INFLUENCE OF LAND USE ON THE CONCENTRATIONS OF ESTROGENS IN WATER AND SEDIMENT WITHIN A WATERSHED

by

QI LUO

(Under the Direction of Paige Adams and Qingguo Huang)

The concentrations of 17β-estradiol, estrone, estriol, and 17α-ethinylestradiol in stream water, influent to and effluent from a sewage treatment plant (STP) receiving wastewater from a broiler processing plant, and sediment within the Upper Satilla watershed were determined for 14 months using LC-MS. The concentrations of 17β-estradiol, estrone, estriol ranged from below detection limits to 62.0 ng L^{-1}, while 17α-ethinylestradiol was not detected in stream water samples but in the STP influent and effluent. High concentrations of estrogens were found in the suspended particles that were separated from the water samples. In sediment, the highest estrogen concentration was 5.0 ng g^{-1}. The estrogen concentrations in STP influent were constantly high. We quantitatively analyzed the temporal and spatial distribution of the estrogens in relation to rainfall, land use, and water quality parameters. The results provide useful information on how agricultural runoff and STP effluent impact the occurrence of estrogens in natural water.

INDEX WORDS: Land use, Estrogens, Poultry litter, Water, Sewage treatment plant, Suspended particles, Sediment

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DEDICATION

I dedicate this work to my lovely family – my mom, Shundi, dad, Zhiping, uncle, Xingping, and, in particular, my late aunt, Bonnie, who passed away in June 2010 after years of courageous fight with cancer. Without their support and encouragement, I cannot possibly make my way to UGA and complete this work.

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CHAPTER 1

INTRODUCTION

Natural and synthetic estrogens are endocrine disrupting compounds. Experimental and epidemiological studies suggest these estrogens have the potential to interrupt normal function of the endocrine systems of humans and wildlife (Jobling et al., 1998; Kidd et al., 2007; Thorpe et al., 2003). Since 1990, detections of these compounds have been reported, and particularly in the past few years these chemicals have drawn increasing concerns from the public and the academic communities. Many studies have reported the detection of estrogens in surface water. During 1999-2000, a survey of organic contaminants in 139 streams across 30 states in the United States reported that endocrine disrupting compounds were found in nearly 40% of water samples (Kolpin et al., 2002). In this survey, the maximum concentrations that have been detected in natural water samples were 200 ng L^{-1} for 17β-estradiol (βE2), 112 ng L^{-1} for estrone (E1), 51 ng L^{-1} for estriol (E3), and 831 ng L^{-1} for 17α-ethinylestradiol (EE2). Concentrations of estrogens that have been measured worldwide ranged from < 0.1 to 2000 ng L^{-1} in rivers, from < 0.05 to 22.8 ng g^{-1} in sediments, and from < 0.3 to 96 ng L^{-1} in sewage treatment plant (STP) effluents (Benotti et al., 2008; Chang et al., 2009; Desbrow et al., 1998; Lai et al., 2002; Petrovic et al., 2004).

The poultry industry in Georgia has continued to grow over the past several decades. The value of poultry and eggs produced in Georgia increased 8% to $3.94 billion for the 2008 production year (Georgia Agricultural Statistics Service, 2009). Because of the rapid growth of poultry industry, the amount of poultry litter produced has increased. Hemmings and Hartel

(2006) estimated that 1.9 million Mg of poultry litter was generated in Georgia in 2006. Most of the poultry litter is applied to agricultural land as fertilizer because it contains plant macronutrients such as N, P, and K (Endale et al., 2002; Moore et al., 1995; Tasistro et al., 2007). Poultry litter also contains appreciable amounts of natural estrogens (Finlay-Moore et al., 2000; Hanselman et al., 2003; Nichols et al., 1997). As a result, the land application of poultry litter may introduce estrogens to surrounding environment. Many factors can potentially influence the transport and fate of estrogens from land applied litters. These factors include the rate and timing of application, the composition of the waste, the intensity of first rainfall, the slope of application site, and soil property (Jenkins et al., 2006; Lee et al., 2003; Nichols et al., 1997; Shore et al., 1995).

In this project, we measured the concentrations of estrogens in stream water, suspended particles, STP influent/effluent, and sediment at selected sites in the Upper Satilla watershed, a watershed heavily impacted by poultry farming and land application, over a 14-month period. We also investigated landuse, rainfall, and many other environmental conditions. The objective was to identify factors governing the spatial and temporal distribution of estrogens and establish quantitative relationships.

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CHAPTER 2

LITERATURE REVIEW

Estrogens have been studied extensively with respect to their physicochemical properties, environmental occurrences and behaviors, and ecological impact. A scientific and accurate assessment of the environmental risks of estrogens requires mechanistic understanding of their transport and transformation in the environment, as well as sensitive and reliable analytical methods that can accurately quantify their concentrations in complex environment samples. This review aims to provide a summary of research results addressing these important areas.

PHYSICOCHEMICAL PROPERTIES OF ESTROGENS

Estrone, βE2, and E3 are natural estrogens excreted by animals and human beings, while EE2 is a synthetic estrogen which is mainly used by humans as a contraceptive. The physicochemical properties of chemicals largely control their behaviors in the environment (Farré Marinel et al., 2008). Some important physicochemical properties of these estrogens are given in Table 2.1 (Lai et al., 2002; Tabak et al., 1981; Hanselman et al., 2003; Syracuse Research Corporation., 2005). As shown in the molecular structures displayed in Table 2.1, all estrogens contain a tetracyclic molecule framework with an aromatic A ring (Tapiero et al., 2002). The phenolic ring and hydroxyl at C-17 are two important structural features for estrogens to exhibit estrogenic activity (Brzozowski et al., 1997; Fang et al., 2001). The βE2 degrades to less potent metabolite, E1, when hydroxyl at C-17 is oxidized to a carbonyl group. Activity reduction was observed by introducing a hydroxyl group to the aromatic A ring of βE2, resulting in E3 (Fang et al., 2001). The 17α-estradiol (αE2) and 17β-estradiol (βE2) are two stereoisomers,

which difference resides in the direction of the hydroxyl at C-17. Only βE2 is present in poultry litter while both βE2 and αE2 are in cattle wastes (Hemmings and Hartel, 2006).

These four estrogens have relatively low solubility in water (0.8-13.3 mg L^{-1}) as measured in a previous study (Lai et al., 2000), although a model calculation yielded an estimate of quite high solubility for E3 (441 mg L^{-1}) (Mansell and Drewes, 2004). Estrogens are weak acids (pk_a, 10.3-10.8) which indicate that they are not ionized under normal environmental conditions. Estrogens are moderately hydrophobic ($LogK_{ow}$ 2.6-4.1), so they tend to sorb to solid phases (Lai et al., 2000; Lee et al., 2003). Their low vapor pressures suggest that volatilization would not be a primary pathway to remove estrogens from water and soil systems (Lai et al., 2002).

BIOLOGICAL EFFECT OF ESTROGENS

The occurrence of estrogens in the environment attracts both public and academic attention because they are ubiquitously present in the environment and their concentrations are usually above the lowest-observed-effect-concentrations (LOECs) for aquatic organism. LOEC is a statistically derived test value defining the lowest concentration of a test chemical that has a significant effect to a target organism measured by a specific endpoint. Table 2.2 compiles the LOECs of estrogens to a few fish species from previous publications (Kang et al., 2002; Kramer et al., 1998; Metcalfe et al., 2001; Mills and Chichester, 2005; Panter et al., 1998; Pawlowski et al., 2004; Renner, 2009; Seki et al., 2002; Sumpter and Johnson, 2005).

The impacts of estrogens on aquatic lives are multiplex. Studies using vitellogenin as a biomarker have shown exposure to estrogens induced vitellogenin production in animals (Desbrow et al., 1998; Irwin et al., 2001; Palmer and Palmer, 1995; Tyler et al., 2005). Routledge et al. (1998) reported βE2 concentration between 1 and 10 ng L^{-1} or E1 concentration between 25

and 50 ng L^{-1} can induce vitellogenin production in rainbow trout. Exposure to a combination of 25 ng L^{-1} E1 and 25 ng L^{-1} βE2 for 3-weeks resulted in higher, despite not significantly, vitellogenin production than exposure to 100 ng L^{-1} E1 alone in rainbow trout (Routledge et al., 1998). Another study reported that 30 days exposure to 200 ng L^{-1} βE2 disturbed the immunity of Japan sea bass (Thilagam et al., 2009). Thorpe et al. (2009) observed that egg production decreased by 28% to 44% after exposure to STP effluents with total estrogenic activity ranged from 0.7 to 21.2 ng L^{-1} of E2 equivalence. EE2 is often found more potent than βE2. Vitellogenin induction occurred in zebrafish after exposure to 21 ng L^{-1} βE2 or 3.0 ng L^{-1} EE2 for 8 days (Rose et al., 2002). Colman et al. (2009) reported that EE2 concentration between 0.5 and 50 ng L^{-1} can reduce male zebrafish aggression and impacted their courtship behavior. A study showed that short term (48 h) exposure to 10 ng L^{-1} of EE2 reduced male zebrafish reproductive success (Coe et al., 2008). Exposure to 4 ng L^{-1} of EE2 for 2 years has resulted in sex reversal in male roach while exposure to 0.03 ng L^{-1} of EE2 in early life of roach could alter the responsiveness of roach to estrogens in later life (Lange et al., 2009).

FATE AND TRANSPORT OF ESTROGENS

Potential Sources of Estrogens in Natural Waters

Sewage treatment plant effluents and land applied animal wastes are two major sources that may introduce estrogens to aquatic systems (Khanal et al., 2006; Ying et al., 2002). Estrogens have been found in STP effluents because traditional sewage treatment systems are not able to completely eliminate estrogens in wastewater (Benotti et al., 2008; Furuichi et al., 2006; Pawlowski et al., 2003; Shappell et al., 2006). Generally, conventional sewage treatment processes can remove about 85-99% βE2 and 77-99% E3, but the ability to remove E1 (25-80%) and EE2 (~50%) is relatively poor (Esperanza et al., 2007; Janex-Habibi et al., 2009; Khanal et

al., 2006; Koh et al., 2009; Layton et al., 2000). Estrogen concentrations in STP influent and effluent were reported ranging from 0.5 ng L^{-1} to 259 ng L^{-1} (Chimchirian et al., 2007). One study reported sewage introduced hormones into urban rivers in Beijing, China (Chang et al., 2009). The type of processes used in STP can impact the concentrations of estrogens in effluent. For example, a system with an oxidation ditch process was more effective in removing E1 and βE2 than one with conventional activated sludge process (Hashimoto and Murakami, 2009). In contrast to natural estrogens, EE2 is resistant to biological treatment. The removal of EE2 was less than 5% in two typical wastewater treatment plants in Australia (Braga et al., 2005).

Land application of animal waste is an important source of estrogens in surface waters. The concentrations of estrogens in animal wastes vary with species, age, gender, and reproductive stage (Hanselman et al., 2003; Shore and Shemesh, 2003). Table 2.3 summarizes the estrogen concentrations in poultry litter (Hanselman et al., 2003; Finlay-Moore et al., 2000; Nichols et al., 1998; Nichols et al., 1997; Shore et al., 1993). In dairy manure, the concentration of βE2 is around 113 to 239 μg kg^{-1} dry matter, αE2 is approximately 25 to 29 μg kg^{-1} dry matter, and E1 is about 58 to 129 μg kg^{-1} dry matter (Hanselman et al., 2003; Shappell et al., 2010). Shore and Shemesh (1993) found estrogens (combined E1 and βE2) in poultry litter ranging from 14 to 65 μg kg^{-1} dry weight while no αE2 was detected. The βE2 concentrations detected in poultry litter ranged from 20 to 35 μg kg^{-1} (Finlay-Moore et al., 2000). The highest concentration of βE2 in poultry litter reported in literature was 133 μg kg^{-1} dry litter (Nichols et al., 1997). Though animal wastes are usually not discharged into water directly, research suggests that runoff from agricultural fields with litters amended are likely to introduce estrogens to water systems. Nichols et al. (1997) found linear relationship between βE2 concentration in agricultural runoff and poultry litter application rate. The highest βE2 concentration in runoff

water was 1280 ng L^{-1} when application rate was 7.05 Mg ha^{-1}. The βE2 concentrations ranged from 20 to 2330 ng L^{-1} in agricultural runoff which was from grassland amended with poultry litter (Finlay-Moore et al., 2000). Transport of estrogens from rangeland influenced by cattle grazing to surface water has been observed (Kolodziej and Sedlak, 2007). Both laboratory and field studies have indicated the possibility of estrogens leaching from lands receiving animal wastes (Arnon et al., 2008; Kjær et al., 2007). Estrogens were detected in shallow groundwater with concentration of E1 up to 20 ng L^{-1} and βE2 up to 80 ng L^{-1} (Swartz et al., 2006; Wicks et al., 2004). The βE2 (0.05 to 1.8 g L^{-1}) was detected in farm pond which received runoff from beef cattle pastures (Irwin et al., 2001).

Degradation of Parent Estrogens

Degradation of estrogens in surface water, sediment, soil, and animal waste is controlled by various factors. E1 and E3 are major metabolites of βE2 (Bolt, 1979). E1 is relatively persistent compared to other degradation products (Sarmah et al., 2008; Skotnicka-Pitak et al., 2008; Xuan et al., 2008). Jürgens et al. (2002) demonstrated that microorganism were able to convert βE2 to E1 with half-lives of βE2 between several hours to 9 days in English river. They also found that environmental conditions such as temperature, pH, dissolved organic carbon, and suspended sediments could impact the biodegradation of βE2. High aqueous salinity (0.17 mM) enhanced estrogens degradation while high alkalinity concentration (120 mM) restrained estrogens degradation in river sediment (Suri et al., 2010).

In river sediment, mineralization of βE2 and E1 has been observed using ^{14}C as a tracer (Bradley et al., 2009). Mineralization rates were 4.2–50.2% for βE2, 2.0–37.6% for E1, and 0.5–2.6% EE2 after 21 days incubation in soil (Stumpe and Marschner, 2009). The composition of microbial populations seemed to be a more important factor influencing the biodegradation rates

than physicochemical properties of the soils such as pH, texture, and soil organic matter (Stumpe and Marschner, 2009). The degradation of βE2 was much slower in autoclaved soil than in micro-active soil, and E1 was quickly stabilized in autoclaved soil (Colucci et al., 2001). An investigation regarding the mineralization of estrogens in breeder litter reported that mineralization of βE2 and E1 were less than 10% at different water potentials and temperatures after 25 weeks of incubation (Hemmings and Hartel, 2006). Another study explored the estrogens degradation in dairy press cake waste at different temperature and acidification (Raman et al., 2001). The results showed that βE2 rapidly transferred to less estrogenic products during the initial 24 h while E1 accumulated during the same 24 h at all tested temperatures. The highest removal rate of E1 was observed when temperature reached 50^{o}C, and the degradation of βE2 was quicker in non-acidified press cake (Raman et al., 2001). The degradation of EE2 was relatively slow (Gaulke et al., 2009; Jürgens et al., 2002; Layton et al., 2000). The half-life for βE2 was 1.2 days while it was 17 days for EE2 in river water under aerobic condition (Jürgens et al., 2002). The primary products of EE2 were monohydroxylated EE2 and 2-methoxy-ethinylestradiol which existed in human urine in conjugated forms (Bolt, 1979; Skotnicka-Pitak et al., 2009).

Sorption of Estrogens

Interaction between estrogens and solid phases can influence their transport from soil to surface water and distribution in water-sediment system. The moderate to high octanol-water partition coefficients (K_{ow}) of estrogens suggest that a significant fraction of estrogens may be retained or dissipated in soil after land application, so the possibility of estrogens being transported from soil to surface water is quite low. However, these values were calculated based on laboratory conditions which are different from real agricultural practices. In actual

agricultural practices, estrogens are present with animal wastes which contain colloids and high concentration of organic matters. The presence of colloids and organic matters could facilitate the transport of estrogens in water-soil systems. It has been shown that application of estrogens mixed with urine to soil enhances the leaching and persistence of estrogens in soil (Lucas and Jones, 2009). The land application of estrogens mixed with wastewater that contained high concentration of organic matter resulted in less sorption of βE2 in soil than the application of βE2 mixed with pure water (Stumpe and Marschner, 2007).

