# THE FATE OF ESTRONE (E1), 17beta-ESTRADIOL (E2), ESTRIOL (E3) and 17alpha-ETHINYLESTRADIOL (EE2) IN SURFACE WATERS

by

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## Abstract

Lakes and rivers receiving wastewater treatment plant effluent contain many different endocrine disrupting compounds. Previous research into the fate of these compounds has focused on laboratory experiments that investigate a single scavenging mechanism, and there has been little research on the overall loss rate constants in receiving waters. This study evaluated the fate of estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol within three different receiving waters (a river, a large lake and a small reservoir) represented by two different mathematical models (plug flow reactor and continuously stirred tank reactor) and three different hydraulic residence times (<8 hours, >50 years and about 1 year). Wastewater treatment plant effluent samples and receiving waters were analysed for the four estrogens over a one year period. E1 and E2 were the only compounds detected and there was only enough data determine the fate of E1. A receiving water loss rate constant for E1 was calculated assuming first-order reaction kinetics. E1 loss was not detectable in the river and the large lake due to a very short and very long residence time, respectively. The time-weighted E1 loss rate constant within the small reservoir was found to be 0.0106 $d^{-1}$. Data suggested that there may be a seasonal component to this loss rate that requires further investigation. The rate constant found suggests that E1 can be transported great distances within rivers.

## Preface

For the sampling of Okanagan River, I received assistance from BC Ministry of Environment (BC MoE) staff in Penticton. I was responsible for planning sample locations, setting sample procedures, scheduling sampling and carrying out a portion of sampling. BC MoE did provide extensive assistance in sampling. For the other locations I planned and conducted all sampling.

Analysis of the samples was carried out with assistance from Dr. Sandra Mecklenburg. I prepared all samples for analysis and collaborated with Dr. Mecklenburg on the analytical method development. Dr. Mecklenburg assisted in the analysis of samples and provided technical expertise.

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## List of Abbreviations

WWTP = wastewater treatment plant
PFR = plug flow reactor
CSTR = continuously stirred mixed tank reactor
E1 = estrone
E2 = 17β – estradiol
E3 = estriol
EE2 = 17α-ethinylestradiol
GC = gas chromatograph
MS = mass spectrometer
LC = liquid chromatograph
ELISA = enzyme linked immunosorbent assay
VTG = vitellogenin
GSI = gonadosomatic index
OSI = ovarian somatic index
USEPA=United States Environmental Protection Agency
SS = suspended sediments
DOM = dissolved organic matter
$MS^2$= tandem mass spectrometer
$GC^2$= tandem gas chromatograph
UV = ultraviolet
FD = fluorescence detection
ND = non detect
HPLC = high performance
ESI = electrospray ionization
UV = ultraviolet treatment
$\tau_w$ = water residence time
OKR = Okanagan River
PNEC = predicted no effect concentration

## Acknowledgements

I would like to first thank my supervisor Dr. Jeff Curtis. Thank you for talking me off an academic ledge numerous times. Your patience, calm guidance and unique perspective have been appreciated and have inspired me. To my other committee members, Drs Deborah Roberts and Bruce Mathieson, your gentle encouragement and understanding have been so important and I truly thank you.

Thank you to Dr. Sandra Mecklenburg for your help during our journey through estrogen analysis. I would have been lost without you. I especially appreciate your help while I was away with a new baby. Thank you David Arkinstall at the Okanagan Regional Chemical Analysis Centre (ORCAC) at UBC Okanagan for your candor, support and help through this project. Yes I am finally finishing.

Thank you to the Cities of Vernon, Kelowna and Penticton for access to your facilities and assistance with sampling. I especially want to thank Dr. Mike Sokal and Daniel St. Hilarie of the Ministry of Environment office. Thank you for assisting me with the Penticton sampling. You have been a great help.

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To my mom, I know that you have not always understood my academic journey, but I appreciate your support and love through the process. I was especially comforted knowing my girls were so happy to go to see Oma while mommy was out. Thank you Kelly for your encouragement during times when I needed it most, you are an amazing husband. I know you will be happy to never help collect another water sample again.

## Dedication

To my incredible family who grew in both numbers and love during this project. To Kelly, 50 Fillion. To mom, the word gratitude is not strong enough. To Peyton and Rowan, you have flipped my world upside down and inside out and brought more love than I ever could have imagined, thanks for spicing up grad studies.

# Chapter 1 - The Behaviour of Introduced Estrogens in Surface Waters Receiving Treated Wastewater Effluent

## 1.1 Introduction

Low concentrations of estrogens are commonly found in urbanized waterways and more specifically within waterways that receive wastewater treatment plant (WWTP) effluent (Li et al., 2011; Zhang & Zhou, 2008). Previous research has focussed on how estrogens enter aquatic systems, potential biological effects and the methods required for analysis (Holthaus et al., 2002; Johnson et al., 2008; Kidd et al., 2007; Panter et al., 1998; Ternes et al., 1999). Most recently, the focus has been on preventing estrogens from entering into natural aquatic systems, primarily by increasing the efficacy of WWTPs (Bolong et al, 2009; Coleman et al., 2010; Kvanli et al, 2008; Ternes et al, 1999). Less research has focussed on the fate of estrogenic substances in receiving waters. The primary objective of this research was to determine an environmental loss rate for specific estrogens within different freshwater systems, in order to better inform policy on the disposal of treated wastewater effluent.

Contaminant estrogenic substances are external or exogenous substances that unnaturally stimulate the endocrine system. There is global evidence that these contaminants have detrimental effects on aquatic organisms (Harries et al., 1997; Jobling, et al., 1998; Sowers et al., 2009). Some of the documented effects in animals include intersex fish and malformations in amphibians (Jobling et al., 1998; Sowers et al., 2009). Estrogenic substances are released into freshwater aquatic systems at alarming rates and primarily through treated wastewater effluent (Jobling et al., 1998; Purdom et al., 1994; Servos et al., 2005; Vajda et al., 2011). In densely human populated areas of Britain, WWTPs contribute up to fifty percent of the river flow, and up to ninety percent during low flow months (Routledge et al., 1998). The impact of these compounds depends on whether or not estrogenic substances are rapidly scavenged or persist in the environment. A better understanding of the environmental loss rate of important estrogens will inform decisions regarding treated wastewater effluent practices.

The first step to determining an environmental loss rate is to quantify the major estrogen inputs and losses within a system. Natural and synthetic steroidal estrogens primarily enter into aquatic

systems through liquid WWTP effluent (Figure 1) (Racz & Goel, 2010). Liquid effluent has been found to be the most estrogenically potent, and is typically released directly into surface waters and therefore it is underlined in Figure 1.1 (Holbrook et al., 2002). As a result, the focus of this research is on the liquid portion of the WWTP effluent (Figure 1.1). The larger arrows in Figure 1.1 represent the higher estrogen potency. Once in the surface waters, there are natural environmental processes that further reduce estrogen concentrations.

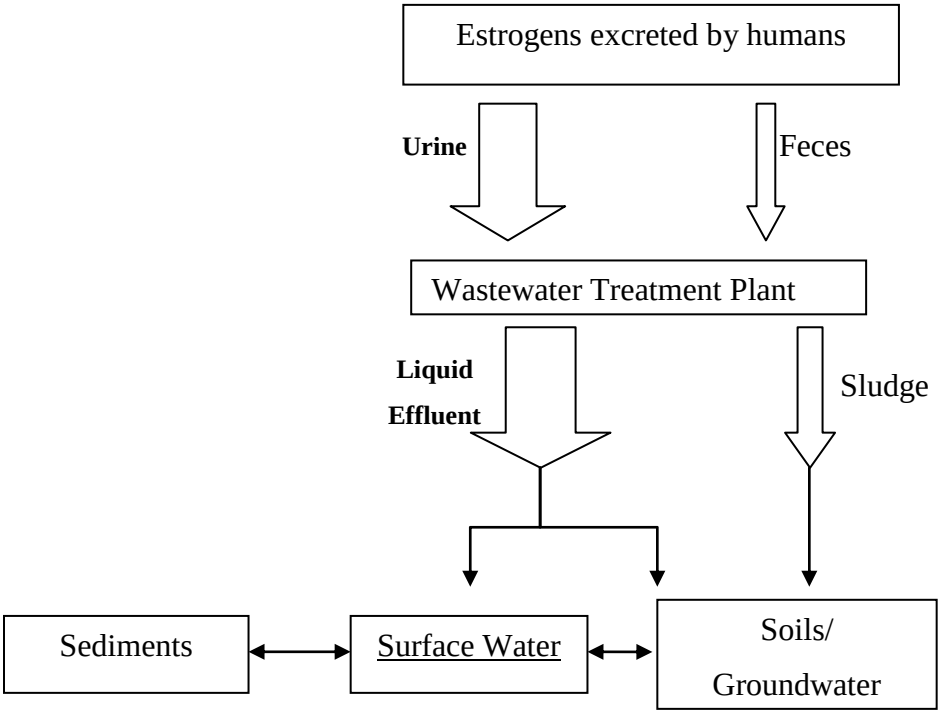



**Figure 1.1. Pathway for Estrogens from WWTPs to enter the environment. The arrows indicate the estrogen potency of the stream and surface water is underlined as the receiving water that is most estrogenically potent.**

Many different compounds have been identified as estrogenic, including natural compounds excreted from humans and synthetic compounds that are unintentionally estrogenic (Hyndman et al., 2010; Jobling et al., 1998; Panter et al., 2010). The most commonly researched estrogens include the natural hormones estrone (E1), 17-β-estradiol (E2) and estriol (E3), and synthetically-produced 17-α-ethinylestradiol (EE2) (Gabet et al., 2007; Gomes et al., 2003; Lei et al., 2009; Muller et al., 2008; Racz & Goel, 2010). Once in freshwater systems, the concentrations of these estrogens can be reduced through dilution, biodegradation, adsorption, photodegradation and volatilization (Figure 1.2). Biodegradation, adsorption, photodegradation and volatilization are scavenging mechanisms (Honeyman & Santschi, 1988). The physico-

chemical properties of the estrogenic compounds determine which mechanisms will play a significant role in the reduction of estrogens. The appropriate quantitative method for analysis of the estrogens is also determined from their physico-chemical properties.

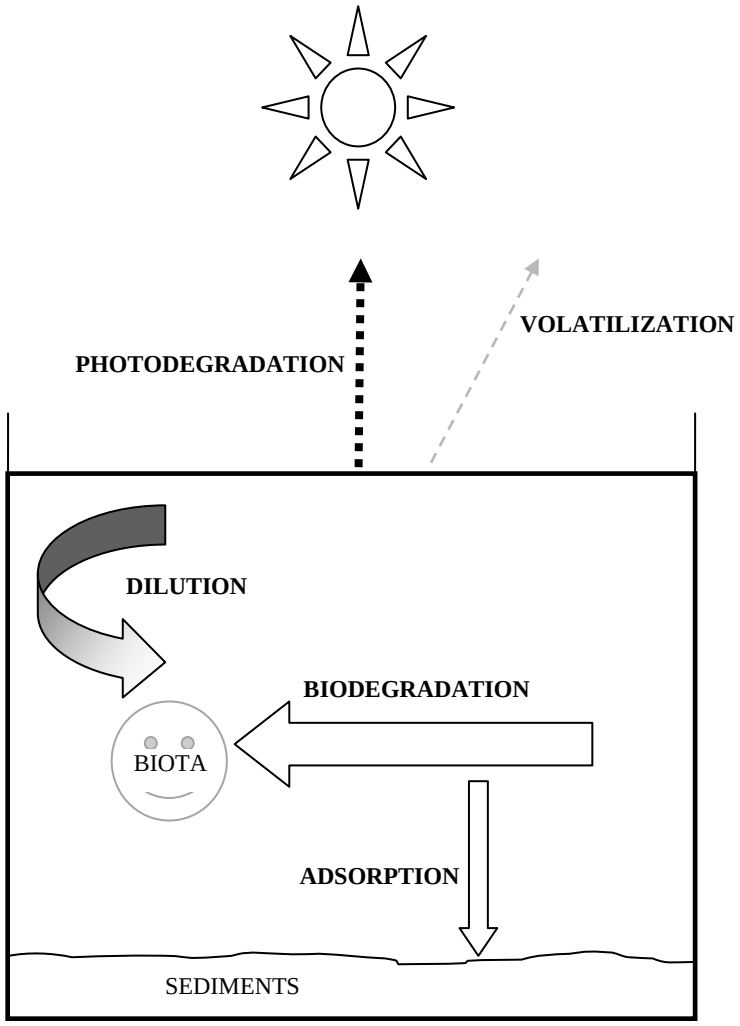



**Figure 1.2. Reduction of estrogen concentration in surface waters through dilution and scavenging mechanisms.**

The techniques used for analysis in both wastewater and freshwater include quantitative and semi-quantitative methods. To date, there has not been a standard analytical method developed for estrogens in treated wastewater or freshwater. Quantitative methods are more precise analytical methods including gas chromatography (GC) and liquid chromatography, both followed by mass spectroscopy (MS) for detection (Pacáková et al., 2009). Semi-quantitative methods include immunochemical methods such as enzyme-linked immunosorbent assay (ELISA) (Pacáková et al., 2009). Immunochemical methods are fast, inexpensive, and can be

performed in the field (Pacáková et al., 2009). This study required more precise quantitative measures, and therefore immunochemical methods were not used.

The next step to determining an environmental loss rate is to calculate the rate at which the estrogens are lost within the system based on the quantified inputs and outputs. Mathematical reactor models can be applied to the receiving water body to make this possible. Since treated wastewater effluent is commonly released into surface water in proximity to the treatment plant, lakes and river systems are key receiving environments. Reactor models have been widely used to describe these aquatic systems (Brezonik, 1994). Continuously stirred tank reactors (CSTRs) and plug flow reactors (PFRs) have been used to determine contaminant fate in lakes and rivers, respectively. These will be further described in the following sections.

Understanding the fate of estrogens in the environment is important to determine long term impact, preventative action and remediation potential. Furthermore, scavenging with respect to different freshwater aquatic systems could suggest a best-practices approach for the release of treated wastewater effluent. The contaminant loss rate should be considered when releasing potentially biologically relevant concentrations into the environment.

Throughout this study, I measured the concentrations of specific estrogen compounds in treated wastewater effluent and corresponding receiving waters. Three different systems were investigated with different receiving water characteristics. By applying a reactor model specifically adapted for each receiving water body, I calculated a loss rate constant for estrogen in receiving waters. From the different systems, I was able to infer what happens to estrogens within different types of freshwater receiving waters and, further, what may happen in generalized systems.

### 1.2 Significance of Endocrine Disruption

A World Wildlife Federation conference in 1991 concluded that "many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife and humans" (Hotchkiss et al., 2008). Affected organisms have been found in aquatic systems receiving pulp and paper mill outflow, treated or untreated human wastewater, and agricultural runoff (Jobling et al., 1998; Panter et al., 1998; Purdom et al.,

1994). Chemical analysis of this water linked the biological effects in these organisms to the presence of estrogenic compounds (Kolpin et al., 2002; Routledge et al., 1998; Servos et al., 2005; Ternes et al., 1999).

### 1.2.1 Estrogenic Effects in Fish

Considerable estrogen impact research has been conducted on vertebrates, with fish being the primary focus due to their high ecological importance (Jukosky et al., 2008; Furuichi et al., 2004; Thorpe et al., 2001). Effects on individuals are most startling, but it is when these effects aggregate to the population level that aquatic ecosystem changes occur (Jukosky, 2008; Kidd et al., 2007; Thorpe et al., 2003).

Male and female fish are affected physically and behaviourally when exposed to estrogenic substances in aquatic systems. Within male fish, there are three effects often observed. First is the expression of the egg protein vitellogenin (VTG). VTG is induced by estrogen and is normally found in female fish during oocyte maturation (Vajda et al., 2011). It is a well-established indicator of disruption due to exposure to exogenous estrogens (Burkhardt-Holm, 2010; Filby et al., 2007). VTG levels found in samples of male flounder in Holland from 1999 to 2002 were higher than in female fish, and this was linked to E1 exposure in water at a concentration of 1.0 ng $L^{-1}$ (Vethaak et al., 2005). The presence of elevated VTG levels in male fish has been linked to mortality and a decrease in fitness in male fish (Thorpe et al., 2009; Vajda et al., 2011) Secondly, the gonadosomatic index (GSI) is used to compare the average size of the gonads to the fish age and size (Garcia-Reyero et al., 2011; Panter et al., 2010). The GSI can indicate potential reproductive success, for example a low GSI found in male fish exposed to estrogens has been found to result in reduced reproductive success (Garcia-Reyero et al., 2011; Panter et al., 2010). Finally, behavioural changes in male fish can demonstrate estrogenic effects which can affect fecundity (Saaristo et al., 2010). It was found that compared to controls, males exposed to estrogens were less proficient at defending their nests until egg hatching (Garcia-Reyero et al., 2011; Hyndman et al., 2010; Saaristo et al., 2010).

In contrast to males, exposure markers within female fish are not easily distinguished. The two main effects in female fish are related to the ovarian somatic index (OSI) and behavioural changes (Lavelle & Sorensen, 2011; Van den Belt et al., 2004). Similar to the GSI in males, the

OSI can be reduced when female fish are exposed to estrogenic substances (Van den Belt et al., 2004). Exposure to E1 and E2 has been demonstrated to dramatically reduce the OSI, resulting in the absence of maturing oocytes thereby affecting reproduction (Van den Belt et al., 2004) Behavioural changes in female fish are subtle, and may be missed or noted as absent (Lavelle & Sorensen, 2011). In one study, it was noted that female stickleback fish exposed to estrogens spent a significant time motionless (Sebire et al., 2011). This subtle lack of movement during key periods of their life cycle could slow down reproduction (Sebire et al., 2011).

The male and female organismal effects can aggregate to the population level, and ultimately the ecosystem level. If organisms in a population have a reduced ability to reproduce, it will result in reduced population fecundity (Crain et al., 1997). The best example of organismal effects aggregating to the population level is in research from the Experimental Lakes Area of northern Ontario, Canada (Kidd et al., 2007). During this experiment, fathead minnows were exposed to 5 ng $L^{-1}$ of E2 over 7 years. Male fish displayed vitellogenin induction and eggs in their testes. While female fish produced fewer eggs overall and released fewer eggs during spawning. Ultimately, the population in this experiment collapsed, demonstrating that chronic low dose exposure of estrogens by fish populations is important to consider (Kidd et al., 2007).

### 1.2.2 Estrogenic Substances

There are four classes of estrogenic substances that can be found within the receiving waters of WWTPs. The first class includes the natural steroidal estrogens, and is the most studied group of exogenous estrogenic substances. The key steroidal estrogens are estrone (E1), 17-β estradiol (E2), estriol (E3). These three steroid hormones are mainly produced by females, but are also synthesized to a lesser extent by males (Barel-Cohen et al., 2006). E2 is a sex hormone that is primarily produced by the ovaries, placenta and the testes (Ying et al., 2002), and is the most common form of estrogen released in urine and feces (Lee & Liu, 2002). E1 is a metabolite of E2 and is commonly found in urine, the ovaries and the placenta (Lee & Liu, 2002; Ying et al., 2002). E3 is produced mainly during pregnancy by the placenta and to a smaller extent by the ovaries (D'Ascenzo et al., 2003). There are various conjugated and deconjugated forms of these three compounds, but their potential effects on organisms are not well understood (Hutchins et al., 2007).

The second class includes synthetic steroid hormones that are specifically designed to mimic natural estrogens within the human endocrine system. The most common synthetic steroid hormone is 17-α-ethinylestradiol (EE2) which is the most commonly prescribed synthetic compound in North America (Kuch & Ballschmiter, 2001). EE2 is derived from E2 and is an orally bio-active estrogen (Servos et al., 2005). EE2 is found in almost all modern formulations of oral contraceptives and hormone replacement therapy pills (Kuch & Ballschmiter, 2001).

The third class includes anthropogenic chemicals known as xenoestrogens, which mimic the chemical structure of steroidal estrogens and are unintentionally estrogenic (Kuch & Ballschmiter, 2001; Falconer et al., 2006) Common xenoestrogens include nonylphenols, octylphenols, atrazine and bisphenol A (Falconer et al., 2006). Some of these chemicals are pesticides, herbicides, plasticizers and industrial by-products. While xenoestrogens are ubiquitous in nature due to the various vectors, they are not designed to be estrogenic and are therefore less potent than the first two classes of estrogenic substances (Kuch & Ballschmiter, 2001; Falconer et al., 2006; Cao et al., 2010).

Finally, the fourth class of estrogenic substances are the phytoestrogens, which are natural substances found in plants such as alfalfa, lavender and soya beans (Aksglaede et al., 2009; Shore et al., 1995). Although phytoestrogens are natural, they can disrupt the natural function of endocrine system similar to other exogenous estrogens (Shore et al., 1995). While these compounds are important to note, they are often not considered within estrogenic substance research because of their lack of potency and their relatively dilute concentrations in the environment (Hotchkiss et al., 2008).

Of the four classes of estrogenic substances noted, the natural and synthetic steroidal forms of estrogen (E1, E2, E3 and EE2) are the most harmful in natural environments (Khanal et al., 2006). These compounds have been shown to be the most potent of the estrogenic substances. E1, E2 and E3 are reported to be 10,000 to 100,000 times more potent than xenoestrogens or phytoestrogens (Cao et al., 2010). The potency of these compounds relates to the estrogen receptors affinity specifically for E1, E2, E3 and EE2 (Segner et al., 2013). There are many estrogen binding sites within the body that trigger a number of different actions. Estrogens regulate metabolism, growth and reproduction among other functions (Segner et al., 2013). Potency in exposed fish was found to be EE2>>E2>E1>E3, based on the binding site affinity

(Segner et al., 2013). Further the affinities for these compounds were much greater than xenoestrogens like nonylphenol (Segner et. al., 2013). Additionally it has been found that approximately 98% of wastewater treatment plant's estrogenic composition is from E1 and E2 (Racz & Goel, 2010). For these reasons, E1, E2, E3 and EE2 were chosen as the target compounds in this study.

#### 1.2.2.1 Physico-chemical Properties of Estrogens

The specific physico-chemical properties of estrogens have an effect on their environmental fate and on analysis techniques. Important physico-chemical properties include molecular weight, molecular structure, the octanol-water partition coefficient (log $K_{ow}$), Henry's Law constant ($K_H$) and the sorption coefficient ($K_D$) (Table 1.1).

The molecular weights (Table 1.1) and structures (Figure 1.3) of E1, E2, E3 and EE2 are very similar. While EE2 is slightly heavier than the other three compounds, they are still considered light compounds. The compounds are also all very weakly polar molecules (Khanal et al., 2006). All of the estrogen molecules are small in size.

