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Antidepressant exposure causes a nonmonotonic reduction in anxiety-related behaviour in female mosquitofish

Jake M. Martin [a,*], Venkatesh Nagarajan-Radha [a,b], Hung Tan [a], Michael G. Bertram [a,c], Jack A. Brand [a], Minna Saaristo [a,d], Damian K. Dowling [a], Bob B.M. Wong [a]

[a] School of Biological Sciences, Monash University, Melbourne, Victoria, Australia
[b] School of Life and Environmental Sciences, The University of Sydney, New South Wales, Australia
[c] Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden
[d] EPA Victoria, Water Sciences Unit, Melbourne, Victoria, Australia

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ABSTRACT

Worldwide, biologically active pharmaceuticals, such as psychoactive drugs, are routinely detected in aquatic ecosystems. In this regard, selective serotonin reuptake inhibitors (SSRIs), a class of antidepressant, are of major environmental concern. Through targeted action on evolutionarily conserved physiological pathways, SSRIs could alter ecologically important behaviours in exposed organisms. Here, using two field-realistic dosages (measured concentrations: 18 and 215 ng/L) of the SSRI fluoxetine (Prozac), we examined the effects of exposure on anxiety-related behaviours in wild-caught female mosquitofish, *Gambusia holbrooki*. Anxiety-related behaviour was assessed using a light/dark transition test, with the swimming activity of fish recorded under two alternating light conditions, complete darkness and bright light, with the shift in light condition used to induce an anxiety-like response. Fluoxetine exposure resulted in a nonmonotonic decrease in anxiety-related behaviour (i.e. nonlinear with dose), with fish in the low-fluoxetine treatment being less responsive to shifts in light condition compared to unexposed fish. There was no such difference between unexposed and high-exposed fish. Further, we detected a significant interaction between exposure treatment and fish weight on general swimming activity, suggesting the presence of a mass-specific effect of fluoxetine. More broadly, contaminant-induced disruption of animal behaviour—as documented here—could have wide-reaching effects on population-level fitness.

Introduction

Pharmaceutical pollution is a rapidly emerging global issue, with large quantities of biologically active drugs making their way into the environment [1,2]. In this regard, psychoactive pharmaceuticals—including selective serotonin reuptake inhibitors (SSRIs)—are frequently detected in aquatic ecosystems [3–5]. Due to non-existent [6] or inadequate [7,8] wastewater treatment, these SSRI antidepressants can enter aquatic systems, and have been repeatably detected in the tissues of wildlife [9–11]. A recent review on global surface water concentrations of SSRIs reported a range of <0.1–351 ng/L in freshwater environments [5], but higher concentrations have been reported in effluent-dominated systems (e.g. [12]).

Selective serotonin reuptake inhibitors bind to, and block, the serotonin transporter molecule [13]. Since the serotonin transporter molecule, and the serotonergic system more generally, are evolutionary conserved across taxa [14], these drugs have the potential to affect non-target wildlife [15]. For example, SSRIs can alter anxiety-related behaviour via the hypothalamic–pituitary–interrenal (HPI) axis by influencing the production of corticotropin-releasing hormone and adrenocorticotropic hormone, as well as cortisol secretion [15]. For this reason, disruption of anxiety-related behaviours is a sensible endpoint to test the risk posed by SSRI exposure. Further, from an ecological perspective, shifts in anxiety-related behaviour can have crucial direct and indirect consequences for health and survival [16], because anxiety is known to regulate ecologically important processes like predator−prey interactions [17].

Currently, however, there appears to be substantial incongruity in the reported effects of SSRIs on anxiety-related behaviours of non-target species, and the primary cause for these differences remain unclear [18]. This is particularly true for fluoxetine [18–21]—the most widespread SSRI pollutant [5]. This variability in reported effects makes assessing the environmental hazards posed by SSRIs difficult. If we are to understand the risks posed by this group of contaminants, and the potential mechanisms driving this heterogeneity, there is a clear need for further research.

* Corresponding author.
E-mail address: jake.martin@monash.edu (J.M. Martin).

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Accordingly, the aim of this study was to test whether fluoxetine, at two environmentally realistic concentrations, affects anxiety-related behaviours of wild-caught female mosquitofish (*Gambusia holbrooki*) using a light/dark transition test. More specifically, we predicted that fluoxetine exposure will reduce the responsiveness of mosquitofish to shifts in environmental light condition (i.e. complete dark to bright light)—an anxiety-inducing stimulus. Further, based on emerging evidence (e.g. [19,22,23]), we predicted that the reduction in anxiety-like behaviour will be nonmonotonic, with stronger effects seen at the lower dosage employed.

Materials and methods

Fish collection, housing, and exposure

Adult female mosquitofish (i.e. individuals >2 cm [24]) were collected using dip nets from a wild population (37° 54′ 28″ S, 145° 08′ 16″ E) and transported to Monash University for housing and experimentation. Given female mosquitofish take 18–70 days to fully mature [24] and have a life span of approximately 18 months [25], the expected age range for those used in this study is 2–18 months. During housing, fish were maintained in glass aquaria (60 × 30 × 30 cm; length × width × height; 30 fish per tank) containing carbon-filtered fresh water at 23–25 °C (12:12 h light:dark cycle), with this water being left to stand for 24 h before being added to tanks. Fish were fed *ad libitum* once daily with commercial fish food (Otohime Hirame larval diet). After one month of acclimation to laboratory conditions, fish were randomly allocated to one of three treatments for 28 days: unexposed (i.e. 0 ng/L), low fluoxetine (nominal level: 30 ng/L) or high fluoxetine (nominal level: 300 ng/L). The low- and high-fluoxetine concentrations were selected based on the typical reported concentrations in freshwater ecosystems (i.e. <0.1–351 ng/L [5]) and dosages employed in previously published studies [18,26,27]. The exposure system followed previously established protocols [28–30]. Specifically, fish were allocated to one of six separate flow-through systems, with each system comprising of four exposure aquaria, resulting in eight replicate tanks per treatment (60 × 30 × 30 cm; water depth: 25 cm).