Once introduced to water systems, estrogens are subject to dilution, biodegradation, and sorption. Desorption of estrogens from sediment may serve as a secondary source for estrogens entering the aquatic phase (Lee et al., 2003). A batch study showed the sorption capacity of charcoal increased with the temperature of the experimental system, and at a neutral pH the solid phase had the highest sorption capacity (Kumar et al., 2009). Suspended particles could impact the distribution of organic contaminants in water systems and their bioavailability to organisms. A few papers have described the relationship between particles sizes and sorption processes (Gao et al., 1998; Johnson et al., 1998). Bioavailability of βE2 in sediment varied with sediment particle sizes (Duong et al., 2009). Organic contaminants have a strong tendency to sorb on suspended particles, because they have large surface areas and are coated with organic matter. It has been reported that sorption of octylphenol and pesticides increased with the decrease of sediment particle size (Gao et al., 1998; Johnson et al., 1998). Similar partitioning trends might be expected for estrogens, because estrogens are moderately hydrophobic compounds. A recent study showed 10-29% of endocrine disrupting compounds (E1, βE2, and EE2) adsorbed to suspended colloids in rivers (Zhou et al., 2006).

NEW METHODS FOR ESTROGENS ANALYSIS

Developing an effective analytical method to measure the concentration of estrogens in the environment is challenging because of the complexity of environmental matrices and extremely low concentrations of these compounds. It requires intensive procedures to concentrate these compounds and remove co-extracted impurities (Díaz-Cruz et al., 2003; Streck, 2009).

Utilizing solid phase extraction (SPE) and mass spectrometry (MS) to detect emerging environmental pollutants has become a trend. Extraction efficiency greatly impacts the accuracy of the chemical analysis. SPE is currently the predominate method in water sample extraction (Benotti et al., 2008; Chang et al., 2009; Lavado et al., 2009; Shappell et al., 2010). Various methods for solid sample extraction have been reported to reach satisfactory recovery (de Alda et al., 2002; Houtman et al., 2007; Petrovic et al., 2001). To remove co-extracted impurities, cleanup is necessary for certain samples such as sewage, agricultural impacted surface water, sediment, and soil (Beck et al., 2008; Hu et al., 2005; Ingrand et al., 2003; Labadie and Hill, 2007; Yamamoto et al., 2006). The high resolution, selectivity, sensitivity, and precision are advantages of MS in confirming the existence and measuring the concentrations of micropollutants.

Extraction

The type of SPE cartridge used is an important factor in water sample extraction. Many studies have evaluated the efficiency of different SPE cartridges. Oasis HLB (hydrophilic-lipophilic balanced copolymer) and C_{18} cartridges are used the most (Chang et al., 2009; Miège et al., 2009; Vulliet et al., 2008). Yamamoto et al. (2006) used the Speclean ENVI-CARB cartridge to extract both free and conjugated hormones from 2 L aqueous sample. Recoveries of E1, βE2, E3, and EE2 were all above 93%. Another study revealed that Strata X and Strata C_{18}

cartridges work well in estrogens extraction (Vulliet et al., 2008). The recoveries for estrogens by using Strata X and Strata C_{18} ranged from 92.7 to 99.9% and 92.9% to 100.3%, respectively.

Optimization of extraction conditions such as pH and volume are necessary. A large volume water sample is concentrated so that estrogen concentrations in the final extract could reach the limits of quantification of the instrument. Different sample volumes were tested to acquire satisfactory extraction efficiencies, because recoveries vary with sample volumes (Gentili et al., 2002; López de Alda and Barceló, 2001). The pKa values of estrogens are around 10, so appropriate pH could help maintain the target chemical in molecular form and therefore enhance the extraction efficiency. A study revealed that at pH 3, the recoveries for the estrogens were higher than at pH 5.5 and 8 (Vulliet et al., 2008).

Extraction of solid samples is usually performed with solvent extraction in conjunction with ultrasonic or shaking (Arnon et al., 2008; Petrovic et al., 2001; Ternes et al., 2002). Soxhlet extraction is recommended by EPA as a standard method for extracting estrogens from solid samples (USEPA, 2007). Viganò et al. (2006) modified soxhlet extraction to extract estrogens from sediment. The recoveries of four estrogens (E1, βE2, E3, and EE2) ranged from 66% to 88%. Other extraction methods have been also developed. For example, microwave-assisted extraction can save extraction time and solvent costs compared to conventional methods (López de Alda and Barceló, 2001; Labadie et al., 2007; Matějíček et al., 2007; Rice and Mitra, 2007; Sanchez-Prado et al., 2010).

Cleanup is necessary to remove co-extracted interferences in crude extracts, especially for samples with high concentration of organic matters. SPE, HPLC, and gel permeation chromatography (GPC) are frequently used for cleanup (Hájková et al., 2007; Isobe et al., 2006; Peng et al., 2006; Ternes et al., 2002). Using hydrophilic and hydrophobic SPE cartridges, e.g.,

C_{18}, florisil, and silica sorbents, to purify samples have been discussed in several papers (Chang et al., 2009; Miège et al., 2009). Using HPLC fractionation technique to purify the extracts is based on the theory that different chemicals have different retention times in the HPLC column (Furuichi et al., 2006; Houtman et al., 2006). A combination of several cleanup steps is necessary sometimes to purify the extract in extremely complex matrices like agriculture impacted water and sediment. Good recoveries for sediment extraction have been achieved by using GPC after florisil cartridge (Isobe et al., 2006). Purification of sediment by Strata-X-AW cartridges following a silica cartridge cleanup was shown successful (Labadie and Hill, 2007).

Analysis of Estrogens in Water

Application of LC-based techniques coupled with MS has been widely used to quantify estrogens in the environment because MS can reach very low detection limits and has high selectivity. Comparison of LC-diode array detector (DAD) and LC-electrospray ionization (ESI)-MS revealed that MS was a more suitable detector than DAD in analyzing estrogens in environmental samples (López de Alda et al., 2002). In this study, the detection limits using MS ranged from 0.4 ng L^{-1} to 10 ng L^{-1} while DAD could only detect estrogens with concentration above 10 to 20 ng L^{-1}.

Viglinò et al. (2008) have proposed an automated online LC-MS-MS method, combined with solid phase extraction. This method was able to rapidly detect estrogens in surface water. The detection limits are 3 ng L^{-1} for βE2, 10 ng L^{-1} for E1, and 50 ng L^{-1} for E3. Extraction recoveries ranged from 85 to 110%. Good reproducibility and repeatability of this method indicated automated operation could help minimize human errors in sample preparation. If lower detection limits need to be achieved, increasing the injection volume could help. This research has shown that by increasing injection volume from 1 mL to 3 mL, the method detection limits

would decrease to 0.9 ng L^{-1} for βE2 from 3 ng L^{-1}, 3.2 ng L^{-1} for E1 from 10 ng L^{-1}, and 25 ng L^{-1} for E3 from 50 ng L^{-1}).

Adding a pre-column derivatization before HPLC-MS-MS enhanced the sensitivity and reliability of the method (Matějíček and Kubáň, 2008). For example, when derivatization was used, the recoveries ranged from 75% to 89% and the detection limits were as low as 70 pg L^{-1} for E1, 78 pg L^{-1} for βE2, 285 pg L^{-1} for E3, and 67 pg L^{-1} for EE2.

Yamamoto et al. (2006) developed a sensitive method to detect estrogens by LC-MS. This study showed that ESI source was less effective in ionizing free estrogens than atmospheric pressure photoionization (APPI), whereas the ESI displayed greater sensitivity to conjugated estrogens and it allowed simultaneous analysis of androgen and estrogen in water. According to this study, APPI was great in ionizing low-polarity molecules such as estrogens, because it had high sensitivity and did not require the addition of any reagents. High recoveries were achieved for the four estrogens (93% to 102%), and detection limits for E1, βE2, E3, and EE2 were 0.51 ng ml^{-1}, 0.41 ng ml^{-1}, 0.72 ng ml^{-1}, and 1.2 ng ml^{-1} respectively.

Qin et al. (2008) described another novel method using column-switching LC-MS-MS. Negative ESI generated better signal than positive ESI because negative ESI was able to yield a higher amount of free estrogens precursor ions than positive ESI. The limits of detection were 0.038 ng L^{-1} for E1, 0.13 ng L^{-1} for βE2, 0.11 ng L^{-1} for E3 with recoveries up to 85.7%, 105.2%, 84.9%, respectively.

Analysis of Estrogen in Solid Samples

GC and LC are frequently used to separate the estrogens for subsequent MS detection. An efficient method for extracting and analyzing estrogens in sediment with GC-MS-MS has been developed (Lei et al., 2009). The detection limits ranged from 0.06 to 0.1 ng g^{-1}. The mean

recoveries were above 79% for most compounds except E3 whose recovery in sediment was 66%. Another method was developed to quantify the concentrations of estrogens by GC-MS. It was able to measure the estrogens in sediment as low as 0.05 ng g^{-1} (Hibberd et al., 2009). A SPE cleanup was utilized after microwave-assisted extraction to reduce the matrix effects.

Compared to GC, LC does not require derivatization which helps reduce potential target chemical loss. Labadie and Hill (2007) developed a rapid method including microwave-assisted extraction, SPE cleanup, and LC-MS-MS to determine estrogen concentrations in river sediments. They reported that LC-MS-MS produce better results than LC-TOF-MS for estrogen analysis.

Matějíček et al. (2007) described a sensitive analytical procedure to determine the concentrations of E1, βE2, E3, EE2 and their conjugates in river sediments. The procedure includes a microwave-assisted extraction with aqueous methanol (25:75, v/v) at 100°C for 10 minutes, followed by SPE cleanup on ion-exchange sorbent Oasis WAX and a HPLC–ion trap-MS with ESI. The recovery for each compound ranged from 83 to 107%. The limits of detection were as low as 1 ng g^{-1}. The good reproducibility of microwave-assisted extraction and high-efficiency of the SPE clean-up in conjunction with the speed and simplicity of the sample treatment distinguishes this method from previous analytical procedures.

A LC-MS-MS method coupled with soxhlet extraction was developed to measure estrogens in sediment (Viganò et al., 2006). The limits of detection ranged from 0.19 ng g^{-1} to 2.45 ng g^{-1}. The lowest recovery was 66.3% for βE2, while good recoveries were obtain for E1 (87.1%), E3 (81.4%), and EE2 (88.7%). Gomes et al. (2004) discussed issues of utilizing chemical analysis to detect the steroid hormones in environmental samples. Concerns included the sample handling and preparation, matrix effects, comparison of LC-MS with GC-MS. They

concluded that LC-MS can achieve adequate low detection limits if sufficient sample preparation is performed. LC-MS gave better recoveries and reproducibility for sediment samples than GC-MS.

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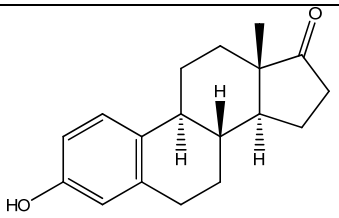
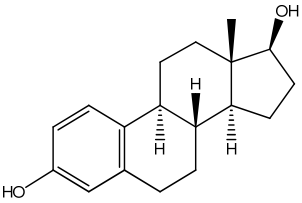
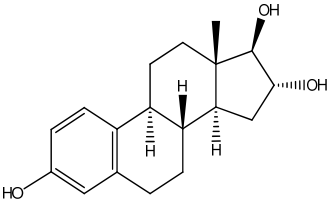
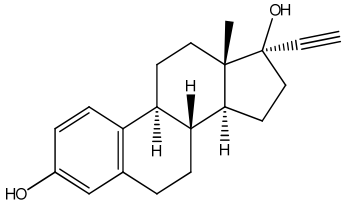
Table 2.1. Physicochemical properties of estrogens

Estrogen	Chemical Structure	MW ($g\ mole^{-1}$)	Solubility in water†‡ ($mg\ L^{-1}$)	$LogK_{ow}$‡	Pk_a§	VP¶ (Pa)
E1		270.37	0.8-12.4	3.1-3.4	10.3-10.8	3×10^{-8}
βE2		272.38	5.4-13.3	3.8-4.0	10.5-10.7	3×10^{-8}
E3		288.38	3.2-13,3	2.6-2.8	10.4	9×10^{-13}
EE2		296.40	4.8	3.7-4.2	nd	6×10^{-9}

†: Tabak et al. (1981). ‡: Lai et al. (2002). §: Hanselman et al. (2003). ¶: Syracuse Research Corporation (2005). MW: molecular weight. VP: vapor pressure. nd: not determined.

Table 2.2. Lowest-observed-effect-concentration (LOEC) in fish exposed in the laboratory to estrogens

Chemical	Fish species	Duration (day)	LOEC (ng L^{-1})	Effect	Refer.
E1	Fathead minnow	21	317.7	reduce testicular growth	Panter et al. (1998)
	Medaka	100	1000	males with testis-ova	Metcalfe et al. (2001)
βE2	Fathead minnow	19	120	inhibition egg production	Kramer et al. (1998)
	Medaka	21	29.3	males with testis-ova	Kang et al. (2002)
E3	Fathead minnow	na	na	na	na
	Medaka	100	10000	males with testis-ova	Metcalfe et al. (2001)
EE2	Fathead minnow	21	10	reduce egg production	Pawlowski et al. (2004)
	Medaka	21	63.9	males with testis-ova	Seki et al. (2004)

na: not applicable

Table 2.3. Reported concentrations of αE2, βE2, and E1 in poultry litter (dry weight basis) †.

Waste type	17α-estradiol	17β-estradiol	Estrone
	μg kg-1	μg kg^{-1}	μg kg^{-1}
broiler litter	nd	33±12‡	nd
broiler litter	nd	133±6§	nd
broiler litter (Al treat)	nd	101±2§	nd
broiler litter	nd	904¶	nd
broiler litter (females)	nd	65±7#	nd
broiler litter (males)	nd	14±4#	nd
layer litter	nd	533±40#	nd
rooster litter	nd	93±13#	nd

†: Hanselman et al. (2003). ‡: Finlay-Moore et al. (2000). §: Nichols et al. (1997). ¶: Nichols et al. (1998). #: Shore et al. (1993).
bdl: below detectable limit.
nd: not determined.
Al: alumina

CHAPTER 3

INFLUENCE OF LAND USE ON THE CONCENTRATIONS OF ESTROGENS IN WATER AND SEDIMENT WITHIN A WATERSHED[1]

[1] Qi Luo, Paige Adams, Junhe Lu, Miguel Cabrera, Qingguo Huang, to be submitted to Environmental Science and Technology.

ABSTRACT

The concentrations of 17β-estradiol, estrone, estriol, and 17α-ethinylestradiol in stream water, influent to and effluent from a sewage treatment plant (STP) receiving wastewater from a broiler processing plant, and sediment within the Upper Satilla watershed were determined for 14 months using LC-MS. The concentrations of 17β-estradiol, estrone, estriol ranged from below detection limits to 62.0 ng L^{-1}, while 17α-ethinylestradiol was not detected in stream water samples but in the STP influent and effluent. High concentrations of estrogens were found in the suspended particles that were separated from the water samples. In sediment, the highest estrogen concentration was 5.0 ng g^{-1}. The estrogen concentrations in STP influent were constantly high. We quantitatively analyzed the temporal and spatial distribution of the estrogens in relation to rainfall, land use, and water quality parameters. The results provide useful information on how agricultural runoff and STP effluent impact the occurrence of estrogens in natural water.

