Evaluating Effects of Bioactive Contaminants Associated with Waste Water Treatment Plant Effluent Discharge to the South Platte River

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Introduction

Previous studies have detected numerous organic contaminants – including biocides and pharmaceuticals – in the South Platte River near Denver, CO, at concentrations that rank among the highest in the nation. Regarded as contaminants of emerging concern (CECs), some of these compounds can disturb biological pathways in exposed species. Bi-monthly sampling and analysis of surface water samples in 2018, documented increased concentrations of multiple CECs downstream of a waste water treatment plant (WWTP). In vitro bioassays conducted on the same samples detected estrogen receptor (ER; 2.9-88ng/L E2-EQ) and glucocorticoid receptor (GR; 17-117 ng/L Dex-EQ)-mediated activities downstream of the WWTP. Peroxisome proliferator activated receptor gamma (PPARɣ)-mediated activities were also detected using a multifactorial Attagene assay, but not a targeted PPARɣ transcriptional activation assay. In addition to bi-monthly monitoring, fathead minnows (*Pimphales promelas*) were exposed in situ for five days at six locations upstream and downstream of the waste water discharge in August of 2018. Despite the ER-mediated biological activity detected in vitro, no significant differences in the expression of male hepatic vitellogenin were found between sites. Consistent with the general lack of detectable PPARɣ-regulated activity, there were no significant effects on PPARɣ-related gene expression in the adipose tissue of females. No site-related differences in GR-related gene expression were detected in females, despite GR-activity in the in vitro assays, suggesting either limited bioavailability or limited potency of GR-active contaminants in vivo.

Objectives

Returning to the S. Platte in 2019, allowed research teams to confirm results seen in 2018, and solidify the two objectives of field work:

1. Characterize the targeted spatial and temporal distribution of GR and PPARɣ-related bioactivity along the river using in vitro bioassays.
2. Analyze GR and PPAR-related gene expression in fathead minnows exposed *in situ* in the S. Platte River to gather evidence concerning potential in vivo impacts in relation to the biological activity detected in vitro.

Recovering caged fish at the Clear Creek Reference site near Golden, CO

A typical caged fish set up in Clear Creek near Denver, CO. Two PVC cages held 12 fathead minnows each. Composite, time integrated, water samples were collected via a co-located autosampler.

The 2019 research team

U.S. Environmental Protection Agency
Office of Research and Development

Methodology

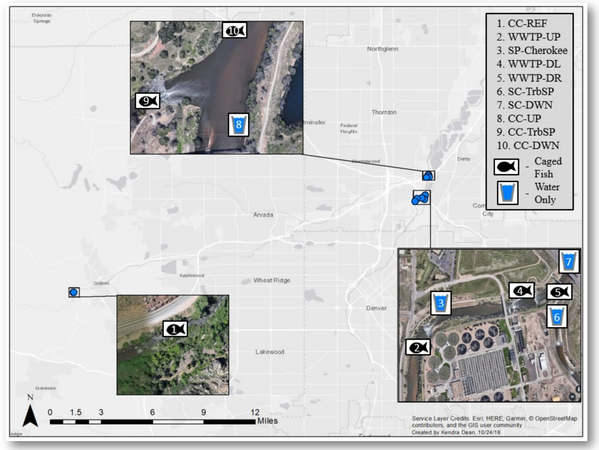

Figure 1. Caged fish locations for the 2019 exposure. Bi-monthly grab samples of surface water were taken at all sites.

Left: Waste water effluent entering the South Platte; Right: Crew working on fish deployments and water quality measurements

Caged Fish

- 24 Fathead minnows were caged using two cages of six males and six females per cage at six sites and a lab control tank for five days of exposure. Deployments were staggered over two days.
- Autosamplers collected water samples at caged fish sites every 15 minutes for the length of the exposure.

Solid Phase Extraction

- Water samples were held on ice and processed within 72 hours of sampling.
- SPE performed using Oasis HLB 5cc 200mg LP Glass Cartridges.
- Samples were eluted, evaporated and reconstituted at 1000x in DMSO for in vitro bioassays.

PPCP and Pesticide Analytics

- Water samples were analyzed by EPA Region 8 for 200 PPCPs (pharmaceuticals and personal care products) and pesticides using LC/MS/MS.

