Stimulated Water

Development of Protocol for Detecting Caffeine and Nicotine in Surface Water Using the Skidmore Analytical Interdisciplinary Laboratory (SAIL)

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Introduction

Ecological Impacts of Urbanization

Globally, urban populations are growing exponentially and in response, the physical infrastructure of cities continues to expand. As of 2010, 50% of all people live in urban areas, up from 10% in 1900 (Grimm et al. 2008) and this percentage is expected to rise to 70% by 2050 (United Nations 2007). In the United States, an estimated 260 million people live in cities and these populations are projected to increase to 370 million by 2050 (United Nations 2009). Urban development impacts the environment in numerous and often negative ways. In stream ecosystems in particular, the proximity of a stream to a developed area can have severe implications for the health of that stream. The volume of impervious surfaces in a watershed can be directly correlated with the degree to which streams in that watershed are degraded (Wengar et al. 2009). The tendency for urban streams to degrade has been termed 'urban stream syndrome' (Walsh et al. 2005, Wenger et al. 2009).

Stream Pollutants

Since the enactment of the Clean Water Act (1972), the U.S. EPA has mandated the regulation of water quality in the United States with a particular focus on drinking water sources (U.S. EPA, Quality, 2010). Biological, nutrient, and sediment criteria must be taken into account when assessing water quality (U.S. EPA, Quality, 2010). Nitrogen and phosphorus are the nutrients most frequently tested for in streams, because their use in fertilizers makes them common contaminants. The input of these nutrients to water bodies can cause eutrophication, harmful algal blooms, and hypoxia (U.S. EPA, Quality, 2010).

In recent years, the variety of pollutants being tested for has increased to include a field of contaminants known as novel contaminants. These pollutants range from estrogen to pharmaceuticals to stimulants and are commonly found downstream of wastewater treatment

plants. Contaminants in this class are found in human waste and are not removed in treatment processes (Daughton and Ternes 1999). Most previous research has focused on the presence of estrogen and other hormones in water downstream of treatment plants (Barnes et al. 2000, Bradley et al. 2007, Kolpin et al. 2002, Kolpin et al. 2004). Increased concentrations of estrogen in surface waters have affected the reproductive health of wild fish populations. Presence of estrogen in the water has been known to lead to the feminization of male fish (Kidd et al. 2007). Because of the relative newness of this field, there remains a wide range of contaminants that have yet to be fully investigated, including caffeine and nicotine, despite their ubiquitous use in society. Additionally, sampling sites have been focused downstream of wastewater treatment plants, but pollutants can also enter streams through stormwater and run-off from impermeable surfaces as well as from leaking septic tanks. Streams that receive high volumes of stormwater and run-off or that are located near high densities of septic tanks have not received as much attention as streams downstream of wastewater effluent.

Run-off and Stormwater

Impermeable surfaces associated with development typically replace surfaces such as fields, forests and wetlands that are considered permeable because water can easily infiltrate into the ground (Brabec et al. 2002). This water can then either be taken up by plants or enter groundwater systems and eventually reach streams. In urban areas, impermeable surfaces prevent the infiltration of water. The rainwater that falls on these surfaces is often rerouted into stormwater management pipes or becomes run-off. The current global estimate of constructed impervious surface coverage is 579,703 km^2 or 0.43% of total land area (Elvidge et al. 2007). The United States contributes a total of 83,337 km^2 (14.4 %) to this global total, and impermeable surfaces account for 1.05% of total land area of the United States (Elvidge et al. 2007). Impermeable surfaces are capable of producing nine times more run-off than a woodlot

of equal size (EPA 1996). As rainwater run-off flows across an impermeable surface, it picks up nutrients and pollutants, including nitrogen, phosphorus, ions, pesticides, bacteria, heavy metals, and pharmaceuticals including stimulants such as caffeine and nicotine (Bannerman et al. 1993, Bradley et al. 2002). This water then either flows from impermeable surfaces directly into water bodies or travels through storm drains and pipes into water systems.

Historically, stormwater in the United States was managed through the construction of drains and pipes designed to collect water and remove it from urban areas as rapidly as possible (Roy et al. 2008). In large cities (populations of 100,000 or more), stormwater is treated in the same plants that treat sanitary waste, but small cities have not historically been required to treat stormwater (Roy et al. 2008). Developers can manage their run-off by implementing Low Impact Design (LIDs), like rain gardens or green roofs or by constructing retention ponds, which collect run-off and filter out many contaminants (Roy et al. 2008). However, retro-fitting of old developments is difficult, expensive, and not always required. This means that run-off from these pre-existing impermeable surfaces still runs into storm drains and consequently into local water bodies.

Septic Tanks

Pollutants can also find their way into ground and surface water though improperly installed septic tanks. Septic systems are responsible for the greatest volume of contaminated wastewater that is discharged into the ground (Carrara 2008). Previous studies have found that leaking from septic tanks has led to increased concentrations of pharmaceuticals and other contaminants in groundwater (Carrara 2008). There is often little regulation regarding the number of septic tanks that can be installed in a town or suburb and high densities of septic tanks is one of the most important contributors to groundwater contamination (Yates 1985). Systems that have been installed in non-suitable soils or that are not maintained with routine cleanings

occurring at least every two to five years are likely to leach pollutants and pathogens into groundwater, which may eventually reach streams, rivers, or lakes (Evans et al. 1999 in Petri 2008).

Although septic tanks are not typically found in urban areas, they are commonly used in surrounding suburban areas and rural residential areas that are not supported by municipal wastewater treatment facilities. As of 2007, approximately 26.1 million homes in the United States were served by septic systems (U.S. EPA, Septic). Petri (2008) estimated that there were approximately 10,000 septic tanks in the Saratoga Lake Watershed as of 2007 and that of these 10,000 tanks, only about 5-10% were properly maintained, which means that portions of the watershed could be at high risk for groundwater contamination.

