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Dedication

To my entire wonderful loving family, who have always been there to give me support and advice throughout my college career, and my life so far.

In loving memory of my grandfather Enrique P. Espinoza.

In loving memory of my good friend and fellow 301st SFS member Siebe A. Bandringa.

To my supportive Air Force Reserve unit, 301st SFS.

ASSESSMENT OF WATER QUALITY AND BENTHIC MACROINVERTEBRATE COMMUNITY IN A WASTEWATER RECEIVING CONSTRUCTED WETLAND

by

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THESIS

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Abstract

In the El Paso region around the 1930s there was a loss of riparian areas and wetlands due to canalization of the Rio Grande. In an attempt to bring back native flora and fauna that once flourished in the floodplain of the Rio Grande region, the creation of Rio Bosque Wetlands Park (RBWP; El Paso Co., TX) was initiated in 1995 (Watts *et al.*, 2002). As part of the construction agreement, El Paso Water Utilities agreed to provide water to fill the wetland upon its completion (Watts *et al.*, 2002). Beginning in 1998, RBWP was filled with treated effluent from the adjacent Roberto Bustamante Wastewater Treatment Plant (WWTP) during the non-irrigation season. Using treated wastewater to fill Rio Bosque's wetland cells is of concern due to contaminants and their metabolites that may remain after treatment. This study assesses the water quality of RBWP by specifically examining pollutants that are known to impact water quality: nutrients and other water chemistry parameters, heavy metals and arsenic, and PPCPs. Further investigations included determining benthic macroinvertebrate (BMI) communities in selected sites within RBWP, and the acute effects of selected pharmaceuticals and personal care products (the stimulant, caffeine; the antibiotic, erythromycin; the analgesic/anti-pyretic, acetaminophen; and the plastics/epoxy resin, BPA) to non-biting midges (Diptera: Chironomidae).

During the course of this study nutrients (ammonia, nitrite + nitrate, phosphate, and chlorophyll-a) exceeded the state water quality criteria. Major factors driving macroinvertebrate community composition may be high levels of nutrients and hydroperiod.

Most heavy metals analyzed were below consensus sediment quality guidelines. However, Cd levels exceeded threshold effect concentration (TEC; below which harmful effects

are unlikely to be observed) levels, suggesting that this metal may have harmful effects to BMI community composition at RBWP.

Of the 9 pharmaceuticals analyzed, ciprofloxacin and codeine were detected in highest concentrations in water and sediment samples. Two additional compounds: caffeine and trimethoprim were detected frequently in sediment samples. Since the concentrations of all compounds were low (ng/L), they do not seem to pose a great risk according to the findings from the 48 hr toxicity tests conducted here as well as other published studies. Supporting the contention that acute toxic effects of many PPCPs are unlikely; however, chronic environmental toxicity cannot be excluded.

During the course of this study, nearly 31,000 benthic macroinvertebrates were collected. Out of the seventeen different taxa that were identified, two groups: midge larvae (Diptera: Chironomidae), and nematodes (Nematoda) were two dominant taxa that accounted for over 50% of the total benthic macroinvertebrates collected on most occasions. This type of community composition is expected because these taxonomic groups represent fauna that are tolerant to poor water quality, which enables them to inhabit the aquatic environment and sediments associated with the study sites at RBWP, an effluent receiving wetland. Water availability is likely the major factor that determines the BMI community that is able to colonize RBWP.

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1 Introduction

Wetlands are characterized by construction (natural or artificial), permanence, source type (freshwater, marine, or treated effluent), and flow (static or flowing) (Scott & Jones, 1995). In the United States wetlands have been receiving much attention, as they are increasingly being recognized for the ecologically important ecosystem services they provide (Dahl, 1990). Ecosystem services provided by wetlands include: flood control, sediment and nutrient retention and export, water purification, and reservoirs of biodiversity (Ramsar, 2013).

Prior to the 1780s, it was estimated that the United States contained approximately 89.4 million hectares of wetlands (Dahl, 1990; Wilen & Bates, 1995). Over the past 200 years, many of these wetlands have been drained, dredged, filled, leveled, and flooded (Dahl, 1990). It is estimated that within 22 states at least 50% of the original wetland land area have been lost, with some states loosing up to 90% (i.e. California) (Dahl, 1990). The state of Texas reported over a 50% loss of wetlands (Dahl, 1990). Around the 1930s in the El Paso region, there was a loss of riparian areas and wetlands due to canalization of the Rio Grande (Watts *et al.*, 2002).

In an attempt to bring back native flora and fauna that once flourished in the floodplain of the Rio Grande region, construction on the Rio Bosque Wetlands Park (RBWP; El Paso Co., Texas) began in 1995 (Watts *et al.*, 2002). As part of the construction agreement, El Paso Water Utilities agreed to provide the water to fill Rio Bosque upon its completion (Watts *et al.*, 2002). Beginning in 1998, RBWP was filled with treated effluent from the adjacent Roberto Bustamante wastewater treatment plant (WWTP) during the non-irrigation season. This season typically begins in September and continues through February with an average of 57×10^6 L/day of treated wastewater entering the wetland (Watts *et al.*, 2002; EPWU, 2010). Any additional water

received, is acquired through annual rainfall that ranges from approximately 0.6 to 5.1 cm per year (Weatherdb, 2013).

Using treated wastewater to fill Rio Bosque's wetland cells is of concern due to contaminants and their metabolites that may remain after treatment. Contaminants in treated effluent can include high levels of nutrients (i.e. nitrogen and phosphorus), synthetic organic chemicals, heavy metals, and compounds of emerging concern (i.e. pharmaceuticals and personal care products (PPCPs)) (Oelsner *et al.*, 2007). These pollutants may impact the aquatic inhabitants that colonize the wetland cells of RBWP, by decreasing fecundity and growth (Brooks *et al*. 2006). This study assesses the water quality of RBWP by specifically looking at pollutants that are known to impact water quality: nutrients and other water chemistry parameters, heavy metals and arsenic, and PPCPs.

General water chemistry parameters such as high nutrients (i.e., nitrogen compounds, ammonia, and phosphorous), and low dissolved oxygen (DO) can affect aquatic biota that depend upon this environment for development of either a few or all life stages (Daughton & Ternes, 1999). Xu *et al.*(2013) conducted a study on the effects of water pollution on benthic macroinvertebrates (BMIs). The study concluded that decreases in DO and increases of total phosphorous (TP) and total nitrogen (TN) were correlated with reductions in pollution intolerant taxa (i.e. stoneflies, and some mayflies) and taxa richness. In addition, pollution tolerant taxa increased, with the dominant taxa including aquatic worms and midges (Oligochaeta: Naididae and Diptera: Chironomidae) (Xu *et al.*, 2013).

Heavy metals present in water can originate from various sources such as geologic weathering, industrial and domestic effluents, and agricultural fertilizers (Smolders *et al.*, 2003). The presence and impacts of heavy metal contamination to aquatic ecosystems has been

documented in recent years (Clements *et al.*, 2000; Smolders *et al.*, 2003; Qu *et al.*, 2010). Qu *et al.* (2010) were interested in determining the effects of heavy metals (released from both active and abandoned mines) to BMI communities in high mountain areas. The authors reported that concentrations detected within their sampling sites were low, on most occasions heavy metals were the following concentrations: Cd: ≤ 0.4 µg/L; Cu: ≤ 7.5 µg/L; Ni: ≤ 3 µg/L; Pb: ≤ 10 µg/L; Zn: ≤ 30 µg/L, with some exceptions at certain sites downstream from mining activities (Qu *et al.*, 2010). Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) (EPT) and dipteran taxa composed 89% of the total abundance of organisms collected. However, as heavy metal concentrations increased (Cd at 0.50 µg/L; Cu at 16.53µg/L; Ni at 4.85 µg/L; Pb at 33.93µg/L, Zn at 40.35 µg/L) there was a decrease in total abundance, and taxa richness of BMIs (Qu *et al.*, 2010). Lagrana *et al.* (2011) investigated acute toxicity of three metals (Cu, Cd, and Pb) on Chironomidae (Diptera) during a 96 hr exposure experiment. The estimated LC_{50} (lethal concentration of 50% mortality of a population) concentrations were the following: 1.37 µg/mL for Cu, 73.09 µg/mL for Cd, and 38.47 µg/mL for Pb.

While water quality (Sharma & Rawat, 2009; Gozalo & Camargo, 2013; Kutcher & Bried, 2014) and heavy metal (Ordonez *et al.*, 2011; Malaj *et al.*, 2012; Protano *et al.*, 2013) impacts on BMIs have been commonly studied; little is known about the effect of PPCPs on these organisms (Brooks *et al.*, 2006). PPCPs are a class of emerging environmental contaminants that are widely used in human and veterinary medicines (Zhou *et al.*, 2009). The Environmental Protection Agency (EPA) defines PPCPs as any product used by individuals for personal health or cosmetic reasons, or used by agribusiness to enhance growth or health of livestock (USEPA, 2010). Some examples include prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, cosmetics, sun-screens, and vitamins (USEPA, 2010).

Pharmaceuticals generally are biologically active compounds that are often water-soluble and are not easily biodegradable (Suarez *et al.*, 2008). Consequently, these compounds can eventually enter surface waters (Zhou *et al.*, 2009). Estimates of PPCPs present in the environment are limited by the lack of analytical methods capable of detecting low concentrations of these compounds (Kolpin *et al.*, 2002). The main route of pharmaceuticals to the environment is via the excretion of the drug or its metabolites from organisms (via urine and defecation), inappropriate disposal of expired or unused medications, incomplete degradation during sewage treatment, and/or leaching from soils following land application of biosolids (Dussault *et al.*, 2008). Many WWTPs are not designed to remove PPCPs and consequently various PPCPs are being released into surface waters (Batt *et al.*, 2006a). Batt *et al.* (2006a) compared the ability of three different wastewater treatment methods to remove caffeine and six antibiotics from effluent. Caffeine was detected in all the effluents, with concentrations ranging from 0.19 to 9.9µg/L, while the antibiotics were detected in concentrations from 0.09 to 6.0µg/L. Their results indicate that the many wastewater management practices are not effective in completely removing the surveyed compounds and these compounds have a high potential to affect the aquatic environment. However, effects on benthic macroinvertebrates in freshwater systems have not been widely studied, despite their key roles in aquatic food webs (Dussault *et al.*, 2008). Daughton & Brooks (2011) report that very little has been published regarding aquatic toxicology of pharmaceuticals, especially exposure data. The published literature regarding pharmaceuticals mostly focuses on analysis, occurrence, and fate of pharmaceuticals in the environment as well as, investigations of WWTP processes and analysis of the treated effluent (Daughton & Brooks, 2011).

PPCPs may lead to abnormal physiological processes and/or reproductive impairment, increase incidences of cancers, and the development of antibiotic-resistant bacteria (Kolpin *et al*., 2002). Kolpin *et al.* (2002) conducted a nationwide study using available analytical methods capable of detecting low concentrations of organic wastewater contaminants (OWCs). Their primary goal was to provide information on the occurrence of a broad suite of 95 OWCs, including antibiotics, prescribed and non-prescribed drugs, as well as reproductive hormones. Eighty-two of the 95 OWCs were detected at least once during the study. Although concentrations of the OWCs were generally low (median detectable concentrations < 1µg/L), they demonstrated that there are detectable quantities of OWCs occurring in effluent receiving streams and that many withstand wastewater treatment and biodegradation processes (Kolpin *et al.*, 2002). A more recent study quantified selected PPCPs at 4 different sites in southern Lake Michigan (Ferguson *et al.*, 2013). The authors reported that all of the selected PPCPs were found in quantifiable amounts at each site (Ferguson et al., 2013). PPCPs that were detected were in mean concentrations of: acetaminophen (5.36 ng/L), caffeine (31.0 ng/L), cotinine (4.03 ng/L), sulfamethazine (0.92 ng/L), and trimethoprim (5.15 ng/L) (Ferguson *et al.*, 2013). In comparison to the study of Kolpin *et al.* (2002), the study of Ferguson *et al.* (2013) detected concentrations of PPCPs in ng/L compared to µg/L. The study of Ferguson *et al.* (2013) demonstrates the advancement of analytical methods for the detection of PPCPs.

Chironomids are widely studied to assess environmental degradation (Brooks *et al.*, 2002; Carew *et al.*, 2007; Lagrana *et al.*, 2011; Tamura *et al.*, 2013). Chironomids are often the earliest colonizers in newly formed or rewetted wetlands (Layton & Voshell, 1991; Batzer & Wissinger, 1996), are common inhabitants of aquatic communities, and are often the dominant aquatic insect in terms of both abundance and species richness (Ferrington, 2008). Soft

sediment-dwelling chironomids are useful bioindicators of water quality because they are typically diverse and abundant in different various bodies of water, and they also have extended contact with the sediment (Carew *et al.*, 2007). Chironomid larvae of particular species have been used successfully as bioindicators of toxicants in aquatic ecosystems as they can provide a direct measure of water quality (Reynolds & Ferrington, 2002). For example, typical responses of BMI communities to polluted water bodies include reduced species richness, diversity, and increased abundances of tolerant organisms (Walters *et al.*, 2009). Tolerance values for chironomids are on average a value of 6 (TCEQ, 2007). The EPA states that the tolerance values scale ranges from 0 to 10, where 0 represents the tolerance value of extremely sensitive organisms and 10 for a tolerant organism (USEPA, 1999). Thus, chironomids have been widely regarded as pollution tolerant organisms (Saether, 1979; Reynoldson & Metcalfe-Smith, 1992; Xu *et al.*, 2013).

The focus of this study was to assess the water quality in selected sites within Rio Bosque Wetlands Park by measuring water chemistry parameters, heavy metal and arsenic concentrations in the sediments, and selected PPCP concentrations in water and sediment over the course of one filling cycle. The BMI community structure was determined over the same time course. Lastly, acute effects of four PPCPs (the stimulant, caffeine; the antibiotic, erythromycin; the analgesic/anti-pyretic, acetaminophen; and the plastics/epoxy resin, BPA) to chironomids were determined.

The research questions investigated in this study were described as follows:

(1) Do the following parameters vary according to phase in the filling cycle in inflow and wetlands cells: a) water chemistry parameters, b) heavy metals and arsenic concentrations, and c) PPCPs.

(2) Do these parameters impact macroinvertebrate diversity at RBWP?

2 Methods

2.1 Study Area

Rio Bosque Wetlands Park is 150.5-hectares including a series of constructed wetland cells located in southeast El Paso Co., TX. The park is managed by University of Texas at El Paso's Center for Environmental Research & Management. The wetlands are enclosed by irrigation canals and drains on three sides (Figure 1), and the western boundary of the park lies adjacent to the Rio Grande, which forms the international border between the U.S. and Mexico. During the non-irrigation season, treated effluent from the Roberto Bustamante WWTP Plant is diverted, and approximately 56.8 million liters of water per day is released into the park (Watts *et al.*, 2002). The Bustamante treatment plant is operated by El Paso Water Utilities, and is classified as a secondary wastewater treatment plant (Bustamante Plant, 2010). It uses extended aeration activated sludge processes, biological nitrification, and caustic air scrubbers for odor control (Bustamante, 2010). Before the effluent is released into the canal system it is disinfected by an ultraviolet light system (Bustamante, 2010).

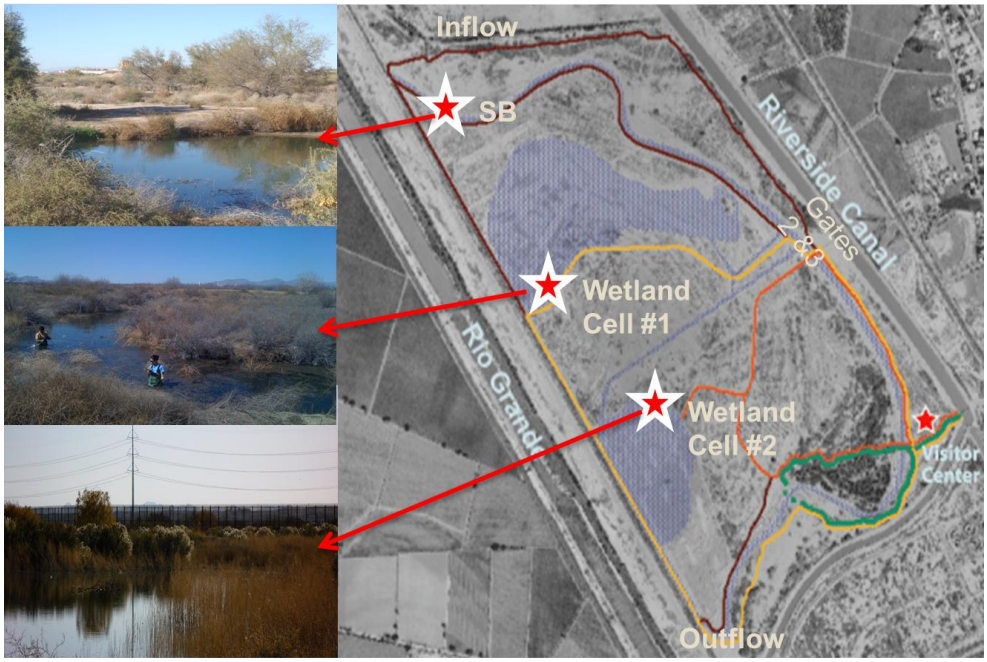


Figure 1. Map of Rio Bosque Wetlands Park and sampling sites (CERM, 2010). *Site 1* is the streambed (SB) (N31°38.789', W106°19.202'), *Site 2* is Cell #1 (N31°38.515', W106°18.501'), and *Site 3* is Cell #2 (31°38.288, W106°18.601').

Once the water is released, it flows into the park by a canal (streambed: SB, Figure 1). It is then delivered through the rest of the park via two delivery gates 2 and 3, which go to Cell #1 (Gate 2) and Cell #2 (Gate 3). More water (up to 3X) is delivered into Cell #2 (0.30m3/s) than Cell #1 (0.09 m3/s) (Rodriguez & Lougheed, 2010). Water leaves the park from Cell #2 from a delivery gate near the outflow as shown in Figure 1. Cell #1 has an inlet with no outlet, and the water level is controlled via Gate 2. The amount of time that the water spends at each location (Cell #1 and Cell #2) is short. As a result Cell #1 drains of water within about two days, whereas Cell #2 drains within 5 days after water release is diverted to the Riverside Canal rather than RBWP during irrigation season (Rodriguez & Lougheed, 2010).

The number of days water was released to Rio Bosque Wetlands Park increased steadily during the first 4 years following completion of the Park in 1998 (Fig. 2). However, in the years following 2002, there was a decline of in water input of up to more than 200 days. In 2013, Rio

Bosque only received water for 33 days, is the least amount since 1998. Relatively low releases occurred during the years of this study (2010-2011), as well.

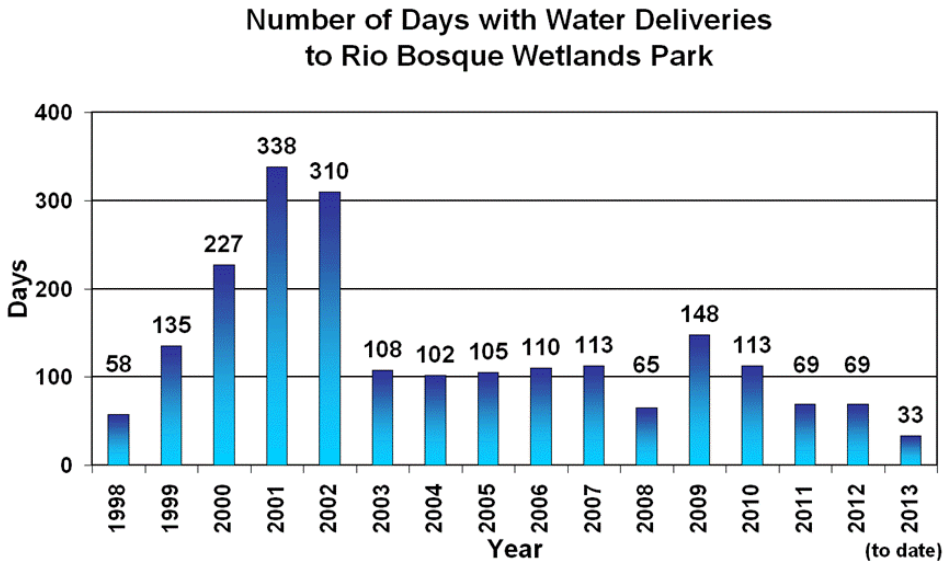


Figure 2. Rio Bosque Wetlands Park water input from 1998 to 3 February 2013. Data provided by John Sproul (Rio Bosque Wetland Park manager).

2.2 Collection and analysis of water samples for water chemistry parameters

Three sampling sites were selected: the water delivery canal (referred to throughout this thesis as streambed, SB) that directly receives inflow of the wastewater effluent, Wetland Cell # 1 (Cell 1), and Wetland Cell #2 (Cell 2) (Fig. 1). Sampling occurred between 16 Oct 2009 to 11 Mar 2011. Basic water quality parameters (temperature (˚C); conductivity (µS/cm); dissolved oxygen (DO) (mg/L and % saturation); pH; Total Dissolved Solids (TDS) (mg/L); oxidation-reduction potential (ORP)) were determined using a hand held YSI® multi-probe system (556 MPS). Prior to each sampling event, the multi-probe was calibrated according to the manufacturer's recommendations. Chlorophyll-a (µg/L) and phycocyanin (RU) were determined using a Turner Designs *Aqua*fluor™ handheld fluorometer.

Water samples for other water chemistry parameters were collected with sterile Nasco WHIRL-PAK® bags. In the laboratory, 250mL of the water collected from each site were filtered with Whatman® glass microfibre filters. Subsequently, filtered and unfiltered water samples were stored at 4 °C, and were analyzed within 24 hrs of field collection. Samples were analyzed using a YSI field photometer and Palintest® kits, which consist of a series of 11 separate tests detecting the concentrations of the following: turbidity (FTU), ammonia (mg/L NH_3), nitrite (NO_2), nitrate (NO_{3-}), phosphate (mg/L P), alkalinity (mg/L $CaCO_3$), hardness (mg/L $CaCO_3$), silica (mg/L SiO_2), chloride (mg/L Cl^-), sulfate (mg/L SO_4^{2-}), and color (mg/L Pt).

