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Impact of the widespread pharmaceutical pollutant fluoxetine on behaviour and sperm traits in a freshwater fish

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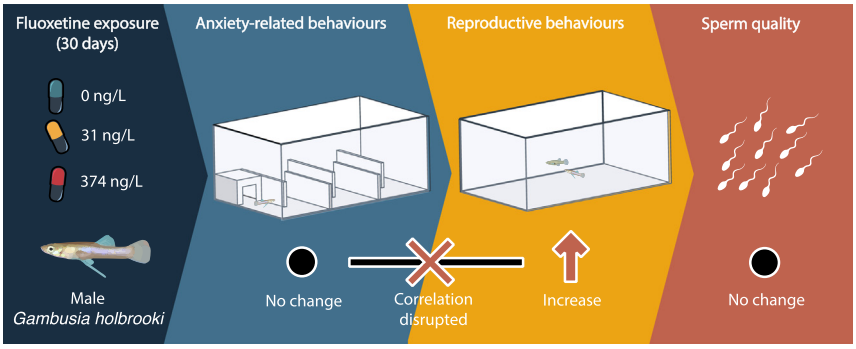
HIGHLIGHTS

- Male mosquitofish (*G. holbrooki*) exposed to fluoxetine at two realistic levels.
- Fluoxetine did not impact anxiety-related behaviours.
- Fluoxetine increased reproductive behaviour.
- Fluoxetine disrupted an across contexts correlation.
- Fluoxetine did not affect sperm quality

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical pollutants are detected in aquatic habitats and wildlife tissues globally. One widespread contaminant of major concern is the antidepressant fluoxetine, which can affect behavioural and physiological processes in non-target species. Despite this, effects of fluoxetine on wildlife behaviour have seldom been investigated across multiple fitness-related contexts, especially at environmentally realistic concentrations. Accordingly, we examined impacts of 35-day fluoxetine exposure at two environmentally relevant concentrations (31 and 374 ng/L) across a suite of fitness-related contexts in wild-caught male mosquitofish (*Gambusia holbrooki*). First, we investigated anxiety-related behaviours (boldness, exploration and activity) in a novel environment (maze arena) and found no significant impacts of exposure. Second, we tested effects of fluoxetine in a reproductive context, including mating behaviour and sperm quality. We found that, relative to controls, fluoxetine exposure resulted in males spending a greater amount of time pursuing females. Further, low-exposed males were more likely to attempt copulation than unexposed males. Lastly, we investigated across-context behavioural correlations, and how fluoxetine exposure might affect such relationships. A significant positive correlation was detected in control fish between activity levels in the maze and time spent pursuing females in the reproductive assay. This relationship was disrupted by fluoxetine at both exposure levels. This is the first evidence that field-detected concentrations of a pharmaceutical pollutant can disturb across-context behavioural correlations in wildlife. Our findings provide clear evidence that fluoxetine can produce context-specific behavioural effects in fish and underscore how pharmaceutical exposure at field-detected concentrations can induce important shifts in wildlife behaviour.

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1. Introduction

Pharmaceutical pollution is a major threat to aquatic ecosystems globally (Arnold et al., 2014; Bernhardt et al., 2017; Saaristo et al., 2018). Hundreds of human and veterinary pharmaceuticals have now been detected in aquatic ecosystems and wildlife tissues around the world (Hughes et al., 2013; Küster and Adler, 2014). One pharmaceutical pollutant of environmental concern is the antidepressant fluoxetine. As with most pharmaceuticals, fluoxetine typically enters the environment via human consumption and excretion (Schultz et al., 2010). Indeed, up to 30% of administered fluoxetine can remain unmetabolised when excreted (van Harten, 1993). This incomplete metabolisation, coupled with insufficient removal by sewage treatment plants (e.g. Vasskog et al., 2006), results in fluoxetine entering aquatic environments in wastewater effluent flows. Consequently, fluoxetine (as well as its primary metabolite norfluoxetine) has been detected in surface waters worldwide at levels ranging from <1–100 ng/L, to as high as 596 ng/L in systems directly receiving wastewater discharge (Hughes et al., 2013; Schultz and Furlong, 2008; Schultz et al., 2010; Vanderford and Snyder, 2006). Once in the environment, fluoxetine can bioaccumulate in wildlife tissues (e.g. Brooks et al., 2005; David et al., 2018; Muir et al., 2017). For example, in an urban wetland receiving treated municipal wastewaters, fluoxetine—relative to 64 other pharmaceuticals present—showed the highest level of bioaccumulation in wild fish (Muir et al., 2017).

In addition to fluoxetine's prevalence in aquatic habitats, its primary pharmacological target, the serotonin transporter molecule, is conserved across a variety of taxa (Gunnarsson et al., 2008; Wang and Tsai, 2006). Consequently, fluoxetine may affect wildlife through its pharmacological action at lower concentrations than are required to induce general toxicity (McDonald, 2017). Moreover, by altering the serotonin system and associated neuroendocrine pathways, fluoxetine can influence multiple fitness-related processes (Kreke and Dietrich, 2008; McDonald, 2017). For example, in fish, pharmacologically relevant dosages of fluoxetine (i.e. ≥100 μg/L) have repeatedly been shown to reduce anxiety-like behaviours (Ansai et al., 2016; Cachat et al., 2010; Wong et al., 2013). By extension, fluoxetine exposure in wildlife could result in alterations to ecologically important behaviours linked to anxiety, such as boldness (i.e. the propensity to take risks), exploration, and activity, which are directly related to fitness and are associated with a range of important processes, such as dispersal (e.g. Cote et al., 2010; Michelangeli et al., 2017) and migration (e.g. Chapman et al., 2011). Moreover, fluoxetine exposure can also disrupt reproduction (reviewed in Kreke and Dietrich, 2008; McDonald, 2017). For example, in aquatic species, fluoxetine has been shown to induce gamete release in mussels (Bringolf et al., 2010; Fong, 1998) increase ovarian growth in crayfish (Kulkarni et al., 1992), and cause shifts in the release of sex hormones in fish species (Foran et al., 2004; Khan and Thomas, 1992; Mennigen et al., 2010).

