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The pharmaceutical pollutant fluoxetine alters reproductive behaviour in a fish independent of predation risk

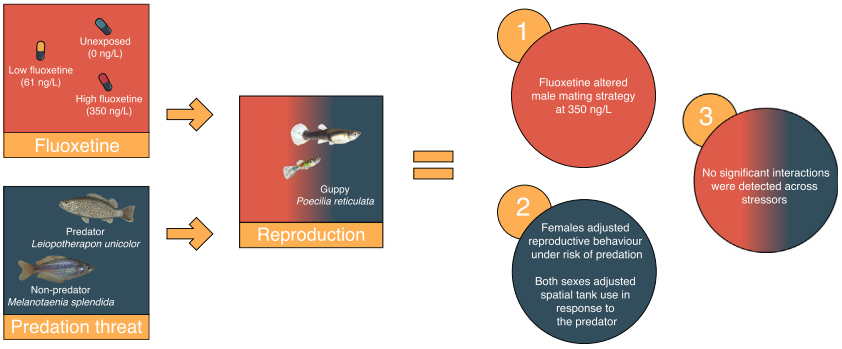
Jack B. Fursdon *, Jake M. Martin, Michael G. Bertram, Topi K. Lehtonen, Bob B.M. Wong

School of Biological Sciences, Monash University, Victoria, Australia

HIGHLIGHTS

- Pharmaceutical pollution represents a major global threat to wildlife and ecosystems.
- Guppies (*P. reticulata*) were exposed to fluoxetine at two field-realistic levels.
- Male and female guppy reproductive behaviour was assessed under predation risk.
- High fluoxetine (350 ng/L) increased male coercive mating behaviour, independent of a predatory threat.
- Highlights importance of considering interactions between natural stressors and pharmaceutical pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical pollutants constitute a major threat to wildlife because of their capacity to induce biological effects at low doses. One such pollutant is the antidepressant fluoxetine, which has been detected in surface waters globally at levels that recent studies suggest can alter physiology and behaviour in aquatic organisms. However, wildlife exposed to pharmaceutical contaminants are typically confronted with multiple stressors simultaneously, including predation risk, which is a particularly important natural stressor that can have direct (e.g. mortality) and indirect (e.g. changed prey behaviour) fitness effects. Accordingly, we investigated potential impacts of environmentally realistic fluoxetine exposure on reproductive behaviour in the guppy (*Poecilia reticulata*) under predation risk. Specifically, we tested whether fluoxetine exposure altered mating behaviour in male and female guppies in the presence of either a predatory spangled perch (*Leiopotherapon unicolor*) or a non-predatory rainbowfish (*Melanotaenia splendida*) control. We found that fluoxetine and the presence of a predatory spangled perch did not interact to affect reproductive behaviour. We also found that, independent of a predatory threat, fluoxetine exposure altered male mating strategy, with males in the high treatment conducting significantly more coercive 'sneak' copulations, whereas the number of courtship displays performed was not significantly affected. Moreover, while fluoxetine exposure did not significantly affect the amount of time that males and females spent following one another, we found that females, but not males, followed a potential partner less when in the presence of the predatory fish. Finally, both sexes reacted to the risk of predation by spending less time in close proximity to a predator than a non-predator. In combination, our findings highlight the capacity of fluoxetine to influence processes of sexual selection at field-realistic concentrations and emphasise the importance of considering multiple stressors when assessing impacts of pharmaceutical pollutants on the behaviour of wildlife.

* Corresponding author at: School of Biological Sciences, Monash University, Melbourne, Victoria 3800, Australia.
E-mail address: jack.fursdon@aapt.net.au (J.B. Fursdon).

