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Impacts of the antidepressant fluoxetine on the anti-predator behaviours of wild guppies (*Poecilia reticulata*)

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ABSTRACT

Chemical pollution from pharmaceuticals is increasingly recognised as a major threat to aquatic communities. One compound of great concern is fluoxetine, which is one of the most widely prescribed psychoactive drugs in the world and frequently detected in the environment. The aim of this study was to investigate the effects of 28-d fluoxetine exposure at two environmentally relevant levels (measured concentrations: 4 ng/L and 16 ng/L) on anti-predator behaviour in wild guppies (*Poecilia reticulata*). This was achieved by subjecting fluoxetine-exposed and unexposed guppies to a simulated bird strike and recording their subsequent behavioural responses. We found that exposure to fluoxetine affected the anti-predator behaviour of guppies, with exposed fish remaining stationary for longer (i.e. 'freezing' behaviour) after the simulated strike and also spending more time under plant cover. By contrast, control fish were significantly more active and explored the tank more, as indicated by the distance covered per minute over the period fish spent swimming. Furthermore, behavioural shifts were sex-dependent, with evidence of a non-monotonic dose-response among the fluoxetine-exposed fish. This is one of the first studies to show that exposure to environmentally relevant concentrations of fluoxetine can alter the anti-predator behaviour of adult fish. In addition to the obvious repercussions for survival, impaired anti-predator behaviour can have direct impacts on fitness and influence the overall population dynamics of species.

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1. Introduction

Contamination of the aquatic environment by pharmaceuticals is a serious problem. Over 4000 human and veterinary pharmaceutical drugs are in use worldwide (Boxall et al., 2012), and with the booming global pharmaceutical market (US$400 billion) (WHO, 2015), the continual input of pharmaceuticals into aquatic environments is particularly concerning. Pharmaceuticals have been designed to treat disease in humans and animals by interacting with specific biological pathways and processes in target organisms (Daughton and Ternes, 1999). Because they were targeted to only modify physiology, traditionally pharmaceuticals were not perceived to pose a threat to aquatic organisms (Daughton and Ternes, 1999; Arnold et al., 2014). Hence, our current knowledge regarding the impacts of pharmaceuticals on wildlife is still rather limited and less is known about the effects on behaviour. This is surprising given that behaviour is often a more ecologically relevant end point of exposure (Brodin et al., 2013; Melvin and Wilson, 2013; Stewart et al., 2014; Wong and Candolin, 2015).

One pharmaceutical of particular concern is fluoxetine. It is one of the world's most widely prescribed pharmaceutical drugs used to treat depression and anxiety disorders in humans (Fent et al., 2006). For example, in 2013, fluoxetine was the 3rd most prescribed anti-depressant in the U.S. with 28 million prescriptions (Grohol 2013), while in 2016, over 500,000 prescriptions of fluoxetine were prescribed in England in a single month (NHS 2016). As a result of its widespread use, fluoxetine is frequently detected in aquatic environments (Metcalfe et al., 2003; Birch et al., 2015). The physicochemical properties of fluoxetine make it a potent, persistent (half-life 112–133 days: Kwon and Armbrust, 2006), and photolytically stable compound, with limited environmental degradation (Benfield et al., 1986; Gram, 1994; Hiemke and Härtter, 2000; Brooks, 2014; Silva et al., 2015). Due to its widespread usage, fluoxe-

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tine has been detected in surface waters at concentrations ranging from <1–600 ng/L around the world (Kolpin et al., 2002; Bratton et al., 2003; Metcalfe et al., 2003; Hughes et al., 2013; Birch et al., 2015; Schlusener et al., 2015). In target organisms, fluoxetine elicits its therapeutic effects by preventing the reuptake of serotonin, which subsequently increases the extracellular serotonin levels in the brain (Frazer, 2001; Fuller et al., 2006). The serotonergic system, which regulates neuroendocrine pathways related to reproduction and behaviour (Ögren et al., 1985; Cunningham et al., 2004; Jørgensen, 2007; Hood et al., 2006; Mennigen et al., 2011), is evolutionary conserved across vertebrates (Kah and Chambolle, 1983; Fuller et al., 2006; Mennigen et al., 2010). Therefore, it is not surprising that fluoxetine has been found to impact physiology and development of non-target aquatic organisms. Specifically, fluoxetine has been shown to decrease milt volume and testosterone levels in goldfish (*Carassius auratus*) (Mennigen et al., 2010), lower spermatozoa and oocyte numbers in zebra mussels (*Dreissena polymorpha*) (Lazzara et al., 2012), and reduce offspring production in mudsnails (*Potamopyrgus antipodarum*) (Pery et al., 2008). Fluoxetine clearly affects the morphology and development of fish, but what about behaviour?

