The impacts of wastewater from municipal waste water treatment plants on fish health in the Quinnipiac River

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Introduction:

Chemicals which affect the reproductive system of fish are omnipresent in the aquatic environment. Municipal wastewater effluent (MMWE) is a major source of these chemicals, as they contain pharmaceuticals and personal care products (PPCPs; e.g. synthetic estrogens and androgens). PPCPs are not effectively treated in standard wastewater treatment plants and therefore many of them enter the environment with potency levels that may impede the reproductive output of fish and other aquatic organisms. One of the main concerns about PCPPs is their ability to affect fish reproduction by disrupting endocrine signaling (Hotchkiss et al., 2008; Burkhardt-Holm, 2010), causing effects such as sex reversal and intersex condition in fish and amphibians (Hutchinson et al., 2006). Effects of PCPPs on reproduction have been observed at different levels of biological organization, including molecular (Garcia-Reyero et al., 2011; Ings et al., 2011), physiological (Dinizi et al., 2010; Ings et al., 2011), organismal (Ma et al., 2005) and population (Jobling et al., 2002a; Jobling et al., 2002b; Kidd et al., 2007).

Interestingly, there has been limited research on the impact of PPCPs on ecosystem health within Connecticut, even though it has the fourth highest population density of all US states, with 738.1 inhabitants/per square mile (Bureau, 2010). The investigators are aware of only one study on the *presence* of PCPPs and non-traditional compounds released from a WWTP into Connecticut water bodies. Scientists from the United States Geological Survey (USGS) conducted a pilot assessment in a reach of the Still River and characterized a subset of persistent pollutants and PCPPs (USGS, unpublished data). This study found the presence of plant and animal steroids, fragrances, personal care products, pesticides, cosmetics, detergent by-products, and flame retardants in the WWTP effluent as well as from downstream samples. Importantly, many of these compounds were not found in detectable concentrations upstream of the MWWE. Currently a second study is being conducted in Southbury, CT. Dr. Allison MacKay (Civil and Environmental Engineering, University of Connecticut [UConn]) is studying the fate and transport of PCPPs discharged from the *Heritage Village* WWTP into the Pomperaug River.

The Quinnipiac River (QR) is a highly urbanized river system. From North Haven, CT, upwards, five WTTPs discharge directly into the Quinnipiac River (EPA, 2011). Understanding the impact of these municipal effluents is of importance to protect the fish living in this system, as the cumulative effect of these discharges might cause increased impacts in organisms. The goal of the proposed project is to characterize the PPCPs present within the MWWE and assess potential impacts to fish health and reproduction by evaluating existing data sets and peer-reviewed literature. We propose to investigate the MWWE from the three uppermost WWTPs in the Quinnipiac River (i.e., Southington, Cheshire and Meriden).

Objectives:

The revised objectives of this study were:

- Measure concentrations of PPCPs in the Quinnipiac River, up and downstream from wastewater treatment plants (WWTP), to determine WWTP contributions.
- Review relevant data from other studies in CT and elsewhere to assess potential impacts of the PPCPs

Methods and Materials:

Study Areas

Ambient water samples were collected from the Quinnipiac River at locations above and below the the Southington, Cheshire and Meriden wastewater treatment plants (Figure 1). One sample was collected upstream from the effluent discharge location, from a location as close to the discharge pipe as feasible taking into account access and safety. Five additional discrete samples were collected downstream from the effluent discharge pipe in a gradient, with approximately 1000 m between collection sites.

