Assessment of endocrine disrupting chemicals in water and sediment samples from British Columbia, Canada

by

Farhana Ali

B.Sc. (Honours), Simon Fraser University, 2011

Research Project Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Environmental Toxicology

in the
Department of Biological Sciences
Faculty of Science

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SIMON FRASER UNIVERSITY

Fall 2015

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Approval

Name:	**Farhana Ali**
Degree:	**Master of Environmental Toxicology**
Title:	***Assessment of endocrine disrupting chemicals in water and sediment samples from British Columbia, Canada***
Examining Committee:	**Chair:** Dr. Felix Breden Professor

Dr. Francis Law
Senior Supervisor
Professor

Dr. Margo Moore
Supervisor
Professor

Dr. Christoper Kennedy
Internal Examiner
Professor

Date Defended/Approved: November 25, 2015

Abstract

Endocrine Disrupting Chemicals (EDCs) can interfere with the endogenous hormone system that leads to adverse health effects in the exposed population of wildlife and humans. Thus, the objective of the present study was to identify and quantify four different classes of EDCs, *i.e.* estrogens, androgens, glucocorticoids and aryl hydrocarbon receptor agonists in the water and sediment samples from 22 sites in British Columbia. All sites were bodies of water that are impacted by agricultural and/or urban activities. Samples were collected during the dry and rainy periods at each sampling location. EDC levels were higher in sediment than in water across all sites. The highest activity was found using the glucocorticoid assay compared to the other two steroid hormone assays. Chemical analysis was performed using gas chromatography-mass spectrometry on a subset of samples to identify specific compounds in the mixture. The chemicals identified were 17β-estradiol, estrone, bisphenol A and dehydroabietic acid. Findings from this study may be used as benchmark levels for future studies in the same region.

Keywords: EDCs, Recombinant yeast bioassays, steroid hormones, AhR agonists

Acknowledgements

I thank Allah for all the help in the completion of this research. I am also grateful to my senior supervisor Dr. Francis Law for entrusting and guiding me throughout on this project. Your guidance and advice on my work has been invaluable. I am equally grateful to my supervisor Dr. Margo Moore for her constructive feedback and guidance on both my research and thesis. My sincere thanks goes to Dr. Chris Kennedy for always providing me with productive feedback in labs and classrooms. I also want to thank Dr. Felix Breden for giving me opportunities to learn and explore science. My special gratitude goes to Dr. Zaheer Khan for providing support and encouragements on my work.

I want to acknowledge the generosity of my colleagues Zeyad Alehaideb, Noor Fageh and Alvin Louie. Many thanks go to Marlene Nguyen for her support during my grad years and Debbie Sandher for her guidance in my undergraduate years. I am forever thankful to Tammy McMullan and Thelma Finlayson for their advice and assistance during my time at the university.

Last but not least, I want to thank Kristina Pohl, Kristen Fay Gorman and Ben Sandkam for giving me a great company and memories that will be cherished forever.

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List of Acronyms

AhR	Aryl Hydrocarbon Receptor
ANOVA	Analysis of Variance
AR	Androgen Receptor
ARNT	Aryl hydrocarbon Receptor Nuclear Translocator
BLD	Below the Limit of Detection
CALUX	Chemical Activated Luciferase gene expression
DHT	Dihydrotestosterone
DHTEQ	Dihydrotestosterone Equivalent
DOC	Deoxycorticosterone
DOCEQ	Deoxycorticosterone Equivalent
E2	17β- Estradiol
EC50	Effective Concentration at 50% of maximal activity
EDC	Endocrine Disrupting Chemicals/Compounds
EEQ	17β- Estradiol Equivalent
ER	Estrogen Receptor
GC/MS	Gas Chromatography- Mass Spectrometry
GR	Glucocorticoid Receptor
HPA	Hypothalamic-Pituitary-Adrenal axis
HPGL	Hypothalamus-Pituitary-Gonadal-Liver axis
HPI	Hypothalamus-Pituitary-Interrenal axis
HPT	Hypothalamus-Pituitary-Thyroid axis
NAP	β- Naphthoflavone
NAPEQ	β- Naphthoflavone Equivalent
PAH	Polyaromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
SEM	Standard Error of the Mean
WWTP	Waste Water Treatment Plant
YAS	Yeast Androgen Screen
YES	Yeast Estrogen Screen
YGS	Yeast Glucocorticoid Screen

1. Introduction

Over the last couple of decades, much has been written about endocrine disrupting compounds (EDCs) and their potential deleterious effects in humans and animals. EDCs are ubiquitous in the environment; they have been found in plastic bottles, metal food cans, detergents, flame retardants, food additives, cosmetics, pesticides, herbicides, etc. Therefore, many living organisms are exposed to EDCs on a daily basis. Evidence is accumulating to indicate that EDCs such as synthetic estrogens, anabolic steroids, anti-inflammatory drugs, polychlorinated biphenyls (PCBs), bisphenol A (BPA), nonylpheol (NP) and some pesticides can disrupt the development and growth of terrestrial and aquatic animals (Damstra et al., 2002; Hayes et al., 2005; Lintelmann et al., 2003). Some of the adverse effects include demasculinization and feminization of fish, decreased hatching success in fish and birds, abnormal thyroid function, and alteration of immune and behavioral functions in fish, birds and mammals (Tierney et al., 2014).

Surprisingly, there have been very few studies on the presence and effects of EDCs in lakes, sloughs, creeks and other small bodies of water (Rosen et al., 2006; Bogdal et al., 2009). A recent experiment in a Canadian lake has shown adverse health effects in a fish population after dosing the lakes with a very low concentration (2 ng/L) of 17 α-ethynylestradiol (EE2) (Kidd et al., 2007; Palace et al., 2009).

1.1 The Endocrine System and Endocrine Disrupting Compounds

The endocrine system (ES) is an organ system that involves similar glands, hormones and secretion patterns in vertebrates from fish to mammals (Campbell et al., 2004). The ES consists of an internal network of signals and responses that are crucial in maintaining and regulating homeostasis and other bodily functions. The endocrine glands include the hypothalamus, pituitary, pineal, thyroid, parathyroid, adrenal cortex and medulla, pancreas, chromaffin tissue (fish), corpuscles of Stannius (fish), the interrenal

organ (fish) as well as male and female reproductive organs. These glands release hormones, chemical messengers that travel in the blood to other parts of the body, to control essential functions such as metabolism, growth, development, reproduction, primary and secondary sexual characteristics, as well as water, calcium, and glucose balance.

U.S. Environmental Protection Agency (USEPA) defines EDC as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.” Thus EDCs act in several ways to interfere with the internal hormonal system. They can mimic hormones and disrupt the normal functioning of an ES. They can cause an over stimulation of certain responses, or initiate a response at an inappropriate time. They can also bind to receptors and block endogenous hormones from binding thus normal signals fail to occur. They may act to alter the metabolism of endogenous hormones and modify the availability of hormone receptors. EDCs can also interfere with the binding proteins that carry/transport the endogenous hormones (Bergman et al., 2012). Overall, EDCs may impact the three axes (i.e. Hypothalamic-Pituitary-Gonadal (HPG) axis (HPG-Liver axis in fish), Hypothalamic-Pituitary-Adrenal (HPA) axis (HP-Interrenal axis in fish), and Hypothalamic-Pituitary-Thyroid (HPT) axis) that balance the sex, stress and thyroid hormones leading to immune function abnormalities (Norris and Lopez, 2011).

1.2 Four classes of EDCs in the environment

In the present study, we examined three groups of natural and synthetic steroid hormones that enter the environment through human/animal excreta, via agricultural waste and Waste Water Treatment Plants (WWTPs). In addition, we studied AhR agonists from industrial wastes and anthropogenic activities.

1.2.1 Estrogenic Compounds

Estrogens are lipid-soluble chemicals that bind to the ER in the cytoplasm after entering the cell. The ligand-receptor complex then enters the nucleus and interacts with

the estrogen response elements located in the promoter regions on DNA (Figure 1.1). This activates specific genes that modulate gene expression that ultimately results in biological effects of estrogens (Bergman et al., 2012).

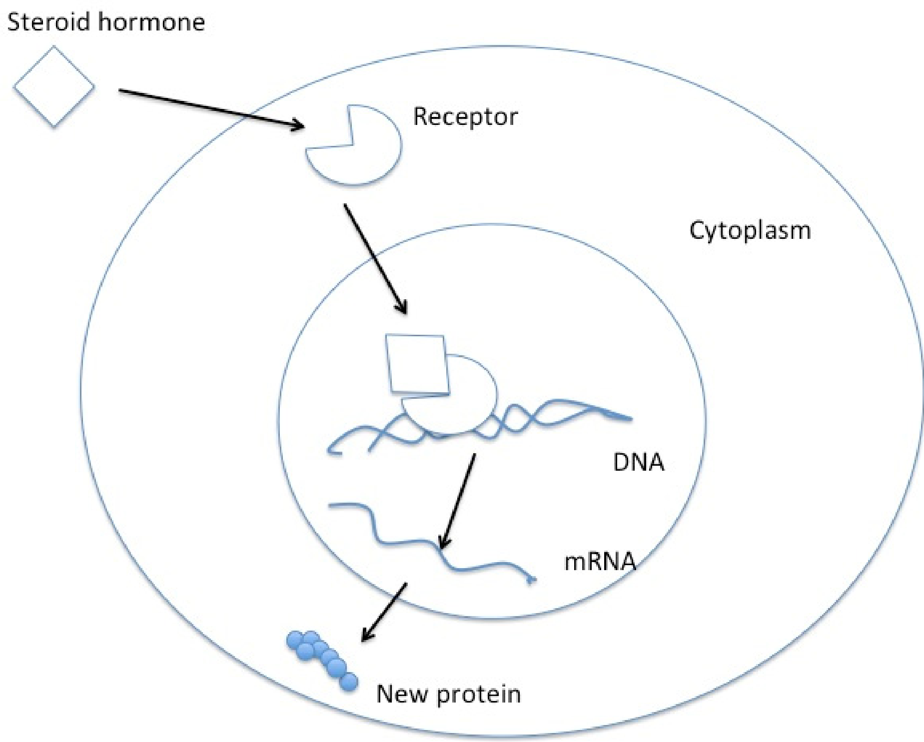


Figure 1.1 Schematic diagram of steroid hormones' mode of action.

The hormone enters the target cell and binds to its receptor in the cytoplasm. The ligand-receptor complex enters the nucleus where it binds to the hormone response element on the DNA. This activates transcription of target genes. The mRNA moves into the cytoplasm where new protein is synthesized.

Naturally occurring estrogenic compounds in mammalian and aquatic species include 17 β-estradiol (E2), estriol (E3) and estrone (E1). E2, a major estrogen, is produced by follicular cells in the ovaries of fish and is involved in gamete production in addition to vitellogenin synthesis. Thus, estrogens are important in reproductive development and female characteristics and behaviours. Production of E2 is controlled by

the hypothalamic-pituitary-gonadal (HPG) axis *via* a negative feedback mechanism that can be modified by xenoestrogens (Hiller-Sturmhöfel and Bartke, 1998).

Synthetic compounds such as ethynylestradiol (EE2) (component of contraceptive pills) and phenolic compounds such as BPA (monomer of plastic used in manufacturing of consumer products) and NP (an industrial surfactant) can mimic the function of estrogens (Krishnan et al., 1993; Knudsen and Pottinger, 1999). Phytoestrogens are estrogenic compounds from plants that possess mammalian sex hormone activity (Miksicek, 1995). Natural and synthetic estrogenic compounds can find their way into the environment through human/animal excreta, industrial/agricultural waste and WWTPs. Table 1.1 shows the structures of some of the estrogenic compounds found in the environment.

Studies in fish have shown that EE2 is about 11 to 27 times more potent in estrogenic activity than E2 (Thorpe et al., 2003). It can lower fertility and egg production in female fish. It also causes feminization and reduces gonad size in male fish (Tilton et al., 2005; Jobling et al., 2003). Studies have shown that BPA is an agonist of estrogen receptor (Kurosawa et al., 2002; Mathews et al., 2001). Thus chronic exposure of male goldfish to 100 to 1000 ng/ml BPA is able to induce vitellogenin production in male goldfish (Ishibashi et al., 2001). NPs also are found to induce vitellogenin in different fish species; it decreases male fertility at concentrations ranging from 100 to 2030 ng/ml (Tabata et al., 2001; Jobling et al., 1996; Kinnberg et al., 2000). Studies have shown that phytoestrogens such as daidzein, enterodiol and genistein are able to inhibit the binding of estrogens to the ER in animals (Whitten et al., 1992) causing infertility (Shutt et al., 1976). An example is the infertility syndrome, known as clover disease, in sheep that graze on subterranean clover (Cheek et al., 1998).

Table 1.1 Example of natural and synthetic estrogenic compounds

Chemical Name	Chemical Structure	Class/Use	References
17 β-estradiol (E2)		Natural female hormone	Kinnberg et al. 2000; Tabata et al. 2001
17 α-ethinyl estradiol (EE2)		Synthetic hormone used as oral contraceptive	Jobling et al. 2003; Tilton et al. 2005; Kidd et al. 2007
Bisphenol A (BPA)		Plasticmonomer in production of certain plastic products	Ishibashi et al. 2001; Jobling et al. 2003
Nonylphenol (NP)		Surfactant used in detergents, paints, pesticides, cosmetics etc.	Kinnberg et al. 2000; Tabata et al. 2001

1.2.2 Androgenic Compounds

Like estrogens, androgens are also lipid-soluble molecules that pass through cell membranes and bind to a specific receptor, the androgen receptor, in the cytoplasm. The ligand-receptor complex enters the nucleus of cells and attaches to the androgen

response element segment of DNA (Figure 1.1). This guides the cell to produce proteins associated with androgens (Bergman et al., 2012).

Androgenic compounds are a group of steroid hormones that stimulate the development of male sex characteristics as well as tissue regeneration in bones and muscles. They also play a subtle role in the female species. Androgens are produced in the ovaries and testes of fish as well as in adrenal cortex of mammals. Natural androgens include testosterone (T), dihydrotestosterone (DHT), androstenedione (AE), dehydroepiandrosterone (DHEA) and 11-ketotestosterone (11-KT). The levels of testosterone in the body are kept in balance through regulation of the HPG axis (Bergman et al., 2012; Hiller-Sturmhöfel and Bartke, 1998).

Synthetic and naturally occurring anabolic steroids are used in cattle farms to promote growth, e.g., trenbolone acetate (Tb), testosterone, zeranol and melengestrol acetate (MGA) (Lange et al., 2002). There has been an increased use of TBA in the cattle industry and as a result, TBA and its metabolites have been detected in the leachate of farms in the USA (Soto et al., 2004; Durhan et al., 2006). Studies have shown that TBA is more potent than testosterone in terms of binding to AR in humans and fish (Bauer et al., 2000; Ankley et al., 2003). Aquatic life exposed to anabolic steroids has shown reduction in plasma vitellogenin levels, masculinization of female fish, reduced fecundity and development of secondary male characteristics (Velasco-Santamaria et al., 2010; Kolok and Sellin*,* 2008; Sellin et al.*,* 2009). Table 1.2 shows some of the androgenic compounds found in the environment.

Table 1.2 Example of natural and synthetic androgenic compounds

Name	Chemical Structure	Class/Use	References
Testosterone (T)		Natural hormone	Bauer et al. 2000; Damstra et al. 2002
Dihydrotestosterone (DHT)		Natural hormone	Bauer et al. 2000; Soto et al. 2004
Trenbolone (Tb)		Synthetic androgen used as anabolic steroid	Ankley et al. 2003; Seki et al. 2006; Sellin et al. 2009

1.2.3 Glucocorticoid Compounds

Glucocorticoids are important in controlling blood glucose levels, metabolism of carbohydrates, proteins and lipids and immune/brain functions. GCs are released from the adrenal cortex after HPA axis activation (Damstra et al., 2002). Endogenous glucocorticoids include cortisol, cortisone and corticosterone. Anti-inflammatory drugs that are widely used in humans and animals include prednisone, dexamethasone, hydrocortisone and cortisone (Iglesias et al., 2014). Table 1.3 shows some of the common anti-inflammatory drugs used in human and veterinary medicine today.

Many anti-biotic and anti-inflammatory drugs are found in calf hutches, lagoons, manure application and aquaculture (Watanabe et al., 2010). Glucocorticoids often are used to induce weight gain in animals since they are found to have synergistic effect with anabolic steroids (Reig et al., 2006). Pharmaceuticals, including GCs, have been detected

in lakes and other bodies of water near farmlands all over the world (Shi et al., 2013). Cherlet et al. (2004) and Reig et al. (2006) detected dexamethasone in bovine milk and feed of livestock. Thus, runoffs from farm fields carrying natural and synthetic drugs and their metabolites are of concern as they can potentially cause adverse health effects in aquatic organisms (Fent et al., 2006; Ziylan and Ince, 2011).

GCs exert their action the same way as estrogens and androgens do. They enter the cell and bind to the glucocorticoid receptor in the cytosol. The complex then translocates to the nucleus and binds to glucocorticoid response element (GRE), a binding domain on DNA, to regulate gene transcription (Figure 1.1) (Bergman et al., 2012).

Table 1.3 Example of natural and synthetic glucocorticoids

Name	Chemical Structure	Class/Use	References
Cortisol		Natural stress hormone	Möstl et al. 1999; Macikova et al. 2014
Cortisone		Natural stress hormone	Möstl et al. 1999; Iglesias et al. 2014
Dexamethasone		Synthetic Glucocorticoid	Cherlet et al. 2004; LaLone et al. 2012
Hydrocortisone		Natural and synthetic Glucocorticoid	Macikova et al. 2014

1.2.4 Aryl Hydrocarbon Receptor (AhR) Agonists

This group of EDC are not hormones but are aromatic hydrocarbons that can activate the aryl hydrocarbon receptors. The majority of the AhR ligands are formed as a result of incomplete combustion of organic materials and anthropogenic activities. These chemicals include halogenated aromatic hydrocarbons (HAHs), polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated furans, organochlorine pesticides (OCPs), polychlorinated dioxins and dioxins-like compounds (Denison et al., 2002). Table 1.4 lists some of the AhR agonists in the environment. These compounds are persistent in the environment due to chemical stability and hydrophobicity. They are released into the environment through incineration of household and industrial waste, as industrial by-products, and incomplete combustion of coal, diesel fuel, tar and plant materials (Boström et al., 2002). AhR activation is usually associated with xenobiotic-induced toxicity and carcinogenicity but it can also disrupt hormonal functions as crosstalk between AhR and nuclear receptors has been observed (Matthews and Gustafsson, 2006).

AhR is bound to a chaperone complex in the cytoplasm. Upon binding of a ligand to AhR, the complex translocates into the nucleus where the chaperone complex dissociates and Ahr-ligand dimerizes with AhR nuclear translocator (ARNT). The AhR-ARNT heterodimer binds to xenobiotic responsive elements (XRE) of the DNA and regulates transcription of its target genes. In general these target genes encode for biotransformation enzymes such as CYP1A1 and CYP1A2 in the CYP450 family members (Pocar et al., 2005).

Endocrine disrupting effects through AhR activation have been reported in animals exposed to PCBs, dioxins and PAHs. For example, whales, exposed to PCBs, were found to have significantly low levels of thyroid hormone, thyroxine (T4) (Villanger et al., 2011). This was due to PCBs binding to AhR and modulating (increasing) gene expression of an enzyme involved in metabolic elimination of T4; another possible mechanism is PCBs interfering with thyroid hormone signalling by binding to thyroid hormone transport proteins (Kato et al., 2004). Rats dosed with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) had premature reproductive senescence and a decrease in E2 level (Shi et al., 2007). TCDD has been shown to lower E2 serum levels by altering steroidogenic enzymes (Takeda et

al., 2009). Benzo [a] pyrene (BAP) can cause a reduction in E2 and ovotoxicity in rats (Xu et al., 2010). BAP exposed killifish have low levels of aromatase which converts androgens into estrogens thus leading to developmental problems (Patel et al., 2006).

Table 1.4 Example of AhR agonists

Name	Chemical Structure	Class/Use	References
Benzo [a] pyrene (BAP)		A PAH, by product of combustion and a known carcinogen	Patel et al. 2006; Xu et al. 2010
Polychlorinated Biphenyl (PCB) 28	Cl Cl Cl	Industrial coolant and plasticizer	Kato et al. 2004; Villanger et al. 2011
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	Cl O Cl Cl O Cl	Industrial waste and through combustion of fossil fuels	Shi et al. 2007; Takeda et al. 2009

1.3 Yeast Screening Bioassays

EDCs in the environment are detected and quantified using various chemical analyses and bioassay procedures. These include *in vitro* cell based assays using yeast strains or mammalian cell lines and chemical analysis using gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography (HPLC). Each of these methods has its own advantages and disadvantages in terms of specificity, sensitivity and cost (Nie et al., 2009; Balsiger et al., 2010)

Yeast based reporter assays have been widely used in the detection of EDCs in the environment samples (Chang et al., 2014; Giesy et al., 2002). Recombinant yeast cells are transformed with a specific receptor, its response element (RE) and a reporter protein such as β-galactosidase or luciferase. Recombinant yeast bioassays measure relative activity of a compound without any prior knowledge about the substance's chemistry. Upon binding of a ligand the receptor is activated and subsequent production of reporter protein is measured (Figure 2.5) (Campbell et al., 2006). Various studies have validated yeast-based bioassays as a sensitive and robust screening tool for the determination of estrogenic (Noguerol et al., 2006; Gaido et al., 1997), androgenic (Bovee et al., 2009; Bhattacharjee and Khurana, 2014), glucocorticoid (Bovee et al., 2011; Antunes-Fernandes et al., 2011) and aryl hydrocarbon agonists (Noguerol et al., 2006; Alnafisi et al., 2007) activities.

1.4 Research Objectives and Study areas

The objective of the present study was to test for EDC levels in water and sediments samples from various water bodies in British Columbia, Canada. Figure 2.1 is the overview map of the region of the Fraser Valley where samples were collected. The current study is the first report on the concentrations of estrogenic, androgenic, glucocorticoid and aryl hydrocarbon agonist compounds present in these sites. The levels for each type of EDCs were obtained using four different yeast bioassays (See Material and Methods). Multiple samples were collected during dry and wet periods from the same locations to assess seasonal and temporal variations in EDCs.

The study area included sloughs, a creek, rivers, canal and ditches that receive runoff from agricultural lands containing manure and fertilizers, untreated water from a pump station, storm sewer and compost facility. These sites were deemed at risk to accumulate natural and synthetic compounds such as 17 β-estradiol, bisphenol A, testosterone, trenbolone, melengestrol acetate, dihydroabietic acid and a multitude of pesticides, herbicides and fungicides used in farms. These chemicals have the potential

to cause adverse effects on the aquatic life present in the sloughs, creek, rivers, canal and ditches.

Fish kills in the tributaries in the Fraser valley have been attributed to runoff from agricultural land (Hall and Schreier, 1996). The sampling location in Agassiz slough is downstream from the town's storm sewer output. Thus, the wastes from urban and rural human population as well as runoff from dairy farms present in the vicinity of the slough impact this site. Another site, Mountain slough, a close-by area, is also impacted by multiple dairy and berry farms. Mountain slough is also a known laying site for Oregon spotted frogs. This frog species has been declared endangered due to urbanization and agricultural activities that have led to its disappearance from 78% of its former range (USFWS, 2014). The surviving populations are in constant threat from chemicals that wash into wetlands from farmlands (Ministry of Environment, B.C. 2002). Another two endangered fish species, the Salish sucker and the Nooksack Dace, reside in Agassiz Slough, Miami River and Pepin creek. Although Pepin creek has good population of fish, they are mainly threatened by agricultural activities in the British Columbia's Fraser Valley (Fisheries and Oceans Canada, 2010).

The sites in Surrey were important in that discharge from the pump station, farms (agricultural and dairy), urban/rural runoffs drain down to Nicomekl river which is home to salmonids and non salmonids fish. Although not threatened, chronic exposure to contaminants draining into the river can lead to bioaccumulation in fish tissues.

The purpose of the present study were to obtain and test environmental samples from areas in the Fraser valley of British Columbia that have never been tested for these contaminants using the yeast-based bioassays and to conduct a preliminary risk assessment for the aquatic species in these areas.

2. Materials and Methods

2.1 Sampling Sites

Figure 2.1 shows the overview map of the areas (circled) of the Fraser Valley where samples were collected. The detailed maps are followed in the sections below.

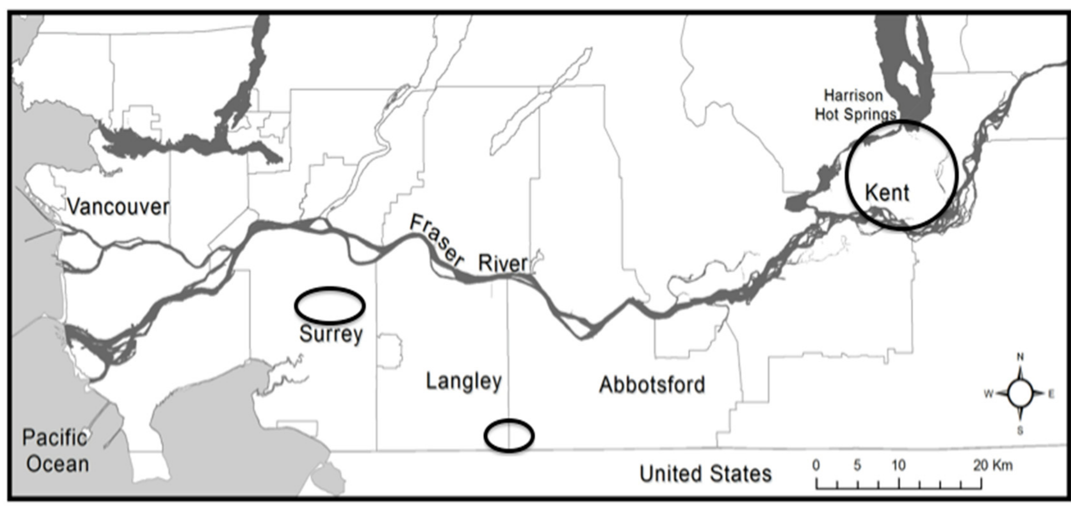


Figure 2.1 Overview map of sampling sites (circled) in Metro Vancouver and Fraser Valley of British Columbia, Canada.

2.1.1 Sampling sites in 2013

There were a total of 9 sampling sites of which 4 were from the District of Kent (AS, MT1, MT2, MT3); 3 from the village of Harrison Hot Springs (MR1, MR2, MR3) and 2 from a creek running through the city of Abbotsford and Langley (PN1, PN2). Each site was sampled in June, August and November for water and sediments. Due to inaccessibility, sediments could not be collected from MR1 in November. Figures 2.2 and 2.3, and Table 2.1 provide more detailed information on these sites.

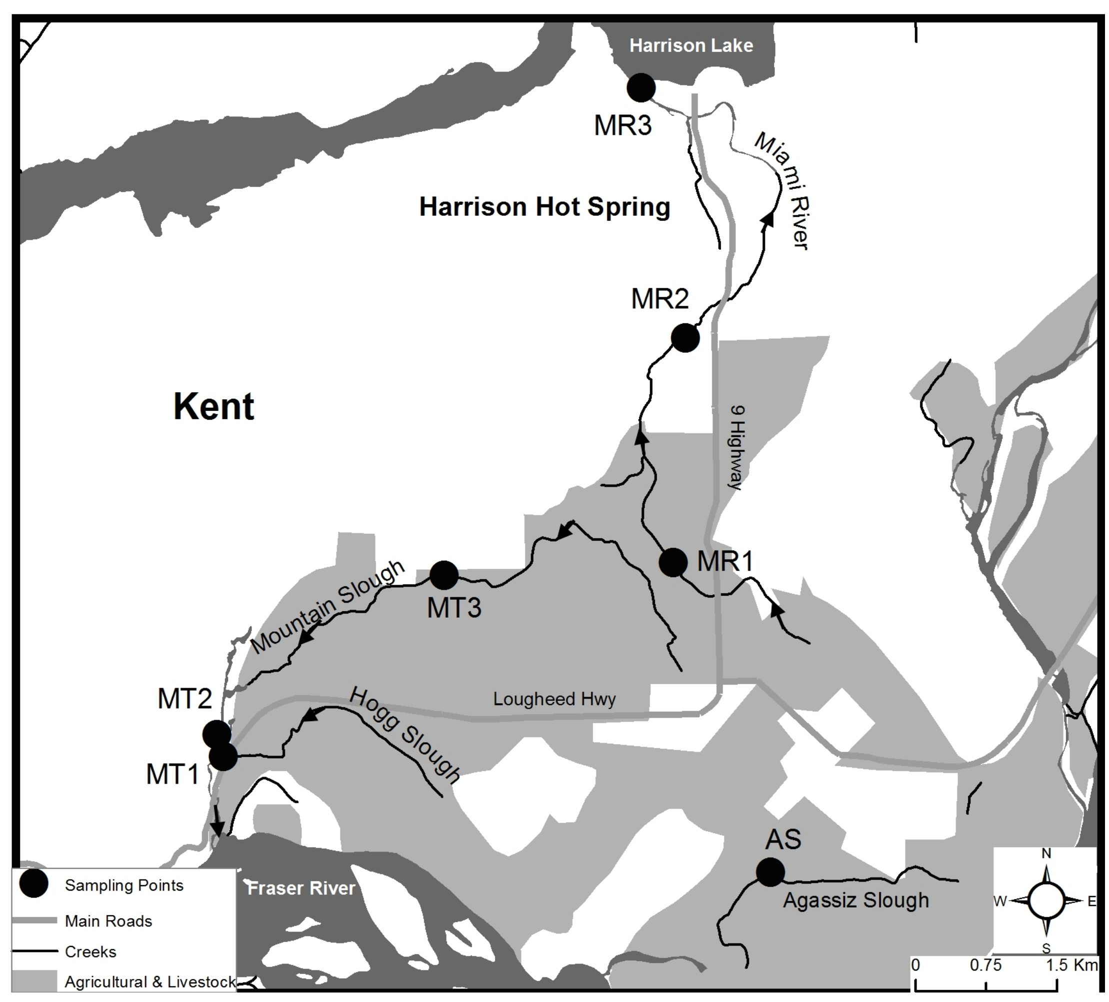


Figure 2.2 Sampling sites in District of Kent and Village of Harrison Hot Springs

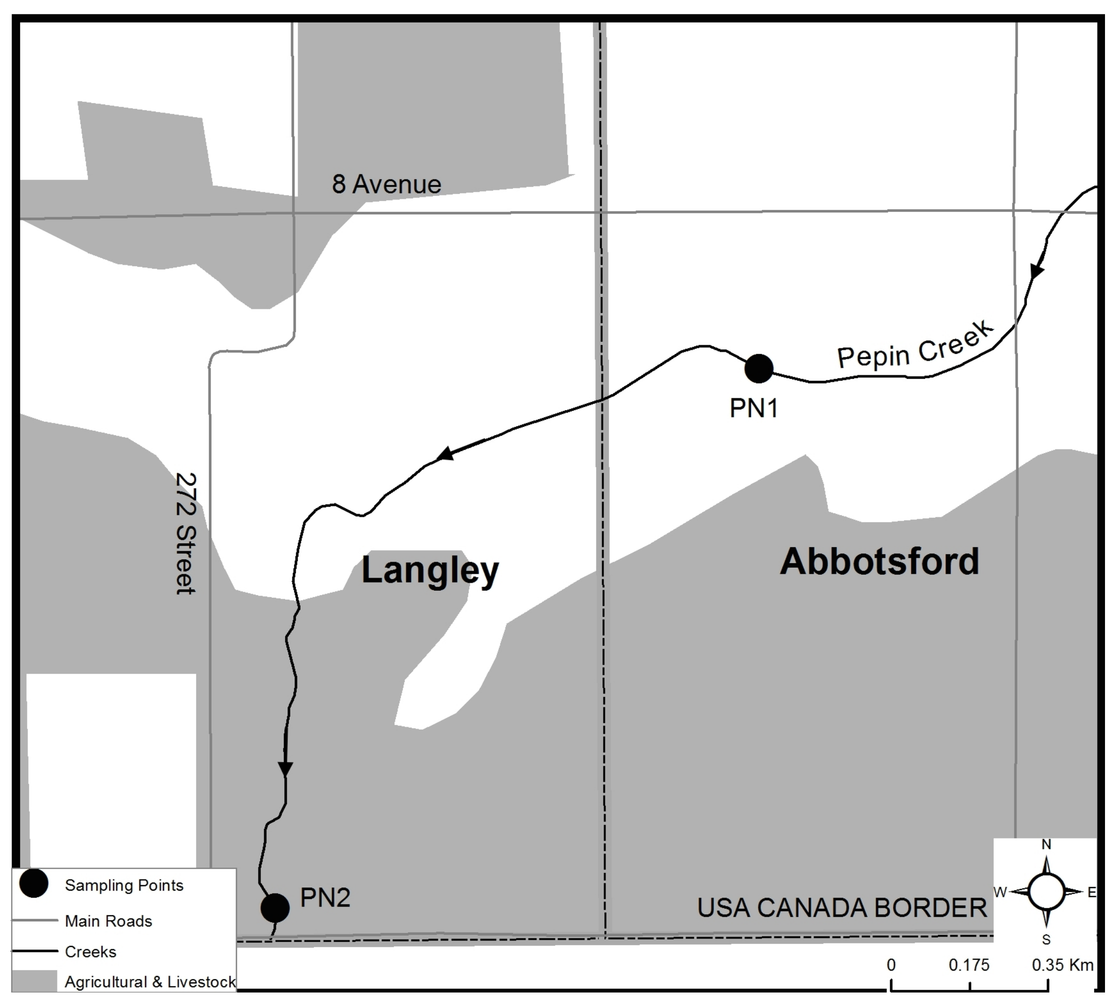


Figure 2.3 Sampling sites located in Pepin Creek

Table 2.1 Sampling locations in 2013

Site ID & Locations	Sampling Dates	UTM		Site Description
		Easting	Northing	
AS – Agassiz Slough Kent Municipality	June 19th August 12th November 4th	589111	5453638	Downstream of the town's storm sewer output; this site is impacted by urban/rural population as well as dairy farms
MT1 – Mountain Slough Kent Municipality	June 19th August 12th November 4th	583320	5454864	Downstream of Hogg Slough inlet which drains multiple dairy farms
MT2 – Mountain Slough Kent Municipality	June 19th August 12th November 4th	583251	5455096	Downstream of Mountain Slough inlet which drains multiple dairy farms and is a known laying site for Oregon spotted frogs
MT3 – Mountain Slough Kent Municipality	June 19th August 12th November 4th	585656	5456787	Downstream of a cranberry/blueberry farm
MR1 – Miami River	June 19th August 12th	588084	5456917	Downstream of a dairy farm

Harrison Hot Springs	November 4th			
MR2 – Miami River Harrison Hot Springs	June 19th August 12th November 4th	588213	5459298	Downstream of a golf course and MR1
MR3 – Miami River Harrison Hot Springs	June 19th August 12th November 4th	587747	5461950	This site is just before entering Harrison Lake; it is impacted by the town of Harrison and several dairy farms. It would capture all upstream contaminants including MR1 and MR2.
PN1 – Pepin Creek Abbotsford	June 17th August 12th November 13th	539879	5423464	Downstream from a permitted compost facility
PN2 – Pepin Creek Langley	June 17th August 12th November 13th	538501	5427856	Last site before creek enters USA, it would capture all upstream contaminants

2.1.2 Sampling sites in 2014/15

There were 13 sites in total for the 2014/15 sampling period – all from the city of Surrey. Each site was sampled twice, once on October 10th, 2014 and again on February 24th, 2015 except S1, S2 and S3 in October because S1 and S3 were inaccessible and no water was found in S2. Figure 2.4 and Table 2.2 provide more detailed information about these sites.

