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Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals

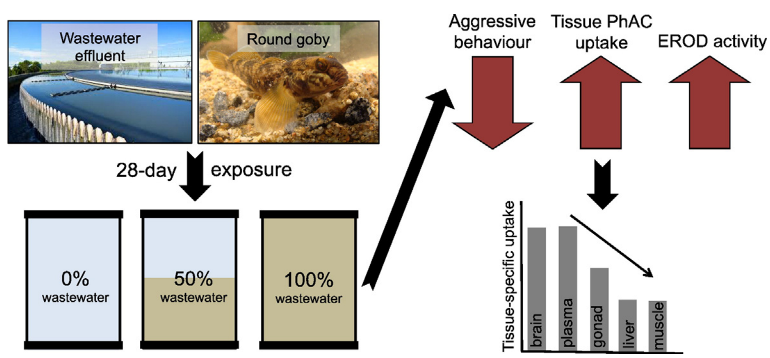
Erin S. McCallum [a,*,1], Emily Krutzelmann [a], Tomas Brodin [b], Jerker Fick [c], Anna Sundelin [c], Sigal Balshine [a]

[a] Department of Psychology Neuroscience & Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON, Canada
[b] Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden
[c] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden

HIGHLIGHTS

- Round goby exposed for 28-days to 0, 50 or 100% wastewater effluent
- Exposure to 100% effluent reduced aggressive acts towards a mirror
- Exposure to 50% and 100% increased tissue uptake of pharmaceuticals
- Pharmaceutical uptake was greatest in brain ≥ plasma > gonads > liver ≥ muscle
- Increased hepatic EROD activity in relation to effluent exposure

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical active compounds (PhACs) are increasingly being reported in wastewater effluents and surface waters around the world. The presence of these products, designed to modulate human physiology and behaviour, has created concern over whether PhACs similarly affect aquatic organisms. Though laboratory studies are beginning to address the effects of individual PhACs on fish behaviour, few studies have assessed the effects of exposure to complex, realistic wastewater effluents on fish behaviour. In this study, we exposed a wild, invasive fish species—the round goby (*Neogobius melanostomus*)—to treated wastewater effluent (0%, 50% or 100% effluent dilutions) for 28 days. We then determined the impact of exposure on fish aggression, an important behaviour for territory acquisition and defense. We found that exposure to 100% wastewater effluent reduced the number of aggressive acts that round goby performed. We complimented our behavioural assay with measures of pharmaceutical uptake in fish tissues. We detected 11 of 93 pharmaceutical compounds that we tested for in round goby tissues, and we found that concentration was greatest in the brain followed by plasma, then gonads, then liver, and muscle. Fish exposed to 50% and 100% effluent had higher tissue concentrations of pharmaceuticals and concentrated a greater number of pharmaceutical compounds compare to control fish exposed to no (0%) effluent. Exposed fish also showed increased ethoxyresorufin-*O*-deethylase (EROD) activity in liver tissue, suggesting that fish were exposed to planar halogenated/polycyclic aromatic hydrocarbons (PHHs/PAHs) in the wastewater effluent. Our findings suggest that fish in effluent-dominated systems may have altered behaviours and greater tissue concentration of PhACs. Moreover, our results underscore the importance of

* Corresponding author at: Rm. 102, Building PC-34, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.
E-mail address: mccalles@mcmaster.ca (E.S. McCallum).
[1] Department of Ecology and Environmental Science, Umeå University, SE-901 87 Umeå, Sweden.

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characterizing exposure to multiple pollutants, and support using behaviour as a sensitive tool for assessing animal responses to complex contaminant mixtures, like wastewater effluent.

1. Introduction

There has been rising concern over the effects that wastewater treatment plant (WWTP) effluents have on wild aquatic animals (Strayer and Dudgeon, 2010; Sumpter, 2009). Part of this concern stems from the fact that WWTP effluents contain anthropogenic, endocrine-active contaminants like steroid hormones, bisphenol-A (BPA), personal care products, and pharmaceutical active compounds (PhACs; Klecka et al., 2010; Pal et al., 2010). Human consumption of PhACs has been increasing in developed countries (Bernhardt et al., 2017; Hemels et al., 2005; OECD, 2013), and traditional WWTPs remain ill-equipped to remove these compounds before wastewater effluent is discharged into the environment (Jelić et al., 2012). Accordingly, reports of PhACs in WWTP effluents and in surface waters have been increasing (Blair et al., 2013; Kolpin et al., 2002; Verlicchi et al., 2012). Although PhACs are specifically designed to modulate human physiology and behaviour, many other vertebrates have well conserved biological targets (e.g., receptors, enzymes) on which PhACs can act (Brown et al., 2014; Gunnarsson et al., 2008). When present in the environment, PhACs are usually found at low concentrations (ng l^{-1} – μg l^{-1}; Blair et al., 2013; Klecka et al., 2010; Kolpin et al., 2002; Pal et al., 2010). However, after chronic and continuous exposure, PhACs can concentrate in fish tissues (Brooks et al., 2005; Ramirez et al., 2009; Schultz et al., 2010). There is therefore a growing awareness that even low concentrations of PhACs may cause sub-lethal changes to animal physiology and behaviours important for survival and reproduction (Brodin et al., 2014; Hellström et al., 2016; Söffker and Tyler, 2012). But, how exposure to the complex PhAC mixtures found in WWTP effluent affects wildlife remains poorly understood (Backhaus, 2014).

