

Influence of shy and bold zebrafish personalities on colonization and olfactory responses in sertraline-contaminated environments

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) such as sertraline are used as antidepressants to treat mental disorders such as depression by blocking serotonin reuptake and thus maintaining high serotonin levels. Currently, increased consumption has led to detection of SSRIs in aquatic systems; however, information about their effects on exposed organisms is limited. This study investigated differences in the colonization response by zebrafish (*Danio rerio*) to sertraline-contaminated environments considering personality traits (shy and bold). Colonization was assessed in terms of success, efficiency, and inhabited time for each sertraline concentration. Fish were tested individually and in groups. In addition, whether these personality traits differed from each other in their olfactory sensitivity to different olfactory stimuli was studied, including sertraline, using the electro-olfactogram (EOG). Bold fish exposed in groups tended to colonize higher concentrations of sertraline than shy fish. These differences were not as significant when fish were exposed individually. Furthermore, analyses in the EOG detected that bold fish reacted between 1.3 and 2.8 times more to all stimuli studied. In contrast, sertraline was not detected by the olfactory system. Varied *D. rerio* colonization responses to sertraline-contaminated environments reveal specific personality-based vulnerabilities. These findings underscore the importance of integrating behavioral phenotypes into environmental risk assessments for pharmaceuticals.

Keywords coping style, colonization, fish behavior, olfaction, personality

Introduction

Animal behavior varies widely among individuals, and identifying differences allows us to understand how individuals interact with their environment, as well as the coping strategies they use to face changes in their habitat (Ibarra-Zatarain et al., 2020; Koolhaas et al., 2010). Individual differences in the behavioral traits of animals include a shyness–boldness continuum, defined by the individual's tendency to take risks (Wilson et al., 1994). This approach has been extensively studied in fish, allowing the identification of behavioral patterns and the establishment of personality traits. Typically, bold individuals tend to be more explorative, take more risks, be more active, and habituate themselves quickly (Budaev & Brown, 2011; Daniel & Bhat, 2020; Dos Santos et al., 2023; Rajput et al., 2022). Furthermore, these fish are usually more aggressive (Yuan et al., 2018), actively cope with stressors, and have lower behavioral flexibility (Dahlbom et al., 2011). Their risk-taking disposition may improve their performance in tasks such as foraging (Ioannou et al., 2008; Sneddon, 2003), as they are more likely to emerge quickly from

shelters, inspect novel objects, and detect predators (Daniel & Bhat, 2020; Dugatkin & Alfieri, 2003). However, shy individuals are risk-averse and present less exploratory behavior in environments (Budaev & Brown, 2011). This personality trait is associated with behaviors of preference regarding refuge zones, areas close to the walls of the fish tank (thigmotaxis), and fast movements with steep turning angles (Dos Santos et al., 2023). In the presence of acute stress, these individuals adopt passive strategies such as freezing and hiding (Rupia et al., 2016). Because of this, individual variation in shy–bold behavior has an impact on biological fitness, as it influences social rank, reproductive success, and survival (Ariyomo & Watt, 2012; Dahlbom et al., 2011; Øverli et al., 2004; Smith & Blumstein, 2008). The exploratory behaviors and activity patterns described above are highly consistent in adult zebrafish (*Danio rerio*; Baker et al., 2018; Thomson et al., 2020). However, some studies have found a lack of consistency in the organism's behavioral traits over time, so context and life stage are important (Alfonso et al., 2020; Réale et al., 2010). The shy and bold traits respond to natural selection (Ariyomo et al., 2013), so the environment also plays an

Received: October 20, 2025. Revised: April 8, 2026. Accepted: April 16, 2026

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important role in the expression of personality. For example, fish tend to show greater boldness in areas of high predation pressure, suggesting that this trait may be an adaptive response to environmental changes (Brown et al., 2005).

