Fish Biomonitoring in an Industrial Environment: The Toxicity Early Warning System (TEWS)

by:

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Abstract

Industrial, agricultural and municipal effluent discharge into receiving waters continues to be a significant environmental problem. Currently, industrial effluent quality is assessed using chemical assays or by evaluating physical parameters, which have multiple limitations. To overcome these limitations, aquatic biomonitoring systems (ABS), which use behavioural responses of living organisms, have been introduced. The Toxicity Early Warning System (TEWS) is a flow-through aquatic biomonitoring system developed by our research group at Lakehead University, which utilizes the behavioural parameters (ventilatory depth, ventilatory rate, body movement and cough rate) of rainbow trout fingerlings as an indicator of potential toxicity. In this thesis, the TEWS is implemented at AbitibiBowater Thunder Bay (ABTB), an industrial pulp and paper company, which has intermittently shown indicators of toxicity in cooling water tests during the Spring season. Prior to implementing the TEWS at ABTB to monitor the Kraft Clean Water Outfall (KCWO), it was necessary to improve the sensitivity and reliability of the system under laboratory conditions. TEWS laboratory results showed that 96-h LC_{50} levels of zinc sulphate ($ZnSO_4$) and leachates from a municipal waste landfill were detected in < 2h by changes in rainbow trout behavioural parameters. The TEWS proved to be effective in detecting Spring toxicity events two consecutive years (2008 and 2009) in a row at the KCWO. Toxicity events were complemented with physical, chemical, biological and theoretical modeling methods, which helped link potential contaminants to the observed toxicity. Aluminum found during 2009 toxicity event (994 μg/L) was well above aquatic guidelines (100 μg/L) and LC_{50} values (310 μg/L), modeled as reactive Al (186 μg/L) and linked to fish mortality via high gill-Al concentrations (217 μg/L).

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Chapter 1 Introduction

Industrial, agricultural and municipal effluent discharge into receiving waters continues to be a significant environmental problem. Effective effluent quality monitoring is a critical first step in evaluating preventative measures, in the identification of possible human health hazards, and in preventing the degradation of the aquatic environment. Growing and diversifying chemical industrialization enhances the risk of pollution catastrophes. As a result, industry requires a rapid, yet sensitive test method for assessing the quality of treated effluents before they are discharged.

Currently, industrial effluent quality is assessed using chemical assays or by evaluating physical parameters. Limitations of these methods include: high costs leading to little repetition, long analysis times, information limited to the contaminants investigated, and no direct measurement of biological toxicity (Girotti et al., 2008). To overcome these limitations, aquatic biomonitoring systems (ABS), which use behavioural responses of living organisms, have been introduced. Advantage of utilizing ABS include: rapid-reacting biological sensors, wide-spectrum response to toxicants, instantaneous data collection, increased efficiency, both in terms of cost and accuracy, and increased ecological relevance (Craig and Laming, 2004).

A wide array of organisms have been utilized in ABS, however fish are commonly selected because they have specialized biological characteristics, such as relatively large body size, long life cycle and chemoreception (Resh, 2008). Most importantly, fish are at the top position in the aquatic food chain and therefore have greater ability to directly affect the health of humans. Several ABS employing fish have been developed in-situ in North America (Smith and Bailey, 1988; Gruber et al., 1989;

Prahacs et al. 1996; Shed et al., 2001; USEPA, 2001). However, no ABS have been established at a pulp and paper site.

The Toxicity Early Warning System (TEWS) is a flow-through aquatic biomonitoring system developed by our research group at Lakehead University (Ingram, 2006), which utilizes the behavioural parameters (ventilatory depth, ventilatory rate, body movement and cough rate) of rainbow trout fingerlings as an indicator of potential industrial effluent toxicity. In this thesis the TEWS is implemented at AbitibiBowater Thunder Bay (ABTB), an industrial pulp and paper (P&P) company in Northwestern Ontario. ABTB utilizes large volumes of river water for manufacturing and cooling processes. The cooling water of ABTB, referred to as the "Kraft Clean Water Outfall" (KCWO) must be tested for acute lethal toxicity with juvenile rainbow trout (*Oncorhynchus mykiss*) and with the water flea (*Daphnia magna)* using standard protocols monthly and weekly, respectively (Environmental Canada 2000a,b). Over the last several years, the KCWO has intermittently failed regulated rainbow trout toxicity tests creating financial and regulatory burdens for the company. Mill environmental managers regularly monitor physicochemical parameter of the KCWO, however these methods have shown limited success in identifying suspect samples and/or potential Spring toxicity sources.

1.1 Research Goal and Objectives

The goal of this thesis was to evaluate the ability of the Toxicity Early Warning System in detecting and determining potential causes of Spring rainbow trout toxicity at AbitibiBowater's Kraft Clean Water Outfall. Specific research objectives undertaken to fulfill this goal are listed below in chronological order:

(1) Improve the sensitivity and reliability of the TEWS under laboratory conditions,

(2) Implement the TEWS at ABTB to monitor cooling water quality in rapid time,

(3) Compliment detected TEWS events at ABTB with laboratory analysis and modeling,

(4) Provide data interpretation and recommendations to AbitibiBowater managers.

1.2 Organization of Thesis

This thesis is composed of six chapters. This first chapter highlights the problem at hand and states the major thesis goal and supporting research objectives. The second chapter provides a detailed literature review on the field of aquatic biomonitoring, i.e., successful systems, technology advancements, advantages of using fish, and system considerations. A TEWS overview is given in the third chapter, which includes details of how fish behavioural responses are quantified and recorded. Chapter four describes laboratory tests conducted prior to implementing the TEWS in-situ at ABTB. In this chapter rainbow trout behavioural responses were determined after exposing fish to a geometrically increasing concentration of zinc sulphate ($ZnSO_4$) and leachate samples from a municipal waste landfill. Chapter five describes the TEWS that was developed and operational at AbitibiBowater during Spring 2008 and 2009. The TEWS was used to detect toxicity events during in-situ deployment. Detected TEWS events were further investigated using chemical analysis, Toxicity Identification Evaluation (TIE) manipulations and chemical equilibrium modeling. The final chapter presents overall conclusions.

Chapter 2 Concept of Aquatic Biomonitoring

Systems that can detect sudden contaminant pulses in surface water are gaining increasing attention worldwide. A major reason for such interest is that discharged effluents of all forms are increasing in frequency, complexity, and severity. Aquatic biomonitoring systems (ABS) assess the toxicity of surface waters by exposing organisms to the environment and measuring distinct endpoints. Endpoints used in ABS have included: behavioural responses, biomarkers, survival, growth, reproduction and genetic damage, all of which can potentially detect developing toxic conditions (Goulden, 1999). The most common endpoint used is the behavioural responses of an organism, which can directly assess the biological quality of surface waters that receive complex effluents (Smolders et al., 2004).

Behavioural responses of an organism can be considered as the integrated result of a diversity of biochemical and physiological processes (Walker et al., 2001). Although they can be difficult to quantify, behavioural responses are more comprehensive than traditional physical, chemical or toxicological methods including short-term and sublethal exposure effects, mechanisms of effect, interaction with environmental variables, and the potential for mortality (Kleerekoper et al. 1973; Giattina et al. 1981; Birtwell and Kruzynski, 1989; Henry and Atchinson, 1986; Little and Finger, 1990; Sagilo and Trajasse, 1998).

The concept of aquatic biomonitoring was proposed more than 40 years ago (Henderson and Pickering, 1963). However, in recent years, several ABS have been developed to rapidly monitor behavioural responses of whole organisms such as fish, protozoa, bacteria and algae to toxic chemicals in both surface and wastewater (van Hoof

et al., 1994; Borcherding and Jantz, 1997; Gerhardt et al., 1998; Pardos et al., 1999; van der Schalie et al., 2001; Cho et al., 2004). Depending on their level of automation and response time, ABS are commonly referred to in literature as Biological Early Warning Systems (BEWS) (Cairns and van der Schalie, 1980; Kramer and Foekema, 2000; van der Schalie et al., 2001). The fundamental components, design and operating parameters of BEWS have been reviewed elsewhere (Kramer and Botterweg, 1991; Gerhardt, 1999).

2.1 Aquatic Biomonitoring Systems (ABS)

Of all the ABS that have been deployed over the last four decades, many have shown difficulty getting out of a laboratory setting. This is reflected in the literature, as relatively few ABS have been tested in-situ in the United States (Smith and Bailey, 1988; Gruber et al., 1989; Shedd et al., 2001, USEPA, 2001) and next to none have been applied to field conditions in Canada (Prahacs et al. 1996). On the other hand, numerous ABS have been thoroughly evaluated and have displayed success under rugged conditions in Europe (Koeman et al., 1978; Evans and Wallwork, 1988; Hendriks and Stouten, 1993; Borcherding, 1994; Borcherding and Jantz, 1997; Kramer and Foekema, 2000; Gerhardt, 1999; Gunatilaka et al., 2000; Gerhardt et al., 1998). In order to highlight the different ABS currently available, this section will investigate four systems that have: (1) utilized organism other than fish, (2) automation, (3) great potential to be established in-situ, and (4) investigated single toxicants as well as complex effluents. The following ABS will be covered: ECOTOX, Dynamic Daphnia Test, Musselmonitor and the Multispecies Freshwater Biomonitor (MFB).

ECOTOX

An ABS by the name of ECOTOX has recently been established that uses different movement parameters (i.e. motility, compactness, speed, alignment and gravitactic orientation) of the motile unicellular flagellate *Euglena gracilis* as endpoints (Streb et al., 2002). These endpoints are recorded with real time image analysis software. A review of the ECOTOX apparatus has been summarized by Tahedl and Hader (1998). The various optical components of this system consist of a custom made microscope, a CCD camera and an IR diode as a light source. A built-in microcomputer controls the lower level functions of the device and acquires the data of the sensory inputs (i.e. temperature, pH, O_2 levels). The signals are transferred to the host computer via a serial port and a frame grabber card digitizes the video images. Finally, statistically significant changes in the measured parameters then indicate a change in the tested water quality.

The ECOTOX has investigated numerous single toxicants at sub-lethal concentrations, which consist of Formaldehyde (0.0025% of EC_{50}), ethanol (0.008% of EC_{50}), cycloxydim (2 mg L^{-1}), $AgNO_3$ (0.6 mg L^{-1}), $HgCl_2$ (1 mg L^{-1}) and $CuSO_4$ (8 mg L^{-1}) (Tahedl and Hader, 1998). Results from this work has shown that gravitactic orientation (based on the r-value) was the endpoint that caused first response for all single toxicants tested except for $HgCl_2$, in which organism speed showed the first response. A seepage water mixture from an industrial waste deposit in central Frankonia (FRG) has also been recently tested by the ECOTOX system. The seepage water consisted of high chemical oxygen demand (COD) (7000mg L^{-1}), high absorbable organic halogenides (AOX) (12mg L^{-1}) and high concentration of NO_2^- (154mg L^{-1}) (Tahedl and Hader, 1998). Findings from this work display that gravitactic orientation was the most sensitive

behavioural parameter, as it responded to highly diluted (Dilution factor of 2048) seepage water in rapid time.

The four major advantages of the ECOTOX system are the rapid organism response time (entire test within 6 to 10 min), test organisms can easily be grown and handled, multiple behaviour parameters are evaluated, and the system is compact and economically feasible. Possible in-situ applications for ECOTOX include the control of wastewater before and after purification to estimate its efficiency, or to monitor seepage water to evaluate its toxic potential (Tahedl and Hader, 1998).

Dynamic Daphnia Test

The "Dynamic Daphnia Test" is one of the oldest automated ABS (established in 1982) and has been employed at approximately 30 river sites in Germany, Austria, Belgium and The Netherlands (Gunatilaka et al., 2000). This system utilizes behavioural responses of highly specialized crustaceans, *Daphnia magna* and *Daphnia pulex*, which are commonly found in a variety of freshwater habitats. These two species are sensitive and have shown rapid acute toxicity responses to environmental toxicants such as heavy metals, chlorine by products, pesticides, herbicides and organic based chemicals (Kovacs et al., 2000; Wogram and Liess, 2001; Pieters et al., 2006). In fact, *Daphnia magna* are currently used in provincially and federally required toxicity tests (lethality) for industrial and commercial effluents in Ontario (Kovacs et al., 2002).

Daphnia responses used to be quantified in this ABS by infrared sensors, however this methodology has recently been replaced by video-based technology to prevent technical errors (Gerhardt et al., 2006). The following "Dynamic Daphnia Test"

apparatus has been summarized from Gunatilaka et al. (2000). Twenty juvenile (6-24h old) daphnia are exposed to test water (20+/-1°C) in two continuous flowing cells. Their swimming behaviour is recorded with video-based technology, which is then quantified and exported to a computer for analysis. If water quality changes occur in the test water via a contaminant, the organisms react by changing their swimming behaviour. Daphnia have been noted to swim slower (retarded activity or death) or react with an increased activity (hyper-active) under toxic conditions (Gunatilaka et al., 2000). When a defined system threshold limit is exceeded, a built in alarm is triggered, which results in early warning.

The "Dynamic Daphnia Test" has investigated single toxicants and complex effluents associated with the heavily industrialized Rhine River in Germany. A case study presented by Gunatilaka et al. (2000) showed the system was established at a water quality monitoring station at Worms (Germany). This station is 10-15 km downstream of a waste water treatment plant (WWTP) of one of the largest chemical industrial complexes in Europe, a WWTP of a cellulose factory in Mannheim, a large communal treatment plant which treats the industrial waste water of chemicals and lastly, two treatment plants of a community. While employed on the Rhine, the ABS recorded few early warning alarms, which have helped link increases (10 times higher than background concentration) of 2-chloropyrine to a communal wastewater plant. Another interesting finding from this case study was that heavy rains lead to increased responses in the "Dynamic Daphnia Test". This was primarily due to increased turbidity in the river water, however on occasions it was found that rain events favoured large discharges of 3-

nitrobenzene sulfonic acid (200 $\mu g\ L^{-1}$). Such contaminant retarded the daphnia activity (drop of close to 500 impulses) causing an alarm situation.

Although the "Dynamic Daphnia Test" has shown to be successful on occasion and has received top ratings by government environmental organizations, it still has several ongoing constraints. A major constraint is the physiological status of the Daphnia. For example, availability of an adequate amount of food (algae and bacteria) in test may influence the physiological status of the daphnia and therefore affect detection levels of toxic substances.

Musselmonitor

One of the more unique ABS is the Musselmonitor®. This instruments development was a result of consistent research on the valve movement of bivalves by Salanki (1964), Salanki and Varanka (1978), Manley and Davenport (1979) and Kramer et al. (1989). It was developed in a laboratory as a flow through system (established in 1988), but within short time became employable under field conditions in six European countries, the USA and Australia (Kramer and Foekema, 2000). Numerous bivalve species have been used in the Musselmonitor®. For example, zebra mussels (*Dreissena polymorpha*) have been used in fresh water and oysters (*Crassostrea gigas*) have been used in marine environments (de Zwart et al., 1995). The Musselmonitor® is based on the concept that bivalves move the two halves of their shells in according to a characteristic pattern in clean water and close their shells during unfavourable toxic conditions (Kramer and Foekema, 2000).

Delta Consult (2009) has summarized how bivalve measurements are recorded and alarms are generated in the Musselmonitor®. Miniature coils are attached to each half of the mussel's shell and a high frequency voltage is passed through the first coil. This creates a magnetic field, which induces a voltage in the other coil. The strength of the induced signal is dependent on the distance between the two coils, and therefore determines the shell position. Microcomputers then process the data and Musselmonitor® alarm notifications (No alarm, Closure alarm, Decreasing Average alarm, Activity alarm and Gaping alarm) are generated (de Zwart et al. 1995).

This bivalve based ABS has been used in a laboratory setting to determine its sensitivity to ammonia (found detection limit of 0.6 mg L^{-1}), copper (found detection limit of 0.005 mg L^{-1}), zinc (found detection limit of 0.5 mg L^{-1}) and various other compounds (Jonson et al., 1991; Kramer et al., 1989). A wide array of field studies have been successful with this ABS such as monitoring the intake of a water supply storage basin, continuous monitoring of the rivers Rhine and Meuses and the most relevant to this research project, monitoring industrial cooling water for optimal chlorination levels (de Zwart et al., 1995).

Multi Species Freshwater Biomonitor

The final ABS that will be reviewed is the Multi Species Freshwater Biomonitor (MFB), which is based on non-optical recording principles, coined "quadropole impedance conversion technique" (Gerhardt, 2007). This system is the first multispecies and multiparameter ABS available (Craig and Laming, 2004). The main components of the MFB are two pairs of steel electrodes. The first pair generates a high frequency

electrical field over a test chamber filled with medium and an organism, and the second pair records the changes in this field based on movements of the organism (Gerhardt et al., 1994). The major advantage of this system is that it can be applied in different media (water, soil and sediment) and therefore is able to simultaneously record different types of organisms in their natural substrates (Gerhardt, 2007). Other advantages of the MFB have been noted as greater sensitivity relative to chemical indicators, its rugged nature, and it ecological relevance and ability to perform long-term biomonitoring (Gerhardt et al., 1994). Similar to the other three ABS, the MFB uses a moving average method in the generation of an alarm. If test data changes significantly from the mean of a previously recorded baseline then an early warning alarm occurs.

Over the last decade, numerous aquatic species have been used in the MFB. For example, Craig and Laming (2004) exposed the three-spined stickleback (*Gasterosteous aculeatus*) to simulated ammonia pollution at two concentrations (0.1 mM and 10 mM NH_4Cl) in order to show the feasibility and effectiveness of using the MFB. Research conducted by Gerhardt (2005) displayed how the mosquitofish (*Gambusia holbrooki*) and water flea (*Daphnia magna*) could be used concurrently to optimize the "early warning" detection of pollution waves from acid mine drainage. Additional work by Ren et al. (2007) has displayed how changes in water flea behaviour can be used as an indicator of early stress response to accidental organophosphorus pesticide contamination. Furthermore, exposing tadpoles (*Xenopus leavis*) to sublethal concentrations (1.25 μg/L) of triphenyltin (TPT) has shown that the MFB can be used as a new tool for automated registration of sublethal toxic effects on tadpole behaviour including recovery (Schriks et al., 2006). A study by Gerhardt (2002) employed the Japanese killifish (*Oryzias latipes*)

in the MFB to monitor both a municipal wastewater treatment plant as well as wastewater from a teramycin producing pharmaceutical industry. MFB monitoring in this study was done before, during and after a pilot laboratory purification process. Results from this research showed the municipal wastewater treatment plant managed to reduce stress and toxicity to fish. However, the pharmaceutical wastewater treatment process still had to be improved, as it negatively affected fish behaviour, ultimately leading to mortality. Finally, current research by Kienle and Gerhardt (2007) investigated the short-term effects of the water accommodated fraction (WAF) of weathered crude oil on the behaviour of the mud shrimp (*Corophhium volutator*) in the MFB. The results of this work showed hyperactivity in the organism at and after 130-min exposure to 50% WAF, solidifying the fact that the MFB could be a successful coastal monitor.

Based on the review of the previous four systems (ECOTOX, Dynamic Daphnia Test, Musselmonitor and MFB), it can be noted that many organisms and technologies have been developed to assess the quality of aquatic environments. Although many organisms have been utilized, the majority of ABS have used fish as sentinel species (Cunha et al. 2008). Especially at their younger developmental stages, fish are ideal sentinels for studying behavioural changes under controlled conditions (van der Schalie et al., 1979).

2.2 Automated Fish Biomonitoring

The first documented aquatic biomonitoring research was conducted with fish. This pioneer work was as simple as placing fish into specific concentrations of known toxicants and visually monitoring their behavioural response prior to mortality (Henderson and Pickering, 1963; Jackson and Brungs, 1966). Early research, conducted

by visual means has documented that several contaminants cause avoidance reactions, but few attract fish: these include detergents (Hara and Thompson, 1978), some metals (Timms et al., 1972; Kleerekoper et al., 1973) and petroleum hydrocarbons (Lawrence and Scherer, 1974).

Although relatively useful, visual monitoring of lethal effects tends to have limitations. The major limitation is that someone must be present continuously to observe the organism, or else there may be a significant delay between the onset of toxicity and death (Cairns and van der Schalie, 1980). In addition, visual observation sacrifices sensitivity as higher toxicant concentrations may be required to cause visually evident changes (Mikol et al, 2007). Due to these drawbacks, research efforts have been put towards advancing technologies, which allowed for the development of automated fish biomonitors that provide more quantifiable, continuous monitoring. The following sections will thoroughly investigate the development, application, advantages and considerations of using fish in ABS.

2.2.1 Developmental Stage

A wide array of technologies has been utilized for automated fish biomonitoring, which includes cinematography (Shelton, 1970), the use of electrodes or transducers attached to fish (Sutterlin, 1969), by immersed non-contact electrodes (Spoor et al. 1971), incorporating light beam sensors (Cairns et al., 1974), by recording pressure changes in the buccal cavity (Schaumberg et al., 1967), as well as quadruple impedance techniques (Gerhardt et al., 1994). Of the variety of methods available, the most heavily researched has been the use of non-contact sensing electrodes to monitor the ventilatory or "breathing" rate of fish (Spoor et al. 1971, Cairns and van der Schalie, 1980). Fish

ventilatory parameters known to be sensitive to toxicity are ventilatory rate (opercular movement), depth or amplitude of ventilation, and coughing or gill purge rates (Shedd et al., 2001). Whole body movements have also been used. The ventilatory movements of fish offer two means of assessing toxic effects. The first being that oxygen consumption is related to the movement of water over the gills produced by the ventilatory movement. The second is that gill tissues are delicate and thus susceptible to toxic materials in water (Cairns and van der Schalie, 1980).

It has been noted by many researchers that non-contact electrode technologies have great promise for industrial based automated biomonitoring systems (Heath, 1972, Gruber et al., 1979, Cairns and Gruber, 1980, Shedd et al., 2001). This is because the technology is relatively robust, provides sensitive measurements, requires little maintenance, eliminates effects of organism fright, operative injury and most importantly, does not restrict the fish being monitored (Spoor et al., 1971).

Spoor et al. (1971) were the first to develop a fish biomonitoring system that utilized non-contact electrodes. This initial research quantified the opercular rhythms and whole body movements of 10 cm bluegill sunfish (*Lepomis macrochirus*) when exposed to copper concentrations of 0.5 mg L^{-1}. The electrodes used by Spoor et al. (1971) were made of stainless steel plates and located at opposite ends of a continuous-flow chamber. These non-contact electrodes were wired and attached to electronic filters and amplifiers in order to record an electrical signal produced by the fish muscle contraction during ventilation and fish body movements.

Morgan (1977) further advanced the work conducted by Spoor et al. (1971). Although similar non-contact electrodes and tank design were utilized, this more recent

study incorporated improved strip chart recorders to monitor the opercular rhythms of three different fish species (*Micropterus salmoides*, *Sarotheroden mossambicus* and *Barbus holubi*). Strip chart recorders were used in this study because they were relatively inexpensive, easily interfaced to electrodes and provided a hard copy of data. This study exposed all three species to various concentrations of cyanide in order to record and determine the lag time of fish response. Key findings were that a 48-h LC_{50} concentration of cyanide could be detected within 1 h 39 min, whereas exposure to a concentration approximately 10 percent of the 48-h LC_{50} resulted in a longer lag time of 12 h 25 min (Morgan, 1977). A further study that combined non-contact electrodes and strip chart recorders to investigate ventilatory responses in fish was that of Drummond and Carlson (1977) which recorded the effects of chlorinated water, heavy metals, coal and kraft mill wastes.

Data acquisition and evaluation is extremely important in automated biomonitoring systems. Data has been recorded by means of event recorders, strip chart recorders, polygraphs, video processors and computers that can integrate signals and generate x-y coordinate data (Kane et al., 2005). An in depth review conducted by Cairns and Gruber (1980) displayed the limitations of using strip chart recorders in automated fish biomonitoring systems. Since a fish very often ventilates at a rate greater than once per second, strip chart data can quickly become exceedingly voluminous. Investigators then have the task of manually quantifying these data by counting the peaks, measuring the amplitudes and/or the areas under the curve (Cairns and Gruber, 1980). In view of the considerable natural variability of such data, the use of computers is a desirable

alternative if ventilatory activity of fish is to be used as an environmental monitor (Thompson et al. 1983)

Gruber et al. (1980) were one of the initial researchers to explore the application of mini computers in automated fish biomonitoring. This research showed that mini computers allowed for a more operator-friendly system that could collect, store and analyze fish behaviour, detecting some sub-lethal toxicants within a 30 minute to 1 hour time period (Gruber et al., 1980). Additional studies by Thompson et al. (1983) thoroughly displayed that sublethal doses of copper and zinc could be detected by ventilatory responses of blue gill (*Lepomis gibbosus)* and recorded in near-real time by mini-computers.

An extensive study by Carlson and Drummond (1978) examined brook trout (*Salvelinus fontinalis*) cough rate for multiple compounds and developed five levels of fish ventilatory response. The five levels are listed as follows: level 1 (normal behaviour) was defined by cough rate, breath rate and amplitude varying, but not increasing significantly, level 2 (chronic effects) was indicated by sole significant increases in cough rate, a level 3 (chronic to acute effects) was defined by significant increases in cough rate accompanied by moderate increases in breath rate and depth, level 4 (acute effects) was defined by significant increases in cough rate, breath rate and amplitude and lastly level 5 (death is imminent) was highlighted by cough rate, breath rate and amplitude becoming greatly reduced or erratic. Because these categories were found on limited data, break-points between each level were not quantified.

2.2.2 Application Stage

As years passed, the feasibility and sensitivity of low-cost computer and automation components increased. Consequently, different chemical compounds were researched, various fish species were used and in-situ automated fish biomonitors became more common. Researchers such as Diamond et al. (1990) ventured into studying more complex toxicants, such as dieldrin (organochlorine insecticide), which showed increased ventilatory frequency in fish at concentrations above 24 μg/L. Another study by Gerhardt (1998) examined how complex effluent from Richards Bay Minerals in Natal, South Africa affected the behavioural responses of rainbow trout. The mining effluent contained a series of metals in varying concentrations and slightly acidic conditions (around pH 6). The copper, zinc and lead concentrations were above guideline levels for freshwater (Jorgensen e al., 1991). Aluminum concentrations in the effluent were around the threshold values reported for toxic effects in aquatic life (Weatherly et al., 1990). Furthermore, salt concentrations in the effluent fell within the range measured in West African lakes, rivers, and reservoirs. Three replicates of eight juvenile fish were exposed to a dilution series of the effluent (0, 5, 10, 20, 50, 75 and 100%) and the behavioural patterns (ventilation, locomotion) were measured with quadropole impedance conversion technology. Results of this research showed decreased activity and increased ventilation frequency within the first 2 h of exposure at ≥96-h LC_{50} value indicating early warning responses (Gerhardt, 1998).

One of the more recent electrode based automated fish biomonitors with good repeatability is that developed by the U.S. Army Center for Environmental Health Research (USACEHR) described in Sarabun et al. (1999). Similar to past work, this

system's electrical signals are generated using the respiratory and body movements of individual fish by electrodes. However, the positioning of the electrodes was modified (above and below each fish) and carbon block electrodes were employed (van der Schalie et al., 2004). Fish signals in this system are amplified, filtered, and analyzed using various algorithms on a personal computer. The muscular electrical output (0.05-1mV) from each fish (1.2-13.3 g) was shown to be independently amplified by a high-gain, true differential input, instrumentation amplifier by a factor of 1000 (Shedd et al., 2001). Signal interference by frequencies above 10 Hz was attenuated by low-pass filters. This ABS has the ability to measure three ventilatory parameters and whole body movements of bluegill (*Lepomis macrochirus*) concurrently. Ventilatory parameters include ventilatory rate, ventilatory depth (mean signal height) and gill purge (cough) rate.

This bluegill based ABS has been tested in laboratory to many unique toxicants (i.e. MS-22 to determine ventilatory anomalies; Sarabun, 1999), but has also been employed and operated continuously at a hazardous waste and ordinance disposal site (Old-Field, Aberdeen Proving Ground (APG), MD). During the 1940's and early 1950s unlined pits and trenches were dug within Old O-Field and used for the disposal of chemical warfare agents, ammunitions, contaminated equipment, and miscellaneous hazardous waste (van der Schalie et al, 2001). The site was selected for continuous ABS monitoring of effluent prior to discharge because of the chemical complexity of the untreated effluent, the need of managers, state and federal regulatory agencies, and for public assurance. Over the five years (1995-1999) of operation at the Old O-Field site, Shedd et al. (2001) reported that abnormal bluegill responses were primarily linked to low dissolved oxygen levels (<5 mg/L). However, this system has detected the metals

arsenic and antimony at levels of 0.27 mg/L and 0.025 mg/L (Shedd et al., 2001). Such concentrations are lower than USEPA water quality criteria levels (0.36 mg/L and 0.088 mg/L; USEPA, 1991), but they are significantly higher than those averaged over the preceding monitoring periods (0.14 mg/L and 0.0007 mg/L), indicating a treatment process change (Shedd et al., 2001). Therefore, this ABS increased treatment facility engineers' awareness of effluent quality and provided an extra measure of assurance to regulators and the public (van der Schalie et al, 2001).

Continuing research with bluegill, van der Schalie et al. (2004) have shown that this species can rapidly detect Zinc (2.80 mg/L, 0.62 fraction of bluegill LC_{50}) within 15 min of exposure. The first bluegill response in this zinc study was a decrease in ventilatory depth followed shortly by and increase in cough rate. Overall, bluegill ventilation and movement responses were found to occur within 1 hour for 12 of 15 chemicals tested at concentrations equal to or less than the 96-h LC_{50} (van der Schalie et al., 2004).

Two studies have recently been described by Mikol et al (2007), which utilized the USACEHR bluegill ABS to monitor two different aquatic environments. The first study took place at a reservoir, which was fed predominantly by a large-scale forested and agricultural watershed. The ABS received untreated effluent from the reservoir for two years (2002-2004) and was operational for 96% of the time (Mikol et al., 2007). Over this time period, fish response alarms were rare. Sudden temperature, fluctuations, physical disturbances of the fish, and drift in fish signal patters triggered occasional alarms, which were classified as non-toxicant related. However, a water quality event occurred while reservoir effluent was offline as a result of construction occurring in the

watershed. Samples taken during this alarm were analyzed in a laboratory and visual inspection of the area between the construction site and reservoir effluent was conducted. A small oil sheen was reported and lab results revealed 47 μg/L of diesel oil in the sample (Mikol et al., 2007). Without the ABS response, the diesel fuel might have been dismissed and could have posed environmental impacts on the watershed.

A second in-situ bluegill study, which was conducted at a small-scale water treatment plant over a nine-month period from January to September in 2004 (Mikol et al., 2007). One ABS systems with two separate testing lines was operational 98% of the time. One line received source water from the agricultural based Monocacy River Watershed (Fredrick Country) and the other received treated (flocculation, sedimentation, sand bed and carbon filtration, chlorination) water that had been dechlorinated before passing through the ABS. It was noted that fluctuations in temperature, dissolved oxygen and turbidity often occurred in the river as a result of heavy storms. Infrequent non-toxicant related alarms in both lines were caused by water quality probe malfunctions, water flow interruptions, and rapid temperature fluctuations. A toxicant related alarm was determined in the line receiving raw Monocacy River water (Mikol et al., 2007). Fish greatly increasing cough rates, which eventually lead to mortality. Significantly, the treated dechlorinated water line showed no increased stress at any time during the event, indicating that the treatment process reduced the toxicity. Raw river water from the episode was chemically analyzed and toxicity tests with *Daphnia magna* were conducted. Analysis of the river water samples using a gas chromatograph/mass spectrometer (GC/MS) showed significant concentrations of the solvent butyl carbitol acetate and the herbicide metolachor. In addition, *Daphnia* toxicity tests showed no mortality after 24

hours (Mikol et al., 2007). Based on the agricultural nature of the watershed and considering the time of the year, it was concluded that chemicals identified by GC/MS, caused the event (Mikol et al., 2007).