INTRODUCTION

The detection of estrogens in the environment is a global issue because estrogens are ubiquitously present in the environment and can have adverse effects on wildlife and human beings (Sumpter and Johnson, 2005). Of particular concerns are βE2, E1, E3, and EE2 because they could disrupt the endocrine system at extraordinarily low concentrations (0.1 to 10 ng L^{-1}) (Thorpe et al., 2003; Tyler et al., 1998). A number of studies reported that βE2 can cause adverse effect at 10-50 ng L^{-1} for a variety of species. The lowest-observed-effect-concentrations (LOECs) for introducing vitellogenin in juvenile female rainbow trout was 3.3 ng L^{-1} for E1 and 14 ng L^{-1} for βE2 (Thorpe et al., 2003). Routledge et al. (1998) reported βE2 concentration between 1 and 10 ng L^{-1} or E1 between 25 and 50 ng L^{-1} can induce vitellogenin production in

rainbow trout. Another study reported that 30 days exposure to 200 ng L^{-1} βE2 disturbed the immunity of Japan sea bass (Thilagam et al., 2009). EE2 is even more potent than βE2. Vitellogenin induction occurred in zebrafish after exposure to 21 ng L^{-1} βE2 or 3.0 ng L^{-1} EE2 for 8 days (Rose et al., 2002). Colman et al. (2009) reported that EE2 concentration between 0.5 and 50 ng L^{-1} can reduce male zebrafish aggression and impacted their courtship behavior. The acute exposure of male zebrafish to 10 ng L^{-1} of EE2 reduced their reproductive success (Coe et al., 2008). Long term exposure (2 years) to 4 ng L^{-1} of EE2 has resulted in sex reversal in male roach and exposure of roach in early life stage to 0.3 ng L^{-1} EE2 altered the responsiveness of roach to estrogens in later life (Lange et al., 2009). The predicted-no-effect-concentrations (PNECs based on annual average) for protecting fresh water life is 0.1 ng L^{-1} for EE2, and 1 ng L^{-1} for βE2 (Young et al., 2004). Potency of E1 is 3-5 times less potent than βE2, so the PNEC for E1 is in the range of 3-5 ng L^{-1} (Routledge et al., 1998).

Published studies have reported observations of steroidal estrogens in different environmental matrices such as surface water, sewage, sediment, and soil (Isobe et al., 2003; López de Alda et al., 2002; Lai et al., 2000; Miège et al., 2009; Xu et al., 2008; Yamamoto et al., 2006). Natural and synthetic estrogens have been measured in river water worldwide ranging from < 0.1 to 2000 ng L^{-1}, and in river sediments ranging from < 0.05 to 22.8 ng g^{-1} (Petrovic et al., 2001; Petrovic et al., 2004). Numerous studies have associated abnormalities of wildlife to exposure from estrogens (Brian et al., 2006; Colman et al., 2009). By coming in contact with environmental sources of these estrogens, animals could assimilate or accumulate the estrogens in their bodies which may disrupt their growth and reproduction, alter sexual differentiation, and enhance chances of getting cancer (Kidd et al., 2007; Lai et al., 2002).

Effluent from sewage treatment plant and agricultural runoff are two major ways for estrogens to enter the environment. Estrogens remain in STP effluents because they cannot be completely removed by conventional sewage treatment systems (Hashimoto and Murakami, 2009; Johnson and Sumpter, 2001). As utilizing reclaimed water becomes more popular, the accumulation of these micropollutants in recycled water might be enhanced (Falconer et al., 2006; Loraine and Pettigrove, 2005). Various advanced treatments have been developed to remove these compounds from sewage (Auriol et al., 2007; Forrez et al., 2009; Mao et al., 2008; Pan et al., 2008).

As a nonpoint source of estrogens, runoff from agricultural field amended with animal wastes, particularly poultry litter, contributes significantly to the loading of estrogens to surface water (Nichols et al., 1998; Nichols et al., 1997; Olsen et al., 2007). Shore et al. (1993) calculated the concentrations of estrogens (14 to 65 μg kg^{-1} dry weight) and androgens (133 μg kg^{-1} dry weight) in poultry litter. In 2008, approximately 13.41 million metric tons of poultry litter was produced in the United States (Jenkins et al., 2009). Most poultry litter is applied on pasture and cropland to increase soil fertility (Jenkins et al., 2009). In the United States, poultry litter is not required to be treated prior to land application as long as it is not discharged directly to water. It thus poses a potential risk to introduce estrogens into aqueous systems through agricultural runoff. Nichols et al. (1997) found a linear relationship between βE2 concentration in agricultural runoff and poultry litter application rate and the highest βE2 concentration was 1280 ng L^{-1} when application rate was 7.05 Mg ha^{-1}. The βE2 concentrations ranged from 20 to 2330 ng L^{-1} in agricultural runoff which was from grassland amended with poultry litter (Finlay-Moore et al., 2000). Transport of estrogens from rangeland influenced by cattle grazing to surface water has been observed (Kolodziej and Sedlak, 2007). Both laboratory and field studies

have indicated the possibility of estrogens leaching from lands receiving animal wastes (Arnon et al., 2008; Kjær et al., 2007). Estrogens were detected in shallow groundwater with concentration of E1 up to 20 ng L^{-1} and βE2 up to 80 ng L^{-1} (Swartz et al., 2006; Wicks et al., 2004). The concentration of βE2 ranged from 0.05 to 1.8 g L^{-1} in farm pond which received runoff from beef cattle pastures (Irwin et al., 2001).

Once released into streams, estrogens may be diluted, degraded, or sorbed. Given their high K_{ow} values, estrogens may be sorbed to sediment or suspended particles and immobilized, thus reducing their concentration in the aqueous phase (Das et al., 2004; Lee et al., 2003). Sorption to sediment or suspended particles raises the concern that the estrogens sorbed on sediment or suspended particles may serve as a secondary source of contamination by dissipating estrogens back to the aqueous phase (Petrovic et al., 2001). While previous studies have focused on investigation of estrogens in STP sludge, limited studies have examined estrogen concentrations in suspended particles separated from natural water. Labadie and Budzinski (2005) reported that the concentration of estrogens associated with suspended particles (< 0.7μm) were lower than their detection limits (0.4-1.9 ng L^{-1}). How suspended particles impact the transport of estrogens needs careful study because at different environmental and experimental conditions, the sorption efficiency of the suspended particles is different.

There have been very few studies that investigated the occurrence of estrogens on a watershed scale (Arikan et al., 2008; Jenkins et al., 2006; Kolodziej and Sedlak, 2007). Kolodziej and Sedlak (2007) reported that estrogen concentrations were above PNECs for fish in 10 to 20% water samples impacted by rangeland grazing. Elevation of estrogen concentrations in runoff from agricultural watersheds was not significant under the condition of drought and conservation tillage (Jenkins et al., 2006). Arikan et al. (2008) conducted a short study to measure the

estrogens within a watershed for only 4 months, while my thesis describes a longer study lasting 14 months. We examined the occurrence and magnitude of four estrogens (βE2, E1, E3, and EE2) in the Upper Satilla watershed, with concentrations of estrogens in water, suspended particles, and sediment measured separately. There are over 440 poultry houses and large areas of cotton/pasture land where poultry litter was applied. Statistical analyses were conducted to probe the temporal and spatial distribution of the estrogen contaminants in relation to landuse and other environmental conditions.

MATERIALS AND METHODS

Site Description

The Upper Satilla watershed is located in southeastern Georgia and occupies a total area of 2,922 km^2 (Fig. 3.1). The study area has dense dendritic stream networks with riparian wetlands along streams. Small tributaries such as Seventeen Mile River, Hurricane Creek, and Pudding Creek flow into the Satilla River. Douglas (population 10,639) is the largest city in the study area. A large broiler processing plant was located in the city. It closed at the end of May 2009, and all the poultry houses in the Upper Satilla watershed were cleaned out before the end of May 2009. Douglas STP has a permitted discharge of 22.7 million liters per day of which about 50% was from the broiler processing plant and another 8% is from a second chicken meat processing facility (Delnee Wilcox, personal communication). Agriculture accounts for about 23% of land use in the Upper Satilla watershed, which indicates a potential impact of agricultural activities on surface water quality, especially where large amounts of animal manure (primarily poultry litter) is landspread.

We delineated the study area into smaller subwatersheds using GIS technology (ArcGIS). The delineation of Upper Satilla watershed was based on the 1998 land cover data from Georgia

GIS Clearinghouse and USGS Hydrologic Units (12-digit) dataset in Georgia (USGS/DNR, 2001). Furthermore, we estimated the area of cotton/pasture land in the subwatersheds where poultry litter was primarily applied based on the 2008 Georgia Cropland Data Layer released by USDA in 2009 (USDA-NASS-RDD, 2009).

Ten sample sites were selected to represent the subwatersheds and three more sites were collected from STP influent, STP effluent, and a pond which STP effluent passed through before entering the river (Table 3.1). Site 1 was Rocky Creek with little or no agricultural activity. It was part of a Nature Conservancy–owned property named Broxton Rocks Preserve, and was used as a control site in this study. Sites 2 and 3 were nested in subwatersheds where poultry litter was applied but few poultry houses existed. Sites 4 and 5 were located in subwatersheds where poultry litter was applied and large numbers of poultry houses existed. Sites 6 and 13 were sewage treatment plant influent and effluent, respectively. Site 7 was from a pond where the sewage treatment plant effluent passed through. Other sites were located along the main channel of the Satilla River and major tributaries. Sites 8 and 9 were on Seventeen Mile River. Sites 10 and 11 were on the Satilla River, respectively above and below the confluence with Seventeen Mile River, and Site 12 was on the main channel of the Satilla River, below the confluence of the Satilla River and Hurricane Creek. Site 1, 2, 3, 4, 5, 8, and 10 were all upstream of the sewage treatment plant while Site 7, 9, 11, and 12 were downstream of the sewage treatment plant.

Sampling and Materials

The thirteen sites were sampled monthly from February 2009 to March 2010, and some sampling events occurred following storm events. Approximately 1-L of water sample was collected in a sterile bottle from the middle of the stream channel for estrogen analysis and another 1 L for nutrient analysis. Water was analyzed for temperature (ºC), pH, dissolved oxygen

(DO, mg L^{-1}), and turbidity (nephlometric turbidity units, NTU) with a YSI® 6600 Multiparameter Sonde (YSI Inc. Yellow Springs, OH). Samples were stored on ice in the field and transported to lab refrigerators within 6 hours of collection. Water samples were immediately analyzed for chloride (Cl), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), orthophosphate (OrthoP), total phosphorus (Total P), and total nitrogen (Total N). Sediment samples were obtained from all but Site 6, 7, and 13 with a sediment coring device (Rickly Hydrologic Inc. Columbus, OH) in January, April, June, and October 2009. Sediment samples were kept frozen in a freezer at -20°C until analyzed. For analysis, the top 8-cm sediment was freeze dried followed by extraction described later in detail.

Pure E1, βE2, E3, and EE2 were obtained from Sigma–Aldrich (St. Louis, MO) to prepare analytical standards. Acetonitrile, dichloromethane, hexanes, and methanol were HPLC grade and obtained from Fisher Scientific (Pittsburgh, PA). Stock solutions of βE2, E1, E3, and EE2 (100 mg L^{-1}) were prepared in methanol and stored at 4ºC. Standard solutions were made from the stock solutions by dilution with methanol. Solid phase extraction cartridges C_{18} (6 mL, 500 mg), florisil cartridges (6 ml, 1000 mg) and silica cartridges (6 mL, 1000 mg) were obtained from Restek (Bellefonte, PA).

Analysis of Estrogens in Stream Water

Aqueous samples were first filtered through 1.0-µm pore size glass fiber filters (Millipore GF/AFPF). The filters were dried at 103°C to achieve constant weight prior to use. A few drops of 3 M sulfuric acid were added to water sample to adjust the pH below 2 to improve recovery. Each sample was then divided into two 400-mL replicate subsamples. To calculate the recovery for each estrogen, we added a 0.9-mL mixture standard solution containing each estrogen at 0.2 mg L^{-1} to a 900-mL water sample. The final concentration was 200 ng L^{-1} for each estrogen

in the spiked samples. And the spiked samples were subjected to the same sample treatment and analysis procedure as described below. The recovery for each estrogen is calculated by the measured concentration of this estrogen in the spiked sample minus its concentration measured in the sample without spiking and then divided by the added concentration of this estrogen.

Before loading aqueous samples into C_{18} cartridges for extraction, the cartridge was conditioned with 3 mL of methanol, 5 mL of deionized water, and a final 5 mL aliquot of deionized water. A 400-mL water sample was loaded onto the solid phase extraction cartridge and vacuum applied to give a flow rate of approximately 5 mL min^{-1}. After the sample was loaded, the cartridge was washed with 5 mL of water and dried for 5 min. Each solid phase extraction cartridge was kept in a freezer at -20^{o}C until eluted. The estrogens were eluted into a 10-mL test tube using 5 mL of methanol. The test tube was placed in a 40^{o}C water bath and blown dry under a gentle stream of nitrogen gas. The sample was then reconstituted in 1 mL dichloromethane/ hexanes (v/v, 1:1).

The extracts were then loaded onto florisil cartridges for cleanup. This cleanup method was reported by Chang et al. (2009). The cartridges were conditioned with 5 mL dichloromethane/methanol (v/v, 4:1) and then dichloromethane/hexanes (v/v, 1:1) prior to use. After loading, the cartridges were rinsed with 10 mL of dichloromethane/hexanes (v/v, 1:1), and the estrogens were eluted using 6 mL dichloromethane/methanol (v/v, 4:1). The solutions were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 ml methanol.

A LC-MS procedure was used to determine estrogen concentrations. An HPLC (Waters Corp, Alliance 2690) with an Ascentic C_{18} reversed phase column (250 × 4 mm, 5 μm; Supelco, St. Louis, MO) was utilized to separate the estrogens. A mass spectrometer (Waters Micromass QuattroMass) with an electrospray source operated in the negative ion mode was used to detect

the estrogens. The selected ion monitoring mode was used to quantify accurate concentrations of estrogens. The LC-MS parameters were optimized. The capillary voltage was -2.98 kV, cone voltage was -53 kV, the source temperature was 100^{o}C and desolvation temperature was 300^{o}C. The nebulizer was set at 36 L h^{-1} and desolvation gas was set at 367 L h^{-1}. The HPLC gradient consisted of water and acetonitrile at flow rate of 0.3 ml min^{-1}. Acetonitrile was 35% at 0 min and increased linearly to 65% at 21 min, held at 65% for 9 min, and increased to 100% at 31 min, then held at 100% for 4 min. A 4-min equilibration at 35% of acetonitrile was used at the end of each run.

Analysis of Estrogens in Sediment

A 5-g freeze dried sediment sample was extracted with 10 mL extraction solvent dichloromethane/methanol (v/v, 2:1) in a 30-mL glass tube. The slurry was placed in an ultrasonic bath (Cavitator Ultrasonic Cleaner) for 30 min, and then centrifuged at 3000×g for 5 min. The supernatant was collected, and the extraction procedure was repeated twice with 5 mL extraction solvent. The three extracts were combined and evaporated under a stream of nitrogen gas in a 40^{o}C water bath, then reconstituted in 10 mL hexanes.

The extract was transferred to a commercial silica cartridge that had already been rinsed with 5 mL hexanes. After the extract passed through the cartridge, 30 mL of hexanes was used to wash out the impurities. The cartridge was then eluted with 5 mL of methanol to collect the target compounds. The eluant was completely evaporated and reconstituted in 1 mL of methanol. The recovery of this method was assessed in a standard addition procedure by spiking 0.3 μg mixture standard containing each estrogen into 3-g sediment. The spiked sediment was treated using the same procedure described above.

Estimation of K_d Values

For those samples with estrogens detected in both aqueous and solid phases, the sorption coefficient K_d was estimated by dividing the concentrations of estrogens sorbed to suspended particles (ng kg^{-1}) by the dissolved concentration in aqueous phase (ng L^{-1}), and $LogK_d$ values thus obtained were compared with results reported by other studies.

Data Analysis

The samples were analyzed in two groups. Group 1 contains Site 6, 7, 13 which were components of STP. The remaining sites were considered as Group 2 which was associated with agricultural activities. A general linear model one way ANOVA with significant differences (SAS; P=0.05) test followed by a least significant difference (LSD) test was used to compare the estrogen concentrations among sites and months and to compare water quality parameters over sites. Again, the program described above was used to determine the impact of closure of poultry houses and broiler processing plant on the occurrence of estrogen and water quality parameters within the watershed. Correlations between estrogen concentrations and rainfall and water quality parameters were analyzed by correlation analysis (SAS). A simple linear regression was fitted between certain water quality parameters and estrogen concentrations for Site 8 and 11 in which estrogen concentrations were detected above the detection limits for at least 5 sampling events. In linear regression analysis, the estrogens sorbed to the suspended particles were taken into account when analyzing the regression between estrogen concentrations in water and water quality parameters.

