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# Contaminants of emerging concern presence and adverse effects in fish: A case study in the Laurentian Great Lakes☆

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The Laurentian Great Lakes are a valuable natural resource that is affected by contaminants of emerging concern (CECs), including sex steroid hormones, personal care products, pharmaceuticals, industrial chemicals, and new generation pesticides. However, little is known about the fate and biological effects of CECs in tributaries to the Great Lakes. In the current study, 16 sites on three rivers in the Great Lakes basin (Fox, Cuyahoga, and Raquette Rivers) were assessed for CEC presence using polar organic chemical integrative samplers (POCIS) and grab water samplers. Biological activity was assessed through a combination of *in vitro* bioassays (focused on estrogenic activity) and *in vivo* assays with larval fathead minnows. In addition, resident sunfish, largemouth bass, and white suckers were assessed for changes in biological endpoints associated with CEC exposure. CECs were present in all water samples and POCIS extracts. A total of 111 and 97 chemicals were detected in at least one water sample and POCIS extract, respectively. Known estrogenic chemicals were detected in water samples at all 16 sites and in POCIS extracts at 13 sites. Most sites elicited estrogenic activity in bioassays. Ranking sites and rivers based on water chemistry, POCIS chemistry, or total *in vitro* estrogenicity produced comparable patterns with the Cuyahoga River ranking as most and the Raquette River as least affected by CECs. Changes in biological responses grouped according to physiological processes, and differed between species but not sex. The Fox and Cuyahoga Rivers often had significantly different patterns in biological response Our study supports the need for multiple lines of evidence and provides a framework to assess CEC presence and effects in fish in the Laurentian Great Lakes basin.



## 1. Introduction

The Laurentian Great Lakes are the largest surface freshwater system in the world and are host to a dynamic ecosystem. Over the past 200 years, the Great Lakes basin has been significantly affected by urban and agricultural development, commercial and recreational fishing, arrival of invasive species, and the physical modification of the watershed. While legacy contamination has been recognized as a limitation to ecosystem sustainability (Bowerman et al., 1995; Crane et al., 2000; Matthiessen and Law, 2002), the relative effects of contaminants of emerging concern (CECs) is insufficiently understood. Multiple studies have reported the omnipresence of CECs, including sex steroid hormones,

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pharmaceuticals, personal care products and industrial chemicals, in freshwater ecosystems (Kolpin et al., 2002; Metcalfe et al., 2003; Gilliom et al., 2006; Barber et al., 2015; Baldwin et al., 2016; Choy et al., 2017). Additionally, the effects of exposure to some of these contaminants have been studied *in vitro* with documented causal linkages to adverse effects in exposed biota (Martinovic et al., 2007; Brodin et al., 2013; Leonard et al., 2017). However, questions remain regarding the potential effects of CEC exposure on resident fish populations and other natural resources. Therefore, the objective of the current study was to determine if fish in the Great Lakes basin are being affected by CEC exposure.

In the current study, two sampling methods were used to evaluate the presence and distribution of CECs in three rivers in the Great Lakes basin, grab water samples and passive samplers. While grab water samples, a common CEC sampling method, provide a measure of chemical concentration, the information is limited to the content of a given parcel of water at a particular moment in time. As a result, it is difficult to use this method to ascertain the variability in the presence of CECs. Passive samplers, such as Polar Organic Chemical Integrative Samplers (POCIS), are an alternative for assessing the presence of CECs (Alvarez et al., 2007; Zhang et al., 2008; Metcalfe et al., 2014). POCIS allow hydrophilic chemicals to permeate the sampler membrane and be taken up by a sorptive sequestering medium over a prolonged exposure period, thus providing a time-integrated record of CEC presence (Alvarez, 2010). However, the information obtained from these samplers is not readily translated to environmental concentrations of detected compounds and also excludes hydrophobic compounds. Though, when combined, these two methods allow for a comprehensive assessment of presence and concentration of CECs in three rivers in the Great Lakes basin.

In addition to instrumental analytical techniques for identifying the presence of CECs, complementary measurements using *in vitro* cell-based assays may provide information regarding the biological activity of collected samples (e.g., Martinovic-Weigelt et al., 2013; Cavallin et al., 2014; Baldigo et al., 2015). These techniques integrate all CECs and other contaminants (both known and unknown) with a common molecular initiating event (e.g., activation of a receptor). In this study, the T47D-KBluc *in vitro* cell line (a stably transfected human breast cancer cell line with an ERα luciferase-reporter gene construct) was used to assess total estrogenicity in water samples. These results were then compared to estrogenicity summation techniques applied to the analytical results. Together, these techniques allow a more thorough evaluation of a subset of estrogenic CECs.

