30-Jan-99 | 10.95 | 10.65 | 10.83 | 10.40 | 10.30 | 10.50 |
| 31-Jan-99 | 10.24 | 10.16 | 10.30 | 10.22 | 10.12 | 10.24 |
| 01-Feb-99 | 10.22 | 10.18 | 10.45 | 10.31 | 10.26 | 10.42 |
| 02-Feb-99 | 10.30 | 10.66 | 10.74 | 10.69 | 10.52 | 10.60 |
| 03-Feb-99 | 10.85 | 10.59 | 10.78 | 10.54 | 10.85 | 10.63 |
| 04-Feb-99 | 10.70 | 10.60 | 10.42 | 10.11 | 10.17 | 10.51 |
| 05-Feb-99 | 10.81 | 10.69 | 10.70 | 10.39 | 10.44 | 10.76 |
| 06-Feb-99 | 10.77 | 10.47 | 10.64 | 10.42 | 10.44 | 10.72 |
| 07-Feb-99 | 11.00 | 10.83 | 10.74 | 10.46 | 10.52 | 10.78 |
| 08-Feb-99 | 10.87 | 10.51 | 10.49 | 10.26 | 10.35 | 10.51 |
| 09-Feb-99 | 10.85 | 10.44 | 10.35 | 9.99 | 10.03 | 10.11 |
| 10-Feb-99 | 11.07 | 10.66 | 10.68 | 10.32 | 10.20 | 10.50 |
| 11-Feb-99 | 10.57 | 10.31 | 10.42 | 10.14 | 10.12 | 10.46 |
| 12-Feb-99 | 11.01 | 10.70 | 10.53 | 10.51 | 10.48 | 10.72 |

Appendix C2. DO measurements (mg/L $O_2$) for nitrate concentration in the test of Lake Superior brook trout with hard water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 10.40 | 10.37 | 10.19 | 10.48 | 10.14 | 9.94 |
| 01-Dec-99 | 10.87 | 10.35 | 10.59 | 10.90 | 10.51 | 10.64 |
| 02-Dec-99 | 10.80 | 10.41 | 10.49 | 10.79 | 10.64 | 10.24 |
| 03-Dec-99 | 10.31 | 10.27 | 10.57 | 10.77 | 10.39 | 10.17 |
| 04-Dec-99 | 9.87 | 10.28 | 10.55 | 10.80 | 10.66 | 10.20 |
| 05-Dec-99 | 11.00 | 10.54 | 10.41 | 10.91 | 10.77 | 10.30 |
| 06-Dec-99 | 10.78 | 10.26 | 10.72 | 10.47 | 10.63 | 10.20 |
| 07-Dec-99 | 10.89 | 10.35 | 10.63 | 10.89 | 10.78 | 10.35 |
| 08-Dec-99 | 10.92 | 10.31 | 10.67 | 10.70 | 10.65 | 10.19 |
| 09-Dec-99 | 10.91 | 10.29 | 10.54 | 10.76 | 10.68 | 10.39 |
| 10-Dec-99 | 10.67 | 9.98 | 10.24 | 10.59 | 10.33 | 10.14 |
| 11-Dec-99 | 10.70 | 10.02 | 10.34 | 10.68 | 10.30 | 10.23 |
| 12-Dec-99 | 11.02 | 10.42 | 10.64 | 10.98 | 10.81 | 10.51 |
| 13-Dec-99 | 10.99 | 10.14 | 10.38 | 10.76 | 10.57 | 10.20 |
| 14-Dec-99 | 10.70 | 10.02 | 10.44 | 10.70 | 10.44 | 10.00 |
| 15-Dec-99 | 10.81 | 10.04 | 10.65 | 10.75 | 10.03 | 10.23 |
| 16-Dec-99 | 10.94 | 10.16 | 10.57 | 10.86 | 10.75 | 10.00 |
| 17-Dec-99 | 10.96 | 10.18 | 10.50 | 10.72 | 10.71 | 9.83 |
| 18-Dec-99 | 10.61 | 10.00 | 10.43 | 10.74 | 10.62 | 9.83 |
| 19-Dec-99 | 10.74 | 10.05 | 10.24 | 10.75 | 10.62 | 10.01 |
| 20-Dec-99 | 10.63 | 10.08 | 10.00 | 10.61 | 10.65 | 10.00 |
| 21-Dec-99 | 10.60 | 10.00 | 10.13 | 10.60 | 10.35 | 9.98 |
| 22-Dec-99 | 10.63 | 9.79 | 10.12 | 10.44 | 10.42 | 10.24 |
| 23-Dec-99 | 10.60 | 10.25 | 10.25 | 10.64 | 10.49 | 10.12 |
| 24-Dec-99 | 10.81 | 9.85 | 10.13 | 10.57 | 10.61 | 9.94 |
| 26-Dec-99 | 10.81 | 10.67 | 10.12 | 10.55 | 10.50 | 10.11 |
| 29-Dec-99 | 10.93 | 11.11 | 9.93 | 11.17 | 11.12 | 10.96 |
| 30-Dec-99 | 11.00 | 10.95 | 10.25 | 10.90 | 10.96 | 11.02 |
| 31-Dec-99 | 11.33 | 11.13 | 11.12 | 10.41 | 11.11 | 10.07 |
| 01-Jan-00 | 11.02 | 10.65 | 7.41 | 10.68 | 11.00 | 10.48 |
| 02-Jan-00 | 10.38 | 10.62 | 9.91 | 10.51 | 10.63 | 10.61 |
| 03-Jan-00 | 10.86 | 10.55 | 10.55 | 10.55 | 10.43 | 10.63 |
| 04-Jan-00 | 10.90 | 10.88 | 10.57 | 10.66 | 10.51 | 10.91 |
| 05-Jan-00 | 10.40 | 10.39 | 10.33 | 10.29 | 10.28 | 10.46 |
| 06-Jan-00 | 11.45 | 10.37 | 11.21 | 10.29 | 10.78 | 10.40 |
| 07-Jan-00 | 11.26 | 11.44 | 11.15 | 11.36 | 11.93 | 11.24 |
| 08-Jan-00 | 10.62 | 10.44 | 10.30 | 10.53 | 10.22 | 10.62 |
| 09-Jan-00 | 9.89 | 9.87 | 9.78 | 9.53 | 9.38 | 9.78 |
| 11-Jan-00 | 10.49 | 10.44 | 10.33 | 10.36 | 10.06 | 10.50 |
| 12-Jan-00 | 10.70 | 10.57 | 10.56 | 10.60 | 10.31 | 10.71 |
| 13-Jan-00 | 10.57 | 10.34 | 10.09 | 10.09 | 9.99 | 10.51 |
| 14-Jan-00 | 10.71 | 10.54 | 10.46 | 10.53 | 10.15 | 10.70 |
| 15-Jan-00 | 11.00 | 10.74 | 10.75 | 10.75 | 10.40 | 10.93 |
| 16-Jan-00 | 10.71 | 10.54 | 10.31 | 10.31 | 10.16 | 10.56 |
| 17-Jan-00 | 11.64 | 11.40 | 11.33 | 11.50 | 11.15 | 11.67 |
| 18-Jan-00 | 10.70 | 10.64 | 10.44 | 10.65 | 10.25 | 10.75 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 19-Jan-00 | 10.68 | 10.41 | 10.25 | 10.40 | 10.07 | 10.44 |
| 20-Jan-00 | 10.60 | 10.30 | 10.31 | 10.33 | 10.11 | 10.43 |
| 21-Jan-00 | 11.00 | 10.74 | 10.73 | 10.43 | | 10.80 |
| 22-Jan-00 | 10.80 | 10.62 | 10.45 | 10.48 | 10.89 | 10.75 |
| 23-Jan-00 | 10.65 | 10.36 | 10.12 | 10.43 | 10.54 | 10.00 |
| 24-Jan-00 | 11.27 | 11.10 | 11.03 | 11.21 | 11.84 | 11.30 |
| 25-Jan-00 | 10.29 | 10.28 | 10.00 | 10.14 | 10.03 | 8.29 |
| 26-Jan-00 | 10.86 | 10.62 | 10.44 | 10.59 | 10.31 | 10.72 |
| 27-Jan-00 | 10.58 | 10.27 | 10.75 | 10.08 | 10.37 | 9.18 |
| 28-Jan-00 | 10.75 | 10.44 | 10.41 | 10.60 | 10.23 | 9.42 |
| 29-Jan-00 | 11.42 | 11.63 | 11.16 | 11.49 | 11.12 | 10.40 |
| 30-Jan-00 | 11.51 | 11.25 | 11.23 | 10.95 | 10.53 | 10.18 |
| 31-Jan-00 | 10.02 | 10.18 | 10.08 | 10.24 | 9.95 | 9.08 |
| 02-Feb-00 | 10.49 | 10.26 | 10.14 | 10.24 | 10.15 | 9.18 |
| 03-Feb-00 | 10.54 | 10.24 | 10.25 | 10.35 | 10.28 | 9.55 |
| 04-Feb-00 | 9.98 | 10.06 | 10.21 | 10.06 | 9.90 | 10.40 |
| 05-Feb-00 | 10.99 | 10.98 | 10.20 | 11.04 | 11.07 | 10.64 |
| 06-Feb-00 | 11.86 | 11.74 | 11.50 | 11.62 | 11.59 | 10.80 |
| 07-Feb-00 | 11.47 | 11.52 | 11.63 | 11.03 | 11.69 | |
| 08-Feb-00 | 11.10 | 10.97 | 10.74 | 10.94 | 11.09 | 10.47 |
| 09-Feb-00 | 12.00 | 11.25 | 11.21 | 11.31 | 10.62 | 11.88 |
| 10-Feb-00 | 10.56 | 10.17 | 10.20 | 10.37 | 9.86 | 9.39 |
| 11-Feb-00 | 11.42 | 10.99 | 10.81 | 11.00 | 10.60 | 10.37 |
| 12-Feb-00 | 11.82 | 10.65 | 10.78 | 10.60 | 10.11 | 10.40 |
| 13-Feb-00 | 11.03 | 10.47 | 10.54 | 10.59 | 10.44 | 9.54 |
| 14-Feb-00 | 11.35 | 10.95 | 10.65 | 11.05 | 10.75 | 9.88 |
| 15-Feb-00 | 9.66 | 10.48 | 10.71 | 10.88 | 10.50 | 9.84 |
| 16-Feb-00 | 10.65 | 9.70 | 10.15 | 10.94 | 9.77 | 10.28 |
| 17-Feb-00 | 10.36 | 10.05 | 9.96 | 10.20 | 9.73 | 9.90 |
| 18-Feb-00 | 11.33 | 10.91 | 10.86 | 11.05 | 11.05 | 10.22 |
| 19-Feb-00 | 10.87 | 10.55 | 10.45 | 10.62 | 10.40 | 10.87 |
| 20-Feb-00 | 10.88 | 10.47 | 10.18 | 10.63 | 10.23 | 9.18 |
| 21-Feb-00 | 10.42 | 10.16 | 10.00 | 10.32 | 9.98 | 10.56 |
| 23-Feb-00 | 10.42 | 10.16 | 10.00 | 10.32 | 9.48 | 10.56 |
| 24-Feb-00 | 10.32 | 10.44 | 10.20 | 10.33 | 10.35 | 10.75 |
| 25-Feb-00 | 10.98 | 11.09 | 11.92 | 10.84 | 11.07 | 10.09 |
| 26-Feb-00 | 10.58 | 10.16 | 8.30 | 9.86 | 10.10 | 9.24 |
| 27-Feb-00 | 10.66 | 11.16 | 11.15 | 11.05 | 11.63 | 10.59 |
| 28-Feb-00 | 9.98 | 10.31 | 10.02 | 9.89 | 10.49 | 9.56 |
| 29-Feb-00 | 10.00 | 10.20 | 10.01 | 10.83 | 10.20 | 9.50 |
| 01-Mar-00 | 10.04 | 10.09 | 9.46 | 9.83 | 10.42 | 9.46 |
| 02-Mar-00 | 9.83 | 10.09 | 10.07 | 9.90 | 10.52 | 9.36 |
| 03-Mar-00 | 10.16 | 10.49 | 10.15 | 10.25 | 10.97 | 10.00 |
| 04-Mar-00 | 10.65 | 10.75 | 10.40 | 10.48 | 10.94 | 11.13 |
| 05-Mar-00 | 10.53 | 10.57 | 10.51 | 10.49 | 11.16 | 11.19 |
| 06-Mar-00 | 10.20 | 10.27 | 10.19 | 10.10 | 10.59 | 10.62 |
| 07-Mar-00 | 11.31 | 11.50 | 11.00 | 11.15 | 11.85 | 11.71 |
| 08-Mar-00 | 10.78 | 11.03 | 10.86 | 10.76 | 11.28 | 11.51 |