All systems received a constant supply of aged, carbon-filtered fresh water (pH measured range: 6.9–7.9), with complete volume renewal every 24 h. The exposed flow-through systems each received a constant supply of fluoxetine (CAS: 56296-78-7) stock solution in reverse osmosis water (low = 6 μg/L and high = 60 μg/L), which was changed daily. Fluoxetine stock solutions were prepared as described in Martin et al. [22] and Bertram et al. [28]. Briefly, on every day of the exposure, a 1 mL solution of fluoxetine hydrochloride and methanol (Sigma-Aldrich; Product Number: F132; low: 18 ug/mL, high: 180 ug/mL) was evaporated to dryness under a gentle nitrogen stream, before being diluted with Milli-Q water to form a 3 L stock solution.

During the exposure, water temperature was maintained at 23.4 ± 0.9 °C (mean ± SD; n = 576) and lighting and feeding conditions were identical to those employed during laboratory housing (see above). Further, weekly water samples (80 mL) were drawn from all exposure tanks to measure fluoxetine concentrations, and fortnightly samples drawn from all unexposed tanks to confirm the absence of contamination. Analytical verification of fluoxetine concentrations was performed using gas chromatography coupled to tandem mass spectrometry, as described in Bertram et al. [28].

Behavioural experiments

A light/dark transition test was used as a proxy for measuring anxiety-like behaviour. The experimental assay used in our study followed similar procedures to those of several published studies addressing the impacts of various toxicants on the locomotor response of juvenile fish [31–34] but were modified for adult fish. Specifically, fish were placed individually into square experimental arenas (18.5 × 18.5 cm; water depth: 5 cm) in fresh water. Experimental arenas were then transferred into a cuboid enclosure (maintained at 24 °C) designed for automated behavioural tracking (ZebraCube; ViewPoint Life Sciences). In total, 112 fish were used in light/dark transition experiments (n: unexposed = 38, low-fluoxetine = 38, high-fluoxetine = 36).

Before the start of each trial, fish were acclimated for 5 min within the experimental arenas in complete darkness. After acclimation, the swimming activity of the fish was measured continuously over a 20-min trial. To measure the response of fish to shifts in light condition, two different light conditions were employed while fish were inside the enclosure, complete darkness or bright light (170–200 lx). This was achieved by turning lights on and off in 5 min intervals, giving four distinct photoperiods (i.e. dark, light, dark, light). Lights and live video tracking were controlled remotely (ZebraLab tracking software v3.22; ViewPoint Life Sciences), with a combination of LED and infrared lighting used to contrast the fish for automated tracking. The swimming activity of fish was measured as the number of pixels moved and was converted to physical distance (cm) using a standard reference (i.e. the length of the arena, 18.5 cm). The following endpoints were recorded for each fish: (1) light/dark response, measured as the difference in activity levels between the light conditions (swimming activity in the light minus swimming activity in the dark), and (2) general swimming activity during each of the four photoperiods.

After completing the trials, fish were euthanised and morphological measurements were taken. Specifically, following previously established protocols [27,35,36], weight (±0.0001 g) and standard length (±0.01 mm; the tip of the snout to the end of the last vertebra) were measured for each fish. Weight and length data for fish used in the present study has been reported as part of Martin et al. [29], thus the main effects of fluoxetine on weight or length will not be tested here (see Figure S1 for data). It is worth highlighting that there was no significant difference in the weight or length of fish across the exposure treatments [29].

Statistics

Data analyses were performed using R version 4.0.0 [37]. To approximate a Gaussian distribution, data were transformed where necessary (see Table S1 for descriptions). Both the light/dark response and general swimming activity were analysed using a linear mixed-effects (LME) model. The light/dark response model included fish weight (g), transition number (the first or second light transition), exposure treatment (unexposed, low fluoxetine or high fluoxetine), and exposure treatment interaction terms as fixed effects, with fish identity included as a random intercept. The model for swimming activity included fish weight, light treatment (i.e. dark or light), transition number, exposure treatment, and exposure treatment interaction terms as fixed effects. In addition, fish identity was included as a random intercept, with transition number and light treatment modelled as random slopes, to account for variability in individual differences in behaviour across transition number and light treatments. Across all models, fish weight was centred to improve the interpretability of main effects. Where interaction terms were non-significant (i.e. $p > 0.05$) and did not improve the fit of the model—assessed using Akaike information criterion (AIC) estimates—they were removed (see Table S1 for final models). For all models, type-II Wald's F-tests with Kenward-Roger Degrees of Freedom Approximation were used to calculate the p-values of fixed effects and interaction terms. Where a significant main effect of exposure treatment was detected, Tukey's p-adjustments were used to investigate pair-wise comparisons. Further, where a significant interaction term was detected between a categorical and continuous predictor, the relationship was investigated with Pearson's correlation tests within each category.

