Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2019, 53, 6035−6043

pubs.acs.org/est

# Antidepressants in Surface Waters: Fluoxetine Influences Mosquitofish Anxiety-Related Behavior at Environmentally Relevant Levels

Jake M. Martin,*[,†] Michael G. Bertram,[†] Minna Saaristo,[†] Jack B. Fursdon,[†] Stephanie L. Hannington,[†] Bryan W. Brooks,[‡,§] S. Rebekah Burket,[‡] Rachel A. Mole,[‡] Nicholas D. S. Deal,[†] and Bob B. M. Wong[†]

[†]School of Biological Sciences, Monash University, Melbourne, Victoria 3800, Australia

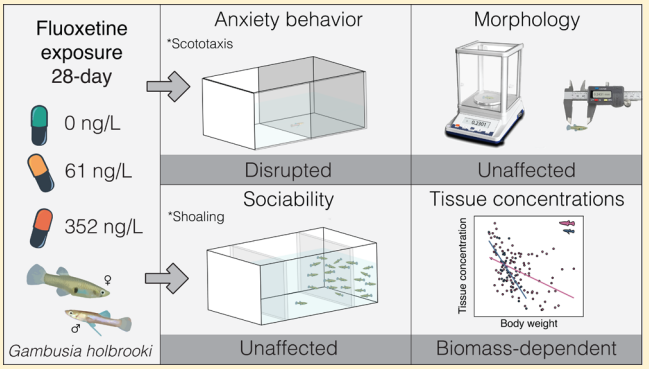
[‡]Department of Environmental Science, Baylor University, Waco, Texas 76706, United States

[§]School of Environment, Jinan University, Guangzhou, 510290 China

Supporting Information



**ABSTRACT:** Pharmaceutical contamination is an increasing problem globally. In this regard, the selective serotonin reuptake inhibitors (SSRIs)—a group of antidepressants—are particularly concerning. By disrupting the serotonergic system, SSRIs have the potential to affect ecologically important behaviors in exposed wildlife. Despite this, the nature and magnitude of behavioral perturbations resulting from environmentally relevant SSRI exposure among species is poorly understood. Accordingly, we investigated the effects of two field-realistic levels of the SSRI fluoxetine (61 and 352 ng/L) on sociability and anxiety-related behaviors in eastern mosquitofish (*Gambusia holbrooki*) for 28 days. Additionally, we measured whole-body tissue concentrations of fluoxetine and norfluoxetine. We found that fluoxetine altered anxiety-related behavior but not sociability. Specifically, female fish showed reduced anxiety-related behavior at the lower treatment level, while males showed an increase at the higher treatment level. In addition, we report a biomass-dependent and sex-specific accumulation of fluoxetine and norfluoxetine, with smaller fish showing higher relative tissue concentrations, with this relationship being more pronounced in males. Our study provides evidence for nonmonotonic and sex-specific effects of fluoxetine exposure at field-realistic concentrations. More broadly, our study demonstrated that neuroactive pharmaceuticals, such as fluoxetine, can affect aquatic life by causing subtle but important shifts in ecologically relevant behaviors.




## 1. INTRODUCTION

Pharmaceutical contamination of the environment is an increasing global problem.[1,2] To date, over 600 different pharmaceuticals have been detected in aquatic ecosystems and wildlife tissues around the world.[3] Further, the release of pharmaceuticals into the environment is likely to escalate due to growing urbanization and a continuing increase in production and diversification of pharmaceutical products.[4] Indeed, recent analyses suggest that the growth in the production of synthetic chemicals, including pharmaceuticals, is equal to or may exceed most other recognized drivers of global change (e.g., climate change, habitat loss, rising atmospheric $CO_2$).[4] Such chemical stressors may result in subtle ecological impacts, including modulation of ecologically relevant behaviors critical to reproduction and survival.

Among emerging environmental contaminants, the selective serotonin reuptake inhibitors (SSRIs) are of increasing concern. This is because SSRIs are among the world's most commonly prescribed antidepressants and are frequently detected in the environment.[5,6] This group of drugs has been found in surface and ground waters globally, typically between <0.1 to 350 ng/L,[6,7] although concentrations as high as 8000 ng/L have been reported.[8] Many countries currently lack regulatory frameworks for monitoring and restricting discharge of SSRIs to the environment (e.g., Australia,[9] European Union,[10] and U.S.A.[11]). Therefore, like most pharmaceutical pollutants, SSRIs commonly enter the environment in wastewater effluent flows due to insufficient removal by sewage treatment plants.[12−14]

Once in the environment, SSRIs have the potential to impact wildlife since the primary therapeutic target is

Received: February 14, 2019
Revised: April 26, 2019
Accepted: April 29, 2019
Published: April 29, 2019




DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

evolutionarily conserved across a diverse range of taxa.[15] Indeed, through disruption of the serotonergic system and associated neuroendocrine pathways, SSRIs have the potential to perturb a number of biologically important functions in nontarget species, such as stress, reproduction, and feeding.[16,17] Moreover, SSRI-induced shifts in behavior could elicit adverse effects on wildlife.[18] For example, SSRI exposure has the potential to influence behavioral traits related to sociability and anxiety,[19−24] which, in wild populations, are known to regulate important inter- and intraspecific interactions (e.g., predator−prey interactions, dispersal, and collective movement).[25−27] Therefore, SSRI-induced shifts in sociability and anxiety-related behaviors could have adverse outcomes for fitness. There is now mounting evidence that SSRI exposure at environmentally realistic levels can alter the behavior of aquatic wildlife,[28−37] although the generality of these findings among species and across various behaviors remains unclear.[38]

The aim of this study was to examine whether one of the most common SSRI contaminants found in surface waters, fluoxetine, can alter anxiety-related behavior and sociability in fish at field-detected levels (nominal: 30 and 300 ng/L). Further, we performed tissue analysis to evaluate the whole-body tissue concentrations of fluoxetine and its primary active metabolite norfluoxetine. On the basis of the reported effects of pharmacologically relevant fluoxetine exposures in other vertebrates,[19,21,23,24] including fish,[22,39,40] we hypothesized that fluoxetine would reduce anxiety-related behaviors and increase sociability in male and female mosquitofish (*Gambusia holbrooki*).

## 2. MATERIALS AND METHODS

**2.1. Animal Collection and Housing.** The eastern mosquitofish—a small, sexually dimorphic, freshwater fish—was selected as a model organism for this investigation. The fish used in experiments (both focal fish and stimulus fish) were wild-caught over two consecutive days ($n$ = 728), sourced from a population at the Monash University Science Centre Lake (37°54′28″S, 145°08′ 16″E), Victoria, Australia. Water samples taken from this lake over consecutive years have indicated no fluoxetine contamination (Envirolab Services, unpublished data). Fish were acclimated to laboratory conditions (23−25 °C; 12:12 h light/dark cycle) for 3 weeks before experimentation in 20 mixed-sex holding tanks (length × width × height: 60 × 30 × 30 cm; ∼35 fish per tank). During housing, fish were fed ad libitum once daily with commercial fish food (Otohime Hirame larval diet).

**2.2. Analytical Verification of Fluoxetine Treatment Levels.** The low treatment (nominal: 30 ng/L) was selected to represent levels reported in surface waters, while the high treatment (nominal: 300 ng/L) was chosen to represent effluent-dominated and dependent systems.[6,7] We selected a 28 day exposure duration as the chronic effects of fluoxetine are manifested in humans, and other mammalian models, after 2−4 weeks of exposure.[41,42] The experimental system followed previously established protocols.[33−37] Briefly, fish ($n$ = 456) were randomly allocated to one of the three experimental treatments: control, low fluoxetine, or high fluoxetine. Each treatment level had two flow-through systems, with each system containing two sex-specific aquaria (45 L; 60 × 30 × 30 cm; water depth: 25 cm), housing 38 fish each. All tanks received complete volume renewal every 24 h (i.e., flow rate ∼1.87 L/h). The fluoxetine experimental systems both had a constant input of fluoxetine stock solution (low: 6 μg/L, high: 60 μg/L) and aged carbon-filtered fresh water (pH measured range from 6.9−7.9), while the control system received only fresh water. Water temperature was maintained at 24.37 ± 0.40 °C (±SD; $n$ = 336), and fish were maintained under the same lighting and feeding conditions as described previously.