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1. Introduction

Pharmaceutical pollution represents a major global threat to humans and wildlife (Arnold et al., 2014; Bernhardt et al., 2017; Boxall et al., 2012; Saaristo et al., 2018). Indeed, in excess of 600 different pharmaceutical contaminants (or their transformation products) have now been detected in the environment across >71 countries spanning all continents (Aus der Beek et al., 2016; Küster and Adler, 2014). In this regard, selective serotonin reuptake inhibitors (SSRIs), a widely prescribed class of antidepressants, are among the most commonly detected pharmaceutical pollutants in the environment (Silva et al., 2012). Acting by limiting reabsorption of the neurotransmitter serotonin into the pre-synaptic nerve cell, SSRIs elevate levels of extracellular serotonin in the synaptic cleft, leading to increased activation of post-synaptic receptors (Stahl, 1998). Serotonin is ubiquitous in all animal phyla possessing nervous systems and is known to play a key role in regulating a range of physiological and behavioural processes (Fent et al., 2006; Weiger, 1997).

One SSRI of particular environmental concern is fluoxetine, which is among the most commonly prescribed antidepressants in the world (Brijnath et al., 2017; Wong et al., 2005). Fluoxetine enters and remains in the environment as a result of excretion by human patients and insufficient removal during wastewater treatment processes (Arnold et al., 2014; Mennigen et al., 2011), with many countries worldwide not presently having regulatory frameworks in place for restricting the discharge of, or monitoring, fluoxetine in drinking water and wastewater flow (e.g. Australia: Department of Agriculture and Water Resources, 2016; European Union: The Council of the European Communities, 2018; New Zealand: Ministry of Health, 2018; United States of America: Environmental Protection Agency, 2016). In this regard, fluoxetine has been detected in surface waters globally, at concentrations typically ranging from <1–100 ng/L (e.g. Batt et al., 2015; Birch et al., 2015; Hughes et al., 2013; Kolpin et al., 2002; Meador et al., 2016; Paíga et al., 2016; Wu et al., 2017), and up to 596 ng/L in systems receiving wastewater discharge (Benotti and Brownawell, 2007). Moreover, levels as high as 929 ng/L have been reported in direct effluent flow (Bueno et al., 2007; Metcalfe et al., 2010).

While levels of fluoxetine found in the environment are not sufficient to induce lethal effects (e.g. 2.89 mg/L LC_{50} for juvenile topmouth gudgeon, *Pseudorasbora parva*: Chen et al., 2018; 198 μg/L LC_{50} for fathead minnow, *Pimephales promelas*: Stanley et al., 2007), many recent studies have found that fluoxetine exposure at close to, and at, environmental concentrations can alter a range of ecologically important traits in non-target species. Reported effects include altered development (Japanese medaka, *Oryzias latipes*: Foran et al., 2004; Northern Leopard Frog, *Rana pipiens*: Foster et al., 2010; western mosquitofish, *Gambusia affinis*: Henry and Black, 2008), growth (guppy, *Poecilia reticulata*: Pelli and Connaughton, 2015; California mussel, *Mytilus californianus*: Peters and Granek, 2016) and survival (guppy: Pelli and Connaughton, 2015). Fluoxetine exposure has also been linked to alterations in various key fitness-related behaviours, including feeding rate (fathead minnow: Weinberger and Klaper, 2014), sociability (Japanese medaka: Ansai et al., 2016; Arabian killifish, *Aphanius dispar*: Barry, 2013), aggression (Arabian killifish: Barry, 2013; Siamese fighting fish, *Betta splendens*: Dzieweczynski and Hebert, 2012), phototaxis (an amphipod, *Echinogammarus marinus*: Guler and Ford, 2010; water flea, *Daphnia magna*: Rivetti et al., 2016), boldness (Siamese fighting fish: Dzieweczynski et al., 2016a, 2016b) and activity (Arabian killifish: Barry, 2013; an amphipod, *Gammarus pulex*: De Lange et al., 2006; Siamese fighting fish: Kohlert et al., 2012), as well as learning and memory retention (common cuttlefish, *Sepia officinalis*: Di Poi et al., 2013). To date, however, investigations of behavioural shifts caused by fluoxetine have focussed on testing effects of exposure independently from other stressors typically found in the environment—as is also true for pharmaceutical pollutants more generally. In nature, however, complex interactions between multiple stressors are likely to be the norm rather than the exception (Blaustein and Kiesecker, 2002; Slocum and Mendelssohn, 2008). Moreover, of the studies that have considered such interactive effects, most have focussed on other abiotic factors (e.g. mixture effects with other pharmaceuticals, see De Castro-Català et al., 2017; Painter et al., 2009), with surprisingly few having examined potential effects of pharmaceutical pollutants in combination with biotic stressors.