Results

Analytical verification of fluoxetine concentrations

During the 28-day exposure period, the average measured concentrations (± SD) of the low- and high-fluoxetine treatments were 18.19 ±

4.98 ng/L (n = 32) and 214.69 ± 38.89 ng/L (n = 32), respectively. Fluoxetine was not detected (i.e. was under the detection limit; less than 2 ng/L, n = 16) in control water samples.

Fish behaviour

There were no significant interactions between any predictors for light/dark response (all $p > 0.05$; see Table S1). We did observe a significant effect of fluoxetine exposure treatment ($F_{2,108} = 3.10$, $p = 0.049$; Fig. 1). Specifically, there was a difference between the light/dark response of unexposed and low-exposed fish, with a comparatively smaller change in swimming activity observed in low-exposed females between the dark and light condition ($z = 2.48, p = 0.035$). However, we saw no significant difference in the light/dark response of fish across the other treatment comparisons ($z = 1.43$, $p = 0.326$ and $z = -1.03$, $p = 0.558$, unexposed–high and low–high, respectively). In addition, there was a significant main effect of transition number ($F_{1,111} = 13.40$, $p < 0.001$). Specifically, on average, fish adjusted their swimming activity more substantially in the first light transition (Δactivity mean ± SE: −134.83 ± 3.47) compared to the second transition (−48.46 ± 3.47). There was no significant main effect of fish weight on light/dark response ($F_{1,108} = 0.12, p = 0.732$). Fish identity accounted for an estimated 15.90 ± 0.09 % (mean ± SE) of the variation in light/dark response.

For swimming activity, we detected a significant interaction between exposure treatment and fish weight ($F_{2,106} = 3.56, p = 0.032$; Fig. 2). For unexposed fish and high-fluoxetine exposed fish, there was a non-significant positive correlation between fish weight and swimming activity ($r = 0.27, p = 0.102$ and $r = 0.27, p = 0.111$), whereas for low-fluoxetine exposed fish there was a non-significant negative correlation ($r = -0.24, p = 0.156$). In addition, there were significant main effects of both light treatment ($F_{1,111} = 40.66$, $p < 0.001$) and transition number ($F_{1,111} = 296.70$, $p < 0.001$). Individual fish identity accounted for an estimated 39.2 ± 3.70 (mean ± SE) of the variation in swimming activity.

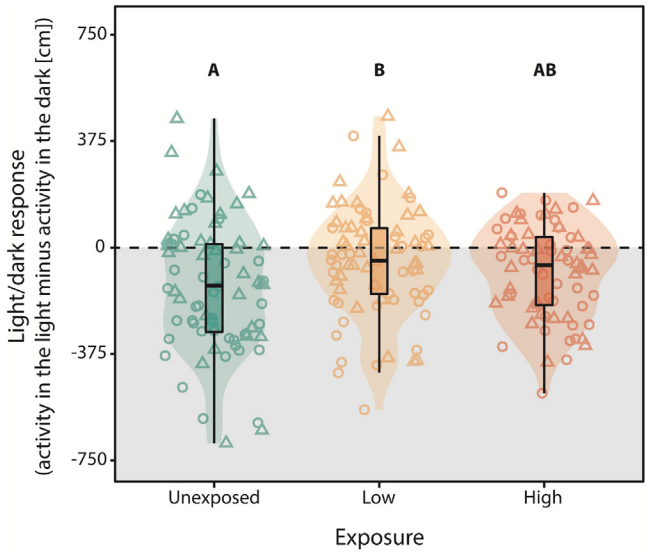

Fig. 1. Light/dark response, measured as the difference in activity levels between the light and dark photoperiods (i.e. light activity minus dark activity; cm), for fish in the unexposed (n = 38), low-fluoxetine (n = 38) and high-fluoxetine (n = 36) treatments. Data from the first light transition and the second light transition are represented by open circles and triangles, respectively. Values below zero indicate increased activity in the dark photoperiod (i.e. area shaded grey), while values above zero indicate increased activity in the light photoperiod (i.e. area shaded white). Box plots show 25th, 50th (median), and 75th percentiles. Groups that share a capital letter are not significantly different from one another.

Discussion

Fluoxetine exposure resulted in a nonmonotonic decrease in light/dark response. Specifically, a significant difference in the light/dark response of low-exposed fish and unexposed fish was observed, while there was no significant difference between the other treatments. Compared to fish from the unexposed treatment, low-exposed fish were less responsive to shifts in environmental light condition, maintaining similar activity levels across both light and dark periods.

This reduction in locomotor-based response to the light condition is interpreted as a shift in anxiety-related behaviour for several reasons. First, a preference for dark environments over bright environments has been validated in a number of teleost species [38–40], and the strength of this preference is commonly used as an indicator of anxiety [41], including in mosquitofish [18,42]. Second, anxiety-like responses to light/dark transition tests have been experimentally confirmed in fish (e.g. juvenile zebrafish [34]). Third, within this study, a reduction in activity levels during the light photoperiods was observed, which is a common response in fish (e.g. [43–45]), including mosquitofish [22], to perceived threatening situations. Lastly, given that fluoxetine acts on evolutionarily conserved neuroendocrine pathways involved in the stress response [15], changes to anxiety-like behaviour are an expected impact on adult fish from an adverse outcome pathway (AOP) perspective [15]. We should highlight that it is possible that fluoxetine-induced changes in general locomotor performance (via muscular/skeletal dysfunctions) may also explain shifts in the light/dark response. However, despite evidence that SSRI exposure can alter bone development in exposed embryos [46], to the best of our knowledge, these effects have not been reported during adult exposure, or at environmentally realistic concentrations. Further, a general impairment of locomotion is unlikely in this study, as we did not detect a general change in locomotor activity across the treatments, only in the relative response to the dark and light photoperiods. Therefore, the most plausible explanation for fluoxetine-induced changes in the light/dark response is disrupted anxiety-related behaviour.