Exposure to pharmaceuticals such as antidepressants in the aquatic environment poses a threat, as these compounds can alter the behavior and personality traits of organisms (see review by Ferreira et al., 2023). Selective serotonin reuptake inhibitors (SSRIs), including sertraline, belong to a class of antidepressants that modulate serotonergic neurotransmission by binding to the serotonin transporter and blocking the reuptake of serotonin from the extracellular space, which generates elevated extracellular serotonin levels (James et al., 2017). Serotonin is a neurotransmitter that has great influence on behavioral parameters such as locomotion, aggressiveness, reproduction, appetite, and social interactions (Saldanha et al., 2023; Winberg & Thörnqvist, 2016). Many antidepressants have been found in the aquatic environment (see review by Corcoran et al., 2010; Ebele et al., 2017). In particular, sertraline has been detected at ng/L concentrations in various environmental compartments, including water, sediment, and biota (Arnnok et al., 2017; Mole & Brooks, 2019; Schultz et al., 2010), but concentrations around $\mu\text{g/L}$ have been also reported in the wastewater from hospitals (Styrishave et al., 2011). Even at microgram levels, numerous effects have been reported in aquatic organisms exposed to this pharmaceutical (see review by Silva et al., 2015). Sertraline produces behavioral alterations in zebrafish, causing a reduction in locomotor activity (Yang et al., 2021) and reductions in sociability and increased anxiety (Al Shuraiqi et al., 2024). In addition, Faria et al. (2022) observed that exposure to this drug during development can cause hypolocomotion and delayed learning. The decrease in swimming distance is associated with a reduction in swimming speed and longer resting times (Richendrer & Creton, 2018; Sehonova et al., 2019). The exposure of zebrafish to sertraline has also produced biochemical and molecular alterations, such as modifications in serotonin and dopamine levels, as well as changes in the expression of key genes associated with their metabolism (serotonergic and dopaminergic systems; Faria et al., 2022; Yang et al., 2021). All these alterations are observed after exposure to this antidepressant in a range between 0.1–100 $\mu\text{g/L}$. Although previous studies have described the behavioral effects of sertraline in zebrafish (examples cited above), the potential role of personality (shy vs. bold) in modulating the colonization of contaminated environments, and how this exposure might modify these behavioral responses according to personality trait, remains understudied.

Besides their personality traits, the way fish behave is also dependent on how they perceive environmental stimuli. Fish use their olfactory system to detect chemicals in the environment that provide crucial information for essential behaviors such as foraging, reproduction, predator detection, migration, and social interaction (Ferrari et al., 2010; Frade et al., 2002; Hubbard, 2024a; Kleerekoper, 1967; Li et al., 2018; Sorensen & Sato, 2005). Fish are able to detect a wide variety of water-soluble olfactory stimuli (odorants), including amino acids, nucleotides, bile acids, steroids, and prostaglandins, even at low concentrations (Belanger et al., 2010; Li et al., 2018; Michel & Lubomudrov, 1995; Valentincic et al., 2005). Contaminants may function as chemical signals but with the potential to affect the

chemosensory systems of fish and may alter the perception of the olfactory stimuli (Tierney et al., 2010). The electro-olfactogram (EOG) is a direct current field potential measured just above the olfactory epithelium and is thought to be a summation of the generator potentials of the olfactory receptor neurons responding to a given odorant (Scott & Scott-Johnson, 2002). Some studies using an electro-olfactogram system have shown that contaminants such as copper, silver nanoparticles, and agricultural pesticides alter the olfactory system of fish at concentrations below the lethal levels (Bilberg et al., 2011; Jarrard et al., 2004; Tierney et al., 2010). These effects can compromise behaviors essential to population survival, such as foraging, reproduction, and predator avoidance (Hubbard, 2024a; Jarrard et al., 2004; Tierney et al., 2010). If behavior is greatly affected by the personality trait of fish, we hypothesized that the EOG signals from shy and bold fish populations under a similar environmental stimulus are different. Furthermore, we also hypothesized that, through its action on the serotonergic system, sertraline could also alter these personality traits and could increase exploratory behavior and colonization of sertraline-contaminated environments in both shy and bold fish. More pronounced effects were expected in shy fish due to their natural predisposition for behavioral inhibition and increased risk aversion.

The aims of the study were, first, to analyze how the personality traits (shy and bold) modulate the impulse of zebrafish to colonize environments with different concentrations of sertraline and to assess whether sertraline exposure alters their exploratory behavior either in groups or individually. Second, we aimed to examine differences in the olfactory sensitivity of shy and bold fish that could explain behavioral responses by using the EOG and common fish odorants (amino acids and bile acids).