Appendix C3. DO measurements (mg/L $O_2$) for nitrate concentration in the test of Lake Superior brook trout in soft water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 10.78 | 10.76 | 10.66 | 10.79 | 8.05 | 10.44 |
| 01-Dec-99 | 10.70 | 10.64 | 10.59 | 10.83 | 10.66 | 10.63 |
| 02-Dec-99 | 11.09 | 10.88 | 10.75 | 10.87 | 10.54 | 10.59 |
| 03-Dec-99 | 11.27 | 10.92 | 10.82 | 10.95 | 10.87 | 10.74 |
| 04-Dec-99 | 11.22 | 10.71 | 10.77 | 11.01 | 10.75 | 10.63 |
| 05-Dec-99 | 10.88 | 10.78 | 10.82 | 10.94 | 10.83 | 10.70 |
| 06-Dec-99 | 10.95 | 10.58 | 10.65 | 10.58 | 10.77 | 10.66 |
| 07-Dec-99 | 11.20 | 10.85 | 10.78 | 10.90 | 10.99 | 10.66 |
| 08-Dec-99 | 10.67 | 10.70 | 10.58 | 10.63 | 10.69 | 10.66 |
| 09-Dec-99 | 10.90 | 10.78 | 10.77 | 10.86 | 10.80 | 10.65 |
| 10-Dec-99 | 10.91 | 10.61 | 10.61 | 10.73 | 10.79 | 10.81 |
| 11-Dec-99 | 10.34 | 10.57 | 10.56 | 10.67 | 10.66 | 10.52 |
| 12-Dec-99 | 10.66 | 11.02 | 11.00 | 11.01 | 11.06 | 10.72 |
| 13-Dec-99 | 11.28 | 10.79 | 10.75 | 10.82 | 10.42 | 10.47 |
| 14-Dec-99 | 10.44 | 10.78 | 10.64 | 10.79 | 10.67 | 10.41 |
| 15-Dec-99 | 10.68 | 11.02 | 10.79 | 10.72 | 10.46 | 10.79 |
| 16-Dec-99 | 10.31 | 10.64 | 10.52 | 10.82 | 10.60 | 10.80 |
| 17-Dec-99 | 10.80 | 10.73 | 10.78 | 10.73 | 10.47 | 10.69 |
| 18-Dec-99 | 11.35 | 10.84 | 10.50 | 10.75 | 10.69 | 10.47 |
| 19-Dec-99 | 11.05 | 10.82 | 10.77 | 10.36 | 10.50 | 10.67 |
| 20-Dec-99 | 11.11 | 11.04 | 10.67 | 10.74 | 10.68 | 10.50 |
| 21-Dec-99 | 10.36 | 10.35 | 10.37 | 10.29 | 10.46 | 10.05 |
| 22-Dec-99 | 10.23 | 10.48 | 10.25 | 10.43 | 10.50 | 10.10 |
| 23-Dec-99 | 10.50 | 10.45 | 10.54 | 10.40 | 10.20 | 10.31 |
| 24-Dec-99 | 10.20 | 10.98 | 10.70 | 10.71 | 10.88 | 10.50 |
| 25-Dec-99 | 10.60 | 10.63 | 10.52 | 10.63 | 10.77 | 10.37 |
| 29-Dec-99 | 10.94 | 10.70 | 10.13 | 10.67 | 10.70 | 10.63 |
| 30-Dec-99 | 10.79 | 10.87 | 10.95 | 10.95 | 11.10 | 10.64 |
| 31-Dec-99 | 11.40 | 11.56 | 11.34 | 11.23 | 11.37 | 10.90 |
| 01-Jan-00 | 10.54 | 10.53 | 9.60 | 10.32 | 10.52 | 10.62 |
| 02-Jan-00 | 10.73 | 10.71 | 10.35 | 10.50 | 10.79 | 10.40 |
| 03-Jan-00 | 11.44 | 11.13 | 10.78 | 10.75 | 10.92 | 10.36 |
| 04-Jan-00 | 11.43 | 11.36 | 10.98 | 11.08 | 10.96 | 9.62 |
| 05-Jan-00 | 10.36 | 10.53 | 10.37 | 10.44 | 10.46 | 10.55 |
| 06-Jan-00 | 11.86 | 11.48 | 11.27 | 11.24 | 11.39 | 10.39 |
| 07-Jan-00 | 11.72 | 11.62 | 11.33 | 11.38 | 11.44 | 10.55 |
| 08-Jan-00 | 10.97 | 10.77 | 10.50 | 10.30 | 10.49 | 10.00 |
| 09-Jan-00 | 10.65 | 10.54 | 10.25 | 8.31 | 10.20 | 9.89 |
| 11-Jan-00 | 10.98 | 10.58 | 10.35 | 10.67 | 10.60 | 10.03 |
| 12-Jan-00 | 11.05 | 10.86 | 10.62 | 11.05 | 10.78 | 8.61 |
| 13-Jan-00 | 11.01 | 10.34 | 10.09 | 10.80 | 10.53 | 8.79 |
| 14-Jan-00 | 11.36 | 10.76 | 10.66 | 10.90 | 10.76 | 9.42 |
| 15-Jan-00 | 11.66 | 11.16 | 10.91 | 11.27 | 11.00 | 10.01 |
| 16-Jan-00 | 11.03 | 10.67 | 10.61 | 11.01 | 10.62 | 9.69 |
| 17-Jan-00 | 11.40 | 11.60 | 11.10 | 11.72 | 11.46 | 9.38 |
| 18-Jan-00 | 10.99 | 11.01 | 10.87 | 11.23 | 10.99 | 9.26 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 19-Jan-00 | 11.20 | 10.57 | 10.53 | 11.01 | 10.57 | 10.57 |
| 20-Jan-00 | 12.14 | 11.00 | 10.73 | 11.32 | 10.98 | 7.54 |
| 21-Jan-00 | 11.30 | 11.37 | 11.12 | 11.51 | 10.97 | 9.19 |
| 22-Jan-00 | 10.74 | 10.86 | 10.56 | 11.06 | 10.80 | 9.46 |
| 23-Jan-00 | 11.12 | 10.85 | 10.65 | 9.98 | 10.60 | 9.70 |
| 24-Jan-00 | 11.55 | 11.73 | 11.45 | 11.40 | 11.50 | 11.70 |
| 25-Jan-00 | 10.93 | 10.89 | 10.65 | 10.70 | 10.54 | 9.71 |
| 26-Jan-00 | 11.34 | 10.90 | 10.82 | 11.58 | 11.02 | |
| 27-Jan-00 | 10.14 | 10.58 | 10.63 | 10.25 | 10.58 | 9.43 |
| 28-Jan-00 | 10.63 | 10.60 | 10.52 | 10.49 | 10.38 | 10.95 |
| 29-Jan-00 | 11.10 | 11.10 | 11.05 | 11.31 | 10.88 | 10.90 |
| 30-Jan-00 | 11.51 | 11.25 | 11.23 | 10.93 | 10.53 | 10.18 |
| 31-Jan-00 | 10.15 | 10.15 | 10.02 | 10.37 | 10.14 | 10.17 |
| 02-Feb-00 | 10.82 | 10.66 | 10.31 | 10.79 | 10.51 | 10.86 |
| 03-Feb-00 | 10.32 | 10.52 | 10.38 | 10.70 | 10.68 | 10.34 |
| 04-Feb-00 | 9.40 | 9.97 | 10.42 | 9.45 | 9.91 | 10.06 |
| 05-Feb-00 | 11.85 | 11.32 | 11.05 | 11.07 | 11.00 | 10.51 |
| 06-Feb-00 | 11.77 | 11.50 | 11.26 | 10.77 | 11.53 | 11.12 |
| 07-Feb-00 | 11.85 | 11.38 | 11.26 | 11.30 | 11.25 | 11.57 |
| 08-Feb-00 | 11.06 | 10.87 | 10.62 | 10.82 | 10.53 | 9.96 |
| 09-Feb-00 | 11.37 | 11.30 | 11.05 | 10.52 | 11.25 | 10.72 |
| 10-Feb-00 | 10.55 | 10.49 | 10.42 | 10.15 | 10.57 | 10.02 |
| 11-Feb-00 | 10.43 | 11.02 | 11.31 | 11.17 | 11.02 | 10.98 |
| 12-Feb-00 | 10.73 | 10.60 | 10.53 | 10.77 | 10.54 | 10.02 |
| 13-Feb-00 | 10.23 | 10.23 | 10.20 | 10.40 | 10.39 | 10.03 |
| 14-Feb-00 | 11.21 | 11.11 | 11.05 | 11.30 | 11.02 | 10.60 |
| 15-Feb-00 | 11.63 | 11.42 | 11.31 | 11.74 | 11.41 | 10.03 |
| 16-Feb-00 | 11.40 | 11.33 | 11.30 | 11.41 | 11.09 | 11.00 |
| 17-Feb-00 | 10.47 | 10.44 | 8.76 | 10.71 | 10.27 | 10.08 |
| 18-Feb-00 | 10.85 | 10.89 | 8.38 | 11.06 | 10.93 | 10.75 |
| 19-Feb-00 | 10.54 | 10.66 | 10.20 | 10.67 | 10.64 | 10.50 |
| 20-Feb-00 | 10.53 | 10.50 | 9.88 | 10.86 | 10.45 | 10.10 |
| 21-Feb-00 | 10.02 | 10.52 | 10.80 | 10.04 | 10.30 | 10.34 |
| 23-Feb-00 | 10.02 | 10.52 | 10.80 | 10.04 | 10.30 | 10.34 |
| 24-Feb-00 | 10.41 | 10.61 | 9.52 | 10.82 | 10.73 | 10.74 |
| 25-Feb-00 | 11.73 | 11.41 | 8.11 | 11.41 | 11.13 | 11.34 |
| 26-Feb-00 | 9.78 | 10.17 | 9.96 | 10.17 | 10.28 | 9.59 |
| 27-Feb-00 | 12.19 | 11.50 | 11.60 | 11.40 | 11.52 | 10.00 |
| 28-Feb-00 | 12.18 | 11.71 | 11.74 | 11.72 | 11.42 | 10.66 |
| 29-Feb-00 | 10.92 | 10.70 | 10.66 | 10.72 | 10.56 | 9.36 |
| 01-Mar-00 | 10.99 | 10.61 | 10.10 | 10.88 | 10.85 | 10.69 |
| 02-Mar-00 | 10.86 | 10.75 | 9.85 | 10.70 | 10.66 | 9.32 |
| 03-Mar-00 | 11.07 | 10.73 | 10.77 | 11.01 | 10.85 | 10.15 |
| 04-Mar-00 | 11.85 | 11.42 | 11.50 | 11.63 | 11.44 | 10.93 |
| 05-Mar-00 | 11.87 | 11.26 | 11.34 | 11.57 | 11.34 | 11.26 |
| 06-Mar-00 | 10.73 | 10.53 | 10.03 | 10.96 | 10.96 | 11.06 |
| 07-Mar-00 | 12.36 | 11.82 | 12.24 | 12.53 | 12.20 | 12.51 |
| 08-Mar-00 | 11.64 | 11.08 | 11.20 | 11.43 | 11.56 | 11.67 |