2.3 Collection and analysis of heavy metal and arsenic in water and sediment samples

A survey was conducted for the following heavy metals: Aluminum (Al), Barium (Ba), Beryllium (Be), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Cadmium (Cd), Manganese (Mn), Nickel (Ni), Lead (Pb), Selenium (Se), Silver (Ag), Vanadium (V), and Zinc (Zn), and arsenic (As). Water samples were collected from each site for all sampling dates with 500mL wide mouth, plastic containers containing either 5% nitric acid or 5% sulfuric acid. In the laboratory water samples were stored at 4°C, and were analyzed within 72 hrs of field collection. Sediment samples were collected from 20 Nov 2009 to 21 Feb 2010 with a kicknet at each of the three sampling sites. Sediment was kicked into the net in a zig-zag manner, alternating kicks of sediment diagonally into the net (1m x 1m), for 1 minute time periods. Sediment was subsequently cleaned to remove any silt from the sediment and a small portion was put into a 250mL wide-mouthed sampling container for metal analysis. Three separate sediment samples were collected near the area where benthic macroinvertebrates were collected,

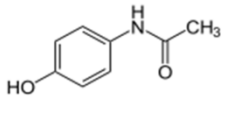
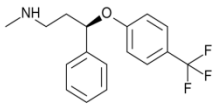
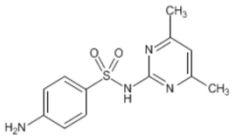
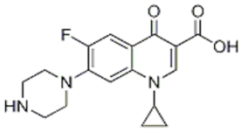
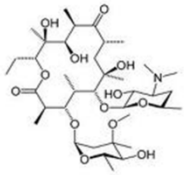
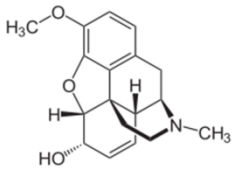
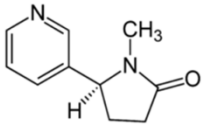
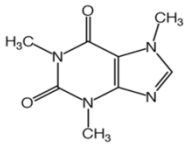
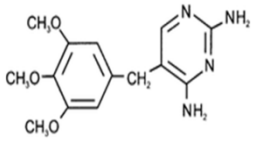
and all three samples were combined into one composite sample. In the laboratory, samples were stored at 4 °C, and were analyzed within 72-hrs of field collection.

Heavy metal and arsenic analyses were conducted according to EPA Method 6010 heavy metal analysis (USEPA, 1996) by using Induced Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Perkin Elmer Optima 4300 DV. Water samples were not pre-treated, and 50mL of water was poured into a 50mL centrifuge tube and subsequently analyzed. Sediment samples were dried in an oven at 60°C for 24-hr, and stored in a plastic bag for subsequent acid digestion. Microwave (CEM MARS 5 (Matthews, NC)) assisted digestion was conducted to disassociate any organic material and to dissolve any metals present according to a modified version of EPA Method 3051 (USEPA, 1994). Approximately 0.25g of dried sediment were digested with a 5mL of aqua regia (3:1 ratio of Hydrochloric Acid (100% HCl) and Nitric Acid (Plasma Pure 67-70% HNO_3)) in a digestion tube and subsequently microwave and heated for 20 minutes at 150 °C. Contents were poured into a 50mL centrifuge tube, and centrifuged to pellet any digested material (typically silicates). Subsequently the supernatant was analyzed by ICP-OES according to EPA Method 6010 (USEPA, 1996).

2.4 Collection and analysis of water and sediment samples for PPCP analysis

A survey of nine commonly detected PPCPs in surface water was investigated to determine the presence and concentrations within water and sediment samples from the three sites at RBWP (SB, Cell #1, and Cell #2). The survey included the following compounds: four antibiotic drugs (ciprofloxacin, erythromycin, sulfamethazine, and trimethoprim), two analgesics (acetaminophen (Tylenol®), and codeine), one anti-depressant (Fluoxetine (PROZAC®)), one stimulant (caffeine), and one metabolite (cotinine (from nicotine)) (Table 1).

Table 1. Group 1 target Pharmaceuticals and Personal Care Products (USEPA, 2007).

Group 1 compounds (Detected in positive electronspray ionization (ESI+) mode)[1]		
Acetaminophen (Analgesic/Antipyretic)	Fluoxetine (Antidepressant)	Sulfamethazine (Antibacterial)
		
Ciprofloxacin (Chemotherapeutic Antibiotic)	Erythromycin (Antibiotic)	Codeine (Pain Reliver)
		
Cotinine (Metabolite of Nicotine)	Caffeine (Stimulant)	Trimethoprim (Antibiotic)
		

Two liters of water were collected in 1L amber glass containers for water sample analyses. Sediment samples were collected with a D-frame kicknet. Sediment was kicked into the net with alternating kicks in a zig-zag manner (1m x 1m) for 1 min. The sediment was cleaned in the net to remove silt and placed into a 500mL amber glass container.

2.4.1 Water Sample Preparation and Analysis

Upon returning to the laboratory, all PPCP water samples were filtered through Whatman® glass microfiber filters. If water samples contained larger particulates, they were filtered twice using glass microfiber filters (GF/F 70 mm), followed by a third filtration to remove fine particulates with Millipore® nitrocellulose membrane filters (0.22 µm). Filtered water was stored at -70 °C. Samples were prepared for analysis by first being adjusted to pH 2.0, followed by the addition 500mg of EDTA, and subsequently spiked with labeled compounds (Table 2) in order to prepare the samples for the extraction of the acid fraction (USEPA, 2007).

Samples were then spiked with labeled standard compounds of known concentrations in order to quantify the amount of target compounds detected in the samples collected (Table 2).

Table 2. List of native and labeled compounds (used as internal controls) and spiked into water and sediment samples.

Native Compound	Stock Concentration (µg/mL)	Spiked Volume (µL)
Acetaminophen	100	1
Caffeine	25	1
Ciprofloxacin	8.75	5
Codeine	5	10
Cotinine	2.5	10
Erythromycin	5	20
Fluoxetine	2.5	20
Sulfamethazine	1	30
Trimethoprim	0.5	30
Labeled Compounds	**Stock Concentration (µg/mL)**	**Spiked Volume (µL)**
$^{13}C_3$-Caffeine	3	10
Cotinine-D_3	2	15
Fluoxetine-D_5	1	50

Approximately 2-hr after spiking samples, solid-phase extraction (SPE) was preformed, using Oasis HLB 20 cc/1g extraction cartridges. SPE involves the process of separating target analytes from any organic particles present within water and sediment samples. The last phase of SPE consisted of elution with 12mL of methanol. Finally, the water samples were analyzed using the Eksigent NanoLC-1D ™ system High-Performance Liquid Chromatography (HPLC) tandem mass spectrometer system (MS/MS) coupled to a linear trap quadrupole (LTQ XL) at UTEP's BBRC Biomolecule Analysis Core Facility according to methods described in EPA Method 1694 (USEPA, 2007).

2.4.2 Sediment sample preparation and analysis

Sediment samples were homogenized and 5g were dried in an oven for a minimum of 12-hrs at 110 ºC (USEPA, 2007). Once the sediment cooled and if particle size was > 1 mm, the sample was ground until particles were < 1 mm in size and 1g was collected for acid digestion (USEPA, 2007). Digestion was conducted to disassociate PPCPs from the sediment. Subsequently, 15mL of pH 2 phosphate buffer (sodium phosphate monohydrate/phosphoric acid: 0.14 M $NaH_2PO_4.H_2O$/85%H_3PO_4 (1.93g NaH2PO4.H2O in 99mL of reagent water + 1mL of 85% H3PO4)) was added and then vortexed for 5 minutes. Sample pH was adjusted to 2.0 ± 0.5 with buffer, and the mixture was vortexed after each addition of buffer. The sample was then spiked with native and labeled compounds (Table 2) and vortexed. The sample was then ready for acid extraction, and this process started with the addition of 20mL of acetonitrile, sonication for 30 minutes, and centrifugation for 5 minutes at about 300 rpm. The supernatant was decanted; 15mL of phosphate buffer was added and adjusted to pH 2.0 ± 0.5 with HCl. The sample was vortexed to re-suspend solids, the pH was checked and readjusted to pH 2.0 ± 0.5 if needed. The same process was performed a second time starting with the addition of 20mL of acetonitrile. A third extraction was done using 15mL of acetonitrile, sonication for 30 minutes, centrifugation for 5 minutes, and retention of the supernatants. The acid extracts were then concentrated to a final volume of 20-30mL by rotary evaporation at 50 °C. Subsequently, 200mL of reagent grade water and 500mg of Na_4EDTA was added to the acid fraction extract and mixed. Finally, the same methods as previously mentioned for SPE in the water preparation and analysis section above were followed (USEPA, 2007).

2.4.3 Target compound analysis and concentration determination

Samples were analyzed using the Eksigent NanoLC-1D ™ system (HPLC) coupled to a linear trap quadrupole (LTQ XL) mass spectrometer system (MS) according to methods described in EPA Method 1694 (USEPA, 2007) in UTEP's BBRC Biomolecule Analysis Core Facility. All standards, field blanks, and processed samples were run through the HPLC/MS/MS system as mentioned above for qualitative and quantitative analysis of the target compounds.

To begin, 1µL of each extract was injected into the Eksigent NanoLC-1D syringe pump into a C18-resine analytical column packed in the laboratory according to EPA methods (USEPA, 2007). The HPLC system allows for target analytes to be separated in the column via the aqueous mobile phases. The LTQ XL mass spectrometer system uses the electrospray ionization (ESI) technique. For this study, ESI mode was set on positive mode (ESI +) to detect the analytes of interest. Using the LTQ XL MS allows for the identification the target compounds via their parent and daughter compounds. This was possible by the LTQ XL MS system measuring the transmitted mass-to-charge ratios (m/z) that is produced during the ionization phase.

Quantitative determination of the analytes in water and sediment samples collected at RBWP was based on the standards that were run at the following concentrations: 1 fmol/µL, 10 fmol/µL, 100 fmol/µL, 200 fmol/µL, 400 fmol/µL, 600 fmol/µL, and 800 fmol/µL. The chromatogram output was used to determine the retention times (RT) for each target compound, as well as the m/z of parent and daughter ions. The peak area of each compound was determined from the chromatogram so it could be subsequently used for identification of target analytes in the samples that were collected.

Once the peaks were selected from the standard, the next step involved selecting the peaks from the chromatograms of the samples that were collected by comparing them to the peak areas of the standards that were created from all the samples run during the analysis. The presence of the target compounds in the chromatogram was determined by following EPA Method 1694, which specifies that the signal to noise ratio should ≥ 3, and in order to quantify the compound it should be ≥ 10 (USEPA, 2007). The peak areas were selected and estimated using Xcalibur 2.0.7 Software (Thermo Electron Corporation, 2006).

Finally, the concentrations of the target analytes present were calculated using the slope-intercept equation, which was obtained from standard calibration curves. When calculating the final concentration of the target compounds in the water samples, the respective field blank samples were subtracted in order to account for possible contamination during processing.

2.5 Benthic macroinvertebrates

Sediment was kicked into a D-frame kicknet (1m x 1m, 500 μm mesh size) in a zig-zag manner, alternating kicks of sediment diagonally into the net, for 1 min. After collection, the sediment was gently rinsed in the kicknet to clean it from the finer silt and debris. Samples were subsequently transferred to a 1L container and preserved with 70% ethanol for quantification.

2.5.1 Laboratory Sample Processing, Sorting, and Identification

Prior to sorting each sample, the sediments were processed with a U.S. Standard sieve #80 (180μm mesh size) and preserved with 70% ethanol. Sorting and identification of organisms was accomplished by using a dissecting microscope at 10X magnification and Merritt *et al.* (2006). In general, insects were identified to family or genus, and nematodes were identified to the Phylum level. However, macroinvertebrates in early life stages, or those individuals that had

key morphological characteristics hard to see or damaged during the collection or processing, were identified to a higher taxonomic level.

When macroinvertebrates were too numerous and when chironomid abundance was too large (>400 individuals), subsampling was conducted according to the area method (Elliott, 1971). This method uses a pan and cylinder with known areas, and quadrants within the pan are randomly selected. A portion of sediment is collected from within the cylinder within the quadrant, and this is performed at least 5 times (according to the stated methods). A pan was first measured to determine the area, and quadrants were measured and add within the pan. Upon completion of picking the sample, the subsampling method was tested using a Chi-square test to determine if the macroinvertebrate distribution in the samples picked were random (Elliott, 1971).

Upon the method was approved according to Elliott (1971), the total number of each taxa within the sample was estimated by the following equation

Estimated total number = (total area of the subsampling pan)

(total area of the cylinder) x (the average number collected in the subsampling cylinder)

During this study, four samples collected were subsampled from the following sampling dates: Cell #1: 10 Dec 09, and 18 Jan10; Cell #2: 18 Jan 10, and 21 Feb 10. Chironomids were subsampled from the following sampling dates: SB: 16 Oct 09, 20 Dec 09; Cell #1: 16 Oct 09, 5 Nov 09, 20 Nov 09, 10 Dec 09, and 18 Jan 10; and from Cell #2: 20 Nov 09.

2.5.2 Chironomidae identification methods

Chironomids were prepared for identification by clearing the larvae in 10% potassium hydroxide (KOH) and mounted in CMC-9 mounting media (Master's Chemical Company, Illinois) on a glass microscope slide following methods by Epler (2001).

2.5.3 Data Evaluation

The number of organisms collected and identified is expressed as the total number of individuals collected per kicknet sample.

Community metric (MVSP: Multi-Variate Stistical Package 3.1, version 3.22, Provalis Research, 2000) was conducted to determine the diversity, species richness, and evenness of the benthic macroinvertebrate community structure at each sampling date over the course of the sampling period.

The diversity of the macroinvertebrate community was determined using Shannon's diversity index. The following equation was used to calculate Shannon's diversity index:

$$H' = -\sum P_i (\log_2 P_i) \qquad \text{(MVSP)}$$

Where:

P_i = total number of individuals in the i^{th} taxa

Species richness (R) was determined based on the number of species found in a collection. The formula is:

$$R = s, \text{ where } s = \text{the number of taxa} \qquad \text{(MVSP)}$$

Shannon's taxa evenness is a measure of taxa distributions within the population.

$$\text{Species Evenness (E)} = \text{Shannon's Diversity} / \log_2 R \qquad \text{(MVSP)}$$

Evenness (E) is a measure of how similar the abundances of different taxa are within a population. When all taxa occur in similar proportions, evenness is one, but when abundances are very dissimilar then the value decreases down to zero.

Bray-Curtis dissimilarity was calculated to determine the difference in the benthic macroinvertebrate composition between the sites. According to the following equation:

$$BC_{ij} = \frac{2C_{ij}}{S_i + S_j}$$

Bray & Curtis, 1957

Where:

C_{ij} is the sum of the lesser value for only the species that are in common between two sites.

S_i and S_j are the total number of individuals counted at the two sites.

The value of the Bray-Curtis dissimilarity index lies between 0 and 1, where 0 means the two sites are the same, and share all the species found, and 1 which means that the two sites dissimilar and do not share any species found.

2.6 Acute: 48-hr LC50 exposure methods and *Chironomus dilutus* culturing methods

2.6.1 *Chironomus dilutus* culturing methods

Chironomus dilutus egg masses were obtained from the Mid-Continent Ecology Division (MED Duluth) USEPA Duluth, MN. *C. dilutus* was chosen because it is the genus most ecologically similar to the genera collected at Rio Bosque and it was used to study toxicity of caffeine by Moore et al. (2008). Prior to the arrival of the egg masses the culturing aquarium was prepared by cleaning a 10-L aquarium with a 5% bleach solution, followed by rinsing with deionized water, and air drying. Once the aquarium was dry, sterile glass beads (4-5 g; 150-212 µm in diameter) were added to form a layer at least a half-cm thickness. 10-L of EPA water was then added to the aquarium followed by the addition of an air-stone attached to an air pump. An air-stone was added not only to add air into the aquarium, but also to allow for water movement. Egg masses were observed daily to observe larval development. Once larvae start hatching, usually within 2-6 days after receiving the egg masses, a Tetra® TetraFin® Plus Goldfish Flakes

food solution (3.5g/62.5mL) made with EPA medium was added to the aquarium. Once *C. dilutus* reached the third instar (13 to 15 days old), larvae were used in the PPCP exposures.

2.6.2 48-hr LC_{50} exposure methods

Acute exposures were conducted according to modified methods of Moore *et al.* (2008). PPCP concentrations for each exposure were determined according to an initial range finder exposure. For all 4 selected PPCPs, acute exposures were 48hrs and consisted of three 150mL replicates of the control and up to 10 different concentrations with 10 *C. dilutus* in each 250mL beaker. All exposures were performed in EPA medium (USEPA, 2003). EPA medium was also used to make PPCP and BPA stock solutions (Moore et al., 2008). Sterile glass beads (4-5 g; 150-212 µm in diameter) were used as the substrate. Exposure conditions consisted of a temperature of 24 ± 1°C in the dark due to photosensitivity of the compounds being tested.

2.7 Statistical Analyses

Statistical analyses of water quality parameters were conducted using General Mixed Model (GLMM) analysis (SAS® version 9.3) to determine significant differences between sites and/or sampling dates for each of the parameters. If there were any significant differences, a Tukey's *post-hoc* tests were conducted to identify where differences occurred. All GLMM analyses and Tukey's *post-hoc* tests were conducted by Dr. Julia Bader (BBRC Statistical Consulting Laboratory, UTEP).

In order to estimate the LC_{50} concentration for each acute toxicity test, Probit analysis via SPSS (IBM© SPSS© Statistics version 19) was used. Probit analysis consists of preforming a probit transformation on the proportion of the observed survival and log transforming the compound concentrations. A linear regression is preformed and the estimated LC_{50} is determined. Subsequently, the NOEC (no observable effect concentration) and LOEC (lowest

observable effect concentration) were determined using a GLM analysis using SPSS (IBM© SPSS© Statistics version 19). If there were any significant differences, a Dunnett's *post-hoc* test was conducted to identify where differences occurred. The estimated NOEC value was determined as the concentration at the lowest concentration where before significance (> p=0.05) was determined between the control and each increasing concentration. The estimated LOEC value was determined as the concentration where significance resulted (< p=0.05).

The benthic macroinvertebrate (BMI) community structure was analyzed using non-metric multi-dimensional scaling (NMDS or NMS). The use of NMDS leads to the discrimination between any variables (McCune & Grace, 2002). BMI values were used to ordinate a Bray-Curtis measure in NMS (PC-ORD, version 5.0; MjM Software Design, Gleneden Beach, OR, USA). Significance of the axes was determined using Monte-Carlo simulation. BMI communities were ordinated in a bi-plot and correlated to the environmental variables. For NMDS, rare species (species values ≤1) were excluded. According to McCune & Grace (2002), if rare species are deleted, then the final stress will normally be quite similar to that in the whole data set. Multi-Response Permutation Procedures (MRPP) was used to compare species composition with the 3 selected sites. MRPP is a group linkage method that is a non-parametric procedure for testing the hypothesis of no difference between two or more groups of entities (McCune & Grace, 2002; Zimmerman *et al.*, 1985; Mielke & Berry, 2001). The groups, in this case, were defined by the values of each of the sites. Sorensen (Bray Curtis) was selected for MRPP, and the distance matrix was rank transformed. The output for MRPP shows the T value, the A value and the p value. T is the test statistic. e A is the agreement statistic that describes within-group homogeneity, compared to the random expectation. When all items are identical within groups, then A=1, while on the other hand if there is heterogeneity

within groups equals expectation by chance, then A=0 (McCune & Grace, 2002). McCune & Grace (2002) report that in community ecology, values for A are commonly below 0.1, and a value of $A > 0.3$ is fairly high.

3 Results

3.1 General water chemistry

The results reported below are from the 16 Oct 2009 to 21 Feb 2010 sampling period. Sampling was initiated two weeks following water release in September 2009, however, results are reported starting the second sampling event (16 Oct 2009), which is one month after water was released due to complications with ease of access of previous selected sites. Water chemistry parameters that are reported (Table 3) are those that generally impact water quality. Other water chemistry parameters measured, but not shown, are given in Appendix A, Table 13.

During the course of this study nutrient levels (Table 3) were above the Texas Surface Water Quality Standards (TSWQS) for the Rio Grande Basin (Segment 2308) (TCEQ, 2010). Ammonia, nitrate + nitrite, phosphate, and Chlorophyll-a exceeded their standards. On most occasions ammonia (57% of the time at SB; 71% of the time at Cell #1; 86% of the time at Cell #2) was above the 0.33 mg/L criteria set by TSWQS (TCEQ, 2010) during the course of the sampling period. Nitrate + nitrite exceeded the 1.95 mg/L criteria set by TSWQS at SB and Cell #2 for all sampling dates, and 85% of the time at Cell #1 over the course of the sampling period (TCEQ, 2010). At Cell #1, one sampling date (5 Nov 09) was an exception where the levels of nitrate + nitrite was below (0.9 mg/L) the state criteria. During the sampling period, at all sites and sampling dates, measured phosphate levels exceeded state criteria levels of 0.46 mg/L (TCEQ, 2010). Chlorophyll-a at both Cell #1 and Cell #2 exceeded the 14.1 μg/L criteria set by TSWQS 71% percent of the time (TCEQ, 2010).

Table 3. Water chemistry parameters (range ± SE) for all sites and sampling dates from 16 Oct 09 to 21 Feb 10.

