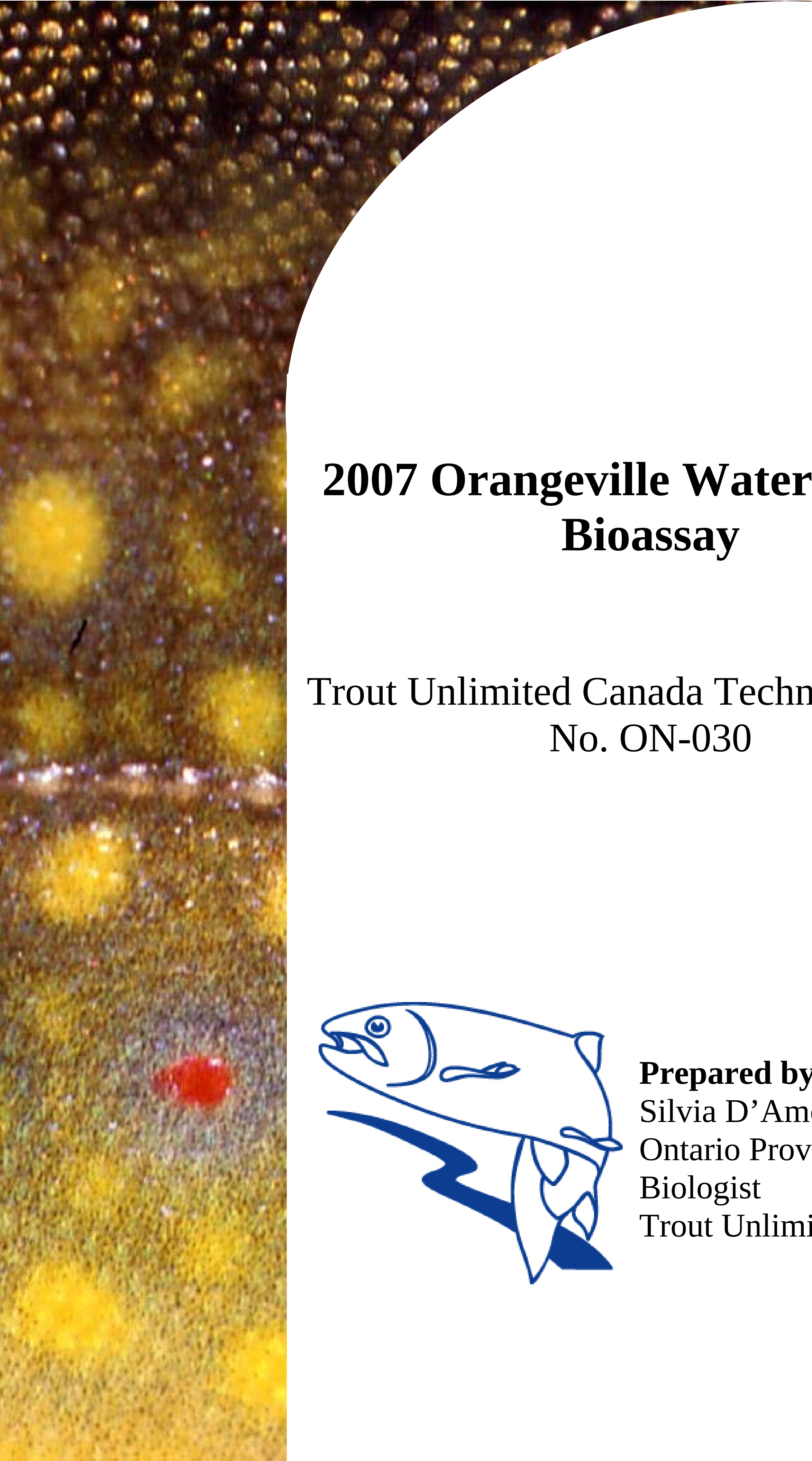

2007 Orangeville Water Quality Bioassay

Trout Unlimited Canada Technical Report
No. ON-030

Prepared by:
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Background

Water quality in our rivers and streams is significantly affected by many anthropogenic activities and sources. Among the most substantial is effluent from sewage treatment facilities which can include suspended nutrients grit, debris, microorganisms and over 200 chemicals. Facilities receive wastewater from a variety of sources including industrial facilities, institutions, households and in some cases storm water management systems. The degree of treatment of this wastewater before it is released to the receiving water body varies greatly across the country. In Ontario, minimum water quality standards for such effluent are regulated, but processes vary dependant on local conditions. As a result, the release of these effluents results in variable impacts to the receiving water and its biological community. Impacts of effluent also vary with climate, seasonality and the bioavailability of the effluent. Generally, impacts include a reduction in dissolved oxygen, influxes of ammonia, chlorine, chlorides and other chemicals, increases in water temperature and flow, increases in suspended solids and introduction of heavy metals and/or organic contaminants and microorganisms.

Changes in water quality due to effluent inputs have been shown to decrease water quality downstream of the inflow, with increases to water quality as distance from the inflow increases (Mukherjee and Jana 2007). This creates a correlated change in populations of fish and macro invertebrates which decrease in numbers and species with proximity to inflows (Monda *et al.* 1995). Many mobile organisms including fish and invertebrates will actively avoid some metal and chemical compounds resulting in changes in animal distribution. As a result populations of animals which are not highly mobile experience significant mortality (Graney *et al.* 1983). High concentrations of a toxin over a short period of time can result in acute effects (Belanger 1991). Low concentrations of toxins which are not detectable or do not elicit a movement response can result in gradual exposure causing bioaccumulation of the substance and delayed effects like impairments in ability to feed, growth, reproduction and/or death (Mukherjee and Jana 2007). While many in lab studies have been conducted on the effects of effluent and individual components of effluent on fish species, onsite toxicity testing with native species is critical to our understanding of impacts (van der Schalie *et al.* 1979). When the factors effecting species disappearance is unknown, in situ testing can aid in the identification of variables correlated to the biotic response, facilitating further testing.