# Appendix D

## Daily Temperature measurements for exposures
## C

Appendix D1. Temperature measurement in °C for exposure treatments of feral brook trout test from Melancthron Creek

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 12-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 13-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 14-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 15-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 16-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 17-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 18-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 19-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 20-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 21-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 22-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 23-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 24-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 25-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 26-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 27-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 28-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 29-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 30-Nov-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 01-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 02-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 03-Dec-98 | 9.0 | 8.7 | 8.7 | 8.5 | 8.5 | 8.5 |
| 04-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 05-Dec-98 | 8.7 | 8.7 | 8.5 | 8.5 | 8.5 | 8.5 |
| 06-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 07-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 08-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 09-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 10-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 11-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 12-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 13-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 14-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 15-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 16-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 17-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 18-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 19-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 20-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 21-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 22-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 23-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 24-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 25-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 26-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 27-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 28-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 29-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 30-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 31-Dec-98 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 01-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 02-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 03-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 04-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 05-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 06-Jan-99 | 8.8 | 8.7 | 8.5 | 8.5 | 8.5 | 8.5 |
| 07-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 08-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 09-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 10-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 11-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 12-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 13-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 14-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 15-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 16-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 17-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 18-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 19-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 20-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 21-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 22-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 23-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 24-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 25-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 26-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 27-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 28-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 29-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 30-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 31-Jan-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 01-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 02-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 03-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 04-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 05-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 06-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 07-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 08-Feb-99 | 8.8 | 8.8 | 8.5 | 8.5 | 8.5 | 8.5 |
| 09-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 10-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 11-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| 12-Feb-99 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |

Appendix D2. Temperature measurements in °C for Lake Superior domestic brook trout test in hard water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 01-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 14-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 16-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 17-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 21-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 22-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 25-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 27-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 30-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 31-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 01-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 14-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 16-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 17-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 21-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 22-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 25-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 27-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 28-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 30-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 31-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 14-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 16-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 17-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 21-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 7.9 | 8.0 |
| 25-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 27-Feb-00 | 8.1 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 28-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 01-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |

Appendix D3. Temperature measurements in °C for Lake Superior domestic brook trout test in soft water treatment.

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 01-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Dec-99 | 8.0 | 8.0 | 7.8 | 8.0 | 8.0 | 8.0 |
| 06-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 14-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 16-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 17-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 21-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 22-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 25-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 28-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 30-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 31-Dec-99 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 01-Jan-00 | 8.0 | 8.0 | 12.0 | 8.0 | 8.0 | 8.0 |
| 02-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Jan-00 | 7.9 | 8.0 | 7.9 | | 7.9 | |
| 14-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 16-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 17-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 13.0 |
| 21-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 22-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 25-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 27-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 28-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 30-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 31-Jan-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 09-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 10-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 11-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 12-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 13-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 14-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 15-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 16-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 18-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 19-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 20-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 21-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 23-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 24-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 25-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 26-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 27-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 28-Feb-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 29-Feb-00 | 8.0 | 8.0 | 8.3 | 8.0 | 8.0 | 8.0 |
| 01-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 02-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 03-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 04-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 05-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 06-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 07-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| 08-Mar-00 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |

# Appendix E

Daily Hardness measurements for exposures
mg/L $CaCO_3$

Appendix E1. Hardness measurements for exposure tanks during the test of feral brook trout from Melancthron Creek. Values are mg/L $CaCO_3$

| control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| 204 | 204 | 200 | 204 | 216 | 208 |
| 208 | 212 | 208 | 200 | 196 | 200 |
| 204 | 196 | 192 | 192 | 196 | 204 |
| 200 | 208 | 192 | 192 | 188 | 192 |
| 204 | 192 | 200 | 200 | 208 | 200 |
| 208 | 196 | 184 | 208 | 192 | 192 |
| 180 | 188 | 200 | 200 | 200 | 192 |
| 204 | 208 | 208 | 212 | 200 | 196 |
| 204 | 204 | 200 | 200 | 196 | 200 |
| 200 | 200 | 192 | 200 | 192 | 188 |
| 208 | 208 | 192 | 208 | 200 | 216 |
| 204 | 200 | 208 | 188 | 208 | 180 |
| 200 | 200 | 192 | 204 | 200 | 204 |
| 196 | 200 | 200 | 200 | 192 | 200 |
| 200 | 208 | 204 | 196 | | |
| | | 208 | | | |

Appendix E2. Hardness measurement of exposure tanks for Lake Superior brook trout in hard water treatments. Values are mg/L $CaCO_3$