The octanol-water partition coefficient is the ratio of the solubility of a compound in octanol to the solubility in water, and is commonly represented as a log value (log $K_{ow}$) (Honeyman & Santschi, 1988). Compounds with a log $K_{ow}$ value greater than 4.0 will tend to adsorb to sediments; a value between 2.5 and 4.0 will have a midrange sorption potential; and a value below 2.5 will have a low potential to adsorb and will tend to remain in aqueous solution (Khanal et al., 2006; Lei et al., 2009; Nghiem et al., 2004). This will be further discussed in the following sections with reference to scavenging of estrogens through adsorption.

Henry's law constant ($K_H$, in atm $m^3$ $mol^{-1}$) can be determined as follows:

$$K_H = \frac{P_{org}}{C_w} \quad , \qquad (1.1)$$

where $P_{org}$ is the partial pressure of the organic compound in the gaseous state (atm), and $C_w$ is the concentration of the compound in water (mol $m^{-3}$) (Alvarez & Illman, 2005). $K_H$ and

compound volatility is considered low if it is less than 1.0 x10 $^{-7}$ atm m$^3$ mol$^{-1}$ (Racz & Goel, 2010). These estrogens have $K_H$ values below 3.80x10$^{-10}$ atm m$^3$ mol$^{-1}$(Table 1.1).

**Table 1.1 Physico-chemical properties of E1, E2 and EE2**

| **Estrogen** | **Molecular weight (MW)** g mol$^{-1}$ | **Octanol-water partition coefficient (log $K_{ow}$)** | **Henry's law constant ($K_H$)** atm m$^3$ mol$^{-1}$ | **Sorption Coefficient ($K_D$)** (log $K_D$) |
|---|---|---|---|---|
| **E1** | 270.37 | 3.13 | 3.80 x10$^{-10}$ | 2.20-2.80 |
| **E2** | 272.39 | 3.57 | 3.64 x10$^{-10}$ | 2.41-2.84 |
| **E3** | 288.4 | 2.81 | 1.3x10$^{-12}$ | - |
| **EE2** | 296.4 | 3.67 | 7.94 x10$^{-12}$ | 2.00-2.84 |
| **References** | Lai et al.,2000 | Leszczynski & Schafer, 1990 | EPISuite, 2000 | Ternes et al., 2004; Xu et al., 2009; Carballa et al., 2007 |

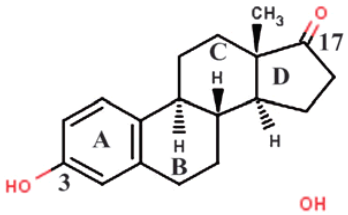
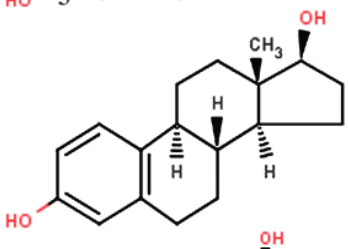
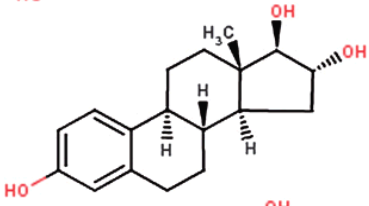
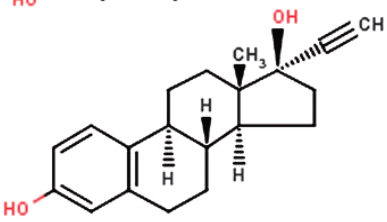
| Compound Class | Compound | Chemical Structure |
|---|---|---|
| | *Estrone (E1)* |  |
| *Class 1* | *17b-Estradiol (E2)* |  |
| | *Estriol (E3)* |  |
| *Class 2* | *17a-Ethinylestradiol (EE2)* |  |

**Figure 1.3. Chemical Structures of Estrogenic Substances**

The adsorption coefficient ($K_D$) is the ratio of the concentration of the compound attached to a solid (adsorbed) to the concentration within the aqueous solution around the solid (United States Environmental Protection Agency, 2012). $K_D$ values are often expressed as log $K_D$ (United States Environmental Protection Agency, 2012). Compounds that have a log $K_D$ value less than 2 show negligible adsorption and values greater than 4 indicate that sorption may be a significant removal process (Clara et al., 2004). In the literature, log $K_D$ values for E1, E2 and EE2 in treatment plants have found values from 2.2 to 2.8 (Carballa et al., 2007), 2.41 to 2.84 (Carballa et al., 2007; Layton, et al., 2000) and from 2.0 to 2.84, respectively (Ternes et al., 2004; Xu et al., 2009; Yi & Harper, 2007).

### 1.3 Scavenging of Estrogens in Surface Water

E1, E2, E3 and EE2 have been consistently identified as key estrogenic compounds that lead to endocrine disruption in aquatic organisms (Routledge et al., 1998). Following this, research has shifted to focus on how to prevent them from entering the environment. Emerging water quality guidelines for effluent estrogen concentrations have focussed the research attention on improving existing WWTPs and developing new or improved technologies (Gabet-Giraud et al., 2010; Nagpal & Meays, 2009). In the absence of effective removal of estrogens in WWTPs, these compounds continue to enter the environment. While there is some research on fate of estrogens in the natural environment, the understanding of loss mechanisms and loss rates remains inadequate.

Research on the fate of estrogen in the environment is just beginning. To date, the majority of this research has been conducted in controlled laboratory settings or within WWTPs (Cao et al., 2008; Jurgens et al., 2002; Liu et al., 2009; Ribeiro et al., 2010; Writer et al., 2011a). Furthermore, estrogen scavenging research has focused on single mechanisms responsible for reducing estrogen concentration. Laboratory findings have limited application in natural systems where multiple processes co-occur, making it difficult to differentiate estrogen scavenging processes outside of the lab (Johnson et al., 2008). For example, where there are high concentrations of suspended sediments (SS), it can be difficult to determine whether these sediments are helping to reduce estrogen concentrations through adsorption or by facilitating biodegradation on the particles (Liu et al., 2009; Writer et al., 2011a). To account for estrogen

scavenging in the environment, modelling exercises have been carried out in waterways (Williams et al., 1999; Williams et al., 2009). These studies do not fully account for the receiving water characteristics and changes in the scavenging mechanisms (Johnson, 2001; Jonkers et al., 2009; Writer et al., 2011).

Further to the modelling studies, there has been some research on natural waters (Writer et al., 2011a; Writer et al., 2011b; Writer et al., 2012). In these studies, estrogen reduction within a single river was investigated. Biodegradation was considered the main cause for scavenging within rivers and was therefore the focus (Writer et al. 2011b). Overall scavenging was investigated by artificially increasing estrogen concentrations in the channel and following the parcel of water downstream to detect degradation (Writer et al., 2012). Artificially increasing the estrogen concentrations above what might be naturally found in the environment could affect the determined loss rate. This is the closest to a natural study that has used to determine loss rates in the environment.

It is evident from the literature that it is difficult to determine exactly what reduces estrogen in natural aquatic systems, but understanding the overall rate is important for determining environmental regulation (Jurgens et al., 2002; Khanal et al., 2006; Lin & Reinhard, 2005; Writer et al., 2011a). Those who make decisions on the discharge practices of WWTPs can use the information on environmental loss rates of estrogens in rivers and lakes to determine the best practices. A best practices approach for estrogens would be to show levels of estrogens below detection downstream of WWTP effluent. The environmental loss rate constant and the dilution factor would help to predict where detectable estrogens might be found in a water body based on estrogen concentrations found in the effluent.

As will be expanded upon in the following sections, dilution, biodegradation and adsorption are likely the primary mechanisms for scavenging, followed by photodegradation.

### 1.3.1 Dilution

Physical dilution of estrogens as they enter into receiving waters is the first mechanism of reduction or attenuation. Dilution is the process of adding a solvent to a solution that ultimately lowers the solute concentration. In this case the solvent is the receiving water body and the solute

is the estrogen. Dilution typically plays a key role in contaminant management (Chin, 2006). This initial dilution of a contaminant in surface water needs to be sufficient to ensure that lethal quantities are not present in the water column (Chin, 2006). For discharge permitting in the United States, the United States Environmental Protection Agency (USEPA) allows a limited area or volume of water designated as a "mixing zone" in which dilution is the dominant attenuation mechanism (Chin, 2006; Writer et al., 2012).

Dilution can be calculated by:

$$C_2 = \frac{C_1 * V_1}{V_2} \quad , \qquad (1.2)$$

where $C_2$ is the contaminant concentration following dilution (ng $L^{-1}$), $C_1$ is the contaminant or estrogen concentration in the WWTP effluent (ng $L^{-1}$), $V_1$ is the volume of water in the loading stream (L), and $V_2$ is the total volume (L). The hydrology of a system, such as inflows of freshwater, precipitation and groundwater inputs and outputs, can concentrate or dilute contaminants.

### 1.3.2 First Order Reaction Kinetics

First order reaction kinetics applies to a reaction of a substance that is irreversible and involves only one reactant (Morel & Herring 1993). First order reaction kinetics can be defined by the integrated first order rate law (Morel & Herring 1993) as follows:

$$\ln[C] = -kt + ln[C_0], \qquad (1.3)$$

where [C] is the concentration following the reaction (mass per volume), [$C_0$] is the initial reactant concentration (mass per volume), k represent the first order rate constant (unitless) and t represents the time of the reaction (s,h,d etc.).

First order kinetics are commonly used define scavenging of estrogens in both the environment and wastewater treatment plants (Campbell et al., 2006; Cao et al., 2010; Johnson & Williams, 2004; Khanal et al., 2006; Liu et al., 2004). First order reaction kinetics has been found to adequately describe the degradation of organics within wastewater treatment systems, and it is assumed to similarly describe scavenging of estrogenic compounds (Hashimoto & Murakami, 2009). There have been a few studies that have experimentally tested this assumption (Johnson & Williams, 2004; Liu et al., 2004; Xu et al., 2009). In these cases first order kinetics best described the scavenging of estrogens. The focus of these experiments was on biodegradation, therefore the first order degradation applied specifically to biodegradation (Johnson & Williams, 2004; Liu et al., 2004; Xu et al., 2009). Other research has suggested that photodegradation of estrogens also follows first order kinetics (Lin & Reinhard, 2005; Zhang et al., 2007). Without further information on dominant scavenging processes or better alternative models, I also assumed first order reaction kinetics in calculating estrogen loss rate constants.

### 1.3.3 Biodegradation

Biodegradation is the breakdown of compounds through microbiological action (Alvarez & Illman, 2005). The bioavailability or accessibility of an organic compound to biodegradation can be determined in part by its size (Alvarez & Illman, 2005). Bioavailable compounds like estrogens are typically small (Table 1.2), which means they are more accessible to microbiota (Alvarez & Illman, 2005).

Biodegradation is considered the most significant process for scavenging organic contaminants from the environment (Alvarez & Illman, 2005; Campbell et al., 2006). The majority of research on biodegradation of estrogens has been done within WWTPs in order to determine the best processes for removal prior to release to receiving waters (Xu et al., 2009). Biodegradation within WWTPs occurs very quickly, with measured half-lives of 0.3 to 0.7 hours for E1to 1.5 to 4.4 hours for EE2 (Ribeiro et al., 2010; Xu et al., 2009). WWTPs are designed for enhanced biodegradation. Within WWTPs, higher temperatures, lower pH, and longer retention times have

been shown to improve rates (Cao et al., 2010). Biodegradation rates in the environment are likely slower than in treatment plants due to lower concentrations of microorganisms and fluctuations in pH, temperature and light (Cao et al., 2008; Ribeiro et al., 2010).

### 1.3.4 Adsorption

Adsorption occurs when matter accumulates on a solid without the development of a three dimensional molecular arrangement (Honeyman & Santschi, 1988). Adsorption is dictated by the octanol-water partition coefficient ($K_{ow}$), the sorption coefficient ($K_D$) and the compound polarity (Tables 1.1 and 1.2) (Alvarez & Illman, 2005). Large log $K_{ow}$ and $K_D$ values would indicate that adsorption to particles would be significant (Khanal et al., 2006).

Since estrogens are mostly non-polar and hydrophobic, they are likely to preferentially adsorb to organic particles (Alvarez & Illman, 2005). Research into how adsorption of estrogens relates to the log $K_{ow}$ values suggests that values near 4 result in very fast sorption rates (<1hr) (Clara et al., 2004; Hashimoto & Murakami, 2009; Suzuki & Maruyama, 2006). $K_D$ values for estrogens have been determined in WWTPs (Carballa et al., 2007; Cicek et al., 2007; Ternes et al., 2004; Xu et al., 2009). $K_D$ values are only applicable to the aqueous environments in which they were determined (United States Environmental Protection Agency, 2012). While it is ideal to use the situation-specific $K_D$ value, literature values can be used for rough estimates.

The majority of the research within WWTPs shows that estrogens, having log $K_D$ values between 2 and 4 results in adsorption to particles present in the treatment plant (Clara et al., 2004; Hashimoto & Murakami, 2009; Suzuki & Maruyama, 2006). High concentrations of the absorbate (estrogens) and organic particles in wastewater compared to natural systems allows for greater adsorption rates (Alvarez & Illman, 2005) For this reason, the log $K_D$ rates reported in the literature may be overestimated for adsorption processes in natural receiving waters.

While adsorption is common and well understood within WWTPs, there have only been a few studies within the natural environment (Racz & Goel, 2010; Zhang & Zhou, 2008). In one study, water and sediment samples were taken from five rivers in Britain and kept in jars under conditions that would inhibit biological degradation (Holthaus et al., 2002). After 1 day, 80-90% of E2 and EE2 adsorbed to the suspended sediments (Holthaus et al., 2002). Estrogen's large log

$K_{ow}$ values, log $K_D$, and hydrophobicity indicate that adsorption is likely important in nature (Racz & Goel, 2010; Zhang & Zhou, 2008).

### 1.3.5 Photodegradation

Photodegradation (photolysis) is the degradation of compounds through the absorption of particular wavelengths of light (Legrini et al., 1993). Photodegradation may occur directly or indirectly. Direct photodegradation occurs when a chemical is transformed or excited through the absorption of photons (Schwarzenbach et al., 1993). Direct photodegradation is more frequent in compounds that contain a high number of conjugated double bonds, such as dissolved organic matter (DOM) (Wetzel, 2001). Indirect photodegradation occurs when another chemical is excited through absorption of photons, and a series of reactions follow causing transformation of the target chemical (photosensitization) (Schwarzenbach et al., 1993). DOM and nitrate/nitrite are common photosensitizers in the environment (Caupos, et al, 2011).

Photodegradation has been investigated in laboratory settings (Caupos et al., 2011; Chowdhury et al., 2010; Leech et al., 2009; Mazellier et al., 2008; Zhang et al., 2007). These experiments primarily expose environmental water, or ultrapure water (laboratory grade water, purified to contain no or few other anions or cations other than $H^+$ and $OH^-$) inoculated with high concentrations (>1mg $L^{-1}$) of estrogens, to UV light that is comparable to peak sunlight (Caupos et al., 2011; Chowdhury et al., 2010; Leech et al., 2009; Mazellier et al., 2008; Zhang et al., 2007). Through these experiments it has been found that both direct and indirect photodegradation of E1, E2, E3 and EE2 occur (Caupos et al., 2011; Chowdhury et al., 2010; Leech et al., 2009; Mazellier et al., 2008; Zhang et al., 2007). Also estrogen photodegradation increases with increasing concentrations of DOM up to 8mg $L^{-1}$ (Chowdhury et al., 2010).

There are significant limitations to applying results from laboratory studies to the environment. Most studies determined that in order to extrapolate laboratory results to the environment, one needed to consider the concentration of DOM, the type of DOM, the concentration of estrogens, solar light intensity, and the attenuation of light within the water body (Chowdhury et al., 2010; Lin & Reinhard, 2005; Mazellier et al., 2008; Racz & Goel, 2010). Without DOM present at appropriate concentrations, photodegradation may not occur at a rate that is measurable (Caupos et al., 2011; Racz & Goel, 2010). The laboratory experiments typically used a light intensity that

mimicked peak sunlight intensity (Caupos et al., 2011; Chowdhury et al., 2010; Liu et al., 2009; Mazellier et al., 2008), but in the environment, solar light intensity varies throughout the day and throughout the seasons. E1, E2, E3 and EE2 do not readily absorb light in the visible spectrum (Racz & Goel, 2010; Writer et al., 2012). They do, however, readily absorb light between 290-400nm with peak photodegradation occurring in the UV-B (290-320nm) spectrum (Leech et al., 2009; Chowdhury et al., 2010). Finally, WWTP effluent which releases estrogens is typically released low in the water column where there is little light penetration (Brezonik, 1994; Hammer & Hammer, 2004).

### 1.3.6 Volatilization

Volatilization is the change in a compound from a liquid or solid state to a gaseous state (Alvarez & Illman, 2005). The volatility of compounds can be determined primarily by the Henry's Law constant (Khanal et al., 2006).

Estrogen compounds have low $K_H$ values (Table 1.1), indicating that they have low volatility (less than 1.0 x10 $^{-7}$ atm $m^3$ $mol^{-1}$ ), suggesting that they would remain in an aqueous solution rather than vaporizing (Racz & Goel, 2010). Therefore, volatilisation losses to the atmosphere are not important to the environmental fate of estrogens.

## 1.4 Measurement Techniques for Estrogens in WWTPs and Aquatic Systems

To determine a loss rate constant for estrogens in aquatic systems, the target compounds need to be measured using quantitative techniques that are reproducible. Since estrogen concentrations are very low in the environment, there are three steps to achieve good quantitative analysis. First, the sample must be pretreated in order to enhance the sensitivity of the analysis. Most analytical techniques for the analysis of estrogens in aqueous samples include a solid phase extraction (SPE) pre-treatment step (Streck, 2009; Tomsíková et al., 2012). Secondly, a separation step is required to isolate the specific compounds required for analysis. Liquid chromatography (LC) and gas chromatography (GC) are the most common separation techniques for estrogens (Tomsíková et al., 2012). Finally, a sensitive detector is required to detect the compounds separated from the sample mixture. The types of detectors that have been used for estrogen determination, in descending order of sensitivity, include tandem mass spectroscopy ($MS^2$), mass

spectroscopy (MS), ultraviolet (UV) detection, and fluorescence detection (FD) (Tomsíková et al., 2012). UV and FD detectors are not sensitive enough for most environmental samples which contain trace levels of estrogens (Tomsíková et al., 2012). Immunochemical methods have been extensively used in the past, but have been largely replaced by LC-MS/LC-$MS^2$ and GC-MS/GC-$MS^2$ for quantitative analysis.

Samples are first filtered to remove particulates which can result in erroneous readings by the mass spectrometer (Streck, 2009). Following filtration, a reverse-phase pre-treatment step is used for estrogens because they are weakly polar or non-polar. Reverse-phase SPE is a process whereby non-polar (hydrophobic) or weakly polar compounds are selected for and adsorbed to a solid material, and polar or hydrophilic compounds pass through, thereby isolating the desired compounds (Hennion, 1999; Simpson, 1998). The SPE step concentrates the desired analytes and lowers the overall detection limit of the method, regardless of which analytical method is chosen. Within the literature surveyed, Oasis HLB and C-18 methods are the most common reverse-phase SPE methods (Pacáková et al., 2009). C-18 columns are readily available commercially and are inexpensive. Florisil columns have been often incorporated in pre-cleanup following HLB or C-18, in order to further reduce matrix effects (Pacáková et al., 2009). In this research, C-18 and florisil columns were used for the initial SPE step.

GC-MS/GC-$MS^2$ and LC-MS/LC-$MS^2$ are the methods of choice throughout the literature for separation and detection of estrogens (de Alda & Barcelo, 2001). Simply put, chromatography is the separation of a mixture. In LC, the mixture is a fluid that contains target compounds to be analysed (mobile phase), passed through an assembly containing a stationary phase or a different material (Benijts et al., 2004; Hennion, 1999). The stationary phase is selected based on its affinity for the target compounds. The target compounds adsorb onto the stationary phase based on their physico-chemical properties. In GC, the fluid mixture is in a gaseous state (mobile phase) (de Alda & Barcelo, 2001). Both methods have advantages and disadvantages for estrogen analysis.

The primary disadvantages of GC are that it is time consuming, costly, and can add further analytical error due to the addition of a derivatization step required to increase the volatility of estrogens for determination (Streck, 2009; Tolgyesi et al., 2010). Derivatization is the process whereby the target analytes are transformed to compounds (derivatives) that are easier to

volatilize (Pietrogrande & Basaglia, 2007). As noted previously, estrogens have low volatility (Table 1.1) and therefore they need to be derivatized to make them more volatile for GC analysis (Tolgyesi et al., 2010). This is essentially forcing a low volatility compound into a gaseous state. There is a risk that, during this process, not all of the analyte will be completely converted to a volatile derivative which will result in incorrectly measured low concentrations (Tomsíková et al., 2012). The primary advantage of GC is that it can be highly selective, and is chosen when there are complex matrices which might result in matrix effects (discussed below with regards to LC) (Heath et al., 2010; Johnson et al., 2008).

The primary disadvantage to using LC is the presence of matrix effects. Matrix effects include ion suppression, where non-target chemical species reduce the ability of the analyte to split into the individual ions (ionization) (Johnson et al., 2008). Ion suppression results in incorrectly low concentrations determined (Johnson et al., 2008). A complex solution may require more than one cleanup step (filtration and multiple SPE steps), which can reduce recovery rates (Johnson et al., 2008). Properly preparing the sample before analysis, and using an internal standard, can reduce the effects of ion suppression and identify sample loss (Gabet et al., 2007). An internal standard is a compound that has a known concentration and a known behaviour in the experimental method (Baronti et al., 2000; Kolpin et al., 2002). Even with its disadvantages, the LC-$MS^2$ method is able to reach detection limits below 1 $ngL^{-1}$ for most estrogens (Gabet et al., 2007).

Both GC and LC methods may be followed by MS or tandem MS ($MS^2$). The preference is for tandem mass spectroscopy when sample matrices are complex such as wastewater effluent and receiving waters (de Alda & Barcelo, 2001). The first MS is typically used to select compounds to be analysed from the sample following LC or GC (de Alda & Barcelo, 2001). The second MS is then used to quantify the ions that have been requested (de Alda & Barcelo, 2001). $MS^2$ has been reported to increase the sensitivity and precision of the analysis for estrogens (Tomsíková et al., 2012).

LC-$MS^2$ with an internal standard is preferable to GC-$MS^2$ when investigating estrogens because of the lower associated costs and greater precision. LC-$MS^2$ is also time conscious in an already time-consuming process. Overall, LC-$MS^2$ is convenient, appropriate to environmental monitoring, and has been widely accepted throughout the literature (Pacáková et al., 2009;

Tomsíková et al., 2012). For these reasons, analysis of estrogens in the receiving waters and the WWTPs was done using LC-$MS^2$.