Many toxins elicit a behavioural response in low concentrations and in high concentrations before mortality allowing for the assessment of animals in situ (Hartwell *et al.* 1989).

The goal of this study is to assess the effectiveness and efficiency of bioassays as a rapid monitoring tool of municipal waste treatment effluent and its potential impact on the biota and water quality of receiving waters. In addition, this study will test a design and determine what water quality and environmental factors would be beneficial in support of a bioassay.

Methods

Brook trout survivorship was monitored at nine sites in the Credit River within the Town of Orangeville, Ontario. This site was chosen based on the recent disappearance of brook trout from biomass monitoring sites within this reach, downstream of the sewage treatment plant outflow (Credit Valley Conservation Authority, pers. comm.).The study was undertaken in the fall November 26th to eliminate potential mortality due to high temperatures. The planned duration of this study was 10 days incorporating two observations per day.

The study assessed survivorship of brook trout through an approximate 1000m stretch of the Credit River between Highway 9 and Dufferin County Regional Road 109. Site 1 was placed approximately 100 meters above the outflow of the town sewage treatment plant. Site 2 was within the outflow. Sites 3 to 8 were placed at 50 meter increments downstream of the outflow (50m, 100m, 150m, 200m, 250m and 300m respectively) and site 9 was placed further downstream where the river crossed Dufferin County Regional Road 109 (Figure 1). At the time of this study the west channel was dry and all flow was directed through the east channel (Figure 1). At each site a soft mesh cage (Promar Collapsible Large Minnow Trap, 3 feet X 1 foot) was fastened via black UV rated cable ties to rebar which had been anchored into the substrate. Water depth at each site was recorded to be between 0.75m and 1.25m in depth with no overhanging vegetation or cover. Cages were anchored outside of the main thalwag to avoid high water velocities. To monitor the correlation between temperature and mortality, a temperature data logger (HOBO Water Temperature Pro ®) was attached to each cage using black UV rated cable ties.

Brook trout (*Salvelinus fontinalas*) were purchased from a local fish farm and transported to the site in large oxygen filled plastic bags containing 6 brook trout each. These fish were within a 6” to 8” size range. Each bag of fish was placed in the river at one of the selected monitoring sites and the brook trout allowed to acclimate to instream temperatures at that site for one hour.