Behaviour is governed by both external stimuli and internal hormonal and neural mechanisms (Bass and McKibben, 2003; Zala and Penn, 2004; Huntingford et al., 2006). Thus, behavioural changes can be immediate, sensitive and observable responses to cues in the surrounding environment (Scott and Slowman, 2004). Impact on behaviour is suggested to be the primary effect of fluoxetine in wildlife, because fluoxetine is designed to alter behaviour in humans (Huggett et al., 2003; Rand-Weaver et al., 2013). Studies conducted on fluoxetine so far have shown a broad range of behavioural effects in fish, such as decreased mating behaviour (fathead minnow, *Pimephales promelas:* Weinberger and Klaper, 2014), weakened aggression (Arabian killifish, *Aphanius dispar*: Barry, 2013), lowered anxiety (zebrafish, *Danio rerio*: Egan et al., 2009) and activity (sheepshead minnow, *Cyprinodon variegatus*: Winder et al., 2012).

Fluoxetine can also have a profound impact on anti-predator behaviours. In many species, behaviours, such as freezing (i.e. remaining motionless), dashing and use of shelter, form a key component of an individual's anti-predator repertoire (Lawrence and Smith, 1989; Godin, 1997; Barber et al., 2004). Apart from the obvious repercussions for survival, anti-predator behaviours can also influence other important fitness components (Abrams and Matsuda, 1993; Magurran and Nowak, 1991; Anholt and Werner, 1998; Lima, 2009). For instance, time spent foraging and time devoted to reproductive behaviours can all be affected by the presence of predators (Kotler et al., 1994; Godin, 1997; Johansson et al., 2004; Winnie and Creel, 2007). Yet, despite this, only a limited number of studies to date have examined the effects of fluoxetine and other SSRIs, on anti-predator responses. And, of those that have, the exposure concentrations used were several orders of magnitude higher than those detected in the environment (Barbosa et al., 2012; Barry 2013; Nilsson et al., 2016).

The guppy (*Poecilia reticulata*) is a small, live-bearing freshwater fish native to north-eastern South America (Houde, 1997; Jirotkul, 1999). Guppies are a well-studied model in behavioural ecology, and are an ecologically relevant species to study the effects of fluoxetine on anti-predator behaviour. This is because they inhabit a wide geographic range (Endler and Houde, 1995; Lindholm et al., 2005) and often encounter and occupy polluted environments close to human habitation, where exposure to contaminants, such as fluoxetine, is likely (Widianarko et al., 2000; Araújo et al., 2009; Willing et al., 2010). Guppies are a sexually dimorphic species, with sexually mature males being vibrantly coloured and smaller than females (Endler 1980; Houde and Endler 1990; Shohet and Watt, 2004). Males also possess a modified anal fin, the gonopodium, which is used as an intromittent organ to inseminate females. Previous research has shown that there are sex differences in the anti-predator responses of guppies. For example, in the presence of a predator, female guppies are known to devote more time to anti-predator behaviours, such as schooling and predator inspections (Magurran and Nowak, 1991; Magurran et al., 1992). Conversely, colourful males either switch from courtship to sneaky mating attempts (Magurran and Nowak, 1991), or take a risk and continue elaborate courtship displays towards females even when the predator is present (Godin and McDonough, 2002). Accordingly, the aim of our study was to investigate the effects of environmentally relevant levels of fluoxetine exposure on anti-predator behaviour in both male and female guppies. We hypothesized that fluoxetine exposure would decrease anti-predator behaviours and inhibit the reaction of exposed fish to a simulated predatory threat. As such, we expected fluoxetine-exposed fish to spend less time under plant cover and, instead, spend more time swimming and exploring their surroundings. Finally, we anticipated the behavioural responses of guppies to differ between the sexes, due to the aforementioned contrasting predator responses between male and female guppies, and because clinical studies in mammals have shown sex-specific differences to fluoxetine treatment (Dalla et al., 2010).

2. Materials and methods

2.1. Exposure setup

Wild adult guppies were collected from Alligator Creek (19°26′17.94″S, 146°57′1.09″E) in Queensland, Australia. The guppies were caught using dip nets and came from a pristine site adjacent to the Bowling Green Bay National Park. Water quality testing at this location confirmed that this guppy population had not been previously exposed to pharmaceuticals (ALS Group, Environmental Division, unpubl. data). Fish were separated by sex and acclimated to laboratory conditions (12:12 h light:dark regime, +24–26 °C) for two months prior to exposure. After acclimation, fish were randomly assigned to one of 12 separate-sex exposure tanks (60 cm × 30 cm × 24 cm; 15 fish per tank). Tanks were allocated to one of three treatments: (1) a low fluoxetine (FLX) exposure treatment (nominal concentration 50 ng/L, n = 10), (2) a high FLX exposure treatment (nominal concentration 500 ng/L, n = 9), and (3) a solvent control treatment (methanol, 0.00004%), with four tanks in total within each treatment. The level of methanol used was negligible and was therefore considered as a water control. A flow-through system following the design of Saaristo et al. (2009, 2013) was used to administer the fluoxetine or water control to the exposure tanks over a 28-d exposure period. Fish were fed *ad libitum* once daily with commercial fish granules (Otohime Hirame).