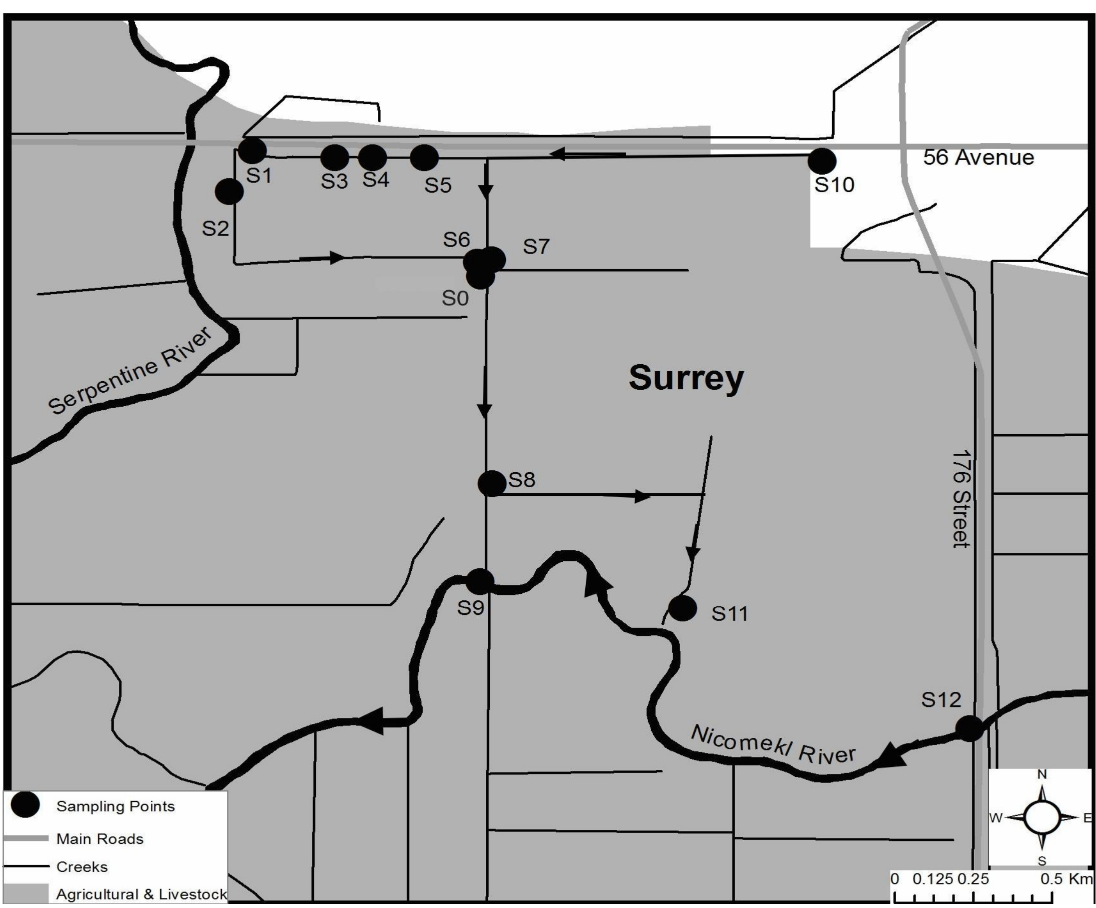


Figure 2.4 Sampling sites in the city of Surrey

Table 2.2 Sampling locations in 2014/15

Site ID	UTM		Site Description
	Easting	Northing	
S0	517755	5438618	Impacted by blueberry farm and not connected to any of the other study sites
S1	517031	5439054	Collects flow from pump station
S2	516957	5438913	Collects flow from pump station
S3	517292	5439029	Collects flow from pump station
S4	517412	5439029	Collects flow from pump station
S5	517576	5439029	Runoff from a blueberry farm
S6	517746	5438666	Runoff from blueberry farms
S7	517791	5438675	Collects flow from pump station and blueberry farms
S8	517792	5437893	Could be impacted by dairy farm

S9	517754	5437553	Nicomekl River site of Salmonidae and non salmonidae, could have impact by dairy farm
S10	518842	5439017	Might be influenced by a Racetrack
S11	518398	5437459	Downstream of all other sites (except S0, S9 and S12), last point before entering Nikomekl River
S12	519310	5437037	In Nikomekl River, not impacted by any other study sites

2.2 Chemicals used in the yeast assays and chemical analyses

The standard compounds used in the yeast screen bioassays were 17β-estradiol (E2), trenbolone (Tb), deoxycorticosterone (DOC) and β-naphthoflavone (NAP). They were purchased from Sigma-Aldrich (Ontario, Canada). Other chemicals used in the preparation of media were: L-histidine, L-leucine and uracil from Sigma, Difco yeast nitrogenous base w/o amino acids and ammonium sulphate from BD Bioscience (ON, Canada), Anhydrous dextrose from Merck Canada, galactose, synthetic complete amino acid dropout mix minus histidine, leucine, and uracil, Tryptophan from MP Biomedicals (OH, USA). Agar from Fisher Scientific. Methanol, acetone, ethyl acetate were from Fisher Scientific. Empore octadecyl C18 47mm solid phase extraction disks were obtained from Supelco Analytical (PA, USA) and Millipore 1.0 μm glass-fiber filters were from Sigma-Aldrich.

Chemicals and other materials used in chemical analysis of samples included 17α-ethynylestridiol (EE2), estriol (E3), estrone (E1), bisphenol A (BPA), nonylphenol (NP) and internal standards β-estradiol-d3 and bisphenol A- d16, all from Sigma Aldrich (ON, Canada). Internal standards 17β-trencolone-d3 and 5α-dihydrotestosterone-d3 were purchased from Cerilliant (Texas, USA). Methanol (HPLC grade), acetonitrile, dicholoromethane, acetone and isooctane were ACS reagent grades and were from Caledon (ON, Canada). Sulphuric acid (ACS reagent) from Anachemia (QC, Canada). Pyridine 99.8% from Sigma-Aldrich. Hexane (HPLC grade) from EMD Chemicals (NJ, USA). Derivatization agents Bis (trimethylsiyl) trifuoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) were from Sigma Aldrich.

Extraction Cartridges used were Oasis HLB (6cc, 50mg) and Sep-Pak Silica (6cc, 500 mg) from Waters Corporation (MA, USA), Agilent sampliQ C18 (6cc, 500 mg) from Agilent Technologies (CA, USA).

2.3 Sample storage and extraction

Water was collected as grab samples by immersing the narrow, open-mouth amber glass 1L bottle just below the surface of water. Amber glass 250 ml jar was used to scoop the top 2-4 cm of sediments under the water while overlaying water was drained off. After sampling, water and sediment samples were placed immediately in a cooler containing ice. The water samples were kept at 4°C while the sediment samples were transferred to -15°C freezer upon arrival at the laboratory and were extracted within 48 h of collection.

Water samples were first filtered using 1.0 μm glass fiber filter to remove particulates, then extracted according to Huang and Sedlak (2001) with modifications. Briefly, Empore 3M C18 extraction disk (ON, Canada) was conditioned with 10 ml methanol (MeOH) and 10 ml distilled water in sequence under the vacuum. Filtered water sample (50 – 250 ml) was then passed through the disk. The disk was eluted with 10 ml MeOH. The methanolic eluent was collected and evaporated down to dryness under a

gentle stream of nitrogen (N_2). The remaining residues were reconstituted in 500 µl of MeOH. The extract was kept in a 4°C refrigerator until used for bioassay.

Sediment samples were extracted using the liquid phase extraction method reported by Ternes et al. (2002) with modifications. About 10-20g of sample was transferred to a centrifuge tube containing 10 ml ethyl acetate. The tube was capped and shaken for 10 min before being centrifuged at 1341g for 10 min. The ethyl acetate layer on top was removed to a separate glass tube. This process was repeated twice with a total of ~ 30 ml of ethyl acetate collected. This solution was evaporated to dryness under a gentle stream of N_2.The remaining residues were reconstituted with 500 µl of MeOH and kept in a 4°C refrigerator until use for bioassay. Since most of the extracts from sediment samples showed toxicity to the yeast cells, an additional silica gel cleaning step was used to reduce the toxicity of the extract according to Bistan et al. (2011). Briefly, the residues were reconstituted in 2 ml ethyl acetate instead of 500 µl MeOH. The silica gel cartridge was conditioned with 2 ml MeOH and then 2ml distilled water. The ethyl acetate solution was passed through the conditioned cartridge and the cartridge was then eluted with 10 ml acetone: ethyl acetate (5:95). This eluate was collected and evaporated to dryness. The residues were reconstituted with 500 µl of MeOH and kept in a 4°C fridge until use for bioassay.

2.4 Protocol for the yeast screen bioassays

The following is the theoretical basis of the yeast screen bioassays: estrogenic, androgenic, glucocorticoid or AhR agonists are bound to the respective receptors in the specific yeast strain causing an up regulation in the production of β-galactosidase. Upon addition of buffer substrate mixture the yeast cells are lysed and β-galactosidase is released in to the medium (Figure 2.5). Addition of a substrate will yield a luminescent signal proportional to the amount of receptors activated in the yeast (Balsiger et al., 2010). The yeast bioassays are abbreviated based on the receptor that is expressed in each e.g. yeast estrogen screen (YES), yeast androgen screen (YAS), yeast glucocorticoid screen (YGS) and aryl hydrocarbon receptor (AhR) assay.

Saccharomyces cerevisiae yeast cells were used for the bioassays; these had been transformed with the human estrogen receptor (ER), the androgen receptor (AR),

the glucocorticoid receptor (GR) and the aryl hydrocarbon receptor (AhR) (Cox and Miller, 2002; Balsiger and Cox, 2009; Balsiger et al., 2010). All yeast cells were generously provided by Dr. Marc Cox's laboratory at the University of Texas, El Paso, USA. Table 2.3 lists the yeast strains, growth media and standard compounds used for each assay. For example, for the estrogenic assay the yeast strain used was DSY-219 in the growth medium SC-UW; SC-UW refers to synthetic complete (SC) media being prepared without uracil (U) and tryptophan (W). Protocols for the preparation of agar plates and growth media are provided in Appendix A.

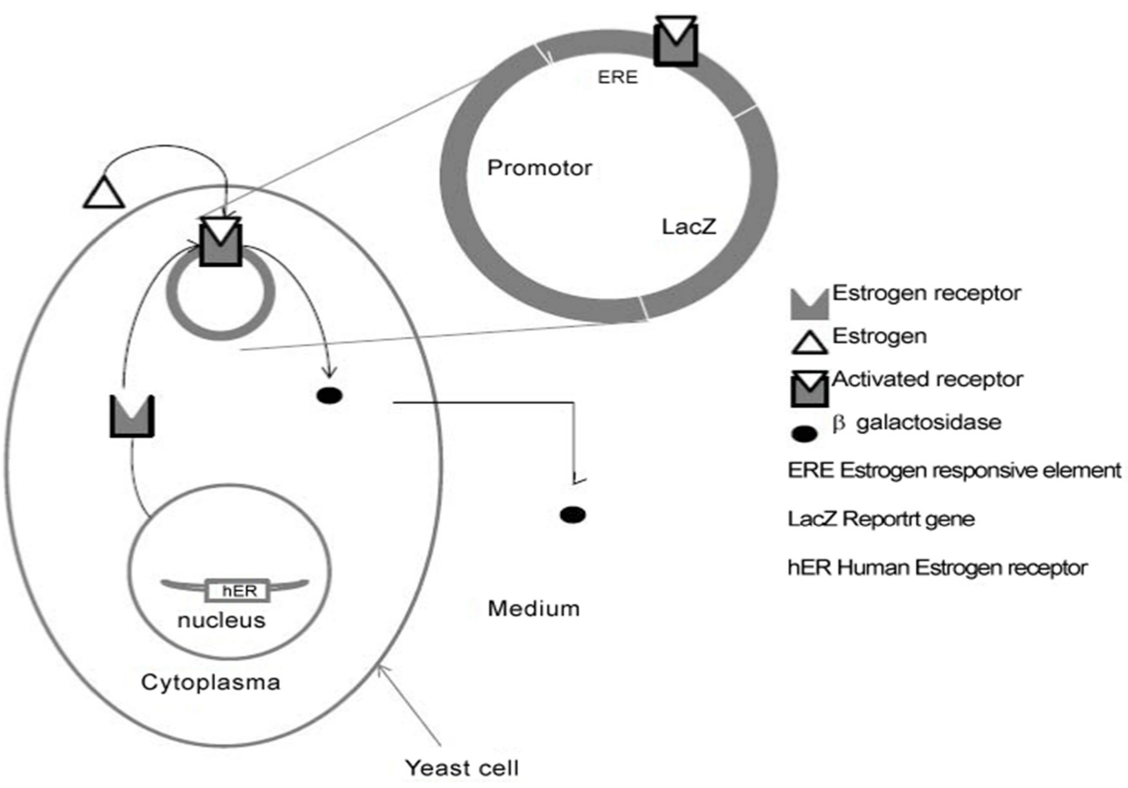


Figure 2.5 Example of Yeast Estrogen Screen mechanism (Bazin et al., 2013 licenced under CC BY 4.0)

Table 2.3 Yeast strains, media and standards for the four yeast screen bioassays

Yeast assay	Standard compound	Yeast strain	Growth media
Yeast estrogen screen (YES)	17β- Estradiol	DSY-219	SC-UW
Yeast androgen screen (YAS)	Trenbolone	DSY-1555	SC-LUW
Yeast glucocorticoid screen (YGS)	Deoxycorticosterone	DSY-1345	SC-UWH
Yeast AhR binding screen	β-Naphthoflavone	MCY-038	SC-W

See Appendix A for Growth Media composition and yeast strain info. SC=synthetic complete; U=Uracil; W=Tryptophan; L=Leucine; H=Histidine

The yeast cells were stored in a -80°C freezer until ready to be grown on an agar plate. Once yeast grew on the agar plate, it was kept at 4°C and used to run bioassays before being discarded after one month. The yeast assay protocol was same for all four bioassays and was adopted from Balsiger et al. (2010) with modifications. Briefly, a few yeast cell colonies were removed from the agar plate and inoculated into 5 ml growth media and grown overnight at 30°C. The next day, the culture was diluted with growth media to prepare a solution with an optical density (O.D.) of 0.08 absorbance at 600 nm. This diluted solution was incubated at 30°C until the culture reached $O.D._{600}$ of 0.1. While culture was incubating, a 1µl aliquot of each standard (as control) and sample extract dilution series (see Table 2.4 for concentrations used) were put into an opaque 96 well culture plate (Fisher Scientific, USA) in triplicates. Once the OD 600 of the culture reached

0.1, 100 µl of culture media was added into each well of a 96 well plate. The plates were sealed with parafilm and kept at 30°C for 2 h. At the end of the incubation period, a solution containing 100 µl of Tropix Gal-screen buffer and substrate (Life Technologies, CA) at a ratio of 1:24 was added into each well and mixed. The plates were covered with aluminum foil and kept at room temperature (~22°C) for an additional 1.5-2 h. Luminescence was measured using a Multilabel plate reader (Perkin Elmer, ON, Canada).

Table 2.4 Dilution series for each standard and test sample

Sample tested	Dilution Series*						
	1	2	3	4	5	6	7
17β- Estradiol (3.00E+6 ng/ml)	300	30	3	0.3	0.03	0.003	-
Trenbolone (4.0E+3 ng/ml)	1.5	0.8	0.5	0.4	0.2	0.1	0.05
Deoxycorticosterone (2.70E+6 ng/ml)	27	13.5	6.8	3.0	1.5	0.8	0.4
β-Naphthoflavone (2.72E+6 ng/ml))	2720	272	27	0.27	0.027	0.0027	-
Test sample extract	1	0.5	0.1	0.01	0.001	0.0001	-

* Each concentration is in ng/ml; dilution factor was used for the test sample extract.

2.5 Calculation and data analysis

Concentration-response curves for the standard and sample dilution series (Table 2.4) were plotted using the Graph pad Prism 6 Software (San Diego, CA, USA) An erratic or reversed dose-response curve indicates that the sample is non-responsive to yeast and those samples were not included in the calculation. EC50s and slopes for the samples were obtained from the concentration-response curves. EC30 and EC20 were calculated from EC_{50} as follow:

EC30 = (0.429^(1/slope))*EC50

EC20 = (0.25^(1/slope))*EC50

The concentration equivalents (EQ) of an EDC standard in each sample was then calculated at EC50, EC30 and EC20 using the following equation by Lorenzen et al. (2004):

EQ at EC50 (ng/ml or g) = [Standard EC50 (ng/ml)/ Sample EC50 (unitless)] x [volume of assay medium (ml)/ volume of extract tested(ml)] x [volume of stock extract/volume or weight of water (ml)/sediment (g) sample]

Where, Volume of assay medium = 201µl

Volume of sample extract = 1 µl

Volume of stock extract = 500 µl

Volume of water or weight of sediment = the amount of sample extracted, i.e., 50-250 ml for water and 10-20 g for sediment.

The final equivalents for each sample were calculated as the average of EC50, EC30 and EC20 obtained from the equation above. Thus, the final amount represents mean ± SEM (standard error of the mean) in ng/ml for water and ng/g dry weight for sediments. For further data analysis, one factor ANOVA was used to determine significant difference at $p < 0.05$ for temporal variations.

2.6 Gas chromatography-mass spectrometry analyses

Methods for the analysis of estrogenic, androgenic and AhR agonists containing samples are followed in sections below. A ddH_2O water sample spiked with standard and deuterated internal standards were also analyzed for estrogens and androgens. For the spiked estrogenic sample, 1 L water was spiked with E2-d3, BPA-d16, E2, EE2, E3, E1, BPA and NP. The extraction and derivatization methods are described below in section 2.6.1. For the spiked androgenic sample, 1 L water was spiked with Tb-d3, DHT-d3, Tb and DHT. The extraction and derivatization protocols applied to samples are described in section 2.6.2.

2.6.1 GC-MS analysis of estrogenic compounds

Three samples with high estrogenic activity were extracted and derivatized using the method described by Nie et al. (2009). Briefly, 1 L of water samples were acidified by 40% H_2SO_4 to the pH of 3. The sample was then filtered using 1.0 µm glass fiber filter. The Oasis HLB cartridge was conditioned with 5 ml of MTBE, 5 ml of MeOH and 5 ml of ultrapure water in sequence before the water sample was passed through at a flow rate of 4-5 ml/min. The cartridge was then washed with 5 ml of 10% MeOH in water, 5 ml of ultrapure water and lastly with 5 ml of 10% MeOH containing 2% NH_4OH. The cartridge was left to dry for 40 min under the vacuum.

For sample cleanup, Sep-Pak Silica cartridge was conditioned with 5 ml of dicholoromethane/acetone (7:3 v/v) and was connected to the bottom of the Oasis cartridge used for extraction. 10 ml of dicholoromethane/acetone (7:3 v/v) was added through the Oasis cartridge that then passed through the Sep-Pak Silica cartridge at a flow rate of 1-2 ml/min. The eluted liquid was evaporated under a gentle stream of N_2 and derivatized for GC-MS analysis.

The purified extract was derivatized by first dissolving the dried extract in 50 µl of pyridine and 50 µl of Bis (trimethylsiyl) trifuoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). The mixed solution was heated in a 4 L ultrasonic water bath for 30 min. The solution was cooled to room temperature before being dried under the stream of N_2. The residues were dissolved in 100 µl of hexane for GC-MS analysis.

2.6.2 GC-MS analysis of androgenic compounds

Two samples with high androgenic activity were extracted using the method described by Durhan et al. (2006). Briefly, 1 L of sample was filtered through a 1.0μm glass fiber and then pumped at a rate of ~5ml/min through an Agilent C18 cartridge which had been washed with 20 ml of acetonitrile and conditioned first with 20 ml of MeOH and then 20 ml of deionized water. The cartridge was then rinsed with 20ml of 50% MeOH in water. The column was dried under vacuum for 2 min and then eluted with 2ml of MeOH twice. The eluents were evaporated under a stream of N_2. The derivatization step was adopted from Parker et al. (2012). Briefly, the dried residues were dissolved in 1 ml dichloromethane and evaporated again using N_2. Residues were then dissolved and vortexed in 50μl of the derivatization reagent *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) with iodine (I_2) (1.4:1000 m/v). The mixture was dried again and extracts were reconstituted in 100μl of MSTFA. This extract was heated at 60°C for 30 min to dryness and dissolved in 100μl of isooctane for GC-MS analysis.

2.6.3 GC-MS analysis of polyaromatic hydrocarbon compounds

Two of the samples with high activity in AhR assay, were extracted using method by Martinez et al. (2004). First, 1L of water was filtered through 1.0μm glass fiber filter before being passed through an Oasis SPE cartridge which had been conditioned with 5ml of ethyl acetate, 5ml of MeOH and 5ml of distilled water containing 2% MeOH in sequence. The sample was placed under vacuum at a flow rate of ~ 5ml/min. The cartridge was rinsed with 5ml ultrapure water, dried under the vacuum and eluted with 5ml of ethyl acetate 5 times. The extract was evaporated using N_2 and residues were reconstituted in 100 μl hexane: cyclohexane: dichloromethane (1:1:1). GC-MS detection was performed at full scan mode to detect a wider range of unknown compounds.

2.6.4 GC-MSD conditions

The system consisted of an Agilent Technologies 6890 series Gas Chromatogram, 5973 Mass Selective Detector and a 7683B series injector. The injector temperature was

set a 250°C, hydrogen gas flow was kept at 0.9 ml/min. 1 µl of each sample was injected into the GC-MSD and samples were analyzed in Selected Ion Monitoring (SIM) and scan mode.

3. Results and Discussion

3.1 Standard dose-response curves for the four bioassays

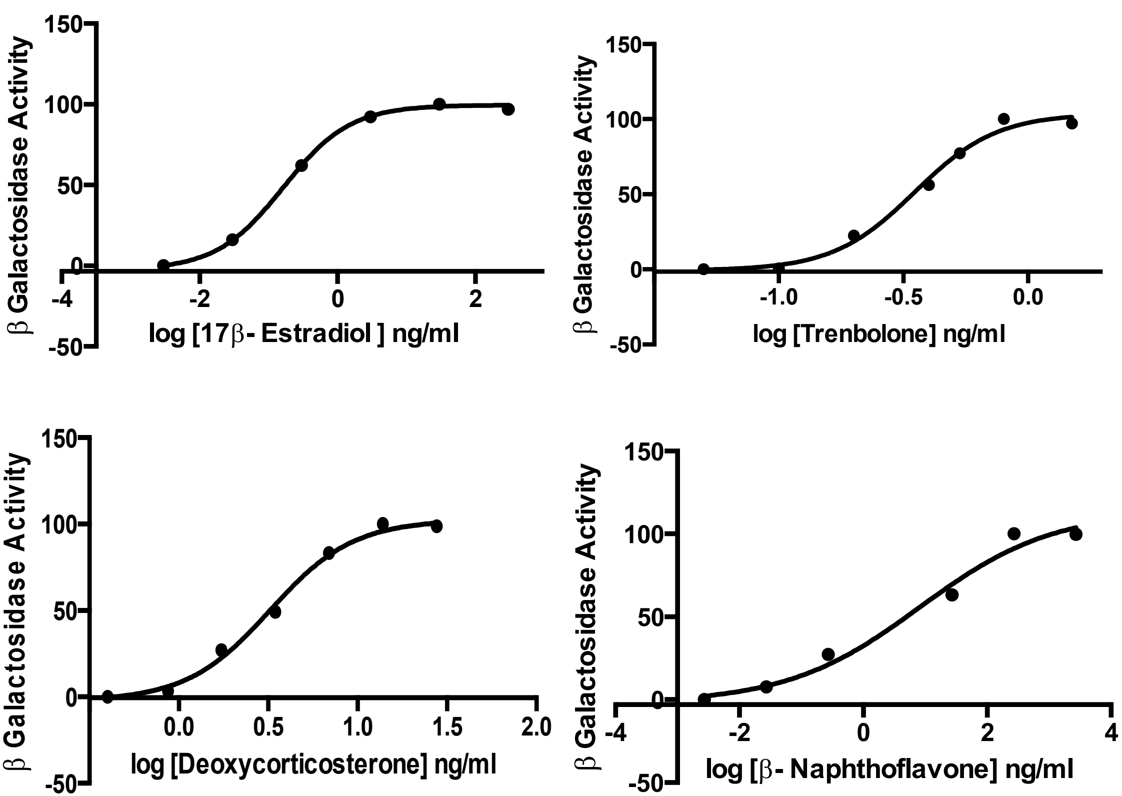


Figure 3.1 Dose-response curves for the four standards used in the yeast bioassays.

* each value on the curve is based on triplicate determination at each dose

Figure 3.1 shows the dose-response curves for the standard compounds in the four yeast bioassays. Each dot on the curve represents mean response (n=3) of that particular dose. The concentrations on the X-axis represent the dilution series from a stock solution as provided in Table 2.4. After determining the EC50 from the curve, EC20 values were calculated for each compound (section 2.5) which set the limits of detection (LOD) of the bioassay. LODs determined for the YES, YAS, YGS and AhR binding bioassays

were 0.025 ng 17β-estradiol equivalents/ml, 0.20 ng trenbolone equivalents/ml, 1.5 ng deoxycorticosterone equivalents/ml and 2.4 ng β-naphthoflavone equivalents/ml respectively. Table 3.1 shows the EC50s obtained for each bioassay compared with the EC50s from other studies. Balsiger et al. (2010) has reported an EC50 of 0.19 ng EEQs/ml for E2 using the same yeast bioassay as in the present study. Sanseverion et al. (2005) obtained EC50s of 0.12 ng EEQs/ml and 0.07 ng EEQs/ml for E2 using colorimetric and bioluminescence yeast estrogen assays, respectively. The EC50 for trenbolone reported by Eldridge et al. (2007) using bioluminescence androgen yeast assay is much higher than ours at 4.87 ng trenbolone equivalents/ml. This is possibly due to BLYAS being less sensitive than the YAS assay. Riggs et al. (2003) had EC50s ranging from 4.9-23.1 ng DOC equivalents/ml in their GR assay. A study by Noguerol et al. (2006) showed an EC50 range of 20 – 140 ng PCBs equivalents/ml which was also higher than the 9.5 ng β-naphthoflavone equivalents/ml EC50 of our study. Probably this was due to use of different yeast strains and plasmids in the recombinant yeast assay (RYA).

Table 3.1 EC50 values for each bioassay compared to other studies

Yeast Bioassay	EC50 current study	EC50 Other studies	References
Yeast estrogen screen (YES)	0.1 ng EEQs/ml	0.19 ng EEQs/ml (YES)	Balsiger et al. 2010
		0.12 ng EEQs/ml (colorimetric YES); 0.07 ng EEQs/ml (bioluminescence YES)	Sanseverion et al. 2005
Yeast androgen Screen (YAS)	0.3 ng TbEQs/ml	4.87 ng TbEQs/ml (bioluminescence YAS)	Eldridge et al. 2007
Yeast glucocorticoid screen (YGS)	3.0 ng DOCEQs/ml	4.9 – 23.1 ng DOCEQs/ml (YGS)	Riggs et al. 2003
Yeast AhR binding screen	9.5 ng NAPEQs/ml	20-140 ng PCB EQs/ml (AhR yeast assay)	Noguerol et al. 2006

EEQs= 17β- estradiol equivalents **TbEQs**= Trenbolone equivalents **DOCEQs**= Deoxycorticosterone equivalents **NAPEQs**= β- Naphthoflavone equivalents **PCB**= polychlorinated biphenyls

3.2 Recovery and accuracy test for the four recombinant yeast bioassays

Efficiency and accuracy of the four bioassays were examined by spiking double distilled water with three different concentrations of the standard chemical. Figure 3.2 shows percent recoveries obtained for the four assays. For each of the spiked yeast assays, the concentrations used were close to the levels of contamination observed in the environmental samples. Overall, the results showed satisfactory recovery and reproducibility for each assay except the glucocorticoid assay of which the recoveries were only ~40%. A revised and improved extraction procedure for glucocorticoid/cortisol compounds is necessary to obtain higher extraction recoveries for glucocorticoids. Average percent recoveries for E2 and Tb were approximately 70% whereas NAP had the highest accuracy and precision with recovery rates close to 92%.

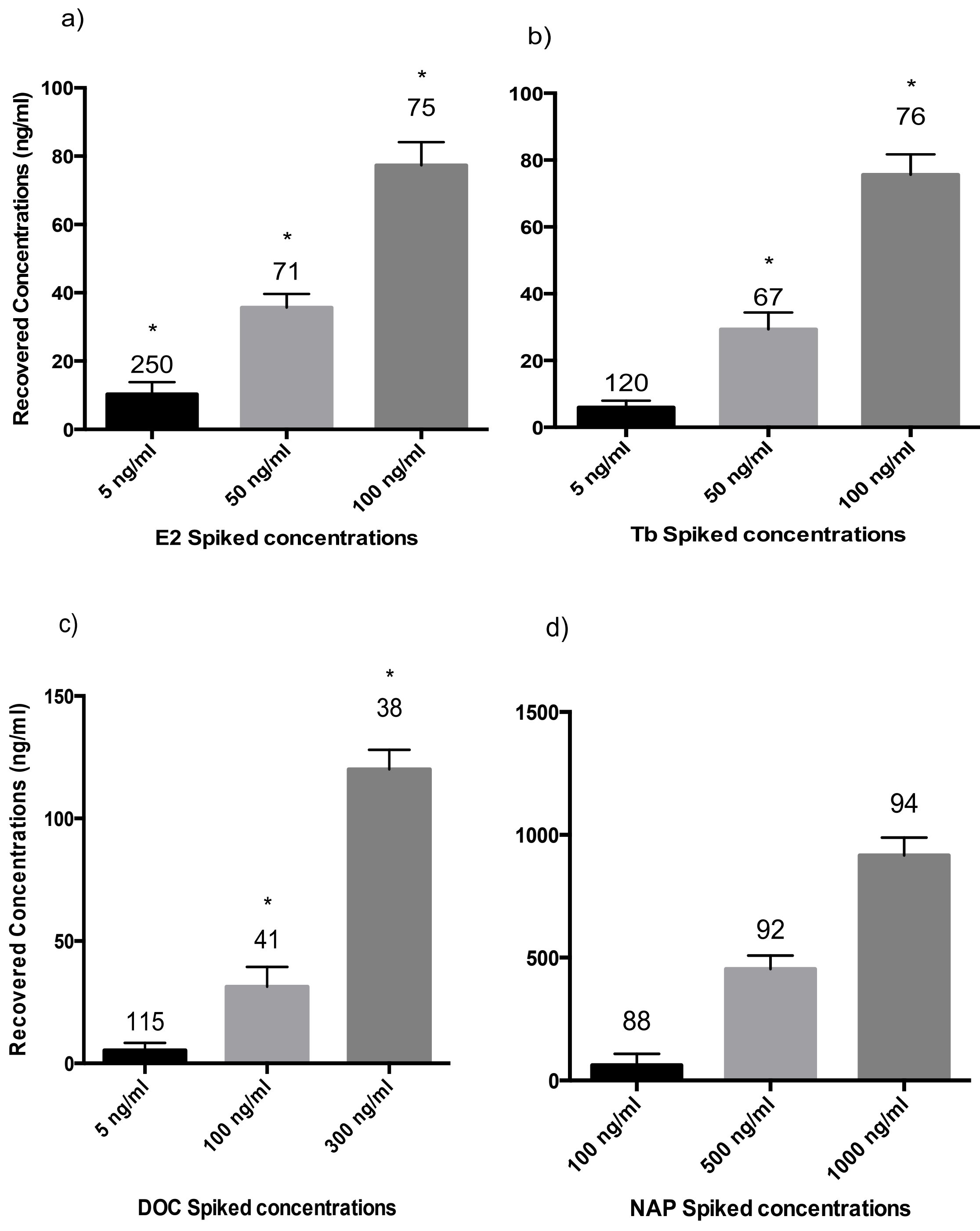


Figure 3.2 Concentrations recovered (± SEM) for each bioassay.

The number above each column denotes percent recovery for the tested concentration. An asterisk indicates that the recovered concentration was significantly different from the spiked concentration at $p<0.05$. Recovery test for **a)** estrogenic (17β-estradiol) **b)** androgenic (Trenbolone) **c)** glucocorticoid (Deoxycorticosterone) and **d)** AhR agonist (β-naphthoflavone) bioassay.

3.3 EDCs levels from sampling sites in 2013

We hypothesized that rainfall levels could significantly influence the levels of contaminants present in water and sediment. In Kent, rainfall levels were the highest in June, lowest in August and intermediate in November. For Pepin Creek area, rainfall levels were about the same in June and November but dry for August. Monthly and daily rainfall levels for each location are presented in Appendix B.

3.3.1 Estrogenic levels in water and sediments

The results of the YES assay expressed as ng EEQs per ml of water and ng EEQs per g of sediment, respectively, are presented in Table 3.2 and Figure 3.3. Figure 3.4 shows EEQs as dot sizes that are proportional to amount of estrogenic activity in water and sediments for all sampling periods. Estrogenic activity in sediments was higher than water for all sites in the three sampling periods, except for pepin creek sites in August where mean water estrogenic concentrations were higher than the sediments. In the dry period of August, four of the nine sampling sites had no detectable E2 activity in water but the highest levels were present in sediments (3617.06 ng EEQs/ml in Miami River). Peck et al. (2004) have reported that surface waters show very low to non-detectable estrogenic activity but sediments are tested positively for estrogenic compounds. In terms of no response of YES, one sediment sample from Mountain Slough (MT2) was non-responsive in June, both water and sediments from Mountain Slough site MT3 showed no response in November and sediments from Pepin Creek (PN2) also were non-responsive in November (Figure 3.3).

3.3.1.1 Estrogenic levels in water

In Agassiz Slough, the mean E2 concentration in water was 2.08 ng EEQs/ml in June, BLD in August, and 8.67 ng EEQs/ml in November. The estrogenic activity in water was slightly higher in November compare to in June. Nonetheless, the higher levels in both rainy periods (June and November) may reflect the impact of rain and high flow of water bringing contaminants from dairy farms as well as urban and rural areas into the slough.

In Mountain Slough locations, mean E2 activity in the water samples was about the same in the months of June and November; about 3.90 ng EEQs/ml in both sampling periods. E2 levels were not very different in MT1 during the three sampling periods; they ranged from 0.31 to 2.72 ng EEQs/ml in both sampling periods of June and August and slightly increased in November to a mean of 4.34 ng EEQs/ml. Mountain slough sites are impacted by runoff from multiple dairy farms (MT1 and MT2) as well as berry farm and possible chicken farm (MT3). E2 activities in MT2 water samples, except in June where it was 9.34 ng EEQs/ml, were much lower (~ 0.60 ng EEQs/ml) compared to MT1. November water and sediment samples from MT3 were non-responsive to bioassay, but levels were not out of range from other Mountain Slough sites for June and August.

The three sites in Miami River showed similar pattern of E2 levels as Agassiz slough *i.e.,* the levels in August were the lowest, a little higher in June and highest in November. The three different locations in Miami River, MR1, MR2 and MR3, showed the same pattern for all three sampling periods. The river flows down from MR1 to MR2 and into MR3 before entering Harrison Lake. MR1 is downstream of a dairy farm, the mean E2 levels in water samples were 5.67 ng EEQs /ml in June, BLD in August and went up again after a rainfall to 25.10 ng EEQs/ml in November. MR2 is downstream of a golf course and receives water from MR1 and dairy farms. The EEQ levels in water were BLD in August, but mean concentrations in June and November were 1.12 and 0.69 ng EEQs/ml respectively. The third site in Miami River, MR3 is the last spot before entering Harrison Lake. This site would capture all upstream sources from the town of Harrison as well as multiple dairy farms. E2 levels were BLD in August, and about the same in June and November *i.e.,* 12.33 and 15.01 ng EEQs/ml respectively. Our results are relatively high compare to those reported by the study of Soto et al. (2004), of which the estrogenic activity in river water close to cattle farms ranged from BLD to 0.99 ng EEQs/ml due perhaps to the difference in water flows.

Unlike other locations where E2 activity was the lowest in August, the concentrations in Pepin Creek were the highest in August with a mean value of 6.99 ng EEQs/ml (Figure 3.3). The levels in June and November were about the same being 1.34 and 0.99 ng EEQs/ml respectively. Pepin Creek sites are impacted by a year round compost facility and the estrogenic activity is 5x higher in August compare to June and

November. A year to year study would confirm if the estrogenic activity are consistently higher in the dry period of August.

The overall pattern of a higher E2 level during rain and a lower level during dry period is in agreement with a study by Zhao et al. (2011) in which a higher estrogenic activity is found during wet period compare to dry period.