Although the majority of recent fish ABS research has been focused on bluegills, current studies show that other species are also being investigated. Ongoing fish ABS research conducted Magalhas et al. (2007) have exposed zebrafish (*Danio rerio*) to sublethal concentration of sodium hypochlorite and noted gradual decrease in swimming activity. This ABS has shown to be a useful tool thus far for detecting sublethal toxicity. It also has great potential to be incorporated into biomonitoring protocols in Brazil.

Fish ABS are not only limited to freshwater, but can also be adapted to marine environments. First results from a new Marine On-line Biomonitor System (MOBS) have shown that seabream (*Sparus aurate*) and turbot (*Scophthalmus maximus*) can be used to detect water quality fluctuations in marine environments (Cunha et al., 2008). This current research has explored the behavior responses of the two marine fish to acute hypoxic test conditions (2 mg O_2/L). Tests were performed for 15 min in small test chambers with isolated fish, as well as in large aquaria with groups of six fish. In both conditions, MOBS recorded significant alterations in fish behaviour. Isolated juvenile seabream increased their ventilation frequency (increase of 20%) and grouped fish decreased their swimming activities by 40% (Cunha et al., 2008).

2.3 Advantages of Using Fish

Fish have been utilized extensively in ABS due to their specialized biological characteristics, such as relatively big body size, long life cycle and chemoreceptors (olfaction and gustation) (Silver and Johnson, 1986: Resh, 2008). The major advantage of

using fish over other organisms is that they are at the top position in the aquatic food chain. Therefore, they have greater ability to directly affect the health of humans. Although, Barbour et al. (1999) recommends using multiple groups of organisms in aquatic biomonitoring programs, this seems to be unachievable most of the time, primarily due to logistical and cost considerations. Monitoring the behavioural parameters of other organisms, such as Daphnia has been found to be problematic (W.J. Keeler, private communication, August 10, 2009). This is primarily because Daphnia tend to cling to surfaces, creating sporadic information with a large degree of variability.

Adopting a fish ABS can reinforce environmental manager's confidence in the operation of traditional physical/chemical based monitoring systems (Shedd, 2001). In summary, major advantages of adopting a fish ABS have been noted by USEPA, (2001) as: (1) early detection and warning of transient, episodic, and developing toxic events, (2) identification of potential toxicity from unsuspected sources and chemicals, (3) integration of the effects of complex chemical mixtures, and (4) acquisition of samples for further chemical analyses based on fish behavioural responses.

2.4 System Considerations

Automated fish biomonitors are used as broad-based toxicity detectors and have various advantages, however it is important to understand the many factors and considerations behind such systems. Automated fish biomonitoring system considerations stated by Cairns and van der Schalie (1980) and more recently by van der Schalie et al. (2001) have been summarized below into three main points: rapid detection, analysis of data and controlling system conditions.

Rapid reliable detection of developing toxic conditions. The speed with which an organism will detect unfavourable condition depends on the type of organism, the particular response being monitored, the concentration of material with respect to the acutely toxic level, the toxicant' s mode of action, and finally the physical-chemical characteristics of the dilution water (temperature, pH, hardness, dissolved oxygen etc.). Fish ventilatory monitoring systems have been shown to respond to a wide variety of organic and inorganic chemicals (American Society for Testing Materials, 1995). Initial fish responses to toxicant concentrations near the 96 h LC_{50} frequently occurs within an hour, but sometimes can take up to several hours (Morgan 1977; Gruber et al. 1980; Evans and Wallwork 1988). Loss of sensitivity to toxicants may occur following long-term exposure to very low levels of the toxic material. A study by Cairns and van der Schalie (1980) that exposed Bluegill for 29 weeks to zinc at 1/100 of the 96 h LC_{50} (0.075 mg L^{-1}) showed some decrease in activity responses to a simulated zinc spill (3.0 mg L^{-1}) zinc).

Appropriate methods for the analysis of data. Recent hardware and software updates have been used to statistically analyze various fish behavioural parameters. The normal range of variation in the parameters being monitored should be statistically determined so that reliable criteria can be established from abnormal responses caused by toxic conditions. For example, baseline data obtained after acclimatization to test conditions are often used to generate confidence intervals by which abnormal responses could be subsequently detected. Nonetheless, one must note that when the parameters being monitored have a diurnal periodicity, it may be necessary to compute a separate normal range of values for several periods of the day (Cairns and van der Schalie, 1980).

The baseline data should also only be conducted after a week acclimatization period, ensuring that species response is only a response to the effluent and not from the stress of moving from one tank to another. Finally, both baseline and test data should be easily digitized in order to increase the amount and accuracy of the data collected (Cairns and Gruber, 1980).

Control of environmental conditions. Similar to any analytical chemistry device, such biomonitors operate within a range of environmental conditions. Certain parameters of water (or waste water) such as temperature, pH, dissolved oxygen, or hardness may independently cause response from organisms when no specific toxicant is present. Furthermore, fluctuations in parameters may make a given amount of toxic material more or less harmful. For example, decreasing pH could ultimately affect the speciation of aluminum, therefore creating the more toxic form of Al to fish (inorganic Al^{3+}) (Gensemer and Playle, 1999). In addition, since fish are poikilotherms, temperature plays an important role in determining exposure effects on a given fish species. Current research conducted on bluegills exposed to brevetoxins shows that respiratory responses were altered at 25°C, but not 19°C (Kane et al., 2005).

Chapter 3 The Toxicity Early Warning System (TEWS)

The aquatic biomonitoring system (ABS) used in this thesis is the Toxicity Early Warning System (TEWS). The TEWS is a flow-through system developed by our research group at Lakehead University, which utilizes the behavioural parameters (ventilatory depth, ventilatory rate, body movement and cough rate) of rainbow trout fingerlings as an indicator of potential effluent toxicity.

3.1 Overview of System

The TEWS is able to quantify fish behavioural parameters by using a Wheatstone bridge circuit to improve sensitivity and nullify the effect of large conductivity changes in the effluent. Details of the bridge system are discussed in Section 3.2.3.

Rainbow trout fingerlings are used in the TEWS, as they are ecologically sensitive species. Furthermore, this species is currently utilized in acute effluent toxicity tests, which are required by the provincial law. Rainbow trout (4-6 g) are acclimatized for three weeks prior to being used in the TEWS to ensure they are disease free. During the acclimatization period fish were exposed to flowing dechlorinated water with a neutral pH (7.1-7.3), maintained at 15 ± 2°C and a moderate intensity of light (250 lux). Average dissolved oxygen levels were from 90% to 100% saturation and the water is classified as soft (< 50 mg/L $CaCO_3$). Fish were fed daily, however they were not fed for 24 h prior to being used for testing.

Before testing the effluent, individual fish are placed into the testing tank and exposed to flowing dechlorinated water (similar chemistry to the acclimatization stage), so that 'normal' fish behaviour could be recorded. Once the baseline is completed, fish

are exposed to flowing effluent and fish behavioural parameters are recorded. To identify changes produced by the effluent exposure, behavioural parameters from both exposures are analyzed, averaged and statistically compared using a two-tailed T-Test. Unequal variance of the data is assumed and significant changes ($p < 0.05$) among any of the four behavioural parameters, indicates a fish response to the effluent. By this approach an individual fish serves as its own baseline control (Kane et al., 2004; ASTM, 2003)

The flow-through design of the TEWS water circuit (seen in Figure 3.1) permits rapid effluent quality monitoring. Dechlorinated water later followed by effluent flows into 4L head tanks in which aeration and cooling begins. Once in the head tank, 1/4 inch tubing and ball flow valves control the flow rate (100 ml/min) into individual experimental tanks. Dechlorinated water/effluent flows into the top of individual tanks, then passes through the tank, exists the tank over an adjustable standpipe, and flows into a collection tank. When testing finite effluent or toxicants, samples are commonly re-circulated from the collection tank back to the head tank via a peristaltic pump (Cole-Parmer Portable Sampler). However, dechlorinated water used in the TEWS flows directly into a common drain.

Now that TEWS testing procedures have been explained, the system components and their functions will be described in further detail. Except for the environmental chamber, the components of the TEWS are relatively inexpensive. The overall system consists of the environmental chamber, experimental tanks and electrodes, a Wheatstone bridge circuit-system and signal processing hardware and software.

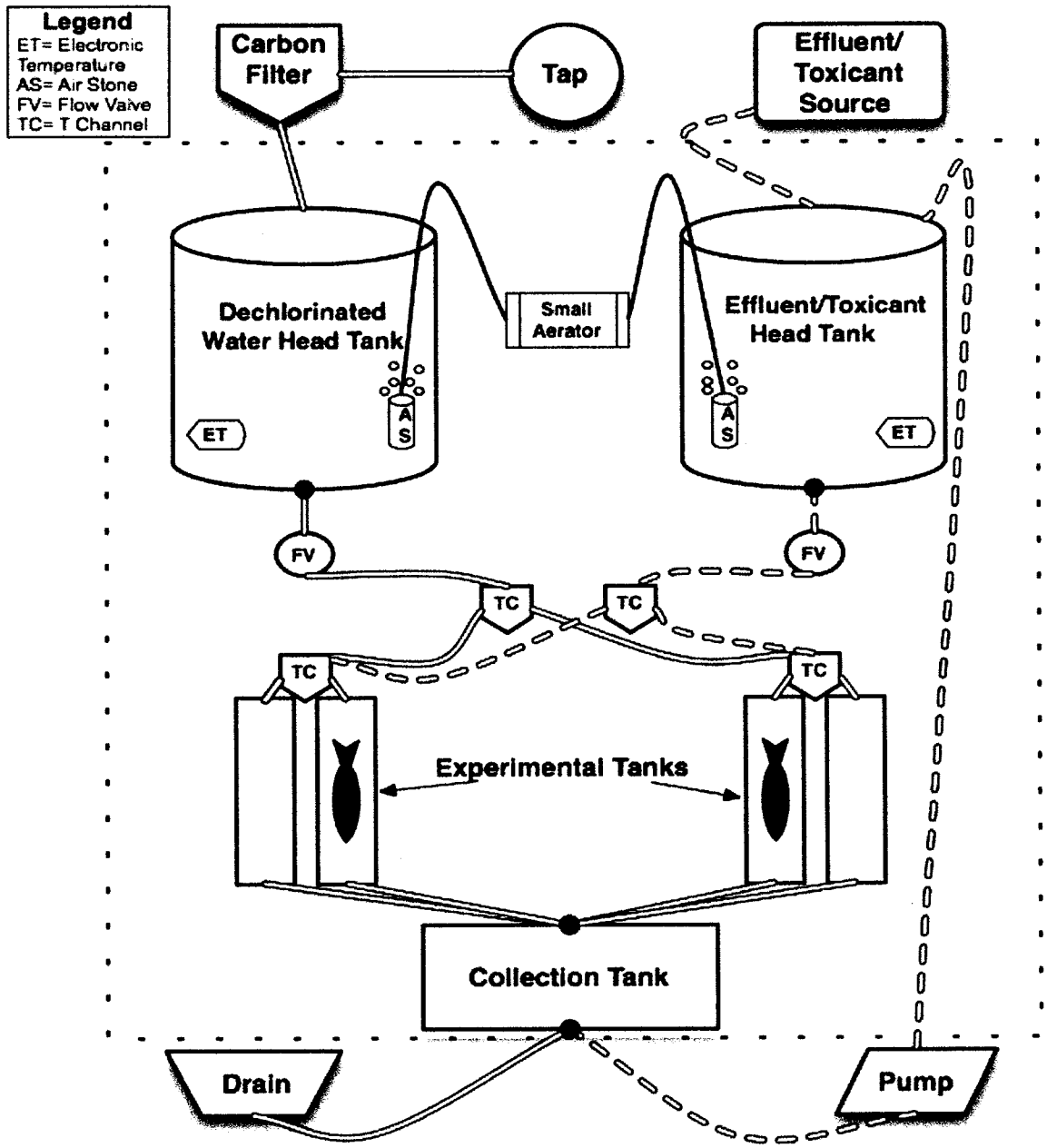


Figure 3.1 Flow-through design of the TEWS. Two fish are tested in the given diagram.

3.2 Main Components

3.2.1 Environmental Chamber

The environmental chamber (Figure 3.2) consists of a Sanyo Versatile Environmental Test Chamber (75 cm x 70 cm x 170 cm). Within the chamber,

temperature is maintained at 15°C and lighting is kept on a continuous cycle of 16 hours light and 8 hours dark with 250 lux. The chamber houses the experimental tanks and electrodes and helps eliminate electrical and mechanical noises.

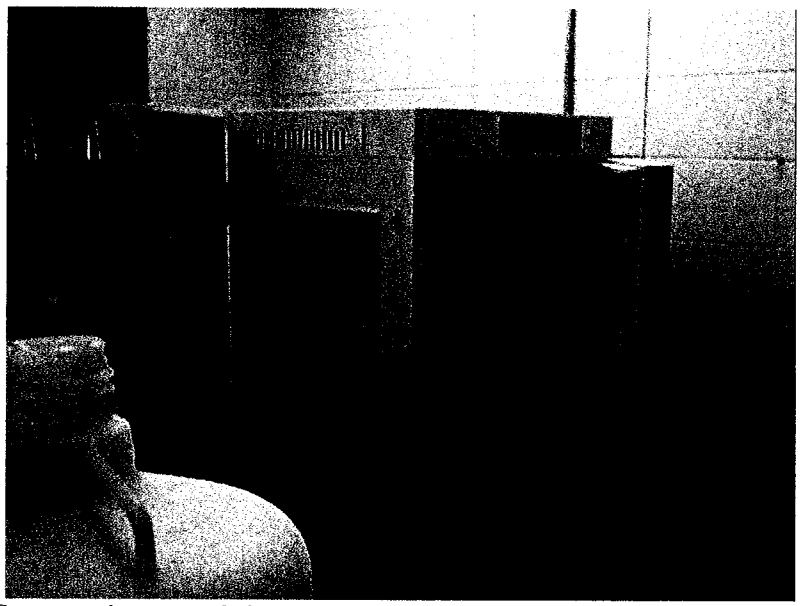
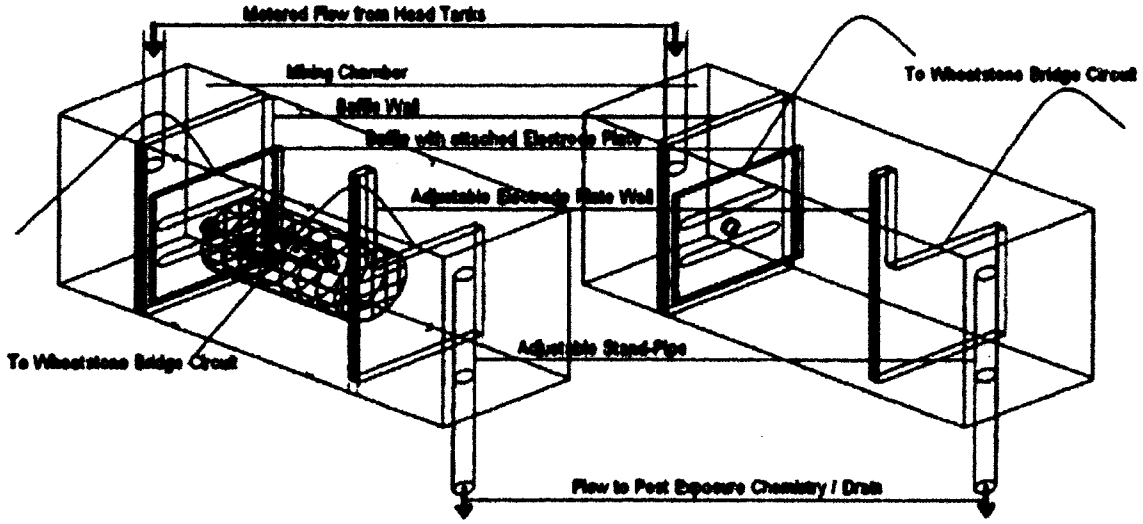


Figure 3.2 Sanyo environmental chamber used to house TEWS components.

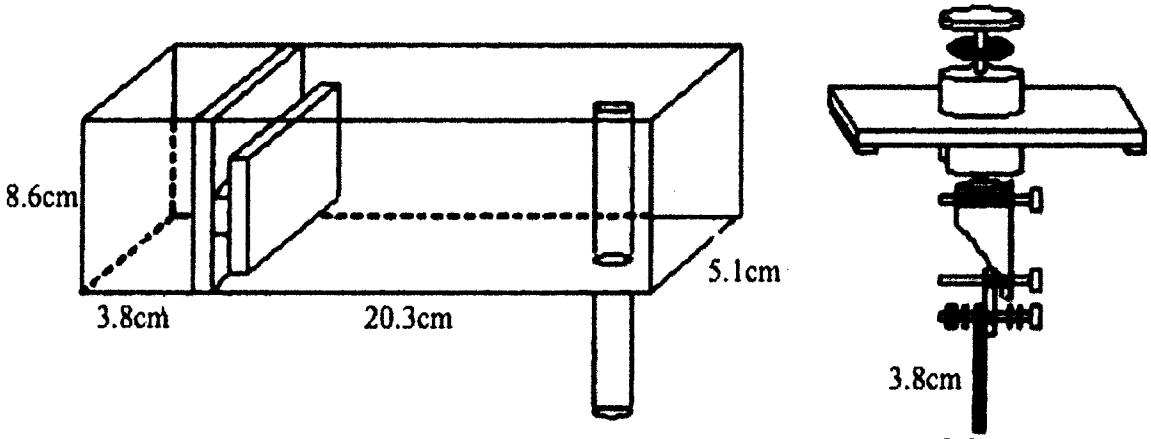
3.2.2 Experimental Tanks and Electrodes

The testing and reference tanks (Figure 3.3A) are flow-through having a flow rate of 100 ml/min. Both tanks consist of inflow and fish-testing compartments. The inflow compartment is 3.8 cm x 5.1 cm x 8.6 cm and contains 167 ml of solution. The fish-testing compartment is 20.3 cm x 5.1 cm x 8.6 cm and contains 891 ml of solution. Gradual flow from one compartment to the other is achieved by removing a small portion of the joining wall. A standpipe located at the end of the fish-testing compartment regulates the water level in each tank. Two stainless steel electrodes (3.8 cm x 3.8 cm x 0.635 cm) fit inside the fish-testing compartment and are suspended at the head and foot of each tank. Electrode holders were designed to slide along the top of the tanks so as to

provide a variable resistance (variable volume fraction), while still allowing access to the testing area. Copper wires were attached to each electrode using small stainless steel washers, machine screws and nuts (Figure 3.3C).



A) Test Tank and Reference Tank of the TEWS



B) Tank measurement and design C) Electrode and Holder

Figure 3.3 Schematic diagram and measurements of tanks and electrodes (Ingram, 2006).

3.2.3 Wheatstone Resistance Bridge Circuitry System

A Wheatstone bridge and preamplifier circuit is used in the TEWS to quantify fish behavioural parameters. This circuit type and design were chosen because it provides

sensitive measurements and accounts for the fact that effluents tested are homogeneous but are still time dependent. The conductivity can change by an order of magnitude during a lengthy monitoring period. Figure 3.4 displays a schematic diagram of the resistance bridge circuit system that was designed for the TEWS.

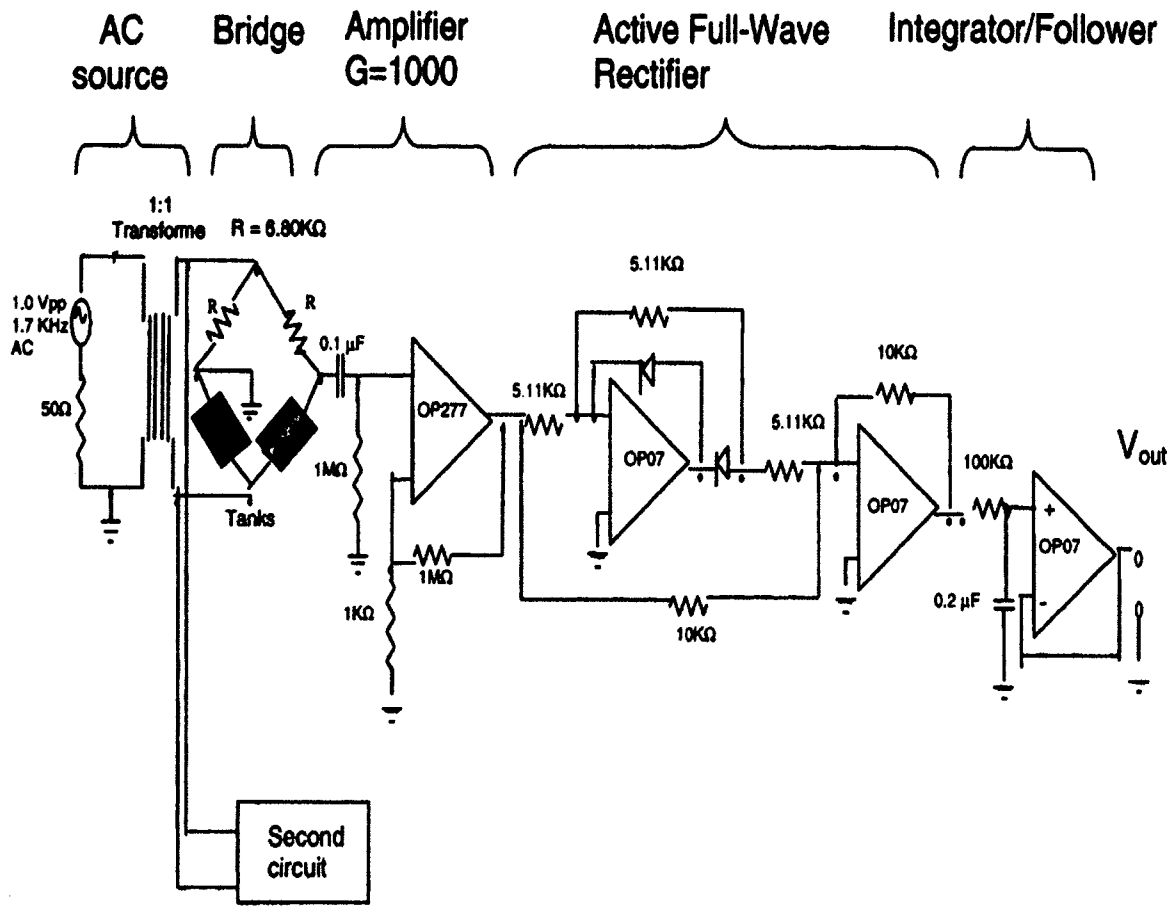


Figure 3.4 Toxicity Monitoring Resistance Bridge Circuit. Detailed layout of the ac source, bridge comparator, electronic amplifier and active ac rectifier section (Ingram, 2006).

An EZ FG-8002 Function Generator powers the ac bridge circuitry. Two of the four resistor arms consist of flow-through water tanks while the other two arms are equal precision resistors. One tank, the test tank contains a rainbow trout fingerling and the other tank, the reference tank, is used to approximately balance the water resistance of the

first as it is a common component in both tanks. The reference tank is electronically grounded at one electrode in order to minimize input offset voltage to the preamp stage of the detector circuit. This is accomplished using a 1:1 600Ω signal transformer, which isolates the single ended ac source output from the bridge circuit. The 600Ω transformer simultaneously provides signal power to two identical bridge circuits. There is negligible cross-talk between the two bridge circuits, as each has an input impedance of about 6 kΩ.

The Wheatstone bridge thus compares the resistance of the water plus fingerling versus that of the water flow alone in the reference tank. These resistances are converted to voltages by the nearly identical electrical currents that flow in the two tanks. The difference in the voltage drops that occur between the tank electrode pairs is fed by a 0.1 μF capacitor into the OP277 pre-amplifying operational amplifier. The gain of this stage was set to 1000x. As an ac current is used to prevent electrode polarization (~2,000 Hz), the amplified ac signal is then rectified after the output stage of the preamplifier, resulting in a variable dc voltage. This is digitized by an external A/D converter and then recorded to a laptop computer hard disk and displayed on the computer screen by the controller software. The electronic system integrity and fish ventilatory signal levels were verified electronically using an EZ OS-5020 Analog Oscilloscope.

In order to eliminate the sizable industrial power line electrical noise factors in the test environment a Sorensen ACR3000 line regulator is used to guarantee a constant 117 VRMS power to the electronics circuits. A high electrical signal to noise ratio is required if small changes in the fish activity are to be observed with a high degree of precision and reproducibility.

3.2.4 Data Acquisition System

A DI-720 data acquisition system available from DATAQ Instruments is utilized by the TEWS. Two pairs of electrodes per fish are plugged into the multichannel DI-720 interface, which then connects to a personal computer via a USB port. WinDaq XL software was used to analyze fish signals by determining voltage values, moving averages, peaks and valley, data compression and frequency domain (Fourier transform). Furthermore, fish signals are instantaneously displayed on a computer and exported to Microsoft Excel for further analysis. Figure 3.5 displays the DI-720 system as well as other TEWS electrical components.

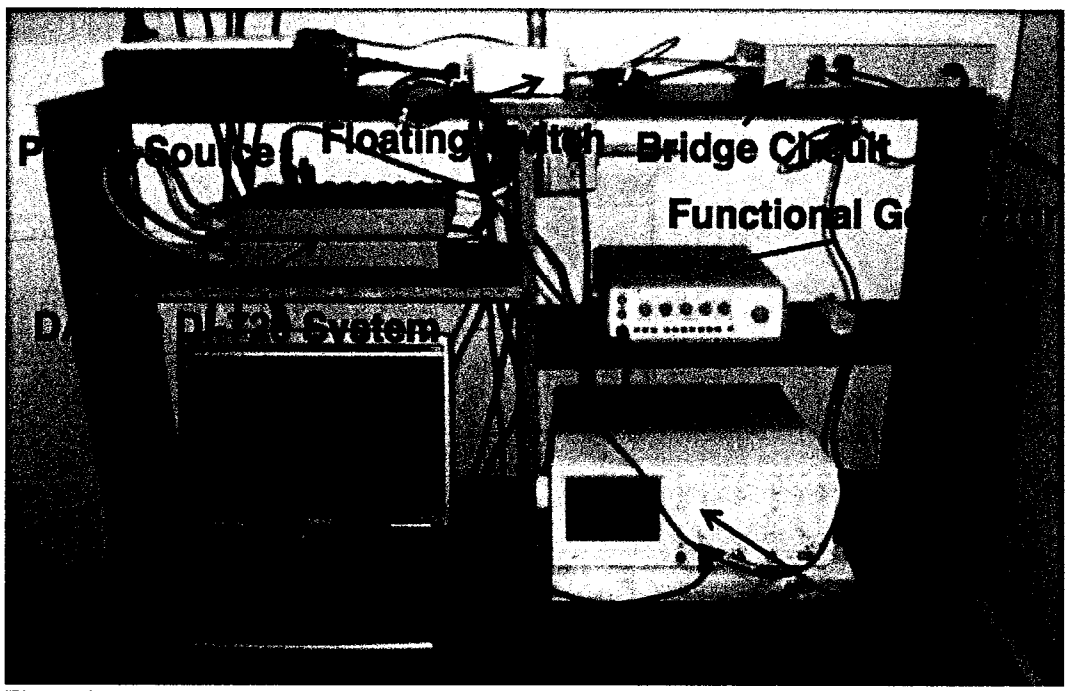


Figure 3.5 DATAQ instrumentation and supporting TEWS electronics.

3.3 Identification of Behavioural Parameters

In order to identify and characterize signal patterns as specific rainbow trout behaviour parameters, several test trials were conducted in dechlorinated water. The

following sections show how ventilatory depth and rate, whole body movements and cough rate were established.

3.3.1 Ventilation Depth and Rate

Rainbow trout ventilation patterns appeared as steady peaks and valleys. Ventilatory depths were measured in volts and on average were found to be 0.03-0.07 V after amplification. Any fish with an initial ventilation depth less than 0.02 V was replaced. Shown in Figure 3.6 is a typical screen shot from the WinDaq XL software, recording the ventilation of a 4.2 g fish in dechlorinated water. This specific fish had a mean breath depth of 0.065 V.

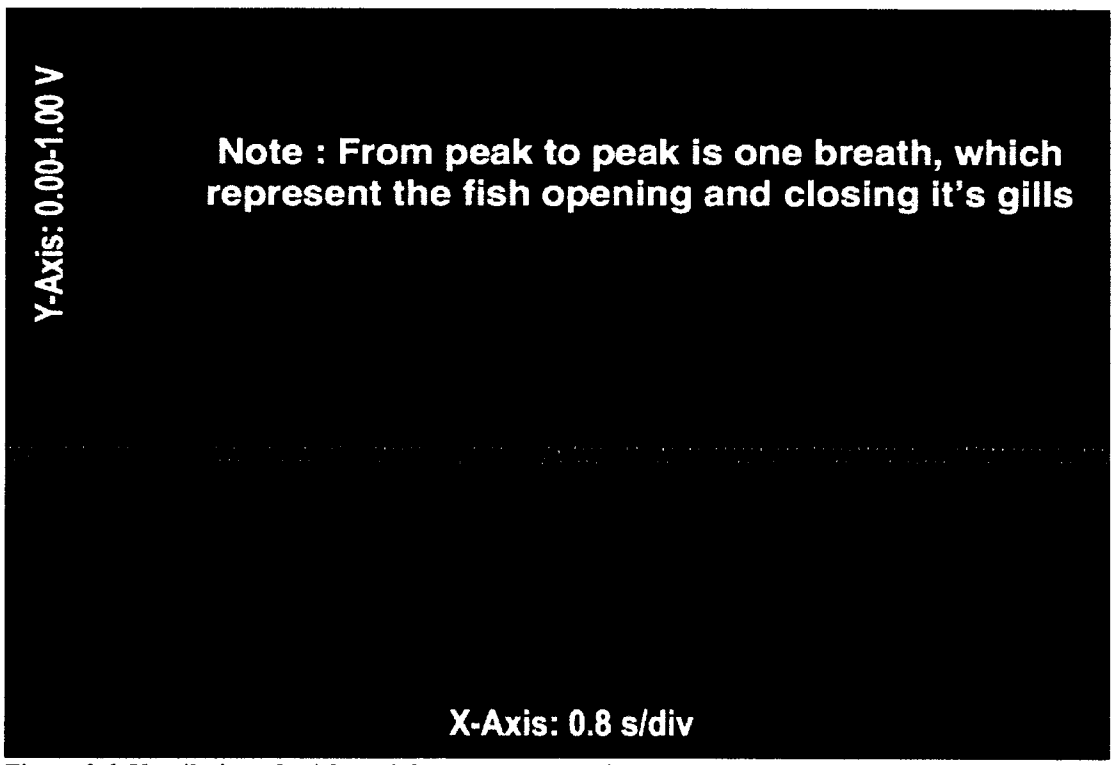


Figure 3.6 Ventilation of a 4.2 g rainbow trout exposed to dechlorinated water.