Materials and methods

Organisms and water parameters

Adult wildtype zebrafish (*D. rerio*) came from a culture at the Center of Marine Sciences (Universidade do Algarve, Faro, Portugal). A total of 92 fish were used, measuring 3.7 ± 0.3 cm in length, weighing 0.57 ± 0.23 g, and aged 1 year. They were kept in a zebrafish rack system with continuous dechlorinated tap water in 2 and 4.5 L tanks (10–15 fish/tank). Fish were fed twice daily (at 9:00 a.m. and 3:00 p.m.) with processed feed (Zebrafeed, SPAROS Lda.) and were maintained in a humid laboratory at 30 ± 1 °C and a 12:12-hr light: dark photoperiod. The water parameters were pH = 7.5; conductivity = 711 μS ; temperature = 28 °C; nitrate (NO_3) = 5 mg/L. All parameters were within the optimal values established by Ribas and Piferrer (2014). Fish care and experimentation were in accordance with the guidelines of the Portuguese legislation for the use of laboratory animals under a “Group-1” license issued by the Veterinary General Directorate of the Ministry of Agriculture, Rural Development and Fisheries of Portugal. The control water was obtained from the recirculating system of the zebrafish racks (Tecniplast, UK).

Chemicals

Sertraline hydrochloride (Chemical Abstracts Service no. 79559-97-0) was provided by Sigma-Aldrich (Steinheim, Germany). A stock solution at 20 mg/L (nominal concentration) of sertraline was prepared with Milli-Q water. Nominal test concentrations (0.01, 0.1, 1, and 10 $\mu\text{g/L}$) were prepared from this stock kept in the laboratory in an opaque glass bottle at 4 °C. These concentrations were selected to cover both environmentally relevant and high-exposure scenarios. Concentrations of 0.01 and 0.1 $\mu\text{g/L}$ are within the range recorded for surface water, treated effluents, and predicted environmental concentrations (Mole & Brooks, 2019), whereas 1 and 10 $\mu\text{g/L}$ represent the exposure scenarios recorded for wastewater and hospital effluents (Styrishave et al., 2011). Samples for all treatments with the chemical were collected to analyze sertraline concentrations during the behavioral experiments and were kept in a freezer at -220 °C until analysis. Chemical analyses were performed at the University of Cadiz by liquid chromatography-mass spectrometry. The limit of detection and limit of quantification were 0.001 $\mu\text{g/L}$ and 0.004 $\mu\text{g/L}$, respectively (see online supplementary material Table S1). The sertraline concentrations of the experimental treatments measured can be seen in online supplementary material Tables S2 and S3.

Personality test

The personality test consisted of exposing individual zebrafish to control water in a nonforced system with five compartments connected to form an “L” shape (Figure 1), in which behavior and ability to explore the system were observed. For this test, version #2 of the heterogeneous multi-habitat assay system (HeMHAS; Salvatierra et al., 2025a) was used. Because colonization behavior was assessed using the HeMHAS, this system was used to classify fish personality; specifically, bold and shy profiles were defined by higher or lower rates of exploratory

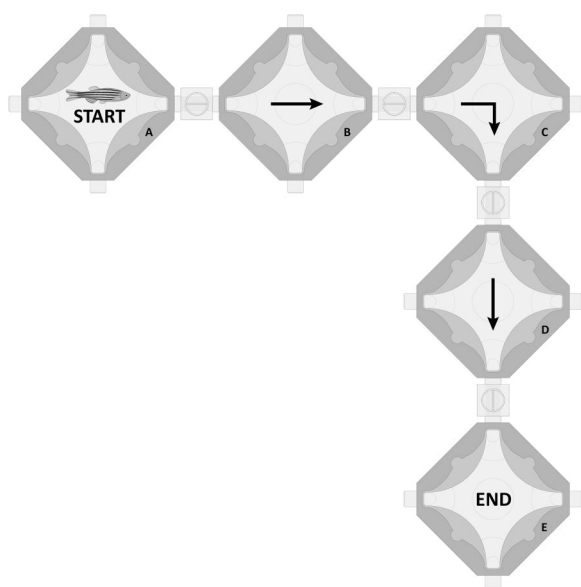


Figure 1 Schematic setup of the five-compartment (A–E) heterogeneous multi-habitat assay system (HeMHAS) used in the personality test. The image includes resources from biorender.com.