	Sites		
Water Chemistry Parameters	**SB**	**Cell #1**	**Cell #2**
pH	6.0-8.1 ± 0.3	6.1-6.8 ± 0.1	6.3-7.7 ± 0.2
Water temp (°C)	22-30 ± 1.2	20-29 ± 1.5	16-20 ± 0.9
Conductivity (mS/cm)	1.4-1.9 ± 0.07	1.4-1.9 ± 0.07	1.5-2.0 ± 0.07
O2 (mg/L)	4.2-7.8 ± 0.5	3.2-8.2 ± 0.7	3.3-11.9 ± 1.3
Ammonia (mg/L NH4-N)	0.1-6.6 ± 1.3	0.2-5.7 ± 0.8	0.3-7.1 ± 1.1
Nitrite (mg/L NO2-N)	0.01-6.4 ± 0.9	0.3-6.5 ± 1.2	2.7 ± 1.2
Nitrate (mg/L NO3-N)	5.1-22 ± 2.1	0.3-18 ± 2.1	5.1-15 ± 1.2
Phosphate (mg/L PO4-P)	2.8-4.0 ± 0.2	3.0-4.6 ± 0.5	2.8-6.8 ± 0.5
Hardness (mg/L CaCO3)	95-250 ± 19	195-250 ± 9	195-355 ± 22
Chlorophyll-a (µg/L)	4.9-8.7 ± 0.6	7.5-40.8 ± 4.4	11.5-36.6 ± 3.4

Water quality parameters were analyzed using GLMM analysis (SAS® version 9.3) to determine significant differences among sites and/or sampling dates for each of the parameters. If there were any, a Tukey's *post-hoc* test was conducted to identify where differences occurred. Most measured water chemistry parameters differed significantly among sampling dates, which is expected due to a decrease in water temperature during the study. Chlorophyll-a was an exception, as it did not differ among sampling dates ($F=2.83$, $p=0.06$). Water temperature, conductivity, O_2, nitrite, phosphate, and chlorophyll-a differed among sites (Table 4, $p<0.05$). While pH, ammonia, nitrate, and hardness were not significantly different among sites ($p>0.05$) (Figure not shown).

Table 4. Tukey's *post-hoc* test results for significance among sites and sampling dates for water chemistry parameters from 16 Oct 09 to 21 Feb 10. F and p values that are in bold indicate significance differences.

Water chemistry parameter	Significantly different among sites: F-value, p-value	Significantly different among sampling dates: F-value, p-value
pH	F=2.4, p=0.13	**F=3.1, p=0.05**
Water temp (°C)	**F=78.6, p<0.0001**	**F=10.7, p=0.0003**
Conductivity (mS/cm)	**F=23.1, p<0.0001**	**F=498.7, p<0.0001**
O2 (mg/L)	**F=4.0, p=0.048**	**F=4.4, p=0.014**
Ammonia (mg/L NH-N)	F=1.8, p=0.20	**F=16.8, p<0.0001**
Nitrite (mg/L NO-N)	**F=7.5, p=0.0078**	**F=7.5, p=0.0005**
Nitrate (mg/L NO-N)	F=0.29, p=7531	**F=4.3, p=0.016**

Phosphate (mg/L PO-P)	**F=4.5, p=0.035**	**F=5.2, p=0.008**
Hardness (mg/L CaCO3)	F=3.2, p=0.07	**F=3.7, p=0.03**
Chlorophyll-a (µg/L)	**F=20.6, p=0.0001**	F=2.8, p=0.06

Water temperature was significantly different between SB and Cell #2 ($p< 0.0001$), and SB and Cell #1 ($p<0.0001$) (Figure 3a). Throughout the sampling period, water temperatures at Cell #2 were generally lower than SB (6 - 13°C) and Cell #1 (4 -10°C) (Table 3, Figure 3a). Only O_2 levels were significantly different between Cell #1 and Cell #2 ($p=0.04$) (Figure 3b). During the sampling period O_2 levels at Cell #2 were generally higher (86% of the time), ranging from 0.17 to 6.5 mg/L (Figure 3b). Conductivity was significantly different among sites ($p<0.01$) (Figure 3c) and, on average, was 1.71 mS/cm at SB; 1.74 mS/cm at Cell #1; and 1.8 mS/cm at Cell #2 (Table 3, Figure 3c).

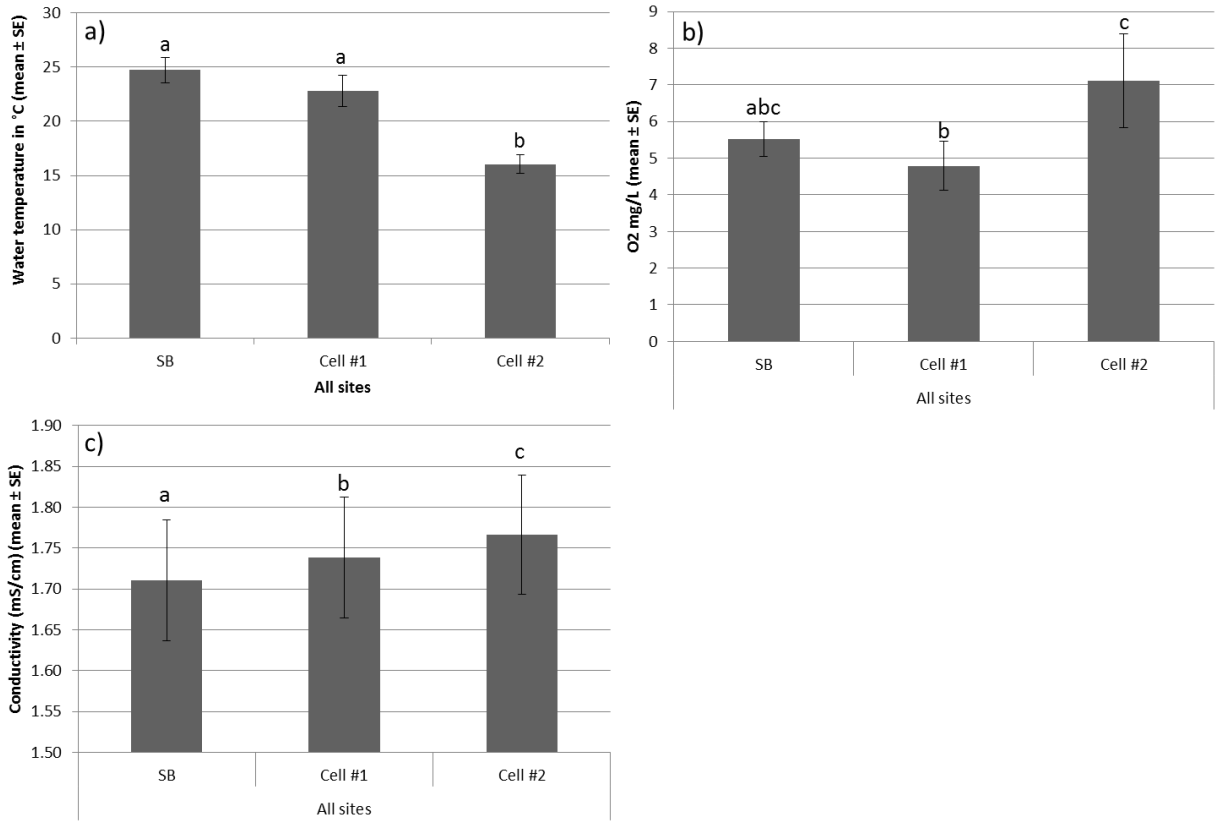


Figure 3. Water temperature (°C) (a); O_2 (mg/L) (b); and Conductivity (mS/cm) (c) measured at all sites and sampling dates from 16 Oct 09. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences

among sites (Tukey's *post-hoc* test via GLMM analysis (Water temperature: F=78.56, p < 0.0001; O_2: F=3.96, p=0.048; and Conductivity: F=23.07, p<0.0001).

There were significant differences among sites for the following water chemistry parameters: nitrite, phosphate, and chlorophyll-a. Nitrate levels were significantly different (F=7.48, p=0.0078) among SB and Cell #1, and SB and Cell #2 (p=0.01) (Figure 4a). Nitrite levels at Cell #1 and Cell #2 were generally higher than SB, ranging from 0.29-5.1 and 0.04-4.2 mg/L NO_2-N, respectively (Figure 4a). Phosphate levels were significantly different (F=4.50, p=0.035) among SB and Cell #1 (p=0.03) only (Figure 4b). Phosphate levels at Cell #1 were generally higher (86% of the time) than SB, and levels ranged from 0.17 to 2.5 mg/L PO_4-P (Figure 4b). Chlorophyll-a was significantly different (F=20.56, p=0.0001) among SB and Cell #1 (p=0.0012), and SB and Cell #2 (p=0.0001) (Figure 4c). Chlorophyll-a levels at Cell #1 (1.4 to 20 μg/L), and Cell #2 (4-32 μg/L) were higher over the course of the sampling period (Figure 4c).

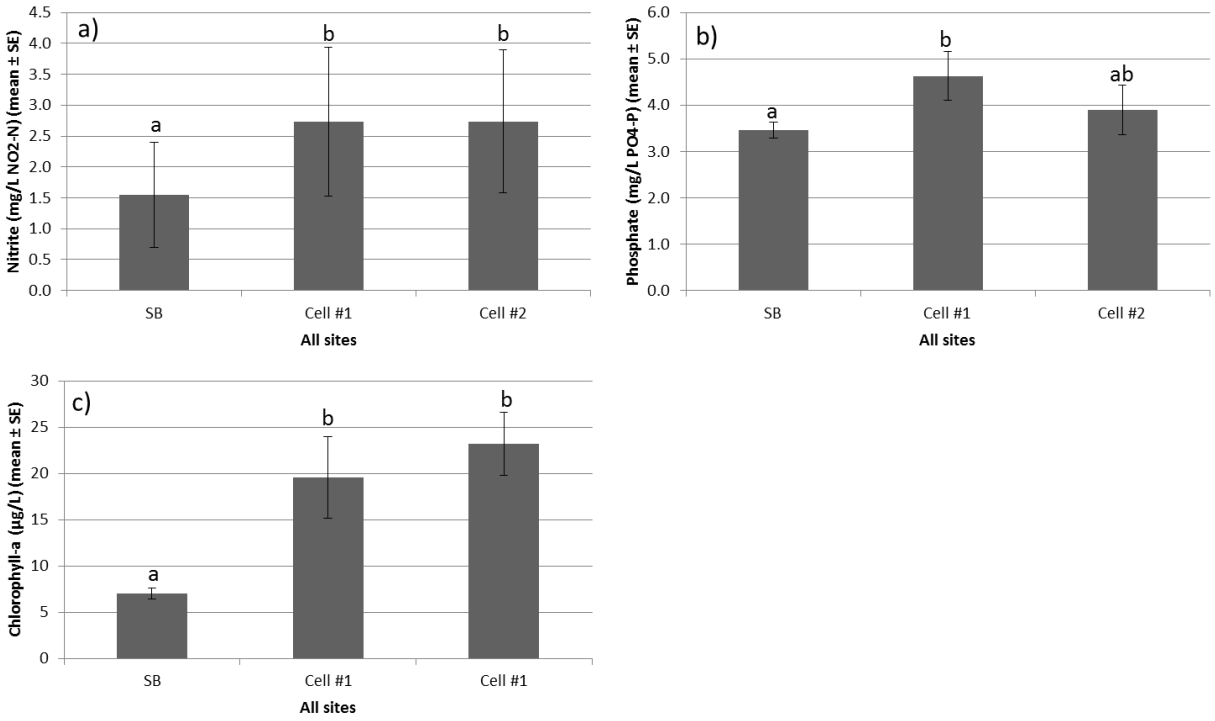


Figure 4. Nitrite (mg/L NO_2-N)) (a); Phosphate (mg/L PO_4-P) (b); and Chlorophyll-a (µg/L) (c) measured at all sites and sampling dates from 16 Oct 09. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences among sites (Tukey's *post-hoc* test via GLMM analysis (Nitrite: F=7.48, p=0.0078; Phosphate: F=4.50, p=0.035; and Chlorophyll-a: F=20.56, p=0.0001).

3.2 Results for heavy metals and arsenic

Of the 14 heavy metals and arsenic analyzed, arsenic and cobalt were below the limit of detection (< 20 ppb). Six (Cd, Cr, Cu, Pb, Ni, and Zn) metals will be discussed in this section as these metals most commonly impact water quality (MacDonald *et al.*, 2000). Heavy metal results not shown are given in Appendix B, Figure 18.

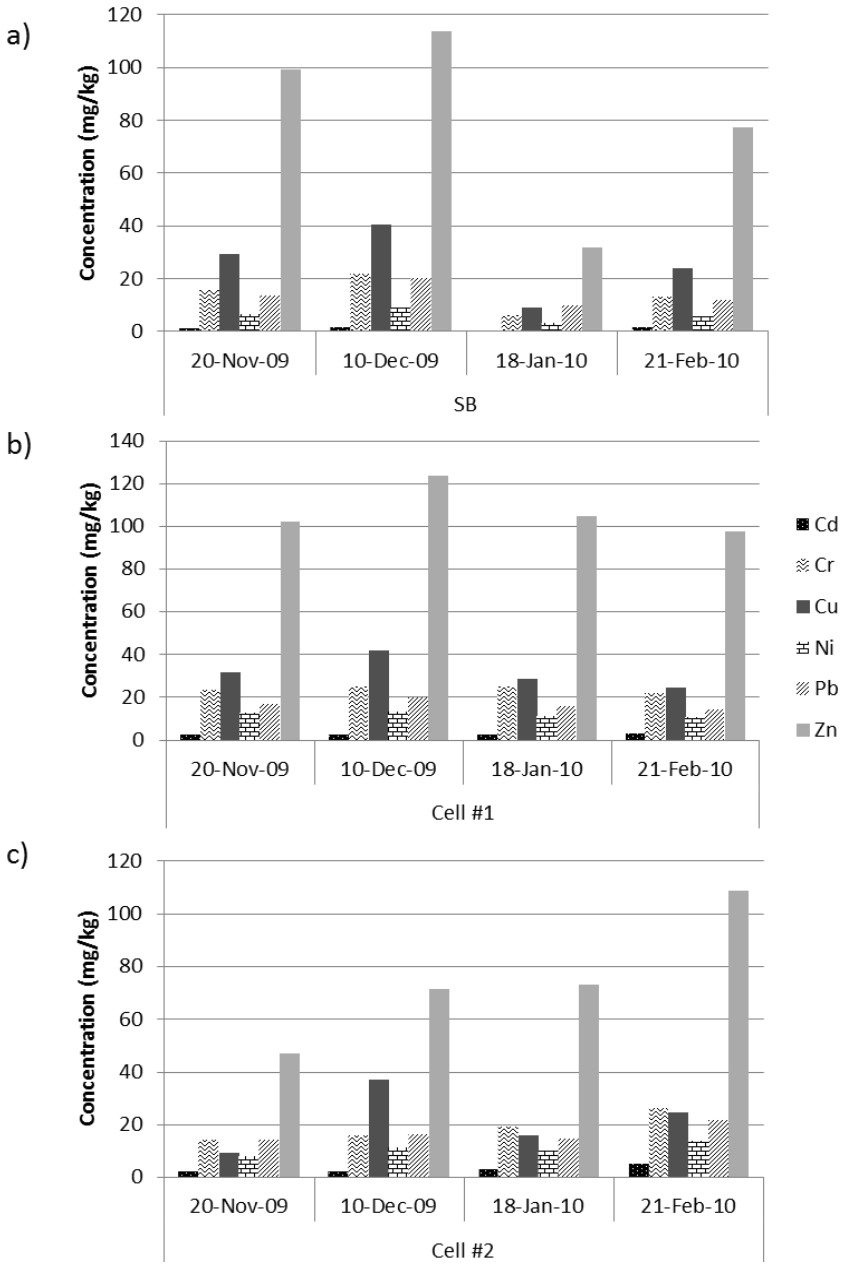


Figure 5. Heavy metal concentrations (mg/kg) in all sites: SB (a), Cell #1 (b), and Cell #2 (c), and sampling dates from 20 Nov 09 to 21 Feb 10.

Of the 6 metals, Cd ($F=6.21$, $p=0.035$) and Ni ($F=9.25$, $p=0.015$) were the metals that were differed significantly in concentration among sites (Figure 3). Cu was the only element that was significantly different among sampling dates (Figure 4). The 4 remaining heavy metals (Cr, Cu, Pb, and Zn) did not differ significantly in concentration among sites or sampling dates. Cd concentrations varied from site to site, with concentrations increasing from SB to Cell #2.

Specifically, Cd levels increased from SB to Cell #1 by 1 to 2 orders of magnitude, and from Cell #1 to Cell #2 there was only a slight increase of about 0.2 mg/kg (Figure 4a). A similar pattern was observed in the concentration levels of Ni, levels increased from 1 to 2 orders of magnitude from SB to Cell #1, however, from Cell #1 to Cell #2 there was a slight decrease by about 2 mg/kg (Figure 4b).

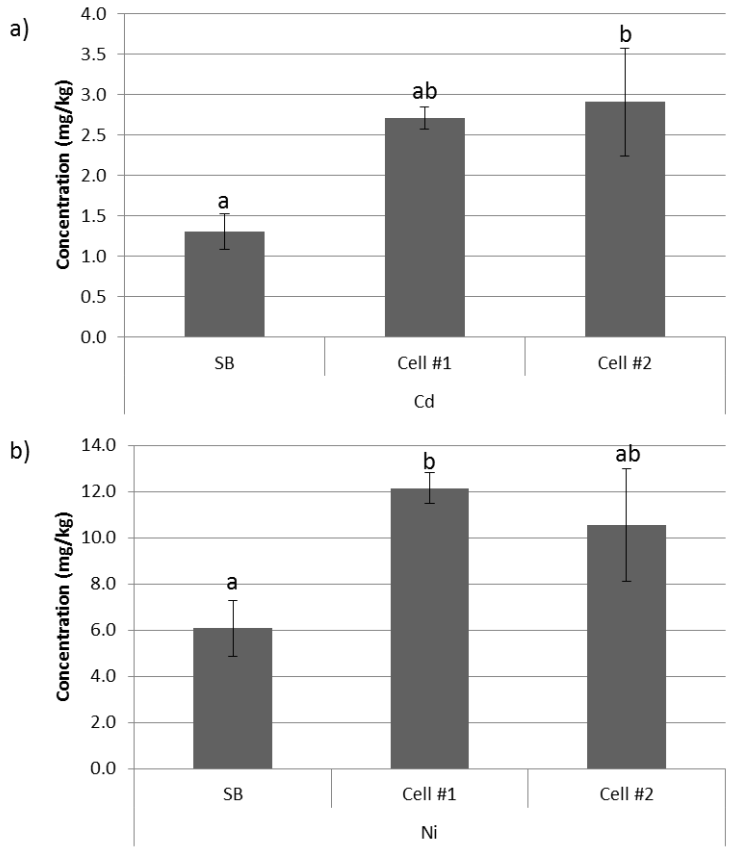


Figure 6. Cadmium (Cd) (a) and Ni (Ni) (b) concentrations (mg/kg) found in the sediment at all sites and sampling dates from 20 Nov 09 to 21 Feb 10. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences among the sites (Tukey's *post-hoc* test via GLMM analysis for Cd: F=6.21, p=0.035; Ni: F=9.25, p=0.015).

Copper (Cu) was the only heavy metal that was significantly different among sampling dates (F=1.92, p=0.044). A Tukey's *post-hoc* test determined that the 18 Jan 10 sampling date was significantly different from the 10 Dec 09 sampling date (p=0.0380). A shift of Cu levels was observed from 20 Nov 09 to 18 Jan 10. Cu concentrations increased by a little over 15

mg/kg from 20 Nov 09 to 10 Dec 09, however, from 10 Dec 09 to 18 Jan 10 concentration levels decreased by about 23 mg/kg (Figure 7).

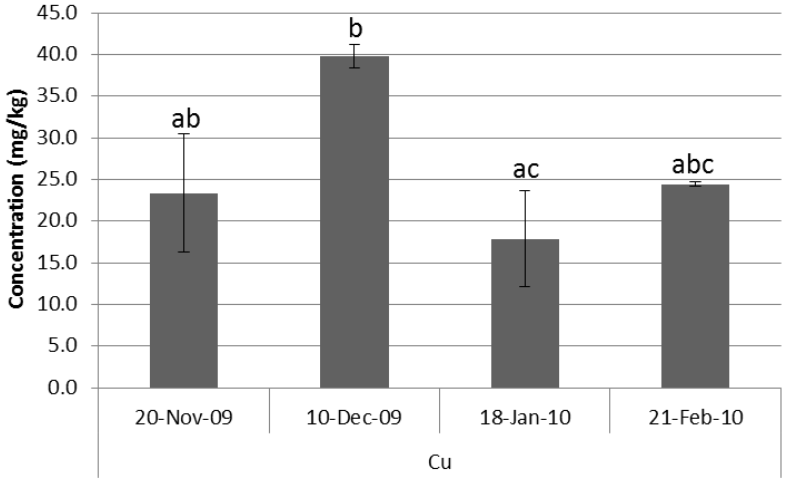


Figure 7. Copper (Cu) concentrations (mg/kg) found in the sediment at all sites and sampling dates from 20 Nov 09 to 21 Feb 10. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences among the sampling dates (Tukey's *post-hoc* test following GLMM analysis (F=1.92, p=0.044).

3.3 Results for target PPCPs in water and sediment

In water samples 8 of the 9 PPCPs were present at least once within each of the sites during the course of the sampling period (Table 5). Erythromycin was the exception, and it was detected below the limit of quantification (Table 5). Water and sediment results reported in this section are over the course of two different water releases at RBWP. It is important to note that only two sediment samples were collected and analyzed during the first water release at RBWP was the same period that BMI samples were collected. Unfortunately, at the beginning of this study PPCP collecting methods were not in place until the second water release at RBWP for water samples, and towards the end of the first water release (sampling dates: 8 Feb 10and 21 Feb 10) at RBWP for sediment samples. Results of PPCPs in water and sediment samples are available in Appendix C: Figures 20 and 21).

Table 5. Concentration (ng/L) ranges of PPCPs detected in water from each site and sampling date from 15 Dec 10 to 11 Mar 11 at Rio Bosque Wetlands Park. Note: n indicates the number of samples collected at each site during the sampling period, and BLQ indicates below the limit of quantification.

	Water Concentration (ng/L)		
Compound	SB (n=2)	Cell #1 (n=3)	Cell #2 (n=3)
Acetaminophen	BLQ	BLQ	BLQ-0.073
Cotinine	BLQ-1.03	BLQ-1.03	BLQ
Caffeine	BLQ-0.23	BLQ-1.6	BLQ
Sulfamethazine	BLQ-0.57	BLQ-0.57	BLQ
Trimethoprim	BLQ-0.41	BLQ-0.17	BLQ
Codeine	BLQ-3.7	BLQ-2.3	BLQ-3.5
Fluoxetine	BLQ-0.11	BLQ-0.47	BLQ-0.11
Ciprofloxacin	44.8-58.3	BLQ-60.6	BLQ-38.3
Erythromycin	BLQ	BLQ	BLQ

In the sediments, acetaminophen and erythromycin were below the limit of quantification, while all other PPCPs analyzed were detected at least one time in each of the sites. The compounds that were the most frequently detected and in high concentrations were ciprofloxacin and codeine, in both water and sediment samples. Two other compounds that were frequently detected in the sediment samples were caffeine and trimethoprim. The concentrations of PPCPs detected were higher in the sediments (Table 5) than in the water (Table 6) by up to 6 orders of magnitude.