On an individual level, anxiety-like behaviour has been linked to a number of ecologically important processes, such as risk of predation, foraging efficiency, and dispersal tendency [47–50]. Wild fish must constantly adjust their behaviour based on perceived level of risk, as many fitness-related behaviours (e.g. foraging, reproduction) are typically associated with increased threat of predation [51]. Therefore, pollutant-induced reductions in anxiety-like behaviour—as seen here—could potentially increase predation risk as a result of suboptimal behaviour in potentially dangerous situations. With that said, it is important to note that the optimal behaviour of any individual depends on a range of social and environmental factors, such as predator presence, population size, and food density [17,51]. As such, the realised impacts of fluoxetine-induced behavioural changes are difficult to predict and warrant further investigation. Despite uncertainty surrounding the magnitude of ecological consequences, it is reasonable to assume that a pollutant-induced perturbation of anxiety-related behaviours could have important fitness consequences for exposed wildlife at the population level [48].

The results of the present study are concordant with five earlier studies [19,22,52–54] that have all reported a decrease in anxiety-related behaviours of fish as a result of environmentally realistic SSRI exposure (see Table S2 for study details). However, our findings contrast with three studies [21,55,56] reporting an increase in anxiety-related behaviour, as well as four [20,36,44,57] that report no effect of SSRI exposure on anxiety-related behaviour (Table S2). It is important to point out that differences in the literature between reported effects of fluoxetine are not limited to anxiety-related behaviour. Indeed, variability between reported effects of fluoxetine on aquatic wildlife has been the subject of debate more generally [58,59].

We contend that the apparent variability across studies is likely the result of one or more of the following factors: differences in study species,

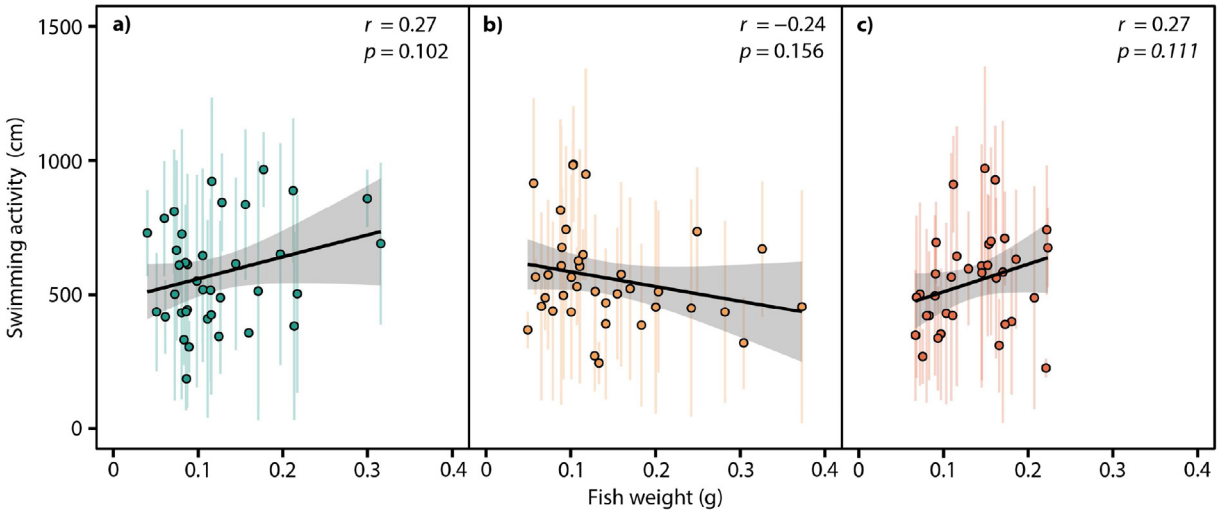

Fig. 2. Mean swimming activity (± SD) across all photoperiods (i.e. pooled for each of the photoperiods) plotted by fish weight for (**a**) unexposed (n = 38), (**b**) low-fluoxetine (n = 38) and (**c**) high-fluoxetine (n = 36) treatments. Pearson's correlation tests were used to calculate r- and p-values. Grey areas around the linear trend lines represent 95 % confidence intervals.

exposure durations, and exposure concentrations. In this regard, there is evidence to suggest that effects of fluoxetine can be mediated by all of the above factors. For example, species-specific sensitivity may contribute to this variability, as many of the neuroendocrine pathways through which fluoxetine alters behaviour vary substantially across species [60]. Further, it is well established that the impacts of fluoxetine are time-dependent, where acute and chronic effects can have different, even conflicting, impacts on behaviour [58]. In addition, previous studies, as well as the present, have reported fluoxetine-induced nonmonotonic dose–response relationships [18,19,22,23,55,61–65]. There is also evidence to suggest that the bioaccumulation of fluoxetine in fish tissue is pH-dependent (e.g. Japanese medaka, *Oryzias latipes* [66]), and among-study variability in bioaccumulation and bioconcentration factors have been linked to differences in water pH (see [18]). With this in mind, it is perhaps unsurprising that studies using different concentrations, exposure durations, species, and water parameters have reported different effects on behaviour. Provided that one or more of the above dose–response relationships (i.e. species-specificity, temporal variation, or nonmonotonicity) are possible at environmentally realistic levels, we must be careful not to disregard low-dose effects as artefacts due to a perceived lack of cross-study repeatability.