3.3.1.2 Estrogenic levels in sediments

Fig. 3.3 also shows the E2 concentrations in the sediment samples from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek. There were no specific patterns of EEQ levels in the sediment samples. Some sites had similar levels in June and November but lower in August while other sites had either higher estrogenic levels or had no response to yeast cells in November than in the other two sampling periods. Sediment samples from Agassiz Slough had mean E2 equivalence of 274.67 ng EEQs/g in June, 8.33 ng EEQ/g in August and 46.65 ng EEQs/g in November (Table 3.2). The significantly higher levels in June than November may be due to the heavy rainfall which preceded the sampling day and had brought with it contaminated soils as this site also receives water from the city's storm sewer. Higher levels in both rainy periods may also be due to runoff from sources further away from the sampling sites.

Sediment E2 levels in Mountain slough showed a somewhat similar pattern in June and August but they were higher in November. These sites are impacted by multiple dairy, chicken and/or berry farms. Mean EEQs were 97.31 ng EEQs/g in June, 68.34 in August and 1938.10 in November for MT1. MT2 June's samples were non responsive to yeast (water data showed EEQs of 9.34 ng/ml), in August the levels were 22.32 ng EEQs/g and November was 34.20 ng EEQs/g. The MT3 location had mean E2 equivalents of 90.33 ng EEQs/g and 26.67 ng EEQs/g in June and August respectively. Sediment samples in November were non responsive to yeast and so did the water sample.

For sediment samples from Miami River, the levels were low in November but high in June with the exception of MR3 where the level was the highest and reached 4198.34 ng EEQs/g in August. The high E2 activity could be due to the type of clay or organic matter in the sample as estrogenic compounds are likely to adsorb onto the sediments (Wang et al., 2012). MR1, the most upstream location, had a mean EEQ of 83.66 ng/g in

June but a lower value, 23.70 ng/g in August. No sediment sample was collected in November. For MR2, downstream from MR1, the mean E2 levels were 17.33 ng EEQs/g in June, 10.69 ng EEQs/g in August and 3.17 ng EEQs/g in November. The last site MR3 which is downstream to the other two sites and also the last point of the river before entering Harrison Lake, had mean E2 levels of 248.35 ng EEQs/g in June, 3305.65 in August and 96.67 in November. The very high levels in August for MR3 could be due to settling down of soil and sediments from upstream and due to dry weather or no flow of water in the area leading to accumulation in sediments.

The two sites in Pepin creek had low sediment EEQs in August whereas the EEQs were the highest in water for August. Low levels in sediments may be due to estrogenic compound degradation as a result of warm temperatures (Tiryaki and Temur, 2010). PN1, which is impacted by a discharge from a year round compost facility, has mean EEQs of 18.79 ng/g in June, 3.10 ng/g in August and significantly higher activity ($p < 0.05$) at 236.50 ng EEQs/g in November. November sediment samples from PN2, which is downstream of PN1, did not respond to yeast. In August, the mean EEQ was not significantly different ($p < 0.05$) in PN2 (3.17 ng EEQs/g) compared to PN1and higher than PN1 concentration in June being 36.73 ng EEQs/g. The high levels could be due to water flow and accumulation of estrogenic compounds from PN1 down to PN2. On its way the contaminants may have accumulated as they run through a Regional park. High activity observed downstream of the park may also be due to pesticide uses as some pesticides have the ability to bind to estrogenic receptors (Kojima et al*.,* 2010; Noguerol et al*.,* 2006).

Table 3.2 Summary of estrogenic levels in water and sediments from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek.

Sampling Period	Sample Type*	Agassiz Slough (AS)			Mountain Slough (MT)			Miami River (MR)			Pepin Creek (PN)		
		L	H	M	L	H	M	L	H	M	L	H	M
June	water	1	3	2	0.3	17	4	0.3	20	6	0.05	4	1
	sediment	199	344	275	44	165	93	3	345	149	8	54	31
August	water	BLD			BLD	4	1	BLD			1.0	16	7
	sediment	5	12	8	8	98	39	3	3617	980	0.03	9	3
November	water	3	15	9	0.06	8	4	BLD	29	14	0.02	3	0.8
	sediment	33	62	47	30	2772	986	0.5	148	50	171	301	237

*Concentrations in water and sediment are presented in ng EEQs/ml and ng EEQs/g respectively. **L**=Low, **H**=High, **M**=Mean. **Note** the values are rounded to the nearest whole number.

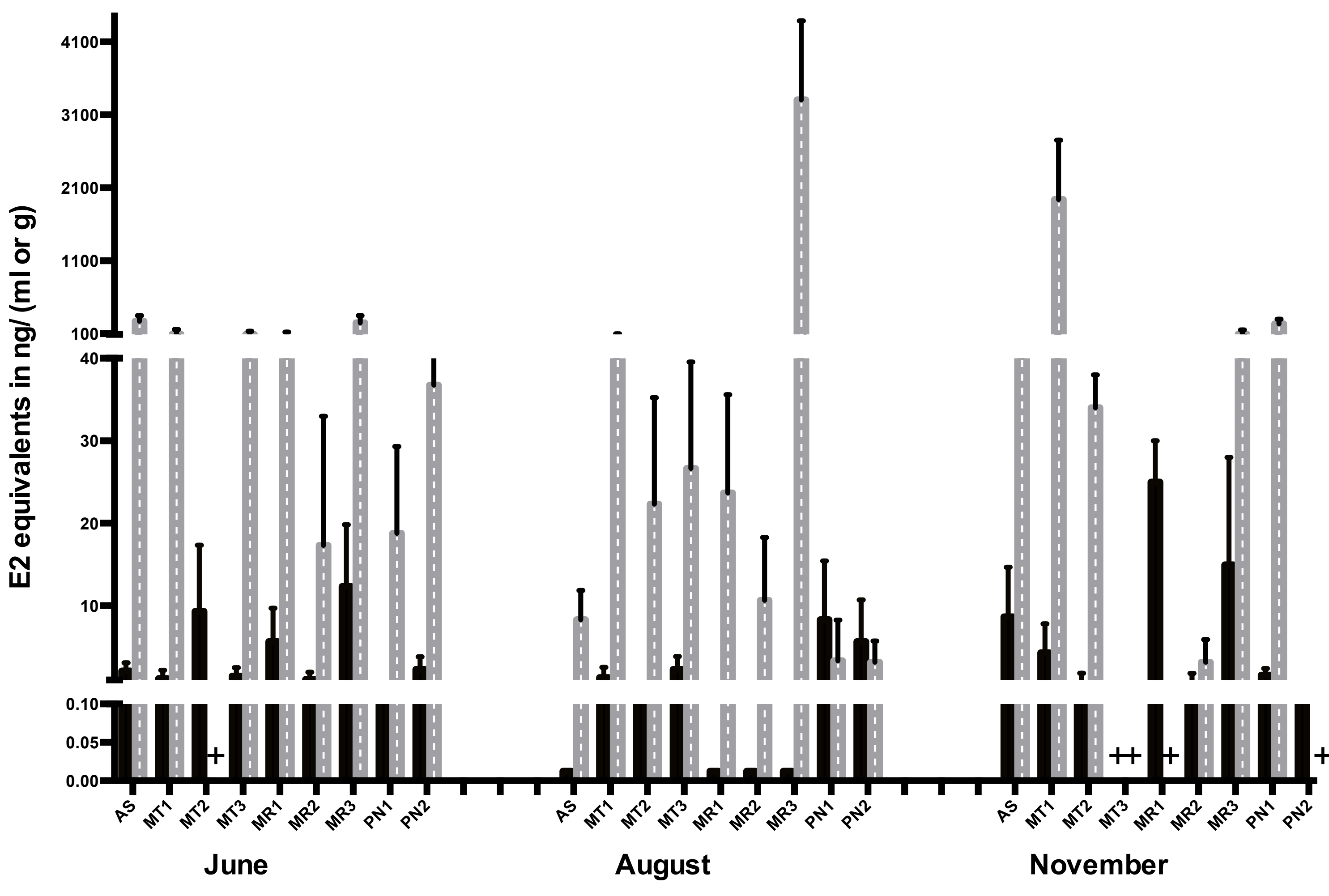


Figure 3.3. YES results for Agassiz Slough (AS), Mountain Slough (MT), Miami River (MR) and Pepin Creek (PN).

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

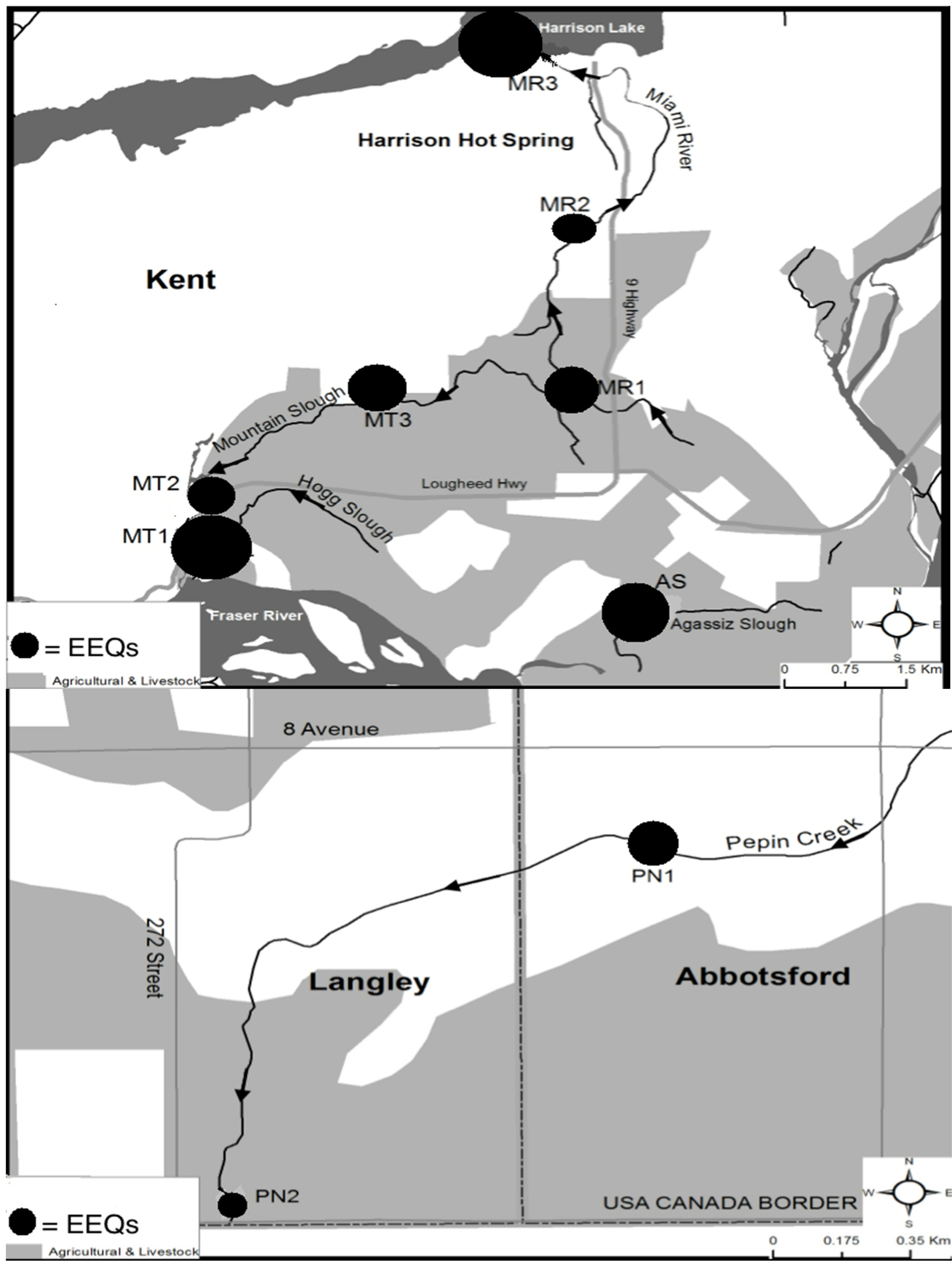


Figure 3.4. Sites in 2013 sampling period with EEQs levels shown as dots.

Sizes of dots are proportional to the amount of EEQs in water and sediments from all sampling periods.

3.3.2 Androgenic levels in water and sediments

Results of the YAS assay are presented in Table 3.3 and Figure 3.5. Figure 3.6 shows TbEQs as dots of sizes that are proportional to amount of androgenic activity in water and sediments for all sampling periods. Androgenic activity in the water and sediment samples was expressed in ng trenbolone equivalents (TbEQs) per ml or g of sample. While non of the water samples were non-responsive to yeast in the three sampling period, there were three sediment samples that had no response which were from different sites; two in August were from Mountain Slough and Miami River and one in November was from Mountain Slough.

3.3.2.1 Androgenic levels in water

The site in Agassiz Slough, which is impacted by dairy farms as well as urban areas, showed mean TbEQs in water of 56.12 ng TbEQs/ml and 33.30 ng TbEQs/ml in June and November respectively but levels were below the limit of detection in August. The undetectable levels in the dry period of August could be due to high temperatures in the slough causing microbial degradation of androgenic compounds (Nichols et al., 1997).

The three sites in Mountain Slough, MT1, MT2 and MT3 showed low TbEQs in water during all three sampling periods, ranging from BLD to 3.11 ng TbEQs/ml, with the exception of MT3 having high Tb equivalents in June, up to 65.21 ng TbEQs/ml (Table 3.3). Chicken and berry farm's influence water in MT3 and flushing of fertilizer and animal waste is due to heavy rain in June which could explain this high levels.

Site MR3 in Miami River showed highest average androgenic activity in June (1236.34 ng TbEQs/ml) and August (531.33 ng TbEQs/ml) compared to all the sites in three sampling period. But Androgenic concentrations were lower in November (7.29 ng TbEQs/ml). Tb equivalents were lower (0.58 ng TbEQs/ml) in June and somewhat same in August (20.81 ng TbEQs/ml) and November (25.45 ng TbEQs/ml) for MR1 which is upstream of MR2 and MR3. MR2 had similar levels in all three periods of sampling, 0.67, 0.37 and 0.86 ng TbEQs/ml in June, August and November respectively. High levels in MR3 could be due to this site being the last point of Miami River thus collecting everything being washed down the river from agricultural and urban land use in the area of Harrison Hot Springs. Low levels observed in the two river sites could be due to high flow rate of

river flushing all the contaminants downstream that's why the lowest point in the river had the highest levels of androgenic activity.

Tb equivalents in Pepin creek were not significantly different ($p < 0.05$) from each other (PN1 vs. PN2) and also not significantly different ($p < 0.05$) in the three rounds of sampling periods (Figure 3.5). Mean TbEQs/ml were 0.88, 0.99 and 0.73 in PN1 for June, August and November respectively. For PN2 the highest concentration was in June of 1.59 ng TbEQs/ml and dropping to 0.41 ng TbEQs/ml and 0.43 ng TbEQs/ml in August and November respectively.

3.3.2.2 Androgenic levels in sediments

Fig. 3.4 shows Tb concentrations in the sediment samples from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek. Sediment androgenic levels were higher than water for all sampling sites except MR3 in June when the sediment concentration was 44.28 TbEQs ng/g compared to 1236.34 TbEQs ng/ml in water. Androgenic levels in sediments from Agassiz Slough were about the same for the three sampling periods, with mean values of 220.27, 270.30 and 217.02 ng TbEQs/g in June, August and November respectively. Slightly higher levels in August could be due to accumulation of androgenic compounds in soil which could not be washed away with water as there was only 3.4 mm of rain leading up to the sampling date in August.

The concentration of androgenic compounds in Mountain Slough were in the range of 21 – 203 ng TbEQ/g in June, 1 – 228 ng TbEQs/g in August, and 31 – 498 ng TbEQs/g in November (Table 3.3). Androgenic concentration in MT1 was higher in June (149.66 ng TbEQs/g) than in August (4.67 ng TbEQs/g) and there was no response to yeast in November. In MT2 the levels were 42.43 ng TbEQs/g in June, and 37.70 ng TbEQs/g in November. The sediments showed no response to yeast in August. Lower activity in dry period possibly is due to increases in ambient temperature, light degradation of androgenic compounds and microbial degradation (Nichols et al., 1997). MT3 showed high levels of contamination in August (TbEQs of 196.51 ng/g) and November (TbEQs of 430.76 ng/g) compared to the other two Mountain Slough locations.

Miami River sites had TbEQs in the range of 13 – 255 ng TbEQs/g in June, 298 – 2010 ng TbEQs/g in August and 13 – 592 ng TbEQs/g in November. The lowest

androgenic levels were in June, about 3-5x higher in November and the highest in August. For MR1, the levels were higher in August (302.96 ng TbEQs/g) compared to June (120.77 ng TbEQs/g) and no sediments were collected in November due to inaccessibility. For MR2, the sediments were non-responsive in August and the levels were 82.01 ng TbEQs/g in June and 543.05 ng TbEQs/g in November. The last site in Miami River, MR3, the levels were lowest in June (45.28 ng TbEQs/g) and November (28.20 ng TbEQs/g) but reached about 2010.09 ng TbEQs/g in August. The very high sediment contamination may be because of accumulation of compounds due to a higher rate of growth hormone use in cattle farms (Lange et al., 2002).

Androgenic activities in sediment samples from Pepin Creek were significantly different at $p < 0.05$ among the three sampling periods and between the two sites PN1 and PN2 (Figure 3.5). Average TbEQs in PN1 were found to be 280.33, 54.67 and only 3.05 ng TbEQs/g in June, August and November respectively. The highest activity in PN2 was also in the rainy month of June at 631.67 ng TbEQs/g. The average concentrations dropped to 9.15 ng TbEQs/g in the dry month of August but rose up again to 25.33 ng TbEQs/g. Highest concentration in PN2 in the raining period of June could be due to flowing of substances down the creek and reaching PN2 from PN1 which collects drainage from a compost facility. The highest levels of androgenic compounds in August at PN1 can be explained by the influence of dry period where there was no washing down of substances down the creek as compared to the raining period of June.

More detectable and/or higher levels in a rainy period are in line with the study by Finlay-Moore et al. (2000) where high concentrations of estrogens and testosterones were reported in both water and soil near agricultural and dairy farm lands. Whereas low androgenic activities observed in some of the sites could be due to anti-androgenic compounds such as PAHs, which may be present in the pesticides, used in the surrounding farmlands.

Table 3.3 **Summary of androgenic levels in water and sediments from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek.**

Sampling Period	Sample Type*	Agassiz Slough (AS)			Mountain Slough (MT)			Miami River (MR)			Pepin Creek (PN)		
		L	H	M	L	H	M	L	H	M	L	H	M
June	water	16	97	56	0.3	65	14	0.2	1577	408	0.3	3	1
	sediment	154	299	220	21	203	94	13	255	87	199	709	456
August	water	BLD			0.2	3	1	0.2	616	184	0.1	1	0.7
	sediment	199	345	270	1	228	101	298	2010	1022	4	58	33
November	water	15	53	33	BLD	2	1	0.2	43	12	0.1	1	0.5
	sediment	196	251	217	31	498	234	13	592	274	1	33	14

***Concentrations in water and sediment are presented in ng TbEQs/ml and ng TbEQs/g respectively. **L**=Low, **H**=High, **M**=Mean. **Note** the values are rounded to the nearest whole number.

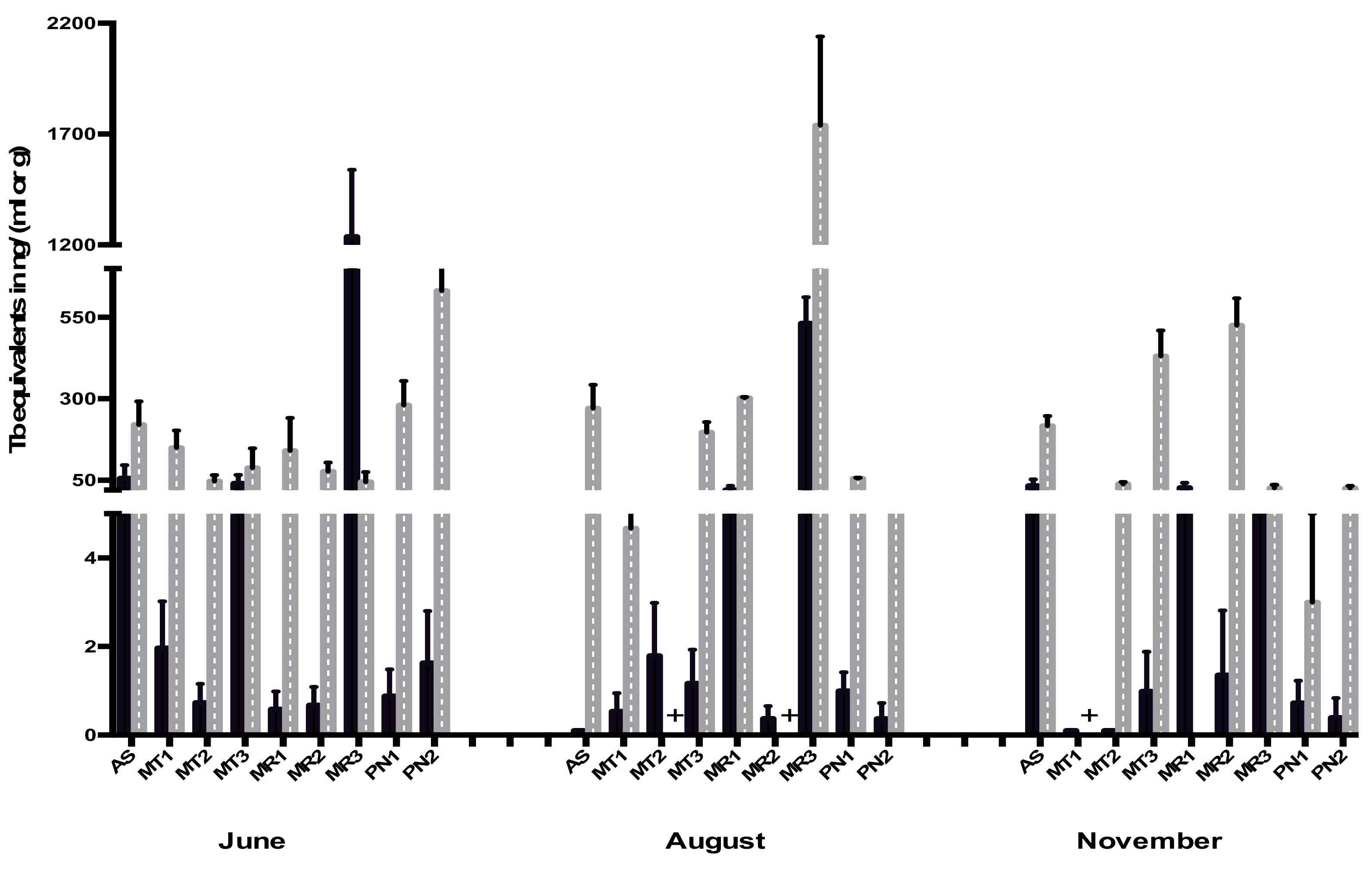


Figure 3.5 YAS assay results for Agassiz Slough (AS), Mountain Slough (MT), Miami River (MR) and Pepin Creek (PN).

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

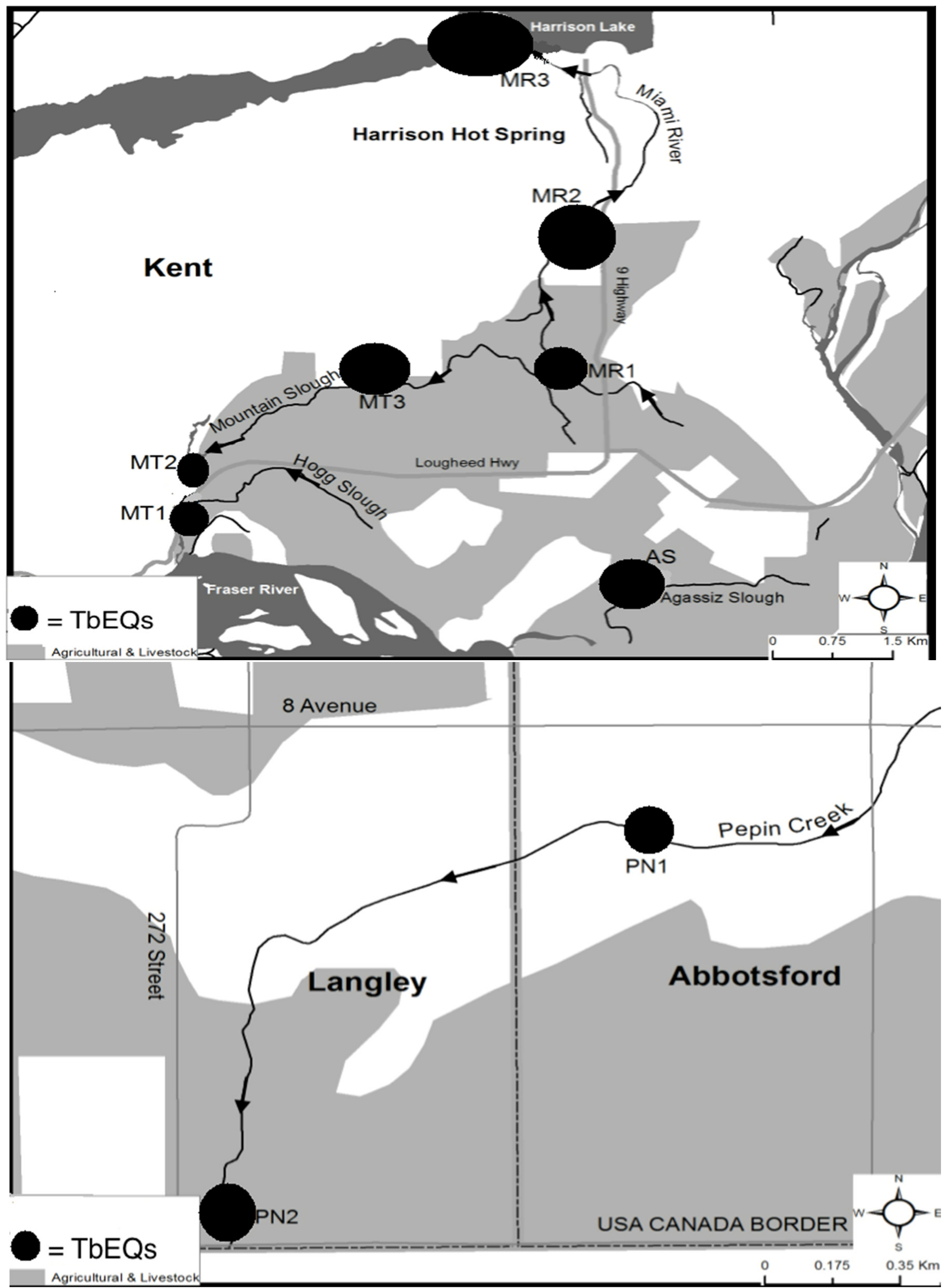


Figure 3.6 Sites in 2013 sampling period with TbEQs levels shown as dots.

Sizes of dots are proportional to the amount of TbEQs in water and sediments from all sampling periods.

3.3.3 Glucocorticoid levels in water and sediments

The results of the YGS assay are presented in Table 3.4 and Figure 3.7. Figure 3.8 shows DOCEQs as dots of sizes that are proportional to amount of glucocorticoid activity in water and sediments for all sampling periods. Glucocorticoid levels in water and sediment samples were expressed in ng of deoxycorticosterone equivalents (DOCEQs) per ml or g of sample. Glucocorticoid assay showed the highest activity when compared to YES and YAS and the number of samples found to be non-responsive to yeast was also higher than in any of the other three bioassays. Our results are in agreement with study by Van Der Linden et al*.* (2008), in which the highest levels detected were from glucocorticoids compared to other EDCs such as E2, progesterone and DHT in surface water.

3.3.3.1 Glucocorticoid levels in water

Water samples from Agassiz Slough in June and August were non-responsive to the yeasts; the mean concentration in water was 11.13 ng DOCEQs/ml in November. Glucocorticoid levels in Mountain Slough sites were higher compared to Agassiz Slough. There was an increase in glucocorticoid levels with time i.e*.,* the levels increased from June to August and from August to November for the two Mountain Slough sites MT1 and MT2. In MT3 the concentrations decreased in August but were the highest among all three Mountain Slough locations in November being at 8209.77 ng DOCEQs/ml. The average concentrations in Mountain Slough were 32.34, 461.42 and 3741.06 ng DOCEQs/ml in June, August and November respectively. Multiple dairy and berry farms impact all three sites in Mountain Slough. The high levels in November reflect the accumulation of Glucocorticoid compounds in these locations over time. As reported in a study by De Clercq et al. (2014), natural and synthetic glucocorticoids remain stable in animal excreta and show no significant loss in the environment.

Glucocorticoid activities in Miami River were high in August and November compared to in June (Table 3.4). MR1 the most upstream site had DOCEQs at 110.20, 7258.35 and 1553.09 ng/ml in June, August and November respectively. Low levels in June could be due to heavy rain fall period which washed away most of the compounds downstream. Whereas in the dry period of August, more compounds could be detected in still water. In November the rainfall levels were moderate. In MR2, the site downstream of

MR1 had mean concentrations at 24.25, 28.04 and 1377.65 ng DOCEQs/ml in June, August and November respectively. This site is downstream of a golf course. The last spot at Miami River, MR3 had undetectable levels of glucocorticoids in June and August but levels were not significantly different from other two Miami River location in November, as 1045.61 ng DOCEQs/ml of activity was detected in November. Low levels in June and August may be due to dilution of water. High levels in the two sites close to dairy farms and agricultural lands possibly be due to use of anti inflammatory drugs in animals leading to the release of cortisol in animal excreta reaching waterways. Courtheyn and Vercammen (1994) demonstrated that residues of corticosteroid were detectable in urine and feces of cattle treated with dexamethasone which becomes part of runoff from farmlands.

Water samples from PN1 had undetectable levels of glucocorticoid activities in June and August and showed mean DOC equivalents of 9.80 ng/ml in November. Samples from PN2 were non-responsive to yeast in June and August but were high at 749.73 ng DOCEQs/ml. No response in June and August samples from PN2 could be due to high contamination during the time of sampling. It is interesting to note that the incidence of sample' no response and high levels were observed in site downstream of PN1 but not in PN1. The contributing factor seems to be the impact from Aldergrove Regional Park that also has horse trails and it is popular with horseback riders. Alexander & Irvine (1998) have reported that social stress in horses causes an increase in free cortisol excretion.

3.3.3.2 Glucocorticoid levels in sediments

For sediment levels of cortisol-like chemicals (Figure 3.7, Table 3.4), there were no results from Agassiz Slough for June and August as the samples showed no response to yeast as did the water samples did for the same sampling period. This could be due to high levels of contaminants present in the slough during the time of sampling. The mean glucocorticoid levels in November were 233.20 ng DOCEQs/g. Sediment samples from the Mountain Slough sites also caused yeast cells death. Non-responsiveness was observed in samples from MT2 and MT3 in June and MT1 and MT2 in November. Thus only one DOCEQs value is available from Mountain Slough from June which was 215.28 ng/g at MT1, and was not significantly different ($p < 0.05$) from a site in a neighbouring Agassiz Sough. The mean concentrations reached 10,010.13 ng DOCEQs/g in August for

MT1. In August the mean DOCEQs for MT2 and MT3 were 17,200.42 and 1933.32 ng/g respectively. Only one DOCEQ value is available from Mountain Slough in November from MT3, which was 8176.98 ng DOCEQs/g which was very close to the water levels of 8209.77 ng DOCEQs/ml from the same location. Very high levels in the dry period may be due to an increase use of anti inflammatory drugs in cattle/dairy farms or mixing of anti inflammatory drugs with growth hormones during the period before sampling (Huetos et al., 1999).

Levels in Miami River were the highest at the most downstream site of MR3 compared to upstream locations of MR1 and MR2. This is due to the site is downstream of urban development as glucocorticoid drug uses by humans also discharge glucocorticoid-like chemicals through urine and feces. The mean DOCEQs increased from 256.96 to 2227.80 to 7902.15 ng/g in June going from upstream to downstream. Sediments were non-responsive to yeasts in August from MR1, but the levels increased from 31.11 and 557.74 ng DOCEQs/g, respectively for MR2 and MR3. In November there were no sediments data from MR1, but MR2 and MR3 showed the same pattern of increased levels from the earlier months as well as increased levels as we moved downstream of the river; levels in MR2 were 637.59 ng DOCEQs/g and in MR3 were 7455.67 ng DOCEQs/g.

Both sediment samples from Pepin creek were non-responsive in June (Table 3.4). The average levels were measured at 111.14 and 517.80 ng DOCEQs/g in August for PN1 and PN2 respectively. In November the mean DOCEQs at PN1 was 2999.89 ng/g whereas samples from PN2 were non-responsive.

Table 3.4 Summary of glucocorticoid levels in water and sediments from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek.

Sampling Period	Sample Type*	Agassiz Slough (AS)			Mountain Slough (MT)			Miami River (MR)			Pepin Creek (PN)		
		L	H	M	L	H	M	L	H	M	L	H	M
June	water	no response			4	76	32	BLD	145	67	BLD/ no response		
	sediment	no response			134	278	215	201	9207	3462	no response		
August	water	no response			4	3618	461	BLD	7981	3640	BLD/ no response		
	sediment	no response			1900	18988	9711	21	736	295	89	621	355
November	water	3	27	11	1345	8999	3740	864	2098	1326	0.3	799	329
	sediment	143	313	233	6895	9005	8210	567	9631	4047	2789	3211	3000

***Concentrations in water and sediment are presented in ng DOCEQ/ml and ng DOCEQ/g respectively. **L**=Low, **H**=High, **M**=Mean. **Note** the values are rounded to the nearest whole number.

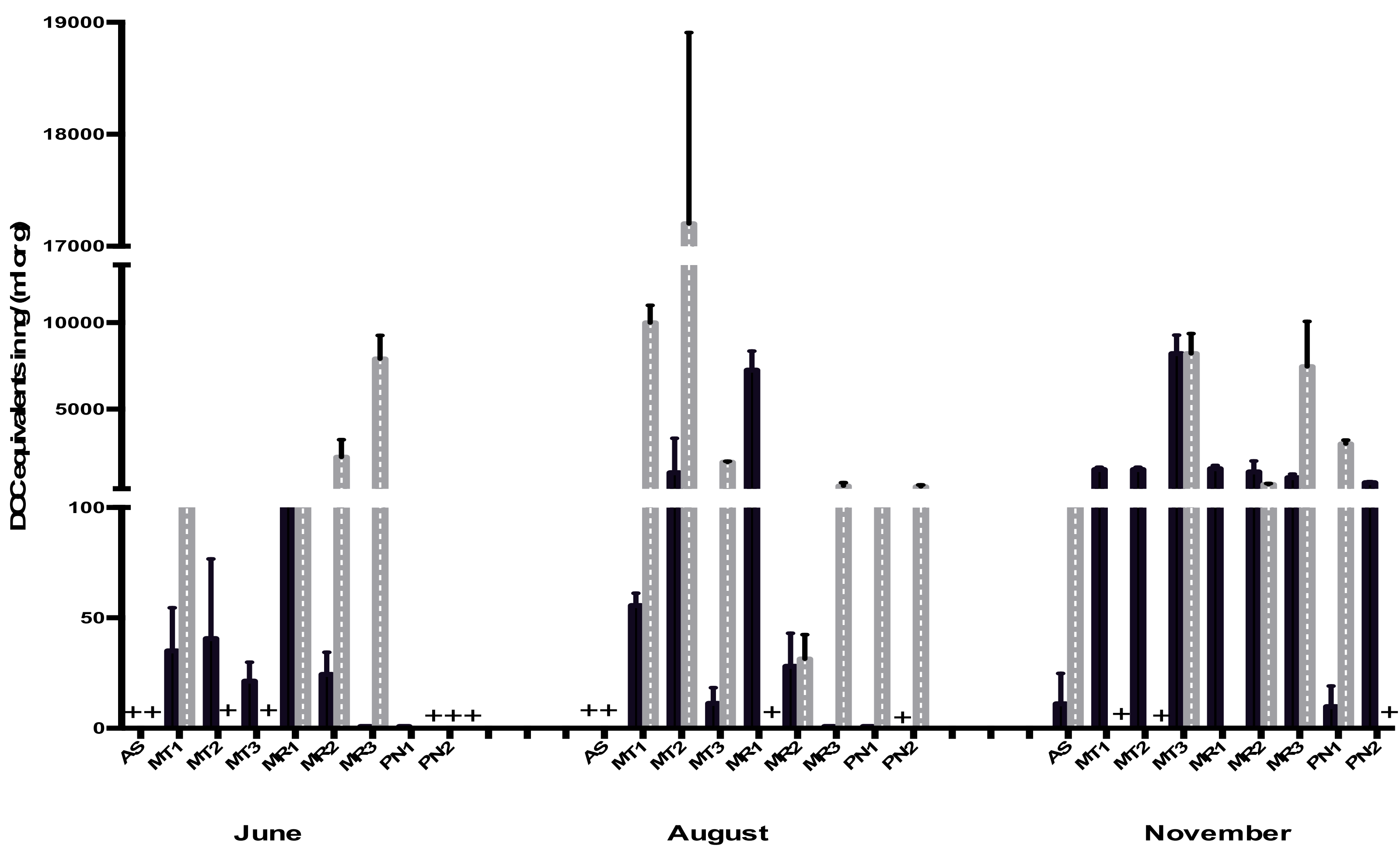


Figure 3.7 YGS assay results for Agassiz Slough (AS), Mountain Slough (MT), Miami River (MR) and Pepin Creek (PN).