Table 6. Concentration of PPCPs detected in the sediments of each site for the first water cycle (a) and the second water cycle (b) at Rio Bosque Wetlands Park. Note: BLQ indicates below the limit of quantification.

	Sediment Concentration (ng/kg)		
Compound	SB (n=3)	Cell #1 (n=4)	Cell #2 (n=4)
Acetaminophen	BLQ	BLQ	BLQ
Cotinine	BLQ	BLQ-15,251	BLQ-10,310
Caffeine	959-45,802	BLQ-178,535	959-117,646
Sulfamethazine	0-15,251	0-15,251	0-3,171
Trimethoprim	0-1,986	603-20,924	0-3,474
Codeine	0	0-13,668	0-13,630
Fluoxetine	0	0-12,106	0-88,003
Ciprofloxacin	0-214,194	0-214,194	0
Erythromycin	BLQ	BLQ	BLQ

3.4 Benthic Macroinvertebrate

3.4.1 General Results

Nearly 31,000 BMIs were collected throughout the course of this study. Seventeen different taxa were identified, however only two taxa groups, midge larvae (Diptera: Chironomidae), and nematodes (Nematoda), accounted for over 50% of the total number of BMIs collected on most occassions (Figure 6). Individual benthic invertebrate identifications, counts and summary statistics for each site are given in Appendix D, Tables 13-14, with trends in total populations, taxon richness and Shannon's diversity illustrated in Figure 8 a-c. Although there are some site and date specific exceptions, in general, individuals representing these two taxonomic groups are usually the dominant taxa found at all sites during the study. Community composition and population trends of individual taxa are illustrated in Figure 10.

3.4.2 Community composition

Overall, immature Chironomidae were the dominant invertebrate accounting for over 50% of the individuals (larval and pupal stages) collected (Figure 6 and Appendix D: Tables 13-15). The second most abundant taxon identified were individuals from Nematoda (Figure 6, Appendix A: Tables 13-15). Individuals found in low abundances (n<5) and on one occasion n=29 were grouped as miscellaneous (Misc) taxa (Figure 6). There were 3 sampling dates when chironomids did not compose of at least 50% of the macroinvertebrate composition. On 21 Feb 10 at SB, only a single individual from Chironomidae was found in that particular sample. On 5 Nov 09 in Cell #2, about 40% of the individuals collected were Chironomidae (Figure 6). On 8 Feb 10 at Cell #2, about 72% of individuals belonged to other taxa. These instances in which non-Chironomidae were numerically dominant may have been due to the changes in life-stage of

chironomids from pupa to adult, mortality, competition, or possibly the area sampled not being suitable for colonization.

The majority of individuals belonged to Chironomidae with few exceptions: SB (21 Feb 10), and Cell #2 (08 Feb 10) where 83% and 72 % of the individuals were other taxa. During the sampling period at SB, 4 out of 7 sampling events composed of only chironomids (05 Nov 09, 20 Nov 09, 10 Dec 09, 08 Feb 10) (Figure 8a). On the last sampling date at SB (21 Feb 10), a shift was observed from chironomid dominating (n=1) to non-chironomid taxa (n=5). However, given the low overall numbers of organisms within this particular sample, this shift may not be biologically significant. In Cell #1, 4 out of 7 sampling events composed of only chironomids (16 Oct 09, 10 Dec 09, 18 Jan 10, and 21 Feb10), while the rest of the samples composed of <10% of non-chironomid taxa (Figure 8b). However, Cell #2 was characterized by a much different community as compared to the other sites (Figure 8c). Cell #2 had a greater overall abundance of individuals other than chironomids, comprising 9 to 72% of the individuals found over the course of the sampling period. At this particular site, there was not an instance where in which only chironomids were found. The three sampling dates that were composed of mostly chironomids were during three subsequent sampling dates: 20 Nov 09 (91%), 10 Dec 09 (83%), and 18 Jan 10 (89%). The only sampling date where non-chironomid taxa were the most abundant was on 08 Feb 10 when macroinvertebrate taxa comprised 72% of the community.

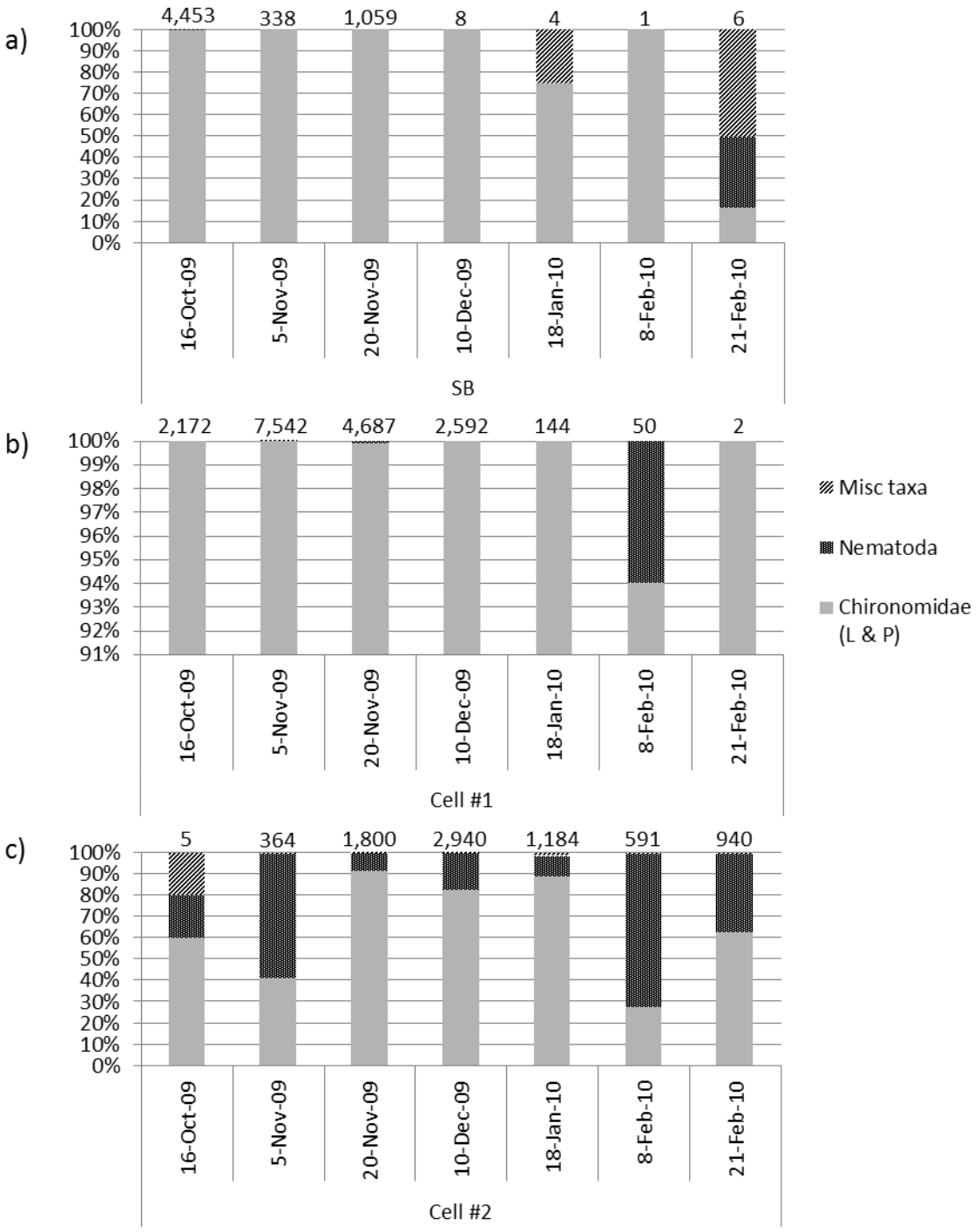


Figure 8. Percent Chironomidae larvae (L) and pupae (P) versus Nematoda and miscellaneous (Misc) taxa within each site and sampling date from 16 Oct 2009 to 21 Feb 2010. The number above each bar indicates the sample size of individuals found at each of the respective sites (a=SB, b=Cell #1, and c=Cell #2) and sampling dates.

3.4.2.1 Dominant Chironomidae taxon

During this study, four genera within the Chironominae subfamily were identified at SB: *Chironomus, Dicrotendipes, Goeldichironomus,* and *Polypedilum* (Figure 9a). The fifth genus belongs to the subfamily within Orthocladinae. This individual was in an early instar stage and could not be identified to the generic level, so it was identified as *Orthocladius/Cricotopus*. The majority of the individuals identified belonged to the genera *Goeldichironomus* and *Chironomus*, with those two genera comprising about 90% of the total. At the beginning of the sampling period *Chironomus* (77%) larvae dominated, while *Goeldichironomus* contributed to 21% of the chironomids found within those two samples. However, on 20 Nov 09 there was a shift in the dominant genera from *Chironomus* (28%) to *Goeldichironomus* (65%), and they continued to be the dominant genus until the end of the sampling period.

During the sampling period in Cell #1 individuals from 2 different subfamilies were identified from: Chironominae, and Tanypodinae (Figure 9b). Four genera were identified from Chironominae: *Chironomus*, *Goeldichironomus*, *Polypedilum*, and *Tanytarsus*. Subfamily (Tanypodinae) was composed of 3 genera: *Ablabesmyia*, *Labrundinia*, and *Tanypus*. The number of Chironomidae individuals in Cell #1 at least doubled after the first sampling date, however, following the second sampling date the number of chironomids decreased steadily until the end of sampling. The two genera were the most frequently found in Cell #1: *Chironomus* and *Goeldichironomus* composing of about 16% and 63% of the individuals, respectively (Figure 9). There was one exception when *Chironomus* and/or *Goeldichironomus* was not found, which was on the last sampling date (21 Feb 10) when only *Polypedilum* was found. Overall *Goeldichironomus* was the dominant genera found (60%-90%) within the sampling period at Cell #1.

During the sampling period at Cell #2, individuals belonging to three different subfamilies were found: Chironomine, Orthocladinae, and Tanypodinae (Figure 9c). Five genera belonged to Chironominae: *Chironomus*, *Dicrotendipes*, *Goeldichirnomus*, *Polypedilum*, and *Tanytarsus*. Orthocladinae was composed of two genera: *Cricotopus* and *Orthocladius*. Some individuals were identified as Orthocladinae and *Orthocladinae/Cricotopus* due to the difficulties in identifying larvae at early instars. Two genera were found within the subfamily Tanypodinae: *Ablabesmyia* and *Tanypus*. The number of Chironomidae found within Cell #2 increased by 3 orders of magnitude within the first three sampling events and continued to increase until the fourth (10 Dec 09) sampling event. Two genera were dominant during the sampling period: *Chironomus*, with an overall average of about 41 %; and *Goeldichironomus*, with an overall average of about 48%.

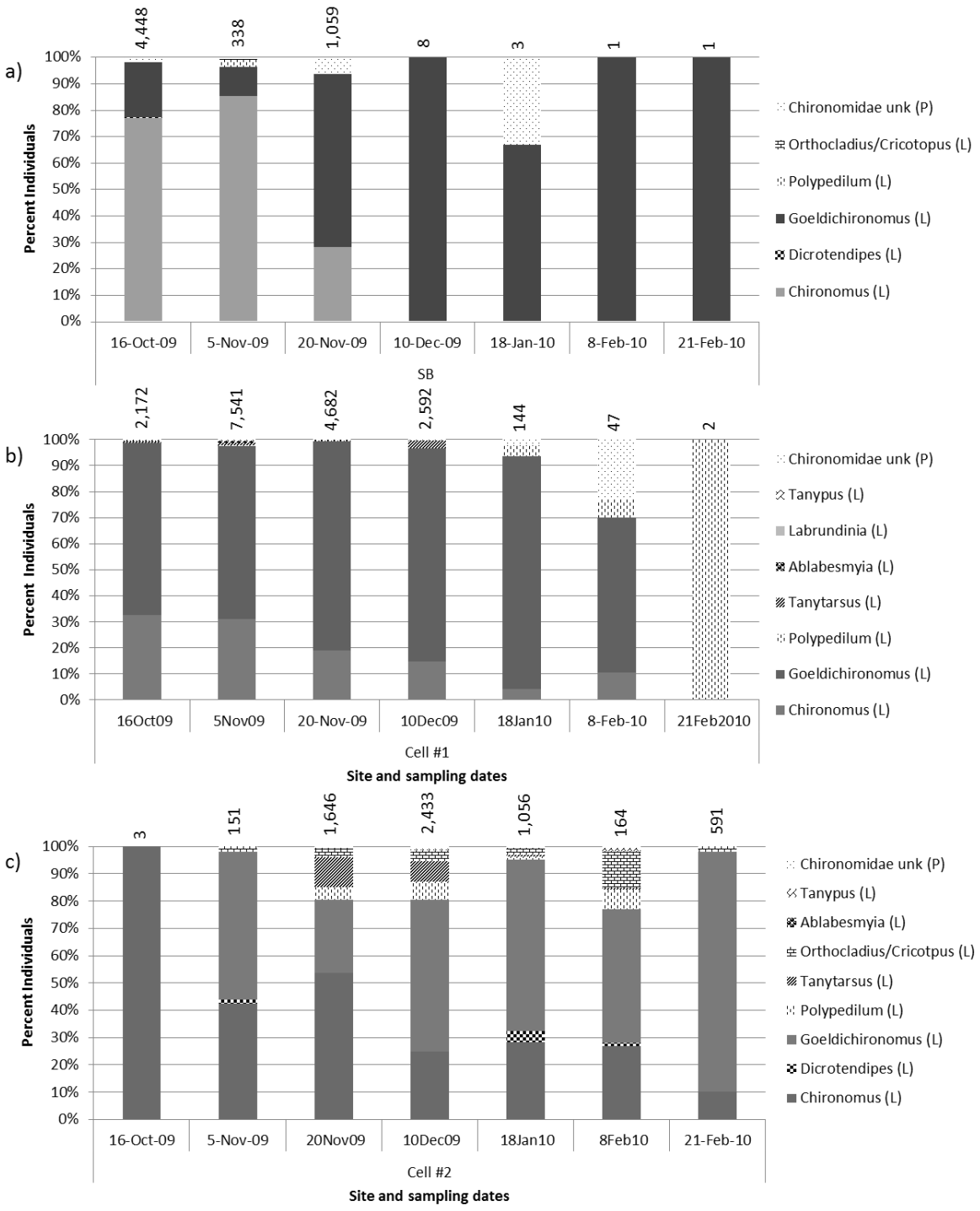


Figure 9. Percent Chironomidae genera at SB (a), Cell #1 (b), and Cell #2 (c) for each sampling date from 16 Oct 09 to 21 Feb 10. The number above the bars indicates the sample size of Chironomidae found within the site. Chironomidae und indicates that genus of (P) was not determined, while (L) indicates larvae.

3.4.2.2 Diversity

MSVP (Multi-Variate Statistical Package) 3.1, version 3.22) was used to analyze trends in taxa diversity, taxa richness, and taxa evenness (Figure 10). Shannon's Diversity, Shannon's Evenness, and Taxa Richness fluctuate between and within the sampling sites, however, all sites have values within expected ranges (Figure 10).

The diversity at SB ranged from 0-1.9 during the study. There were two occasions at SB when the diversity index (H') had a value of zero and taxa richness of 1, which was on 10 Dec 09 and 08 Feb 10 (Figure 10a). The highest diversity at SB occurred on 21 Feb 10 (H'=1.9) with a taxa richness of 4. In Cell #1, the majority of organisms consisted of chironomids. There was one occasion when the diversity was zero, which occurred on 21 Feb 10 when only one taxon was found (Figure 10b). The highest diversity (1.8) in Cell #1 occurred on 8 Feb 10. Initially evenness had a zero value, but as the sampling period progressed it peaked by the 8 Feb 10 sampling date (J=0.76). Taxa richness was highest (9), at Cell #1 during the first sampling date, and had the lowest taxa richness (1) on 21 Feb 10. In Cell #2, diversity, evenness, and taxa richness were all higher than the other two sites (Figure 10c). Diversity values at Cell #2 ranged from 1.4 to 2.2. The lowest diversity (H'=1.4) occurred on the first sampling date when 3 taxa were found, and also when the taxa abundances were more similar (J=0.87) within the sample. The highest diversity at Cell #2 occurred during the middle of the sampling period (10 Dec 09) when 14 taxa were found.

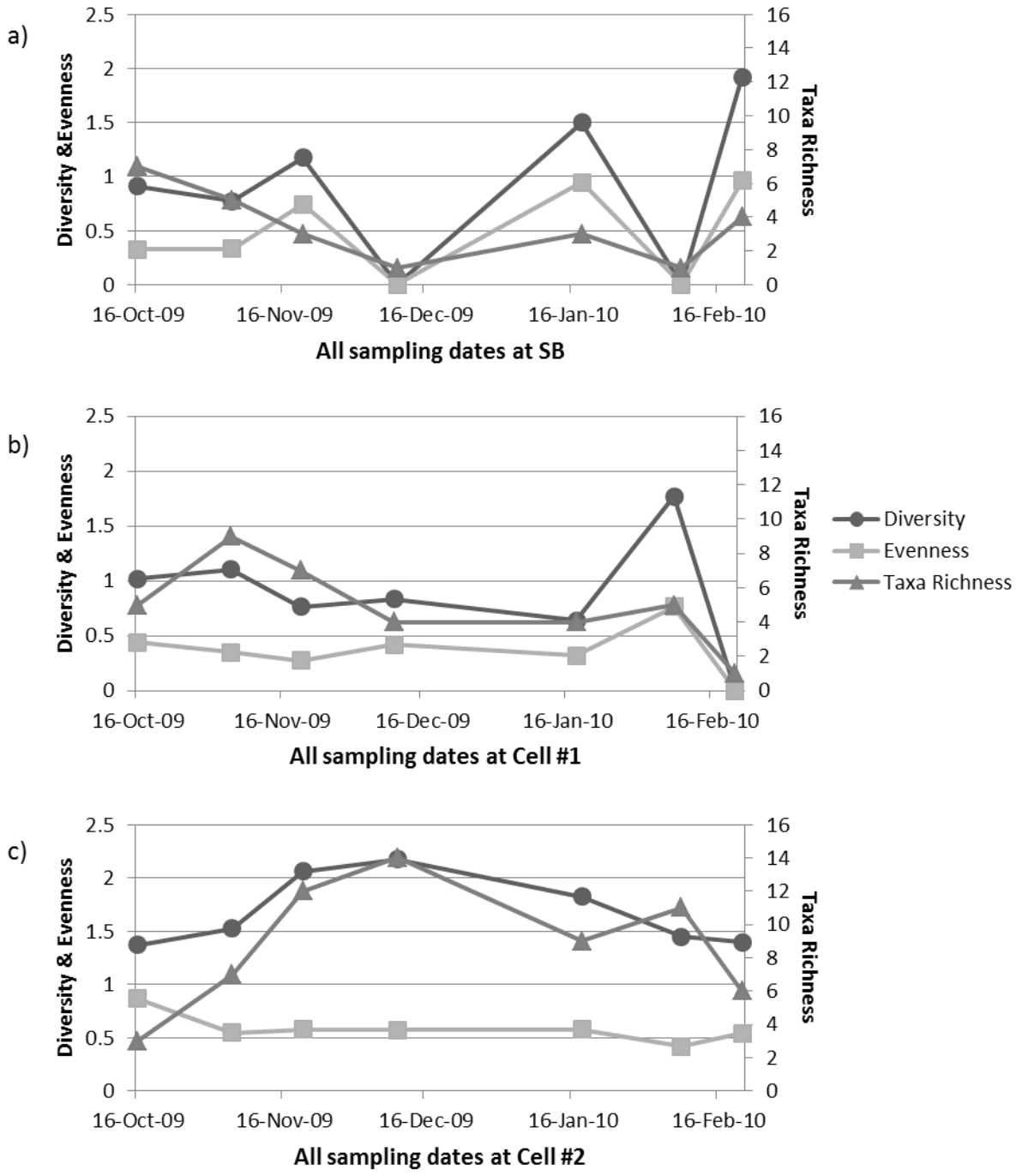


Figure 10. Each of the three sites: SB (a), Cell #1 (b), and Cell #2 (c) showing the Shannon-Diversity Index (circles), Evenness (squares), and Taxa Richness (triangles) for macroinvertebrates occurring in Rio Bosque Wetlands Park collected from 16 Oct 09 to 21 Feb 10.

Bray Curtis dissimilarity analysis was used to determine community similarity among the sites at RBWP from 16 Oct 09 to 21 Feb 10 (Figure 11). The matrix for the Bray Curtis analysis is available in Table 7. According to the analysis, Cell #2 was found to be most similar according to BMI composition (i.e. abundance and taxa richness) throughout the sampling period (B-C index values ranging from 0.19 to 0.67) (Figure 11). While SB and Cell #1 were found to have a more similar BMI community composition throughout the sampling period (B-C index values ranging from 0.25 to 0.55) (Figure 11).

