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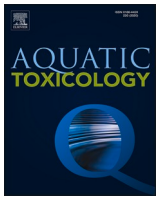

Effects of long-term fluoxetine exposure on morphology, but not behaviour or metabolic rate, in male guppies (*Poecilia reticulata*)

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ABSTRACT

Contamination of aquatic ecosystems by pharmaceuticals is a growing threat worldwide. The antidepressant fluoxetine is one such pharmaceutical that is frequently detected in aquatic ecosystems, and has been found to alter the behaviour and physiology of exposed wildlife. Few studies, however, have investigated potential combined effects on behaviour and metabolic rate. In addition, exposures are often short in duration and rarely conducted under ecologically relevant conditions. Here, we examined the impacts of long-term fluoxetine exposure on boldness (exploration, activity, and antipredator behaviour), metabolic rate, and morphology in male guppies (*Poecilia reticulata*). Specifically, fish were exposed for 8 months (corresponding to approximately two overlapping generations) in semi-natural mesocosms to one of three treatments: an unexposed control (0 ng L^{-1}), or low or high fluoxetine (mean measured concentrations: 30 ng L^{-1} and 292 ng L^{-1}, respectively). Following exposure, we quantified male exploratory behaviour and activity in a novel environment (maze arena) and antipredator behaviour in the presence or absence of a live predator (spangled perch, *Leiopotherapon unicolor*), as well as metabolic rate and morphology (mass, standard length, and scaled mass index). Fluoxetine exposure did not significantly alter boldness, metabolic rate, mass, or standard length. However, fluoxetine exposure did alter body condition, whereby fish in the high treatment had a higher scaled mass index than control fish. Our results, considered alongside previous work, underscore the importance of exposure duration in mediating the effects of fluoxetine on fitness-related traits. Continued research under extended exposure periods (i.e., spanning multiple generations) is essential if we are to accurately predict the ecological impacts of fluoxetine on exposed wildlife, and their underlying mechanism(s).

1. Introduction

Pharmaceutical pollution is increasingly recognised as a major environmental threat, with over 900 active pharmaceutical compounds detected in aquatic environments around the globe (Patel et al., 2019; Graumnitz and Jungmann, 2021). Pharmaceuticals are often not completely metabolised by humans or other animals, and most current wastewater treatment plants are not equipped to adequately remove drug residues (Baresel et al., 2019; Saaristo et al., 2023). As a result, the effluent from wastewater treatment plants is a leading source of pharmaceutical contamination, along with industrial effluent, and run-off from livestock operations (Wronski and Brooks, 2023). Further, many

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drugs persist in the environment for long periods of time due to resistance to environmental degradation (O'Flynn et al., 2021; Maculewicz et al., 2022), or continued replenishment (i.e., 'pseudo-persistence'; Arnold et al., 2014; Correia et al., 2023).

Antidepressants are frequently detected in aquatic ecosystems globally as a result of their widespread usage (Wilkinson et al., 2022). This includes the selective serotonin re-uptake inhibitor (SSRI) fluoxetine (sometimes marketed as Prozac), which is among the most heavily prescribed drugs used to treat depression and anxiety-related disorders in both humans (Hurst and Lamb, 2000; Wong et al., 2005) and domesticated animals (Chutter et al., 2019). Fluoxetine inhibits the re-uptake of serotonin, increasing extracellular serotonin levels in the brain and, in doing so, elicits behavioural changes (McDonald, 2017; Gould et al., 2021). As a result of its pervasive use, fluoxetine is frequently detected in aquatic environments, with concentrations typically ranging between <1–330 ng L^{-1} in freshwater systems (Mole and Brooks, 2019; Gould et al., 2021; Sumpter and Margiotta-Casaluci, 2022). It has high potential to affect wildlife because its primary target receptor (the serotonin transporter) is evolutionarily conserved across a broad range of taxa (Gunnarsson et al., 2008; Correia et al., 2023). Indeed, across a wide range of aquatic species, fluoxetine exposure has been attributed to physiological and morphological effects (e.g., Bidel et al., 2016; Thoré et al., 2020), as well as the perturbation of ecologically important behaviours including, aggression (e.g., McCallum et al., 2017; Peters et al., 2017), antipredator and anxiety-related behaviours (e.g., Saaristo et al., 2017; Martin et al., 2019a,b; Al Shuraiqi et al., 2021), reproductive behaviours (e.g., Fursdon et al., 2019; Thoré et al., 2020), and social behaviours (e.g., Hong et al., 2021; Martin et al., 2019c).

Given that fluoxetine can cause changes to anxiety-related behaviours, one ecologically important behavioural trait likely to be affected is boldness (Ferreira et al., 2023), which is defined as the tendency for individuals to take risks in return for potential fitness gains (Sih et al., 2004; Wilson and Godin, 2009). Altered risk-taking behaviours can have important ecological consequences, including those related to predator-prey interactions (Smith and Blumstein, 2008; Hulthén et al., 2017), and reproductive behaviours (Croft et al., 2003). In order to fully explore the potential effects of fluoxetine on anxiety-related behaviours, experiments must be performed under varying ecological contexts (e.g., in novel environments or in the presence of predation risk), which can offer more robust insights into behavioural responses than assays performed in isolation (Bertram et al., 2022; Martin et al., 2024). Research is also lacking on potential effects on metabolic rate (but see Tan et al., 2020), despite evidence that fluoxetine can disrupt a range of physiological processes (Campos et al., 2016; Hird et al., 2016). In addition, the serotonergic system is known to regulate various physiological functions, including energy expenditure (McGlashon et al., 2015; Moon et al., 2022). Metabolism also influences all biological activities and is strongly associated with other ecologically important behavioural and physiological traits (Mathot et al., 2019; Wu and Seebacher, 2022). For instance, metabolic rate has been linked to boldness, with previous studies observing increased boldness in fish with higher metabolic rate (Killen et al., 2011; Myles-Gonzalez et al., 2015; Behrens et al., 2020). However, in the context of fluoxetine exposure, few studies have examined behaviour and metabolic rate in combination (but see Tan et al., 2020). Furthermore, while efforts to understand the consequences of fluoxetine exposure over longer time periods are increasing (Mason et al., 2021; Polverino et al., 2021; Thoré et al., 2021a; Polverino et al., 2023; Thoré et al., 2023), the majority of studies to date have employed relatively short exposure durations (i.e., <1 month). This is because the therapeutic effects of fluoxetine typically occur within 2–4 weeks in humans (Gardier et al., 1996; Hensler, 2003). While these short-term exposure studies have been useful in understanding the effects of acute exposure, they might not reflect circumstances seen in nature. In particular, the continued resupply of wastewater effluent, coupled with the persistence of fluoxetine in the environment (Souza et al., 2022), underscore the importance of longer-term exposure studies.

Here, we investigated whether and how long-term (8-month) exposure to environmentally relevant concentrations of fluoxetine impacted boldness, metabolic rate, and morphology in male guppies (*Poecilia reticulata*). Boldness was examined in two contexts: in a maze arena, as well as in the presence of a predator (spangled perch, *Leiopotherapon unicolor*). We then measured oxygen consumption as a proxy for metabolic rate, and recorded mass and standard length as morphological measurements. If chronic effects of fluoxetine exposure would reflect those reported in acute exposure studies (Pelli and Connaughton, 2015; Martin et al., 2017; Martin et al., 2019b), we would expect to observe increased boldness, activity, and exploration in a novel maze in fluoxetine-exposed fish. We would similarly expect fluoxetine-exposed fish to be bolder and more active in the presence of a predator. On the other hand, if longer-term exposure leads to greater habituation or tolerance to the drug (e.g., due to neuroadaptive changes; Targum, 2014), it is also possible that we may not observe any differences between the boldness of chronically exposed and unexposed fish. Regarding metabolic rate, acute exposure to fluoxetine has been shown to increase metabolic rate in both humans and exposed wildlife (Hird et al., 2016), while long-term exposure has indicated a decrease in metabolic rate (Tan et al., 2020). As such, we hypothesised that fluoxetine-exposed fish would have a lower metabolic rate than unexposed fish. Finally, in addition to behavioural and physiological endpoints, we quantified potential differences in mass, standard length, and body condition (scaled mass index) in fluoxetine-exposed and unexposed fish. Previous studies on both acute and long-term exposure in guppies have found no impact of fluoxetine on morphology (Fursdon et al., 2019; Wiles et al., 2020). We therefore predicted that there would be no effect of fluoxetine exposure on mass, standard length, or body condition.

2. Materials and methods

2.1. Study species

Guppies are small, live-bearing freshwater fish native to Trinidad and the north of South America (Rosen and Bailey, 1963) that have been introduced to various tropical and sub-tropical regions worldwide (Deacon et al., 2011). In many parts of the world, guppies are known to occur in environments close to human habitation, where exposure to contaminants—including pharmaceuticals, such as fluoxetine—is likely (Widianarko et al., 2000; Araújo et al., 2009). More generally, the guppy is a common model in behavioural ecology (Burns, 2008) and ecotoxicology (Norrgren, 2012; Thoré et al., 2021c), and thus represents an ideal model species for this study. We used male guppies because boldness, activity, and exploration can have important fitness consequences for males, as previous studies have shown that these traits can facilitate dispersal and male reproductive success (Gasparini et al., 2019; Herdegen-Radwan, 2019).

2.2. Animal collection and housing

2.2.1. Guppies

The guppies used in this study were sourced from a wild population collected in November 2016 from Alligator Creek (19°26′18″ S, 146°57′01″ E), a rainforest-fed stream located within Bowling Green Bay National Park in Townsville, Australia (collection permit: WITK17685216). Analysis of water samples from the collection site indicated that it was free from fluoxetine contamination (Envirolab Services; see Section 2.3. '*Chemical exposure and analyses*'). The founding population consisted of 3600 adult guppies of both sexes, which were randomly distributed across 12 stainless steel mesocosm tanks (648 L; 180 × 60 × 60 cm; water depth: 30 cm; Fig. S1), with 300 fish introduced into each tank (50:50 sex ratio) as part of a broader research program investigating the long-term impacts of fluoxetine on the behaviour, ecology, and evolution of guppies. The size of mesocosm

tanks fell within the range of natural pool sizes that guppies typically inhabit in the wild, including the source population of guppies used in this study (*pers. obvs.*). The mesocosm system was kept under natural light:dark cycle (average 12:12 h light:dark) in a temperature-controlled greenhouse facility at Monash University, where tanks were monitored weekly for temperature (mean = 23.26 °C, SD = 0.91 °C, $n = 520$) and pH (mean = 7.15, SD = 0.68, $n = 515$; see Table S1 for summary statistics of each mesocosm tank). Each tank contained carbon-filtered tap water, aerated by commercial air pumps (Resun LP100), a 2 cm layer of pebble substrate (grain size: 7 mm), and aquatic plants (Java moss, *Taxiphyllum barbieri*) for refuge. Fish were fed to apparent satiation once daily with commercial fish pellets (Aquasonic Nutra Xtreme C1 pellets, 0.8 mm). The fluoxetine exposure commenced five months after the founder population had been introduced into the mesocosms (see Section 2.3. '*Chemical exposure and analyses*', below).

