2016

Pharmaceutical and personal care products concentrations in the Belgrade Lakes: A possible threat to aquatic ecosystems and human health

Serena Haver
Colby College

Recommended Citation

Haver, Serena, "Pharmaceutical and personal care products concentrations in the Belgrade Lakes: A possible threat to aquatic ecosystems and human health" (2016). *Honors Theses.* Paper 810.
https://digitalcommons.colby.edu/honorstheses/810

Pharmaceutical and personal care products concentrations in the Belgrade Lakes:

A possible threat to aquatic ecosystems and human health

Serena M. Haver
Environmental Studies Program
Colby College
Waterville, Maine
May 6, 2016

A thesis submitted to the faculty of the Environmental Studies Program in partial fulfillment of the graduation requirements for the Degree of Bachelors of Arts with honors in Environmental Studies

William McDowell, Advisor	Gail Carlson, Reader	Philip Nyhus, Reader

ABSTRACT

Pharmaceuticals and active ingredients from personal care products (PPCPs) include prescription and over-the-counter drugs, sunscreen, fragrances, and soaps. Recently researchers have detected PPCPs in surface waters, posing a threat to aquatic biota and human health. These compounds can enter the environment through dumping, direct excretion, or sewage effluent streams. We tested 14 water samples from East Pond, Great Pond, and Long Pond in Maine for the presence of 23 different PPCPs. Of those, we only detected significant levels of caffeine, 1,7-dimethylxanthine, and amphetamine all below 1µg/L. There were no significant differences between any of the three lakes for any of the compounds. The levels of these three compounds were relatively similar to more urban lakes, but the prevalence of discrete PPCPs was much lower, indicating low overall PPCP pollution. Additionally, there was higher concentration of PPCPs detected at public boat launches opposed to private residences. Due to our small sample size and the timing of our sampling, further research should be directed towards gaining more samples around the lake to gain a more holistic picture of PPCPs in the Belgrade watershed, particularly during summer months when seasonal population increases could increase the abundance and types of PPCPs present within the lakes. Additionally, detailed land use patterns of the region and an assessment of PPCP concentrations in freshwater mussels could reveal important PPCP consumption patterns and temporal trends around the watershed.

ACKNOWLEDGMENTS

First, I would like to extend thanks to my advisor, Faculty Fellow William McDowell, for helping get this project off the ground, going sampling with me in the middle of December, and the endless support you have given. I would also like to thank Professor Gail Carlson, one of my readers, for her enthusiasm about PPCPs and human health as well as for all the work she did helping out with the logistics of testing for PPCPs and discussing the possible implications for our research. Thank you to Philip Nyhus, another one of my readers, for giving me advice along the way. I would also like to extend gratitude to the following individuals and organizations whose invaluable efforts made this project possible:

Goldfarb Center for student-faculty collaborative research
Daniel Snow, Director of Water Sciences Laboratory at the University of Nebraska
Homeowners on Great Pond, East Pond, and Long Pond
Manuel Gimond, GIS & Quantitative Analysis Specialist; Research Scientist
    Environmental Studies, Analysis
Charlie Baeder (BRCA), Executive director of Belgrade Regional Conservation Alliance

TABLE OF CONTENTS

FIGURES

TABLES

APPENDICIES

INTRODUCTION

Maine is renowned for its natural landscapes and plethora of outdoor recreational activities. Its lakes cover over one million acres and generate $3.5 billion dollars through recreation, residential living, and tourism (Maine DEP). In order to maintain the health of these resources, the Maine Department of Environmental Protection (DEP) and the Maine Volunteer Lake organization have been dedicated to protecting Maine's lakes since 1971, utilizing both water quality specialists and citizen science (DEP 2013, McCullough et al. 2013). Due to the ecological and economic importance of the Belgrade Lakes to the region and the state of Maine, it is vital to explore the different causes of water quality decline and assess the risk to the aquatic ecosystems and local human populations (Bowles 2010).

In the past few years, anthropogenic intensification and eutrophication have caused a decline the water quality resulting in milfoil invasions, algal blooms, hypoxia, and fish kills (Liang and Guillory 2015). Recently, researchers around the world have also begun to explore pharmaceuticals and personal care products (PPCPs) as another category of contaminant concern for freshwater ecosystems. This project aims to document the prevalence of PPCPs in East Pond, Great Pond, and Long Pond, located in the Belgrade Lakes Watershed in central Maine. We also aim to assess the possible risks to aquatic organisms and human health associated with PPCPs at these concentrations (Bruton et al. 2010b).

Pharmaceuticals and Personal Care Products: A Growing Concern

Pharmaceuticals and personal care products (PPCPs) are cosmetics, drugs, or other consumer products used to cleanse, beautify, or treat humans or animals (Nutrition Center for Food Safety and Applied Food and Drug Administration 2015). Examples of PPCPs include prescription and over the counter drugs, soaps, fragrances, solvents, non-ionic and anionic surfactants, bleaches, dyes, and sunscreen agents (Caldwell 2015). In order to achieve their desired effects, PPCPs contain chemical compounds that are specially designed to interact with physiological systems (Boxall et al. 2012). These reactive components have been shown to persist in sediments, soils, and surface waters (Adolfsson-Erici et al. 2002, Benotti et al. 2009, Banerjee et al. 2016). Recent technological advances, including solid phase extraction followed by liquid chromatography-mass spectrometry, have allowed for detection of PPCPs in the ng/L range in aquatic samples (Lam et al. 2004). Given the widespread use of PPCPs, it is important to consider how we dispose of them and the possible effects on both the environment and human health (Daughton and Ternes 1999, Enick and Moore 2007, Schirmer and Schirmer 2008, Caldwell 2015).

Research on PPCPs as micro-pollutants, or pollutants found in the μg/L or ng/L range, only began about 15 years ago. Most of the literature focuses on the presence of certain compounds in sewage effluent, medical waste effluent, groundwater, drinking water, landfills, and surface waters. Recently, researchers have also begun to study psychoactive and illicit drugs in the environment. Large data gaps exist documenting the occurrence, fate, or activity of PPCPs or their metabolites in any of these types of water (Kümmerer 2009). Furthermore, there is no current data about the presence of PPCPs or their impacts on the environment or human health in central Maine lakes.

Current Knowledge on PPCPs in the Environment

Sources of PPCPs

The majority of PPCPs found in aquatic systems are the result of consumer use, excretion, disposal of unused products flushed down toilets, or from wastewater treatment facilities (Caldwell 2015). Wastewater treatment facilities treat water, typically from homes, manufacturing sites, or runoff, by removing suspended particles and pollutants (Perlman 2016). Since neither municipal wastewater treatment plants, nor onsite wastewater treatment systems (including septic systems) can effectively treat the complex mixture of PPCPs present in sewage, PPCPs are often released directly into the environment (Figure 1) (Jones et al. 2004, Vieno et al. 2005, Carrera et al. 2008, Benotti et al. 2009, Schaider et al. 2013, Papageorgiou et al. 2016, Banerjee et al. 2016). For example, a study of 18 different antibiotics in sewage treatment effluent demonstrated that none of them were readily biodegradable (Alexy et al. 2004). Other sources include aquaculture facilities and releases to soils and subsequently groundwater from bio solid and manure application (Boxall et al. 2012). In aquatic ecosystems, sewage effluent is often cited as the primary influence on detection frequencies and concentrations of PPCPs (Fairbairn et al. 2016).

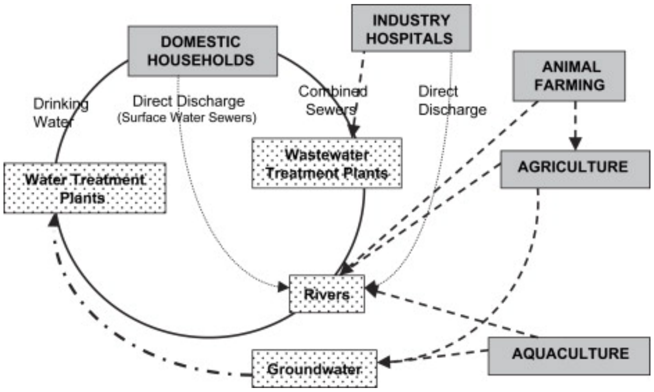


Figure 1. Sources and pathways of the urban PPCP (Ellis 2006).

Some technologies such as the membrane-based bioreactors (MBRs) have the potential to remove PPCPs from sewage effluent. Banerjee et al. (2016) found that MBR treated sewage effluent could remove 98.56% of triclosan and 99.74% of surfactant. In order to treat wastewater containing large amounts of biomass, MBRs add membrane separation to traditional activated sludge processing. This additional step removes the requirement for sedimentation, a problem for conventional treatment, and produces water with fewer dissolved constituents like organic matter and ammonia (Santos et al. 2011). Today, MBRs are primarily used for large-scale industrial, domestic, and municipal wastewater treatment (Yang et al. 2006). Their effectiveness, especially on smaller scales, is still not fully understood (Yang et al. 2006; Banerjee et al. 2016).

Ubiquity in the Aquatic Environment

PPCPs are frequently detected in freshwater samples from around the world, even in supposedly pristine bodies of water (Vieno et al. 2005, Fairbairn et al. 2016, Banerjee et al. 2016). For example, atrazine was detected in surface waters far from agricultural application in the United States, with a drinking water treatment plant as the only known source of contamination (Benotti et al. 2009). According to Benotti et al. (2009), researchers have detected atrazine in food. Subsequent disposal of this food may be the source of atrazine loading into the surface waters.

A national study spanning 139 streams in 30 different states found measureable amounts of one or more medications in 80% of the water samples drawn. Of the types of compounds tested, the most prevalent were steroids and nonprescription drugs. Detergent metabolites had the highest percentage concentration in the locations detected (Kolpin et al. 2002). Fairbairn et al. (2016) found that PPCPs like erythromycin, an antibiotic, reached levels as high as 10 μg/L downstream of wastewater treatment plants. In another study, Ferrey (2013) detected 56 different PPCPs and other chemicals downstream of a wastewater treatment plant. Bodies of water close to intense urbanization or livestock production are especially susceptible to PPCP contamination (Koplin et al. 2004). Since these attributes are not characteristic of the Belgrade watershed, we would expect to see lower concentrations of PPCPs in East Pond, Great Pond, and Long Pond.

Breakdown of PPCPs in Surface Waters

Once in the aquatic environment, PPCPs may be eliminated through processes such as biodegradation, sorption, photodegredation, and sedimentation (Vieno et al. 2005). By studying the persistence of eight different pharmaceuticals in aquatic outdoor field microcosms, Lam et al. (2004) found that half-lives ranged from about 1 day with acetaminophen, a pain reliever, to 82 days with carbamazepine, an anticonvulsant, in sunlit microcosms of exposed pond water and natural autoclaved water. Over the 30 day experiment, Lam et al. (2004) also found that these eight pharmaceuticals did not break down in the dark control microcosms, suggesting that photodegradation, not biodegradation, may be a limiting factor in their persistence.

Fluctuation in PPCP Prevalence

Researchers have also found that PPCP concentrations vary considerably with seasonal or population changes. A study of 23 different stream locations in Iowa showed that organic wastewater contaminants, many of which are also PPCPs, varied with stream flow (Koplin et al. 2004). Koplin et al. (2004) observed that organic wastewater contaminant concentrations decreased as stream flow increased. Other studies have attributed this phenomenon to reduced dilution, slowing the degradation processes of PPCPs and allowing them to persist longer in aquatic environments (Musolff et al. 2009, Luo et al. 2011, Veach and Bernot 2011). Koplin et al. (2004) also noted a correlation between the frequent detection of methyl salicylate, a common ingredient in UV sunscreens, and high summer temperatures.

PPCPs in aquatic ecosystems vary based on societal influences such as source proximity and population fluctuations as well as physicochemical and environmental influences. Fairbain et al. (2016) noted that increased PPCP concentrations have been associated with cold, low flow conditions due to reduced degradation and in warm, high-flow conditions due to increased wastewater treatment flow, reduced retention time and removal efficiency. They also described the seasonal changes in agricultural herbicides in surface waters, spiking in the early summer, when application rates and precipitation are the highest. While studying the Aura River in Finland, located near a wastewater treatment facility, Vieno et al. (2005) found that PPCP concentrations increased during winter months and decreased during spring and summer months. Another study of the Upper White River watershed in Indiana demonstrated the same seasonal trend with increased PPCP concentrations in the winter and decreased concentrations in the spring and summer months (Veach and Bernot 2011). A study of seasonal variation of stimulatory drugs in the Llobregat River in Spain near a drinking water plant indicated that nicotine, caffeine, and paraxanthine had the opposite trend, with the highest concentrations detected in the spring and summer (Huerta-Fontela et al. 2008). These studies demonstrate that there are several factors that contribute to PPCP concentrations, which must be reviewed to predict their impact in aquatic ecosystems.

General Effects of PPCPs on Aquatic Organisms

PPCPs can have a variety of effects on biological processes in aquatic environments. Some contain anti-bacterial or anti-fungal properties which are helpful in treating certain infections and illnesses in humans or livestock, but can have dire consequences for aquatic organisms, even those at high taxonomic levels, such as fish (Table 1) (Kümmerer 2004, Sumpter et al. 2006). Synthetic estrogen from birth control pills, antihistamines from allergy medication, pain relievers like ibuprofen and acetaminophen, anti-depressants, triclosan (an antimicrobial agent), caffeine, bisphenol A (found in durable plastics), and illicit drugs, have been shown to have a wide range of biological impacts, including lethal toxicity at very high concentrations, feminization of fish and amphibians, and changes in bacterial communities in aquatic ecosystems (Crain et al. 2007, Drury et al. 2013, Ferrey 2013, Rosi-Marshall et al. 2015).

Most of the literature regarding PPCPs and their impact on aquatic organisms focuses on the impacts of exposure at high concentrations, with a particular emphasis on mortality. However, a number of significant sub-lethal effects including histological changes, behavioral effects, biochemical responses, and gene regulation can occur at low concentration (Klaper and Welch 2011). By synthesizing available information to generate an effect level diagram for selected PPCPs in fish and invertebrates, Boxall et al. (2012) describes the effects of some PPCPs at varying concentrations (Figure 2). They demonstrated that the effect level for different organisms varies based on the level of exposure (Boxall et al. 2012).

PPCPs can also alter the microbial communities of aquatic ecosystems. Microbial communities serve as the basis of aquatic food webs, a resource for higher trophic levels, and the decomposition of organic matter. Any disturbance in this microbial community as a result of PPCP pollution could alter the structure of aquatic ecosystems (Shaw et al. 2015). Diphenhydramine, an antihistamine, has been shown to cause significant increases in *Pseudomonas sp*. and decreases in *Flavobacterium sp.* in stream biofilms (Rosi-

Marshall et al. 2013, MedlinePlus 2015a). These studies demonstrate the importance of considering PPCP prevalence when assessing lake health.

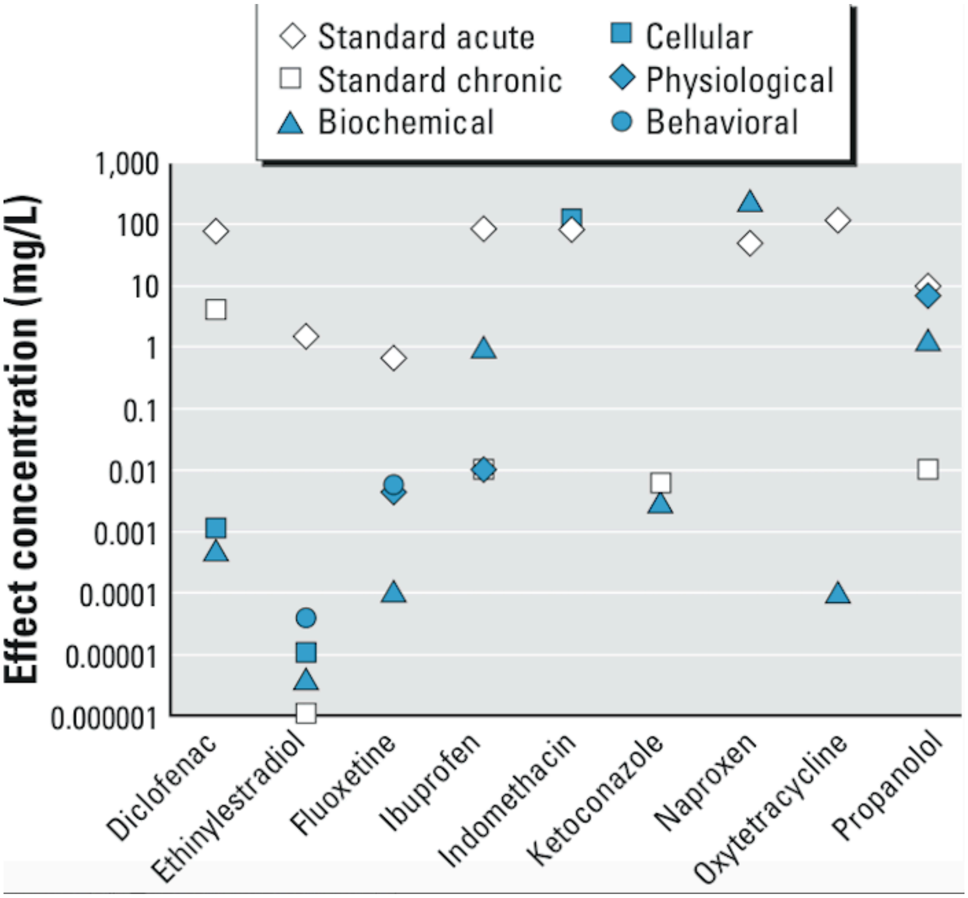


Figure 2. Acute and chronic effects documenting the effects of PPCPs on certain organisms (e.g., fish and invertebrate mortality, reproduction, and growth) (Boxall et al. 2012). Conversion: 1mg/L=1000μg/L.