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng

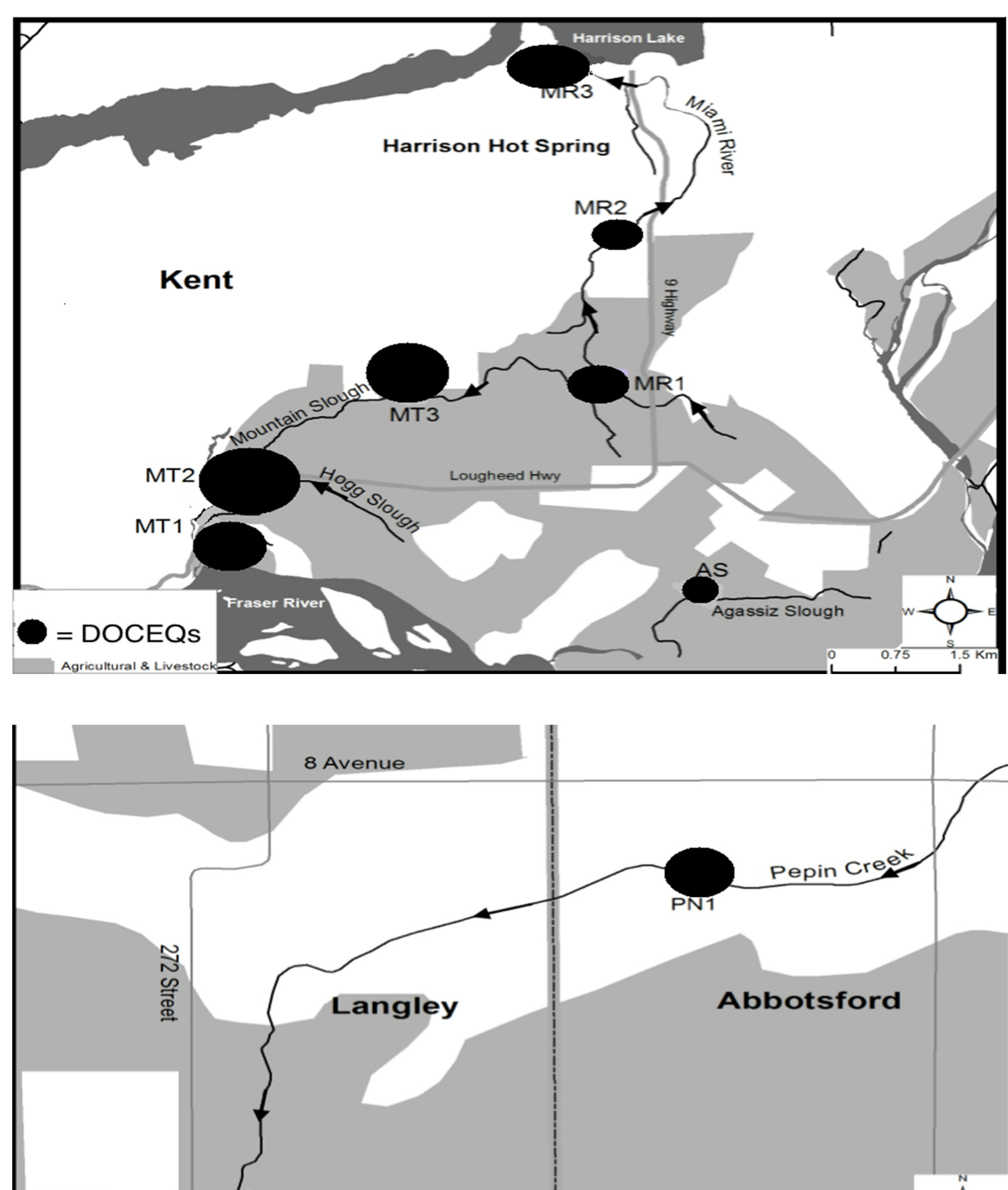


Figure 3.8 Sites in 2013 sampling period with DOCEQs levels shown as dots.

Sizes of dots are proportional to the amount of DOCEQs in water and sediments from all sampling periods.

3.3.4 Aryl hydrocarbon receptor agonists levels in water and sediments

Results of the AhR binding assay expressed as ng of *β*-naphthoflavone equivalents (NAPEQs) per ml or g sample of water and sediment respectively, are presented in Table 3.5 and Figure 3.9. Figure 3.10 shows NAPEQs as dots of sizes that are proportional to amount of AhR activity in water and sediments for all sampling periods. The NAPEQ levels dropped in November and were the highest in June with the exception of a few sites. June was also the wettest month and it was expected to see a higher AhR activity after rainfall. There was only one site, MT2, which was non responsive to yeast in November.

3.3.4.1 AhR agonists levels in water

AhR agonists' levels in water samples from Agassiz Slough were the highest in June (mean NAPEQs of 2849.80 ng/ml), dropped to 11.12 ng NAPEQs/ml in August and were undetectable in November. The M1 site in Mountain Slough had the same pattern of the highest mean concentrations in June (5182.65 ng NAPEQs/ml), dropping in August (1014.03 ng NAPEQs/ml) and the lowest were calculated in November (95.66 ng NAPEQs/ml). MT2 had the same pattern of the highest levels of 11048.32 ng NAPEQs/ml in June, dropping to very low 28.67 ng NAPEQs/ml in August and November but these samples were non responsive to the yeasts so it could not be confirmed if it followed the same pattern of lowest activities in November just like AS and MT1. Concentrations in MT3 were the highest in August of 25,825.33 ng NAPEQs/ml, 356.71 ng NAPEQs/ml in November and lowest in June of 39.01 ng NAPEQs/ml. The highest levels in August for MT3 is supported by findings by Qin et al. (2014) of which the highest level of PAHs are detected in summer months near agricultural lands. This may be due to increased solubility of PAHs in higher temperatures along with water evaporation leading to concentrated PAHs in surface water.

Levels in Miami River were about the same for the three sites in June, 156.59 ng NAPEQs/ml in MR1 and 149.30 ng NAPEQs/ml in MR2 but slightly higher at 245.03 ng NAPEQs/ml in MR3 (Figure 3.9). However MR3 is affected by urban development and impact by urbanization on MR1 and MR2 is small. The concentrations of aromatic hydrocarbon-like chemicals were higher in August at MR1 and MR2 being at 1849.11 ng

NAPEQs/ml and 3579.24 ng NAPEQs/ml respectively. But concentrations decreased in MR3 to 47.30 ng/ml. During the last sampling period in November, the AhR agonists' levels MR3 dropped even further down to 6.28 ng NAPEQs/ml, whereas levels were 165.30 and 274.41 ng NAPEQs/ml in MR1 and MR2 respectively. Comparison of NAP equivalents in water from June to August to November reveal that activities went from high to low from June to November in the two Sloughs with the exception of one site, MT3. On the other hand activities in the river were highest in August possibly due to water evaporation causing an increase in the concentration of PAHs. Also, an increase in temperature increased the solubility of AhR agonists in water (Qin et al., 2014). Contamination levels are about the same in June and November, with the exception of MR3 which is the last location in the river before Miami River enters Harrison Lake, where the levels are very low ranging from 4.10 – 247.55 ng NAPEQs/ml. The lowest levels in MR3 may be due to dilution of the compounds in the river and also PAHs being settled down in sediments along the way to entering Harrison Lake.

Pepin Creek data clearly show that urban impact increases AhR agonists in the environment as NAP equivalents are 10 to 200 times higher in PN2 compared to PN1 in all three sampling periods (Figure 3.9). AhR agonists' levels in PN1 were highest in June (286.67 ng NAPEQs/ml), dropped to 57.64 ng NAPEQs/ml in August and were lowest in November (17.19 ng NAPEQ/ml). This site has the same pattern of high and low levels at a given sampling period as site AS, MT1 and MR3. PN2 is impacted by the Aldergrove Regional Park, thus has influence of urbanization. The park has horse and cycling trails as well. The highest concentrations in PN2 were in August at 5336.33 ng NAPEQs/ml, lower in November of 3671.23 ng NAPEQs/ml and lowest at 3110.55 ng NAPEQs/ml in June. The lowest levels in the raining period of June and November could be due to an increase in water level in the creek and the dilution of AhR agonists.

3.3.4.2 AhR agonists levels in sediments

Fig. 3.9 shows the concentrations of NAP equivalents in sediment samples from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek. Only 19% of the sediment samples had lower levels of NAP activity compared to the water samples from the same location. Detection of a higher level of NAP-like contaminants in sediments is due to preferential adsorption of hydrocarbons onto soil particles rather than being

dissolved in water (Hiller et al., 2008). Average NAP concentration activities in Agassiz Slough were 36,525.91 ng NAPEQs/g in June, dropped to 6357.88 ng NAPEQs/g in August, and dropped further in November to 1311.72 ng NAPEQs/g. These levels were significantly different ($p < 0.05$) from each other. This is the same pattern we observe for NAP concentrations in the water samples where activities decreased with time. In Mountain Slough the levels were the highest in MT3 which was impacted by poultry and berry farms. This may be due to the solvents used to apply pesticide and/or herbicide to the fields, as residues of pesticides are found in wash water from farms and this could increase the AhR activity in the waterways (Atwater et al., 1998). Activities in MT3 sediments were 34451.15 ng NAPEQs/g in June, this is comparable to levels in Agassiz Slough in the same month. Levels in August rose to average NAPEQs of 38,263.40 ng/g but dropped in November to 1236.22 ng NAPEQs/g. The low activity in November could be the result of selection of sediments from a site a little further away from the farm. The NAPEQs for the other two Mountain Slough sites were not significantly different from each other in August, being 3750.05 ng NAPEQs/g for MT1 and 3779.05 ng NAPEQs/g for MT2. MT had lowest levels at 203.12 ng NAPEQs/g in November and also low at 662.50 ng NAPEQs/g in June. On the other hand levels in MT2 were high at 16,648.83 ng NAPEQ/g in June and sediments were non responsive to yeast in November which was the case with water during the same sampling period. Overall highest levels in MT3 in all sampling periods suggest influence of an abundance use of pesticides and herbicides in the nearby fields.

In Miami River, the NAPEQs in August were very close to each other for all three sites, MR1, MR2 and MR3, being at 1059.19, 1096.20 and 952.55 ng NAPEQs/g, respectively. All these sites are impacted by runoff from dairy farms, MR2 is also impacted by a nearby golf course and MR3 is by town of Harrison as well. Zhao et al. (2013) have detected PAHs and organochlorine pesticides in manure samples in China. Our results are consistent with their findings.

NAPEQ levels in the sediments of Pepin Creek were the highest in November for both PN1 and PN2 sites; they were 8794.99 ng NAPEQs/g and 3465.75 ng NAPEQs/g, respectively. For PN1, the levels were not different significantly between June (730.30 ng NAPEQs/g) and August (853.06 ng NAPEQs/g) ($p < 0.05$). On the other hand the levels

in PN2 varied; mean NAPEQs was 1313.42 ng/g and 149.93 ng/g in June and August, respectively.

Urban developments such as a golf course (MR2), Harrison town centre (MR3) and Aldergrove regional park (PN2) may contribute to AhR activity due to combustion, leachate of construction material and oil/grease into waterways, and presence of high traffic (Qin et al., 2014; Cailleaud et al., 2007; Jalova et al., 2013)

Overall, 75% of the sites from the same body of water had very similar levels of AhR agonists' contamination in the month of August. Our results are very close to ones reported by Tam et al. (2001) from China where the AhR agonists were in the range of 356 to11098 ng/g.

Table 3.5 Summary of AhR agonists levels in water and sediments from Agassiz Slough, Mountain Slough, Miami River and Pepin Creek.

Sampling Period	Sample Type*	Agassiz Slough (AS)			Mountain Slough (MT)			Miami River (MR)			Pepin Creek (PN)		
		L	H	M	L	H	M	L	H	M	L	H	M
June	water	2105	3498	2851	10	12990	5423	98	301	184	245	3199	1710
	sediment	33005	39578	36528	345	38789	17254	88	28780	8849	689	1396	1025
August	water	4	19	11	17	28008	8856	34	3999	1825	41	6808	2995
	sediment	4976	7274	6349	3001	44789	15130	817	1345	1037	114	887	508
November	water	BLD			45	398	226	4	335	148	6	3711	1843
	sediment	915	1540	1311	155	1409	719	177	1123	594	2077	10459	6130

*Concentrations in water and sediment are presented in ng NAPEQs/ml and ng NAPEQs/g respectively. **L**=Low, **H**=High, **M**=Mean. **Note** the values are rounded to the nearest whole number for clarity.

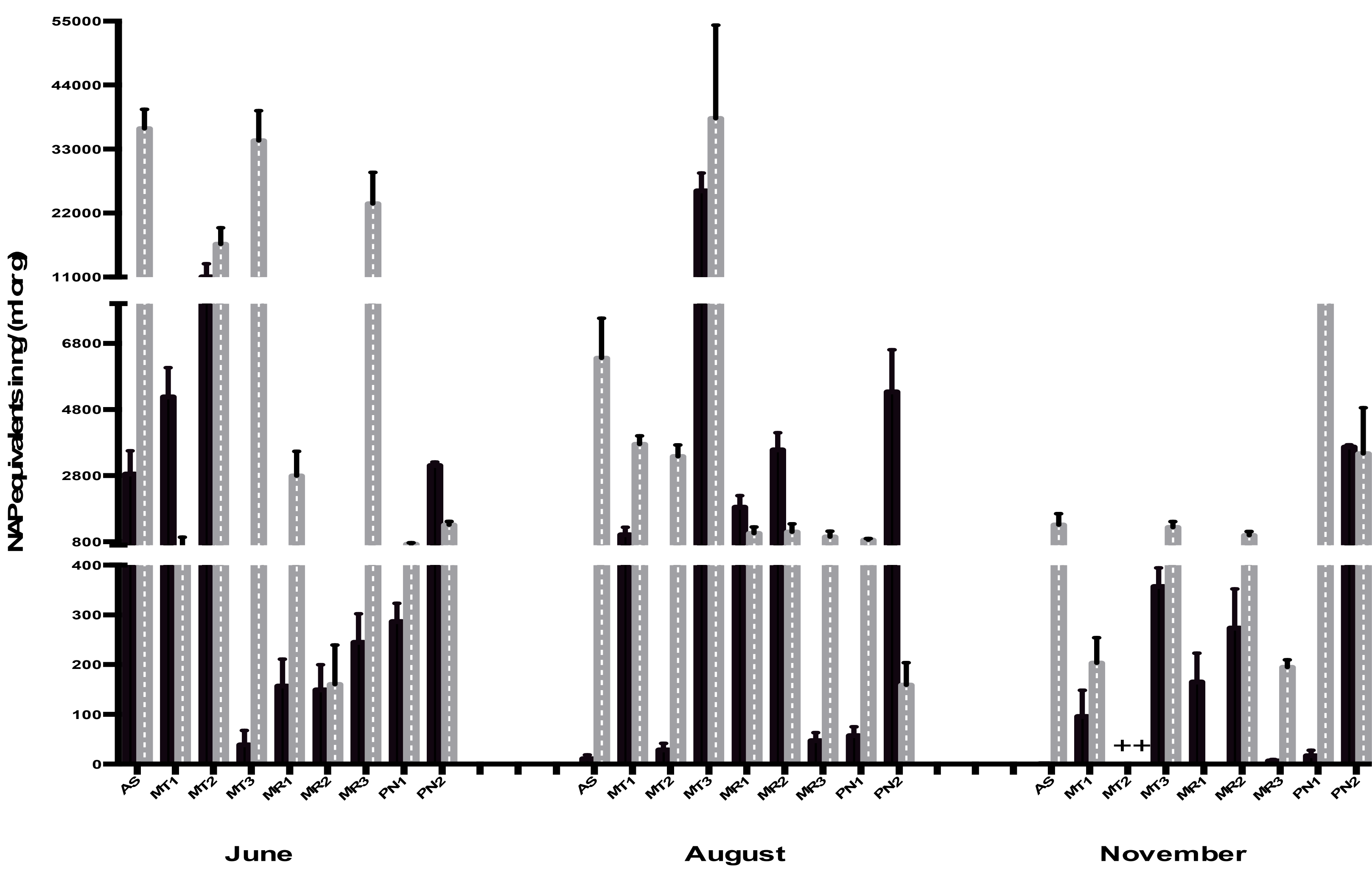


Figure 3.9 AhR assay results for Agassiz Slough (AS), Mountain Slough (MT), Miami River (MR) and Pepin Creek (PN).

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in

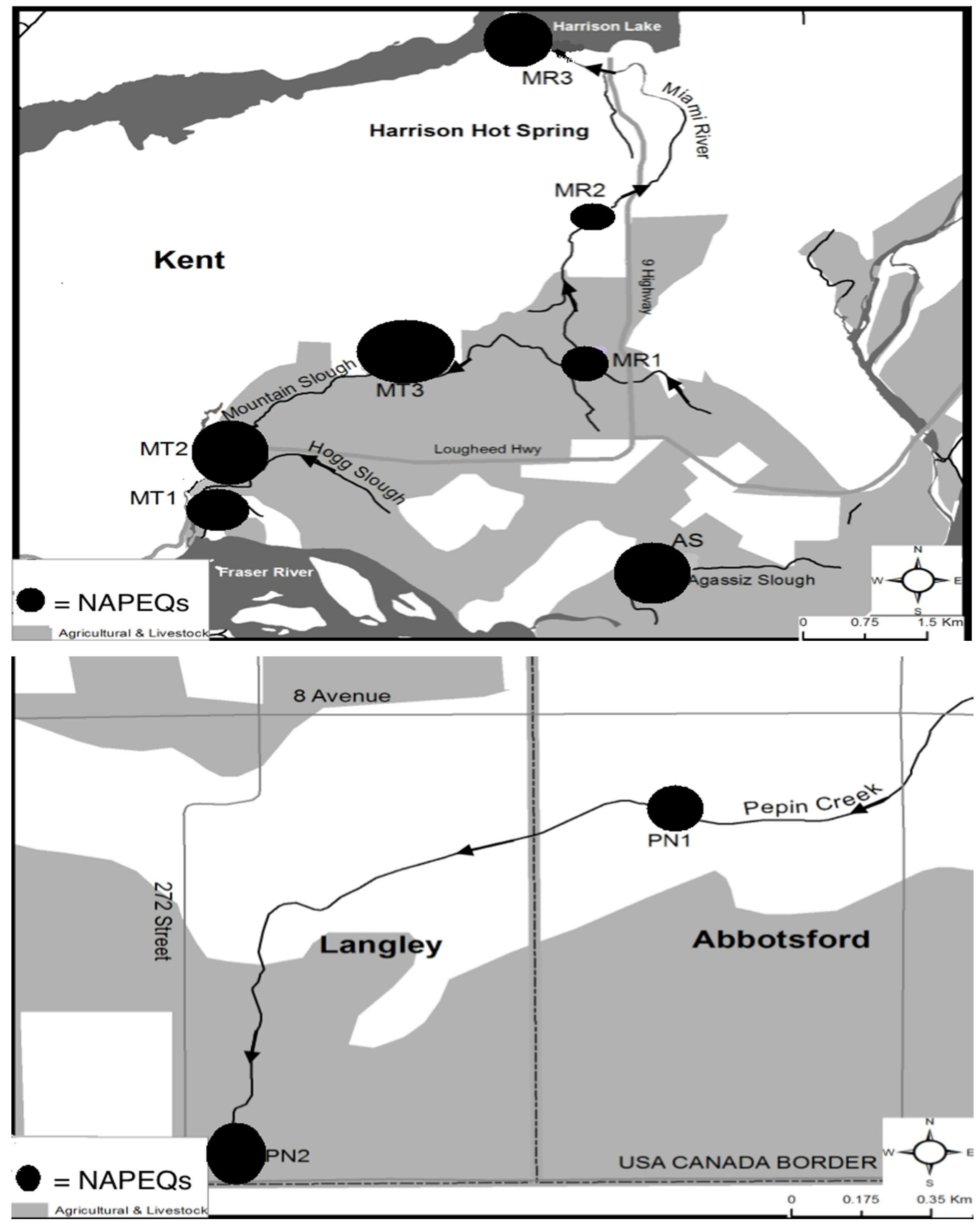


Figure 3.10 Sites in 2013 sampling period with NAPEQs levels shown as dots.

Sizes of dots are proportional to the amount of NAPEQs in water and sediments from all sampling periods.

3.4 EDCs levels from sampling sites in 2014/15

An area in Surrey was selected to test for EDCs since these sites are impacted by anthropogenic activities while draining into a Nicomekl river, a fish-bearing watercourse. Table 2.2 summarizes each sampling site and a description of possible impacts on the sites. S0 site is not connected to any other site; S1-S4 all catch water flows from the pump station; S5 and S6 are impacted by berry farms as well as flows from the pump station; S10 is downstream from a horse racetrack; S7 collects everything coming down from S1-S6 and S10; S8 is downstream of all the sites mentioned above plus there may be some influence from more dairy farmlands in between; S11 collects everything from the sites mentioned above and it is also the last site before water enters Nicomekl river; S9 and S12 are located in the Nicomekl river.

Since the ditches had very little or no water in October no results are presented from site S2; and due to inaccessibility, no water and sediments were collected from sites S1 and S3 in October.

3.4.1 Estrogenic levels in water and sediments

Results from the YES assay are presented in Table 3.6 and Figure 3.11 show the results of YES bioassay on water and sediment samples, respectively in ng EEQs/ml and ng EEQs/g. Figure 3.12 shows EEQs as dots of sizes that are proportional to amount of estrogenic activity in water and sediments for both sampling periods.

3.4.1.1 Estrogenic levels in water

All water samples were responsive to the yeast cells in both sampling periods with the exception of site S5 in February. Estrogenic activity was found to range from BLD to 3.97 ng EEQs/ml in October and from BLD to 6.02 ng EEQs/ml in February. In October the mean EEQs were BLD, *i.e.,* <0.0125 ng/ml for site S8 and very low (~ 0.04 ng EEQ/ml) at sites S4, S5, S6, S9 and S11. The highest average concentrations were measured at S7 (3.59 ng EEQs/ml) and S12 (1.90 ng EEQs/ml). In February, the estrogenicity in water was very close to what had been measured in October's samples. With higher water levels in February, the estrogenicity for S1, S2 and S3 were very low; BLD for S2 and ~ 0.09 ng

EEQ/ml for S1 and S3. EEQs levels at S0 were the same for both sampling periods. Compare to October, average EEQs levels in February were higher for S4, S6, S8, S9 and S11 and were lower at S7, S10 and S12. The highest activities were found in S8 (5.42 ng EEQ/ml) and S11 (4.85 ng EEQ/ml) in February. A possible explanation for the variation in estrogenic activity near dairy farms is a change in rate of excretion during pregnancy and lactation periods in cattle. In addition, an increase or decrease in the number of animals during the time of sampling can influence the levels of EEQs detected in the runoffs (Hanselman et al., 2003).

3.4.1.2 Estrogenic levels in sediments

Figure 3.11 shows the estrogenic levels for all sites in sediment samples. Unlike water samples, there were 4 sites where sediments were non-responsive to the yeasts. These sites included S2, S4, S5 and S10 in October and S10 in February. In October the highest estrogenic levels were at S6 (524.20 ng EEQ/g) that had collected all the flow from the pump station and berry farms, followed by site S7 (233.34 ng EEQ/g) which received flow from pump station, berry farms and horse racetrack. S0 site also had high mean estrogenic levels at 125.48 ng EEQ/g. The levels in Nicomekl River were 32.90 ng EEQ/g and 9.03 ng EEQ/g for S9 (downstream) and S12 (upstream) respectively. Dilution could be a factor in decrease levels down the river. Most of the sites had lower estrogenic levels in sediments in February compared to in October. This could be due to more rainfall causing dilution.

On average, the estrogenic contamination was much lower in water in both seasons compare to levels in the sediments. Concentrations in sediments were either, on average, higher or had no response in October compare to the sediment samples in February. Higher levels in rainy period were expected as estrogens are degraded more rapidly in warmer temperature. Less sunlight to cause abiotic degradation and lower rates of microbial breakdown are other explanations for higher levels in sediment in October (Tiryaki and Temur, 2010).

Table 3.6 Summary of mean estrogenic levels from sites in Surrey.

Site	October		February	
	water (ng EEQs/ml)	sediment (ng EEQs/g)	water (ng EEQs/ml)	sediment (ng EEQs/g)
S0	0.34	125.48	0.34	0.85
S1, S2, S3, S4	0.08[1]	no response [2]	0.14	2.90
S5, S6	0.05	no response (S5) 524.20 (S6)	no response (S5) 2.70 (S6)	4.22
S10	0.31	no response	0.05	no response
S7	3.59	233.34	0.18	1.17
S8	BLD	25.32	5.42	38.20
S11	0.02	BLD	4.85	34.12
S9, S12	0.98	21.0	0.23	4.94

[1]No water samples were collected from S1, S2 and S3
[2]No sediment samples were collected from S1 and S3

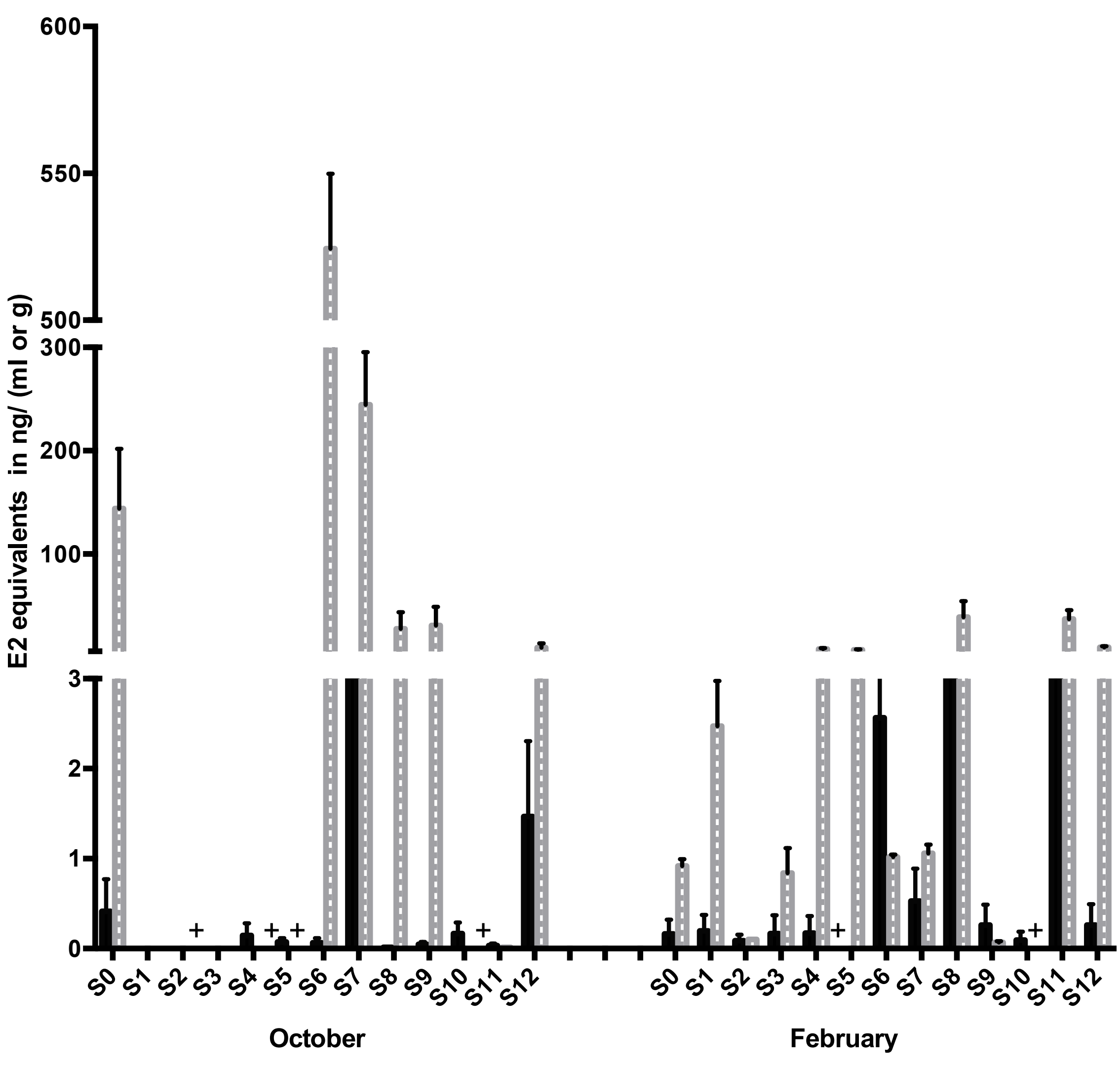


Figure 3.11 YES assay results for sites in Surrey.

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

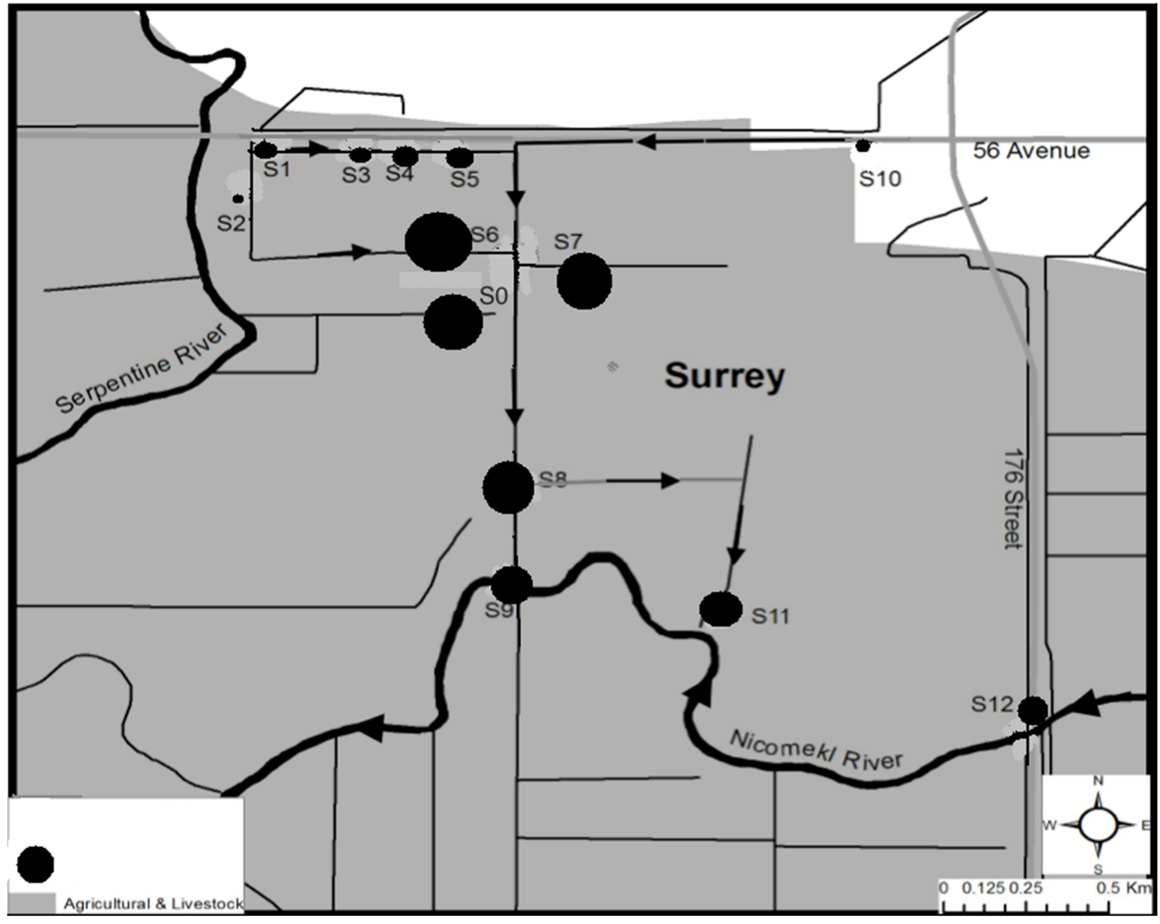


Figure 3.12 Sites in Surrey with EEQs levels shown as dots.

Sizes of dots are proportional to the amount of EEQs in water and sediments from all sampling periods.

3.4.2 Androgenic levels in water and sediments

Results from the YAS assay are presented in Table 3.7 and Figure 3.13; they are expressed as ng of TbEQs per ml and g of water and sediments, respectively. Figure 3.14 shows TbEQs as dots of sizes that are proportional to amount of androgenic activity in water and sediments for both sampling periods.

3.4.2.1 Androgenic levels in water

About 30% of the sites in October were BLD of 0.10 ng/ml of the YAS assay. In addition, two sites were non responsive to yeast and the remaining sites had very low TbEQs of approximately 1.18 ng TbEQ/ml. However, S8 and S9 stood out as having very high levels at 150.58 ng TbEQs/ml and 115.05 ng TbEQs/ml respectively. The high levels of androgenic activity at S8 possibly are due to runoff from the nearby dairy farms. There is also a nursery and a horseback riding school close to these two locations. Cows were also spotted in a close by field in October. Schiffer et al. (2001) found androgenic compounds, Tb and MGA, in dairy farms runoffs and determined that the stability of Tb can be up to 8 weeks after application on the field in manure. Only two sites had no response to yeast in October (Table 3.7). In February, most sites had androgenic contamination levels at around 0.60 ng TbEQs/ml. There was a decrease in TbEQs in the S0, S8 and S9 sites in February compared to levels in October. Nevertheless S8 still had the highest level out of all locations in February at 23.90 ng TbEQ/ml. Other locations where androgenicity increased were S4 (23% up), S7 (33%), S10 (7%), S11 (3.5%) and S12 (1.5%).

3.4.2.2 Androgenic levels in sediments

Androgenic contamination in sediments was higher than in water for both sampling period (Figure 3.13). There was one sediment sample (S6) which was non responsive to yeast from October sampling period. Androgenicity ranged from an average of 2.72 to 260.25 ng TbEQs/g were found in the sediments. The highest activity was found at S4. This may be due to the application of fertilizer on the berry field from which runoffs get into S4. In February the levels were lower compared to October sediment samples. There were three samples (S1, S4, S8) found to be non responsive to yeast and one (S3) where levels were BLD. The highest activity was at site S2 at 31.73 ng TbEQs/g; this site is the closest point receiving flow from the pump station. The same site also had a high level of 84.37 ng TbEQs/g in October. According to Phillips et al. (2012) concentrations of androgens are 10x higher in sewer output compared to discharge from a treated wastewater plant. The site with the second highest activity was S9 at 27.78 ng TbEQ/g. The rest of the sites were averaged at 4.56 ng TbEQ/g.

Table 3.7 Summary of mean androgenic levels from sites in Surrey.