Despite fluoxetine's capacity to influence a range of biological processes, few studies have investigated the effects of environmentally realistic fluoxetine exposure on non-reproductive and reproductive behaviours concomitantly—which is also true for pharmaceutical pollutants more generally. Fewer still have considered the importance that behavioural and physiological alterations can have on individuals across multiple ecological contexts, despite growing appreciation that functionally unrelated behaviours are often correlated, whereby a shift in one trait can correspond with a shift in another (i.e. behavioural syndromes, Sih et al., 2004, 2012).

Here, we set out to test the hypothesis that 35-day fluoxetine exposure at two environmentally realistic levels (average measured concentrations: 31 and 374 ng/L) would disrupt behaviour across two ecologically important contexts in wild-caught male mosquitofish (*Gambusia holbrooki*). First, we tested the effect of fluoxetine on anxiety-related behaviours (boldness, exploration, and activity) in a novel environment (maze arena). Second, using the same males, we tested the impact of fluoxetine exposure in a reproductive context, in terms of both reproductive behaviour and sperm quality. Lastly, we tested for potential across-context behavioural correlations and the effects of fluoxetine on such relationships.

2. Methods

2.1. Animal collection and housing

The present research was approved by the Biological Sciences Animal Ethics Committee of Monash University (BSCI/2015/2). Sexually mature male (mean weight: 0.1999 ± 0.0406 g, mean length: 22.31 ± 1.26 mm; $n = 105$) and female (mean weight: 0.4036 ± 0.1990 g, mean length: 26.14 ± 3.81 mm; $n = 105$) mosquitofish were collected from a wild population at Science Centre Lake (37°54′28″ S, 145°08′16″ E), Monash University, Australia. Water samples taken from the site over consecutive years indicated no fluoxetine contamination (unpublished data). Before experimentation, fish were acclimated to laboratory conditions (24–26 °C; 12:12 h light:dark cycle) in single-sex holding tanks (80 × 45 × 45 cm, water depth: 30 cm) for 1 month. Fish were fed daily on an *ad libitum* diet of commercial fish food (Otohime Hirame). The mosquitofish was selected as a model because its life-history is well characterised, including reproductive behaviour and sperm traits (Bisazza et al., 2001; Locatello et al., 2008; McPeek, 1992). Mosquitofish have a largely coercive polyandrous mating system and internal fertilisation, with males using a modified anal fin as an intromittent organ during copulation (McPeek, 1992). Due to the species' coercive mating system (Bisazza et al., 2001), and capacity for females to store sperm (Locatello et al., 2008), sperm quality is likely to play an important role in predicting reproductive success of male mosquitofish under sperm competition (Locatello et al., 2008).

2.2. Chemical exposure and monitoring

Male mosquitofish were randomly allocated to three treatment groups for 35 days: unexposed (i.e. fresh water), low fluoxetine and high fluoxetine. A 35-day exposure duration was selected because the full therapeutic effects of fluoxetine typically take 2–4 weeks to manifest in humans (Gardier et al., 1996; Hensler, 2003), and the spermatogenic cycle of *G. holbrooki* takes 30 days (Koya and Iwase, 2004). The nominal fluoxetine exposure concentration of the low treatment (40 ng/L) was selected to represent levels repeatedly detected in surface waters, while the nominal high concentration (400 ng/L) was selected to represent the higher end of surface water detections (reviewed in Hughes et al., 2013). The design of the chemical exposure followed previously published protocols (Bertram et al., 2018a; Martin et al., 2017). Briefly, exposure involved three identical flow-through systems (24 h cycling), one per treatment, with each system comprising 4 aquaria (60 × 30 × 30 cm, water depth: 25 cm), housing 30 fish each. The low- and high-fluoxetine exposure systems both received a constant supply of fluoxetine stock solution (replaced daily) and fresh water, whereas the unexposed system received fresh water only. The low- and high-fluoxetine stock solutions (6 and 60 μg/L, respectively) were prepared following methods described in Bertram et al. (2018a). Weekly water samples (200 mL) were taken from all of the low and high exposure tanks to measure fluoxetine concentrations. Additionally, water samples were collected from each unexposed tank fortnightly to ensure the absence of fluoxetine. Water samples were analysed by Envirolab Services using gas chromatography coupled to tandem mass spectrometry (7000C Triple Quadrupole GC–MS/MS, Agilent Technologies, Delaware, USA), based on methods described in Bertram et al. (2018a). Mean measured concentrations for the low- and high-fluoxetine treatments were 30.61 ng/L (SD = 6.28, $n = 24$) and 374.50 ng/L (SD = 62.91, $n = 24$). No fluoxetine contamination was detected in the unexposed system ($n = 12$), with the limit of quantification for fluoxetine being 2 ng/L.