### 1.5 Surface Water Characteristics affecting Loss Rate

Modelling the environmental fate of a contaminant is very common (Wania & Mackay, 1999). These models start with the principles of mass balance, which was originally introduced by Lavoiser ("Lavoisier's Law). This principle states that in a closed system mass is not created or destroyed. In aquatic pollution research, this law enables accounting of a contaminant in a water body through:

$$\begin{array}{l}\text{Rate of}\\ \text{accumulation in}\\ \text{system}\end{array} = \begin{array}{l}\text{Rate of}\\ \text{reactant inflow}\end{array} - \begin{array}{l}\text{Rate of reactant}\\ \text{outflow}\end{array} + \begin{array}{l}\text{Rate of change}\\ \text{from reaction}\\ \text{within system}\end{array} \quad , (1.4)$$

Applying the mass balance equation (1.4) to a river or lake is useful for tracking contaminants. The river or lake becomes the boundaries over which mass balance applies. The assumption that rivers and lakes behave like reactor models is reasonable when considering contaminant behaviour (Brezonik, 1994). Rivers are analogous to plug flow reactor (PFR) models and most lakes and reservoirs can be considered to behave like continuously stirred tank reactor (CSTR) models (Brezonik, 1994). Using mass balance principles with PFR and CSTR reactors, the environmental fate of E1, E2, E3 and EE2 in rivers and lakes/reservoirs can be determined.

#### 1.5.1 Plug Flow Reactor

In an ideal PFR (Figure 1.4), a reactant mixture moves downstream in a plug, and within that plug the mixture is uniform (Thibodeaux, 1996). Mixing only occurs within the plug and does not occur up or downstream. The contaminant or reactant flows through the reactor at a uniform rate with concentration decreasing over the length of the pathway as a result of scavenging processes (Hammer & Hammer, 2004). Since the assumption is that within a PFR the flow velocity is uniform throughout, time and distance down the flow path are equal. In principle, the

longer the PFR, the longer the contaminant spends in the reactor and the greater the chance for scavenging.

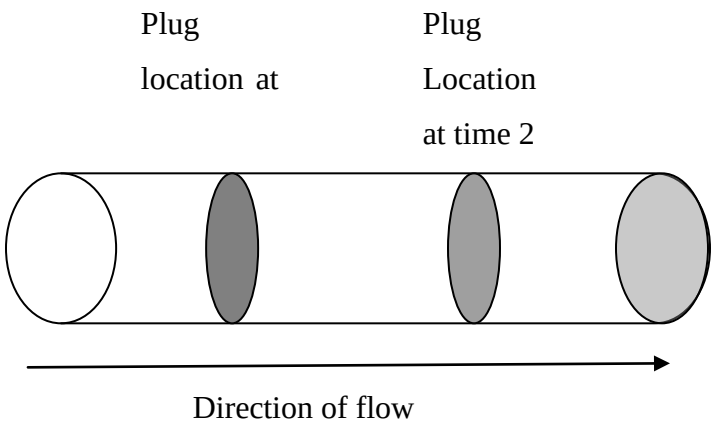



**Figure 1.4. Plug Flow Reactor (PFR) adapted from (Hammer & Hammer, 2004)**

Environmental scavenging rates in a PFR model can be calculated by:

$$\frac{C_x}{C_o} = e^{-(\frac{kA}{Q})l} \quad (1.5)$$

where $C_x$ is the estrogen concentration at time x (ng $L^{-1}$), $C_o$ is the initial estrogen concentration in the river (ng $L^{-1}$), k is the first order reaction rate constant ($s^{-1}$) A is the cross-sectional area of the PFR ($m^2$), Q is the discharge of the PFR ($m^3$ $s^{-1}$), and l is the length of the channel (m). In a PFR, t =(A*l)/Q so equation (1.4) becomes:

$$\frac{C_x}{C_o} = e^{-kt} \quad (1.6)$$

In reality, uniform movement is hindered by friction on the side of the channel and resulting turbulent flow of the water causes short circuiting or non-uniform movement (Hammer & Hammer, 2004).

### 1.5.2 Continuously Stirred Tank Reactor

In an ideal CSTR, it is assumed that complete mixing occurs and that the reactant concentrations are the same throughout the volume of the tank (Brezonik, 1994). The mass of the contaminant (estrogen) will enter into the tank (lake) and be instantly mixed throughout the whole volume of the lake (Figure 1.5). Contrary to the PFR, the contaminants in a CSTR are transported through dispersion only and the contaminant concentration decreases through initial dilution and scavenging within the reactor (Brezonik, 1994).

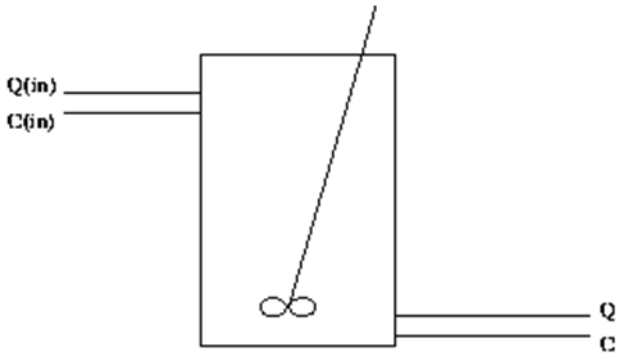



**Figure 1.5. Continuously Stirred Tank Reactor (CSTR) (Hammer & Hammer, 2004) (Q is the flow and C is the concentration)**

Applying mass balance and first order reaction kinetics into the CSTR the equations are as follows:

$$\frac{dm}{dt} = m_{in} - m_{out} + m_{rxn} \quad (1.7)$$

$$\frac{dC}{dt} = -(\frac{Q}{V} + k)C \quad (1.8)$$

$$\ln(Ct) - \ln(Ci) = -(\frac{Q}{V} + k)t \quad (1.9)$$

$$\frac{Ct}{Ci} = e^{-(\frac{Q}{V}+k)t} \quad (1.10)$$

Equation 1.7 demonstrates the mass balance of the reactor where change in mass within the reactor is as a result of the mass into the reactor ($m_{in}$) the mass out of the reactor ($m_{out}$) plus the mass of the reaction either decreasing or increasing concentrations of the contaminant ($m_{rxn}$). In Equation 1.8 the change in contaminant concentration (C ng $L^{-1}$) over time (s) is derived from the discharge of the contaminant (Q,L $s^{-1}$) the volume of the reactor (V,L) the reaction rate constant ($s^{-1}$) and the concentration of the contaminant in the reactor. Incorporating first order reaction kinetics into 1.8 gives 1.9 where Ct is the contaminant concentration at time t (ng $L^{-1}$) and Ci is the initial contaminant concentration (ng $L^{-1}$) and rearranging the equation result in 1.10.

The homogeneity assumption is not always true in lakes, as they can be stratified based on temperature or salinity differences into essentially separate layers. Stratified lakes in most cases do completely mix periodically and the CSTR theory is applicable during this mixing time scale (Brezonik, 1994). The longer the time a contaminant spends in the reactor, the greater the potential for scavenging and therefore the hydraulic residence time of the CSTR is fundamental to the reduction of contaminants (Brezonik, 1994).

### 1.6 Objectives

Previous work has been done on the environmental fate of estrogens and this research has determined that estrogens do degrade and are scavenged by primarily biodegradation and

adsorption (Holthaus et al., 2002; Jurgens et al., 2002; Writer et al., 2011a; Writer et al., 2012). These studies primarily incorporated laboratory techniques that did not sufficiently encompass variability within the natural systems.

The focus of the literature has been on the effect of estrogens on fish, the methods used for analysis and the optimization of WWTPs. Through this research it has been determined that fish are negatively impacted by the potent E1, E2, E3 and EE2 estrogens ultimately leading to the potential collapse of populations. While advances have been made on which treatment processes can reduce estrogens, investigations of WWTP effluents show that estrogens continue to enter aquatic systems. Within treatment plants biodegradation and adsorption are key scavenging mechanisms. Unfortunately there are no definite answers to how estrogens are reduced in the environment. The research that I carried out was a first step in addressing the literature gap on environmental loss rates of E1, E2, E3 and EE2 in surface freshwater.

The goal of this research was to determine an environmental loss rate constant for E1, E2, E3 and EE2 in three different freshwater systems which receive treated wastewater. Mass balance and mathematical reactor modelling was used to account for the individual characteristics of each water body. By sampling treated effluent and receiving water concentrations, the loss rate constants of the estrogens could be quantified. Three WWTPs with different discharge practices were investigated in Penticton, Kelowna and Vernon, British Columbia, Canada. The WWTPs all utilize tertiary treatment focused on removing nitrogen and phosphorous. Since the treatment mechanisms are similar and the contributing waste streams are similar, it was expected that they would be releasing similar levels of estrogens. The main difference between the plants was the use of ultraviolet (UV) treatment at the end of the treatment process. The Penticton WWTP did not have UV during the time of this study. In Kelowna, UV is used 100% of the time, and in Vernon UV is used only during irrigation season. The effect of UV was briefly investigated as part of this study. These loss rate constants could potentially inform policy for the disposal of treated effluent in receiving waters to ensure that biologically relevant concentrations are not found in surface waters.

# Chapter 2 – The Determination of an Environmental Loss Rate Constant for Estrogens in Waters Receiving Treated Wastewater

## 2.1 Introduction

The objective of this study was to determine environmental loss rates of estrogens in lakes and rivers by first measuring estrogens in treated effluent and receiving waters, and then applying mass balance and reactor model theory to find a loss rate constant. E1, E2, E3 and EE2 were chosen based on their prevalence in wastewater treatment plant effluent and their potency (Li et al., 2011; Writer et al., 2012; Zhang & Zhou, 2008). The feminization of male fish has been the most striking result of exposure to estrogens, in particular E1, E2 and EE2 (Jobling et al., 1998; Panter et al., 2000). Effects such as the reduction in male gonad size, changes in spawning behaviour and the presence of eggs in the testes are examples of how these estrogens are disrupting the reproductive success of fish (Garcia-Reyero et al., 2011; Hyndman et al., 2010; Saaristo et al., 2010;Vajda et al., 2011; Kidd et al.,2007)

Three wastewater treatment plants (WWTPs) were selected based on their similar treatment plant design and the corresponding different effluent receiving waters. From the receiving waters concentrations, loss rates were examined in a river, lake and reservoir corresponding to a plug flow reactor (PFR) and a continuously stirred tank reactor (CSTR) respectively. The receiving waters represent a wide range of water residence times ($\tau_w$) in order to account for a range in loss rates. Okanagan River has a short $\tau_w$ (<1 hour to 8 hours), Okanagan Lake has a very long $\tau_w$ (>50 years), and MacKay reservoir has a $\tau_w$ of about 1 year. These systems were chosen for their sensitivity to detect rates of scavenging that might be from hours to decades. In this design it was unlikely that rates could be estimated from two systems, but very likely that one system would be useful. Samples were collected from the wastewater effluent and the receiving waters approximately monthly during 2008 and 2009.

## 2.2 Methods

### 2.2.1 Site Descriptions

The wastewater treatment plants (WWTPs) and receiving waters used in this study are situated in the Okanagan Valley, British Columbia, Canada (Figure 2.1). The valley is oriented approximately north-south, and is dominated by a series of large lakes in the valley bottom. The largest lake is Okanagan Lake, which drains south through the Okanagan River, a headwater tributary to the Columbia River. The Okanagan Valley bottom is a semi-arid desert with average precipitation of 315 mm annually (Nordin, 2005). Evaporation from Okanagan Lake is significant and estimated at 1 m per year (Nordin, 2005).

The three research sites are located in the south (Penticton), central (Kelowna), and north Okanagan Valley (Vernon) (Figure 2.2). The receiving waters for the Penticton, Kelowna, and Vernon WWTPs are the Okanagan River, Okanagan Lake, and MacKay Reservoir, respectively. Though the population size contributing to each WWTP differs, the age and gender demographics are the same (Statistics Canada 2012 a,b,c). The age and gender demographics are noted in case there is a relationship between population composition and effluent estrogen concentrations.

The Penticton WWTP discharges an average of 12 ML $d^{-1}$ of liquid effluent into a channelized section of Okanagan River (OKR) downstream of Okanagan Lake (City of Penticton, 2009). The river flows into Skaha Lake, approximately 3.3km south of the WWTP. Flow in the river is managed to maintain water levels in Okanagan Lake. During this study discharge ranged from 5 to 40 $m^3$ $s^{-1}$, with the lowest flows occurring during the winter months (Environment Canada, 2011). For range of discharge during this study, OKR could contain 0.3 to 3% treated wastewater. During 2009-2010 the travel time between the WWTP outfall and Skaha Lake was calculated between 0.9 to 7.8 hours, depending on flow rates. As with most rivers, the Okanagan River below the Penticton WWTP conforms to a plug flow reactor (PFR) model (Brezonik, 1994). Okanagan River is an important salmonid spawning channel, and Skaha Lake is also home to many important resident fish species (Lawrence, 2003).

The Kelowna WWTP discharges approximately 29 ML $d^{-1}$ of liquid effluent into Okanagan Lake at 60m depth, and is the largest single source of wastewater in the valley (City of Kelowna,

2009). There are currently two other known WWTPs discharging into Okanagan Lake, but both are from very small communities. Okanagan Lake is the largest lake in the drainage basin, and has a hydraulic residence time >50 years (City of Kelowna, 2009).Wastewater inflow to Okanagan Lake from the Kelowna WWTP accounts for about 1.2% of all inputs on an annual basis, however, during natural low-flow periods (August to February), the proportion increases to about 3% (Guy, 2010). Okanagan Lake is a warm monomictic lake with turnover occurring between October and May (Nordin, 2005). Very little mixing occurs between layers in the lake, but there can be some mixing within layers (Brezonik, 1994). A conventional CSTR assumes mixing is complete and fast (Brezonik, 1994), and therefore Okanagan Lake cannot be considered a CSTR over short periods of time (<1 year), but it can be considered completely mixed over the period in which stratification and then complete mixing occurs.

The Vernon WWTP produces an average of 13 ML $d^{-1}$ liquid effluent which is pumped into MacKay reservoir (Manwell & Geller, 2010). MacKay reservoir is a landlocked pond with no natural surface water inflow or outflow. The average depth is approximately 18 m and the average surface area is 324,704 $m^2$. The reservoir does not contain an important fishery, and was designed specifically to store wastewater effluent. For this study, the MacKay reservoir water balance was calculated using a previous water balance updated with current values (Manwell & Geller, 2010). Since the reservoir is not lined, about 20% of total water inflows of water is lost and gained from groundwater (Manwell & Geller, 2010). There are some contributions from precipitation over the reservoir (~4% of total water inflows). The primary inflow is from the treatment plant and the primary outflow is through an irrigation system. The reservoir is operated so that filling occurs all year round, but the outfall pipe is only open from April to September. Ideally, the reservoir inflows are equal to outflows on an annual basis so that net storage is zero. Fine bubblers within the reservoir prevent stratification, resulting in a system that is analogous to a CSTR (Brezonik, 1994).

The three receiving waters studied had different flow characteristics and water residence time ($\tau_w$). To account for individual characteristics, reactor models were used. The PFR and CSTR reactor models will treat contaminants differently.

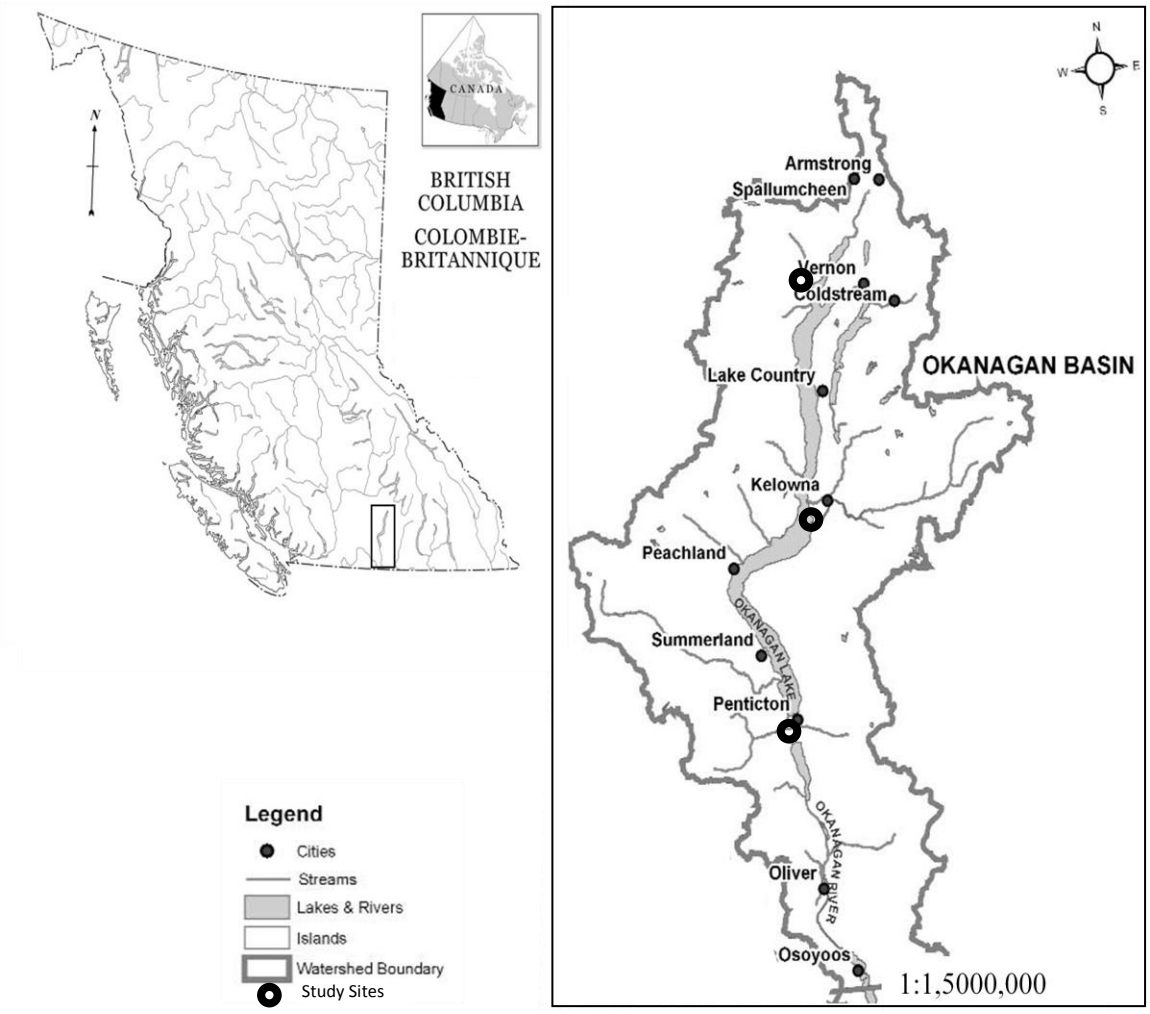



**Figure 2.1. Map of study area showing locations of the cities where sampling occurred within the Okanagan Basin. Maps of British Columbia reproduced with permission of Natural Resources of Canada (2012), courtesy of Atlas Canada. Map of Okanagan Basin adapted from Rae (2005)**

### 2.2.2 Sample Collection

Water samples were collected using 1 or 4 L amber glass bottles that had been pre-cleaned with solvent and using Teflon lined caps. Samples were stored at approximately 4$^{o}$C without using preservatives for a maximum of 4 days. Samples were collected approximately monthly from July 2009 to October 2010 (Table 2.1 and 2.2).

**Table 2.1 Wastewater Treatment Plant Sample Days**

| Wastewater Treatment Plant | Sample Date |
|---|---|
| Penticton | Jul 16, 2009 |
| Penticton | Sep 9, 2009 |
| Penticton | Oct 7, 2009 |
| Penticton | Nov 18, 2009 |
| Penticton | Jan 21, 2010 |
| Penticton | Mar 11, 2010 |
| Penticton | May 7, 2010 |
| Penticton | Jun 1, 2010 |
| Penticton | Jul 7, 2010 |
| Penticton | Aug 17, 2010 |
| Penticton | Oct 20, 2010 |
| Kelowna Pre-UV | Jul 20, 2009 |
| Kelowna Pre-UV | Aug 20, 2009 |
| Kelowna Pre-UV | Sep 22, 2009 |
| Kelowna Pre-UV | Oct 23, 2009 |
| Kelowna Pre-UV | Nov 17, 2009 |
| Kelowna Pre-UV | Dec 8, 2009 |
| Kelowna Pre-UV | Jan 19, 2010 |
| Kelowna Pre-UV | May 5, 2010 |
| Kelowna Pre-UV | Jun 17, 2010 |
| Kelowna Pre-UV | Jul 27, 2010 |
| Kelowna Pre-UV | Aug 17, 2010 |
| Kelowna Pre-UV | Oct 28, 2010 |
| Kelowna Post-UV | Jul 20, 2009 |
| Kelowna Post-UV | Aug 20, 2009 |
| Kelowna Post-UV | Sep 22, 2009 |
| Kelowna Post-UV | Oct 23, 2009 |
| Kelowna Post-UV | May 5, 2010 |
| Kelowna Post-UV | Jun 17, 2010 |
| Kelowna Post-UV | Jul 27, 2010 |
| Kelowna Post-UV | Aug 17, 2010 |
| Kelowna Post-UV | Oct 28, 2010 |
| Vernon Pre-UV | Jul 6, 2009 |
| Vernon Pre-UV | Aug 20, 2009 |
| Vernon Pre-UV | Sep 28, 2009 |
| Vernon Pre-UV | Oct 21, 2009 |
| Vernon Pre-UV | Nov 13, 2009 |
| Vernon Pre-UV | Dec 4, 2009 |
| Vernon Pre-UV | Jan 19, 2010 |
| Vernon Pre-UV | May 17, 2010 |
| Vernon Pre-UV | Jun 29, 2010 |
| Vernon Pre-UV | Aug 9, 2010 |
| Vernon Pre-UV | Sep 9, 2010 |

| Wastewater Treatment Plant | Sample Date |
|---|---|
| Vernon Pre-UV | Oct 26, 2010 |
| Vernon Post-UV | Jun 29, 2010 |
| Vernon Post-UV | Aug 9, 2010 |
| Vernon Post-UV | Sep 9, 2010 |

**Table 2.2 Receiving water sample dates**

| Receiving Water | Sample Date |
|---|---|
| Okanagan River | Dec 8, 2009 |
| Okanagan River | Jan 21, 2010 |
| Okanagan River | Feb 6, 2010 |
| Okanagan River | Mar 11, 2010 |
| Okanagan River | May 7, 2010 |
| Okanagan River | Jul 7, 2010 |
| Okanagan River | Aug 17, 2010 |
| Okanagan River | Oct 15, 2010 |
| Okanagan Lake | Jul 30, 2009 |
| Okanagan Lake | Aug 24, 2009 |
| Okanagan Lake | Sep 23, 2009 |
| MacKay Reservoir | Aug 20, 2009 |
| MacKay Reservoir | Sep 28, 2009 |
| MacKay Reservoir | Nov 27, 2009 |
| MacKay Reservoir | Apr 26, 2010 |
| MacKay Reservoir | May 28, 2010 |
| MacKay Reservoir | Jun 22, 2010 |
| MacKay Reservoir | Jul 30, 2010 |
| MacKay Reservoir | Sep 2, 2010 |
| MacKay Reservoir | Oct 15, 2010 |

In Penticton, grab samples were collected from the outlet of the WWTP. Okanagan River was sampled at two locations upstream (2500 and 900 m) and four sites downstream (50, 1000, 1800 and 3300 m) of the effluent pipe (Table 2.3). River samples were collected midstream at approximately 50 cm depth to avoid sediment.