2.2. Monitoring of fluoxetine

During the exposure period, 1L water samples from each of the exposure tanks were collected weekly in glass bottles, preserved with 1 g of sodium azide, and filtered through a 0.45 μm cellulose filters (Whatman, England) before storage at 4 °C until extraction. The levels of fluoxetine in the exposure tanks were quantified with liquid chromatography-mass spectrometry (LC/MS) following the protocol of Anumol et al. (2013). Briefly, samples were spiked with isotopically labeled surrogate (100 ng/L) standard before solid-phase extraction using Agilent Plexa cartridge (200 mg). Analysis was performed with an Agilent 1210 Ultra High Performance Liquid Chromatography (UHPLC) connected to an Agilent 6410 triple quadruple mass spectrometer (QQQ). The following transitions were monitored for fluoxetine (310 > 148) and fluoxetine_d6

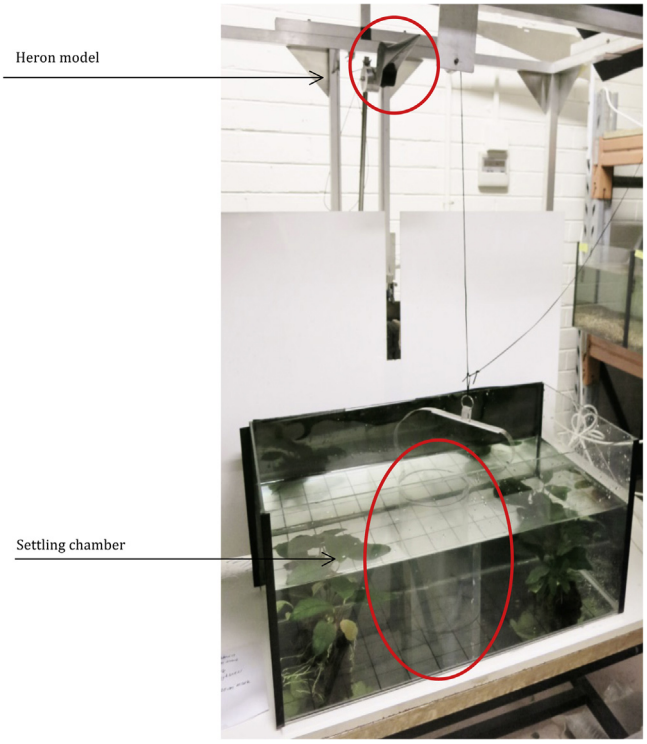

Fig. 1. Experimental setup. Individual guppies were introduced into a settling chamber (20 cm long, 12 cm diameter), which was then lifted via a remote mechanism after the fish had acclimated. The heron model was then released, and struck at a central point inside the tank, before being immediately retracted. The subsequent behavioural responses of the test subject were then filmed.

(316 > 154). The concentration of fluoxetine in the control tanks was below detection limit (< 1 ng/L) throughout the exposure period.