Similar to chemical evaluation, a variety of laboratory and field assessment techniques have been developed to examine the effects of CEC exposure on organisms. Exposure of biota to CECs *in vivo* have had documented changes in biologic indices (Ashfield et al., 1998; Huggett et al., 2002; Conners et al., 2009), morphometric endpoints (Panter et al., 1998; van Aerle et al., 2002; Sowers et al., 2009), physiological parameters (Thompson et al., 2000; Hemmer et al., 2001; Teles et al., 2004) and behavioral characteristics (Nassef et al., 2010; Hazelton et al., 2013; Weinberger and Klaper, 2014). A limitation of many of the aforementioned studies is their focus on a single compound (or simple mixture) that may not capture biological responses to complex chemical mixtures representative of conditions in natural aquatic systems.

While *in situ* studies are able to document effects as a result of exposure to complex mixtures, it is often difficult to ascertain whether the observed effects are causally linked to CEC exposure. *In situ* studies have focused on resident fish populations downstream of point sources, such as wastewater treatment plants (WWTP) and pulp and paper mill effluents, documenting detrimental changes to biological indices (Sandstrom et al., 1988; McMaster et al., 1991; Tetreault et al., 2011). In the context of point source investigations, alterations to gonad (Jobling et al., 1998; Kavanagh et al., 2004; Vajda et al., 2008) and liver (Andersson et al., 1988; Simpson et al., 2000; Schlacher et al., 2007; Kahl et al., 2014) structure and function are particularly noteworthy as they may have immediate implications for reproductive fitness and detoxification, respectively. In addition, wastewater effluent exposure has been linked to adverse physiological effects (McMaster et al., 1991; Van der Kraak et al., 1992; Burki et al., 2006). A few studies have used environmental water samples collected downstream of point sources to assess behavioral changes in laboratory settings (Schoenfuss et al., 2002; Martinovic et al., 2007; Garcia-Reyero et al., 2011; Kovacs et al., 2011; Minarik et al., 2014; Martel et al., 2017). In the current study, a combination of resident fish collections and laboratory exposures to site water were used to assess a range of biological endpoints.

Previous laboratory studies have frequently focused on model species, including zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), and fathead minnow (*Pimephales promela*). In contrast, many field-based studies assess longer-lived resident species that may be selected because of their economic or ecological value. The use of a variety of species with differing life histories adds further complexity in determining the biological effects of CEC exposure (Jorgenson et al., 2015). This challenge is exemplified by the sometimes-conflicting results observed in the same species in different studies. For example, reproductive impairment has been documented in white suckers collected immediately downstream of point sources in Boulder Creek (Colorado, USA) (Woodling et al., 2006; Vajda et al., 2008) and Jackfish Bay (Ontario, Canada) (McMaster et al., 1991; Van der Kraak et al., 1992). However, other studies have not observed similar effects in white suckers collected immediately downstream of point sources in the Colorado River basin (Hinck et al., 2009) or Delaware River drainage (Blazer et al., 2014) despite the fact that histological changes were documented in other species in the same studies. The current study evaluated responses in three wild-caught and one laboratory exposed species to assess if fish in the Great Lakes basin are being affected by exposures to CECs.

## 2. Methods

### 2.1. Ethics statement

The U.S. Fish and Wildlife Service obtained permission for access to field sites and specimen collections. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committees of St. Cloud State University (permit number: 0213).

### 2.2. Site selection

Sixteen sites across the Fox River (Wisconsin), Cuyahoga River (Ohio), and Raquette River (New York) were sampled in the Spring of 2014 (Fig. 1, Table 1). Sites in the three rivers included agricultural settings, urban areas and putative reference sites. In each river, sites were identified to represent upstream areas with limited agricultural or urban influences to areas downstream with increasing anthropogenic influence. In addition, some sites were located immediately downstream of wastewater outfalls. An effort was made to identify putative reference sites in each river (usually upstream sites). For resident fish collections, each river was divided into three reaches (upstream, middle, downstream) with reaches separated by an impediment to migration (e.g., dam or distance