2.2.2. Spangled perch

Adult spangled perch (standard length: 5.53 ± 0.08 cm, $n = 11$), used as predatory stimuli in behavioural trials (see Section 2.4.2. '*Exploratory behaviour in the presence of a predator*' below), were sourced from a commercial supplier (Australian Native Fish Enterprise). The supplier collected the perch from a wild population in Kallangur, Queensland, before they were transported to Monash University via air freight. Spangled perch were acclimated to laboratory conditions for two weeks prior to the commencement of experiments, housed in large, aerated tanks filled with carbon-filtered tap water (60 × 30 × 30 cm, water depth: 20 cm; 2 fish per tank) at 24 °C under a 12:12 h light:dark cycle. They were fed to apparent satiation once daily with Hikari cichlid gold pellets (3.2–3.7 mm). Perch were not exposed to fluoxetine so that we could determine the effects of exposure treatment on male guppy behaviour without any potential interactive effects of predator exposure. The spangled perch is an opportunistic carnivore, with smaller fish species comprising a considerable portion of their diet (Medeiros and Arthington, 2014). They are also known to exist sympatrically with guppies in Australia, including the source population of guppies used in this study. Previous research has shown that guppies can differentiate between a spangled perch and a non-predatory heterospecific (rainbowfish, *Melanotaenia splendida*) by associating less with the former (Fursdon et al., 2019).

2.3. Chemical exposure and analyses

Fluoxetine exposure commenced five months after the founder population had been introduced to the mesocosm tanks (April 2017). Each of the 12 independent mesocosm populations were randomly assigned to one of three treatments: solvent control (i.e., unexposed; $n =$ 4 mesocosms), low fluoxetine (nominal concentration: 30 ng L^{-1}; $n = 4$ mesocosms), or high fluoxetine (nominal concentration: 300 ng L^{-1}; $n =$ 4 mesocosms). The low-fluoxetine treatment was chosen to represent fluoxetine concentrations commonly detected in surface waters, while the high treatment was chosen to represent the higher range of environmental detections (reviewed in Mole and Brooks, 2019). Guppies were exposed to these concentrations for eight months, representing 2–3 overlapping generations (Reznick et al., 1997).

To achieve the nominal low and high fluoxetine concentrations, stock solutions were created by dissolving fluoxetine hydrochloride (Sigma Aldrich; product number: F132, CAS: 56296-78-7) at the desired concentrations depending on the treatment (low fluoxetine: 10 µg in 1 mL methanol; high fluoxetine: 100 µg in 1 mL methanol) and diluting these solutions in 1 L of reverse osmosis water. The stock solutions were then mixed into the appropriate mesocosm tanks twice per week. As fluoxetine was dissolved using methanol (HPLC grade ≥99.9%), each of the control tanks received 1 mL of methanol mixed into 1 L of reverse osmosis water twice per week to act as a solvent control. Partial water changes (20%) were conducted once per week prior to dosing. Similar water changes were also done in the control tanks.

Water samples were analysed monthly to measure fluoxetine concentrations and to check for potential contamination in the control tanks. This involved collecting two replicate water samples (100 mL) from each fluoxetine exposure tank, and half of the control tanks (selected at random), using a serological pipette. Samples were then analysed using solid phase extraction (SPE) and liquid chromatography coupled to tandem mass spectrometry (LC-MS) by Envirolab Services (MPL Laboratories; NATA accreditation: 2901; accredited for compliance with ISO/IEC: 17025), with a minimum detection limit of 2 ng L^{-1}. A detailed description of this protocol can be found in Bertram et al. (2018).

2.4. Behavioural trials

One week prior to behavioural trials, adult male guppies were separated from the main populations in the mesocosms and placed in perforated stainless steel cages (diameter × height: 35 cm × 32 cm) within their respective mesocosm tank. This was done by catching all males in the mesocosms with nets, and then placing them in the steel cages. Isolating males prior to behavioural testing was necessary because catching them directly from the mesocosm may have indirectly biased samples towards certain behavioural types (e.g., less bold individuals may be more likely to seek refuge in the aquatic plants during sampling; Diaz Pauli et al., 2015). For all behavioural trials, an ID number tag (not revealing treatment history) was placed in view of the camera on each observation tank, for each trial, to enable later blind analysis of the videos. Following completion of behavioural trials, guppies were returned to the mesocosm tanks.

2.4.1. Exploratory behaviour in a maze

We first examined boldness, activity, and exploration, which involved observing male guppies from each fluoxetine treatment (control $n = 41$, low fluoxetine $n = 39$, high fluoxetine $n = 39$) in a maze set-up following the protocol of Lucon-Xiccato and Bisazza (2017). The maze was positioned inside a 54 L (60 × 30 × 30 cm) observation tank and consisted of an acclimation chamber (9 × 5 cm) that was separated from the rest of the maze by a removable, opaque partition (Fig. 1). The observation tank was filled with 10 cm of filtered tap water (i.e., water that did not contain any fluoxetine) in a controlled temperature room set to 24 °C (water temperature mean ± SD = 25.5 °C ± 0.92). The external surfaces of the observation tanks were frosted to prevent visual disturbance during trials. In addition, light regimes were kept as similar as possible across observation tanks to control for any potential effects of light on behaviour (Endler, 1991; Smith et al., 2002). Observation tanks were drained and thoroughly rinsed in-between each trial to avoid any potential cross-contamination of conspecific chemical cues between trials.

The male guppies were collected (using a net) from the cages in the mesocosms and transported by car in aerated buckets to the nearby laboratory facility where the behavioural experiments were performed.

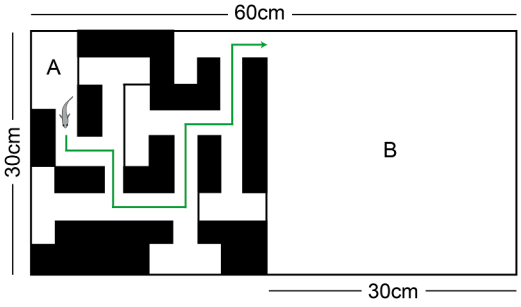

Fig. 1. Diagram depicting the maze arena, which comprised (A) an acclimation chamber (9 × 5 cm) leading into a series of interconnected T-junctions (width = 3 cm), and (B) the exit leading to open water.

At the start of each trial, an individual focal male was placed into the acclimation chamber in the maze (Fig. 1) for a 30 min acclimation period. The opaque partition was then lifted manually, and the focal fish was free to explore the maze for 45 min. All trials were filmed from above (using Panasonic HC-V180 cameras). The behaviours of the guppies were later quantified from the videos using an open-source behavioural analysis key-logging software (BORIS version 5.1.3; Friard and Gamba, 2016). Specifically, we measured latency to emerge from the acclimation chamber (sec), which is a well-studied and validated measure of boldness in a wide range of species (Myles-Gonzalez et al., 2015; Näslund et al., 2015; Kerman et al., 2016; Collins et al., 2019), including guppies (Brown and Braithwaite, 2004; Burns, 2008). For the individuals that emerged (control $n = 39$, low fluoxetine $n = 36$, high fluoxetine $n = 36$), we quantified latency to complete the maze (sec) as a measure of the fish's tendency to explore a novel environment. Individuals that did not emerge from the starting chamber were excluded from analysis. Finally, we measured general activity levels by calculating the average speed (cm sec^{-1}). This was achieved by tracing the path of the fish and then measuring the total distance covered during the trial by using the open-source image analysis software ImageJ (version 1.51k; Schneider et al., 2012). All videos were scored blind (by J.L.T) regarding fluoxetine treatment to avoid any potential observer bias, by using the male ID number that did not disclose treatment history.

2.4.2. Exploratory behaviour in the presence of a predator

Exploratory behaviour of male guppies from each fluoxetine treatment was observed either in the presence or absence of a predatory spangled perch in a 3 × 2 independent factorial design (predator absent: control $n = 31$, low fluoxetine $n = 30$, high fluoxetine $n = 29$; predator present: control $n = 30$, low fluoxetine $n = 32$, high fluoxetine, $n = 30$). Behavioural trials were conducted in observation tanks (60 × 30 × 30 cm, water depth: 20 cm) that each had a separate glass compartment (8 × 30 × 30 cm, water depth: 20 cm) on one end (hereafter referred to as the 'predator compartment'; Fig. S2). This allowed for visual access between the guppies and spangled perch without physical interaction during the trial. The predator compartment either housed a spangled perch in the predator treatments or was left empty in the non-predator treatments. Both the observation tank and predator compartment were filled with filtered tap water (free from fluoxetine) in a controlled temperature room set to 24 °C (water temperature mean ± SD = 24.78 ± 0.99) and contained a 3 cm layer of white sand lining the bottom. The tanks were frosted on all sides, excluding the two sides abutting one another, to prevent visual disturbance during trials. All tanks were lit from above using fluorescent lights. Observation tanks were drained and thoroughly rinsed in-between each trial to avoid any potential cross-contamination of conspecific chemical cues between trials.

The male guppies were collected (using a net) from the cages in the mesocosms and transported by car in aerated buckets to the nearby laboratory facility where the behavioural experiments were performed. At the beginning of each trial, an individual guppy was placed inside an opaque, cylindrical chamber (diameter × height: 12 cm × 30 cm) in the centre of the observation tank and allowed to acclimate for 20 min (Fig. S2). Following acclimation, the chamber was lifted manually, and behaviour was filmed from above for 20 min (using Panasonic HC-V180 cameras). Behaviour was subsequently quantified using BORIS (version 5.1.3; Friard and Gamba, 2016). We measured latency of the focal fish to enter a pre-determined predator strike zone (sec), as well as total time spent in the strike zone (sec). The 'strike zone' refers to a 6 × 30 cm zone closest to the predator compartment and was based on the striking range of another, similar-sized predator of guppies, the pike cichlid (*Crenicichla alta*; Walker et al., 2005). Consequently, entering the strike zone was considered inherently bold (Godin and Dugatkin, 1996; Ward et al., 2004). We also measured activity levels during the trial by dividing the tank into 50 grid squares (6 × 6 cm each) with markers, which were superimposed over videos during analysis. Activity was calculated as the total number of grid squares crossed during the trial. All videos were scored blind (by J.L.T) regarding fluoxetine treatment to avoid any potential observer bias, by using the male ID number that did not disclose treatment history. The side of the tank where the predator compartment was placed was randomised between trials to control for any potential side bias (Jeswiet and Godin, 2011).