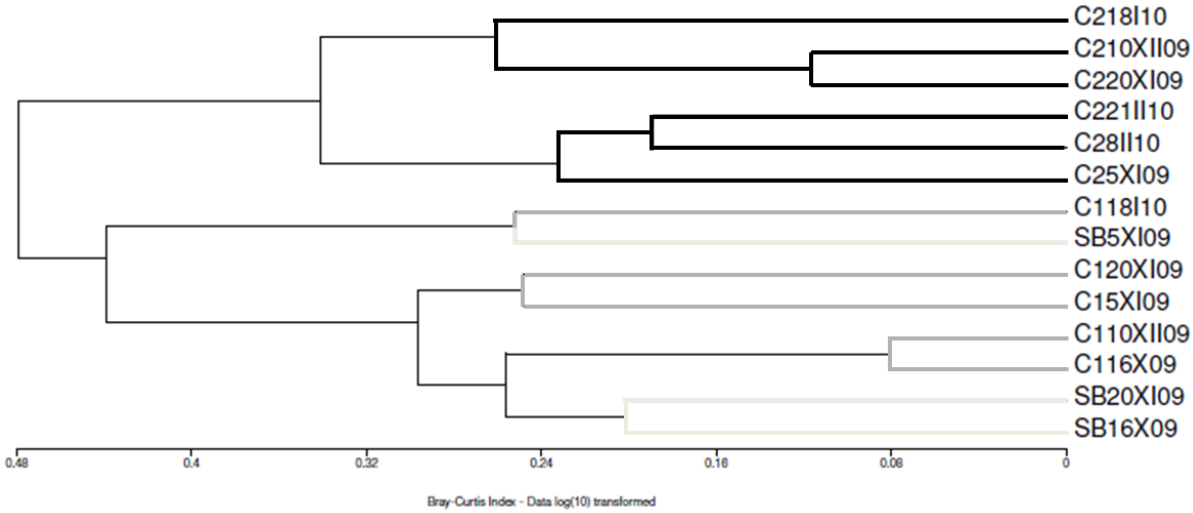


Figure 11. Bray Curtis (B-C) dissimilarity analysis for all sites and sampling dates from 16 Oct 09 to 21 Feb 10 for n>100. Values range from 0 (groups are identical in taxonomic composition) to 1(no taxa in common). Sites are abbreviated as SB= Streambed (in light gray), C1= Cell #1 (in dark gray), and Cell #2 (in black). The values that follow indicate the sampling date formatted with the day first followed by the month in roman numerals finally followed by the two-digit year.

Table 7. Bray Curtis Dissimilarity (MVSP) analysis output matrix (includes only values with n>100). Values range from 0 (groups are identical in taxonomic composition) to 1(no taxa in common). The values that follow indicate the sampling date formatted with the day first followed by the month in roman numerals finally followed by the two-digit year.

	SB16X09	SB5XI09	SB20XI09	C116X09	C15XI09	C120XI09	C110XII09	C118I10	C25XI09	C220XI09	C210XII09	C218I10	C28II10	C221II10
SB16X09	0.00													
SB5XI09	0.45	0.00												
SB20XI09	0.20	0.29	0.00											
C116X09	0.30	0.36	0.20	0.00										
C15XI09	0.38	0.47	0.38	0.28	0.00									
C120XI09	0.29	0.43	0.28	0.23	0.25	0.00								
C110XII09	0.33	0.37	0.20	0.08	0.27	0.28	0.00							
C118I10	0.53	0.25	0.39	0.45	0.55	0.52	0.46	0.00						
C25XI09	0.48	0.46	0.50	0.54	0.67	0.51	0.55	0.55	0.00					
C220XI09	0.48	0.48	0.49	0.36	0.32	0.39	0.37	0.58	0.44	0.00				
C210XII09	0.46	0.53	0.49	0.39	0.31	0.41	0.39	0.63	0.47	0.12	0.00			
C218I10	0.38	0.42	0.43	0.40	0.46	0.45	0.40	0.53	0.38	0.24	0.28	0.00		
C28II10	0.55	0.43	0.58	0.58	0.56	0.53	0.58	0.50	0.23	0.28	0.30	0.24	0.00	
C221II10	0.50	0.42	0.46	0.51	0.57	0.48	0.51	0.46	0.23	0.33	0.38	0.25	0.19	0.00
	SB16X09	SB5XI09	SB20XI09	C116X09	C15XI09	C120XI09	C110XII09	C118I10	C25XI09	C220XI09	C210XII09	C218I10	C28II10	C221II10

3.4.3 Non-Multidimensional Scaling (NMDS) Analysis

In order to examine patterns in BMI community structure and environmental variables within sites at RBWP NMDS was used. NMDS identifies axes that describe biologically meaningful, multivariate gradients in community data (McCune & Grace, 2002). Rare species (species values ≤1) were excluded prior to running NMDS analysis. According to McCune & Grace (2002), if rare species are deleted, then the final stress will normally be quite similar to that in the whole data set. BMI communities were ordinated in a bi-plot and correlated with the environmental variables: water chemistry parameters, heavy metals and arsenic present, and PPCPs present (Figure 12). NMDS of the BMI community indicated that a solution incorporating two axes, with a final stress of 11.16, was the most appropriate. It is suggested that the stress level of ecological data sets tend to have final stress levels between 10 and 20, and the half closer to 10 can be meaningfully interpreted (McCune & Grace, 2002).

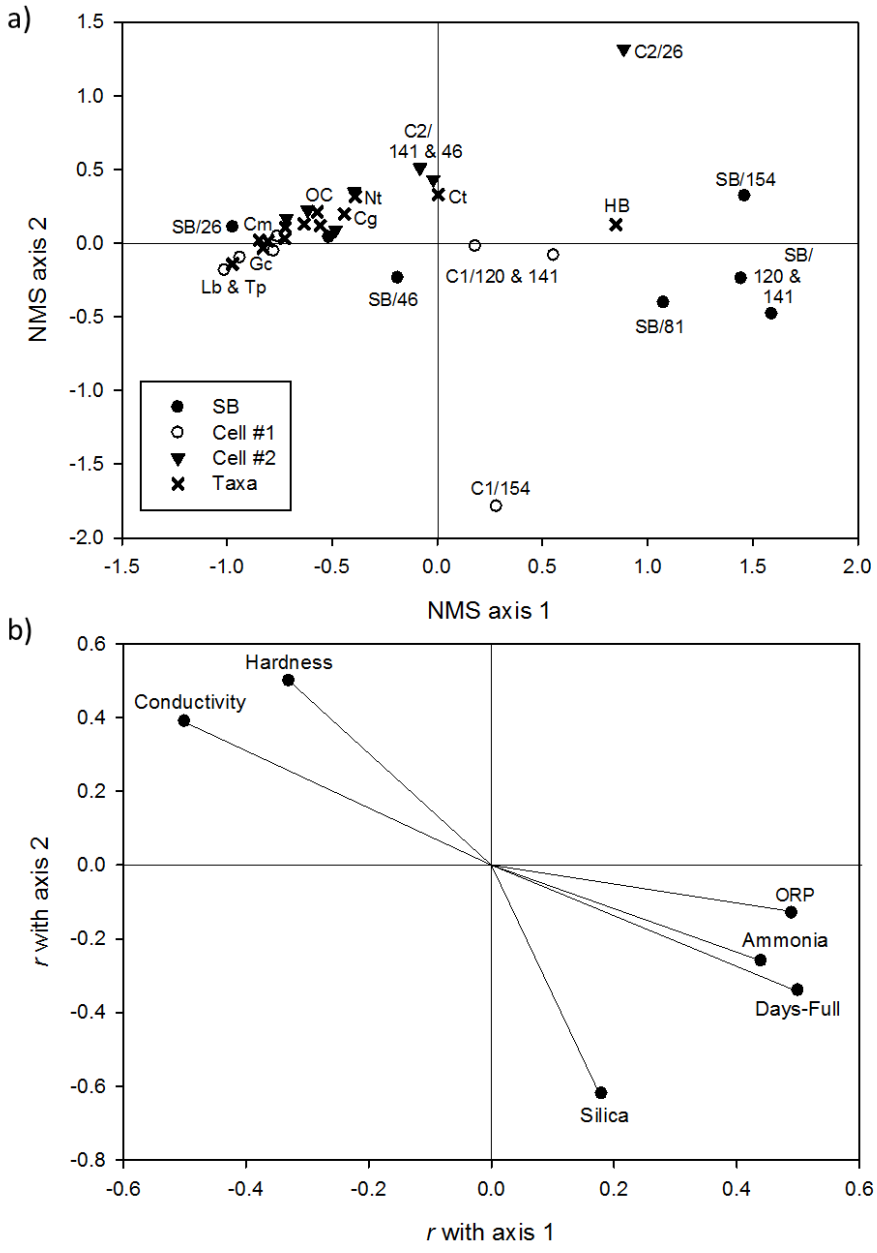


Figure 12. Non-metric multi-dimensional scaling (NMDS) analysis of BMI communities (taxa) in 3 selected sites at RBWP, including site and scores of BMI species (panel a, top), and environmental variables (panel b, bottom). Note: Only variables that were significantly correlated with either axis 1 or 2 are displayed. Note: The site followed by the number indicates the site location and the days-full, and is related to the site and sampling date.

A correlation analysis (via IBM© SPSS© Statistics version 19) with the NMS axis scores and environmental variables (water chemistry parameters, heavy metals values, and PPCP values) was conducted to determine if there was any significant relationship. The correlation

analysis resulted in the ammonia, conductivity, days-full, hardness, ORP, and silica being significantly correlated. The correlation with the above mentioned variables (Table 8) indicated that NMS axis 1 was significantly and positively correlated with ammonia (r =0.44), days-full (r =0.50), and ORP (r =0.49), while conductivity (r = -0.50) was negatively correlated. While NMS axis 2 was significantly and positively correlated with hardness (r =0.50), and silica (r = -0.62) was negatively correlated. The variables that most explain the pattern that is in the NMS plot are conductivity and Days-full on axis 1, and hardness and silica with axis 2. It is important to note that although water temperature was not significantly correlated with any of the NMS axis scores, water temperature and conductivity were highly significantly negatively correlated (r=-0.91, p=0.0001). No significant relationship was found between heavy metal and arsenic values and any NMS axis scores. Unfortunately, during this study not enough PPCP data were available to run a correlation analysis with the NMS axis scores.

Table 8. Pearson's correlation coefficients (r) of NMDS axes with environmental variables days-full (# of days since water has been released). ** indicates that correlation is significant at p<0.05 level, ** indicates correlation is significant at p<0.01, and without asterisks indicates there was no significance. The values that are highlighted are the variables that most explain the pattern of the BMI community composition.

Variable	r with axis 1	r with axis 2
Ammonia (mg/L NH4-N)	0.44*	-0.26
Conductivity (mS/cm)	-0.50*	0.39
Days-full (# of days)	0.50*	-0.34
Hardness (mg/L CaCO3)	-0.33	0.50*
ORP	0.49*	-0.13
Silica (mg/L) SiO2	0.18	-0.62**

MRPP results indicate that each of the 3 sites x 14 species were significantly different (T=-3.18, A=0.13, p=0.01). Resulting in SB being significantly different from Cell #2 (T= -2.76, p=0.02). Results from the pairwise comparisons are given in Table 9. Each of the 3 selected sites was found to be grouped in different areas of the NMS plot. Most of SB was plotted on the right side (4 out of 7 sampling dates) of the NMS plot (Figure 13, panel a). Most of Cell #1 (4

out of 7 sampling dates) and Cell #2 (6 out of 7 sampling dates) were plotted on the left side of the NMS plot (Figure 13, panel a).

Table 9. Pairwise comparisons for Multi-Response Permutation Procedures (MRPP). 1 is coded for SB, 2 is coded for Cell #1, and 3 is coded for Cell #2. T is the test statistic. A is the agreement statistic that describes within-group homogeneity, compared to the random expectation.

Group codes compared	T	A	p
1 vs. 2	-1.836	0.088	0.06
1 vs. 3	-2.760	0.129	0.02
2 vs. 3	-1.687	0.063	0.07

3.5 Results for *Chironomus dilutus* acute 48-hr LC_{50} exposure experiments

The effects of PPCPs were determined using four PPCPs (the stimulant, caffeine; the antibiotic, erythromycin; the analgesic/anti-pyretic, acetaminophen; and the plastics/epoxy resin, BPA) to determine the acute effects to chironomids. As expected, it was found that most of the test organisms survived in the lower concentrations, while the in the highest concentrations the survivability percentage of the test organisms greatly declined.

In the first toxicity study, *C. dilutus* was exposed to erythromycin concentrations ranging from no erythromycin to 750 mg/L. During the last day of the erythromycin exposure, over 80% of the chironomids survived in treatments up to 384 mg/L. At the next highest concentration (576 mg/L), survival decreased to about 50%. In the highest concentration there was another decrease in survival to about 20%. The Probit analysis was significant ($Z= -6.105$, $p \leq 0.0001$) and resulted in a 48 hr LC_{50} of 732.5 mg/L. A Dunnett's *post-hoc* test ($F=20.23$, $p \leq 0.0001$) resulted in the NOEC of 384 mg/L ($p=0.717$) and a LOEC of 576 mg/L ($p=0.001$).

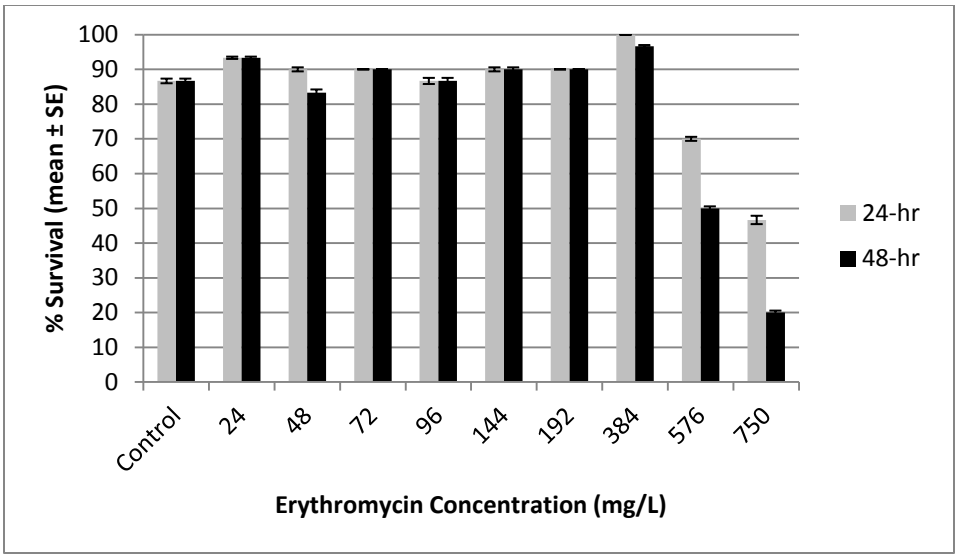


Figure 13. Exposure of erythromycin for 48 hr LC_{50} exposure using third instar *C. dilutus*. Survival is in percent ± SE.

In the subsequent acute 48 hr LC_{50} study *C. dilutus* was exposed to bis-phenol A (BPA) ranging in concentrations from no BPA to 14 mg/L. During the last day of BPA exposure, with each increase of concentration there was a decrease in survival of the test species. However, there was one exception, at concentrations of 5 and 5.5 mg/L of BPA midges had similar survival percentages (about 60%). At the highest concentration of BPA (14 mg/L), about 10% of midges survived. The Probit analysis for the BPA exposure was significant ($Z= -7.95$, $p \leq 0.0001$) and resulted in a 48-hr LC_{50} of 6.09 mg/L. A Dunnett's *post-hoc* test ($F=43.09$, $p \leq 0.0001$) resulted in a NOEC of 3.5 mg/L ($p=0.244$) and a LOEC of 4 mg/L ($p=0.032$).

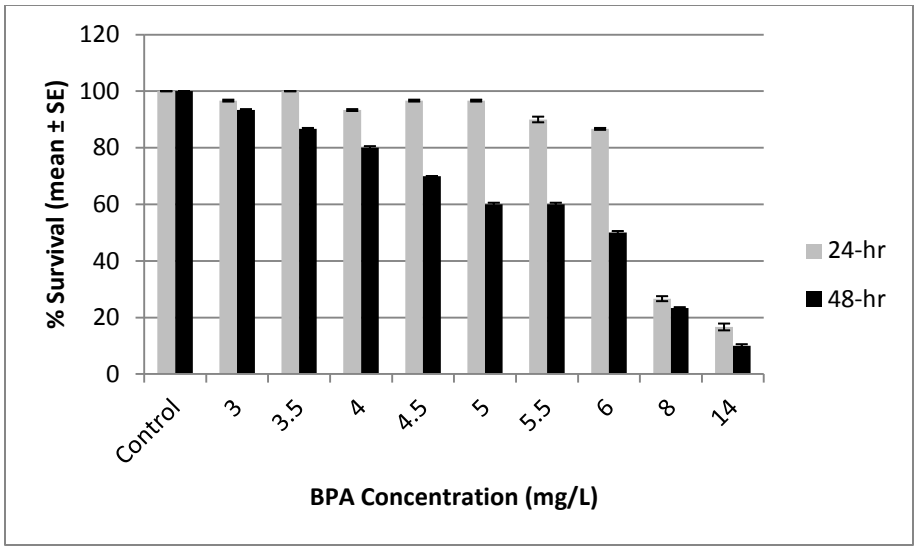


Figure 14. Exposure of BPA for 48 hr LC_{50} to third instar *C. dilutus*. Survival is in percent ± SE.

To determine effects of caffeine, *C. dilutus* was exposed to caffeine ranging concentrations up to 4.5 g/L. During the last day of the study it was observed that as the concentration of caffeine increased, survival of *C. dilutus* decreased. It was observed that survival percentage decreased from about 70% to a little over 20% from caffeine concentrations of 2.1 to 2.5 g/L. In the final two caffeine concentrations (3.5 and 4.5 g/L) none of the test organisms survived. The Probit analysis for the caffeine exposure was significant ($Z = -9.64$, $p \leq 0.0001$) and resulted in a 48 hr LC_{50} of 2.03 g/L of caffeine. A Dunnett's *post-hoc* test ($F=26.55$, $p \leq 0.0001$) resulted in the NOEC of 1.5 mg/L ($p=0.793$) and a LOEC of 1.9 mg/L ($p=0.003$).

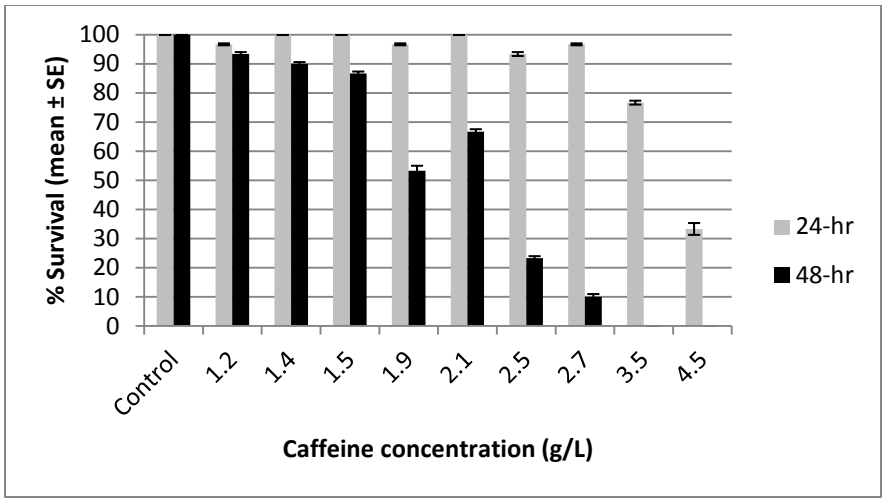


Figure 15. Exposure of caffeine for 48 hr LC_{50} to third instar *C. dilutus*. Survival is in percent ± SE.

Finally, *C. dilutus* were exposed to in concentration up to 3.5 mg/L of acetaminophen. In concentrations of 1.2 to 1.4 mg/L, there was the decline in survival of about 30%. In the highest concentration (3.5 mg/L), about 18% of the test organisms survived after 48 hrs. Probit analysis for acetaminophen exposure was significant (Z= -8.18, $p \leq 0.0001$) and resulted in an LC_{50} of 1.69 g/L. A Dunnett's *post-hoc* test (F=10.47 $p \leq 0.0001$) resulted in the NOEC of 1.2 g/L (p=0.539) and a LOEC of 1.4 g/L (p=0.003).

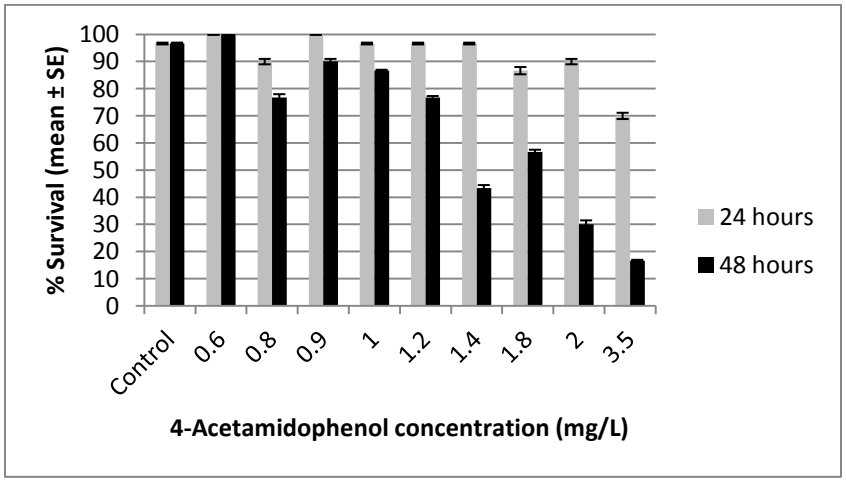


Figure 16. Exposure of 4-acetamidophenol for 48 hr LC_{50} to third instar *C. dilutus*. Survival is given as percent ± SE.

4 Discussion

This study was important as it improved our understanding of water quality at RBWP and the potential impact on the BMI community from contaminant found in wasterwater effluent. At RBWP, this was the first seasonal survey of benthic macroinvertebrate diversity, the first baseline study for heavy metals and arsenic concentrations, and the first study to determine the presence and concentrations of selected PPCPs present within the wastewater entering the wetland.

4.1 PPCPs

The survey of PPCPs at RBWP resulted in detection of 8 of the 9 compounds in water and 7 of 9 in sediment samples. Acetaminophen was detected in the water only one time during the sampling period; however, it was detected below the limit of quantification in the sediments. Erythromycin was typically below the limit of quantification in both water and sediment samples. The compounds that were the most frequently detected in high concentrations were ciprofloxacin and codeine in both water and sediments. Two other compounds that were frequently detected in the sediment samples were caffeine and trimethoprim. Not unexpectedly, the concentrations of PPCPs detected were higher in the sediments than in the water (by up to 6 orders of magnitude, Tables 5,6) which is likely due to sedimentation as the water flows through the wetland cells. Compounds detected in this survey are discussed below according to categories defined in the methods section.