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 240 | | | | | |
| 01-Dec-99 | | | | | 200 | |
| 02-Dec-99 | | | 200 | | | |
| 03-Dec-99 | | | | | 196 | |
| 04-Dec-99 | | | | 192 | | |
| 05-Dec-99 | | | | | | |
| 06-Dec-99 | 200 | | | | | |
| 07-Dec-99 | | 208 | | | | |
| 08-Dec-99 | | | 200 | | | |
| 09-Dec-99 | | | | 208 | | |
| 10-Dec-99 | | | | | 212 | |
| 11-Dec-99 | | | | 220 | | |
| 12-Dec-99 | 216 | | | | | |
| 13-Dec-99 | | 220 | | | | |
| 14-Dec-99 | | | 200 | | | |
| 15-Dec-99 | | | | 208 | | |
| 16-Dec-99 | | | | | 200 | |
| 17-Dec-99 | | | | 212 | | |
| 18-Dec-99 | 208 | | | | | |
| 19-Dec-99 | | 200 | | | | |
| 20-Dec-99 | | | 216 | | | |
| 21-Dec-99 | | | | | | |
| 22-Dec-99 | | | | 200 | | |
| 23-Dec-99 | | | | | 216 | |
| 24-Dec-99 | | | | | | 200 |
| 25-Dec-99 | | | | | | |
| 26-Dec-99 | 216 | | | | | |
| 27-Dec-99 | | | | | | |
| 28-Dec-99 | | | | | | |
| 29-Dec-99 | 200 | | | | | |
| 30-Dec-99 | | 200 | | | | |
| 31-Dec-99 | | | 200 | | | |
| 01-Jan-00 | | | | 212 | | |
| 02-Jan-00 | | | | | 204 | |
| 03-Jan-00 | | | | | | 200 |
| 04-Jan-00 | 212 | | | | | |
| 05-Jan-00 | | 240 | | | | |
| 06-Jan-00 | | | | | | |
| 07-Jan-00 | | | | | | 200 |
| 08-Jan-00 | | 212 | | | | 200 |
| 09-Jan-00 | | | | | | |
| 10-Jan-00 | | | | | | 200 |
| 11-Jan-00 | 200 | | | | | |
| 12-Jan-00 | | 200 | | | | |
| 13-Jan-00 | | | 200 | | | |
| 14-Jan-00 | | | | | | |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 15-Jan-00 | 200 | | | | | |
| 16-Jan-00 | | 220 | | | | |
| 17-Jan-00 | | | 200 | | | |
| 18-Jan-00 | | | | 204 | | |
| 19-Jan-00 | | | | | 200 | |
| 20-Jan-00 | 200 | | | | | |
| 21-Jan-00 | | | | | | |
| 22-Jan-00 | 212 | | | | | |
| 23-Jan-00 | | 212 | | | | |
| 24-Jan-00 | | | | 200 | | |
| 25-Jan-00 | | | | | 256 | |
| 26-Jan-00 | | | | | | |
| 27-Jan-00 | | | | | | |
| 28-Jan-00 | | | | | | 200 |
| 29-Jan-00 | 200 | | | | | |
| 30-Jan-00 | | 200 | | | | |
| 31-Jan-00 | | | | | | |
| 01-Feb-00 | | | | | | |
| 02-Feb-00 | 200 | | | | | |
| 03-Feb-00 | | 200 | | | | |
| 04-Feb-00 | | | 240 | | | |
| 05-Feb-00 | | | | 200 | | |
| 06-Feb-00 | | | | | 200 | |
| 07-Feb-00 | | | | | | 200 |
| 08-Feb-00 | 200 | | | | | |
| 09-Feb-00 | | 204 | | | | |
| 10-Feb-00 | | | 200 | | | |
| 11-Feb-00 | | | | 212 | | |
| 12-Feb-00 | | | | | 200 | |
| 13-Feb-00 | | | | | | 200 |
| 14-Feb-00 | 200 | | | | | |
| 15-Feb-00 | | 192 | | | | |
| 16-Feb-00 | | | 200 | | | |
| 17-Feb-00 | | | | | | |
| 18-Feb-00 | | | | 200 | | |
| 19-Feb-00 | | | | | 200 | |
| 20-Feb-00 | | | | | | 200 |
| 21-Feb-00 | 200 | | | | | |
| 22-Feb-00 | | | | | | |
| 23-Feb-00 | 200 | | | | | |
| 24-Feb-00 | | 200 | | | | |
| 25-Feb-00 | | | 200 | | | |
| 26-Feb-00 | | | | 200 | | |
| 27-Feb-00 | | | | | 200 | |
| 28-Feb-00 | | | | | | 200 |
| 29-Feb-00 | 200 | | | | | |
| 01-Mar-00 | | 200 | | | | |
| 02-Mar-00 | | | 220 | | | |
| 03-Mar-00 | | | | 212 | | |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 04-Mar-00 | | | | | 200 | |
| 05-Mar-00 | | | | | | 200 |
| 06-Mar-00 | 200 | | | | | |
| 07-Mar-00 | | 212 | | | | |
| 08-Mar-00 | | | 200 | | | |

Appendix E3. Hardness measurement of exposure tanks for Lake Superior brook trout test in soft water treatments. Values are mg/L $CaCO_3$

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 30-Nov-99 | 60 | | | | | |
| 01-Dec-99 | | | | | 40 | |
| 02-Dec-99 | | | 40 | | | |
| 03-Dec-99 | | | | 53 | 40 | |
| 04-Dec-99 | | | | | | |
| 05-Dec-99 | 40 | | | | | |
| 06-Dec-99 | | | | | | |
| 07-Dec-99 | | 48 | | | | |
| 08-Dec-99 | | | 44 | | | |
| 09-Dec-99 | | | | 54 | | |
| 10-Dec-99 | | | | | 40 | |
| 11-Dec-99 | | | | 54 | | |
| 12-Dec-99 | 44 | | | | | |
| 13-Dec-99 | | 44 | | | | |
| 14-Dec-99 | | | 48 | | | |
| 15-Dec-99 | | | | 56 | | |
| 16-Dec-99 | | | | | 40 | |
| 17-Dec-99 | | | | | | 48 |
| 18-Dec-99 | 52 | | | | | |
| 19-Dec-99 | | 48 | | | 56 | |
| 20-Dec-99 | | | 56 | | | |
| 21-Dec-99 | | | | | | |
| 22-Dec-99 | | | | 60 | | |
| 23-Dec-99 | | | | | 60 | |
| 24-Dec-99 | | | | | | 60 |
| 25-Dec-99 | | | | | | |
| 26-Dec-99 | 60 | | | | | |
| 27-Dec-99 | | | | | 40 | |
| 28-Dec-99 | | | | | | |
| 29-Dec-99 | 52 | | | | | |
| 30-Dec-99 | | 52 | | | | |
| 31-Dec-99 | | | 40 | | | |
| 01-Jan-00 | | | | 40 | | |
| 02-Jan-00 | | | | | 40 | |
| 03-Jan-00 | | | | | | 40 |
| 04-Jan-00 | 40 | | | | | |
| 05-Jan-00 | | 40 | | | | |
| 06-Jan-00 | | | 52 | | | |
| 07-Jan-00 | | | | | | 52 |
| 08-Jan-00 | 40 | | | | | |
| 09-Jan-00 | | 40 | | | | |
| 10-Jan-00 | | | | | | |
| 11-Jan-00 | 40 | | | | | |
| 12-Jan-00 | | 40 | | | | |
| 13-Jan-00 | | | 40 | | | |
| 14-Jan-00 | | | | 56 | | |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 15-Jan-00 | 40 | | | | | |
| 16-Jan-00 | | 48 | | | | |
| 17-Jan-00 | | | 40 | | | |
| 18-Jan-00 | | | | 40 | | |
| 19-Jan-00 | | | | | 56 | |
| 20-Jan-00 | | | | | | 40 |
| 21-Jan-00 | 40 | | | | | |
| 22-Jan-00 | | 40 | | | | |
| 23-Jan-00 | 40 | | | | | |
| 24-Jan-00 | | 44 | | | | |
| 25-Jan-00 | | | 56 | | | |
| 26-Jan-00 | | | | 44 | | |
| 27-Jan-00 | | | | | 40 | |
| 28-Jan-00 | | | | | | 40 |
| 29-Jan-00 | 40 | | | | | |
| 30-Jan-00 | | 40 | | | | |
| 31-Jan-00 | | | 40 | | | |
| 01-Feb-00 | | | | | | |
| 02-Feb-00 | 44 | | | | | |
| 03-Feb-00 | | 40 | | | | |
| 04-Feb-00 | | | 40 | | | |
| 05-Feb-00 | | | | 40 | | |
| 06-Feb-00 | | | | | 40 | |
| 07-Feb-00 | | | | | | 40 |
| 08-Feb-00 | 40 | | | | | |
| 09-Feb-00 | | 44 | | | | |
| 10-Feb-00 | | | 40 | | | |
| 11-Feb-00 | | | | 40 | | |
| 12-Feb-00 | | | | | 44 | |
| 13-Feb-00 | | | | | | 40 |
| 14-Feb-00 | 44 | | | | | |
| 15-Feb-00 | | 40 | | | | |
| 16-Feb-00 | | | 40 | | | |
| 17-Feb-00 | | | | 40 | | |
| 18-Feb-00 | | | | | | |
| 19-Feb-00 | | | | | 40 | |
| 20-Feb-00 | | | | | | 40 |
| 21-Feb-00 | 40 | | | | | |
| 22-Feb-00 | | | | | | |
| 23-Feb-00 | 40 | | | | | |
| 24-Feb-00 | | 40 | | | | |
| 25-Feb-00 | | | 40 | | | |
| 26-Feb-00 | | | | 40 | | |
| 27-Feb-00 | | | | | 40 | |
| 28-Feb-00 | | | | | | 44 |
| 29-Feb-00 | 40 | | | | | |
| 01-Mar-00 | | 40 | | | | |
| 02-Mar-00 | | | 40 | | | |

| Date | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 03-Mar-00 | | | | 44 | | |
| 04-Mar-00 | | | | | 40 | |
| 05-Mar-00 | | | | | | 40 |
| 06-Mar-00 | 40 | | | | | |
| 07-Mar-00 | | 40 | | | | |
| 08-Mar-00 | | | 40 | | | |

