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Assessment of biomarkers for contaminants of emerging concern on aquatic organisms downstream of a municipal wastewater discharge☆

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HIGHLIGHTS

- Fish and mussels were caged up- and down-stream of wastewater treatment discharge.
- The aim was to determine the best biomarkers for contaminants of emerging concern.
- Several emerging contaminants were elevated downstream of wastewater discharge.
- Biological effects were measured downstream of wastewater discharge.
- Biomarkers of emerging contaminants were elevated in fish exposed to effluent.

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ABSTRACT

Contaminants of emerging concern (CECs), including pharmaceuticals, personal care products and estrogens, are detected in wastewater treatment plant (WWTP) discharges. However, analytical monitoring of wastewater and surface water does not indicate whether CECs are affecting the organisms downstream. In this study, fathead minnows (*Pimephales promelas*) and freshwater mussels *Pyganodon grandis* Say, 1829 (synonym: *Anodonta grandis* Say, 1829) were caged for 4 weeks in the North Saskatchewan River, upstream and downstream of the discharge from the WWTP that serves the Edmonton, AB, Canada. Passive samplers deployed indicated that concentrations of pharmaceuticals, personal care products, an estrogen (estrone) and an androgen (androstenedione) were elevated at sites downstream of the WWTP discharge. Several biomarkers of exposure were significantly altered in the tissues of caged fathead minnows and freshwater mussels relative to the upstream

Abbreviations: AHTN, Tonalide® synthetic musk; ALP, alkali labile phosphate; AND, androstenedione; BCA, bicinchoninic acid; BFC, benzoxy-4-trifluoromethyl-coumarin; BSA, bovine serum albumin; CAT, catalase; CBZ, carbamazepine; CEC, contaminants of emerging concern; CFU, colony forming units; CYP1A1, cytochrome P450 1A1; CYP3A, cytochrome P450 3A; DO, dissolved oxygen; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid) or Ellman's reagent; EC, electrical conductivity; EDCs, endocrine disrupting compounds; EDTA, ethylenediaminetetraacetic acid; EPCOR, Edmonton Power Corporation; EROD, ethoxyresorufin-O-deethylase; EST, Estrone; GC, gas chromatography; GMZ, gemfibrozil; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-*S*-transferase; HFC, 7-hydroxy-4-trifluoromethylcoumarin; HHCB, Galaxolide® synthetic musk; IBU, ibuprofen; KPB, potassium phosphate buffer; LC, liquid chromatography; LOD, below detection limits; LOQ, below limits of quantitation; LPO, lipid peroxidation; MDA, malonaldehyde; MOPS, 3-(N-morpholino) propanesulfonic acid; MS, mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; NSR, North Saskatchewan River; POCIS, polar organic contaminants integrated sampler; PPCPs, pharmaceuticals and personal care products; SDS, sodium dodecyl sulfate; SI, supplementary information; SMX, sulfamethoxazole; SOD, superoxide dismutase; SPMD, semi-permeable membrane device; TBARS, THIOBARBITURIC acid reactive substances; TCS, triclosan; Temp, water temperature; TGSH, TOTAL glutathione; TPM, trimethoprim; Vtg, vitellogenin; WWTP, waste water treatment plant.

☆ Roles of authors: EJJ conducted the field research and drew all figures except for the multivariate analyses; GGG was responsible for field research, logistics, site selection, tissue sample collection and initial processing; AASM did the multivariate analyses and corresponding figures; PG, AASM and MG performed analysis of oxidative stress for mussel tissues; GGG analyzed VTG expression, TWM and AM measured glutathione and oxidative stress activity and mRNA, MG and JW performed BFC and EROD analysis in fathead minnows. CDM and TS performed chemical analyses of pharmaceuticals in water. CDM was lead PI on the project. All authors played a role in writing and editing of the MS.

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reference sites. Biomarkers altered in fish included induction of CYP3A metabolism, an increase in vitellogenin (Vtg) gene expression in male minnows, elevated ratios of oxidized to total glutathione (i.e. GSSG/TGSH), and an increase in the activity of antioxidant enzymes (i.e. glutathione reductase, glutathione-*S*-transferase). In mussels, there were no significant changes in biomarkers of oxidative stress and the levels of Vtg-like proteins were reduced, not elevated, indicating a generalized stress response. Immune function was altered in mussels, as indicated by elevated lysosomal activity per hemocyte in *P. grandis* caged closest to the wastewater discharge. This immune response may be due to exposure to bacterial pathogens in the wastewater. Multivariate analysis indicated a response to the CECs Carbamazepine (CBZ) and Trimethoprim (TPM). Overall, these data indicate that there is a 1 km zone of impact for aquatic organisms downstream of WWTP discharge. However, multiple stressors in municipal wastewater make measurement and interpretation of impact of CECs difficult since water temperature, conductivity and bacteria are also inducing biomarker responses in both fish and mussels.