As mentioned above, the present findings are suggestive of a nonmonotonic dose–response relationship, as the magnitude of the reported effect did not increase linearly with dosage. Evidence for nonmonotonic effects is increasingly reported at environmentally realistic dosages of fluoxetine across a wide range of species, including studies investigating anxiety-like behaviour and antipredator behaviour in fish [18,19,22,55,63,67]. Consistent with the result observed in the present study, Martin et al. [18] reported a significant reduction in anxiety-related behaviour in mosquitofish at the lower of two environmentally realistic fluoxetine dosage (61 ng/L for 28 days) using a scototaxis assay, but did not detect a difference at the higher dosage (352 ng/L). Similarly, Painter et al. [19] reported evidence for a nonmonotonic effect of fluoxetine exposure on fish behaviour (fathead minnow, *Pimephales promelas*; 25–250 ng/L for 5 days as embryos), with more pronounced reductions in antipredator behaviour detected at lower dosages. More broadly, nonmonotonic dose–response relationships could result from a number of different mechanisms, such as receptor desensitisation, negative feedback with increasing dose, dose–dependent metabolism modulation, or opposing effects induced by an analyte binding to multiple receptors that differ in their affinity [68]. It is possible that such mechanisms are driving the reported nonmonotonic effects of fluoxetine in aquatic wildlife. With increasing evidence for fluoxetine-induced nonmonotonic effects, the potential mechanisms driving this relationship clearly warrant further investigation.

Interestingly, we detected an interaction between fish size (i.e. weight) and fluoxetine exposure treatment on swimming activity. This interaction appeared to be driven by a positive relationship between fish size and swimming activity in the unexposed and high-fluoxetine treatments, compared to a negative relationship in the low-fluoxetine treatment. On further investigation, the relationship between fish size and swimming activity was not statistically significant within each treatment (i.e. unexposed, low-fluoxetine or high-fluoxetine). Therefore, despite detecting a statistically significant interaction, we suggest that fluoxetine-induced changes in the relationship between fish size and anxiety-like behaviour be verified with additional studies. Still, this is not the first time such an interaction has been shown, with Martin et al. [18] reporting that fluoxetine disrupted the relationship between fish size and anxiety-like behaviour in mosquitofish. We contend that a mass-specific uptake of fluoxetine could be a mechanism driving fluoxetine-induced changes in the relationship between behaviour and body size. Indeed, a negative relationship between the tissue concentration of fluoxetine and fish body size has previously been documented [18]. Although the generality of mass-specific effects of fluoxetine remains to be tested, it is important to highlight this result, as the presence of a mass-dose relationship for fluoxetine exposure may provide insights when comparing reported effects across previous studies.

Conclusions

Here we report that antidepressant exposure at field-detected levels can disrupt the behaviour of non-target species, like fish. Further, these effects appeared to be nonmonotonic, with stronger effects reported at the lower of two fluoxetine dosages. Importantly, contaminant-induced changes in anxiety-related behaviour, as reported here, could alter predator-prey interactions with consequences for population fitness.

Associated content

Data and R code associated with this article is publicly available in an Open Science Framework (OSF) project, supported by the Centre for Open Science (COS). See Martin, J. (2020, September 10). Antidepressant exposure causes a nonmonotonic reduction in anxiety-related behaviour in female mosquitofish. Retrieved from osf.io/8g5tm.

Conflict of 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.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hazl.2020.100004.

References

Küster, A., Adler, N., [1]. Pharmaceuticals in the environment: Scientific evidence of risks and its regulation. Philos. Trans. R. Soc. B Biol. Sci. 369 https://doi.org/10.1098/rstb.2013.0587.

Mezzelani, M., Gorbi, S., Regoli, F., [2]. Pharmaceuticals in the aquatic environments: Evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60. https://doi.org/10.1016/j.marenvres.2018.05.001.

Silva, L.J.G., Lino, C.M., Meisel, L.M., Pena, A., [3]. Selective serotonin re-uptake inhibitors (SSRIs) in the aquatic environment: an ecopharmacovigilance approach. Sci. Total Environ. 437, 185–195. https://doi.org/10.1016/j.scitotenv.2012.08.021.

Hughes, S.R., Kay, P., Brown, L.E., [4]. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47, 661–677. https://doi.org/10.1021/es3030148.

Mole, R.A., Brooks, B.W., [5]. 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.

Sato, T., Qadir, M., Yamamoto, S., Endo, T., Zahoor, A., [6]. Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric. Water Manag. 130, 1–13. https://doi.org/10.1016/j.agwat.2013.08.007.

Bueno, M.J.M., Gomez, M.J., Herrera, S., Hernando, M.D., Agüera, A., Fernández-Alba, A. R., [7]. Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring. Environ. Pollut. 164, 267–273. https://doi.org/10.1016/j.envpol.2012.01.038.