# Appendix F

Daily conductivity measurements for exposures
umohms/$cm^3$

Appendix F1. Feral brook trout from Melancthron Creek conductivity measurements in umohms/cm$^3$

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 15-Nov-98 | 320 | 360 | 410 | 480 | 580 | 900 |
| 16-Nov-98 | 325 | 340 | 375 | 450 | 320 | 880 |
| 17-Nov-98 | 330 | 370 | 390 | 440 | 590 | 910 |
| 18-Nov-98 | 320 | 370 | 410 | 480 | 595 | 910 |
| 19-Nov-98 | 310 | 350 | 400 | 460 | 560 | 900 |
| 20-Nov-98 | 310 | 350 | 380 | 450 | 560 | 900 |
| 21-Nov-98 | 400 | 450 | 400 | 460 | 440 | 890 |
| 22-Nov-98 | 350 | 490 | 500 | 450 | 380 | 900 |
| 23-Nov-98 | 310 | 320 | 380 | 440 | 540 | 810 |
| 24-Nov-98 | 290 | 310 | 340 | 390 | 520 | 790 |
| 25-Nov-98 | 330 | 340 | 390 | 410 | 490 | 740 |
| 26-Nov-98 | 280 | 320 | 370 | 430 | 590 | 910 |
| 27-Nov-98 | 310 | 340 | 380 | 420 | 600 | 960 |
| 28-Nov-98 | 310 | 340 | 440 | 400 | 580 | 860 |
| 29-Nov-98 | 310 | 350 | 370 | 430 | 560 | 890 |
| 30-Nov-98 | 280 | 330 | 350 | 450 | 560 | 900 |
| 01-Dec-98 | 310 | 370 | 390 | 470 | 600 | 900 |
| 02-Dec-98 | 330 | 360 | 390 | 470 | 600 | 900 |
| 03-Dec-98 | 320 | 340 | 390 | 470 | 610 | 940 |
| 04-Dec-98 | 300 | 350 | 360 | 470 | 590 | 920 |
| 05-Dec-98 | 300 | 320 | 340 | 420 | 520 | 820 |
| 06-Dec-98 | 280 | 320 | 370 | 400 | 540 | 880 |
| 07-Dec-98 | 310 | 330 | 370 | 460 | 590 | 920 |
| 08-Dec-98 | 280 | 310 | 330 | 420 | 530 | 820 |
| 09-Dec-98 | 290 | 310 | 370 | 400 | 530 | 910 |
| 10-Dec-98 | 310 | 340 | 350 | 450 | 540 | 800 |
| 11-Dec-98 | 290 | 320 | 390 | 420 | 520 | 800 |
| 12-Dec-98 | 300 | 340 | 380 | 450 | 540 | 860 |
| 13-Dec-98 | 300 | 330 | 390 | 420 | 540 | 820 |
| 14-Dec-98 | 300 | 340 | 390 | 475 | 520 | 800 |
| 15-Dec-98 | 290 | 320 | 390 | 440 | 560 | 880 |
| 16-Dec-98 | 290 | 320 | 360 | 420 | 570 | 840 |
| 17-Dec-98 | 300 | 330 | 360 | 440 | 540 | 880 |
| 18-Dec-98 | 300 | 330 | 380 | 450 | 570 | 900 |
| 19-Dec-98 | 280 | 300 | 340 | 420 | 520 | 890 |
| 20-Dec-98 | 280 | 310 | 330 | 420 | 490 | 740 |
| 21-Dec-98 | 300 | 320 | 350 | 420 | 520 | 940 |
| 22-Dec-98 | 250 | 300 | 360 | 360 | 580 | 890 |
| 23-Dec-98 | 270 | 300 | 360 | 480 | 560 | 920 |
| 24-Dec-98 | 300 | 330 | 360 | 470 | 580 | 890 |
| 25-Dec-98 | 295 | 310 | 380 | 480 | 600 | 940 |
| 26-Dec-98 | 310 | 330 | 380 | 460 | 560 | 920 |
| 27-Dec-98 | 280 | 310 | 380 | 400 | 540 | 860 |
| 28-Dec-98 | 300 | 320 | 340 | 500 | 590 | 900 |
| 29-Dec-98 | 310 | 330 | 350 | 480 | 540 | 920 |
| 30-Dec-98 | 300 | 350 | 380 | 500 | 610 | 940 |

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 31-Dec-98 | 290 | 340 | 390 | 460 | 620 | 950 |
| 01-Jan-99 | 300 | 330 | 380 | 450 | 600 | 960 |
| 02-Jan-99 | 310 | 330 | 360 | 460 | 580 | 940 |
| 03-Jan-99 | 310 | 340 | 380 | 480 | 600 | 920 |
| 04-Jan-99 | 310 | 320 | 380 | 450 | 580 | 900 |
| 05-Jan-99 | 300 | 340 | 400 | 480 | 600 | 900 |
| 06-Jan-99 | 310 | 350 | 400 | 480 | 600 | 890 |
| 07-Jan-99 | 310 | 340 | 390 | 450 | 550 | 900 |
| 08-Jan-99 | 320 | 350 | 400 | 470 | 550 | 890 |
| 09-Jan-99 | 320 | 360 | 400 | 460 | 570 | 900 |
| 10-Jan-99 | 280 | 315 | 350 | 480 | 560 | 900 |
| 11-Jan-99 | 300 | 340 | 350 | 520 | 580 | 895 |
| 12-Jan-99 | 295 | 340 | 370 | 500 | 570 | 910 |
| 13-Jan-99 | 285 | 320 | 370 | 540 | 580 | 930 |
| 14-Jan-99 | 305 | 330 | 370 | 550 | 610 | 910 |
| 15-Jan-99 | 305 | 330 | 370 | 540 | 620 | 940 |
| 16-Jan-99 | 325 | 340 | 380 | 560 | 635 | 890 |
| 17-Jan-99 | 300 | 325 | 355 | 450 | 550 | 880 |
| 18-Jan-99 | 300 | 330 | 380 | 550 | 600 | 940 |
| 19-Jan-99 | 290 | 320 | 380 | 520 | 600 | 960 |
| 20-Jan-99 | 290 | 330 | 370 | 500 | 570 | 910 |
| 21-Jan-99 | 300 | 330 | 400 | 480 | 600 | 910 |
| 22-Jan-99 | 285 | 310 | 360 | 470 | 580 | 900 |
| 23-Jan-99 | 280 | 320 | 380 | 450 | 580 | 895 |
| 24-Jan-99 | 285 | 315 | 350 | 440 | 560 | 895 |
| 25-Jan-99 | 305 | 340 | 380 | 430 | 520 | 860 |
| 26-Jan-99 | 295 | 350 | 390 | 445 | 530 | 890 |
| 27-Jan-99 | 290 | 330 | 380 | 510 | 580 | 860 |
| 28-Jan-99 | 280 | 320 | 350 | 450 | 550 | 900 |
| 29-Jan-99 | 320 | 340 | 330 | 450 | 580 | 840 |
| 30-Jan-99 | 300 | 300 | 350 | 520 | 600 | 900 |
| 31-Jan-99 | 290 | 320 | 380 | 460 | 580 | 940 |
| 01-Feb-99 | 310 | 340 | 380 | 470 | 580 | 940 |
| 02-Feb-99 | 280 | 320 | 370 | 450 | 600 | 900 |
| 03-Feb-99 | 300 | 320 | 350 | 450 | 550 | 900 |
| 04-Feb-99 | 295 | 335 | 380 | 440 | 580 | 900 |
| 05-Feb-99 | 290 | 330 | 360 | 420 | 560 | 880 |
| 06-Feb-99 | 300 | 320 | 350 | 450 | 560 | 880 |
| 07-Feb-99 | 300 | 330 | 380 | 440 | 550 | 900 |
| 08-Feb-99 | 320 | 340 | 380 | 440 | 550 | 890 |
| 09-Feb-99 | 320 | 370 | 400 | 450 | 580 | 940 |
| 10-Feb-99 | 300 | 330 | 370 | 440 | 550 | 880 |
| 11-Feb-99 | 320 | 330 | 380 | 480 | 600 | 920 |
| 12-Feb-99 | 290 | 320 | 380 | 450 | 560 | 910 |

Appendix F2. Conductivity measurements for Lake Superior domestic brook trout test in hard water treatment as umohms/cm$^3$.