Site	October		February	
	water (ng TbEQs/ml)	sediment (ng TbEQs/g)	water (ng TbEQs/ml)	sediment (ng TbEQs/g)
S0	0.74	18.53	0.28	3.43
S1, S2, S3, S4	0.90[1]	172.31[2]	5.42	no response (S1, S4) 15.87
S5, S6	no response	16.09 (S5) no response (S6)	0.20	8.12
S10	BLD	8.98	0.70	3.15
S7	BLD	43.21	3.31	0.50
S8	150.58	15.69	23.90	no response
S11	BLD	2.72	0.35	2.42
S9, S12	58.11	20.31	4.30	15.59

[1]No water samples were collected from S1, S2 and S3
[2]No sediment samples were collected from S1 and S3

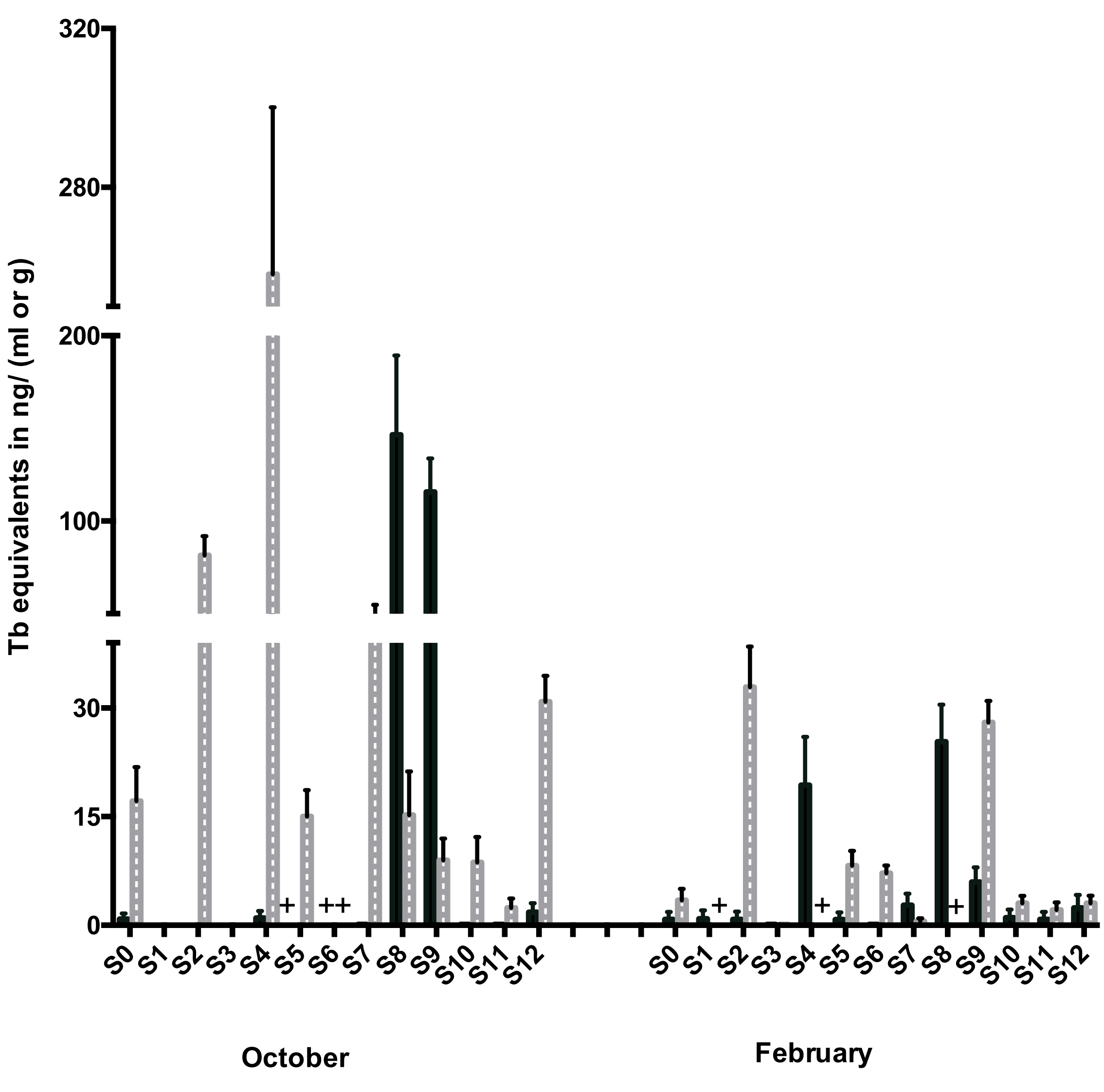


Figure 3.13 YAS assay results for sites in Surrey.

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

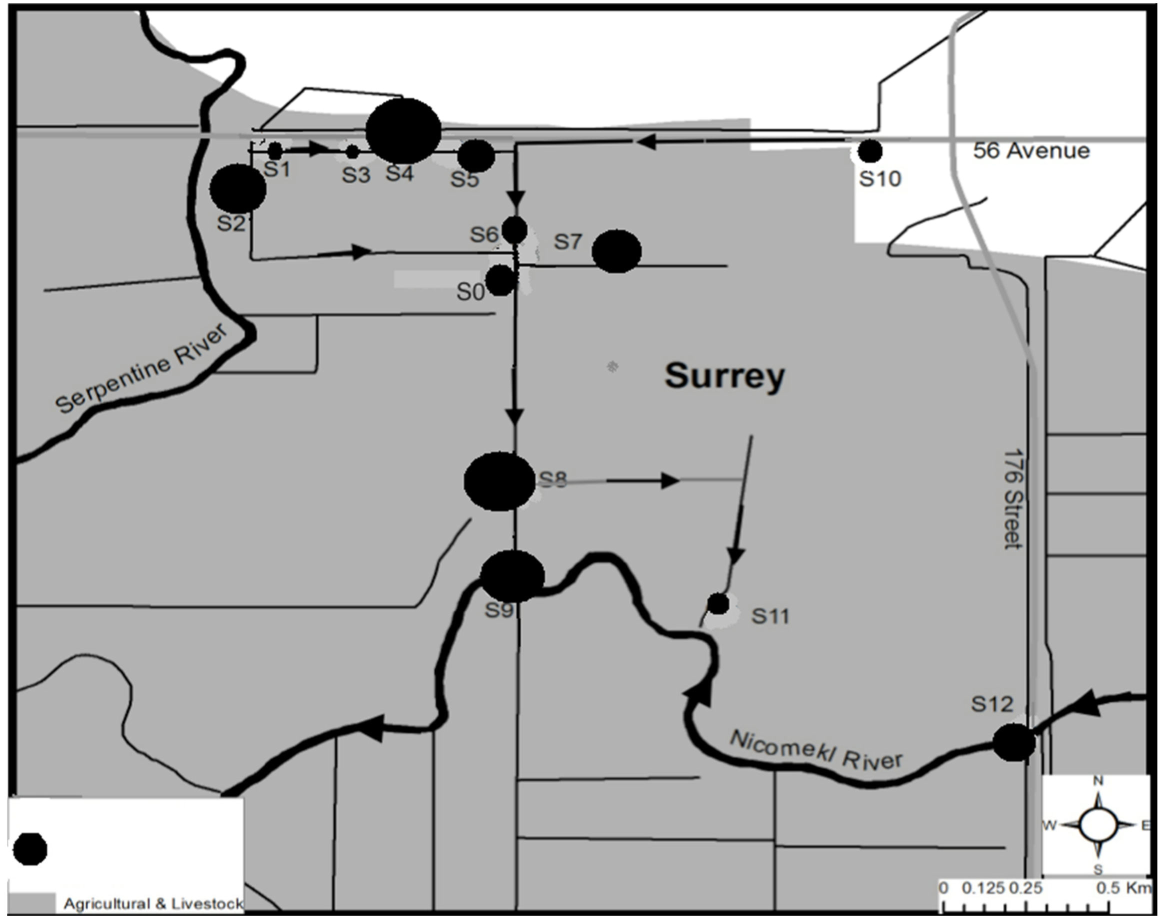


Figure 3.14 Sites in Surrey with TbEQs levels shown as dots.

Sizes of dots are proportional to the amount of TbEQs in water and sediments from all sampling periods.

3.4.3 Glucocorticoid levels in water and sediments

Results from YGS assay are presented in Table 3.8 and Figure 3.15. The results are expressed in ng of DOCEQs per ml and g of water and sediment sample, respectively. Figure 3.16 shows DOCEQs as dots of sizes that are proportional to amount of glucocorticoid activity in water and sediments for both sampling periods.

3.4.3.1 Glucocorticoid levels in water

Glucocorticoid levels in water ranged from BLD of 0.88 ng DOCEQs/ml at S11 to 22.28 ng DOCEQs/ml at S12 in October. Only S10 sample was non responsive to yeast. The DOCEQs at Nikomekl River were highest upstream at S12 and dropped downstream

(S9) to 3.40 DOCEQs in ng/ml. Location S8, which is close to dairy farms, horse riding school and an animal care facility, had the second highest levels of 18.59 ng DOCEQs/ml. S0 site also had high levels at 17.21 ng DOCEQs/ml. The rest of the sites were on average 5.41 ng DOCEQs/ml. In February, only S12 sample was non responsive to yeast whereas four samples were BLD from sites S0, S5, S7 and S11. This could be due to dilution factor because of rainy period as these sites had higher water levels in October. The highest level in February was detected in S2 (243.0 ng DOCEQs/ml), which receives effluent directly from a pump station. Macikova et al. (2014) reported the highest glucocorticoid receptor activity using GR-CALUX assay from an untreated wastewater (387.5 ng DOCEQs/ml) compared to treated wastewater (35.0 ng DOCEQs/ml) and river water (1.9 ng DOCEQs/ml). My results are consistent with their findings in that the receiving end of pump station had high levels compared to levels in the river and during rainy period.

3.4.3.2 Glucocorticoid levels in sediments

Table 3.8 shows that the sediments samples in October and February are similar. For example, S0 site had no detectable glucocorticoid activity in both sampling period; S6, S7 and S12 samples were non responsive to yeast in both time periods. Two more sites' (S2 & S10) samples were also non responsive to yeast in October. The highest mean activity was in S4 (776.50 ng DOCEQ/g), S5 (133.88 ng DOCEQ/g) and S8 (124.08 ng DOCEQ/g). S4 receives untreated water from a liquid waste pump station that collects sewer from storm drain. S5 gets water from S4 as well as runoff from a berry farm, and S8 is influenced by dairy farms close by. In February, glucocorticoid activity was lower in two sites but increased in two others. Thus, levels in S4 and S5 dropped to 55.20 ng DOCEQs/g and 40.10 ng DOCEQs/g respectively. Glucocorticoid levels rose in S8 to 245.10 ng DOCEQs/g and in S9 to 57.70 ng DOCEQs/g in February. These two sites are close to dairy farms, a horse riding school and also a dog and cat boarding site. The three sites, S2, S3, S4, receiving water from the pump station had levels not significantly different from each other at $p < 0.05$; all average at ~ 44.50 ng DOCEQs/g each site. Sediments from site 10, which is influenced by a horse race track nearby, had levels at 24.26 ng DOCEQs/g very close to what was found in water (24.52 ng DOCEQs/ml)

High levels of glucocorticoid activity in sites close to land with cows, horses and other small animals (animal care facility) suggest either use of anti-inflammatory drugs in animals which are excreted in urine and feces (Mostle et al., 1999; Popot et al., 2006) or social stress in animals which causes an increase in free cortisol in animal excreta (Alexander & Irvine 1998) or use of fertilizers laden with antibiotics.

Table 3.8 Summary of mean glucocorticoid levels from sites in Surrey.

Site	October		February	
	water (ng DOCEQs/ml)	sediment (ng DOCEQs/g)	water (ng DOCEQs/ml)	sediment (ng DOCEQs/g)
S0	17.21	BLD	BLD	BLD
S1, S2, S3, S4	6.02[1]	no response (S2) 776.50 (S4)	63.05	47.29
S5, S6	9.98	133.88 (S5) no response (S6)	7.04	40.10 (S5) no response (S6)
S10	no response	no response	24.52	24.26
S7	3.03	no response	BLD	no response
S8	18.59	124.08	143.53	245.10
S11	BLD	2.10	BLD	2.73
S9, S12	12.84	28.87 (S9) no response (S12)	25.23 (S9) no response,	57.70 (S9) no response (S12)

[1]No water samples were collected from S1, S2 and S3

[1]No water samples were collected from S1, S2 and S3

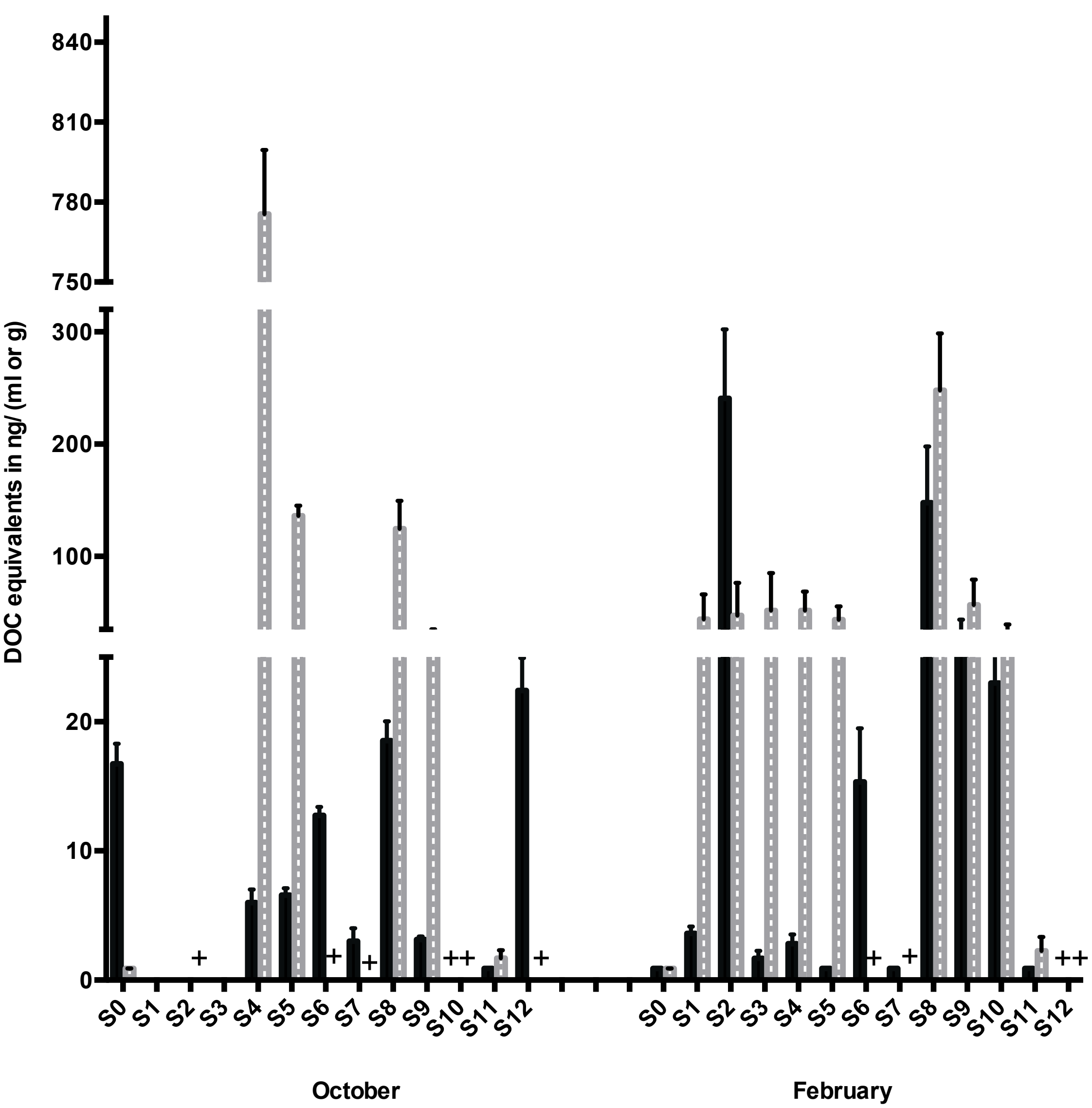


Figure 3.15 YGS Assay results for sites in Surrey.

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

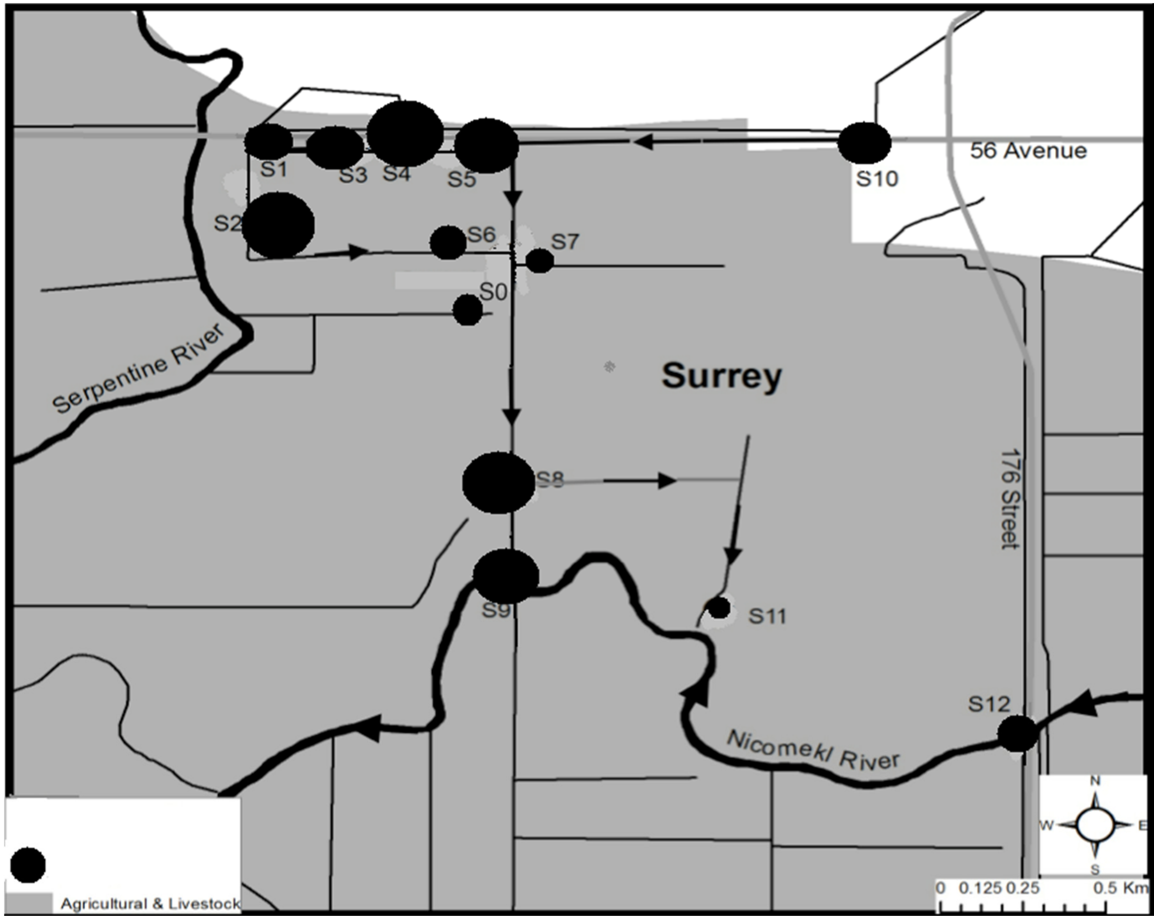


Figure 3.16 Sites in Surrey with DOCEQs levels shown as dots.

Sizes of dots are proportional to the amount of DOCEQs in water and sediments from all sampling periods.

3.4.4 Aryl Hydrocarbon receptor agonists levels in water and sediments

Results from AhR binding assay are presented in Table 3.9 and Figure 3.17. The results are expressed in ng of β-Naphthoflavone equivalents (NAPEQs) per ml and g of water and sediments, respectively. Figure 3.18 shows NAPEQs as dots of sizes that are proportional to amount of AhR activity in water and sediments for both sampling periods.

3.4.4.1 AhR agonists levels in water

For water samples, 9 out of 10 tested positive for AhR activity in October and 11 out of 13 were positive in February. There was one S4 site that had no response to yeast in October otherwise the NAPEQs ranged from 14.77 – 374.80 ng/ml. The three highest levels of contamination were found at S10 (232.34 ng NAPEQs/ml), S11 (374.80 ng NAPEQs/ml) and S12 (337.05 ng NAPEQs/ml). All three sites are close to roadways; especially S10 and S12 are on the highway. This is due to vehicle emission and asphalt contaminating the waterways (Qin et al., 2014). In February, AhR activity was the highest at only one location, S8, at 626.10 ng NAPEQ/ml. This was much higher than what was observed in October at being only 14.77 ng NAPEQs/ml. S8 is also on the street and higher traffic in February is one explanation. This site is also influenced by nearby farmlands that may have been using pesticides on the fields. The levels either dropped or increased in February compared to results in October and that could be due to other factors such as temperature, PAHs solubility, dilution or use of pesticides or contaminated manure in the surrounding farmlands (Tremblay et al*.,* 2005).

3.4.4.2 AhR agonists levels in sediments

Figure 3.17 shows that levels in sediments were higher than water. This was expected since PAHs are more likely to adsorb onto organic matter than remain in the water phase. None of the samples showed non-responsiveness to the yeasts in both sampling periods (Table 3.9). The average NAPEQs ranged from 126.91 – 4489.21 ng/g in October. The highest at the S12 location was near a busy highway. The NAPEQs for S12 were not significantly different in February sitting at 4945.34 ng NAPEQs/g. In February 85% of the sites had lower NAPEQs compared to values in October. This is explained by higher ambient temperature in February that caused decrease in sorption of PAHs to sediments (Hiller et al., 2008).

Table 3.9 Summary of mean AhR agonists' levels from sites in Surrey.

Site	October		February	
	water (ng NAPEQs/ml)	sediment (ng NAPEQs/g)	water (ng NAPEQs/ml)	sediment (ng NAPEQs/g)
S0	15.67	1574.39	3.71	335.00
S1, S2, S3, S4	no response[1]	2134.50[2]	27.92	681.24
S5, S6	49.02	523.73	32.55	132.21
S10	232.34	1913.44	21.99	63.04
S7	32.26	332.82	85.10	7727.43
S8	14.77	176.63	626.10	436.89
S11	374.80	545.98	20.60	514.11
S9, S12	179.11	2308.06	75.40 (S9) no response (S12)	2524.5

[1]No water samples were collected from S1, S2 and S3

[2]No sediment samples were collected from S1 and S3

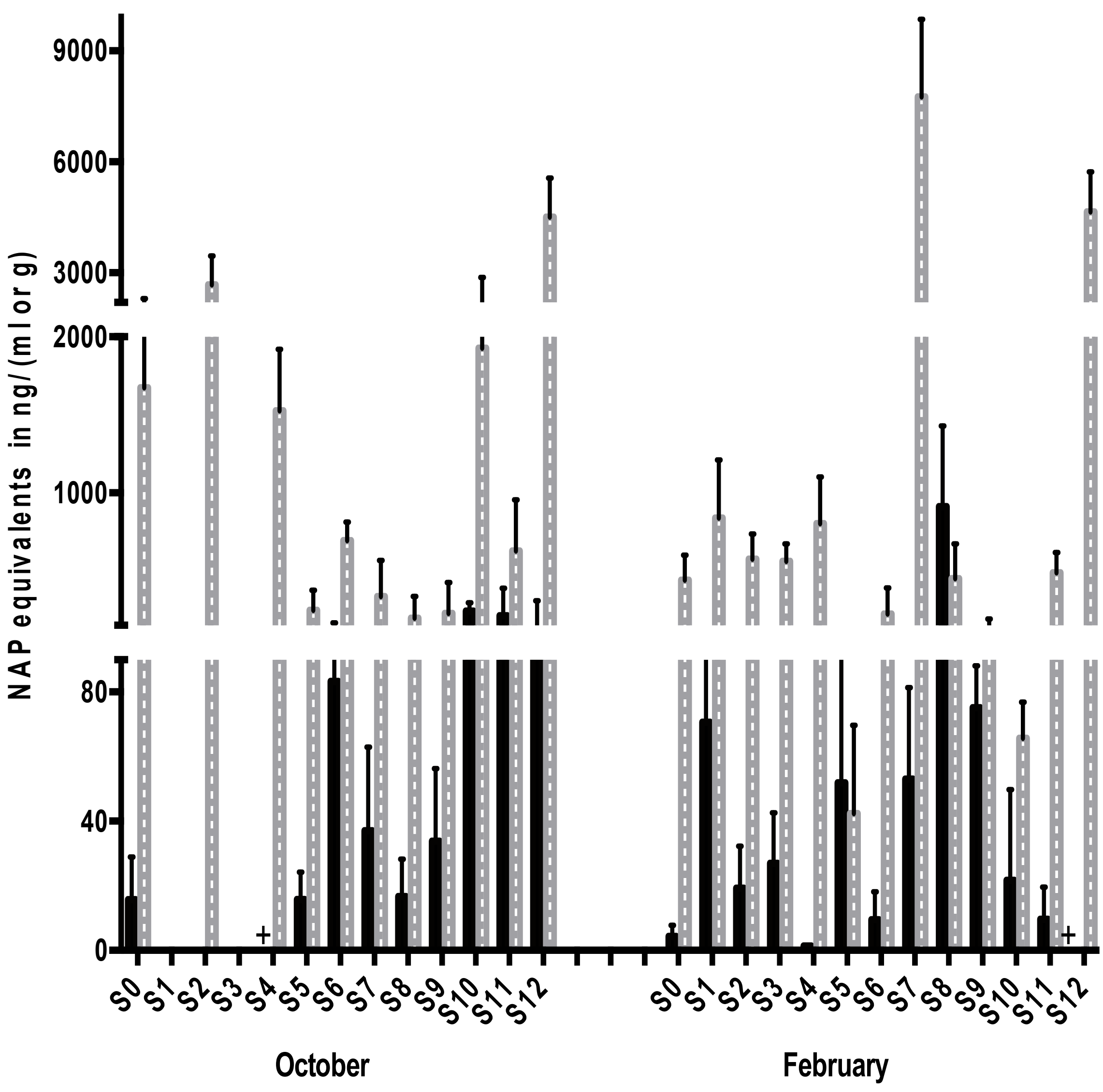


Figure 3.17 AhR Assay results for sites in Surrey.

+ indicates no response to yeast; black bars=water values in ng/ml, grey bars=sediment values in ng/g

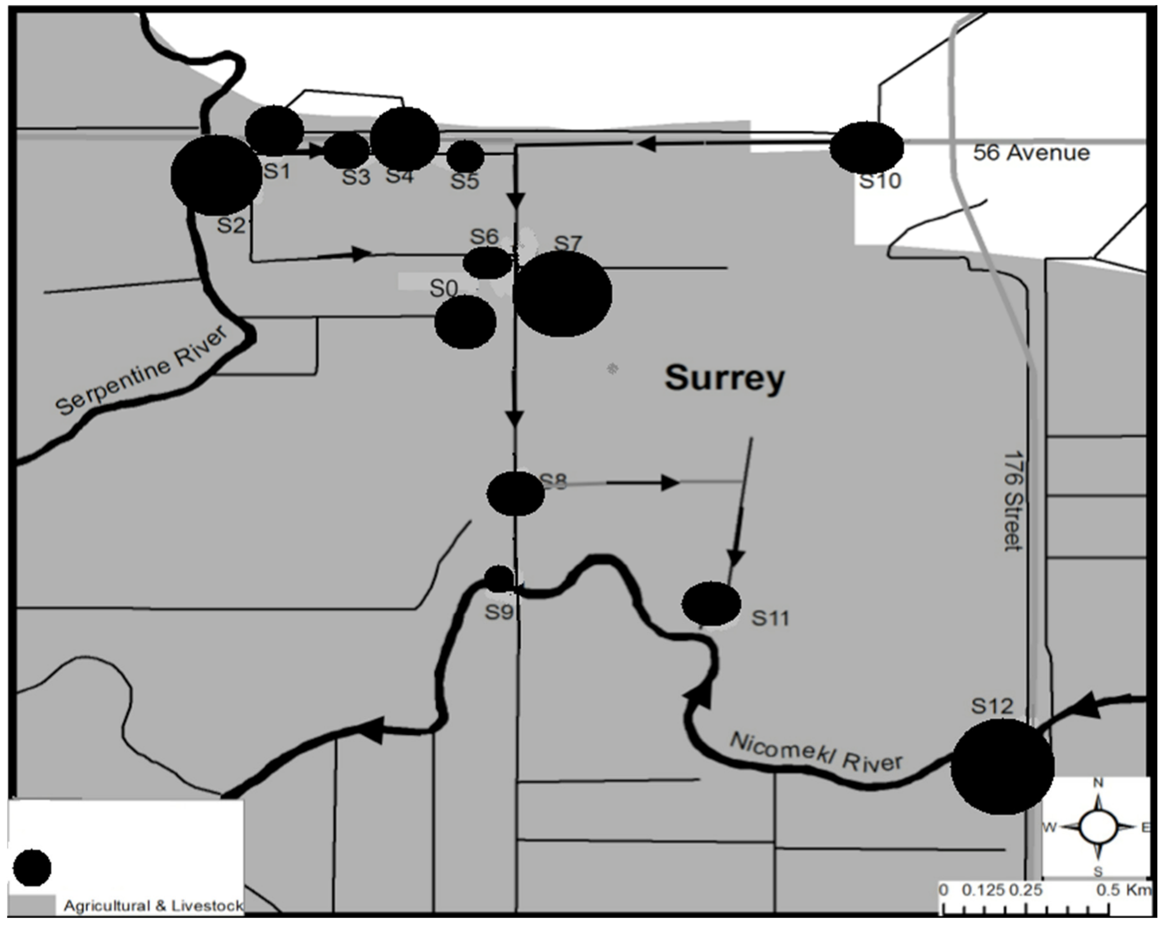


Figure 3.18 Sites in Surrey with NAPEQs levels shown as dots.

Sizes of dots are proportional to the amount of NAPEQs in water and sediments from all sampling periods.

3.5 Results from Chemical Analyses

Chemical analysis using GC-MS was performed to identify compounds that were contributing to EDC activity in the estrogenic, androgenic and AhR agonist bioassays.

The three water samples (AS, MR1, MR3) with high levels of estrogenic activity in the YES assay were analyzed for estrogenic compounds using GC-MSD. The Total Ion Current (TIC) chromatogram for the six standards is shown in Figure 3.19. The six standard peaks, visible in the chromatogram, are of nonylphenol (NP), bisphenol A (BPA), estrone (E1), 17β-estradiol (E2), 17β- ethynylestradiol (EE2) and estriol (E3). The

concentrations used were 1452.2 ng NP/ml, 1780.5 ng BPA/ml, 1614.1 ng E1/ml, 1875.6 ng E2/ml, 1599.0 ng EE2/ml and 1465.5 ng E3/ml. The calibration curves for each standard are available in Appendix G. Results of the analyses showed the presence of two steroidal chemicals, E2, E1 and one industrial EDC, BPA (Figure 3.20 & Appendix G). The EEQs of each detectable compound were calculated using estradiol equivalency factor (EEF) that were 1.0, 0.11 and 0.0004 for E2, E1 and BPA respectively (Vega-Morales et al., 2013). The total EEQs obtained through chemical analysis was 11.6 ng EEQs/ml whereas the total EEQs calculated from the yeast bioassay was 48.8 ng EEQs/ml. The higher concentrations obtained from the yeast assay is likely because recombinant yeast responds to any chemical that can activate the estrogen receptor whereas only six estrogenic chemicals were targeted in the chemical analysis.

Two water samples (MT3 and MR3) with high androgenic activity in the YAS assay were also selected for analysis using GC/MSD; in particular, to detect trenbolone as it is often used as a growth promoter in dairy farms (Schiffer et al., 2001). Concentrations for standards were 2389.4 ng DHT/ml and 1564.8 ng Tb/ml. TIC of the standard androgenic compounds, dihydrotestosterone (DHT) and trenbolone (Tb), showed visible peaks for each standard (Figure 3.21). However, no androgenic compounds were detected in the water samples other than dehydroabietic acid (DHAA) (Appendix G). DHAA is a component of resin acid found in coniferous trees and is detected in effluents of pulp and paper mills. DHAA can be reduced by microorganisms to retene, a PAH, that can activate the AhR receptor (Scott et al*.,* 2011). Likewise, these samples also had activity in the AhR assay. DHAA in the samples with positive results in the YAS bioassay suggests that DHAA has androgenic activity since androgen receptor is activated. Other studies have reported that DHAA may have endocrine disrupting properties. Masculinization of female mosquitofish was observed in pulp mills effluents in the USA (Howell et al., 1980); female guppies exposed to mill effluent developed secondary male sex characteristics (Larsson et al., 2002) and in a study by Wartman et al. (2009), the androgenic potency to 3-spined stickleback of pulp and paper mill effluent was found to be 4 times higher than the estrogenic potency. Finally, an *in vivo* and *in vitro* study with goldfish and mosquitofish by Ellis et al. (2003) have suggested androgenic activity in a pulp and paper mill effluent that contained high amounts of DHAA.

No PAHs were detected through GC-MSD analysis possibly due to use of the full scan mode rather than the selective ion monitoring mode (SIM) of analysis. Higher alkanes such as tetra-, penta-, octadecanes; ei-, tri-, hexa-, octacosane; cyclohexane, cyclotetracosane etc. were detected (see Appendix G). Possible sources of these compounds are industrial lubricants, greases, diesel and aviation fuel (Sarker, M., 2011).

Although no chemical analysis was performed for samples from the city of Surrey, a report by Metro Vancouver (2015) has confirmed presence of Brominated Diphenyl Ethers (BDEs), Polyaromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), phenols and nonylphenols in the influent and effluent of the pump station. The pump station (Figure 3.18) is located between S1 and S2. The detected PAHs that exceeded the guideline values included pyrene, benzo(a)pyrene, anthracene and benzo (a) anthracene. Detected PCBs included PCB 77, PCB 105, PCB 126 and PCB 169. Whereas phenols as well as nonylphenol and ethoxylates had the highest levels compared to other organic compounds of concern. Their report confirms our results from the AhR assay, as the average NAPEQs were 14.50 – 71.13 ng/ml. The higher activity observed in the bioassay is due to response of a mixture of chemicals including synergistic and potentiation effects whereas data from Metro Vancouver (2013, 2015) is based on individually detected compounds.

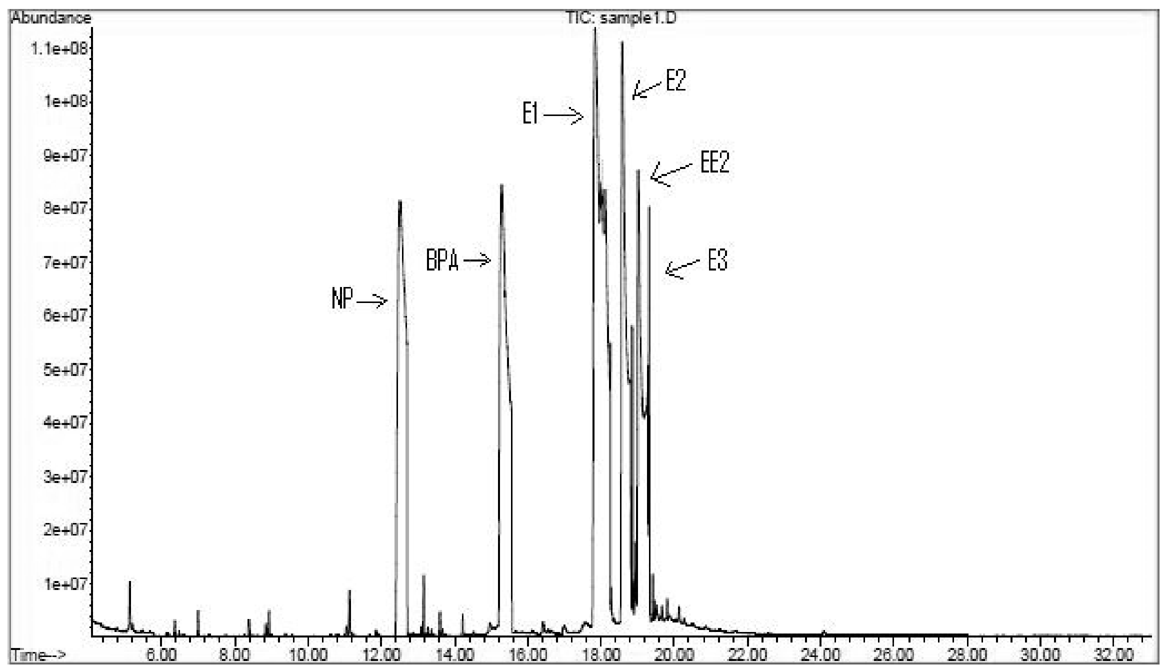


Figure 3.19 TIC of Estrogenic Standards: Nonylphenol (NP), Bisphenol A (BPA), Estrone (E1), 17β- Estradiol (E2), 17β- ethynylestradiol (EE2) and Estriol (E3).