4.1.1 Antibacterial

Ciprofloxacin

The frequency detection of ciprofloxacin in this study was similar to that found by Lindberg *et al.* (2005). They reported that fluroquinolone antibiotics, such as ciprofloxacin, are frequently detected above analytical quantification in WWTP effluent. Golet *et al.* (2003) investigated the wastewater treatment process and found that this type of antibiotic was removed by up to 88-92% from the aqueous phase due to its adsorption to sludge. Other studies support that this is the predominant removal mechanism for this compound as opposed to biodegradation (Batt *et al.*, 2007; Zorita *et al.*, 2009). However, it is also clear, that even though a high percentage of ciprofloxacin is adsorbed to sludge in the treatment process, some amount of this compound is still released into surface waters. For instance, in a study conducted in New Jersey investigating the occurrence of antibiotics in water and in sediments of a stream receiving treated effluent by Gibs *et al.* (2013), ciprofloxacin was detected in the water at a concentration of 0.08 µg/L (80 ng/L) and 10 µg/kg (10,000 ng/kg) in the sediments downstream of the WWTP. In the current ciprofloxacin was detected in water at 60 ng/L, slightly lower than the 80 ng/L found by Gibs *et al.*, (2013), however, the concentration in the sediments was an order of magnitude higher at RBWP (561, 381 ng/kg versus 10, 000 ng/kg). Similar to this study, Gibs *et al.* (2013) found higher concentrations of ciprofloxacin in sediments as compared to water. A possible explanation for why concentrations of ciprofloxacin may be higher at RBWP is the potential difference in the percentage of residents being prescribed ciprofloxacin. Both studies support the contention that ciprofloxacin has a high binding affinity to sludge/sediment.

Robinson *et al.* (2005) determined the effects of ciprofloxacin to *Daphnia magna*. A 48-hr exposure of 10 mg/L of ciprofloxacin to *Daphnia magna* was conducted. The exposure

concentration of 10 mg/L ciprofloxacin was chosen because it was about two orders of magnitude higher that the concentration that was detected in hospital wastewaters (125 μg/L/ 125,000 ng/L) (Robinson *et al.*, 2005). A 10 mg/L 48-hr exposure of ciprofloxacin resulted in <10% mortality to *D. magna* (Robinson *et al.*, 2005). These results suggest that even though concentrations of ciprofloxacin are being released into surface waters, concentrations have to be at least >3 orders of magnitude higher than what is typically reported in wastewater receiving waters. In this study ciprofloxacin was detected at a concentration as high as 60 ng/L, which is about half as much as reported in the hospital effluent in the study of Robinson *et al*. (2005). The findings of Robinson *et al.* (2005) suggest that the concentrations being detected in water and sediments at RBWP may not pose an acute toxicity risk to its aquatic inhabitants.

Erythromycin

During the sampling period at RBWP, erythromycin was detected below the limit of quantification in both water and sediment samples. Yan *et al.* (2014) investigated the occurrence and fate of erythromycin at various WWTPs. The authors reported that the overall removal efficiency varied at each WWTP, and ranged from 26 to 86% (Yan *et al.*, 2014). It was stated that differences in removal efficiency was due to the different treatment methods in place at each WWTP. In the current study, erythromycin was detected below the limit of quantification and these findings suggest that it is possible that the Roberto Bustamante wastewater treatment plant is employing a method that has a high removal efficiency for erythromycin.

A 48 hr exposure was conducted in this study to determine the acute toxicity of erythromycin to *C. dilutus*. The 48 hr LC_{50} exposure to *C. dilutus* resulted in an estimated LC_{50} of 732.5 mg/L, the NOEC of 384 mg/L, and the LOEC of 576 mg/L of erythromycin. Solomon *et al.* (1996) reported that the estimated LC_{50} concentration for erythromycin after 48 hr for

Daphnia is 0.94 mg/L, which is at more than three orders of magnitude lower than the LC_{50} for *C.dilutus*. These findings suggest that *C. dilutus* may be more tolerant to erythromycin than *Daphnia*. Since erythromycin was detected below the limit of quantification at RBWP, it is likely that it does not pose a risk to the benthic macroinvertabrate community. Future studies should include a further investigation of erythromycin for multiple water cycles at RBWP.

Sulfamethazine

During the sampling period at RBWP, sulfamethazine was detected in concentrations in the water ranging from below the limit of quantification to 0.57 ng/L, and in the sediments ranging from 2,473 to 15,251 ng/kg. Yan *et al.* (2014) investigated the occurrence of sulfamethazine at various WWTPs, and found that after wastewater treatment the antibiotic was detected in the effluent an average of 20 ng/L, and in the sludge an average of 2 ng/g. The findings of Yan *et al.* (2014) suggest that sulfamethazine is being released into surface waters. The impact of sulfamethazine to benthic macroinvertebrates is unknown. In this assessment at RBWP, the detection frequency of sulfamthazine was low, and detection was less than half of the samples collected in the water and in the sediments. These findings suggest that sulfamethazine may not pose a great risk to the benthic macroinvertebrate community. However, further studies should include more sampling events over the course of multiple water cycles.

Trimethoprim

Perez *et al.* (2005) conducted experiments to investigate the biodegradation of trimethoprim in different steps of the treatment process. The authors determined that the step that involved activated sludge treatment along with a nitrification process was the only step that had the ability to eliminate trimethoprim (Perez *et al.*, 2005). However, they further stated that

the time that would be best for this elimination to occur (about 3 days) exceeds the general amount of time of 8-12 hours in a conventional wastewater treatment plant (Perez *et al.*, 2005). As a result of these findings, they suggest that a significant amount of this compound may be released to surface waters or other water recipients (Perez *et al.*, 2005). However, the concentrations that were detected in RBWP showed that as the water flowed through the wetland there was a reduction in trimethoprim concentrations (Figures 13,14). These findings are further supported by other published studies (Batt *et al.*, 2006b; Perez *et al.*, 2005; Le-Minh *et al.*, 2010), which report that microorganisms capable of nitrification processes have the ability to degrade trimethoprim. Waiser *et al.* (2011) reported the LC_{50} of trimethoprim to Cladoceran was 100 mg/L. The findings of the concentrations of trimethoprim and what was reported by Waiser *et al.* (2011) that trimethoprim may not pose a risk to the aquatic community at RBWP.

4.1.2 Analgesics

Acetaminophen

During this study at RBWP, acetaminophen was only detected one time within water samples at concentrations ranging from BLQ to 0.073 ng/L. As discussed above Yan *et al.* (2014) examined the occurrence of pharmaceutical active compounds in the influent and effluent from various WWTPs in Chonquing, China. The authors found after the wastewater treatment process, acetaminophen concentrations were detected below or close to the limit of quantification in the effluent (Yan *et al.*, 2014). It was further reported that acetaminophen had a high removal percentage during the treatment process due to it being easily biodegradable in water (Yan *et al.*, 2014). As a result of the high removal efficiency it was also observed that acetaminophen did not accumulate in sludge regardless of the treatment process used (Yan *et al.*, 2014). In this current study at RBWP the findings were comparable to the study of Yan *et al.*

(2014). Acetaminophen was detected at a very low concentration only one time in the water, and it was detected below the limit of quantification in the sediments.

An acetaminophen exposure was conducted on *Daphnia* and it was estimated that the EC_{50} (effect concentration) was 9.2 mg/L (Quinn *et al.*, 2008). Another study reported the LC_{50} of acetaminophen to fish and it was estimated at 378 mg/L after being exposed >96 hr (Solomon *et al.*, 1996). In the current study, an acetaminophen 48 hr LC_{50} exposure study resulted in an estimated LC_{50} of 1.69 g/L(1690 mg/l) of 4-acetamidophenol, the NOEC of 1.2 g/L (1200 mg/L), and the LOEC of 1.4 g/L (1400 mg/L). These findings suggest that even though a low concentration of acetaminophen was detected within the water at RBWP benthic macroinvertebrates are at a low risk for toxic effects.

Codeine

Codeine was the second frequently most detected compound within the water and sediment samples collected at RBWP. Concentrations of codeine in the water ranged from below the limit of quantification to 3.7 ng/L, and in the sediments concentrations ranged from below the limit of quantification to 79, 309 ng/kg. An investigation was conducted to determine the occurrence of legal and illicit drugs in the influent and effluent from a WWTP in Verona, Italy (Repice *et al.*, 2013). Codeine concentrations were determined to be 288 ng/L in the influent, and 115 ng/L in the effluent (Repice *et al.*, 2013). Based on these findings, the authors estimated that the average efficiency of codeine removal was about 65%. In another study, conducted by Lin *et al.* (2010), it was reported that codeine was detected as high as 57 mg/L in a wastewater-receiving river in Taipei, Taiwan. Lin *et al.* (2010)'s study further supports that codeine is being released into surface waters. At RBWP, the concentration of codeine detected was much less in the water and sediments at RBWP than that found by Lin *et al*. The impact of

codeine on aquatic invertebrates is largely unknown. Daughton & Brooks (2011) state that there is a particular concern of the extent of knowledge regarding the linkage between exposure to pharmaceuticals and adverse effects in aquatic organisms.

4.1.3 Stimulant

Caffeine was the third highest detected compound at RBWP, however, it was detected in a high concentration only in the sediments. Caffeine was detected in the water in concentrations ranging from below the limit of quantification to 1.59 ng/L, while in the sediments, caffeine was detected ranging from below the limit of quantification to 258,615 ng/kg. A study in Korea detected caffeine in treated effluent in concentrations of 0.024 μg/L (Sim *et al.*, 2010). Furthermore, it was reported by the authors that the concentrations of caffeine were reduced from the concentrations detected in the influent, which indicated a high decrease rate of this compound during the treatment process at the WWTP.

Moore *et al.* (2008) conducted a 48 hr exposure study using *Ceriodaphnia dubia* (*Daphnia*), *Pimephales promelas* (fathead minnow), and *Chironomus dilutus* (non-biting midge). The 48 hr LC_{50} exposure responses were at the following concentrations: LC_{50}=60 mg/L for *C. dubia*; LC_{50}=100 mg/L for *P. promelas*; and LC_{50}=1230 mg/L for *C. dilutus* (Moore *et al.*, 2008). The authors also conducted a 7-day exposure and the responses of *C. dubia* and *P. promelas* were LC_{50}=46 and 55 mg/L, respectively (Moore *et al.*, 2008). In the current study, a 48 hr exposure resulted in an estimated LC_{50} of 2.03 g/L of caffeine, which is almost double that of Moore *et al.*'s (2008) findings. Again, the concentration detected within the water at RBWP at the highest concentration was 1.59 ng/L of caffeine. The concentration that is needed to elicit an effect on *C. dilutus* would be 6 orders of magnitude higher within the waters at RBWP. Moore *et al.* (2008) further stated that their findings further suggest from their study and with

data from previous studies that caffeine may pose a negligible acute risk for most aquatic organisms (Moore *et al.*, 2008).

4.1.4 Anti-depressant

Fluoxetine is a serotonin re-uptake inhibitor, and is usually prescribed as an anti-depressant. During the sampling period at RBWP fluoxetine was detected at concentrations ranging from BLQ to 0.47 ng/L, and on one occasion it was detected at 1 ng/L. Fluoxetine was detected more often within the water samples than in the sediments. In the sediments, fluoxetine was detected only one time during the sampling period at a concentration of 12,106 ng/kg. However, fluoxetine was not detected during period when benthic macroinvertebrates were collected. Péry *et al.* (2008) investigated the effects of fluoxetine on the life-cycle of *Chironomus riparius*. The authors report that no effect was observed on *C. riparius* to fluoxetine concentrations up to 59.5 mg/kg (Péry *et al.*, 2008). The concentration of fluoxetine in the RBWP sediment was in such a low concentration (0.012 mg/kg) and given the findings of Péry *et al.* (2008), is very unlikely that fluoxetine would have a negative effect on *C. riparius* in the field.

4.1.5 Metabolite of nicotine

During the sampling period, cotinine was found at concentrations ranging from BLQ to 1.03 ng/L at RBWP. However, cotinine was detected in water samples collected after the period of benthic macroinvertebrates collection. In the sediments, cotinine was detected twice during during the period when benthic macroinvertebrates were collected, at concentrations of 2,744 and 7, 138 ng/kg. During the next sampling period cotinine was detected in concentrations ranging from 1,345 to 10, 311 ng/kg. In the study of Waiser *et al.* (2011) concentrations of cotinine of 0.18 µg/L were found in Wascana Creek, and cotinine was commonly detected over

the course of the sampling period. The concentration of cotinine detected in the water at RBWP is comparable to concentrations was found by Waiser *et al.* (2011). The toxicity of cotinine to benthic macroinvertebrates is unknown, thus concentrations of cotinine at RBWP may pose a risk to the aquatic community.

4.1.6 Bisphenol-A

In a small-scale unpublished study investigating the presence of estrogenic compounds at RBWP, detectable levels of 4-tert-octylphenol, nonylphenol, and BPA were found (R. De La Torre-Roche, pers. com.). Specifically, BPA was detected at the following concentrations during De La Toree-Roche's study: 1, 345 ng/L Bustamante Plant effluent, 3,340 ng/L at Cell #1, 377 ng/L at Cell #2, and BLQ at Gate 1 (See Figure 1) ((R. De La Torre-Roche, pers. com.). Since it BPA had been detected at RBWP, a 48-hr exposure was conducted to *C. dilutus* to determine the LC_{50}. The estimated LC_{50} for BPA was 6.09 mg/L, with a NOEC of 3.5 mg/L and a LOEC of 4 mg/L. Mihaich *et al.* (2009) investigated the 96-hr acute toxicity of BPA to a midge (*Chironomus tetans*) and a snail (*Marisa cornuarietis*). The estimated LC_{50} for the 96-hr acute toxicity study was 2.7 mg/L of BPA, with a NOEC of 1.4 mg/L, and a LOEC of 2.1 mg/L for *C. tetans*. For *M. cornuarietis* the estimated LC_{50} was 2.24 mg/L, the NOEC was 1.18 mg/L, which was based on survival (Mihaich *et al.*, 2009). Even though the BPA 48-hr exposure in this study was half as long as that used by Mihaich et al. (2009), the findings suggest that as chironomids are exposed for longer periods of time the LC_{50} concentration decreases. Since BPA was not analyzed in water or sediments in this study, future studies at RBWP should include the detection of BPA.

4.2 Water chemistry parameters

During the course of this study at RBWP, ammonia, nitrate + nitrite, phosphate, and Chlorophyll-a. On most occasions ammonia (57% of the time at SB; 71% of the time at Cell #1; 86% of the time at Cell #2) was above the 0.33 mg/L criteria set by TSWQS (Table 3) for the Rio Grande Basin, segment 2308 (TCEQ, 2010). However, there were some exceptions: (TCEQ, 2010) during the course of the sampling period. Nitrate + nitrite exceeded the 1.95 mg/L criteria set by TSWQS at SB and Cell #2 during the whole sampling period, and 85% of the time at Cell #1 over the course of the sampling period (TCEQ, 2010). On the 5 Nov 09 sampling date, at Cell #1, was the exception where nitrate + nitrite levels were below (0.9 mg/L) the state criteria. Chlorophyll-a at both Cell #1 and Cell #2 exceeded the 14.1 μg/L criteria set by TSWQS 71% percent of the time (TCEQ, 2010). During the course of the sampling period, at all sites and sampling dates, measured phosphate levels exceeded state criteria levels of 0.46 mg/L (TCEQ, 2010). In addition, chlorophyll-a at Cell #1 and Cell #2, on most occasions, exceeded the 14.1 μg/L criteria set by TSWQS (TCEQ, 2010). According to IBWC (2011), high levels of Chlorophyll-a for long periods indicate low water quality, and may be indicative of excess nutrient levels. These findings are not surprising since RBWP is a wastewater receiving wetland, and treated effluent generally contains increased levels of nutrients (Castro, pers. com; TCEQ, 2006). According to the IBWC (2011), high levels of nutrients (i.e. nitrogen compounds, ammonia, and phosphorous) may cause excessive plant growth, which can lead to reduced DO levels. Furthermore, elevated ammonia levels can be toxic to aquatic organisms (IBWC, 2011). It is important to note that during the sampling period at RBWP, DO levels were >3 mg/L (Table 3), which is above the TCEQ criteria set for DO levels and is still considered an acceptable level (TCEQ, 2010). The effects of low DO (>2 mg/L) can lead to a reduction of the abundance of

aquatic organisms in a body of water (IBWC, 2011). It is possible that the high levels of nutrients, chlorophyll-a, and DO levels during the sampling period may likely have effected BMI composition over the course of sampling.

As part of their study, Rodriguez & Lougheed (2010) examined water quality at RBWP, and the potential role of it improving the quality of the water (Rodriguez & Lougheed, 2010). They reported that wastewater effluent contributed significantly to total phosphorous, nitrate, and ammonia levels as they exceeded state criteria levels downstream of the WWTP (Rodriguez & Lougheed, 2010 and IBWC, 2010). Overall findings of Rodriguez and Lougheed (2010) led to determining that through the processes of denitrification, which can be accomplished through plant uptake and even sedimentation, the wetlands of Rio Bosque were effective at reducing nitrate concentrations in the water as it flowed through the wetland cells. Even though RBWP was not created to act as a secondary method of treatment to improve water quality, this study demonstrated some functionality in nutrient processing (Rodriguez & Lougheed, 2010). As in the case of Rodriguez & Lougheed (2010), nitrate and ammonia levels within RBWP were found to be one order of magnitude higher than the levels implemented by state screening for regional water bodies (Rodriguez & Lougheed, 2010). In this more recent study conducted at RBWP, nitrate levels showed a similar pattern of decreasing as the water flowed through wetland cells (Table 3). However, during this study NO_3-N (nitrate) levels did not differ significantly among sites ($F=0.29$, $p=0.753$) (Table 4), as in the study of Rodriguez & Lougheed. Findings regarding ammonia were also comparable to the study of Rodriguez & Lougheed (2010). In this study, ammonia levels were similar in Cell #2 and SB. Ammonia levels did not differ significantly among sites ($F=1.82$, $p=0.205$). In both studies, ammonia levels did not decrease from inflow to Cell #2 or outflow.

4.3 Benthic Macroinvertebrates

In this study benthic macroinvertebrates were collected to determine the BMI community at RBWP, and if the measured water quality parameters impacted the BMI community. During the course of this study at RBWP, nearly 30,900 benthic macroinvertebrates were collected (Table 8-10). Although 17 different taxa were identified, two taxa groups: midge larvae (Diptera: Chironomidae), and nematodes (Nematoda), accounted for over 75% at of the total number of invertebrates collected (Appendix D: Tables 12-14). The community was dominated by chironomids, and the two genera that were most often found were *Goeldichironomus* and *Chironomus*; with *Goldichironomus* being the dominant genus overall (Figure 9 and Appendix D: Figures 12-14). Although there were some site and date specific exceptions, in general, individuals representing these taxonomic groups were usually the dominant taxa found at all the sites during the study period from 16 Oct 09 to 21 Feb 10. This type of community composition is expected because these taxonomic groups represent fauna that are considered tolerant to poor quality (i.e. high nutrient levels) that enable them to inhabit the aquatic environment and sediments associated with the study sites at RBWP a wastewater receiving wetland. RBWP is very unique in that it is not only a wastewater receiving constructed wetland, but it is also a wetland that does not receive water continually, and this might further explain the dominance of chironomids.

Becerra Jurado *et al.* (2009) examined the taxa composition in natural ponds (NP) and constructed ponds (CP) used for treating agricultural wastewater. The authors reported that few studies have investigated macroinvertebrate communities of constructed wetlands and the driving environmental factors that determine their community structure (Becerra Jurado *et al.*, 2009). It was reported that the macroinvertebrate community was dominated by two taxa: Coleoptera

(35% in natural ponds NP, and 45% in CP), and Hemiptera (22% in NP, and 17% in CP). The authors further reported that chironomids were highly associated with the constructed ponds, which were characterized by low pH and high molybdate reactive phosphorous (MRP), while the less tolerant organisms, EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera orders) were found in the natural ponds. The findings of their study does support that constructed wetlands do provide habitats for macroinvertebrates, however, the percentage of tolerant versus intolerant taxa are much different in each of the respective ponds (Becerra Jurado *et al.*, 2009). This study is comparable to the cuurent study at RBWP in that although BMI community composition was not dominated by beetles; it was dominated by chironomids. The levels of pH at RBWP were not low during the study period, however, nutrient levels were high, and over the course of the study it is likely that elevated nutrient concentrations contributed to reduced diversity, thus suggesting that the water quality at RBWP may be impacting the community composition.

In another study, Pires *et al.* (2000) investigated benthic macroinvertebrate communities of intermittent streams. The authors reported that the number of macroinvertebrates were consistently high. Dipteran members (Chironomidae and Simulidae) comprised of 73.2% of the taxa, followed by Ephemeroptera (10.3%), Coleoptera (4.1%), and Trichoptera (3.1%). The authors further mentioned that Dipteran larvae dominated where there was poorer water quality, which was during dry weather conditions, which also coincided with reduced DO levels (as low as 2 mg/L) as compared to wet weather conditions (8 -12 mg/L). The study conducted at RBWP is comparable to that of Pires *et al.* (2000) in that a higher percentage of chironomids were found during what was categorized by Pires *et al.* (2000) to be drought conditions, when DO levels were reduced (i.e. <5 mg/L). During the collecting period at RBWP, DO levels ranged from 3-5 mg/L during the first half of the sampling period (16 Oct 09 to 10 Dec 09), and during the last

half of the sampling period (18 Jan 10 to 21 Feb 10) DO levels ranged from 6-12 mg/L. It is important to note that during the last half of the sampling period the abundance of macroinvertebrates was much less than during the first half of the sampling period. The findings of Pires *et al.* (2000) suggest that hydroperiod plays a major role in macroinveretebrate community composition. Water availability to RBWP is likely a factor driving BMI community composition. Even though hydroperiod may also contribute to BMI community composition another factor that should be considered is water temperature.