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 10-Dec-99 | 270 | 350 | 380 | 500 | 620 | 820 |
| 11-Dec-99 | 310 | 350 | 400 | 500 | 680 | 860 |
| 12-Dec-99 | 280 | 350 | 400 | 500 | 660 | 860 |
| 13-Dec-99 | 280 | 350 | 380 | 500 | 610 | 860 |
| 14-Dec-99 | 280 | 350 | 400 | 500 | 640 | 900 |
| 15-Dec-99 | 280 | 350 | 390 | 470 | 700 | 900 |
| 16-Dec-99 | 280 | 350 | 400 | 500 | 680 | 940 |
| 17-Dec-99 | 300 | 330 | 400 | 500 | 620 | 940 |
| 18-Dec-99 | 250 | 310 | 380 | 500 | 660 | 920 |
| 19-Dec-99 | 300 | 340 | 420 | 520 | 700 | 980 |
| 20-Dec-99 | 300 | 350 | 400 | 500 | 680 | 960 |
| 21-Dec-99 | 300 | 350 | 400 | 520 | 660 | 980 |
| 22-Dec-99 | 300 | 350 | 400 | 550 | 650 | 1200 |
| 23-Dec-99 | 300 | 350 | 400 | 600 | 680 | 100 |
| 24-Dec-99 | 270 | 350 | 400 | 500 | 650 | 990 |
| 26-Dec-99 | 280 | 360 | 400 | 540 | 660 | 960 |
| 29-Dec-99 | 300 | 350 | 400 | 510 | 640 | 1100 |
| 30-Dec-99 | 300 | 350 | 410 | 520 | 700 | 1150 |
| 31-Dec-99 | 290 | 360 | 390 | 500 | 650 | 1020 |
| 01-Jan-00 | 300 | 350 | 400 | 500 | 650 | 1040 |
| 02-Jan-00 | 300 | 370 | 400 | 540 | 700 | 1040 |
| 03-Jan-00 | 280 | 340 | 390 | 560 | 700 | 1010 |
| 04-Jan-00 | 300 | 330 | 410 | 510 | 660 | 1005 |
| 05-Jan-00 | 280 | 340 | 380 | 500 | 660 | 1010 |
| 06-Jan-00 | 310 | 360 | 370 | 520 | 600 | 1005 |
| 07-Jan-00 | 300 | 330 | 380 | 480 | 660 | 1100 |
| 08-Jan-00 | 300 | 330 | 380 | 490 | 660 | 1000 |
| 09-Jan-00 | 300 | 340 | 390 | 500 | 660 | 1000 |
| 11-Jan-00 | 300 | 350 | 380 | 500 | 640 | 1010 |
| 12-Jan-00 | 300 | 350 | 400 | 500 | 640 | 1015 |
| 13-Jan-00 | 290 | 340 | 390 | 480 | 660 | 1015 |
| 14-Jan-00 | 300 | 350 | 400 | 500 | 660 | 1010 |
| 15-Jan-00 | 300 | 340 | 410 | 510 | 700 | 1010 |
| 16-Jan-00 | 320 | 360 | 400 | 520 | 660 | 1100 |
| 17-Jan-00 | 310 | 350 | 390 | 520 | 680 | 1010 |
| 18-Jan-00 | 310 | 350 | 390 | 540 | 660 | 1010 |
| 19-Jan-00 | 300 | 310 | 350 | 500 | 660 | 990 |
| 20-Jan-00 | 300 | 340 | 400 | 510 | 660 | 1010 |
| 21-Jan-00 | 300 | 350 | 410 | 520 | 660 | 1010 |
| 22-Jan-00 | 310 | 360 | 400 | 500 | 660 | 1010 |
| 23-Jan-00 | 300 | 340 | 400 | 520 | 660 | 1010 |
| 24-Jan-00 | 300 | 350 | 400 | 500 | 640 | 1008 |
| 25-Jan-00 | 300 | 350 | 400 | 500 | 600 | 590 |
| 26-Jan-00 | 310 | 370 | 400 | 520 | 660 | 900 |
| 27-Jan-00 | 320 | 360 | 400 | 550 | 700 | 660 |
| 28-Jan-00 | 300 | 370 | 410 | 560 | 700 | 620 |

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 29-Jan-00 | 300 | 350 | 420 | 520 | 660 | 770 |
| 30-Jan-00 | 300 | 380 | 420 | 560 | 700 | 660 |
| 31-Jan-00 | 400 | 380 | 420 | 600 | 800 | 700 |
| 02-Feb-00 | 300 | 370 | 450 | 540 | 700 | 700 |
| 03-Feb-00 | 300 | 390 | 450 | 560 | 710 | 700 |
| 04-Feb-00 | 350 | 400 | 480 | 580 | 780 | 760 |
| 05-Feb-00 | 300 | 410 | 500 | 600 | 700 | 760 |
| 06-Feb-00 | 300 | 400 | 450 | 540 | 700 | 780 |
| 07-Feb-00 | 310 | 380 | 420 | 540 | 700 | 720 |
| 08-Feb-00 | 310 | 340 | 400 | 540 | 700 | 840 |
| 09-Feb-00 | 300 | 350 | 400 | 500 | 600 | 800 |
| 10-Feb-00 | 330 | 400 | 440 | 560 | 720 | 780 |
| 11-Feb-00 | 310 | 400 | 440 | 580 | 740 | 1020 |
| 12-Feb-00 | 300 | 370 | 410 | 500 | 660 | 1010 |
| 13-Feb-00 | 300 | 350 | 420 | 500 | 660 | 800 |
| 14-Feb-00 | 320 | 360 | 400 | 510 | 700 | 820 |
| 15-Feb-00 | 300 | 350 | 420 | 540 | 700 | 820 |
| 16-Feb-00 | 300 | 350 | 400 | 500 | 700 | 1009 |
| 18-Feb-00 | 320 | 400 | 440 | 540 | 720 | 880 |
| 19-Feb-00 | 330 | 390 | 450 | 550 | 720 | 900 |
| 20-Feb-00 | 320 | 370 | 450 | 520 | 700 | 1008 |
| 21-Feb-00 | 350 | 370 | 430 | 520 | 660 | 900 |
| 22-Feb-00 | 350 | 400 | 450 | 580 | 800 | 1400 |
| 23-Feb-00 | 350 | 400 | 450 | 580 | 800 | 1400 |
| 24-Feb-00 | 300 | 340 | 390 | 500 | 700 | 1100 |
| 25-Feb-00 | 320 | 380 | 430 | 520 | 700 | 1000 |
| 26-Feb-00 | 370 | 420 | 480 | 600 | 800 | 1200 |
| 27-Feb-00 | 310 | 350 | 410 | 540 | 700 | 1000 |
| 28-Feb-00 | 320 | 350 | 400 | 500 | 700 | 1020 |
| 29-Feb-00 | 330 | 380 | 420 | 500 | 700 | 1100 |
| 01-Mar-00 | 320 | 370 | 420 | 520 | 700 | 1100 |
| 02-Mar-00 | 350 | 400 | 420 | 520 | 640 | 1040 |
| 03-Mar-00 | 360 | 420 | 470 | 600 | 760 | 1220 |
| 04-Mar-00 | 310 | 360 | 410 | 500 | 640 | 1120 |
| 05-Mar-00 | 340 | 380 | 420 | 540 | 700 | 1180 |
| 06-Mar-00 | 350 | 400 | 430 | 550 | 720 | 1190 |
| 07-Mar-00 | 350 | 400 | 450 | 560 | 720 | 1190 |
| 08-Mar-00 | 340 | 390 | 430 | 540 | 710 | 1100 |

Appendix F3. Conductivity measurements for Lake Superior domestic brook trout test in soft water treatment as umohms/$cm^3$.

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 10-Dec-99 | 150 | 180 | 200 | 250 | 350 | 250 |
| 11-Dec-99 | 140 | 170 | 200 | 250 | 350 | 560 |
| 12-Dec-99 | 160 | 180 | 200 | 250 | 350 | 600 |
| 13-Dec-99 | 150 | 180 | 200 | 250 | 370 | 640 |
| 14-Dec-99 | 150 | 170 | 200 | 230 | 360 | 660 |
| 15-Dec-99 | 150 | 200 | 220 | 250 | 380 | 620 |
| 16-Dec-99 | 150 | 170 | 210 | 250 | 370 | 620 |
| 17-Dec-99 | 150 | 190 | 210 | 250 | 400 | 600 |
| 18-Dec-99 | 150 | 180 | 200 | 250 | 400 | 620 |
| 19-Dec-99 | 150 | 180 | 190 | 260 | 400 | 650 |
| 20-Dec-99 | 140 | 170 | 200 | 140 | 350 | 600 |
| 21-Dec-99 | 150 | 250 | 200 | 280 | 350 | 610 |
| 22-Dec-99 | 150 | 180 | 230 | 280 | 350 | 630 |
| 23-Dec-99 | 150 | 180 | 200 | 270 | 350 | 610 |
| 24-Dec-99 | 140 | 170 | 210 | 250 | 370 | 660 |
| 26-Dec-99 | 150 | 180 | 220 | 260 | 360 | 600 |
| 29-Dec-99 | 140 | 160 | 210 | 260 | 350 | 600 |
| 30-Dec-99 | 150 | 200 | 230 | 270 | 380 | 670 |
| 31-Dec-99 | 140 | 180 | 200 | 270 | 400 | 660 |
| 01-Jan-00 | 130 | 170 | 150 | 240 | 380 | 650 |
| 02-Jan-00 | 140 | 180 | 180 | 250 | 390 | 650 |
| 03-Jan-00 | 130 | 180 | 200 | 250 | 350 | 620 |
| 04-Jan-00 | 140 | 170 | 200 | 240 | 350 | 610 |
| 05-Jan-00 | 130 | 150 | 180 | 240 | 380 | 600 |
| 06-Jan-00 | 130 | 160 | 180 | 250 | 400 | 250 |
| 07-Jan-00 | 130 | 150 | 180 | 250 | 350 | 250 |
| 08-Jan-00 | 130 | 160 | 170 | 220 | 370 | 300 |
| 09-Jan-00 | 130 | 150 | 190 | 150 | 350 | 660 |
| 10-Jan-00 | 130 | 150 | 170 | 250 | 400 | 350 |
| 11-Jan-00 | 140 | 150 | 170 | 250 | 340 | 140 |
| 12-Jan-00 | 130 | 140 | 160 | 220 | 400 | 150 |
| 13-Jan-00 | 115 | 140 | 180 | 250 | 450 | 180 |
| 14-Jan-00 | 115 | 140 | 200 | 270 | 450 | 220 |
| 15-Jan-00 | 125 | 185 | 220 | 280 | 460 | 220 |
| 16-Jan-00 | 120 | 160 | 200 | 260 | 490 | 600 |
| 17-Jan-00 | 120 | 130 | 200 | 270 | 420 | 180 |
| 18-Jan-00 | 130 | 170 | 210 | 280 | 480 | 660 |
| 19-Jan-00 | 140 | 180 | 220 | 280 | 440 | 140 |
| 20-Jan-00 | 140 | 180 | 220 | 310 | 450 | 170 |
| 21-Jan-00 | 130 | 180 | 225 | 310 | 460 | 180 |
| 22-Jan-00 | 130 | 180 | 210 | 220 | 470 | 220 |
| 23-Jan-00 | 140 | 170 | 220 | 300 | 460 | 810 |
| 24-Jan-00 | 140 | 170 | 220 | 310 | 460 | 230 |
| 25-Jan-00 | 140 | 180 | 240 | 300 | 470 | 820 |
| 26-Jan-00 | 140 | 160 | 220 | 290 | 440 | 320 |
| 27-Jan-00 | 140 | 180 | 230 | 280 | 480 | 800 |