**Table 2.3 Okanagan River sample sites**

| River Sample Site (distance relative to the WWTP outfall pipe) | Geographic Coordinates | |
|---|---|---|
| -2500m | 49°30’01.42”N | 119°36’50.76” |
| -900m | 49°29’14.93”N | 119°36’35.71” |
| 50m | 49°28’54.28”N | 119°36’00.71” |
| 1000m | 49°28’25.12”N | 119°35’47.04” |
| 1800m | 49°27’56.83”N | 119°35’40.32” |
| 3300m | 49°27’12.36”N | 119°35’50.59” |

The Kelowna WWTP uses ultraviolet light treatment (UV) for disinfection purposes. When possible (Table 2.1), grab samples were taken pre-UV and post-UV.

Grab samples were collected from Okanagan Lake under stratified conditions (Table 2.4) using a Van Dorn water sampler. Water samples were collected near the outfall pipe (Latitude 49° 51' 42.74"N, Longitude 119° 30'26.48"W).

Specific conductance measurements were also taken in the approximate area of the wastewater plume in Okanagan Lake, estimated using a plume flow model designed for the City of Kelowna (Hay and Company Consultant Inc, 2001). Conductivity measurements were taken on July 6, 2010 using a YSI 600R water quality sampling sonde and a 650MDS multi-display meter, in order to confirm the location of the effluent plume (Figure 2.3). The conductivity readings were obtained along an irregular grid pattern that was determined by trolling through the area looking for high conductivity readings.

**Table 2.4. Okanagan Lake sites where water samples were taken (Figure 2.2)**

| | Geographic Coordinates | | Depth (m) |
|---|---|---|---|
| S1 | 49° 51' 3.33"N | 119° 30'26.44"W | 35 |
| S2 | 49° 52'02.85"N | 119° 30'27.45"W | 25 |
| S3 | 49° 52'15.79"N | 119° 30'28.11"W | 25 |
| S4 | 49° 52'16.23"N | 119° 30'15.01"W | 25 |
| S5 | 49° 53'10.27"N | 119° 30'53.20"W | 25 |

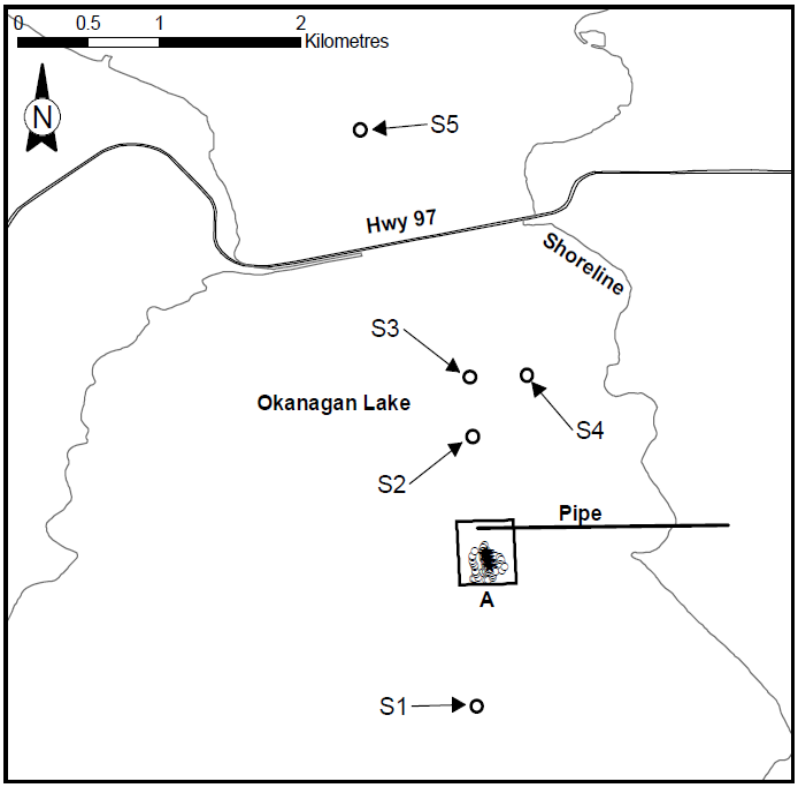



**Figure 2.2 Study area with sites shown where water samples for estrogens taken**

In Vernon, UV disinfection is used from April to October. When possible, effluent samples were taken pre-UV and post-UV (Table 2.1). Water samples were collected directly from MacKay reservoir by hand dipping a sample bottle over the side of a boat while moving to prevent contamination. The same approximate location (Latitude 50°11'48.82" N, Longitude 119°22' 05.52"W) was sampled during the ice-free period (April to November). Temperature profiles were taken (YSI 600R water quality sampling sonde and a 650MDS multi-display meter) in the reservoir to verify that there was no stratification.

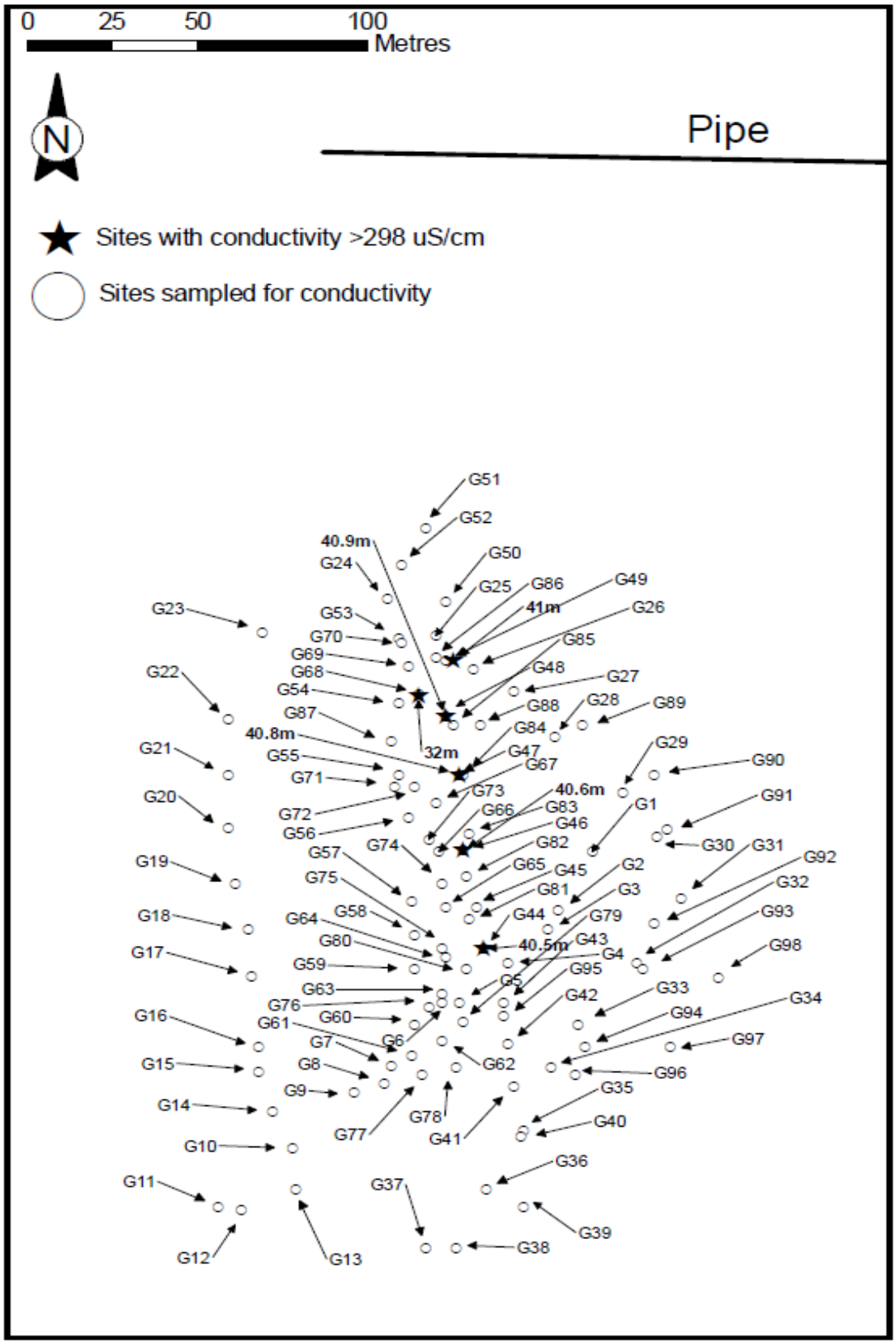



**Figure 2.3. Zoomed on section "A" of figure 2.2, showing conductivity sites**

### 2.2.3 Chemical Analysis

Estrogens in WWTP effluent and receiving waters are difficult to measure because they are present at concentrations in the parts per trillion (Briciu et al., 2009). For this reason, the analytical techniques evolved through the sampling period to improve detection and minimize sample matrix effects. A standard method has not been developed for the analysis of E1, E2, E3 and EE2 in water and wastewater. A widely accepted method involves a cleanup stage of the raw sample and analysis by Liquid Chromatography Tandem Mass Spectrometry (LC-$MS^2$) (Alda & Barcelo, 2001). The methods for sample preparation and analysis were similar to those outlined by Viglino et al. (2008), where samples are prepared using solid phase extraction (SPE) and analysed with a LC-$MS^2$.

### 2.2.4 Sample Preparation

Prior to analysis, water samples ranging between 1L and 4 L were first filtered using Whatman GF/C 47mm glass microfiber filters (nominal pore size 0.45 μm) (Millipore,MA, USA) that had been ashed in a muffle furnace at 400$^{o}$C for 60 minutes. Next, 61% of the samples were spiked with 200 μL of 60 μg $L^{-1}$ deuterated estradiol (2,4,16,16-D4 estradiol) (Sigma-Aldrich, Oakville, ON, Canada) internal standard. The deuterated estradiol was chosen based on previous research (Reddy et al., 2005). Not all of the samples were spiked with this internal standard. Then the estrogens were extracted from the sample by solid phase extraction.

Similar to other research, the estrogen extraction was done using Thermo Scientific Hypersept C-18 500 mg (6 mL) SPE columns (Varian Inc., Palo Alto, California) prior to instrumental analysis (de Alda & Barcelo, 2001). The SPE process included six steps:

1. The majority of the SPE columns were prepared using 10 mL methanol, 10 mL ethyl acetate, and 10 mL e-pure water (all HPLC grade), respectively. Ethyl acetate was used because, in December 2009, a supply shortage made it difficult and expensive to obtain acetonitrile, which is commonly used (Clara, et. al, 2004; D'Ascenzo et al., 2003; Williams et al., 2009). Samples analysed prior to the supply shortage (25% of samples) were prepared using acetonitrile.

2. The sample was passed through the SPE cartridge under a vacuum at a flow rate of approximately 10 mL $min^{-1}$.
3. The SPE cartridges were rinsed with 15 mL e-pure water and dried under vacuum for 30 minutes.
4. The cartridges were rinsed with 10 mL hexane and dried again for 10 minutes.
5. The analyte was eluted off the columns into a vial using 2 times 1.5 mL ethyl acetate (acetonitrile for samples processed prior to December 2009).
6. The solvent was evaporated from the vial gently with a heat plate and airflow for approximately 40 minutes.

Near the end of the study, Thermo Scientific Hypersep Florisil SPE columns were used to further "cleanup" the samples (used for 18% of samples). The florisil columns were incorporated based on further literature review in order to refine the methodology (Gabet-Giraud et al., 2010). The florisil SPE columns were prepared by rinsing with hexane. The dried samples in the vials were re-dissolved in 0.2 mL of DCM (dichloromethane, $CH_2Cl_2$) and 0.8 mL of hexane. Dissolved samples were then poured through the florisil SPE column; the vial was rinsed with 0.75 mL hexane and the rinse added to the column. The column was then rinsed with 6 mL of a mixture of hexane and 2% DCM. The analyte was eluted from the column into a vial using 3.75 mL of 75% acetone and 25% DCM mixture. The solvent was evaporated from the vial with gentle heating and air flow, resulting in a dried sample. All of the samples were then prepared for LC $MS^2$ by dissolving the dried sample in 200 µL of HPLC methanol.

All standards, reagents and solvents were obtained from Sigma-Aldrich or Fisher Scientific (Oakville, ON, Canada). Solvents used were HPLC-grade (methanol, ethyl acetate) or Optima-grade (n-hexane) for the solid phase extraction (SPE) prep procedure, and HPLC-grade (dichloromethane, acetone) or Optima-grade (n-hexane) for the florisil cleanup procedure. Calibration standards and spike stock solutions were prepared in HPLC-grade acetonitrile or methanol, depending on availability.

### 2.2.5 Instrumental Analysis

The separation of the analytes from the solution was achieved using a mixture of 60% methanol and 40% e-pure water at a flow rate of 200 µl $min^{-1}$. Cleanup of the column and reconditioning

was performed before each subsequent sample. Electro spray ionization (ESI) (negative mode) using flow injection technique was used to produce ions. The full-scan mass spectra and the $MS^2$ spectra of the selected compounds were obtained from 10µL injections of each compound at a flow rate of 200 µL $min^{-1}$.

The detection limit for the method was determined by spiking various concentrations of estrogens in e-pure water, and using the area of the lowest peak that was three times higher than the noise.

Instrumental and process errors were accounted for using the percent recovery of the internal standards (Fernandez et al., 2007). For the other samples, percent recoveries were calculated using spiked process blanks.

As mentioned previously, analytical techniques evolved during this study. The samples were categorised according to three main methods. Method 1 did not incorporate the use of an internal standard, and acetonitrile was used in the SPE pre-treatment. Method 2 included the use of the internal standard and ethyl acetate. Method 3 added the additional florisil step to Method 2.

### 2.2.6 Treatment Comparison

Kelowna and Vernon WWTP samples were examined for the effect of UV on analyte concentrations. A paired two-tailed student's t-test was used to compare the samples.

Box plots were used to summarize the estrogen concentration data from all three wastewater treatments plants. Plots were constructed for each year, and for the total number of samples. The box plots were used to display differences between treatments. A single factor ANOVA was carried out to compare concentrations of E1 and E2 in three treatment plants for all of the years and 2009 and 2010 separately. To further test differences between the treatment plants post-hoc Tukey-Kramer HSD analyses were used.

### 2.2.7 Loss Rate Constants in Each Receiving Water

The environmental loss rate constants for estrogens within river and lake systems were investigated by applying PFR and CSTR modelling. Okanagan River was treated as a PFR, while

Okanagan Lake and MacKay reservoir were treated as CSTRs. In all cases, dilution was first accounted for, and then loss due to bulk scavenging processes. The loss rate constants were separated out by seasons and a single factor ANOVA was used to test the difference between seasons. To further test differences between the treatment plants post-hoc Tukey-Kramer HSD analyses were used.

#### 2.2.7.1 Penticton

The dilution in Okanagan River was found by using the inflows into Okanagan River and the inflow volumes from the WWTP effluent. The inflows into Okanagan River include Shingle Creek and Ellis Creek, which enter approximately 360 m and 640 m downstream of the wastewater treatment plant, respectively. Shingle Creek flows were available, and Ellis Creek flows were estimated using Penticton Creek flows as they share similar watershed area and characteristics (Environment Canada, 2011). The upstream river discharge data was found using Environment Canada hydrometric data (Environment Canada, 2011). A dilution factor (DF, unitless) was calculated for a point 3300 m downstream of the WWTP (Equation 2.2). Where dilution at 3300m downstream is calculated as follows:

$$\text{Dilution}_{\text{at 3300m downstream of effluent}} = Q_{\text{WWTP Effluent}} / (Q_{\text{Okanagan River Upstream of WWTP}} + \quad , \qquad (2.1)$$

and the DF is calculated by:

$$DF_{3300m} = \frac{Q_{WWTP}}{(Q_{OKR} + Q_{WWTP} + Q_{SC} + Q_{EC})}, \qquad (2.2)$$

where Q is discharge ($m^3 s^{-1}$), and the subscripts WWTP, OKR, SC and EC specify outflow from the Penticton WWTP, Okanagan River upstream of the WWTP, Shingle Creek and Ellis

Creek, respectively . The 3300m location was chosen for two reasons. First, it was located at the end of the river, right before entering Skaha Lake. Second, it was far enough downstream of the effluent so that complete mixing of the effluent and the river water was likely.

In a plug flow reactor such as a river, the environmental loss rate constant may be calculated using the equation (Brezonik, 1994):

$$k = \frac{-ln\left(E/E_0\right)}{\tau_w} \quad (2.3)$$

where k is the first-order scavenging rate ($s^{-1}$), E is concentration measured at 3300m effluent concentration (ng $L^{-1}$),$E_0$ is the concentration leaving the WWTP influent concentration (ng $L^{-1}$), and $\tau_w$ is the water residence time in the PFR (seconds or minutes). In a PFR, $\tau_w$ is calculated as follows:

$$\tau_w = \frac{Al}{Q} \quad (2.4)$$

where A is the cross sectional area of the channel at 3300m below the WWTP ($m^2$), l is the distance travelled in the PFR (3300 m), and Q is the discharge in the channel ($m^3\ s^{-1}$) (Brezonik, 1994).

#### 2.2.7.2 Kelowna and Vernon

Assuming Okanagan Lake and the MacKay Reservoir behave like CSTRs, dilution can be determined by the proportion of the inflow of wastewater effluent to the outflow from the water body (Brezonik, 1994). With the exception of the water balance, there was no major dilution to consider within the Vernon system.

In a CSTR, the environmental loss rate constant (k, $day^{-1}$) can be calculated using (Brezonik, 1994):

$$k = \frac{\left(E_0/E\right)-1}{\tau_w}, \quad (2.5)$$

where $E_0$ is the initial concentration following dilution (ng $L^{-1}$), E is the final concentration (ng $L^{-1}$). $E_0$ was found by (Brezonik, 1994):

$$E_0 = \frac{m_{E_1}}{Q}, \quad (2.6)$$

where $m_{E1}$ is the mass of contaminant over time entering into the water body (ng) and Q is the daily discharge exchange between the WWTP, other inflows into the CSTR and outflows from the CSTR (L $day^{-1}$). The mass of estrogen $ME_1$ within MacKay Reservoir was calculated using:

$$m_{E_1} = m_{reservoir} + m_{WWTP} - m_{irrigation} - m_{scavenged}, \quad (2.7)$$

where m is the mass of estrogen (ng) within the reservoir ($m_{reservoir}$), discharged daily from the WWTP ($m_{WWTP}$), removed daily through irrigation pumping ($m_{irrigation}$) and lost to scavenging processes ($m_{scavenging}$). The daily water discharge exchange (Q, L $day^{-1}$) in the reservoir was calculated as the balance of storage, inflows and outflows:

$$Q = Q_{WWTP} + Q_{precip} + Q_{netGW} - Q_{outflow} - Q_{irrigation} - Q_{evap}; \quad (2.8)$$

where Q is the discharge of water (L day$^{-1}$) discharged daily from the WWTP ($Q_{WWTP}$), added daily from precipitation inputs ($Q_{precip}$) and net groundwater (inflow or outflow, $Q_{GW}$), and daily losses to outflow ($Q_{outflow}$), irrigation pumping ($Q_{irrigation}$) and evaporation ($Q_{evap}$).

An environmental loss rate constant for the MacKay Reservoir was calculated between sample dates. The pre- and post-UV values were both used in order to calculate the loss rate constant, as the samples were considered to be replicates and not different samples.

In order to calculate an annual environmental loss rate constant in the CSTRs, the results of the individual sample days were temporally weighted based on the sampling dates. The temporally weighted loss rate constants ($k_{annual}$) were assumed to represent the period of time centred on that sampling date, beginning and ending halfway between the previous and subsequent sampling dates. The weighted loss rate constants were calculated as follows:

$$k_{annual} = \frac{\sum_{i=0}^{n} \left( \frac{\left(\mathrm{JD} - (\mathrm{JD}-1)\right)/2}{\left((\mathrm{JD}+1) - \mathrm{JD}\right)/2} \right) k}{JDn - JDi}, \quad (2.9)$$

$$k_{annual} = \left( \frac{\left(\mathrm{JD} - (\mathrm{JD}-1)\right)/2}{\left((\mathrm{JD}+1) - \mathrm{JD}\right)/2} \right) k, \quad (2.10)$$

where JD is the Julian sample date, (JD-1) is the previous Julian sample date, (JD+1) is the subsequent Julian sample date, i is the initial sample date and n is the final sample date.

#### 2.2.7.2.1 Interpolation

There were instances when sampling of the WWTP effluent and MacKay reservoir did not occur on the same day. In these cases interpolation was used to find the concentrations of E1 in the corresponding water body. Interpolated values were calculated by:

$$E1_{day\ x} = \frac{E1_{day\ x-1} + (E1_{day\ x+1} - E1_{day\ x-1})}{(JD_{day\ x+1} - JD_{day\ x-1}) * (JD_{day\ x} - JD_{day\ x-1})}, \quad (2.11)$$

where E1 is the concentration of estrone within the water body (MacKay or Vernon WWTP) and day x is the date that the E1 concentration is unknown.

## 2.3 Results

### 2.3.1 Measurement Methods

The detection limit for all three analytical methods for E1 was 0.02 ng $L^{-1}$ and for E2 was 0.07 ng $L^{-1}$. The recovery rates ranged for each method (Table 2.5). The Okanagan Lake samples are not represented in this table because there were no estrogens detected.

**Table 2.5. Percent recoveries for each method, separated by sample site**

| Sample Type | Sample Site | Method 1 | Method 2 | Method 3 |
|---|---|---|---|---|
| WWTP Effluent | Penticton WWTP | 75 % | 78% | 8.7% |
| | Kelowna WWTP | 50% | 75% | 54% |
| | Vernon WWTP | 75% | 21% | 37% |
| Receiving Water | Okanagan River | not used | 6% | 41% |
| | MacKay Reservoir | 75% | 61% | 8.7% |

.