RESULTS AND DISCUSSION

Analytical Method Performance

A total of 166 water samples, 166 suspended particle samples, and 32 sediment samples were analyzed from February 2009 to March 2010. The detection limits and recoveries for water

and solid samples were summarized in Table 3.2. Satisfactory recoveries were obtained for four estrogens in both water and solid samples. For water samples, the recovery ratio was 66%±5.9 for E1, 96%±8.7 for βE2; 68%±6.6 for E3, and 98±8.0 for EE2. In sediment and suspended particles, the recovery ratios were 89%±4.9, 101%±2.0, 74±2.6%, and 91%±7.8 for E1, βE2, E3, and EE2, respectively. The relatively small standard deviations indicated good reproducibility. Detection limits were calculated by standard deviations obtained through analyzing 20 blanks. For water samples, the detection limits (DLs) were at ng L^{-1} level (DL_{E1}=0.2 ng L^{-1}, $DL_{\beta E2}$=0.8 ng L^{-1}, DL_{E3}=0.6 ng L^{-1}, DL_{EE2}=1.0 ng L^{-1}). The detection limits for solid samples were 0.04, 0.16, 0.12, and 0.2 ng g^{-1} for E1, βE2, E3, and EE2, respectively. Target compounds were identified by comparing the retention times with the standard solution containing the mixture of the four target compounds. Fig. 3.2 shows a typical chromatograph for E1, βE2, E3, and EE2, respectively. Quality control samples include field blanks, trip blanks, and method blanks that were prepared along each sampling time, which were analyzed together with all other samples that were collected at the same sampling time. During LC-MS analysis, a mixture standard solution containing each chemical at 10 μg L^{-1} and one solvent blank were measured after runs of every 10 samples.

Estrogen Levels in the Environment

The concentrations of estrogens in water, suspended particles, and sediment samples are summarized in Table 3.3 to Table 3.5. In most samples, estrogen concentrations were below the detection limits. Estrogen concentrations above the detection limits were found in 23 out of 124 stream water samples (excluding STP related samples at Site 6, 7, and 13) with maximum concentrations of E1, βE2, and E3 up to 8.1, 62.1, and 34.4 ng L^{-1}, respectively. EE2 was never detected in the stream water samples. Estrogens were detected in STP influent samples (Site 6)

with concentrations ranged from 2.9 to 46.4 ng L^{-1} for E1, < 0.8 to 67.2 ng L^{-1} for βE2, 4.6 to 124.9 ng L^{-1} for E3, and < 1.0 to 8.6 ng L^{-1} for EE2. In the STP effluent (Site 13), the concentrations of estrogens were much lower than the influent ($P < 0.05$). High concentrations of estrogens were found to be sorbed on the suspended particles that were separated from the water samples. In suspended particles, the highest concentration was 554.5 ng g^{-1} for E1, 3832.3 ng g^{-1} for βE2, and 676.1 ng g^{-1} for E3, but EE2 concentrations were below detection limit for all suspended particle samples from stream water. Concentrations of estrogens in sediment are much lower than in suspended particles, with the highest concentrations of E1 and E3 at 2.1 ng g^{-1} and 4.9 ng g^{-1}, respectively, while no βE2 and EE2 were detected.

The PNECs are calculated values based on the dataset of LOECs (Young et al., 2002). Generally, the maximum acceptable toxicant concentrations (MATC) were calculated based on the LOEC values. The PNEC equals to MATC (8.5-11.1 ng L^{-1} for E2 and 0.57 ng L^{-1} for EE2) divided by a safety factor which varied with chemicals (5 for E2 and 10 for EE2) (Young et al., 2002). Our data showed that the natural estrogens were present at tens ng L^{-1} level in many stream water samples which were much higher than the PNECs for wildlife in water (0.1 ng L^{-1} for EE2, 1 ng L^{-1} for βE2, and 3-5 ng L^{-1} for E1). Similar estrogen levels in surface water have been reported by many earlier studies. In an extensive survey in the U.S., the maximum concentrations of βE2 and E1 in the surface water were found to be at 200 ng L^{-1} and 112 ng L^{-1} respectively (Kolpin et al., 2002). Even in shallow ground water, 80 ng L^{-1} of βE2 was identified (Wicks et al., 2004). The occurrence of βE2 (0.5-17 ng L^{-1}) in a drinking water source was documented by Benotti et al. (2008).

Although the control site (Site 1) was relatively pristine, estrogens were still detected in the March, 2009, storm sample while it was not observed in any of the other 13 samples. The

estrogens detected following the Marchstorm event were possibly from an incidental excretion by wildlife such as deer, hog, resident duck, and aquatic fauna (fish and turtle). Similar observations have been reported in other studies (Irwin et al., 2001; Kolodziej et al., 2004; Shore and Shemesh, 2003). High concentrations of *E. coli* and *fecal coli* were also measured in the same sample which further supports our assumption (Appendix C). The very low streamflow (accordingly low dilution) may also be responsible for the high concentrations of estrogens at this site. A study reported that only one cattle excretion could elevate the concentrations of estrogens above PNECs to fish in a stream with flow rate of 0.012 $m^3 s^{-1}$ (Kolodziej and Sedlak, 2007). Verification of this assumption would require long term monitoring at this site.

Estrogens were frequently detected at certain sites in both stream water and suspended particles (Fig. 3.3). For example, estrogens were found in 1/3 of stream water samples and 1/4 of suspended particle samples collected from Site 4. At Site 8, estrogens were detected in about 39% stream water samples with concentrations ranging from 7.06 to 25.98 ng L^{-1} and in 14.3% suspended particle samples with concentration ranging from 7.8 to 280.4 ng g^{-1}. Estrogens were present in more than 1/4 samples collected at Site 10 which was the downstream of Site 4. Higher concentrations of estrogens were observed at Site 11 (8.10 to 44.17 ng L^{-1} in stream water samples and below detection limits to 276.8 ng g^{-1} in suspended particles samples). Site 11 is the confluence of Site 9 and 10 and estrogens were found in 33.3% of stream water samples collected from Site 11.

The repetitive detections of estrogen concentrations above PNECs in the same sampling sites implicate a concern because continuous exposure from such high concentrations could result in adverse effect to animals. For some samples, more than two natural estrogens were simultaneously presented in stream water samples. Earlier studies have shown that multiple

estrogens together might cause more severe consequences for animals than individually (Bergeron et al., 1999; Brian et al., 2006; Routledge et al., 1998; Thorpe et al., 2003). Brian et al. (2006) reported that male fish exposed from mixture of estrogens exhibited higher vitellogenin level than any other treatment only exposure to individual estrogen. Routledge et al. (1998) also pointed out that exposure to a combination of 25 ng L^{-1} E1 and 25 ng L^{-1} βE2 for 3-week resulted in higher, despite not significantly, vitellogenin production than exposure to 100 ng L^{-1} E1 alone in rainbow trout. Since high concentrations of estrogens were detected in suspended particles at these sits, desorption of estrogens from suspended particles might be another problem. Though there is no direct information about the estrogenic activities of suspended particles sorbed with estrogens, the release of sorbed estrogens to the aqueous phase could occur during the transport of suspended particles downstream to water bodies having lower aqueous phase estrogen concentrations.

Site 12 is the only site among all sites where estrogens have not been detected even once either in water, suspended particles, or sediment. Site 12 is the most downstream site with the highest flow rate and further away from direct estrogen contamination sources or agricultural lands. Estrogens might have been diluted or degraded during transport. This suggests that estrogens can be effectively dissipated during transport, to reduce their concentrations to below PNEC levels. Moreover, this also indicates that based on the results of this study, the net export of estrogens from the Upper Satilla watershed to downstream areas is minimal during the study period.

Impact of Land Use on Estrogen Concentrations

The mean concentrations for each estrogen was calculated by averaging those measured at all sites except sites 6, 7, and 13 for each month (hereafter, referred to as means by month), or

those measured in all sampling months for each site (hereafter, means by site). Sites 6, 7 and 13 are excluded because they are closely associated with STP and will be analyzed together in later sections. Table 3.6 summarizes P values from Analysis of variance (ANOVA) on the means by month and the means by site. The results do not indicate statistically significant differences ($P > 0.05$) among the means by site, regardless of the sample matrices (water, particles or sediment), whereas significant differences can be found between the means by month for E1 and βE2 in water samples.

The fact that no significant differences were found between sites is interesting. It suggests that the relatively different landuses (e.g. agricultural vs. poultry) among different subwatersheds did not make a significant difference in estrogen concentrations among these subwatersheds.

Several factors need to be taken into account when interpreting the above results from statistical analysis. First, the means by site are calculated by averaging estrogen concentrations over 14 months. Our analysis in Table 3.6 and discussion in later sections suggest a significant influence of time-dependent factors (i.e., rainfall events, closure of poultry houses) on the measured estrogen concentrations. The large variance associated with time could have obscured the variance, if any, with sites. Our data do not support a statistical analysis independently on time and site factors. Second, although there is relative difference in landuses among different subwatersheds, it is indeed not clearly defined. For example, cotton/pasture lands are scattered over the entire watershed, and poultry houses are also present in most areas despite numbers and density may be different. So all the sampling sites excepted Site 1 have potential of being impacted by poultry litter land application.

Estrogen Concentration Variation with Time

The 14-month study period gave us an opportunity to monitor the variation of estrogens in the environment over long time. There are significant differences in the means of estrogen concentrations by months (Table 3.6). Occurrence of high estrogen concentrations coincided with heavy rainfall events (Fig. 3.4). When heavy rainfall was experienced in March, May, June, July, and August 2009, elevation of estrogen concentrations in the watershed was observed. The occurrence of βE2 in surface water correlated fairly well with the rainfall amounts at Site 1, 2, 3, and 10 by regression analysis (Table 3.7). However, regression analysis did not show a significant correlation ($r < 0.3$) between the sums of all estrogen concentrations at all sites with rainfall amounts, indicating that rainfall alone is not sufficient to justify all time-dependent variations.

Another factor which could have contributed the time-dependent variation in estrogen concentrations is the closure of poultry houses. All poultry houses in Upper Satilla watershed were closed at the end of May 2009 due to the closure of the broiler processing plant. In order to address the impact of this closure, we grouped February to August 2009 as pre-closure time and from September 2009 to the end of study (March 2010) as post-closure time. The borderline was chosen in August because the closure occurred in May and it has been reported that impacts of animal waste land application can last up to 3 months (Kjær et al., 2007). The means of the sum of each estrogen during pre-closure and post-closure times are presented in Figure 3.5 with 95% confidence intervals. It is evident that the concentrations of E1 and βE2 are significantly different before and after the closure event, which is confirmed by ANOVA (Table 3.8). This indicates that poultry activity and/or land application can influence the estrogen concentrations at a significant level. It is to be noted that almost no estrogen was detected in any stream water samples after November 2009, which is 6 months after the closure event.

Our data collected from May to August represent a worst case scenario because all the poultry houses were emptied around that point of time due to the closure of the broiler processing plant. Farmers likely applied all the poultry litter resulting from cleanout of the last flocks to the surrounding agricultural land just prior to closure of the processing plant. The one time release of large amount of poultry litter into agricultural land would increase the possibility for estrogens entering surface water with agricultural runoff as estrogen concentrations in agricultural runoff increased with application rate of poultry litter (Nichols et al., 1997). In June samples, which were collected after a rainfall event (28.3 mm), estrogen concentrations above the PNECs were observed at Site10 (2.3 ng L^{-1} βE2) and 11 (8.6 ng L^{-1} βE2). The impact of over loading of poultry litter to agricultural land lasted over 3 months. Through August, estrogens were still detected above the PNECs in 5 out 10 stream water samples.

Estrogen Concentrations and Water Quality Parameter

Many water quality parameters were measured and documented (Appendix C). The results of ANOVA suggested there is no significant difference among sites in temperature, DO, pH, turbidity, solid, and total P, whereas NO_3-N, chloride, NH_4-N, orthophosphate, and total N changed with sampling sites (Table 3.9). The concentration of NO_3-N was higher in Site 3, 4, 5 than Site 9, 11, 8, 12, and 2 ($P < 0.05$). Total N concentrations ranged from 0.62 to 1.26 mg L^{-1}. Site 1 contains lowest mean concentration of total N while Site 4 was constantly above 1 mg L^{-1} ($P < 0.05$). The comparison of water quality parameters (Table 3.9) between pre-closure time (February to August 2009) and post-closure time (September 2009 to March 2010) reveals that the NO_3-N, NH_4-N, and total N were lower in post-closure samples than in pre-closure samples ($P < 0.05$).

Poultry litter contains a significant amount of phosphorus, part of which is present in orthophosphate form. Phosphate tends to attach to solid soil particles, so it tends to be retained by soil. However, when litter is applied on the surface of pasture, contact between litter and soil is limited, and the phosphates can be carried into stream waters with storm runoff. Soil erosion of poultry litter amended fields can potentially introduce a considerable amount of particulate phosphate to streams (Pierson et al., 2001). Our regression analysis shows that estrogen concentrations seemed to increase as orthophosphate concentration increases at Site 8 (R^2=0.9) and 11 (R^2=0.45) (Fig. 3.6). Animal wastes contain high concentration of NH_4-N that can easily transfer into water with runoff. The relationship between NH_4-N and estrogen concentrations was found at 11 (R^2=0.62) but not at Site 8 (R^2=0.38) (Fig. 3.6). It needs to be noted that the above regression analysis only include those data above detection limits. If we included estrogen concentrations below detection limits into the regression analysis, no linear relationship was found between any water quality parameters and estrogen concentration. Therefore, whether orthophosphate or NH_4-N can be used as indicators of estrogen concentration cannot be determined based on the data we collected.

Estrogens in STP Influent and Effluent

Estrogens detected in all STP influent samples (Site 6) were constantly high. It is the only site which was significantly different from the other sites ($P < 0.05$). Estrogen concentrations in STP influent was in the same magnitude (several $\mu g\ L^{-1}$ to a few $ng\ L^{-1}$) reported by previous papers (Lee et al., 2004; Solé et al., 2000; Ternes et al., 1999b; Wicks et al., 2004; Williams et al., 2003). However, in contrast to previous results which showed E1 was usually the predominant compound, our analysis revealed that concentrations of βE2 and E3 were higher than E1 in the STP influent. Several studies reported similar trend as ours. Clara et al. (2005)

found the concentration of βE2 was higher than E1 in several STP influents. Viglinò et al. (Viglinò et al., 2008) also reported a similar trend (243 ng L^{-1} of E3, 125 ng L^{-1} of βE2, and 80 ng L^{-1} of E1) in STP influent in Montreal, Canada. They proposed that the low temperature during sampling time hindered the biodegradation of βE2 to E1.

We analyzed how the closure of broiler processing plant may influence the estrogen concentrations in STP influent by conducting ANOVA on the means of total estrogen concentration for pre-closure time vs that for post-closure time (Table 3.8). This result did not show significant difference ($P > 0.05$), which may suggest that the contribution of the poultry processing plant to the large amounts of estrogens in STP influent is limited. The fact that EE2 was consistently detected in STP influent samples indicates that the estrogens in these samples may be primarily of human origin.