During the 28 day experiment, weekly water samples were drawn from all exposure tanks to measure fluoxetine concentrations and from half of the control tanks—selected at random—to confirm the absence of contamination. Water samples were analyzed by Envirolab Services, Perth, using gas chromatography coupled to tandem mass spectrometry (GC-MS/MS), following analytical methods reported by Bertram et al.[35] The mean measured fluoxetine concentration was 60.7 ± 29.7 ng/L (±SD; $n$ = 16) for the low treatment and 351.9 ± 102.2 ng/L ($n$ = 16) for the high treatment. No fluoxetine contamination was detected in the control tanks (all samples under quantification limit of 2 ng/L, $n$ = 8).

**2.3. Behavioral Assays.** To investigate the potential impacts of fluoxetine on sociability and anxiety-related behaviors, focal fish completed one of two separate behavioral assays, both of which commenced immediately after the 28 day exposure period. All experiments were performed blind to treatment. Trials were video-recorded, and behavioral end points were quantified from this footage using the event-recording software JWatcher v1.0.[43] All trials were conducted in aged, carbon-filtered fresh water. Between trials, the tanks were emptied and dried to prevent residual conspecific chemical cues.

Scototaxis trials were used to test whether fluoxetine influenced anxiety-related behaviors, following the methods of Maximino et al.[44] Scototaxis trials offer a clear conflict situation using the innate aversion of many animals to brightly lit environments and are a broadly applicable approach for measuring the anxiolytic effects of pharmacological agents.[44] More specifically, these trials offer the subject a choice to enter either a white area (anxiety-invoking) or a black area. Individual fish from one of the three experimental treatments (females: control $n$ = 35, low fluoxetine $n$ = 35, high fluoxetine $n$ = 38; males: control $n$ = 37, low fluoxetine $n$ = 37, high fluoxetine $n$ = 34) were selected at random and placed in a scototaxis trial tank (60 × 30 × 30 cm; water depth: 10 cm; Figure S1). Scototaxis tanks were divided transversely into black and white halves of equal size, with each of two dividers being placed 5 cm from the center of the tank, forming a compartment (10 × 30 × 30 cm) in which the fish was acclimated before the beginning of the assay. After a 5 min acclimation, the dividers were removed and the fish were left to explore the tank for 15 min. The following behavioral endpoint were scored: the time taken to first enter the white area of the tank and the total time spent in the white area.

The potential influences of fluoxetine on sociability were assessed using a shoaling assay, following the design of Cote et al.[26] In this assay, sociability was measured as an individual's spatial position in an observation tank (60 × 30 × 30 cm; water depth: 20 cm) relative to an unexposed same-sex shoal of 17 conspecifics (total of 16 different shoals, comprising 8 female shoals and 8 male shoals). The observation tank was divided into three compartments using transparent perforated dividers—one large central compartment (40 × 30 × 30 cm) and two smaller side compartments (each compartment: 10 × 30 × 30 cm)—which allowed visual and olfactory communication but not physical interaction. The stimulus shoal (1 of 16) was randomly allocated to one of the two side



DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

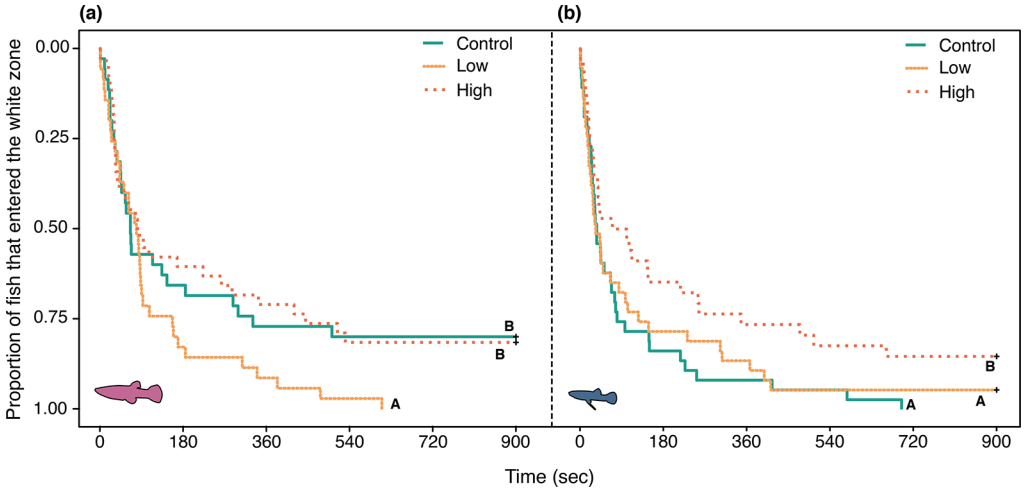



**Figure 1.** Proportion of fish that entered the white area over time for control (green; $n$ = 72), low-fluoxetine (light orange short-dash; $n$ = 72), and high-fluoxetine (dark orange long-dash; $n$ = 72) females (a) and males (b). Groups within either panel that share a capital letter are not significantly different from one another. '+' represents right-censored data.

compartments 15 min before the beginning of each trial. A single focal fish from one of the three exposure treatment groups (females: control $n$ = 33, low fluoxetine $n$ = 34, high fluoxetine $n$ = 34; males: control $n$ = 37, low fluoxetine $n$ = 36, high fluoxetine $n$ = 35) was confined to an opaque holding container (17 × 5 × 12 cm; water depth: 3 cm) within the central compartment for a 5 min acclimation period. After acclimation, the focal fish was released, and its behavior was video-recorded for 15 min. To score the position of the focal fish relative to the shoal, the central compartment was divided into three equally sized zones ("social", "neutral", and "asocial" zones; Figure S1b). A sociability score was calculated by summing the weighted proportion of time the focal fish spent within the three sociability zones (i.e., [proportion of time in the "social" zone × 1] + [proportion of time in the "neutral" zone × 0] + [proportion of time in the "asocial" zone × −1]). This score indicates the use of the entire central compartment relative to the position of the stimulus shoal, with a higher score indicating a more social individual (maximum: 1, minimum: −1). In addition, we recorded the proportion of time a fish spent shoaling within one body length as the total time spent within 2 cm of the stimulus shoal.

**2.4. Fish Size and Condition Measurements.** Immediately upon completion of both behavioral assay types, the fish were euthanized with an overdose of tricaine methanesulfonate (40 mg/L) and their weights (±0.0001 g) and standard lengths (±0.01 mm) were measured. Using weight and standard length measures, a condition index was calculated following previously established protocols.[33,35−37]

**2.5. Fluoxetine and Norfluoxetine Tissue Analysis.** After size and condition measurements, fish were immediately stored at −18 °C until the samples were homogenized and whole-body tissue concentrations of both fluoxetine and norfluoxetine were measured. Specifically, fish were stored at −18 °C within 5 min of being euthanized. Whole-body tissue concentrations of fluoxetine and norfluoxetine for individual fish were analyzed using isotope dilution liquid chromatography−tandem mass spectrometry (LC-MS/MS), following previously established protocols.[45,46] Fluoxetine-*d*6 and norfluoxetine-*d*6 were used as internal standards, purchased from Cerilliant (Round Rock, TX, U.S.A.; > 98% pure). Tissue extraction methods, chemical vendors for extraction solvents and reagents, as well as instrument parameters and detection limits have been previously described.[46] The method detection limit (MDL) for this analysis was 0.36 ng/g for fluoxetine and 0.71 ng/g for norfluoxetine. Whole-body tissue concentrations of fluoxetine and norfluoxetine were measured for a total of 272 fish, 73 males (control: $n$ = 21, low fluoxetine: $n$ = 28, high fluoxetine: $n$ = 24) and 199 females (control: $n$ = 62, low fluoxetine: $n$ = 66, high fluoxetine: $n$ = 71). Fewer males were analyzed than females because a proportion of males were required for a different, unrelated study.