E1 was measured in the largest concentrations in the most samples, and E2 was only detected in some of the samples. E3 and EE2 were not detected in any of the samples and so are excluded from the presentation of results, but will be discussed briefly in the last section of this chapter.

### 2.3.2 Wastewater Treatment Plants

E1 concentrations measured in samples from the Penticton WWTP ranged from non detectable (ND) to 27.1 ng $L^{-1}$ (Table 2.6), while E2 concentrations ranged from ND to 1.83 ng $L^{-1}$ (Table 2.7). With the exception of March 11, 2010, E1 concentrations were higher than E2 in samples from the Penticton WWTP effluent (Figure 2.4). Both E1 and E2 concentrations peaked on the final sample date in October 2010 (Figure 2.4). Note that, during the period of this study, the Penticton WWTP did not have UV in operation.

The highest E1 concentrations were measured in the Kelowna WWTP pre- and post-UV effluent (range 0.9 to 77.2 ng $L^{-1}$ and 1.5 to 69.9 ng $L^{-1}$, respectively (Table 2.6, Figure 2.5). The E2 concentrations ranged between ND and 4.0 ng $L^{-1}$ in the pre-UV samples, and between ND and 8.0 ng $L^{-1}$ in the post-UV samples (Table 2.7).

E1 concentrations measured in the Vernon pre-UV samples ranged between ND and 36.1 ng $L^{-1}$, while post-UV concentrations ranged between ND and 1.5 ng $L^{-1}$ (Table 2.6, Figure 2.6). E2 concentrations measured in pre-UV samples from the Vernon WWTP ranged from ND to 6.1 ng $L^{-1}$, and not detectable following UV (Table 2.7).

**Table 2.6 E1 concentrations in treated wastewater effluent (ND denotes non-detectable concentration)**

| | Min ($ng\ L^{-1}$) | Max ($ng\ L^{-1}$) | Median ($ng\ L^{-1}$) | Mean ($ng\ L^{-1}$) | SD | N |
|---|---|---|---|---|---|---|
| **Vernon WWTP Pre-UV** | ND | 36.1 | 14.5 | 14.8 | 11 | 12 |
| **Vernon WWTP Post-UV** | ND | 1.5 | 0.7 | 1.1 | 0.7 | 3 |
| **Kelowna WWTP Pre-UV** | 0.9 | 77.2 | 34.1 | 30.6 | 25.4 | 12 |
| **Kelowna WWTP Post-UV** | 1.5 | 69.9 | 26.4 | 27 | 24.5 | 9 |
| **Penticton WWTP No UV** | ND | 27.1 | 0.58 | 4.2 | 8.8 | 11 |

**Table 2.7 E2 concentrations in treated wastewater effluent (ND denotes non-detectable concentration)**

| | Min ($ng\ L^{-1}$) | Max ($ng\ L^{-1}$) | Median ($ng\ L^{-1}$) | Mean ($ng\ L^{-1}$) | SD | N |
|---|---|---|---|---|---|---|
| **Vernon WWTP Pre-UV** | ND | ND | ND | ND | - | 3 |
| **Vernon WWTP Post-UV** | ND | 6.1 | ND | 3.9 | 3.1 | 12 |
| **Kelowna WWTP Post-UV** | ND | 8 | 1.1 | 5.8 | 3.6 | 9 |
| **Kelowna WWTP Pre-UV** | ND | 4 | 1 | 2.9 | 1.6 | 12 |
| **Penticton WWTP No UV** | ND | 1.83 | ND | 1.1 | 0.8 | 11 |

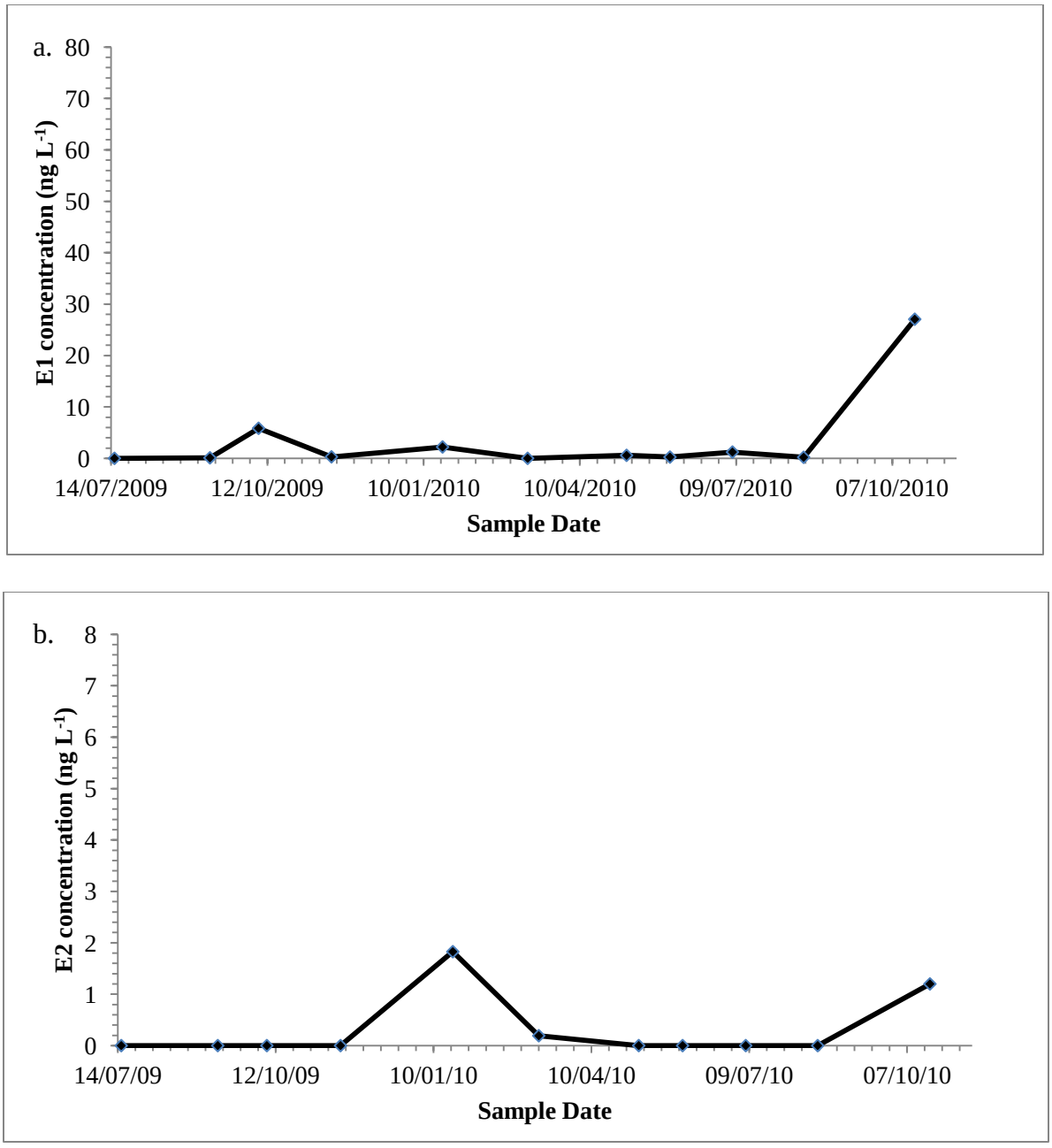



**Figure 2.4. E1(a) and E2 (b) concentrations measured in effluent at the Penticton WWTP**

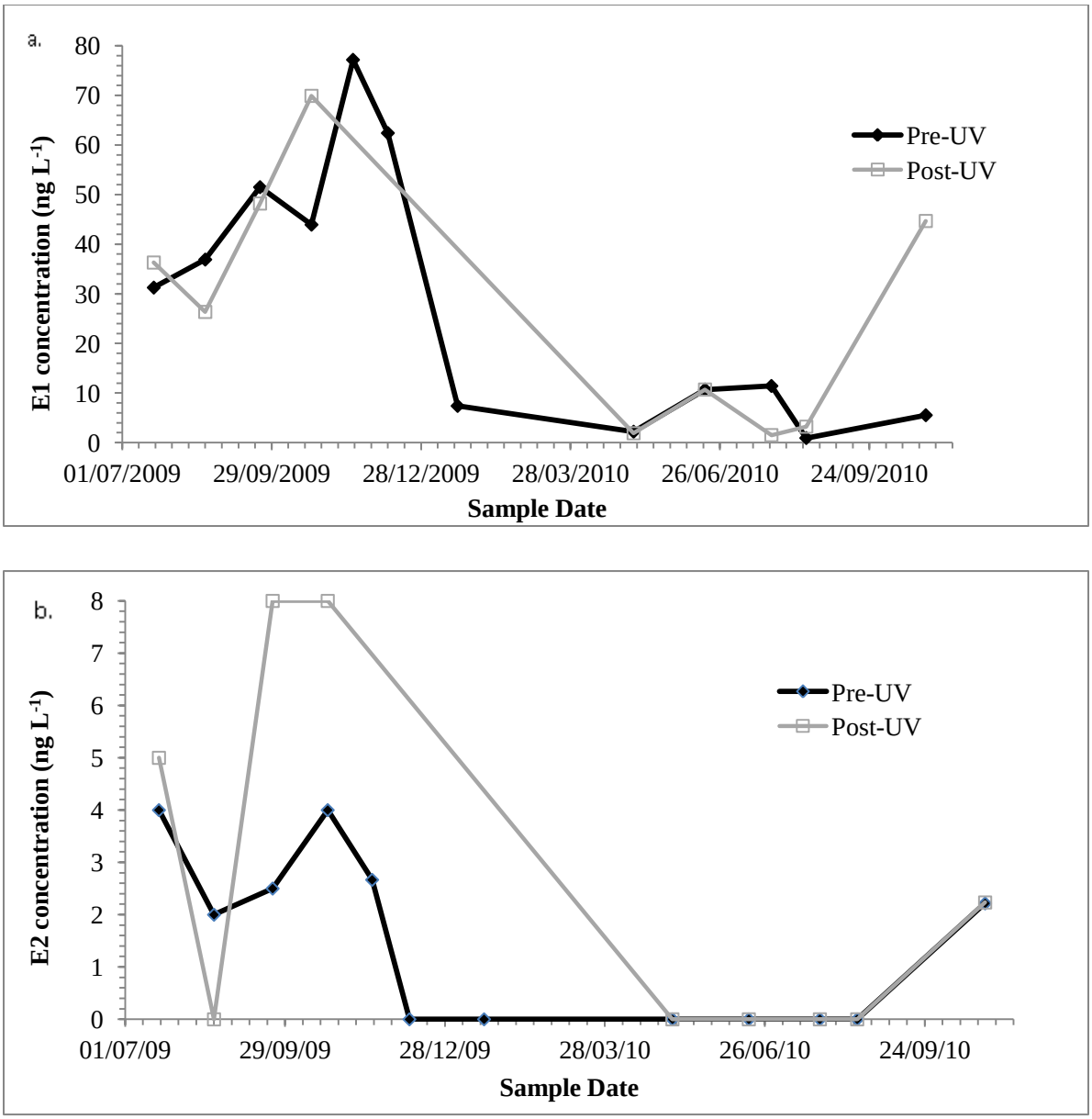



**Figure 2.5. E1 (a) and E2 (b) concentrations measured pre and post-UV at the Kelowna** WWTP

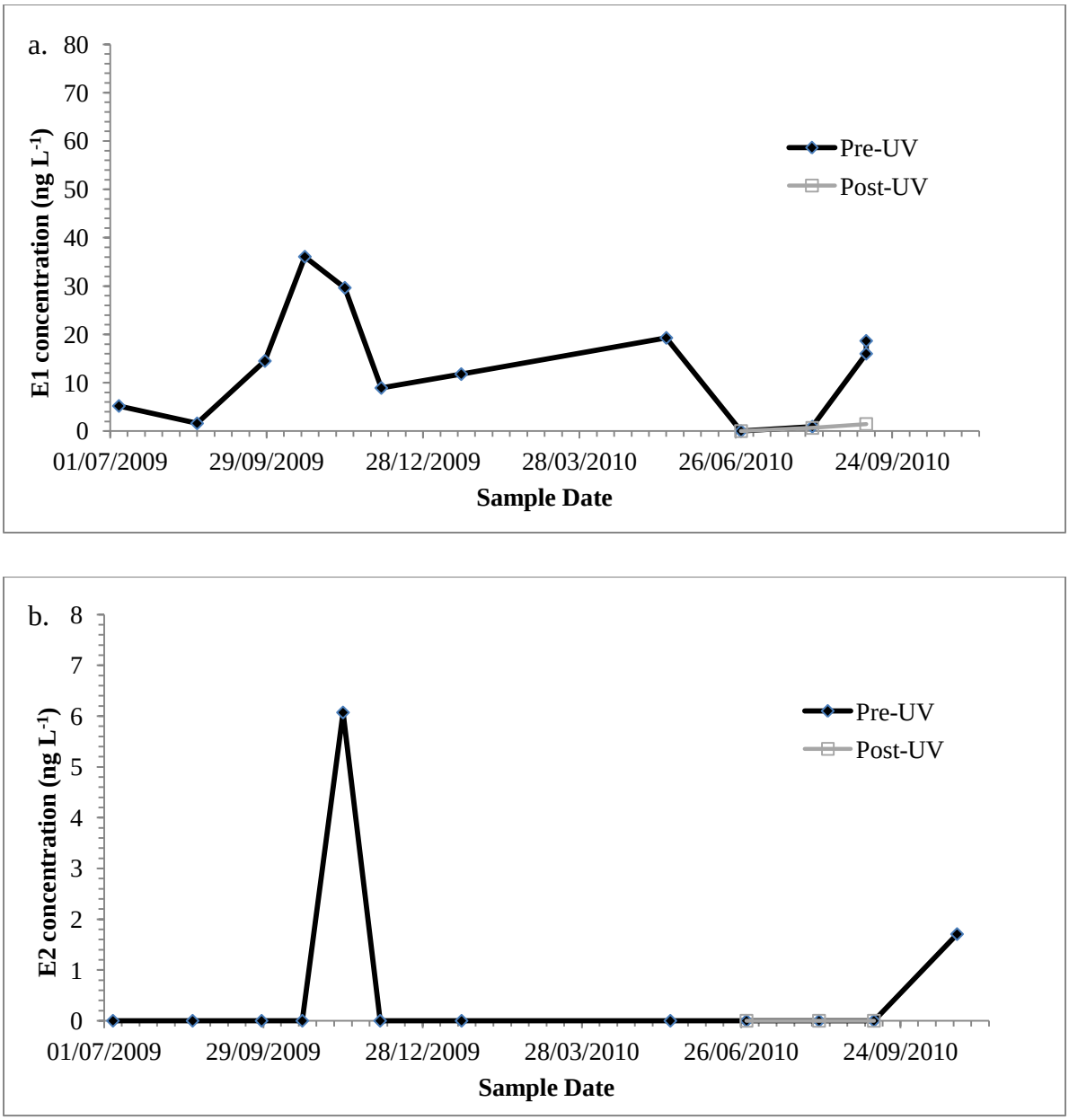



**Figure 2.6. E1 (a) and E2 (b) concentrations measured pre- and post-UV at the Vernon WWTP**

A single factor ANOVA was used to test if the E1 concentrations from the treatment plant samples were different. Non-detect values were set to the detection limit. A significant difference was found between all three treatment plants ($p=0.002$). Further analysis using a post-hoc Tukey-Kramer HSD found that the Penticton WWTP samples were significantly lower than the Kelowna WWTP samples (Tukey-Kramer HSD; $p<0.05$) (Figure 2.7a). There was no significant

difference between the Kelowna and Vernon samples or between the Penticton and Vernon samples. A single factor ANOVA was also used to compare the E2 concentrations using the detection limit for the non-detectable values. E2 concentrations between the treatment plants differed significantly ($p=2x10^{-5}$). Kelowna WWTP E2 concentrations were significantly higher than both the Vernon and Penticton effluents (Tukey-Kramer HSD; $p<0.05$)(Figure 2.7b). There was no significant difference between the Vernon and Penticton samples. 2009 and 2010 E1 results were split for comparison based on an apparent difference (Figures 2.8). The single factor ANOVA showed a significant difference between the treatment plants ($p = 5.3 x10^{-5}$). Kelowna samples were significantly higher than both the Penticton and Vernon WWTPs (Tukey-Kramer HSD:$p<0.05$) (Figure 2.8b). There was no significant difference noted between Penticton and Vernon. This pattern was not repeated in 2010 (Figure 2.8c). The single factor ANOVA for the 2010 E1 concentrations did not show any difference between the WWTPs ($p=0.48$). Although there were many non-detects in the E2 readings for the WWTPs (Figures 2.4b to 2.5b), the ANOVA showed similar results. The 2009 E2 concentrations were different between treatment plants ($p=0.04$). A significant difference between each treatment plant could not be determined through a Tukey-Kramer HSD. The 2010 E2 results were not different when compared using an ANOVA ($p=0.93$).

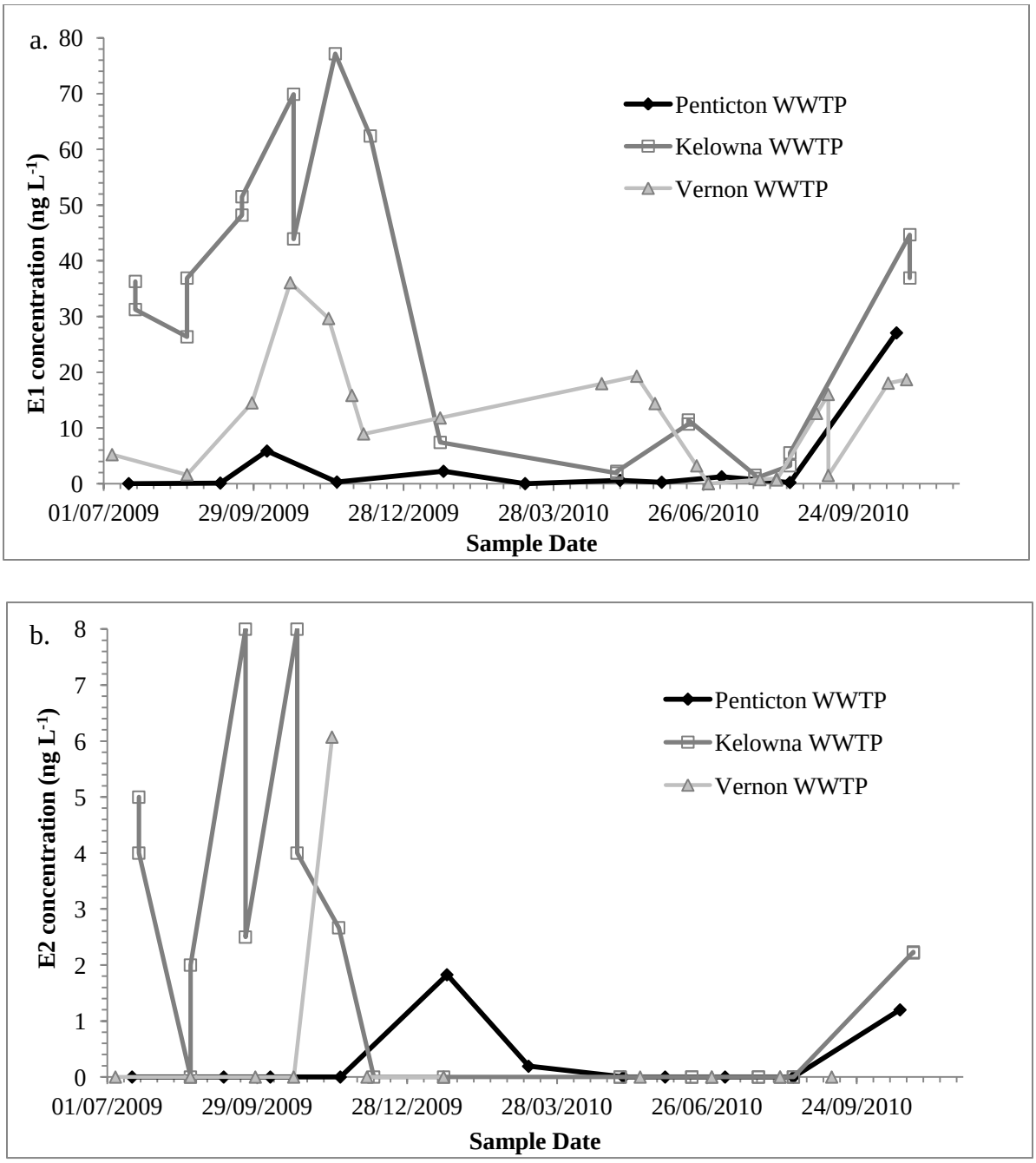



**Figure 2.7. E1 (a) and E2 (b) concentrations measured at the three WWTPs**

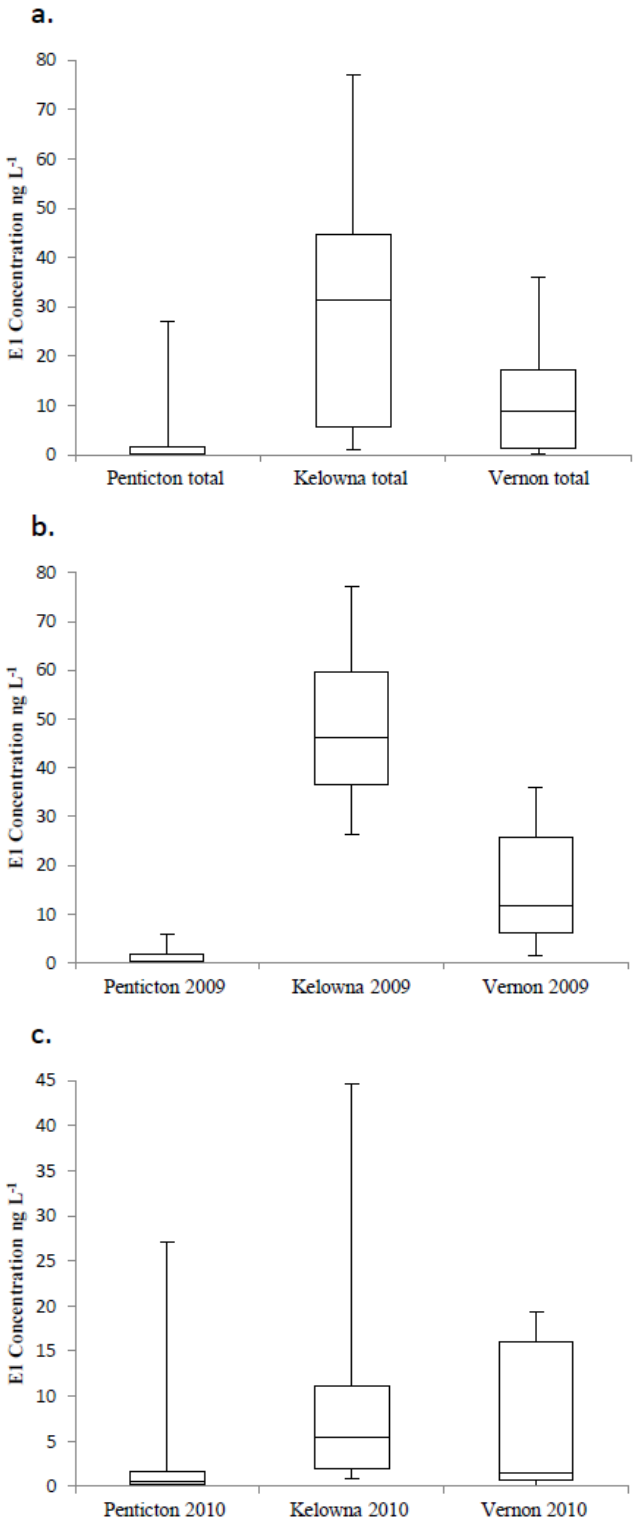



**Figure 2.8. Box plots comparing E1 concentrations measured at the three WWTPs for all data (a), 2009 data (b) and 2010 data (c.)**

#### 2.3.2.1 Ultraviolet Treatment Comparison

To compare the pre- and post-treatment results, a paired student's t-test was used. The pre- and post-UV E1 concentrations at the Kelowna WWTP were not found to be statistically different, and the null hypothesis could not be rejected (p=0.49, n=9). The same was true for E2 concentrations at this location (p=0.13, n=8). The Vernon pre- and post-UV E1 concentrations were also not found to be statistically different (p=0.41, n=3). The pre and post-UV concentrations of E2 could not be compared for the Vernon WWTP as only the pre-UV samples contained detectable (>0.07 ng $L^{-1}$) concentrations of E2.