Fish ventilation rates were calculated using a Fast Fourier Transform (FFT). Generally, ventilation rate of rainbow trout fingerlings generated peaks at frequencies

between 1.5 to 3 Hz. Displayed in Figure 3.7 is a FFT calculated from Figure 6. A major peak was established at 2.662 Hz. As Hz is defined as the number of cycles per second, we can therefore state this value as breaths per second.

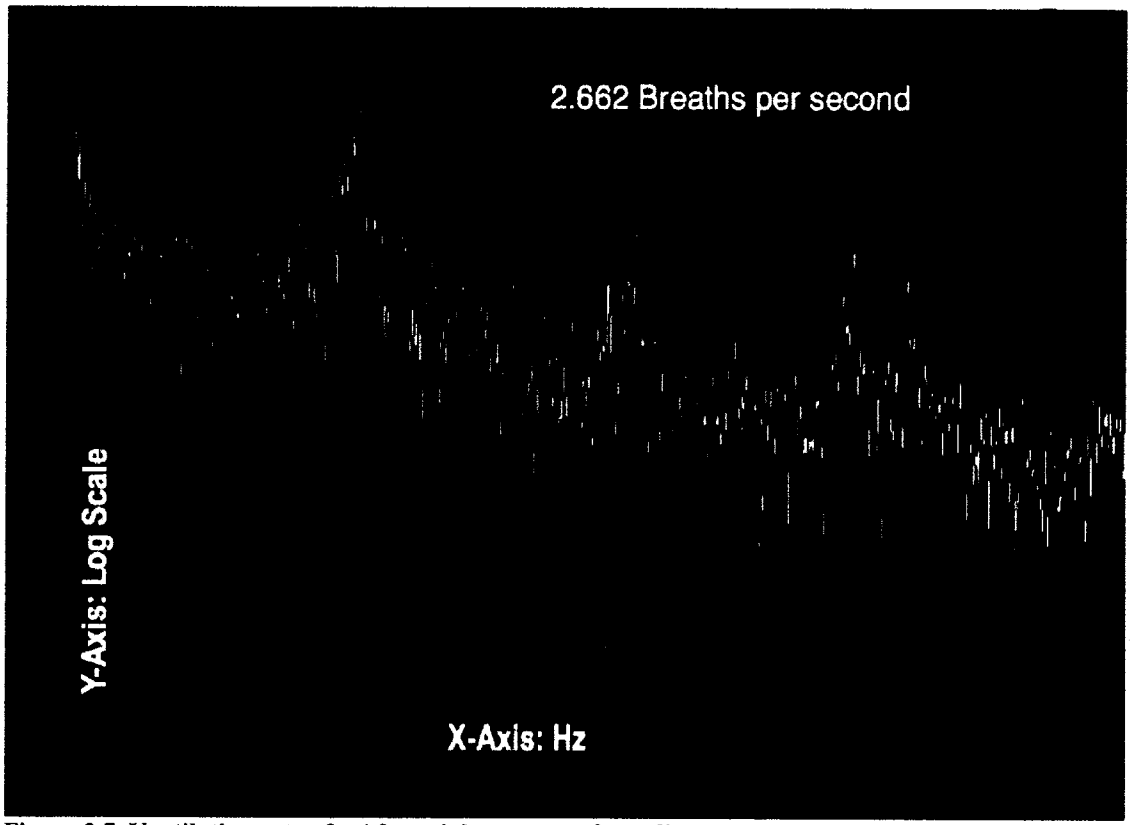


Figure 3.7 Ventilation rate of a 4.2 g rainbow trout after a Fast Fourier Transformation. A clear peak can be identified; the corresponding frequency is used as a measurement.

3.3.2 Body Movements

Fish body movements were detected by TEWS as an erratic signal presented in bursts in the time domain. Signal bursts covered a broad spectrum, however most of the energy was located in the range between 0.05 and 2 V. Figure 3.8 shows signal bursts of different magnitude for the same 4.2 g fish used in Figure 3.6, 3.7. Based on the magnitude and shape of the signal, body movements were generally classified into whole body movements and fin movements.

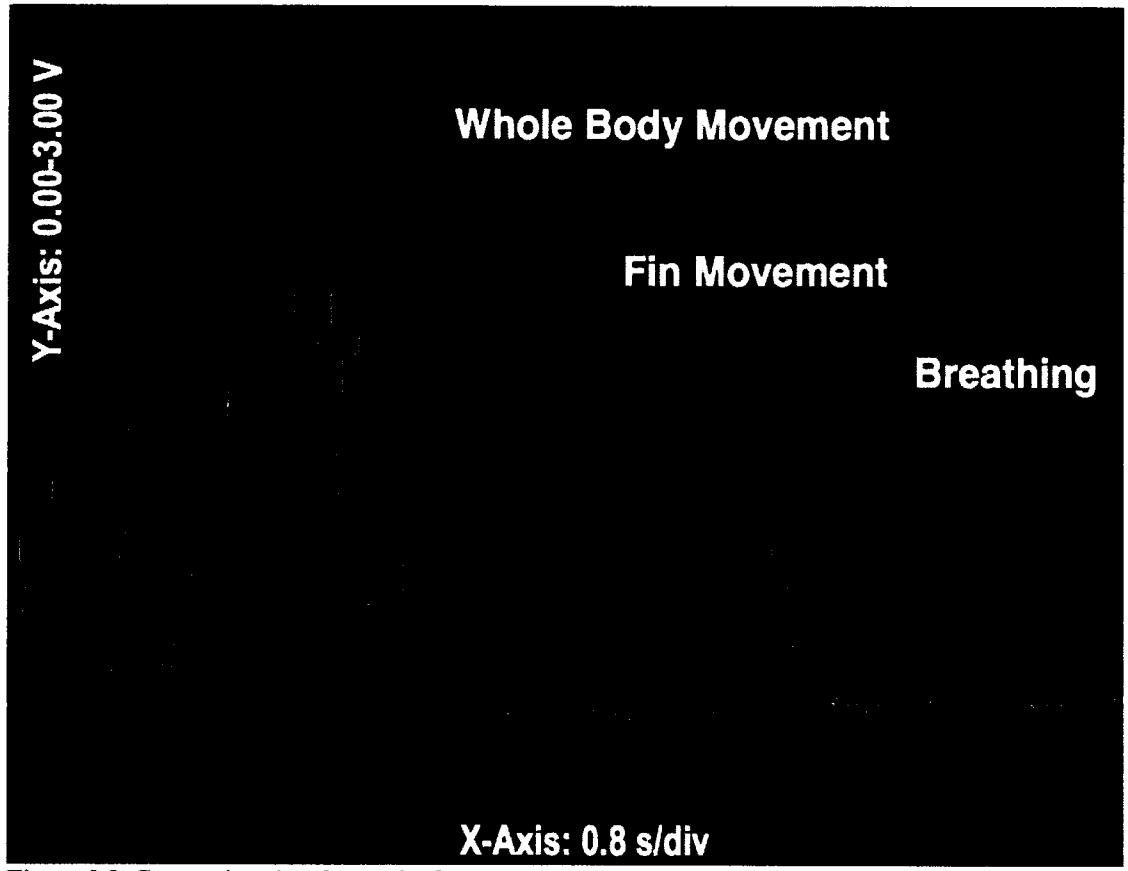


Figure 3.8 Comparing signal magnitude and shape of whole body movements, fin movements and breathing.

WinDaq XL software was used to compress files, so that overall movement (whole body and fin movement) could be established for any given time period. Shown in Figure 3.9 is a compressed one-hour fish test in dechlorinated water, displaying sections of very low movement, moderate movement and major movement. In this situation, the major movement was provoked by gently tapping the side of the testing tank.

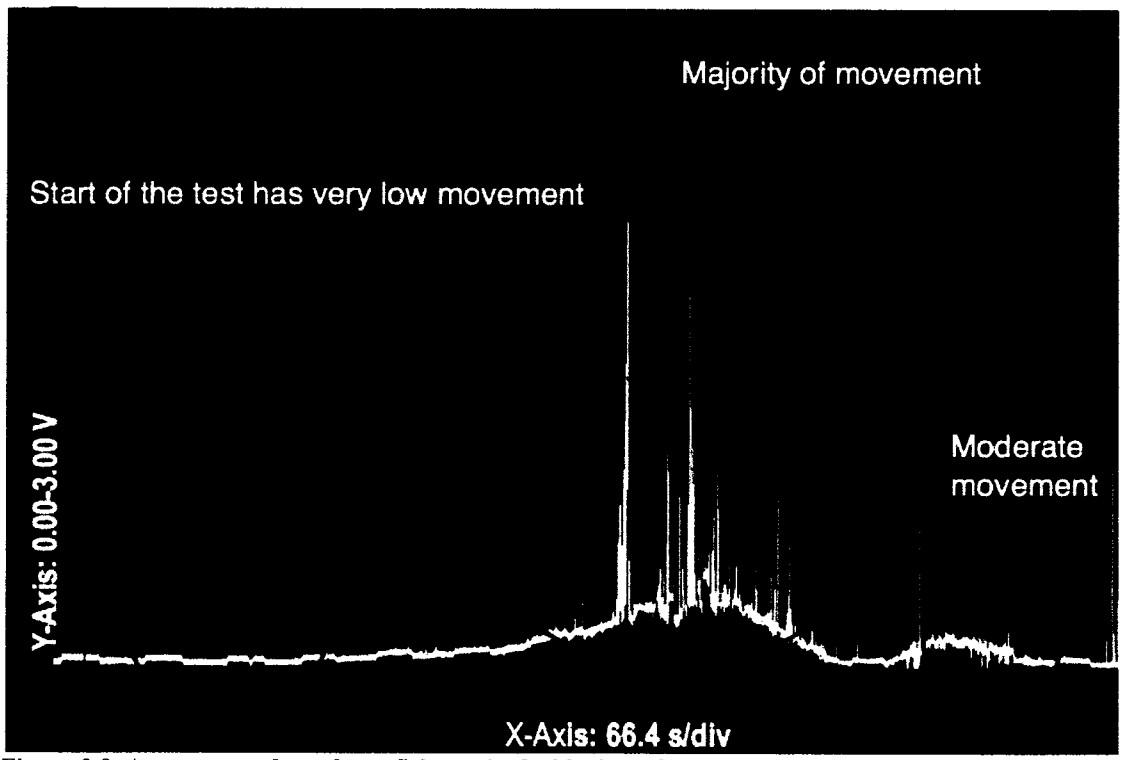


Figure 3.9 A compressed one-hour fish test in dechlorinated water, showing movements.

3.3.3 Cough Rate

Gill purging, commonly referred to as coughing was defined as any interruption in the normal ventilatory cycle of the fish. Coughing was commonly displayed as a double hitch in the normal breathing patterns and was caused by a reversal of water backwards over the fishes gill. Fish cough rates were analyzed and established manually. Shown in Figure 3.10 is the repetitive coughing pattern of a fish exposed to a zinc solution.

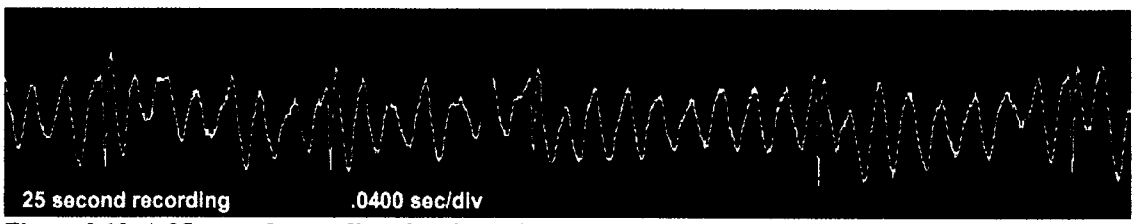


Figure 3.10 A 25 second recording showing rainbow trout coughing pattern.

Chapter 4 Laboratory TEWS Testing

The TEWS was evaluated under laboratory conditions for an extended period of time (January 2008 through February 2009) at Lakehead University, Thunder Bay, Ontario. During this period, rainbow trout were exposed to a geometrically increasing concentration of zinc sulphate ($ZnSO_4$) and leachate samples from a municipal waste landfill (see sections 4.1.1 and 4.2.1). Laboratory studies were conducted to further improve the TEWS before it was employed in-situ (Chapter 5). Both laboratory studies focus on detecting rainbow trout responses to samples with identified chemical composition and investigating fish responses relative to corresponding 96-h LC_{50} levels. LC_{50} is defined as the Lethal Concentration of a sample that would kill 50% of the test organisms in a given time period. Historically, the LC_{50} has been the standard acute toxicity benchmark used in aquatic toxicity testing, and there are 96-h LC_{50} values for literally hundreds of chemicals for widely used test species such as rainbow trout (van der Schalie et al., 2004).

4.1 Detecting Sublethal Zinc Concentrations

4.1.1 Zinc Toxicity

There is a growing concern about the increased concentration of zinc in both inland and coastal waters. Zinc may originate naturally in the environment from the leaching of soil/rock, or from anthropogenic sources, such as the discharge of industrial wastewater and mining byproducts (Zhou et al, 2008). The toxicity of zinc to fish has been widely studied during the past few decades, and a considerable amount of experimental data is available (USEPA, 1980; Spear, 1981).

Zinc 96-hr-LC_{50} values for rainbow trout are found to vary with fish size and physical-chemical water conditions, mainly hardness and pH (Holcombe and Andrew, 1978). Svecevicius (1999) found the zinc 96-hr-LC_{50} for fingerling rainbow trout in hard water (270-300 mg/L as $CaCo_3$) to be 3.79 mg/L. More relevant to this study, Hebert and Shurben (1963) found the zinc 96-hr-LC_{50} for fingerling rainbow trout in soft water (25-44 mg/L as $CaCo_3$) to be 0.91 mg/L.

Fish biomonitoring systems offer an appealing tool for the rapid detection of zinc in aquatic environments (Zhou et al, 2008). Zinc has been shown to affect multiple ventilation parameters of fish (summarized in Table 4.1). The most recent study, conducted by van der Schalie et al. (2004) showed that bluegills detected 2.80 mg/L zinc within an hour under laboratory conditions. This rapid detection was observed as an increase in fish ventilation depth and increase in cough rate.

The purpose of this lab research was to develop the TEWS further. For the purpose of improving the system, it seemed best to use a toxicant that was easily dosed and measured and whose toxicity to fish was well documented: zinc sulphate (Sparks et al., 1972). This study investigates the toxic effects of zinc sulphate on rainbow trout fingerlings by correlating 96-hr-LC_{50} values with ventilation response of trout exposed to a geometrically increasing concentration of zinc sulphate.

Table 4.1 Summary of literature on the effects of zinc on fish cough rate (CR), ventilation rate (VR) and ventilation depth (VD). In all tests the rate of ventilation or coughing increased after exposure.

LOEC µg/L	Response Type	Reaction (%)	Hardness (mg/L $CaCO_3$)	pH	Species	Average Weight (g)	Source
2800	CR	Increase	156-272	6.7-7.4	bg	1.6-14.7	van der Schalie et al., 2004
1000	CR	Increase (150%)	-	7.6-7.8	t	320	Yang & Wong, 1994
1390	CR	Increase	45	7.5	bc	-	Drummond & Carlson, 1977
20000	CR	Increase (1150%)	-	-	rbt	-	Hughes, 1975
6000	CR	Increase (200%)	-	-	bg	-	Sparks et al., 1972
2000	VR	Increase (40%)	-	7.6-7.8	bh	190	Yang & Wong, 1994
144	VR	Increase	25	7.0	rbt	122.4	Cairns et al., 1982
2500	VR	Increase	34-68	7.0-8.5	bg	87.1	Sparks et al., 1972
2550	VR	Increase	50	7.8	rbt	-	Cairns and Sparks, 1971
2800	VD	Increase	156-272	6.7-7.4	bg	1.6- 14.7	van der Schalie et al., 2004

Abbreviations: bc= brook charr (*Salvelinus fontinalis*); rbt= rainbow trout (*Onchorhynchus mykiss*); bh= big head (*Aristichthys nobilis*) bg= bluegill (*Lepomis macrochirus*); t= tilapia (*Sarotherodon mossambicus); LOEC=lowest observed effect concentration*

4.1.2 Analytical Methods

In order to determine an appropriate concentration of $ZnSO_4$ to use for the ventilation response experiments, preliminary laboratory toxicity tests were conducted and analyzed using probit analysis. Acute lethal toxicity to rainbow trout was assessed using a 96h LC_{50} toxicity test based on the EPS1/RM/13 protocol by Environment Canada. Fish used in these tests were 4-6 g.

Toxicant solutions for the LC_{50} and ventilation response experiments were prepared by dissolving reagent grade $ZnSO_4 \cdot 7H_2O$ in dechlorinated water to create a desired stock solution (1 mg/L). Toxicant concentrations, as mg/L of metal ion, were

measured using a Jarrell Ash Inductively Coupled Argon Plasma 9000 Spectrophotometer (ICP). Samples were collected, acidified with concentrated nitric acid, sealed with parafilm, and held until the end of a test for analysis.

During the ventilation response experiments, five rainbow trout were exposed to each of the following measured zinc concentrations: 161, 315, 637, 1274 and 2548 μg/L. The preparation of each sample is shown in Table 4.2. TEWS technology was used to measure ventilation depth, rate and cough rate of individual fish (refer to chapter 3 for details).

Table 4.2 Preparation of Zinc Sulfate Solution.

Conc. Level	Equivalent $ZnSO_4$ (μg/L)	Equivalent Zn (μg/L)	Dilution Water Volume (L)	1 mg/L Zinc Sulphate Stock Volume (ml)
1	700	161	25	17.5
2	1400	315	25	35
3	2800	637	25	70
4	5600	1274	25	140
5	11200	2548	25	280

Prior to zinc being introduced, fish were placed into a testing tank and exposed to dechlorinated water for two hours to establish a baseline. Throughout the first hour of the baseline test, no measurements were recorded, however during the second hour ventilation parameters were recorded. Baseline analysis consisted of randomly sampling ten, thirty-second data sets for ventilatory rate and depth and ten one-minute data sets for cough rate.

After the baseline, zinc concentrations were introduced for a six-hour period. A five-minute data set was analyzed hourly for all three ventilation parameters. Fish behavioural parameters determined during the baseline and zinc exposure were then averaged and compared (refer to Chapter 3 for details).

4.1.3 Results and Discussion

This study determined the zinc 96-hr-LC_{50} for rainbow trout fingerlings to be 1131 µg Zn/L with a 95% confidence interval of 793-1614 µg Zn/L. The hardness of the water during the test was approximately 46.7 mg/L expressed as $CaCO_3$ and pH was neutral (7.1-7.3). Such results fall in the typical zinc 96-hr LC_{50} range (240 to 7210 µg/L) for rainbow trout found in the U.S EPA ECOTOX Database.

Averaged rainbow trout responses to the five zinc concentrations are shown in Table 4.3. No mortality was seen during any of the 6-hour zinc exposures. The highest zinc concentration (2548 µg/L) produced a significant increase in fish cough rate (421%) within 1 hour and a moderate increase in ventilatory rate (24%) after 3 hours of exposure. Hughes (1975) also determined that rainbow trout increased their cough rate (1150%) when exposed to zinc (20 mg/L). Although the zinc concentrations in their study was much higher, this signifies that cough rate has the potential to reach even greater levels than what was seen in this study. Cairns and Sparks (1971) support the increased ventilation finding, as they showed that rainbow trout ventilation rate tended to increase when exposed to 2550 µg/L of zinc.

The 1274 µg/L concentration was the closest to the calculated LC_{50} value. At this concentration fish showed a significant increase in cough rate (340%), however the increase in ventilation rate (21%) was not deemed significant. The lowest detected zinc concentration was 637 µg/L (56% of LC_{50}), which significantly increased fish cough rate (260%). Fish ventilation rate, depth and/or cough rate did not respond to the concentrations of 315 and 161 µg Zn/L within the six-hour exposure period.

Table 4.3 Ventilation Response to zinc concentrations in comparison to 96-hr-LC_{50}.

Zinc (µg/L)	Fraction of 96-h LC_{50}	Response Type	Time to first Response (h)	Percent Change (%)
161	0.14	NR	NR	NR
315	0.28	NR	NR	NR
637	0.56	Cough Rate	5 hours	Increase 260.1 ± 56.1 *
1274	1.13	Cough Rate, Ventilation Rate	2 hours 3 hours	Increase 340.3 ± 87.5 * Increase 21.4 ± 1.3
2548	2.25	Cough Rate, Ventilation Rate	1 hour 3 hours	Increase 421.0 ± 77.8 * Increase 24.4 ± 3.2

*** Indicates significant percent change (p-value <0.05), NR = No Response**

Fish coughing was found to be the most sensitive ventilatory parameter to zinc concentrations of 2548, 1274 and 637 µg/L. This supports the finding of Sprague (1971) who stated that cough rate showed more promise as a significant response indicator to sublethal concentrations than did ventilation rate.

Although fish parameter responses (increasing/decreasing) can be compared, actual zinc response times from this study are difficult to compare to those found in Table 4.1 for two reasons. First, many of the older studies neglected to record fish size, water hardness and pH, all of which have a major effect on the bioavailability of the free zinc ion. Secondly, test chamber size and the flow rate of each biomonitoring system plays an important role in response times, but are not given in detail in earlier publications.

4.2 Toxicity Assessment of Landfill Leachate

4.2.1 Environmental Impacts of Leachate

Landfills are one of the most commonly used methods for the management of municipal solid wastes, due to their economic appeal (Hermosilla et al., 2009). However, the inevitable generation of landfill leachate is an environmental drawback. Leachate is a complex mixture of chemicals generated from groundwater or rain events that pass

through the landfill, are absorbed by the waste and eventually exceed the holding capacity of the porous medium within the landfill (Parkes et al, 2007).

Due to the decomposition of the solid waste in the landfill, the leachate generated may contain high concentrations of heavy metals, large ammonium concentrations and many other toxic substances (Thomas et al., 2009). The effects of this leachate reaching a water system can include eutrophication and poisonous impacts from ammonia, heavy metals and organic compounds on aquatic organisms (Haarstad and Maehlum, 1999).

It has been shown that landfill leachate has both acute and chronic toxicity (Deng and Englehardt, 2006). The toxicity of leachate is a product of the waste landfilled, the type of landfill operated, and the stage of degradation within a landfill (Thomas et al., 2009). Leachate can be treated biologically, chemically, or by physical technologies (Robinson et al., 1992; Kurniawan et al., 2006a, 2006b), but this does not always remove the toxicity from landfill leachate (Osaki et al., 2006). Aeration of leachate holding lagoons is a common physical method for the oxidation of organic material, the volitization of organic compounds and the removal of heavy metals (Martin and Johnson, 1995).

Toxicity assessment of landfill leachate, both treated and untreated, is necessary to monitor the impact leachate discharges exert on the aquatic environment (Thomas et al., 2009). Although many different methods are available to carry out direct toxicity assessments, fish biomonitoring is an attractive method because it integrates synergistic or antagonistic effects of chemicals in the leachate. Furthermore, it uses behavioural parameters as test endpoints, rather than mortality, which accounts for the concept of ecological death. The concept of ecological death is defined as the chemical effect of low

toxicant exposures that are not sufficient to kill the organism, but enables the organism to function in an ecological context (Scott and Sloman, 2004).

The aim of this study was to evaluate the water quality and toxicity of two leachate samples from a municipal waste landfill using rainbow trout as a sensor in the Toxicity Early Warning System (TEWS). Direct behavioural responses as well as toxic effects after short-term exposure were measured.

4.2.2 Materials and Methods

Study Site

The test water was taken from two leachate lagoons of the City of Thunder Bay, John Street Municipal Landfill, Ontario. The landfill has two lagoons constructed down-slope from the waste collection area (Figure 4.1) . The primary (non-aerated) lagoon is a collection pool for the leachate as it moves from the solid waste area and the secondary (aerated) is a treatment lagoon, equipped with two floating type aerators of five horsepower each.

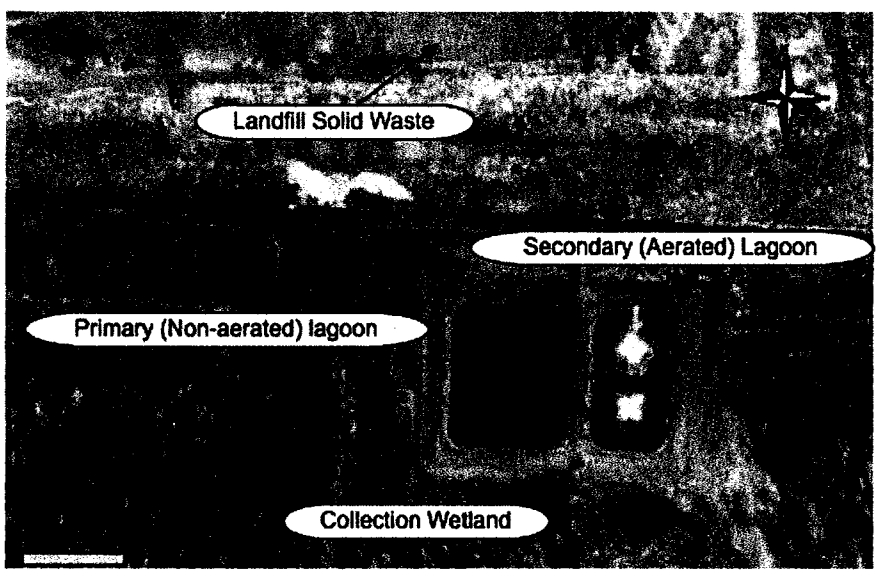


Figure 4.1 Aerial of leachate lagoons at the John St. landfill in Thunder Bay, Ontario.

Analytical methods

Samples were collected from the two lagoons in mid January 2008. Five holes were cut in the surface ice of each lagoon and 50 L was taken from each hole, totalling 250 L collected per lagoon. Leachate samples were kept in 25 L pails lined with polyethylene bags, which were transferred to a refrigerator for storage. Analytical and toxicological analyses were completed within 2 weeks of sample collection. Background water quality tests were conducted at the Lakehead University Environmental Lab: conductivity was measured using the Accumet XL60 probe, alkalinity and pH were measured by titration with HCl using a Mettler automatic titrator, anions were measured by IC/ICP, and total metals were measured using ICAP (for use when target element levels are expected to be above ICP detection limits). Water samples were first digested with nitric acid and incubated at 95°C for 3 hours. Biological Oxygen Demand (BOD) was determined by incubating water samples in an airtight container at 20°C for 5 days. BOD was calculated from the difference in dissolved oxygen at the beginning and end of incubation.

Toxicological Assessment

Acute lethal toxicity to rainbow trout was assessed for each leachate using a 96-hr-LC_{50} toxicity test based on the EPS1/RM/13 protocol by Environment Canada. Fish used in the toxicity tests were 4-6 g and LC_{50} values were computed using probit analysis.

In order to quantify rainbow trout behavioural responses to both leachates, TEWS technology was employed. Test solutions for the LC_{50} and ventilation response experiments were prepared from leachate diluted with dechlorinated tap water to the

following concentration levels: 6.25, 12.5, 25, 50, 100% leachate. For the behavioural response experiments, five rainbow trout were exposed to each of the five concentrations, totaling 25 fish tested per leachate sample.

Individual rainbow trout were exposed to a 2-hour baseline composed of dechlorinated water. The purpose of the first hour was to acclimatize the fish to the testing tanks. During the second hour ventilation parameters were recorded. Baseline analysis consisted of randomly sampling ten, thirty-second data sets for ventilatory rate and depth and ten one minute data sets for cough rate and body movements.

After the baseline, individual leachate concentrations were introduced for a two-hour exposure period and fish behavioural responses were recorded. Data analysis was performed by randomly sampling twenty, thirty-second data sets for ventilatory rate and depth and twenty one-minute data sets for cough rate and body movements. Fish behavioural parameters determined during the baseline and leachate exposure were then averaged and compared.

4.2.3 Results and Discussion

Leachate Water Quality

Water quality results for each leachate and reference data from Lake Superior and the Ontario Provincial Water Quality Objectives (PWQO) are shown in Table 4.4. Apart from conductivity and alkalinity, parameters with the greatest discrepancies were found to be chloride, potassium, sodium, phosphorus and nitrogen.

Chloride was found to be 562 times higher (786.9 mg/L) in the aerated leachate and 628 times higher (870.4 mg/L) in the non-aerated leachate when compared to Lake Superior concentrations (1.4 mg/L). Such leachate concentrations are above water quality

guidelines and if discharged may pose negative impacts on freshwater fish and invertebrates. When chloride levels become high or fluctuate, osmoregulation of organisms can be disrupted leading to inhibition of growth and reproduction (Kemp and Keegan, 1985). Since sampling was conducted during the winter it was suspected that elevated chloride concentration were linked to the City's use of road salt, which may originate from snow dump runoff (Oberts, 1994).

Table 4.4 Values for all parameters in both leachates with reference data from Lake Superior and Provincial Water Quality Objectives (PWQO). All parameters except conductivity and pH are in units of mg/L .

Analysis	Aerated	Non-Aerated	Lake Superior	PWQO
Conductivity µS/cm	4419.455	5873.417	115.39	
pH	8.403	7.766	7.36	8.5
Alkalinity mg/L	1254.505	1911.811	41.55	
Br	7.669	26.231		
NO2-N	n/a	n/a		
NO3-N	2.001	0.0025		
PO4-P	0.201	0.005		
SO4	24.702	25.383	3.44	
Cl	786.873	879.392	1.4	0.002
Al	0.107	0.067	0.031	0.075
As	0.003	0.003	0.005	0.1
B		3.5		0.2
Ba	0.072	0.324	0.011	
Be	0.0003	0.0003	0.002	
Ca	42.963	86.43	13.66	
Cd	0.001	0.001	0.001	0.0002
Co	0.007	0.005	0.01	0.001
Cr	0.004	0.009	0.002	0.009
Cu	0.008	0.01	0.002	0.005
Fe	0.284	5.307	0.051	0.3
K	191.754	222.538	0.52	
Mg	122.340	131.885	2.88	
Mn	0.037	0.218		
Mo	0.025	0.025		0.04
Na	783.270	631.308	1.58	
Ni	0.053	0.066	0.002	0.025
Pb	0.015	0.055		
S	11.816	13.543		
Se	0.025	0.025	0.01	0.1
Sr	0.719	1.335		
Ti	n/a	n/a		
V	0.006	0.005	0.01	0.006
Zn	0.008	0.009	0.002	0.02
T Phosphorus	0.866	0.616	0.003	0.03
T Nitrogen	38.278	62.342	0.505	

***Note: n/a = below detectable limit, blank = no data**

Both leachates showed potassium and sodium concentrations between 368 and 495 times higher than levels found in Lake Superior. There are no guidelines established for either of these elements and little literature exists on the possible health and environmental effects of these particular elements. However, the EPA has listed sodium to the contaminant candidate list since a high sodium diet, has been linked to increasing blood pressure in humans (USEPA, 2003).

Total phosphorus was 279 times higher (0.866 mg/L) in the aerated leachate and 199 times higher (0.616 mg/L) in the non-aerated leachate when compared to Lake Superior (3 μg/L). Both leachates were above the total phosphorus PWQO of 0.03 mg/L. Total nitrogen was 76 times higher (38.3 mg/L) in the aerated leachate and 123 times higher (62.3 mg/L) in the non-aerated leachate when compared to Lake Superior.