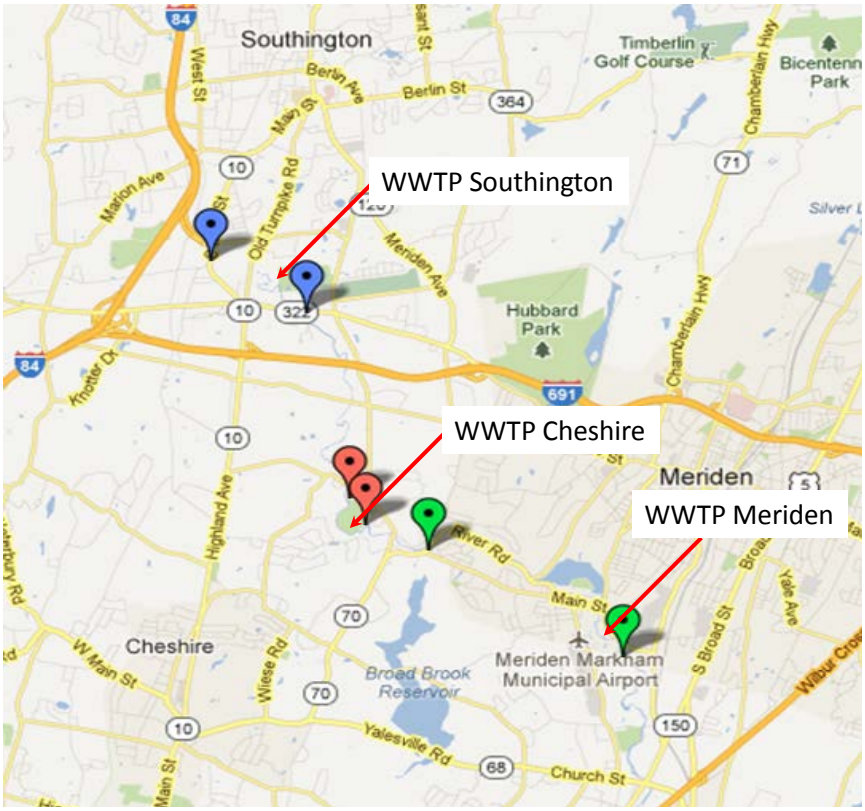

Figure 1: Collection sites at three waste water treatment plants (WWTP) in the Quinnipiac River.

Water Collection

Sampling of the effluent for each WWTP occurred at the same relative time during the day (10 AM-2 PM), which reduced temporal variability (Salgado et al., 2011). Also, the facilities were not sampled within 48 hours of a significant precipitation event (> 1" rain) to minimize any dilution in WWTPs that have combined sewer and storm water systems. A single composite 1-L grab sample was be collected from the main flow, to obtain as representative a sample as possible. Samples were placed in a cooler kept at <4°C, transported back to the Center for Environmental Sciences and Engineering at the University of Connecticut, and stored in a walk-in cooler until analyzed. From each sampling event, extra samples were analyzed for quality control, including field blanks, field duplicates and triplicates, and equipment blanks.

Chemical Analysis

MWWE samples were quantified for common pharmaceuticals using ultra-performance liquid chromatography, followed by tandem mass spectrometry (UPLC/MS/MS) according to EPA method 1694 (EPA, 2007). In summary, a 500 mL sample was acidified with hydrochloric acid, loaded onto a solid phase extraction (SPE) cartridge, the SPE was then eluted with methanol, followed by concentration with nitrogen. The sample is then reconstituted with methanol, and internal standards were added, and followed by a final SPE clean-up procedure before analysis by UPLC/MS/MS.

Results and Discussion:

A total of 58 samples were collected, including quality control samples, during three discrete sampling events from the Quinnipiac River on July 20th, September 25th, and December 2nd. Only eight of the compounds analyzed for were detected in samples collected from the Quinnipiac River (QR), with only one compound, Caffeine, detected in greater than 50% of the samples (Table 1). Sulfamethoxazole, an antibiotic, was the only other compound detected in greater than 40% of the samples collected and analyzed.

Table 1. PPCPs detected in water samples collected from the Quinnipiac River.

Compound	% Detected	Range (ug/L)
Acetaminophen	8.6%	ND-0.065
Caffeine	87.9%	ND-1.005
Metoprolol	13.8%	ND-0.241
Trimethoprim	25.9%	ND-0.248
Ciprofloxacin	5.2%	ND-0.700
Sulfamethoxazole	44.8%	ND-0.227
Ketoprofen	0.0%	ND
Propranolol	20.7%	ND-0.170
Diltiazem	24.1%	ND-0.064
Ibuprofen	0.0%	ND
Naproxen	0.0%	ND
Gemfibrozil	0.0%	ND
Triclosan	0.0%	ND
Diclofenac	0.0%	ND