Estrogens were also measured in STP effluent (Site 13). The concentration of E1, βE2, and E3 were significantly lower in effluent than in influent ($P=0.046$ for E1, $P=0.0012$ for βE2, $P= 0.0001$ for E3, and $P=0.0749$ for EE2) while the removal efficiency for EE2 was low (Fig. 3.7). The highest concentrations of estrogens in the effluent were 11.79 ng L^{-1} for E1, 19.78 ng L^{-1} for βE2, 7.73 ng L^{-1} for E3, and 5.69 ng L^{-1} for EE2. The concentrations in STP effluent were in the same range reported by some other studies (Desbrow et al., 1998; Gentili et al., 2002). The removal efficiency of a STP is influenced by numerous parameters, including microbial activity, temperature, and rainfall (Rodgers-Gray et al., 2000; Ternes, 1998). In this study, the detection of estrogens in effluent seems to coincide with 6 rain events in March storm, June, July, August, November, and December 2009. For other months, the concentrations of βE2, E1, and E3 in effluent were below detection limit, while the removal efficiencies dropped to 64.25%±17.12% for E1, 63.8%±20.4% for βE2 in heavy rainfall months (March storm, June,

July, August, November, and December 2009). Strong correlations were observed between rainfall and E1, βE2, and E3 concentration detected in the STP effluent (Table 3.10). This coincidence suggests heavy rainfall could impact the sewage treatment efficiency by increasing flow rate and consequently reducing the retention time as well as washing more pollutants to sewage influent than normal days.

On two occasions, elevation of E1 (November 2009) and EE2 (September 2009) were found after the sewage treatment process. Similar results were observed by Clara et al (2005). E1 concentration was higher in effluent than influents in 4 of 30 STP samples (Baronti et al., 2000). Carballa et al. (2007) detected higher mass fluxes of E1 in output than input of STP. There are two possible explanations for this phenomenon. One is the transformation of βE2 to E1 and E3 during the treatment process (Andersen et al., 2003; Williams et al., 2003). Another is the deconjugation of conjugated estrogens. Estrogens are indeed primarily excreted in conjugated form (D'Ascenzo et al., 2003; Reddy et al., 2005). The conjugated estrogens may serve as precursors and generate free estrogens with the help of microorganisms in the treatment systems (D'Ascenzo et al., 2003; Isobe et al., 2003; Ternes et al., 1999a; Tyler and Routledge, 1998).

The K_d Values and Comparison with Literature

The $LogK_d$ values are listed in Table 3.11. No $LogK_d$ information for EE2 was determined since the concentration of EE2 in the suspended particles was too close to the detection limit. The averaged $LogK_d$ values for E1 (3.55±0.50) are in good agreement with other studies, while the $LogK_d$ values for βE2 and E3 were 2- to 3-fold higher than some other studies (ref), but are close to the results reported by Carbella et al. and Braga et al. (2005). Variation in hydrologic conditions, characteristics of suspended particles, and stream water states may cause heterogeneity in $LogK_d$ from different studies. The sorption of estrogens to sediments has been

shown to increase with the increase of total organic carbon and salinity of aqueous phase (Lai et al., 2000). The initial concentrations of estrogens also affected the adsorption coefficient (Ying et al., 2003). Joss et al. (2004) observed that when the initial estrogen concentration was around 1 to 50 ng L^{-1}, the K_d values were 2 to 3 factor greater than the K_d values measured under 1.7 μg L^{-1} of estrogens. In our study, the estrogen concentrations were generally within the 1-50 ng L^{-1} range.

Estrogen Concentrations in Sediment

The concentrations of estrogens in sediment were in a range of < 0.04 to 2.4 ng g^{-1} for E1 and < 0.12 to 5.0 ng g^{-1} for E3, whereas no βE2 and EE2 were ever detected (Appendix B). These concentrations were in agreement with other reports (Isobe et al., 2006; López de Alda et al., 2002; Labadie and Hill, 2007). Viganò et al. conducted a study of estrogens in river sediment which showed that E1 and E3 were identified whereas βE2 and EE2 were not detected (Viganò et al., 2008). Jürgens et al. (Jürgens et al., 2002) demonstrated that biodegradation of βE2 occurred in sediment under both aerobic and anaerobic condition. The very low estrogen concentration suggested that sediment is not a sink for estrogens, and corroborates that effective dissipation occurs to estrogens in natural waters.

CONCLUSIONS

In most samples, estrogens concentrations were below the detection limits of this study. Samples with concentrations above the detection limits ranged in the levels of ng L^{-1} in water or subng g^{-1} in sedimentswhich is comparable to many other studies examining general surface waters. Significant differences were found in estrogen concentrations before and after the closure of the broiler processing plant and houses. And the decrease in frequency of detection and low concentration after closure indicate that watershed can effectively recover. These facts suggest

poultry litter land application in a watershed with a dense population of poultry houses could impact the occurrence of estrogens in surface water. However, ANOVA did not show statistically significant differences among the site means of estrogen concentrations averaged over 14 months for each site. This suggests that the occurrence of estrogens in surface water is not significantly impacted by factors such as number of poultry houses and area of landuse. ANOVA indicated significant differences in the means of estrogen concentrations by month. Rainfall is an important factor contributing to the time-dependent variation Concentrations at certain sites were frequently present at levels beyond PNECs immediately after rainfall events. Such pulses of high estrogen concentrations may cause acute toxicity to vulnerable species, which represents a point of concern from a management point of view.

At the most downstream site (Site 12), estrogen was not detected in any of the matrices evaluated during this study. This, in combination with very rare and low-level detections of estrogens in sediment samples (< 0.4-5.0 ng g^{-1}), indicates effective dissipation of estrogens. Effective dissipation downstream from this dense area of poultry farming indicates the Upper Satilla watershed would not serve as an estrogens source to downstream areas.

The sites associated with the STP had higher concentrations of estrogens, and these estrogens are likely primarily of human origin, while the contribution of broiler processing plant was limited. The STP was effective in reducing estrogen concentrations, but high estrogen concentrations still occurred at times, particularly following heavy rainfall events. Advanced treatment may be required to ensure consistent complete removal of estrogens from the wastewater.

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Table 3.1. Description of sampling sites in the Upper Satilla watershed[†]

Site No.	Stream Name (Sampling location)	Area (km^2)	Upstream/ Downstream To STP	Poultry Houses Number	Agricultural[‡] Land use (%)	Forest[§] Land use (%)	Other[¶] Land use (%)
1	Rocky Creek (control site)	9.10	Upstream	0	5	83	12
2	Little Hurricane (County Rd 552)	65.37	Upstream	0	22	65	13
3	Upper Satilla Creek (Whitley Rd)	45.17	Upstream	0	52	31	17
4	Pudding Creek (US Hwy 441)	204.4	Upstream	50	30	52	18
5	Hurricane Creek (GA Hwy 32)	142.0	Upstream	35	20	67	14
6	Influent to STP (pipeline)	na[#]	na[#]	na[#]	na[#]	na[#]	na[#]
7	Pond	na[#]	Downstream	0	na[#]	na[#]	na[#]
8	Seventeen Mile River 1 (GA Hwy 32)	433.5	Upstream	98	33	50	17
9	Seventeen Mile River 2 (GA Hwy 158)	462.1	Downstream	98	32	51	17
10	Satilla River 1 (GA Hwy 64)	927.1	Upstream	173	32	51	17
11	Satilla River 2 (GA Hwy 158)	2306	Downstream	368	25	57	17
12	Satilla River 3 (GA Hwy 1)	2922	Downstream	440	23	59	18
13	Effluent from STP (Sear Rd)	14.54	Downstream	0	23	45	33

† Landcover is based on 1998 land use classification (Georgia GIS Clearinghouse)

‡ Includes row-crops and pasture land

§ Includes deciduous, evergreen, mixed, forested wetlands

¶ Includes urban, open water, transportation, utility, vegetation, and golf courses

Not applicable

STP Sewage treatment plant

Table 3.2. Detection limits and analytical recoveries of estrogens in water and solid samples (sediment and suspended particles)

Compound	DL_w	DL_s	Recovery $_w$	Recovery $_s$
	ng L^{-1}	ng g^{-1}	%±SD	%±SD
E1	0.200	0.0400	66±5.9	89±4.9
βE2	0.800	0.160	96±8.7	101±2.0
E3	0.600	0.120	68±6.6	74±2.6
EE2	1.00	0.200	98±8.0	91±7.8

DL: detection limit. W: water sample. S: solid sample. SD: standard deviation.

Table 3.3. Minimal, maximum, and mean concentration of each estrogen in water samples for each site

Site	Water sample											
	E1 ng L^{-1}			βE2 ng L^{-1}			E3 ng L^{-1}			EE2 ng L^{-1}		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Site 1	< 0.2	-	-	< 0.8	62.0	6.16	< 0.6	-	-	< 1.0	-	-
Site 2	< 0.2	-	-	< 0.8	11.2	1.75	< 0.6	-	-	< 1.0	-	-
Site 3	< 0.2	-	-	< 0.8	21.9	4.65	< 0.6	-	-	< 1.0	-	-
Site 4	< 0.2	3.09	0.315	< 0.8	21.9	4.00	< 0.6	-	-	< 1.0	-	-
Site 5	< 0.2	-	-	< 0.8	-	-	< 0.6	-	-	< 1.0	-	-
Site 6	< 0.2	46.4	14.3	< 0.8	67.2	25.4	4.6	125	51.0	< 1.0	8.61	2.69
Site 7	< 0.2	7.72	1.41	< 0.8	7.32	1.29	< 0.6	-	-	< 1.0	-	-
Site 8	< 0.2	3.71	0.889	< 0.8	15.5	2.52	< 0.6	23.0	3.36	< 1.0	-	-
Site 9	< 0.2	8.09	0.752	< 0.8	-	-	< 0.6	-	-	< 1.0	-	-
Site 10	< 0.2	3.27	0.349	< 0.8	36.5	4.32	< 0.6	-	-	< 1.0	-	-
Site 11	< 0.2	2.25	0.459	< 0.8	22.7	4.05	< 0.6	34.4	3.27	< 1.0	-	-
Site 12	< 0.2	-	-	< 0.8	-	-	< 0.6	-	-	< 1.0	-	-
Site 13	< 0.2	11.8	3.09	< 0.8	19.8	3.04	< 0.6	7.73	0.942	< 1.0	5.69	1.41

Table 3.4. Minimal, maximum, and mean concentration of each estrogen in suspended particle samples for each site

Site	Suspended Particles											
	E1 ng g^{-1}			βE2 ng g^{-1}			E3 ng g^{-1}			EE2 ng g^{-1}		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Site 1	< 0.04	554	50.4	< 0.16	19555	1778	< 0.12	-	-	< 0.2	-	-
Site 2	< 0.04	-	-	< 0.16	939	104	< 0.12	676	75.2	< 0.2	-	-
Site 3	< 0.04	8.03	0.830	< 0.16	297	40.3	< 0.12	52.5	18.9	< 0.2	-	-
Site 4	< 0.04	125	9.94	< 0.16	3.832	266	< 0.12	-	-	< 0.2	-	-
Site 5	< 0.04	26.2	3.30	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 6	< 0.04	15.0	2.37	< 0.16	105	17.5	< 0.12	26.4	3.15	< 0.2	-	-
Site 7	< 0.04	280	22.2	< 0.16	182	21.5	< 0.12	-	-	< 0.2	-	-
Site 8	< 0.04	-	-	< 0.16	247	18.3	< 0.12	167	12.0	< 0.2	-	-
Site 9	< 0.04	32.7	2.54	< 0.16	200	15.5	< 0.12	-	-	< 0.2	-	-
Site 10	< 0.04	27.5	1.86	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 11	< 0.04	-	-	< 0.16	277	18.6	< 0.12	-	-	< 0.2	-	-
Site 12	< 0.04	-	-	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 13	< 0.04	45.3	3.27	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-

Table 3.5. Minimal, maximum, and mean concentration of each estrogen in sediment samples for each site

Site	Sediment											
	E1 ng g^{-1}			βE2 ng g^{-1}			E3 ng g^{-1}			EE2 ng g^{-1}		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Site 1	< 0.04	-	-	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 2	< 0.04	2.41	1.22	< 0.16	-	-	< 0.12	4.95	2.5	< 0.2	-	-
Site 3	< 0.04	0.17	0.08	< 0.16	-	-	< 0.12	0.97	0.4	< 0.2	-	-
Site 4	< 0.04	1.06	0.29	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 5	< 0.04	-	-	< 0.16	-	-	< 0.12	1.74	1.3	< 0.2	-	-
Site 6	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Site 7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Site 8	< 0.04	1.13	0.3	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 9	< 0.04	0.32	0.1	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 10	< 0.04	-	-	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 11	< 0.04	-	-	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 12	< 0.04	-	-	< 0.16	-	-	< 0.12	-	-	< 0.2	-	-
Site 13	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: no sample.

Table 3.6. P values result from ANOVA on the means of estrogen concentrations over all sites for each month (means by month) or those over all months for each site (means by site)

Chemical	P-value					
	Stream water		Suspended Particles		Sediment	
	Month	Site	Month	Site	Month	Site
E1	0.009*	0.473	0.558	0.414	0.380	0.284
βE2	0.0410*	0.568	0.579	0.392	nd	nd
E3	0.417	0.325	0.477	0.192	0.555	0.127
EE2	nd	nd	nd	nd	nd	nd

*: significant difference at the 0.05 probability level. nd: not determined.

Table 3.7. Results of regression analysis between rainfall amounts and estrogen concentrations at selected sites

Site	Chemical	24 h		72 h		120 h	
		R	Pr > F	R	Pr > F	R	Pr > F
Site 1	βE2	0.900	<0.0001	0.870	<0.0004	0.820	<0.0019
Site 2	βE2	0.910	0.0008	0.880	0.0017	0.820	0.0060
Site 3	βE2	0.530	0.115	0.700	0.024	0.780	0.00790
Site 10	βE2	0.820	0.0002	0.810	0.0002	0.750	0.00130

R: correlation coefficient. Pr > F: probability.

Table 3.8. P values result from ANOVA on the means of the estrogen concentrations summed for pre-closure against that for post-closure periods

Event	p-value			
	E1	βE2	E3	EE2
Broiler processing Plant[#]	0.248	0.0503	0.659	0.439
Poultry Houses[$]	0.0225*	0.00650*	0.934	nd

*: significant difference at the 0.05 probability level.
#: the sum of concentrations at Sites 6, 7, and 13, reflecting the influence of broiler processing plant.
$: the sum of concentrations at Site 1, 2, 3, 4, 5, 8, 9, 10, 11, and 12, reflecting the influence of poultry houses.
nd: not determined.

Table 3.9. P values result from ANOVA on the means of water quality parameters over all months for each site and estrogen concentrations summed for pre-closure against that for post-closure periods

P	T	DO	pH	Turb	Solid	NO_3-N
Site	0.0772	0.980	0.627	0.524	0.569	0.0012*
Closure	< 0.0001*	0.0827	0.9883	0.0577	0.672	0.0001*

P	Cl	OrthoP	NH_4-N	Total P	Total N
Site	0.00650*	0.0482*	0.0252*	0.123	< 0.0001*
Closure	0.0624	0.681	< 0.0001*	0.0521	< 0.0001*

*: significant difference at the 0.05 probability level. T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen Total P: total phosphorus. Total N: total nitrogen.

Table 3.10. Correlation coefficient (R) from linear regression analysis of estrogen concentrations in sewage treatment plant effluent against 24 hours, 72 hours, and 120 hours rainfall amount before sampling date

Chemical	Rainfall					
	24 h		72 h		120 h	
	R	Pr > F	R	Pr > F	R	Pr > F
E1	0.800	0.0006	0.800	0.006	0.710	0.0042
βE2	0.320	0.270	0.370	0.190	0.340	0.230
E3	0.890	< 0.0001	0.866	< 0.0001	0.820	0.0003
EE2	-0.220	0.450	-0.290	0.320	-0.230	0.430

Pr > F: probability

Table 3.11. LogK_d values estimated in this study and reported by other references

Estrogen	Our study	Koh et al.†	Carballa et al.‡	Braga et al.§	Joss et al.¶	Holthaus et al.#
E1	2.63-4.03	1.99-2.53	2.77-2.90	2.73	2.90-3.00	nd
βE2	2.39-5.50	1.11-2.78	4.02-4.54	~3.50	nd	1.32-2.09
E3	2.68-3.86	1.46-2.79	nd	nd	nd	nd
EE2	nd	2.00-3.35	nd	nd	nd	1.28-2.41

†: Koh et al. (2009). ‡: Carballa et al. (2007). §: Braga et al. (2005). ¶: Joss et al. (2004).
#: Holthaus et al. (2002). nd: not determined.