Following one hour acclimation, the fish were released into each cage starting from the most upstream (control), moving downstream. Fish were monitored every hour until 4:30 pm at which point monitoring ceased due to diminishing light levels. The cages were checked again at 9 am on November 27, 2007. Monitoring was scheduled for twice daily from November 27 to December 4, but due mortality rate monitoring ended at 9 am on November 27. At each monitoring interval the number of mortalities, number of fish in distress (determined by changes in respiration and/or swimming ability) and number of living fish were recorded until 100% mortality was observed.

Mortalities were recorded and removed from the cage and placed on ice in a cooler for pathological testing. Once trials were complete, all fish and equipment were removed from the stream and the temperature data logger data were plotted using Microsoft Excel.

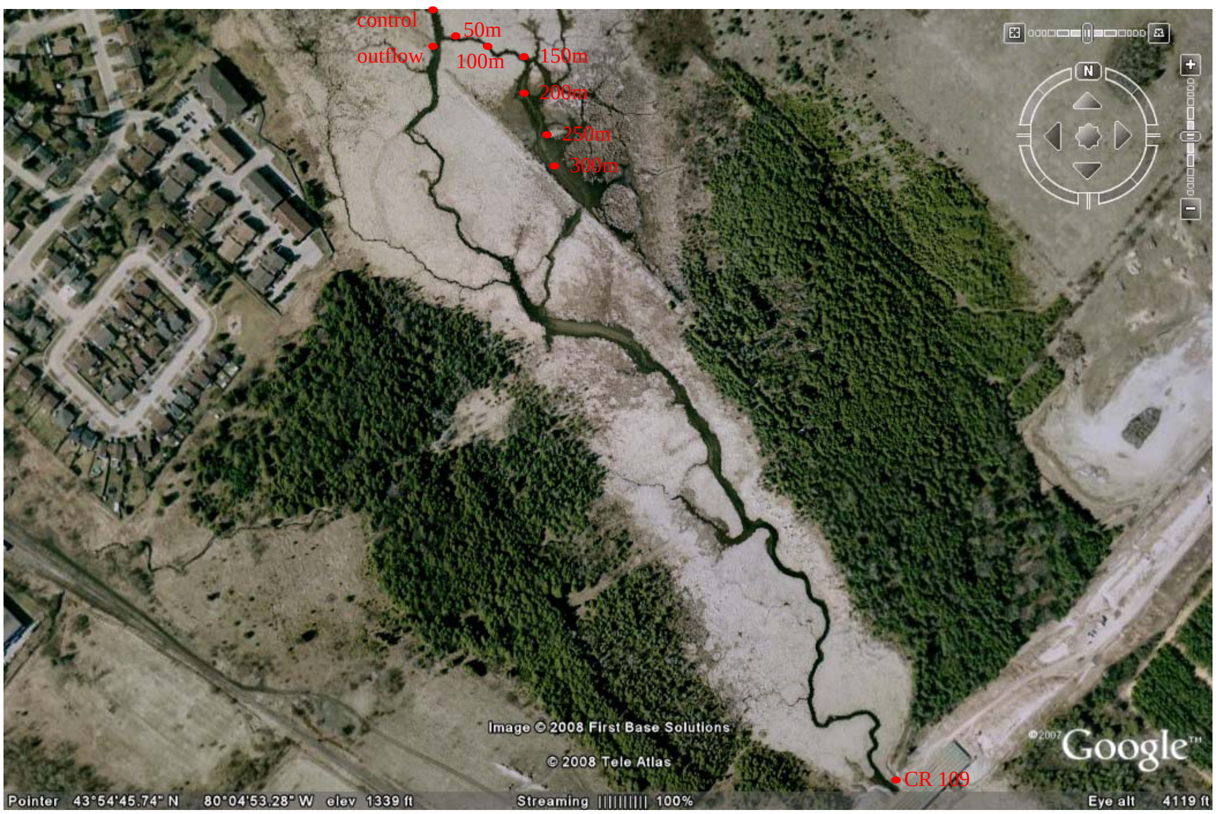

Figure 1: Sampling Site – Locations are appropriate.