For caffeine and sulfamethoxazole, the only compounds with greater than 40% detected, the upstream concentrations were significantly lower than the downstream concentrations (Figures 2 and 3). The downstream collections generally showed the highest concentrations at the collection location closest to the WWTP discharge, with concentrations decreasing as the further away from the discharge. The baseline concentrations for both caffeine and sulfamethoxazole, collected upstream of the discharge, significantly lower than the concentrations from the first collection point downstream of the inlet pipe, denoting that the WWTPs are sources of these two compounds. There were no detectible concentrations of any of the other monitored compounds from the upstream samples. The antibiotic trimethoprim was the only other compound that was detected in the first two downstream samples, but was not detected in the third through the fifth locations at any of the three WWTP sites (Table 2). Propanolol and diltiazem

were not detected upstream from the WWTP, but were only found in samples collected immediately downstream of the Cheshire (0.017 and 0.017 ug/L, respectively) and Meriden (0.094 and 0.043 ug/L, respectively) WWTPs. Acetaminophen was only detected immediately downstream of the Southington WWTP (0.035 ug/L) and was not detected upstream nor in the 2-5th samples collected downstream.

Table 2. Concentration of trimethoprim measured upstream and downstream from the WWTPs.

	Cheshire (ug/L)	Meriden (ug/L)	Southington (ug/L)
Upstream	Not Detected	Not Detected	Not Detected
1st location	0.136	0.176	0.105
2nd location	0.117	0.111	0.082

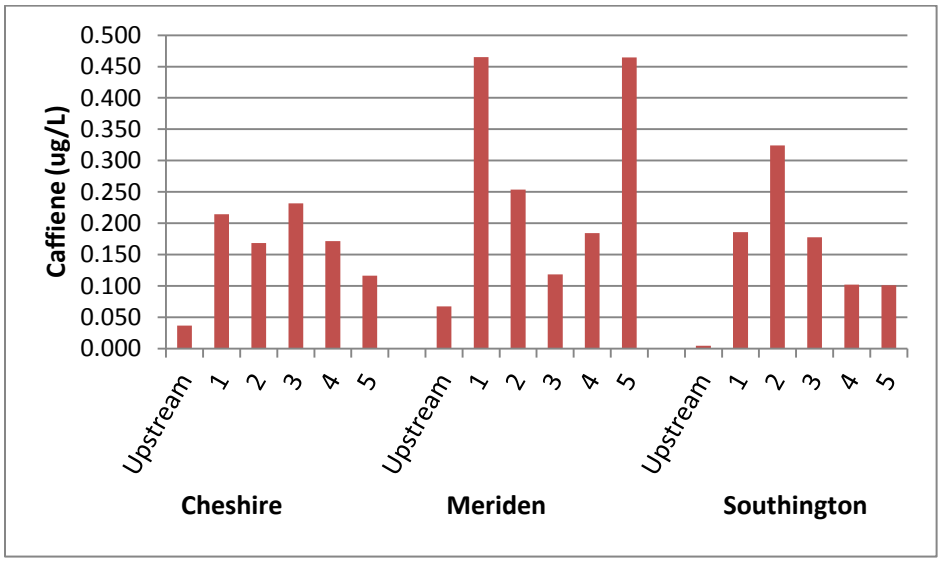

Figure 2. Mean caffeine concentrations measured upstream and the 5 downstream sites from each of the three WWTPs.