behavior, respectively. The system was filled with a control water (see the *Organisms and water parameters* section) volume of 3.5 L, which was completely renewed for every seven fish to minimize possible interferences due to fish-released compounds during the experimentation; 92 fish were used. The fish were not sexed prior to the experiment, and adult zebrafish from the main culture were haphazardly put into the first closed compartment using a net. The last compartment was enriched with small stones due to this species' preference for enriched areas, which promote territorial behavior (Schroeder et al., 2014; Woodward et al., 2019). Once the fish was placed in the first compartment, we waited 3 min for the fish to rest and de-stress. Then the rotatory door was opened carefully, and the fish was allowed to explore the system for 5 min. This time was established based on preliminary tests performed with 10 fish that proved that it was enough time for this species to move through the total system (five compartments), as well as previous studies using *D. rerio* in this system (Salvatierra et al., 2025b). The criteria used to separate the two personalities depended on the total number of compartments explored during those 5 min: (1) shy fish were those that only moved through the first two compartments and (2) bold fish were those who reached the last two compartments. Fish that only reached the third compartment (corner of the L-shaped experimental setup; Figure 1) were classified as having an intermediate (undefined) personality and therefore were not used in experiments. During the experiment, the behavior was recorded by a camera (Tapo C225 V2, TP-Link, Shenzhen, China) and analyzed in real time using the Tapo App (version 3.5.103, TP-Link Global Inc.). All personality tests were performed on one day between ~ 9:00 a.m. and 7:00 p.m. The fish were then separated into different aquaria by personality (shy and bold) for further analysis. Finally, the video recordings were analyzed for the following parameters: residence time (time each fish spent in each of the compartments) and the total number of compartments visited per fish during the 5 min.

Colonization test

The ability to colonize environments contaminated with sertraline was analyzed for shy ($n = 32$) and bold ($n = 32$) fish, previously separated according to the preceding test. For colonization, the behavior was studied in (a) groups and (b) as individuals (Figure 2). These experiments involved exposing *D. rerio* to a linear gradient of sertraline using version #2 of the HeMHAS. This gradient had an uncontaminated compartment (0 $\mu\text{g/L}$), one low concentration of sertraline (0.01 $\mu\text{g/L}$), two intermediate concentrations (0.1 and 1 $\mu\text{g/L}$), and one high concentration (10 $\mu\text{g/L}$). All these concentrations were carefully introduced into the system with rotatory doors closed to avoid mixing. The experiments were carried out under continuous illumination at 30 ± 1 °C. Additionally, the fish were subjected to a 24-hr fasting period prior to the study. These colonization tests were spaced 2 to 3 days apart from the personality tests to minimize any possible short-term habituation effects.

The group colonization study consisted of introducing four individuals from the same personality trait (shy or bold) into the first noncontaminated compartment. After allowing the fish to de-stress for 3 min, the rotatory door was opened and their movement throughout the compartments was recorded for

5 min. A total of 16 replicates were performed, eight replicates for each personality trait. To maintain the concentrations in each compartment, the uncontaminated water of the first compartment and sertraline solution of the remaining compartments was renewed every two replicates.

Individual colonization trials comprised introducing a single fish into the noncontaminated compartment. The fish were acclimated for 3 min to reduce handling stress; the colonization assay then lasted 5 min. A total of 64 replicates were performed, 32 for each personality trait (shy and bold), and the medium was changed every four replicates. All the replicates were recorded for subsequent video analysis. The video analysis evaluated colonization success, colonization efficiency, and the inhabited time of each concentration.