The parameters that varied most between leachate are demonstrated in Figure 4.2A and B. Nitrate (NO_3-N) had the greatest difference between leachate; it was 800 times higher in the aerated leachate. Phosphate (PO_4-P) had the next greatest difference being 40 times higher in the aerated leachate. Nitrate and phosphate are removed from Figure 4.2B, so the lesser fold differences are visible. Most parameters tend to be higher in the non-aerated leachate including (in order of magnitude) iron, manganese, barium, lead, and bromine. Total nitrogen and ammonia (NH_3-N) are slightly higher in the non-aerated leachate. The parameters higher in the aerated leachate include (in order of magnitude) aluminum, total phosphorus, cobalt and sodium.

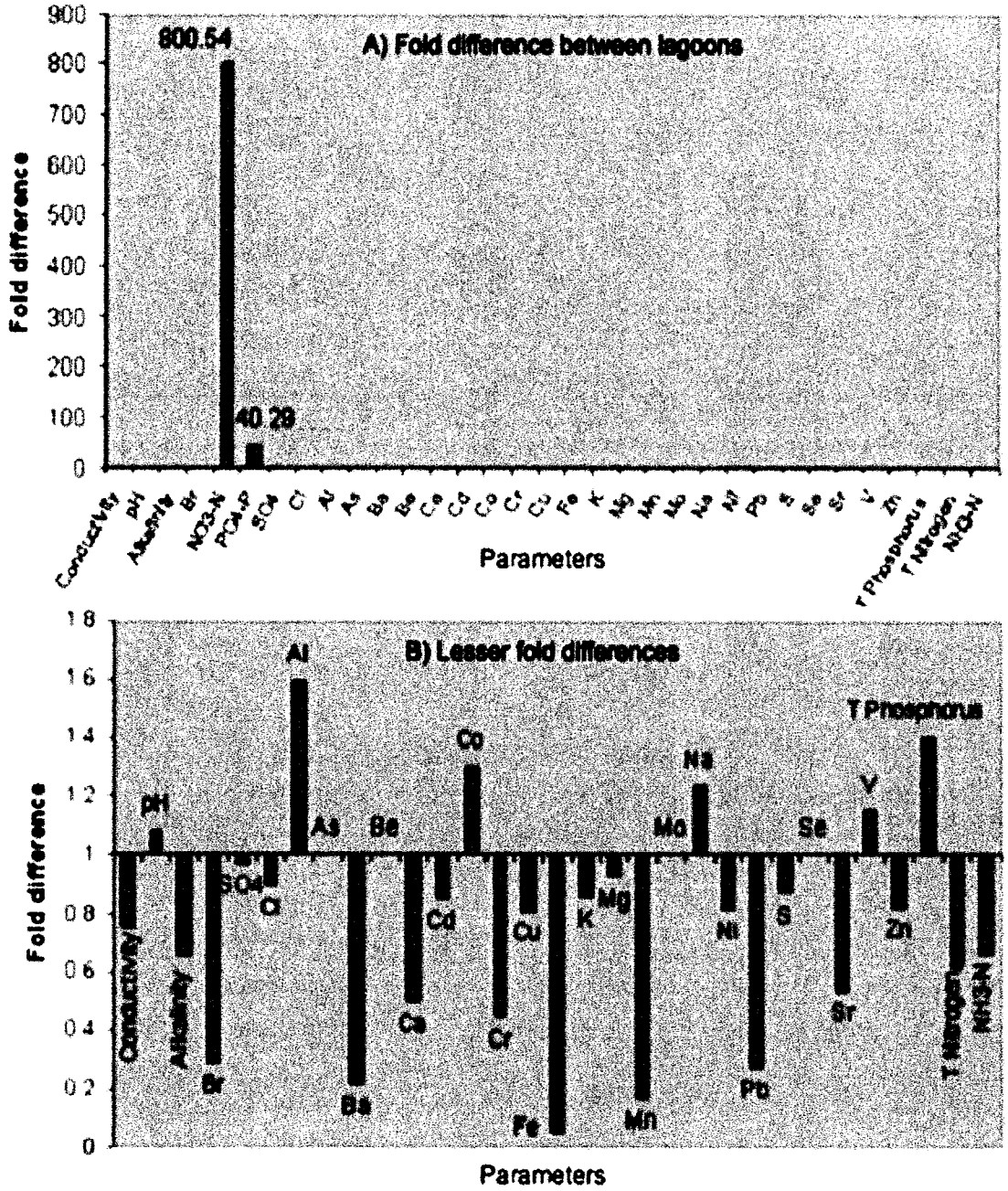


Figure 4.2 Fold differences in water quality parameters between leachate. Values above 1 fold are greater in the aerated leachate and between 0-1, greater in the non-aerated leachate.

Rainbow Trout Survival

The 96-hr-LC_{50} value of the non-aerated leachate was found to be 10.2% (95% confidence interval: 8.5 - 12.1%). The hardness of this leachate was 758.9 mg $CaCO_3$/L, which is very high in comparison to hard water defined by Svecevicius (1999) as 270-300 mg $CaCO_3$/L.The pH of the non-aerated sample tested was 8.06.

The aerated leachate was found to be slightly less toxic to rainbow trout having a higher 96-hr-LC_{50} value of 13.4% (95% confidence interval: 10.8 - 16.6%). The hardness of this leachate was 611.1 mg $CaCO_3$/L, which is also high, but lower than the non-aerated leachate. The pH of the aerated leachate was found to be higher than the non-aerated leachate, having a value of 8.47.

Direct Behavioural Responses

Fish were exposed for 2 hours to a dilution series of non-aerated and aerated leachate and behavioural responses were recorded using TEWS technology. Result expressed as percent change from baseline behaviour for the non-aerated and aerated leachate can be viewed in Table 4.5 and Table 4.6 respectively.

Table 4.5 Rainbow trout response to varying concentrations of the non-aerated leachate (96-hr-LC_{50} value = 10.2%).

Non-Aerated Concentration (% Leachate)	Ventilation Rate (% change)	Ventilation Depth (% change)	Body Movements (% change)	Cough Rate (% change)
6.25	16.5 ± 3.4	-64.1 ± 18.3	-82.9 ± 3.3	172.5 ± 32.9
12.5	-20.1 ± 2.4	-85.2 ± 9.2	-88.8 ± 1.5	528.4 ± 74.7
25	-27.7 ± 4.5	-89.7 ± 4.3	-92.4 ± 2.2	1009.0± 200.6
50	-42.8 ± 7.5	-88.2 ± 6.0	-97.2 ± 0.7	284.5 ± 105.1
100	N/A	N/A	-96.3 ± 2.4	N/A

N/A = Response could not be determined due to low signal

Table 4.6 Rainbow trout response to varying concentrations of the aerated leachate (96-hr-LC_{50} value = 13.4%).

Aerated Concentration (%) Leachate	Ventilation Rate (% change)	Ventilation Depth (% change)	Body Movements (% change)	Cough Rate (% change)
6.25	8.9 ± 3.7	-43.4 ± 7.7	-61.7 ± 22.7	167.6 ± 55.6
12.5	-11.3 ± 5.7	-79.3 ± 12.2	-91.4 ± 7.1	237.3 ± 101.9
25	-24.3 ± 10.2	-62.3 ± 18.0	-94.8 ± 3.8	923.8 ± 163.4
50	-35.4 ± 7.5	-66.8 ± 19.8	-93.8 ± 1.6	651.5 ± 218.1
100	N/A	N/A	-98.3 ± 1.7	N/A

N/A = Response could not be determined due to low signal

Fish mortality was observed while testing the top concentration (100%) of both leachates. During 100% concentration exposures, fish ventilation signals were extremely low (< 1 breath per second and depths < 0.01V) making it difficult to differentiate between levels of electrical noise and actual signal. Therefore, fish body movement was the only measurable parameter from the 100% exposures.

Fish behaviour varied with increasing leachate concentrations, but did not follow a linear relationship. All leachate concentrations tested reduced fish ventilation rate, depth and body movements, except for the lowest concentrations. The 6.25% exposure concentration of the non-aerated and aerated leachate showed an increase in fish ventilation rate of approximately 17 % and 9% respectively. This result is similar to the findings of Gerhardt (1998) who showed that a mining effluent (LC_{50}=10%) with elevated metals and salts increased rainbow trout ventilation at low effluent concentrations (20%), indicating early warning response.

Body movements showed the greatest decrease out of all behavioural parameters ranging from 83% to 97% in the non-aerated and from 62% to 98% in the aerated leachate. It is suspected that fish may be drastically reducing movements in order to

allocate energy resources to other physiological processes, such as osmo-and ionregulation, and/or respiration (Triebskorn et al., 1997).

The 12.5 % leachate concentration was the closest to the calculated LC_{50} values of the non-aerated (10.2%) and aerated leachate (13.4%). For both leachates, the 12.5% concentration showed an increase in cough rate, a sudden decrease in ventilation rate and a continued decrease in ventilation depth and body movements.

Fish cough rate hit a maximum at both non-aerated and aerated 25% leachate concentrations showing increases of 1009% and 924% respectively. Such increases are comparable to findings of Yang and Wong (1994), who exposed Big head *(Aristichthys nobilis)* to digested sewage sludge and observed cough rates as high as 1500% .For both leachates, cough rate was found to peak at the 25% concentration exposure, then decline at the 50% concentration. This supports Carlson and Drummond (1978) who stated that cough rate would increase at different magnitudes indicating chronic to acute toxicity, however when death is imminent cough rate would be greatly reduced or erratic.

In summary, both landfill leachates, which were high in salts, nutrients and few metals showed acute toxicity to rainbow trout fingerlings. Non-aerated and aerated leachate concentrations were found to degrade fish ventilation (Figure 4.3) possibly indicating osmrregulatory and ionoregulatory defects caused by pollutants and/or salts (Gerhardt, 1998). Furthermore, leachate concentrations decreased fish body movements, which may have ecological consequences, such as impaired migration or altered predation. This study shows that treatment beyond aeration is recommended if leachate is to be discharged into aquatic environments.

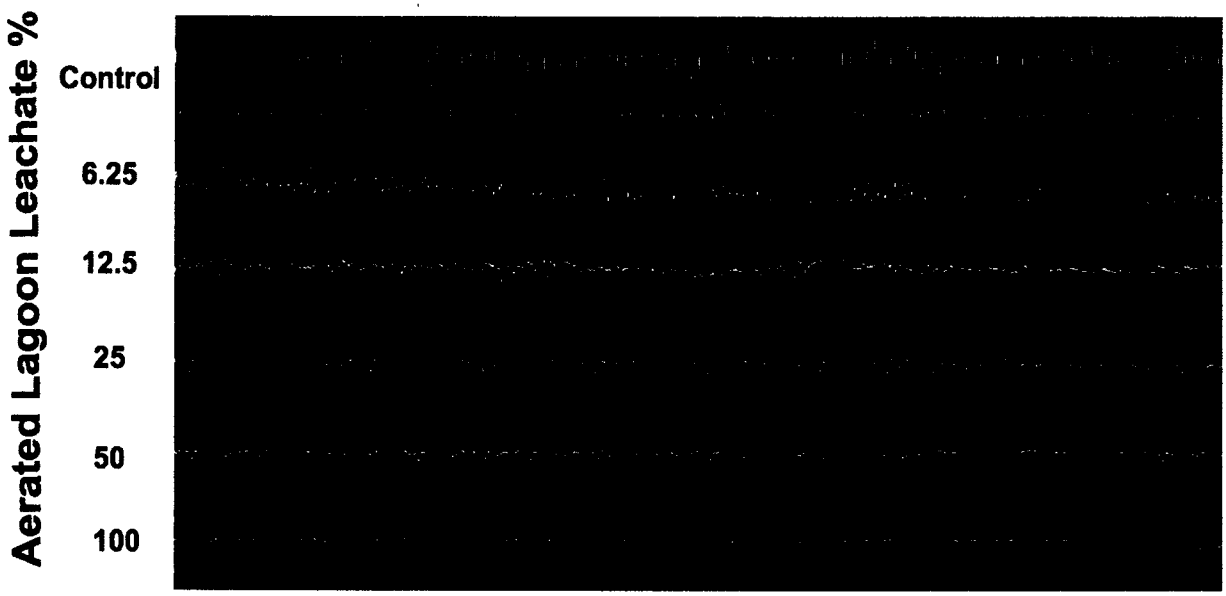


Figure 4.3 Degradation of fish Breath

4.3 Laboratory Summary

Overall, the TEWS operated very reliably throughout the laboratory testing period. The purpose of the zinc sulphate and landfill leachate laboratory studies was to further improve the TEWS before it was employed in an industrial setting. Laboratory results demonstrate that rainbow trout behavioural responses (ventilatory depth, ventilatory rate, body movement and cough rate) are able to provide rapid (<2 h) detection of contaminants at 96-h LC_{50} levels.

Observed toxicant response times for rainbow trout in the TEWS are comparable to responses reported for other fish ventilatory monitoring systems. The USACHER bluegill biomonitor described by van der Schalie et al. (2004) found that changes in ventilatory patterns and/or movement detected 12 of 15 chemicals equal to or less than 96-h LC_{50} values within ≤ 1 h. Morgan and Kuhn (1984) found that changes in ventilatory patterns of largemouth bass could be detected within 2-6 h at 96-h LC_{50}

values. Results from Sloof (1979) showed changes in rainbow trout respiration within 24 h of exposure at concentrations of 0.01-0.30 of the 48-h LC_{50} value. Furthermore, Baldwin et al. (1994) demonstrated that, to achieve 40-80 min response times in rainbow trout ventilatory rates, toxicant concentrations of 0.1-2.5 of the 96-h LC_{50} value was necessary.

Laboratory results showed that approximately half the 96-h LC_{50} zinc sulphate value was detected by a significant increase in cough rate indicating possible long-term (chronic) effects. At or above 96-h LC_{50} zinc sulphate values fish cough rate significantly increased, with moderate increases in ventilation rate indicating possible sublethal to lethal effects. Both landfill leachates were found to increase cough rate and ventilation rate, but decreased ventilation depth and body movements at concentrations below the 96-h LC_{50} values indicating possible lethal (acute) effects. Concentrations at or above the 96-h LC_{50} leachate values were found to increase cough rate, however ventilation rate, depth and body movements were drastically reduced indicating that death was imminent. From both laboratory tests it was found that an increase in fish cough rate was the most consistent indicator of developing toxic rainbow trout conditions

Understanding the relationship between regulated measures of acute toxicity (rainbow trout 96-h LC_{50}) and TEWS response will allow industries to employ similar biomonitoring techniques for effluent screening and the prediction of acute toxicity. The subsequent chapter describes the TEWS that was established in-situ to monitor industrial cooling water toxicity.

Chapter 5 Field TEWS Testing

5.1 Rationale

AbitibiBowater Thunder Bay (ABTB) is an industrial pulp and paper (P&P) company in Northwestern Ontario. ABTB utilizes large volumes of water from the Kaministiquia River for manufacturing and cooling processes. Before this water can be discharged back into the natural environment as effluent, it must be tested for acute lethal toxicity with juvenile rainbow trout (*Oncorhynchus mykiss*) and with the water flea (*Daphnia magna)* using standard protocols monthly and weekly, respectively (Environmental Canada 2000a,b). An effluent is considered out of compliance with provincial and federal guidelines when there is more than 50% mortality in a 100% effluent exposure at the conclusion of the test.

The majority of pulp and paper regulatory toxicity episodes are associated with manufacturing effluents and seldom with industrial cooling water discharges. However, a study conducted by Kovacs et al. (2002) showed that 10 of 28 P&P mills studied in Canada have experienced episodes of cooling water toxicity. Reasons for such failures were for the most part unknown, however a select few mills identified the lack of treatment, unwanted contamination and disinfection agents as possible reasons for the observed toxicity (Kovacs et al., 2002).

ABTB's Kraft Clean Water Outfall (KCWO) has intermittently failed rainbow trout toxicity tests during the Spring over the last several years. Although mill environmental managers monitor the KCWO's total suspended solids (TSS), five-day biological oxygen demand (BOD_5), pH and conductivity on a daily basis and dissolved

organic carbon (DOC) weekly, they have been unable to determine the source of these cooling water episodes. This is primarily because physical/chemical effluent monitoring methods are not suited for detecting toxicity. In order to mitigate this problem, ABTB has collaborated with our research group at Lakehead University to develop a Toxicity Early Warning System (TEWS) that uses rainbow trout fingerlings as a biomonitoring species.

Presented in this paper are results from three separate studies examining Spring toxicity episodes at the KCWO. The first study outlines the development of the TEWS at ABTB to detect possible Spring toxicity events. The second study combines the TEWS with other techniques to investigate possible causes of Spring toxicity. The third study applies chemical modeling of the KCWO as a compliment to laboratory sample analysis. The overall goal was to develop a better understanding of how Spring events might influence toxicity at the KCWO.

5.2 Background

5.2.1 Spring Flood

Spring flood is one of the most defining events in Northern latitudes especially in the boreal ecosystem (Laudon et al., 2000). It has been documented in areas of North America and Europe during the last decades (Carline et al., 1992; Davies et al., 1992). Atmospheric deposition of toxic chemicals, nutrients, and solids accumulate throughout the winter in snow from sources such as fossil fuel combustion, refuse incineration, chemical processing, metal refining and atmospheric pollution originating globally (Oberts, 1994). When snow melts, the release of the accumulated substance may have dramatic consequences for soils, surface waters and biota (Baker et al., 1996).

Surface water acidification associated with Spring flood events is a major problem for aquatic environments. Spring flood can decline the pH of surface water by anthropogenic SO_4^{2-} and NO_3^- deposition (Laundon et al., 2001). Natural sources such as the dilution of acid neutralization capacity (ANC) (Laudon et al., 2000), organic acidity (Campbell et al., 1992) and SO_4^{2-} derived from sediments or bedrock (Jansson and Ivarsson, 1994) may also lead to pH decline. The flushing of contaminants such as metals and anions in combination with acidification of ABTB's intake water (Kaministiquia River) is a potential cause of Spring KCWO non-compliance.

5.2.2 Metal Mixtures

In river systems, metals can be found in different physio-chemical forms (Salbu and Oughton, 1995). They can vary from ions and molecules to high molecular mass species such as colloids and particles (Teien et al., 2006). Metals such as nickel, aluminum, cobalt, zinc, lead and copper have been documented to have additive toxicity effects to fish (Roy and Campbell, 1995; Brotheridge et al. 1998; Wang et al., 2009). Relatively new metal mixture toxicity research associated with the Biotic Ligand Model (BLM) has investigated metal-metal interactions like that of cadmium and lead (Playle, 2004). The mechanisms of metal mixture toxicity in the environment depends on the chemistry of the individual compounds, environment-specific bioavailability, toxicological modes of action, and possible interactions among metals once bioaccumulated (Gust, 2006).

5.2.3 Aluminum

In Spring flood acidified water, metals commonly found in the environment such as aluminum (Al) are generally present in more dissolved, inorganic forms (Al_i) (Teien et

al., 2006). The chemical speciation of aluminum in natural waters is complicated because Al_i readily forms complexes with OH^-, F^-, SO_4^{2-}, organic compounds and is dependent on physical-chemical water parameters (Shuping et al., 1997).

From pH 6.8 and higher and under controlled conditions, Al is relatively insoluble (Figure 5.1). Solid forms of aluminum would be expected to begin to precipitate in this pH range, initially as unstable colloids, then as recognizable minerals, like that of Gibbsite (Gardner and Comber, 2003).

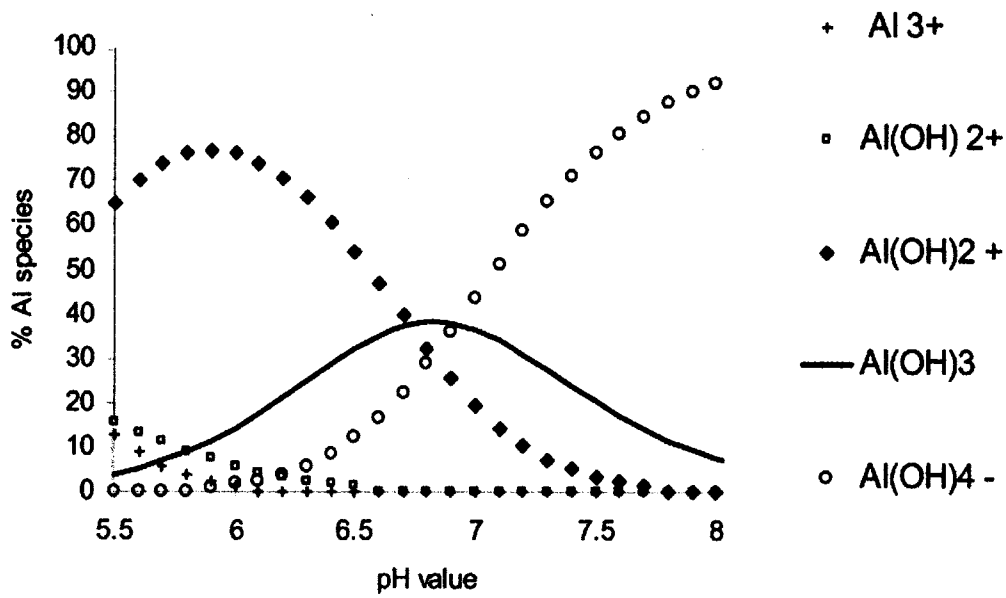


Figure 5.1 Displays typical solubility and speciation of aluminum in freshwater (Gardner and Comber, 2003)

In the presence of complexing ligands and under acidic conditions (pH<6.8), aluminum solubility is increased. At low pH values, dissolved aluminum is present mainly in the aqueous form Al^{3+} (Shuping et al. 1997). As pH rises, aluminum in fresh water undergoes a series of hydrolysis reactions producing hydroxide complexes such as $AlOH^{2+}$, $Al(OH)_2^+$ (Kwak, 1997).

Temperature also plays a major role in aluminum speciation. It has been documented that a decrease in temperature of about 15°C has the equivalent effect on Al speciation and solubility as a decrease in pH by one unit (Lydersen, 1990). Water temperature is frequently ignored in Al toxicological studies, but it has been shown that Al toxicity to Atlantic salmon, *Salmo salar* is temperature dependent (Poleo et al., 1991)

The toxicity of Al to fish in acidified waters has been well documented (Gensemer and Playle, 1999). Aluminum is a gill toxicant to fish, causing both ion-regulatory effects and respiratory disturbances due to the precipitation and polymerization of Al at the gill microenvironment. Ion-regulatory effects of Al predominate at low pH where positively charged Al species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$) bind to negatively charged sites at the gill surface (Poleo, 1995). Respiratory disturbances caused by Al may result from the accumulation of an Al-based precipitate on the gills formed by the precipitation or polymerization of Al as acidic, Al-rich water passes into the more basic gill microenvironment (Gensemer and Playle, 1999). Although aluminum toxicity to fish has been documented under various conditions, few have shown how Spring flood events may alter the aluminum speciation of industrial intake waters. It is possible that Spring flood may be responsible for non-compliance with regulatory rainbow trout toxicity requirements for industries such as ABTB.

In addition to temperature and pH, the presence of binding ligands influences the toxicity of Al to fish. In organic rich boreal waters like that of the Kaministiquia River, a common ligand is dissolved organic carbon (DOC). During Spring runoff events DOC is commonly found at higher concentrations and plays a role in decreasing pH (Serrano and

Buffam, 2008). DOC binds to Al_i, which creates less toxic forms of Al to interact at gill microenvironments (Gensemer and Playle, 1999).

Although associated with toxicity under acidic conditions, aluminum is used in numerous industrial practices. A current concern is the use of Al-base coagulants in the water treatment process (Zhang and Zhou, 2005). Aluminum sulfate ($Al_2(SO_4)_3$), commonly referred to in industry as alum and polyaluminum chloride (PAC) are coagulants that are used worldwide (Yan et al., 2008). The cooling water process at ABTB uses either alum or PAC depending on the turbidity of their intake river water to remove suspended solids, nutrients and organic matter (Zhang and Zhou, 2005). KCWO system parameters (pH, temperature and the presence of other ligands) may prevent a portion of the Al-based coagulant added to cooling water from being removed during treatment. This portion may remain as residual Al in the treated water (Zhang and Zhou, 2005). Therefore, the concentration and speciation of the Al residual left in KCWO effluent line may also be associated to Spring toxicity events.

5.3 Materials and Methods

The investigation of non-compliance incidents at the KCWO at ABTB consisted of three individual studies. The first focused on the development of the TEWS at ABTB to detect possible Spring toxicity events. Detecting toxicity was conducted by the TEWS established the final KCWO on the Kaministiquia River at ABTB from March 29 - May 5, 2008. Critical TEWS modifications had to be made from the laboratory based design in order to deal with the much higher levels of electrical noise found in the field conditions. The second study combined the TEWS with other techniques to investigate possible

causes of Spring toxicity. This study was carried out from March 1 - May 4, 2009. The third study concentrated on modeling the chemical speciation of Al at the KCWO.

5.3.1 Site Description

Monitoring, sampling and modeling for studies I, II, and III were carried out on site at ABTB (48°21'N, 89°18'W) located in Northwestern Ontario (Figure 5.2). The site location was approximately 9 km upstream from the mouth of the Kaministiquia River at its entry point into Lake Superior. The climate in this region is influenced by Lake Superior, resulting in cooler summer temperatures and warmer winter temperatures for an area extending inland as far as 16 km (Environment Canada, 2009). The daily air-temperatures for Spring 2009 ranged from a high of 16.0°C to a low of -12.3°C (Environment Canada, 2009). The surrounding geology is typical of the Canadian Shield with gently sloping hills, and thin soil lying on top of bedrock with many bare outcrops.

ABTB draws its cooling water from the Kaministiquia River immediately upstream of the mill. River water is drawn at a rate of approximately 160,000 m^3/day (A. Nicholson, private communication, June 22, 2009). KCWO effluent is discharged through a submerged diffuser and enters into the Kaministiquia River. ABTB's final KCWO characteristics vary and depend on process, production rate, operating procedures and quality of the intake river water.

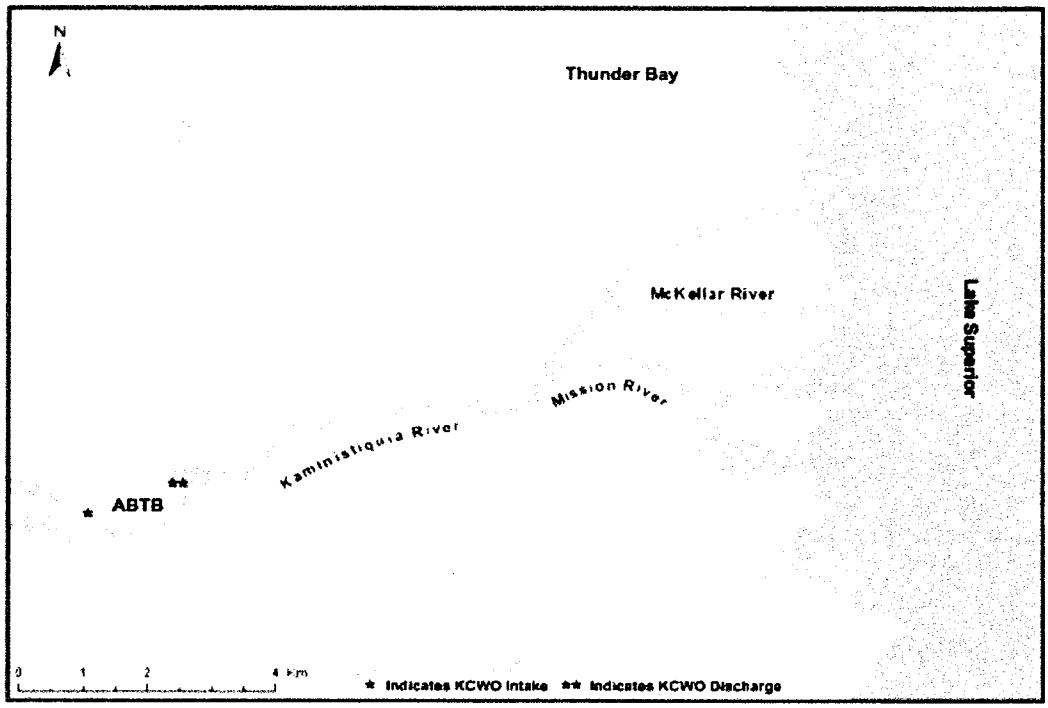


Figure 5.2 ABTB's KCWO and its location on the Kaministiquia River.

5.3.2 Detecting Toxicity using TEWS (Study I)

Aquatic Biomonitoring Systems (ABS) have been applied in-situ in North America on very few occasions (Smith and Bailey, 1988; Gruber et al., 1989; Prahacs et al. 1996; Shed et al., 2001; EPA, 2001), none of which have been established at a pulp and paper industry. Therefore, most of the on-site research in Spring of 2008 was focused on optimizing and troubleshooting the TEWS apparatus for use at the KCWO at ABTB.

TEWS Field Design

For the detection of toxicity episodes, cooling water was diverted from the final KCWO into two separate paths (Figure 5.3).

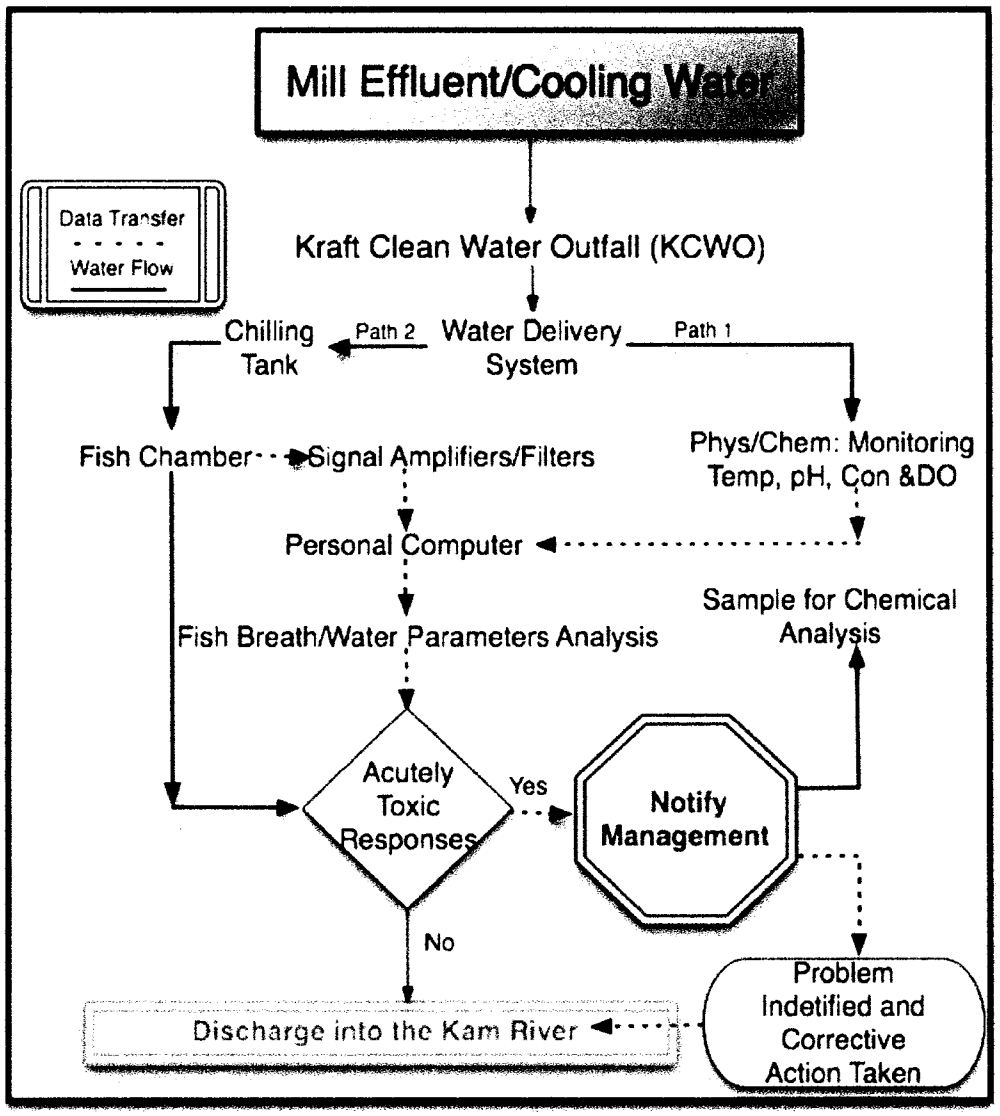


Figure 5.3 TEWS operations at ABTB. Note the solid lines indicate the flow of water and the dotted lines show the flow of electronic data transfer.