Yuan, S., Jiang, X., Xia, X., Zhang, H., Zheng, S., [8]. Detection, occurrence and fate of 22 psychiatric pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing, China. Chemosphere. 90, 2520–2525. https://doi.org/10.1016/j.chemosphere.2012.10.089.

Brooks, B.W., Kevin Chambliss, C., Stanley, J.K., Ramirez, A., Banks, K.E., Johnson, R.D., Lewis, R.J., [9]. Determination of select antidepressants in fish from an effluent-dominated stream. Environ. Toxicol. Chem. 24, 464. https://doi.org/10.1897/04-081R.1.

Muir, D., Simmons, D., Wang, X., Peart, T., Villella, M., Miller, J., Sherry, J., [10]. Bioaccumulation of pharmaceuticals and personal care product chemicals in fish exposed to wastewater effluent in an urban wetland. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-15462-x.

Grabicová, K., Grabic, R., Fedorova, G., Kolářová, J., Turek, J., Brooks, B.W., Randák, T., [11]. Psychoactive pharmaceuticals in aquatic systems: A comparative assessment of environmental monitoring approaches for water and fish. Environ. Pollut. 261 https://doi.org/10.1016/j.envpol.2020.114150.

Benotti, M.J., Brownawell, B.J., [12]. Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions. Environ. Sci. Technol. 41, 5795–5802. https://doi.org/10.1021/es0629965.

Frazer, A., [13]. Serotonergic and noradrenergic reuptake inhibitors: prediction of clinical effects from in vitro potencies 62.

Mennigen, J.A., Stroud, P., Zamora, J.M., Moon, T.W., Trudeau, V.L., [14]. Pharmaceuticals as neuroendocrine disruptors: lessons learned from fish on prozac. J. Toxicol. Environ. Health. B. Crit. Rev. 14, 387–412. https://doi.org/10.1080/10937404.2011.578559.

McDonald, M.D., [15]. An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 197, 19–31. https://doi.org/10.1016/j.cbpc.2017.03.007.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., [16]. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc. R. Soc. B Biol. Sci. 28520181297 https://doi.org/10.1098/rspb.2018.1297.

Biro, P.A., Abrahams, M.V., Post, J.R., Parkinson, E.A., [17]. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc. R. Soc. B Biol. Sci. 271, 2233–2237. https://doi.org/10.1098/rspb.2004.2861.

Martin, J.M., Bertram, M.G., Saaristo, M., Fursdon, J.B., Hannington, S.L., Brooks, B.W., Burket, S.R.R., Mole, R.A., Deal, N.D.S., Wong, B.B.M., [18]. 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.

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., [19]. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas). Environ. Toxicol. Chem. 28, 2677. https://doi.org/10.1897/08-556.1.

Margiotta-Casaluci, L., Owen, S.F., Cumming, R.I., De Polo, A., Winter, M.J., Panter, G.H., Rand-Weaver, M., Sumpter, J.P., [20]. Quantitative cross-species extrapolation between humans and fish: The case of the anti-depressant fluoxetine. PLoS One. 9 https://doi.org/10.1371/journal.pone.0110467.

Dzieweczynski, T.L., Campbell, B.A., Kane, J.L., [21]. Dose-dependent fluoxetine effects on boldness in male Siamese fighting fish. J. Exp. Biol. 219, 797–804. https://doi.org/10.1242/jeb.132761.

Martin, J.M., Saaristo, M., Bertram, M.G., Lewis, P.J., Coggan, T.L., Clarke, B.O., Wong, B. B.M., [22]. The psychoactive pollutant fluoxetine compromises antipredator behaviour in fish. Environ. Pollut. 222, 592–599. https://doi.org/10.1016/j.envpol.2016.10.010.

Nielsen, S.V., Kellner, M., Henriksen, P.G., Olsén, H., Hansen, S.H., Baatrup, E., [23]. The psychoactive drug Escitalopram affects swimming behaviour and increases boldness in zebrafish (Danio rerio). Ecotoxicology. 27, 485–497. https://doi.org/10.1007/s10646-018-1920-x.

Pyke, G.H., [24]. A review of the biology of Gambusia affinis and G. holbrooki. Rev. Fish Biol. Fish. 15, 339–365. https://doi.org/10.1007/s11160-006-6394-x.

Haynes, J.L., Cashner, R.C., [25]. Life history and population dynamics of the western mosquitofish: a comparison of natural and introduced populations. J. Fish Biol. 46, 1026–1041. https://doi.org/10.1111/j.1095-8649.1995.tb01407.x.

Wiles, S.C., Bertram, M.G., Martin, J.M., Tan, H., Lehtonen, T.K., Wong, B.B.M., [26]. Long-term pharmaceutical contamination and temperature stress disrupt fish behavior. Environ. Sci. Technol. 54, 8072–8082. https://doi.org/10.1021/acs.est.0c01625.

Tan, H., Polverino, G., Martin, J.M., Bertram, M.G., Wiles, S.C., Palacios, M.M.M., Bywater, C.L., White, C.R., Wong, B.B.M., [27]. Chronic exposure to a pervasive pharmaceutical pollutant erodes among-individual phenotypic variation in a fish. Environ. Pollut. 263114450 https://doi.org/10.1016/j.envpol.2020.114450.

Bertram, M.G., Ecker, T.E., Wong, B.B.M., O'Bryan, M.K., Baumgartner, J.B., Martin, J.M., Saaristo, M., [28]. 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.