General Effects of PPCPs on Humans

Scientists and health advocates have also drawn attention to the possible human health implications of PPCPs. Persistent organic pollutants, many of which come from PPCPs, are known to bio-accumulate in the food chain (Klaper and Welch 2011).

Widespread exposure to these pollutants through product consumption or use has been linked to impaired neurodevelopment, immune and reproductive function, and endocrine system disruption, causing inflammation, birth defects, and certain cancers. Even low-level exposure to endocrine disrupting chemicals can impact fetal, neonatal, and childhood development (Damstra 2002). Most of the data describing the impacts of PPCPs on humans focuses on consumption or use of the initial PPCP, not environmental exposure.

Table 1. Acute effects of selected pharmaceutical compound on aquatic organisms (Enick and Moore 2007)

Chemical name	Species	Endpoint measure	Concentration (μg/L)	Reference
Acetaminophen	Daphnia magna (water flea)	Swimming ability 48 h EC50	9200	Kuhn et al. (1989)
Ibuprofen	Lepomis macrochirus (bluegill sunfish)	1.5 h LOEC Significant decrease in activity	0.001	Reported in De Lange et al. (2006)
Carbamazepine	Brachionus calyciflorus (rotifer)	Reproduction 48 h NOEC	377	Ferrari et al. (2004)
Gemfibrozil	Carassius auratus (goldfish)	1) Plasma parameters and 2) Plasma testosterone levels by 50% after 14d exposure	1500	Mimeault et al. (2005)

Humans can also be exposed to PPCPs in water via direct contact from recreation or through drinking water sources (Kümmerer 2004). According to Jones et al. (2004), pharmaceuticals from sewage effluent can cause drug-resistant pathogens in the water. Exposure to these pathogens while recreating polluted bodies of water may have health implications. Studies have shown that PPCPs are also prevalent in drinking water. Loraine and Pettigrove (2006) studied parts of the Colorado River that were severely impacted by septic systems and tested the river water entering and leaving a drinking water treatment facility sourced from the Sacramento-San Joaquin River Basin. The pre-treatment water contained phthalate esters, sunscreens, clofibrate, clofribic acid, ibuprofen, triclosan and diethyltoluamide (DEET). The treated water, delivered to humans for consumption, still contained many of these compounds, including di (ethylhexyl) phthalate, benzophenone, ibuprofen, and triclosan, indicating that water treatment facilities are unable to effectively or completely remove these compounds (Loraine and Pettigrove 2006). In addition to surface water, groundwater is a widespread source for drinking water, and thus, the prevalence of PPCPs in groundwater should also be considered. According to the USGS, PPCPs can move from septic systems into groundwater (Phillips et al. 2015). However, few studies have documented this phenomenon. Exposure to high concentrations or chronic low levels could pose a tremendous threat to human health.

The Study Area

Physical Characteristics of the Belgrade Lakes Watershed in Maine

The Belgrade Lakes watershed is a chain of seven lakes and ponds located in the Kennebec River Valley region of central Maine. The watershed spans an area of 46,676 hectares (ha) across 13 townships including Augusta, Belgrade, Manchester, Mercer, Mount Vernon, New Sharon, Norridgewock, Oakland, Readfield, Rome, Sidney, Smithfield, and Vienna, ME. There are two major flow paths through the lakes. The first begins in Salmon Lake and McGrath Pond, flows through Hatchery Brook into Great Pond, and then into Long Pond. The water then flows into Messalonskee Stream, and empties into the Kennebec River. An alternative flow path beginning in East Pond travels through the Serpentine Stream into North Pond and then drains via Great Meadows Stream into Great Pond, followed by Long Pond, and lastly, Messalonskee Stream (McCullough 2010, Figure 5).

Possible Sources of PPCP Pollution in the Belgrade Lakes Watershed in Maine

In the Belgrade lakes watershed, septic systems are the primary mode of sewage treatment (Figure 3) (Reed and Haver 2015). Septic systems consist of a septic tank and leach field. Septic tanks are watertight receptacles that are placed underground and receive wastewater from the home. They account for 45% of the treatment of household wastewater which involves settling particles to the bottom of the tank, trapping grease and oils, allowing scum to rise to the top, and utilizing bacteria to break down some of the solids. The effluent then flows onto a leach field, consisting of a bed of crushed gravel and absorbent soil (Reed 1999, McDowell et al. 2005, Reed and Haver 2015). Its ability to effectively treat sewage is highly dependent on soil type and hydrology as well as maintenance (Cole and Firmage 1997). In Maine, residents are encouraged to pump their septic tanks every 2-3 years for year-round residences or 4-5 years for seasonal residences (O'Hara and Luoma 2015). However, there is no strict enforcement of these policies, and many septic systems have not been updated or maintained in over 20 years (Figure 3).

In assessing the presence and impact of PPCPs on the Belgrade Lakes, we must consider the possible sources. Due to the high prevalence of PPCPs in sewage effluent and infrequent maintenance of septic systems, their effluent provides a logical avenue for pollution into the watershed. Schaider et al. (2013), found that working septic systems were able to remove 99% of acetaminophen but less than 50% of carbamazepine. Additionally, leaking septic systems due to a lack of maintenance, improper installation, and overflow during storms can exacerbate the likelihood pollution (O'Hara and Luoma 2015). Phillips et al. (2015) detected numerous prescription pharmaceuticals, a floor cleaner, detergent degradation products, fragrances, insect repellent, and sunscreen additives downgradient[1] of septic systems in New York and New England.

In recent years, the Belgrade Lakes watershed has undergone residential and commercial development in response to a growing tourist population attracted to nature and outdoor recreation (Burgess and Nelson 2009). These population and development swells may impact the number of septic systems on the lake as well as the amount of effluent that is discharged. As a result, septic tanks may serve as an increasingly important source of PPCP pollution into the watershed.

---

[1] Down gradient is a term used to describe how groundwater flows under the ground,

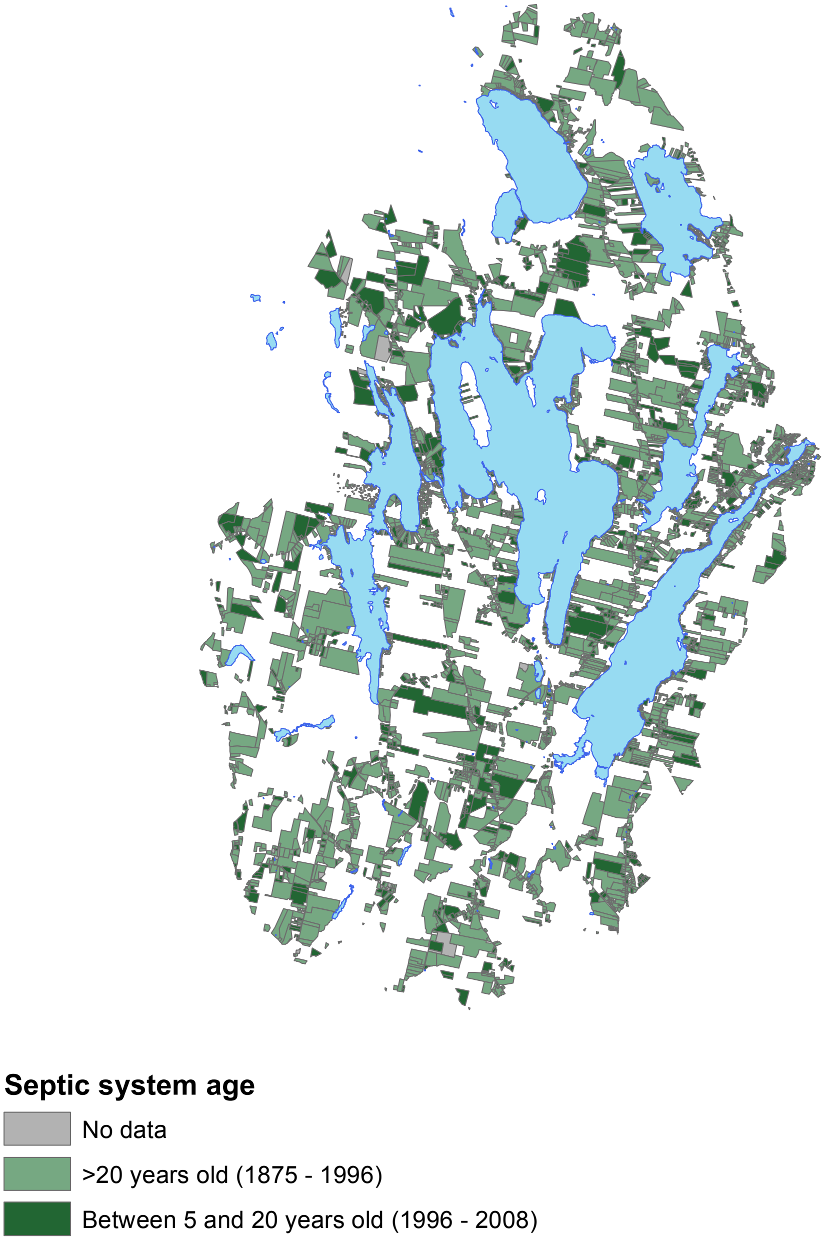


Figure 3. Septic systems coded by age in the Belgrade Watershed. Date refers to last documents septic permit (Data collected from McCullough 2010).

Twenty-Three Common PPCPs

A number of PPCPs are prevalent in aquatic ecosystems and may have important impacts on the environment and human health. This study focused on a pre-selected suite of 23 common PPCPs (Table 2). Below is a review of the current literature of each compound, its probable source, prevalence in aquatic ecosystems, and reported effects on the environment and human health. Most of the available data on prevalence focuses solely on sewage effluent or river water in densely populated areas. The literature primarily documents effects on aquatic organisms and humans at high, lethal levels. Few studies examine PPCP concentrations in more rural lakes, like those in the Belgrade watershed, or the effects on organisms at chronic, low level exposures.

Stimulants: Caffeine, 1,7-Dimethylxanthine, and Cotinine

Caffeine is one of the most widely consumed drugs in the world, present in coffee, tea, cocoa, and many pharmaceutical drugs for its stimulant and analgesic (pain-relieving) effects (Bruton et al. 2010a). Agricultural runoff and landfill leachate may also be important inputs (Figure 4) (Hollingsworth et al. 2003, Buszka et al. 2009, Bruton et al. 2010a). In humans, only a small fraction of caffeine is excreted as the unchanged molecule. Most is excreted as 1,7-dimethylxanthine (paraxanthine) (Vanderveen et al. 2001). In a report of 50 randomly selected Minnesota lakes and rivers, Ferrey (2015) found a maximum caffeine concentration of 0.067μg/L in some lakes.

Paraxanthine (1,7-dimethylxanthine) is one of the primary breakdown products of caffeine. In humans, paraxanthine has many of the same effects as caffeine including increased systolic blood pressure, plasma epinephrine levels, and free fatty acids (Benowitz et al. 1995). Recent studies using polar organic integrative samplers, a passive diffusion method, have detected concentrations of paraxanthine as high as 0.0234 μg/L upstream of a wastewater treatment plant to 0.0019 µg/L downstream in receiving bodies of water including rivers and creeks (Bartlet-Hunt et al. 2009). Driesen (2015) reported concentrations of 1672 µg/L in the wastewater from the Experimental Center of Carrión de los Céspedes in Seville, Spain. Caffeine has been shown to impact a number of freshwater species including water flea intoxication, brine shrimp mortality, and growth changes in the fathead minnow (Table 9) (Bruton et al. 2010a). In lentic biofilms, caffeine can stimulate gross primary production by 39% (Shaw et al. 2015). The impacts of 1,7-dimethylxanthine are not as well understood. One study reported an LC50 in the Cladocerans order (water fleas), the concentration required to kill 50% of the population, exceeding 100,000 µg/L (Fernández et al. 2010).

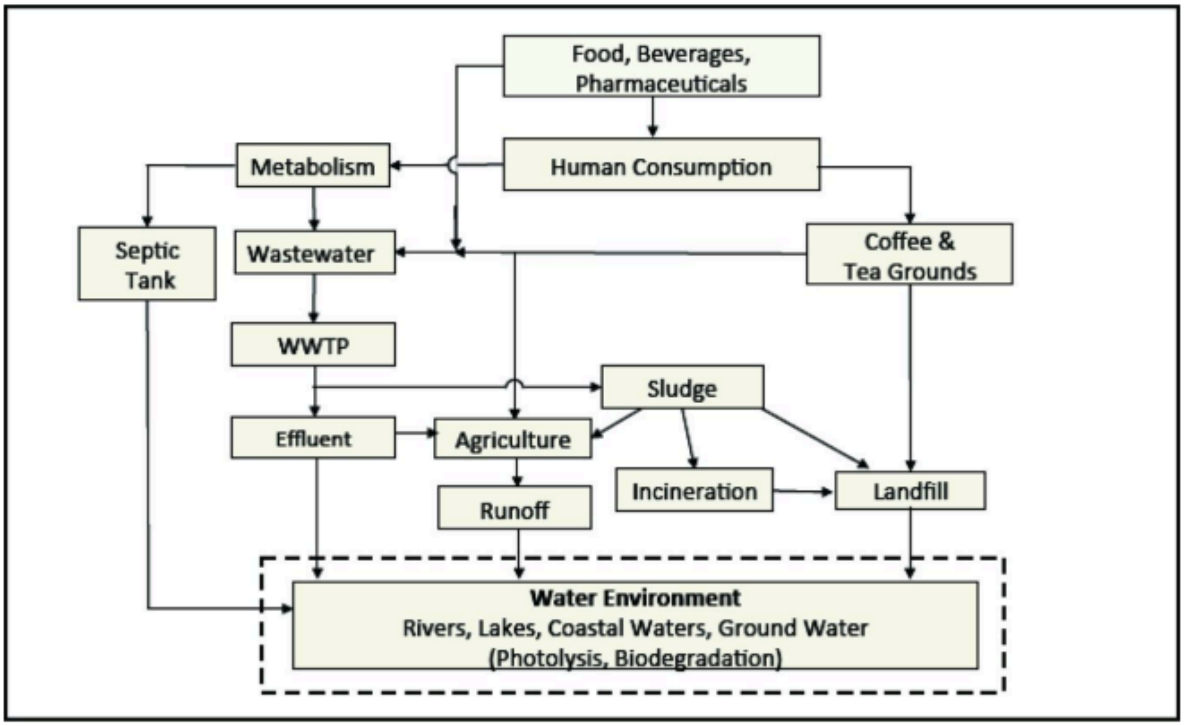


Figure 4. Caffeine routes into the environment (Bruton et al. 2010a).

Cotinine is a breakdown product of nicotine. In humans approximately 70-80% of nicotine is excreted as cotinine. Previous studies have found cotinine concentrations in lake waters as high as 0.0421 μg/L (Benowitz et al. 1995, Ferrey 2015). The effects of cotinine on aquatic organisms are not well understood. According to Crane et al. (2006), the effects of cotinine should be similar to those of nicotine, which include binding to nicotinic-Ach receptors, facilitating release of Ach, dopamine and glutamate neurotransmitters in the water flea, *Daphnia pulex*. Other researchers have linked cigarette butts in aquatic ecosystems to mortality in *Daphnia* species. Slaughter 2011 found an LC50 of one smoked cigarette butt per liter of water for *Atherinops affins*, marine topsmelt, and *Pimephales promelas,* fathead minnow.

Over-the-Counter Medications: Acetaminophen, Cimetidine, Diphenhydramine, Ibuprofen, and Naproxen

Acetaminophen is typically used for its analgesic and antipyretic (fever reducing) properties. Humans excrete less than 5% of acetaminophen unchanged, the rest is excreted as metabolites (Mazaleuskaya et al. 2015). Bartlet-Hunt et al. (2009) found acetaminophen in all river and creek samples except for one site upstream and one site downstream of a wastewater treatment plant. It was detected in all sewage effluent samples except for one. Acetaminophen concentrations downstream of a wastewater treatment plant reached 0.064 μg/L, compared to 0.0044 μg/L upstream (Bartlet-Hunt et al. 2009). At very high concentrations, acetaminophen can be lethal to the zooplankton *Daphnia magna* (Kim et al. 2010). Kim et al. (2007) found that in *Daphnia magna,* the EC50 (the concentration of a drug that elicits a half-maximal response) was 8200 μg/L after 96hours of exposure.