**2.6. Statistical Analysis.** Data analysis was conducted in R version 3.5.1. For all models analyzing behavioral responses, experimental treatment level, sex, focal fish standard length, as well as the interactions among them were used as predictors. In addition, tank ID—as a measure of tank effects—was included in all models as a random effect. For models used to test behavioral responses in the sociability assay, shoal ID was also included as a random effect. For all models, collinearity was checked across variables using correlation matrices, and variables that exceeded a correlation coefficient of 0.70 were considered to be highly correlated and were therefore not included in the same model. Forward-backward stepwise model comparisons based on the Akaike Information Criterion (AIC) were used for model selection. Where necessary, data were transformed to approximate normality (Tables S1−4 for descriptions). For all models, Wald tests were used to calculate the $p$-values of fixed effects (*Anova* function, *car* package).

For the scototaxis assay, the time taken by the fish to first enter the white area (right-censored at 15 min) was compared across treatments using a Cox proportional hazard mixed effect (COXME) model (*coxme* function, *coxme* package). The assumption of proportionality was met for both COXME models, tested by examining the interaction between Schoenfeld residuals and log time (*coxph* and *cox.zph* functions, *survival* package). For all other behavioral responses, linear mixed effect (LME) models (*lmer* function, *lme4* package) were used.

To investigate effects of experimental treatment levels on length, weight, and condition index, data from focal fish used in the scototaxis and shoaling assays were pooled ($n$ = 425) and LME models were used, with treatment level and sex as predictors.



DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

To investigate the accumulation of fluoxetine and norfluoxetine in the tissue of exposed fish (i.e., low- and high-fluoxetine treatments; $n$ = 189), LMEs were used. Control fish were not included in this analysis as no fluoxetine or norfluoxetine were detected in their tissues. Sex, exposure treatment, and weight were used as fixed effects in these models, and tank ID was included as a random effect. Tissue concentrations of each of these compounds approximated a log-normal distribution, and thus a natural log transformation was performed. To account for left-censoring of fluoxetine and norfluoxetine measurements due to the method detection limit (MDL: fluoxetine = 0.36 ng/g, norfluoxetine = 0.71 ng/g), all samples below the MDL were included as the MDL divided by 2, following Antweiler and Taylor.[47]

## 3. RESULTS

**3.1. Behavioral Endpoints.** A significant three-way interaction was detected between treatment, sex, and fish length (COXME: $F$ = 7.44, $p$ = 0.031) on the time to first enter the white area in the scototaxis assay.

For females, fish from the low-fluoxetine treatment level were significantly faster to enter the white area than both control and high-fluoxetine fish (COXME: low−control: $z$ = −2.06, $p$ = 0.039, low−high; $z$ = −1.97, $p$ = 0.049; Figure 1a). Specifically, low-fluoxetine females of average size (22.02 mm) were predicted to be 1.72 ± 0.26 (±SE) and 1.65 ± 0.25 times more likely to enter the white area at any given time, relative to control and high-fluoxetine females, respectively. High-fluoxetine and control females showed no significant difference from one another in this regard (COXME: $z$ = −0.16, $p$ = 0.873). For males, a significant difference was observed in the time taken to first enter the white area between high-fluoxetine fish and both control and low-fluoxetine fish (COXME: high−control: $z$ = 3.04, $p$ = 0.002, high−low: $z$ = 2.30, $p$ = 0.022; Figure 1b). Specifically, high-fluoxetine males of average size (22.02 mm) were predicted to be 2.30 ± 0.27 and 1.87 ± 0.27 times less likely to enter the white area at any given time compared to control and low-exposed males, respectively. There was no significant difference in the time taken to enter the white area between control males and low-fluoxetine males (COXME: $z$ = −0.85, $p$ = 0.395). Interestingly, when comparing the time taken to enter the white area across sexes, there was a statistically significant difference between control males and control females (COXME: $z$ = 3.03, $p$ = 0.002; Figure 1), with males being 2.19 ± 0.26 times more likely to enter the white area at any given time than females. This sex-specific difference was not detected in either low-fluoxetine or high-fluoxetine males and females (COXME: $z$ = −0.17, $p$ = 0.870 and $z$ = 0.35, $p$ = 0.727, respectively; Figure 1). In addition, fluoxetine treatment significantly influenced the relationship between fish length and time to enter the white area (Figure S2; for details and discussion, see Supporting Information pages S6, S10, and S17).

No significant interactions were detected between any predictors on the total time spent in the white area (all $p$ > 0.05; Table S1). Additionally, we found no effect of fluoxetine treatment (LME: $F_{2,5.9}$ = 2.28, $p$ = 0.187; Figure S3) or fish sex (LME: $F_{1,7.9}$ = 0.38, $p$ = 0.554; Figure S3) on total time spent in the white area. In general (i.e., across both sexes and all treatments), fish showed a strong preference for the black side of the tank (paired $t$-test: $df$ = 215, $t$ = 105.65, $p$ < 0.001), spending, on average, 844.60 ± 3.73 s in the black area, as opposed to 55.39 ± 3.73 s in the white area.

In the sociability assay, we found no significant interactions between any predictors on weighted sociability score or the proportion of time fish spent associating within one body length (LME: all $p$ > 0.05; Table S2). Additionally, there was no significant effect of treatment (LME: $F_{2,5.4}$ = 0.94, $p$ = 0.445 and $F_{2,5.7}$= 0.81, $p$ = 0.491; Figure S4) or sex (LME: $F_{1,4.6}$= 0.02, $p$ = 0.960 and $F_{1,5.9}$ = 0.13, $p$ = 0.726; Figure S4). There was, however, a significant effect of focal fish length on weighted sociability score, with larger fish exhibiting greater sociability (LME: $F_{1,195.3}$= 12.66, $p$ < 0.001) and spending significantly more time within 2 cm of the stimulus shoal (LME: $F_{1,194.0}$ = 8.12, $p$ = 0.005).

In all models used to investigate behavioral responses, exposure tank ID accounted for less than 3% of the variation in the response. For end points in the sociability trial, shoal ID accounted for <1% of the variation in the response.

**3.2. Fish Size and Condition.** There was no significant interaction between experimental treatment and sex on any measures of fish size and condition (LME: all $p$ > 0.05; Table S3). Further, there was no significant effect of fluoxetine treatment on any size or condition measurements (LME: all $p$ > 0.05; Table S3). There was a significant effect of sex on fish length (LME: $F_{1,6.9}$ = 10.40, $p$ = 0.015; Figure S5a) and weight (LME: $F_{1,6.9}$= 28.47, $p$ = 0.001; Figure S5b) but not on condition index (LME: $F_{1,7.7}$ = 0.01, $p$ = 0.922; Figure S5c). Across all size and condition models, tank ID accounted for <2% of the variation in the response.

**3.3. Fluoxetine and Norfluoxetine Accumulation.** No fluoxetine or norfluoxetine was detected in the tissue of control fish (0 of 87). Fluoxetine was detected in 89.47% of fish tested from the high-fluoxetine treatment (85 of 95) and in 61.70% of fish from the low-fluoxetine treatment (58 of 94). Norfluoxetine was detected in 93.68% of fish from the high-fluoxetine treatment (89 of 95) and in 81.91% of fish from the low-fluoxetine treatment (77 of 94). Fish in the high-fluoxetine exposure treatment had significantly higher levels of both fluoxetine and norfluoxetine in their tissue than fish from the low-fluoxetine exposure treatment (LME: $F_{1,24.0}$ = 12.4, $p$ = 0.002 and $F_{1,24.0}$ = 22.0, $p$ < 0.001, respectively). Mean concentrations of fluoxetine in fish from the low- and high-fluoxetine treatments were 5.63 ± 1.05 and 10.32 ± 0.88 (ng/g ± SE), respectively. Higher mean concentrations of norfluoxetine were observed in fish from the low- and high-fluoxetine treatment at 10.24 ± 1.56 and 26.81 ± 2.21 (ng/g), respectively. Bioaccumulation factors for fluoxetine were subsequently calculated at 184.1 ± 31.8 (mean ± SE; male: 198.3 ± 48.6; female: 178.0 ± 40.8) and 33.1 ± 2.7 L/kg (male: 36.2 ± 5.5; female: 32.2 ± 3.1) for the low and high treatments, respectively.