Many classes of PhACs are present in wastewater effluent, but psychiatric pharmaceuticals are particularly concerning because of their potential effects on animal behaviour and physiology in the wild (Brodin et al., 2014; Calisto and Esteves, 2009; Corcoran et al., 2010). Compounds such as anxiolytics or antidepressants that are prescribed for treatment of human behavioural disorders are commonly measured in wastewater effluents and in surface waters (typically in low ng l^{-1}; Calisto and Esteves, 2009; Fick et al., 2017; Klaminder et al., 2015; Metcalfe et al., 2010; Verlicchi et al., 2012). Psychiatric pharmaceuticals are known to bioconcentrate in fish tissues, especially in the brain (Brooks et al., 2005; Grabicova et al., 2014), but also in fish plasma, liver, and muscle tissue (Heynen et al., 2016; Schultz et al., 2010). In the laboratory, fish behaviours have been altered following exposures to environmentally relevant concentrations of psychiatric pharmaceuticals. For example, the anxiolytic oxazepam increased boldness in European perch (*Perca fluviatilis*), making exposed fish more active and exploratory (Brodin et al., 2013). Likewise, the antidepressant fluoxetine reduced predator response behaviours in guppy (*Poecilia reticulata*), making them slower to respond to a threat (Pelli and Connaughton, 2015). While the number of studies connecting pharmaceutical exposures to changes in fish behaviour has grown (e.g., Dzieweczynski and Hebert, 2012; Greaney et al., 2015; Hedgespeth et al., 2014; Olsén et al., 2014; Painter et al., 2009; Weinberger and Klaper, 2014), there are also studies that have reported few changes to behaviour following exposure to environmentally relevant concentrations (Holmberg et al., 2011; Margiotta-Casaluci et al., 2014; McCallum et al., 2017a). The behavioural effects documented in the laboratory may not generalize to the wild because wastewater effluent contains a mixture of PhACs and other compounds.

To better address how wastewater effluent might affect fish behaviour, several studies have now measured behavioural endpoints following exposure to wastewater effluent in the laboratory or in the field. In the laboratory, Sebire et al. (2011) found that male three-spine stickleback (*Gasterosteus aculeatus*) exposed to 50% and 100% wastewater built fewer nests and spent less time courting female mates. Garcia-Reyero et al. (2011) and Martinović et al. (2007) found that male fathead minnow (*Pimphales promelas*) exposed to 100% wastewater effluent were less aggressive and less successful at securing a nesting site against unexposed competitors. In one of the only studies on fish collected directly from a wastewater-exposed field site, Saaristo et al. (2014) found that exposed male mosquitofish (*Gambusia holbrooki*) courted females more than males from a reference location. The few studies reviewed here have focused primarily on reproductive behaviours, and they were partnered with measures of the estrogenic or anti-androgenic activity of the wastewater effluent as a potential cause of behavioural disruption. However, many other behaviours impact animal fitness (e.g., foraging, territory defense; Brodin et al., 2014; Zala and Penn, 2004). For instance, Melvin (2016) showed that short-term exposure to wastewater effluent reduced activity and swimming performance in empire gudgeons (*Hypseleotris compressa*). It is also worth noting that two studies have reported no change to fish behaviour following chronic exposures to WWTP effluent (McCallum et al., 2017b; Schoenfuss et al., 2002).

Of the handful of studies that have addressed the impacts of WWTP effluents on behaviour to date, none have measured PhAC uptake into fish tissues to characterize the extent of exposure. In this study, we used an invasive fish species, the round goby (*Neogobius melanostomus*), to test how chronic (28-day) exposure to wastewater effluent (at 0%, 50%, or 100% dilutions) affected fish behaviour alongside tissue-specific uptake of pharmaceuticals. We measured aggression as an ecologically relevant behavioural endpoint because round goby use aggression to acquire and defend sheltered territories from conspecifics and heterospecifics (Balshine et al., 2005; Bergstrom and Mensinger, 2009; Dubs and Corkum, 1996; Janssen and Jude, 2001). They use these shelters to reproduce and care for offspring during the breeding season (Corkum et al., 1998; MacInnis and Corkum, 2000), and without shelter they are susceptible to avian and aquatic predators (Belanger and Corkum, 2003; King et al., 2006; Reyjol et al., 2010; Somers et al., 2003). Round goby aggressiveness is also thought to have contributed to their invasion success in the Laurentian Great Lakes, Western Europe, and the Baltic Sea (Corkum et al., 2004; Kornis et al., 2012). We then investigated in which bodily tissues the pharmaceuticals concentrated the most, and predicted that fish exposed to 50% and 100% effluent would concentrate a greater number of PhACs and higher concentrations of PhACs in their tissues. We further hypothesized that round goby exposed to 50% and 100% effluent would exhibit reduced aggressive responses when compared to controls (0%) if we also detected psychiatric medications like antidepressants or anxiolytics in the exposed fish tissues. Such medications alter monoamine signaling to ameliorate depressive or anxious behaviours in humans (e.g., antidepressants improve mood via increased serotonergic signaling, anxiolytics sedate via increased GABA signaling: Argyropoulos and Nutt, 1999; Stahl, 1998), and prior laboratory exposures have accordingly found that psychiatric pharmaceuticals reduced fish aggression and altered social interactions (Brodin et al., 2013; McCallum et al., 2017a; Paula et al., 2015; Perreault et al., 2003).

Finally, because wastewater effluent is a complex mixture of PhACs and other pollutants, we also assessed 7-ethoxyresorufin-*O*-deethylase (EROD) activity in fish liver tissue. Cytochrome P450 enzymes are involved in Phase 1 metabolism of planar halogenated/polycyclic aromatic hydrocarbons (PHHs/PAHs) and other similarly structured xenobiotic