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The antidepressant fluoxetine alters mechanisms of pre- and post-copulatory sexual selection in the eastern mosquitofish (*Gambusia holbrooki*)☆

Michael G. Bertram [a, *], Tiarne E. Ecker [a], Bob B.M. Wong [a], Moira K. O'Bryan [a, b], John B. Baumgartner [c], Jake M. Martin [a], Minna Saaristo [a, d]

[a] School of Biological Sciences, Monash University, Victoria, Australia
[b] The Development and Stem Cells Program of Monash Biomedicine Discovery Institute and the Department of Anatomy and Developmental Biology, Monash University, Victoria, Australia
[c] Department of Biological Sciences, Macquarie University, New South Wales, Australia
[d] Department of Biosciences, Åbo Akademi University, Turku, Finland

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ABSTRACT

Contamination of aquatic habitats with pharmaceuticals is a major environmental concern. Recent studies have detected pharmaceutical pollutants in a wide array of ecosystems and organisms, with many of these contaminants being highly resistant to biodegradation and capable of eliciting sub-lethal effects in non-target species. One such pollutant is fluoxetine, a widely prescribed antidepressant, which is frequently detected in surface waters globally and can alter physiology and behaviour in aquatic organisms. Despite this, relatively little is known about the potential for fluoxetine to disrupt mechanisms of sexual selection. Here, we investigate the impacts of 30-day exposure to two environmentally realistic levels of fluoxetine (low and high) on mechanisms of pre- and post-copulatory sexual selection in the eastern mosquitofish (*Gambusia holbrooki*). We tested 1) male mating behaviour in the absence or presence of a competitor male, and 2) sperm quality and quantity. We found that high-fluoxetine exposure increased male copulatory behaviour in the absence of a competitor, while no effect was detected under male-male competition. Further, fluoxetine exposure at both concentrations increased total sperm count relative to males from the control group, while no significant change in sperm quality was observed. Lastly, low-fluoxetine males showed a significant reduction in condition index (mass relative to length). Our study is the first to show altered mechanisms of both pre- and post-copulatory sexual selection in an aquatic species resulting from environmentally realistic fluoxetine exposure, highlighting the capacity of pharmaceutical pollution to interfere with sensitive reproductive processes in wildlife.

1. Introduction

Numerous pharmaceutical pollutants are capable of altering ecologically important traits and behaviours in wildlife (Boxall et al., 2012; Arnold et al., 2014; Brodin et al., 2014). Pharmaceutically active compounds enter the environment via multiple pathways, including the excretion of chemicals used for human and veterinary healthcare, discharge from manufacturing and disposal of unused medications (Heberer, 2002). Of the approximately 5000 actively marketed pharmaceutical products, over 600 have now been detected in the environment globally (Küster and Adler, 2014). Worldwide consumption of pharmaceuticals is also increasing due to a growing and ageing human population (Khetan and Collins, 2007; Arnold et al., 2014). Antidepressant pharmaceuticals pose a distinct threat to wildlife as they are specifically designed to induce physiological effects at low concentrations (Khetan and Collins, 2007) and have a particularly strong potential to alter behaviour (Arnold et al., 2014; Brodin et al., 2014). The most frequently

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* Corresponding author. School of Biological Sciences, Monash University, Melbourne, Victoria 3800, Australia.
E-mail address: michael.g.bertram@monash.edu (M.G. Bertram).

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prescribed class of antidepressants is the selective serotonin reuptake inhibitors (SSRIs) (Fong and Ford, 2014), which act by inhibiting the reuptake of the monoamine neurotransmitter serotonin (5-hydroxytryptamine) by the pre-synaptic nerve cleft, thereby increasing the effect of serotonin on the post-synaptic nerve (Stahl, 1998). Serotonin is a ubiquitous neurotransmitter, present in all phyla possessing nervous systems (Weiger, 1997). As such, SSRIs have the potential to alter a range of ecologically important traits and behaviours in wildlife.

One SSRI of environmental concern is fluoxetine, which is used to treat major depression and other psychiatric disorders in humans, and is among the most commonly prescribed pharmaceuticals (Wong et al., 2005). Present in aquatic environments globally, fluoxetine has been detected in surface waters at concentrations typically ranging from <1 to 100 ng/L (e.g., Kolpin et al., 2002; Fernández et al., 2010; Gardner et al., 2012; Hughes et al., 2013), although levels as high as 596 ng/L have been reported in systems receiving wastewater discharge (Benotti and Brownawell, 2007). In addition, fluoxetine has been found to bioaccumulate in fish tissues, especially in the brain (Brooks et al., 2005; Schultz et al., 2010). Exposure to fluoxetine can influence a range of ecologically important traits, including development (Japanese medaka, *Oryzias latipes*, Foran et al., 2004; western mosquitofish, *Gambusia affinis*, Henry and Black, 2008), reproduction (zebrafish, *Danio rerio*, Lister et al., 2009) and survival (guppy, *Poecilia reticulata*, Pelli and Connaughton, 2015), as well as various morphological and physiological characteristics (e.g., altered growth in *P. reticulata*, Pelli and Connaughton, 2015; impaired cardiovascular and ventilatory response to hypoxia in Gulf toadfish, *Opsanus beta*, Panlilio et al., 2016). Fluoxetine exposure has also been linked with alterations in a variety of behaviours in fish, such as activity (Siamese fighting fish, *Betta splendens*, Kohlert et al., 2012; Arabian killifish, *Aphanius dispar*, Barry, 2013), feeding and foraging (fathead minnow, *Pimephales promelas*, Stanley et al., 2007; *P. promelas*, Weinberger and Klaper, 2014), aggression (*B. splendens,* Lynn et al., 2007; *B. splendens*, Dzieweczynski and Hebert, 2012; *A. dispar,* Barry, 2013), sociability (*A. dispar*, Barry, 2013; *O. latipes*, Ansai et al., 2016) and antipredator behaviour (*P. reticulata*, Pelli and Connaughton, 2015; eastern mosquitofish, *Gambusia holbrooki*, Martin et al., 2017). However, variability in fluoxetine sensitivity reported across studies, model species and biological responses has made ascertaining what fluoxetine concentrations pose a risk to aquatic wildlife challenging (Stewart et al., 2014; Sumpter et al., 2014), highlighting the need for further research investigating the impacts of environmentally realistic concentrations of fluoxetine on ecologically relevant traits. Having received little attention relative to other endpoints, this is especially true for the effects of exposure to fluoxetine on mechanisms of sexual selection.