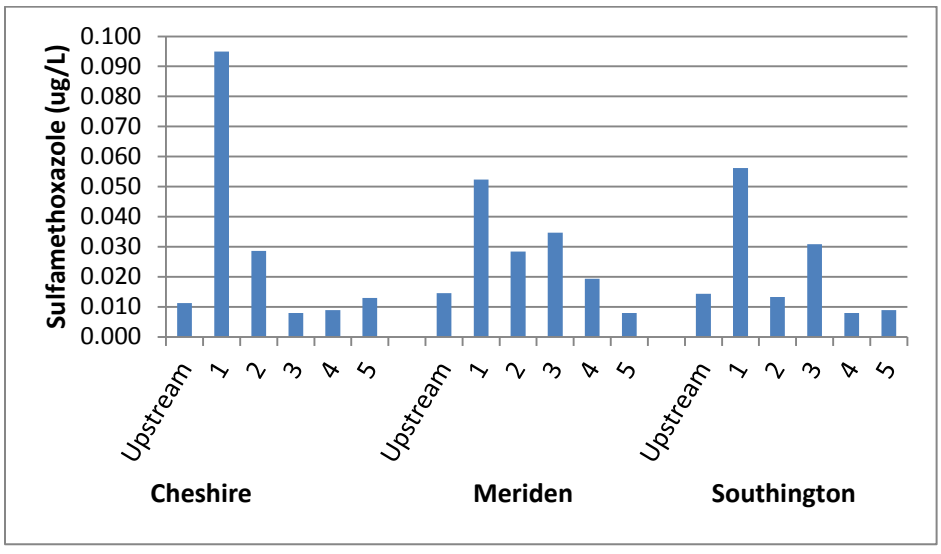

Figure 3. Mean sulfamethoxazole concentrations measured upstream and the 5 downstream sites from each of the three WWTPs.

There is minimal data on PPCPs in rivers in Connecticut, however USGS conducted a very small pilot study on organic compounds in the Farmington and Still Rivers (USGS, *unpublished data*), including several PPCPs, where they collected one sample upstream of the WWTP and one sample downstream. They did not examine the same compounds as this study, with the exception of triclosan, but found similar results for a few of the compounds measured. Several PPCPs were not detected upstream, but were detected immediately downstream of the WWTP at very low concentrations (0.02 – 0.06 g/L), including triclosan, several fragrances, cosmetics, and detergents.

The concentrations found in this study are extremely low (part per trillion) and infrequently found with the exception of caffeine and sulfamethoxazole. WWTPs are definitely sources of these types of compounds to the QR, but in all cases concentrations of each of these compounds decrease the further downstream from the WWTP input. In the case of trimethoprim the concentration decreased to below detection by the third monitoring location, approximately 3000m downstream, while for acetaminophen, propanolol and diltiazem they decreased to below detection level by the second monitoring location.

References:

Burkhardt-Holm, P. 2010. Endocrine disruptors and water quality: A state-of-the-art review. International Journal of Water Resources Development 26:477-493.

Dinizi, M. S., R. Mauricio, M. Petrovic, M. J. L. De Alda, L. Amaral, I. Peres, D. Barcelo, and F. Santana. 2010. Assessing the estrogenic potency in a Portuguese wastewater treatment plant using an integrated approach. Journal of Environmental Sciences-China 22:1613-1622.

Garcia-Reyero, N., C. M. Lavelle, B. L. Escalon, D. Martinovic, K. J. Kroll, P. W. Sorensen, and N. D. Denslow. 2011. Behavioral and genomic impacts of a wastewater effluent on the fathead minnow. Aquatic Toxicology 101:38-48.

Hotchkiss, A. K., C. V. Rider, C. R. Blystone, V. S. Wilson, P. C. Hartig, G. T. Ankley, P. M. Foster, C. L. Gray, and L. E. Gray. 2008. Fifteen years after "Wingspread" - Environmental endocrine disrupters and human and wildlife health: Where we are today and where we need to go. Toxicological Sciences 105:235-259.

Hutchinson, T. H., G. T. Ankley, H. Segner, and C. R. Tyler. 2006. Screening and testing for endocrine disruption in fish - Biomarkers as "signposts," not "traffic lights," in risk assessment. Environmental Health Perspectives 114:106-114.

Ings, J. S., M. R. Servos, and M. M. Vijayan. 2011. Hepatic Transcriptomics and Protein Expression in Rainbow Trout Exposed to Municipal Wastewater Effluent. Environmental Science & Technology 45:2368-2376.

Jobling, S., N. Beresford, M. Nolan, T. Rodgers-Gray, G. C. Brighty, J. P. Sumpter, and C. R. Tyler. 2002a. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biology of Reproduction 66:272-281.

Jobling, S., S. Coey, J. G. Whitmore, D. E. Kime, K. J. W. Van Look, B. G. Mcallister, N. Beresford, A. C. Henshaw, G. Brighty, C. R. Tyler, and J. P. Sumpter. 2002b. Wild intersex roach (Rutilus rutilus) have reduced fertility. Biology of Reproduction 67:515-524.

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

Ma, T. W., X. Q. Wan, Q. H. Huang, Z. J. Wang, and J. K. Liu. 2005. Biomarker responses and reproductive toxicity of the effluent from a Chinese large sewage treatment plant in Japanese medaka (*Oryzias latipes*). Chemosphere 59:281-288.