A significant interaction was detected between sex and weight for fluoxetine, and a similar near significant interaction was observed for norfluoxetine (LME: $F_{1,179.1}$ = 5.60, $p$ = 0.019 and $F_{1,179.1}$ = 3.77, $p$ = 0.054, respectively). Specifically, males showed a significant decrease in tissue concentration with increasing weight (LME: fluoxetine, $\beta$ = −1.75 ± 0.58, $F_{1,179.3}$ = 8.93, $p$ = 0.003; norfluoxetine, $\beta$ = −1.13 ± 0.52, $F_{1,179.3}$ = 4.67, $p$ = 0.032). For females, this effect was less pronounced with regards to fluoxetine (LME: $\beta$ = −0.283 ± 0.119, $F_{1,180.7}$ = 5.56, $p$ = 0.019) and was not significant for norfluoxetine (LME: $\beta$ = −0.050 ± 0.106, $F_{1,180.7}$= 0.22, $p$ = 0.638). To further investigate the relationship between body burden and fish weight, data below the MDL was excluded and a series of Spearman's rank correlation tests were used. These tests


DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

revealed statistically significant negative relationships across both sexes for fluoxetine (female: $n$ = 97, $rho$ = −0.47, $p$ < 0.001; male: $n$ = 36, $rho$ = −0.63, $p$ < 0.001; Figure 2a) and norfluoxetine (female: $n$ = 113, rho = −0.44, $p$ < 0.001; male: $n$ = 40, rho = −0.59, $p$ < 0.001; Figure 2b).

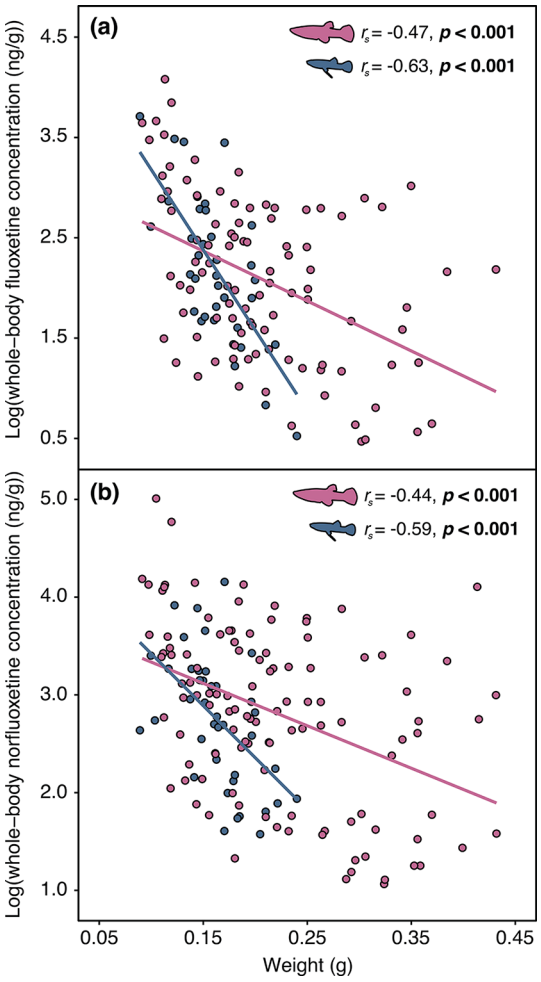



**Figure 2.** (a) Natural log of whole-body tissue concentration of fluoxetine (ng/g) plotted against fish weight with females represented by pink circles (n = 97) and males by blue circles (n = 36). (b) Natural log of whole-body tissue concentration of norfluoxetine (ng/g) plotted against fish weight with females represented by pink circles (n = 113) and males by blue stars (n = 40).

## 4. DISCUSSION

Here, using wild-caught mosquitofish, we report evidence that exposure to field-detected concentrations of fluoxetine can alter anxiety-related behavior. In females, low-fluoxetine exposure (61 ng/L) resulted in a decrease in time taken to enter the white area during a scototaxis assay, indicating a decrease in anxiety.[44] For males, high-fluoxetine exposure (352 ng/L) caused an increase in the time taken to first enter the white area, indicating an increase in anxiety.[44] Further, in terms of time taken to first enter the white area, mosquitofish from the control treatment showed a significant difference between the sexes (with males entering more rapidly), whereas no such difference was seen between the sexes in low- or high-fluoxetine treatments. Therefore, we hypothesize that the sex-specific effects seen here could, in part, be a result of base differences in anxiety behavior across the sexes. In addition, sex differences in SSRI pharmacokinetics (i.e., absorption, distribution, and metabolism) are commonly reported in mammalian models[48,49] and may have also contributed to the sex-specific effects seen here. Natural sex differences in anxiety-related behavior of fish have been reported previously.[50−52] Indeed, in a number of different fish species (e.g., *Poecilia reticulata*,[50] *Gasterosteus aculeatus*,[51] *Brachyrhaphis terrabensis*, and *Brachyrhaphis roseni*[52]), males have been shown to be bolder than females, which is consistent with the results seen in the present study. It has been hypothesized that differences between the sexes, like those seen here, may result from males experiencing greater variance in reproductive success than females, and thus males may benefit from engaging in more risky behavior to gain higher fitness returns (i.e., engaging in more frequent foraging or mating behavior).[51] In addition, for sexually dimorphic species, where the female is the larger of the two sexes, females can experience higher levels of perceived/realized predation threat, as larger fish are a more valuable food source for predators.[53]

Prey fish, like mosquitofish, must constantly balance the risk of predation with the need to forage and/or locate potential mates.[54] Therefore, shifts in anxiety-related behavior, as seen here, could alter the propensity of individuals to take risks—with implications for ecological fitness. For female mosquitofish, decreased anxiety resulting from fluoxetine exposure could lead to increased predation risk and an associated increase in mortality rate.[54] Conversely, for males, increased anxiety as a result of fluoxetine exposure may result in reduced feeding and mating opportunities and hence lower reproductive success.[54] However, in reality, the optimal behavioral strategy and the ecological implications of a behavioral strategy are likely to depend on a range of environmental factors that can vary over time, such as species composition and resource availability (e.g., predator presence; food density).[54] For example, a whole lake experiment using a domestic and wild type strain of rainbow trout (*Oncorhynchus mykiss*) reported that the domestic strain, which shows higher levels of risk-prone behavior (e.g., propensity to forage) compared to the wild type strain,[54] had higher growth rates in low-predation environments. However, in environments with higher predation threat, the domestic strain suffered higher mortality than wild type trout.[55] Therefore, perturbation of anxiety-related behavior could alter the fitness of exposed fish, though the magnitude of these implications may depend on complex interactions with environmental factors.

Interestingly, shifts in anxiety-related behavior observed here for females, but not males, suggest a nonmonotonic-like dose−response (i.e., a nonlinear relationship between fluoxetine dosage and the response). It is important to highlight that the present study was not specifically designed to examine a broader dose−response relationship, given the experimental design and resource considerations. That said, evidence for a nonmonotonic dose−response phenomenon appears to be increasingly reported with low-dose fluoxetine exposure.[28,33−35,56,57] More broadly, the nonmonotonicity of responses seen at low-dose fluoxetine exposure is similar to those reported for endocrine-disrupting chemicals (EDCs).[58] Indeed, some of the general explanations proposed for nonmonotonic effects of EDCs (e.g., receptor competition, receptor down-regulation, and desensitization)[58] could apply to neuroendocrine disruptors, like fluoxetine. Future research


DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

is necessary to examine potential nonmonotonic influences of serotonergically active chemicals on fish behavior.

