Environmental Pollution 239 (2018) 706–713

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Evaluation of targeted and untargeted effects-based monitoring tools to assess impacts of contaminants of emerging concern on fish in the South Platte River, CO☆

Drew R. Ekman [a,*], Kristen Keteles [b,**], Jon Beihoffer [b], Jenna E. Cavallin [c], Kenneth Dahlin [d], John M. Davis [a], Aaron Jastrow [e], James M. Lazorchak [f], Marc A. Mills [g], Mark Murphy [d], David Nguyen [d], Alan M. Vajda [h], Daniel L. Villeneuve [i], Dana L. Winkelman [j], Timothy W. Collette [a]

[a] U.S. EPA, National Exposure Research Laboratory, 960 College Station Rd., Athens, GA 30605, USA
[b] U.S. EPA, National Enforcement Investigations Center, Denver Federal Center Building 25, E-3, Denver, CO 80225, USA
[c] Badger Technical Services, U.S. EPA, Duluth, MN 55804, USA
[d] U.S. EPA, Region 8, 1595 Wynkoop St., Denver, CO 80202, USA
[e] U.S. EPA, Region 5, Ralph Metcalfe Federal Building, 77 West Jackson Blvd., Chicago, IL 60605, USA
[f] U.S. EPA, National Exposure Research Laboratory, 26 Martin Luther King Blvd., Cincinnati, OH 45268, USA
[g] U.S. EPA, National Risk Management Research Laboratory, 26 Martin Luther King Blvd., Cincinnati, OH 45268, USA
[h] University of Colorado Denver, Department of Integrative Biology, Campus Box 171, P.O. Box 173364, Denver, CO 80217, USA
[i] U.S. EPA, National Health and Environmental Effects Research Laboratory, Duluth, MN 55804, USA
[j] U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit, Department of Fish, Wildlife and Conservation Biology, 201 J.V.K. Wagar Building, 1484 Campus Delivery, Colorado State University, Fort Collins, CO 80523, USA

A R T I C L E I N F O

Article history:
Received 2 January 2018
Received in revised form
27 March 2018
Accepted 11 April 2018

Keywords:
Effects-based monitoring
Contaminants of emerging concern
Estrogens
Metabolomics
Fish
Vitellogenin

A B S T R A C T

Rivers in the arid Western United States face increasing influences from anthropogenic contaminants due to population growth, urbanization, and drought. To better understand and more effectively track the impacts of these contaminants, biologically-based monitoring tools are increasingly being used to complement routine chemical monitoring. This study was initiated to assess the ability of both targeted and untargeted biologically-based monitoring tools to discriminate impacts of two adjacent wastewater treatment plants (WWTPs) on Colorado's South Platte River. A cell-based estrogen assay (*in vitro*, targeted) determined that water samples collected downstream of the larger of the two WWTPs displayed considerable estrogenic activity in its two separate effluent streams. Hepatic vitellogenin mRNA expression (*in vivo*, targeted) and NMR-based metabolomic analyses (*in vivo*, untargeted) from caged male fathead minnows also suggested estrogenic activity downstream of the larger WWTP, but detected significant differences in responses from its two effluent streams. The metabolomic results suggested that these differences were associated with oxidative stress levels. Finally, partial least squares regression was used to explore linkages between the metabolomics responses and the chemical contaminants that were detected at the sites. This analysis, along with univariate statistical approaches, identified significant covariance between the biological endpoints and estrone concentrations, suggesting the importance of this contaminant and recommending increased focus on its presence in the environment. These results underscore the benefits of a combined targeted and untargeted biologically-based monitoring

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author. U.S. EPA, National Exposure Research Laboratory, 960 College Station Rd., Athens, GA 30605, USA.
** Corresponding author. U.S. EPA, National Enforcement Investigations Center, Denver Federal Center Building 25, E-3, Denver, CO 80202 USA.
E-mail addresses: ekman.drew@epa.gov (D.R. Ekman), keteles.kristen@epa.gov (K. Keteles), Beihoffer.jon@epa.gov (J. Beihoffer), cavallin.jenna@epa.gov (J.E. Cavallin), dahlin.kenneth@epa.gov (K. Dahlin), davis.john@epa.gov (J.M. Davis), jastrow.aaron@epa.gov (A. Jastrow), lazorchak.jim@epa.gov (J.M. Lazorchak), mills.marc@epa.gov (M.A. Mills), murphy.mark@epa.gov (M. Murphy), nguyen.david@epa.gov (D. Nguyen), alan.vajda@ucdenver.edu (A.M. Vajda), villeneuve.dan@epa.gov (D.L. Villeneuve), dana.winkelman@colostate.edu (D.L. Winkelman), collette.tim@epa.gov (T.W. Collette).

https://doi.org/10.1016/j.envpol.2018.04.054
0269-7491/Published by Elsevier Ltd.

strategy when used alongside contaminant monitoring to more effectively assess ecological impacts of exposures to complex mixtures in surface waters.