2.3. Behaviour trials

Behaviour trials began after the 28-d exposure period. For each trial, an individual fish was gently transferred from its exposure tank into a settling chamber in the centre of the experimental aquarium (60 cm × 30 cm × 24 cm; 25 °C ± 0.5; *Anubias barteri* plant located at either end of tank) (Fig. 1). A permanent marker was used to mark the bottom of the experimental tank with 5 cm × 5 cm grid squares to allow us to track the movement of the test subjects during experimental trials. After allowing the fish to settle for five minutes (sensu Barber et al., 2004), the settling chamber (an open cylinder) was slowly raised vertically out of the tank using a pulley system and moved behind a curtain. The bird model was then quickly released, striking at a point 5 cm above the bottom of the tank, before being immediately retracted. The model was based on the heron (*Nycticorax caledonicus*), a species that preys on guppies from the study population (pers. obs.). Any fish that swam away from the centre when the chamber was lifted, and was behind the plant when the bird model was released, was removed from the data set. The responses of the fish were filmed for a total of 15 min (i.e. 5 min acclimation period followed by a further 10 min after the bird model was released), with the experimenter leaving the room during the actual filming. We used two digital cameras (Canon PowerShot S110): one at the front and one above the tank, and the films were later quantified blind to the treatment using JWatcher event recording software (V1.0). Three anti-predator behaviours were investigated: (1) freezing, where the fish ceased movement and remained stationary immediately after the heron strike, (2) time spent under plant cover, and (3) activity, where the fish was swimming around the tank. Furthermore, we analyzed exploratory behaviour as the total surface area covered during the 10 min trial, as well as the distance covered per minute. For the total area covered, we used a grid made up of 5 cm × 5 cm squares drawn on the bottom of the experimental tank and calculated the total number of squares that the fish swam over during 10 min. The distance travelled per minute was calculated by dividing the total number of grid squares traversed by the fish with the amount of time it spent swimming. Distance travelled per unit time was quantified to control for the fact that different individuals varied in the time they took to commence swimming. A total of 80 replicates were conducted (low FLX treatment: n = 12 males and n = 17 females; high FLX treatment: n = 17 males and n = 16 females; and control treatment: n = 9 males and n = 9 females). Each fish was tested once. Following each trial, the fish was weighed (Scientech ZSA-210) and its body length measured with digital callipers (Kincrome 150 mm). Males and females measured, on average (standard length ± SE), 16.53 ± 0.16 mm and 21.80 ± 0.41 mm, respectively, with no difference in length between treatments for either of the sexes (males: one-way ANOVA: $F_{2,38} = 0.007$, $p = 0.993$; females: Kruskal-Wallis: H = 3.953, $p = 0.139$). After the morphological measurements, fish were euthanized with an overdose (40 mg/L) of anesthetic clove oil (Cunha and Rosa 2006).

2.4. Statistical analyses

Behavioural data were checked for normality and heterogeneity of variance, and, where appropriate, rank-transformed. We then used multiple models (structural equation modeling (SEM), random forest, GLM) to test for differences in behavioural responses (freezing, total time under cover, activity) among the treatment groups (control, low and high FLX), and between the sexes (male and female). The results for Random Forests and GLMs are not included because they substantively agreed with the results for SEMs. Results for SEMs are presented because this modelling approach allows for the influence of direct and indirect effects to be accounted for in the results. Initially, both length and weight were included in the models. Because neither length nor weight (nor their interaction terms) had a significant effect on the response variables, they were excluded from the final models. To compare specific treatment combinations and sex differences, we used a Mann-Whitney *U* test. Finally, in order to identify the total area and distance covered per minute, we used ANCOVA with fish standard length as the covariate. This was followed by Tukey HSD post-hoc tests to compare between the treatment groups. All statistical analyses were performed using RStudio (0.99.467).

2.5. Ethics statement

The methods for animal housing, handling and experimental protocols were assessed and approved by the Biological Sciences Animal Ethics Committee at Monash University (AEC number: BSCI/2013/09) and the Department of Environment and Primary Industries (PM/21/0005). The study complies with all the relevant State and Federal laws of Australia.

3. Results

3.1. Fluoxetine measurements

The measured concentrations of fluoxetine in the exposure tanks were as follows: (1) low fluoxetine (4 ng/L, SE = 0.39, n = 10), and (2) high fluoxetine (16 ng/L, SE = 2.73, n = 9). The concentration of fluoxetine in the control tanks was below the detection limit throughout the exposure period (see details in Table S1).

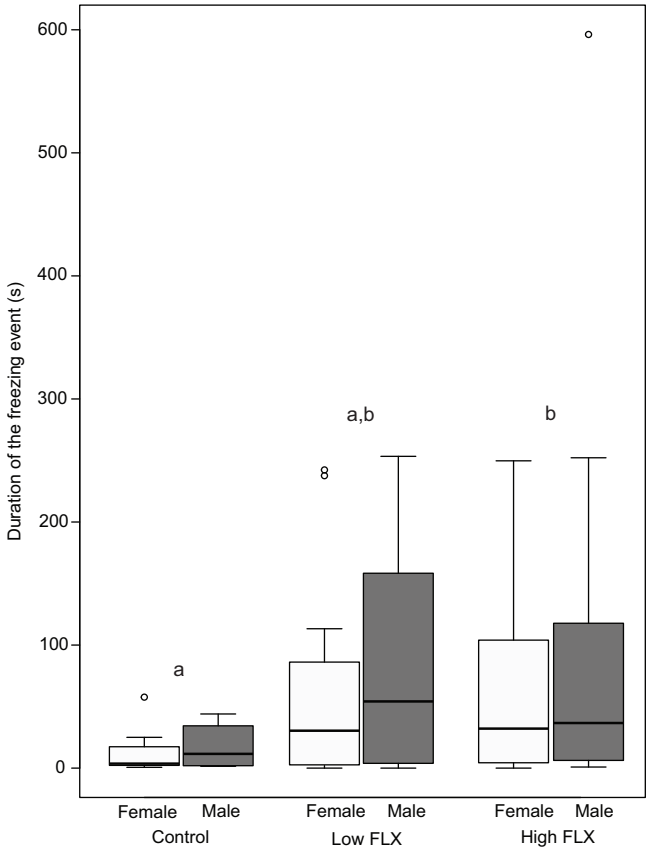

Fig. 2. The total amount of time (s) fish froze after the simulated bird strike. White bars represent females (control = 9, low fluoxetine (FLX) = 15, high FLX = 12), and grey bars males (control = 9, low FLX = 12, high FLX = 17). Boxplots show the upper and lower quartiles and the median of the data. Treatments without letters in common are significantly different to each other ($p < 0.05$).