In the present study, we did not observe an effect of fluoxetine on the total time spent in the white area across exposure treatments or sexes, despite seeing shifts in the time taken to first enter the white area. Similarly, using the same scototaxis protocol, Porseryd et al.[59] reported that zebrafish (*Danio rerio*) exposed to a mixture of 17$\alpha$-ethinylestradiol (0.9 and 1 ng/L for 14 days) and citalopram (100 and 400 ng/L for 14 days) did not differ in the total time spent in the white area; however, they did report a significant reduction in the time to first enter the white area. This could suggest that the accumulative time spent in the white area is perceived as less stressful than the initial entry into the white area, and thus the accumulative time spent in the white area is less sensitive to disruption.

To date, a handful of studies have investigated impacts of SSRIs on anxiety-related behavior of different fish species with varied water chemistry conditions at concentrations less than 1000 ng/L.[30−34,37,56,59−62] However, results from these recent studies carried out on a diverse range of species have yielded seemingly contradictory results (see Table S5 for a full list of SSRIs, dosages, durations, assay types, end points, study species, and nature of behavioral responses). In summary, three studies reported an increase in anxiety-related end points (*Betta splendens* at 500 ng/L for 1−15 days,[31,32] *Poecilia reticulata* at 4 and 16 ng/L for 28 days[34]), four reported a decrease (*Pimephales promelas* embryonic exposure at 25 ng/L for 5 days,[56] *P. promelas* exposure at 1000 ng/L for 28 days,[62] *P. reticulata* at 30 and 500 ng/L for 28 days,[30] *G. holbrooki* at 8, 25, and 97 ng/L for 28 days[33]), and four studies reported no effect on anxiety-related end points (*Poecilia wingei* at 200 ng/L for 21 days,[61] *P. promelas* at 100 ng/L for 28 days,[60] *D. rerio* at 100 and 400 ng for 14 days,[59] and *G. holbrooki* at 31 and 374 ng/L for 35 days[37]). Indeed, some inconsistencies have been reported in behavioral perturbation within the same study species (e.g., *P. reticulata*[34,30] and *G. holbrooki*[33,37]). For example, in male *G. holbrooki*, fluoxetine has previously been shown to reduce antipredator behavior at two environmentally realistic concentrations (8 and 97 ng/L for 28 days),[33] whereas in contrast, male *G. holbrooki* in the present study showed an increase in anxiety at the higher dosage (352 ng/L for 28 days). However, the aforementioned study also documented a nonmonotonic decrease in antipredator behavior in female *G. holbrooki*, with a reduction at the lower concentration (8 ng/L for 28 days) but not at the higher concentration (97 ng/L for 28 days),[33] which is consistent with the effects reported in the present study. In a separate study, using male *G. holbrooki* only, no effect of fluoxetine was reported on boldness-related traits (i.e., time to first emerge from a refuge and time to complete a maze) at 31 and 374 ng/L for 35 days.[37] However, in that study,[37] a statistically nonsignificant marginal trend was observed where at the highest dosage tested, males appeared to take longer to emerge from a refuge zone, similar to the results seen here for males.

At this stage, there does not seem to be a straightforward pharmacokinetic or pharmacodynamic consensus on whether fluoxetine—and SSRIs more generally—can disrupt anxiety-related behaviors among various fish species at environmentally realistic concentrations.

We suggest that the apparent absence of repeatability across studies may be explained by differences in exposure conditions, study species, behavioral endpoints, and/or the complex mechanisms by which fluoxetine may differentially influence behavioral responses with age. For example, it is well established that the impacts of fluoxetine are time-dependent, where acute and chronic effects can have different, even conflicting, impacts on behavior.[16,63,64] Further, as seen in the present study and alluded to above, SSRIs have been reported to induce sex-specific and nonmonotonic effects. If we consider one or more of the above response relationships (i.e., temporal variation, sex-specificity, or nonmonotonicity) to be valid at environmentally realistic levels, we must be careful not to disregard low-dose effects as artifacts due to a perceived lack of a cross-study repeatability among various behaviors and species. Instead, we should consider such factors when interpreting the likely consequences of environmental fluoxetine exposure. Undoubtedly, the effects of SSRI exposure on anxiety-related behavior in wildlife warrant further comparative investigations.

Here, we report no effect of fluoxetine exposure at the tested concentrations on sociability. To date, only four previous studies have addressed effects of SSRIs on sociability at environmentally relevant concentrations.[31,32,65,66] Of these, Dzieweczynski et al.[32] was the only study to report an SSRI-induced effect on sociability, showing a reduction in social interest in *B. splendens*. This difference between these studies, however, could be the result of Dzieweczynski et al.[31] using female *B. splendens* as the social stimulus for males, which may have introduced a reproductive motivation into the sociability assay. Hence, based on the current body of evidence, it appears that fluoxetine at field-detected levels may not induce substantial shifts in the sociability of fish.

In the present study, we observed mean BAF values for fluoxetine at the low and high treatment levels to be 184 and 33 L/kg, respectively. In previously reported laboratory experiments and field studies across various fish species (see Table S6 for details), BAF and bioconcentration factor (BCF) values for fluoxetine have been reported from 9 up to 500 L/kg.[67−73] The variability of BAFs and BCFs reported across these studies (see Table S6 for details) appears to correspond with pH, which is known to influence bioavailability and uptake of fluoxetine[68] and other ionizable bases,[74] by fish, as well as their subsequent responses to pharmaceuticals.[22,77] In fact, when these previous laboratory BCF studies with fluoxetine are considered,[67−73] pH significantly (Spearman's correlation: $\rho = 0.81$, $p = 0.014$) influences BCF values among previously examined fish species (Figure S6). Thus, future bioaccumulation and toxicology studies with fluoxetine and other ionizable contaminants should account for pH influences on internal doses and toxicological response thresholds.

In the present study, we observed a statistically significant correlation between norfluoxetine and fluoxetine body burden and fish weight. Specifically, we observed a decrease in tissue concentration as the weight of both sexes increased. This negative relationship between tissue concentration and weight may have resulted from smaller fish having relatively higher metabolic rates (i.e., higher specific oxygen consumption rates).[75] With fish gills suggested as the primary integrating organ for uptake of organic contaminants[76] and particularly ionizable pharmaceuticals,[46,74] it is possible that higher concentrations of fluoxetine and norfluoxetine in smaller fish could have resulted from differences in uptake during respiration. Indeed, a positive correlation between oxygen consumption and organic contaminant uptake has previously been demonstrated.[77−79] Additional research is necessary to



DOI: 10.1021/acs.est.9b00944
Environ. Sci. Technol. 2019, 53, 6035−6043

understand metabolic influences on contaminant uptake and bioaccumulation, especially in multistressed systems with depressed dissolved oxygen. Although a significant negative relationship between weight and body burden was observed in both sexes, the relationship was shallower for female fish, resulting in females of greater mass accumulating higher levels of fluoxetine than males of similar weights (Figure 2). Though explanations for such observations are not clear, internal disposition in females, compared to males, possibly due to differential protein binding (e.g., bases like fluoxetine bind to $\alpha$1-acid glycoprotein, though protein amounts and binding differences with ionizables by sex, with age, or among fish species, remain poorly understood),[80] may have occurred in the present study.

In summary, we provide evidence that fluoxetine exposure at environmentally relevant levels can disrupt anxiety-related behavior in fish, while, in contrast, exposure did not alter sociability. Effects of fluoxetine on anxiety-related behavior were sex-dependent and, for females, appeared to follow a nonmonotonic dose−response. Importantly, fluoxetine-induced shifts in anxiety-related behaviors may have consequences for the fitness of aquatic wildlife, with organisms inhabiting fluoxetine-contaminated sites responding less optimally to potentially threatening novel stimuli, including predatory threats.[81] In addition, we also observed, for the first time, evidence for biomass-dependent accumulation of fluoxetine and norfluoxetine in the tissue of exposed fish, with smaller fish showing higher relative tissue concentrations. More broadly, our results support a growing body of literature which demonstrates that fluoxetine and other pharmaceutical contaminants can cause subtle but important shifts in the behavior of aquatic wildlife at concentrations presently found in urban aquatic systems, with potential impacts on the health and fitness of exposed populations.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00944.