### 2.3.3 Penticton and Okanagan River

Estrogen concentrations within Okanagan River were variable over the study period (Figure 2.10). E1 concentrations ranged from ND to 1.4 ng $L^{-1}$, and E2 concentrations ranged from ND to 44.7 ng $L^{-1}$ (Table 2.5). There were detectable levels of estrogen measured upstream of the WWTP outfall pipe on 21 January 2010, 7 July 2010, and 15 October 2010, suggesting inputs other than Penticton WWTP effluent. For these dates, concentrations downstream of the outfall were corrected by subtracting the upstream concentrations (Figure 2.10). Most notably, apparent E2 concentrations in the river channel were higher than the WWTP effluent (Tables 2.7 and 2.8).

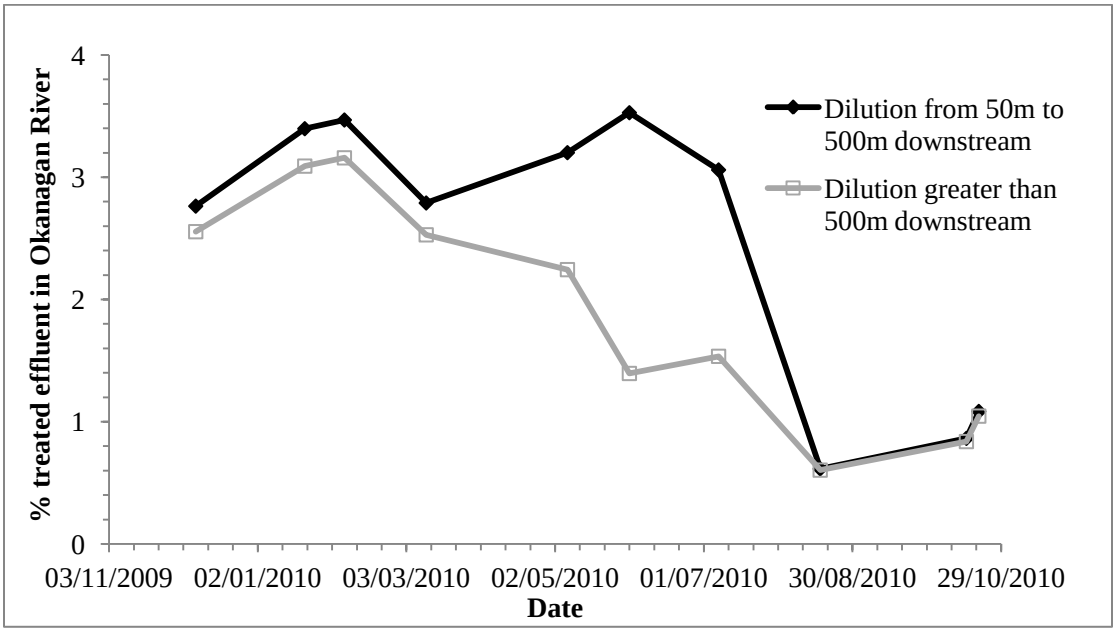



**Figure 2.9. Percent of treated effluent at two downstream distances in Okanagan River during sample period**

The dilution factor calculated using Equation 2.2 in Okanagan River ranged from 0.6% to 3% during the days sampled (Figure 2.9). The fluctuations in the dilution factor within the river can be accounted for through natural hydrologic variations in the river as well as reservoir releases. The river is controlled by a dam at the outfall of Okanagan Lake used to control the water levels upstream.

E1 concentrations following dilution would be expected to range between 0.0018 ng $L^{-1}$ and 0.009 ng $L^{-1}$, using the median E1 concentration in the effluent (0.3 ng $L^{-1}$) (Table 2.6). The majority of the results in the river should be below detection based on dilution. This is consistent with what was found in samples. The maximum concentration detected in the river was 0.33 ng $L^{-1}$, which occurred when the WWTP effluent E1 concentration was 27.1 ng $L^{-1}$ on October 20, 2010. These results indicated a 1.2% proportion of effluent within the channel. Using river and wastewater treatment plant discharge for the same date, WWTP inputs made up 0.95% of the total river discharge, which is comparable to 1.2% found from the E1 concentrations (Figure 2.9). This establishes dilution in the river.

During the sample period, Okanagan River travel time in the section between the WWTP and Skaha Lake (3300 m) ranged between 1.9 hours to 7.6 hours. An environmental loss rate constant could not be calculated for this section because there were no detectable levels of estrogens downstream of the WWTP that could not be accounted for through dilution.

**Table 2.8. E1 and E2 concentrations measured in Okanagan River**

| Estrogen | Min (ng $L^{-1}$) | Max (ng $L^{-1}$) | Median (ng $L^{-1}$) | SD | N |
|---|---|---|---|---|---|
| E1 | ND | 1.4 | 0.7 | 0.4 | 24 |
| E2 | ND | 44.7 | 0.18 | 14.9 | 24 |

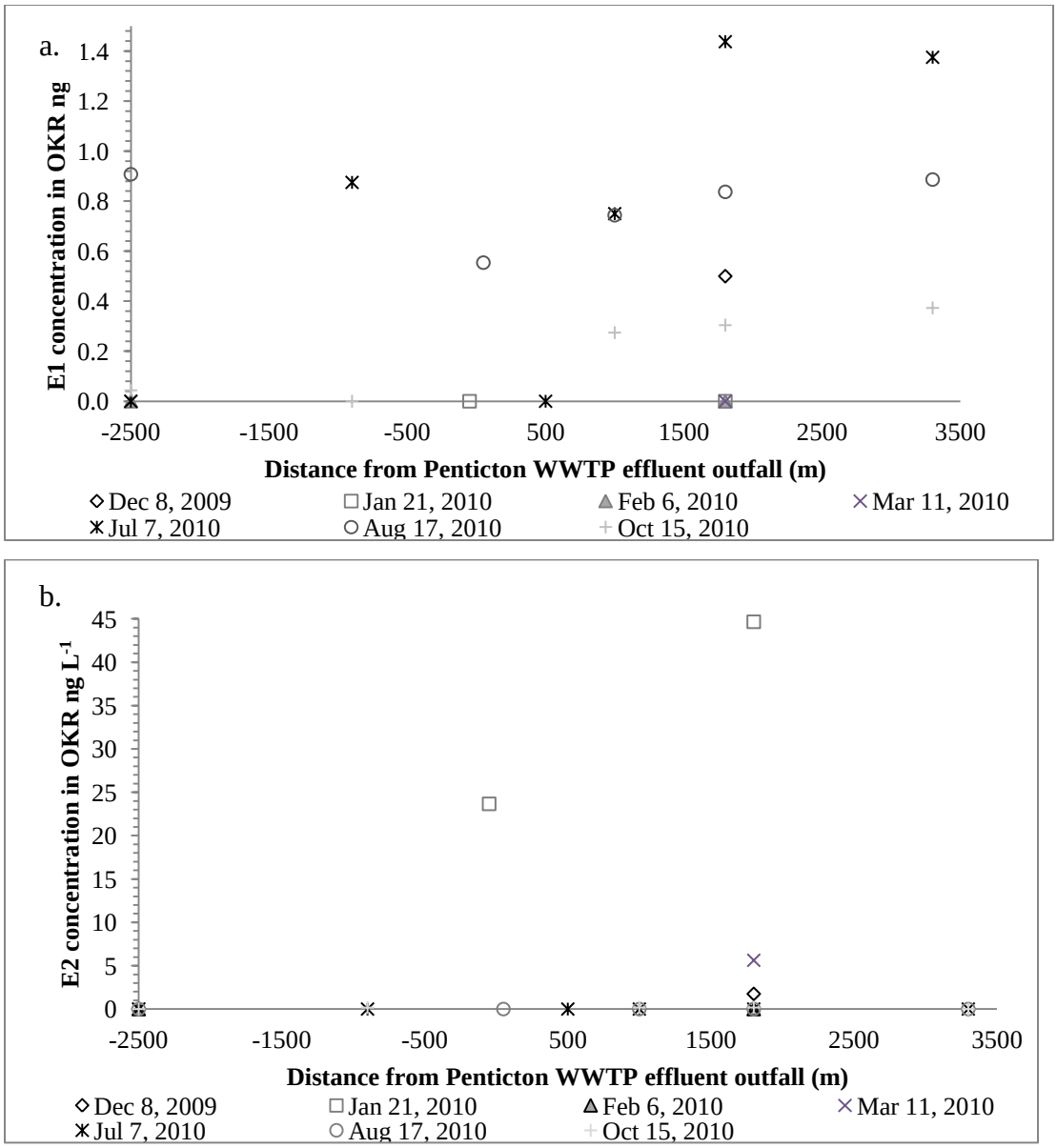



**Figure 2.10. E1 (a) and E2 (b) concentrations measured in the Okanagan River**

### 2.3.4 Kelowna and Okanagan Lake

There were no detectable concentrations of estrogens in Okanagan Lake. As a result, a loss rate constant could not be calculated.

In order to verify that samples were collected from the plume of the WWTP outfall pipe, conductivity measurements were made at different depths and locations in the area proximal to the pipe (Figure 2.3). The conductivity of WWTP effluent is approximately 900 μS $cm^{-1}$, while

the conductivity of Okanagan Lake is approximately 281 µS cm $^{-1}$ (Mitchell & Hansen, 2011). This large contrast in values was expected to produce a detectable spike in conductivity in the vicinity of the effluent pipe.

Of the 99 measurements made, 6 were considered high outliers (2.2 times the interquartile range) for conductivity (>296 µS cm $^{-1}$) (Figure 2.11). From the measurements, there is a localized area between 40 and 41m depth below the lake surface that had elevated conductivity. The conductivity in this area was approximately 16 to 25 µS cm$^{-1}$ higher than the background level of Okanagan Lake. Assuming that conductivity behaves conservatively, this change from background corresponds to a 1.8 to 3.9% concentration of treated wastewater effluent. Using the mean E1 concentration from Kelowna post-UV WWTP of 27 ng L$^{-1}$ (Table 2.6), a concentration in these areas would be expected to be detected. Further investigation is required to investigate this.

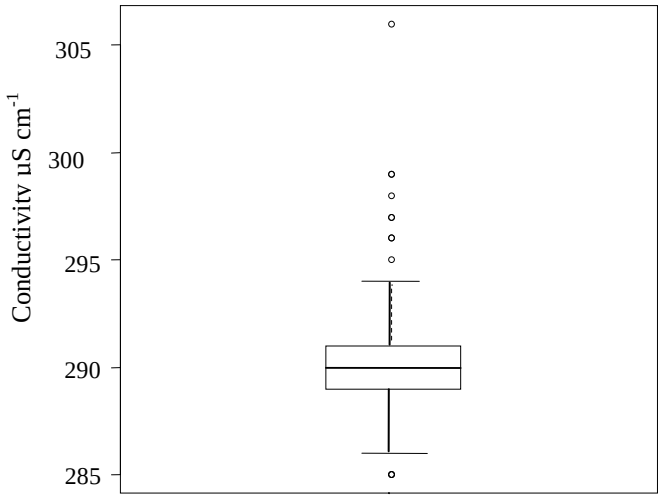



**Figure 2.11 Box plot summarizing conductivity measurements taken in Okanagan Lake**

### 2.3.5 Vernon and MacKay Reservoir

E1 and E2 concentrations measured in the MacKay Reservoir ranged from 0.12 to 5.2 ng L$^{-1}$ and ND to 0.4 ng L$^{-1}$, respectively (Table 2.9). E1 and E2 concentrations were generally lower in

MacKay Reservoir samples than those from the Vernon WWTP (Figures 2.12 a and b). Since the reservoir and effluent samples were not always sampled on the same day, interpolation was used to determine the E1 concentrations that match the corresponding sample dates in each water body (Figure 2.13). Interpolation was not used for the E2 concentrations because there were too many readings below detection to make interpolation valid. The loss rate constant was calculated between sample dates using equations 2.6 to 2.11. The loss rate constants calculated between sample dates ranged between 0.0008 $d^{-1}$ and 0.0502 $d^{-1}$ (Figure 2.14). The variation was further investigated by separating the loss rates based on the seasons they were sampled in (Table 2.10).

**Table 2.9. E1 and E2 concentrations measured in MacKay Reservoir**

| Estrogen | Min (ng $L^{-1}$) | Max (ng $L^{-1}$) | Median (ng $L^{-1}$) | SD | N |
|---|---|---|---|---|---|
| E1 | 0.12 | 5.2 | 0.39 | 2.1 | 9 |
| E2 | ND | 0.4 | 0.43 | 0.2 | 9 |

The lowest seasonal loss rate constant was calculated for the winter, at 0.0008 $d^{-1}$ (880 d), and the highest loss rate constant was calculated for the summer, at 0.0502 $d^{-1}$ (14 d) (Table 2.10). The 95% confidence intervals for fall and spring and spring and summer overlap (Table 2.10). The confidence intervals for fall and summer do not overlap and none of the confidence intervals encompass the Winter $K_{E1}$ value. A one-way ANOVA on the fall, spring and summer data showed that the difference between the seasonal data is not statistically significant ($p = 0.4$). There were insufficient samples with detectable concentrations to calculate a loss rate constant for E2. The average annual environmental loss rate constant for E1 in MacKay Reservoir was 0.0106 $d^{-1}$, which corresponds to a half life ($T_{1/2}$) of 65 days

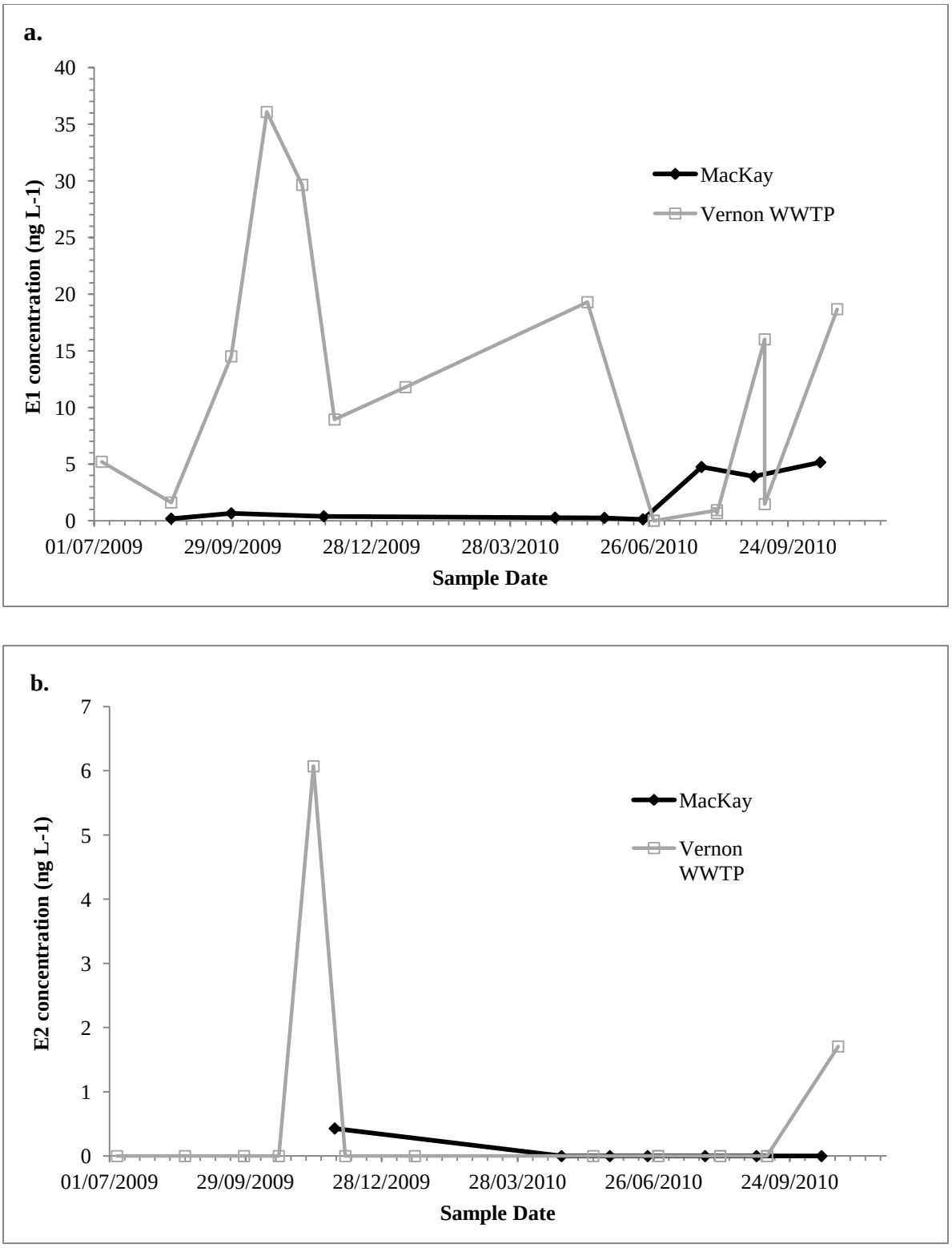



**Figure 2.12 E1 (a) and E2 (b) concentrations measured in Vernon WWTP effluent and MacKay Reservoir measured**

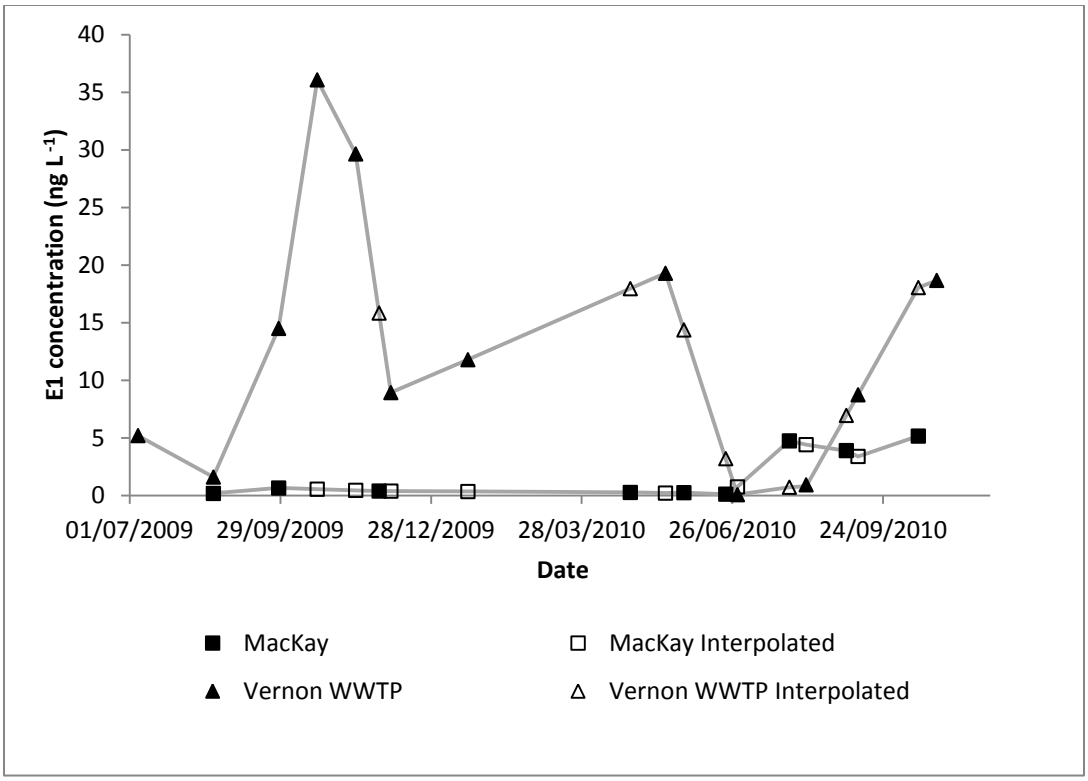



**Figure 2.13 Interpolated and measured E1 concentrations**

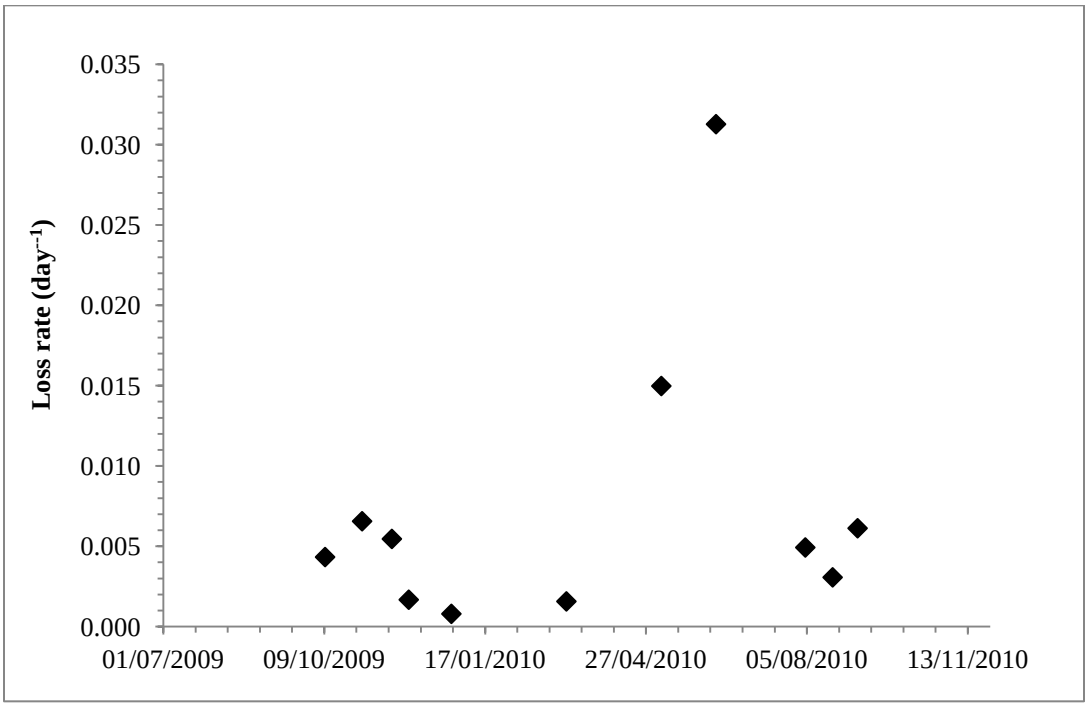



**Figure 2.14 Loss rate constants found in MacKay Reservoir for E1**

**Table 2.10 Seasonal and annual environmental loss rate constants for E1 within MacKay Reservoir**

| | Fall (Sept 21-Dec 21) | Winter (December 22 - March 21) | Spring (March 22-June 21) | Summer (June 22 - September 20) | Annual |
|---|---|---|---|---|---|
| $K_{E1\,(d^{-1})}$ | 0.0136 | 0.0008 | 0.0321 | 0.0502 | 0.0106 |
| 95% Confidence Interval (±) | 0.003 | | 0.016 | 0.013 | |
| N | 4 | 1 | 2 | 3 | 11 |
| $T_{1/2}$ (d) | 51 | 880 | 22 | 14 | 65 |

### 2.3.6 General CSTR Model for E1 Fate in Okanagan Receiving Waters

By applying the annual average E1 loss rate constant calculated for MacKay Reservoir, Equation 2.5 can be used to estimate an average residence time required to reduce concentrations of E1 in Okanagan receiving waters that act like CSTRs:

$$\tau_W = \frac{\left(E1_0/E1_c\right)-1}{0.0106} \quad , \qquad (2.12)$$

where $E1_c$ is the desired concentration of E1 in the receiving water body (ng $L^{-1}$). The term $E1_0$, as noted in equation 2.6, is the concentration of E1 in the WWTP effluent diluted into the waterbody.