The first path flowed directly into a stainless steel sampling box (90 x 40 x 30 cm, volume ~108 L) where temperature, pH and conductivity measurements were taken by instantaneous probes and recorded by ABTB's Process Information Management (PIM) computer system.

Dissolved Oxygen (DO) was measured using an Accumet AP64 handheld DO meter to ensure that the cooling water had acceptable DO levels for fish exposures (90 to

100% saturation). DO levels were monitored prior to testing. An aeration system composed of a standard aquatic air pump and air stone was readily available in the event that the cooling waters DO level dropped below 3 mg/L. Throughout the duration of Study I, the cooling water DO did not drop lower than 8 mg/L and was not a factor in the interpretation of the monitoring results.

The second path directed cooling water to the TEWS (Figure 5.3). To satisfy the requirements for testing with rainbow trout, the temperature of the cooling water was adjusted and maintained at 15°C ± 2°C. Due to the normally large fluctuation in cooling water temperature (10°C to 45°C), a Frigidunit Model D1-100 coil chiller and cylindrical stainless steel chilling tank (volume ~118 L) were used to maintain 15±2°C (Figure 5.4). The chiller coil was made of stainless steel, and was approximately 22.5 cm in length and 11.5 cm in diameter. The chiller had an internal thermometer, programmed to the desired temperature. Once the desired temperature was reached, cooling water was released by a manual ball valve. Cooling water flowed by gravity into a peristaltic pump (Cole-Parmer Portable Sampler). The pump propelled the cooling water (200 ml/min) to the top of the TEWS testing chamber where it entered a head tank. Once in the head tank, Hoffman Swinging Jaw Clamps #299-600 controlled the flow rate (100 ml/min) into individual fish cells. Cooling water flowed into the top of individual fish testing cells, then passed through the cell, exited the top of the cell over an adjustable standpipe and then returned back into ABTB's main cooling water line. Between each test, the entire TEWS apparatus was thoroughly scrubbed and rinsed three times with dechlorinated water.

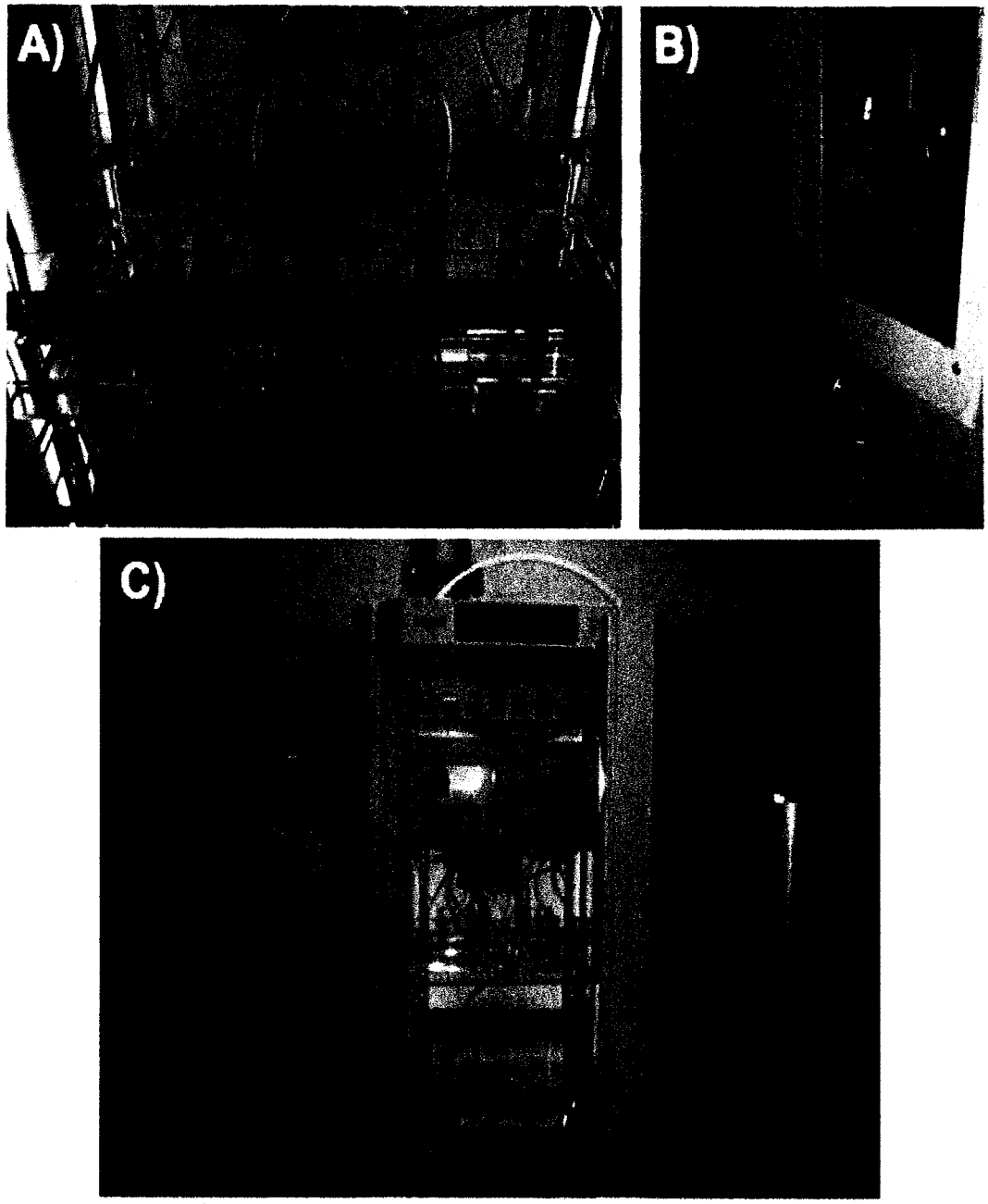


Figure 5.4 TEWS system components (A) Individual fish cells (B) ABTB physical/chemical sampling box (C) TEWS electronics, TEWS testing chamber and chiller (from left to right).

TEWS Test Procedures

Juvenile rainbow trout (2-4g) were acclimated on site directly in the TEWS chamber for a minimum of one week prior to testing. During the acclimatization period

fish were exposed to continuous but low-level light (LUX 200), fed commercial trout chow daily, and held at 15°C ±2°C. Water levels in the acclimatization tank always exceeded 1.0 L of dechlorinated water per 10 grams of fish. Fish were only fed during the acclimation period to prevent signal disruptions in testing associated with feeding.

TEWS tests (5 hours in length) were conducted every other day for the entire Spring 2008 study period. Prior to effluent exposure, a two-hour baseline test was conducted for each fish. During the baseline test, fish were exposed to dechlorinated water. Throughout the first hour of the baseline test, no measurements were recorded. This first hour period allowed the fish to become acclimatized to the test tank. During the second hour, fish ventilatory rate (measured in Hz), ventilatory depth (mean signal height measured in volts), cough rate (measure as cough/min) and body movements (measured as a percent) were recorded to a laptop using a DATAQ Di-720 instrument. Baseline data analysis consisted of randomly sampling ten, thirty-second data sets for ventilatory rate and depth and ten, one-minute data sets for cough rate and whole body movements

After the baseline test, fish were exposed to full strength cooling water for 3 hours. This time length was chosen as van der Schalie et al. (2004) showed that bluegill ventilatory and movement responses were found to occur within approximately 1h for 12 of 15 tested chemicals at concentrations equal to or less than half the 96-h LC_{50}. A LC_{50} value is defined as the Lethal Concentration that would kill 50% of the test organisms in a given time period.

Ventilatory parameters of exposed fish were recorded as described above. Fish with extremely low ventilatory rates (<1 breath per second) or depths (< 0.01 V) were considered to be severely stressed or dead. An absolute measurement of time-of-death is

not possible because very low levels of electrical noise are present in the system. Cooling water data analysis consisted of randomly sampling thirty, thirty-second data sets for ventilatory rate and depth and thirty, one-minute data sets for cough rate and whole body movements.

To identify changes produced by the cooling water exposure, behavioural parameters from both exposures were analyzed, averaged and statistically compared using a two-tailed T-Test. Unequal variance of the data was assumed and behavioural responses were deemed significant when $p< 0.05$ and very significant when $p<0.01$. By this approach an individual fish serves as its own baseline control (Kane et al., 2004; ASTM, 2003). Final results were expressed as percent change from the baseline value.

Metal Analysis

Total metal concentrations in the cooling water were assessed during TEWS events in 2008 study. Samples were acid digested, in which HCl and HNO_3 (3:1) were added to a 50ml sample. Each sample was placed in a glass tube, sealed, heated on a hotplate for 3-4 hours, mixed thoroughly and examined using inductively coupled plasma (ICP).

Supporting Data

A record log of all chemicals used in the Kraft cooling system was established in order to determine when chemical additions were at highs, lows and if such additions might be linked to TEWS events. Included in the chemical log was the addition of

coagulation aids, alum/PAC and the addition and concentration of Cl_2 in the cooling water.

Mill operational parameters such as intake river temperature, BOD and KCWO flow rate were collected from the PIM database. Surrounding area precipitation and snow cover data for the 2008 study period was obtained from Environment Canada (2009).

5.3.3 Investigating Causes of Spring Toxicity (Study II)

ABS like the TEWS is a powerful tool for detecting early warning signs of toxicity. However, the information to be drawn from it is limited. The TEWS cannot determine causes of toxicity or specific chemical concentrations. As a result, the 2009 study combined the TEWS with a wide array of supporting techniques to provide ABTB with a more complete analysis of potential causes of toxicity.

TEWS Test Procedures

The TEWS was the main component used to detect acute levels of toxicity at the KCWO. TEWS test procedures were conducted as described above with the following modifications. Due to the high sedimentation load associated with Spring flood, the cooling water entering the TEWS testing chamber was filtered. Although filtration of a sample could reduce toxicity, not filtering the cooling water would have led to clogged system lines and false TEWS warning. Therefore, Nitex bolting cloth with a pore size of 100 microns was put on the cooling water line entering the TEWS testing chamber and was cleaned as needed. If a TEWS event was detected, 150 L of effluent was immediately collected and brought back to the lab for phase 1 Toxicity Identification Evaluation (TIE).

Kraft Cooling System Sample Sites

The Kraft cooling system at ABTB is isolated from all other mill processes (A. Nicholson, private communication, May 20, 2009). In order to determine how the KCWO discharge becomes toxic to rainbow trout over the course of the Spring, five distinct sites were examined in the cooling water system over a 65-day period. The five sites selected were: (A) Kaministiquia River, (B) Raw Water, (C) Graver Treated, (D) Chemical Plant and (E) the Final KCWO (Figure 5.5). The cooling system intakes water from upstream of the Kaministiquia River, where it is chlorinated with elemental chlorine and flows into one of two paths. The first path flows into the graver where Al-based coagulants (alum or PAC) are added to remove impurities. The path then enters the Kraft mill where it is used as non-contact cooling water. The second path travels to a neighboring independent industrial company Erco Worldwide, which also uses the river water for non-contact cooling. Under normal operating conditions a portion of the second path flows into the Kraft mill at ABTB for non-contact cooling water, however this line was shut off during the 2009 study period. Finally, both paths combine prior to the final KCWO and are discharged downstream into the Kaministiquia River.

Sampling Procedures

Each site was sampled at the same time, on a weekly basis from March 1 to May 4, 2009. The sampling technique used for Site A varied over the duration of the study due to the melting of the Kaministiquia River. During the first half of the study, Site A was sampled by cutting a hole in the ice approximately 1 m deep with a gas powered ice auger. During the second half of the study, when the ice was thin or absent, samples were

taken from just off the banks of the river. The remaining four sites were sampled directly from cooling water system lines. A minimum of 25 L was collected from each study site, with typically larger samples taken from the final KCWO (225L). Chemical analysis and standard toxicology tests were conducted on each sample.

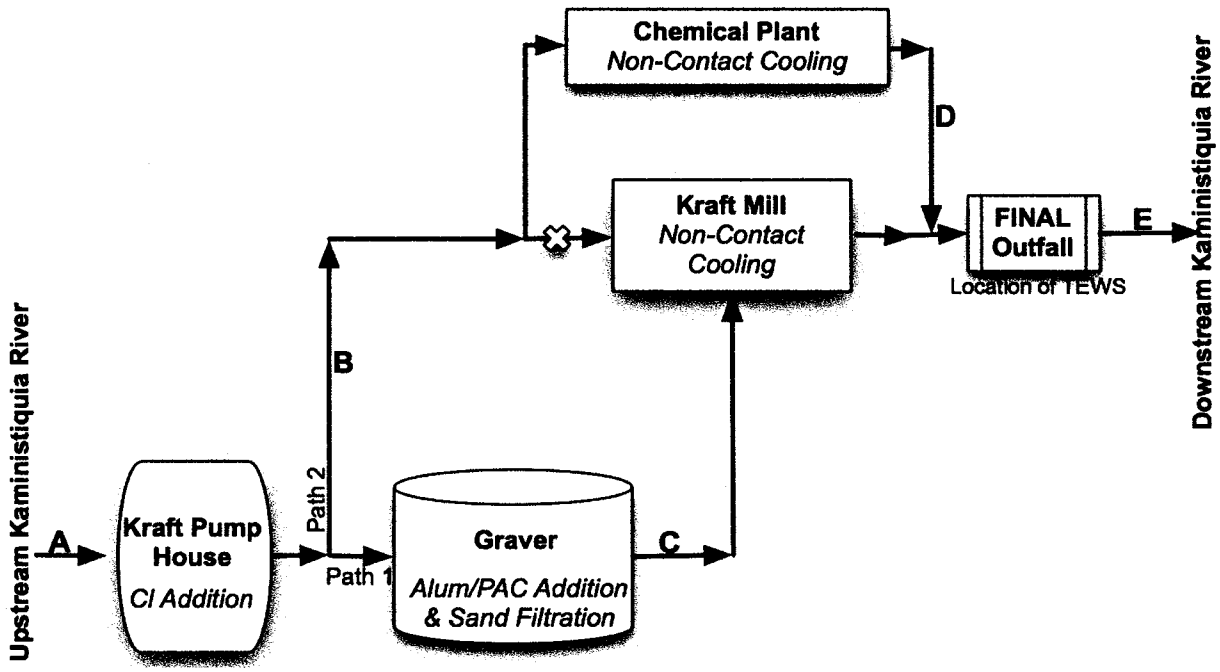


Figure 5.5 Shows the five sampling sites and flow direction of the KCWO system. The X indicates a closed effluent line during the 2009 sample period.

Chemical Analysis

Chemical analysis of samples consisted of the following: pH, total alkalinity, conductivity, dissolved organic carbon (DOC), metal concentrations and inorganic anion concentrations.

Alkalinity and pH were measured using a Mettler DL53 Automatic Titrator. The conductivity was determined using the Accumet XL60 Multimeter System. DOC was

assessed for site A and E every two weeks by a Skalar SAN^{plus} System using persulfate UV digestion with colorimetry measurements. Inorganic anions (chloride, nitrate and sulphate) were measured using a Dionex DX-120 ion chromatograph (IC).

Total metal concentrations were measured as described above. Dissolved metal concentrations were measured following the filtration of each sample using a 0.45-micron filter. Metals associated with particulates were found by determining the difference between total and dissolved metal concentrations (Teien et al., 2006). Fish gill tissue was assessed for total metal concentrations using ICP. A total of 0.5 g of wet gill weight was extracted from 20 cooling water and 10 dechlorinated water exposed fish, put in a glass vial, dissolved in 10 ml of concentrated HCl and HNO_3, diluted with DDW and analyzed using ICP.

Toxicity Analysis

Acute lethal toxicity to rainbow trout was assessed using a pass/fail toxicity test design based on the EPS1/RM/13 protocol by Environment Canada. In summary, ten fish were placed in a lined bucket containing either dechlorinated water as a control or 100% sample for a period of 96 hours. Each solution was aerated for 30 minutes prior to the addition of fish and was continually aerated for the duration of the test. Toxicity tests were performed on all samples the day the samples were taken. Each test was assessed for mortality every 3 hours, in order to allow for the prompt initiation of TIE testing upon the detection of toxicity.

When an adequate amount of effluent was obtained from a toxicity episode, a phase 1 TIE analysis was carried out. The goal of a TIE test was to identify the toxic

component(s) of the cooling water. TIE analysis was based on USEPA (1991b), but was adapted for cooling water. Four TIE manipulations aimed at studying metals, chlorine, sample aging and volatiles were conducted (Figure 5.6). Approximately 80 mg/L EDTA (10% of LC_{50}) was added to toxic samples to chelate metals. Sodium thiosulphate ($Na_2S_2O_3$) at a concentration of 6.6 g/L (29% of LC_{50}) was added to toxic samples to neutralize oxidants (such as chlorine) and other chemicals used in water disinfection (such as chlorine dioxide), chemicals formed during chlorination (such as mono and dichloramines), bromide, iodine, and some electophile organic chemicals. Samples were aged for a week prior to testing to determine if toxicity was linked to Al polymerization/ precipitation reactions rapidly occurring in the sample. Vigorous aeration was conducted one hour prior to testing in order to sparge volatiles components from the sample, thereby reducing toxicity. Following each manipulation, a pass/fail toxicity test was conducted as describes above, to evaluate whether or not the manipulation altered the toxicity of the sample.

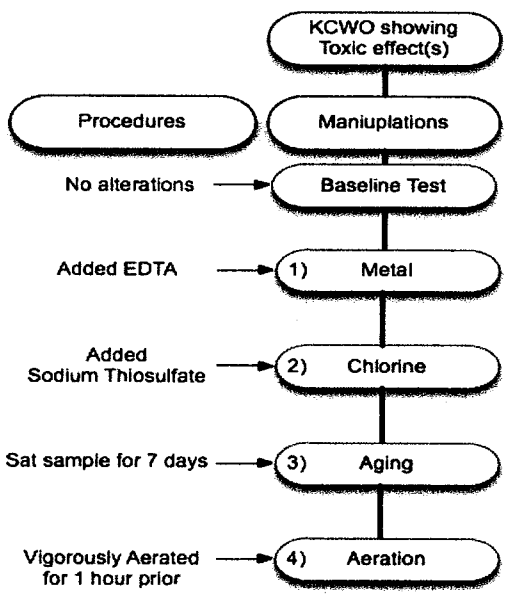


Figure 5.6 Displays TIE manipulations and procedures.

Scanning Electron Microscope (SEM)

Precipitates formed during toxicity tests were analyzed using SEM to determine if they were aluminum based. A JEOL JSM-5900 LV SEM with Control User Interface Version 5.27 was used for all imaging. The X-ray analysis used a back scatter electron (BSE) detector, which detects the primary electrons deflected off the nuclei. These electrons produce a spectrum containing an atomic number contrast. Elements with high atomic numbers show up as bright white spots on the SEM images, whereas elements with low atomic numbers, such as carbon, appear as black.

5.3.4 Aluminum Modeling (Study III)

Chemical equilibrium modeling can predict the composition and stability of solutions. Chemical equilibrium models can be employed to calculate concentrations of different components, such as soluble or insoluble forms of compounds at equilibrium, based on the initial conditions and knowledge of the chemical reactions involved. Modeling the chemical speciation of aluminum at the KCWO was carried out using Visual MINTEQ version 2.53. The chemical modeling conducted in this study was broken into two sections: comparing aluminum speciation of toxic samples to non-toxic samples and altering physical-chemical parameters of toxic samples.

Comparing Events

Aluminum concentration, supporting element concentrations, anion concentrations and chemical parameters found at Site E on the April 15, 2009 rainbow trout toxicity event were inputted into Visual MINTEQ. Aluminum speciation modeling was conducted for this event and compared to the aluminum speciation of a non-toxic

sample from Site E (April 20, 2009). Table 5.1 presents the chemical compositions inputted into Visual MINTEQ. The modeling assumes that humic substances consist of humic and fulvic acids. As fulvic acids are more mobile, they are assumed to make up the majority of dissolved organic matter. Furthermore, in all simulations the system was open to the atmosphere and oversaturated solids were allowed to precipitate.

Altering Parameters

The chemical speciation and toxicity of aluminum is dependent on pH, temperature and binding ligands (Shuping et al., 1997). Therefore, the temperature of the toxicity event at Site E (April 15, 2009) was altered to 15°C (temperature maintained during regulated toxicity tests) and modeled for aluminum speciation. Furthermore, temperature was reduced to 0°C and DOC was removed from the model in order to show how Al speciation would be affected.

Table 5.1 Summary of chemical compositions inputted into Visual MINTEQ.

Chemical Parameter All (mg/L) except Temp (°C) and pH	4/15/09 Site E Event	4/20/09 Site E Non-Event
DOC	4.3	9.9
Cl	12.28	12.86
NO_3	0.285	0.207
Al	0.994	0.771
As	0.006	<DL
Ba	0.022	0.019
Ca	15.349	11.535
Cu	<DL	0.003
Fe	0.664	1.097
K	1.83	1.65
Mg	4.90	3.49
Mn	0.0622	0.0715
Na	5.99	5.47
Ni	0.003	0.003
S	9.74	1.61
Zn	0.004	0.033
PH	6.615	6.732
Temp.	24.2	28.46

5.4 Results and Discussion

5.4.1 Study I: Detecting Toxicity at the KCWO in 2008

ABTB traditional KCWO monitoring methods have shown little success in detecting Spring toxicity. Development of a sensitive biomonitoring system at ABTB may allow for toxicity events to be detected early and thus, further examined.

Two toxicity events were detected at the KCWO during the 2008 study period (March 29- May 4). Event 1 (April 4-8) was supported by TEWS response and a failing acute rainbow trout toxicity test. Event 2 (April 26-28) was only supported by the TEWS and therefore may not be truly representative of KCWO toxicity. In addition to detecting toxicity using the TEWS, this study also examined KCWO physical/chemical trends and specific metal concentrations that were found during Event 1.

TEWS Response

Event 1 occurred April 4-8, in which TEWS first indicated abnormal fish behaviour. Significant TEWS responses ($p<0.01$) led to the early warning on April 4 (Figure 5.7). These responses include: (A) an increase in ventilation rate (47.1%), (B) a decrease in ventilation depth (-21.3%), (C) an increase in coughing rate (350%) and (D) a decrease in whole body movements (-42.6%). TEWS fish response values and p-values are presented in Table 5.2. Event 1 was confirmed by a failing acute rainbow trout toxicity test conducted on April 7 (LC_{50} =19.018%).

Event 2 was reported by TEWS on April 26-28, however an acute rainbow trout toxicity test was not conducted at this time. Significant TEWS responses ($p<0.01$) led to early warning on April 26 (Figure 5.7). These responses included: (A) a decreased ventilation rate (-26.1%), (B) a decreased ventilation depth (-14.7%) and (C) decreased whole body movements (-24.4%). Cough rate was not significantly different on April 26, however on April 28 cough rate significantly differed by increasing 300%. TEWS fish response values and p-values are presented in Table 5.3.

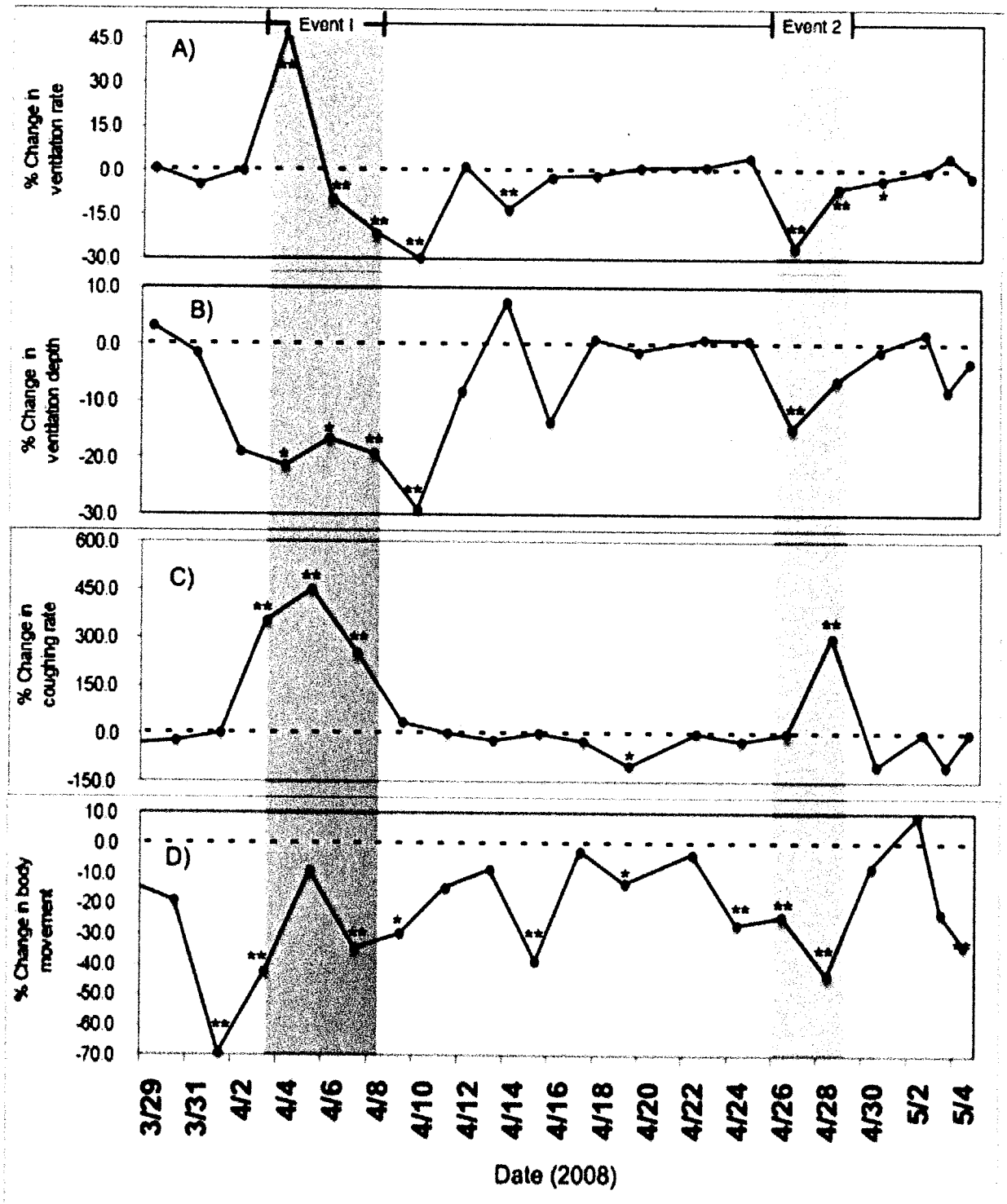


Figure 5.7 Spring 2008 TEWS behavioural responses. Expressed as percent change from baseline exposure. The red (April 4-8 event) indicates when toxicity occurred at the KCWO and blue (April 26-28 event) indicates a possible toxicity event. Significant percent change from baseline is indicated by * and very significant is indicated by **. (A) change in ventilation rate, (B) change in ventilation depth, (C) change in coughing rate, (D) change in body movement.

Table 5.2 Summary of TEWS responses and p-values for Event 1 (April 4-8)

(n=10)	Baseline	KCWO Effluent	P-Value
April 4, 2008			
Ventilation Depth (V)	0.027 ± 0.007	0.021 ± 0.006	0.037*
Ventilation Rate (Hz)	2.12 ± 0.34	3.12 ± 0.40	1.6E-06**
Cough Rate (cough/min)	2.1 ± 0.99	9.4 ± 0.84	1.3E-12**
Body Movements (%)	59.38 ± 9.68	16.75 ± 4.22	2E-08**
April 6, 2008			
Ventilation Depth (V)	0.031 ± 0.009	0.026 ± 0.004	0.019*
Ventilation Rate (Hz)	2.70 ± 0.09	2.44 ± 0.03	1.7E-06**
Cough Rate (cough/min)	2.5 ± 0.71	13.7 ± 1.34	2E-12**
Body Movements (%)	60.34 ± 13.31	50.79 ± 9.91	0.087
April 8, 2008			
Ventilation Depth (V)	0.032 ± 0.009	0.021 ± 0.005	0.003**
Ventilation Rate (Hz)	1.71 ± 0.11	1.34 ± 0.06	2.1E-07**
Cough Rate (cough/min)	3.1 ± 0.57	10.7 ± 0.823	5.7E-14**
Body Movements (%)	50.17 ± 23.86	22.07 ± 4.88	0.005**

Significant ($p<0.05$) indicated by *, Very Significant ($p<0.01$) indicated by **

Table 5.3 Summary of TEWS responses and p-values for Event 2 (April 26-28)

(n=10)	Baseline	KCWO Effluent	P-Value
April 26, 2008			
Ventilation Depth (V)	0.020 ± 0.002	0.017 ± 0.002	0.001**
Ventilation Rate (Hz)	3.51 ± 0.12	2.59 ± 0.06	1.8E-11*
Cough Rate (cough/min)	3.0 ± 0.82	3.0 ± 0.82	1
Body Movements (%)	35.81 ± 7.74	11.46 ± 3.45	8E-07*
April 28, 2008			
Ventilation Depth (V)	0.019 ± 0.003	0.018 ± 0.002	0.623
Ventilation Rate (Hz)	2.48 ± 0.05	2.33 ± 0.05	1.9E-06**
Cough Rate (cough/min)	2.0 ± 0.67	8.0 ± 0.82	1.2E-12**
Body Movements (%)	68.75 ± 11.75	25.14 ± 4.4	2E-07**

Significant ($p<0.05$) indicated by *, Very Significant ($p<0.01$) indicated by **

The detection of Event 1 by the TEWS showed that rainbow trout behaviour parameters could be used as indicators for early detection of developing toxic conditions at the KCWO. Multiple fish behaviour parameters indicated toxicity in Event 1. This is supported by the finding of van der Schalie et al. (2004) who showed that a combination of bluegill ventilatory parameters indicated first alarms for contaminants such as cyanide (increase in ventilatory rate, decrease in ventilatory depth and increase in cough rate) and

tricaine methane sulfonate (increase in ventilatory rate, increase in ventilatory depth and increase in movement).