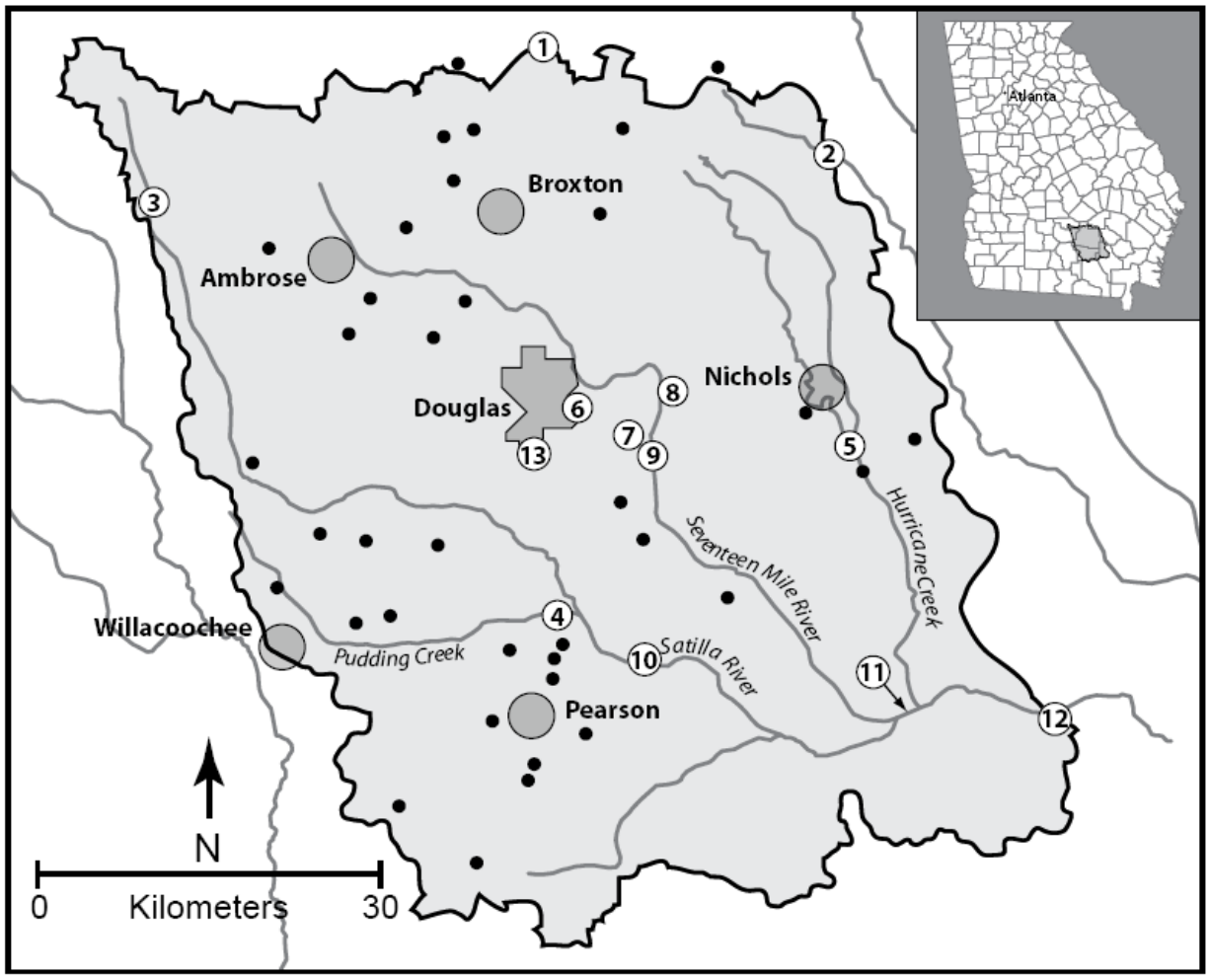

Fig. 3.1. Map of Upper Satilla watershed study area

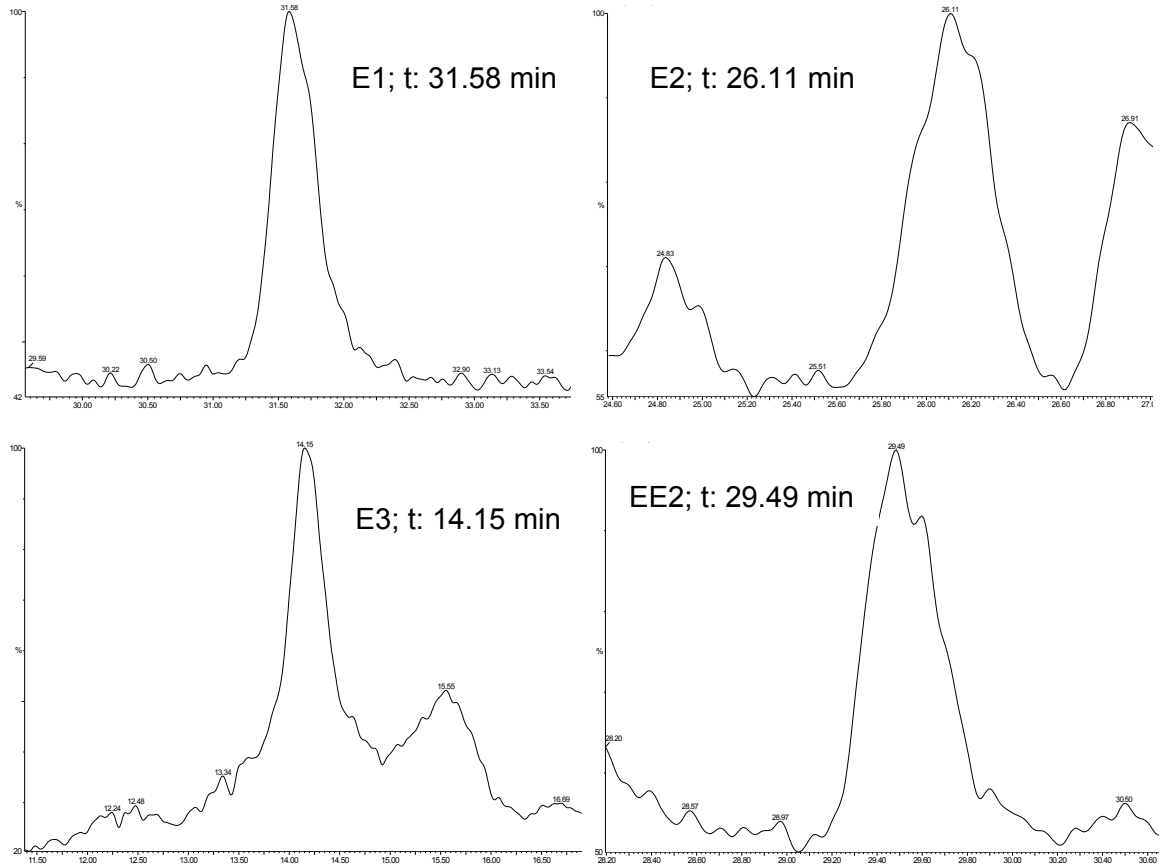

Fig. 3.2. Typical chromatograph and retention times of E1, βE2, E3, and EE2. The concentration of E1 is 12.73 ng L^{-1}, βE2 is 8.22 ng L^{-1}, E3 is 23.73 ng L^{-1}, and EE2 is 7.63 ng L^{-1} in this figure.

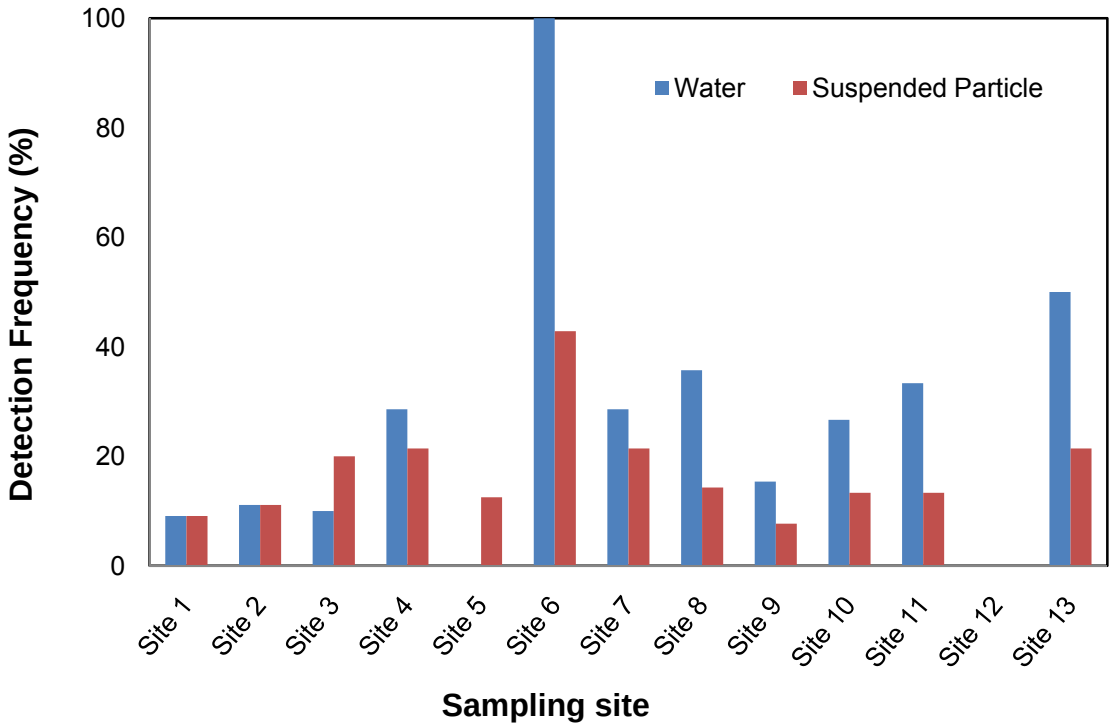

Fig. 3.3. Detection frequency of estrogens in water and suspended particle samples

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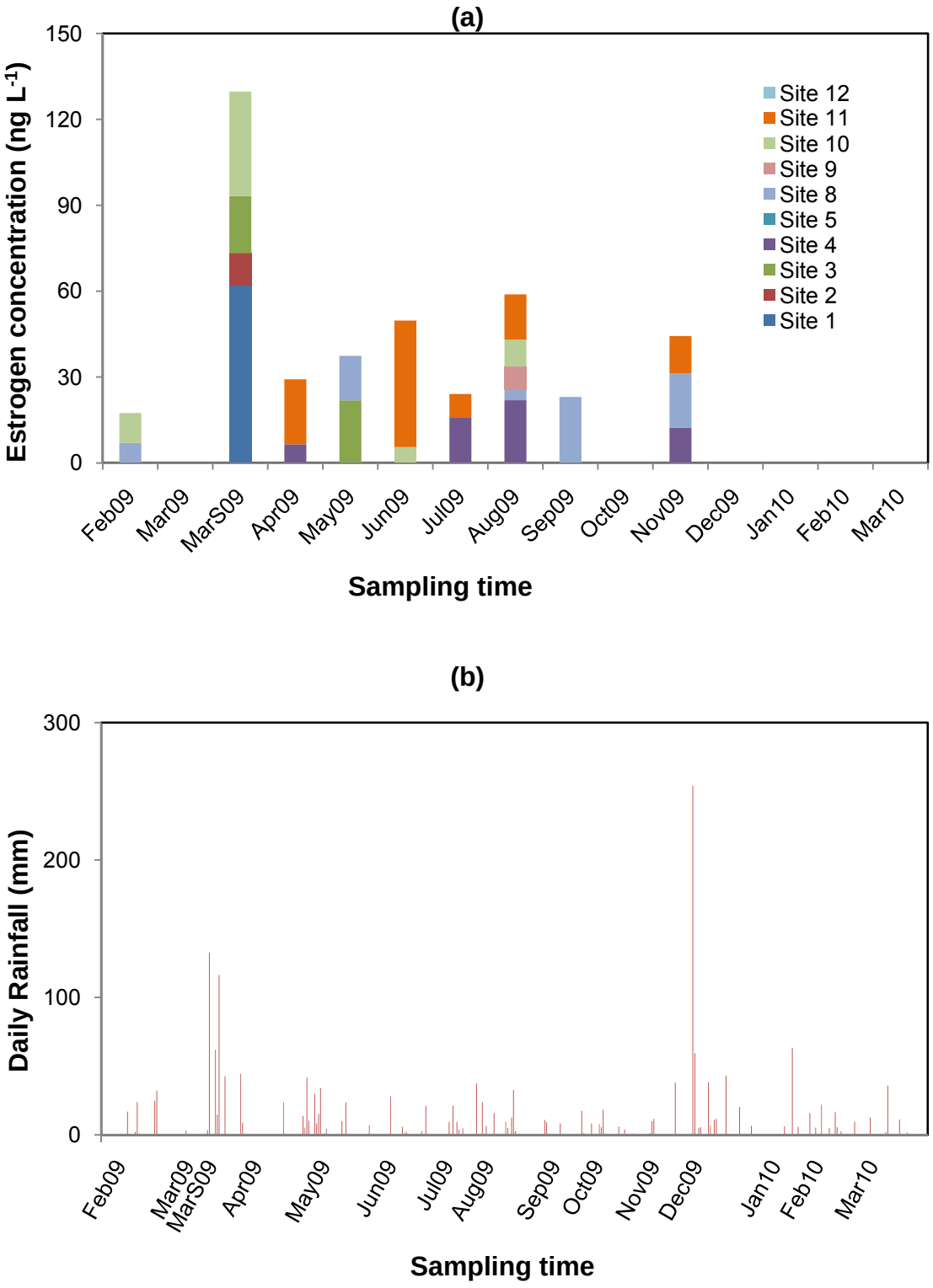

Fig. 3.4. Estrogen concentrations detected at the whole watershed (a) and daily rainfall amount across the study period (b). MarchS09: March storm event in 2009.