Caffeine

Caffeine is a naturally occurring stimulant which 80% of the world's population consumes daily for its ability to increase alertness (Heckman et al. 2010). According to the Food and Agriculture Organization, the United States is ranked 10th in caffeine consumption, behind countries such as the U.K., Brazil, Canada, Australia, and Japan, countries recognized for heavy coffee and tea consumption (Heckman et al. 2010). Though 71% of caffeine consumed is in the form of coffee, recently developed and heavily advertised energy drinks, sport drinks, and fortified waters have created a new branch of the caffeine market (Heckman et al. 2010). Caffeine also plays an important pharmaceutical role as cough, cold, and headache medicine as well as a cardiac, cerebral, and respiratory stimulant (Buerge et al. 2003).

The abundance of caffeine in our culture increases the likelihood that it will act as an environmental contaminant. Kolpin et al. (2002) previously studied the presence of pharmaceuticals in 139 streams across 30 states by analyzing 95 different pharmaceuticals and

found caffeine to be the 4th most frequently found, occurring in 70% of the samples. Since the mean half-life of caffeine is relatively short, approximately 1.5 days, (Moore et al. 2007) it will not likely be environmentally hazardous if its addition to water systems is not consistent. In the human body, caffeine is rapidly filtered from the blood stream by the kidneys and excreted in urine (Heckman et al. 2010). As a consequence of caffeine's high consumption and excretion rates, the compound is consistently prevalent in wastewater. Because of its consistent presence, caffeine can be quantitatively used as an anthropogenic marker when found in surface waters to indicate septic tank leakage or run-off contamination (Buerge et al. 2003).

Nicotine

The nicotine found in tobacco plants is another widely used stimulant that also has the potential to act as a pervasive pollutant. Despite an overwhelming volume of published information about its detrimental health impacts, tobacco remains the source of the highest number of preventable fatal illnesses in the United States. Approximately 440,000 deaths each year and $157 billion in medical costs can be attributed to prolonged tobacco use (Surgeon General). As of 2001, 46.2 million adults in the United States, more than 15% of the country's population, were cigarette smokers (Surgeon General). Nicotine is a dependence-inducing toxin found in tobacco products which, like caffeine, is a stimulant (Jacobsen et al. 2004). Though it receives the most attention for its ability to create habitual cravings for tobacco products, nicotine also can act similarly to caffeine in its ability to increase focus and promote brain function (Jacobsen et al. 2004).

Although there are other anthropogenic input sources, the most substantial portion of nicotine's introduction to the natural environment can be attributed to human excretion. When metabolized, nicotine is converted to cotinine, indicating that high levels of human nicotine consumption would yield high presences of the metabolite cotinine in wastewater (Bradley et al.

2007). This form of nicotine is a non-point source of pollution which has the potential to be processed by microbial activity in aquatic ecosystems (Bradley et al. 2007). Agricultural input from tobacco cultivation is another non-point source of nicotine but this input source is both regionally limited and absent in urban settings. Alternatively, cigarette butt waste is heavily influential in urban settings and densely populated residential areas (Novotny et al. 2009). Since the introduction of filtered cigarettes in the 1950s, consumer preference for this alternative has increased to account for 99% of cigarettes purchased (Novotny et al. 2009). The plastic-like cellulose acetate filters prevent a large amount of nicotine and tar inhalation because the carcinogens are retained by the non-consumed portion of the cigarette (Novotny et al. 2009), but the dense concentrations of toxins contained in the environmentally persistent filters have detrimental environmental impacts (Novotny et al. 2009). Globally, an estimated 1.69 billion pounds of non-biodegradable cigarette filters are littered each year (Carlozo 2008).

Study Site: Saratoga Lake Watershed

Saratoga County currently has a population of 220,069 people (U.S. Census Bureau 2009), and with an annual growth rate of 30.5%, it is the fastest growing county in upstate New York (U.S. Census Bureau 2000). An expanding population demands increased infrastructure and the volume of impermeable surfaces, septic systems, and wastewater in the area is likely to grow. This expansion could have important implications for surface water contamination in the Saratoga Lake Watershed. The Saratoga Lake Watershed covers a total of 244 square miles and includes 13 municipalities (SLIPID 2000). In the summer, large quantities of tourists are drawn to the watershed both by the track and by the natural beauty of the area. Overall, water quality in the watershed is considered to be relatively good. The degradation of these water bodies could lead to a loss of industry for the city of Saratoga Springs and for the rest of the watershed.

Spring Run, a stream that is piped under several major roadways and then flows aboveground into Lake Lonely, has proven to be an exception to the overall good water quality based on its State 303 (d) designation, a title reserved for streams in need of remediation (DEC 2009). Spring Run contains higher concentrations of nitrogen and phosphorus than the other main Lake Lonely tributary, Bog Meadow Brook, or Lake Lonely itself (Halstead et al. 2007). Spring Run receives inputs of stormwater and run-off from impermeable surfaces throughout its journey underneath Broadway. Citizens of Saratoga Springs may be unaware that a stream runs under their feet and not realize the far reaching consequences of certain actions, such as casually throwing a cigarette butt to the ground. These small actions may have larger implications for the health of the environment.