Data and Results

Brook trout were placed in observation cages on November 26th at 12 pm. Fish at each site showed no behaviour indicative of temperature shock and settled into cages, orienting themselves facing upstream. Cages were observed for 3 to 5 minutes following instillation during which time all individuals continued to respire at a normal rate. Distress behaviours were noted within the outflow site at the first observation (1:30 pm). Distress behaviours included irregular swimming, inability to maintain a vertical position and exaggerated gasping.

Distress behaviours were quickly followed by the first mortality at this site at 2:30 pm. By 3 pm, all individuals in the outflow site were dead. Mortalities were observed in the site 50m downstream within 3 hours of the study initiation. Sites at 50m, 100m and 150m downstream displayed a progressive increase in individuals displaying distress behaviours. Upon return to the site at 9am, November 27, observed mortality was 100% at all sites with the exception of the control site. The control subjects, upstream of the outflow, displayed 0% mortality during the course of this study. All mortalities observed on November 27th were not considered recent in relation to the time of observation as carcases displayed discolouration and rigor. As a result all test sites displayed 100% mortality in less than 18 hours (Figure 2 and 3).

Temperatures monitoring indicated the control site maintained the coldest temperatures. The outflow displayed highest temperatures. Downstream of the discharge the site at 50m displayed temperatures significantly higher than the control. Sites 100m and further downstream display temperatures similar to one another but remain warmer than the control (Figure 4). Temperature monitoring did not show any correlation between temperature variability and rate of mortality.

On November 26, dissolved oxygen at the outflow site and 50m downstream was measured at 8 mg/l and 11 mg/l respectively by XCG Consultants Ltd. (pers. com.). Total chlorine for November 26 and 27 was reported as 1.23mg/l and 1.24mg/l respectively by the

Public Works Department of the City of Orangeville (pers. com.). Un-ionized ammonia was recorded on November 27th as 0.003mg/L in the outflow (pers. com.).

Due to the acute mortality, toxicity testing was not advised as the short time frame did not allow for bioaccumulation. However, mortalities were sent to a pathology lab for testing. All results were negative (see appendix 1).