Cimetidine is typically used to treat peptic ulcer disease in patients with renal failure and gastro esophageal reflux disease, a condition in which a backflow of stomach acid causes heartburn and damages the esophagus (MedlinePlus 2010). In a study of 9 patients with normal renal function after a single intravenous dose of cimetidine, 47.3% was excreted unchanged (Larsson et al. 1982). In surface waters in the Han River in South Korea, cimetidine has been detected at levels as high as 5.38 μg/L (Choi et al. 2008), the highest concentration reported in the world as of 2008. According to Shaw et al. (2015), cimetidine can stimulate gross primary production in lentic biofilms by 46%. For *Daphnia magna,* the predicted no effect level concentration (PNEC)[2], or concentration below which there is no effect, is 35 μg/L (Buth et al. 2007).

Diphenhydramine is used as an antihistamine, antiemetic, sleep aid, sedative, and central nervous system depressant. In the human body, diphenhydramine breaks down in gastrointestinal tract to a number of metabolites. Humans only excrete 1.9% of diphenhydramine unchanged (Couper and Logan 2014). The Minnesota lakes report, published in 2015, tested for different PPCPs in 50 lakes in Minnesota in 2008 and 2013. They found that diphenhydramine reached levels of 0.0357 μg/L in 2008 (Ferrey 2015). In lentic biofilms, exposure to diphenhydramine can decrease primary production by 24% (Shaw et al. 2015). Xie et al. (2016) found that exposure to diphenhydramine at 21.7 μg/L caused enhanced swimming and decreased feeding rates in the crustacean *Carassius auratus.*

---

[2] PNECs are calculated using ECSAR Analysis, a computer program that predicts toxicity based on the known acute toxicity of similar compounds in various aquatic organisms (Buth et al. 2007).

Ibuprofen is one of the world's most widely consumed pharmaceuticals, used to treat pain, inflammation, and fevers. It is almost completely broken down in the body with little or no unchanged drug found in urine (Paíga et al. 2013, Mazaleuskaya et al. 2014). A study of 42 water samples from Portugal found ibuprofen in landfill leachates, wastewater treatment plant influents and effluents, hospital effluents, and surface waters. In the surface waters of the Lima River, researchers detected concentrations of 0.723µg/L. Paíga et al. (2013) attributed these high concentrations to the widespread consumption of ibuprofen among the Portuguese population, noting how wastewater treatment plants, landfills, and hospitals were importance sources of pollution. They also ran an environmental risk assessment on the concentration of ibuprofen leached from landfills, concluding that it does pose a significant ecotoxicological threat to aquatic organisms including fish, daphnids, and algae (Paíga et al. 2013). In a different study, *Daphnia magna* exposed to levels of ranging from 0.5 µg/L to 50 µg/L of Ibuprofen experienced a decrease in the total amount of eggs produced per female, total number of brood per female, and body length (Wang et al. 2016).

Naproxen is an over the counter medication used to relieve pain, tenderness, swelling, and stiffness caused by several different types of arthritis (MedlinePlus 2015b). Vree et al. (1993) recovered 50.8% of unchanged naproxen in human urine. Naproxen is stable in water for 14 days and can partially degrade in activated sludge (Qurie et al. 2014). Naproxen can be fatal at high concentrations. The plankton B. *calyciflorus* has an LC50 (24h), lethal dose at which 50% of the population is killed, of 6248 µg/L. *Daphnia magna* has an EC50 (48h), concentration of half-maximal response, of 1740 µg/L (Ornelas et al. 2010).

Prescription medications: Amphetamine, Carbamazepine, Gemfibrozil, Morphine, Phenazone, Sulfamethoxazole, Trimethoprim, and Warfarin

Amphetamine and amphetamine-type stimulants, a larger class of drugs, are psychoactive drugs that stimulate the central nervous system (De la Torre et al. 2004). Physicians prescribe d-amphetamine for the treatment of ADHD, narcolepsy, and as an appetite suppressant. Adverse effects include anorexia, weight loss, insomnia, and addition (Heal et al. 2013). The number of ADD/ADHD stimulant prescriptions, including amphetamine, has annually increased from 2007-2011 by 39%, from 34.8 million to 48.4 million prescriptions in the United States (NFLIS 2011). Amphetamine is also commonly used as a recreational drug for its psych-stimulant effects on the central nervous system (Heal et al. 2013). Amphetamine users experience increased alertness, wakefulness, insomnia, energy, self-confidence, decreased appetite, enhanced mood, well-being, and euphoria (De la Torre et al. 2004). According to the European Monitoring Center for Drugs and Drug Addition (2013), 12.4% of young adults aged 16-34 abused amphetamine in 2011-2012 in the UK. Methamphetamines may also be an important source of amphetamines, which is a metabolite (Barnes et al. 2008).

In the Minnesota lakes report, amphetamines were detected at a maximum concentration of 0.0291μg/L (Ferrey 2015). High levels of amphetamines have been shown to impact microbial communities by altering chemotaxic responses in certain bacteria, and stimulating behavioral changes, interfering with catecholamine production, photosynthesis, and nitrogen capabilities in aquatic algae (Chet et al. 1973, Rosi-Marshall et al. 2015). Although some effects in aquatic organisms have been described, there appear to be large data gaps regarding how environmentally relevant levels of amphetamines may be affecting aquatic ecosystems (Rosi-Marshall et al. 2015). Huerta-

Fontela et al. (2008) have demonstrated that amphetamines can be effectively treated through drinking water treatment processes.

Carbamazepine is an anticonvulsant for patients suffering from seizures (MedlinePlus 2012). More recently it has also been used to treat bipolar depression. Bertilsson and Tomson (1986) found that 90% of a single oral dose was excreted in urine in the form of metabolites. A screening of 27 different surface waters in Germany revealed concentrations of 0.05 μg/L to 3.2 μg/L. Highest reported concentrations were those found near wastewater treatment plants. In surface waters, carbamazepine degrades slowly by photo-degradation with a half-life of about 100 days (Bahlmann et al. 2009). Ecotoxicological studies have demonstrated that high carbamazepine can cause immobilization of *Daphnia magna,* which had an EC50 of 0.11 μg/L[3]. For humans, the predicted no observed effect level, generated using geo-referenced models P*h*ATE™, of carbamazepine through drinking water and fish consumption is 226,000 μg/L (Cunningham et al. 2010).

Gemfibrozil is a lipid regulating agent prescribed to patients undergoing diet changes to reduce their cholesterol or fat intake (MedlinePlus 2014, DailyMed 2015). Less than 2% is excreted unchanged in the urine (Citron Pharma LLC 2015). The maximum concentration detected in the Minnesota Lakes study was 0.00207μg/L (Ferrey 2015). Fang et al. (2012) detected gemfibrozil in influent, effluent, and groundwater. They noted that land application of sewage containing gemfibrozil, following treatment in a wastewater treatment plant, was a source of groundwater pollution. Studies of goldfish

---

[3] Converted from 475 μg/M using a molecular weight of carbamazepine of 236.27 g/mol (Kim et al. 2016b)

reported that gemfibrozil between 1.5 µg/L and 1,500 µg/L taken up from the water reduced plasma testosterone levels by 49-72% (Mimeault et al. 2005).

Morphine is an opiate prescribed for pain relief. Additionally, morphine is found in poppy seeds and is a metabolite of heroin (Boleda et al. 2009). In the human body, less than 10% is excreted unchanged (Buclin et al. 2009). Wastewater treatment plants can remove up to 73% of morphine from untreated sewage (Boleda et al. 2009). In one article, Zuccato and Castiglioni (2009) synthesized data on selected illicit drugs in surface waters all over the world. They found that morphine levels ranged from zero µg/L in Belgium to 0.010 µg/L in Germany. High levels of morphine have been shown to have a stimulating effect on certain fish such as *Macropodus opercularis,* resulting in erratic swimming and circling (Csanyi et al. 1984). Morphine has also been shown to reduce the phagocytic activity in mussel hemocytes, potentially weakening the immune system (Gagné et al. 2006). Little data exists on the ability of water treatment facilities to clear morphine.

Phenazone is an analgesic and antipyretic administered as mouth and eardrops ("Chemical: Drug antipyrine" 2016). In humans, approximately 3.3% is excreted unchanged with the rest breaking down into 4-hydroxy-antipyrine, norantipyrine, 3-hydroxymethl-antipyrine, and 3-carboxy-antipyrine (Danhof and Breimer 1979). Reddersen et al. (2002) routinely detected phenazone in groundwater samples in Berlin, Germany at 3 µg/L, suspected to have originated from a nearby pharmaceutical plant. High levels of phenazone did not have any acute effects on fish, daphnia, or algae, but chronic effects are still unknown. Redderson et al. (2002) also found that the treatment process at the local water treatment plant was able to effectively remove 90% of phenazone from the drinking water. The last 10% remaining in drinking water posed no toxicological threat for humans at such low concentrations (Reddersen et al. 2002). Methodology for toxilogical threat assessment was not discussed.

Sulfamethoxazole and trimethoprim are used to treat bacterial infections in humans. Physicians prescribe them individually or together in a drug called Sulfatrim (PharmGKB 2015). Sulfamethoxazole can also be used as an antibiotic agent for animals. Cribb and Speilberg (1992) found that humans excrete 54% of ingested sulfamethoxazole unchanged. In source and finished water sites from the Scioto River Basin in Ohio, sulfamethoxazole was detected in 16 samples at levels below 0.005 µg/L (Finnegan et al. 2010). Humans metabolize trimethoprim, another antibiotic, and excrete 80% unchanged. The Minnesota lakes report found trimethoprim at a maximum concentration of 0.00175 µg/L in 2013 (Ferrey 2015). Liguoro et al*.* (2012) found that high levels of trimethoprim caused growth inhibition in *Lemna minor*, swimming activity inhibition in *Poecilia reticulata*, and reproduction and growth inhibition in *Daphnia magna*. However, researchers concluded that environmental concentrations below 1 µg/L are unable to evoke appreciable biological effects in various aquatic organisms (De Liguoro et al. 2012).

Warfarin is an anticoagulant, commonly administered to patients with deep vein thrombosis, atrial fibrillation, and recurrent stroke or heart valve prosthesis. Less than 1% is excreted unchanged in the urine and none is found in the faces (Merad 1988). Carmona et al. (2014) detected warfarin in wastewater treatment effluent, surface water, and drinking water in the Turia River basin in Spain. They cited septic systems, domestic solid wastes, wastewater treatment plants, commercial-industrial discharges, and animal agriculture as possible source of warfarin pollution into these bodies of water. In surface water, warfarin levels reached 0.015 µg/L, which was consistent with levels detected in other surface waters in Spain. Little data from either toxicity or QSAR studies regarding the effects of warfarin on aquatic organisms. In regards to human health, Carmona et al. (2014) also noted low level warfarin contamination in mineral and drinking waters, posing a possible threat to human health.

Recreational Drugs: MDA, MDMA, and Methamphetamine

MDA (Methylenedioxyamphetamine) is a psycho-stimulant that can be consumed directly as a drug of abuse or result from the metabolism of MDMA (Huerta-Fontela et al. 2008, Medline Plus 2016). Zuccato and Castiglioni (2009) found MDA in concentrations ranging from zero μg/L in Belgium, Germany, and Ireland, to 0.010 μg/L in the Llobregat River in Spain. According to Huerta-Fontela (2014), MDA, along with other amphetamine-type stimulants, were removed through conventional water treatment processes. The effects of MDA have not been thoroughly researched and large data gaps remain regarding aquatic biota and human health (Huerta-Fontela et al. 2008).

MDMA (methylenedioxyamphetamine, also known as ecstasy) is another psycho-stimulant. Humans excrete about 15% of MDMA unchanged (Abraham et al. 2009). Zuccato and Castiglioni (2009) found concentrations ranging from 0.0011μg/L in Italian rivers to 0.003μg/L in Spain's Llobregat River. MDMA has also been detected in drinking water sources in Spain. Huerta-Fontela (2008) found that MDMA is not effectively removed during regular water treatment. This may raise some concern for human health via drinking water. A large data gap exists for the effects of MDMA on aquatic organisms and humans at all levels of exposure.

The last recreational drug is methamphetamine. Recently, headlines have described the discovery of methamphetamine laboratories all over the state of Maine, including the Belgrade watershed (Burns 2015). Methamphetamine is a psycho-stimulant and a sympathomimetic[4] drug. Researchers have found that humans excrete 40-50% of methamphetamines unchanged (Toxnet 2016). In the Zuccato and Castiglioni (2009) study, methamphetamines were detected at low levels around 0.0001 μg/L in surface waters in Spain and Italy. Researchers have demonstrated that biological mechanisms are the predominant method for degradation of methamphetamine, which is photo-stable (Bagnall et al. 2013). Methamphetamine at levels of 45 μg/L to 450 μg/L[5] has also been shown to enhance memory in *Lymnaea stagnalis,* a pond snail, in laboratory experiments (Kennedy et al. 2010). It is unknown water treatment facilities can remove methamphetamines from water.

Agricultural or Veterinary Chemicals: Sulfamethazine, Sulfrachlorpyridazine, and Thiabendazole

Sulfamethazine is an antimicrobial and antibacterial agent used in veterinary medicine. Sulfamethazine is typically excreted in the urine as a combination of the unchanged compound and several metabolites (Bevill et al. 1977). It is not used in human medication. Manured fields are a point source of pollution for sulfamethazine in surface waters (Hirsch et al. 1999). According to Carstens et al. (2013)*,* sulfamethazine has a 2.7-day half-life in pond water, broken down by photodegredation and sorption to sediment. Carstens et al. (2013) found sulfamethazine in 26 out of 52 surface water samples at levels as high as 0.48 μg/L. Another study found that *Daphnia magna* had a NOEC, no observed effect level, of 3,300 μg/L. Concentrations exceeding this caused growth

---

[4] Sympathomimetic drugs produce physiological effects similar to those caused by the activity or stimulation of the sympathetic nervous system.

[5] Converted from 0.3 μmol^{-1} and 3.3 μmol^{-1} using a molar mass for methamphetamine, 150g/mol.

inhibition, immobilization, and reproductive problems after exposure from 96 hours to 7 days (Ji et al. 2012).

Sulfrachlorpyridazine is a broad-spectrum sulfonamide antibiotic used in swine and cattle industries. In a study of 20 river waters samples from River Trent at Shardlow, Derbyshire, UK, no sulfrachlorpyridazine was found (Blackwell et al. 2004). Few literature sources describe sulfrachlorpyridazine in surface waters or their effects on aquatic ecosystems or human health. Only one study explored the presence of sulfrachlorpyridazine in seafood and found that exposure to 0.020µg/L had a 91.2% recovery rate (Gehring et al. 2006).

Thiabendazole is a fungicide and parasiticide primarily used in veterinary medicine and agriculture. In humans, little thiabendazole is excreted in either urine or feces following metabolism. Runoff is a likely source. In surface waters in the Suerte River Basin in Costa Rica, nearby several banana plantations, researchers reported a range of 1 µg/L to 3 µg/L (Castillo et al. 2000). In Trenton, New Jersey, a more urban environment, researchers found concentrations of thiabendazole below 0.0011 µg/L in sewage effluents (Albrecht and Franco-Paredes 2014, Kim et al. 2016a). In a review of thiabendazole as a potential seed treatment, Moore et al. (2006) noted that thiabendazole is persistent and immobile in aquatic environments. Its only mode for degradation is photolysis. Moore et al. (2006) also reported that rainbow trout and bluegill sunfish had a NOAEC of 12 µg/L and Daphnids had an EC50 310 µg/L. Thiabendazole was shown to interfere with growth and reproduction of these organisms. According to the US EPA (2014), data gaps exist for the effects on aquatic plants (Moore et al. 2006). The US EPA Re-registration Eligibility Decision (RED) (2002) concluded that the presence of thiabendazole in food or drinking water does not pose a threat to humans.

Other compounds: Triclosan

Triclosan is a common antibacterial agent, has been found in soaps, has been detected in river water, groundwater, sediments, biota samples of fish, and human breast milk (Adolfsson-Erici et al. 2002, Banerjee et al. 2016). In the Minnesota lakes report, triclosan was detected at a maximum concentration of 0.00575µg/L. High levels of triclosan have also been shown to cause sub-lethal effects in certain fish including jaw locking, quiescence, and erratic swimming movements, which can significantly affect their ability to obtain food and evade predators (Orvos et al. 2002, Fritsch E. Werner I., Davies R., Beggeli S., Feng W., Pessah I. 2013). Triclosan is also a significant environmental source of dioxins, which are unintentional byproducts of organochlorines manufacturing that have carcinogenic and endocrine-disrupting properties (Ferrey 2015).

Project Goals

This project aims to examine for the first time whether the Belgrade Lakes are contaminated with PCPPs. We will measure of a suite of 23 different PPCPs (Table 2) in three Belgrade Lakes, model how PPCP concentrations relate to other stressors in aquatic ecosystems, such as nutrient loading from septic systems, and to characterize possible threats to public health, either through drinking water or recreation. We will determine if these compounds are present, what concentrations they are in, and if there is any variation between the lakes. Based on the toxicity of the compounds present, we will assess if there may be any concern for the possible impacts on health of aquatic ecosystems or humans. We hypothesize that lakes with higher septic system and cesspool density and low residence time or flushing rate will have the highest rates of PPCP concentrations.

Table 2. The 23 compounds tested for using liquid chromatography-gas spectrometry, positive ion detection and their common uses. An asterisk denotes negative ion detection.