Skidmore Analytical Interdisciplinary Laboratory (SAIL)

The Skidmore Analytical Interdisciplinary Laboratory (SAIL) is a new facility that was established at Skidmore College in fall 2010 through funding from the National Science Foundation. This laboratory primarily serves the Environmental Studies program and the Chemistry, Biology, and Anthropology departments, and is available to any student on campus who wishes to conduct research. It includes several state of the art instruments that are not frequently available at undergraduate institutions. Since the laboratory is new to the school, very few student projects have utilized this resource. Of all of the instruments supported by the laboratory, the three instruments that are most capable of detecting novel contaminants at low concentrations are the High Performance Liquid Chromatograph (HPLC), the Gas Chromatograph with Mass Spectrometer (GC), and the Ion Chromatograph (IC).

Research Purpose

Our ultimate aim in this project was to develop methods that could be used to detect caffeine and nicotine in surface water using SAIL. However, these methods can also be used to

detect a wide variety of other novel contaminants in addition to caffeine and nicotine. In order to determine the best methods possible, we experimented with the HPLC, IC, and GC in order to determine which would be the best suited for this variety of research. Our hope is that these methods will contribute to the advancement of understanding of whether novel contaminants are present in the Saratoga Lake Watershed, in what concentrations they are present, and from what sources they are contributed. For the purposes of this project, we focused on streams that are potentially being impacted by leaking septic tanks and Spring Run, which receives high volumes of stormwater run-off.

Methods and Protocol Development

Site Identification and Sample Collection

To identify and select the stream sites for sample collection within the Saratoga Lake Watershed, we used a hotspot map of improperly installed septic systems (LA Group in Petri 2008). This map identified septic tanks that are located on improper soils or within 200 feet of a stream and created hotspots based on the relative density of septic tanks (Figure 1).

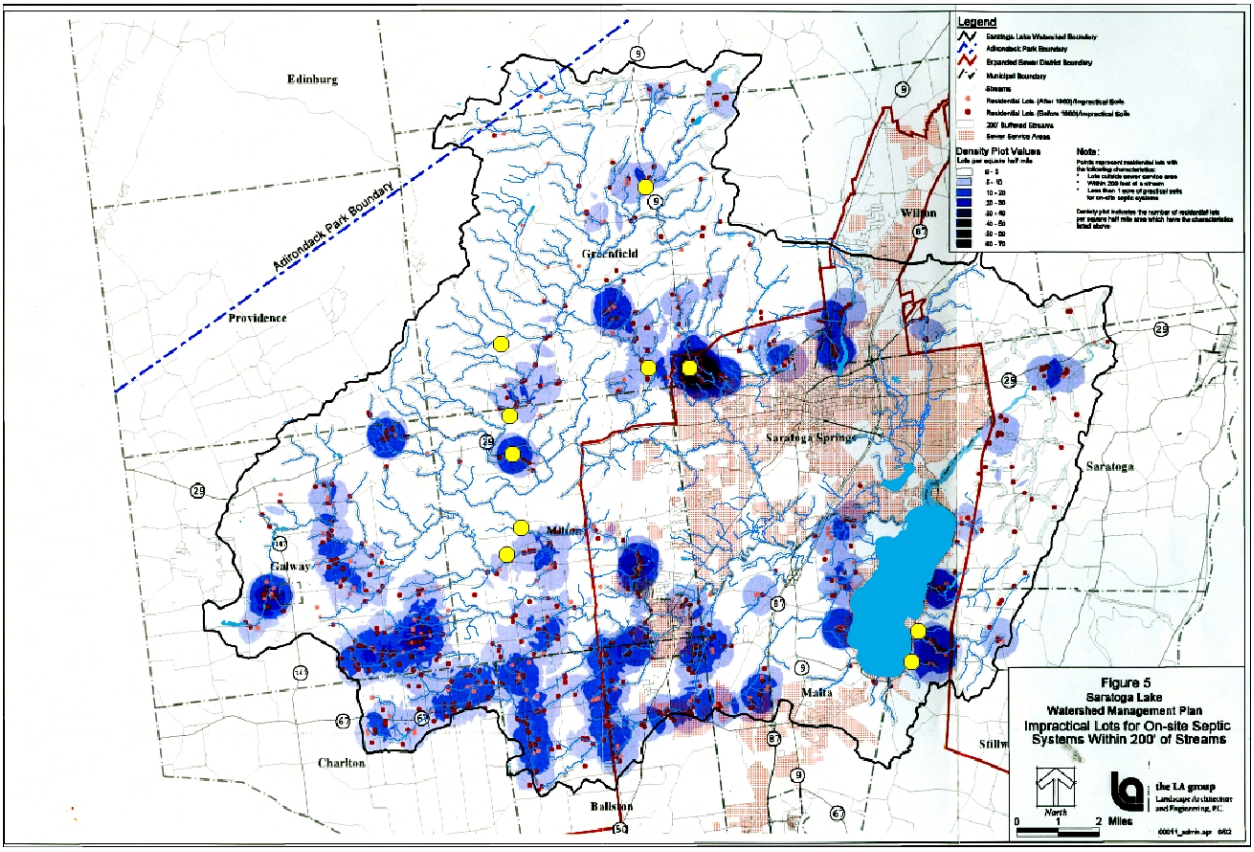

Figure 1. Ten sampling sites in the Saratoga Lake Watershed selected along septic tank density continuum (based on LA Group in Petri 2008) indicated by yellow circles.

We selected streams along a continuum ranging from low density to high density of septic systems. To serve as a control, we choose a non-urban stream site that was not near improperly installed septic systems. Additionally, we selected two locations along Spring Run,

one in Congress Park, upstream of downtown Saratoga Springs, and one adjacent to EBI, downstream of downtown Saratoga Springs.