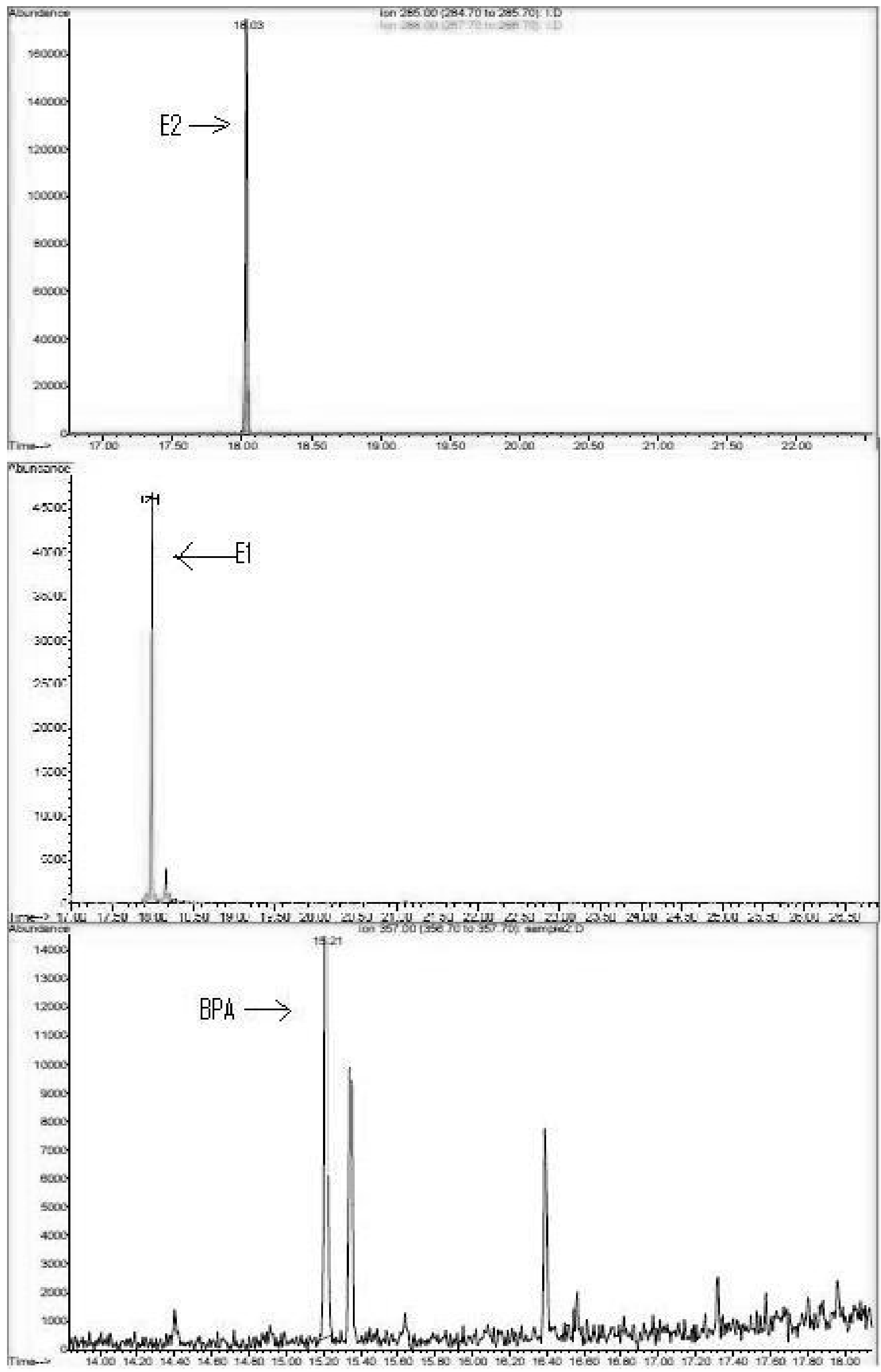


Figure 3.20 TIC of E2, E1 and BPA detected in water samples

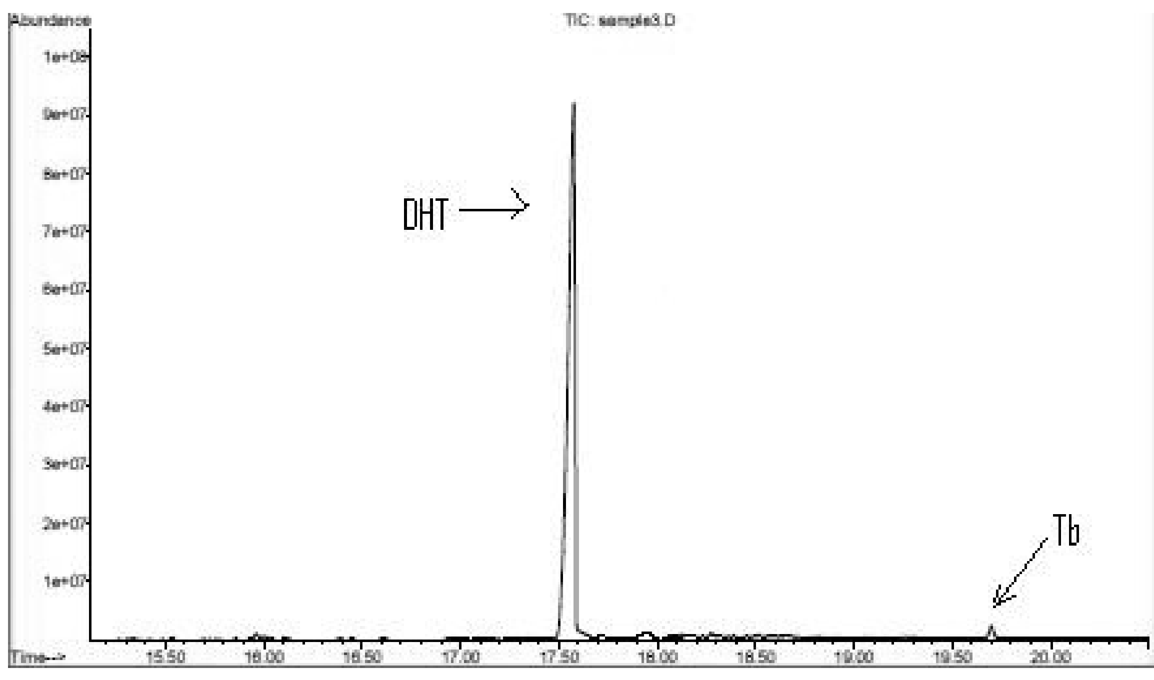


Figure 3.21 TIC of Androgenic Standards Dihydrotestosterone (DHT) and Trenbolone (Tb).

4. Risk to exposed species

Water and sediment quality objectives/guidelines for the protection of aquatic species are based on exposure to single compounds. Thus, it is challenging to develop guidelines based on the results of an effect-related yeast bioassay on chemical mixtures that may interact with one another. However, concentrations obtained from the current study can be compared to the levels set as guidelines for the purposes of risk assessment if toxic equivalency factors (TEFs) are available for specific groups of EDCs.

Since there is no objective/guideline value available for NAPEQs, the concentrations obtained through the yeast assay were converted into benzo [a] pyrene equivalents (BAPEQs). Environment Canada has water and sediment quality guidelines for the protection of aquatic life for some PAHs including benzo [a] pyrene (BAP). The guideline for the protection of aquatic life is 0.015 ng/ml and 31.9 ng/g of BAP in water and sediment, respectively (CCME, 1999). The concentrations measured in water and sediment samples in the present study ranged from 0.34 - 7982.28 ng BAPEQ/ml and 3.71 - 12764.87 ng BAPEQ/g, for water and sediment, respectively. Thus levels in the current study exceed the guideline values from Canadian Council of Ministers of the Environment (CCME).

There are no objective/guideline for estradiol, androgens or glucocorticoid equivalents for the protection of aquatic life under Canadian or British Columbia water/sediment quality guidelines. A predicted no effect E2 concentration for the protection of aquatic life derived by the European union is of 0.0004 ng/ml (SCHER, 2011) whereas concentrations higher than 0.001 ng EEQ/ml are associated with adverse effects (UK Environmental Agency, 2004). Other predicted no effect concentrations (PNECs) and hazard concentrations based on no observed adverse effect levels (NOAEL) range from 0.00073 to 0.002 ng/ml of E2 (Wu et al., 2014). E2 values from the current study were 0.0125 – 29.3 ng EEQs/ml and therefore potentially may cause adverse effects on the aquatic life in these sites.

As for the androgenic compounds, the reported toxicity in literature varies depending on the type of androgens, sex of the fish, species tested and the endpoint

measured. One study found no effect of trenbolone at 0.02 ng/ml in courtship behavior of zebrafish (Larsen & Baatrup, 2010) but 0.009 ng/ml of the same compound induced irreversible masculinization in female zebrafish (Morthorst et al., 2010). Other studies have reported LOAELs at 0.03 ng TbEQs/ml can reduce fecundity and at 0.4 ng TbEQs/ml can affect secondary sex characteristics in different species of female fish (Ankley et al., 2003; Seki et al., 2006). The range of TbEQs in the current study is 0.1 – 1577.30 ng/ml, higher than the reported LOAELs and NOAELs in the literature. A proper risk assessment with weight of evidence (WoE) approach is required to establish risk to the species inhabiting the study areas.

Studies reporting the effects of synthetic glucocorticoids are rare. The study by Kugathas and Sumpter (2011) reported that synthetic glucocorticoid concentration of 0.1 ng/ml (NOAEC) did not have statistically significant effects in aquatic organisms although the LOAEC was found to be 1 ng/ml. Other studies have reported lethal effects on reproduction, growth and development of fish exposed to 500 ng/ml of synthetic glucocorticoids (LaLone et al., 2012). Glucocorticoid levels derived from the YGS bioassay in the water and sediment samples from the current study were the highest among the three steroid hormones; levels ranging from 0.75 – 8999.32 ng DOCEQs/ml in water and 0.75 – 18988.10 ng DOCEQs ng/g in sediments were found. These high concentrations in the current study may cause adverse effects such as high plasma glucose concentrations and compromised immune system in fish leading to susceptibility to disease and other contaminants in the environment (Kugathas & Sumpter, 2011).

EDCs can alter a living organism in many ways depending on numerous factors. Thus it is unreasonable to characterize a risk or no risk to the species living in these waterways. In addition, as dose response is not always a monotonic relationship and high concentrations have not been studied, it is uncertain if the high levels obtained in current study reflect real exposure to these compounds because of the indirect nature of bioassays. Overall one has to look at exposure levels, hazard, species susceptibility, life stage and potency of the chemical mixture, to name a few, for a proper risk assessment of the EDCs (Testai et al., 2013). In addition, other factors like pH, salinity, dissolved oxygen concentration and presence of metals in the environment should also be taken into consideration.

5. Study Limitations and Future Directions

Yeast bioassays are fast, cost effective and reliable way of testing for EDCs in the environment. As there is an increase in environmental contamination and pollution with rapid industrialization, these bioassays can provide a rapid screen for potential exposure of wildlife to EDCs. Nevertheless these results do not directly predict the biological response an animal would have to these compounds as we are dealing with mixtures of EDCs and the pharmacokinetics (absorption, distribution, metabolism and elimination) of these chemicals in aquatic species and wildlife are still not known.

Some suggested research studies in the future may include the following: (a) improvement on the extraction methods to better recover EDCs from the samples (b) spatial and temporal variation of EDC levels should be examined closely with regards to the sources in order to protect the aquatic species in these areas; this can be achieved by increasing sampling, examining reproducibility and validating results with chemical analyses (c) more information such as habitat preference, foraging behaviour and other characteristics on the species living in these sloughs, creeks, lake and river will enable a site specific risk assessment of exposure.

References

Alexander, S. L., & Irvine, C. H. G. (1998). The effect of social stress on adrenal axis activity in horses: The importance of monitoring corticosteroid-binding globulin capacity. *Journal of Endocrinology*, *157*(3), 425–432.

Alnafisi, A., Hughes, J., Wang, G., Miller, C. (2007). Evaluating polycyclic aromatic hydrocarbons using a yeast bioassay. *Environmental Toxicology and Chemistry,* 26(7), 1333-1339.

Ankley, G. T., Jensen, K. M., Makynen, E., Kahl, M. D., Korte, J. J., Hornung, M. W., Gray, L. E. (2003). Effects of the androgenic growth promoter 17-beta-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environmental Toxicology and Chemistry / SETAC*, *22*(6), 1350–1360.

Antunes-Fernandes, E., Bovee, T., Daamen, F, Helsdingen, R., van den Berg, M., van Duursen, M. (2011). Some OH-PCBs are more potent inhibitors of aromatase activity and (anti-) glucocorticoids than non-dioxin like (NDL)-PCBs and $MeSO_2$-PCBs. *Toxicology Letters,* 206, 158-165.

Atwater, J. W., Whalen, T. F., Dasika, R. (1998). Mushroom Waste Management Project. Liquid Waste Management-Phase I & Phase II. Environment Canada.

Balsiger, H. and Cox, M. (2009). Yeast-Based reporter assays for the functional characterization of cochaperone interactions with steroid hormone receptors. The *Nuclear Receptor Superfamily*, 505, 141-156.

Balsiger, H., de la Torre, R., Lee, W.Y., Cox, M. B. (2010). A Four-Hour Yeast Assay for the Direct Measure of Estrogenic Activity in Wastewater Without Sample Extraction, Concentration, or Sterilization. *Science of the Total Environment*, *408*(6), 1422–1429.

Bazin I., Hassine A., Mnif W., Gonzalez, C. (2013). YES as a Tool for Detecting Estrogenic Activity of Some Food Additives Compounds: E 104, E 122, E 124, E 132 and E 171. *Journal of Ecosystem & Ecography,* 3(3), 128.

Bauer, E. R., Daxenberger, A, Petri, T., Sauerwein, H., Meyer, H. H. (2000). Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. *Apmis*, *108*(12), 838–846.

Bergman, Å., Heindel, J., Jobling, S., Kidd, K., Zoeller, R. T. (2012). *State of the science of endocrine disrupting chemicals, 2012. United Nations Environment Programme and World Health Organization.* (Vol. 211).

Bhattacharjee, G. & Khurana, S. (2014). In vitro Reporter Assays for Screening of Chemicals that disrupt Androgen Signaling. *Journal of Toxicology*, 1-7.

Bistan, M., Logar, R., Tišler, T. (2011). Detection of estrogenic activity in Slovenian wastewaters by bioassay. *Open Life Sciences*, *6*(5), 829–837.

Bogdal, C., Naef, M., Schmid, P., Kohler, M., Zennegg, M., Bernet, D., Hungerbühler, K. (2009). Unexplained gonad alterations in whitefish (*Coregonus spp*.) from Lake Thun, Switzerland: Levels of persistent organic pollutants in different morphs. *Chemosphere*, *74*(3), 434–440.

Boström, C.E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environmental Health Perspectives*, *110*, 451–488.

Bovee, T., Bor, G., Heskamp, H., Lasaroms, J., Sanders, M., Nielen, M. (2009). Validation and application of a yeast bioassay for screening androgenic activity in calf urine and feed. *Analytica Chimica ACTA,* 673, 225-234.

Bovee, T., Helsdingen, R., Hamer, A., Brouwer, B., Nielen, M. (2011). Recombinant cell bioassays for the detection of (gluco) corticosteroids and endocrine-disrupting potencies of several environmental PPCB contaminants. *Anal Bioanal Chem,* 401, 873-882.

Cailleaud, K., Forget-Leray, J., Souissi, S., Hilde, D., LeMenach, K., Budzinski, H. (2007). Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species Eurytemora affinis (*Calanoïda, copepoda*). Part 1: PCBs and PAHs. *Chemosphere*, *70*(2), 270–280.

Campbell, R. K., Satoh, N., Degnan, B. M. (2004). Piecing together evolution of the vertebrate endocrine system. *Trends in Genetics*, *20*(8), 359–366.

Campbell, C., Borglin, S., Green, F., Grayson, A., Wozei, E., Stringfellow, W. (2006). Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere,* 65, 1265-1280.

Canadian Council of Ministers of the Environment. (CCME 1999a). Canadian Sediment Quality Guidelines for the Protection of Aquatic Life - Polycyclic Aromatic Hydrocarbons. *Canadian Environmental Quality Guidelines* 1–16.

Canadian Council of Ministers of the Environment. (CCME 1999b). Canadian water quality guidelines for the protection of aquatic life - Polycylic aromatic hydrocarbons. *Canadian Environmental Quality Guidelines*, 1–13.

Chang, H., Choo, K., Lee, B., Choi, S. (2009). The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water. *Journal of Hazardous Materials,* 172, 1-12.

Cheek, A. O., Vonier, P. M., Oberdörster, E., Burow, B. C., McLachlan, J. (1998). Environmental signaling: A biological context for endocrine disruption. *Environmental Health Perspectives*, *106*(1), 5–10.

Cherlet, M., De Baere, S., De Backer, P. (2004). Quantitative determination of dexamethasone in bovine milk by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *805*(1), 57–65.

Courtheyn, D., Vercammen, J., De Brabander, H. F., Vandenreyt, I., Batjoens, P., Vanoosthuyze, K., Van Peteghem, C. (1994). Determination of dexamethasone in urine and faeces of treated cattle with negative chemical ionization-mass spectrometry. *The Analyst*, *119*(12), 2557–64.

Cox, M. and Miller, C. (2002). The p23 co-chaperone facilitates dioxin receptor signalling in a yeast model system. *Toxicology Letters,* 129, 13-21.

Damstra, T., Barlow, S., Bergman, A., Kavlock, R., Kraak, G. Van Der. (2002). International Programme on Chemical Safety. Global Assessment of the State of the Science of Endocrine Disruptors. World Health Organization.

De Clercq, N., Bussche, J. Vanden, Croubels, S., Delahaut, P., Vanhaecke, L. (2014). Development and validation of a high resolution mass spectrometry based method to study the long-term stability of natural and synthetic glucocorticoids in faeces. *Journal of Chromatography A*, *1336*, 76–86.

Denison, M. S., Pandini, A, Nagy, S. R., Baldwin, E. P., Bonati, L. (2002). Ligand binding and activation of the Ah receptor. *Chem Biol.Interact.*, *141*, 3–24.

Durhan, E. J., Lambright, C. S., Makynen, E., Lazorchak, J., Hartig, P. C., Wilson, V. S., Ankley, G. T. (2006). Identification of metabolites of trenbolone acetate in androgenic runoff from a beef feedlot. *Environmental Health Perspectives*, *114*(April), 65–68.

Eldridge, M. L., Sanseverino, J., Layton, C., Easter, J. P., Schultz, T. W., Sayler, G. S. (2007). *Saccharomyces cerevisiae* BLYAS, a New Bioluminescent Bioreporter for Detection of Androgenic Compounds. *Applied and Environmental Microbiology*, *73*(19), 6012–6018.

Ellis, R. J., van den Heuvel, M. R., Bandelj, E., Smith, M., McCarthy, L. H., Stuthridge, T. R., Dietrich, D. R. (2003). In vivo and in vitro assessment of the androgenic potential of a pulp and paper mill effluent. *Environmental Toxicology and Chemistry / SETAC*, *22*(7), 1448–1456.

Fent, K., Weston, A, Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*, *76*(2), 122–159.

Finlay-Moore, O., Hartel, P. G., Cabrera, M. L. (2000). 17β-estradiol and Testosterone in Soil and Runoff from Grasslands Amended with Broiler Litter. *Journal of Environmental Quality*, 29, 1604-1611.

Fisheries and Oceans Canada. (2010). Recovery strategy for the Salish Sucker (*Catostomus sp.*) in Canada. *Species at Risk Act Recovery Strategy Series*, Vancouver, vii +64

Gaido, K., Leonard, L., Lovell, S., Gould, J., Babai, D., Portier, C., McDonnell, D. (1997). Evaluation of Chemicals with Endocrine Modulating Activity in a Yeast-Based Steroid Hormone Receptor Gene Transcription Assay. *Toxicology and Applied Pharmacology,* 143, 205-212.

Giesy, J., Hilscherova, K., Jones, P., Kannan, K., Machala, M. (2002). Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen (ER) mediated activity in environmental samples. *Marine Pollution Bulletin,* 45, 3-16.

Hall, K. J., & Schreier, H. (1996). Urbanization and agricultural intensification in the Lower Fraser River valley : Impacts on water use and quality. *GeoJournal*, *40*, 135–146.

Hanselman, T., Graetz, D., Wilkie, A. C. (2003). Manure-Borne Estrogens as Potential Environmental Contaminants: A Review. *Environmental Science and Technology*, *37*(24), 5471–5478.

Hayes, T. B. (2005). Welcome to the revolution: integrative biology and assessing the impact of endocrine disruptors on environmental and public health. *Integrative and Comparative Biology*, *45*(2), 321–329.

Hiller, E., Jurkovič, Ľ., Bartaľ, M. (2008). Effect of Temperature on the Distribution of Polycyclic Aromatic Hydrocarbons in Soil and Sediment, *2008*(1), 231–240.

Hiller-Sturmhöfel, S., & Bartke, A. (1998). The Endocrine System: An overview. *Alcohol Health and Research Worlds*, 22(3), 153-164.

Howell, W. M., Black, D. A., Bortone, S. A. (1980). Abnormal Expression of Secondary Sex Characters in a Population of Mosquitofish, *Gambusia affinis holbrooki:* Evidence for Environmentally-induced Masculinization. *COPEIA*, 4, 676-681.

Huang, C. H., & Sedlak, D. L. (2001). Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. *Environmental Toxicology and Chemistry / SETAC*, *20*(1), 133–139.

Huetos, O., Ramos, M., Martín de Pozuelo, M., San Andrés, M., Reuvers, T. B. (1999). Determination of dexamethasone in feed by TLC and HPLC. *The Analyst*, *124*(11), 1583–7.

Iglesias, A., Nebot, C., Vázquez, B. I., Miranda, J. M., Abuín, C. M. F., Cepeda, A. (2014). Detection of veterinary drug residues in surface waters collected nearby farming areas in Galicia, North of Spain. *Environmental Science and Pollution Research*, *21*(3), 2367–2377.

Ishibashi, H., Tachibana, K., Tsuchimoto, M., Soyano, K., Ishibashi, Y., Nagae, M., Arizono, K. (2001). In vivo testing system for determining the estrogenic activity of endocrine-disrupting chemicals (EDCs) in goldfish (*Carassius auratus)*. *Journal of Health Science*, *47*(2), 213–218.

Jálová, V., Jarošová, B., Bláha, L., Giesy, J. P., Ocelka, T., Grabic, R., Hilscherová, K. (2013). Estrogen-, androgen- and aryl hydrocarbon receptor mediated activities in passive and composite samples from municipal waste and surface waters. *Environment International*, *59*, 372–383.

Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, S., Tyler, C. (2003). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquatic Toxicology*, *65*(2), 205–220.

Jobling, S., Sheahan, D., Osborne, J., Matthiessen, P., Sumpter, J. P. (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry*, *15*(2), 194–202.

Kato, Y., Ikushiro, S., Haraguchi, K., Yamazaki, T., Ito, Y., Suzuki, H., Kimura, R., Yamada, S., Inoue, T., Degawa, M. (2004). A Possible Mechanism for Decrease in Serum Thyroxine Level by Polychlorinated Biphenyls in Wistar and Gunn Rats. *Toxicological Sciences,* 81, 309-315.

Kidd, K., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(21), 8897–8901.

Kinnberg, K., Korsgaard, B., Bjerregaard, P., Jespersen, A. (2000). Effects of nonylphenol and 17beta-estradiol on vitellogenin synthesis and testis morphology in male platyfish *Xiphophorus maculatus*. *The Journal of Experimental Biology*, *203*, 171–81.

Knudsen, F. R., & Pottinger, T. G. (1999). Interaction of endocrine disrupting chemicals, singly and in combination, with estrogen-, androgen-, and corticosteroid-binding sites in rainbow trout (*Oncorhynchus mykiss)*. *Aquatic Toxicology*, *44*(3), 159–170.

Kojima, H., Takeuchi, S., Nagai, T. (2010). Endocrine-disrupting Potential of Pesticides via Nuclear Receptors and Aryl Hydrocarbon Receptor. *Journal of Health Science*, *56*(4), 374–386.

Kolok, A. S., & Sellin, M. K. (2008). The environmental impact of growth-promoting compounds employed by the United States beef cattle industry: history, current knowledge, and future directions. *Reviews of Environmental Contamination and Toxicology*, *195*, 1–30.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L., Feldman, D. (1993). Bisphenol-A: Estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology*, *132*(6), 2279–2286.

Kugathas, S. & Sumpter, J. (2011). Synthetic glucocorticoids in the Environment: First Results on Their Potential Impacts on Fish. *Environmental Science & Technology,* 45, 2377–2383.

Kurosawa, T., Hiroi, H., Tsutsumi, O., Ishikawa, T., Osuga, Y., Fujiwara, T., Taketani, Y. (2002). The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocrine Journal*, 49(4), 465-471.

LaLone, C., Villeneuve, D. L., Olmstead, W., Medlock, E. K., Kahl, M. D., Jensen, K. M., Ankleyy, G. T. (2012). Effects of a glucocorticoid receptor agonist, dexamethasone, on fathead minnow reproduction, growth, and development. *Environmental Toxicology and Chemistry*, *31*(3), 611–622.

Lange, I. G., Daxenberger, A., Schiffer, B., Witters, H., Ibarreta, D., Meyer, H. H. D. (2002). Sex hormones originating from different livestock production systems: Fate and potential disrupting activity in the environment. *Analytica Chimica Acta*, *473*, 27–37.

Larsen, M. G., & Baatrup, E. (2010). Functional behavior and reproduction in androgenic sex reversed zebrafish (*Danio rerio*). *Environmental Toxicology and Chemistry*, *29*(8), 1828–1833.

Larsson, D. G. J., Kinnberg, K., Sturve, J., Stephensen, E., Skön, M., Förlin, L. (2002). Studies of masculinization, detoxification, and oxidative stress responses in guppies (*Poecilia reticulata*) exposed to effluent from a pulp mill. *Ecotoxicology and Environmental Safety*, *52*(1), 13–20.

Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., Wenzel, A. (2003). Endocrine disruptors in the environment (IUPAC Technical Report). *Pure Appl. Chem., 75*(5), 631–681.

Lorenzen, A., Hendel, J. G., Conn, K. L., Bittman, S., Kwabiah, A. B., Lazarovitz, G., Topp, E. (2004). Survey of hormone activities in municipal biosolids and animal manures. *Environmental Toxicology*, *19*(3), 216–225.

Macikova, P., Groh, K. J., Ammann, A., Schirmer, K., Suter, M. J.-F. (2014). Endocrine disrupting compounds affecting corticosteroid signaling pathways in Czech and Swiss waters: potential impact on fish. *Environmental Science & Technology*, *48*(21), 12902–11.

Martinez, E., Gros, M., Lacorte, S., Barceló, D. (2004). Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels. *Journal of Chromatography A*, *1047*(2), 181–188.

Matthews, A. & Gustafsson, J. (2006). Estrogen receptor and aryl hydrocarbon receptor signaling pathways. *Nuclear Receptor Signaling*, 4, e016.

Matthews, J. B., Twomey, K., Zacharewski, T. R. (2001). In Vitro and in Vivo Interactions of Bisphenol A and Its Metabolite, Bisphenol A Glucuronide, with Estrogen Receptors α and β. *Chemical Research in Toxicology, 14*(2), 149-157.

Metro Vancouver (2013). Sanitary sewer overflow risk assessment of interim management options: Lynn branch siphon, Katzie pump station and Cloverdale pump station. Report by Tetra Tech, Vancouver, B.C., Canada.

Metro Vancouver (2015). Update of Screening level human health and ecological risk assessment for sanitary sewer overflows: Cloverdale pump station, Lynn Branch siphon and Katzie pump station. Report prepared by SLR global environmental solutions, Vancouver, B.C., Canada.

Miksicek, R. J. (1995). Estrogenic Flavonoids: Structural Requirements For Biological Activity. *Proc. Soc. Exp. Biol. Med*, (208), 44–50.

Ministry of Environment, British Columbia (BC). (2002). Wildlife in British Columbia at Risk: Oregon Spotted Frog. http://www.gov.bc.ca/.

Morthorst, J. E., Holbech, H., Bjerregaard, P. (2010). Trenbolone causes irreversible masculinization of zebrafish at environmentally relevant concentrations. *Aquatic Toxicology*, *98*(4), 336–343.

Möstl, E., Messmann, S., Bagu, E., Robia, C., Palme, R. (1999). Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. *Journal of Veterinary Medicine A*, *46*(10), 621–631.

Nichols, D. J., Daniel, T. C., Moore, P., Edwards, D. R., Pote, D. H. (1997). Runoff of Estrogen Hormone 17β-Estradiol from Poultry Litter Applied to Pasture. *Journal of Environment Quality*, *26*(4), 1002.

Nie, Y., Qiang, Z., Zhang, H., Adams, C. (2009). Determination of endocrine-disrupting chemicals in the liquid and solid phases of activated sludge by solid phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography. A*, *1216*(42), 7071–80.

Noguerol, T. N., Boronat, S., Casado, M., Raldúa, D., Barceló, D., Piña, B. (2006). Evaluating the interactions of vertebrate receptors with persistent pollutants and antifouling pesticides using recombinant yeast assays. *Analytical and Bioanalytical Chemistry*, *385*(6), 1012–1019.

Norris, D. & Lopez, K (2011). Hormones and Reproduction of Vertebrates. Fishes, Volume 1. Academic Press. San Diego, CA.

Palace, V., Evans, R., Wautier, K., Mills, K., Blanchfield, P., Park, B., Kidd, K. (2009). Interspecies differences in biochemical, histopathological, and population responses in four wild fish species exposed to ethynylestradiol added to a whole lake. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(11), 1920–1935.

Parker, J., Webster, J. P., Kover, S. C., Kolodziej, E. P. (2012). Analysis of trenbolone acetate metabolites and melengestrol in environmental matrices using gas chromatography-tandem mass spectrometry. *Talanta*, *99*, 238–46.

Patel, M. R., Scheffler, B. E., Wang, L., Willett, K. L. (2006). Effects of benzo(a)pyrene exposure on killifish (*Fundulus heteroclitus*) aromatase activities and mRNA. *Aquatic Toxicology (Amsterdam, Netherlands)*, *77*(3), 267–78.

Peck, M., Gibson, R. W., Kortenkamp, A., Hill, E. M. (2004). Sediments are major sinks of steroidal estrogens in two United Kingdom rivers. *Environmental Toxicology and Chemistry / SETAC*, *23*(4), 945–952.

Phillips, P. J., Chalmers, T., Gray, J. L., Kolpin, D. W., Foreman, W. T., Wall, G. R. (2012). Combined sewer overflows: An environmental source of hormones and wastewater micropollutants. *Environmental Science & Technology*, *46*, 5336–5343.

Pocar, P., Fischer, B., Klonisch, T., Hombach-Klonisch, S. (2005). Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. *Reproduction*, *129*, 379–389.

Popot, M., Donval, A, Bonnaire, Y., Huau, J. (2006). Use of accelerating solvent extraction for detecting non-steroidal anti-inflammatory drugs in horse feces. *Journal of Analytical Toxicology*, *30*(5), 323–30.

Qin, N., He, W., Kong, X.-Z., Liu, W.-X., He, Q.-S., Yang, B., Zhao, X.-L. (2014). Distribution, partitioning and sources of polycyclic aromatic hydrocarbons in the water-SPM-sediment system of Lake Chaohu, China. *The Science of the Total Environment*, *496*, 414–23.

Reig, M., Mora, L., Flores, M., Toldrá, F. (2006). HPLC Method for the Analysis of Dexamethasone in feed and water for Livestock. *52nd International Congress of Meat Science and Technology: Harnessing and Exploiting Global Opportunities.* Wageningen. Academic Publishers. Netherlands. 365-366.

Riggs, D. L., Roberts, P. J., Chirillo, S. C., Cheung-Flynn, J., Prapapanich, V., Ratajczak, T., Smith, D. F. (2003). The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling in vivo. *The European Molecular Biology Organization Journal*, *22*(5), 1158–67.

Rosen, M. R., Goodbred, S. L., Patiño, R., Leiker, T. J., Orsak, E. (2007). Investigations of the Effects of Synthetic Chemicals on the Endocrine System of Common Carp in Lake Mead, Nevada and Arizona. *Publications of the US Geological Survey*. Paper 80.

Sanseverino, J., Gupta, R. K., Layton, A., Patterson, S. S., Ripp, S., Saidak, L., Simpson, M. L., Schultz, T., Sayler, G. S. (2005). Use of. *Saccharomyces cerevisiae* BLYES Expressing Bacterial Bioluminescence for Rapid, Sensitive Detection of Estrogenic Compounds. *Applied and Environmental Microbiology*, *71*(8), 4455–4460.

Sarker, M. (2007). Municipal Waste Plastic Conversion into Different Category of Liquid Hydrocarbon Fuel. *Chemistry, Emission Control, Radioactive Pollution and Indoor Air Quality.* InTech. 45-80.

SCHER (Scientific Committee on Health and Environmental Risks). (2011). Opinion on draft environmental quality standards under the Water Framework Directive – 17β-estradiol (E2), European Union (30 March).

Schiffer, B., Daxenberger, A., Meyer, K., Meyer, H. H. D. (2001). The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: Environmental studies. *Environmental Health Perspectives*, *109*(11), 1145–1151.

Scott, J., Incardona, J. P., Pelkki, K., Shepardson, S., Hodson, P. V. (2011). AhR2-mediated, CYP1A-independent cardiovascular toxicity in zebrafish (*Danio rerio*) embryos exposed to retene. *Aquatic Toxicology*, *101*(1), 165–174.

Seki, M., Fujishima, S., Nozaka, T., Maeda, M., Kobayashi, K. (2006). Comparison of response to 17 beta-estradiol and 17 beta-trenbolone among three small fish species. *Environmental Toxicology and Chemistry / SETAC*, *25*(10), 2742–2752.

Sellin, M. K., Snow, D. D., Gustafson, S. T., Erickson, G. E., Kolok, S. (2009). The endocrine activity of beef cattle wastes: do growth-promoting steroids make a difference? *Aquat Toxicol*, *92*(4), 221–227.

Shi, J., Liu, X., Cao, J., Bo, T., Li, Y. (2013). Occurrence and Risk Assessment of Estrogens and Anti-Inflammatories in Baiyangdian Lake, North China. *Environmental Engineering and Management Journal*, *12*(7), 1437–1445.

Shi, Z., Valdez, K. E., Ting, A. Y., Franczak, A., Gum, S. L., Petroff, B. K. (2007). Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biology of Reproduction*, *76*(2), 198–202.

Shutt, D.A. (1976). The effects of plant oestrogens on animal reproduction. *Endeavor,* 35, 110-113.

Soto, A. M., Calabro, J. M., Prechtl, N. V., Yau, A. Y., Orlando, E. F., Daxenberger, A., Sonnenschein, C. (2004). Androgenic and Estrogenic Activity in Water Bodies Receiving Cattle Feedlot Effluent in Eastern Nebraska, USA. *Environmental Health Perspectives*, *112*(3), 346–352.