2.5. Standard metabolic rate

To assess the potential impacts of fluoxetine exposure on metabolic rate in male guppies, we used fluorescence-based closed respirometry to measure the rate of oxygen consumption ($\dot{V}O_2$) as a proxy for standard metabolic rate (hereon referred to as 'metabolic rate'; Pettersen et al., 2015; Nelson, 2016). All trials were conducted in a dark room kept at 24 °C to reduce fish stress (Maximino et al., 2010) and minimise disturbance to the respirometry readings. Guppies were not fed for 24 h prior to trials to ensure individuals were in a post-absorptive state, as recent feeding can affect metabolic rate in fish (Jobling, 1981). Adult focal fish from each exposure treatment (control $n = 34$, low fluoxetine $n = 32$, high fluoxetine $n = 34$) were randomly selected from the mesocosms and transported in aerated buckets to the nearby laboratory facility where metabolic rate was measured. The guppies were placed in individual 100 mL glass vials containing pasteurised tap water at 24 °C, with a pre-calibrated, non-consumptive oxygen sensor spot on the base of each vial. We then measured oxygen saturation in 18 vials simultaneously (12 experimental and six control vials) using six 24-channel PreSens sensor dish readers (Sensor Dish Reader, SDR2, PreSens Precision Sensing, Germany). The control vials (100 mL) contained only pasteurised water, where one vial per sensor plate was measured simultaneously along with three experimental vials to account for any potential background microbial activity. Oxygen saturation measurements were taken at 1-min intervals for 3 h, with the first 30 min being excluded from analysis to allow the fish to acclimate (Olito et al., 2017).

For each trial, $\dot{V}O_2$ was calculated from the change in oxygen saturation over time using $\dot{V}O_2 = -[(m_a - m_c)/100] \times \dot{V} \times \beta O_2$; where m_a is the rate of change of oxygen saturation for a focal guppy, m_c is the rate of change in control vials (i.e., vials only containing water), βO_2 is the oxygen capacitance of air saturated water at 24 °C (Cameron, 1986), and $\dot{V}$ is the water volume of the vials (0.1 L). The parameters m_a and m_c were estimated by ranking local linear regressions (*rankLocReg* function; *LoLinR* package; Olito et al., 2017) to provide a calculation for $\dot{V}O_2$. This was then converted to metabolic rate (mJ h^{-1}) using the calorific conversion factor of 20.08 J mL^{-1} O_2 (Lighton, 2018). For a detailed list of methodological information following the guidelines of Killen et al. (2021) for reporting methods of aquatic respirometry, see Table S2.

2.6. Morphological measurements

After completing behavioural trials and metabolic rate measurements, standard length (from the tip of the snout to the caudal peduncle; Howe, 2002) was measured using digital callipers (±0.01 mm), and mass (±0.0001 g) was measured using digital scales (Scientech ZSA-210). We also calculated scaled mass index because body condition is closely associated with fitness and life history traits (Karametsidis et al., 2023) and linked to behaviours such as boldness (Bjornson et al., 2018) and exploration (Brown and Braithwaite, 2004).

2.7. Statistical analysis

Data were analysed using R version 4.2.1 (R Core Team, 2022). Across all models, data were log transformed where necessary to approximate a Gaussian error distribution, and continuous predictors were scaled to improve the interpretability of main effects. Behavioural and morphological variables, as well as metabolic rate data, were analysed using linear mixed-effects models (LME; *lmer* function, *lme4* package; Bates et al., 2015). Where a significant main effect was

detected, we conducted pair-wise comparisons using the *emmeans* package (Lenth et al., 2018). The global models for the maze assay included fluoxetine treatment (control, low fluoxetine, and high fluoxetine), mass (g), experimental day (1–8), and water temperature as fixed effects. Experimental day was included to account for differences in the order fish underwent trials. We also included an interaction term for fluoxetine treatment and mass. Mass was included as a covariate because it has previously been linked with behaviours, such as boldness, in fish (Brown et al., 2007; Si et al., 2023). Global models for the predator assay included fluoxetine treatment, predator treatment (predator present or absent), mass, time of day (minutes since first trial of the day commenced), water temperature, and predator side (left or right) as fixed effects. While predator ID was included in initial models, it was excluded from final analysis because it did not explain any variation in the data. As trials were conducted over a single day, time of day was included to account for differences in the time of day fish underwent, and the order in which they underwent, trials. We also included the two-way interaction between fluoxetine treatment and predator treatment, as well as fluoxetine treatment and mass. Where the inclusion of covariates did not improve model fit, as tested by Akaike information criterion (AIC) comparisons, they were removed from the model (see Table S3 for final models). All models included mesocosm tank as a random effect. For some models, mesocosm variance was estimated as zero, although mesocosm was maintained as a random intercept in the model as its inclusion would not affect the fixed effects estimates.

To analyse the possible effect of fluoxetine on metabolic rate, we used a LME model with fluoxetine treatment, mass, the two-way interaction between fluoxetine treatment and mass, and oxygen trial (morning or afternoon) as fixed effects, and mesocosm was included as a random effect. Mass and metabolic rate were analysed in a $\log_{10}$-$\log_{10}$ framework to satisfy the assumption of homoscedasticity.

We also used LME models to analyse the potential effects of fluoxetine exposure on guppy mass, standard length, and body condition. Scaled mass index was calculated as a proxy for body condition, whereby a standard major axis regression was performed on the log of body mass and standard length of fish (*sma* function, *smatr* package; Warton et al., 2012). We then calculated a beta coefficient, which was used to obtain the scaled mass for each fish.

3. Results

3.1. Chemical analyses

Mean measured exposure concentrations for the low- and high-fluoxetine treatments were 30 ng L^{-1} (SD = 12.84, n = 36) and 291.90 ng L^{-1} (SD = 156.59, n = 36), respectively. No fluoxetine was detected in the control tanks (all under the limit of detection; <2 ng L^{-1}, n = 18). Refer to Table S4 for raw values.

3.2. Exploratory behaviour in a maze

There was no significant effect of fluoxetine exposure on latency to emerge from the starting chamber ($\chi^2 = 0.769$, $df = 2$, $p = 0.681$; Fig 2A). There was, however, an effect of experimental day ($\chi^2 = 13.335$, $df = 1$, $p < 0.001$), whereby guppies that were tested later in the experimental period were slower to emerge from the starting chamber (Fig. S3). Fluoxetine exposure did not significantly affect latency to complete the maze ($\chi^2 = 3.502$, $df = 2$, $p = 0.174$; Fig. 2B). There was, again, a significant effect of experimental day ($\chi^2 = 6.620$, $df = 1$, $p = 0.010$), whereby guppies that were tested later in the experimental period took longer to complete the maze (Fig. S4). Finally, there was a marginally non-significant effect of fluoxetine exposure on average speed ($\chi^2 = 5.597$, $df = 2$, $p = 0.061$; Fig. 2C).

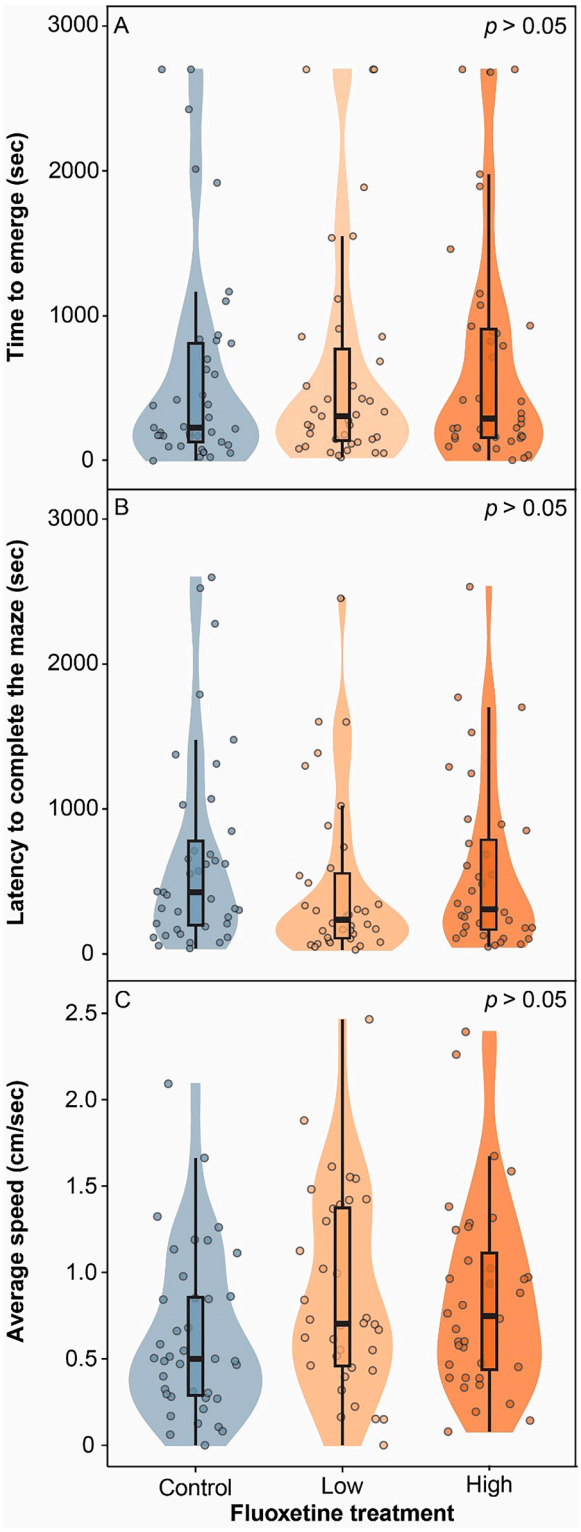

Fig. 2. Boxplots and violin plots showing (A) time to emerge from the starting chamber (sec), (B) time to complete the maze (sec), and (C) average speed (cm sec^{-1}) during the trial, plotted for unexposed (blue; n = 41), low-fluoxetine (light orange; n = 39), and high-fluoxetine (dark orange; n = 39) male guppies. Boxplots show the 25th, 50th (median) and 75th percentiles. The coloured area surrounding the boxplot (violin plot) shows the probability density at different values smoothed by a kernel density estimator, while the points are the raw data (randomly spaced along the x-axis).

3.3. Exploratory behaviour in the presence of the predator

Fluoxetine exposure did not significantly affect latency to enter the predator strike zone ($\chi^2 = 1.292$, $df = 2$, $p = 0.524$; Fig. 3A), total time spent in the predator strike zone ($\chi^2 = 0.707$, $df = 2$, $p = 0.702$; Fig. 3B), or activity levels ($\chi^2 = 0.896$, $df = 2$, $p = 0.639$; Fig. 3C). However, we found a significant effect of predator treatment, whereby guppies, irrespective of fluoxetine exposure, entered the strike zone sooner ($\chi^2 = 17.656$, $df = 1$, $p < 0.001$), and subsequently spent less time overall in this area ($\chi^2 = 103.786$, $df = 1$, $p < 0.001$) when the predator was present compared to when the predator was absent (Fig. S5). Activity levels, however, were not significantly altered by predator treatment ($\chi^2 = 2.278$, $df = 1$, $p = 0.131$; Fig. S5). Time of day had a significant impact on time spent in the predator strike zone, as guppies tested later in the day spent less time in the predator strike zone ($\chi^2 = 11.873$, $df = 1$, $p < 0.001$; Fig. S6). Finally, we detected an effect of the side of the predator compartment on activity ($\chi^2 = 5.875$, $df = 1$, $p = 0.015$). Specifically, guppies were slightly more active (i.e., crossed a greater number of grid squares) when the predator compartment was on the left side of the experimental tank compared to the right (Fig. S7).