Chemical name	Common Uses
STIMULANTS AND METABOLITES	
Caffeine	Stimulant
1,7-dimethylxanthine	Caffeine metabolite
Cotinine	Cigarettes
OVER THE COUNTER MEDICATIONS	
Acetaminophen	Pain and fever
Cimetidine	Ulcers, GERD
Diphenhydramine	Hay fever, allergies, common cold
Ibuprofen*	Pain, tenderness, swelling, stiffness
Naproxen*	Pain, tenderness, swelling, stiffness
PRESCRIPTION MEDICATION	
Amphetamine	ADD, Narcolepsy
Carbamazepine	Seizures and epilepsy
Gemfibrozil*	Reduce cholesterol and triglycerides
Morphine	Severe pain
Phenazone	Ear pain and swelling
Sulfamethoxazole	Antibiotic
Trimethoprim	Antibiotic
Warfarin*	Prevent blood clots
RECREATIONAL DRUGS	
MDA	Psychoactive drug
MDMA	Psychoactive drug
Methamphetamine	Psychoactive drug
AGRICULTURAL OR VETRINARY COMPOUNDS	
Sulfamethazine	Antibacterial agent for farm animals
Sulfrachlorpyridazine	Antibiotic for swine and cattle
Thiabendazole	Fungicide
OTHER DRUGS	
Triclosan*	Antibacterial agent

Study Sites

East Pond, Great Pond, and Long Pond, three lakes within the Belgrade watershed, have recently undergone high development density and increasing year-round use of lake homes (Burgess and Nelson 2009). East Pond covers 1,823 acres and has an average depth of 18m. Great Pond covers 8239 acres and has an average depth of 21m (Michael H. 1996). Long Pond covers 1334 acres in the northern basin and 1334 acres in the southern basin. Long Pond has an average depth of 8m (Table 3).

Table 3. General characteristics of the Belgrade Lakes (McCullough 2010)

Lake	Watershed Area (ha)	Lake Surface Area (ha)	Mean depth (m)	Volume (m^3)	Flushing rate (flushes/year)
East Pond	1060	677	5.48	33,848,120	0.29
Great Pond	21471	3313	6.40	209,160,000	0.52
Long Pond	6232	1043	10.67	81,113,391	3.70

MATERIALS AND METHODS

Sample Collection

We collected samples at 14 different sites, selected to provide a spatially diverse sampling of each lake, while taking into account property access, due to the large number of private properties in the region. Distance between sampling sites was also considered to ensure that sites were far enough apart to be considered independent measures of PPCP compound concentrations within a lake. The smallest distance between sampling sites was approximately 840 m. Collection occurred on December 11, 2015 and December 14, 2015. We sampled a total of five sites on East Pond, six on Great Pond, and three on Long Pond. We collected samples anywhere from 2 ft. to 40 ft. offshore where the water was approximately 1 m deep (Figure 5). We collected samples in either new, pre-cleaned, amber glass bottles or reused amber glass bottles rinsed with methanol (Appendices 1, 2). During collection, we rinsed each bottle three times with lake water and then filled them by inverting the bottle into the water about 6 inches beneath the surface. After collection, we stored the samples in a cooler until returning them to the laboratory, where we refrigerated them at 2 C degrees until extraction.

Solid Phase Extraction

Like many other researchers testing PPCPs in environmental waters, I performed on-site solid phase extraction (SPE) to prepare the samples for liquid chromatography-mass spectrometry (LC-MS) three to six days following collection (Roberts and Thomas 2006, Paíga et al. 2013, Papageorgiou et al. 2016). Using vacuum filtration, I ran the samples through a 25mm GMF (glass microfiber, 1μm nominal pore size) filter into an SPE cartridge. Prior to each run, I washed the system with 5-10mL of methanol followed by 5-10mL of deionized water. I ran the samples at an average flow rate of 4.26mL/min

(Appendix 1). Afterwards, I removed the cartridges and refrigerated them at approximately 2 degrees C.

Cartridge Processing

Following solid phase extraction, I shipped the samples to the Director of Laboratory Services, Dan Snow at the Water for Food Laboratories at the University of Nebraska for liquid chromatography-mass spectrometry analysis followed by positive and negative ion detection for a suite of 23 different compounds common in PPCPs (Table 2).

A Suite of 23 compounds Commonly Found in PPCPs

The 23 different compounds tested for are found in a wide variety of PPCPs and are frequently detected in surface waters. Director Snow tested water samples for three stimulants (caffeine, 1,7-dimethylxanthine, and cotinine), five over-the-counter medications (acetaminophen, diphenhydramine, cimetidine, naproxen, and ibuprofen), three agricultural or veterinary chemicals (sulfamethazine, sulfrachlorpyridazine, thiabendazole), eight prescription medications (sulfamethoxazole, trimethoprim, carbamazepine, d-Amphetamine, morphine, phenazone, gemfibrozil, and warfarin), three recreational drugs (MDMA, MDA and methamphetamine), and one other drug (triclosan) (Table 2).

Data Analysis

I used RStudio version 0.99.484 to analyze the data (RStudio 2015). First, I compared concentrations of the 23 PPCP compounds among lakes (East Pond, Great Pond, and Long Pond) using a one-way ANOVA (Analysis of Variance) followed by a Tukey HSD test. A Bonferroni corrected alpha value of 0.0016 was used to determine significance. I then generated a plot of the concentrations of each compound detected against the other

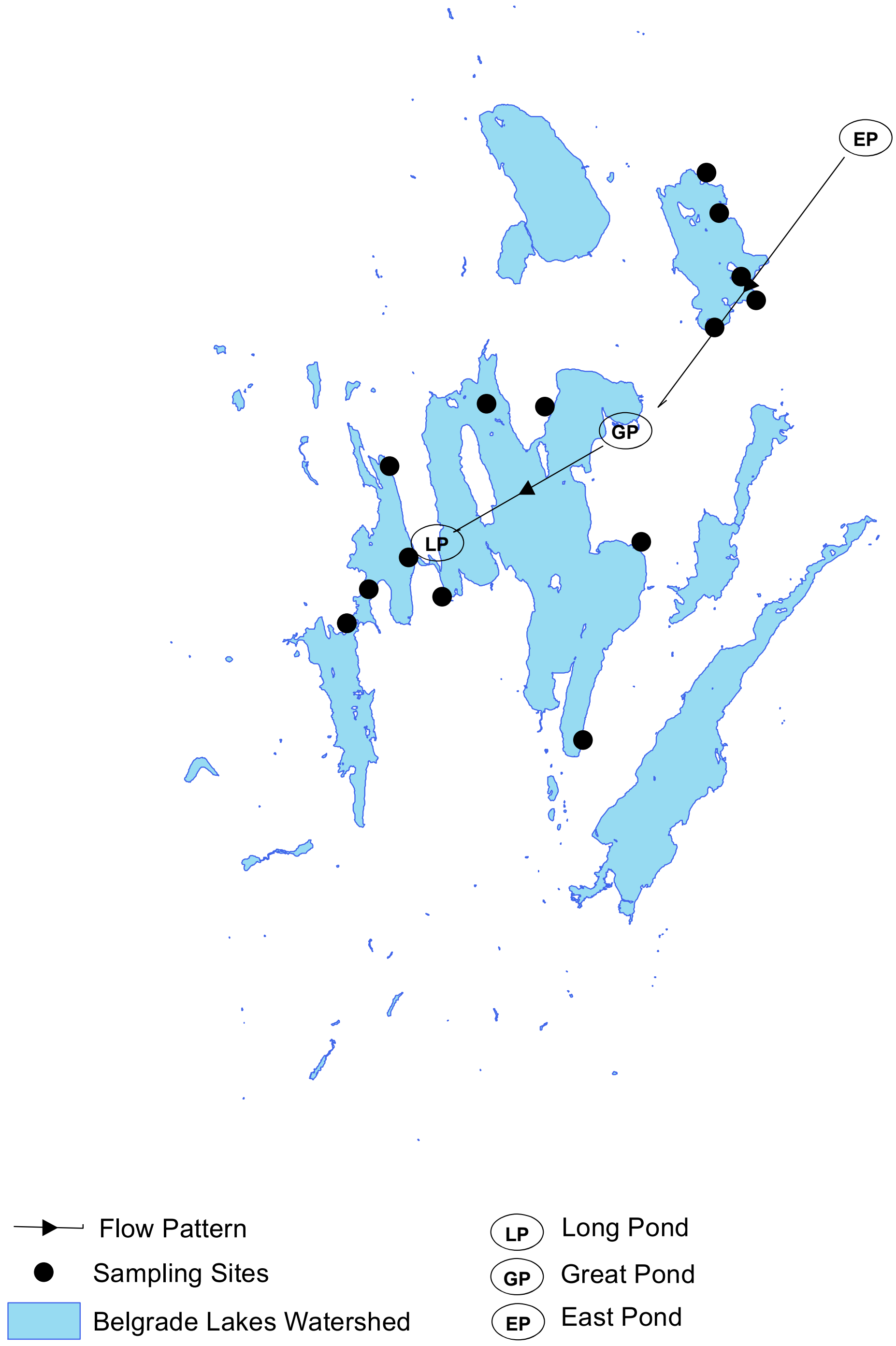


Figure 5. Sampling sites and flow path for the Belgrade Watershed generated using ArcGIS. (Belgrade lakes shape file from McCullough 2010, sampling site coordinates from GoogleMaps 2016).

compounds (caffeine and 1,7-dimethylxanthine, caffeine and amphetamine, 1,7-dimethylxanthine and amphetamine) using Microsoft Excel 14.6.2. To quantify the linear relationships observed in the plots, I then ran a linear regression for each pair in RStudio and recorded the adjusted r^2 and p-value. I also generated a correlation matrix using the Pearson correlation, another measure of linear relationships, for the three detected compounds.

In order to help isolate hotspots for PPCPs within the watershed, I also classified each sample site as a year round residence, seasonal residence, or a public boat launch. I generated a bar chart of the concentration of each detected compound at each sample site color coded by land use. Next, I then ran a one-way ANOVA comparing a given compound concentration with the land use classification. I also generated a bar chart of for the average concentrations for all 3 lakes organized by land use type with error bars to denote significant differences.

RESULTS

In our 14 samples from East Pond, Great Pond, and Long Pond, we detected 3 of 23 compounds above the detection limit: caffeine, 1,7-diethmethylxanthine (a caffeine metabolite), and amphetamine (Figure 6, Table 4). Caffeine was detected in all 14 samples and with a maximum concentration of 0.021 μg/L and an average concentration of 0.006 μg/L. We detected 1,7-dimethyxanthine in 8 out of 14 samples, with the highest concentration at 0.013 μg/L and an average concentration of 0.003 μg/L. Lastly, we detected amphetamine in all 14 samples with a maximum concentration of 0.0100 μg/L and an average concentration of 0.003 μg/L. We also detected cotinine at one site at the detection limit of 0.002 μg/L. However, since the average amount of cotinine within the samples was below the detection limit, it was not included in the following analyses. The other 19 of the 23 compounds that we tested for yielded nonzero results well below detection limit (Figure 6).

The one-way ANOVA of the each detected compound among East Pond, Great Pond, and Long Pond revealed that there was no significant difference in concentrations of caffeine, 1,7-dimethylxanthine, or amphetamine between the three lakes. All p-values exceeded the Bonferroni corrected alpha value of 0.0016 (Table 5). Further analysis comparing the physical (lake size and flushing rate) or anthropogenic characteristics (septic system prevalence and age) among East Pond, Great Pond and Long Pond was not preformed (Figure 3, Table 3).

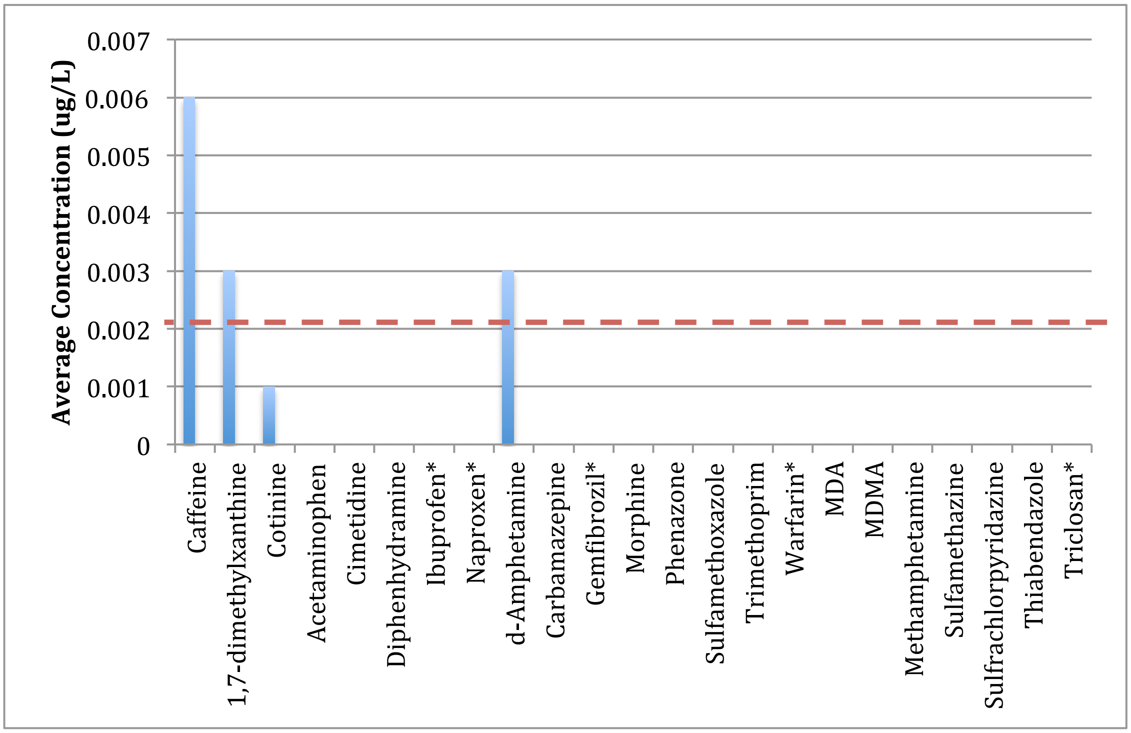


Figure 6. Average concentrations of target compounds. The red line indicates the detection limit for the positive ion analysis, 0.002 µg/L. The asterisk (*) signifies compounds detected using the negative ion analysis, which has a detection limit of 0.005 µg/L (n=14 samples).

Table 4. Watershed wide average and maximum concentrations for the 4 detected compounds in East Pond, Great Pond, and Long Pond. All concentrations are in µg/L. 'Samples' denotes the number of samples that each compound was detected in.

	Caffeine	1,7-Dimethylxanthine	Cotinine	Amphetamine
Average	0.006	0.0025	0.002	0.003
Maximum	0.021	0.013	0.002	0.01
Samples (n=14)	14	8	1	14

Table 5. Tukey HSD p-values between lakes for compounds with concentrations above the detection limit. All p-value exceed 0.0016, the Bonferroni corrected level of significance.

Compound	GP-EP	LP-EP	LP-GP
1,7-dimethylxanthine	0.461	0.887	0.839
Caffeine	0.693	0.624	0.961
Amphetamine	0.728	0.169	0.393

Plots of the concentrations of each compound detected against the other in conjunction with a linear regression analysis indicated a linear trend for each pair of compounds (Figure 7, 8, 9). I found an adjusted r^2 value of 0.8737 with p-value $5.973*10^{-7}$ for caffeine and 1,7-dimethylxanthine (Figure 7), an adjusted r^2 value of $7.06*10^{-7}$ with a p-value $2.152*10^{-5}$ for caffeine and amphetamine (Figure 8), and an adjusted r^2 value of 0.5988 with a p-value 0.000706 for 1, 7-dimethylxanthine and amphetamine (Figure 9). All p-values were below the accepted level of significance (p=0.05). The relationship between caffeine and its metabolite 1,7-dimethylxanthine was the strongest. The correlation matrix further supports the linear relationship between all three compounds (Table 6).

Land use categorization and spatial distribution of these three compounds revealed that the highest concentrations were detected at two of the three boat launch sites tested and at one seasonal residence (Figure 10, 11, 12). An ANOVA of the land use categorization with each compound indicated that the differences for caffeine and 1,7-dimethylxanthine were insignificant and the difference for amphetamines was marginally significant (Table 7).