## 2.4 Discussion

### 2.4.1 Measurement Method

At the time of this research, there were no standard methods for the determination of E1, E2, E3 or EE2 concentrations within wastewater or the environment. The analytical methods used in this research evolved to reducing matrix effects. In order to incorporate data between methods the

sample recoveries were used. Although uncertainty of the results would be reduced by using the same analytical methods, to get the best results within the time constraints the methods improved throughout the study. The addition of the florisil SPE column improved peak definition within the mass spectrum of the sample. Prior to the florisil addition the peaks were not as defined. It was believed that the complex sample matrices were making the spectrum difficult to read. Florisil helped remove more interfering compounds prior to the instrumental analysis. While the addition of the florisil step helped to define peaks, it did reduce the recovery rate of the target compounds. For this reason method 2 would be preferred to balance the clarity of the spectrum and the loss of target compounds. The methods were not rigorously compared based on time constraints however all three methods have been used throughout the literature and have been considered feasible.

### 2.4.2 Estrogen Levels

E1 was the most common and concentrated estrogen species detected in both WWTP effluent and receiving water samples, while E2 was the second most common. This is similar to the literature (Brix et al., 2010; LoPez De Alda et al., 2002; Rodriguez-Mozaz et al., 2005; Sole et al., 2000), and is attributed to the rapid oxidation of E2 to E1 during the wastewater treatment process (Chimchirian et al., 2007; D'Ascenzo et al., 2003; Johnson, 2001; Ternes et al., 1999).

The literature has revealed that E1 and E2 are similar in potency (Segner et. al., 2013) It was noted with respect to VTG production in male fish, that E1 has similar effect levels to E2 (Dammann et al., 2011; Van den Belt et al., 2004). The predicted no effect concentration (PNEC) has been determined to be 3 ng $L^{-1}$ for E1 and 1 ng $L^{-1}$ for E2 (Gross-Sorokin et al., 2006). 3 samples taken in MacKay reservoir had E1 concentrations greater than the PNEC and 4 samples in Okanagan River had E2 concentrations greater than the PNEC.

EE2 and E3 were not detected in any samples. Since other studies have found EE2, it was also expected to be found in these receiving waters. Further research is required in order to determine the reason for this. E3 may have not been detected as a result of the analytical method. Samples that were spiked with E3 during method development demonstrated ion suppression of E3. In addition, E3 retention time was less than the other 3 compounds and it came off of the column at the same time as a slug of contaminants. It may have been masked in this large peak. It is also

possible that E3 was already scavenged within the wastewater treatment process because removal efficiency of E3 is greater than the removal efficiency of E1 (Chimchirian et al., 2007).

### 2.4.2.1 Wastewater Treatment Plant Effluent

The ranges of E1 and E2 concentrations measured in WWTP effluent in this study were 0.3 to 77.2 ng $L^{-1}$, and <0.07 to 8.0 ng $L^{-1}$, respectively (Table 2.6 and 2.7), which was comparable to reported ranges of 0.1 to 70 ng $L^{-1}$ for E1, and <1 to 64 ng $L^{-1}$ for E2 (Table 2.11) from the literature. While it appears that E2 levels measured in this study were lower than those reported in the literature, the mean reported values (1.5 to 4 ng $L^{-1}$) are comparable to the mean of this study <0.07-5.8 ng $L^{-1}$.

**Table 2.11 Typical concentrations of E1 and E2 exiting wastewater treatment plants**

| E1 (ng $L^{-1}$) | E2 (ng $L^{-1}$) | Treatment Details | Reference |
|---|---|---|---|
| range = 0.3 – 77.2 | range = <0.07 – 8.0 | | This study |
| mean = 2, range = 1–3 | mean = 3, range = 2–3 | Activated sludge treated effluent | Muller et al., 2008 |
| mean = 6.3 +/- 0.9 (n=3) | mean = 1.5 +/- 0.5 (n=3) | Activated sludge treated effluent | Chimchirian et al., 2007 |
| median = 9, max = 70 | median <1, max = 3 | Treated effluent of 16 WWTPs in Germany (preliminary and final clarification and aeration and phosphate elimination using Fe(III)Cl3 or Fe(II)Cl2) | Ternes et al., 1999 |
| median = 3, max = 48 | median = 6, max = 64 | Treated effluent of 10 WWTPs in Canada | Ternes et al., 1999 |
| mean = 4, max = 58 (n=27) | mean = 4, max = 19 (n=27) | Activated sludge treated effluent | Schusener & Clean 2008 |
| mean = 17, range = 1–96 | mean = 1.8, range = 0.2–14.7 | Treated effluent of 18 WWTPs | Servos et al., 2005 |

Three (12.5%) of the samples taken in Okanagan River downstream of the treatment plant had higher E2 concentrations than in the effluent (Figure 2.4 and 2.10). For these sample dates dilution or loss rate constants could not be determined because it appeared that there was a generation of E2 in the river. E1, E2 and E3 have been found to increase in concentration within WWTPs as a result of a change from their conjugated forms, which were not tested for in this study (Reddy et al., 2005; Zhou et al., 2012). While this has been primarily noted to occur within treatment plants, this conversion may occur in the receiving waters. Further investigation is required to determine the source of higher E2 concentrations in the receiving water.

#### 2.4.2.1.1 Pre- and Post-UV Samples

Results from this study are consistent with the finding that WWTP UV irradiation has a negligible effect on both E1 and E2 (Racz & Goel, 2010). UV within treatment plants uses UV-C irradiation at 254nm, while natural sunlight contains UV-B, UV-A and visible radiation (290-700nm) (Chowdhury et al., 2010). UV is designed for the inactivation of microorganisms and not for the reduction of estrogens. UV within WWTPs appears to be effective if an oxidant such as titanium dioxide ($TiO_{2)}$ is added, or the process is coupled with ozone ($O_3$) treatment (Koh et al., 2008; Racz & Goel, 2010). The treatment plants in this study did not use added oxidants in the UV process. Since the difference between the pre- and post-UV samples was found to be insignificant in the t-test performed, the results were combined when using them for further analysis.

#### 2.4.2.1.2 Comparison of Treatment Plant Effluent

E1 concentrations at all three WWTPs follow a similar trend (Figure 2.7). E1 concentrations appear to be higher between September and December 2009 than during other months. This increase in effluent estrogen concentrations during cold months is consistent with the literature (Fernandez et al., 2008; Zhou et al., 2012). When specifically tested, a temperature decrease during wastewater treatment has been found to decrease estrogen removal (Koh et al., 2008). It has been assumed that this is due to a decrease in the biological activity within the treatment process (Fernandez et al., 2008). This is also in agreement with variability in the seasonal loss rate constants found for MacKay Reservoir (Table 2.10). Further investigation is required to test the hypothesis that there is a seasonal component to estrogen concentrations in treated effluent.

When the treatment plant manager was interviewed, it was discovered that there was an equipment change in December of 2009 (M. Gosselin personal communication, 4 February, 2013). The filters used to separate total suspended solids from the liquid effluent prior to UV were changed. The dual media sand filters containing anthracite coal and fine sand were switched to a cloth disc filter designed for the same purpose. Further investigation would be required to determine whether this was an important change or simply a coincidence. A literature investigation did not show any relevant studies.

#### 2.4.2.2 Receiving Waters

The majority of the values on receiving water estrogen concentrations in the literature are taken from large rivers with inputs from multiple treatment plants (Baronti et al., 2000; Cargouët et al., 2004; Lei et al., 2009; Luna-Pabello et al., 1992; Ternes et al., 1999; Zhang & Zhou, 2008; Zhao, Zhang et al., 2010). The range in concentrations within the literature is considerable; E1 ranges between ND and 112 ng $L^{-1}$ while E2 ranges between ND and 200 ng $L^{-1}$ (Table 2.12). In this research, the measured range was from ND to 5.2 ng $L^{-1}$ for E1 and between ND to 44.7 ng $L^{-1}$ for E2 (Tables 2.8 and 2.9). The results found within these receiving water bodies are consistent with what is found in other literature.

In Okanagan River there appears to be another source of E1 and E2 (Figure 2.10). An investigation through interviews with the local authority resulted in no definitive answer into a possible source. Potentially the E1 and E2 could be coming from septic systems located on the plateau above Okanagan River. There is a possibility that there are a few non-point sources for estrogens into Okanagan River upstream of the WWTP effluent.

**Table 2.12 Published E1 and E2 concentrations found in rivers that receive treated wastewater effluent**

| E1 (ng $L^{-1}$) | E2 (ng $L^{-1}$) | Reference |
|---|---|---|
| mean=16.9,<br>range = 2.2-55.3 | mean =7.8,<br>range = 0.9-32.4 | Lei, Huang et al. 2009 |
| mean =3.6<br>range = 1.7-5.0 | ND | Kim, Cho et al. 2007 |
| mean =not reported,<br>median= ND<br>range = 0.7-1.6 | ND | Ternes, Stumpf et al. 1999 |
| mean =not reported | mean=not reported | Kolpin, Furlong et al. |

| E1 (ng $L^{-1}$) | E2 (ng $L^{-1}$) | Reference |
|---|---|---|
| median= 27<br>range = ND-112 | median= 160<br>range = ND-200 | 2002 |
| mean=2.9<br>median= 3.0<br>range = ND-8.2 | mean=1.5<br>median= 1.5<br>range = ND-1.7 | Gong, Ran et al. 2009 |
| mean =2.1<br>median= 2.9<br>range = ND-3.1 | mean =1.6<br>median= 1.7<br>range = ND-1.6 | Zhao, - Ying et al. |
| mean=2.7<br>median= 1.9<br>range = 0.9-7.8 | mean=1.3<br>median= 1.3<br>range = ND-1.8 | Zhao, - Ying et al |
| mean=35.2<br>median= 33.4<br>range = 5.0-78.7 | mean =3.5<br>median= 2.8<br>range = ND-7.7 | Zhao, - Ying et al. |

### 2.4.3 Environmental Loss Rate Constants

The E1 half lives calculated in this study are longer than those reported in the literature for E2. In this study, the average half life for E1 was calculated at 65 days. The shortest seasonal half life (during the summer) was found to be 14 days and the longest half life (winter) was 880 days (Table 2.10). Jurgens et al. (2002) found half lives for the conversion of E2 to E1 ranging from 0.2 to 9 days. Further degradation of E1 occurred at similar half lives. In their experiment, they dosed river water with E2 concentrations ranging from 20 ng $L^{-1}$ to 500 μg $L^{-1}$ and incubated samples at 20$^{o}$C. In another experiment, the half life of E2 in river sediments was found to be 2 days (Holthaus et al., 2002). Further degradation was not detected (Holthaus et al., 2002). Yet another study found that E1 completely degraded from a sample of river water in 5 days when incubated at 28$^{o}$C, and in 7 days at 15$^{o}$C (Matsuoka, 2005). While there can't be a direct comparison made between the half lives for E1 and E2, there is much less literature on the E1 rate. There is some suggestion that E1 degrades much slower than E2 (Holthaus et al., 2002). This is consistent with the dramatic difference between the literature E2 half lives and the E1 half lives found in this study.

Laboratory studies like these can overestimate the actual environmental degradation rate because of the controlled experimental conditions, specifically high initial concentrations of estrogens, consistent temperature, consistent light availability and consistent aeration (Cao et al., 2008). In the environment, there are fluctuations in temperature, light availability and oxygen availability that would affect the loss rate constants by affecting degradation mechanisms (Cao et al., 2008). Maintaining temperature, light and oxygen at levels optimum for biological activity would likely increase biodegradation rates. Biologic activity is generally limited by these three parameters, and, therefore, biological degradation would be slower in the environment where these conditions are not optimized. Water temperatures of 20$^{o}$C in cooler climates would be limited to a short window during the summer, thereby reducing the average loss rate over a year. Since biological degradation is important to the loss rate of estrogens, artificially enhancing it in the laboratory skews the determined loss rate constant. Furthermore, a slower loss rate would be expected in the environment as the microbial population may not be adapted to fluctuations in wastewater effluent or even adapted to utilizing estrogens (Jurgens et al., 2002; Khanal et al., 2006; Ying et al., 2002).

While not concluded in this study, there is suggestion that seasonal variations affect the estrogen loss rate. The winter loss rate constant was well below the confidence limits of the other seasonal data suggesting it is different (Table 2.10). There is also some evidence that the summer seasonal rate is higher than the fall rate constant (Table 2.10). The seasonal variations in the data can be explained through the seasonal environmental variations affecting biological activity, particle availability and sunlight availability. Changes would therefore affect biodegradation, adsorption and photodegradation

The loss rate constant determined for E1 in this study was smaller than the one found in the literature for E1. The main differences between the two rates is that this study determined a rate within a CSTR (0.0106 d $^{-1}$) and the other rate (0.6 d$^{-1}$) was determined in a PFR (Writer et al., 2011a; Writer et al., 2012). Scavenging dynamics within a river channel can be much faster than in a reservoir or a lake because of increased light penetration, aeration and increased contact with sediments. This is consistent with the dynamics in a PFR and a CSTR, and could explain why the reaction rates found in the literature for rivers are much faster than was found for MacKay Reservoir in this study (Jurgens et al., 2002; Writer et al., 2011b; Writer et al., 2012).

An environmental loss rate constant was not detectable within Okanagan River (the only PFR analogue used in this study) as a result of low levels in the WWTP effluent and a very short channel or "reactor" length. Higher loss rate constants would have been detectable within Okanagan River. For example, if it is assumed that a minimum of a 5% change in diluted treatment plant E1 concentrations would be detectable, a loss rate constant between 0.47 and 2.74 $h^{-1}$ ($t_{1/2}$ = 1.47h and 0.25 h, respectively) would have been detectable. During the sample period, the length of the channel would have to be a minimum of 0.85 to 3.27 km to detect a 0.6 $d^{-1}$ loss rate constant for E1.

When the E1 concentrations in the effluent peaked, the subsequent downstream concentration could be accounted for by dilution. For most of the sample days, E1 or E2 were not detectable in Okanagan River because the low levels in the effluent were diluted below detection limits. The non-detect was determined following a background correction for the upstream levels that were measured (Figure 2.10). This is consistent with what has been done in the literature (Writer et al., 2012). If the effluent concentrations were at the top of the range measured at all three plants (77.2 ng $L^{-1}$ for E1 and 8.0 ng $L^{-1}$ for E2) (Table 2.6), and the dilution of the river channel was at its lowest noted 0.6% (Figure 2.9), the resultant concentration in the river would be 0.5 ng $L^{-1}$ and 0.05 ng $L^{-1}$ for E1 and E2, respectively. Correcting for background E1 levels, the result may approach the PNEC levels. Since this river channel is important for spawning salmonids, further investigation should be considered to ensure that estrogen concentrations in the WWTP effluent concentrations do not increase (Lawrence, 2003).

It was assumed that Okanagan River behaves like a plug flow reactor, and in order for this reactor to sufficiently reduce analyte concentrations there needs to be adequate retention time for degradation to occur. This retention time is directly related to the length of the channel and volumetric flow within the channel. With respect to estrogens, Okanagan River is not an effective PFR as it is simply too short. The retention time in the channel during the time of this study was between 1.9 hours to 7.6 hours. The fact that there was no loss detected within the short river channel is consistent with the literature (Labadie, et.al. 2007; Williams et al., 2003). In order for a channel of this length to be effective, the loss rate must be fast enough to scavenge estrogens in less than 8 hours.

Similar to the Okanagan River site, an environmental loss rate constant could not be calculated for Okanagan Lake. Unlike Okanagan River, the non-detectable estrogen concentrations were the result of the long residence time of the lake. As calculated for an average year, the total wastewater contribution into Okanagan Lake makes up approximately 1.2% of volume of water in Okanagan Lake (Guy, 2010). Therefore the median concentration of E1 (31.3 ng $L^{-1}$) from Kelowna WWTP would dilute to 0.38 ng $L^{-1}$. This would have been detectable in our samples had dilution been the only factor in reducing E1 concentrations in Okanagan Lake. Since no estrogen was detectable, the water residence time of >50 years allows for the wastewater to be scavenged in Okanagan Lake. The size of the lake and long residence time are important to the non-detectable concentrations of E1 and E2 within the system.

If complete lake mixing does not occur, or when a wastewater plume is not buoyant due to temperature or density conditions, there is potential that the released effluent could pool in the hypolimnion. Within this bottom layer, the water is cooler and there is little light penetration, resulting in less scavenging. Due to the lack of fast or turbulent mixing within the lake, there is also the potential for effluent plumes to pool higher up in the water column, in areas that are ecologically important to fish or other aquatic species, resulting in extended exposure to estrogen.

MacKay Reservoir is much different than Okanagan Lake. Okanagan Lake is classified as an oligotrophic lake and therefore has high clarity and low nutrient concentrations, resulting in potentially low rates of biodegradation and adsorption. In contrast, MacKay Reservoir was observed to be a very productive system as observed through algae blooms. MacKay Reservoir is relatively shallow, receives mainly wastewater with little to no dilution from external sources, and is completely mixed. Previous research explains that the rate in which estrogens are removed from aquatic systems is controlled by biodegradation, adsorption and photodegradation (Atkinson et al., 2011; Jurgens et al., 2002; Lin & Reinhard, 2005). Therefore, it is expected that the average estrogen loss rate constant in MacKay Reservoir (0.0106 $d^{-1}$) is higher than what might be found in Okanagan Lake.

### 2.4.4 A General Environmental Loss Rate Constant Model for E1

Since the potential effects of even low estrogen concentrations in freshwater aquatic systems are detrimental, the ultimate target should be non-detectable concentrations. This involves optimizing wastewater treatment processes and determining the best practices for disposing of the treated effluent. Comparing the environmental CSTR rate found in this study to the environmental PFR rate found in the literature, a best practices approach for WWTP effluent can be suggested.

Equation 2.12 is a useful model to predict the time required for desired degradation of E1 in natural aquatic systems, or to predict resulting concentrations in different receiving water environments where the residence times are known. For example, the residence times required for effluent degradation (after accounting for dilution) to selected target E1 concentrations in a CSTR can be calculated (Figure 2.15). In the absence of site specific E1 loss rates, existing systems which include the WWTP effluent and the receiving water can be evaluated by equation 2.12. Literature values for the concentrations of E1 out of the treatment plant can be used in the absence of known values. If the diluted E1 concentration in the CSTR is about 1 ng $L^{-1}$ it would take approximately 100 days to be reduced to 0.5 ng $L^{-1}$. In order to reduce 5.2 ng $L^{-1}$ E1, which is the peak concentration in MacKay reservoir to 1 ng $L^{-1}$ it would take approximately 390 days. These calculated residence times are high, and suggest that a large lake or reservoir would be required to effectively ensure a low resulting E1 concentration. Even using higher loss rates calculated during the summer season, a long residence time would be required to reduce E1 concentrations to 0.05 ng $L^{-1}$ (Figure 2.16). To reduce 5.2 ng $L^{-1}$ to 0.05 ng $L^{-1}$ it would take over 5 years using the winter rate. To reduce the same concentration to 1 ng $L^{-1}$ it would take about 85 days using the summer rate.

It is important to note that, in this study, the loss rate constant for E1 was found within a CSTR system, and that it may not apply to flowing systems such as rivers and streams. The dynamics within a CSTR are not the same as in a PFR. For example the initial concentration of the E1 is mixed into the entire volume of the CSTR and only into the plug of the PFR (Brezonik, 1994). If one assumes similar dynamics occurs in a river as in MacKay Reservoir, the E1 loss rate constant can be applied to PFR systems (Figure 2.17). A shorter residence time is needed in a PFR (or river) than a CSTR (or lake) (Figure 2.15 and Figure 2.17). For example to reduce 5.2

ng $L^{-1}$ to 1 ng $L^{-1}$ it would take approximately 150 days. This is less than half of the time of the CSTR. This suggests that a river is preferential for effluent disposal based on its increased efficiency. However, it would be impossible to find a river with this residence time. The global average for the water residence time in rivers is about 19 days (Schlesinger, 1997). Rivers are not long enough to facilitate estrogen scavenging and estrogen loss only occurs through dilution. In contrast, the global average water residence time in lakes is about 4.4 years (Schlesinger, 1997). Most lakes have the residence time required to scavenge estrogens following dilution.