Tabata, A., Kashiwada, S., Ohnishi, Y., Ishikawa, H., Miyamoto, N., Itoh, M., Magara, Y. (2001). Estrogenic influences of estradiol-17β, *p*-nonylphenol and bis-phenol-A on Japaneses Medaka (*Oryzias latipes*) at detected environmental concentrations. *Water Science and Technology,* 43(2), 109-116.

Takeda, T., Matsumoto, Y., Koga, T., Mutoh, J., Nishimura, Y., Shimazoe, T., Ishii, Y., Ishida, T., Yamada, H. (2009). Maternal exposure to Dioxin disrupts gonadotropin production in fetal rats and imprints defects in sexual behavior. *The Journal of Pharmacology and Experimental Therapeutics*, 329, 1091-1099.

Tam, N. F. Y., Ke, L., Wang, X. H., Wong, Y. S. (2001). Contamination of polycyclic aromatic hydrocarbons in surface sediments of mangrove swamps. *Environmental Pollution*, *114*(2), 255–263.

Ternes, T., Andersen, H., Gilberg, D., Bonerz, M. (2002). Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. *Analytical Chemistry*, *74*(14), 3498–3504.

Testai, E., Galli, C. L., Dekant, W., Marinovich, M., Piersma, A. H., Sharpe, R. M. (2013). A plea for risk assessment of endocrine disrupting chemicals. *Toxicology*, *314*(1), 51–59.

Thorpe, K. L., Cummings, R. I., Hutchinson, T. H., Scholze, M., Brighty, G., Sumpter, J. P., Tyler, C. R. (2003). Relative Potencies and Combination Effects of Steroidal Estrogens in Fish. *Environmental Science & Technology*, *37*(6), 1142–9.

Tierney, K., Farrell, A., Brauner, C. (2014). Organic Chemical Toxicology of Fishes. Fish Physiology, Volume 33. Academic Press. San Diego, CA.

Tilton, S. C., Foran, C. M., Benson, W. H. (2005). Relationship between ethinylestradiol-mediated changes in endocrine function and reproductive impairment in Japanese medaka (*Oryzias latipes)*. *Environmental Toxicology and Chemistry / SETAC*, *24*(2), 352–359.

Tiryaki, O., & Temur, C. (2010). The Fate of Pesticide in the Environment. *J. Biol. Environ. Sci*, *4*(10), 29–38.

Tremblay, L., Kohl, S. D., Rice, J., Gagné, J.-P. (2005). Effects of temperature, salinity, and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. *Marine Chemistry*, *96*, 21–34.

UK Environment Agency. 2004. Proposed Predicted-No-Effect-Concentrations (PNECs) for Natural and Synthetic Steroid Oestrogens in Surface Waters. Technical Report.

US Environmental Protection Agency. http://www.epa.gov/endocrine-disruption

United States Fish and Wildlife Service (USFWS) Department of the Interior. (2014). Endangered and Threatened Wildlife and Plants; Threatened Status for Oregon Spotted Frog. 79(168), 51658-51710.

Van Der Linden, S. C., Heringa, M. B., Man, H. Y., Sonneveld, E., Puijker, L. M., Brouwer, A., Van Der Burg, B. (2008). Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environmental Science and Technology*, *42*(15), 5814–5820.

Vega-Morales, T., Sosa-Ferrera, Z., Santana-Rodríguez, J. J. (2013). Evaluation of the presence of endocrine-disrupting compounds in dissolved and solid wastewater treatment plant samples of Gran Canaria Island (Spain). *BioMed Research International*, *2013*, Article ID 790570.

Velasco-Santamaría, Y. M., Madsen, S. S., Bjerregaard, P., Korsgaard, B. (2010). Effects of 17β-trenbolone in male eelpout Zoarces viviparus exposed to ethinylestradiol. *Analytical and Bioanalytical Chemistry*, *396*, 631–640.

Villanger, G. D., Lydersen, C., Kovacs, K. M., Lie, E., Skaare, J. U., Jenssen, B. M. (2011). Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales (*Delphinapterus leucas*) from Svalbard. *The Science of the Total Environment*, *409*(13), 2511–24.

Wang, L., Ying, G.-G., Chen, F., Zhang, L.-J., Zhao, J.-L., Lai, H.-J., Tao, R. (2012). Monitoring of selected estrogenic compounds and estrogenic activity in surface water and sediment of the Yellow River in China using combined chemical and biological tools. *Environmental Pollution*, *165*, 241–249.

Wartman, C., Hogan, N. S., Hewitt, L. M., McMaster, M. E., Landman, M. J., Taylor, S., van den Heuvel, M. R. (2009). Androgenic effects of a Canadian bleached kraft pulp and paper effluent as assessed using threespine stickleback (*Gasterosteus aculeatus*). *Aquatic Toxicology*, *92*(3), 131–139.

Watanabe, N., Bergamaschi, B., Loftin, K., Meyer, M. T., Harter, T. (2010). Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. *Environmental Science & Technology*, *44*(17), 6591–600.

Whitten, P., Russell, E., Naftolin, F. (1992). Effects of a normal, human-concentration, phytoestrogen diet on rat uterine growth. *Steroids,* 57, 98-106.

Wu, F., Fang, Y., Li, Y., Cui, X., Zhang, R., Guo, G., Giesy, J. (2014). Predicted No-Effect Concentration and Risk Assessment for 17-(Beta)-Estradiol in Waters of China. *Reviews of Environmental Contamination and Toxicology*, 228, 31-56.

Xu, C., Chen, J.-A., Qiu, Z., Zhao, Q., Luo, J., Yang, L., Shu, W. (2010). Ovotoxicity and PPAR-mediated aromatase downregulation in female Sprague-Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl) phthalate. *Toxicology Letters*, *199*(3), 323–32.

Zhao, J.-L., Ying, G.-G., Yang, B., Liu, S., Zhou, L.-J., Chen, Z.-F., Lai, H.-J. (2011). Screening of multiple hormonal activities in surface water and sediment from the Pearl River system, South China, using effect-directed in vitro bioassays. *Environmental Toxicology and Chemistry*, *30*(10), 2208–2215.

Zhao, L., Dong, Y.-H., Wang, H. (2013). Residues of organochlorine pesticides and polycyclic aromatic hydrocarbons in farm-raised livestock feeds and manures in Jiangsu, China. *The Science of the Total Environment*, *450-451*, 348–55.

Ziylan, A., & Ince, N. H. (2011). The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: Treatability by conventional and non-conventional processes. *Journal of Hazardous Materials*, *187*(1-3), 24–36.

Appendix A Yeast strains and Media preparations

a) Yeast stains of *Saccharomyyces cerevisiae* were kindly provided by Dr. Marc Cox, Department of Biological Sciences, University of Texas, El Paso, USA.

Yeast strain	Description
DSY-219	Estrogen Receptor + Response Element with lacZ
DSY-1555	Androgen Receptor + Response Element with lac Z + FKBP52 immunophilin
DSY-1345	Glucocorticoid Receptor + Response Element with lacZ + FKBP52 immunophilin
MCY-038	Aryl Hydrocarbon Receptor + Response Element with lacZ

b) Growth Media Preparations* for each assay for a 100 ml Total volume

Compounds	Estrogen assay	Androgen assay	Glucocorticoid assay	AhR binding assay
	SC-UW	**SC-LUW**	**SC-UWH**	**SC-W**
Yeast Nitrogen Base	0.67 g	0.67 g	0.67 g	0.67 g
Anhydrous Dextrose**	2.0 g	2.0 g	2.0 g	2.0 g
Synthetic Complete	0.2 g	0.2 g	0.2 g	0.2 g
Histidine	0.2 ml	0.2 ml	-	0.2 ml
Leucine	1.0 ml	-	1.0 ml	1.0 ml
Uracil	-	-	-	1.0 ml
dd H_2O	23.8 ml	24.8 ml	24.0 ml	22.8 ml

Add 75.0 ml of dd H_2O to each preparation to bring the final volume up to 100 ml

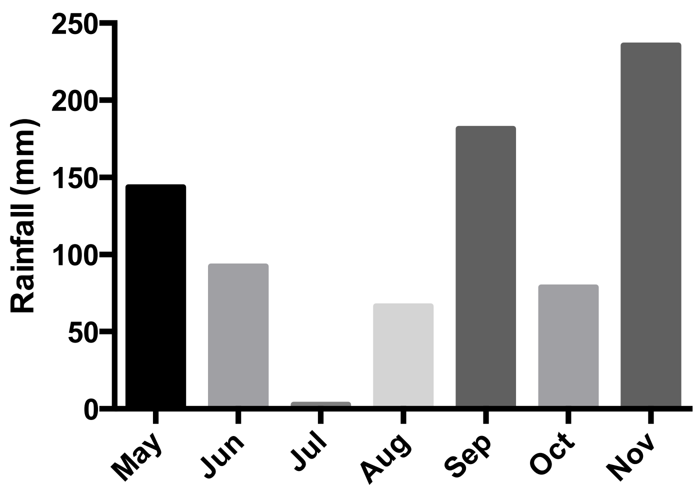
* Liquid culture media reagents are the same as growth media except that agar was added at 2% to medium.
** Galactose, not Dextrose, is used to prepare SC-W growth media.
SC= synthetic Complete; U= Uracil; W= Tryptophan; L= Leucine; H= Histidine

Appendix B Rainfall data

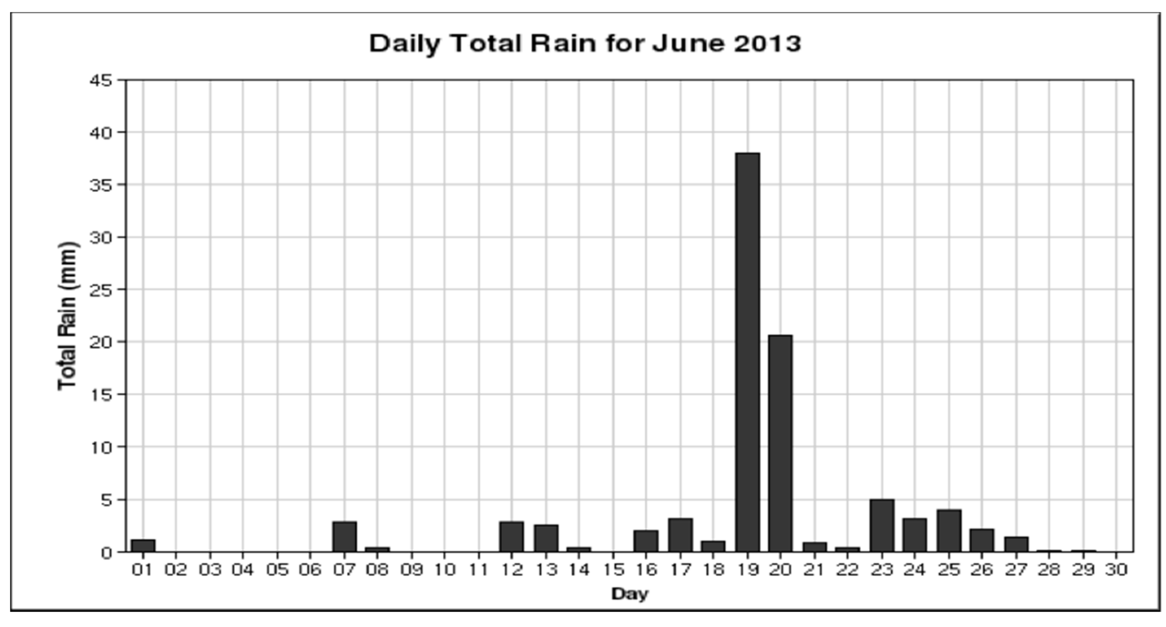
(Data from Environment Canada)

A) Rainfall during the sampling period in 2013

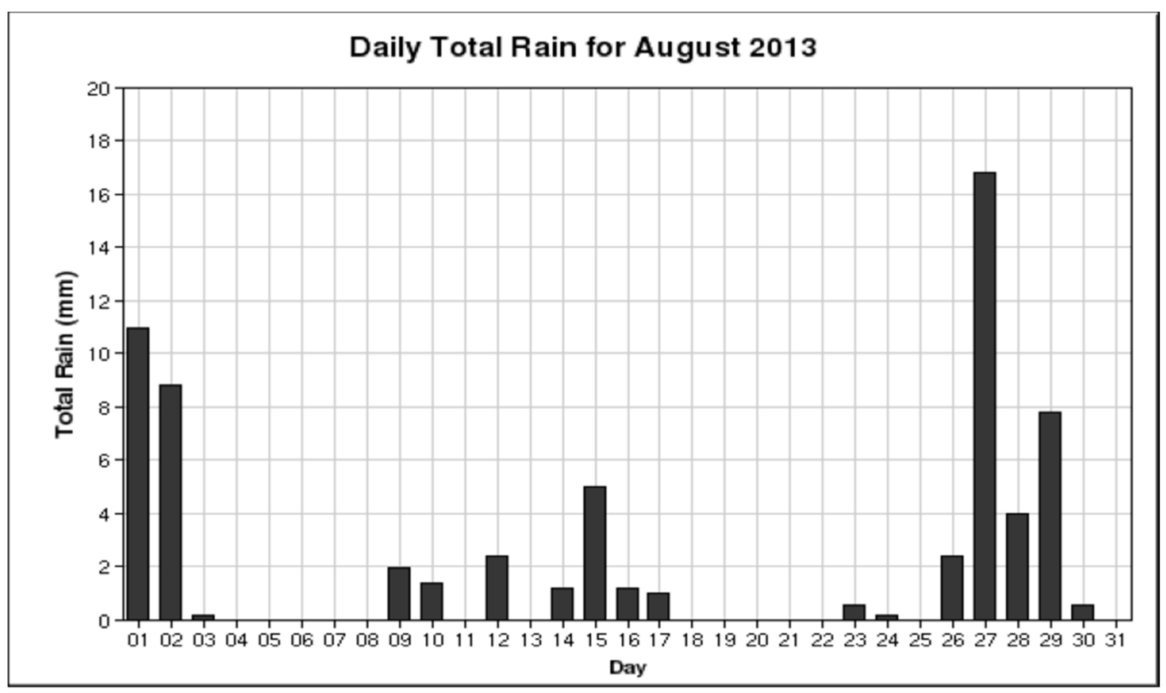
i) Total monthly Rainfall (mm)



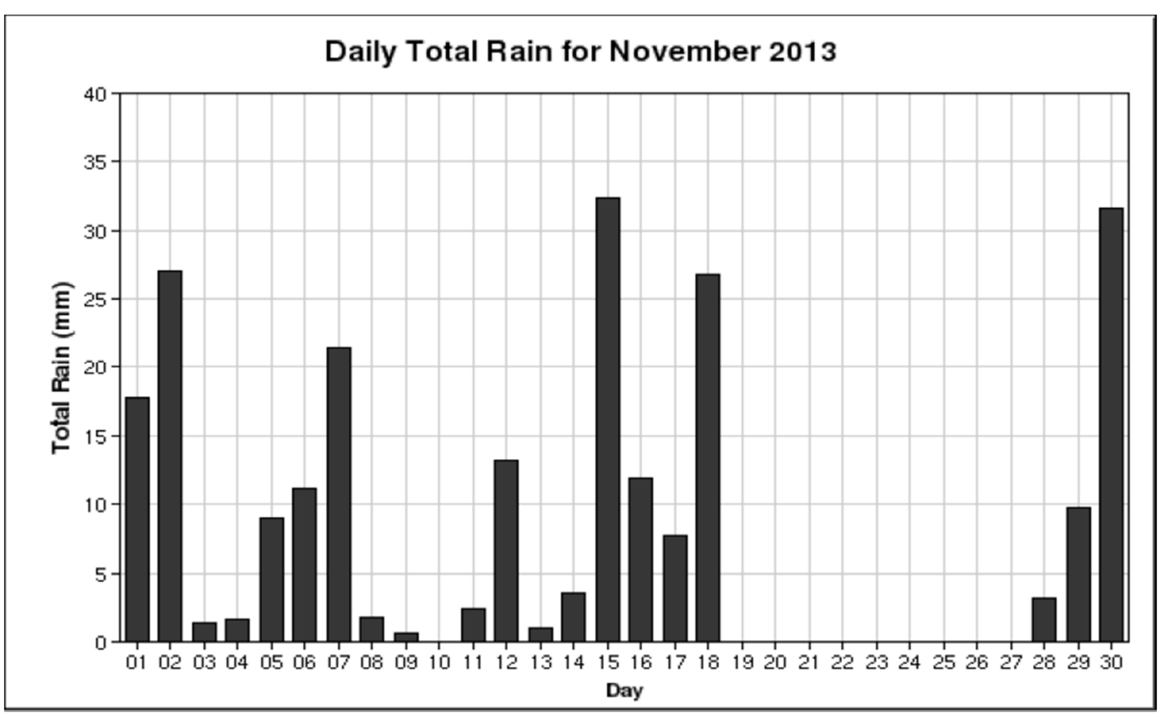
ii) Daily Rainfall levels in June, samples were collected on June 17th & 19th



iii) Daily Rainfall levels in August, samples were collected on August 12th



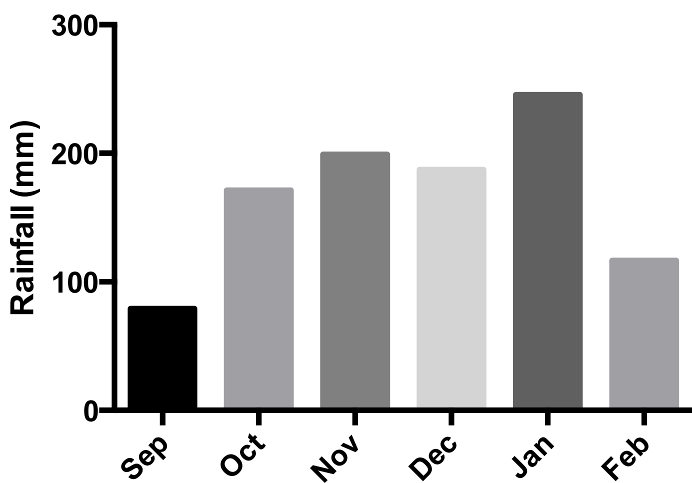
iv) Daily Rainfall levels in November, samples were collected on November 4th and 13th



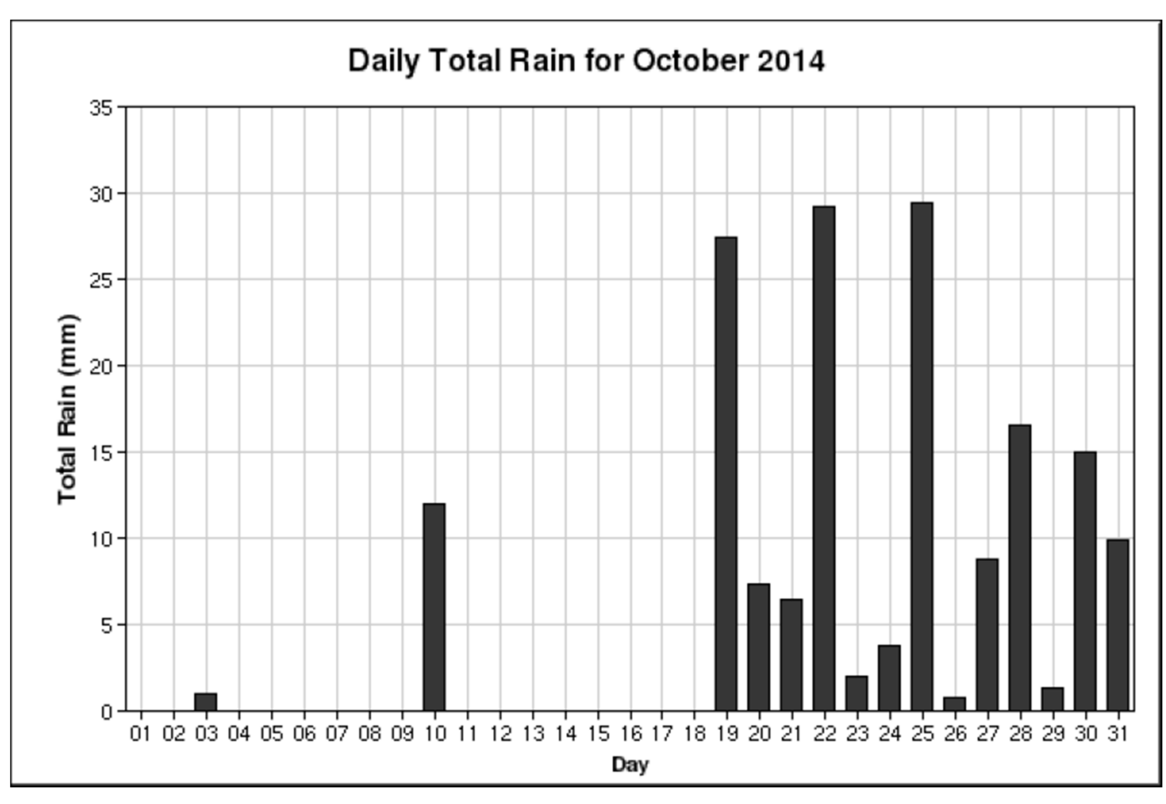
data obtained from http://climate.weather.gc.ca

B) Rainfall during the sampling period in 2014/15

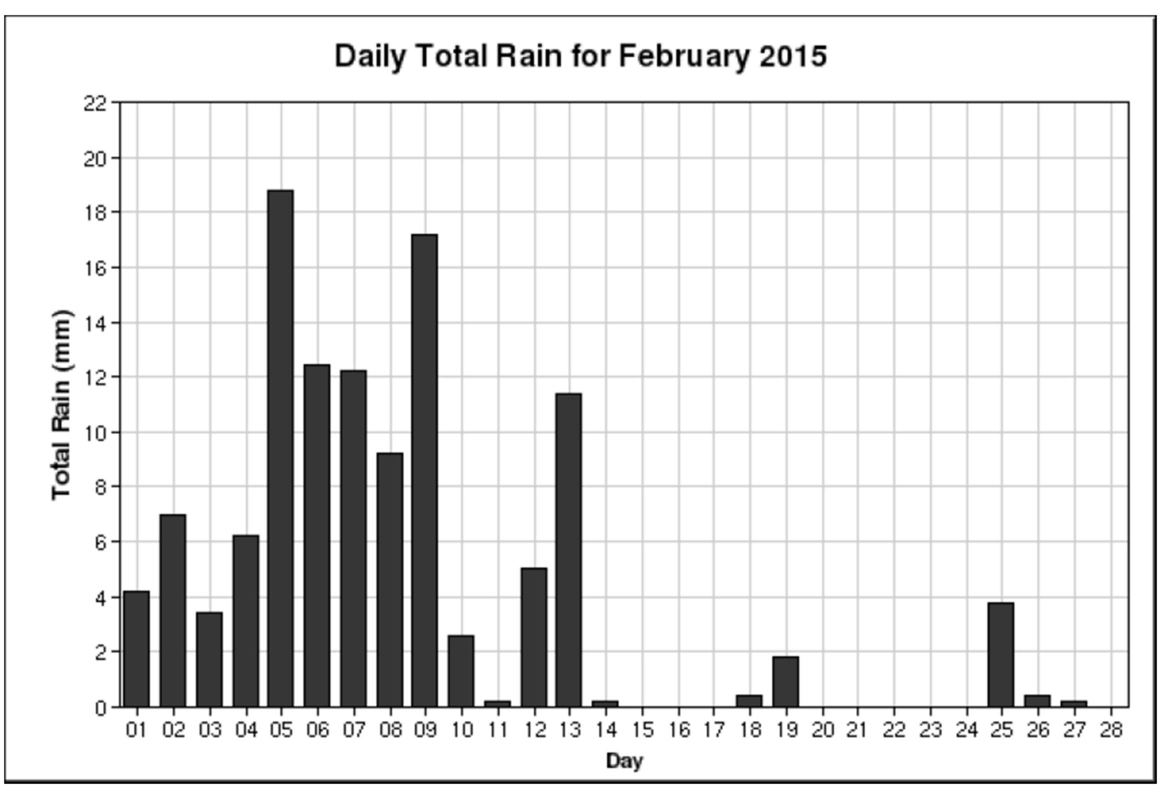
i) Total monthly Rainfall (mm)



ii) Daily Rainfall levels in October, samples were collected on October 10th



iii) Daily Rainfall levels in February, samples were collected on February 24th



data obtained from http://climate.weather.gc.ca

Appendix C Estradiol Equivalents (EEQs) in water and sediments

a) EEQs, mean (n=3) ± SEM, in water from sampling sites in 2013

Site ID	June	August	November
AS	2.08 ± 0.58	BLD	8.67 ± 3.48
MT1	1.22 ± 0.59	1.32 ± 0.70	4.34 ± 2.03
MT2	9.34 ± 4.63	0.4 ± 0.31	0.79 ± 0.61
MT3	1.50 ± 0.58	2.30 ± 0.91	no response
MR1	5.67 ± 2.33	BLD	25.10 ± 2.89
MR2	1.12 ± 0.49	BLD	0.69 ± 0.65
MR3	12.33 ± 4.33	BLD	15.01 ± 7.51
PN1	0.40 ± 0.26	8.33 ± 4.10	1.61 ± 0.45
PN2	2.29 ± 0.89	5.67 ± 2.91	0.36 ± 0.32

BLD= Below the Limit of Detection

b) EEQs, mean (n=3) ± SEM, in sediments from sampling sites in 2013

Site ID	June	August	November
AS	274.67 ± 41.91	8.33 ± 2.03	46.65 ± 8.11
MT1	97.31 ± 36.27	68.34 ± 18.28	1938.10 ± 470.35
MT2	no response	22.32 ± 7.45	34.20 ± 2.31
MT3	90.33 ± 25.71	26.67 ± 7.45	no respose
MR1	83.66 ± 22.24	23.70 ± 6.89	NS
MR2	17.33 ± 9.02	10.69 ± 4.41	3.17 ± 1.59
MR3	248.35 ± 58.09	3305.65 ± 625.89	96.67 ± 30.33
PN1	18.79 ± 6.07	3.10 ± 2.85	236.50 ± 37.82
PN2	36.73 ± 9.82	3.17 ± 1.48	no response

NS= No Sediment sample was collected due to inaccessibility

c) EEQs, mean (n=3) ± SEM, in water and sediments from sampling sites in 2014/15

Site ID	October		February	
	water	**sediment**	**water**	**sediment**
S0	0.34 ± 0.23	125.48 ± 33.38	0.34 ± 0.19	0.85 ± 0.54
S1	NW	NS	0.10 ± 0.10	2.42 ± 2.29
S2	NW	no response	BLD	BLD
S3	NW	NS	0.08 ± 0.08	0.52 ± 0.16
S4	0.08 ± 0.07	no response	0.39 ± 0.11	8.55 ± 4.61
S5	0.06 ± 0.04	no response	no response	7.33 ± 3.29
S6	0.05 ± 0.04	524.20 ± 54.72	2.70 ± 1.30	1.05 ± 0.98
S7	3.59 ± 1.29	233.34 ± 69.40	0.18 ± 0.17	1.17 ± 0.76
S8	BLD	25.32 ± 9.32	5.42 ± 3.29	38.20 ± 8.96
S9	0.05 ± 0.01	32.90 ± 10.44	0.23 ± 0.13	0.08 ± 0.03
S10	0.31 ± 0.17	no response	0.05 ± 0.05	no response
S11	0.02 ± 0.02	BLD	4.85 ± 0.64	34.12 ± 4.90
S12	1.90 ± 1.48	9.03 ± 2.33	0.24 ± 0.13	9.79 ± 3.58

BLD= Below the Limit of Detection; NS= No Sediment sample was collected due to inaccessibility; NW= No water sample was collected due to inaccessibility

Appendix D Trenbolone Equivalents (TbEQs) in water and sediments

a) TbEQs, mean (n=3) ± SEM, in water from sampling sites in 2013

Site ID	June	August	November
AS	56.12 ± 23.39	BLD	33.30 ± 11.32
MT1	1.97 ± 0.61	0.53 ± 0.24	BLD
MT2	0.73 ± 0.25	1.79 ± 0.69	BLD
MT3	40.33 ± 15.07	1.17 ± 0.44	0.98 ± 0.52
MR1	0.58 ± 0.23	20.81 ± 7.37	25.45 ± 8.99
MR2	0.67 ± 0.24	0.37 ± 0.17	0.86 ± 0.84
MR3	1236.34 ± 174.80	531.33 ± 46.66	7.29 ± 4.91
PN1	0.88 ± 0.35	0.99 ± 0.25	0.73 ± 0.29
PN2	1.59 ± 0.67	0.41 ± 0.21	0.43 ± 0.26

BLD= Below the Limit of Detection

b) TbEQs, mean (n=3) ± SEM, in sediments from sampling sites in 2013

Site ID	**June**	**August**	**November**
AS	220.27 ± 41.70	270.30 ± 42.18	217.02 ± 17.16
MT1	149.66 ± 30.32	4.67 ± 1.20	no response
MT2	42.43 ± 10.90	no response	37.70 ± 4.06
MT3	87.33 ± 35.03	196.51 ± 18.19	430.76 ± 45.48
MR1	120.77 ± 57.95	302.96 ± 2.08	NS
MR2	82.01 ± 16.44	no response	543.05 ± 48.19
MR3	45.28 ± 17.90	1739.35 ± 231.32	28.20 ± 6.24
PN1	280.33 ± 42.96	54.67 ± 1.76	3.05 ± 1.15
PN2	631.67 ± 49.31	9.15 ± 3.21	25.33 ± 4.33

NS= No Sediments were collected due to inaccessibility

c) TbEQs, mean(n=3) ± SEM, in water and sediments from sampling sites in 2014/15

Site ID	October		February	
	water	sediment	water	Sediment
S0	0.74 ± 0.67	18.53 ± 4.72	0.28 ± 0.28	3.43 ± 0.87
S1	NW	NS	0.24 ± 0.24	no response
S2	NW	84.37 ± 26.00	0.37 ± 0.35	31.73 ± 7.21
S3	NW	NS	BLD	BLD
S4	0.90 ± 0.55	260.25 ± 44.55	21.10 ± 5.85	no response
S5	no response	16.09 ± 6.08	0.30 ± 0.31	8.68 ± 3.20
S6	no response	no response	BLD	7.55 ± 2.67
S7	BLD	43.21 ± 6.35	3.31 ± 1.91	0.50 ± 0.50
S8	150.58 ± 54.62	15.69 ± 3.48	23.90 ± 5.96	no response
S9	115.05 ± 40.40	8.95 ± 2.73	5.90 ± 2.15	27.78 ± 3.73
S10	BLD	8.98 ± 3.02	0.70 ± 0.66	3.15 ± 1.60
S11	BLD	2.72 ± 1.99	0.35 ± 0.35	2.42 ± 1.99
S12	1.90 ± 0.92	31.67 ± 4.53	2.70 ± 2.02	3.18 ± 1.89

BLD= Below the Limit of Detection; NS= No Sediment sample was collected due to inaccessibility; NW= No water sample was collected due to inaccessibility

Appendix E Deoxycorticosterone Equivalents (DOCEQs) in water and sediments

a) DOCEQs, mean(n=3) ± SEM, in water from sampling sites in 2013

Site ID	June	August	November
AS	no response	no response	11.13 ± 8.00
MT1	35.01 ± 11.27	55.67 ± 3.18	1506.11 ± 80.88
MT2	40.67 ± 20.80	1317.33 ± 1152.67	1507.30 ± 76.30
MT3	21.33 ± 4.91	11.27 ± 4.05	8209.77 ± 614.24
MR1	110.20 ± 20.21	7258.35 ± 632.51	1553.09 ± 106.79
MR2	24.25 ± 5.78	28.04 ± 8.66	1377.65 ± 361.59
MR3	BLD	BLD	1045.61 ± 120.63
PN1	BLD	BLD	9.80 ± 5.40
PN2	no response	no response	749.73 ± 28.86

BLD= Below the Limit of Detection

b) DOCEQs, mean (n=3) ± SEM, in sediments from sampling sites in 2013

Site ID	June	August	November
AS	no response	no response	233.20 ± 49.33
MT1	215.28 ± 42.53	10010.13 ± 577.35	no response
MT2	no response	17200.42 ± 986.58	no response
MT3	no response	1933.32 ± 33.33	8176.98 ± 668.90
MR1	256.96 ± 30.07	no response	NS
MR2	2227.80 ± 579.15	31.11 ± 6.39	637.59 ± 38.62
MR3	7902.15 ± 782.97	557.74 ± 109.30	7455.67 ± 1505.93
PN1	no response	111.14 ± 13.00	2999.89 ± 121.82
PN2	no response	517.80 ± 55.01	no response

NS= No Sediments were collected due to inaccessibility

c) DOCEQs, mean (n=3) ± SEM, in water and sediments from sampling sites in 2014/15

Site ID	**October**		**February**	
	water	sediment	water	sediment
S0	17.21 ± 3.43	BLD	BLD	BLD
S1	NW	NS	3.81 ± 1.11	42.40 ± 5.57
S2	NW	no response	243.00 ± 59.89	44.10 ± 13.21
S3	NW	NS	2.02 ± 0.78	47.67 ± 15.45
S4	6.02 ± 1.24	776.50 ± 59.80	3.40 ± 1.14	55.20 ± 8.90
S5	6.75 ± 0.98	133.88 ± 13.24	BLD	40.10 ± 10.99
S6	13.21 ± 0.79	no response	14.40 ± 4.97	no response
S7	3.03 ± 1.09	no response	BLD	no response
S8	18.59 ± 2.76	124.08 ± 22.45	143.53 ± 36.56	245.10 ± 98.66
S9	3.40 ± 0.44	28.87 ± 7.87	25.23 ± 11.23	57.70 ± 9.89
S10	no response	no response	24.52 ± 7.16	24.26 ± 12.01
S11	BLD	2.10 ± 0.78	BLD	2.73 ± 1.43
S12	22.28 ± 3.90	no response	no response	no response

BLD= Below the Limit of Detection; NS= No Sediment sample was collected due to inaccessibility; NW= No water sample was collected due to inaccessibility

Appendix F β- Naphthoflavone Equivalents (NAPEQs) in water and sediments

a) NAPEQs, mean (n=3) ± SEM, in water from sampling sites in 2013

Site ID	June	August	November
AS	2849.80 ± 407.23	11.12 ± 4.36	BLD
MT1	5182.65 ± 509.32	1014.03 ± 132.20	95.66 ± 30.37
MT2	11048.32 ± 1300.78	28.67 ± 7.62	no response
MT3	39.01 ± 16.46	25825.33 ± 1764.48	356.71 ± 21.86
MR1	156.59 ± 31.52	1849.11 ± 203.03	165.30 ± 33.17
MR2	149.30 ± 29.16	3579.24 ± 299.56	274.41 ± 45.32
MR3	245.03 ± 32.92	47.30 ± 9.21	6.28 ± 1.86
PN1	286.67 ± 21.17	57.64 ± 10.14	17.19 ± 6.35
PN2	3110.55 ± 58.90	5336.33 ± 736.00	3671.23± 35.31

b) NAPEQs, mean (n=3) ± SEM, in sediments from sampling sites in 2013

Site ID	June	August	November
AS	36525.91 ± 1913.64	6357.88 ± 692.65	1311.72 ± 199.09
MT1	662.50 ± 163.31	3750.33 ± 147.05	203.12 ± 29.24
MT2	16648.83 ± 1641.72	3779.05 ± 201.10	no response
MT3	34451.15 ± 2961.29	38263.40 ± 9254.58	1236.22 ± 101.61
MR1	2794.67 ± 426.19	1059.19 ± 107.22	NS
MR2	160.33 ± 45.74	1096.20 ± 142.89	993.67 ± 71.54
MR3	23593.33 ± 3118.55	952.55 ± 96.61	194.67 ± 8.84
PN1	730.30 ± 23.13	853.06 ± 27.30	8794.99 ± 960.71
PN2	1313.42 ± 59.12	149.93 ± 25.98	3465.75 ± 801.36

NS= No Sediments were collected due to inaccessibility

c) NAPEQs, mean (n=3) ± SEM, in water and sediments from sampling sites in 2014/15