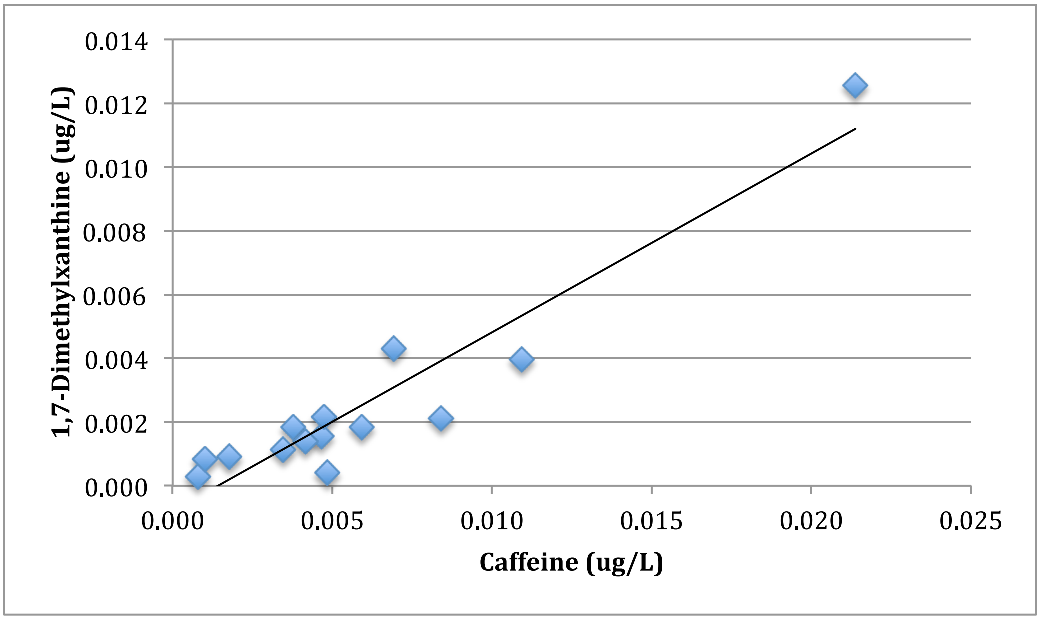


Figure 7. Linear relationship between Caffeine and 1,7-Dimethylxanthine, a metabolite of caffeine (Adjusted r^2=0.8737, p-value $5.973*10^{-7}$).

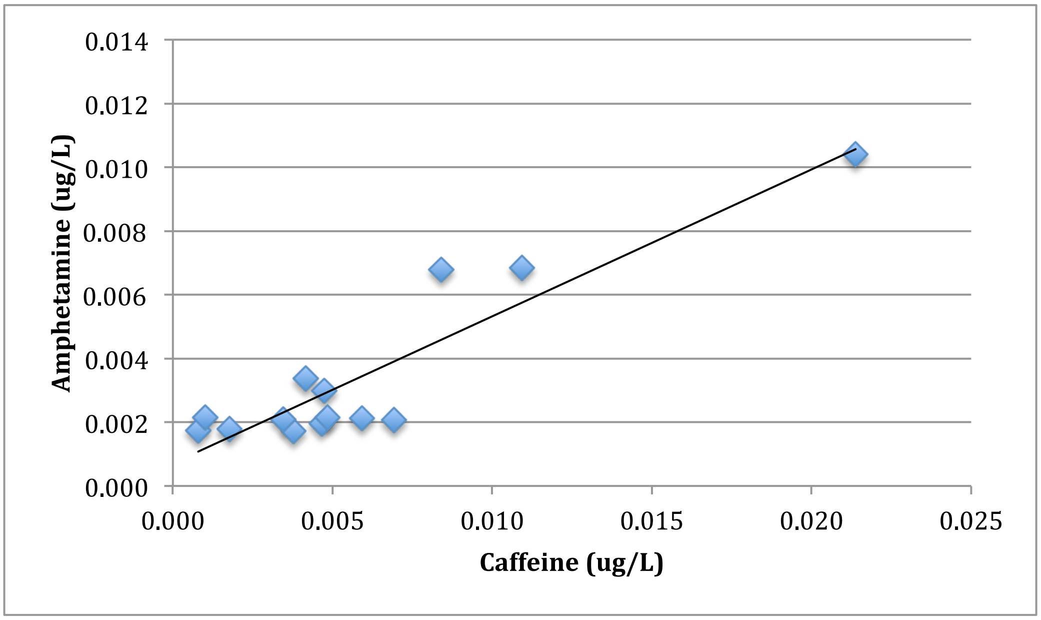


Figure 8. Linear relationship between caffeine and amphetamine. (Adjusted r^2 value = 0.7722, p-value $2.152*10^{-5}$).

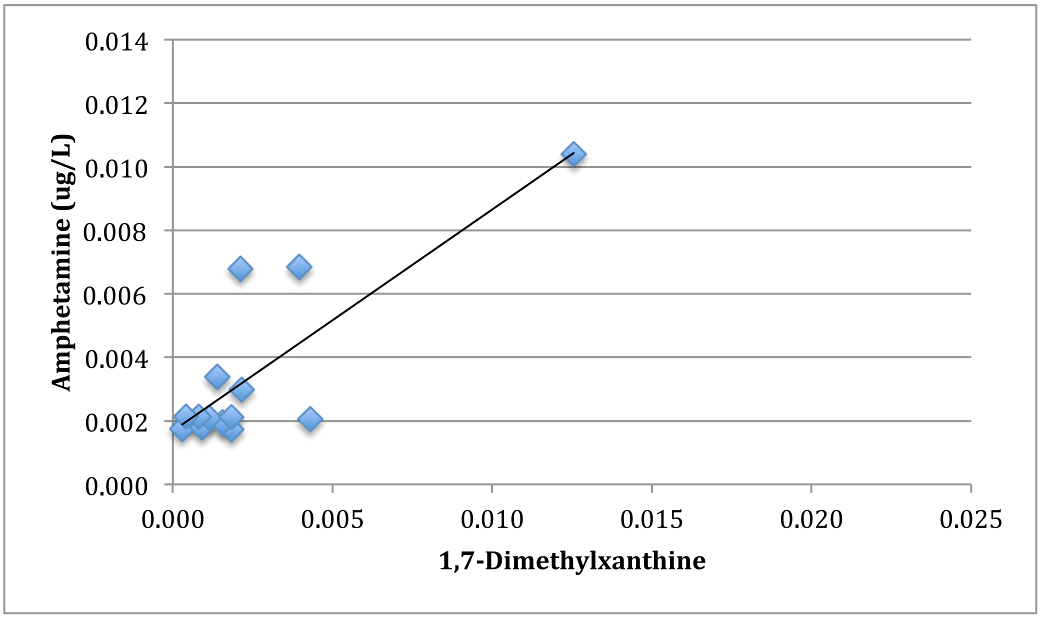


Figure 9. Linear relationship between 1,7-Dimethylxanthine and amphetamine (Adjusted $r^2 = 0.5988$. p-value $7.06*10^{-4}$).

Table 6. Correlation matrix calculated using Pearson's correlation coefficient.

	Caffeine	1,7-Dimethylxanthine	Amphetamine
Caffeine	1.00	0.940	0.889
1,7-Dimethylxanthine	0.940	1.00	0.793
Amphetamine	0.889	0.793	1.00

An average of the concentrations from all sites categorized by land use for each compound supported this trend of higher PPCP concentrations at boat launches than both year round or seasonal residences. Error bars denoting standard deviation also highlight that in our small sample pool, this difference is not significant (Figure 13).

Table 7. Results of one-way ANOVA for each compound and land use. All values exceed the standard level of significance, alpha = 0.05.

Compound	P-value
Caffeine	0.15
1,7-Dimethylxanthine	0.165
Amphetamine	0.0627

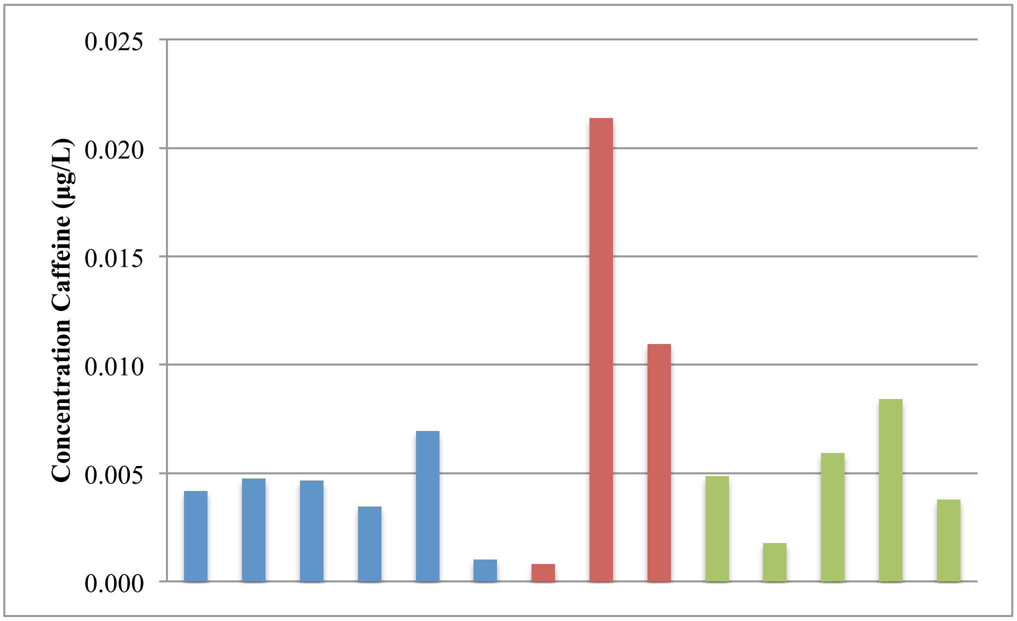


Figure 10. Concentration of caffeine at each sample site categorized by land use type. Blue signifies year round residences, red signifies public boat launches, and green signifies seasonal residences.

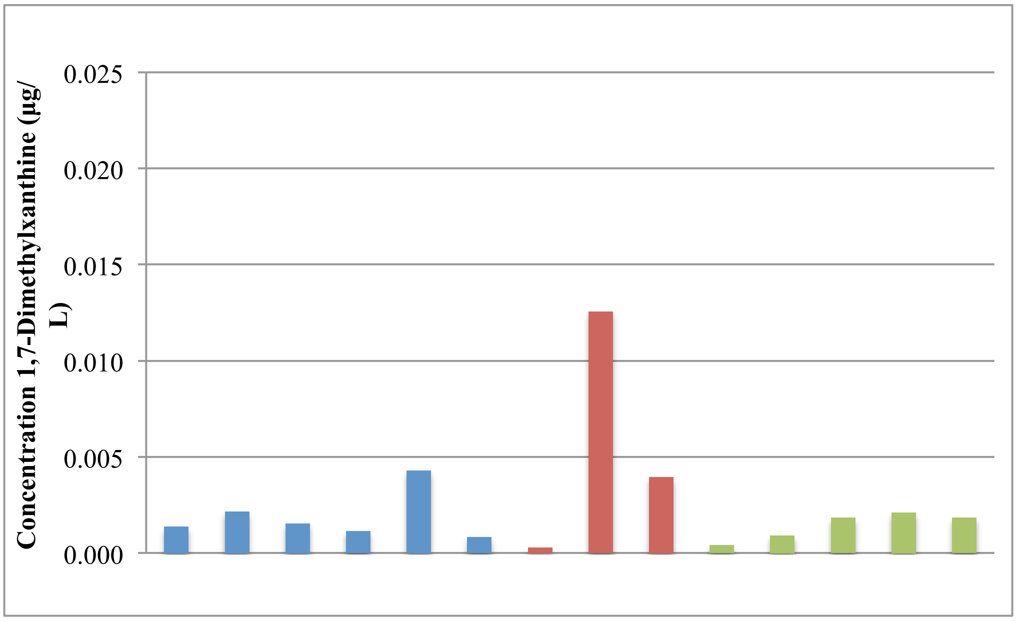


Figure 11. Concentration of 1,7-dimethylxanthine at each sample site categorized by land use type. Blue signifies year round residences, red signifies public boat launches, and green signifies seasonal residences.

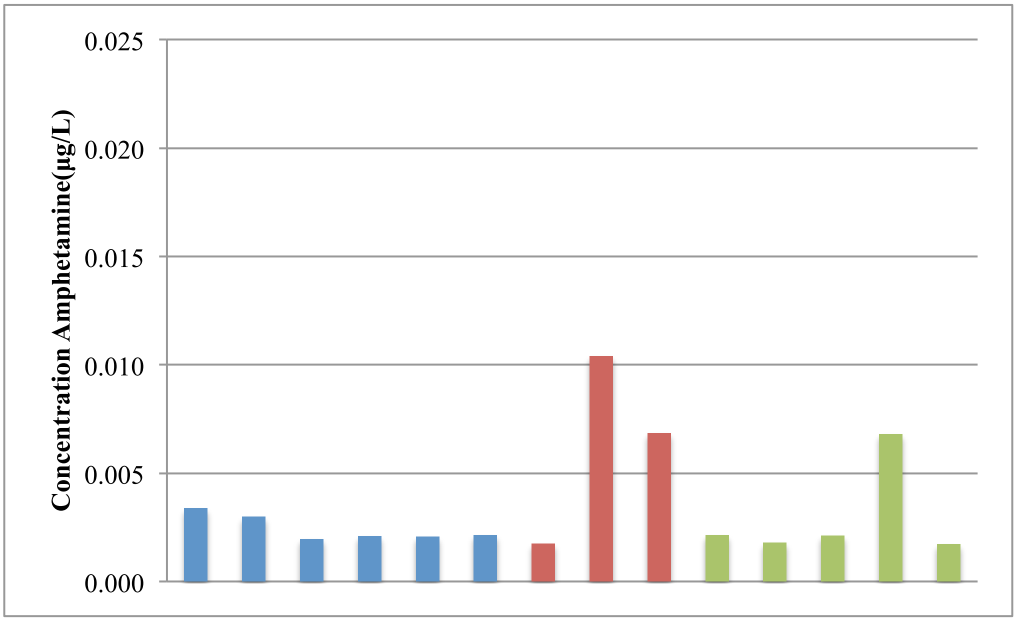


Figure 12. Concentration of amphetamines at each sample site categorized by land use type. Blue signifies year round residences, red signifies public boat launches, and green signifies seasonal residences.

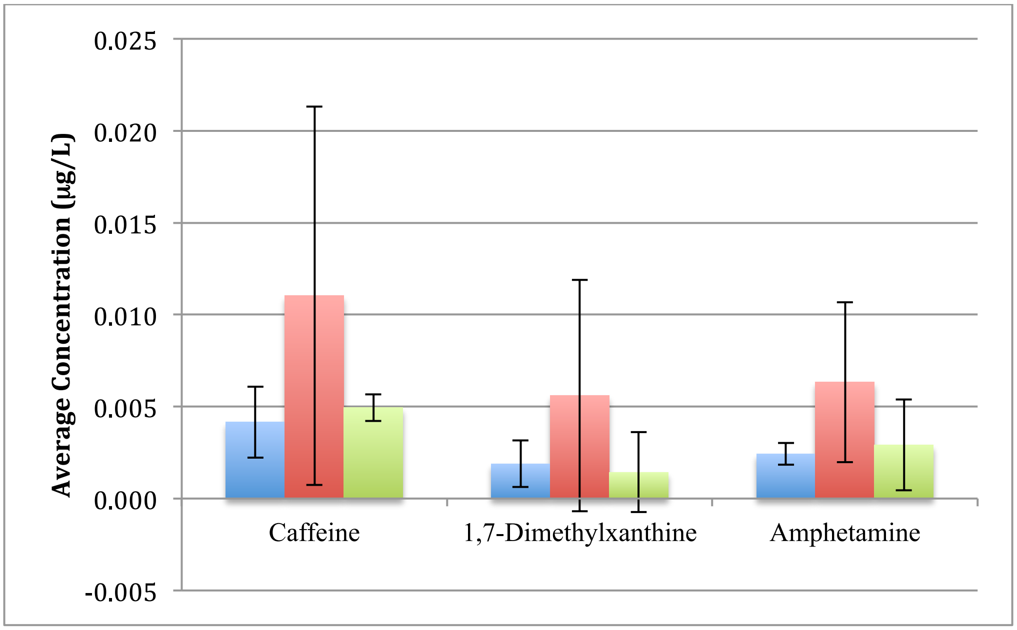


Figure 13. Average concentrations of each compound for all sites, organized by land use type. Blue signifies year round residences, red signifies public boat launches, and green signifies seasonal residences.

DISCUSSION

Of the 23 compounds that we tested for, only 3 were above the detection limit and one was close. The other 19 compounds were nonzero but well below the detection limit. All 23 compounds have been previously detected in either sewage water effluent, surface waters, or river waters (Koplin et al. 2002, Zuccato and Castiglioni 2009, Ferrey 2015, Fairbairn et al. 2016). The low concentrations and lack of discrete PPCPs in the Belgrade Watershed may be attributed to its distance from commonly cited sources such as municipal wastewater treatment plants, urban centers, or large industrial or agricultural sites (Buerge et al. 2006, Carmona et al. 2014). The nearest wastewater treatment plant to any of our sample sites is about 10 miles away (GoogleMaps 2016).

As sewage effluent from wastewater treatment plants was reported to be a significant driver of PPCP concentrations in more urban watersheds, we hypothesized that septic systems may be an important source of pollution into the Belgrade watershed. Despite the prevalence and age of shoreline septic systems in the region, the number of discrete PPCPs in the Belgrade watershed is much lower than those detected in more urban environments or nearby wastewater treatment plants (Figure 3). Of the 11 lakes explicitly mentioned in the Minnesota lakes report, six were noted to have septic input rather than an urban sewer system. These lakes have comparable numbers of unique PPCPs to those found in the Belgrade watershed, but higher concentrations of certain compounds (Table 8) (Ferrey 2015). Unlike Ferrey et al. (2015), we did not detect diphenhydramine or triclosan in any of our samples.