| Date | control | 6.26 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| 28-Jan-00 | 150 | 180 | 210 | 300 | 450 | 880 |
| 29-Jan-00 | 140 | 170 | 250 | 300 | 480 | 350 |
| 30-Jan-00 | 130 | 170 | 280 | 350 | 450 | 900 |
| 02-Feb-00 | 160 | 200 | 240 | 320 | 500 | 900 |
| 03-Feb-00 | 150 | 190 | 250 | 310 | 500 | 560 |
| 04-Feb-00 | 150 | 180 | 220 | 310 | 560 | 560 |
| 05-Feb-00 | 150 | 200 | 250 | 320 | 490 | 490 |
| 06-Feb-00 | 150 | 180 | 230 | 280 | 510 | 480 |
| 07-Feb-00 | 140 | 180 | 240 | 240 | 450 | 450 |
| 08-Feb-00 | 150 | 180 | 250 | 310 | 450 | 540 |
| 09-Feb-00 | 150 | 200 | 260 | 300 | 450 | 520 |
| 10-Feb-00 | 150 | 190 | 240 | 280 | 500 | 540 |
| 11-Feb-00 | 150 | 190 | 250 | 290 | 470 | 800 |
| 12-Feb-00 | 140 | 180 | 180 | 260 | 400 | 520 |
| 13-Feb-00 | 160 | 190 | 220 | 260 | 350 | 540 |
| 14-Feb-00 | 160 | 180 | 220 | 280 | 410 | 600 |
| 15-Feb-00 | 150 | 180 | 230 | 290 | 400 | 350 |
| 16-Feb-00 | 140 | 180 | 210 | 270 | 400 | 640 |
| 18-Feb-00 | 160 | 210 | 250 | 320 | 500 | 500 |
| 19-Feb-00 | 160 | 250 | 290 | 370 | 540 | 500 |
| 20-Feb-00 | 150 | 200 | 240 | 340 | 510 | 510 |
| 21-Feb-00 | 150 | 220 | 250 | 340 | 500 | 500 |
| 22-Feb-00 | 160 | 300 | 270 | 350 | 500 | 900 |
| 23-Feb-00 | 160 | 300 | 270 | 350 | 500 | 900 |
| 24-Feb-00 | 150 | 250 | 250 | 340 | 450 | 880 |
| 25-Feb-00 | 150 | 270 | 250 | 330 | 500 | 900 |
| 26-Feb-00 | 170 | 300 | 250 | 360 | 540 | 740 |
| 27-Feb-00 | 150 | 230 | 250 | 330 | 460 | 640 |
| 28-Feb-00 | 150 | 210 | 220 | 300 | 450 | 650 |
| 29-Feb-00 | 160 | 210 | 200 | 350 | 500 | 700 |
| 01-Mar-00 | 150 | 210 | 250 | 310 | 510 | 900 |
| 02-Mar-00 | 160 | 200 | 240 | 310 | 500 | 700 |
| 03-Mar-00 | 170 | 220 | 250 | 380 | 540 | 800 |
| 04-Mar-00 | 140 | 190 | 210 | 300 | 450 | 740 |
| 05-Mar-00 | 140 | 180 | 220 | 300 | 470 | 880 |
| 06-Mar-00 | 140 | 190 | 200 | 320 | 480 | 900 |
| 07-Mar-00 | 150 | 190 | 210 | 330 | 520 | 960 |
| 08-Mar-00 | 140 | 190 | 230 | 330 | 490 | 900 |

# Appendix G

Raw data for embryo mortality and egg totals

Appendix G1

Total number of feral brook trout embryos from Melancthron Creek embryos exposed to nitrate.

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 88 | 53 | 43 | 79 | 62 | 61 |
| B | 79 | 46 | 57 | 65 | 58 | 76 |
| C | 74 | 63 | 56 | 70 | 70 | 62 |
| D | 78 | 57 | 60 | 70 | 69 | 100 |
| E | 75 | 57 | 65 | 67 | 65 | 57 |
| F | 74 | 59 | 55 | 57 | 67 | 65 |
| G | 82 | 62 | 60 | 57 | 59 | 45 |
| H | 65 | 55 | 54 | 49 | 50 | 46 |

Mortality of feral brook trout embryos from Melancthron Creek embryos exposed to nitrate until eye-up stage.

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 1 | 0 | 0 | 5 | 3 | 1 |
| B | 3 | 0 | 0 | 2 | 2 | 0 |
| C | 1 | 1 | 0 | 1 | 4 | 5 |
| D | 3 | 2 | 0 | 3 | 2 | 1 |
| E | 2 | 1 | 3 | 2 | 1 | 2 |
| F | 2 | 1 | 0 | 1 | 1 | 1 |
| G | 0 | 6 | 3 | 5 | 1 | 1 |
| H | 1 | 0 | 1 | 3 | 0 | 1 |

Mortality of feral brook trout embryos from Melancthron Creek embryos exposed to nitrate until hatch.

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 1 | 0 | 1 | 6 | 6 | 1 |
| B | 4 | 1 | 1 | 2 | 4 | 2 |
| C | 2 | 2 | 1 | 3 | 4 | 5 |
| D | 4 | 4 | 1 | 3 | 2 | 3 |
| E | 2 | 2 | 3 | 5 | 6 | 2 |
| F | 2 | 4 | 1 | 3 | 3 | 3 |
| G | 0 | 8 | 3 | 6 | 3 | 2 |
| H | 1 | 2 | 2 | 4 | 0 | 1 |

Mortality of feral brook trout embryos from Melancthron Creek embryos exposed to nitrate until yolk-sac absorption.

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 3 | 2 | 4 | 7 | 6 | 1 |
| B | 4 | 4 | 4 | 2 | 4 | 3 |
| C | 3 | 3 | 2 | 4 | 5 | 5 |
| D | 4 | 4 | 4 | 3 | 3 | 3 |
| E | 2 | 3 | 3 | 6 | 6 | 2 |
| F | 2 | 7 | 2 | 3 | 4 | 3 |
| G | 0 | 8 | 5 | 6 | 3 | 2 |
| H | 2 | 2 | 3 | 6 | 0 | 3 |

Appendix G2

Lake Superior brook trout embryo mortality at yolk-sac absorption in hard water treatment

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 74 | 76 | 91 | 98 | 100 | 85 |
| B | 76 | 89 | 86 | 97 | 110 | 86 |
| C | 79 | 87 | 98 | 99 | 114 | 92 |
| D | 69 | 89 | 88 | 84 | 99 | 102 |
| E | 78 | 82 | 76 | 92 | 108 | 103 |
| F | 59 | 99 | 87 | 77 | 115 | 103 |
| G | 58 | 104 | 88 | 83 | 92 | 98 |
| H | 77 | 111 | 79 | 89 | 109 | 99 |

Lake Superior brook trout embryo mortality at eye-up in hard water treatment

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 39 | 38 | 55 | 58 | 68 | 59 |
| B | 39 | 38 | 49 | 49 | 78 | 46 |
| C | 44 | 38 | 50 | 67 | 67 | 69 |
| D | 31 | 36 | 47 | 47 | 51 | 68 |
| E | 39 | 50 | 38 | 53 | 47 | 56 |
| F | 33 | 39 | 28 | 35 | 55 | 60 |
| G | 22 | 43 | 47 | 40 | 39 | 50 |
| H | 31 | 53 | 33 | 43 | 53 | 54 |

Lake Superior brook trout embryo mortality at hatch in hard water

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 62 | 64 | 80 | 83 | 96 | 75 |
| B | 57 | 71 | 71 | 70 | 96 | 66 |
| C | 67 | 70 | 89 | 95 | 102 | 79 |
| D | 53 | 65 | 73 | 74 | 73 | 92 |
| E | 60 | 69 | 71 | 89 | 87 | 89 |
| F | 44 | 69 | 64 | 62 | 83 | 92 |
| G | 40 | 78 | 84 | 63 | 71 | 84 |
| H | 63 | 88 | 61 | 71 | 96 | 82 |