Of the four-parameter responding to Event 1, ventilatory rate and cough rate showed the highest significant deviation from the baseline. Walden et al. (1970) also found rainbow trout cough frequency was the most significantly altered behavioural parameter when Kraft pulp mill effluent concentration approached lethal levels.

During the 2008 study period, fish body movements were lower during the cooling water exposure. On average, body movements were reduced by 40.3%. A possible reason for decreased movements could be that fish were more acclimatized to the TEWS testing cell after the two-hour baseline.

The detected events established by the TEWS, show its potential as a toxicity indicator at the KCWO. In order to further understand why such events occurred, physical and chemical parameters associated with the KCWO were investigated.

Physical and Chemical Trends

Over the Spring 2008 study period, physical/chemical KCWO parameters fluctuated. Fluctuations corresponded with moderate to heavy precipitation and increasing air temperature, which led to the ice on the Kaministiquia River to melt. Relationships among parameters were graphed against the 2008 study period (Figure 5.8).

An inverse relationship was found between flow rate of the KCWO system and conductivity. The conductivity reached a maximum of 188.9 μS on April 16, in which the flow rate was found to be at a near minimum of 1576.7 GPM. When examining KCWO

intake river temperature it was seen that intake river water began to drop in temperature (3.2°C-0.2°C) between April 16 and April 18, indicating that the ice covering the river began to melt and mix with the underlying water causing the temperature to drop. Before the river-ice break up, pH of the KCWO was relatively low (ranging from 6.1-6.8). When the river-ice break up occurred (April 16 to April 18) the pH of the KCWO dropped from 6.8 to 6.5 then rose and stayed above 7.1 for the remained of the study period (April 19 to May 5).

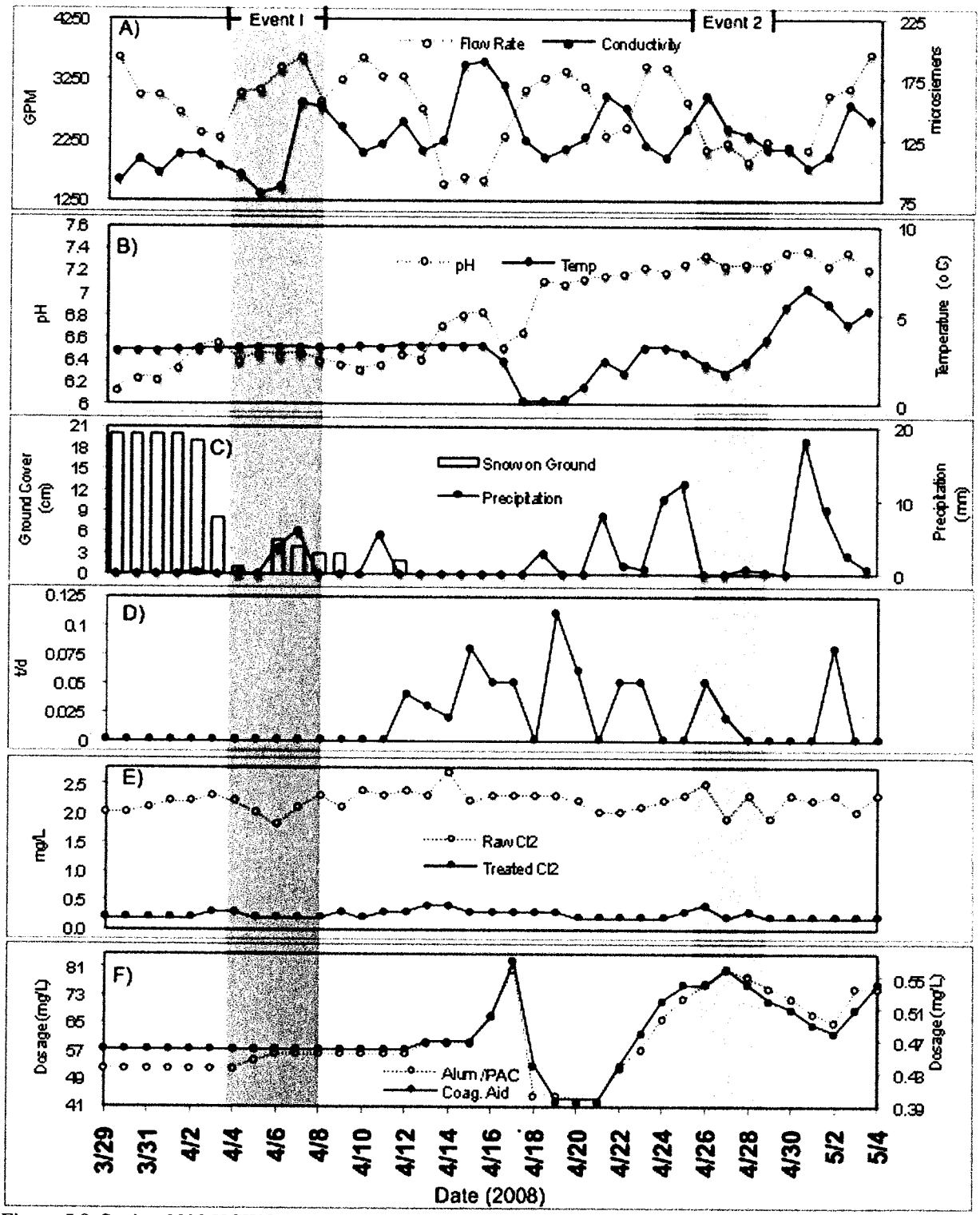


Figure 5.8 Spring 2008 KCWO physical/chemical fluctuations. The red (April 4-8 Event) indicates when toxicity occurred at the KCWO and blue (April 26-28 Event) indicates a possible toxicity event. (A) KCWO flow rate and KCWO conductivity, (B) KCWO pH and KCWO river intake temperature, (C) Local snow cover and precipitation, (D) KCWO Biological Oxygen Demand (BOD), (E) Raw (Site B) Cl_2 and treated (Site C) Cl_2, (F) Aluminum sulfate and coagulation aid additions.

When comparing area snow cover and precipitation, it was seen that during the time the river-ice broke up there was no snow covering the ice. The first major rain event (5.9 mm) on April 7, 2008 melted the majority of the snow cover. Precipitation events later in the Spring study period hit a peak of 18 mm on May 2. BOD was below the detection limit during the first half of the study period (March 29- April 11), however BOD was significantly higher during the second half of the study (April 12- May 4) reaching a maximum of 0.11 t/d on April 19.

When comparing Raw (Site B) Cl_2 to Treated (Site C) Cl_2 concentrations over the study period it was found that Cl_2 in the Raw line (1.8-2.7 mg/L) and Treated line (0.2-0.4 mg/L) were both fairly constant. The Raw was always significantly higher than the Treated. This is likely because the raw line is directly after chlorination and the treated goes through the coagulation process, which would reduce Cl_2 concentrations.

Examining the relationship between the alum/PAC addition and coagulation aid showed that both were proportionately added over the entire study period. The mill switched from alum to PAC on April 17. Alum concentrations were highest (67 mg/L) on April 16 and PAC additions hit a high April 17 and April 27 (80 mg/L). The change from alum to PAC was most likely due to the river-ice break up occurring April 16-18. This natural event would potentially increase the turbidity of the river, therefore, requiring a stronger coagulant such as PAC. It is important to note that TEWS Event 1 occurred while the mill was using alum as a coagulant.

In order to determine the potential conditions in which Spring toxicity occurred, physical/chemical parameters were measured for the April 7 KCWO detected toxicity event (Table 5.4). Flow rate, conductivity and precipitation were found to be high during

the event. All other parameters are within one standard deviation of the mean taken from the entire study period.

During Event 1, both the conductivity and the flow rate of the KCWO system were high. Such results contradict the relationship found throughout the 2008 study period. It was first suspected that increased flow rate of the KCWO was linked to Spring melt events, however ABTB environmental managers clarified that KCWO flow rate is regulated by ambient air temperature (C. Walton, private communication, June 29, 2009). Therefore, as air temperature rises in the Spring, so does the KCWO flow rate. The increased conductivity may be related to the snow melting, which would be accelerated by the precipitation (5.9mm) on April 7. This would ultimately depend on the ion concentrations found in the snow. A full metal scan was conducted for the April 7 KCWO toxicity event to determine if increased conductivity was related to metal concentrations coming from Spring runoff. The results are presented below.

Table 5.4 Summary of all KCWO parameters during April 7, 2008 toxicity event.

Parameter	**April 7**	**Study Mean ± ST DEV**
KCWO Flow Rate (GPM)	**3605.3**	**2815.17± 824.9**
KCWO Conductivity (µS)	**182**	**112 ± 29.1**
River Intake Temperature (°C)	3.1	2.73± 1.06
KCWO Temperature (°C)	13.1	18.4± 7.9
KCWO pH	6.86	6.61 ± 0.45
Precipitation (mm)	**5.9**	**1.57 ± 3.5**
BOD (t/d)	0	0.0097 ± 0.02
Raw Cl_2 (mg/L)	2.1	2.2 ± 0.26
Treated Cl_2 (mg/L)	0.2	0.3 ± 0.1
Alum dosage (mg/L)	56	52.78 ± 3.7
Coagulation Aid addition (mg/L)	0.46	0.47 ± 0.04
TEWS Response (YES/No)	YES	N/A
Rainbow Trout LC_{50} Value	19.018	N/A
Daphnia LC_{50} Value	Non toxic	N/A
Occurred Before or After River-ice breakup?	Before	N/A
Hardness (mg/L $CaCO_3$)	54.5	N/A
Ammonia (mg/L N)	0.083	N/A

Metal Concentrations

Samples were collected from the KCWO on April 4, 6, 7, 11 and June 2 and assessed for metals using ICP (Figure 5.9). It was found that metal concentrations (Cr, Cu, As, Ti, Mn, Al and Fe) were higher on April 4, 6, 7 (Event 1), when compared to April 11 and June 2 (Non-event). More specifically, Event 1 showed total Al concentrations in the range of 0.713-1.132 mg/L, whereas April 11 and June 2 showed lower total Al concentrations of 0.407 mg/L and 0.1108 respectively. Metals such as Arsenic (6 μg/L), chromium (3 μg/L), titanium (11 μg/L) and copper (9 μg/L) were present during Event 1 in low concentrations. These metals are below detection limits (As = 5 μg/L, Cr = 2 μg/L, Ti = 5 μg/L) during April 11 and June 2, except for extremely low levels of copper (1 μg/L) on June 2. Hardness varied slightly among all samples (45-59 mg/L $CaCO_3$), however cooling water may be classified as soft.

Analysis and comparison of metal concentrations during Event 1 to non-events and the Canadian water quality guidelines for the protection of freshwater aquatic organisms (Table 5.5) indicates that metal concentrations from Spring runoff may be a source of the increased KCWO conductivity and toxicity. However, more data is needed before such increased metal concentrations can be linked directly to rainbow trout toxicity events.

Table 5.5 Canadian metal guidelines for the protection of freshwater aquatic life (CCREM, 2007).

Metal	Concentration (μg/L)	Date Implemented
Trivalent chromium (Cr (III))	8.9	1997
Hexavalent chromium (Cr (VI))	1.0	1997
Copper	2.0-4.0 (hardness dependent)	1987
Arsenic	5.0	1997
Aluminum	5-100 (pH dependent)	1987
Iron	300	1987

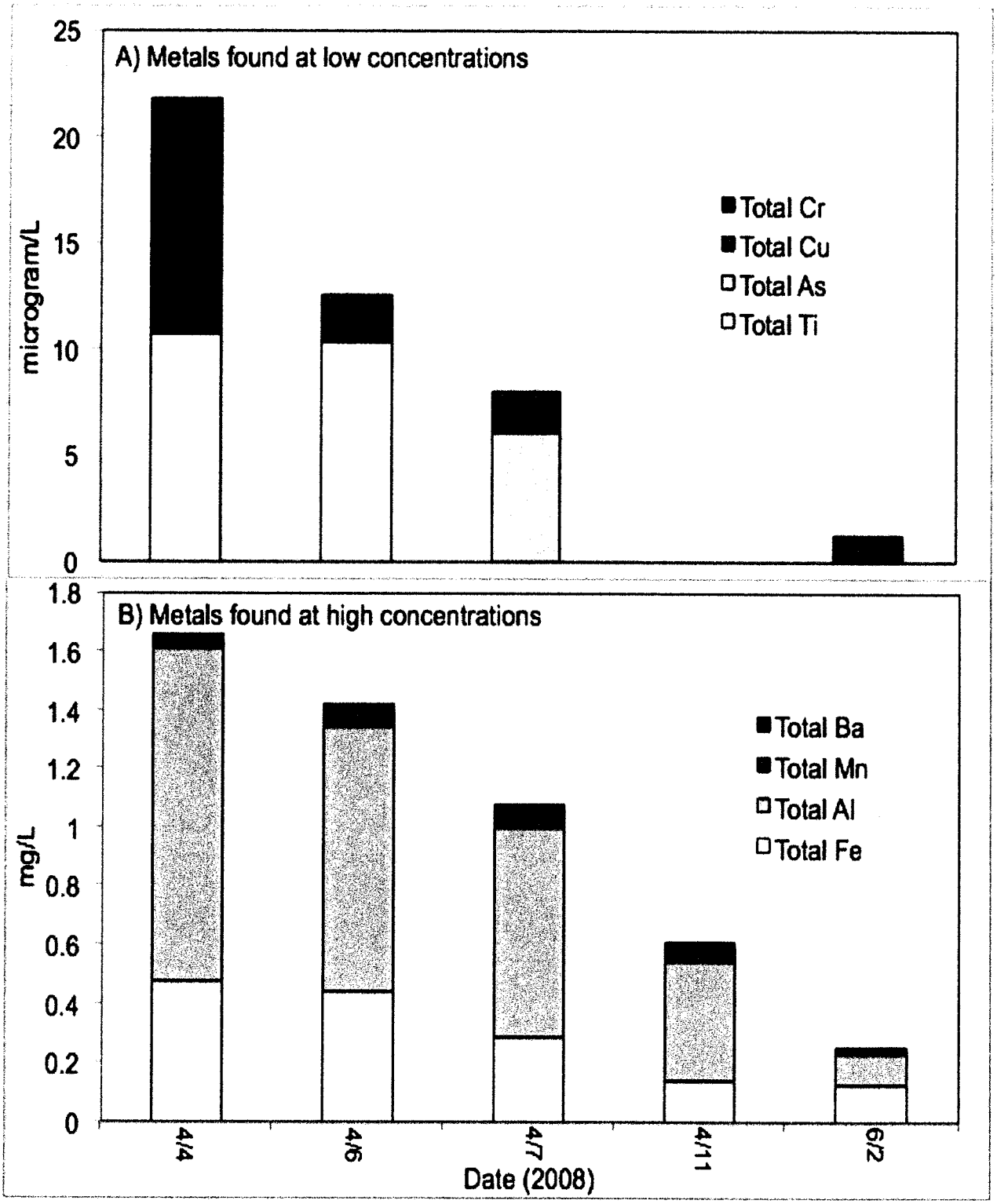


Figure 5.9 Total metal concentrations found at the KCWO during Event 1 (April 4-10) and after Spring runoff period. (A) Metals found at low concentrations (μg/L), (B) Metals found at high concentrations (mg/L).

5.4.2 Study II: Investigating Causes of Spring Toxicity in 2009

Although detecting toxicity events at the final KCWO (Site E) is important, understanding the source of the Spring toxicity is the ultimate goal. Understanding the cause of Spring toxicity will allow ABTB to design and implement system changes aimed at preventing this intermittent toxicity from occurring. One toxicity event was detected at Site E using the TEWS during the 2009 study period (March 1 to May 4). This event occurred on April 15 and was confirmed by a pass/fail rainbow trout toxicity test, in which 100 % fish mortality was seen within the first 12 hours of effluent exposure. Once toxicity was detected, the failing effluent was assessed using TIE methods (as described above). In addition to analyzing Site E (Final KCWO), four other sample sites: Kaministiquia River (Site A), Raw Water (Site B), Graver Treated (Site C) and the Chemical Plant (Site D) were analyzed for toxicity, inorganic anions and metal concentrations in order to determine where the source of the toxicity originates.

TEWS Fish Behaviour

An event was reported by TEWS on April 15, and confirmed by a acute rainbow trout toxicity test. Significant TEWS responses ($p < 0.01$) led to early warning of toxicity on April 15 (Figure 5.10). These responses include: (A) an increase in ventilation rate (21.2%), (B) a decrease in ventilation depth (-39.4%) and (C) an increase in coughing rate (176.5%). A decrease in whole body movements (D) (-42.6%) was noted, but did not significantly differ from the baseline. TEWS fish response values and p-values are presented in Table 5.6.

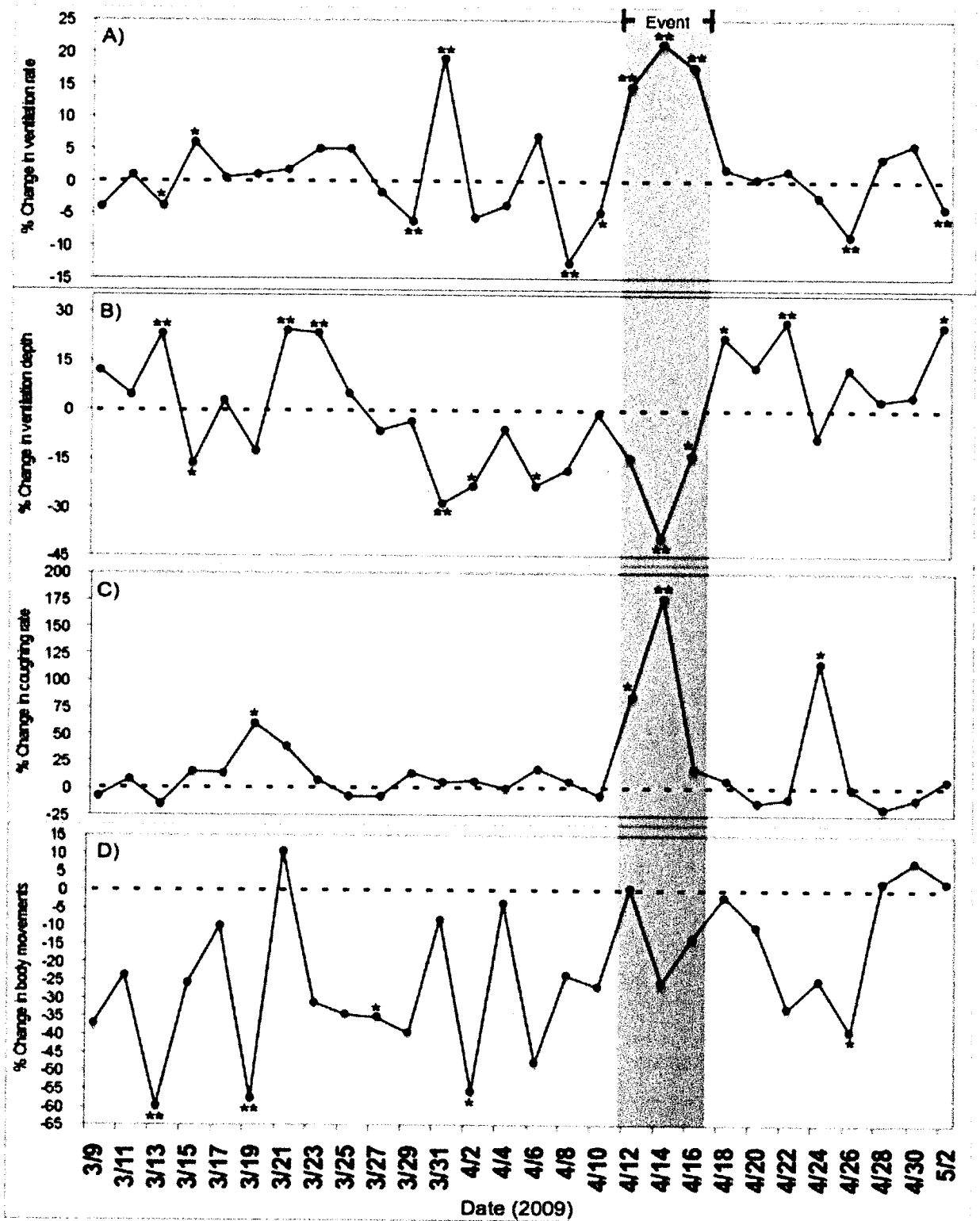


Figure 5.10 Spring 2009 TEWS behavioural responses. The red (April 15 Event) indicates when toxicity occurred at the KCWO. Significant percent change from baseline is indicates by * and very significant indicated by **. (A) change in ventilation rate, (B) change in ventilation depth, (C) change in coughing rate, (D) change in body movement.

Table 5.6 Summary of TEWS responses and p-values for Spring Event (April 15)

(n=10)	Baseline	KCWO Effluent	P-Value
April 15, 2009			
Ventilation Depth (V)	0.065 ± 0.008	0.039 ± 0.003	1.1E-06**
Ventilation Rate (Hz)	2.010 ± 0.125	2.44 ± 0.143	1.3E-06**
Cough Rate (cough/min)	1.70 ±0.48	4.70 ±1.34	3.1E-05**
Body Movements (%)	22.71 ± 7.82	16.91 ± 9.76	0.160

Significant ($p<0.05$) indicated by *, Very Significant ($p<0.01$) indicated by **

The TEWS accurately detected toxicity events in 2009 as it did in 2008. Similar to results from the 2008 study, multiple rainbow trout parameters (ventilation depth, ventilation rate and cough rate) were indicative of toxicity. Fish body movements were seen to be lower (-23.04) during all effluent exposures. KCWO physical and chemical parameters were recorded in order to support the detected TEWS event.

Establishing Physical/Chemical Relationships

Several relationships were determined for KCWO physical/chemical parameters over the study period (Figure 5.11). Similar to the previous study, an inverse relationship was found between KCWO flow rate and conductivity. The conductivity reached a maximum of 139.7 μS on April 9, when flow rate was found to be at a near low of 902.8 GPM.

The Kraft cooling system intake river temperature first began to fluctuate on April 14, indicating that the river ice began to melt and mix with underlying water. Before the river ice breakup, the KCWO pH was noted to be neutral (average of 7). During the break up the KCWO dropped from 7.4 to a low of 6.8. After the break up the pH of the KCWO was seen to be higher, having an average pH of 7.3.

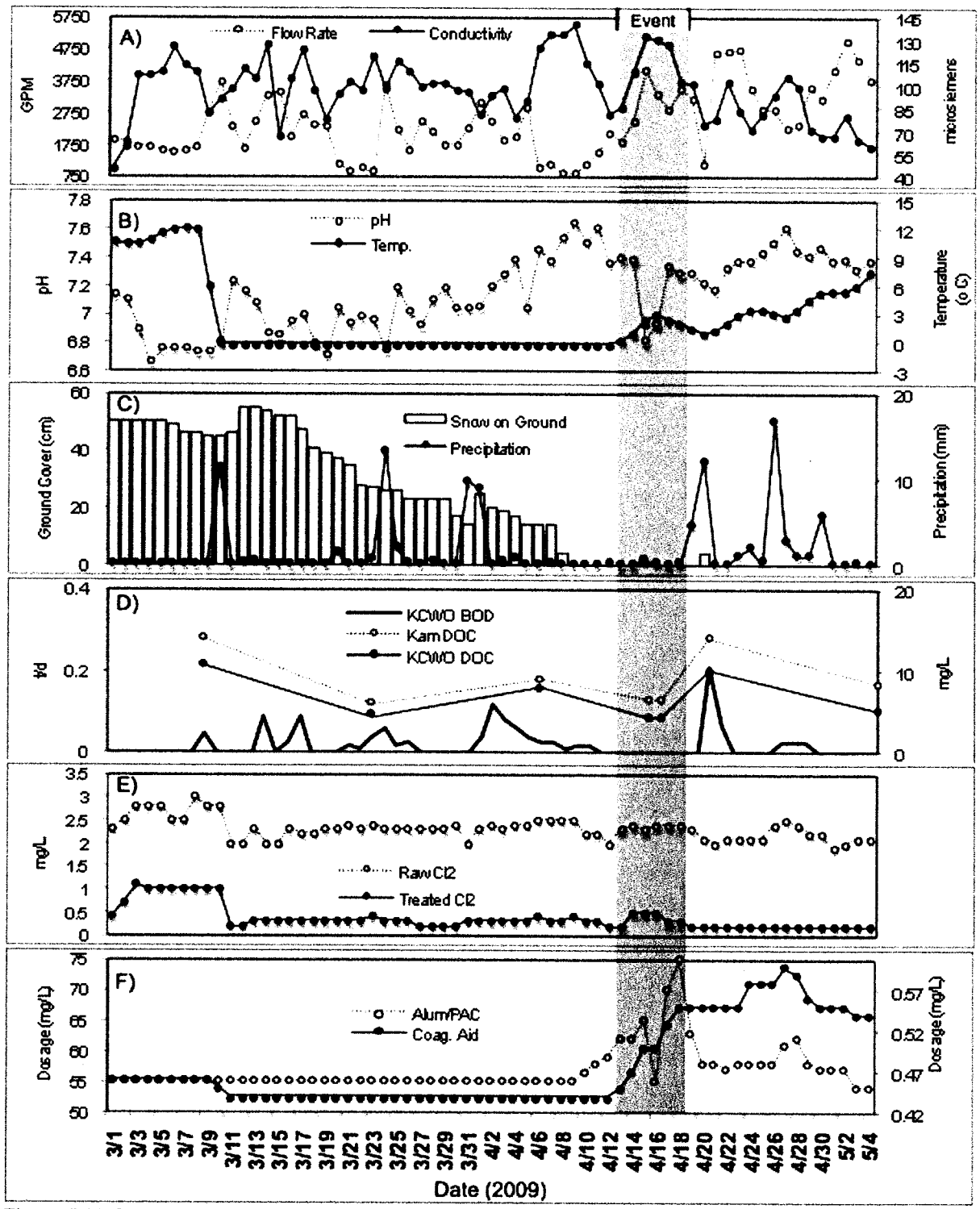


Figure 5.11 Spring 2009 KCWO physical/chemical fluctuations. The red (April 15 Event) indicates when toxicity occurred at the KCWO: (A) KCWO flow rate and KCWO conductivity, (B) KCWO pH and KCWO river intake temperature, (C) Local snow cover and precipitation, (D) KCWO Biological Oxygen Demand (BOD), KCWO Dissolved Organic Carbon (DOC) and Kaministiquia River DOC, (E) Raw (Site B) Cl_2 and Treated (Site C) Cl_2, (F) Aluminum sulfate/PAC and coagulation aid additions.

An early rain event of 13.3 mm on March 23 led to the melting of snow. Similar to the 2008 study, there was no snow cover during the river-ice break up. Precipitation reached a maximum value of 16.8 mm on April 26 and had an average of 1.5 mm throughout the study period.

Unlike the 2008 study, BOD was detected throughout the entire 2009 period. BOD values were found to be highest (0.21 t/d) on April 20. The Kaministiquia River DOC was found to be in the range of 5.9 - 14 mg/L and were higher than DOC at the KCWO (4.3 – 10.7 mg/L) at all sampling intervals. In the cooling water system, cooling water goes through the coagulation process in the graver before reaching the KCWO, thus reducing the DOC.

The concentrations of Cl_2 found in the Treated (Site C) and Raw (Site B) lines followed similar trends to what was found in the 2008 study. The Cl_2 was consistently higher in the Raw line (1.9-2.5 mg/L) when compared to the Treated line (0.2-0.5 mg/L). This is expected since the Treated line goes through the coagulation process, which would remove a portion of the residual Cl_2 concentration.

The addition of alum/PAC and their coagulation aids were recorded on a daily basis for the 2009 study period. The mill switched from alum to PAC on April 15 due to the high turbidity level of the incoming river water, however changed would not be reflected until April 16. Similar to the 2008 study, the increase in river turbidity can be linked to the river ice breakup noted to begin on April 14. Alum was added from March 9 to April 4 and ranged from 55 - 62 mg/L. The alum coagulant aid was added in the range 0.44- 0.5 mg/L. PAC and its coagulant aid were added from April 15 to May 4 and ranged from 54 – 65 mg/L and 0.5 – 0.58 mg/L respectively.

KCWO physical/chemical parameters for the detected toxicity event on April 15 are summarized in Table 5.7. Similar to 2008, flow rate and conductivity were found to be high during the detected toxicity event. The pH of the KCWO during the toxicity event was relatively low (pH 6.6) compared to the mean of the entire study (7.15). All other parameters are within one standard deviation of the mean taken from the entire study period. Additional chemical parameters were established throughout the 2009 study in order to further determine the source of Spring toxicity events.

Table 5.7 Summary of all KCWO parameters during April 15, 2009 toxicity event

Parameter	April 15	Study Mean ± ST DEV
KCWO Flow Rate (GPM)	**4088.51**	**2510.28.± 1268.93**
KCWO Conductivity (µS)	**169.6**	**97.6 ± 27.7**
River Intake Temperature (°C)	0.97	1.28 ± 1.988
KCWO Temperature (°C)	24.2	25.38± 10.13
KCWO pH	**6.6**	**7.15± 0.31**
Precipitation (mm)	0.6	1.54 ± 3.6
BOD (t/d)	0	0.019 ± 0.037
Raw Cl_2 (mg/L)	2.3	2.3 ± 0.23
Treated Cl_2 (mg/L)	0.5	0.37 ± 0.26
Alum/PAC dosage (mg/L)	65	56.78 ± 3.70
Coagulation Aid addition (mg/L)	0.5	0.48 ± 0.05
TEWS Response (YES/No)	YES	N/A
Rainbow Trout toxicity test (Pass/Fail)	Fail	N/A
Daphnia LC_{50} Value	Non toxic	N/A
Occurred Before or After River-ice breakup?	During	N/A
Hardness (mg/L $CaCO_3$)	58.5	49.8±8.62

Inorganic Anions

Increased conductivity could be associated with inorganic anions, which are commonly found during Spring melt. Therefore, inorganic anion concentrations were determined at each of the five sample sites.

Chloride, nitrate and sulphate concentrations found at each of the 5 Kraft cooling system sample sites are shown in Figure 5.12. Site E (Final KCWO) chloride

concentrations were found to be 12.28 mg/l during the April 15 event, which is low compared to the average concentration (13.78 ± 4.16 mg/L) found at Site E over the study period. The highest chloride concentration found at all sites was 23.82 mg/L at Site E. This is much lower than the 600 mg/L guideline set out for the protection of aquatic life (BCMOE, 2009). In comparison, average chloride levels found in five rivers and two creeks during Spring melt events in St. Paul, MN was 49 mg/L and 116 mg/L respectively (Oberts, 1994).

Site E was found to have a nitrate concentration of 0.29 mg/L during the April 15 event, which is higher than the average concentration (0.25 ± 0.09 mg/L) determined at Site E over the duration of the study period. Despite the increase in nitrate, the levels present at Site E (maximum of 0.456 mg/L) remain much lower than the 200mg/L guideline set out for the protection of aquatic life (BCMOE, 2009). When comparing Site E nitrate concentrations to the average concentrations of 2,300 rainfall events in the US (0.96 mg/L), such values are relatively low (Oberts, 1994).