3.4. Standard metabolic rate

We found no significant main effect of fluoxetine exposure ($\chi^2 = 0.242$, $df = 2$, $p = 0.886$; Fig. S7) and no significant interaction between mass and fluoxetine exposure ($\chi^2 = 4.247$, $df = 2$, $p = 0.988$) on metabolic rate. There was, however, a significant main effect of mass on metabolic rate of male guppies, with males of higher mass having, on average, higher metabolic rates (estimate ± SE = 0.696 ± 0.132, $\chi^2 = 27.877$, $df = 1$, $p < 0.001$). There was also a main effect of oxygen trial (i.e., time of day) whereby metabolic rate was slightly higher in the afternoon compared to the morning ($\chi^2 = 4.247$, $df = 1$, $p = 0.039$; Fig. S8).

3.5. Morphology

Fluoxetine exposure did not significantly affect guppy mass ($\chi^2 = 1.968$, $df = 2$, $p = 0.374$; Fig. 4A) or standard length ($\chi^2 = 5.571$, $df = 2$, $p = 0.062$; Fig. 4B). However, fluoxetine did have a significant effect on scaled mass index ($\chi^2 = 12.440$, $df = 2$, $p = 0.002$; Fig. 4C). Specifically, guppies exposed to the high-fluoxetine treatment had a higher scaled mass index than those in the unexposed control group (t-ratio = 3.386, $df = 5.410$, $p = 0.039$). By contrast, there was no significant difference in scaled mass index between the controls and low-fluoxetine treatment (t-ratio = –1.769, $df = 5.800$, $p = 0.260$), or between the low- and high-fluoxetine treatments (t-ratio = –3.303, $df = 5.090$, $p = 0.292$).

4. Discussion

We tested whether long-term (8-month) exposure to environmentally realistic concentrations (30 and 292 ng L^{-1}) of the pharmaceutical pollutant fluoxetine altered boldness, standard metabolic rate, and morphology in male guppies. Importantly, this was conducted under ecologically relevant conditions in a semi-natural mesocosm system. We found that fluoxetine exposure did not alter boldness or anxiety-related behaviours in a novel environment (i.e., maze arena) or under the perceived threat of predation. Moreover, fluoxetine exposure did not significantly alter metabolic rate, mass, or standard length. However, we detected an effect on body condition, whereby guppies in the high-fluoxetine treatment had a higher scaled mass index than those in the control group.

4.1. Boldness and anxiety-related behaviours

Given that serotonin is involved in mediating the stress response by inhibiting the secretion of adrenocorticotropic-releasing hormone

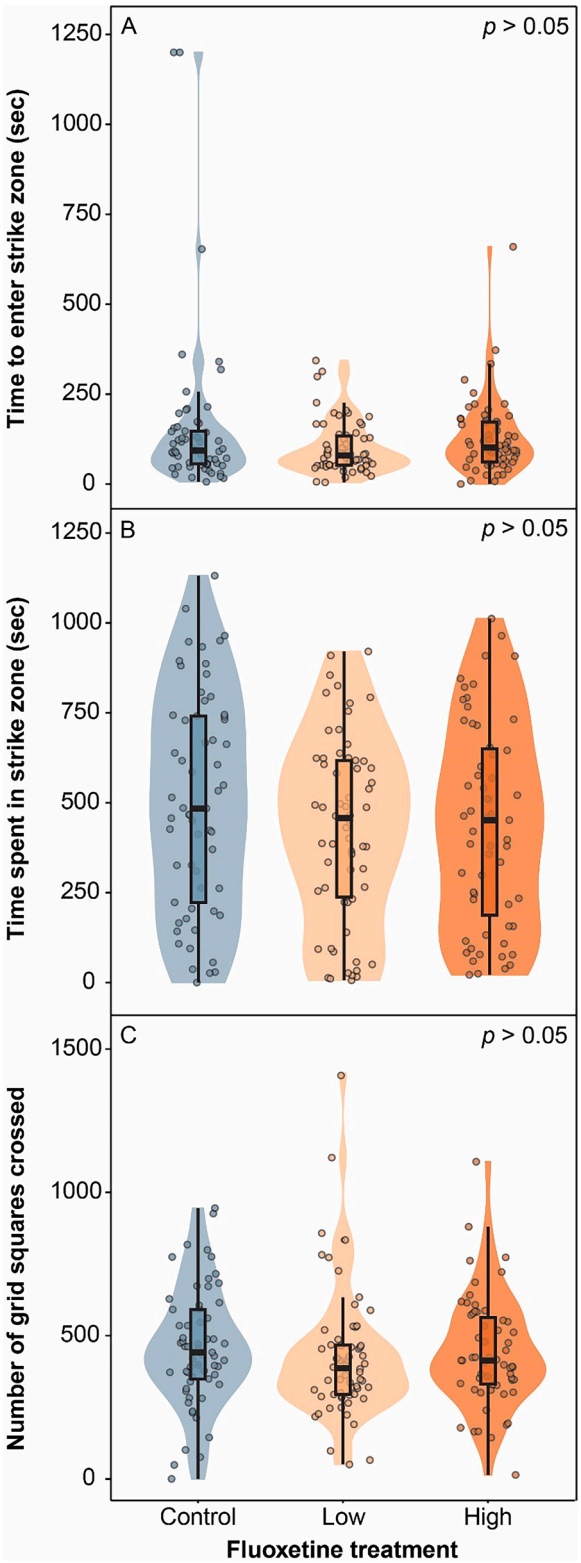

Fig. 3. Boxplots and violin plots showing (A) latency to enter the predator strike zone (sec), (B) total time spent in the predator strike zone (sec), and (C) total number of grid squares crossed during the trial, plotted for unexposed (blue; $n = 61$), low-fluoxetine (light orange; $n = 62$), and high-fluoxetine (dark orange; $n = 59$) male guppies. Boxplots show the 25th, 50th (median) and 75th percentiles. The coloured area surrounding the boxplot (violin plot) shows the probability density at different values smoothed by a kernel density estimator, while the points are the raw data (randomly spaced along the x-axis).

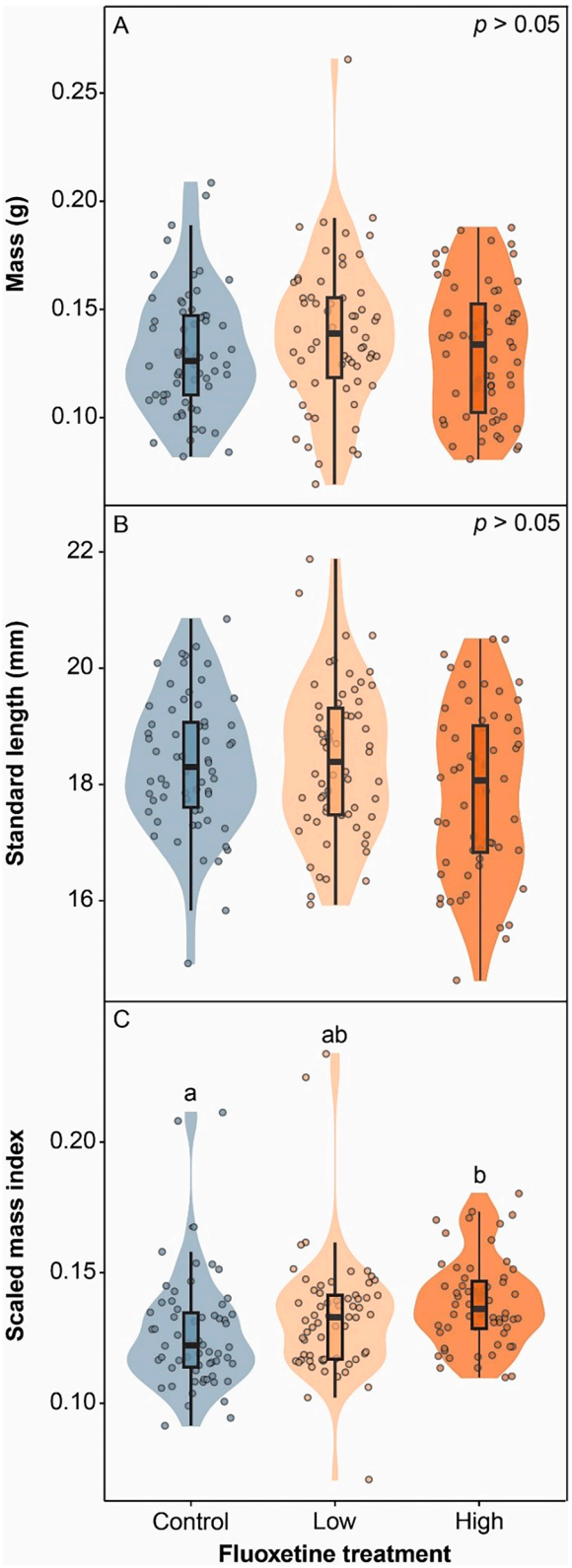

Fig. 4. Boxplots and violin plots showing (A) mass (g), (B) standard length (mm), and (C) scaled mass index, plotted for unexposed (blue; $n = 62$), low-fluoxetine (light orange; $n = 62$), and high-fluoxetine (dark orange; $n = 60$) male guppies. Boxplots show the 25th, 50th (median) and 75th percentiles. The coloured area surrounding the boxplot (violin plot) shows the probability density at different values smoothed by a kernel density estimator, while the points are the raw data (randomly spaced along the x-axis).

(ACTH; McDonald, 2017; Miranda et al., 2023; Correia et al., 2023), fluoxetine exposure could be expected to affect anxiety and, thereby, influence boldness. Indeed, previous studies have found altered boldness and antipredator behaviour in fluoxetine-exposed fish following acute (<1-month) exposure (Pelli and Connaughton, 2015; Singer et al., 2016; Martin et al., 2017; Saaristo et al., 2017; Vera-Chang et al., 2019; Correia et al., 2022; but see Martin et al., 2019a). However, consistent with the prediction that longer-term exposure may lead to tolerance that could ameliorate short-term effects, we found no impact of fluoxetine on boldness or exploratory behaviour in a maze, or in the presence of a predator, following 8 months of exposure. This aligns with previous research on long-term exposure in various aquatic species, including snails (*Physa acuta*; Henry et al., 2022), daphnids (*Daphnia magna*; Heyland et al., 2020), and guppies (Tan et al., 2020; Polverino et al., 2023), which found no clear effect of fluoxetine exposure on mean-level expression of behaviours (but see Thoré et al., 2021a; Polverino et al., 2021). This supports the idea that the average effects of fluoxetine exposure can vary depending on the exposure period (Polverino et al., 2023). The mechanism of action differs between short- and long-term fluoxetine exposure, and long-term effects are often only seen after weeks of exposure due to altered expression of serotonin receptors (Stewart, 2014). Furthermore, transgenerational studies (i.e., exposed parents, unexposed offspring) on fluoxetine and other SSRIs have demonstrated that the effects of exposure can differ over successive generations (Minguez et al., 2015; Vera-Chang et al., 2018; Heyland et al., 2020). In these studies, parental exposure to an SSRI saw effects such as low cortisol levels and reduced exploratory behaviour (Vera-Chang et al., 2018), increased size (Heyland et al., 2020), and altered fecundity (Minguez et al., 2015; Heyland et al., 2020) in the unexposed offspring. This contrasts with the abovementioned long-term exposure (i.e., spanning multiple generations) studies, where there were no observed effects of fluoxetine. The loss of efficacy of a drug following chronic administration in human patients (known as tachyphylaxis; Katz, 2011; Targum, 2014; Fornaro et al., 2019) could be a possible explanation for why we observe behavioural alterations following acute exposure, but not long-term exposure. Taking the results of the present study and those of the abovementioned studies into consideration, further investigation into the impacts of long-term fluoxetine exposure on the serotonergic system would therefore be valuable in identifying the underlying mechanisms driving such findings.