Martin, J.M., Saaristo, M., Tan, H., Bertram, M.G., Nagarajan-Radha, V., Dowling, D.K., Wong, B.B.M., [29]. Field-realistic antidepressant exposure disrupts group foraging dynamics in mosquitofish. Biol. Lett. 1520190615 https://doi.org/10.1098/rsbl.2019.0615.

Fursdon, J.B., Martin, J.M., Bertram, M.G., Lehtonen, T.K., Wong, B.B.M., [30]. 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.

Chiffre, A., Clérandeau, C., Dwoinikoff, C., Le Bihanic, F., Budzinski, H., Geret, F., Cachot, J., [31]. Psychotropic drugs in mixture alter swimming behaviour of Japanese medaka (Oryzias latipes) larvae above environmental concentrations. Environ. Sci. Pollut. Res. 23, 4964–4977. https://doi.org/10.1007/s11356-014-3477-4.

Bachour, R.-L., Golovko, O., Kellner, M., Pohl, J., [32]. Behavioral effects of citalopram, tramadol, and binary mixture in zebrafish (Danio rerio) larvae. Chemosphere. 238124587 https://doi.org/10.1016/j.chemosphere.2019.124587.

Kristofco, L.A., Cruz, L.C., Haddad, S.P., Behra, M.L., Chambliss, C.K., Brooks, B.W., [33]. Age matters: developmental stage of Danio rerio larvae influences photomotor response thresholds to diazinion or diphenhydramine. Aquat. Toxicol. 170, 344–354. https://doi.org/10.1016/j.aquatox.2015.09.011.

Peng, X., Lin, J., Zhu, Y., Liu, X., Zhang, Y., Ji, Y., Yang, X., Zhang, Y., Guo, N., Li, Q., [34]. Anxiety-related behavioral responses of pentylenetetrazole-treated zebrafish larvae to light-dark transitions. Pharmacol. Biochem. Behav. 145, 55–65. https://doi.org/10.1016/j.pbb.2016.03.010.

Lagesson, A., Saaristo, M., Brodin, T., Fick, J., Klaminder, J., Martin, J.M., Wong, B.B.M., [35]. Fish on steroids: temperature-dependent effects of 17β-trenbolone on predator escape, boldness, and exploratory behaviors. Environ. Pollut. 245, 243–252. https://doi.org/10.1016/J.ENVPOL.2018.10.116.

Bertram, M.G., Martin, J.M., Saaristo, M., Ecker, T.E., Michelangeli, M., Deal, N.D.S., Lim, S.L., O'Bryan, M.K., Wong, B.B.M., [36]. Context-specific behavioural changes induced by exposure to an androgenic endocrine disruptor. Sci. Total Environ. 664, 177–187. https://doi.org/10.1016/j.scitotenv.2019.01.382.

R Core Team, [37]. R: A Language and Environment for Statistical Computing. https://www.r-project.org/.

Maximino, C., de Brito, T.M., Colmanetti, R., Pontes, A.A.A., de Castro, H.M., de Lacerda, R.I.T., Morato, S., Gouveia, A., [38]. Parametric analyses of anxiety in zebrafish scototaxis. Behav. Brain Res. 210, 1–7. https://doi.org/10.1016/j.bbr.2010.01.031.

Serra, E.L., Medalha, C.C., Mattioli, R., [39]. Natural preference of zebrafish (Danio rerio) for a dark environment. Brazilian J. Med. Biol. Res. 32, 1551–1553. https://doi.org/10.1590/S0100-879X1999001200016.

Maximino, C., Marques, T., Dias, F., Cortes, F., Taccolini, I., Pereira, P., Colmanetti, R., Gazolla, R., Tavares, R., Rodrigues, S., Valéria, S., Pontes, A., Romão, C., Prado, V., [40]. A. Jr., A comparative analysis of the preference for dark environments in five teleost. Int. J. Comp. Psychol. 20.

Maximino, C., Marques de Brito, T., Dias, C.A.G.D.M., Gouveia, A., Morato, S., [41]. Scototaxis as anxiety-like behavior in fish. Nat. Protoc. 5, 209–216. https://doi.org/10.1038/nprot.2009.225.

Meijide, F.J., Da Cuña, R.H., Prieto, J.P., Dorelle, L.S., Babay, P.A., Lo Nostro, F.L., [42]. Effects of waterborne exposure to the antidepressant fluoxetine on swimming, shoaling and anxiety behaviours of the mosquitofish Gambusia holbrooki. Ecotoxicol. Environ. Saf. 163, 646–655. https://doi.org/10.1016/j.ecoenv.2018.07.085.

Pompermaier, A., Kirsten, K., Soares, S.M., Fortuna, M., Kalichak, F., Idalencio, R., Koakoski, G., Barreto, R.E., Barcellos, L.J.G., [43]. Waterborne agrichemicals compromise the anti-predatory behavior of zebrafish. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-09862-2.

Olsén, K.H., Ask, K., Olsén, H., Porsch-Hällström, I., Hallgren, S., [44]. Effects of the SSRI citalopram on behaviours connected to stress and reproduction in Endler guppy, Poecilia wingei. Aquat. Toxicol. 148, 113–121. https://doi.org/10.1016/j.aquatox.2013.12.032.

Godin, J.-G.J., [45]. Behavioural Ecology of Teleost Fishes. Oxford University Press.