Trinstad *et al.* (2010) examined the growth rates of chironomids from an ephemeral floodplain wetland. The authors reported that the average development time was 17 days. The capability of rapid growth occurred at water temperatures >18°C, with a completion of their life cycle if water remained at least 2 to 3 weeks (Tronstad *et al.*, 2010). During the time period that the benthic macroinvertebrates were collected at RBWP the average water temperature at each site was: 24.7 °C at SB, 22.8°C at Cell #1, and 16.1°C at Cell #2. Tronstad *et al.*, (2007) reported that chironomids are capable of completing their life cycle within about 2 weeks at water temperatures >23°C. Another study reported that at water temperatures ranging from 10-18 °C, during winter and spring, growth rates are probably lower and developments times are likely to be longer (Entrekin *et al.*, 2001). These findings suggest that the water temperature at SB and Cell #1 are more suitable conditions for a rapid life cycle, and that in it may take longer for chironomids to go through a complete life cycle in Cell #1. This may explain why a shift was seen in the abundance of the dominant genera from the beginning the sampling period from *Chironomus* and towards the middle of the sampling period to *Goeldichironomus*. *Chironomus* abundance was significantly positively correlated ($r=0.484$, $p=0.03$) with water temperature, while *Goeldichironomus* was not correlated with water temperature ($r=0.32$, $p=0.16$). It is

important to note that during this study, the effluent used to fill the park was warm as water exiting the Bustamante WWTP is generally 30 C (Castro, pers. com.). This may explain why water temperature was generally warmer in SB than in both Cell #1 and Cell #2 (Tables 3, 4). This may also explain why higher abundances of the BMI community was found within the first three sampling dates due to the water temperature being warmer, and on the fourth sampling date BMI abundances dramatically dropped (Table 8). Another possible factor that could potentially effect BMI community composition, but not examined, could be habitat suitability.

RBWP can be characterized as a temporary wetland due to water only being available during non-irrigation season, and having some occasional rain contributions. Williams (1996) reported on the factors (physicochemical and biological) that are common to many temporary bodies of water and what factors most strongly influence insect inhabitants. The main physicochemical parameters that influence the insect community are pH, water temperature and dissolved oxygen. Biological factors include succession of species, seasonal influx of aerial colonizers, and changes in food availability. Williams (1996) further mentions that habitat duration is likely the most important factor. Early investigations also indicated that water bodies that are temporary do not support as diverse of an insect community as what is observed in permanent water bodies (Wiggins *et al.*, 1980; Beaver, 1985; Williams & Feltmate, 1992).

Although during the sampling period plants were not collected, it was observed that the amount of vegetation within each of the sites differed from site to site. SB had little riparian vegetation, however, no vegetation was growing within the canal. Cell #1 had more riparian vegetation than SB, and some vegetation growth within the wetland. Cell #2 had more vegetation growing within the wetland cell than the other two sites. Cell #2 may be more suitable habitat for BMI taxa as there may have had greater food availability than the other two sites. Cell #2

was characterized by having higher benthic macroinvertebrate diversity and taxa richness than the other two sites (see in Figure 13). Many studies have examined the role macrophytes play in a water body (Schriver *et al.*, 1995, Campeau *et al.*, 1994, and Lodge, 1985). It is generally accepted that macrophytes can influence the distribution of aquatic organisms by affecting predation susceptibility and food resources (de Szalay & Resh, 2000). Macrophytes also can provide attachment areas and materials to build refuges (de Szalay & Resh, 2000). Future studies should include an analysis of the vegetation within the wetland cells at RBWP to better characterize the habitats and food resources provided by each site.

Although substrate was not analyzed it was observed that the substrate differed from site to site. Watts *et al.* (2002) reported that RBWP contains many different types of depositional soils that range from sandy loams to silty clays with varying permeability according to a soil survey conducted by the U.S. Bureau of Reclamation at RBWP. The area where SB is located was characterized as having a mixture of Vinton fine sandy loam and Harkey loam while the area where Cell #1 is located is characterized as having Sanelli silty loam. Cell #2 is is characterized as having made land Gila material (Watts *et al.*, 2002). Furthermore, it was reported that Gila material soil type is the prominent soil type where the former river channel was filled during the 1930s (Watts *et al.*, 2002). Future studies should include an analysis of the substrate within each site to better characterize the macroinvertebrate community from site to site, which may better explain differences in the taxa collected.

4.3.1 NMDS analysis

The pattern of BMI community structure was ordinated in a bi-plot correlated with environmental variables (Figure 12). The correlation analysis resulted in ammonia, conductivity, days-full, hardness, ORP, and silica being significantly correlated. The correlation (Table 11)

analysis indicated that NMS axis 1 was significantly and positively correlated with ammonia (r =0.44), days-full (r =0.50), and ORP (r =0.49), while conductivity (r = -0.50) was negatively correlated. While NMS axis 2 was significantly and positively correlated with hardness (r =0.50), and silica (r =-0.62) was negatively correlated. The variables that most explain the pattern that is in the NMS plot are conductivity and Days-full on axis 1, and hardness and silica with axis 2. Again, it is important to note that although water temperature was not significantly correlated with either of the two NMS axis scores, water temperature and conductivity were highly significantly negatively correlated (r=-0.91, p=0.0001). It is believed that the amount of days-full is a major driver of the community composition at RBWP. Most of the higher abundances of organisms that were collected were found during the first three sampling events at RBWP, when levels of conductivity and ammonia were higher. As time progressed and as the days-full increased (during the last half of the sampling period), water temperature decreased, ammonia levels increased, and organism abundance decreased.

A study that was conducted in the Forgotten Stretch of the Rio Grande River investigated the effects of metals to BMI community composition as the water flowed from the beginning to the end of the stretch of the river (Ordonez *et al.*, 2011). The study also used NMDS analysis to determine the BMI community structure with measured environmental variables (Ordonez *et al.*, 2011). The authors reported that NMDS results supported, that as the water flowed down river to the end of the Forgotten Stretch, the BMI community composition shifted from tolerant taxa (lower diversity) to sensitive taxa (higher diversity) via the pattern of BMI associations with the environmental variables (Ordonez *et al.*, 2011). This study conducted at RBWP is comparable to the study of Ordonez *et al.* (2011) in that heavy metals were detected in low concentrations, with the exception of Cd (noted by the authors). The authors further reported that the NMDS

analysis suggested that other factors may be driving the BMI community composition not heavy metals (Ordonez *et al.*, 2011). However, in this study conducted at RBWP Cd levels are likely impacting the BMI community.

4.4 Heavy metals & arsenic

During the course of this study 12 of the 14 heavy metals and arsenic were detected (Figure 5 and Appendix B: Figure 18). Arsenic and cobalt were below the limit of detection (< 20 ppb).

Rios-Arana *et al.* (2003) conducted survey of heavy metals (Chromium (Cr), Copper (Cu), Cadmium (Cd), Nickel (Ni), Lead (Pb), and Zinc (Zn)), and arsenic (As) in water and sediments at seven sites along the Rio Grande in the El Paso-Juarez region. The authors reported the presence of As, Cr, Cu, Ni, Pb, and Zn in the water column and all but two elements (Co and As) were above the detection limit (<200 ppb) in the sediments. As expected, concentrations of heavy metals and As were lower in the water column than in the sediments (Rios Arana *et al.*, 2003). Zn and Pb concentrations in water exceeded the USEPA's Quality Criteria freshwater chronic criteria values. Overall, Zn was found in the highest concentration, which ranged from 5-18 mg/kg. In this survey conducted at RBWP, Al was detected in the highest concentration, 21,220 mg/kg (Appendix B: Figure 18a). In comparison to Zn concentrations detected at the Rio Grande sites, in this study, Zn concentrations were up to 99 mg/kg, which was almost 20 times as high. It was stated by Rios-Arana *et al.* (2003) that the concentrations of heavy metals and arsenic detected in the sediments in their survey was less than what was detected in other similar studies conducted along the river. The survey conducted by Assadian *et al.* (1998) found detected concentrations of the following metals within the sediments at a location in San Elizario, TX: 35.1 mg/kg of Zn, 13.0 mg/kg of Cr, 9.6 mg/kg of Ni, 9.6 mg/kg of Pb, and 0.7

mg/kg of Cd. In comparison to the study of Assadian *et al.* (1998) the concentration of zinc detected in their survey was about a third less than the concentration detected within the sediments at RBWP. A possible explanation of the difference in concentrations of the heavy metals and arsenic detected in this study versus the two previously mentioned studies could be the method of heavy metal and arsenic analysis used, the geological composition of the sediment: naturally occurring metals within RBWP, point and non-point source contributions. While Arana *et al.* (2010) investigated certain heavy metals and arsenic concentrations as a baseline for concentrations within the Rio Grande in the El Paso/Ciudad Juárez area, another study in the Rio Grande examined the impact of heavy metals to BMI community composition.

Ordonez *et al.* (2011) investigated the effect of heavy metal concentrations and their effect on BMI community composition along the Forgotten Stretch of the Rio Grande River. Heavy metals that were analyzed were detected in the following concentrations: Cd: 2 mg/kg; Cr: 3 mg/kg; Cu: 5 mg/kg; Ni: 4 mg/kg; Pb: 5 mg/kg; and Zn: mg/kg (Ordonez *et al.*, 2011). The authors reported that they observed a change in the BMI community composition from a higher abundance of more tolerant taxa in the upper stretch to a higher abundance of more sensitive taxa in the lower stretch of the river (Ordonez *et al.*, 2011). Since heavy metal concentrations were consider low, the authors suggest that other variables (i.e., levels of nutrients and PPCPs) not measured most likely contributed to the change in BMI composition (Ordonez *et al.*, 2011). The levels of heavy metals detected at RBWP were higher (in some cases up to almost 4 times, Appendix B: Table 12) than those reported by Ordonez *et al.* (2011). It is important to note that although metals were considered low, Ordonez et al (2011) that Cd levels were above the reported threshold effect concentration (TEC; MacDonald *et al.*, 2000). Results from the study of Ordonez *et al.* (2011) were comparable to this study conducted at RBWP in that Cd levels

were similar, with a mean concentration of 3 mg/kg observed at Cell #2 (Appendix B: Table 12).

MacDonald *et al.* (2000) developed consensus-based sediment quality guidelines for freshwater ecosystems. They reported that the threshold effect concentration (TEC), which is the concentration that is below which harmful effects to aquatic communities are unlikely to be observed (MacDonald *et al.,* 2000). The reported TECs for the following heavy metals and arsenic are as follows: As: 9.79 mg/kg; Cd: 0.99 mg/kg; Cr: 43.4 mg/kg; Cu: 31.6 mg/kg; Pb: 35.8; Mercury (Hg): 0.18 mg/kg; Ni: 22.7 mg/kg; and Zn: 121 mg/kg (MacDonald *et al.,* 2000). In this study at RBWP the following metals were below the TECs reported by MacDonald *et al.* (2000): Cr, Pb, Ni, and, Zn. The exception was Cd at it exceeded the 0.99 mg/kg TEC at every site over the course of the sampling period (MacDonald *et al.,* 2000). During the course of this study Cu exceeded the 31.6 mg/kg TEC (25% of the time at SB; 50% of the time at Cell #1; 25% of the time at Cell #2) (Figure 5). These findings suggest that Cd and Cu may have harmful effects to BMIs within RBWP. MacDonald *et al.* (2000) further reported consensus based heavy metal and arsenic concentrations that above which a harmful effect is likely to be observed, which is called probable effect concentration (PEC). The following PEC values were reported as follows: As: 33.0 mg/kg; Cd: 4.98 mg/kg; Cr: 111 mg/kg; Cu: 149 mg/kg; Pb: 128 mg/kg; Hg: 1.06 mg/kg; Ni: 48.6 mg/kg; and Zn: 459 mg/kg (MacDonald *et al.,* 2000). It is important to note that one time during this study Cd (4.87 mg/kg) approached the PEC of 4.98 mg/kg at Cell #2. Lagrana *et al.* (2011) determined acute LC_{50} toxicity to chironomids for the following heavy metals: estimated 96-hr LC_{50s} of 1.37 μg/mL for Cu, 73.09 μg/mL for Cd, and 38.47 μg/mL for Pb. It is important to note that although results were not obtained for water samples, analyzed sediment samples had a higher concentration of Cd than the LC_{50} value reported by Lagrana *et*

al. (2011). The findings of this study at RBWP as compared to the reported TEC, PEC, and LC_{50} values suggest that Cd is likely to have harmful effects to the BMI community.

5 Future Directions

Future work should include a collection of water and sediment samples over the course of multiple water releases for further PPCP and heavy metal analyses. A further investigation of erythromycin and BPA should be conducted, since limited results were available (i.e. erythromycin was detected BLQ, and BPA was only detected during a preliminary study). The levels of Cd should be further examined, since during the course of this study Cd levels consistently exceeded TEC levels.

Additional collections of BMIs should be conducted over the course of multiple water releases to obtain a better idea of the BMI community composition and how it changes over time. Findings in the present study suggest that high nutrient levels may be impacting the community composition. In order to better determine how water quality impacts BMI communities, a comparison of multiple sites representing high and low water quality would be beneficial. Since nutrient concentrations also affect food availability to BMIs, measuring coarse and fine organic particulate in the sediment may also help better define habitat suitability. Future work should also include a determination of a life cycle of the chironomids that inhabit RBWP under environmentally relevant conditions to determine how many generations inhabit the sediments in a given season. Further exposure studies should be included to determine the chronic effects of PPCPs, and the toxic effect of mixtures of PPCPs to chironomids. Exposures to metals detected in this study should be further investigated, especially Cd, since it was consistently high.

6 Conclusions

Overall, levels of PPCPs at RBWP are not likely to cause acute toxic effects to the BMI community. However, it is important to note that chronic environmental toxicity cannot be excluded. More samples should be collected over multiple filling events at RBWP to really determine what is happening with concentration levels.

Over the course of this study nutrient (ammonia, nitrate + nitrite, phosphate, and chlorophyll-a) levels (Table 3) were consistently above the TSWQS values (TCEQ, 2010). Ammonia, nitrate + nitrite, phosphate, and Chlorophyll-a exceeded their standards. Factor that could be driving the macroinvertebrate community composition may be high nutrient levels and hydroperiod (days-full) (Figure 2), as further supported by the NMDS analysis (Figure 12) by looking at day-full.

Most of the heavy metals analyzed were below consensus sediment quality guidelines as reported by MacDonald *et al.* (2000). The exception was Cd, and throughout the course of this study levels repeatedly exceeded TEC levels reported by MacDonald et al. (2000). The findings of this study suggest that Cd may have harmful effects to BMI community composition.

Out of the 9 PPCPs analyzed, Ciprofloxacin and codeine, were frequently detected in high concentrations in water and sediment samples. Since detected concentrations of all compounds were detected in low concentrations (ng/L), they do not seem to pose a great risk according to the findings from published studies and the 48 hr toxicity tests conducted. However, it is important to note that chronic effects from the present compounds, to the aquatic community cannot be excluded.

During the sampling period, nearly 30,900 benthic macroinvertebrates were collected at RBWP. Overall, only two taxa groups, midge larvae (Diptera: Chironomidae), and nematodes

(Nematoda), were the two dominant taxa out of 17 identified taxa. Individuals from these taxa accounted for over 50% at of the total number of invertebrates collected.

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Appendix

Appendix A: Water Chemistry Results

Water quality parameters were analyzed using GLMM (SAS® version 9.3) Analysis to determine significant differences between sites and/or sampling dates for each of the parameters. If there were any significant differences, a Tukey's *post-hoc* test was conducted to identify where differences occurred. Most of the measured water chemistry parameters, from Table 4, were significantly different ($p<0.05$) among sampling dates, which is expected due to a decrease in water temperature during the study.

Water chemistry parameters that were measured, but not previously mentioned are shown below. Most of the water chemistry parameters measured were below the state criteria (Appendix A: Table 10). Measured total dissolved solids (TDS) levels at all sites and sampling dates were below the 1,400 mg/L standard set by TSWQS (TCEQ, 2010). All measured chloride levels were below the 250 mg/L standard set by TSWQS (TCEQ, 2010). The standard of 450 mg/L of Sulfate was never exceeded during the sampling period at each site.

Table 10. Other measured water chemistry parameters (mean ± SE) for all sites and sampling dates from 16 Oct 2009 to 21 Feb 2010.

	Sites		
Water Chemistry Parameters	SB	Cell #1	Cell #2
ORP	302.1 ± 33	89.6 ± 17	118.5 ± 57
TDS (g/L)	1.116 ± 0.05	1.130 ± 0.05	1.149 ± 0.05
Alkalinity (mg/L CaCO3)	110.4 ± 21	92.7 ± 10	98.6 ± 8
Chloride (mg/L Cl-)	205.6 ± 5	205.7 ± 12	200.9 ± 10
Sulfate (mg/L SO4)	211.4 ± 26	220.0 ± 25	201.4 ± 32
Silica (mg/L SiO2)	33.2 ± 1	32.5 ± 1	27.6 ± 1.7

However, there were a few water chemistry parameters that were significantly different among sites: ORP, TDS, and silica (Appendix A: Table 11).

Table 11. Tukey's *post-hoc* test results for significance among sites and sampling dates for water chemistry parameters from 16 Oct 09 to 21 Feb 10. F and p values that are in bold shown if there was any significance among sites and/or sampling dates.

Water chemistry parameter	Significance among sites: F-value, p-value	Significance among dates: F-value, p-value
ORP	**F=10.6, p=0.0022**	F=2.1, p=0.13
TDS (g/L)	**F=12.0, p=0.0014**	**F=314.0, p<.0001**
Alkalinity (mg/L CaCO3)	F=0.30, p=0.75	F=2.2, p=0.12
Chloride (mg/L Cl-)	F=0.30, p=0.75	**F=5.4, p=0.0065**
Sulfate (mg/L SO4)	F=0.81, p=0.47	**F=19.8, p=<.0001**
Silica (mg/L SiO2)	**F=19.4, p=0.0002**	**F=9.6, p=0.0005**

ORP was significantly different (F=10.6, p=0.0022) among SB and Cell #1 (p=0.006), and SB and Cell #2 (p=0.00) (Appendix A: Table 14 and Figure 17a). TDS was significantly different (F=12.0, p=0.0014) among SB and Cell #2 (p=0.001), and Cell #1 and Cell #2 (p=0.03) (Appendix A: Table 14 and Figure 17b). Silica was significantly different (F=19.4, p=0.0002) among SB and Cell #2 (p=0.0003), and Cell #1 and Cell #2 (p=0.0008) (Appendix A: Table 14 and Figure 17c).

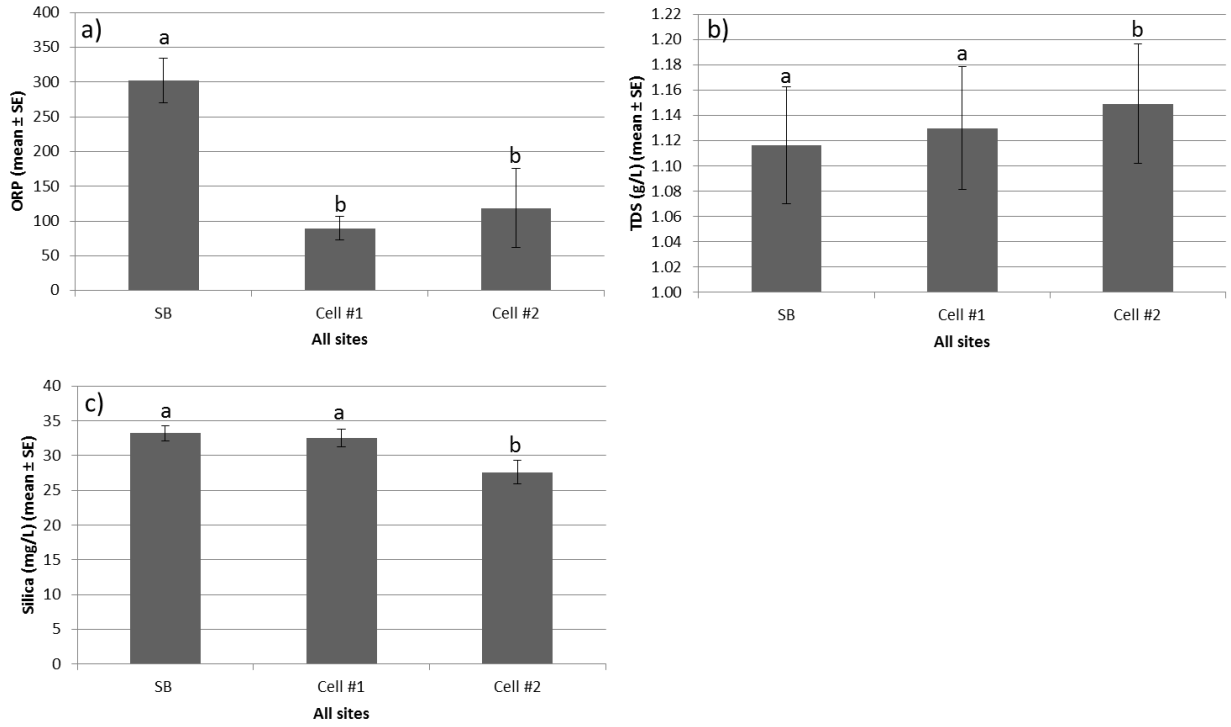

Figure 17. ORP (a); TDS (g/L) (b); and Silica (mg/L SiO2) (c) measured at all sites and sampling dates from 16 Oct 09. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences among sites (Tukey's *post-hoc* test via GLMM analysis (ORP: F=10.59, p=0.0022; TDS: F=12.01, p=0.0014; and Silica: F=19.44, p=0.0002).

Appendix B: Heavy Metals

Six (Cd, Cr, Cu, Pb, Ni, and Zn) of the 14 metals analyzed will be discussed in this section due to these metals most commonly impacting water quality (Figure 3 and Appendix B: Table 12).

Table 12. Mean concentrations (mg/kg) for the 6 commonly impacting water quality heavy metals at each of the 3 sites.

	Sites		
Metal	SB	Cell #1	Cell #2
Cd	1.3	2.7	3
Cr	14	24	19
Cu	26	32	22
Ni	6.1	12	10
Pb	14	17	16
Zn	80	107	75

Heavy metals analyzed, but not mentioned above (Appendix C: Figure 18 a,b). The element that was detected in the sediments with the highest concentration was Al (21,220 mg/kg) (Appendix C: Figure 18a). The element that was detected in the lowest concentration was Ag (0.165 mg/kg) (Appendix C: Figure 18b).