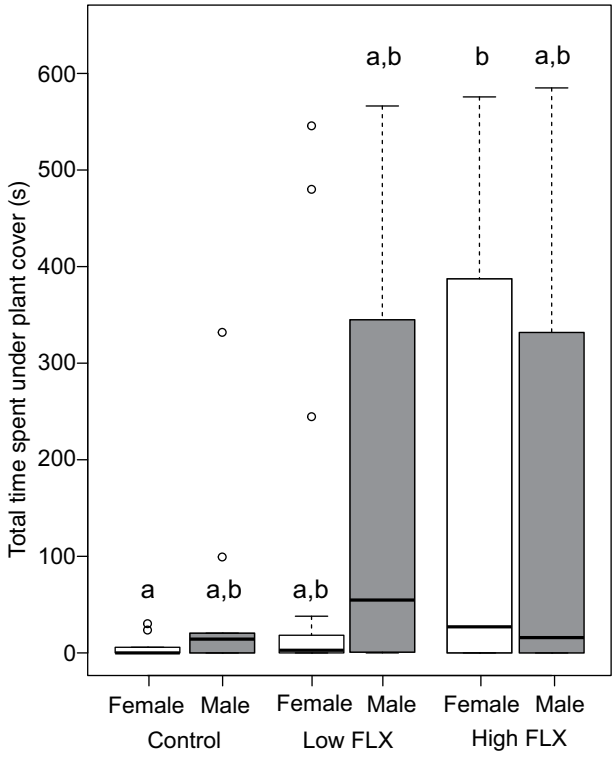

Fig. 3. The total amount of time (s) fish spent under plant cover. White bars represent females (control = 9, low FLX = 17, high FLX = 16), and grey bars males (control = 9, low FLX = 12, high FLX = 17). Boxplots show the upper and lower quartiles and the median of the data. Treatments without letters in common are significantly different to each other ($p < 0.05$).

3.2. Freezing

Fluoxetine exposure had an impact on freezing behaviour (SEM: $z = 2.231$, $p = 0.026$). Specifically, high FLX fish froze for significantly longer than control fish (Mann-Whitney: $U = 183$, $p = 0.024$), while the duration of the first freezing event did not differ between control and low FLX ($U = 185$, $p = 0.072$), nor between low and high FLX fish ($U = 475.5$, $p = 0.794$) (Fig. 2). There were no sex differences between the treatment groups in the duration of freezing behaviour (SEM: $z = 0.977$, $p = 0.328$).

3.3. Time spent under plant cover

Fluoxetine exposure had an effect on time spent under plant cover (SEM: $z = 2.103$, $p = 0.035$, $n = 81$). Specifically, high FLX fish spent significantly more time under cover than control fish ($U = 198$, $p = 0.047$) (Fig. 3). Other treatment groups, however, did not differ significantly from each other (low FLX versus control: $U = 236$, $p = 0.456$; low versus high FLX fish: $U = 575$, $p = 0.262$) (Fig. 3). Furthermore, there was no difference among treatments in the number of times fish visited plant cover (SEM: $z = 1.022$, $p = 0.307$). In regard to sex-specific effects, males showed no significant differences between treatment groups (SEM: $z = 0.797$, $p = 0.426$), while females did (SEM: $z = 2.396$, $p = 0.017$). Specifically, high FLX females spent more time under cover than control females ($U = 35$, $p = 0.034$), while there was no difference between low and high FLX females ($U = 100$, $p = 0.125$), or between control and low FLX females ($U = 65$, $p = 0.545$) (Fig. 3).

3.4. Activity

Fluoxetine exposure made fish less active (SEM: $z = -2.459$, $p = 0.014$). Specifically, high FLX fish were significantly less active than control fish ($U = 408$, $p = 0.028$) (Fig. 4). However, there was no difference in activity between either low or high ($W = 388$, $p = 0.144$), or control and low, FLX fish ($U = 310$, $p = 0.404$) (Fig. 4). Furthermore, there was no significant sex difference in activity levels (SEM: $z = -1.147$, $p = 0.251$).