During the April 15 event, sulphate concentrations at Site E were found to be 24.58 mg/L, which was higher than the average concentration (14.87 ± 7.69 mg/L) found at this site over the duration of the study period. The highest sulphate concentration found among all sites was well below the 100 mg/L guideline set for the protection of aquatic life (BCMOE, 2009). It is interesting to note that the level of sulphate decreased by approximately 72% at Site E when coagulants were changed from alum to PAC. This is likely because when alum $Al_2(SO_4)_3$ is added to water it dissociates, thereby increasing sulphate concentrations. Inorganic anions are related to increased conductivity, however they may not be significant in toxicity events as they are clearly below guidelines levels.

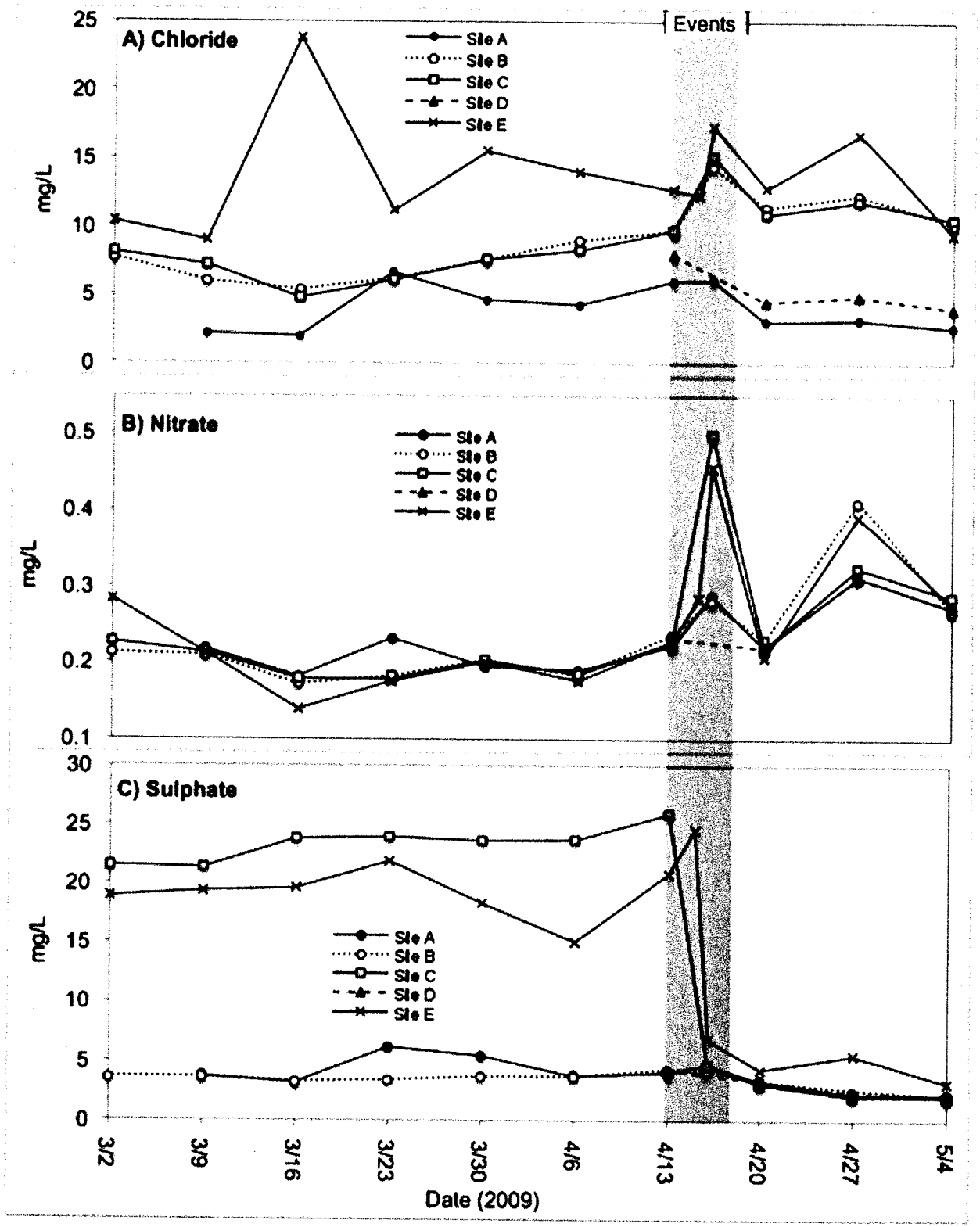


Figure 5.12 Anion concentrations found at the KCWO during Spring 2009. The red (April 15 Event) indicates when toxicity occurred at the KCWO. (A) Total Chloride, (B) Total Nitrate, (C) Total Sulphate.

Site Specific Rainbow Trout Toxicity

Results from the weekly rainbow trout toxicity tests are shown in Table 5.8. Site C showed toxicity on 6 occasions, which was the highest of all sites. Site B had two toxicity events, Site D and E had one toxicity event and Site A showed no signs of toxicity. Based on the order in which toxicity was revealed, it is assumed that Sites B, C, D and E would have failed concurrently on April 15, however only Site E was tested at that specific time point.

Table 5.8 Summary of Rainbow Trout bioassay results over the Spring study period.

Sample Date	Sample Time 24- h	Site A (Kam River)	Site B (Raw)	Site C (Graver)	Site D (Chem Plant)	Site E (Final KCWO)
03/02/09	11:30	*	N	N	*	N
03/09/09	13:00	N	N	**T (100%)**	*	N
03/16/09	15:30	N	N	**T (60%)**	*	N
03/23/09	11:30	N	N	**T (60%)**	*	N
03/25/09	19:30	N	**T (90%)**	**T (100%)**	*	N
03/30/09	11:00	N	N	N	*	N
04/06/09	9:30	N	N	**T (70%)**	*	N
04/13/09	10:00	N	**T (100%)**	**T (100%)**	**T (100%)**	N
04/15/09	14:00	*	*	*	*	**T (100%)**
04/16/09	15:00	N	N	N	*	N
04/20/09	11:00	N	N	N	N	N
04/27/09	11:30	N	N	N	N	N
05/04/09	11:00	N	N	N	N	N

Note: * represents no sample being tested, N represents no toxicity shown (<50% mortality) and T represents toxicity shown (>50% mortality).

5.4.2.1 Investigating Site C (Graver) Toxicity

Dissolved Aluminum

An inverse relationship was found at Site C between dissolved Al and pH (Figure 5.13). The highest concentration of dissolved Al recorded at Site C was 68 μg/L, which corresponded to a pH of 6. The lowest concentration of dissolved Al was found to be 4 μg/L and corresponded with a pH of 6.6. On April 15 the coagulant used at Site C was changed from alum (aluminum sulphate) to PAC (polyaluminum chloride), however the change did not come into effect until April 16, due to the hydraulic retention time of the Kraft cooling system. While using alum the average dissolved Al concentration was 51 μg/L and the average pH was 6.1. While using PAC the average Al concentration was lower (6.3 μg/L) and the average pH was higher (6.5).

It should be noted that pH began to increase before the coagulant change; however after the change the pH remained relatively high. The gradual pH increase prior to the switch to PAC may be linked to a natural increase in the pH of the intake water. Furthermore, all 6 toxicity events recorded at Site C occurred while alum was being used.

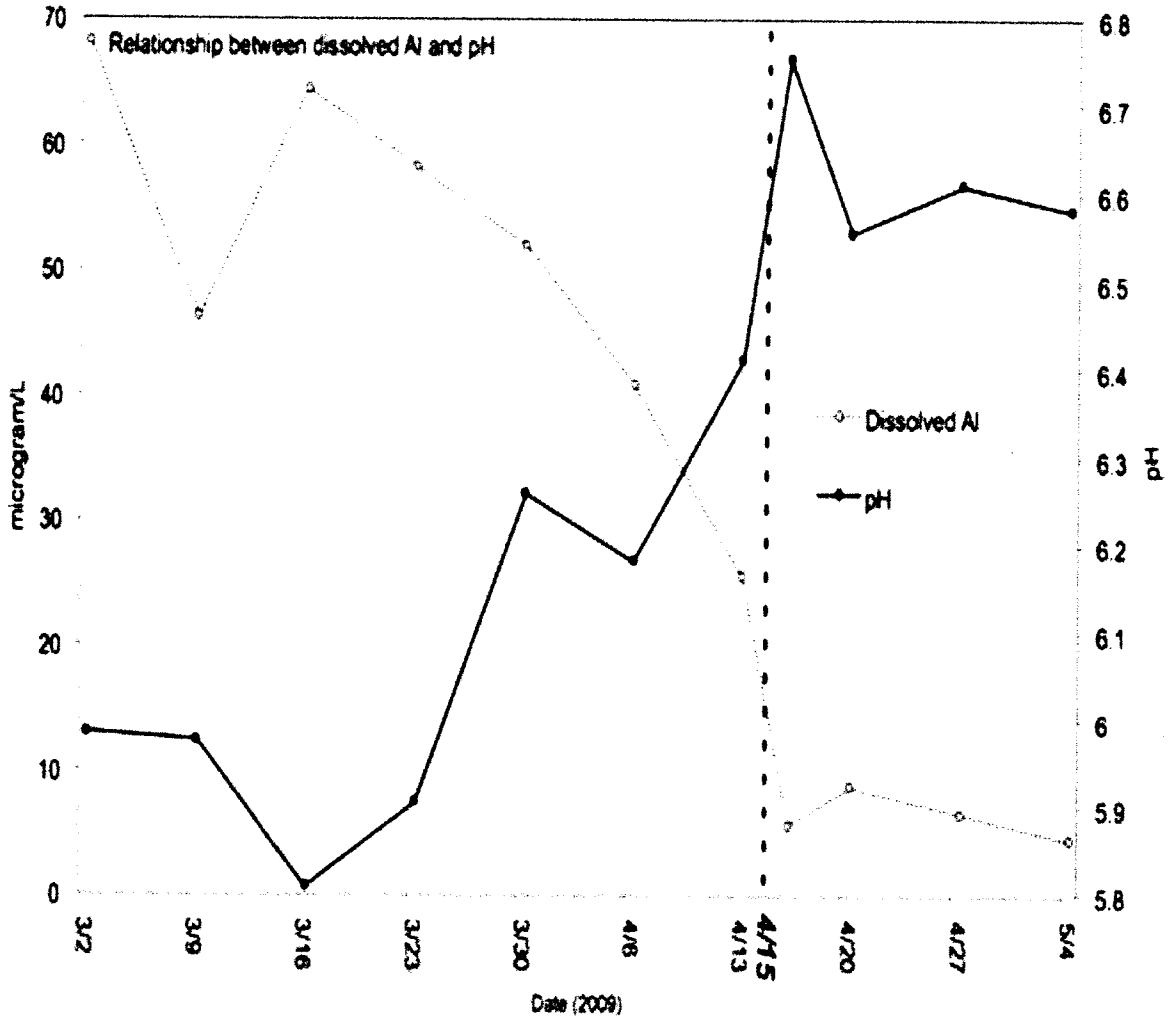


Figure 5.13 Inverse relationship between dissolved Al and pH at Site C (Graver). ABTB changed from Alum to PAC on April 15, 2009, which is indicated by the dotted line (- - - -).

Aluminum Precipitation

While conducting toxicity tests for Site C, the formation of a precipitate was commonly observed. Figure 5.14 shows the precipitate that formed in the March 9, 2009 sample, in which rainbow trout toxicity was detected. The pH of this sample was 5.9 pre-aeration and increased to 7.4 post-aeration. The increase in pH may have changed Al speciation in the sample from dissolved (Al_i) to solid forms (i.e. gibbsite).

The composition of the precipitate that formed in the Site C sample on March 9, 2009 was investigated using SEM (Figure 5.15). Elemental analysis revealed that the precipitate was composed of three main elements: (A) silicon, (B) aluminum and (C) iron. Aluminum represented the majority of the sample as seen in Figure 17 A.

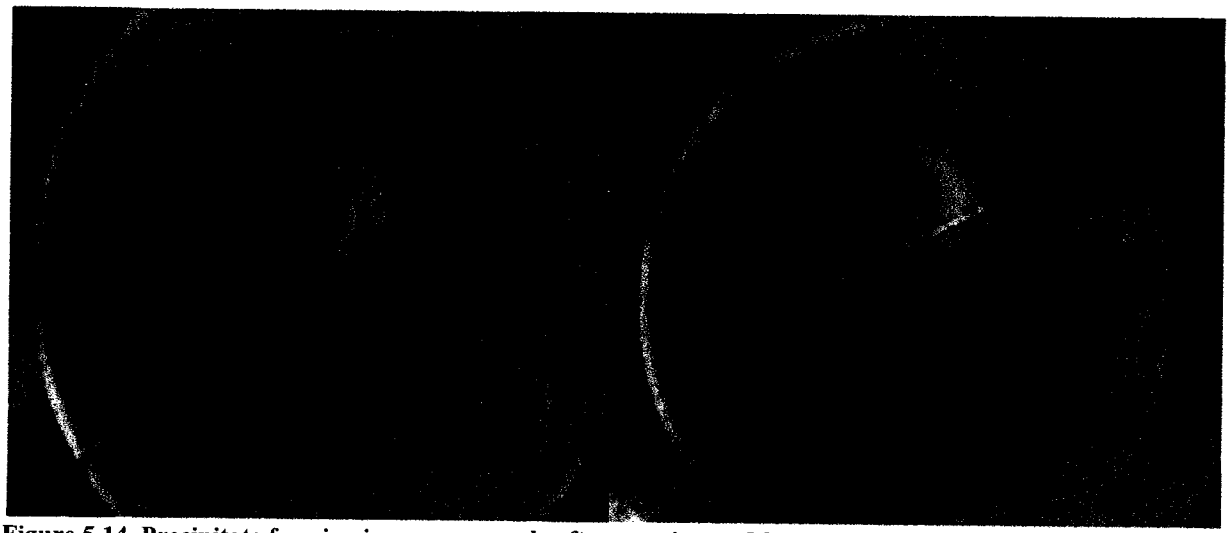


Figure 5.14 Precipitate forming in graver sample after aeration on March 23 2009. (A) Non-aerated graver sample prior to rainbow trout bioassays test (pH=5.91), (B) Aerated graver sample after 96 hours rainbow trout bioassay (pH=7.36).

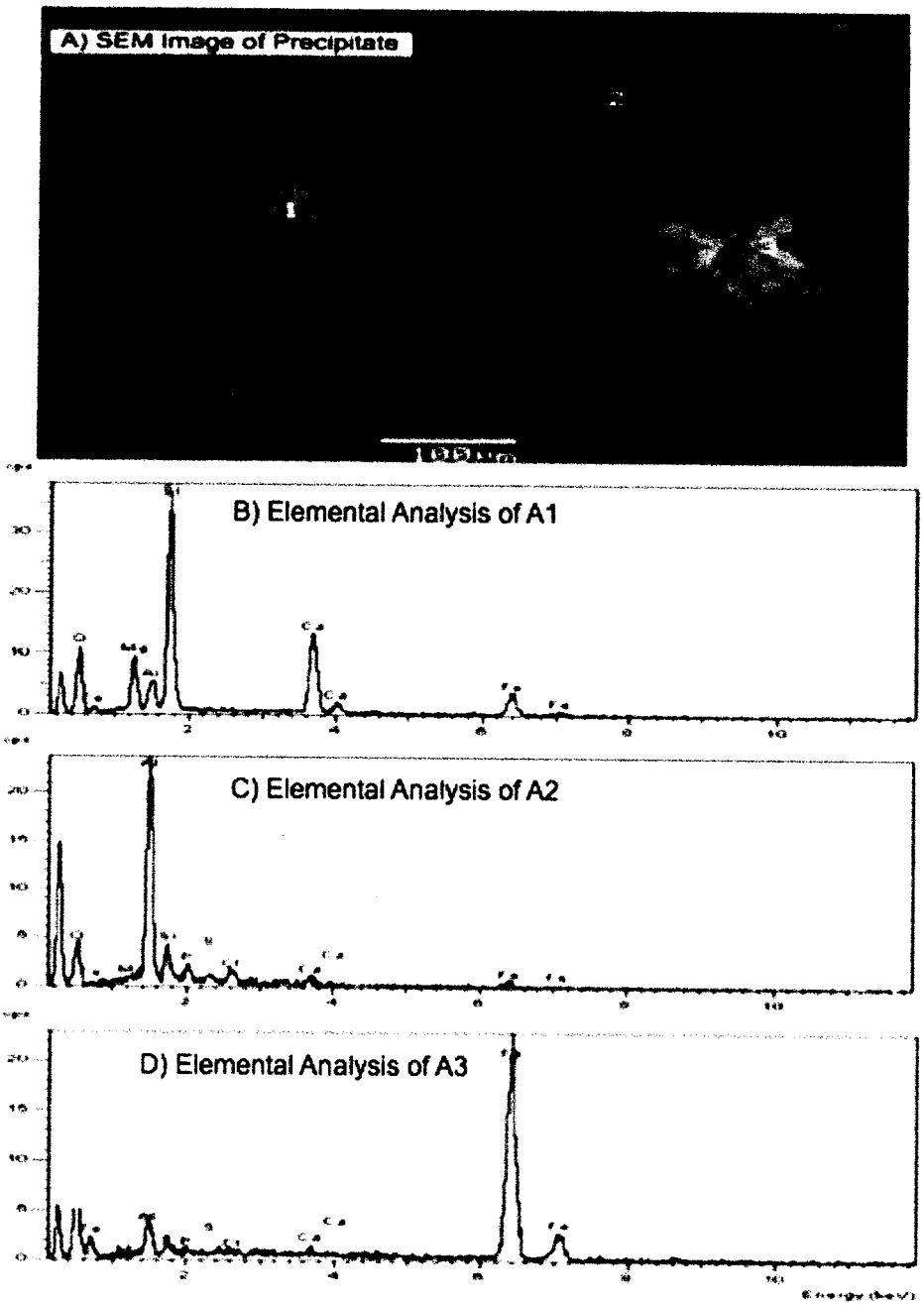


Figure 5.15 Examining the graver precipitate. (A) SEM image of graver precipitate, (B) elemental analysis corresponding to image A1, (C) elemental analysis of image A2, (D) elemental analysis of image A3.

Analysis of Fish Gills

Gills excised from fish exposed to Site C samples showing toxicity were analyzed to determine if Al was accumulating on the gills of rainbow trout (n=10) during toxicity events. Fish exposed to the failing March 9 sample were found to have approximately 217 μg Al/g wet weight accumulate on their gills (Figure 5.16). The March 9 sample had a total Al concentration of 0.778 in which 6% was in the dissolved form. Fish exposed to a Site C sample that showed no mortality (April 25 sample), only had approximately 16 μg Al/g wet weight accumulate on their gills. The April 25, sample had a total Al concentration of 0.868 in which 0.5% was in the dissolved form. The higher dissolved Al concentration found in the March 9 sample is suspected to be associated with its lower pH value (pH 6). Fish exposed to dechlorinated control water only had 5 μg Al/g wet weight accumulate on their gills. The control water showed to have Al concentrations below the detection limit (5 μg/L) and had a pH of 7.2.

Based on these findings, fish toxicity events observed in Site C may be linked to the accumulation of Al on fish gills. One might hypothesize that the degree of Al accumulated in rainbow trout gills may be dependent on the dissolved concentration of Al in the source water. It could also depend on a portion of the particulate Al depending on the pH and or temperature of the source water (Rodushkin et al. 1995)

This may partially explain why in this study fish gills accumulated 219 μg Al/g wet weight despite having only approximately 50 μg /L of dissolved Al in the March 9 sample. The accumulation of Al in the gills reflects the bioavailability of Al in the sample.

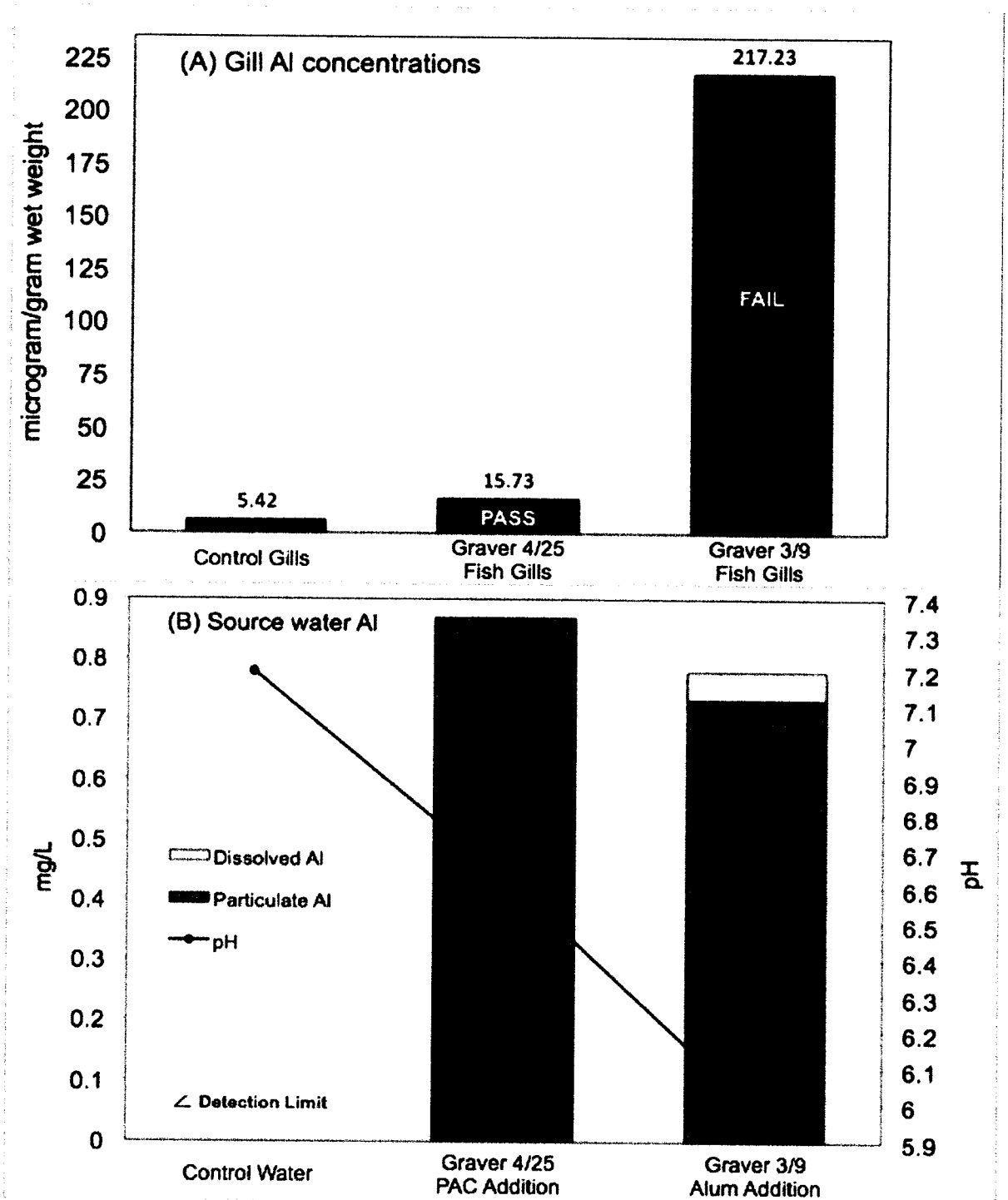


Figure 5.16 Relationship between source water Al and Al found on gills of rainbow trout. (A) Gill Al concentrations found in control gills, gills exposed to April 25, 2009 graver sample and gills exposed to March 3, 2009 graver sample, (B) Total Al and pH of dechlorinated control water, April 25, 2009 graver sample (pass) and March 3, 2009 graver sample (fail) expressed as dissolved and particulate aluminum.

5.4.2.2 Investigating Site E (KCWO) Toxicity

Metal Concentrations

Samples were collected from Site E and assessed weekly for metal concentrations from March 2 to May 4 using ICP (Figure 5.17). Metals found at low concentrations like that of Cr, Ni, Cu, As peaked during the April 15 event, having a combined concentration of 11.4 μg/L The average combined concentration of these metals throughout the sample period was approximately 4.6 mg/L. Similar to the 2008 findings, arsenic (6 μg/L) was only found during the detected toxicity event. Metals found in higher concentrations like that of Ba, Mn, Al, Fe were also found to be high during the April 15 event, having a combined concentration of 2.3 mg/L. The average combined concentration of these metals throughout the sample period was approximately1.5 mg/L. Total aluminum concentrations increased greatly during the event, reaching a near high concentration of 0.994 mg/L. The KCWO is a soft effluent having an average hardness level of 49.8 ± 8.6 mg/L $CaCO_3$ for the 2009 study period.

Comparing metal concentrations during the April 15 TEWS event to the findings from the 2008 study, shows that metal concentrations were at high levels during both events. Aluminum is consistently found at high concentrations during toxicity events, therefore investigating its form and speciation during events is of great importance.

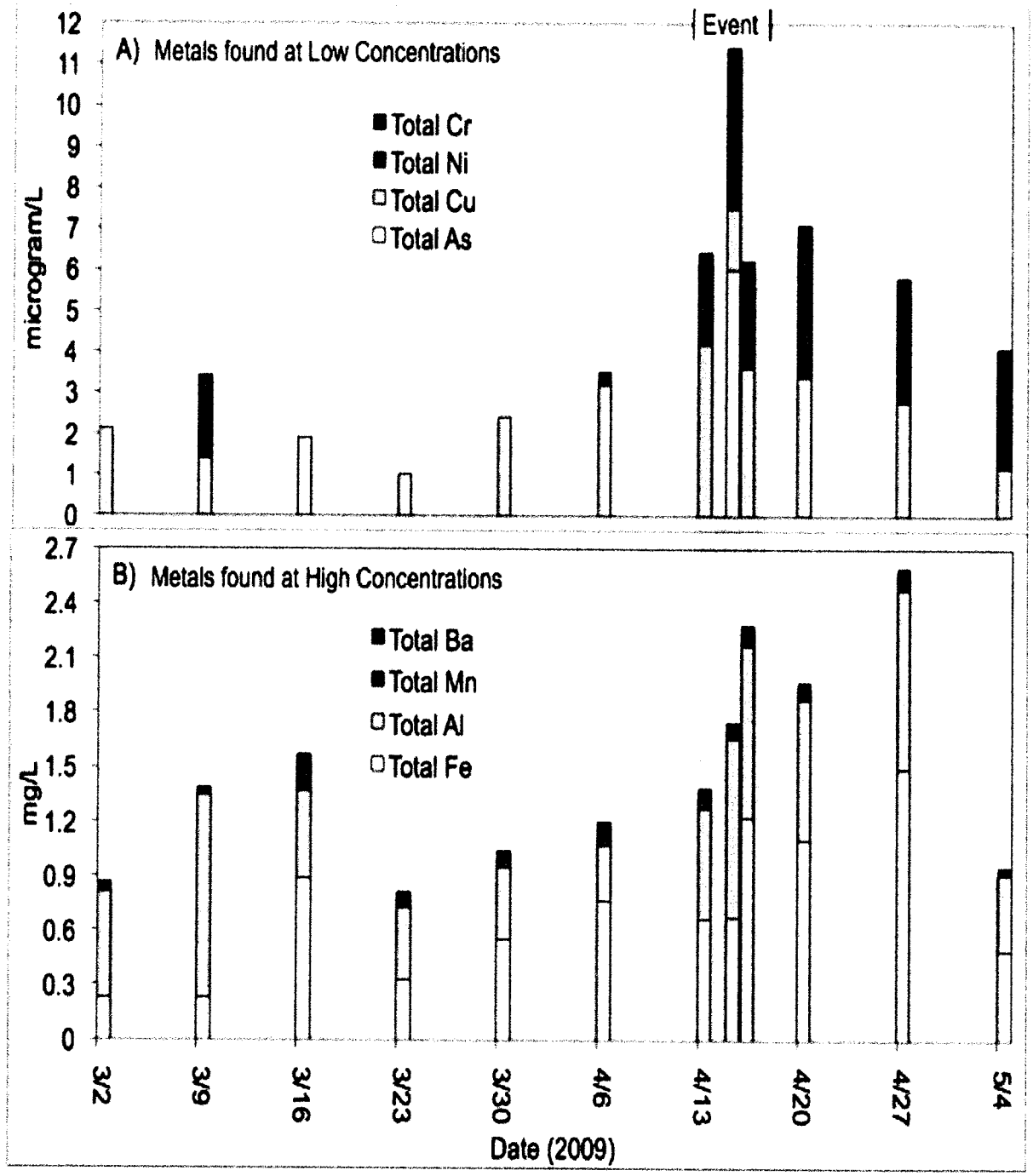


Figure 5.17 Spring 2009 KCWO total metal concentrations. The red (April 15 Event) indicates when toxicity occurred at the KCWO. (A) Metals found at low concentrations (µg/L), (B) Metals found at high concentrations (mg/L).

Total Aluminum

Total aluminum concentrations at Site E are expressed as particulate (>0.45 micron) and dissolved (<0.45 micron) Al (Figure 5.18). On average, dissolved Al accounted for 18.3% of the total Al concentration and represents the more reactive forms of Al, such as Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^{+}$. Particulate Al accounted for 81.7% of the total Al and represents Al associated with clays (aluminum silicates), minerals (diaspore and gibbsite) and Al bound to organic or inorganic matter.

Water quality parameters such as DOC, pH and temperature affect the amount of dissolved Al concentration in a sample (Gensmer and Playle, 1999). During the April 15 event, pH and DOC are 6.6 and 4.3 mg/L respectively. One might expect this to lead to increased dissolved Al in the sample. However, dissolved Al found during April 15 was 113 μg/L, which was slightly lower than the average found throughout the study (125 μg/L). This may be due to a temperature effect as the KCWO can vary from 10° C to 45°C. Furthermore, this result may be time dependent, as peaks in dissolved Al were found to be the highest shortly after the April 15 event. Nonetheless, total aluminum was found at high concentrations during detected toxicity events. In order to establish where the increased concentration of Al originates five sample sites within the cooling system were examined.