Interestingly, these low numbers of unique PPCPs are not universal among septic system areas. According to Loraine et al. (2009), who studied parts of the Colorado River severely impacted by septic systems, they detected esters, sunscreens, clofibrate, clofribic acid, ibuprofen, triclosan and diethyltoluamide (DEET). Some of the variation between these septic influenced watersheds may be attributed to varying consumption patterns throughout the United States. Lentic and lotic ecosystems may also contribute to these discrepancies.

Table 8. Lakes categorized with septic input of PPCPs and the concentrations of compounds in our suite of 23 chemicals present in each lake from the Minnesota lakes report on PPCP prevalence in comparison to our Belgrade Lakes results. Ferrey et al. (2015) reported concentrations in 2008 and 2013. The highest concentration is recorded here (Amphet = Amphetamine). Maximum concentrations are also recorded for the Belgrade watershed. Since the Minnesota lakes report did not test for 1,7-Dimethylxanthine, it was omitted from this table.

Lake	Caffeine	Amphet.	Cotinine	Diphenhydramine	Triclosan
Belgrade	0.021	0.003	-	-	-
White Sand	-	-	6.9	-	-
Red Sand	-	-	3.6	14	5.8
Sullivan	-	-	6.1	-	-
Stewart	<15.2	-	1.83	-	-
Shingobee	<15	2.91	-	35.7	-
Kabetogama	-	-	-	-	-

Caffeine

Although caffeine is relatively well metabolized in the human body, with only about 0.5-10% excreted in urine, its prevalence in our samples is not surprising (Ferreira 2005). As with other PPCPs, the primary mode of input into the environment is sewage, a result of excreted caffeine through urine or feces as well as from unconsumed caffeinated products from washing machines, dirty dishes, or coffee and tea appliances (Figure 13). According to Buerge et al. (2006), 16,000 μg/person day^{-1} of caffeine ends up in raw wastewater. Its widespread consumption in conjunction with its high loads in wastewater can help explain their ubiquitous prevalence in the Belgrade Lakes and other aquatic ecosystems (Ferreira 2005, Buerge et al. 2006, Benotti et al. 2009, Sun et al. 2016).

Recently, researchers have used caffeine and its metabolites as long-term tracers of municipal wastewater into lacustrine (lake) systems (Benotti et al. 2009). Edward et al. (2015) noted that caffeine's insignificant volatility, water solubility due to its low octanol-water coefficient, and long-half life, between 10 and 20,000 years, make it a good chemical marker of anthropogenic pollution. Wastewater treatment influents contained levels as high as 72 μg/L (Buerge et al. 2003). Edward et al. (2015) reported caffeine concentrations up to 6.8 μg/L, with an average concentration of 2.0 μg/L in surface waters in Barbados, West Indies (Buerge et al. 2006, Edwards et al. 2015). In Swiss Lakes and rivers, Buerge et al. (2003) detected caffeine in almost every sample, at concentrations ranging from 0.006 μg/L to 650 μg/L. Remote mountain lakes were the only exception with concentrations below 0.002 μg/L. Compared to these studies, the Belgrade Lakes concentrations are closest to those detected in remote mountain lakes (Table 9).

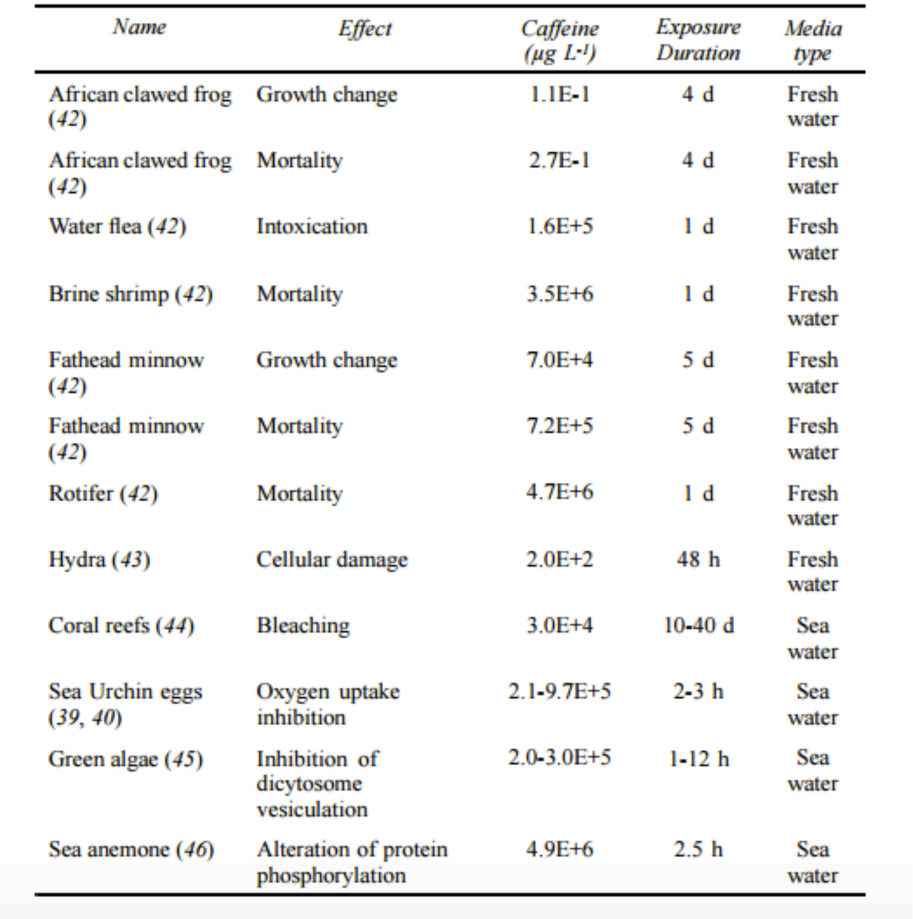
Table 9. Comparison of caffeine concentrations in the Belgrade Lakes watershed to other bodies of water around the world.

	Concentration (μg/L)	Type of water	Source
Maine	Avg: 0.006, Max: 0.021	Lakes	This study
West Indies	Avg: 2, Max: 6.8	Surface water	Edward et al. 2015
Switzerland	Range: 0.006 to 650	Lakes	Buerge et al. 2003
Switzerland	<2	Remote lakes	Buerge et al. 2003
Switzerland	Range: 7 to 73	WWTP Effluent	Buerge et al. 2003

However, its presence in all 14 samples and its long half-life due to slow photochemical degradation merits consideration of the possible aquatic and human health effects (Figure 14) (Buerge et al. 2006). Moore et al. (2008) studied the responses of *Ceriodaphnia dubia*, *Pimephales promelas,* and *Chironomus dilutes*, three common freshwater organisms found in lakes, to caffeine. After exposing each organism to various concentrations of caffeine from zero µg/L to 200,000 µg/L, they observed acute responses including impaired reproduction and growth after 48-hours. Moore et al. (2008) concluded that environmental concentrations of caffeine pose a negligible risk to aquatic vertebrate and invertebrate organism. In a different study, Aguirre-Martínez et al. (2015) found that exposure to caffeine at 0.10 µg/L to 50 µg/L over the course of 14 days caused general stress and changes in certain biomarkers in *C. fluminea,* more commonly known as the Asian clam. Bruton et al. (2010) also described a number of effects on aquatic organisms in both freshwater and sea water ecosystems (Table 10). The levels we found in the Belgrade watershed were well below those shown to have a significant effect on aquatic biota.

Caffeine has also been shown to impact aquatic ecosystems on a microbial level (Shaw et al. 2015). Gibson et al. (2009) performed aquarium experiments looking at the effects of caffeine on *Pseudomonas*, a bacterium commonly found in aquatic habitats. In response to caffeine, they observed increased growth, bacterial colony count, and the development of a bio-film like sheen on the glass of the experimental aquarium. Gibson et al. (2009) also observed that increased ammonia concentrations correlated with the metabolic activity of the bacteria, which is highly toxic to fish. Shaw et al. (2015) found that caffeine stimulated gross primary production by 39% in algal biomass after 21 days in agar with $3.73*10^5$ μg/L[6]. However, researchers concluded that these changes in the microbial community do not significantly impact the ecosystem as a whole (Moore et al. 2008, Rosi-Marshall et al. 2015).

Table 10. Some effects of caffeine in aquatic species (Bruton et al. 2010a).



Name	*Effect*	*Caffeine* $(\mu g\ L^{-1})$	*Exposure Duration*	*Media type*
African clawed frog (*42*)	Growth change	1.1E-1	4 d	Fresh water
African clawed frog (*42*)	Mortality	2.7E-1	4 d	Fresh water
Water flea (*42*)	Intoxication	1.6E+5	1 d	Fresh water
Brine shrimp (*42*)	Mortality	3.5E+6	1 d	Fresh water
Fathead minnow (*42*)	Growth change	7.0E+4	5 d	Fresh water
Fathead minnow (*42*)	Mortality	7.2E+5	5 d	Fresh water
Rotifer (*42*)	Mortality	4.7E+6	1 d	Fresh water
Hydra (*43*)	Cellular damage	2.0E+2	48 h	Fresh water
Coral reefs (*44*)	Bleaching	3.0E+4	10-40 d	Sea water
Sea Urchin eggs (*39, 40*)	Oxygen uptake inhibition	2.1-9.7E+5	2-3 h	Sea water
Green algae (*45*)	Inhibition of dicytosome vesiculation	2.0-3.0E+5	1-12 h	Sea water
Sea anemone (*46*)	Alteration of protein phosphorylation	4.9E+6	2.5 h	Sea water

In humans caffeine is typically consumed as a socially acceptable stimulant. Some studies have identified adverse human health such as general toxicity and cardiovascular

---

[6] Conversion from 2.5mM using a molar mass for caffeine of 149g

effects from consuming caffeine products. These effects are only associated with levels above a moderate consumption of $4.0 * 10^5$ μg/day (Nawrot et al. 2003). As a result, low concentrations of caffeine in the Belgrade watershed through drinking water or recreation do not likely pose a direct threat to human health. In regards to indirect effects, pathogenic bacteria may raise some concern. Linden et al. (2015) found that there was weak correlation between the caffeine and fecal coliforms in the Sinos River watershed in Brazil. However, other researchers have not been able to correlate the two (Jagoda et al. 2015).

These studies suggest that caffeine at the levels detected in the East Pond, Great Pond, and Long Pond pose a negligible acute risk to the environment and human health. However, most researchers focus on high levels of contamination. Little is understood about chronic exposure to low levels of caffeine in aquatic ecosystems. Efforts should be made to reduce caffeine inputs into the Belgrade Lakes on the basis of the precautionary principle, reducing the risk of potential harm from widespread caffeine even without a complete scientific basis.

1,7-Dimethylxanthine (Paraxanthine)

The compound 1,7-dimethylxanthine, also known as paraxanthine, is one of the primary breakdown products of caffeine. The high consumption of caffeinated consumer products and the fact that caffeine is well metabolized in the body can help explain its prevalence in the Belgrade watershed. About 80% to 84% of caffeine undergoes demethylation into 1,7-dimethylxanthine (Fernández et al. 2010, Thorn et al. 2012, Driesen 2015). Paraxanthine is extremely prevalent in sewage effluent. Fernández et al. (2010) found ubiquitous paraxanthine at concentrations ranging from 0.0003 μg/L 0.0278 μg/L in the Henares-Jarama-Tajo River, one of the most densely populated areas of Spain, lined with wastewater treatment plants. Interestingly, the concentrations detected in the Belgrade lakes paralleled these concentrations. In regards to aquatic organisms, one pharmokinetics study reported that the metabolism of paraxanthine in tilapia is concentration-dependent (Gómez-Martínez 2011). Like caffeine, responses in humans are seen on a magnitude of mg/kg (Benowitz et al. 1995). It is unlikely that paraxanthine at these concentrations has any direct influence on human health.

Amphetamine

Unlike caffeine, amphetamine is not pervasive in our consumer products. Rather, in the United States, amphetamine is a "Controlled Drug," prescribed only for the treatment of ADHD, narcolepsy, and as an appetite suppressant. Amphetamine is also a recreational drug and a breakdown product of methamphetamine. As a result, it is surprising that among all of the prescription and over the counter medications we tested, many of which are much easier to obtain, we only found significant levels of amphetamine in Belgrade watershed. In 2001, the National Drug Intelligence Center reported that methamphetamine is not a significant threat in the Maine (National Drug Intelligence Center 2001). As of 2013, 1 in 20 Maine high school students reported using a stimulant like methamphetamine, which includes amphetamine. From 2011 to 2012, the number of reported methamphetamine laboratory related incidents increased from 6 to 13 in the state of Maine, two of which were in Kennebec County (Maine MethWatch 2013).

Researchers have found that humans excrete 40% to 50% of methamphetamines unchanged and only 4% to 7% as amphetamine (Toxnet 2016). In our study, we did not detect any significant amounts of methamphetamine and did not test for phydroxymethamphetamine. The small percentage of excreted amphetamine from methamphetamine usage leads me to believe that amphetamine presence in the Belgrade Lakes reflects excretion of prescription drugs or direct amphetamine abuse rather than methamphetamine abuse. However, it is also possible that methamphetamines break down more quickly in the soil or aquatic environments than amphetamine. The only paper that I was able to find regarding this topic described the persistence of methylamphetamine sulfate in soils, which does not break down (Janusz et al. 2003). Currently, little data exists about the mobility or breakdown products of methamphetamines in the soil or water.

As with other PPCPs, amphetamines can enter the watershed through human excretion directly into the lakes, through sewage effluent, or dumping (Pal et al. 2013). There is no regulation of amphetamine or other illicit drugs in wastewater, surface waters, drinking water or the atmosphere (Pal et al. 2013). Despite efforts to control amphetamine usage by the public, studies around the world have also detected amphetamines in surface waters. In the Llobregat River in Spain, amphetamine was found at 0.009 μg/L. In the UK, in the Taff and Ely, concentrations reached 0.0035 μg/L (Zuccato and Castiglioni 2009). In the Minnesota lakes report, researchers detected a maximum concentration of 0.00291 μg/L (Ferrey 2015). Levels detected in the Belgrade watershed exceeded some more urban surface waters, but remain much lower than levels detected in river water near wastewater treatment plants effluent (Table 11) (Huerta-Fontela et al. 2008).

From our results, we cannot distinguish between amphetamines from prescription drugs or from illegal use, through methamphetamine or amphetamine abuse. Additionally, there is no readily available data about the consumption patterns for amphetamine or demographics specific to the Belgrade Watershed (NCBDD 2016). ADD is a common disorder in both children and adults, so prescription drug use cannot be ruled out (Faraone 2005). More research combining data from local hospitals about prescription medications containing amphetamines or from police records documenting arrests for amphetamine abuse may help explain our data.

Table 11. Comparison of the Belgrade Lakes concentrations of amphetamines to other bodies of water around the world.

Lake	Max concentration (μg/L)	Source
Belgrade Lakes	0.003	This study
Llobregat River (Spain)	0.009	Zuccato et al. 2009
Taff and Ely River (UK)	0.0035	Zuccato et al. 2009
Minnesota	0.00291	Ferrey 2015
Llobregat River near WWTPs	$6.5*10^8$	Huerta-Fontela et al. 2008

In regards to human health, amphetamines in raw water can be eliminated through a drinking water treatment consisting of pre-chlorination, coagulation/flocculation, sand filtration, ozonation, GAC filtration and post-chlorination (Huerta-Fontela et al. 2008). However, the concentrations and effects of PPCPs in well water in the Belgrade watershed are unknown. Adverse health effects due to recreation containing levels on the μg/L level of amphetamines are also not well understood. In addition to possible toxicity in aquatic biota and drinking water, amphetamine presence can also serve as a useful tool in assessing drug usage in the region. After detecting amphetamine, methamphetamine, and MDMA, in wastewater, Nowicki et al. (2014) used sewage epidemiology to estimate the level of drug consumption in Poznán, Poland. They found that levels of consumption and concentration in wastewater were lower than other reported parts of Europe.

Patterns in PPCP Concentrations

The linear relationships between each pair of compounds detected demonstrated a strong linear trend line in all three cases, caffeine and paraxanthine, caffeine and amphetamine, and paraxanthine and amphetamine (Figure 3, 4, 5, Table 6). This indicates that some parts of the lake had higher concentrations than other parts of the lakes, either due to a common source or a hotspot for bioaccumulation. Classification of each sample site by land use, year round residence, public boat launch, and seasonal residence (Figure 10, 11, 12) revealed that the highest concentrations were at two of the three public boat launches. While analyzing the land use results, it is important to keep in mind that our sample size was severely limited with only three public boat launches, six year-round residences, and five seasonal residences. Despite the insignificant figures, the fact that two of the three boat launches had the highest overall concentrations of PPCPs is worthy of consideration.

There are two potential explanations for these high levels. The first is relative neighborhood density. A relative observational comparison of the neighborhood while sampling noted that the two highest public boat launches had high and moderate surrounding camp density. The third boat launch located on Castle Island in Rome, ME, with much lower concentrations, appeared to be slightly more isolated from its neighbors. More neighbors could indicate more leaking septic systems causing the increased PPCP pollution. However, in our samples, we also found that the concentrations at these boat launches exceed those at year-round and seasonal residence with septic systems greater than 20 years old. As a result, septic systems may not be the predominant input. Recent preliminary research on East Pond focused on septic systems and nutrient loading suggest that septic systems may not be a predominant source of pollution (Reed and Haver 2015).