Lake Superior brook trout embryo mortality at yolk-sac absorption in hard water treatment

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 62 | 66 | 80 | 83 | 98 | 77 |
| B | 58 | 72 | 73 | 72 | 99 | 68 |
| C | 68 | 71 | 89 | 97 | 103 | 81 |
| D | 54 | 66 | 74 | 74 | 73 | 94 |
| E | 63 | 69 | 73 | 90 | 88 | 90 |
| F | 45 | 70 | 67 | 63 | 86 | 98 |
| G | 40 | 80 | 84 | 63 | 71 | 89 |
| H | 66 | 90 | 61 | 71 | 98 | 85 |

**Appendix G3**

Total Lake Superior brook trout eggs in soft water treatment

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 99 | 119 | 90 | 101 | 105 | 93 |
| B | 100 | 101 | 86 | 105 | 105 | 103 |
| C | 98 | 104 | 91 | 93 | 102 | 115 |
| D | 100 | 104 | 88 | 98 | 84 | 87 |
| E | 98 | 91 | 77 | 97 | 95 | 105 |
| F | 107 | 95 | 86 | 97 | 71 | 104 |
| G | 100 | 95 | 91 | 103 | 86 | 96 |
| H | 113 | 107 | 77 | 94 | 94 | 95 |

Lake Superior brook trout embryo mortality at eye-up in soft water

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 46 | 63 | 41 | 52 | 53 | 42 |
| B | 44 | 57 | 48 | 49 | 47 | 48 |
| C | 51 | 45 | 47 | 42 | 45 | 51 |
| D | 55 | 58 | 40 | 60 | 29 | 45 |
| E | 56 | 42 | 35 | 48 | 31 | 43 |
| F | 47 | 36 | 45 | 52 | 32 | 42 |
| G | 45 | 35 | 45 | 42 | 37 | 47 |
| H | 46 | 56 | 32 | 50 | 37 | 43 |

Lake Superior brook trout embryo mortality at hatch in soft water treatment

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 80 | 105 | 80 | 78 | 78 | 83 |
| B | 81 | 87 | 73 | 86 | 74 | 85 |
| C | 82 | 88 | 85 | 79 | 85 | 105 |
| D | 94 | 97 | 79 | 93 | 60 | 79 |
| E | 90 | 84 | 74 | 85 | 72 | 96 |
| F | 85 | 83 | 77 | 82 | 55 | 89 |
| G | 77 | 77 | 77 | 71 | 64 | 75 |
| H | 90 | 90 | 63 | 79 | 70 | 71 |

Lake Superior brook trout embryo mortality at yolk-sac absorption in soft water treatment.

| Replicate | control | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| A | 86 | 112 | 83 | 84 | 82 | 92 |
| B | 81 | 87 | 75 | 91 | 87 | 90 |
| C | 88 | 100 | 89 | 82 | 89 | 110 |
| D | 96 | 98 | 80 | 93 | 67 | 84 |
| E | 96 | 87 | 77 | 87 | 81 | 102 |
| F | 89 | 86 | 78 | 85 | 62 | 95 |
| G | 87 | 86 | 83 | 88 | 75 | 86 |
| H | 105 | 96 | 66 | 86 | 94 | 95 |

# Appendix H.

Raw data for growth and biomass

Raw data of growth and biomass for exposures.

| concentration (Replicate) | Feral Growth | Lake Superior Growth | Lake Superior Growth | Feral Biomass | Lake Superior Biomass | LakeSuperior Biomass |
|---|---|---|---|---|---|---|
| Control a | 0.012550 | 0.011108 | 0.013747 | 0.012550 | 0.011108 | 0.013747 |
| Control b | 0.011871 | 0.012398 | 0.014149 | 0.009497 | 0.012398 | 0.014149 |
| Control c | 0.014291 | 0.012372 | 0.011987 | 0.011433 | 0.011135 | 0.011987 |
| Control d | 0.014331 | 0.012172 | 0.013251 | 0.012898 | 0.012172 | 0.013251 |
| Control e | 0.015591 | 0.011302 | 0.012298 | 0.014032 | 0.011302 | 0.012298 |
| Control f | 0.015665 | 0.011781 | 0.013149 | 0.009399 | 0.011781 | 0.013149 |
| Control g | 0.015360 | 0.012730 | 0.012981 | 0.010752 | 0.012730 | 0.012981 |
| Control h | 0.013763 | 0.012944 | 0.013120 | 0.012387 | 0.012944 | 0.013120 |
| 6.26 a | 0.010533 | 0.012035 | 0.014860 | 0.010533 | 0.012035 | 0.011888 |
| 6.26 b | 0.011921 | 0.013072 | 0.013009 | 0.011921 | 0.013072 | 0.013009 |
| 6.26 c | 0.013264 | 0.012723 | 0.014173 | 0.013264 | 0.012723 | 0.014173 |
| 6.26 d | 0.013281 | 0.012271 | 0.013242 | 0.013281 | 0.012271 | 0.013242 |
| 6.26 e | 0.013507 | 0.011957 | 0.014348 | 0.013507 | 0.011957 | 0.014348 |
| 6.26 f | 0.014296 | 0.013143 | 0.014735 | 0.014296 | 0.013143 | 0.014735 |
| 6.26 g | 0.013550 | 0.013598 | 0.014066 | 0.013550 | 0.013598 | 0.014066 |
| 6.26 h | 0.014756 | 0.012962 | 0.013828 | 0.014756 | 0.012962 | 0.011062 |
| 12.5 a | 0.012320 | 0.013262 | 0.013013 | 0.012320 | 0.013262 | 0.013013 |
| 12.5 b | 0.013078 | 0.012509 | 0.012719 | 0.013078 | 0.012509 | 0.012719 |
| 12.5 c | 0.013545 | 0.013109 | 0.014204 | 0.013545 | 0.013109 | 0.011363 |
| 12.5 d | 0.013372 | 0.013845 | 0.014023 | 0.013372 | 0.013845 | 0.014023 |
| 12.5 e | 0.013337 | 0.013290 | 0.015303 | 0.012003 | 0.013290 | 0.013773 |
| 12.5 f | 0.013174 | 0.013611 | 0.014446 | 0.011857 | 0.013611 | 0.014446 |
| 12.5 g | 0.013184 | 0.013388 | 0.015311 | 0.013184 | 0.013388 | 0.015311 |
| 12.5 h | 0.012422 | 0.013846 | | 0.012422 | 0.013846 | |
| 25 a | 0.012800 | 0.012833 | 0.011970 | 0.012800 | 0.012833 | 0.011970 |
| 25 b | 0.012277 | 0.012703 | 0.012638 | 0.012277 | 0.011433 | 0.012638 |
| 25 c | 0.013667 | 0.011886 | 0.012913 | 0.013667 | 0.010697 | 0.012913 |
| 25 d | 0.013398 | 0.012648 | 0.013946 | 0.013398 | 0.012648 | 0.013946 |
| 25 e | 0.013806 | 0.010081 | 0.012790 | 0.013806 | 0.010081 | 0.012790 |
| 25 f | 0.012697 | 0.015490 | 0.013812 | 0.012697 | 0.012392 | 0.013812 |
| 25 g | 0.014364 | 0.014006 | 0.011245 | 0.014364 | 0.012605 | 0.011245 |
| 25 h | 0.014892 | 0.012598 | 0.015526 | 0.014892 | 0.012598 | 0.013973 |
| 50 a | 0.013005 | 0.011811 | 0.012688 | 0.013005 | 0.009449 | 0.012688 |
| 50 b | 0.011462 | 0.013270 | 0.013252 | 0.011462 | 0.013270 | 0.013252 |
| 50 c | 0.011708 | 0.013545 | 0.013424 | 0.011708 | 0.013545 | 0.013424 |
| 50 d | 0.012720 | 0.013750 | 0.014216 | 0.012720 | 0.013750 | 0.014216 |
| 50 e | 0.011974 | 0.013609 | 0.013018 | 0.011974 | 0.013609 | 0.011716 |
| 50 f | 0.012161 | 0.014149 | 0.014157 | 0.012161 | 0.014149 | 0.012741 |
| 50 g | 0.012340 | 0.013940 | 0.013233 | 0.012340 | 0.013940 | 0.013233 |
| 50 h | 0.012088 | 0.012465 | 0.010732 | 0.010879 | 0.012465 | 0.005366 |
| 100 a | 0.013268 | 0.012621 | 0.013608 | 0.013268 | 0.011359 | 0.012247 |
| 100 b | 0.011137 | 0.013185 | 0.012297 | 0.010023 | 0.013185 | 0.007378 |
| 100 c | 0.012454 | 0.013508 | 0.014501 | 0.012454 | 0.013508 | 0.013051 |
| 100 d | 0.011859 | 0.014258 | 0.013140 | 0.010673 | 0.012832 | 0.013140 |
| 100 e | 0.012407 | 0.014013 | 0.012566 | 0.012407 | 0.012612 | 0.012566 |
| 100 f | 0.011520 | 0.013407 | 0.012780 | 0.011520 | 0.012066 | 0.005112 |
| 100 g | 0.013244 | 0.013162 | 0.014343 | 0.013244 | 0.011846 | 0.010040 |
| 100 h | 0.012506 | 0.013097 | | 0.012506 | 0.011787 | |