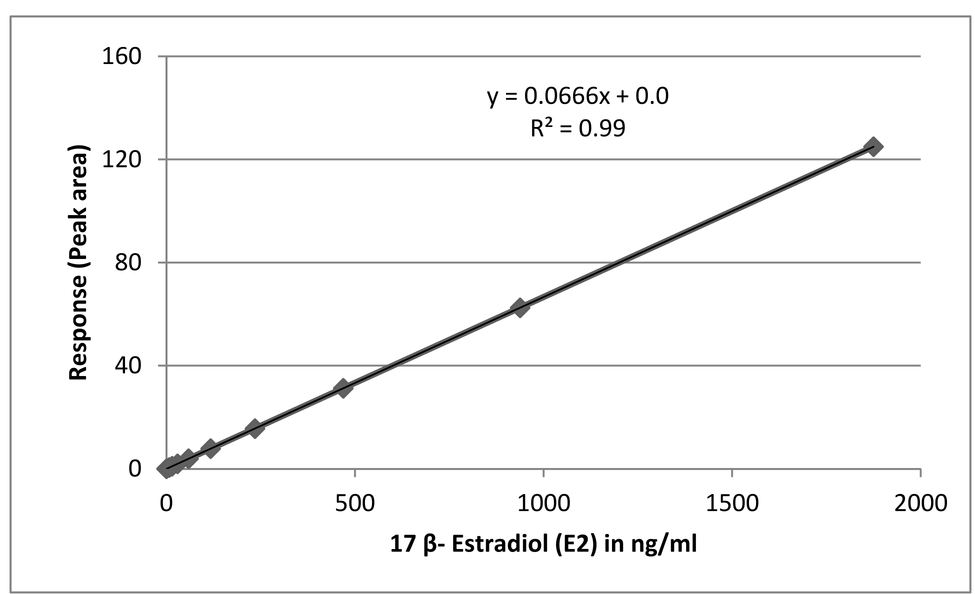
Site ID	October		February	
	water	sediment	water	sediment
S0	15.67 ± 7.62	1574.39 ± 362.79	3.71 ± 2.03	335.34 ± 92.64
S1	NW	NS	71.13 ± 21.36	858.50 ± 215.02
S2	NW	2673.35 ± 452.06	14.50 ± 7.53	509.50 ± 93.31
S3	NW	NS	26.06 ± 8.96	550.11 ± 64.33
S4	no response	1596.39 ± 227.66	BLD	806.06 ± 173.57
S5	16.03 ± 5.91	293.00 ± 74.53	57.70 ± 23.28	42.30 ± 15.76
S6	82.39 ± 47.98	714.63 ± 68.08	7.40 ± 5.04	222.13 ± 97.77
S7	32.26 ± 16.69	332.82 ± 134.81	85.10 ± 16.37	7727.43 ± 1410.27
S8	14.77 ± 6.69	176.63 ± 80.83	626.10 ± 298.09	436.89 ± 128.34
S9	34.31 ± 12.99	126.91 ± 114.80	75.40 ± 7.51	102.99 ± 41.33
S10	232.34 ± 31.69	1913.44 ± 847.21	21.99 ± 16.23	63.04 ± 6.70
S11	374.80 ± 101.70	545.98 ± 189.68	20.60 ± 5.92	514.11 ± 76.90
S12	337.05 ± 108.83	4489.21 ± 609.44	no response	4945.34 ± 622.34

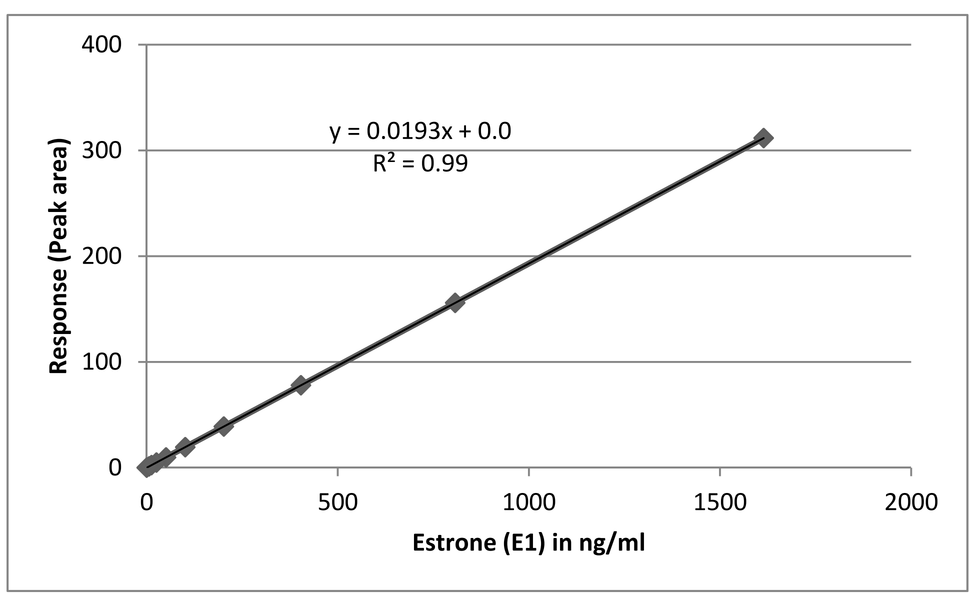
BLD= Below the Limit of Detection; NS= No Sediment sample was collected due to inaccessibility; NW= No water sample was collected due to inaccessibility

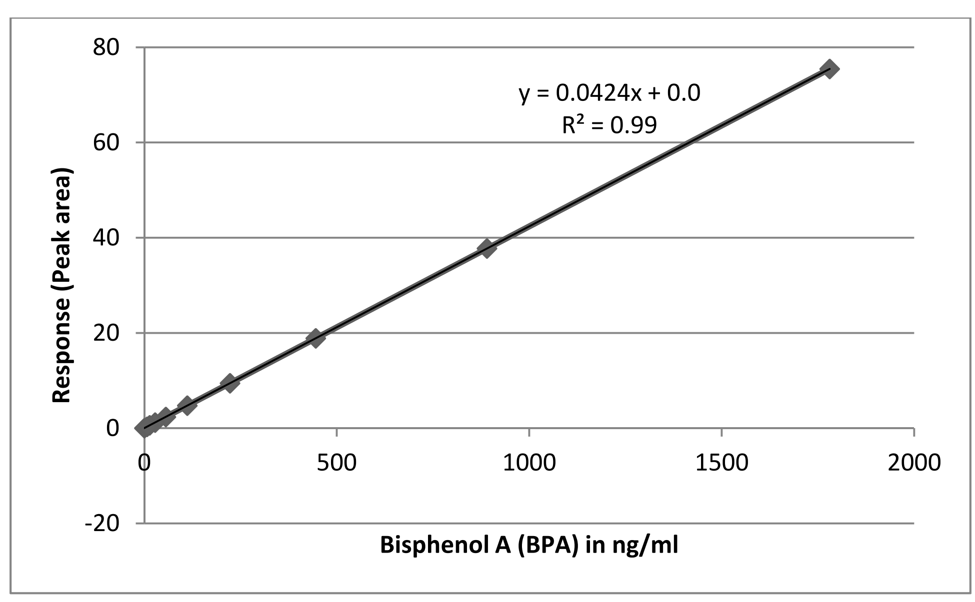
Appendix G GC-MS Analysis Results

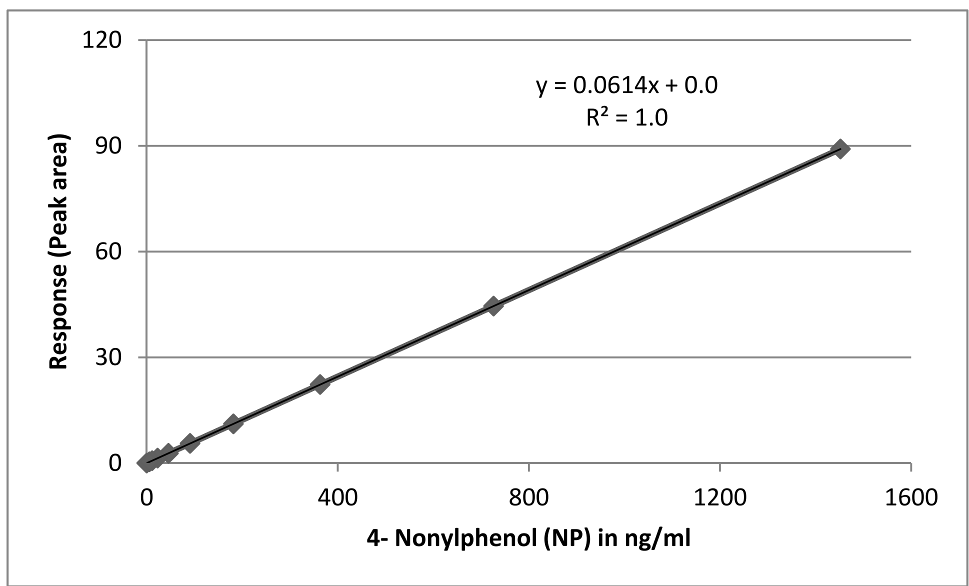
Calibration Curves

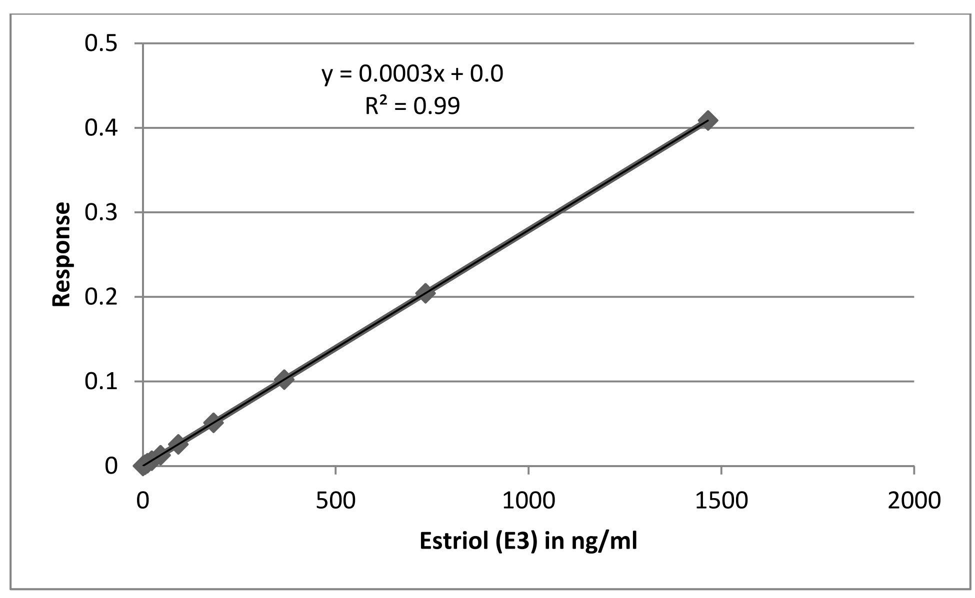
a) Calibration curves for the Estrogenic compounds obtained from GC-MSD

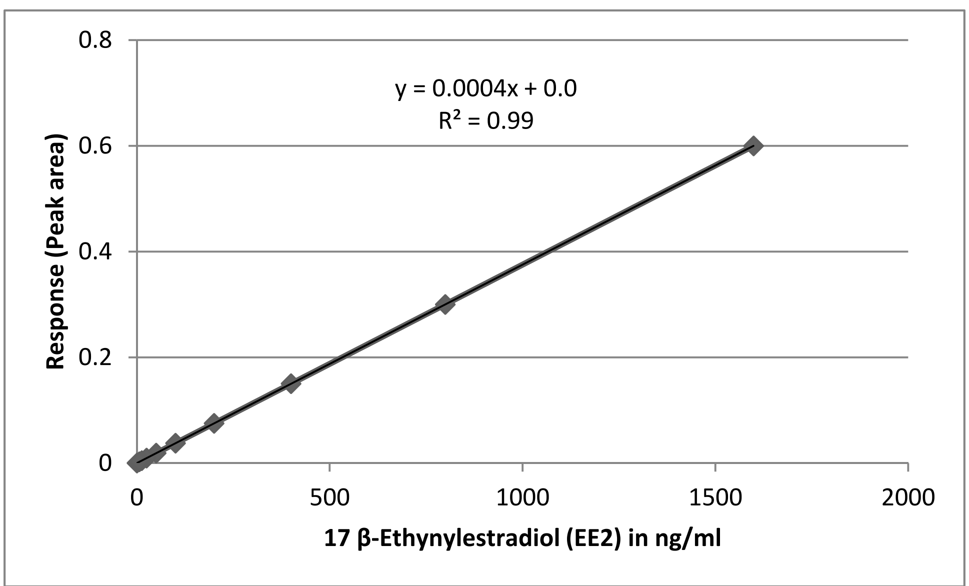




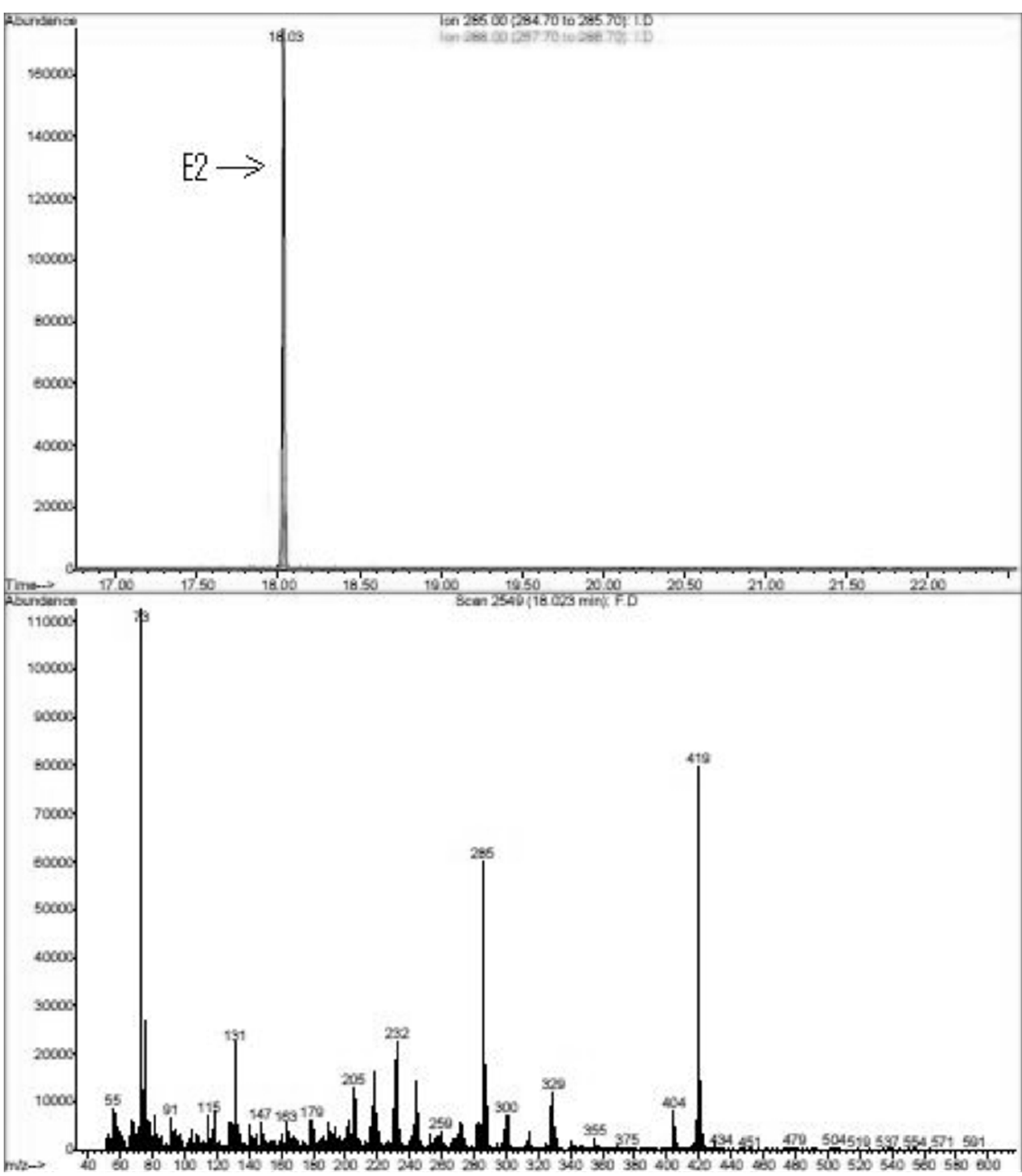

80
60
40
20
0
-20
0
500
1000
1500
2000
y = 0.0424x + 0.0
R² = 0.99
Response (Peak area)
Bisphenol A (BPA) in ng/ml


120
90
60
30
0
0
400
800
1200
1600
y = 0.0614x + 0.0
R² = 1.0
Response (Peak area)
4- Nonylphenol (NP) in ng/ml

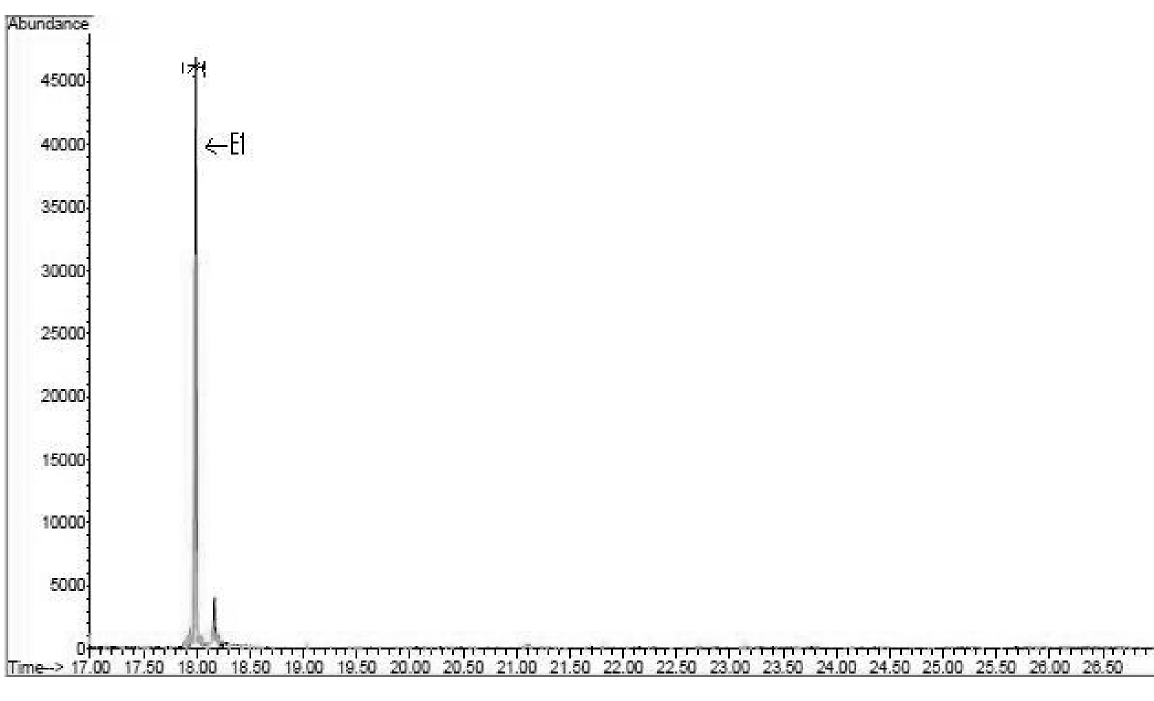

y = 0.0003x + 0.0
R² = 0.99
Response
Estriol (E3) in ng/ml
0
0.1
0.2
0.3
0.4
0.5
0
500
1000
1500
2000

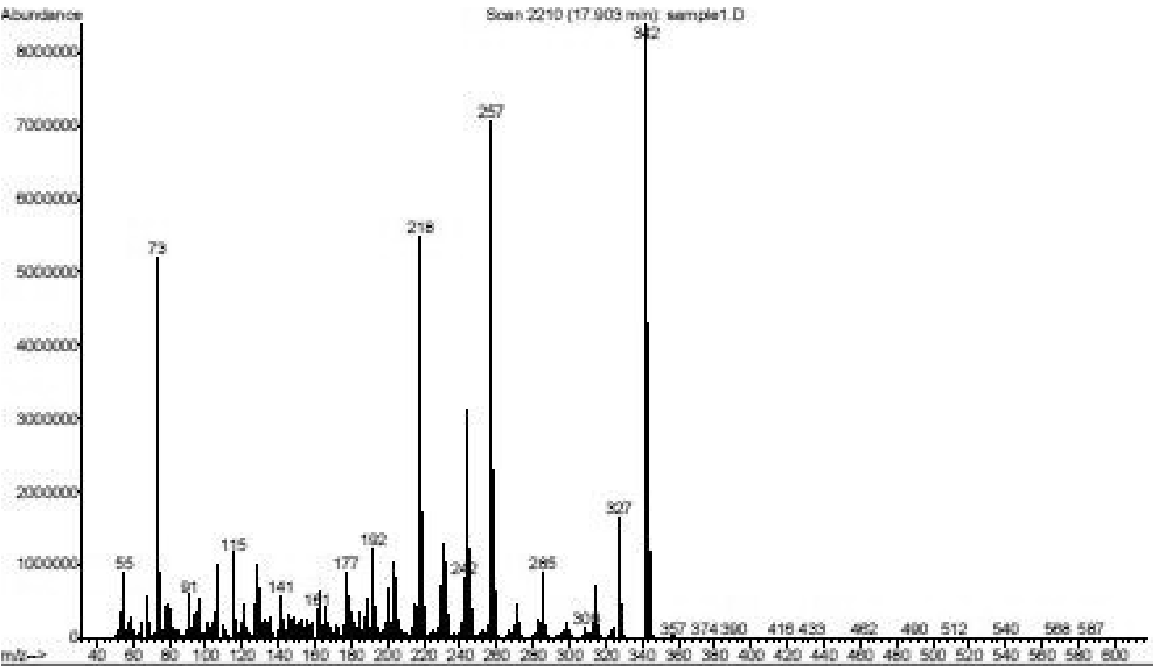

y = 0.0004x + 0.0
R² = 0.99
Response (Peak area)
17 β-Ethynylestradiol (EE2) in ng/ml
0
0.2
0.4
0.6
0.8
0
500
1000
1500
2000

GC-MS Library Searches for E2, E1, BPA, DHAA and poly hydrocarbons

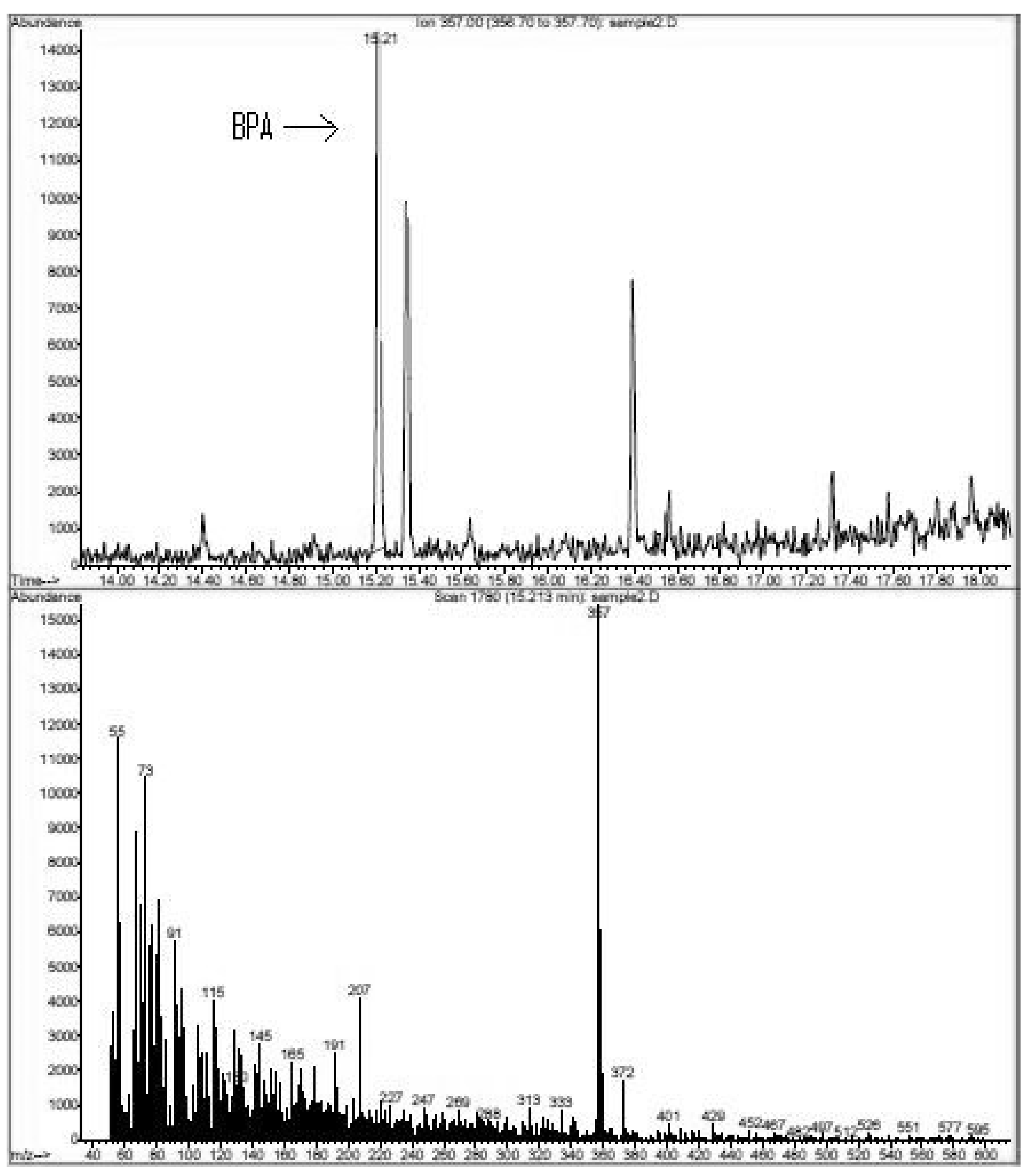


a) TIC and Mass Spectrum of E2 in water sample

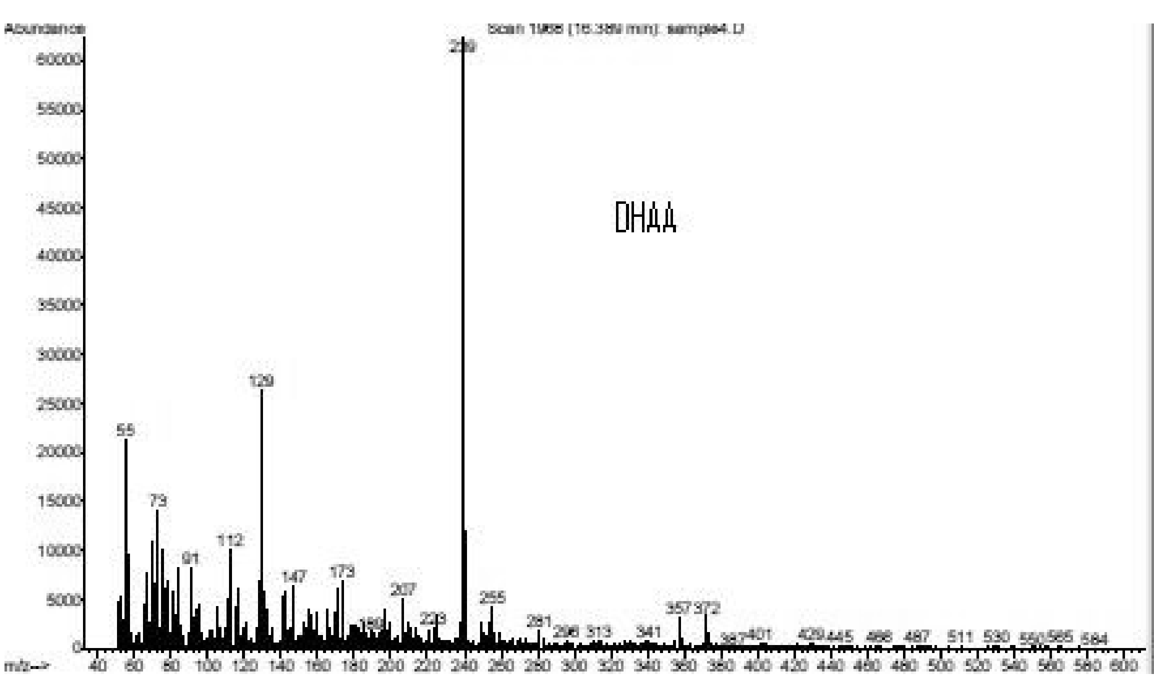


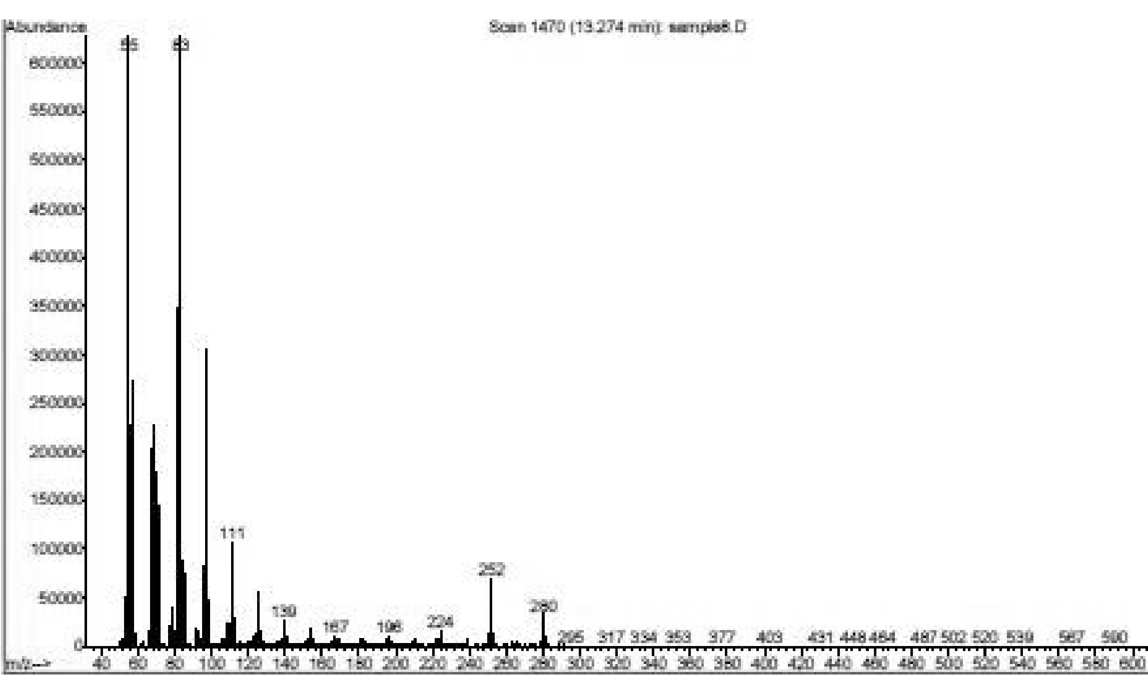


b) TIC and Mass Spectrum of E1 in water sample

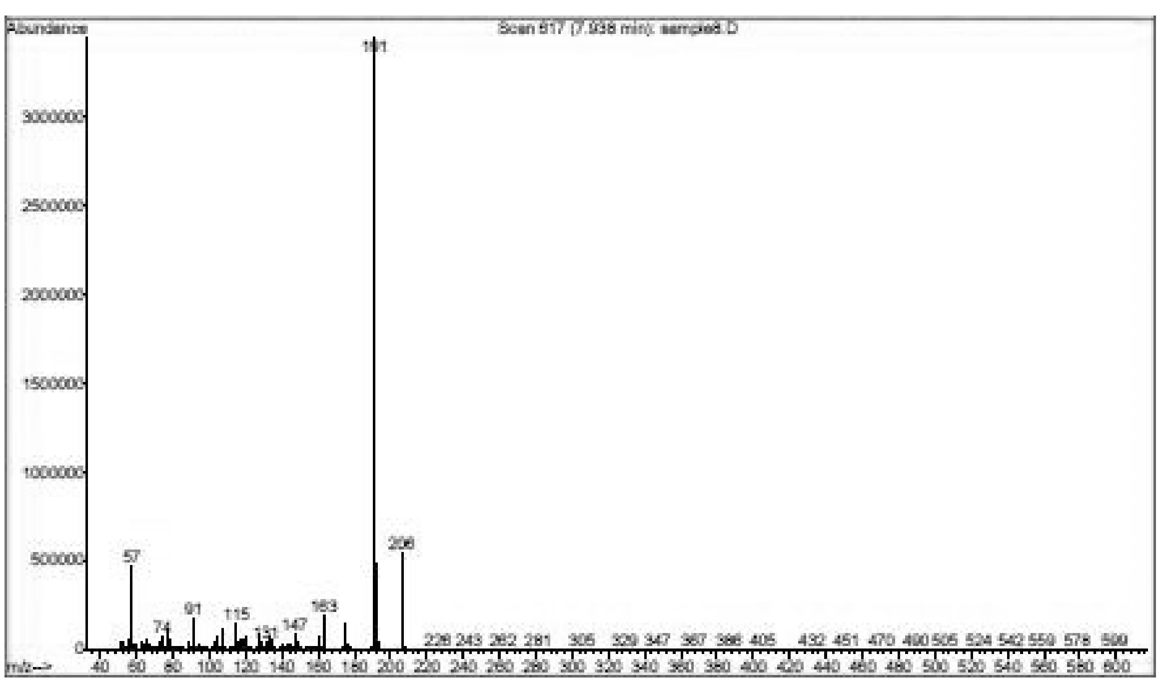


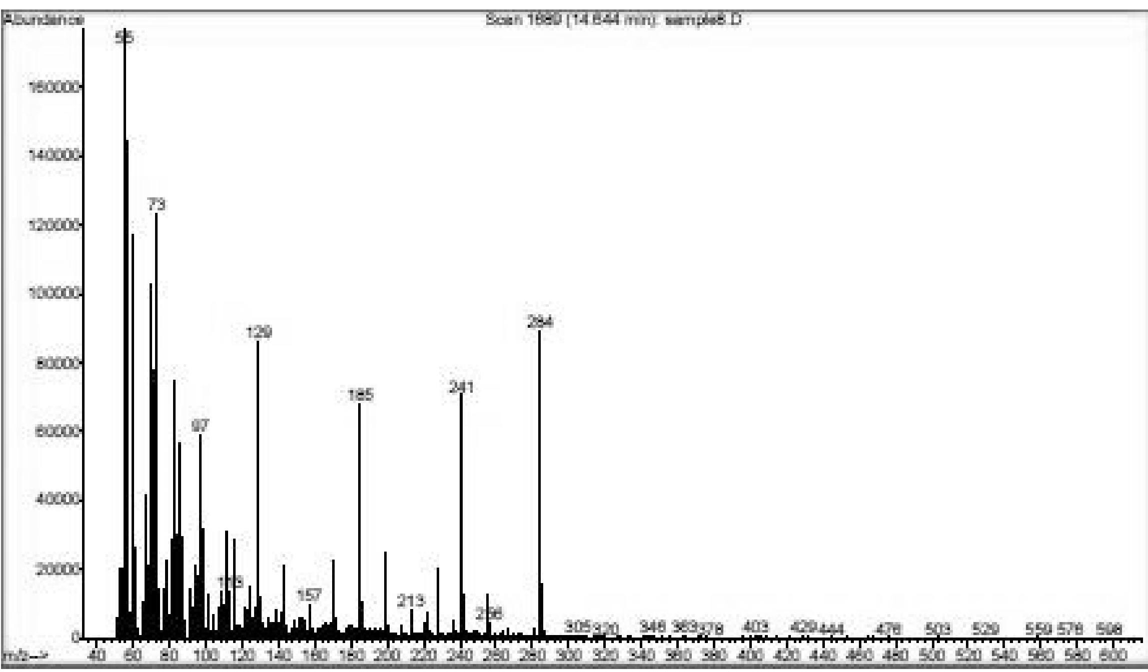
c) TIC and Mass Spectrum of BPA in water sample





d) SIM of Dehydroabietic acid (DHAA)





e) Mass spectra of Polyhydrocarbons