While we did not detect an effect of fluoxetine exposure on boldness and exploratory behaviour in the presence of a predator, we did find that guppies behaved differently in the presence of a predator compared to an empty compartment. Specifically, guppies entered the predator strike zone faster and spent less time overall in the strike zone in the presence of a predator, and this was true regardless of fluoxetine exposure. In alignment with our findings, previous research has also observed changes in guppy behaviour in response to predators (Fursdon et al., 2019; Tan et al., 2020; Mason et al., 2021). This result is unsurprising given that male guppies are known to closely approach a potential predator (i.e., predator inspection behaviour) and subsequently flee back to safer habitats based on the level of perceived risk (Pitcher, 1991; Fishman, 1999). Such behaviour is known to allow individuals to better gauge the level of threat posed by potential predators (Godin et al., 1995). In this respect, we rule out the possibility that guppies simply entered the strike zone sooner due to increased activity, because we found no difference in activity levels between predator and non-predator treatments. Moreover, guppies spent less time in the strike zone overall when the predator was present compared to an empty compartment, indicating that the fish inspected the predator, perceived the threat, and adjusted their behaviour accordingly. These findings are therefore consistent with individuals engaging in predator inspection and avoidance behaviour, and, more generally, contribute to the experimental validity regarding the use of spangled perch in this study. Lastly, while we did not expose spangled perch to fluoxetine in this study in order to focus on guppy behaviour, we acknowledge the possibility

that both the predator and prey species could be exposed to contaminants in the wild. Given this may influence the interactions between the species (Bose et al., 2022), future studies may examine the potential interactive effects of pharmaceutical exposure in both predator and prey species.

4.2. Standard metabolic rate

Although we had hypothesised that fluoxetine-exposed guppies would have a lower metabolic rate than unexposed guppies, we found no significant effect of fluoxetine exposure on metabolic rate. Given that fluoxetine can decrease cortisol levels (Miranda et al., 2023) and cortisol levels influence metabolic rate (Mommsen et al., 1999), it is surprising that we found no effect of fluoxetine exposure on metabolic rate in male guppies. Methodological differences may explain the lack of any effect of fluoxetine on metabolism. First, it is important to note that we measured metabolic rate under non-stressful conditions. It may, therefore, be beneficial to understand how fluoxetine possibly affects metabolic rate under conditions that more closely reflect the behavioural trials that were conducted, where fish were potentially exposed to higher levels of stress. Palacios et al. (2016), for instance, found that oxygen uptake of juvenile damselfish (*Pomacentrus ambionensis*) increased by 38% in the presence of a predator compared to when the predator was absent. Second, there may be sex differences in the response of metabolism to fluoxetine. In addition to reporting an increase in the metabolic rate of guppies in the presence of a predator regardless of fluoxetine treatment, Tan et al. (2020) showed that female guppies in the low-fluoxetine treatment were found to have a lower average metabolic rate than unexposed females, which does not support the results of our study measuring males. Past studies have reported sex-specific effects of fluoxetine exposure (e.g., Martin et al., 2017; Saaristo et al., 2017; Thoré et al., 2021b; Martin et al., 2019b; Wiles et al., 2020; Polverino et al., 2023), which may be attributed to differences in the serotonergic system between the sexes (Zucker and Prendergast, 2020). While it was beyond the scope of this study to identify the exact mechanism(s) underpinning such an effect, this may, in part, explain the difference in results between studies. Finally, differences in respirometry methodology may explain the lack of any observed effect of fluoxetine on metabolism. Our study used a closed respirometry system with no mixing device, which may have impacted precision compared with intermittent flow respirometry (Clark et al., 2013). Yet, the relatively long measurement period allowed a robust calculation of oxygen concentration over time (Svendsen et al., 2016), while a high respirometer-to-fish volume ratio ensured that fish did not reach a critical oxygen pressure in the vials. Despite no effect of fluoxetine, this method was able to detect significant differences in metabolism across time of day and body mass was found to be a reliable predictor of metabolic rate ($R^2 = 0.51$), with a significant allometric scaling relationship in line with previous research on other taxa (White and Kearney, 2014; White et al., 2019).

4.3. Morphology

Given that previous acute and long-term fluoxetine exposure studies on guppies have found no impact of fluoxetine on morphology, we hypothesised that fluoxetine exposure would not impact mass, standard length, or body condition. In support of this hypothesis, we found no effect of fluoxetine exposure on mass or standard length in male guppies. This is consistent with previous studies that found no effect of fluoxetine on the same morphological measurements in eastern mosquitofish (*Gambusia holbrooki*; Bertram et al., 2018) and guppies (Fursdon et al., 2019; Wiles et al., 2020) under similar exposure concentrations. We did, however, detect an effect of fluoxetine exposure on body condition. Specifically, we found that high-fluoxetine males had a higher scaled mass index than unexposed males, while there was no difference between the low- and high-fluoxetine males, or between the low-fluoxetine and unexposed males. Body condition in fish is closely associated with species life history and fitness (Karametsidis et al., 2023). Given that body condition has been linked to consistent differences in a range of behaviours (Brown and Braithwaite, 2004; Bjornson et al., 2018; Kanno et al., 2023), identifying such morphological changes following exposure is important when monitoring responses to environmental contaminants. In contrast to our findings, Bertram et al. (2018) found that body condition in male mosquitofish was reduced in the low-fluoxetine treatment compared to the control. Such a reduction in body condition has been attributed to a reduction in food consumption (Bertram et al., 2018), which has previously been observed in fluoxetine-exposed fish (reviewed in Correia et al., 2023). Differences in exposure duration could again be a driver for this discrepancy, as discussed above regarding behaviour. However, after 15 months of exposure, Wiles et al. (2020) found no impact of fluoxetine on the body condition of male guppies. The differing results seen between the abovementioned and present study support the idea that the effects of fluoxetine, and other SSRIs, may not only be duration-dependent (Stewart, 2014) but also generation-specific, as reported in previous transgenerational and multigenerational exposure studies (Minguez et al., 2015; Vera-Chang et al., 2018; Heyland et al., 2020; Thoré et al., 2021a). The mechanism (s) that may drive these duration- and generation-dependent effects are not yet understood. It is clear that further research is warranted to provide further insights into the mechanism(s) underpinning such effects.

While we measured fluoxetine concentrations in the water throughout the 8-month exposure period, we did not measure the accumulation of fluoxetine in the tissues of our study fish. Previous studies have shown that fluoxetine—and its metabolite norfluoxetine—accumulates in the brain, muscle, and plasma of fish (Pan et al., 2018; Martin et al., 2019b; Yan et al., 2020; McCallum et al., 2023). Such accumulation has been found to be biomass dependent. For instance, in fluoxetine-exposed eastern mosquitofish, smaller fish exhibited higher relative tissue concentrations, likely due to smaller fish having relatively higher metabolic rates (Martin et al., 2019b). Mass was included in all models initially as a covariate but did not substantially account for variation in the response variable (and was subsequently removed). Thus, if there were mass-specific effects of bioaccumulation, they were unlikely to influence the behavioural responses.

5. Conclusion

In summary, we found no impact of 8-month fluoxetine exposure on boldness and exploratory behaviour in male guppies. Moreover, we found no effect of fluoxetine on metabolic rate, mass, or standard length. We did, however, detect an effect of fluoxetine exposure on body condition, whereby guppies in the high-fluoxetine treatment had a higher scaled mass index than did control males. Taken together with past research, our findings suggest that the impacts of fluoxetine exposure may be duration- or generation-dependent, highlighting the importance of exposure duration in mediating the effects of fluoxetine on fitness-related traits. There is a growing number of studies reporting the effects of fluoxetine on wildlife, yet still, relatively few studies employ long-term (>1-month) environmentally realistic exposures. Further research under extended time periods (i.e., spanning multiple generations) is therefore essential if we are to accurately predict the ecological impacts of fluoxetine—and other pharmaceutical pollutants—on exposed wildlife, as well as their underlying mechanism(s).

CRediT authorship contribution statement

Kate N. Fergusson: Writing – review & editing, Writing – original draft, Visualization, Project administration, Formal analysis, Data curation. **James L. Tanner:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jack A. Brand:** Writing – review & editing, Visualization, Formal analysis, Data

curation. **Stephanie L. Hannington:** Writing – review & editing, Investigation. **Amanda K. Pettersen:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **Josefin Sundin:** Writing – review & editing, Investigation, Funding acquisition. **Minna Saaristo:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Michael G. Bertram:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Jake M. Martin:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bob B.M. Wong:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data and associated R scripts associated with this paper are available from the Open Science Framework repository: https://doi.org/10.17605/OSF.IO/HSBJE.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2024.107082.

References

Al Shuraiqi, A, Al-Habsi, A, Barry, MJ, 2021. Time-, dose- and transgenerational effects of fluoxetine on the behavioural responses of zebrafish to a conspecific alarm substance. Environ. Pollut. 270, 116164 https://doi.org/10.1016/j.envpol.2020.116164.

Araújo, FG, Peixoto, MG, Pinto, BC, Teixeira, TP, 2009. Distribution of guppies *Poecilia reticulata* (Peters, 1860) and *Phalloceros caudimaculatus* (Hensel, 1868) along a polluted stretch of the Paraíba do Sul River, Brazil. Braz. J. Biol. 69, 41–48. https://doi.org/10.1590/s1519-69842009000100005.

Arnold, KE, Brown, AR, Ankley, GT, Sumpter, JP, 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos. Trans. R. Soc. B-Biol. Sci. 369, 20130569 https://doi.org/10.1098/rstb.2013.0569.

Baresel, C, Ek, M, Ejhed, H, Allard, A-S, Magnér, J, Dahlgren, L, Westling, K, Wahlberg, C, Fortkamp, U, Söhr, S, Harding, M, Fång, J, Karlsson, J, 2019. Sustainable treatment systems for removal of pharmaceutical residues and other priority persistent substances. Water Sci. Technol. 79, 537–543. https://doi.org/10.2166/wst.2019.080.

Bates, D, Mächler, M, Bolker, B, Walker, S, 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Behrens, JW, von Friesen, LW, Brodin, T, Ericsson, P, Hirsch, PE, Persson, A, Sundelin, A, van Deurs, M, Nilsson, PA, 2020. Personality- and size-related metabolic performance in invasive round goby (*Neogobius melanostomus*). Physiol. Behav. 215, 112777 https://doi.org/10.1016/j.physbeh.2019.112777.