3.5. Exploratory behaviour

Fluoxetine exposure affected the exploratory behaviour of guppies and the impact was sex-specific. Fluoxetine treatment had a significant effect on the total surface area covered during the trial (ANCOVA: $F_2 = 4.836$, $p = 0.014$), as well as on the distance traversed per minute (ANCOVA: $F_2 = 4.583$, $p = 0.017$). Specifically, low FLX males swam over fewer squares than control males (Tukey HSD test: $p = 0.009$) (Fig. 5A), and also at a lower speed (Tukey HSD test: $p = 0.012$) than control males (Fig. 5B). There was no significant difference between any of the other treatments (Tukey HSD tests: $p > 0.05$).

In contrast to males, females showed no significant difference in swimming activity across treatments. Specifically, there was no significant difference in the total number of squares traversed among treatments (ANCOVA: $F2 = 0.611$, $p = 0.548$) (Fig. 5A), or the number of squares traversed per minute (ANCOVA: $F2 = 1.186$, $p = 0.316$) between treatments (Fig. 5B).

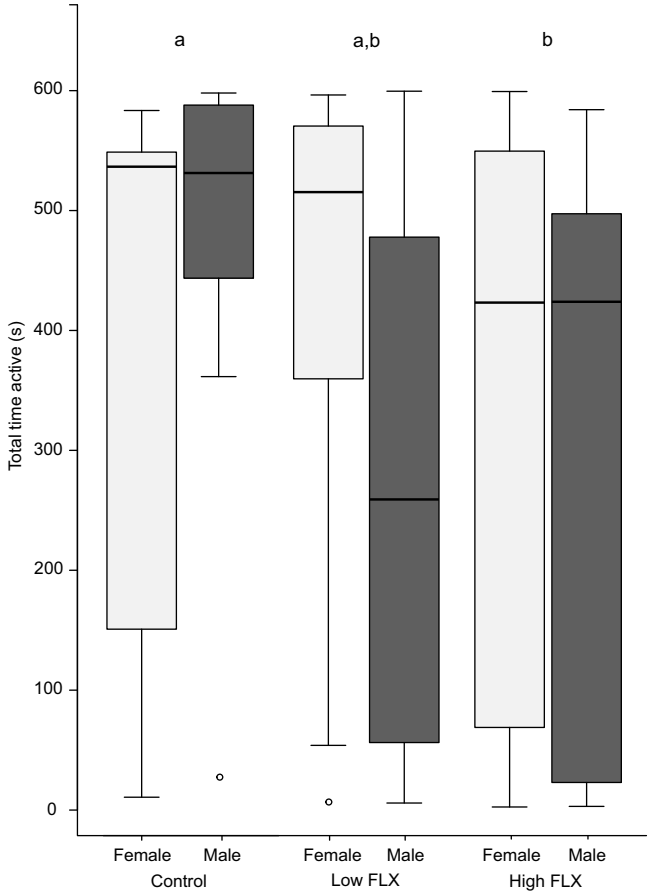

Fig. 4. The total amount of time (s) fish were active. White bars represent females (control = 9, low FLX = 17, high FLX = 16), and grey bars males (control = 9, low FLX = 12, high FLX = 17). Boxplots show the upper and lower quartiles and the median of the data. Treatments without letters in common are significantly different to each other ($p < 0.05$).

4. Discussion

Our study demonstrates that short-term exposure to environmentally relevant concentrations of fluoxetine affects anti-predator behaviours of wild fish. Specifically, after a simulated bird strike, fluoxetine-exposed fish froze for longer, spent more time under plant cover, and were less active. Furthermore, fluoxetine exposure caused sex-specific effects and showed evidence of non-monotonic dose response.

Freezing is an effective anti-predator response when the predator is within close proximity (Godin, 1997). In our study, high fluoxetine concentration-exposed fish took longer to cease freezing behaviour and resume activity. Recent studies investigating the impact of fluoxetine on freezing behaviour have yielded contrasting results. For example, chronic exposure to fluoxetine increased freezing behaviour in some studies (guinea pig, *Cavia porcellus*: Olsen et al., 2002; zebrafish: Maximino et al., 2011), but not in others (piaucu fish, *Leporinus macrocephalus*: Barbosa et al., 2012; zebrafish: Wong et al., 2013; Eastern mosquitofish, *Gambusia holbrooki*: Martin et al., 2016). There are several potential reasons for these differences. First, studies often employ different assays to test anti-predator responses, which could potentially influence the level of threat perceived by test subjects and, hence, their behavioural responses. Another possibility is that it takes 2–4 weeks for fluoxetine to produce therapeutic effects (Gardier et al., 1996; Matuszczyk et al., 1998; Le Poul et al., 2000) and therefore acute experiments using high doses are more likely to trigger