In total, we collected samples from each of the 12 locations on four occasions during February and March 2011, twice after high input events had occurred and twice after minimal input had occurred. Water collection dates were determined based on the weather conditions of the three consecutive days prior to the sampling date. For the high input sampling rounds, we waited for precipitation events, preferably rain storms or heavy snowfall followed by overnight temperatures above freezing before sampling. We believed that rain combined with warm temperatures would melt high volumes of snow, causing increased water flow over impervious surfaces and through the soil. We predicted that this increased flow might bring more caffeine and cotinine into the streams. Before collecting the baseline samples, we waited for colder periods without precipitation during which minimal snow melt occurred. During the baseline sampling rounds, the stream flows were lower but we believed that we might detect higher concentrations of caffeine and cotinine because of the lower volume of water in the channels. All water samples were stored in a freezer until they could be analyzed for chemical content.

SAIL Protocol

We used the Skidmore Analytical Interdisciplinary Laboratory (SAIL) to develop our standard protocol for testing for the presence and concentrations of caffeine and cotinine in streams. To determine which instrument would best detect caffeine and cotinine, we ran standard solutions with known concentrations of caffeine and cotinine on three instruments in the laboratory: the high performance liquid chromatograph (HPLC), the ion chromatograph on liquid chromatography mode (IC), and the gas chromatograph with mass spectrometer (GC).

High Performance Liquid Chromatograph (HPLC)

The first instrument we used was the HPLC because stream water samples require very little preparation to be processed by this instrument. Also, we believed we would be able to detect caffeine, cotinine, and nicotine using the same wavelength setting. This meant that we would have been able to detect caffeine, cotinine, and nicotine in the same sample rather than running three samples for each contaminant. The HPLC works by separating the components of water samples as a solvent, in our case water and methanol, carries each sample through a retention column. The amount of time it takes each component of the sample to move through the retention column varies based on particle size and ratio of solvents used. We ran the instrument at a maximum wavelength of 275 nm with a solvent ratio of 75% water to 25% methanol. Based on this solvent ratio and previous studies, we predicted that caffeine, nicotine, and cotinine would not be retained in the column for longer than nine minutes.

Our standard solutions were composed of 1 mg/L dilutions of liquid caffeine, cotinine, and nicotine standards in methanol. We injected approximately 0.1 mL of each of these standard solutions through the HPLC column to verify that they could be detected at 275 nm within a nine minute time frame. After running separate standards for caffeine, nicotine, and cotinine, we were able to detect caffeine and cotinine, but not nicotine. The HPLC output displays the beginning of the injection as a peak at around 2 minutes. Nicotine may have a very short retention time, in which case its peak would be integrated the detection peak. Since we believed we were more likely to find cotinine than its non-metabolized parent form, we decided to proceed in testing only for cotinine. We ran a mix of equal ratios of each of the diluted solutions to ensure that the resolution between the caffeine and cotinine peaks was high enough for definitive identifications. We were able to detect and identify both caffeine, at around 3.5 minutes, and cotinine, at approximately 6 minutes (Figure 2).

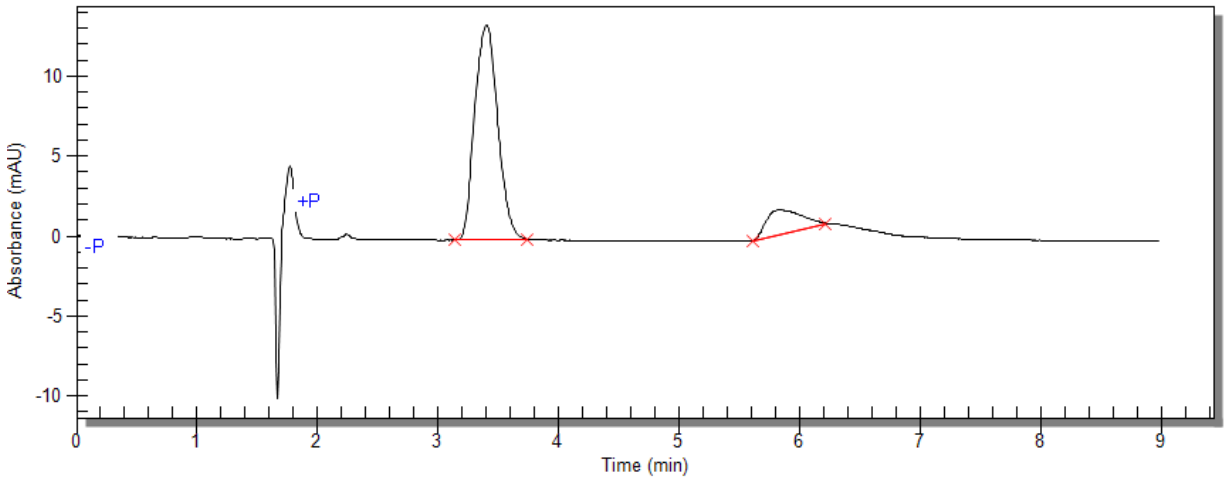

Figure 2. HPLC results of caffeine and cotinine standard mix; the injection peak appears just before 2 minutes, the caffeine peak appears around 3.5 minutes, and the cotinine peak appears around 6 minutes.

To prepare our stream water samples for the HPLC, we filtered 5 mL of each sample through a 20 micron filter to remove all suspended particles. We ran a sample from Spring Run at the downstream location by EBI following the same methods used for the standard solutions. Because of its State 303 (d) List designation, we expected Spring Run to be highly likely to contain at least one of our novel contaminants of interest. However, we did not detect caffeine or cotinine in the Spring Run at EBI sample.