Summary Plots

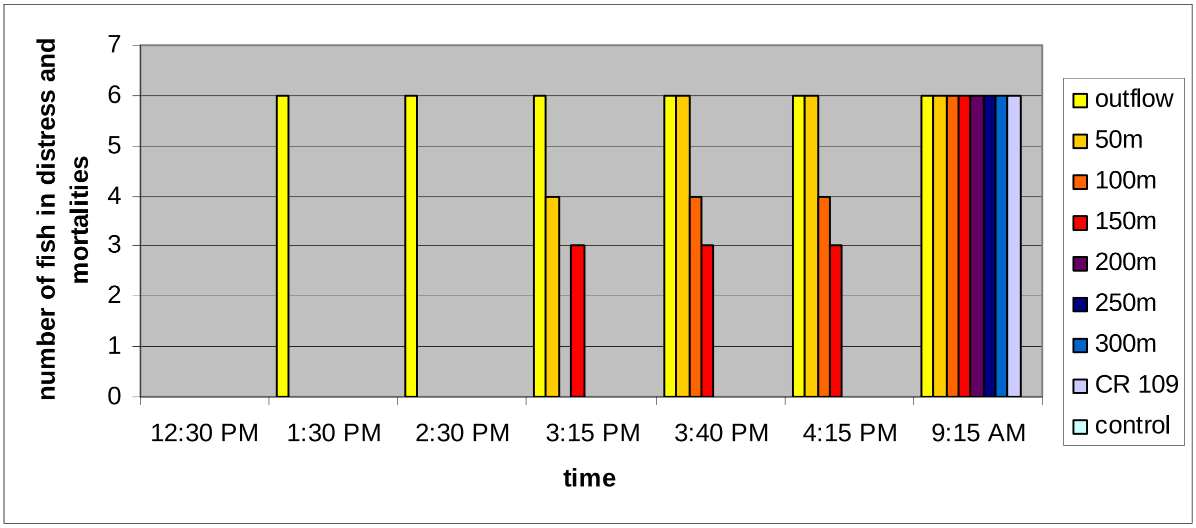

Figure 2: Distress and mortalities over time by site

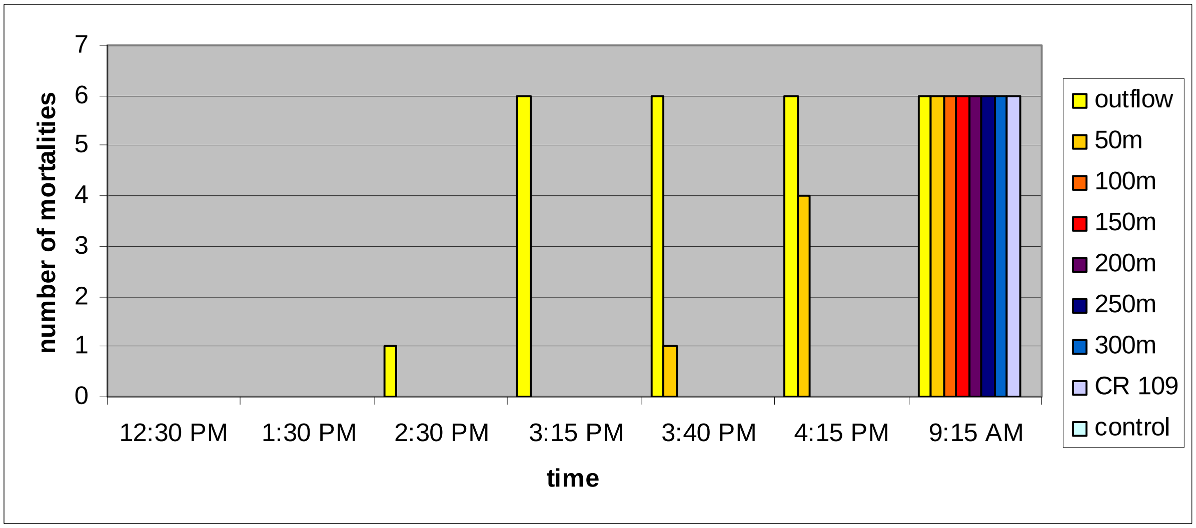

Figure 3: Mortalities over time by site

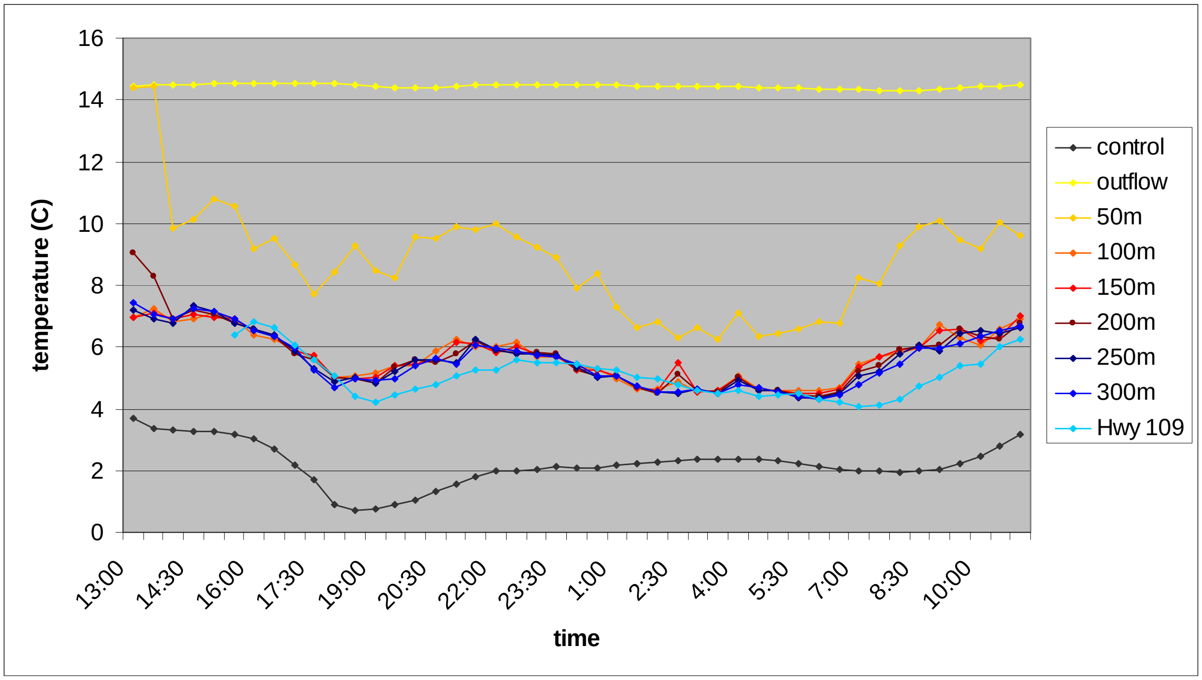

Figure 4: Temperature by Site

Implications

The use of brook trout in the coarse assessment of water quality downstream of a sewage treatment outflow can be very effective. This effectiveness is drawn from the sensitivity of brook trout as a species to many factors including chemical composition and temperature of surrounding water. The subjects of this study displayed extremely acute mortality within the outflow and every site downstream. The speed of distress and mortality of all test subjects combined with the lack of distress and 100% survival of control subjects suggests acute toxicity as a result of the effluent discharge. This result is consistent with the findings of a study initiated by the Credit Valley Conservation Authority in 2006 in which caged fathead minnows displayed 100% mortality within 24h within the outflow of the plant (Credit Valley Conservation Authority, pers. com.).

Temperature monitoring during the bioassay indicated that temperatures did not reach lethal limits for brook trout (Power 1980). This fact, combined with the significant acclimation period, leads to the conclusion that temperature shock is not likely the cause of mortality. It could be speculated that sustained high winter temperatures within the outflow could negatively affect brook trout, but temperatures in the lower sites should be within a reasonable range for long term survivorship and therefore does not explain the 100% mortality in all study sites. Temperature may act as a compounding variable, making individuals more susceptible to compounds within the combined river and outflow waters.