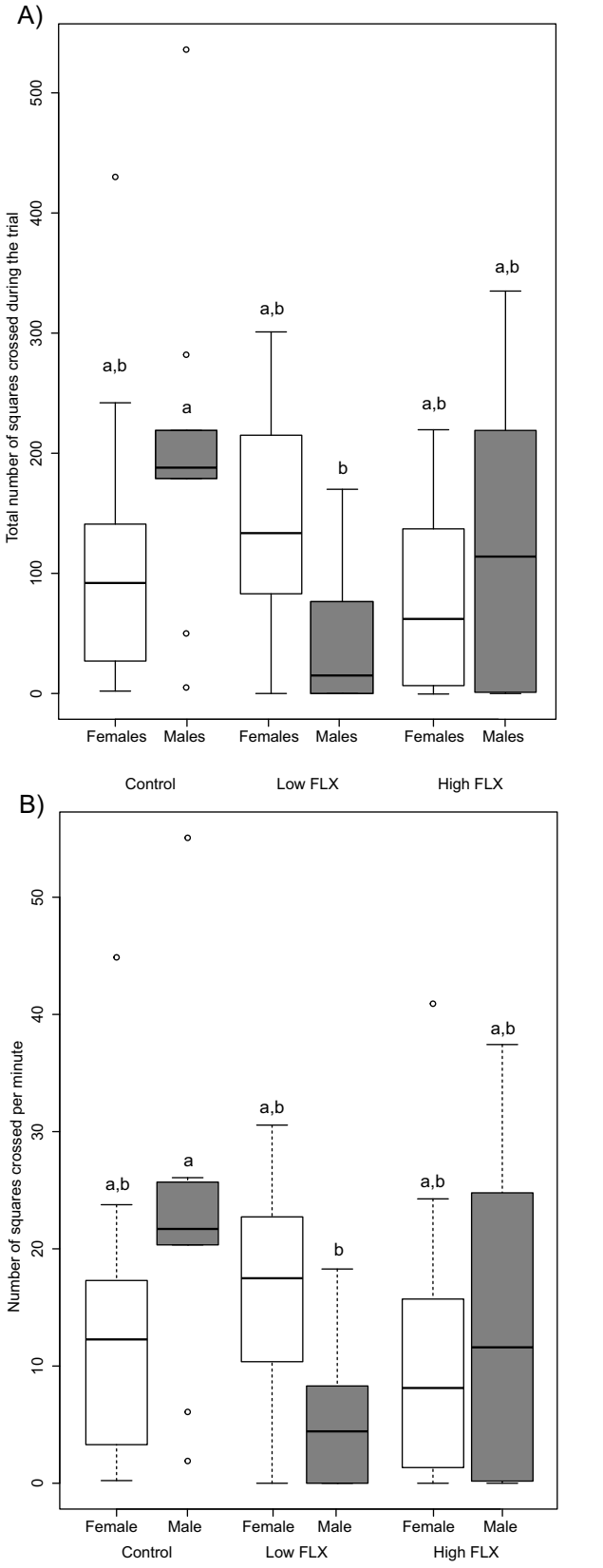

Fig. 5. (A) The total number of squares crossed during the trial, and (B) number of squares crossed per minute. White bars represent females (control = 9, low FLX = 17, high FLX = 16), and grey bars males (control = 9, low FLX = 12, high FLX = 17). Boxplots show the upper and lower quartiles and the median of the data. Treatments without letters in common are significantly different to each other ($p < 0.05$).

toxic responses (Kalueff et al., 2008; Stewart et al., 2013; Stewart et al., 2014). Lastly, differences in freezing behaviour could also reflect a non-monotonic dose-response. When low concentrations of fluoxetine cause effects that higher concentrations do not, as has been demonstrated in various taxa (Sánchez-Argüello et al., 2009; Conners et al., 2009; Guler and Ford, 2010; Di Poi et al., 2013; Barry, 2013), studies using multiple exposure concentrations are needed to distinguish the chronic effects from the toxic.

