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No evidence of exposure to environmental estrogens in two feral fish species sampled from the Yarra River, Australia: A comparison with Northern Hemisphere studies

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ABSTRACT

Environmental estrogens originate from a variety of sources including sewage treatment plant (STP) effluents and adverse physiological effects (endocrine disruption) have been observed in several fish species sampled downstream of STP discharges. In this study we examined common carp (*Cyprinus carpio*) and roach (*Rutilis rutilis*) for signs of exposure to environmental estrogens in the iconic Yarra River, Melbourne, Australia. The Yarra River flows through the city of Melbourne and more than 2 million people live within the catchment. Two STPs discharge water into the Yarra River within the middle reaches, and the areas immediately downstream of these discharge locations were the focus of this study. Carp and roach were chosen as test species since both have been utilised extensively for endocrine disruption research throughout Europe, North America and Asia, and data from various international studies was used for comparison with the results of the present study.

Neither species showed evidence of exposure to environmental estrogens, with no elevation of plasma vitellogenin levels in males and no incidence of intersex gonads. Most physiological endpoints in both species from this study were within ranges reported in carp and roach from reference sites in other studies, however some degenerative histological changes in both male and female gonads were observed. Surface water samples showed no estrogenic activity (measured by the yeast-estrogen screen, YES), but did display strong anti-estrogenic and weak androgenic activity (measured by the yeast-androgen screen, YAS).

Whilst the results show no evidence of impacts from environmental estrogens in the Yarra River, the presence of both anti-estrogenic and androgenic activity in water samples, as well as some gonadal changes in carp is concerning and indicates that our focus needs to broaden, in order to look for biological impacts in resident fauna that might be due to environmental pollutants other than environmental estrogens.

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1. Introduction

Endocrine disrupting chemicals (EDCs) are compounds that interfere with an organism's endocrine system by modulating hormone receptors and subsequently affecting the production, storage and uptake of hormones, or the action of a hormone within a specific target tissue or organ (Colborn et al., 1993). There are a number of known or suspected EDCs already present in aquatic environments worldwide, and a major source is sewage treatment plant (STP) effluent discharge (Desbrow et al., 1998; Snyder et al., 1999; Ternes et al., 1999; Tan et al., 2007). These effluents may contain mixtures of chemicals and especially for domestic effluents, compounds such as natural estrogens (17β-estradiol, estrone, estriol), synthetic estrogens (17α-ethinylestradiol) and compounds with estrogenic activity (i.e. alkylphenol ethoxylates, some pesticides, BPA, plasticizers) are commonly reported (Jobling et al., 1995; Meesters and Schroder, 2002; Ying et al., 2002a, 2002b). Collectively, these compounds that interact with estrogen receptors are called environmental estrogens or xenoestrogens, and their occurrence in STP effluents has been correlated with adverse physiological effects in resident fishes (Folmar et al., 1996; Lye et al., 1997; Jobling et al., 1998).

The reproductive axis is a major target of EDCs in fish (and

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other organisms), and a variety of adverse effects have been reported including changes in sex steroid hormone synthesis, induction of the yolk precursor vitellogenin (Vtg), degenerative changes in the ovaries and testes including intersex conditions (oocytes in testicular tissue; spermatogenic cells in ovarian tissue), abnormal gametogenesis, reduced sperm viability, reduced gonad size (and gonadosomatic index – GSI), lowered fertility and changes in the timing of sexual reproduction (i.e. Jobling and Tyler, 2003; Matthiessen, 2003; Mills and Chichester, 2005). Evidence of endocrine disruption in wild fish has been found in rivers receiving STP discharges (Purdom et al., 1994; Sumpter and Jobling, 1995; Folmar et al., 1996; Hamilton et al., 2015), pulp and paper mill discharges (Van der Kraak et al., 1992; Hewitt et al., 2008) and agricultural (pesticide) chemical runoff (Lavado et al., 2004; Schmitt et al., 2005). As such, wild fish have become a valuable monitoring tool for assessing aquatic environments for evidence of endocrine disruption. Two cyprinids, the common carp (*Cyprinus carpio* Linnaeus, 1758) and roach (*Rutilis rutilis* Linnaeus, 1758) are freshwater species that have been studied extensively in Europe, North America and parts of Asia to monitor their responses to EDC exposure.