Colonization success was defined as the number of fish that reached each concentration during each 1-min interval of the 5-min trial. Colonization efficiency was defined as the time it took for the fish to colonize each compartment normalized to the residence time observed for that personality type under noncontaminated conditions. In this case, colonization was either positive (they took less time to move among the compartments) or negative (they took longer to reach each compartment) depending on the residence time, which was 5 min for shy fish and 0.2 min for bold fish. This response was calculated for all sertraline concentrations. For group colonization trials, the latency for the first individual to enter each compartment and the time required for the four individuals to arrive were recorded. Colonization efficiency was then calculated for each concentration using the following formula:

$$\text{Colonization efficiency (\%)} = 100 - \left(\frac{\text{Observed time}}{\text{Expected time}} \times 100 \right)$$

where the observed time was the time taken to colonize each compartment and the expected time was the median residence time obtained for each population (shy and bold personalities). In the case of shy fish that did not colonize the subsequent compartments, it was decided to set the maximum expected time to

reach each compartment as the observed time. For instance, if a fish failed to move from the first to the second compartment, it was assigned the maximum residence time (5 min) as the observed time. Consequently, the observed times for the subsequent compartments (3–5) were recorded as 10, 15, and 20 min, respectively. Because the majority of bold fish colonized the final compartment, the maximum experimental duration (5 min) was assigned as the observed time for individuals that failed to colonize. Thus, for bold fish that did not colonize a given compartment, regardless of the compartment, the observed time was 5 min. These differences in the calculations used to define the observed time for shy and bold fish that did not colonize all the compartments occur because the shy fish took longer (all the experimental time, 5 min) to colonize only one compartment whereas the bold fish were potentially able to colonize all the compartments in that time.

Finally, inhabited time was defined as the time during which at least half of the group (two or more fish) occupied a specific compartment. For individual trials, it was calculated as the total time spent by each fish in each concentration.

EOG recording

After the behavioral tests, the fish were left to rest in their culture tanks for at least 2 days. During this period, the fish were not handled and were kept in culture conditions (see the *Organisms and water parameters* section). The EOG recordings of these adult zebrafish followed the methodology described by Li et al. (2018). Before analysis, the fish were anesthetized by immersion in a solution containing 250 mg/L of MS-222 (3-amino-benzoic acid ethyl ester, Sigma-Aldrich) buffered with sodium bicarbonate at a concentration of 500 mg/L. In addition, during the examination, the fish were given this anesthetic through the mouth with a continuous flow of ~2–3 m/min (by gravity), as well as dechlorinated water over the olfactory epithelium to keep it wet (Li et al., 2018). Each fish was placed in the system and secured with wet cloths, leaving only the head uncovered. The EOG responses were recorded using borosilicate

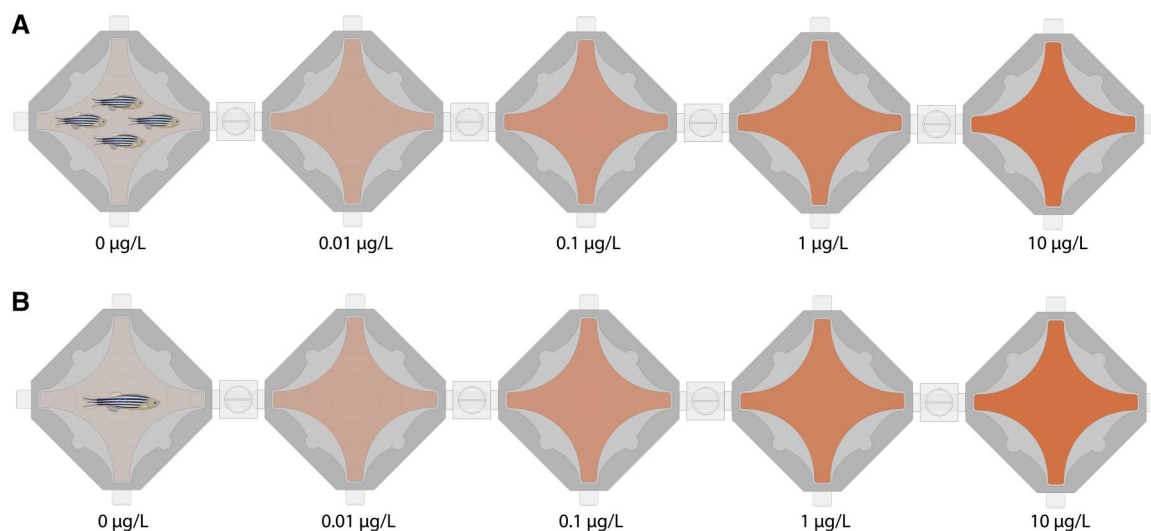


Figure 2 Schematic setup of the heterogeneous multi-habitat assay system (HeMHAS) used in (A) group (B) individual colonization tests with a sertraline concentrations gradient (from 0–10 µg/L).