Dissolved oxygen is typically observed at reduced levels in effluent discharge causing changes in distribution of fish and invertebrate species as well as hampering growth and survival (Belanger 1991; Lowell and Culp 1999). In this study, dissolved oxygen at the outflow site and 50m downstream fell within reasonable limits and was consistent with the Provincial Water Quality Objective (PWQO) of 5-6 mg/l (Ontario Ministry of Environment and Energy 1994). Brook trout are known to preferably select waters where dissolved oxygen is greater than 5 mg/l and these values certainly place the caged fish within preferred ranges (Spoor 1990).

The distress behaviours observed included changes in respiration rates and in some cases distinct gasps. Drummond (Drummond *et al.* 1974) calls this 'coughing' and describes these behaviours as "regularly recurring breaks in the ventilating rhythm of the organism". This behaviour is highly correlated with toxicity and exposure to many chemicals including chlorine, ammonia and metals (Drummond, Olson, and Batterman 1974). Where possible, most fish species actively avoid chlorine at levels of 0.02 mg/l to 0.61 mg/l (dependant on species) (Fava and Tsai 1976; Cherry *et al.* 1977; Larrick et al 1978; Giattina et al 1981). Brook trout are the most sensitive fish species in relationship to chlorine toxicity displaying a LC_{50} (toxicity at which 50% mortality is observed) of less than 0.01 mg/l (Dandy 1972). Their susceptibility to chlorine has been shown to increase at high temperatures (Thatcher *et al.* 1976). Information provided by the Public Works Department of the Town of Orangeville revealed extremely high levels of total chlorine, well above the PWQO guidelines for a healthy aquatic environment (Ontario Ministry of Environment and Energy 1994). The observed behaviour and staggered mortality downstream of the outflow is consistent with both the increase in mixing with receiving waters as the effluent moves downstream and the augmenting effect of temperatures on chlorine toxicity upstream.