In vitro Bioassay

- Indigo Biosciences Human Glucocorticoid Receptor Assay System
- Indigo Biosciences Human Peroxisome Proliferator-Activated Receptor Gamma Reporter Assay System
- T47D Estrogen Receptor Agonist Assay using methodology adapted from Wilson et al (2004)

mRNA Analysis

- Extraction of hepatic total RNA using Qiagen RNeasy Mini Kit.
- qPCR using SYBR green and Taqman reagents completed on male hepatic RNA for vitellogenin, cyp1a1, and GR-related genes; nr3c1, sgk1, and nfkbiaa.
- qPCR analyzed using a one-way ANOVA, followed by a Duncan's post-hoc test, differences considered significant at $p < 0.05$

Results

Caged Fish Exposure

Recovery Data	
Location	Caged Fish
WWTP Upstream	13M/11F
WWTP Downstream Right	12M/10F
WWTP Downstream Left	13M/10F
Clear Creek Tributary Wth S. Platte	15M/9F
Clear Creek Downstream	12M/12F
Clear Creek Reference	13M/11F
CSU Lab Control	15M/9F

Figure 2. Large differences in male to female ratio due to immature males, sex was confirmed by gonad post exposure.

Male and female fathead minnows

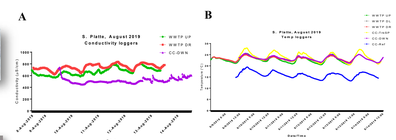

Figure 3. (A) Conductivity loggers placed at caged fish sites show a decrease in conductivity after Clear Creek enters the S. Platte. **(B)** Temperature loggers show a substantial difference in temperature at the Clear Creek reference site.

In Vitro Bioassay

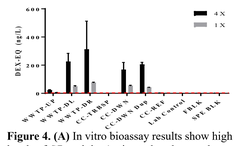

Figure 4. (A) In vitro bioassay results show high levels of GR activity (estimated as dexamethasone equivalents) downstream of the WWTP.

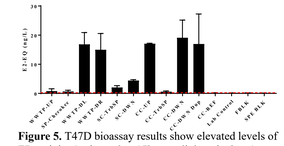

Figure 5. T47D bioassay results show elevated levels of ER activity (estimated as 17b-estradiol equivalents) downstream of the WWTP.

Post-exposure collection of mucus, plasma and muscle tissue

mRNA qPCR

Gene-specific Targets	
ER-Related	
vtg	Vitellogenin
AhR-Related	
cyp1a1	Cytochrome P450
GR-Related	
nr3c1	Glucocorticoid Receptor
sgk1	Serum/glucocorticoid regulated kinase 1
nfkbiaa	NF-kappaB inhibitor alpha-like protein A

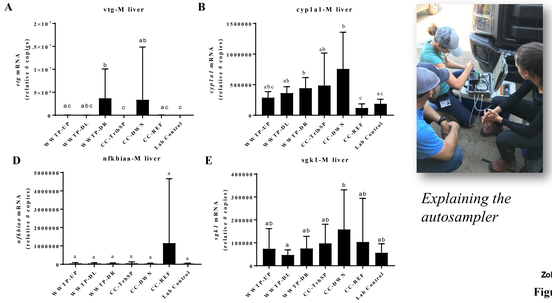

Figure 6. (A) qPCR results show elevated hepatic vitellogenin directly downstream of the WWTP. **(B)** There was no significant differences between upstream and downstream of the WWTP in Cyp1a1. **(C, D, E)** No significant differences in GR-related gene expression occurred.

Explaining the autosampler

PPCP & Pesticides

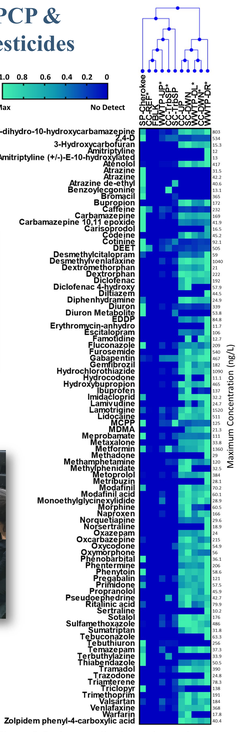

Figure 7. Ratio normalization to the maximum concentration of each respective chemical shows elevated concentrations of CECs downstream of the WWTP. No detects are labeled as "0".

Conclusions & Acknowledgements

- The S. Platte River continues to be an area of great interest; containing high concentrations of CECs (Fig. 7) and elevated levels of GR (Fig. 4; 5-75 ng/L Dex-EQ) and ER (Fig. 5; 0.78-16 ng/L E2-EQ) bioactivity, as indicated by in vitro bioassay.
- Based on two consecutive years of caged fish studies, the concentrations of ER- and GR-bioactive chemicals did not appear to elicit in vivo effects on gene expression. Results suggest the bioactive compounds may have limited bioavailability, are rapidly metabolized, and/or have insufficient cumulative potency to provoke short term effects in vivo.
- Further in vivo and in vitro results are still pending.

Wilson, VS, Bobseine, K, Gray, L.E.J. 2004. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen agonist and antagonists. *Toxicol Sci* 81; 69-77.

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