Seeking plant cover is another anti-predator behaviour commonly utilised by fish. In our study, fluoxetine-exposed fish (high FLX) spent more time under plant cover than control fish. In orb-web spiders (*Larinioides cornutus*), direct addition of serotonin has been known to cause amplified anti-predator responses following a mock predator attack (Jones et al., 2011). In this regard, given that anxiety and fear, which are triggered by stress, are modulated by the neurotransmitter serotonin (Leonardo and Hen, 2006), amplified anti-predator response, such as remaining extended periods under plant cover, could be a sign of stress. Indeed, an extensive review on selective serotonin reuptake inhibitors found that 38% of the reviewed studies reported an increase in anxious responses in animals (Griebel, 1995). The apparently anxious response observed in our study, however, is contrary to the many clinical studies that have reported serotonin and SSRIs as causing reduced anxiety (anxiolytic effects) (Salchner and Singewald 2002; Spennato et al., 2008; Ravinder et al., 2011; Burghardt et al., 2013; Deschauz et al., 2013). One possibility for these different results is that the behavioural effects of fluoxetine change over time. That is, acute exposure to fluoxetine causes effects different from chronic exposure. For example, a study by Pittman and Ichikawa (2013) showed that although zebrafish performed anxious (fear) behaviour after the first week of exposure to fluoxetine, at the end of the second week, anxiety (anxiolytic effects) was (were) reduced. Hence, in the context of the current study, it is possible that fluoxetine had yet to exert therapeutic effects on guppies and what we documented was, in fact, the initial anxiogenic (fear) effect. Furthermore, the fact that we used wild guppies could have played a role, as laboratory and wild animals have been shown to respond differently to stress (Künzl et al., 2003; Augustsson 2004; Oosthuizen et al., 2013; Modlinska et al., 2015). Overall, overestimating the risk of predation by remaining under plant for longer could also be costly because it provides limited opportunities for other fitness-related activities (Cooper and Frederick, 2007).

Fluoxetine-exposed fish (high FLX) were less active than control fish. These results are consistent with decreased locomotion and lethargy, and subsequent reduced activity levels reported in several other aquatic species, including other species of fish (Perreault et al., 2003; De Lange et al., 2006; Airhart et al., 2007; Clements and Schreck, 2007; Henry and Black, 2008; Guler and Ford, 2010 Guler and Ford, 2010). It is worth noting, however, that these earlier studies used exposure concentrations (ug/L) of fluoxetine that are much higher than those detected in the environment. Thus, our findings are significant in showing that fluoxetine can have effects on fish activity even at environmentally relevant (i.e. lower) concentrations and give further support to the use of swimming activity as a sensitive biomarker of chemical pollution (for review, see Little and Finger, 1990).

Another anti-predator behaviour linked to activity is exploratory behaviour (i.e. an individual's behavioural response to novel environments, resources and objects: *sensu* Reale et al., 2007; Smith and Blumstein, 2008). In our study, exposure to fluoxetine affected the exploratory behaviour of guppies. Specifically, low fluoxetine fish swam over fewer squares and at slower speed than control fish. This is in accordance with prior research, which reported reduced average swimming velocity (Barry, 2013), swimming speed (Painter et al., 2009), and shorter predator evasion in fluoxetine-exposed fish in response to the presence of a predator cue (Weinberger and Klaper, 2014). The potential of SSRIs affecting the feeding in fish can be ruled out, because the food given to the exposed and control fish was measured and we did not observe any differences in the feeding rates. This suggests that fluoxetine (at the exposure levels used in our study) is not affecting the appetite of guppies. Furthermore, high- and low-predation risk is known to drive exploratory behaviour and a recent study showed that guppies that co-occurred with large predators were less exploratory than those present in sites with small predators (Burns et al., 2016).

We found sex differences in several of the quantified anti-predator behaviours. Seeking of plant cover, for example, revealed that high fluoxetine concentration-exposed females spent more time under cover than control females. This is consistent with a previous study, which found that maternal fluoxetine exposure in mice increased depressive-like behaviour in adult females, but not in males (Lisboa et al., 2007). We also found sex differences in exploratory behaviour, with low fluoxetine concentration-exposed males swimming over fewer squares and at lower speed than control males. This sensitivity to fluoxetine among males is in accordance with a recent multigenerational study, which demonstrated that male rat offspring showed increased anxiety-like behaviours, while females showed no such response (Gobinath et al., 2016). Our study highlights the need for studies to investigate impacts of fluoxetine on a broad range of behaviours in both sexes.

Altered anti-predator behaviours, as a result of fluoxetine exposure, could have important fitness consequences. Responding to predation threat by, for example, freezing or staying under plant cover for longer, is likely to be costly, because time and energy used in remaining stationary is diverted from other fitness-related activities, such as foraging and finding mates (Lima and Dill, 1990; Sih, 1992; Lima and Bendekoff, 1999; Welton et al., 2003). Further, decreased exploratory behaviour could negatively impact the reproductive success of males, which, in the case of guppies are known to benefit from being bold and exploring new territories in search for mating opportunities (Magurran and Seghers, 1994; Croft et al., 2003).

In conclusion, our study is one of the first to show that exposure to environmentally relevant concentrations of fluoxetine alters anti-predator behaviour in wild guppy. We demonstrate that fluoxetine exposure causes sex-specific effects in a non-monotonic manner, with females spending more time under cover and males becoming less exploratory depending on the level of exposure. In addition to the obvious repercussions for survival, the impaired anti-predator behaviours reported in our study could have direct impacts on fitness and underscore the importance of considering the effects of pharmaceutical pollutants on behaviour at environmentally relevant exposures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2016.12.007.

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