Sexual selection can occur both before (i.e., pre-copulatory) and after (i.e., post-copulatory) mating (Andersson and Simmons, 2006), with both of these processes being vulnerable to disruption by pharmaceutical pollution. Studies using pharmacological dosages have demonstrated that treatment with fluoxetine can induce male sexual dysfunction in humans (Gregorian et al., 2002; Serretti and Chiesa, 2009) and rodents (Taylor et al., 1996; Matuszcyk et al., 1998). However, findings from the handful of studies that have examined the impacts of environmentally realistic concentrations of fluoxetine on reproductive behaviour have been mixed. Specifically, while some studies have reported an increase in certain reproductive behaviours following fluoxetine exposure (Weinberger and Klaper, 2014), others have reported a decrease (Forsatkar et al., 2014), or no significant effect (Schultz et al., 2011; Dzieweczynski and Hebert, 2012). Further, the effects of fluoxetine on male mating behaviour under male-male competition, where males compete for the opportunity to reproduce, has received very little attention, despite being a central component of pre-copulatory sexual selection (Andersson, 1994). Clearly, the potential impacts of fluoxetine on mating and reproductive behaviours in wildlife require further investigation.

In species where females mate multiply (polyandry), an important component of post-copulatory sexual selection is sperm competition, where the sperm of multiple males compete to fertilise available ova (Andersson and Simmons, 2006). In polyandrous species, a key predictor of each male's fertilisation success is his proportional contribution to the sperm pool (Parker, 1998), with elevated sperm production allowing males to copulate more often and allocate more sperm to each ejaculate (Parker, 1982). Sperm quality traits such as viability and speed can also influence fertilisation success under sperm competition (Snook, 2005). Because treatment with SSRIs, including fluoxetine, can reduce fertility in human males (reviewed in Brezina et al., 2012; Nørr et al., 2016), considerable attention has been paid to the impacts of fluoxetine at pharmacological levels on fertility in rodent models (e.g., Bataineh and Daradka, 2007; Alzahrani, 2012; Monteiro Filho et al., 2014). In addition, research in aquatic species has reported reproductive dysfunction in species as diverse as male goldfish (*Carassius auratus*, Mennigen et al., 2010) and zebra mussels (*Dreissena polymorpha*, Fong, 1998). Despite this, the potential effects of exposure to environmentally realistic levels of fluoxetine on both sperm quality and quantity remain to be investigated in *any* aquatic vertebrate.

The eastern mosquitofish is a small, internally fertilising poeciliid fish with a widespread geographic distribution (Pyke, 2005, 2008) that is attracting increased interest as a model for investigating the impacts of chemical pollutants (e.g., Saaristo et al., 2013, 2014; Magellan et al., 2014; Martin et al., 2017; Melvin et al., 2017). Mosquitofish have a coercive mating system, where males copulate with females by 'sneaking' from behind and thrusting the tip of their gonopodium—a modified anal fin used for internal fertilisation—into the female's genital pore (Bisazza et al., 2001). No courtship occurs and, although females may exert some control over the outcome of unsolicited mating attempts by spending more time associating with preferred males, male sexual coercion and male-male competition are the primary modes of pre-copulatory sexual selection in this species (Bisazza et al., 2001). Wild mosquitofish females are typically inseminated by multiple males (Zane et al., 1999) and are capable of storing sperm for several months (Evans et al., 2003), putting the sperm of multiple males in direct competition. Further, in this species, approximately ninety-percent of all broods are sired by multiple males, making sperm competition a major source of post-copulatory sexual selection (Zane et al., 1999). These attributes make mosquitofish an excellent system for investigating the effects of pollutants on sexually selected traits and behaviours.

Here, we investigated the effects of 30-day exposure to two environmentally realistic levels of fluoxetine—nominal low and high concentrations of 40 and 400 ng/L, respectively—on mechanisms of pre- and post-copulatory sexual selection in mosquitofish. Utilising two separate flow-through exposures, we experimentally investigated the impact of fluoxetine on 1) male mating behaviour in the absence or presence of a competitor, and 2) total sperm count and sperm quality. In addition, all fluoxetine-exposed and control (i.e., unexposed) males were tested for differences in their morphological characteristics.

2. Materials and methods

2.1. Animal collection and housing

Mosquitofish were wild-caught from the Science Centre Lake at Monash University (37° 54′ 28″ S, 145° 08′ 16″ E), Victoria, Australia.