Bertram, MG, Ecker, TE, Wong, BBM, O'Bryan, MK, Baumgartner, JB, Martin, JM, Saaristo, M, 2018. The antidepressant fluoxetine alters mechanisms of pre- and post-copulatory sexual selection in the eastern mosquitofish (*Gambusia holbrooki*). Environ. Pollut. 238, 238–247. https://doi.org/10.1016/j.envpol.2018.03.006.

Bertram, MG, Martin, JM, McCallum, ES, Alton, LA, Brand, JA, Brooks, BW, Cerveny, D, Fick, J, Ford, AT, Hellström, G, Michelangeli, M, Nakagawa, S, Polverino, G, Saaristo, M, Sih, A, Tan, H, Tyler, CR, Wong, BBM, Brodin, T, 2022. Frontiers in quantifying wildlife behavioural responses to chemical pollution. Biol. Rev. 97, 1346–1364. https://doi.org/10.1111/brv.12844.

Bidel, F, Di Poi, C, Imarazene, B, Koueta, N, Budzinski, H, Van Delft, P, Bellanger, C, Jozet-Alves, C, 2016. Pre-hatching fluoxetine-induced neurochemical, neurodevelopmental, and immunological changes in newly hatched cuttlefish. Environ. Sci. Pollut. Res. 23, 5030–5045. https://doi.org/10.1007/s11356-015-4591-7.

Bjornson, F, Anderson, GW, 2018. Body condition, rather than size, predicts risk-taking and resource holding potential in hatchery reared juvenile lake sturgeon *Acipenser fulvescens*. J. Fish. Biol. 93, 1188–1196. https://doi.org/10.1111/jfb.13840.

Bose, APH, McCallum, ES, Avramović, M, Bertram, MG, Blom, E-L, Cerveny, D, Grønlund, SN, Leander, J, Lundberg, P, Martin, JM, Michelangeli, M, Persson, L, Brodin, T, 2022. Pharmaceutical pollution disrupts the behavior and predator-prey interactions of two widespread aquatic insects. iScience 25, 105672. https://doi.org/10.1016/j.isci.2022.105672.

Brown, C, Braithwaite, VA, 2004. Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. Anim. Behav. 68, 1325–1329. https://doi.org/10.1016/j.anbehav.2004.04.004.

Brown, C, Jones, F, Braithwaite, VA, 2007. Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. J. Fish. Biol. 71, 1590–1601. https://doi.org/10.1111/j.1095-8649.2007.01627.x.

Burns, JG, 2008. The validity of three tests of temperament in guppies (*Poecilia reticulata*). J. Comp. Psychol. (Baltim) 122, 344–356. https://doi.org/10.1037/0735-7036.122.4.344.

Cameron, JN, 1986. The solubility of carbon dioxide as a function of temperature and salinity (appendix table). In: Cameron, JN (Ed.), Principles of Physiological Measurement. Academic Press, London, UK, pp. 254–259.

Campos, B, Rivetti, C, Kress, T, Barata, C, Dircksen, H, 2016. Depressing antidepressant: fluoxetine affects serotonin neurons causing adverse reproductive responses in *Daphnia magna*. Environ. Sci. Technol. 50, 6000–6007. https://doi.org/10.1021/acs.est.6b00826.

Chutter, M, Perry, P, Houpt, K, 2019. Efficacy of fluoxetine for canine behavioral disorders. J. Vet. Behav. 33, 54–58. https://doi.org/10.1016/j.jveb.2019.05.006.

Clark, TD, Sandblom, E, Jutfelt, F, 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782. https://doi.org/10.1242/jeb.084251.

Collins, SM, Hatch, SA, Elliott, KH, Jacobs, SR, 2019. Boldness, mate choice and reproductive success in *Rissa tridactyla*. Anim. Behav. 154, 67–74. https://doi.org/10.1016/j.anbehav.2019.06.007.

Correia, D, Domingues, I, Faria, M, Oliveira, M, 2022. Chronic effects of fluoxetine on *Danio rerio*: a biochemical and behavioral perspective. Appl. Sci. 12, 2256. https://doi.org/10.3390/app12042256.

Correia, D, Domingues, I, Faria, M, Oliveira, M, 2023. Effects of fluoxetine on fish: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 857, 159486 https://doi.org/10.1016/j.scitotenv.2022.159486.

Croft, DP, Albanese, B, Arrowsmith, BJ, Marc, B, Webster, M, Krause, J, 2003. Sex-biased movement in the guppy (*Poecilia reticulata*). Oecologia 137, 62–68. https://doi.org/10.1007/s00442-003-1268-6.

Deacon, AE, Ramnarine, IW, Magurran, AE, 2011. How reproductive ecology contributes to the spread of a globally invasive fish. PLoS One 6, e24416. https://doi.org/10.1371/journal.pone.0024416.

Diaz Pauli, B, Wiech, M, Heino, M, Palm, A, 2015. Opposite selection on behavioral types by active and passive fishing gears in a simulated guppy *Poecilia reticulata* fishery. J. Fish. Biol. 86, 1030–1045. https://doi.org/10.1111/jfb.12620.

Endler, JA, 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. Vision. Res. 31, 587–608. https://doi.org/10.1016/0042-6989(91)90109-I.

Ferreira, CSS, Soares, SC, Kille, P, Oliveira, M, 2023. Identifying knowledge gaps in understanding the effects of selective serotonin reuptake inhibitors (SSRIs) on fish behaviour. Chemosphere 335, 139124. https://doi.org/10.1016/j.chemosphere.2023.139124.

Fishman, MA, 1999. Predator inspection: closer approach as a way to improve assessment of potential threats. J. Theor. Biol. 196, 225–235. https://doi.org/10.1006/jtbi.1998.0834.

Fornaro, M, Anastasia, A, Novello, S, Fusco, A, Pariano, R, De Berardis, D, Solmi, M, Veronese, N, Stubbs, B, Vieta, E, Berk, M, de Bartolomeis, A, Carvalho, AF, 2019. The emergence of loss of efficacy during antidepressant drug treatment for major depressive disorder: an integrative review of evidence, mechanisms, and clinical implications. Pharmacol. Res. 139, 494–502. https://doi.org/10.1016/j.phrs.2018.10.025.

Friard, O, Gamba, M, 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7, 1325–1330. https://doi.org/10.1111/2041-210X.12584.

Fursdon, J, Martin, JM, Bertram, MG, Lehtonen, T, Wong, BBM, 2019. The pharmaceutical pollutant fluoxetine alters reproductive behaviour in a fish independent of predation risk. Sci. Total Environ. 650, 642–652. https://doi.org/10.1016/j.scitotenv.2018.09.046.

Gardier, A, Malagié, I, Trillat, A, Jacquot, C, Artigas, F, 1996. Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic antidepressant drugs: recent findings from in vivo microdialysis studies. Fundam. Clin. Pharmacol. 10, 16–27. https://doi.org/10.1111/j.1472-8206.1996.tb00145.x.

Gasparini, C, Speechley, EM, Polverino, G, 2019. The bold and the sperm: positive association between boldness and sperm number in the guppy. R. Soc. Open. Sci. 6, 190474 https://doi.org/10.1098/rsos.190474.

Godin, JG, Davis, SA, 1995. Who dares, benefits: predator approach behaviour in the guppy (*Poecilia reticulata*) deters predator pursuit. Proc. R. Soc. B Biol. Sci. 259, 193–200. https://doi.org/10.1098/rspb.1995.0028.

Godin, JG, Dugatkin, LA, 1996. Female mating preference for bold males in the guppy, *Poecilia reticulata*. Proc. Natl. Acad. Sci. U.S.A. 93, 10262–10267. https://doi.org/10.1073/pnas.93.19.10262.

Gould, SL, Winter, MJ, Norton, WHJ, Tyler, CR, 2021. The potential for adverse effects in fish exposed to antidepressants in the aquatic environment. Environ. Sci. Technol. 55, 16299–16312. https://doi.org/10.1021/acs.est.1c04724.

Graumnitz, S, Jungmann, D, 2021. The Database "Pharmaceuticals in the Environment". Umweltbundesamt, Germany.

Gunnarsson, L, Jauhiainen, A, Kristiansson, E, Nerman, O, Larsson, DGJ, 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ. Sci. Technol. 42, 5807–5813. https://doi.org/10.1021/es8005173.

Henry, J, Brand, JA, Bai, Y, Martin, JM, Wong, BBM, Wlodkowic, D, 2022. Multi-generational impacts of exposure to antidepressant fluoxetine on behaviour, reproduction, and morphology of freshwater snail *Physa acuta*. Sci. Total Environ. 814, 152731 https://doi.org/10.1016/s0024-3205(02)02482-7.

Hensler, JG, 2003. Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. Life Sci. 72, 1665–1682. https://doi.org/10.1016/s0024-3205(02)02482-7.

Herdegen-Radwan, M, 2019. Bolder guppies do not have more mating partners, yet sire more offspring. BMC. Evol. Biol. 19, 211. https://doi.org/10.1186/s12862-019-1539-4.

Heyland, A, Bastien, T, Halliwushka, K, 2020. Transgenerational reproductive effects of two serotonin reuptake inhibitors after acute exposure in *Daphnia magna* embryos. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 238, 108875 https://doi.org/10.1016/j.cbpc.2020.108875.

Hird, CM, Urbina, MA, Lewis, CN, Snape, JR, Galloway, TS, 2016. Fluoxetine exhibits pharmacological effects and trait-based sensitivity in a marine worm. Environ. Sci. Technol. 50, 8344–8352. https://doi.org/10.1021/acs.est.6b03233.

Hong, X, Zhao, G, Zhou, Y, Chen, R, Li, J, Zha, J, 2021. Risks to aquatic environments posed by 14 pharmaceuticals as illustrated by their effects on zebrafish behaviour. Sci. Total Environ. 771, 145450 https://doi.org/10.1016/j.scitotenv.2021.145450.

Howe, J, 2002. Standard length: not quite so standard. Fish. Res. 56, 1–7. https://doi.org/10.1016/S0165-7836(01)00312-5.

Hulthén, K, Chapman, BB, Nilsson, PA, Hansson, L-A, Skov, C, Brodersen, J, Vinterstare, J, Brönmark, C, 2017. A predation cost to bold fish in the wild. Sci. Rep. 7, 1239. https://doi.org/10.1038/s41598-017-01270-w.

Hurst, M, Lamb, HM, 2000. Fluoxetine: a review of its use in anxiety disorders and mixed anxiety and depression. CNS. Drugs 14, 51–80. https://doi.org/10.2165/00023210-200014010-00005.

Jeswiet, SB, Godin, J-GJ, 2011. Validation of a method for quantifying male mating preferences in the guppy (*Poecilia reticulata*). Ethology 117, 422–429. https://doi.org/10.1111/j.1439-0310.2011.01891.x.

Jobling, M, 1981. The influences of feeding on the metabolic rate of fishes: a short review. J. Fish. Biol. 18, 385–400. https://doi.org/10.1111/j.1095-8649.1981.tb03780.x.