Predation is a ubiquitous biotic stressor that can impact fitness directly via mortality or indirectly by producing changes in prey morphology, life-history and/or behaviour (Creel and Christianson, 2008; Sih et al., 1985). Previous studies have shown that fluoxetine can alter behavioural responses of fish to visual (e.g. Martin et al., 2017; Pelli and Connaughton, 2015; Saaristo et al., 2017) and chemical (e.g. Barry, 2014) predator cues. However, to date, potential interactive effects of fluoxetine exposure and predation risk on reproductive behaviours have not been investigated. Such behaviours include conspicuous mating displays, which often communicate an individual's phenotypic and genetic quality, such as health, ability to sire young, and quality of parental care (Barber et al., 2001; Hoikkala et al., 1998; Lindström et al., 2006; Sargent, 1982). However, conspicuous sexual displays can also be costly, as they often elevate an individual's vulnerability to predators by increasing detectability and rate of predator-prey encounters (Hoefler et al., 2008; reviewed in Lima and Dill, 1990), and by limiting escape potential from would-be predators (Cooper, 1999; Killian et al., 2006). In light of such costs, individuals often adjust their reproductive behaviour according to perceived predation risk (Sih, 1994). For example, to minimise the likelihood of detection, male cross-banded tree frogs (*Smilisca sila*) reduce their calling rate—a behaviour used to attract females—when in the presence of a predator (Tuttle and Ryan, 1982). Therefore, it is important to consider potential interactions between pharmaceutical pollutant exposure and predation risk on reproductive behaviour in wildlife (reviewed in Saaristo et al., 2018).

The guppy (*Poecilia reticulata*) is a small, internally fertilising poeciliid native to north-eastern South America (Rosen and Bailey, 1963) that is now found in over 69 countries around the world (Deacon et al., 2011). Guppies inhabit freshwater habitats, many of which are exposed to wastewater contaminants (Araújo et al., 2009; reviewed in Magurran, 2005), such as fluoxetine (Hughes et al., 2013). Guppies have also been the focus of extensive behavioural research examining mating tactics under predation risk (reviewed in Houde, 1997), which, in combination with their presence in polluted environments, makes them an ideal model for investigating potential effects of fluoxetine contamination and predation risk on reproductive behaviour. Indeed, guppies have recently received increasing attention as a model species in behavioural ecotoxicology (Bertram et al., 2015; Holmberg et al., 2011; Pelli and Connaughton, 2015; Saaristo et al., 2017; Tomkins et al., 2017). Male guppies engage in two alternative mating strategies, either soliciting copulations from females by performing elaborate courtship displays or engaging in surreptitious 'sneak' copulations without first courting the female (Houde, 1997). When under threat of predation, males typically favour sneaking behaviour as the conspicuous nature of courtship displays increases the likelihood of detection by predators (Endler, 1987). Moreover, sneak copulations circumvent some of the energetic costs associated with courtship displays, although sneaking also carries a relatively low probability of successful insemination, with approximately one third as many sperm being transferred during sneak copulations compared to copulations following courtship (Matthews and Magurran, 2000; Pilastro and Bisazza, 1999; Pilastro et al., 2007). Given these trade-offs, males should favour sneaking in situations where courtship displays are less effective or are relatively costly, such as in environments with high predation risk (reviewed in Houde, 1997).

Here, we examined impacts of short-term (28-day) exposure to two environmentally relevant levels of fluoxetine—nominal low and high concentrations of 40 and 400 ng/L, respectively—on male and female guppy reproductive behaviour in the presence or absence of a predatory