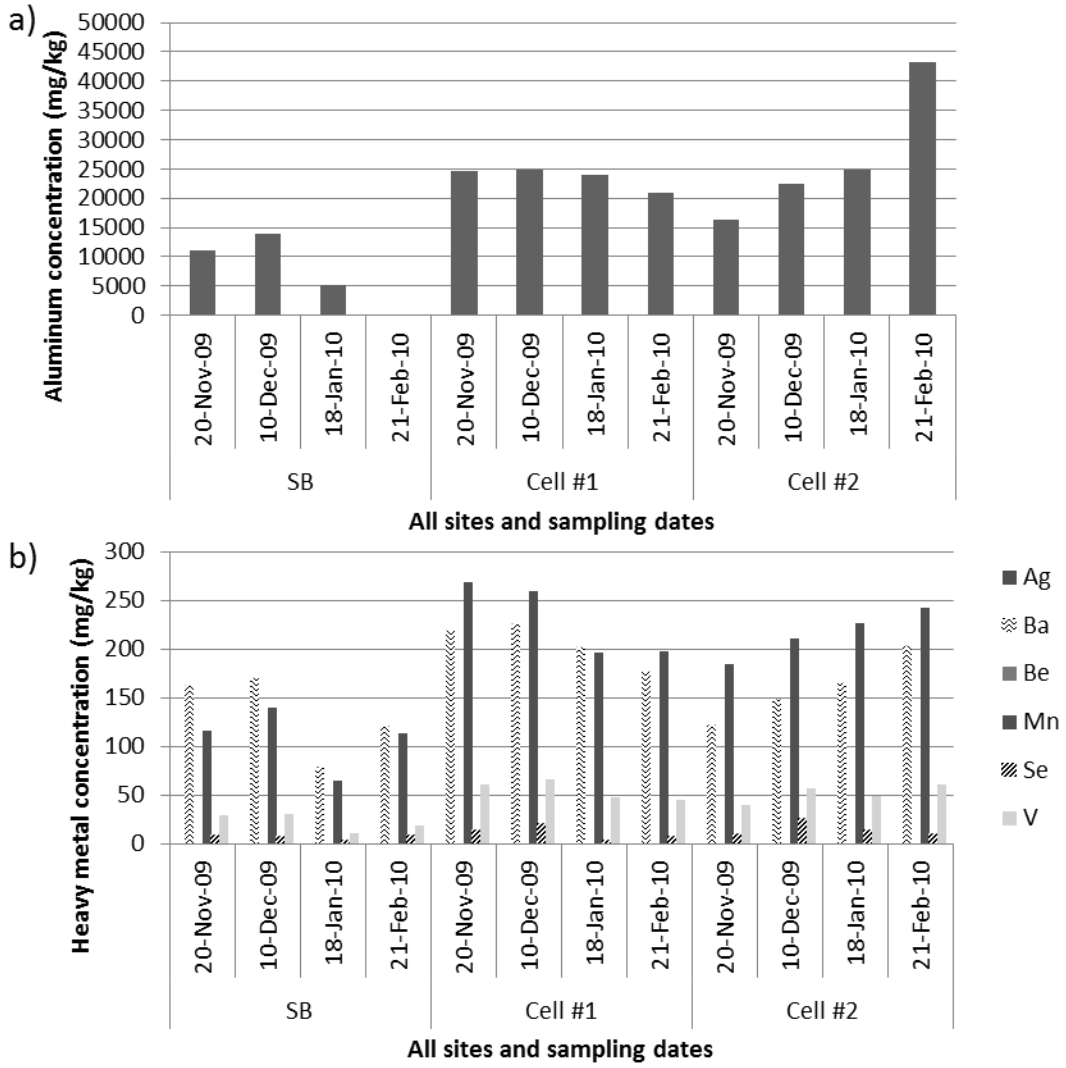

Figure 18. Aluminum (Al) (a) and all other heavy metals: Ag, Ba, Be, Mn, Se, and V; concentrations detected at all sites and sampling dates from 20 Nov 09 to 21 Feb 10.

The following metals (out of the 14 and arsenic) were significantly different among sites: Be, Mn, and V (Appendix C: Figure 19 a-c). The remaining heavy metals did not differ significantly in concentration among sites or sampling dates. Be concentration levels were significantly different among sites ($F=5.19$, $p=0.049$) concentration levels increased from SB to Cell #1 by 0.7 times, and from Cell #1 to Cell #2 there was only a slight increase by 0.10 mg/kg. Be was significantly different among SB and Cell #2 ($p=0.049$) only (Appendix C: Figure 19a).

Mn was significantly different (F=15.99, p=0.0039) among SB and Cell #1 (p=0.0049), and SB and Cell #2 (p=0.0092) (Appendix C: Figure 19b). Mn concentrations increased a little over one order of magnitude from SB to Cell #1, however, concentration levels from Cell #1 to Cell #2 only decreased by about 10 mg/kg. Vanadium (V) was significantly different (F=15.24, p=0.0045) among SB and Cell #1 (p=0.0057), and SB and Cell #2 (p=0.0099) (Appendix C: Figure c). The concentration levels of V increased by about 30 mg/kg from SB to Cell #1. However, the concentration levels from Cell #1 to Cell #2 decreased slightly by about 4 mg/kg.

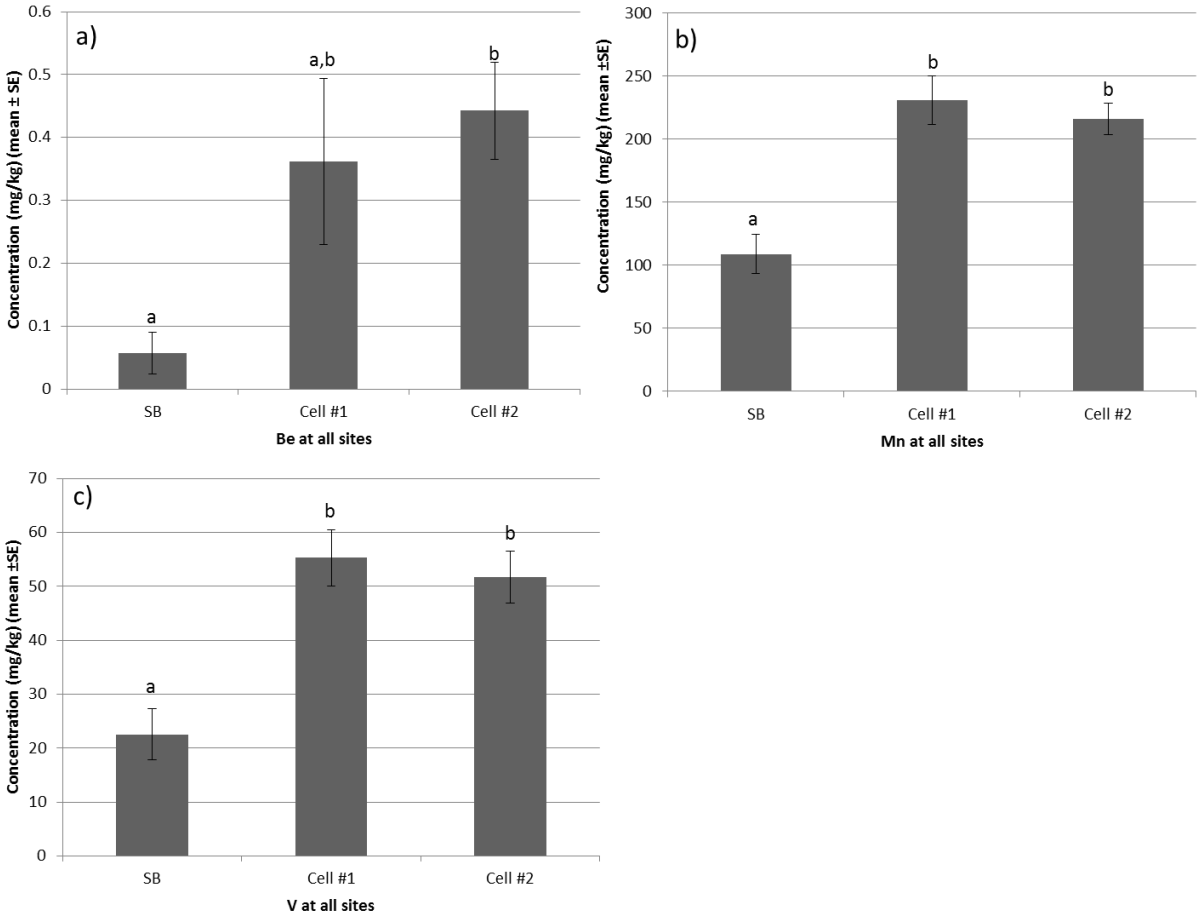

Figure 19. Beryllium (Be) (a), Manganese (Mn) (b), and Vanadium (V) (c) concentrations (mg/kg) found in the sediment at all sites and sampling dates from 20 Nov 09 to 21 Feb 10. All values are given as mean +/- 1SE. The letters above the bars indicate significant differences among the sites (Tukey's *post-hoc* test via GLMM analysis (Be: F=5.19, p=0.049 ; Mn: F=15.99, p=0.0039; V: F=15.24, p=0.0045).

Appendix C: PPCPs

In water samples collected 8 of the 9 PPCPs were detected at least once in each of the sites (Table 5 and Appendix C: Figure 20). The one exception was erythromycin it was below the limit of quantification (Table 5). The compounds that were the most frequently detected and in high concentrations were ciprofloxacin (Appendix C: Figure 20a), and codeine (Appendix: Figure 20b) in water samples. PPCPs in higher concentrations were separated from the others for better viewing (Appendix C: Figures 20a and 21a and b). Water and sediment results are over the course of two different water releases at RBWP. Unfortunately, at the beginning of this study PPCP collecting methods were not in place until the second water release at RBWP for water samples, and towards the end of the first water release (sampling dates: 8 Feb 10 and 21 Feb 10) at RBWP for sediment samples. During the second water release water was diverted to Riverside Canal on 7 Mar 11, for irrigation season, and unfortunately by 11 Mar 11 SB was dry and no samples were collected (Appendix: Figure 20 and 21). Water was still available at Cell #1 and Cell #2, and water and sediment samples were still collected.

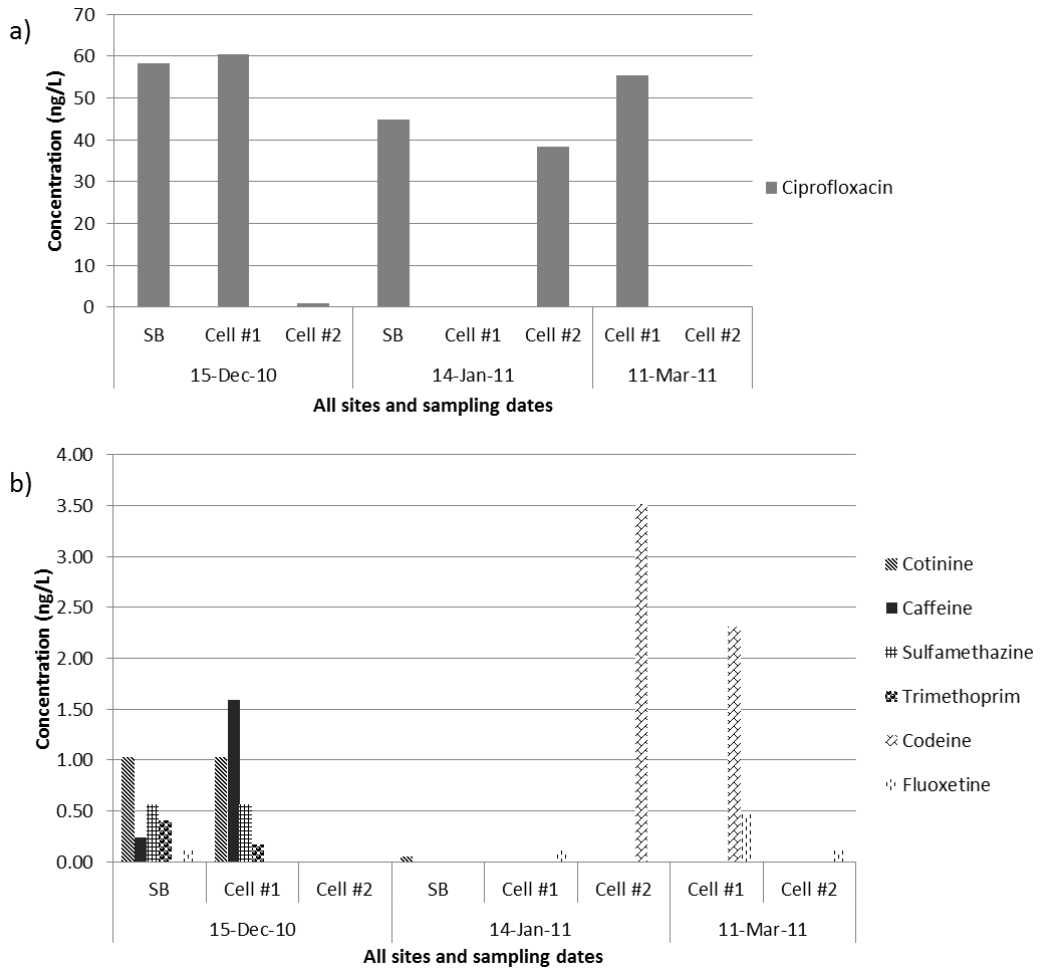

Figure 20. Concentrations of PPCPs detected within water samples collected from each site from 15 Dec 10 to 11 Mar 11 during the second water release for Ciprofloxacin (a), and all other PPCPs (b) at Rio Bosque Wetlands Park. Note: Missing SB values from 11 Mar 11 indicates that a water sample was not collected.

In the sediments, 7 of 9 PPCPs were detected at least once at each site during the sampling period (Appendix C: Figure 21). Acetaminophen and erythromycin were the two compounds that were detected below the limit of quantification. The compounds that were most frequently detected in high concentrations were: ciprofloxacin (Appendix: Figure 21a and 21b), caffeine (Appendix C: Figure 21a and 21b). Concentrations of PPCPs detected were higher in the sediments (Table 5 and Appendix C: Figures 20 and 21) than in the water (Table 6) by up to 6 orders of magnitude, which could be due to sedimentation as the water flows through the

wetland cells. It is important to note that during the course of this study only two sediment samples were collected and analyzed during the first water release at RBWP, and corresponded to the same period that BMI samples were collected.

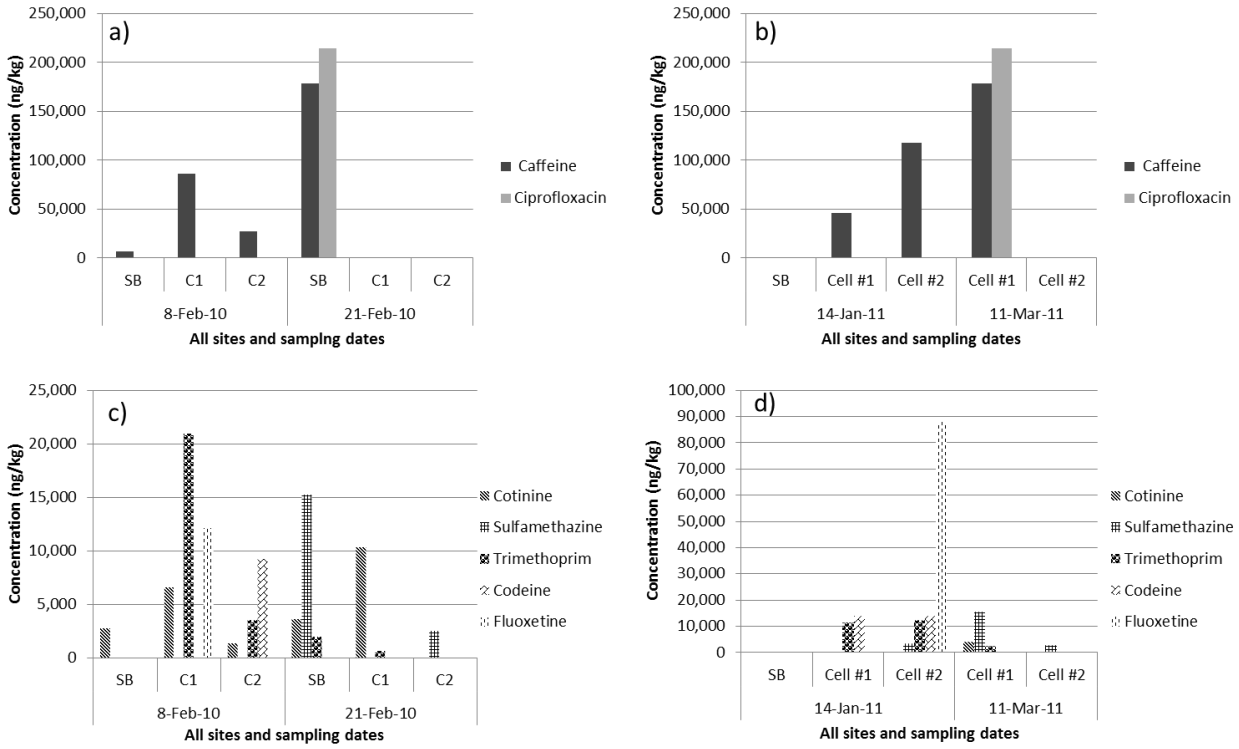

Figure 21. Concentrations of PPCPs detected within sediment samples collected from each site from the first water release 8 Feb 10 and 21 Feb 10 (a and c), and the second water release from 15 Dec 10 to 11 Mar 11 (c and d) at Rio Bosque Wetlands Park. Note: Missing SB values from 11 Mar 11 indicates that a water sample was not collected.

Appendix D: Benthic Macroinvertebrates

Individual benthic invertebrate identifications, counts and summary statistics for each site (Appendix D: Tables 13-15). BMI composition is discussed in sections 3.4.2 and 3.4.2.1 above.

Table 13. Total numbers and percentage of individuals collected at SB from 16 Oct 09 to 21 Feb 10. Counts for the family Chironomidae are a sum of the subfamilies: Chironominae, and Orthocladinae. Chirnomidae genera are in bold. Note: L, P, and N indicates: larvae, pupae, and nymph.

Taxa	Total number	Percent of total
Ceratopogonidae (L)	1	0.02
Chironomidae (L)	5,695	97
Chironomus	**4,007**	**68.3**
Dicrotendipes	**12**	**0.20**
Goeldichironomus	**1,666**	**28.4**
Polypedilum	**9**	**0.15**
Othocladius/Cricotopus	**1**	**0.02**
Chironomidae (P)	163	2.8
Hydrophilidae Berosus (L)	4	0.07
Gomphidae (N)	1	0.02
Nematoda	5	0.09
Total collected	5869	100.00

Table 14. Total numbers and percentage of individuals collected at Cell #1 from 16 Oct 09 to 21 Feb 10. Counts for the family Chironomidae are a sum of the subfamilies: Chironominae, and Tanypodinae. Chirnomidae genera are in bold. Note: L, P, and N indicates: larvae, pupae, and nymph.

Taxa	Total number	Percent of total
Chironomidae (L)	17,092	99
Chironomus	**4353**	**25**
Goeldichironomus	**12,477**	**73**
Polypedilum	**41**	**0.2**
Tanytarsus	**157**	**1**
Ablabesmyia	**48**	**0.3**
Labrundinia	**2**	**0.01**
Tanypus	**14**	**0.1**
Chironomidae (P)	89	1
Ephidridae (L)	1	0.01
Nematoda	7	0.04
Total collected	17,189	100.00

Table 15. Total numbers and percentage of individuals collected at Cell #2 from 16 Oct 09 to 21 Feb 10. Counts for the family Chironomidae are a sum of the subfamilies: Chironominae, Orthocladinae, and Tanypodinae. Chirnomidae genera are in bold. Note: L, P, and N indicates: larvae, pupae, and nymph.

Taxa	Total number	Percent of total
Ceratopogonidae (L)	4	0.05
Chironomidae (L)	5,997	76.6
Chironomus	**1,955**	**25.0**
Dicrotendipes	**49**	**0.6**
Goeldichironomus	**3,135**	**40.1**
Polypedilum	**270**	**3.5**
Tanytarsus	**369**	**4.7**
Othocladius/Cricotopus	**206**	**2.6**
Ablabesmyia	**4**	**0.05**
Tanypus	**9**	**0.1**
Chironomidae (P)	47	0.6
Dolichopodidae (L)	2	0.03
Baetidae (L)	1	0.01
Corixidae (N)	1	0.01
Notonectidae (N)	1	0.01
Coenagrionidae (N)	29	0.4
Nematoda	1,741	22.3
Triops longicaudatus	1	0.01
Total collected	7,824	100.00

Curriculum Vitae

Prior to beginning her college career, Jennifer Martinez, enlisted in the Air Force reserves August 1999 as a Security Forces Member, to present. While in the AF reserves she has deployed to Saudi Arabia (2003 and 2012) and Iraq (2008). She began her college career in 2000, completing her Associates in Arts degree at Tarrant County Community College- NW Campus (May 2003). During this time she worked as a math tutor at TCCC-NW campus. She also became a member of Phi Theta Kappa honor society. Upon graduating from TCCC-NW she continued her college career at University of North Texas and completed her Bachelor degree in Biology and Chemistry (May 2007). During her time at UNT she entered the Forensic Science Program after its inception in 2005. She was the first President of UNT's Forensic Science Club. In the summer of 2007 she attended an undergraduate internship by attending a Forensic Genetics class at UNT Health Science Center. Prior to graduating from UNT she worked as an undergraduate research assistant in Dr. James Kennedy's benthic ecology lab, where she gained lab and field experience. The knowledge and skills gained while working in the lab guided the future direction of Jennifer's college career. Jennifer began her pursuit of a Master of Science degree in biology under the guidance of Dr. Elizabeth Walsh at the University of Texas at El Paso in August of 2009. As a Masters student, Jennifer has served as a teaching assistant for Introductory Biology (BIOL 1107). She has presented preliminary results from her research at the Southwestern Association of Naturalists Meeting (April 2010), the Joint Meeting of American Society of Limnology and Oceanography and North American Benthological Society (June 2010), and annual Society of Freshwater Science meeting (May 2011). Following graduation she will begin her position as an Environmental Scientist with the environmental consulting firm, Conestoga-Rovers & Associates.

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