Like our study, Reed and Haver (2015) was also limited by sample size and seasonal variation. A closer analysis of nearby land usage, updated septic system density, and distance to each sampling site from possible sources in conjunction with more sampling sites could help elucidate a clearer pattern.

The other possible explanation is that septic systems are not a point source of pollution for PPCPs. Rather the high prevalence of input at boat launches, which lack septic systems, is attributable to visitor usage and direct defecation or dumping from the boat launch. Documentation of boat launch uses and human traffic would help discern if PPCPs detected in this study are more likely the result of defecating or dumping PPCPs directly into the lakes or from septic system leakage.

The next highest concentration was found at a year round private residence. Compared to the other private residences, both year-round and seasonal residences, this property did not appear to have higher camp density or a significant amount of visitors. The PPCPs present at this site may be the result of defecation by the homeowners or the movement of certain compounds in the watershed. An overlay of shoreline household and septic system density as well as a greater understanding about the fates of PPCPs in the watershed would aid in interpreting these results.

Conclusion and Future Directions

This project has demonstrated that certain PPCPs are present at detectable concentrations throughout East Pond, Great Pond, and Long Pond. The levels of caffeine and its metabolite are much lower than more urban areas or areas closer to wastewater treatment facilities. At these low levels, caffeine and its metabolite are not likely a significant threat to the aquatic ecosystem or human health at this time. However, the concentration of amphetamines matches those found in more populated bodies of water.

A greater understanding of the effects due to chronic exposure of PPCPs at these environmental levels is vital to understanding their impact in the Belgrade Lakes (Jones et al. 2004).

Testing the tissues of freshwater mussels in the Belgrade Lakes may provide some insight into PPCP bioaccumulation in the lakes over time. Since freshwater mussels are filter feeders, PPCPs can bio-accumulate in the their tissues. An assessment of PPCPs in the tissues of freshwater mussels in conjunction with their age could elucidate important temporal trends. Future research should also explore seasonal variation. The Belgrade Lakes area experiences significant population fluxes between summer and winter seasons. Researchers have found that PPCP concentrations, including caffeine and amphetamines, can vary greatly between seasons, from weekday to weekend, winter to summer, and even if there is a large event drawing tourists (Buerge et al. 2006, Edwards et al. 2015). In the Belgrade Lakes, July Fourth may be a peak weekend for study. Our findings regarding the widespread prevalence of significant levels of amphetamine reveal an interesting public health phenomenon and raise questions about drug usage, both legal and illegal, around the lakes. A closer examination of prescribed medications containing amphetamine could help illuminate the type of amphetamine entering the watershed to better direct preventative PPCP pollution efforts.

The data collected throughout the course of this thesis project provides preliminary results of PPCP concentrations in the Belgrade Lakes, documenting a few select sample sites at a single point in time. The literature cited here demonstrates the novelty of this field and highlights the lack of general understanding of the fate and effects of PPCPs in the aquatic environment. The presence of caffeine, 1,7-dimethylxanthine, and amphetamine indicate that PPCPs are entering the Belgrade Lakes watershed demonstrate the importance for more holistic research, with larger sample sizes and seasonal variability.

APPENDICIES

Appendix 1. Summary table of collection details.

Sample	New bottle=1, old=0	Distance from shore (ft.)	Approximate depth (m)	Land use (PBL=public boat launch)
EP1	0	15	1	Year round
EP2	1	10	1	Year round
EP3	1	20	1	PBL
EP4	1	10	1	Seasonal
EP5	1	8	1	Seasonal
GP1	1	15	1	Year round
GP2	1	10	1	Year round
GP3	0	20	1	Seasonal
GP4	1	30	1	Year round
GP5	1	1	1	Seasonal
GP6	1	40	1	PBL
LP1	1	2	1	Year round
LP2	1	10	1	Seasonal
LP3	1	10	1	PBL

Appendix 2. Continuation of summary table of collection details.

Sample	Substrate descript	Date
EP1	Soft sediment with boulders	12/11/15
EP2	Soft sediment with boulders	12/11/15
EP3	Sandy beach	12/11/15
EP4	Sandy substrate, course gravel/cobble, w/ intake pipe?	12/11/15
EP5	Rocky, private metal grate boat launch	12/11/15
GP1	Course gravel, cobble beach	12/11/15
GP2	Sand, gravel, cobble	12/11/15
GP3	Concrete slab, fine sand	12/11/15
GP4	Shallow	12/11/15
GP5	Not recorded	12/11/15
GP6	Soft sediment, shallow	12/14/15
LP1	Rip rap, protected point, cobble/boulders, steep drop off, lots of algae on rock surface	12/14/15
LP2	Sand, gravel, cobble	12/14/15
LP3	Some rocks	12/14/15

LITERATURE CITED

Abraham, T. T., A. J. Barnes, R. H. Lowe, E. A. Kolbrich Spargo, G. Milman, S. O. Pirnay, D. A. Gorelick, R. S. Goodwin, and M. A. Huestis. 2009. Urinary MDMA, MDA, HMMA, and HMA excretion following controlled MDMA administration to humans. Journal of analytical toxicology 33:439–46.

Adolfsson-Erici, M., M. Pettersson, J. Parkkonen, and J. Sturve. 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere 46:1485–1489.

Albrecht, H., and C. Franco-Paredes. 2014. Cutaneous Larva Migrans - Infectious Disease and Antimicrobial Agents. http://www.antimicrobe.org/b08.asp#top.

Alexy, R., T. Kümpel, and K. Kümmerer. 2004. Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57:505–12.

Bagnall, J., L. Malia, A. Lubben, and B. Kasprzyk-Hordern. 2013. Stereoselective biodegradation of amphetamine and methamphetamine in river microcosms. Water research 47:5708–18.

Bahlmann, A., M. G. Weller, U. Panne, and R. J. Schneider. 2009. Monitoring carbamazepine in surface and wastewaters by an immunoassay based on a monoclonal antibody. Analytical and bioanalytical chemistry 395:1809–20.

Banerjee, P., T. K. Dey, S. Sarkar, S. Swarnakar, A. Mukhopadhyay, and S. Ghosh. 2016. Treatment of cosmetic effluent in different configurations of ceramic UF membrane based bioreactor: Toxicity evaluation of the untreated and treated wastewater using catfish (Heteropneustes fossilis). Chemosphere 146:133–44.

Barnes, A. J., M. L. Smith, S. L. Kacinko, E. W. Schwilke, E. J. Cone, E. T. Moolchan, and M. A. Huestis. 2008. Excretion of methamphetamine and amphetamine in human sweat following controlled oral methamphetamine administration. Clinical chemistry 54:172–80.

Bartlet-Hunt, S., T. Damon, J. Schokley, K. Hoagland, and D. Snow. 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environmental Pollution 157:786–791.

Benotti, M., R. Trenholm, B. Vanderford, J. Holady, B. Standforn, and S. Snyder. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. Environ. Sci. Technol. 43:597–603.

Benowitz, N. L., P. Jacob, H. Mayan, and C. Denaro. 1995. Sympathomimetic effects of paraxanthine and caffeine in humans. Clinical pharmacology and therapeutics 58:684–91.

Bevill, R. F., R. M. Sharma, S. H. Meachum, S. C. Wozniak, D. W. Bourne, and L. W. Dittert. 1977. Disposition of sulfonamides in food-producing animals: concentrations of sulfamethazine and its metabolites in plasma, urine, and tissues of lambs following intravenous administration. American journal of veterinary research 38:973–7.

Blackwell, P. A., H.-C. H. Lützhøft, H.-P. Ma, B. Halling-Sørensen, A. B. . Boxall, and P. Kay. 2004. Fast and robust simultaneous determination of three veterinary antibiotics in groundwater and surface water using a tandem solid-phase extraction with high-performance liquid chromatography–UV detection. Journal of Chromatography A 1045:111–117.

Boleda, M. A. R., M. A. T. Galceran, and F. Ventura. 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water research 43:1126–36.

Bowles, A. 2010. Abstracts of presentations. Colby College Undergraduate Research Symposium. Digital Commons @ Colby, Colby College.

Boxall, A. B. A., M. A. Rudd, B. W. Brooks, D. J. Caldwell, K. Choi, S. Hickmann, E. Innes, K. Ostapyk, J. P. Staveley, T. Verslycke, G. T. Ankley, K. F. Beazley, S. E. Belanger, J. P. Berninger, P. Carriquiriborde, A. Coors, P. C. DeLeo, S. D. Dyer, J. F. Ericson, F. Gagné, J. P. Giesy, T. Gouin, L. Hallstrom, M. V. Karlsson, D. G. J. Larsson, J. M. Lazorchak, F. Mastrocco, A. McLaughlin, M. E. McMaster, R. D.

Meyerhoff, R. Moore, J. L. Parrott, J. R. Snape, R. Murray-Smith, M. R. Servos, P. K. Sibley, J. O. Straub, N. D. Szabo, E. Topp, G. R. Tetreault, V. L. Trudeau, and G. Van Der Kraak. 2012. Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? Environmental Health Perspectives 120:1221–1229.

Bruton, T., A. Alboloushi, B. de la Garza, B.-O. Kim, and R. U. Halden. 2010a. Fate of Caffeine in the Environment and Ecotoxicological Considerations. Pages 257–273.

Bruton, T., A. Alboloushi, B. de la Garza, B.-O. Kim, and R. U. Halden. 2010b. Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations. (R. U. Halden, Ed.). American Chemical Society, Washington, DC.

Buclin, T., M. Nicod, S. Kellenberger, K. Martin, and M. Guidoux. 2009. Morphine. http://sepia.unil.ch/pharmacology/index.php?id=85.

Buerge, I. I., T. Poiger, M. D. Müller, and H.-R. Buser. 2003. Caffeine, an anthropogenic marker for wastewater comtamination of surface waters. Environmental science & technology 37:691–700.

Buerge, I. J., T. Poiger, M. D. Müller, and H.-R. Buser. 2006. Combined Sewer Overflows to Surface Waters Detected by the Anthropogenic Marker Caffeine. Environmental Science & Technology 40:4096–4102.

Burgess, I., and M. Nelson. 2009. Belgrade Lakes Maine: The history of a summer community and its effect on the enviornment. http://belgradelakesassociation.org/Portals/0/PDFs/General/Belgrade Lakes History1.pdf.

Burns, C. 2015. Meth in Maine: A decade of thwarting supply but little to show for it | BDN Maine. http://bangordailynews.com/2015/04/09/the-point/meth-in-maine-a-decade-of-thwarting-supply-but-little-to-show-for-it/.

Buszka, P. M., D. J. Yeskis, D. W. Kolpin, E. T. Furlong, S. D. Zaugg, and M. T. Meyer. 2009. Waste-indicator and pharmaceutical compounds in landfill-leachate-affected ground water near Elkhart, Indiana, 2000–2002. Bulletin of Environmental Contamination and Toxicology 82:653–659.

Buth, J. M., W. A. Arnold, and K. McNeill. 2007. Unexpected Products and Reaction Mechanisms of the Aqueous Chlorination of Cimetidine. Environmental Science & Technology 41:6228–6233.

Caldwell, D. J. 2015. Pharmaceuticals in the Environment. (R. E. Hester and R. M. Harrison, Eds.) Issues in Environmental Science and Technology. Royal Society of Chemistry, Cambridge.

Carmona, E., V. Andreu, and Y. Picó. 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water. The Science of the total environment 484:53–63.

Carrera, C., W. Robertson, D. Blowes, M. Moncur, E. Sverko, S. Backus, and C. Ptacek. 2008. Fate of pharmaceutical and trace organic compounds in three septic plumes, Ontario, Canada. Environ. Sci. Technol. 42:2805–2811.

Carstens, K. L., A. D. Gross, T. B. Moorman, and J. R. Coats. 2013. Sorption and photodegradation processes govern distribution and fate of sulfamethazine in freshwater-sediment microcosms. Environmental science & technology 47:10877–83.

Castillo, L. E., C. Ruepert, and E. Solis. 2000. Pesticide residues in the aquatic environment of banana plantation areas in the North Atlantic Zone of Costa Rica. Environmental Toxicology and Chemistry 19:1942–1950.

Chemical: Drug antipyrine. 2016. . https://www.pharmgkb.org/chemical/PA448453#tabview=tab1&subtab=31.

Chet, I., Y. Henis, and R. Mitchell. 1973. Effect of Biogenic Amines and Cannabinoids on Bacterial Chemotaxis. J. Bacteriol. 115:1215–1218.

Choi, K., Y. Kim, J. Park, C. K. Park, M. Kim, H. S. Kim, and P. Kim. 2008. Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. The Science of the total environment 405:120–8.

Citron Pharma LLC. 2015. Gemfibrozil. http://www.drugs.com/pro/gemfibrozil.html.

Cole, R., and D. Firmage. 1997. Land use patterns in relation to lake water quality in the North Pond watershed. Waterville, ME.

Couper, F., and B. Logan. 2014. Drugs and Human Performance FACT SHEETS - Diphenhydramine. http://www.nhtsa.gov/people/injury/research/job185drugs/diphenhydramine.htm.

Crain, D., T. Iguchi, S. Jobling, H. Laufer, G. LeBlanc, L. Guillette, and M. Eriksen. 2007. An ecological assessment of bisphenol-A: evidence from comparative biology. Reproductive toxicology 24:225–239.

Csanyi, V., A. Doka, C. Castellano, S. Puglisi-Allegra, and A. Oliverio. 1984. An ethopharmacological analysis of morphine effects in fish (Macropodus Opercularis). Behavioural processes 9:315–21.

Cunningham, V. L., C. Perino, V. J. D'Aco, A. Hartmann, and R. Bechter. 2010. Human health risk assessment of carbamazepine in surface waters of North America and Europe. Regulatory toxicology and pharmacology : RTP 56:343–51.

DailyMed. 2015. Gemfibrozil. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=a68f430e-7c98-4bd9-8902-758706713996.

Damstra, T. 2002. Potential Effects of Certain Persistent Organic Pollutants and Endocrine Disrupting Chemicals on the Health of Children. Journal of Toxicology: Clinical Toxicology 40:457–465.

Danhof, M., and D. D. Breimer. 1979. Studies on the different metabolic pathways of antipyrine in man. I. Oral administration of 250, 500 and 1000 mg to healthy volunteers. British journal of clinical pharmacology 8:529–37.

Daughton, C. G., and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environmental health perspectives 107 Suppl :907–38.

DEP, M. 2013. Lakes, Maine Department of Environmental Protection. Maine Department of Environmental Protection.

Driesen, K. 2015. Acute aquatic toxicity test of caffeine with Daphnia magna Straus and biomonitoring of PRB and Intensive Green Filter wastewater treatment systems.

Drury, B., E. Rosi-Marshall, J. Kelly, and J. Scott. 2013. Triclosan exposure increases triclosan resistance and influences taxonomic composition of benthic bacterial communities. Environmental science and technology 47:8923–8930.

Edwards, Q. A., S. M. Kulikov, and L. D. Garner-O'Neale. 2015. Caffeine in surface and wastewaters in Barbados, West Indies. SpringerPlus 4:57.

Ellis, J. 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environmental Pollution 144:184–189.

Enick, O., and M. Moore. 2007. Assessing the assessments: Pharmaceuticals in the environment. Environmental Impact Assessment Review 27:707–729.

Fairbairn, D. J., M. E. Karpuzcu, W. A. Arnold, B. L. Barber, E. F. Kaufenberg, W. C. Koskinen, P. J. Novak, P. J. Rice, and D. L. Swackhamer. 2016. Sources and transport of contaminants of emerging concern: A two-year study of occurrence and spatiotemporal variation in a mixed land use watershed. The Science of the total environment 551-552:605–613.

Fang, Y., A. Karnjanapiboonwong, D. A. Chase, J. Wang, A. N. Morse, and T. A. Anderson. 2012. Occurrence, fate, and persistence of gemfibrozil in water and soil. Environmental toxicology and chemistry / SETAC 31:550–5.

Faraone, S. V. 2005. What Is the Prevalence of Adult ADHD? Results of a Population Screen of 966 Adults. Journal of Attention Disorders 9:384–391.

FDA (Food and Drug Administration). 2015. Consumers - Cosmetics Safety Q&A:

Personal Care Products. Center for Food Safety and Applied Nutrition.

Fernández, C., M. González-Doncel, J. Pro, G. Carbonell, and J. V Tarazona. 2010. Occurrence of pharmaceutically active compounds in surface waters of the Henares-Jarama-Tajo River system (Madrid, Spain) and a potential risk characterization. The Science of the total environment 408:543–51.

Ferreira, A. P. 2005. Caffeine as an environmental indicator for assessing urban aquatic ecosystems. Cadernos de Saúde Pública 21:1884–1892.

Ferrey, M. 2013. Pharmaceuticals and endocrine active chemicals in Minnesota Lakes. Minnesota Pollution Control Agency, Saint Paul, MN.

Ferrey, M. 2015. Pharmaceuticals, personal care products, and endocrine active chemical monitoring in lakes and rivers: 2013. file:///Users/serenahaver/Downloads/Minnesota 2015 study on pharmaceuticals in lakes (1).pdf.