1. Introduction

Contaminants of emerging concern (CECs) have been detected in discharges from municipal wastewater treatment plants (WWTPs) and these include pharmaceuticals, personal care products and estrogens (Anderson et al., 2003; Metcalfe et al., 2003; Carballa et al., 2004; Servos et al., 2005; Lishman et al., 2006; Chen et al., 2006; Brun et al., 2006; Kerr et al., 2008; Yargeau et al., 2008; Metcalfe et al., 2010). Direct water analysis monitoring of CECs in receiving waters is expensive and does not provide direct information on the biological impacts of exposure to these contaminants (Hecker and Hollett, 2009). Monitoring biomarker expression in aquatic organisms can be an effective way to evaluate whether CECs in wastewater effluents affect the receiving environment. Biomonitoring has many advantages: it integrates the response temporally, accounts for bioavailability and more directly assesses exposure under ambient conditions (Schmitt and Dethloff, 2000). In rivers, caging appropriate organisms provides consistent and reliable sample numbers with a defined exposure.

Widespread sexual disruption and reduced fertility in wild fish was studied and documented downstream of wastewater effluents (Jobling et al., 1998, 2002). More recent studies of fish endocrine responses to wastewater include intersex (occurrence of ova-testes) (Bahamonde et al., 2013), altered gene expression and physiology (altered steroid production) (Bahamonde et al., 2014; Tanna et al., 2013; Tetreault et al., 2011). These responses, including induction of vitellogenin (Vtg) are consistent with the exposure of fish to an environmental estrogen (17α-ethinylestradiol) that led to a population collapse of fathead minnows (*Pimephales promelas*) during a whole lake experiment (Kidd et al., 2007, 2014). Larsson et al. (1999) found distinct induction of Vtg in caged juvenile rainbow trout (*Oncorhynchus mykiss*) downstream of Swedish sewage treatment works. Harries et al. (1997) found estrogenic activity in caged rainbow trout downstream of sewage treatment works in the UK. Ings et al. (2011, 2012) observed changes in gene expression and stress responses in juvenile rainbow trout exposed to a tertiary treated wastewater plant in Ontario. Cazenave et al. (2014) observed activation of antioxidant enzymes and lipid oxidative damage, among other biomarkers of fish health in a neotropical fish species caged downstream of wastewater discharges in Argentina.

In mussels, likewise, biomarker responses to CECs have been observed. Gagné et al. (2004) observed several biomarker responses in freshwater mussels (*Elliptio complanata*) caged for a year downstream of a municipal WWTP that indicated exposure to both estrogenic and serotonergic compounds. In marine mussels (*Mytilus edulis*) collected from intertidal regions impacted by municipal wastewater, biomarker responses included reduced levels of Vtg-like proteins in females and elevated lipid content in males (Hellou et al., 2003). Gillis et al. (2014a) observed induction of oxidative stress biomarkers and modulation of immune function in freshwater mussels (*Lasmigona costata*) caged in a river influenced by wastewater. Bianchi et al. (2014) found that in the freshwater mussel, *Diplodon chilensis*, gGST and gCAT were suitable biomarkers for high fecal bacteria pollution.

In a survey of pharmaceuticals and endocrine disrupting compounds (EDCs) in Alberta, Canada, measurable concentrations of a number of EDCs were identified in the treated effluents from the Gold Bar WWTP (serves the City of Edmonton). These EDCs included estrone, bisphenol A and nonyphenol (Sosiak and Hebben, 2005). Therefore, aquatic organisms in the North Saskatchewan River (NSR) downstream of this WWTP discharge could be impacted by exposure to EDCs and other contaminants released in the treated effluent. However, sampling of fish and mussels at this site would be very difficult because of the nature of the plume and river volume and velocity. Caging studies are particularly useful because natural exposure gradients can be studied by placing cages at varying distances downstream from the source of contamination and upstream sites can be used for reference locations. This strategy has been used in variety of studies to examine the effects environmental contamination and responses in organisms to exposure to municipal wastewaters (e.g. Stien et al., 1998) seldom, however, with both fish and bivalves exposed at the same time.

In this study, we used an essentially sedentary invertebrate species (freshwater mussel) and a highly mobile vertebrate (fathead minnow) to increase the likelihood of identifying sensitive and robust biomarkers of CECs downstream of the discharge from the Gold Bar WWTP. Fish and mussels were caged for 4 weeks at sites over an exposure gradient downstream of the WWTP discharge, as well as at two upstream reference sites. Biological effects in caged fish and mussels were assessed using biomarkers of oxidative stress, immunomodulation and endocrine disruption, as well as induction of the microsomal Phase I detoxification enzymes, CYP3A and CYP1A1, two enzyme activities involved in metabolism of pharmaceuticals, polycyclic aromatic hydrocarbons and coplanar polychlorinated biphenyls. In addition, passive samplers were deployed in the river at the fish and mussel caging sites to assess the distributions of selected CECs in the receiving waters.

2. Materials and methods

2.1. Study sites

The study sites were located upstream and downstream of the effluent discharge from the Edmonton Power Corporation (EPCOR) Gold Bar WWTP that serves the city of Edmonton. The WWTP serves approximately 730,000 people and has an average daily flow of 255 ML/day. It has tertiary treatment facilities for biological nutrient removal and UV disinfection. Full operating conditions can be found at: http://corp.epcor.com/watersolutions/operations/edmonton/goldbar/pages/goldbar-wastewater-treatment-plant.aspx.

The satellite imagery of the study area obtained using Google Earth (taken in 14 Sep 2008) showed a dark effluent plume on the south side of the river that extended at least 10 km downstream of the WWTP discharge. Two reference sites, NSR1 and NSR2, were established on the south side of the river, 1.25 and 1.10 km, respectively upstream of the WWTP discharge. There are no WWTPs upstream of the reference sites, only stormwater inputs. Downstream of the WWTP discharge