In addition to the Spring Run sample, we ran samples from Wheeler Creek and Mud Creek, two locations included within the intermediate range of the septic influence continuum. We used a different solvent ratio (70% water and 30% methanol) and a different HPLC retention column to alter retention time in hopes of achieving a higher resolution between contaminants. The standards must be run using the same methods that are used for the stream water samples to ensure accurate identification of the peaks as their hypothesized contaminants, so we ran each standard and the mix of standards again following the new methods. The HPLC functions best when the solvent ratio is close to the sample it is processing, so we diluted the standards with

methanol in a 70:30 ratio. Unfortunately, we did not detect caffeine or cotinine in the Wheeler or Mud Creek samples (Figure 3).

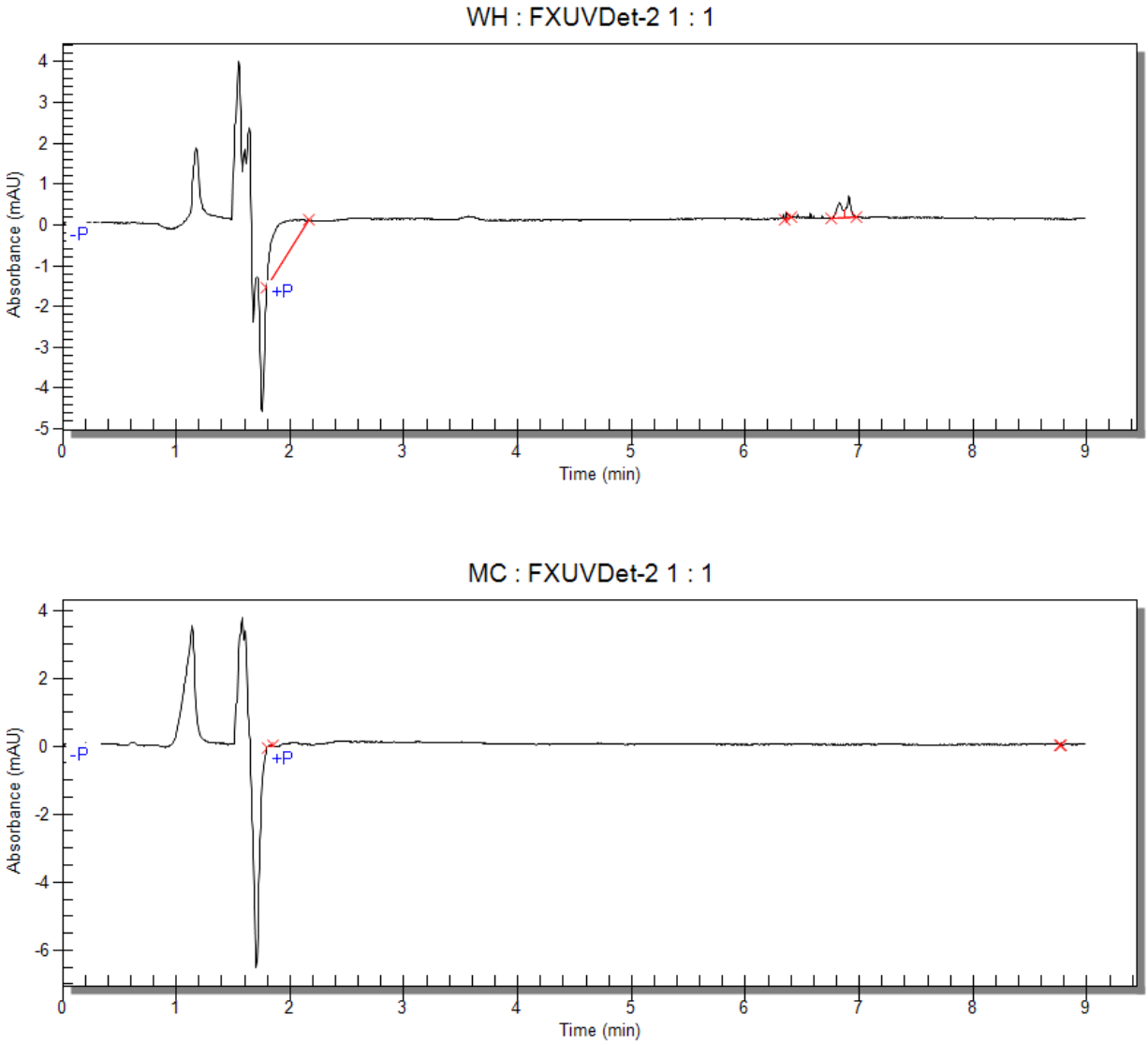

Figure 3. Based on the methods used for caffeine and cotinine standard solutions at 1 mg/L concentrations, neither caffeine nor cotinine were in the Wheeler Creek (top) or Mud Creek (bottom).

We determined that the HPLC was not an ideal instrument for our research purposes. We were not able to separate the nicotine peak from the injection peak. Previous research into novel contaminants has tested for cotinine instead of nicotine (Barnes et al. 2002, Kolpin et al. 2002, Kolpin et al. 2004) justifying our decision to proceed without identifying nicotine in a standard

solution. However, we would ideally be able to detect both cotinine and nicotine using the same methods and same instrument.

There are several possibilities for why we were unable to detect our novel contaminants using the HPLC. The column that is currently installed on the instrument may not be designed to process stream water samples that potentially contain numerous contaminants and can stress the instrument. By filtering our water samples, we anticipated being able to avoid this problem. Another possibility is that the instrument's pump is not appropriate to process stream water. The pump pressure builds to too high of a level, causing the instrument to shut down. We could not alter the pump pressure from its default setting, which may have been why we could not interpret the results of our sample. The HPLC that is currently installed in SAIL functions best with a higher methanol to water solvent ratio. To procure results with a high enough resolution to confirm identity of caffeine, cotinine, and nicotine peaks, we needed to alter the solvent ratio. Since we were having many technical difficulties and since we were not confident in the limited results we were getting, we moved on to another instrument.