The common carp is a widely distributed freshwater fish found in several countries. It is long- lived and highly fecund and has been used widely in biomonitoring studies. Downstream of some STPs, male carp have been shown to have elevated plasma Vtg levels, reduced plasma estrogen (17β-estradiol) and androgen levels (testosterone or 11-ketotestosterone) and alterations in estrogen/androgen ratios (Folmar et al., 1996; Sakamoto et al., 2003; Sole et al., 2003b; Mitchelmore and Rice, 2006). In addition, histological changes in male gonads, such as intersex, testicular atrophy, macrophage aggregates, necrosis, fibrosis and a change in gonad staging have been reported in carp caught downstream of some STPs (Hassanin et al., 2002; Sole et al., 2003b; Lavado et al., 2004; Mitchelmore and Rice, 2006; Stansley and Washuta, 2007). Similarly, female carp sampled downstream of some STPs have also shown altered hormone levels (Sakamoto et al., 2003) and histological changes including increased rates of atresia and shifts in gonad staging (Lavado et al., 2004; Mitchelmore and Rice, 2006), whilst female carp sampled from areas polluted with diffuse agricultural and industrial chemicals, have displayed histological changes including oocyte atresia, calcified follicles, fibrosis and ovarian tumours (Patiño et al., 2003; Baldigo et al., 2006; Hinck et al., 2007, 2008). This information demonstrates that carp can be affected by endocrine disruption, and wild carp from several countries have been shown to display Vtg induction, alterations in sex steroid levels and gonadal changes in response to exposure to STP-derived EDCs (Appendix Table A1 and A2).

Roach are another long-lived species that have been used widely in biomonitoring studies (Tyler et al., 2007). A study of juvenile roach exposed to treated sewage effluents in the UK demonstrated dose-dependent induction of plasma Vtg, as well as dose-dependent feminisation of the reproductive ducts in males, however no fish displayed intersex (testicular oocytes) (Rodgers-Gray et al., 2001). In a subsequent study, Beresford et al. (2004) reported high rates ($>80\%$) of reproductive duct feminisation in juvenile roach collected from 5 of 7 rivers sampled in south-west UK, which was attributed to varying contributions of STP effluent to overall flows in the different rivers. As is the case with carp, roach have been shown to be sensitive to environmental estrogens, and assessments of wild fish from various locations have demonstrated adverse physiological effects (Appendix Table A3 and A4).

In Australia, only a limited number of studies have measured the concentrations of estrogenic chemicals in STP effluents and surface waters (e.g. Williams et al., 2007; Ying et al., 2009; Ferguson et al., 2013; Scott et al., 2014; Vadja et al., 2015). In studies undertaken in Victoria between 2003–2007, concentrations of natural estrogens or estrogenic activity (expressed as 17β-estradiol equivalents, EEQ) have been reported in the range of <0.1–73 ng/L (Mispagel et al., 2009). Seasonal differences in concentrations have been reported, with the estrogen levels being slightly higher in the summer months than in winter (Allinson et al., 2010). These values are within a similar range to values reported in STP effluents elsewhere in the world (Desbrow et al., 1998; Snyder et al., 1999; Ternes et al., 1999), and the concentrations are within the range known to elicit adverse biological effects in fishes (reviewed by Mills and Chichester (2005)). Few studies have been conducted to assess wild fish populations for evidence of endocrine disruption in Australia (Batty and Lim, 1999; Game et al., 2006; Leusch et al., 2006; Codi King et al., 2008; Rawson et al., 2008; Kellar et al., 2014).

In the 1860s, both carp and roach were introduced to Australian rivers for angling purposes and have since established self-sustaining populations (Brumley, 1991). Following these introductions, carp have undergone a huge range expansion and are now considered the most abundant large freshwater fish in southern Australia (Koehn, 2004). Carp are a declared pest species in several countries, including Australia where they are considered noxious, due to their negative impacts on stream habitat and other species. Roach are also an introduced species, however they are not considered a major threat to native fishes, and therefore are not a formally listed noxious species in Australia. Roach have not undergone a widespread expansion so their distribution tends to be restricted to Victorian inland and coastal waters (Rowe et al., 2008). A major Victorian river in which both species have established populations is the Yarra River.

The Yarra River originates near Warburton, 76 km north-east of Melbourne in the Yarra Ranges National Park, and flows in a south-westerly direction where it eventually drains into the northern end of Port Phillip Bay (Melbourne Water, 2007). The river is 245 km in length, and more than 2 million people live within the 4078 km^2 Yarra Catchment. Several areas in the upper reaches are reserved for water supply, with numerous major reservoirs and water holding facilities, and these forested and mountainous areas are considered undisturbed and in a pristine condition. Moving downstream, the trend is for a decline in water quality and overall condition, largely due to pollution that is introduced via urbanised tributaries and creeks which join the Yarra River at several points along its length. In addition, there are four STPs that discharge either directly into the Yarra River, or into its tributaries.

The aims of this study were to assess possible endocrine disruption in a Victorian wild fish population, by examining carp and roach from the Yarra River and comparing the results to findings from international studies. In lieu of having reference sites, we compared our findings with published information on the same types of endocrine disruption-related endpoints in wild caught carp and roach from European, North American and Asian studies (Appendix Table A1–A4). There are obviously challenges in trying to directly compare physiological endpoints in fish caught from different environments during different seasons; so rather, we used this information as a guide to determine acceptable ranges of values for fish collected from so-called reference sites.

2. Methods

2.1. Sampling sites

Fish, and/or water samples were collected from six different sites along a 30 km section of the Yarra River between Yarra Glen and Warrandyte, throughout which several creeks and tributaries enter the river, and the degree of urbanisation increases (Fig. 1).

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