Fraher, D., Hodge, J.M., Collier, F.M., McMillan, J.S., Kennedy, R.L., Ellis, M., Nicholson, G.C., Walder, K., Dodd, S., Berk, M., Pasco, J.A., Williams, L.J., Gibert, Y., [46]. Citalopram and sertraline exposure compromises embryonic bone development. Mol. Psychiatry. 21, 656–664. https://doi.org/10.1038/mp.2015.135.

Smith, B.R., Blumstein, D.T., [47]. Fitness consequences of personality: a meta-analysis. Behav. Ecol. 19, 448–455. https://doi.org/10.1093/beheco/arm144.

Wolf, M., Weissing, F.J., [48]. Animal personalities: consequences for ecology and evolution. Trends Ecol. Evol. 27, 452–461. https://doi.org/10.1016/j.tree.2012.05.001.

Smith, B.R., Blumstein, D.T., [49]. Behavioral types as predictors of survival in Trinidadian guppies (Poecilia reticulata). Behav. Ecol. 21, 919–926. https://doi.org/10.1093/beheco/arq084.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., Sih, A., [50]. Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). Proc. R. Soc. B Biol. Sci. 277, 1571–1579. https://doi.org/10.1098/rspb.2009.2128.

Mittelbach, G.G., Ballew, N.G., Kjelvik, M.K., [51]. Fish behavioral types and their ecological consequences. Can. J. Fish. Aquat. Sci. 71, 927–944. https://doi.org/10.1139/cjfas-2013-0558.

Weinberger, J., Klaper, R., [52]. 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. 151, 77–83. https://doi.org/10.1016/j.aquatox.2013.10.012.

Pelli, M., Connaughton, V.P., [53]. 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.

Bláha, M., Grabicova, K., Shaliutina, O., Kubec, J., Randák, T., Zlabek, V., Buřič, M., Veselý, L., [54]. Foraging behaviour of top predators mediated by pollution of psychoactive pharmaceuticals and effects on ecosystem stability. Sci. Total Environ. 662, 655–661. https://doi.org/10.1016/j.scitotenv.2019.01.295.

Saaristo, M., McLennan, A., Johnstone, C.P., Clarke, B.O., Wong, B.B.M., [55]. 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.

Dzieweczynski, T.L., Kane, J.L., Campbell, B.A., Lavin, L.E., [56]. Fluoxetine exposure impacts boldness in female Siamese fighting fish, Betta splendens. Ecotoxicology. 25, 69–79. https://doi.org/10.1007/s10646-015-1568-8.

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., [57]. 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.

Herculano, A.M., Maximino, C., [58]. Serotonergic modulation of zebrafish behavior: Towards a paradox. Prog. Neuro-Psychopharmacology Biol. Psychiatry. 55, 50–66. https://doi.org/10.1016/j.pnpbp.2014.03.008.

Sumpter, J.P., Donnachie, R.L., Johnson, A.C., [59]. The apparently very variable potency of the anti-depressant fluoxetine. Aquat. Toxicol. 151, 57–60. https://doi.org/10.1016/j.aquatox.2013.12.010.

Kreke, N., Dietrich, D.R., [60]. Physiological endpoints for potential SSRI interactions in fish. Crit. Rev. Toxicol. 38, 215–247. https://doi.org/10.1080/10408440801891057.

De Lange, H.J., Noordoven, W., Murk, A.J., Lürling, M., Peeters, E.T.H.M., [61]. Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat. Toxicol. 78, 209–216. https://doi.org/10.1016/j.aquatox.2006.03.002.

Guler, Y., Ford, A.T., [62]. Anti-depressants make amphipods see the light. Aquat. Toxicol. 99, 397–404. https://doi.org/10.1016/j.aquatox.2010.05.019.

Barry, M.J., [63]. Effects of fluoxetine on the swimming and behavioural responses of the Arabian killifish. Ecotoxicology. 22, 425–432. https://doi.org/10.1007/s10646-012-1036-7.

Bossus, M.C., Guler, Y.Z., Short, S.J., Morrison, E.R., Ford, A.T., [64]. Behavioural and transcriptional changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and sertraline. Aquat. Toxicol. 151, 46–56. https://doi.org/10.1016/j.aquatox.2013.11.025.

de Farias, N.O., Oliveira, R., Sousa-Moura, D., de Oliveira, R.C.S., Rodrigues, M.A.C., Andrade, T.S., Domingues, I., Camargo, N.S., Muehlmann, L.A., Grisolia, C.K., [65]. Exposure to low concentration of fluoxetine affects development, behaviour and acetylcholinesterase activity of zebrafish embryos. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 215, 1–8. https://doi.org/10.1016/j.cbpc.2018.08.009.

Nakamura, Y., Yamamoto, H., Sekizawa, J., Kondo, T., Hirai, N., Tatarazako, N., [66]. The effects of pH on fluoxetine in Japanese medaka (Oryzias latipes): acute toxicity in fish larvae and bioaccumulation in juvenile fish. Chemosphere. 70, 865–873. https://doi.org/10.1016/j.chemosphere.2007.06.089.

Nielsen, S.V., Frausing, M., Henriksen, P.G., Beedholm, K., Baatrup, E., Baatrup, E., [67]. The psychoactive drug escitalopram affects foraging behavior in zebrafish (Danio rerio). Environ. Toxicol. Chem. 38, 1902–1910. https://doi.org/10.1002/etc.4474.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., [68]. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.