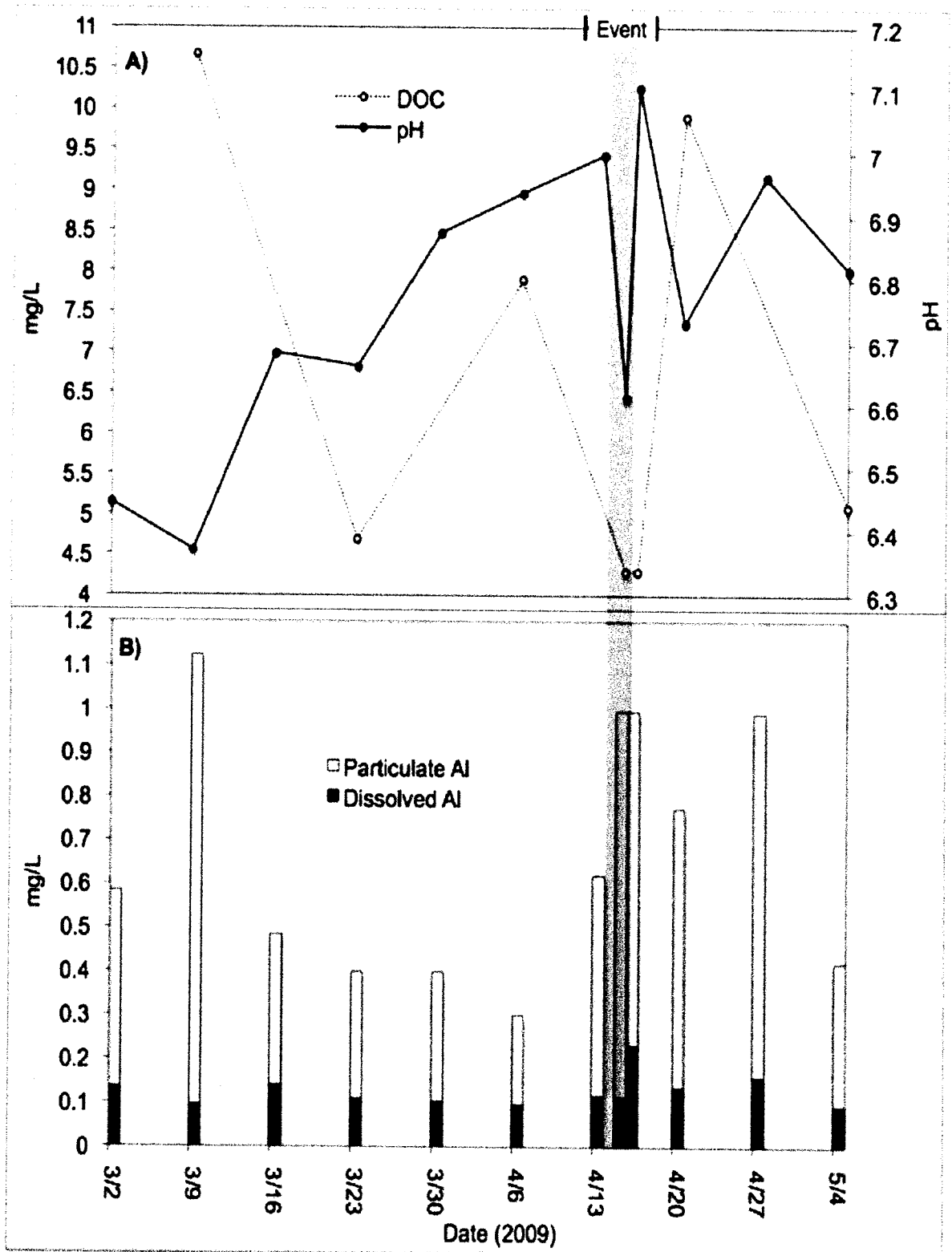


Figure 5.18 Spring 2009 aluminum concentrations. The red (April 15 Event) indicates when toxicity occurred at the KCWO. (A) DOC and pH, (B) Total aluminum expressed as particulate (≥ 0.45 μm) and dissolved aluminum (≤ 0.45 μm).

The Source of Site E Aluminum

Aluminum concentrations and pH values for each of the five cooling system sample sites are shown in Figure 5.19. Site C was determined to be the main source of Al found at Site E for the first half of the study (March 2 to April 13). The average concentration of Al found at Site C during this period was 0.74 mg/L, with a high of 0.89 mg/L (April 13) and a low of 0.56 mg/L (March 23). The majority (94%) of Al coming from Site C was in the particulate form.

The main source of Al at Site E changed from Site C to Site B for the second half of the study (April 13 to May 5). The average concentration of Al found at Site B during this period was 4.02 mg/L, with a high of 8.13 mg/L (April 27) and a low of 0.72 mg/L (April 13). The majority (96%) of Al coming from Site B was also in particulate form.

It is suspected that aluminum concentrations at Site A are not similar to that of Site B because of sampling procedures. The weekly samples conducted at Site A were taken at the surface of the Kaministiquia River, and therefore are not truly representative of the intake river water coming into the mill. The river water coming into the mill is taken very close to the bottom of the river. This is important to note as during Spring runoff events concentrations of colloidal metals are found to be higher due to erosion and resuspension of sediments located at the bottom of river basins (Smith et al., 2003). Site A was found to peak in total Al (5.5 mg/L) on March 9. This sample was noted to be heavily composed of sediments in comparison to the other Site A weekly samples. This shows that Site A has the potential to have high Al concentrations, however it may have been limited by surface water sampling techniques.

It is suspected that low pH values influenced Al speciation leading to fish toxicity events. The lowest pH was consistently found at Site C, which showed the most toxicity events. The highest pH was consistently found at Site A, which showed no toxicity events.

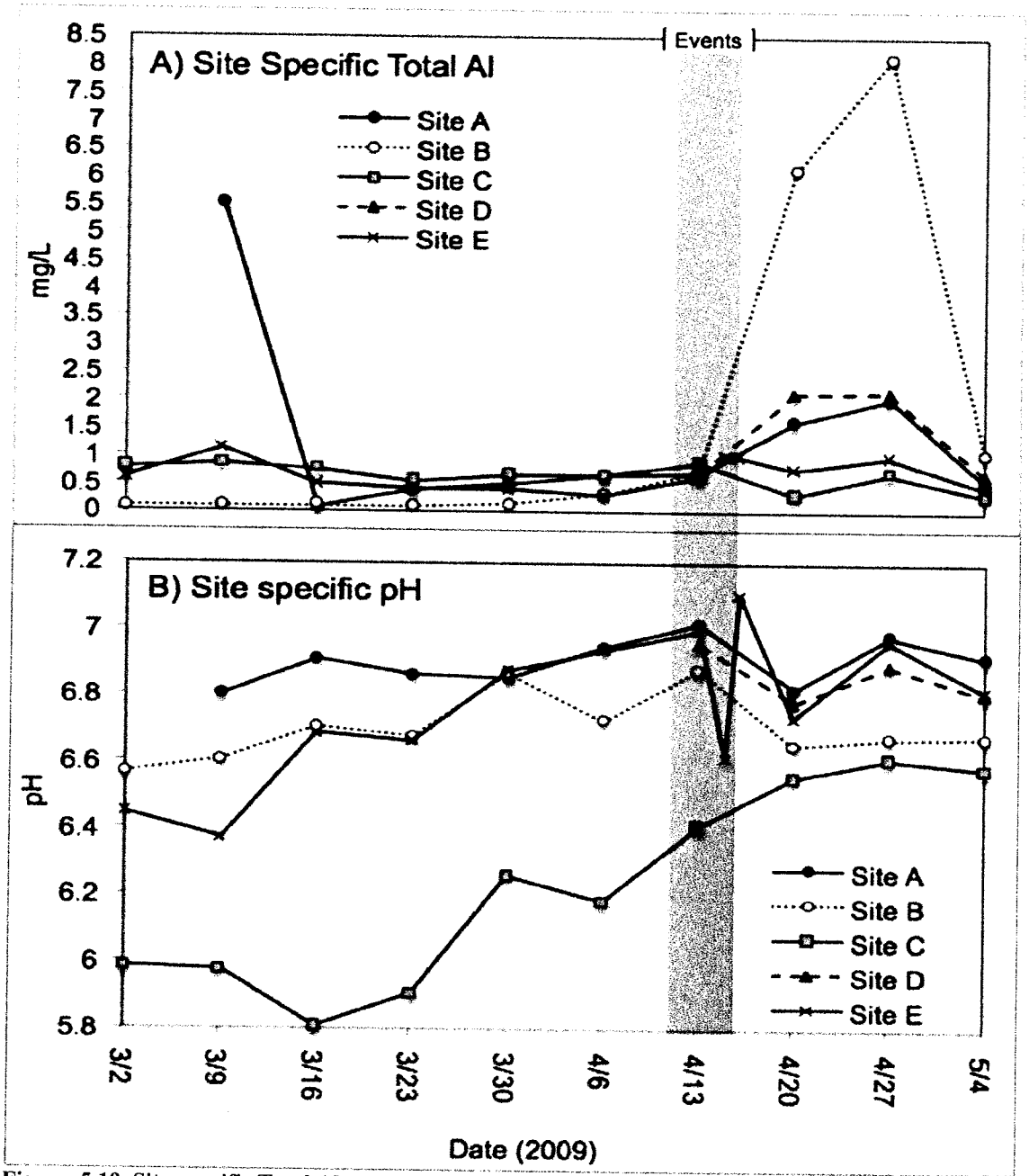


Figure 5.19 Site-specific Total Al concentrations and pH. The red indicates when toxicity occurred at Site B, Site C, Site D (April 13) and Site E (April 15, 2009). (A) Sources of Total Al at each site, (B) pH fluctuations at each site.

Toxicity Identification Evaluation (TIE)

The TIE manipulations conducted for the toxicity event that occurred on April 15 at Site E are shown in Table 5.9. The original test on the April 15 cooling water showed 100% mortality, however a reduction in toxicity (only 30% mortality) was observed in the TIE baseline test conducted after 24 hours of sample storage. Results from the TIE manipulations showed that the addition of sodium thiosulphate aimed at eliminating toxicity due to chlorine, completely reduced the toxicity of the April 15 sample. Therefore, it is possible that the toxicity may be linked to chlorine. Sodium thiosulphate increased the pH of the sample from 6.82 to 7.08, which may have influence other potential modes of toxicity, such as aluminum toxicity.

The addition of EDTA (metal manipulation) increased the toxicity of the April 15 effluent to 80% mortality compared to the 30% mortality of the baseline test. This may be due to several reasons: (1) EDTA added to the sample may cause toxicity directly to the fish, or (2) the addition of EDTA reduces the pH of the sample thereby influencing other potential modes of toxicity that are pH dependent. Given that only 10% of the EDTA LC_{50} for rainbow trout was added for this manipulation, it is not likely to have caused the observed toxicity. The EDTA did however reduce the pH of the sample, which may have resulted in the increased mortality observed.

Aging the original April 15 Site E sample for a total of 7 days resulted in the total elimination of toxicity. Based on this finding, it is suspected that toxicity may have dissipated if it was either linked to a volatile toxicant or Al that has had extended time to undergo rapid polymerization and precipitation phenomena, leading to less toxic forms of

Al to be up taken via fish gills. Vigorous aerating the sample for one hour prior to the toxicity test drastically increased the pH and resulted in the elimination of toxicity.

Table 5.8 TIE manipulations conducted for the April 15 toxicity event at Site E

Manipulation	Initial				After 96-hours			
	Temp	DO	pH	COND	Temp	DO	pH	Mortality
April 15 Event	14.0	9.5	6.82	175	15.0	10.1	7.72	10
Baseline	15.0	9.8	6.88	179	14.0	9.9	7.26	3
Chlorine	14.9	10.0	7.08	4700	14.1	9.9	7.76	0
Metal	15.0	10.0	6.49	172	14.5	10.2	7.65	10
Aging	15.0	10.2	6.90	175	14.5	10.0	7.31	0
Aeration	15.0	10.3	7.55	178	14.0	10.2	7.75	0

5.4.3 Study III: Modeling Events in 2009

Aluminum Speciation

Modeling the chemical speciation of aluminum at the KCWO was conducted using Visual MINTEQ version 2.53. The species of aluminum that were present at Site E during toxicity events and non-toxicity events are displayed in Table 5.9. The most toxic form of aluminum (Al^{3+}) was present during the Site E toxicity event, but not during the non-toxicity event. Although Al^{3+} concentrations are found to be very low (1 μg/L) during toxicity events, the combined reactive Al (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$) concentration were found to be high (186 μg/L). It is suspected that such high levels of reactive Al resulted in rainbow trout mortality, considering the levels are significantly higher than the proposed aquatic guideline for total reactive Al. The proposed guideline is 25 μg/L when sample pH > 6.5 (Gardner and Comber, 2003).

Table 5.9 Aluminum species that would be present at Site E

Site E Toxicity Event (4/15/09)		Site E Non-Toxicity Event (4/20/09)	
% of total Al concentration	Species name	% of total Al concentration	Species name
0.034	**Al^{+3}**	0.061	Al DOM1
0.144	Al DOM1	0.258	$AlOH^{+2}$
1.062	$AlOH^{+2}$	8.841	$Al(OH)_2^{+}$
17.59	$Al(OH)_2^{+}$	23.621	$Al(OH)_3$ (aq)
26.091	$Al(OH)_3$ (aq)	67.214	$Al(OH)4^{-}$
55.046	$Al(OH)_4^{-}$		
0.031	$AlSO_4^{+}$		

Altering KCWO Parameters

Altering the temperature, pH and binding ligands of toxic samples helped determine how the chemical speciation of Al may change. A pH sweep (5.5-7.5) was conducted for the April 15 Site E toxicity event (Figure 5.20). The original toxicity event occurred at a pH 6.6, which correlates to approximately 1 μg/L Al^{3+} and 186 μg/L of total reactive Al bioavailable to fish. If the pH of this sample was increased to pH 7 and pH 7.5 the total reactive Al concentration would decrease to approximately 44 μg/L and 5 μg/L respectively. Based on this model, Al^{3+} concentrations would be eliminated at pH 7.

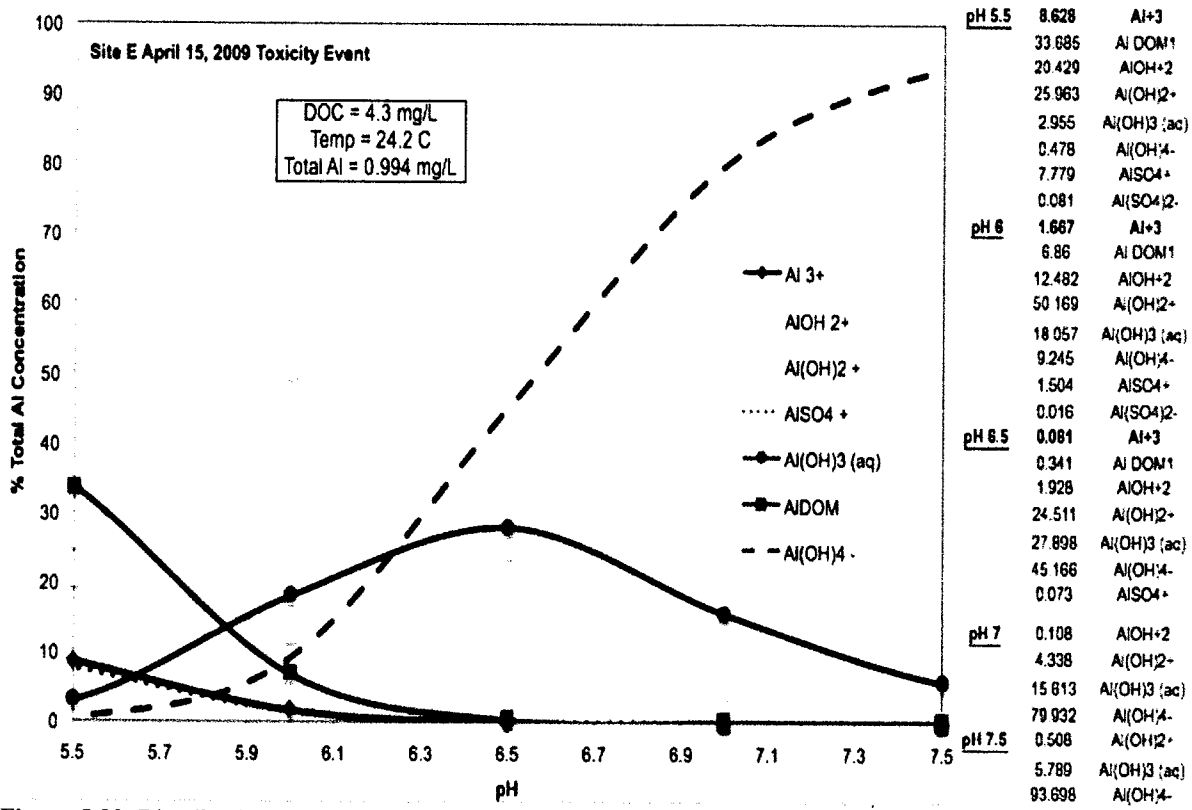


Figure 5.20 Distribution of Al species for the April 15 Site E toxicity event. Note: Red lines are most harmful to fish, yellow lines are harmful to fish and green lines are not harmful to fish.

Temperature also plays a major role in metal speciation. The April 15 Site E toxicity event was modeled at 15°C in order to replicate regulatory rainbow trout toxicity test conditions. A pH sweep from 5.5 -7.5 was conducted at this temperature (Figure 5.21). Based on this model, a sample with pH 6.6 at 15°C would have an Al^{3+} concentration of 3 μg/L. The amount of reactive Al would be increased to approximately 343 μg/L, creating even more toxic conditions. Based on this model, Al^{3+} concentrations would be eliminated at pH of 7.5.

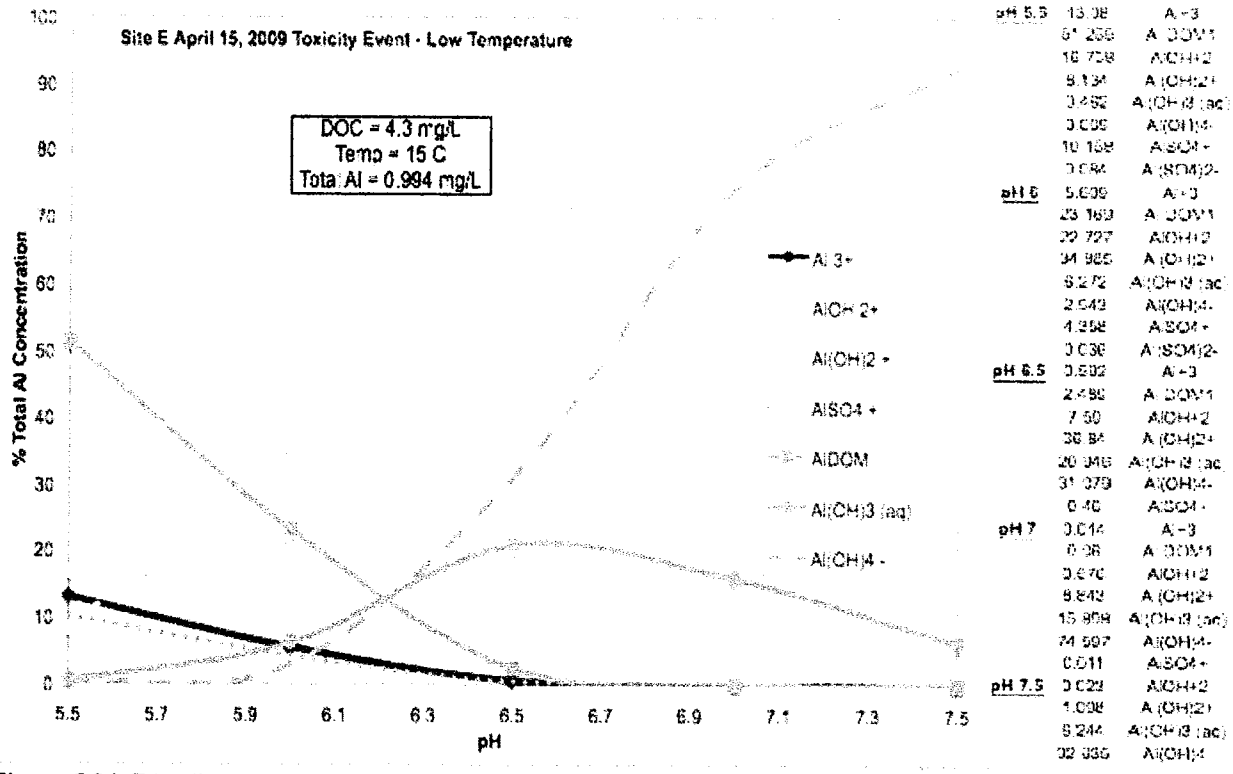


Figure 5.21 Distribution of Al species under modified temperature conditions (15 °C). Note: Red lines are most harmful to fish, yellow lines are harmful to fish and green lines are not harmful to fish.

The April 15 toxicity Site E event was modeled at 0°C with the removal of al DOC from the system in order to show how temperature and DOC would influence concentrations of reactive Al. A pH sweep from 5.5 -7.5 was conducted (Figure 5.22). If the sample remained at pH 6.6 approximately 58 μg/L Al^{3+} and 756 μg/L of reactive Al would be bioavailable to fish. Such levels of reactive Al are much higher than the guideline (25 μg/L) and would likely lead to increased fish toxicity. Based on this model, Al^{3+} concentrations would be eliminated at pH 8.

Based on these three models it is clear that Al speciation at the KCWO is greatly affected by pH, DOC and temperature. A combination of unfavorable conditions, such as low pH, DOC and temperature could ultimately increase the frequency of toxicity events seen at the KCWO.

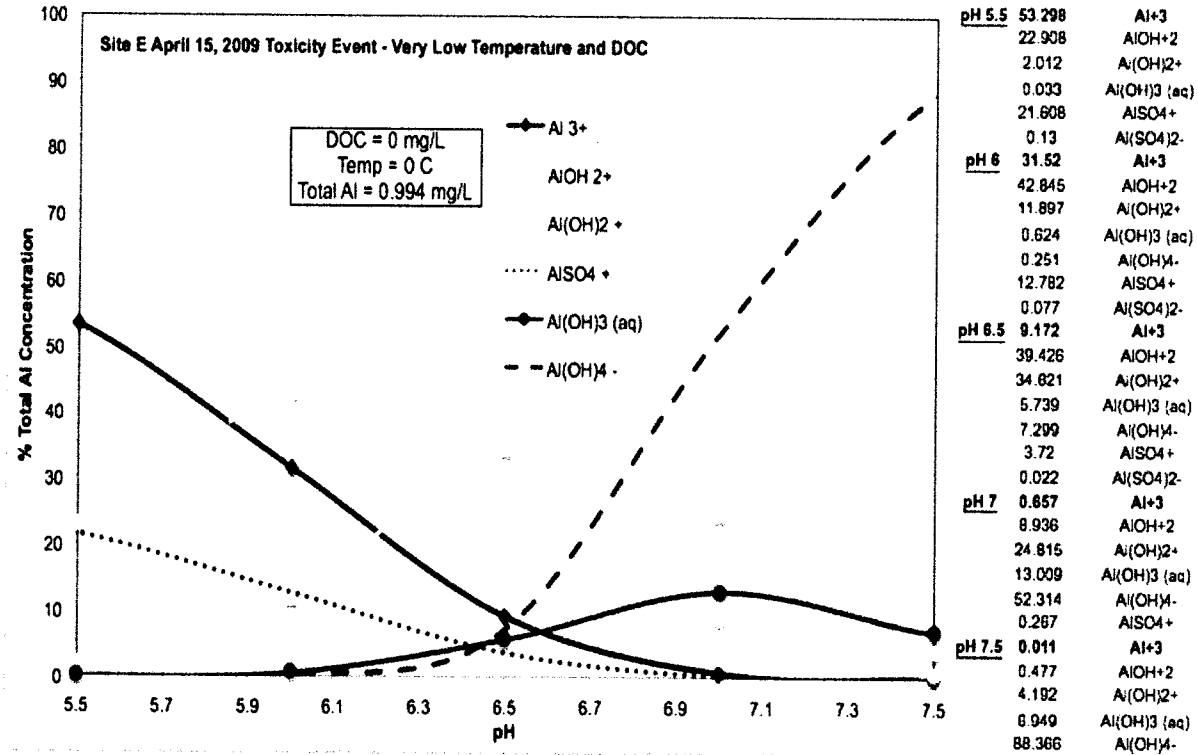


Figure 5.22 Distribution of Al species under modified temperature (0°C) and DOC (0 mg/L) conditions. Note: Red lines are most harmful to fish, yellow lines are harmful to fish and green lines are not harmful to fish

5.5 Spring Toxicity Findings

The TEWS proved to be effective in detecting Spring toxicity events (April 7, 2008 and April 15, 2009) at ABTB. Both events were supported by laboratory toxicity tests. By adopting the TEWS AbitibiBowater has been able to: (1) establish early detection and warning of transient, episodic, and developing toxic conditions, (2) identify potential toxicity from other ABTB system sources, (3) respond to the effects of metal mixtures, and (4) acquire adequate samples for further chemical analyses and Toxicity Identification Evaluation (TIE).

Increased flow rate and conductivity of the Kraft cooling system were found to coincide with detected rainbow trout toxicity events in 2008 and 2009. Such a relationship is common among Spring flood events in Northern Latitudes (Laudon et al.,

2000). It was suspected that high conductivity was associated with atmospheric fallout of chemicals, cations, anions and nutrients, which accumulated in the snowpack throughout the winter. This melts during the Spring flood event and causes changes in intake river for the cooling system at ABTB.

Based on the 2008 and 2009 ICP metal analysis, it was found that total metal concentrations were increased during TEWS events compared to non-events. Metals not normally detected in the KCWO such as arsenic, chromium and nickel were present during toxicity events in both 2008 and 2009. Such trace metal concentrations may not be toxic to rainbow trout individually, however they may have an additive effect in the soft-water conditions of the KCWO (Marr et al., 1998; Playle, 2004;Birceanu et al., 2008; Borgmann et al., 2008). It is suspected that the increased metal concentrations originate from two external sources; (1) fine-grained particulate washed through the snowpack during the Spring melt (Schondorf and Herrmann, 1987), (2) concentrations of colloidal metals eroded and resuspended from the bottom of the Kaministiquia river during Spring runoff events. (Smith et al., 2003).

In addition to the trace metals appearing during failures, aluminum concentrations that are normally present in the KCWO at moderate levels were found at high concentrations during events. Such levels are well above the LC_{50} range of 0.161 to 0.310 mg/L (EPA ECOTOX database) for rainbow trout and the Canadian guidelines for the protection of aquatic life (5 μg/L at pH < 6.5 and 100 μg/L ≥ 6.5). Based on this study, it is suspected that aluminum is the primary cause of rainbow trout toxicity at the KCWO.

From the results presented in Study II, it is suspected that aluminum was also the main cause of rainbow trout toxicity at Site C (Graver). High levels of aluminum were

found to accumulate on the gills of fish during graver toxicity events. Of all sampling sites, Site C showed the most toxicity. This was because of high concentrations of dissolved Al, the low pH, a low temperature and minimal binding ligands due to the coagulation process. It was found that when the graver switched from alum to PAC the pH was increased and the amount of dissolved Al was reduced, thereby reducing toxicity events.

The Kaministiquia River samples taken from the surface of the river were determined to be non-representative of the actual sediment-water interactions occurring at the bottom of the river where the intake line is located. It was found that Site B (Raw) was most representative of the intake river water. The Site B was found to be low in total Al prior to the river ice breakup, however during and shortly after the break up the total Al spiked to concentrations as high as 8 mg/L. Such spikes in Al can be associated with the mobilization of river transported colloidal aluminum and tended to occur concurrently with the Site E (KCWO) toxicity failures.

TIE manipulations conducted on the April 15 KCWO did not entirely support metal toxicity as the cause of KCWO toxicity. However, it was determined that the toxicity was pH related and that toxicity was greatly reduced with aging of the sample. This finding could further support Al toxicity, as fresh Al solutions could be undergoing rapid polymerization and precipitation phenomena in bulk solution and in the water passing over the gills, causing highly toxic conditions to fish. In contrast, if a solution is aged for some time these polymerization/precipitation reactions have already occurred in the bulk solution, so that water passing over the gills would contain more Al in a less toxic form (Gensemer and Playle, 1999).

5.6 Industry Recommendations

Based on the results of this project and related efforts, three recommendations are proposed to ABTB in order to prevent future Spring toxicity events at the KCWO. It must be noted that these three recommendations are based on limited knowledge of ABTB overall processes.

Recommendation 1: ***Increase the pH of the final KCWO to at least 7.5***

Rationale:

As mentioned throughout the report, aluminum has been established as the primary toxicant and therefore should be controlled. Aluminum can be toxic to fish in two ways: (1) ion-regulatory and (2) the polymerization and precipitation of Al on fish gills, both of which are pH dependent. Ion-regulatory effects are associated with reactive Al (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$) which are found generally at pH <6.8. The polymerization and precipitations occurs when slightly acidic effluent (pH 5 to 6) with high total Al levels enters the more basic gill environment causing neutralization and precipitate formation on the gills. The pH of 7.5 was chosen because it would eliminate the majority of reactive Al at the KCWO. This was supported by Al speciation modeling at the KCWO under extreme conditions (low temperature and no DOC)

Actions:

- Adding a strong base, such as NaOH or lime would increase the pH of the KCWO
- Using PAC in the coagulation process earlier in the Spring may keep the pH of the Graver and KCWO higher

Recommendation 2: ***Increase the temperature of the Graver and KCWO***

Rationale:

Temperature is commonly neglected in terms of Al speciation, however it was shown to play an important role in analysis of Al speciation at the KCWO. If the temperature of the Graver and the KCWO were increased less aluminum would be present in its reactive form to bind with fish gills.

Actions:

- The temperature of the KCWO is already relatively high (10-45° C). Instead of discharging this already treated water containing considerable thermal energy into the Kam River, the KCWO effluent could be at least partially diverted back to the Graver for heating purposes. This would also reduce treatment consumable costs.
- Furthermore, the cooling system should become a nearly closed system, in which seasonal river fluctuations would not be a threat. This would depend on the overall heating/cooling requirements and the effects of chemical additions.

Recommendation 3: ***Further investigate the intake river water***

Rationale:

At this time little is know about the sediment-water interactions occurring at the river intake, especially during Spring flood events.

Actions:

- Improved monitoring of the river intake is highly suggested.

Chapter 6 Conclusions

The research conducted in this thesis evaluated the ability of the TEWS to detect and help determine potential causes of Spring toxicity at AbitibiBowater. This study characterized the responses of the TEWS relative to standard acute toxicity testing benchmark concentrations and provided a method for rapid toxicity monitoring at AbitibiBowater Thunder Bay's Kraft Clean Water Outfall.

From laboratory tests it was found that an increase in fish cough rate was the most consistent indicator of developing toxic rainbow trout conditions using the TEWS. The pattern of change in rainbow trout ventilatory and cough rate determined in laboratory tests was consistent with the five levels of fish ventilatory response described by Carlson and Drummond (1978). When the TEWS was optimized, it was found that changes in rainbow trout behavioural parameters were detected within <2 h for contaminant concentrations at 96-h LC_{50} values. Observed toxicant response times for rainbow trout in the TEWS are comparable to responses reported for other fish biomonitoring systems.

Laboratory testing optimized the signal collection procedure for implementing the TEWS at ABTB, however several TEWS structural modifications were necessary to facilitate the handling of the cooling water flow at the field site. Modifications such as the addition of a stainless steel chiller and tank were used to maintain cooling water at 15°C ± 2°C in order to satisfy the requirements for testing with rainbow trout. A Sorenson 120 volt ac power line regulator was required at the site, as there was a substantial amount of electrical switching and relay noise present at the ABTB outflow building. A high spurious electrical noise rejection was required because the flow tank monitoring voltages were about 1 volt RMS and the Wheatstone bridge imbalance signal was

typically in the microvolt to millivolt range prior to amplification by a factor of 1000. A high electrical signal to noise ratio was required to observe small changes in the fish activity with a high degree of sensitivity and reproducibility.

The TEWS proved to be effective in detecting Spring toxicity events at ABTB, which were supported by laboratory toxicity tests. Physical, chemical, biological and modeling methods determined the most likely cause of the observed TEWS events. Aluminum in the cooling water appears to have been the cause of the observed TEWS events. Elevated aluminum concentrations found at the KCWO during events, which originate as particulates in ABTB intake water, and/or from the use of Al-based coagulants in the ABTB coagulation process seemed to be the source of this primary toxicant. Using observed cooling water pH, temperature, water hardness and/or binding ligands and metal concentrations as data, the Visual MINTEQ model predicted a large increase in reactive aluminum. This was verified with analytical measurements and fish gill analysis.

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