Ion Chromatograph (IC) on Liquid Chromatography Mode

Using the IC, we processed our standard solutions and a stream water sample from Spring Run. To prepare the samples for the instrument, we poured approximately 5 mL of each of the standards and of the mix into plastic vials and capped them. These vials can be loaded into an auto-sampler which rotates through each of the samples, draws up a fraction of each sample, and injects the sample into the instrument in a mobile phase. Based on the polarity of each molecule, the instrument separates each component of the solution and yields the time and wavelength at which each molecule can be detected.

We ran trials of the dilutions of caffeine and cotinine standards at 1 mg/L. We also ran a mix of equal parts of the caffeine and cotinine standard solutions to see if we could detect and

identify both contaminants in one trial run. Although we were able to detect caffeine and cotinine on the IC in our standard solutions and in the mix of the two standard solutions, we could not conclusively identify caffeine or cotinine in our stream water samples based on the IC yield. There were peaks that encompassed both the time at which we expected to see caffeine and the time at which we expected to see cotinine based on the standards, but these peaks were very broad and could have encompassed a variety of other contaminants. Since the resolution of the stream water sample was poor, we did not continue to pursue use of this instrument.

Gas Chromatograph (GC) with Mass Spectrometer

To prepare the standards and stream samples for the GC, we concentrated 10 mL of each of the standards and stream water samples using solid phase extraction. To complete this type of extraction, we used solid phase extraction (SPE) cartridges. We completed the initial preparation of the cartridges by syringing 6 mL of methanol followed by 6 mL of double de-ionized water (DDW) through the SPE column at a rate of approximately 1 drip per second. Then we syringed 10 mL of the standard solution and stream water samples through their respective cartridges at the same rate to extract our contaminants of interest, thereby concentrating our samples. Following completion of the extraction, we eluted the molecules extracted by the SPE cartridge by syringing 1 mL of methanol through the cartridge into a 2 mL glass vial. We determined that 1 mL of methanol would be an appropriate volume of elution solvent to use to avoid diluting our standard solutions or stream samples while still recovering high proportions of any target molecules we may detect. We ran the concentrated samples using an auto-sampler for 22.53 minutes, the length of time necessary to detect caffeine and cotinine. Following the 10 mL extraction methods, we were able to detect caffeine (before 13 minutes) and cotinine (11 minutes) with good resolution at concentrations of 1 mg/L (Figure 4).

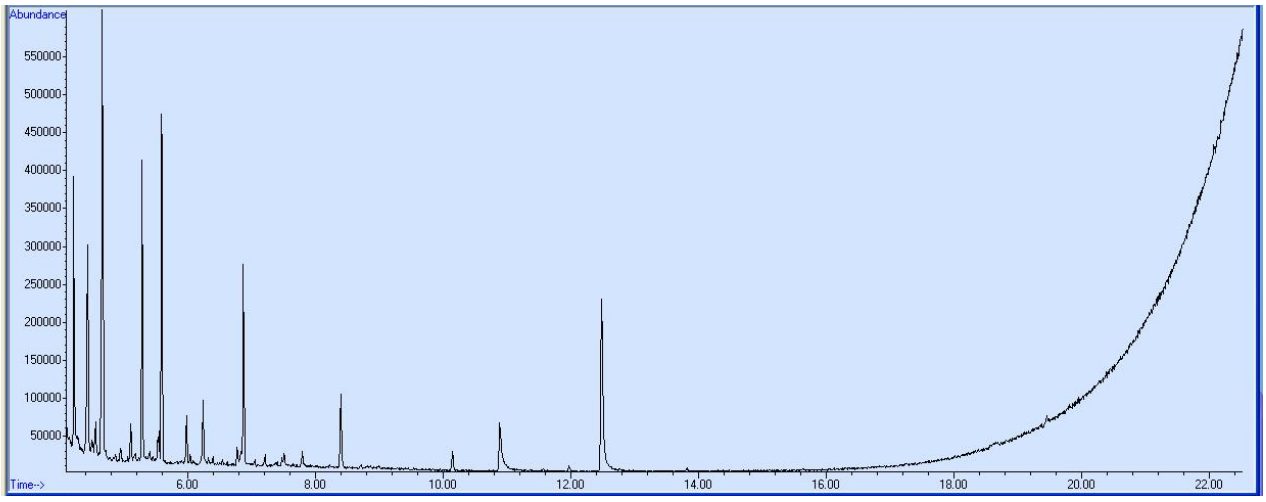

Figure 4. GC output display of caffeine, just before 13 minutes, and cotinine, around 11 minutes, both at 1 mg/L concentrations.

Our initial standard solutions were at concentrations of 1 mg/L. Once we verified that the GC would be able to detect this concentration of our contaminants, we further diluted our caffeine standard to 100 µg/L. Since we aimed to detect a lower concentration, we needed to increase the volume of standard solution we concentrated using the SPE cartridge. We syringed 100 mL of the caffeine standard solution through the SPE cartridge and then eluted the retained caffeine with 1 mL of methanol. We were able to detect caffeine at just before 13 minutes at 100 µg/L. Due to time constraints, we did not run a combined solution of caffeine and cotinine standards at 100 µg/L.

The next step was to dilute the standard solutions to concentrations of 10 µg/L to determine if the GC is capable of detecting caffeine and cotinine at these concentrations. Since we decreased the concentration of the standards by a factor of 10, we needed to increase the volume of solution (also by a factor of 10) to 1 L for the extraction. Our team syringed 1 L of a mix of caffeine and cotinine standards diluted to 10 µg/L through a prepared SPE cartridge and again eluted the retained contaminants using 1 mL of methanol. We were able to detect a small

concentration of caffeine but we did not successfully detect cotinine at this concentration (Figure 5).