Diagrams of behavioral experiments, scototaxis and sociability assays, linear mixed effect model (LME) and Cox proportional hazard mixed effect (COXME) model testing results, literature investigating effects of SSRIs, literature investigating bioconcentration and bioaccumulation factor of fluoxetine, relationship between fluoxetine bioconcentration factor (BCF) and pH, brief discussion of results (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: jake.martin@monash.edu.

### ORCID

Jake M. Martin: 0000-0001-9544-9094

Minna Saaristo: 0000-0002-9632-8611

Bryan W. Brooks: 0000-0002-6277-9852


### Author Contributions

J.M.M., M.G.B., M.S., and B.B.M.W. conceived and designed the experiments, which J.M.M. and J.B.F. carried out. Video and statistical analysis were performed by J.M.M., S.L.H., and N.D.S.D. Tissue analysis was coordinated by B.W.B., which S.R.B. and R.M. conducted. The manuscript was drafted by J.M.M. All authors contributed critically to drafts and gave final approval for publication.

### Notes

The present research was approved by the Biological Sciences Animal Ethics Committee of Monash University (permit number: BSCI/2016/21) and complies with all relevant State and Federal laws of Australia.

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We would like to thank James Tanner, Victoria Cockle, Lee Martin, Warren Martin, and Samuel Haddad for their assistance during the project. This study was supported by a Monash University Dean's Scholarship (to J.M.M.), Australian Postgraduate Award scholarships (to J.M.M. and M.G.B.), and the Australian Research Council (DP130100385 and DP160100372, both to B.B.M.W.). Additional research funding was provided by Baylor University.

## ■ REFERENCES


(1) Boxall, A. B. A.; Rudd, M. A.; Brooks, B. W.; Caldwell, D. J.; Choi, K.; Hickmann, S.; Innes, E.; Ostapyk, K.; Staveley, J. P.; Verslycke, T.; Ankley, G. T.; Beazley, K. F.; Belanger, S. E.; Berninger, J. P.; Carriquiriborde, P.; Coors, A.; Deleo, P. C.; Dyer, S. D.; Ericson, J. F.; Gagne, F.; Giesy, J. P.; Gouin, T.; Hallstrom, L.; Karlsson, M. V.; Larsson, D. G. J.; Lazorchak, J. M.; Mastrocco, F.; Mclaughlin, A.; Mcmaster, M. E.; Meyerhoff, R. D.; Moore, R.; Parrott, J. L.; Snape, J. R.; Murray-Smith, R.; Servos, M. R.; Sibley, P. K.; Straub, J. O.; Szabo, N. D.; Topp, E.; Tetreault, G. R.; Trudeau, V. L.; Van Der Kraak, G. Pharmaceuticals and personal care products in the environment: What are the big questions? *Environ. Health Perspect.* **2012**, *120*, 1221−1229.

(2) Arnold, K. E.; Brown, A. R.; Ankley, G. T.; Sumpter, J. P. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. *Philos. Trans. R. Soc., B* **2014**, *369*, 20130569.

(3) Küster, A.; Adler, N. Pharmaceuticals in the environment: scientific evidence of risks and its regulation. *Philos. Trans. R. Soc., B* **2014**, *369*, 20130587.

(4) Bernhardt, E. S.; Rosi, E. J.; Gessner, M. O. Synthetic chemicals as agents of global change. *Front Ecol. Environ.* **2017**, *15*, 84−90.

(5) Silva, L. J. G.; Lino, C. M.; Meisel, L. M.; Pena, A. Selective serotonin re-uptake inhibitors (SSRIs) in the aquatic environment: an ecopharmacovigilance approach. *Sci. Total Environ.* **2012**, *437*, 185−195.

(6) Hughes, S. R.; Kay, P.; Brown, L. E. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environ. Sci. Technol.* **2013**, *47*, 661−677.

(7) Mole, R. A.; Brooks, B. W. Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. *Environ. Pollut.* **2019**, 118.

(8) Fick, J.; Söderström, H.; Lindberg, R. H.; Phan, C.; Tysklind, M.; Larsson, D. G. J. Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ. Toxicol. Chem.* **2009**, *28*, 2522−2527.

(9) Department of Agriculture and Water Resources, (2016). National Water Quality Management Strategy http://www.agriculture.gov.au/water/quality/nwqms, Accessed date: 12 August 2018.

(10) The Council of the European Communities, (**2018**). *Council Directive 91/271/EEC Concerning Urban Waste-water Treatment* https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31991L0271, Accessed date: 12 August 2018.

(11) Environmental Protection Agency, 2016. Contaminant Candidate List (CCL) and Regulatory Determination https://www.epa.gov/ccl/chemical-contaminants-ccl-4, Accessed date: 12 August 2018.