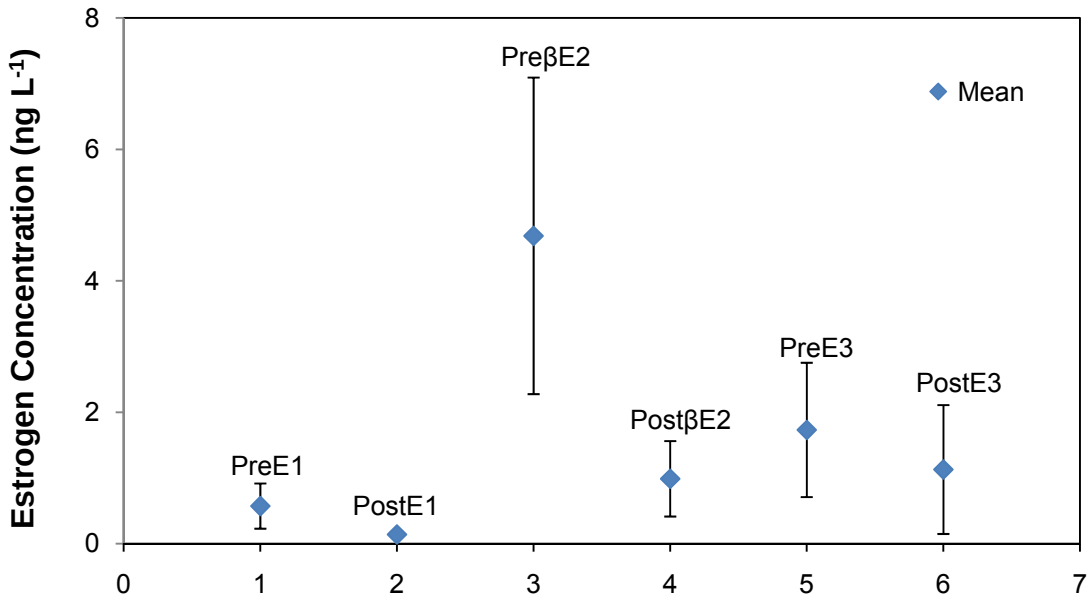

Fig. 3.5. Means of the sum of each estrogen during pre-closure and post-closure times with 95% confidence intervals

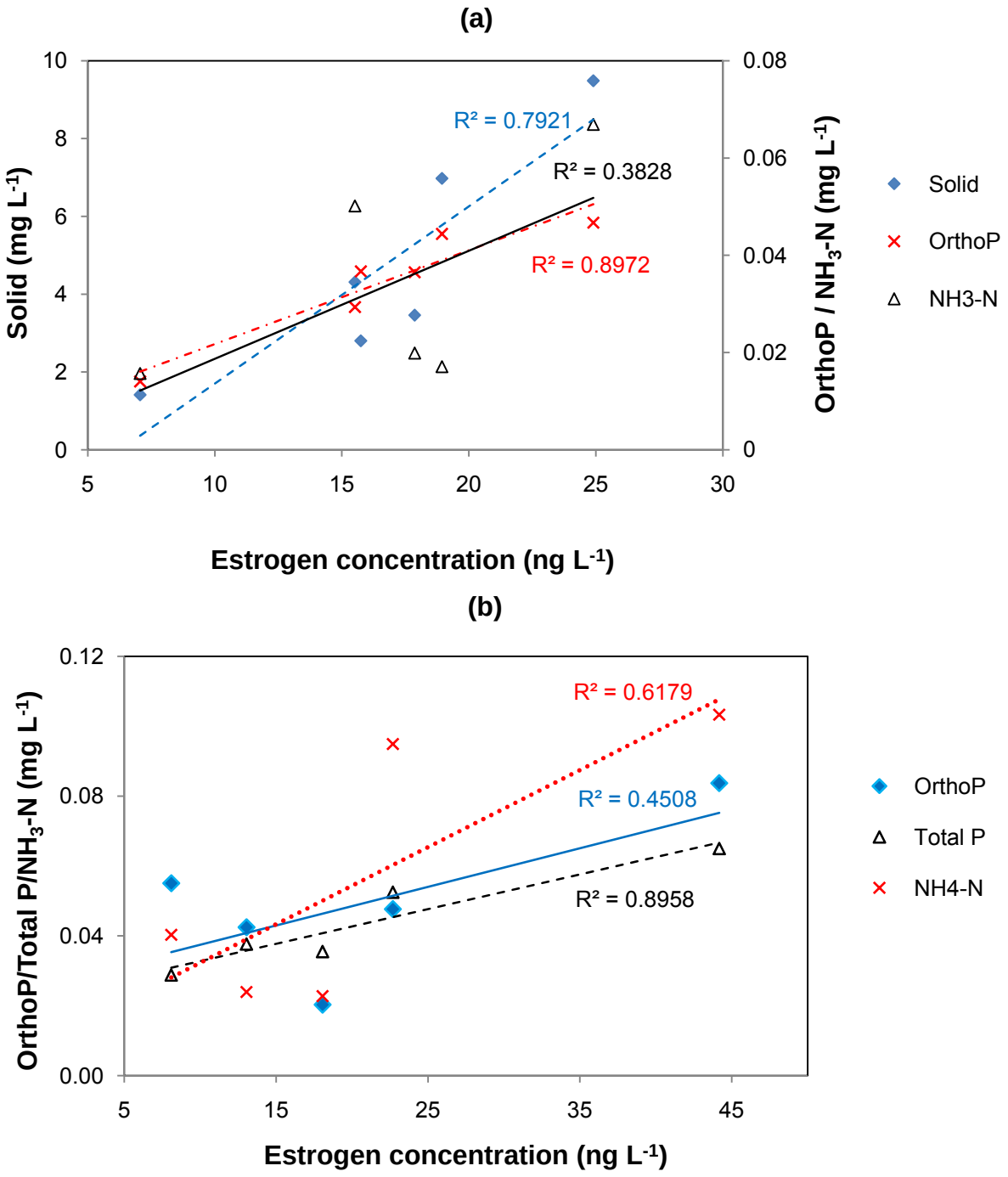

Fig. 3.6. Simple linear regression between water quality parameters and estrogen concentrations for Site 8 (a) and Site 11 (b). OrthoP: orthophosphate. Total P: total phosphorus. NH_4-N: ammonia-nitrogen.

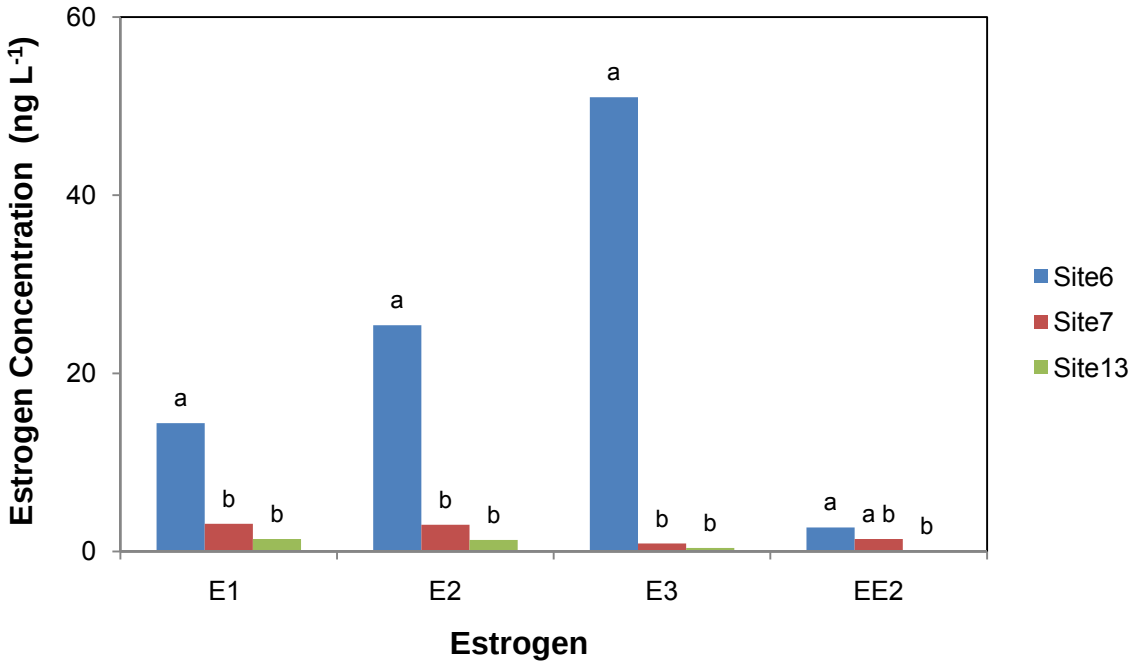

Fig. 3.7. Mean concentration of each estrogen at Site 6, 7, 13 averaged by month. Means followed by different letters indicates a significant difference at $P \leq 0.05$.

APPENDICES

APPENDIX A.

CONCENTRATIONS OF ESTROGENS IN WATER SAMPLES AND SUSPENDED PARTICLE SAMPLES

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Feb09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	10.2	67.2	125	bdl	bdl	bdl	bdl	bdl
7	5.68	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	7.06	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	ns	ns	ns	ns	ns	ns	ns	ns
10	bdl	10.4	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns	ns	ns	ns	ns
Mar09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	bdl	8.89	4.63	bdl	bdl	bdl	bdl	bdl
7	7.72	7.32	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009.

APPENDIX A. Continuation from before

Month	Water				Suspended particles			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
MarS09								
1	bdl	62.0	bdl	bdl	555	19554	bdl	bdl
2	bdl	11.20	bdl	bdl	bdl	939	676	bdl
3	bdl	20.01	bdl	bdl	8.03	297.3	52.5	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	26.2	bdl	bdl	bdl
6	19.1	63.0	19.6	4.62	bdl	104.5	bdl	bdl
7	ns	ns	ns	ns	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	36.49	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	11.8	3.09	7.73	bdl	Trace	bdl	bdl	bdl
Apr09					bdl	bdl	bdl	bdl
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	6.52	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	ns	ns	ns	ns	ns	ns	ns	ns
7	2.18	2.74	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	22.7	bdl	bdl	bdl	bdl	Trace	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009.

APPENDIX A. Continuation from before

Month	Water ($ng\ L^{-1}$)				Suspended particles ($ng\ g^{-1}$)			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
May09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	21.9	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	125	3832	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	2.92	30.8	81.7	bdl	7.94	37.3	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	182	bdl	bdl
8	bdl	15.5	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	45.3	bdl	bdl	bdl
Jun09								
1	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns
3	bdl	bdl	bdl	bdl	bdl	104	135	bdl
4	bdl	bdl	bdl	bdl	bdl	Trace	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	11.6	15.5	54.8	bdl	12.2	48.3	26.4	bdl
7	2.74	1.70	bdl	bdl	29.7	118	Trace	bdl
8	bdl	bdl	bdl	bdl	bdl	247	bdl	bdl
9	bdl	bdl	bdl	bdl	32.7	199	Trace	bdl
10	3.27	2.27	bdl	bdl	27.5	Trace	Trace	bdl
11	1.25	8.56	34.4	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	3.26	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX A. Continuation from before

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Jul09								
1	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns
4	2.7	12.9	bdl	bdl	23.5	148	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	12.7	8.2	23.7	7.6	bdl	51.6	19.8	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	ns	ns	ns	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns	ns	ns
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	1.70	6.40	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	3.60	bdl	bdl	5.50	bdl	bdl	Trace	bdl
Aug09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	21.9	bdl	bdl	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	46.4	25.2	47.2		bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	3.71	13.0	bdl	bdl	bdl	7.78	bdl	bdl
9	8.09	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	Bdl	9.33	bdl	bdl	bdl	276	bdl	
11	2.25	4.30	9.27	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	8.19	13.43	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX A. Continuation from before

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Sep09								
1	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	11.3	30.1	65.1	5.19	bdl	6.62	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	23.0	bdl	bdl	bdl	167	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	Trace	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	5.69	bdl	bdl	bdl	bdl
Oct09								
1	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	7.23	15.7	75.7	bdl	bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX A. Continuation from before

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Nov09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	12.3	bdl	bdl	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	3.12	14.9	84.7	bdl	bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	18.9	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	13.1	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	6.59	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Dec09								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns	ns	ns	ns	ns
6	20.2	45.8	27.2	8.6	15.0	13.3	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	8.80	19.8	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX A. Continuation from before

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Jan10								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	25.3	29.7	61.2	bdl	bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Feb10								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	26.5	bdl	37.6	5.24	bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	280	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX A. Continuation from before

Month	Water (ng L^{-1})				Suspended particles (ng g^{-1})			
Site	E1	βE2	E3	EE2	E1	βE2	E3	EE2
Mar10								
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
6	4.07	bdl	5.75	bdl	bdl	bdl	bdl	bdl
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl

ns: no sample. Trace: concentration closes to detection limit. MarS09: March storm 2009

APPENDIX B.

CONCENTRATIONS OF ESTROGENS IN SEDIMENT

Month	Sediment (ng g^{-1})			
Site	E1	βE2	E3	EE2
Jan09				
1	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl
3	bdl	bdl	0.970	bdl
4	1.06	bdl	bdl	bdl
5	bdl	bdl	1.74	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns
Apr09				
1	bdl	bdl	bdl	bdl
2	2.41	bdl	4.95	bdl
3	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl
5	bdl	bdl	0.75	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	1.13	bdl	0.23	bdl
9	0.28	bdl	1.62	bdl
10	0.31	bdl	0.34	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns

ns: no sample. Trace: concentration closes to detection limit

APPENDIX B. Continuation from before

Month	Sediment (ng g^{-1})			
Site	E1	βE2	E3	EE2
Jun09				
1	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl
3	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	bdl	bdl	0.300	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns
Oct09				
1	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl
3	Trace	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl
5	bdl	bdl	bdl	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	0.240	bdl	0.220	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns

ns: no sample. Trace: concentration closes to detection limit

APPENDIX C.

WATER QUALITY PARAMETERS

Month Site	T °C	DO mg L^{-1}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Feb09													
1	11.42	1.42	0.00	9.05	2.10	0.02	6.87	0.008	0.019	0.09	0.40	61.8	21.3
2	12.75	0.85	0.00	9.32	2.23	0.00	13.29	0.025	0.002	0.04	0.81	291	81.8
3	10.81	1.14	0.00	13.50	4.77	0.02	16.85	0.007	0.023	0.26	0.61	181.4	100
4	10.95	2.18	0.00	13.30	1.34	0.10	18.19	0.015	0.097	0.07	0.86	223.8	230
5	12.60	0.84	0.00	9.04	2.76	0.06	14.49	0.057	0.057	0.05	0.79	139.4	108
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	52000	5200000
7	17.84	1.04	0.00	11.56	21.05	4.71	118.22	0.855	0.170	0.90	4.33	121.8	99
8	9.73	2.25	0.00	13.52	1.41	0.05	13.38	0.014	0.016	0.06	0.67	176.4	70
9	8.74	1.95	0.00	14.14	1.78	0.03	13.33	0.016	0.009	0.06	0.62	109.4	54
10	9.36	2.15	0.00	9.47	1.67	0.02	15.30	0.003	0.014	0.01	0.74	132.6	66
11	9.25	2.51	0.00	14.33	1.44	0.03	16.59	0.035	0.011	0.08	0.67	79.4	70
12	9.55	2.39	0.00	9.84	1.54	0.03	15.64	0.038	0.014	0.34	0.67	109.4	54
13	18.16	0.67	56.10	11.59	1.38	13.28	122.66	1.075	0.069	1.43	10.13	2187	901
Mar09													
1	13.94	11.26	4.00	7.86	3.32	0.00	6.69	0.017	0.021	0.05	0.50	344.4	110
2	15.84	5.51	4.00	7.79	7.00	0.01	14.97	0.056	0.117	0.06	1.23	142.4	108
3	15.15	7.19	4.00	7.92	3.00	0.02	16.36	0.008	0.126	nd	nd	215.2	200
4	18.60	139.65	1.10	-	3.08	0.05	16.42	0.077	0.167	nd	nd	49.2	66
5	15.90	9.24	5.60	7.99	3.31	0.06	17.04	0.105	0.117	0.40	0.97	305.8	310
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0	0
7	20.25	12.95	1.50	nd	21.65	1.60	96.88	0.795	0.132	0.98	2.38	172.4	182
8	17.54	142.68	0.80	nd	4.00	0.06	14.46	0.021	0.158	0.04	1.01	140.6	88
9	17.58	154.06	1.20	nd	3.67	0.08	14.47	0.023	0.151	0.03	1.09	229	100
10	18.44	181.23	1.40	nd	4.49	0.03	15.88	0.017	0.111	0.08	1.21	120.2	88
11	18.74	204.55	0.30	nd	3.61	0.04	17.49	0.076	0.059	0.40	0.98	24.4	40
12	18.67	207.63	1.80	7.76	4.50	0.04	16.68	0.057	0.060	0.12	0.92	31.6	34
13	20.16	10.93	578.5	7.77	1.41	5.57	125.03	0.251	0.071	0.35	5.02	10.4	90

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENIX C. Continuation from before