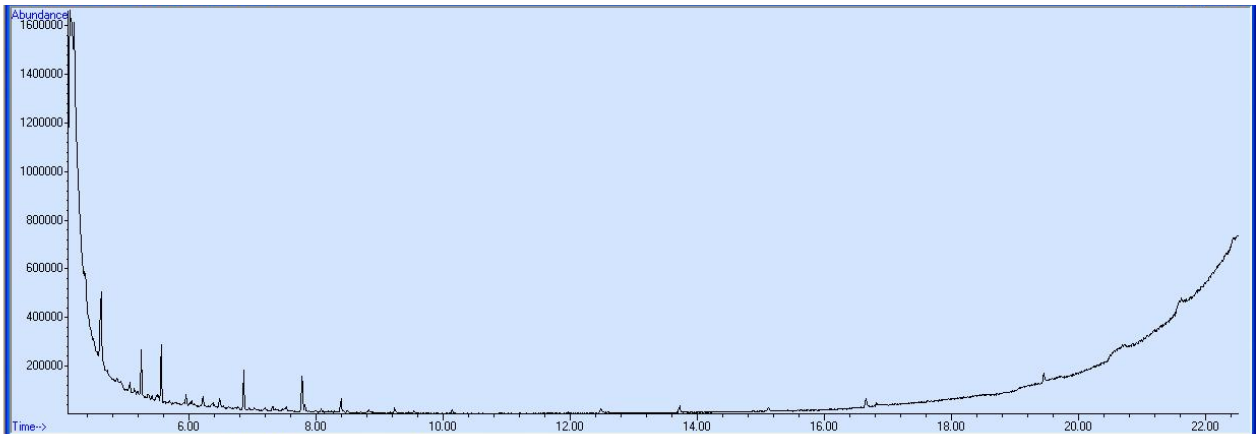

Figure 5. GC output of caffeine and cotinine 10 µg/L concentrations. Caffeine was detected at a very low abundance just before 13 minutes but cotinine was not detected.

There are several possible reasons for why we were unable to detect cotinine at 10 µg/L. The type of SPE cartridges we used might not retain cotinine molecules as well as a different type of cartridge might. If this is the case, other extraction methods will have to be pursued. A liquid to liquid phase extraction process could be investigated or different extraction protocol could be developed. Also, methanol might not be the ideal solvent to remove cotinine from the cartridge during elution. For this reason, another type of solvent might be more effective. Despite our inability to detect cotinine at 10 µg/L, we were able to detect caffeine at 10 µg/L, so we decided to run a stream water sample on the GC.

We concentrated 1 L of a stream water sample from Bell Brook, which runs through the highest density of the improperly installed septic tanks found within the Saratoga Lake Watershed. The maximum concentration of cotinine found in stream water samples in the literature we reviewed for this project was 1.03 µg/L (Glassmeyer et al. 2005). Since we were unable to detect cotinine at 10 µg/L using the GC and following the extraction methods we had

developed, we did not expect to detect cotinine in the concentration of the 1 L Bell Brook sample. When we ran the stream water sample from Bell Brook, we did not detect caffeine or cotinine (Figure 6).

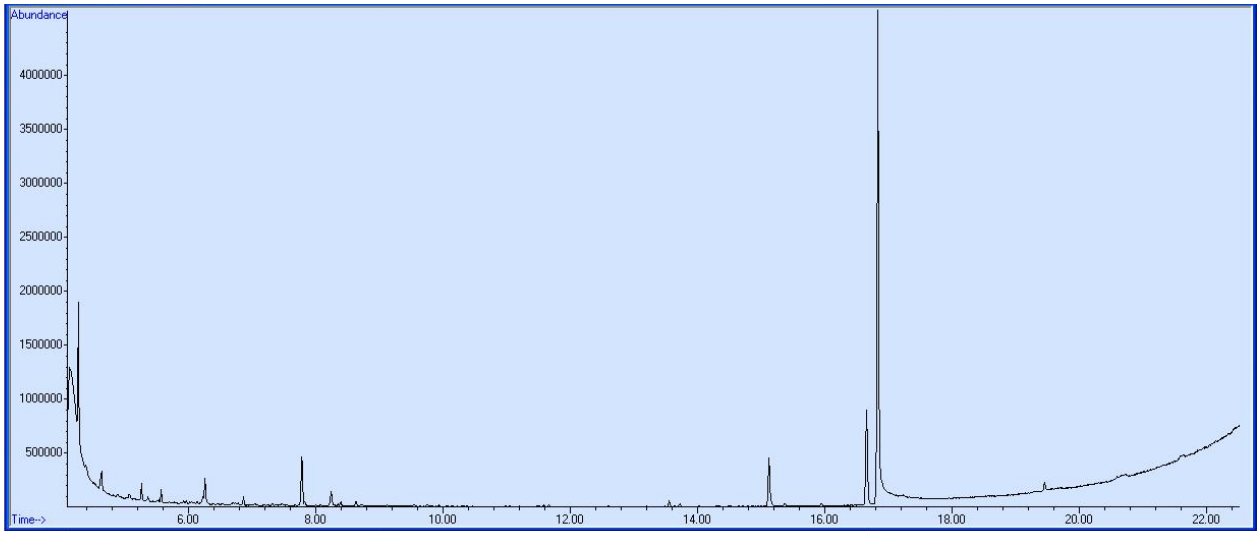

Figure 6. Following a 1 L concentration and extraction from a stream sample from Bell Brook, caffeine and cotinine were not detected.