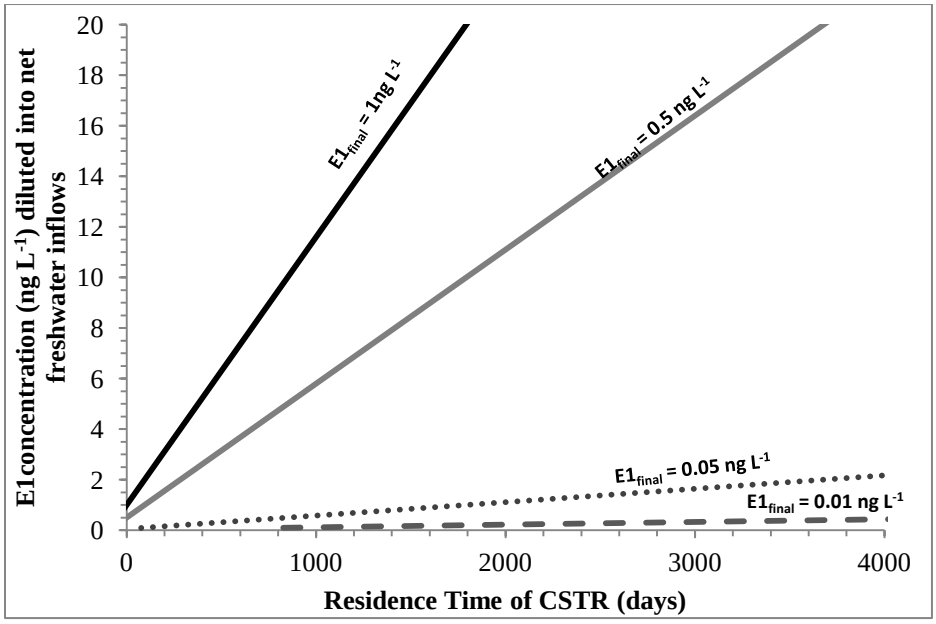



**Figure 2.15 Residence times required to scavenge diluted E1 concentrations to selected target E1 concentrations based on an average degradation rate of 0.0106 $d^{-1}$**

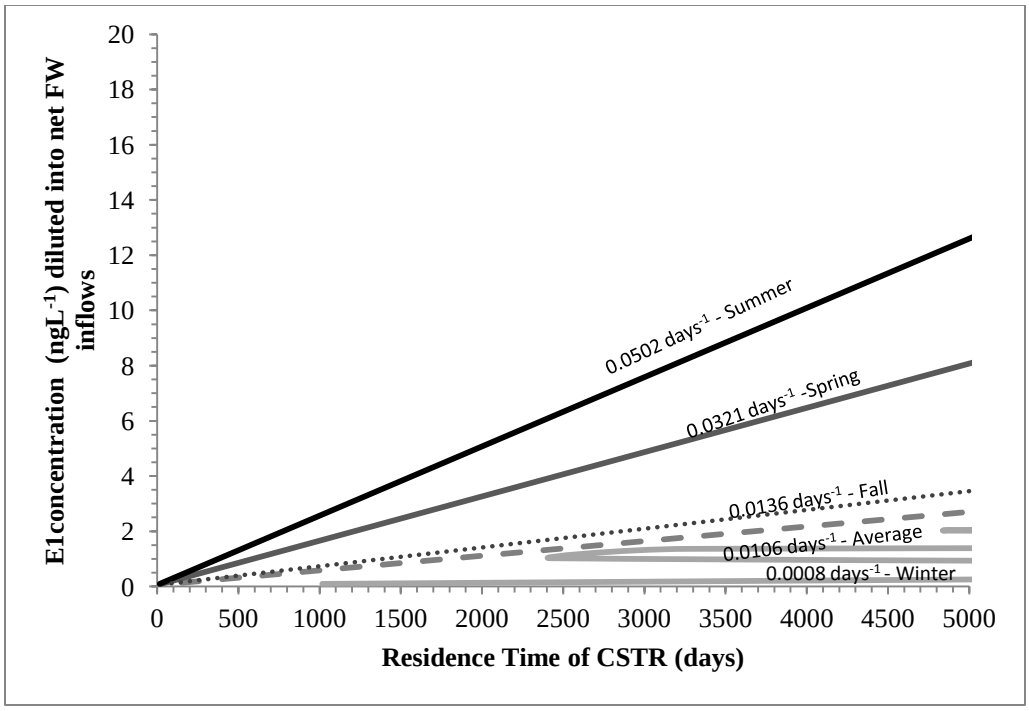



**Figure 2.16 CSTR residence time curves required to reduce E1 concentrations to 0.05 ng $L^{-1}$ for different seasonal loss rate constants**

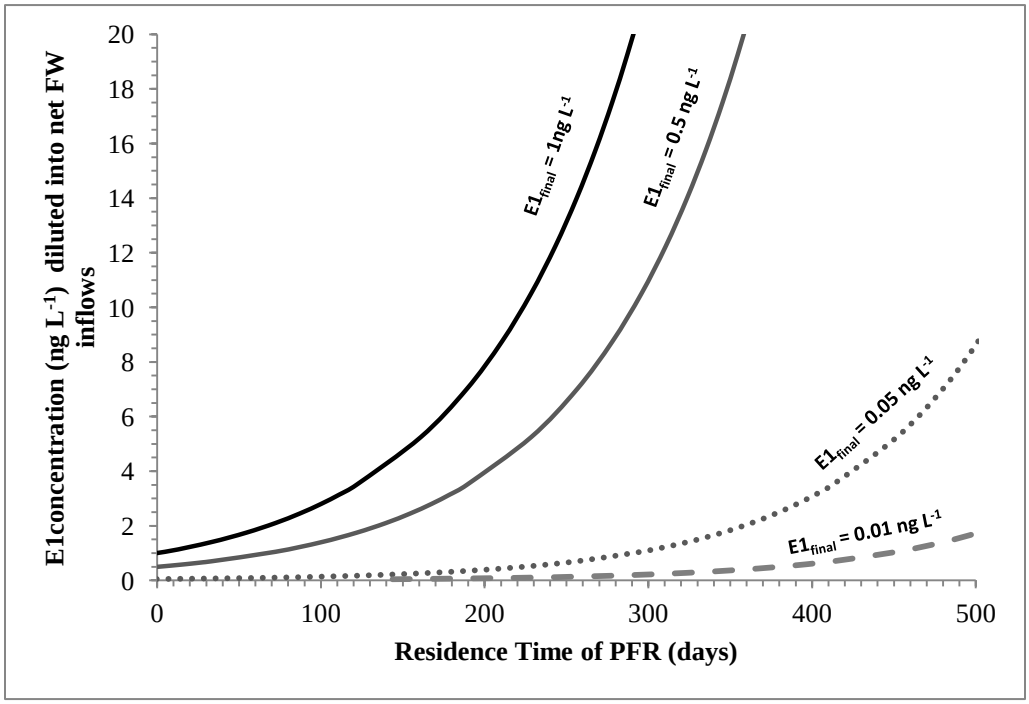



**Figure 2.17 The MacKay Reservoir loss rate constant applied to a PFR model, to calculate residence time curves for different target concentrations of E1**

At some distances in rivers there is little time for appreciable degradation, and dilution is the primary factor in reducing estrogen concentrations (Labadie, et al., 2007; Writer et al., 2011; Writer et al., 2012). One study found that even 10km downstream of effluent discharge, there was no estrogen scavenging (Labadie,et al., 2007). In this study, concentrations in Okanagan River were found up to 3.3 km downstream of the WWTP which is where the river discharges into Skaha Lake. The impact of this may be ecologically significant in some rivers. For example, this section of Okanagan River is used by lake fish populations for spawning (Lawrence, 2003). In addition to fish spawning habitat, there are many human settlements around rivers and drinking water extraction. With their ability to transport contaminants downstream without having been scavenged, rivers are a problematic location for treated wastewater discharge. Options for discharge are often limited to the easiest and most inexpensive method, and therefore proximity to a water body is usually imperative. For these reasons, rivers are common receiving environments for WWTP effluent. Since estrogens are known to have an ecological effect on aquatic organisms, and aquatic organisms heavily rely on river systems, it makes sense to look to alternative receiving waters for effluent release. In situations where a river discharges to a lake in proximity to the WWTP, the lake should be chosen for the receiving water if economically feasible. Given the requirement for residence time for the reduction of estrogens, discharging to a lake would allow scavenging and reduce impacts to rivers.

The best way to ensure that estrogens do not have an impact of aquatic organisms would be to further polish treatment plant effluent prior to release into the natural environment. An example of this is MacKay Reservoir. As demonstrated in this study, MacKay is another step of wastewater treatment by taking already treated wastewater and through naturalized scavenging mechanisms further polishes it with respect to estrogens. While lakes are better options to rivers for effluent disposal, there are still potential problems. A CSTR models assume that, once the contaminant enters the system, it is instantaneously mixed throughout the entire volume. As noted in the literature, this means a reduced reaction rate (Xu et al., 2009). This means aquatic organisms could be chronically exposed to low doses of estrogens. In addition there might be potential for mixing zones in lakes that contain high concentrations of estrogens. For these reasons it is desirable to prevent estrogen release into the environment through polishing reservoirs.

# Chapter 3 – Conclusions, Management Implications and Future Directions

## 3.1 Conclusions

Continued concerns about estrogen-related effects on freshwater animals prompted this research on how effectively estrogens are scavenged in the environment. While research into environmental estrogens (specifically estrone (E1), estradiol (E2), estriol (E3) and ethinylestradiol (EE2)) has been moving forward since the mid 1990's, the environmental fate of these estrogens has not been established. By investigating three different receiving waters, this study acquired useful information regarding the persistence of estrogens in the environment.

From wastewater treatment plant (WWTP) effluent grab samples, it was found that primarily E1 is released into receiving water bodies. This is consistent with most of the research (Falconer et al., 2006; Garcia-Reyero et al., 2011; Johnson, 2001; Servos et al., 2005; Thorpe et al.,2009), and suggests that E1 is potentially the most important estrogen of concern. Given the concentration of E1 in treated effluent and its biological potency it would be prudent to identify a guideline for E1 within receiving waters.

Through investigating estrogen concentrations in three different receiving waters, it was determined that the resultant concentrations of E1 are determined by the volume of water available for dilution and water residence time. Within a plug flow reactor or river system, there is insufficient time within each discrete plug to reduce E1 concentrations below detection and, therefore, E1 can be found at great distances downstream of wastewater treatment plants. While an acceptable concentration for E1 has not been determined, previous studies have indicated that the predicted no effect concentration in aquatic organisms (PNEC) is 3ng $L^{-1}$ (Gross-Sorokin et al., 2006).

Rivers are important ecosystems; they provide habitat, food, transport and a natal environment to many different vertebrates and invertebrates. For example anadromous fish like some salmonids require rivers for spawning habitat and for emerging fry (Lister & Genoe, 1970). Detectable concentrations of estrogens within river systems could mean catastrophic effects on the longevity of species that use rivers for the same purpose. Rivers are also conduits to estuaries and oceans, which have significant ecological roles as important fish habitat and nutrient transport (Bianchi,

2006). By transporting estrogens downstream without scavenging, there is the potential for contamination of these systems. Based on an annual average scavenging rate for E1 of 0.0106 $d^{-1}$ ($t_{1/2}$ = 65 d) and limited dilution, it would be difficult to find a river channel long enough to attenuate estrogen concentration to acceptable levels. Even using the 0.6 $d^{-1}$ rate for E1 found by Writer et al. (2012), a large area of aquatic habitat could still be affected prior to effective scavenging. If river channels were long enough to allow for estrogen loss within the water column, there would still be a significant distance over which estrogens were potentially high enough to cause detrimental ecological effects. It would be advisable to pipe effluent downstream to a larger water body with sufficient volume for dilution, rather than use rivers for disposal of treated effluent.

Within most large lakes that assume CSTR conditions, dilution is the most important process to reach non-detectable levels of E1. An ideal CSTR is assumed to be completely mixed, where reactant concentrations are the same throughout the control volume (Brezonik, 1994). This assumption does not always apply to lake systems, and especially large lakes. In many cases, lake homogeneity is disrupted by thermal or density stratification, in which distinct layers form. Under stratified conditions, vertical mixing by advection (thermal heating, seiching etc.), dispersion (plume, boundary mixing etc) and diffusion is usually slow (Fischer, 1979). Turbulent mixing does occur in lake environments due to thermal or density differences, seiches, surface waves and wind driven currents, but it does not persist for long (Fischer, 1973; Fischer, 1979). While a lake is stratified, these mixing mechanisms often occur within limnetic layers, but rates of mixing between layers are so small that the layers can be considered as distinct water bodies (Fischer, 1973; Fischer, 1979; Welch & Lindell, 2004). This could mean that effluent is diluted and mixed in a much smaller volume of water than the entire lake. This is not only a problem for estrogens, but it is a problem for more conventional contaminants like nutrients and biological oxygen demand (BOD). A pooling of effluent could result in localized problems within a lake.

In general a long residence time and dilution make large lakes desirable to reduce estrogen concentrations. Further polishing of wastewater through reservoirs like MacKay, is the best option to prevent potential problems lakes.

Between the three systems a scavenging rate could only be determined for MacKay Reservoir. The average time weighted scavenging rate for E1 was calculated at 0.0106 $d^{-1}$, indicating a half

life of about 65 d. Sampling was carried out in spring, summer, fall and winter months and an apparent seasonal loss rate constant was found (Table 2.10) resulting in much longer half-lives in the winter and shorter half-lives in the summer. The winter loss rate constant (0.0008 $d^{-1}$) was below the confidence intervals for the other seasonal constants (Table 2.10). With small data set it was difficult to statistically prove a difference. A difference in the seasonal loss rate constants is supported by the seasonal variability in dominant degradation mechanisms. Temperature, light availability and intensity, and the availability of oxygen affect the potential for biological degradation, adsorption and photodegradation.

A slow winter scavenging rate can be due to many variables. For most of the winter months, there is ice covering MacKay reservoir and the temperatures are very cold. Ice cover suggests that there is little oxygen exchange within the water column, which combines with cold temperatures to slow down aerobic biodegradation, which has been shown to be important degradation mechanism in WWTPs (Burkhardt-Holm, 2010; Jurgens et al., 2002; Khanal et al., 2006; Ribeiro et al., 2010; Xu et al., 2009).

Peak sunlight availability is required to be effective at reducing estrogen concentrations in water through direct photodegradation and by indirectly promoting biodegradation. (Chowdhury et al., 2010; Mazellier et al., 2008; Racz & Goel, 2010). During the winter months peak sunlight is not present due to short photoperiod and decreased sunlight intensity. In addition to this MacKay Reservoir is covered with ice and snow which greatly reduces or eliminates sunlight penetration into the water column. Photodegradation would be very minimal during winter months.

During winter months, adsorption is likely to be less prevalent as well. There is less biological activity and therefore less suspended organic material for adsorption. In the case of MacKay Reservoir the aeration systems is turned off eliminating any turbulent mixing. The reservoir is also ice covered and therefore there is no wind action. Without mixing there is no energy for inorganic particle suspension from bottom sediments which also reduces adsorption mechanisms.

Conversely, in the summer there are sufficiently high temperatures and oxygen exchange with the atmosphere and an aeration system to maximise biological degradation rates. This is consistent with the fastest scavenging rate calculated for MacKay Reservoir (Table 2.10). Peak

available UV radiation is available during the summer months which increase biological activity and photodegradation. Finally, unlike winter, there is increased mixing in MacKay in the summer months through the aeration system and wind action. This would allow for increased adsorption onto suspended particles.

It is difficult to isolate scavenging mechanisms in the natural environment as they are all linked. Further sampling for different parameters during this study might have allowed for the determination of the most significant mechanism. To test for biological activity, measuring biological oxygen demand would be useful. This would confirm the assumption that there is less biological activity during winter months and therefore less potential for biological degradation of estrogens. Testing for total suspended solids, turbidity and or particulate organic matter would identify the availability of particulate for adsorption. Observations were made on algal blooms in MacKay Reservoir, but there was no quantitative measure. This study was not designed to identify which mechanism is most significant in the environment and therefore there was no evidence of which is dominant.

### 3.2 Management Implications

This research identified important factors that can have implications on how treated effluent is released in the environment to reduce estrogen concentrations. With respect to the receiving water bodies (rivers, lakes, reservoirs), there are three main observations. Firstly, rivers require a long distance to reduce the concentrations of estrogens from WWTP effluent through scavenging. This suggests that rivers should not be used for the disposal of treated wastewater effluent unless dilution of effluent is sufficient to reduce estrogen concentrations below detection or below effect levels. This is especially true during cooler seasons in cold climate regions. Because there was a seasonal component to the scavenging of E1, this should be considered in designing WWTP effluent outfall. If release to rivers is the only option, it would be optimal to do it during a time when dilution and scavenging rates are highest in order to reduce impacts on the ecosystem.

Secondly, it is advisable that wastewater be further polished following wastewater treatment and before final release in to the environment. This can be done through polishing ponds like

MacKay Reservoir. MacKay Reservoir allows for scavenging mechanisms to attenuate potentially detrimental concentrations of estrogens prior to them entering into natural aquatic ecosystems. While reservoirs may not be required if releasing into large receiving water bodies where dilution dominates, the use of a reservoir would prevent any potential concerns with pooling of effluent plumes or pockets of high estrogen concentrations in large, stratified lakes. Allowing natural attenuation to occur in a reservoir is inexpensive in comparison to an engineered system, and is an additional treatment step that would only require a reservoir with sufficient residence time to degrade estrogens to a level that is acceptable prior to release. This residence time can be determined using the information presented in Chapter 2.

Finally, the loss rate constants calculated in this research could be used by managers or regulators when planning new effluent release. The loss rate constant could be used to be used to predict what might happen in a river or PFR. Although the scavenging rates are approximations, they are the first available in natural systems, and if they are applied to the dynamics of individual receiving water (river, lake, reservoir), regulators could determine potential impacts of E1 downstream. This may have an impact on the placement and timing of wastewater discharge. While this may be impractical for large urban populations, the potential impacts of E1 could lead to innovative polishing solutions. For example the use of engineered wetlands that rely on both scavenging and biodegradation to polish effluent.

### 3.3 Future Directions

This research set out to answer a first order question on the rate at which estrogens are scavenged in the environment following release from wastewater treatment plants. Through this research, knowledge was gained about estrogen fate in 3 different WWTP receiving waters. This research describes, for the first time, the fate of WWTP estrogens in natural systems and thus provides more valuable data than the existing published reports which examined artificial (laboratory) settings. Finally a discussion of the remaining gaps in knowledge was made to help direct future research in this important field.

To begin with, the lack of standard methods available for the examination of estrogens in freshwater aquatic systems needs to be resolved. The introduction of USEPA method 539 in

2010 is a starting point to address this weakness (United States Environmental Protection Agency, 2010). This method is similar to the one used in this research, employing pre-treatment using a C-18 SPE column followed by LC-$MS^2$ analysis. Although this method is designed for concentrations found in drinking water, it could be used for more complex matrices such as wastewater.

Water quality sampling and analysis comes with an associated variability and error. Further investigation into the persistence of estrogens in receiving waters should attempt to mitigate this error through analysis and sampling. First, the analytical method used should be consistent for all of the samples taken. This would eliminate one aspect of the variability in this study. Next, the variability that has been noted in the literature in wastewater effluent should be accounted for (Nelson et al., 2011). Grab sampling involves a certain amount of error when used to represent the average concentration of a substance within an entire, large body of water (Martin et al., 1992; Vermeirssen et al., 2005). The temporal variation of WWTP effluent and receiving water would be accounted for through temporal composite sampling of the wastewater effluent. A similar type of sampling within the receiving water could include spatial composite sampling. In a uniform body of water like MacKay Reservoir, grab sampling is adequate; however the lack of homogeneity in river and lake systems justifies a different sampling technique. In order to mitigate the sampling error in large lakes or river systems, effort needs to be made to follow the parcel of treated wastewater in the receiving water. An example of this is the research of Writer et al. (2012), where bromide was used as a conservative tracer to monitor the degradation of 4-Nonylphenol and 17β-Estradiol in the Redwood River. In order to mimic this research in a lake, a conservative tracer could be followed from the wastewater treatment plant effluent, through the effluent pipe and into the receiving water. There is a potential high cost and effort to this, but such a study could assist in clarifying how estrogens behave in a large, natural CSTR where stratification occurs.

While the above steps would help to reduce errors associated with the present research design, it is likely that the conclusions drawn here would not change with these improvements. This conclusion being that lake environments, in most cases, are superior receiving waters to rivers. Based on the loss rate constant determined (0.0106 $d^{-1}$), the global average of 19 days for river

residence time is insufficient to reduce estrogens through scavenging. Conversely, the global average residence time of 4.4 years for lakes allows for scavenging of estrogens.

At the onset of this project, there were three identified gaps in the current body of research that needed to be investigated further. The first gap was the lack of conclusive evidence of which scavenging mechanisms explain estrogen losses in receiving waters. As outlined in this research, the literature shows, there is evidence that biodegradation, adsorption and photodegradation are all occurring in the environment (Caupos et.al., 2011; Chowdhury et al., 2010; Leech et al., 2009; Racz & Goel, 2010; Writer et al., 2011a). These mechanisms are difficult to isolate in the environment, and environmental conditions are difficult to reproduce in the laboratory. Mechanism determination was not an objective within this study. It would be a prudent next step to include sediment analysis in subsequent research.

The second gap was the need to identify a seasonal component to estrogen scavenging, which was partially addressed in this research. Seasonal patterns could be further clarified by data collection over multiple years, and sampling under ice. Seasonality is interesting as it suggests that the summer rates are faster than winter rates, and biological mechanisms are most important to environmental scavenging of estrogens. In addition, this research could be more broadly applicable to different regions of the globe if the temperature-dependence and biological activity dependence of the scavenging rate could be calculated. Ultimately, a seasonal degradation rate could suggest optimal timing for release of treated effluent into the environment, if this option was possible to control.

Finally, there was a need to address the importance of E1 in relation to other estrogens, and to establish its effects on aquatic organisms. Since E1 is the compound most often detected in effluent and in receiving waters, its potency at various relevant concentrations needs to be determined. If the effects of estrogens at the outfall of wastewater treatment plants are observed, and research suggests that E1 is the highest in concentration, then it should be confirmed that E1 is an important endocrine disruptor in aquatic systems. This relationship needs to be explored further.

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