Kanno, Y, Locklear, ML, Platis, NM, Lewis, ST, 2023. Body condition metrics explain fish movement in experimental streams. J. Zool. 320, 18–28. https://doi.org/10.1111/jzo.13049.

Karametsidis, G, Rueda, L, Bellido, JM, Esteban, A, García, E, Gil de Sola, L, Pennino, MG, Pérez-Gil, JL, Hidalgo, M, 2023. The trade-off between condition and growth shapes juveniles' survival of harvested demersal fish of the Mediterranean sea. Mar. Environ. Res. 184, 105844 https://doi.org/10.1016/j.marenvres.2022.105844.

Katz, G, 2011. Tachyphylaxis/tolerance to antidepressive medications: a review. Israel J. Psychiatry Relat. Sci. 48, 129–135.

Kerman, K, Sieving, K, St Mary, C, Avery, M, 2016. Evaluation of boldness assays and associated behavioral measures in a social parrot, monk parakeet (*Myiopsitta monachus*). Behaviour 153, 1817–1838. https://doi.org/10.1163/1568539X-00003356.

Killen, SS, Christensen, EAF, Cortese, D, Závorka, L, Norin, T, Cotgrove, L, Crespel, A, Munson, A, Nati, JJH, Papatheodoulou, M, McKenzie, DJ, 2021. Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. J. Exp. Biol. 224 https://doi.org/10.1242/jeb.242522.

Killen, SS, Marras, S, McKenzie, DJ, 2011. Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. J. Anim. Ecol. 80, 1024–1033. https://doi.org/10.1111/j.1365-2656.2011.01844.x.

Lenth, R, Singmann, H, Love, J, Buerkner, P, Herve, M, 2018. Emmeans: estimated marginal means, aka least-squares means. Am. Stat. 34, 216–221. https://doi.org/10.1080/00031305.1980.10483031.

Lighton, JR, 2018. Measuring Metabolic Rates: A Manual For Scientists. Oxford University Press, Oxford, United Kingdom.

Lucon-Xiccato, T, Bisazza, A, 2017. Sex differences in spatial abilities and cognitive flexibility in the guppy. Anim. Behav. 123, 53–60. https://doi.org/10.1016/j.anbehav.2016.10.026.

Maculewicz, J, Kowalska, D, Świacka, K, Tański, M, Stepnowski, P, Białk-Bielińska, A, Dołżonek, J, 2022. Transformation products of pharmaceuticals in the environment: their fate, (eco)toxicity and bioaccumulation potential. Sci. Total Environ. 802, 149916 https://doi.org/10.1016/j.scitotenv.2021.149916.

Martin, JM, Bertram, MG, Saaristo, M, Ecker, TE, Hannington, SL, Tanner, JL, Michelangeli, M, O'Bryan, MK, Wong, BBM, 2019a. Impact of the widespread pharmaceutical pollutant fluoxetine on behaviour and sperm traits in a freshwater fish. Sci. Total Environ. 650, 1771–1778. https://doi.org/10.1016/j.scitotenv.2018.09.294.

Martin, JM, Bertram, MG, Saaristo, M, Fursdon, JB, Hannington, SL, Brooks, BW, Burket, SR, Mole, RA, Deal, NDS, Wong, BBM, 2019b. Antidepressants in surface waters: fluoxetine influences mosquitofish anxiety-related behavior at environmentally relevant levels. Environ. Sci. Technol. 53, 6035–6043. https://doi.org/10.1021/acs.est.9b00944.

Martin, JM, Michelangeli, M, Bertram, MG, Blanchfield, PJ, Brand, JA, Brodin, T, Brooks, B, Cerveny, D, Fergusson, KN, Lagisz, M, Lovin, LM, Ligocki, IY, Nakagawa, S, Ozeki, S, Sandoval-Herrera, N, Kendall, S, Sundin, J, Tan, H, Thoré, E, Wong, BBM, McCallum, ES, 2024. Evidence of the Impacts of Pharmaceuticals on Aquatic Animal Behaviour (EIPAAB): a systematic map and open access database. EcoEvoRxiv. https://doi.org/10.32942/X2NG9R.

Martin, JM, Saaristo, M, Bertram, MG, Lewis, PJ, Coggan, TL, Clarke, BO, Wong, BBM, 2017. The psychoactive pollutant fluoxetine compromises antipredator behaviour in fish. Environ. Pollut. 222, 592–599. https://doi.org/10.1016/j.envpol.2016.10.010.

Martin, JM, Saaristo, M, Tan, H, Bertram, MG, Nagarajan-Radha, V, Dowling, DK, Wong, BBM, 2019c. Field-realistic antidepressant exposure disrupts group foraging dynamics in mosquitofish. Biol. Lett. 15, 20190615 https://doi.org/10.1098/rsbl.2019.0615.

Mason, RT, Martin, JM, Tan, H, Brand, JA, Bertram, MG, Tingley, R, Todd-Weckmann, A, Wong, BB, 2021. Context is key: social environment mediates the impacts of a psychoactive pollutant on shoaling behavior in fish. Environ. Sci. Technol. 55, 13024–13032. https://doi.org/10.1021/acs.est.1c04084.

Mathot, KJ, Dingemanse, NJ, Nakagawa, S, 2019. The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. Biol. Rev. 94, 1056–1074. https://doi.org/10.1111/brv.12491.

Maximino, C, de Brito, TM, Dias, CAGD, Gouveia, A, Morato, S, 2010. Scototaxis as anxiety-like behavior in fish. Nat. Protoc. 5, 209–216. https://doi.org/10.1038/nprot.2009.225.

McCallum, ES, Bose, APH, Warriner, TR, Balshine, S, 2017. An evaluation of behavioural endpoints: The pharmaceutical pollutant fluoxetine decreases aggression across multiple contexts in round goby (*Neogobius melanostomus*). Chemosphere 175, 401–410. https://doi.org/10.1016/j.chemosphere.2017.02.059.

McCallum, ES, Cerveny, D, Bose, APH Fick, J, Brodin, T, 2023. Cost-effective pharmaceutical implants in fish: validating the performance of slow-release implants for the antidepressant fluoxetine. Environ. Toxicol. Chem. 42, 1326–1336. https://doi.org/10.1002/etc.5613.

McDonald, MD, 2017. An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish. Comp. Biochem. Physiol. Toxicol. Pharmacol. 197, 19–31. https://doi.org/10.1016/j.cbpc.2017.03.007.

McGlashon, JM, Gorecki, MC, Kozlowski, AE, Thirnbeck, CK, Markan, KR, Leslie, KL, Kotas, ME, Potthoff, MJ, Richerson, GB, Gillum, MP, 2015. Central serotonergic neurons activate and recruit thermogenic brown and beige fat and regulate glucose and lipid homeostasis. Cell Metab. 21, 692–705. https://doi.org/10.1016/j.cmet.2015.04.008.

Medeiros, E, Arthington, A, 2014. Fish diet composition in floodplain lagoons of an Australian dryland river in relation to an extended dry period following flooding. Environ. Biol. Fishes. 97, 797–812. https://doi.org/10.1007/s10641-013-0180-0.

Minguez, L, Ballandonne, C, Rakotomalala, C, Dubreule, C, Kientz-Bouchart, V, Halm-Lemeille, M-P, 2015. Transgenerational effects of two antidepressants (sertraline and venlafaxine) on *Daphnia magna* life history traits. Environ. Sci. Technol. 49, 1148–1155. https://doi.org/10.1021/es504808g.

Miranda, JPGA, Isaac, ABJ, Silva, RB, Toledo, LCS, Barcellos, LJG, Delicio, HC, Barreto, RE, 2023. Acute effects of fluoxetine on stress responses and feeding motivation in Nile tilapia. Fishes. 8, 348–361. https://doi.org/10.3390/fishes8070348.

Mole, RA, Brooks, BW, 2019. Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. Environ. Pollut. 250, 1019–1031. https://doi.org/10.1016/j.envpol.2019.04.118.

Mommsen, TP, Vijayan, MM, Moon, TW, 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish. Biol. Fish. 9, 211–268. https://doi.org/10.1023/A:1008924418720.

Moon, JH, Oh, CM, Kim, H, 2022. Serotonin in the regulation of systemic energy metabolism. J. Diabetes. Investig. 13, 1639–1645. https://doi.org/10.1111/jdi.13879.

Myles-Gonzalez, E, Burness, G, Yavno, S, Rooke, A, Fox, M, 2015. To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. Behav. Ecol. 26, 1083–1090. https://doi.org/10.1093/beheco/arv050.

Näslund, J, Bererhi, B, Johnsson, JI, 2015. Design of emergence test arenas can affect the results of boldness assays. Ethology 121, 556–565. https://doi.org/10.1111/eth.12368.

Nelson, JA, 2016. Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. J. Fish. Biol. 88, 10–25. https://doi.org/10.1111/jfb.12824.

Norrgren, L, 2012. Fish models for ecotoxicology. Acta Vet. Scand. 54, S14. https://doi.org/10.1186/1751-0147-54-S1-S14.

O'Flynn, D, Lawler, J, Yusuf, A, Parle-McDermott, A, Harold, D, McCloughlin, T, Holland, L, Regan, F, White, B, 2021. A review of pharmaceutical occurrence and pathways in the aquatic environment in the context of a changing climate and the COVID-19 pandemic. Anal. Methods 13, 575–594. https://doi.org/10.1039/D0AY02098B.

Olito, C, White, CR, Marshall, DJ, Barneche, DR, 2017. Estimating monotonic rates from biological data using local linear regression. J. Exp. Biol. 220, 759–764. https://doi.org/10.1242/jeb.148775.

Palacios, MM, Killen, SS, Nadler, LE, White, JR, McCormick, MI, 2016. Top predators negate the effect of mesopredators on prey physiology. J. Anim. Ecol. 85, 1078–1086. https://doi.org/10.1111/1365-2656.12523.

Pan, C, Yang, M, Xu, H, Xu, B, Jiang, L, Wu, M, 2018. Tissue bio-concentration and effects of fluoxetine in zebrafish (*Danio rerio*) and redcrucian cap (*Carassius auratus*) after short-term and long-term exposure. Chemosphere 205, 8–14. https://doi.org/10.1016/j.chemosphere.2018.04.082.

Patel, M, Kumar, R, Kishor, K, Mlsna, T, Pittman Jr, CU, Mohan, D, 2019. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299.

Pelli, M, Connaughton, VP, 2015. Chronic exposure to environmentally-relevant concentrations of fluoxetine (Prozac) decreases survival, increases abnormal behaviors, and delays predator escape responses in guppies. Chemosphere 139, 202–209. https://doi.org/10.1016/j.chemosphere.2015.06.033.

Peters, JR, Granek, EF, de Rivera, CE, Rollins, M, 2017. Prozac in the water: chronic fluoxetine exposure and predation risk interact to shape behaviors in an estuarine crab. Ecol. Evol. 7, 9151–9161. https://doi.org/10.1002/ece3.3453.

Pettersen, AK, White, CR, Marshall, DJ, 2015. Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proc. R. Soc. B Biol. Sci. 282, 20151946 https://doi.org/10.1098/rspb.2015.1946.