DOI: 10.1021/acs.est.9b00944
*Environ. Sci. Technol.* 2019, 53, 6035−6043

(12) Lajeunesse, A.; Gagnon, C.; Sauve, S. Determination of basic antidepressants and their N-desmethylmetabolites in raw sewage and wastewater using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **2008**, *80*, 5325−5333.
(13) Vasskog, T.; Anderssen, T.; Pedersen-Bjergaard, S.; Kallenborn, R.; Jensen, E. Occurrence of selective serotonin reuptake inhibitors in sewage and receiving waters at Spitsbergen and in Norway. *J. Chromatogr.* **2008**, *A1185*, 194−205.
(14) Arnnok, P.; Singh, Ra.; Burakham, R.; Pérez-Fuentetaja, A.; Aga, D. Selective Uptake and Bioaccumulation of Antidepressants in Fish from Effluent-Impacted Niagara River. *Environ. Sci. Technol.* **2017**, *51*, 10652−10662.
(15) Caveney, S.; Cladman, W.; Verellen, L.; Donly, C. Ancestry of neuronal monoamine transporters in the Metazoa. *J. Exp. Biol.* **2006**, *209*, 4858−4868.
(16) Herculano, A. M.; Maximino, C. Serotonergic modulation of zebrafish behavior: towards a paradox. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2014**, *55*, 50−66.
(17) McDonald, D. M. An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2017**, *197*, 19−31.
(18) Saaristo, M.; Brodin, T.; Balshine, S.; Bertram, M. B.; Brooks, B. W.; Ehlman, S. M.; McCallum, E. S.; Sih, A.; Sundin, J.; Wong, B. B. M.; Arnold, K. E. Direct and indirect effects of chemical contaminants on the behavior, ecology and evolution of wildlife. *Proc. R. Soc. London, Ser. B* **2018**, *285*, 20181297.
(19) Tse, W. S.; Bond, A. J. Serotonergic intervention affects both social dominance and affiliative behavior. *Psychopharmacology.* **2002**, *161*, 324−330.
(20) Dulawa, S. C.; Hollick, K. A.; Gundersen, B.; Hen, R. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* **2004**, *29*, 1321−1330.
(21) Santos, J. M.; Martinez, R. C.; Brandao, M. L. Effects of acute and subchronic treatments with fluoxetine and desipramine on the memory of fear in moderate and high-intensity contextual conditioning. *Eur. J. Pharmacol.* **2006**, *542*, 121−128.
(22) Valenti, T. W.; Gould, G. G.; Berninger, J. P.; Connors, K. A.; Keele, N. B.; Prosser, K. N.; Brooks, B. W. Human therapeutic plasma levels of the selective serotonin reuptake inhibitor (SSRI) sertraline decrease serotonin reuptake transporter binding and shelter-seeking behavior in adult male fathead minnows. *Environ. Sci. Technol.* **2012**, *46*, 2427−2435.
(23) Golub, M. S.; Hogrefe, C. E.; Bulleri, A. M. Peer social interaction is facilitated in juvenile rhesus monkeys treated with fluoxetine. *Neuropharmacology* **2016**, *105*, 553−560.
(24) Payet, J. M.; Burnie, E.; Sathanantha, N. J.; Russo, A. M.; Lawther, A. J.; Kent, S.; Lowry, C. A.; Hale, M. W. Exposure to acute and chronic fluoxetine has differential effects on sociability and activity of serotonergic neurons in the dorsal raphe nucleus of juvenile male BALB/c mice. *Neuroscience* **2018**, *386*, 1−15.
(25) Brown, C.; Jones, F.; Braithwaite, V. In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Anim. Behav.* **2005**, *70*, 1003−1009.
(26) Cote, J.; Fogarty, S.; Weinersmith, K.; Brodin, T.; Sih, A. Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proc. R. Soc. London, Ser. B* **2010**, *B277*, 1571−1579.
(27) Sumpter, D. J. T.; Szorkovszky, A.; Kotrschal, A.; Kolm, N.; Herbert-Read, J. E. Using activity and sociability to characterize collective motion. *Philos. Trans. R. Soc., B* **2018**, *B373*, 20170015.
(28) Guler, Y.; Ford, A. T. Anti-depressants make amphipods see the light. *Aquat. Toxicol.* **2010**, *99*, 397−404.
(29) Barry, M. J. Effects of fluoxetine on the swimming and behavioral responses of the Arabian killifish. *Ecotoxicology* **2013**, *22*, 425−432.
(30) Pelli, M.; Connaughton, V. P. Chronic exposure to environmentally-relevant concentrations of fluoxetine (Prozac) decreases survival, increases abnormal behaviors, and delays predator escape responses in guppies. *Chemosphere* **2015**, *139*, 202−209.
(31) Dzieweczynski, T. L.; Kane, J. L.; Campbell, B. A.; Lavin, L. E. Fluoxetine exposure impacts boldness in female Siamese fighting fish, *Betta splendens*. *Ecotoxicology* **2016**, *25*, 69−79.
(32) Dzieweczynski, T. L.; Campbell, B. A.; Kane, J. L. Dose-dependent fluoxetine effects on boldness in male Siamese fighting fish. *J. Exp. Biol.* **2016**, *219*, 797−804.
(33) Martin, J. M.; Saaristo, M.; Bertram, M. G.; Lewis, P. J.; Coggan, T. L.; Clarke, B. O.; Wong, B. B. M. The psychoactive pollutant fluoxetine compromises antipredator behavior in fish. *Environ. Pollut.* **2017**, *222*, 592−599.
(34) Saaristo, M.; McLennan, A.; Johnstone, C. P.; Clarke, B.; Wong, B. B. M. Impacts of the antidepressant fluoxetine on the anti-predator behavior of wild guppies (*Poecilia reticulata*). *Aquat. Toxicol.* **2017**, *183*, 38−45.
(35) Bertram, M. G.; Ecker, T. E.; Wong, B. B. M.; O'Bryan, M. K.; Baumgartner, J. B.; Martin, J. M.; Saaristo, M. The antidepressant fluoxetine alters mechanisms of pre- and post-copulatory post-copulatory sexual selection in the eastern mosquitofish (*Gambusia holbrooki*). *Environ. Pollut.* **2018**, *238*, 238−247.
(36) Fursdon, J. B.; Martin, J. M.; Bertram, M. G.; Lehtonen, T. K.; Wong, B. B. M. The pharmaceutical pollutant fluoxetine alters reproductive behavior in a fish independent of predation risk. *Sci. Total Environ.* **2019**, *650*, 642−652.
(37) Martin, J. M.; Bertram, M. G.; Saaristo, M.; Ecker, T. E.; Hannington, S. L.; Tanner, J. L.; Michelangeli, M.; O'Bryan, M. K.; Wong, B. B. M. Impact of the widespread pharmaceutical pollutant fluoxetine on behavior and sperm traits in a freshwater fish. *Sci. Total Environ.* **2019**, *650*, 1771−1778.
(38) Sumpter, J. P.; Donnachie, R. L.; Johnson, A. C. The apparently very variable potency of the anti-depressant fluoxetine. *Aquat. Toxicol.* **2014**, *151*, 57−60.
(39) Cachat, J.; Stewart, A.; Grossman, L.; Gaikwad, S.; Kadri, F.; Chung, K. M.; Wu, N.; Wong, K.; Roy, S.; Suciu, C.; Goodspeed, J.; Elegante, M.; Bartels, B.; Elkhayat, S.; Tien, D.; Tan, J.; Denmark, A.; Gilder, T.; Kyzar, E.; DiLeo, J.; Frank, K.; Chang, K.; Utterback, E.; Hart, P.; Kalueff, A. V. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nat. Protoc.* **2010**, *5*, 1786−1799.
(40) Wong, R. Y.; Oxendine, S. E.; Godwin, J. Behavioral and neurogenomic transcriptome changes in wild-derived zebrafish with fluoxetine treatment. *BMC Genomics* **2013**, *14*, 348−361.
(41) Gardier, A. M.; Malagié, I.; Trillat, A. C.; Jacquot, C.; Artigas, F. Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam. Clin. Pharmacol.* **1996**, *10*, 16−27.
(42) Hensler, J. G. Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. *Life Sci.* **2003**, *72*, 1665−1682.
(43) Blumstein, D. T., Daniel, J. C., 2007. *Quantifying Behavior the JWatcher Way*; Sinauer Associates, MA.
(44) Maximino, C.; De Brito, T. M.; Dias, C. A. G. D.; Gouveia, A.; Morato, S. Scototaxis as anxiety-like behavior in fish. *Nat. Protoc.* **2010**, *5*, 209−216.
(45) Du, B.; Perez-Hurtado, P.; Brooks, B. W.; Chambliss, C. K. Evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for pharmaceuticals in fish. *J. Chromatogr.* **2012**, *A1253*, 177−183.
(46) Bean, T. G.; Rattner, B. A.; Lazarus, R. S.; Day, D.; Burket, S. R.; Brooks, B. W.; Haddad, S. P.; Bowerman, W. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. *Environ. Pollut.* **2018**, *232*, 533−545.
(47) Antweiler, R. C.; Taylor, H. E. Evaluation of Statistical Treatments of Left-Censored Environmental Data using Coincident Uncensored Data Sets: I. Summary Statistics. *Environ. Sci. Technol.* **2008**, *42*, 3732−3738.
(48) Bigos, K. L.; Pollock, B. G.; Stankevich, B. A.; Bies, R. R. Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: An updated review. *Gender medicine.* **2009**, *6*, 522−543.