Month Site	Temp °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
MarS09													
1	15.81	5.97	12.90	5.65	5.16	0.05	5.09	0.018	0.032	0.04	0.75	754	570
2	nd	nd	nd	nd	24.03	0.08	6.48	0.050	0.050	0.09	0.87	7701	5100
3	16.21	6.51	112.9	7.34	26.36	0.26	8.33	0.051	0.071	0.08	1.00	5794	5700
4	16.99	0.02	42.60	6.33	28.87	0.12	8.79	0.066	0.053	0.11	1.16	14136	TNTC
5	16.20	4.35	611.6	5.50	15.45	0.60	10.10	0.179	0.078	0.23	1.70	14136	TNTC
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17300	190909.1
7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
8	16.59	0.34	30.10	6.54	15.32	0.12	7.47	0.020	0.033	0.05	0.82	12033.	TNTC
9	16.64	0.03	22.00	6.42	14.13	0.08	5.88	0.011	0.032	0.04	0.87	17328.	TNTC
10	17.31	0.00	38.00	6.84	18.43	0.23	8.43	0.100	0.045	0.14	1.21	17328.	TNTC
11	17.61	0.01	19.80	6.48	14.91	0.12	10.36	0.039	0.040	0.07	0.96	6488	TNTC
12	18.02	0.00	28.00	6.29	24.25	0.11	8.37	0.031	0.036	0.06	0.88	17328.	TNTC
13	19.22	0.01	57.30	6.99	24.34	1.10	23.19	2.532	2.131	3.18	3.90	111985	180000
Apr09													
1	15.37	9.03	2.00	7.22	3.51	0.02	4.08	0.015	0.029	0.01	0.53	544.6	173
2	16.24	5.35	6.30	7.31	4.93	0.02	7.19	0.069	0.277	0.06	1.20	222.4	81.1
3	16.08	6.35	5.60	7.77	3.74	0.27	9.78	0.012	0.178	0.02	1.07	148.8	117
4	19.00	5.85	5.20	6.93	3.44	0.16	8.00	0.100	0.192	0.09	1.28	146	135
5	16.27	7.87	4.30	7.41	4.57	0.11	7.63	0.078	0.120	0.10	1.14	262.6	171
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	217800	2100000
7	23.36	8.41	2.80	7.63	73.18	2.44	68.14	0.280	4.620	0.48	6.19	313	1300
8	17.68	6.46	345.6	7.74	6.66	0.12	7.84	0.050	0.130	0.03	1.04	86	72.1
9	17.60	6.16	9.10	7.61	6.36	0.12	7.72	0.038	0.136	0.03	1.07	74	27
10	21.58	9.67	0	7.76	9.87	0.08	7.61	0.024	0.170	0.03	1.17	86	63.1
11	18.46	5.45	338.7	7.17	5.65	0.08	7.47	0.048	0.095	0.05	1.05	20	54
12	18.72	5.59	5.80	6.84	5.79	0.07	7.35	0.031	0.076	0.04	1.01	20	36
13	21.13	8.73	786.2	7.50	2.91	4.85	85.60	0.027	6.885	0.13	8.73	1607	3700

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

Month Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
May09													
1	20.93	7.52	234.9	7.02	5.42	0.09	6.63	0.021	0.051	0.01	0.77	324.8	118
2	21.63	5.47	13.10	6.65	7.19	0.17	6.44	0.067	0.120	0.09	1.10	357	220
3	21.41	6.11	98.70	7.14	4.52	0.32	10.41	0.017	0.097	0.02	0.99	151.8	191
4	22.04	5.29	7.70	6.91	3.79	0.11	6.95	0.125	0.073	0.14	1.25	204.4	260
5	21.53	6.10	7.00	6.64	6.75	0.07	8.96	0.036	0.046	0.09	1.10	434.4	700
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	193500	600000
7	25.59	5.66	6.20	7.23	23.05	1.99	25.54	0.488	0.146	0.61	1.48	48.6	390
8	21.41	6.05	227.1	7.35	4.31	0.09	8.40	0.029	0.050	0.06	1.07	176.8	164
9	21.41	5.71	10.70	7.02	4.42	0.09	8.20	0.016	0.040	0.05	1.07	202.8	230
10	22.05	5.51	8.70	6.96	4.96	0.11	7.07	0.050	0.054	0.10	1.17	278.2	270
11	22.16	4.74	5.50	6.71	2.48	0.06	7.85	0.019	0.033	0.06	1.14	176	106
12	22.51	4.70	5.80	6.55	2.76	0.06	7.82	0.014	0.028	0.07	1.11	99.2	82
13	23.83	8.08	32.70	7.18	7.59	2.44	29.86	0.508	0.096	0.72	3.03	167.2	1545
Jun09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	25.49	1.69	8.60	5.89	16.05	0.03	12.45	0.026	0.106	0.02	0.97	nd	nd
4	26.86	1.66	7.00	6.72	12.95	0.04	10.37	0.110	0.211	0.12	1.74	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	30.69	8.82	192.8	6.47	15.51	17.37	91.86	1.823	17.302	2.03	32.80	nd	nd
8	27.56	2.25	34.00	5.94	2.80	0.04	11.84	0.037	0.401	0.01	1.28	nd	nd
9	25.29	3.65	8.60	5.90	4.20	0.16	26.43	0.153	0.143	0.15	0.98	nd	nd
10	nd	nd	nd	nd	6.19	0.03	12.13	0.065	0.471	0.03	1.41	nd	nd
11	28.40	3.02	nd	6.90	0.55	0.10	13.27	0.084	0.103	0.07	1.32	nd	nd
12	30.29	3.85	nd	5.67	3.57	0.10	13.77	0.077	0.082	0.06	1.16	nd	nd
13	28.31	7.05	199.7	6.30	4.40	17.75	98.93	1.647	0.042	1.61	16.74	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

Month Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Jul09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	26.41	2.73	15.40	6.69	20.85	0.02	9.09	0.077	0.024	0.16	1.45	19.4	72.1
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	313000	3500000
7	27.26	7.06	7.00	7.93	30.47	3.93	99.95	1.439	0.027	1.43	1.33	41	1273
8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	24.92	1.76	2.30	6.28	2.51	0.04	7.46	0.057	0.181	0.01	1.03	12.6	19.7
11	26.68	3.94	1.80	5.50	4.69	0.07	9.57	0.055	0.040	0.03	1.16	23.6	46
12	27.36	4.92	2.90	5.60	5.67	0.04	9.88	0.067	0.027	0.03	0.96	22	16.4
13	27.41	8.01	0.20	8.13	8.76	9.57	99.39	1.081	0.033	1.19	7.67	880	1364
Aug09													
1	24.55	4.34	6.60	5.48	2.26	0.02	12.19	-0.005	0.008	0.02	0.63	54.4	56
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	24.99	4.54	1.70	5.87	2.71	0.18	11.20	0.044	0.038	0.07	1.40	209.8	310
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	410600	4900000
7	27.86	5.88	4.00	7.10	50.88	4.89	42.90	0.170	0.232	0.35	5.37	341	1909
8	24.18	4.68	4.40	6.56	3.46	0.06	8.04	0.037	0.020	0.02	0.97	172.5	164
9	24.15	4.45	3.90	6.10	3.03	0.05	8.89	0.011	0.013	0.02	0.93	71.4	173
10	24.52	4.83	2.10	5.75	2.96	0.11	9.27	0.002	0.028	0.02	1.18	123.6	104
11	25.29	5.02	7.40	5.52	4.84	0.05	14.38	0.020	0.023	0.04	1.03	111.2	270
12	26.67	5.26	4.10	5.70	6.78	0.08	10.48	0.031	0.040	0.06	1.24	116	470
13	28.08	7.86	6.70	8.06	2.27	1.15	54.97	0.115	0.003	0.14	1.01	5710	5700

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

Month Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Sep09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	27.04	1.22	9.90	6.47	3.34	0.02	24.99	0.112	0.175	0.10	1.15	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	26.82	2.95	9.50	6.95	16.21	9.40	66.35	1.002	0.152	1.03	6.35	nd	nd
8	25.88	4.74	18.10	6.28	9.49	0.03	11.08	0.047	0.067	0.01	1.17	nd	nd
9	27.28	6.57	2.90	7.05	10.26	0.15	45.71	0.649	0.007	0.66	0.62	nd	nd
10	27.33	2.13	11.70	5.96	4.60	0.11	9.67	0.041	0.031	0.04	1.16	nd	nd
11	28.26	5.05	3.70	5.96	2.33	0.08	9.86	0.060	0.036	0.05	1.16	nd	nd
12	28.00	5.88	8.50	5.87	4.32	0.10	10.38	0.060	0.033	0.11	1.07	nd	nd
13	27.32	8.58	0.60	7.82	0.39	14.54	73.67	0.869	0.016	0.88	13.32	nd	nd
Oct09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	23.55	3.26	11.30	6.67	9.53	0.01	23.26	0.082	0.164	0.05	1.26	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	24.36	2.73	2.90	7.15	3.76	5.88	58.81	0.880	8.583	0.96	12.56	nd	nd
8	22.40	4.37	30.00	6.14	156.45	0.01	5.92	0.031	0.016	0.02	1.21	nd	nd
9	21.14	4.33	10.70	6.74	25.81	0.03	40.40	0.506	0.006	0.46	0.60	nd	nd
10	21.94	2.31	5.90	6.37	6.39	0.03	6.34	0.045	0.089	0.03	0.91	nd	nd
11	23.68	3.86	16.50	6.02	3.22	0.06	10.09	0.084	0.054	0.06	0.92	nd	nd
12	23.93	5.91	5.40	5.94	2.71	0.02	8.94	0.061	0.027	0.04	0.72	nd	nd
13	25.39	8.07	1.60	7.77	1.44	2.66	68.95	0.108	3.035	0.13	4.66	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

Month Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Nov09													
1	18.23	6.81	5.80	6.58	5.53	0.01	15.56	0.023	0.018	0.00	0.65	nd	nd
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	18.64	4.56	20.40	6.77	14.23	0.04	21.91	0.032	0.110	0.06	1.13	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	21.08	4.57	12.50	7.20	134.00	2.77	52.33	0.567	1.572	0.60	4.28	nd	nd
8	18.38	2.86	7.40	6.77	6.97	0.02	13.00	0.044	0.017	0.05	0.95	nd	nd
9	18.49	4.39	4.40	6.82	17.13	0.02	21.67	0.106	0.014	0.13	0.71	nd	nd
10	17.22	3.60	3.20	6.56	2.43	0.03	8.07	0.035	0.015	0.02	0.80	nd	nd
11	18.93	7.15	3.50	6.92	1.47	0.02	18.34	0.042	0.024	0.04	0.63	nd	nd
12	19.48	8.22	3.00	6.80	1.21	0.02	18.22	0.034	0.013	0.03	0.57	nd	nd
13	22.89	8.84	3.00	7.90	1.87	1.03	90.66	0.767	0.011	0.77	0.68	nd	nd
Dec09													
1	13.99	8.97	6.90	6.12	2.98	0.35	11.49	0.027	0.019	0.02	0.66	nd	nd
2	14.23	3.73	22.00	5.73	8.42	0.02	9.12	0.033	0.020	0.01	0.81	nd	nd
3	13.80	7.33	9.80	6.39	4.10	0.09	15.19	0.009	0.013	0.01	0.74	nd	nd
4	11.93	7.97	18.70	6.68	6.11	0.21	14.97	0.019	0.026	0.02	1.02	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	17.46	7.42	23.20	6.58	9.79	3.81	22.17	0.211	0.776	0.81	4.61	nd	nd
8	11.44	8.22	10.50	6.09	8.72	0.02	10.95	0.025	0.015	0.04	0.68	nd	nd
9	11.91	7.43	8.70	5.99	7.93	0.04	11.71	0.010	0.017	0.05	0.75	nd	nd
10	12.68	8.15	15.10	6.61	8.65	0.24	11.65	0.008	0.019	0.00	1.00	nd	nd
11	13.50	8.18	18.70	6.24	8.68	0.07	11.22	0.015	0.016	0.02	0.84	nd	nd
12	13.71	8.55	18.10	6.51	9.05	0.09	12.34	0.023	0.016	0.04	0.77	nd	nd
13	19.84	9.65	11.00	7.21	10.15	4.88	45.09	0.230	0.307	0.32	5.14	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

11 Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Jan10													
1	2.80	13.13	4.00	7.21	13.58	0.42	7.09	0.027	0.014	0.00	0.69	nd	nd
2	1.98	11.65	2.50	6.66	12.10	0.12	14.28	0.038	0.020	-0.01	0.73	nd	nd
3	1.08	13.26	3.10	8.77	13.71	0.82	13.61	0.014	0.038	0.00	1.06	nd	nd
4	2.63	12.23	6.80	7.41	12.68	1.10	13.76	0.009	0.033	0.01	1.46	nd	nd
5	2.31	12.88	4.30	6.68	13.33	0.41	14.66	0.026	0.025	0.01	0.97	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	9.93	12.66	4.20	7.06	2.19	4.11	46.72	0.036	0.065	0.11	3.92	nd	nd
8	2.64	13.47	3.10	6.67	1.20	0.19	11.84	0.007	0.016	0.00	0.63	nd	nd
9	2.93	13.35	3.20	6.50	1.30	0.19	11.82	0.028	0.022	0.00	0.64	nd	nd
10	2.17	13.03	4.30	6.99	1.55	0.33	11.90	0.002	0.031	0.01	0.86	nd	nd
11	2.61	13.12	4.00	6.40	1.61	0.21	12.39	0.017	0.021	0.01	0.84	nd	nd
12	2.65	12.57	4.00	6.31	1.65	0.19	12.37	0.001	0.019	0.00	0.82	nd	nd
13	14.00	10.76	0.80	7.48	0.67	4.58	57.75	0.003	0.010	0.04	4.41	nd	nd
Feb10													
1	9.28	10.93	17.00	6.33	1.93	0.26	5.45	0.031	0.039	0.01	0.73	nd	nd
2	9.64	8.74	3.40	5.40	1.81	0.09	9.00	0.044	0.041	0.03	0.92	nd	nd
3	9.15	10.33	8.60	7.89	2.82	0.61	9.43	0.018	0.045	0.01	1.05	nd	nd
4	10.81	9.16	7.90	6.03	3.10	0.52	9.44	0.023	0.061	0.04	1.26	nd	nd
5	10.82	10.08	4.40	6.18	2.79	0.26	9.94	0.037	0.048	0.05	1.06	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	16.11	8.95	4.30	6.90	3.40	4.43	26.19	0.091	0.635	0.13	5.28	nd	nd
8	10.43	10.68	7.10	6.70	3.17	0.18	8.73	0.017	0.038	0.02	0.70	nd	nd
9	11.23	10.46	7.60	6.53	2.53	0.16	8.75	0.012	0.038	0.02	0.76	nd	nd
10	10.66	9.28	6.90	6.30	2.55	0.23	8.80	0.008	0.046	0.08	0.83	nd	nd
11	10.73	8.88	5.50	5.99	2.34	0.16	8.64	0.009	0.041	0.03	0.90	nd	nd
12	10.90	8.93	5.60	5.95	2.10	0.14	8.63	0.022	0.042	0.02	0.93	nd	nd
13	15.07	10.87	117.6	6.79	0.94	4.03	32.85	0.034	0.122	0.03	4.10	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

APPENDIX C. Continuation from before

Month Site	T °C	DO mg L^{-}	Turb NTU	pH	Solid mg L^{-1}	NO_3-N mg L^{-1}	Cl mg L^{-1}	OrthoP mg L^{-1}	NH_4-N mg L^{-1}	Total P mg L^{-1}	Total N mg L^{-1}	*E. coli* MPN	*fecal coli* MPN
Mar10													
1	7.74	11.62	4.50	6.18	1.72	0.11	4.84	0.026	0.022	0.00	0.48	nd	nd
2	8.04	10.71	3.50	5.85	1.93	0.02	9.13	0.043	0.024	0.02	0.75	nd	nd
3	7.47	10.56	6.40	7.95	3.74	0.48	10.28	0.019	0.034	0.01	0.89	nd	nd
4	8.91	10.63	28.00	6.21	3.50	0.57	10.04	0.020	0.040	0.03	1.20	nd	nd
5	8.95	10.78	6.10	6.49	2.90	0.26	11.15	0.043	0.030	0.03	1.01	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	13.35	11.32	16.80	6.79	34.50	3.18	27.44	0.071	0.440	0.12	3.92	nd	nd
8	9.18	10.68	6.00	6.70	2.48	0.16	9.04	0.021	0.026	0.01	0.65	nd	nd
9	10.01	11.61	52.00	6.72	2.70	0.15	9.17	0.017	0.022	0.02	0.71	nd	nd
10	8.79	10.37	26.90	6.37	3.10	0.23	9.29	0.016	0.026	0.01	0.85	nd	nd
11	9.52	9.94	6.10	6.04	2.71	0.13	8.94	0.016	0.026	0.02	0.88	nd	nd
12	9.49	9.85	10.50	6.08	2.85	0.12	8.92	0.037	0.031	0.02	0.81	nd	nd
13	14.95	11.22	4.20	7.13	1.81	6.67	47.60	0.064	0.022	0.04	6.18	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.