Pitcher, T, 1991. Who dares, wins: the function and evolution of predator inspection behaviour in shoaling fish. Netherlands J. Zool. 42, 371–391. https://doi.org/10.1163/156854291X00397.

Polverino, G, Aich, U, Brand, JA, Bertram, MG, Martin, JM, Tan, H, Soman, VR, Mason, RT, Wong, BBM, 2023. Sex-specific effects of psychoactive pollution on behavioral individuality and plasticity in fish. Behav. Ecol. 34, 969–978. https://doi.org/10.1093/beheco/arad065.

Polverino, G, Martin, JM, Bertram, MG, Soman, VR, Tan, H, Brand, JA, Mason, RT, Wong, BBM, 2021. Psychoactive pollution suppresses individual differences in fish behaviour. Proc. R. Soc. B Biol. Sci. 288, 20202294. https://doi.org/10.1098/rspb.2020.2294.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reznick, DN, Shaw, FH, Rodd, FH, Shaw, RG, 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science (1979) 275, 1934–1937. https://doi.org/10.1126/science.275.5308.1934.

Rosen, DE, Bailey, RM, 1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. Bull. Am. Mus. Nat. Hist. 126, 1–176.

Saaristo, M, McLennan, A, Johnstone, CP, Clarke, BO, Wong, BBM, 2017. Impacts of the antidepressant fluoxetine on the anti-predator behaviours of wild guppies (*Poecilia reticulata*). Aquat. Toxicol. 183, 38–45. https://doi.org/10.1016/j.aquatox.2016.12.007.

Saaristo, M, Sharp, S, Zhang, S, Taylor, MP, 2023. Emerging Contaminants in Recycled Water. EPA Publication, 2054, 42p, ISBN: 978-0-6457569-0-6.

Schneider, CA, Rasband, WS, Eliceiri, KW, 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.

Si, MD, Zhang, WW, Li, CL, Su, L, Zhang, XY, 2023. Risk predictability in early life ahapes personality of mosquitofish in adulthood. Animals 13, 1214. https://doi.org/10.3390/ani13071214.

Sih, A, Bell, AM, Johnson, JC, Ziemba, RE, 2004. Behavioral syndromes: an intergrative overiew. Q. Rev. Biol. 79, 241–277. https://doi.org/10.1086/422893.

Singer, ML, Oreschak, K, Rhinehart, Z, Robison, BD, 2016. Anxiolytic effects of fluoxetine and nicotine exposure on exploratory behavior in zebrafish. PeerJ. 4, e2352. https://doi.org/10.7717/peerj.2352.

Smith, BR, Blumstein, DT, 2008. Fitness consequences of personality: a meta-analysis. Behav. Ecol. 19, 448–455. https://doi.org/10.1093/beheco/arm144.

Smith, EJ, Partridge, JC, Parsons, KN, White, EM, Cuthill, IC, Bennett, ATD, Church, SC, 2002. Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). Behav. Ecol. 13, 11–19. https://doi.org/10.1093/beheco/13.1.11.

Souza, LP, Carneiro, JGM, Lastre-Acosta, AM, Ramos, B, Teixeira, ACSC, 2022. Environmental persistence of the antidepressant fluoxetine and its pharmaceutical alternative: kinetics of oxidation and mathematical simulations. Water (Basel) 14, 3536–3549. https://doi.org/10.3390/w14213536.

Stewart, AM, Grossman, L, Nguyen, M, Maximino, C, Rosemberg, DB, Echevarria, DJ, Kalueff, AV, 2014. Aquatic toxicology of fluoxetine: understanding the knowns and the unknowns. Aquat. Toxicol. 156, 269–273. https://doi.org/10.1016/j.aquatox.2014.08.014.

Sumpter, JP, Margiotta-Casaluci, L, 2022. Environmental occurrence and predicted pharmacological risk to freshwater fish of over 200 neuroactive pharmaceuticals in widespread use. Toxics. 10, 233. https://doi.org/10.3390/toxics10050233.

Svendsen, MBS, Bushnell, PG, Christensen, EAF, Steffensen, JF, 2016. Sources of variation in oxygen consumption of aquatic animals demonstrated by simulated constant oxygen consumption and respirometers of different sizes. J. Fish. Biol. 88, 51–64. https://doi.org/10.1111/jfb.12851.

Tan, H, Polverino, G, Martin, JM, Bertram, MG, Wiles, SC, Palacios, MM, Bywater, CL, White, CR, Wong, BBM, 2020. Chronic exposure to a pervasive pharmaceutical pollutant erodes among-individual phenotypic variation in a fish. Environ. Pollut. 263, 114450 https://doi.org/10.1016/j.envpol.2020.114450.

Targum, SD, 2014. Identification and treatment of antidepressant tachyphylaxis. Innov. Clin. Neurosci. 11, 24–28.

Thoré, ESJ, Brendonck, L, Pinceel, T, 2021b. Neurochemical exposure disrupts sex-specific trade-offs between body length and behaviour in a freshwater crustacean. Aquat. Toxicol. 237, 105877 https://doi.org/10.1016/j.aquatox.2021.105877.

Thoré, ESJ, Philippe, C, Brendonck, L, Pinceel, T, 2020. Antidepressant exposure reduces body size, increases fecundity and alters social behavior in the short-lived killifish *Nothobranchius furzeri*. Environ. Pollut. 265, 115068 https://doi.org/10.1016/j.envpol.2020.115068.

Thoré, ESJ, Philippe, C, Brendonck, L, Pinceel, T, 2021c. Towards improved fish tests in ecotoxicology – efficient chronic and multi-generational testing with the killifish *Nothobranchius furzeri*. Chemosphere 273, 129697. https://doi.org/10.1016/j.chemosphere.2021.129697.

Thoré, ESJ, Van Hooreweghe, F, Philippe, C, Brendonck, L, Pinceel, T, 2021a. Generation-specific and interactive effects of pesticide and antidepressant exposure in a fish model call for multi-stressor and multigenerational testing. Aquat. Toxicol. 232, 105743 https://doi.org/10.1016/j.aquatox.2021.105743.

Thoré, ESJ, Vanden Berghen, B, Brendonck, L, Pinceel, T, 2023. Long-term exposure to a pharmaceutical pollutant affects geotaxic behaviour in the adult but not juvenile life stage of killifish. Sci. Total Environ. 876, 162746 https://doi.org/10.1016/j.scitotenv.2023.162746.

Vera-Chang, MN, St-Jacques, AD, Gagné, R, Martyniuk, CJ, Yauk, CL, Moon, TW, Trudeau, VL, 2018. Transgenerational hypocortisolism and behavioral disruption are induced by the antidepressant fluoxetine in male zebrafish *Danio rerio*. Proc. Natl. Acad. Sci. 115, E12435–E12442. https://doi.org/10.1073/pnas.1811695115.

Vera-Chang, MN, St-Jacques, AD, Lu, C, Moon, TW, Trudeau, VL, 2019. Fluoxetine exposure during sexual development disrupts the stress axis and results in sex- and time- dependent effects on the exploratory behavior in adult zebrafish Danio rerio. Front. Neurosci. 13, 1015. https://doi.org/10.3389/fnins.2019.01015.

Walker, J, Ghalambor, C, Griset, O, McKenney, D, Reznick, D, 2005. Do faster starts increase the probability of evading predators? Funct. Ecol. 19, 808–815. https://doi.org/10.1111/j.1365-2435.2005.01033.x.

Ward, AJ, Thomas, P, Hart, PJ, Krause, J, 2004. Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). Behav. Ecol. Sociobiol. 55, 561–568. https://doi.org/10.1007/s00265-003-0751-8.

Warton, DI, Duursma, RA, Remko, A, Falster, DS, Taskinen, S, 2012. smatr 3 - an R package for estimation and inference about allometric lines. Methods Ecol. Evol. 3, 257–259. https://doi.org/10.1111/j.2041-210X.2011.00153.x.

White, CR, Kearney, MR, 2014. Metabolic scaling in animals: methods, empirical results, and theoretical explanations. In: Comprehensive Physiology, pp. 231–256. https://doi.org/10.1002/cphy.c110049.

White, CR, Marshall, DJ, Alton, LA, Arnold, PA, Beaman, JE, Bywater, CL, Condon, C, Crispin, TS, Janetzki, A, Pirtle, E, Winwood-Smith, HS, Angilletta, MJ, Chenoweth, SF, Franklin, CE, Halsey, LG, Kearney, MR, Portugal, SJ, Ortiz-Barrientos, D, 2019. The origin and maintenance of metabolic allometry in animals. Nat. Ecol. Evol. 3, 598–603. https://doi.org/10.1038/s41559-019-0839-9.

Widianarko, B, Van Gestel, CA, Verweij, RA, Van Straalen, NM, 2000. Associations between trace metals in sediment, water, and guppy, *Poecilia reticulata* (Peters), from urban streams of Semarang, Indonesia. Ecotoxicol. Environ. Saf. 46, 101–107. https://doi.org/10.1006/eesa.1999.1879.

Wiles, SC, Bertram, MG, Martin, JM, Tan, H, Lehtonen, TK, Wong, BBM, 2020. Long-term pharmaceutical contamination and temperature stress disrupt fish behaviour. Environ. Sci. Technol. 54, 8072–8082. https://doi.org/10.1021/acs.est.0c01625.

Wilkinson, JL, Boxall, ABA, Kolpin, DW, Leung, KMY, Lai, RWS, Galbán-Malagón, C, Adell, AD, Mondon, J, Metian, M, Marchant, et al., 2022. Pharmaceutical pollution of the world's rivers. Proc. Natl. Acad. Sci. 119, e2113947119 https://doi.org/10.1073/pnas.2113947119.

Wilson, ADM, Godin, J-GJ, 2009. Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behav. Ecol. 20, 231–237. https://doi.org/10.1093/beheco/arp018.

Wong, DT, Perry, KW, Bymaster, FP, 2005. Case history: the discovery of fluoxetine hydrochloride (Prozac). Nat. Rev. Drug Discov. 4, 764–774. https://doi.org/10.1038/nrd1821.

Wronski, AR, Brooks, BW, 2023. Global occurrence and aquatic hazards of antipsychotics in sewage influents, effluent discharges and surface waters. Environ. Pollut. 320, 121042 https://doi.org/10.1016/j.envpol.2023.121042.

Wu, NC, Seebacher, F, 2022. Physiology can predict animal activity, exploration, and dispersal. Commun. Biol. 5, 109. https://doi.org/10.1038/s42003-022-03055-y.

Yan, ZH, Zhang, XD, Bao, XH, Ling, X, Yang, HH, Liu, JC, Lu, GH, Ji, Y, 2020. Influence of dissolved organic matter on the accumulation, metabolite production and multi-biological effects of environmentally relevant fluoxetine in crucian carp (*Carassius auratus*). Aquat. Toxicol. 226, 105581 https://doi.org/10.1016/j.aquatox.2020.105581.

Zucker, I, Prendergast, BJ, 2020. Sex differences in pharmacokinetics predict adverse drug reactions in women. Biol. Sex. Differ. 11, 32. https://doi.org/10.1186/s13293-020-00308-5.