Finnegan, D., L. Simonson, and M. Meyer. 2010. Occurrence of Antibiotic Compounds in Source Water and Finished Drinking Water from the Upper Scioto River Basin, Ohio, 2005–6.

Fritsch E. Werner I., Davies R., Beggeli S., Feng W., Pessah I., C. R. 2013. Triclosan impairs swimming behavior and alters expression on excitation-contraction coupling proteins in fathead minnow (Pimephales promelas). Environ. Sci. Technol. 47:2008–2017.

Gagné, F., C. Blaise, M. Fournier, and P. D. Hansen. 2006. Effects of selected pharmaceutical products on phagocytic activity in Elliptio complanata mussels. Comparative biochemistry and physiology. Toxicology & pharmacology : CBP 143:179–86.

Gehring, T. A., B. Griffin, R. Williams, C. Geiseker, L. G. Rushing, and P. H. Siitonen. 2006. Multiresidue determination of sulfonamides in edible catfish, shrimp and salmon tissues by high-performance liquid chromatography with postcolumn derivatization and fluorescence detection. Journal of Chromatography B 840:132–

138.

Gibson, A., R. Morgan, and A. Nikitin. 2009. The effect of caffeine on the bacterial populations in a freshwater aquarium system. Student Summer Scholars at Grand Valley State University:paper 31.

Gómez-Martínez, L. E. 2011. Disposition Kinetics of Caffeine and Paraxanthine in Nile Tilapia (Oreochromis niloticus): Characterization of the Main Metabolites. Archives of Environmental Contamination and Toxicology 60:654–664.

GoogleMaps. 2016. Map data. https://www.google.com/maps/search/wastewater+treatment+plant/@44.5445601,-69.7723179,10.99z.

Heal, D. J., S. L. Smith, J. Gosden, and D. J. Nutt. 2013. Amphetamine, past and present--a pharmacological and clinical perspective. Journal of psychopharmacology (Oxford, England) 27:479–96.

Hirsch, R., K. Haberer, L. Kratz, and T. Ternes. 1999. Occurence of antibiotics in the aquatic environment. Science of the Total Environment 225:109–118.

Hollingsworth, R. G., J. W. Armstrong, and E. Campbell. 2003. Caffeine as a novel toxicant for slugs and snails. Annals of Applied Biology 142:91–97.

Huerta-Fontela, M., M. T. Galceran, and F. Ventura. 2008. Stimulatory Drugs of Abuse in Surface Waters and Their Removal in a Conventional Drinking Water Treatment Plant. Environmental Science & Technology 42:6809–6816.

Jagoda, A., W. Żukowski, and B. Dąbrowska. 2015. Investigations of the presence of caffeine in the Rudawa River, Kraków, Poland. Environmental monitoring and assessment 187:566.

Janusz, A., K. P. Kirkbride, T. L. Scott, R. Naidu, M. V. Perkins, and M. Megharaj. 2003. Microbial degradation of illicit drugs, their precursors, and manufacturing by-products: implications for clandestine drug laboratory investigation and environmental assessment. Forensic Science International 134:62–71.

Ji, K., S. Kim, S. Han, J. Seo, S. Lee, Y. Park, K. Choi, Y.-L. Kho, P.-G. Kim, J. Park, and K. Choi. 2012. Risk assessment of chlortetracycline, oxytetracycline, sulfamethazine, sulfathiazole, and erythromycin in aquatic environment: are the current environmental concentrations safe? Ecotoxicology 21:2031–2050.

Jones, O., J. Lester, and N. Voulvoulis. 2004. Potential ecological and human health risks associated with the presence of pharmacuetically active compounds in the aquatic environment. Critical Reviews in Toxicology 34:335–350.

Kennedy, C. D., S. W. Houmes, K. L. Wyrick, S. M. Kammerzell, K. Lukowiak, and B. A. Sorg. 2010. Methamphetamine enhances memory of operantly conditioned respiratory behavior in the snail Lymnaea stagnalis. The Journal of experimental biology 213:2055–65.

Kim, J., J. Park, P.-G. Kim, C. Lee, K. Choi, and K. Choi. 2010. Implication of global environmental changes on chemical toxicity-effect of water temperature, pH, and ultraviolet B irradiation on acute toxicity of several pharmaceuticals in Daphnia magna. Ecotoxicology (London, England) 19:662–9.

Kim, S., P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, and S. H. Bryant. 2016a. Thiabendazole, PubChem Substance and Compound databases. Nucleic Acids Research 44:D1202–D1213.

Kim, S., P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, and S. H. Bryant. 2016b. Carbamazepine, PubChem Substance and Compound databases. Nucleic Acids Research 44:D1202–D1213.

Kim, Y., K. Choi, J. Jung, S. Park, P.-G. Kim, and J. Park. 2007. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environment international 33:370–5.

Klaper, R., and L. Welch. 2011. Emerging contaminant threats and the Great Lakes: Existing science, estimating relative risk and determining policies. (C. S., Ed.). Alliance for the Great Lakes.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999−2000: A National Reconnaissance. Environmental Science & Technology 36:1202–12.

Koplin, D., E. Furlong, M. Meyer, E. Thurman, S. Zaugg, L. Barber, and H. Buxton. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999−2000: A National Reconnaissance. Environ. Sci. Technol. 36:1202–1211.

Koplin, D., M. Skopec, M. Meyer, E. Furlong, and S. Zaugg. 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Science of the Total Environment 328:119–130.

Kümmerer, K., editor. 2004. Pharmaceuticals in the Environment. Springer Berlin Heidelberg, Berlin, Heidelberg.

Kümmerer, K. 2009. The presence of pharmaceuticals in the environment due to human use--present knowledge and future challenges. Journal of environmental management 90:2354–66.

De la Torre, R., M. Farré, M. Navarro, R. Pacifici, P. Zuccaro, and S. Pichini. 2004. Clinical pharmacokinetics of amfetamine and related substances-Monitoring in conventional and non-conventional matrices. Clinical pharmacokinetics 43:167–185.

Lam, M. W., C. J. Young, R. A. Brain, D. J. Johnson, M. A. Hanson, C. J. Wilson, S. M. Richards, K. R. Solomon, and S. A. Mabury. 2004. Aquatic persistance of eight pharmaceuticals in a microcosm study. Environmental Toxicology and Chemistry 23:1431.

Larsson, R., P. Erlanson, G. Bodemar, A. Walan, A. Bertler, L. Fransson, and B. Norlander. 1982. The pharmacokinetics of cimetidine and its sulphoxide metabolite in patients with normal and impaired renal function. British journal of clinical pharmacology 13:163–70.

Liang, J., and B. Guillory. 2015. Eutrophication: Sources, consequences and solutions.

Pages 1–31 Belgrade Lakes water quality report -Problems, Consequences, and solutions. Waterville, ME.

De Liguoro, M., V. Di Leva, M. Dalla Bona, R. Merlanti, G. Caporale, and G. Radaelli. 2012. Sublethal effects of trimethoprim on four freshwater organisms. Ecotoxicology and environmental safety 82:114–21.

Loraine, G. A., and M. E. Pettigrove. 2006. Seasonal Variations in Concentrations of Pharmaceuticals and Personal Care Products in Drinking Water and Reclaimed Wastewater in Southern California. Environmental Science & Technology 40:687–695.

Luo, Y., L. Xu, M. Rysz, Y. Wang, H. Zhang, and P. J. J. Alvarez. 2011. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environmental science & technology 45:1827–33.

Maine MethWatch. 2013. Maine methamphetamine prevention project. Margaret Chase Smith Policy Center, Maine Drug Enforcement Agency.

Mazaleuskaya, L., K. Sangkuhl, C. Thorn, G. FitzGerald, R. Altman, and T. Klein. 2015. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenetics and genomics.

Mazaleuskaya, L., K. Theken, L. Gong, C. Thorn, FitzGerald, R. Altman, and T. Klein. 2014. PharmGKB summary: ibuprofen pathways. Pharmacogenetics and genomics.

McCullough, I. 2010. The impacts of land use and development patterns on water quality of the Belgrade Lakes. Waterville, ME.

McCullough, I., C. Loftin, and S. Sader. 2013. TB207: A manual for remote sensing of Maine lake clarity. http://digitalcommons.library.umaine.edu/cgi/viewcontent.cgi?article=1012&context=aes_techbulletin.

McDowell, W., M. Clifford, M. Frode-Hutchins, J. Harvala, K. Knudsen, and C. Brick. 2005. Septic system impact on surface waters: A review of the Inland Northwest.

Tristate Water Quality Council.

Medline Plus. 2016. Club Drugs. National Library of Medicine. https://www.nlm.nih.gov/medlineplus/clubdrugs.html.

MedlinePlus, (NIH). 2010. Cimetidine: MedlinePlus Drug Information. https://www.nlm.nih.gov/medlineplus/druginfo/meds/a682256.html.

MedlinePlus, (NIH). 2012. Carbamazepine: MedlinePlus Drug Information. https://www.nlm.nih.gov/medlineplus/druginfo/meds/a682237.html.

MedlinePlus, (NIH). 2014. Gemfibrozil: MedlinePlus Drug Information. https://www.nlm.nih.gov/medlineplus/druginfo/meds/a686002.html.

MedlinePlus, (NIH). 2015a. Diphenhydramine: MedlinePlus Drug Information. https://www.nlm.nih.gov/medlineplus/druginfo/meds/a682539.html.

MedlinePlus, (NIH). 2015b. Naproxen: MedlinePlus Drug Information. https://www.nlm.nih.gov/medlineplus/druginfo/meds/a681029.html.

Merad, R. 1988. Warfarin. http://www.inchem.org/.

Mimeault, C., A. J. Woodhouse, X.-S. Miao, C. D. Metcalfe, T. W. Moon, and V. L. Trudeau. 2005. The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, Carassius auratus. Aquatic toxicology (Amsterdam, Netherlands) 73:44–54.

Moore, K., D. Rieder, and B. Madden. 2006. Review of new use for thiabendazole as a seed treatment on dry peas. United States Environmental Protection Agency, Office of Prevention Pesticides and Toxic Substances.

Moore, M. T., S. L. Greenway, J. L. Farris, and B. Guerra. 2008. Assessing caffeine as an emerging environmental concern using conventional approaches. Archives of environmental contamination and toxicology 54:31–5.

Musolff, A., S. Leschik, M. Möder, G. Strauch, F. Reinstorf, and M. Schirmer. 2009.

Temporal and spatial patterns of micropollutants in urban receiving waters. Environmental pollution (Barking, Essex : 1987) 157:3069–77.

National Drug Intelligence Center. 2001. Maine Drug Threat Assessment. Johnstown, PA.

Nawrot, P., S. Jordan, J. Eastwood, J. Rotstein, A. Hugenholtz, and M. Feeley. 2003. Effects of caffeine on human health. Food additives and contaminants 20:1–30.

NCBDD. 2016. Treatment patterns for children ages 2-5 with ADD. http://www.cdc.gov/ncbddd/adhd/stateprofiles-psychological/stateprofile-psychological-maine.pdf.

NFLIS (National Forensic Laboratory Information System). 2011. ADD/ADHD Stimulants in NFLIS.

O'Hara, F., and J. Luoma. 2015. Septic systems how they work and how to keep them working. Maine.

Ornelas, R., M. Nardone, and A. Machado. 2010. Pharmaceuticals and Personal Care Products: NSAIDS. http://www.csun.edu/~vchsc006/nsaid.pdf.

Orvos, D., J. Inauen, M. Capdevielle, A. Rothenstein, V. Cunningham, and D. Versteeg. 2002. Aquatic toxicity of triclosan. Enviornmental Toxicology and Chemistry 21:1338–1349.

Paíga, P., L. H. M. L. M. Santos, C. G. Amorim, A. N. Araújo, M. C. B. S. M. Montenegro, A. Pena, and C. Delerue-Matos. 2013. Pilot monitoring study of ibuprofen in surface waters of north of Portugal. Environmental science and pollution research international 20:2410–20.

Pal, R., M. Megharaj, K. P. Kirkbride, and R. Naidu. 2013. Illicit drugs and the environment--a review. The Science of the total environment 463-464:1079–92.

Papageorgiou, M., C. Kosma, and D. Lambropoulou. 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Science of The Total Environment 543:547–569.

Perlman, H. 2016. Water Use: Wastewater treatment. http://water.usgs.gov/edu/wuww.html.

PharmGKB. 2015. Sulfamethoxazole. https://www.pharmgkb.org/chemical/PA451544?previousQuery=Sulfamethoxazol.

Phillips, P., C. Schubert, D. Argue, I. Fisher, E. Furlong, W. Foreman, J. Gray, and A. Chalmers. 2015. Concentrations of hormones, pharmaceuticals and other micropollutants in groundwater affected by septic systems in New England and New York 512-513:43–54.

Qurie, M., M. Khamis, F. Malek, S. Nir, S. A. Bufo, J. Abbadi, L. Scrano, and R. Karaman. 2014. Stability and Removal of Naproxen and Its Metabolite by Advanced Membrane Wastewater Treatment Plant and Micelle-Clay Complex. CLEAN - Soil, Air, Water 42:594–600.

Reddersen, K., T. Heberer, and U. Dünnbier. 2002. Identification and significance of phenazone drugs and their metabolites in ground- and drinking water. Chemosphere 49:539–544.

Reed, E., and S. (ES494) Haver. 2015. Belgrade lakes water quality report: Problems, consequences, and solutions. Waterville, ME.

Reed, M. 1999. Decentralized Systems Technology Fact Sheet-Septic Tank-Soil Absorption Systems. Office of Water Washington D.C.

Roberts, P. H., and K. V Thomas. 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. The Science of the total environment 356:143–53.

Rosi-Marshall, E. J., D. W. Kincaid, H. A. Bechtold, T. V. Royer, M. Rojas, and J. J. Kelly. 2013. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecological Applications 23:583–593.

Rosi-Marshall, E. J., D. Snow, S. L. Bartelt-Hunt, A. Paspalof, and J. L. Tank. 2015. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. Journal of hazardous materials 282:18–25.

RStudio, T. 2015. RStudio: Integrated development environment for R. RStudio, Inc, Boston, MA.

Santos, A., W. Ma, and S. J. Judd. 2011. Membrane bioreactors: Two decades of research and implementation. Desalination 273:148–154.

Schaider, L., R. Rudel, and Rodgers K. 2013. Contaminants of emerging conern and septic systems: A synthesis of scientific literature and application to groundwater quality on Cape Cod. Silent Spring Institute, Newton, MA.

Schirmer, K., and M. Schirmer. 2008. Who is chasing whom? A call for a more integrated approach to reduce the load of micro-pollutants in the environment. Water science and technology : a journal of the International Association on Water Pollution Research 57:145–50.

Shaw, L., C. Phung, and M. Grace. 2015. Pharmaceuticals and personal care products alter growth and function in lentic biofilms. Environmental Chemistry 12:301.

Sumpter, J. P., A. C. Johnson, R. J. Williams, A. Kortenkamp, and M. Scholze. 2006. Modeling Effects of Mixtures of Endocrine Disrupting Chemicals at the River Catchment Scale. Environmental Science & Technology 40:5478–5489.

Sun, Q., Y. Li, M. Li, M. Ashfaq, M. Lv, H. Wang, A. Hu, and C.-P. Yu. 2016. PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater. Chemosphere 150:596–604.

Thorn, C. F., E. Aklillu, E. M. McDonagh, T. E. Klein, and R. B. Altman. 2012. PharmGKB summary: caffeine pathway. Pharmacogenetics and genomics 22:389–95.

Toxnet. 2016. d-Methamphetamine.

Vanderveen, J. (Committee on M. N. R., M. (Staff) Poos, C. (Food and N. B. Garza, and A. (Staff) Yates. 2001. Pharmacology of Caffeine. National Academies Press (US), National Academy of Sciences.

Veach, A. M., and M. J. Bernot. 2011. Temporal variation of pharmaceuticals in an urban and agriculturally influenced stream. The Science of the total environment 409:4553–63.

Vieno, N. M., T. Tuhkanen, and L. Kronberg. 2005. Seasonal Variation in the Occurrence of Pharmaceuticals in Effluents from a Sewage Treatment Plant and in the Recipient Water. Environmental Science & Technology 39:8220–8226.

Vree, T. B., M. Van Den Biggelaar-Martea, C. P. Verwey-Van Wissen, M. L. Vree, and P. J. Guelen. 1993. The pharmacokinetics of naproxen, its metabolite O-desmethylnaproxen, and their acyl glucuronides in humans. Effect of cimetidine. British journal of clinical pharmacology 35:467–72.

Wang, L., Y. Peng, X. Nie, B. Pan, P. Ku, and S. Bao. 2016. Gene response of CYP360A, CYP314, and GST and whole-organism changes in Daphnia magna exposed to ibuprofen. Comparative biochemistry and physiology. Toxicology & pharmacology : CBP 179:49–56.

Xie, Z., G. Lu, K. Hou, D. Qin, Z. Yan, and W. Chen. 2016. Bioconcentration, metabolism and effects of diphenhydramine on behavioral and biochemical markers in crucian carp (Carassius auratus). The Science of the total environment 544:400–9.

Yang, W., N. Cicek, and J. Ilg. 2006. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. Journal of Membrane Science 270:201–211.

Zuccato, E., and S. Castiglioni. 2009. Illicit drugs in the environment. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 367:3965–3978.