DOI: 10.1021/acs.est.9b00944
*Environ. Sci. Technol.* 2019, 53, 6035−6043

(49) Kokras, N.; Dalla, C.; Papadopoulou-Daifoti, Z. Sex differences in pharmacokinetics of antidepressants. *Expert Opin. Drug Metab. Toxicol.* **2011**, *7*, 213−226.
(50) Harris, S.; Ramnarine, I. W.; Smith, H. G.; Pettersson, L. B. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos* **2010**, *119*, 1711−1718.
(51) King, A. J.; Fürtbauer, I.; Mamuneas, D.; James, C.; Manica, A. Sex-differences and temporal consistency in stickleback fish boldness. *PLoS One* **2013**, *8*, No. e81116.
(52) Ingley, S. J.; Rehm, J.; Johnson, J. B. Size doesn't matter, sex does: a test for boldness in sister species of *Brachyrhaphis* fishes. *Ecol. Evol.* **2014**, *4*, 4361−4369.
(53) Britton, R. H.; Moser, M. E. Size specific predation by herons and its effect on the sex-ratio of natural populations of the mosquito fish *Gambusia affinis*. *Oecologia* **1982**, *53*, 146−151.
(54) Mittelbach, G. G.; Ballew, N. G.; Kjelvik, M. K. Fish behavioral types and their ecological consequences. *Can. J. Fish. Aquat. Sci.* **2014**, *71*, 927−944.
(55) Biro, P. A.; Abrahams, M. V.; Post, J. R.; Parkinson, E. A. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc. R. Soc. B Biol. Sci.* **2004**, *271*, 2233−2237.
(56) Painter, M. M.; Buerkley, M. A.; Julius, M. L.; Vajda, A. M.; Norris, D. O.; Barber, L. B.; Furlong, E. T.; Schultz, M. M.; Schoenfuss, H. L. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales promelas*). *Environ. Toxicol. Chem.* **2009**, *28*, 2677−2684.
(57) Bossus, M. C.; Guler, Y. Z.; Short, S. J.; Morrison, E. R.; Ford, A. T. Behavioral and transcriptional changes in the amphipod *Echinogammarus marinus* exposed to two antidepressants, fluoxetine and sertraline. *Aquat. Toxicol.* **2014**, *151*, 46−56.
(58) Vandenberg, L. N.; Colborn, T.; Hayes, T. B.; Heindel, J. J.; Jacobs, D. R., Jr.; Lee, D.-H.; et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr. Rev.* **2012**, *33*, 378−455.
(59) Porseryd, T.; Kellner, M.; Caspillo, N. R.; Volkova, K.; Elabbas, L.; Ullah, S.; Olsén, H.; Dinnétz, P.; Hällström, I. P. Combinatory effects of low concentrations of 17$\alpha$-etinylestradiol and citalopram on non-reproductive behavior in adult zebrafish (*Danio rerio*). *Aquat. Toxicol.* **2017**, *193*, 9−17.
(60) Margiotta-Casaluci, L.; Owen, S. F.; Cumming, R. I.; De Polo, A.; Winter, M. J.; Panter, G. H.; Rand-Weaver, M.; Sumpter, J. P. Quantitative cross-species extrapolation between humans and fish: the case of the anti-depressant fluoxetine. *PLoS One* **2014**, *9*, No. e110467.
(61) Olsén, K. H.; Ask, K.; Olsen, H.; Porsch-Hallstrom, I.; Hallgren, S. Effects of the SSRI citalopram on behaviors connected to stress and reproduction in Endler guppy, *Poecilia wingei*. *Aquat. Toxicol.* **2014**, *148*, 113−121.
(62) Weinberger, J.; Klaper, R. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish *Pimephales promelas* (fathead minnow). *Aquat. Toxicol.* **2014**, *151*, 77−83.
(63) Silva, R. C. B.; Brandao, M. L. Acute and chronic effects of gepirone and fluoxetine in rats tested in the elevated plus-maze: An ethological analysis. *Pharmacol., Biochem. Behav.* **2000**, *65*, 209−216.
(64) Stewart, A. M.; Grossman, L.; Nguyen, M.; Maximino, C.; Rosemberg, D. B.; Echevarria, D. J.; Kalueff, A. V. Aquatic toxicology of fluoxetine: under- standing the knowns and the unknowns. *Aquat. Toxicol.* **2014**, *156*, 269−273.
(65) McCallum, E. S.; Bose, A. P. H.; Warriner, T. R.; Balshine, S. An evaluation of behavioral endpoints: the pharmaceutical pollutant fluoxetine decreases aggression across multiple contexts in round goby (*Neogobius melanostomus*). *Chemosphere* **2017**, *175*, 401−410.
(66) Meijide, F. J.; Da Cuna, R. H.; Prieto, J. P.; Dorelle, L. S.; Babay, P. A.; Lo Nostro, F. L. Effects of waterborne exposure to the antidepressant fluoxetine on swimming, shoaling and anxiety behaviors of the mosquitofish *Gambusia holbrooki*. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 646−655.
(67) Paterson, G.; Metcalfe, C. D. Uptake and depuration of the anti- depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). *Chemosphere* **2008**, *74*, 125−130.
(68) Nakamura, Y.; Yamamoto, H.; Sekizawa, J.; Kondo, T.; Hirai, N.; Tatarazako, N. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* **2008**, *70*, 865−873.
(69) Zhang, X.; Oakes, K. D.; Cui, S. F.; Bragg, L.; Servos, M. R.; Pawliszyn, J. Tissue-Specific In Vivo Bioconcentration of Pharmaceuticals in Rainbow Trout (*Oncorhynchus mykiss*) Using Space-Resolved Solid-Phase Microextraction. *Environ. Sci. Technol.* **2010**, *44*, 3417−3422.
(70) Lajeunesse, A.; Gagnon, C.; Gagne, F.; Louis, S.; Cejka, P.; Sauve, S. Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and after ozone treatment - Evidence of biological effects. *Chemosphere* **2011**, *83*, 564−571.
(71) Togunde, O. P.; Oakes, K. D.; Servos, M. R.; Pawliszyn, J. Optimization of solid phase microextraction for non-lethal in vivo determination of selected pharmaceuticals in fish muscle using liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2012**, *1261*, 99−106.
(72) Bostrom, M. L.; Huang, C. X.; Engstrom, H.; Larsson, E.; Berglund, O.; Jonsson, J. A. A specific, highly enriching and "green" method for hollow fiber liquid phase microextraction of ionizable pharmaceuticals from fish tissue. *Anal. Methods* **2014**, *6*, 6031−6037.
(73) Bostrom, M. L.; Ugge, G.; Jonsson, J. A.; Berglund, O. Bioaccumulation and trophodynamics of the antidepressants sertraline and fluoxetine in laboratory-constructed, 3-level aquatic food chains. Environ. *Environ. Toxicol. Chem.* **2017**, *36*, 1029−1037.
(74) Nichols, J. W.; Du, B.; Berninger, J. P.; Connors, K. A.; Chambliss, C. K.; Erickson, R. J.; Brooks, B. W. Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. *Environ. Toxicol. Chem.* **2015**, *34*, 1425−1435.
(75) Mitz, S. V.; Newman, M. C. Allometric relationship between oxygen consumption and body weight of mosquitofish, *Gambusia affinis*. *Environ. Biol. Fishes* **1989**, *24*, 267−273.
(76) Randall, D. J.; Connell, D. W.; Yang, R.; Wu, S. S. Concentrations of persistent lipophilic compounds in fish are determined by exchange across the gills, not through the food chain. *Chemosphere* **1998**, *37*, 1263−1270.
(77) Brauner, C. J.; Randall, D. J.; Neuman, J. F.; Thurston, R. V. The effect of exposure to 1,2,4,5-tetrachlorobenzene and the relationship between toxicant and oxygen uptake in rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **1994**, *13*, 1813−1820.
(78) Yang, R.; Brauner, C.; Thurston, V.; Neuman, J.; Randall, D. J. Relationship between toxicant transfer kinetic processes and fish oxygen consumption. *Aquat. Toxicol.* **2000**, *48*, 95−108.
(79) Blewett, T.; MacLatchy, D. L.; Wood, C. M. The effects of temperature and salinity on 17-alpha-ethynylestradiol uptake and its relationship to oxygen consumption in the model euryhaline teleost (*Fundulus heteroclitus*). *Aquat. Toxicol.* **2013**, *127*, 61−71.
(80) Armitage, J. M.; Erickson, R. J.; Luckenbach, T.; Ng, C. A.; Prosser, R. S.; Arnot, J. A.; Schirmer, K.; Nichols, J. W. The effects of temperature and salinity on 17-alpha-ethynylestradiol uptake and its relationship to oxygen consumption in the model euryhaline teleost (*Fundulus heteroclitus*). Assessing the bioaccumulation potential of ionizable organic compounds: current knowledge and research priorities. *Environ. Toxicol. Chem.* **2017**, *36*, 882−897.
(81) Lima, S. L.; Dill, L. M. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **1990**, *68*, 619−640.


DOI: 10.1021/acs.est.9b00944
*Environ. Sci. Technol.* 2019, 53, 6035−6043