The detection level of 10 µg/L is close to the high end of the range that caffeine has been detected in the environment. The maximum concentrations we found in our literature review were 7.99 µg/L at low flow (Glassmeyer et al. 2005) and 6.00 µg/L at high flow (Kolpin et al. 2002). While we cannot definitively say that caffeine is not present in Bell Brook, we can say that the concentration must be relatively low. Since Bell Brook is in the most densely concentrated area of septic tanks in the watershed, it is unlikely that caffeine would be found in higher concentrations in other streams. While we had hoped to conduct a survey of streams in the watershed, the time-consuming nature of our methods limited us to running only one sample.

Suggestions for Future Research:

In order to continue research on novel contaminants in the Saratoga Lake Watershed, we believe that SAIL should invest in a carboy apparatus, which would filter samples through the cartridge via gravity. This would make it possible to lower the standard detection levels to 1.0 µg/L, which is closer to what has been previously detected. Our project was limited to collecting samples in the winter and early spring, but we believe that collecting samples at baseline during the summer might yield higher concentrations of novel contaminants.

We also recommend that future researchers pursue investigation of how caffeine and cotinine are transported into surface water through a comparison of stormwater and run-off, septic system influence, and wastewater effluent. Samples from Spring Run at Congress Park and EBI could be analyzed because this stream received high input from stormwater and urban run-off. Water running into storm drains during rain events can be collected and analyzed. Samples from the wastewater treatment plants can also be analyzed. While no wastewater is discharged in the Saratoga Lake Watershed, wastewater is discharged into the Hudson River and samples could be collected downstream of these locations. Furthermore, the 10 sampling sites (Figure 1) identified in this project can be used to examine septic tank influence.

While we limited our study to caffeine and cotinine, future research could also look into the possible presence of other novel contaminants in the watershed. Additionally, the ecological impacts of novel contaminants has been largely uninvestigated. We believe that an analysis of the ecological impacts of caffeine, cotinine and other novel contaminants is important, considering their prevalence in water bodies across the United States. There are many potential ways to quantify these impacts. We outlined methods for assessing changes in foraging behavior of fish exposed to caffeine and nicotine (Appendix A).

Based on our methods development process, we created a list of information that should be obtained prior to starting a project involving the use of the instruments in the SAIL facilities. This list of recommendations for future research endeavors is included (Appendix B).

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Appendix A

Methods for Assessing Changes in Fish Foraging Habits after Exposure to Caffeine and Cotinine

Adapted from Moore, M. T., Greenway, S. L., Farris, J. L., and Guerra, B. 2007. Assessing caffeine as an emerging environmental concerns using conventional approaches. Arch. Of Environmental Contamination and Toxicology

Study Species: Fathead Minnows (*Pimephalus promelas*)

- common toxicological test organism
- Benthic and mid-level water column species
- Blackworms recommended as a feed (We found frozen red worms available at PetCo)

Experiment 1 (Foraging): 4 total runs, two tanks per treatment

- **Treatments**: Varying concentrations of caffeine and nicotine in tap water
  - Recommended Concentrations[1]
    - Cotinine—0.13 micrograms/L, 0.06 micrograms/L, 0.01 micrograms/L
    - Caffeine—6.00 micrograms/L, 3.00 micrograms/L, 1.00 micrograms/L
- Fish exposed to treatments for 10 days, then transferred to non-treatment aquariums for 7 experimental days of foraging trials
  - Recommended 5 fish per tank
- Daily experimental trials
  - 12 dead, equally distributed, partially buried black worms in a vegetated habitat
    - "Vegetation" can be as minimal as sand but can include plants
  - Fish were placed in the foraging tank individually and allowed to enter a feeding area for 5 minutes
  - Number of encounters with food were counted during each trial
  - All remaining prey were counted after each trial
- Trials can be filmed to ensure number of encounters with food are recorded accurately
  - To characterize movement, video monitor screen was separated into four quadrants and number of visits to each quadrant was recorded
  - Foraging efficiency over time could be monitored along with differences in movement/search strategy

[1] Concentrations determined from the following sources: Cain, T.G., D.W. Kolpin, J.D. Vargo, and M.D. Wichman. Occurrence of Antibiotics, Pharmaceuticals, and Sterols at Select Surface, and Wastewater Sites in Iowa.
Kolpin et al. 2004. Urban Contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Science of the Total Environment 328: 119-130.
Glassmeyer, S.T. et al. 2005. Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination. Enviro. Sci. Technol. 39: 5157-5169
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- Since fish are initially naïve about their habitat, foraging habits will automatically improve over time

Experiment 2 (Appetite): Testing the ability of fish to the learn the relative food availability of each patch

- Two patches—one with 22 partially buried worms, one with 4 partially buried worms
- Fish allowed to forage for ten minutes
- After the 7th day, relative food values of each patch were switched
  - Determine how fish responded to change in environmental condition
- Video analysis
  - Total time fish were in each patch
  - First patch sampled
  - Number of visits to and exits from each patch
  - More time spent in a patch means that patch is preferred

Appendix B

Guide for Conducting Novel Contaminant Research in the Skidmore Analytical Interdisciplinary Laboratory (SAIL)

☐ Identify your contaminant(s) of interest.

☐ Research past studies related to this contaminants, focusing on the methods used.

☐ Research literature values for your contaminant of interest in surface water.

☐ Meet with SAIL Instrumentation Manager as early as possible to share the results of your literature review and establish a research goal.

☐ With the help of the SAIL Instrumentation Manager, identify which instruments are best suited to your research.

☐ Determine necessary equipment (bottles, syringes, cartridges etc.) that will need to be ordered.

☐ Determine necessary chemicals and standards that will have to be ordered.

☐ If necessary, meet the Skidmore College Laboratory Safety Officer to discuss the safety protocol for handling your chemicals and standards.

☐ Keep careful documentation of methods development throughout your research process.