Ammonia is highly toxic to aquatic animals and at low concentrations also elicits a "cough" response in fish. Though ammonia levels were recorded well below the brook trout LC_{50} of 1.005 mg/l (Constable 2003), it is unknown what the concentrations were when the behaviour was observed. According to Environment Canada 62,000 tonnes of ammonia are released into our aquatic environments per year in Canada resulting in an average of 13.89 mg/l in discharges (Constable 2003). Lethal concentrations of ammonia range from 0.083 mg/l for molluscs to 0.4 mg/l for sunfish to 1.5 mg/l for channel catfish (Roseboom and Richey 1977). Brook trout are considered a sensitive species and it is not unreasonable to expect mortalities at levels below 0.4 mg/l.

The results identified in this study along with data provided by XCG Consultants Ltd. and the Public Works Department of the City of Orangeville suggest that the mortalities observed may be the result of unusually high chlorine levels in this system. However, chlorine levels were not monitored in conjunction with sites and observation of study subjects.

It is evident that the methods tested in this study are an effective way of handling fish in these conditions and executing a valuable bioassay. The acute effects observed did however require more frequent observation. Additional sample sites and control sites were planned for this pilot study, however the need to record observations precluded the installation of additional sites. It is recommended that additional observers are deployed in future tests.

The observed results warrant duplication of the study to further explain the original observations. It is suggested that this study be re-run utilizing three repetitions of this same sample design at three random times during the fall of 2008. In addition, as the bioassays are conducted, water temperatures should be monitored as well as additional measurements including pH, ammonia, chlorine, and dissolved oxygen. It is strongly recommended that data loggers are utilized for continuous monitoring of these variables. An additional bioassay during July or August may aid in the assessment of temperature dependant variables like ammonia.

This study does not represent or depict the ongoing operational conditions at this site, but a single, short observed time period. To better understand the water quality conditions at this site it is necessary to repeat this study and compile all data over multiple bioassays.

Appendix A

Ministry of
Natural Resources

Fish Health Inspection Report

Shaded areas for Lab use only

Culture Station Trout Unlimited Canada (Silver Creek Hatchery)	**Case No.** 5673
Date Received Nov. 27, 2007	**Reason for Examination:** *Monitoring* / *Mortality Diagnosis* ✓ / *Other (Specify)*

Samples Collected/Shipped by	**Condition and Type of Samples**	**Previous Disease History**
Sylvia Damelio U of G X52760	Whole fish on ice.	

Fish Samples						**Number of Fish Examined**				**Disease Agent Analysis and Results** (Positive + / Negative - / Not Done O)								
Species Name	Lot No.	History Code	Quantity in Lot	Age (in months)	Rearing Unit Type or No.	Bacteriology	Virology	Parasitology	Other	IPN	IHN	VHS	BKD	ERM	FUR	WD	CS	Other (specify)
Brook trout	???					6	6	-	-	-	-	-	O	-	-	O	O	

Comments - Culture Station

Fish caged in Credit River above (CTL) and below a sewer treatment plant as sentinel fish. 18 fish submitted in total from 6 cages. 1 cage located upstream from the plant (CTL) and 5 downstream as test cages. Several fish died in the downstream cages within an hour of being introduced to the cages.

Comments - Fish Lab

1 fish processed for each cage.
No evidence of virus. No bacterial pathogens cultured.
In cases of acute mortality such as this etiological agents are less likely to be the cause than are environmental factors. Since these deaths occurred with in one hour of introduction factors such as temperature shock or Bohr effect (a sort of bends for fish due to CO2 buildup during shipment) are possibilities.

IPN - infectious pancreatic necrosis
IHN - infectious hematopoietic necrosis
VHS - viral hemorrhagic septicemia
BKD - bacterial kidney disease
ERM - enteric redmouth disease
FUR - furunculosis
WD - whirling disease
CS - ceratomyxosis

Signature of Fish Lab Official	**Date**	
S. Lord	**Jan. 7, 2008**	For virus sheets see case 5672 White Lake FCS

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