1. Introduction

There is growing recognition that chemical monitoring alone does not provide sufficient information to adequately assess the risks from anthropogenic chemicals in the natural environment. This is particularly true for contaminants of emerging concern (CECs), some of which have been shown to cause endocrine disruption in laboratory experiments at levels below their analytical detection limits (Parrott and Blunt, 2005). Many CECs, including widely-used pharmaceuticals and personal care products, are not always effectively removed by wastewater treatment plants (WWTPs) (Ternes, 1998); thus, they occur in the aquatic environment as constituents of complex mixtures whose composition changes over time and location. It is important for risk assessors to better understand the cumulative impacts of these mixtures, and this information cannot be provided by periodic chemical monitoring alone.

The need to understand the biological impacts of CECs is particularly pressing in the arid Western region of the U.S., where the flow in many rivers can be dominated by WWTP effluent (Patten, 1998; Woodling et al., 2006), particularly in years with low snowfall. In these situations, the levels of CECs and other stressors can increase dramatically, potentially producing adverse impacts on fish and other aquatic wildlife. For these reasons, we have undertaken a multi-year integrated biological and chemical field investigation, where fathead minnows (FHM; *Pimephales promelas*) were cage-deployed for five days below and above two urban WWTPs, and also at a reference site in the South Platte River watershed in Colorado, during low flow periods. A primary goal of these studies was to test the efficacy of both targeted and untargeted effects-based monitoring (EBM) tools for characterizing potential ecological impacts. Because the sites were expected to be estrogenic, vitellogenin (vtg) mRNA abundance in the livers of male FHM was examined. We also applied a recombinant *in vitro* bioassay using T47D-KBluc cells to quantify the estrogenic activity of water collected from the various study locations (Wilson et al., 2004). In addition to these targeted approaches focused on estrogenic activity, an untargeted metabolomics analysis that could detect perturbation of a broader range of biochemical pathways was employed.

Here we present results from these analyses for the field study conducted in September 2013. The exposure scenario from this study provided a rich opportunity to evaluate these EBM approaches for assessing the impacts of CECs. For example, one of the WWTPs serves a relatively smaller population and also uses biological nutrient removal prior to discharge. The other WWTP serves a much larger population and employs less advanced treatment technology (at the time this study was conducted). In addition, this WWTP discharges using two separate outfalls that receive different levels of influent and also utilize different levels of treatment. Our deployments were designed to test whether these EBM tools would discriminate biological activities associated with these different treatment scenarios.

In addition to carrying out EBM, chemical monitoring was conducted using water collected at all of these sites. Analyses for CECs (pharmaceuticals, personal care products, pesticides, etc.), common wastewater indicators, steroid hormones, and inorganic chemicals were performed. In addition, we measured a group of water quality parameters, which, if out of normal ranges, could serve as non-chemical stressors for fish (e.g., pH, temperature, dissolved oxygen, etc.). Finally, partial least squares (PLS) regression was used to determine the extent of covariance between these chemical and water quality measurements and the metabolite profiles. This approach is effective at prioritizing stressors according to the extent to which they are eliciting biological responses at a given site (Davis et al., 2016). Such information could help inform a wide range of environmental monitoring programs, and may allow improved decision making regarding the prioritization of contaminant monitoring and remediation efforts.

2. Materials and methods

2.1. Study design

Two WWTPs that release effluent into the South Platte River were selected for this study (Fig. 1). Both WWTPs (W1 and W2) used primary and secondary treatment (activated sludge), solids removal, and disinfection with chlorine. Site W1 received approximately 18 million gallons per day (MGD) and has aerobic nitrifying trickle filters and a de-nitrification process for nutrient removal. W2 received approximately 100 MGD and has two treatment complexes (South and North). At the time of this study, the North complex (W2-N) received twice the amount of influent as the South complex (W2-S). W2-N treated influent nitrogen using nitrification whereas W2-S employed ammonification.

To assess fish responses to the effluents from these WWTPs, sexually-mature (6-8 month-old) male fathead minnows (*Pimephales promelas*) were deployed in cages in proximity of the two WWTPs, and at a relatively unimpacted reference site (REF). Specifically, after acclimation (approximately 48hrs. following overnight shipment from the U.S. EPA Aquatic Research Laboratory in Cincinnati, OH), fish were deployed at W1, W2-UP, W2-N, W2-S, and REF using PVC cages (15 fish per cage; one cage per site) with mesh on both ends (Fig. 1). Note that previous chemical and hydrologic analyses at these sites have shown that very little mixing of the effluents occurs between W2-N and W2-S, and that REF was located in a large, relatively unimpacted, creek that flows into the South Platte (Fig. 1). There are no major WWTPs upstream of REF, and no evidence of estrogenic endocrine disruption at this site (Woodling et al., 2006); thus it was used as the "control" for evaluating the impact from the WWTPs in that it takes into account any stresses on the fish as a result of being cage-deployed, and it most closely reproduces the (non-chemical) field conditions of the various test sites. Deployments occurred from September 5th through September 10th, 2013 during seasonal low-flow conditions. Flow data at each site including the percent contribution to total river flow from the W2-N and W2-S effluent streams (data for the W1 percent contributions were not available) are provided in the Supporting Information (Table S1). A storm event occurred on the last day of the deployment. However, fish were removed from the sites and the five-day grab samples were taken prior to increases in flow.