micropipettes (outer diameter = 1.50 mm, inner diameter = 1.17 mm) filled with 3M sodium chloride in 4% agar. These were coupled to solid-state electronics via Ag/AgCl electrodes and a 3 M potassium chloride solution as in previous studies (Belanger et al., 2010; Hubbard et al., 2002; Michel & Lubomudrov, 1995). The recording electrode was placed (usually) between the two largest olfactory lamellae and the reference electrode was placed on the head. The signal was digitized using DigiData 1322A (Axon Instruments, Inc.), amplified 1,000 times with a D.C. amplifier and recorded in AxoScope software (Axon Instruments, Inc.) as in Fatsini et al. (2017) and Li et al. (2018). Responses were measured in millivolts.

Dechlorinated water filtered with activated carbon (blank) was used as a negative control. As a positive control, a 10^{-5} M solution of L-serine was used, with which the optimal position of the electrode was adjusted, considering a response between -1 and -2 mV as optimal (Li et al., 2018). Responses to the positive and negative controls were recorded regularly during the experiment (after every six stimuli). The olfactory stimuli studied were, first, the amino acids L-alanine, L-arginine, L-histidine, and L-methionine at concentrations of 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, and 10^{-7} M; and second, the bile acids taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDC) at concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M. Finally, the highest sertraline concentration used in the behavioral tests (10 µg/L) served as a stimulus in addition to two higher sertraline concentrations, 100 µg/L and 1 mg/L. Once the analyses were completed, the fish were overdosed with anesthetic, decapitated, and sexed according to the presence or absence of eggs. A total of 15 fish were used (eight shy and seven bold fish).

Data analysis

Statistical analyses were performed using SigmaPlot software (v.14.0). The normal distribution of the data was assessed by the Shapiro–Wilk test and the homogeneity of variances was checked by the Brown–Forsythe test. Differences between the residence time spent in each compartment by individual fish in the personality test were assessed using one-way ANOVA for normally distributed data ($p > 0.05$) or the Kruskal–Wallis test for nonnormal data ($p < 0.05$). Additionally, differences between the number of compartments visited during the time of the experiment by shy and bold populations were analyzed using the Mann–Whitney U test, as the data followed a nonnormal distribution ($p < 0.05$).

Regarding the colonization parameters, success was compared between shy and bold fish at each concentration and time point. For normally distributed data, Student's t -test (when variances were homogeneous) and Welch's t -test (when variances were nonhomogeneous) were used. For nonnormally distributed data, a Mann–Whitney U test was performed. For colonization efficiency, the observed time in each sertraline concentration was compared with the expected time by using a one-sample Student's t -test (parametric test) or a Wilcoxon signed-rank test (nonparametric test). Finally, a Student's t -test for homogeneous variances or a Welch's t -test for nonhomogeneous variances (parametric tests), or a Mann–Whitney U test (nonparametric test) was performed to test for differences between the time each concentration of sertraline (including the

uncontaminated compartment) was inhabited by shy and bold fish. The differences in the use of each concentration by each personality type were analyzed using a one-way ANOVA (for normal data) or a Kruskal–Wallis test (for nonnormal data), followed by Tukey's post hoc test.

For the EOG measured, all the amplitude data for each of the concentrations of the stimuli studied were blank-subtracted (negative control). Subsequently, a two-way ANOVA was performed using personality (shy/bold) and the different concentrations of each of the stimuli as factors. To observe any differences among the different treatments of each stimulus, the Holm–Sidak post hoc analysis (pairwise multiple comparison procedures) was performed. In addition, a linear regression of the log-transformed data was performed to calculate odorant detection thresholds followed by a two-way ANOVA using the different odorants and personalities as factors.

Online [supplementary material Table S4](#) provides a table summarizing the statistical tests performed for each parameter and the corresponding assumptions as well as the results of all tests (see online [supplementary material Tables S5–21](#)).

Results

Personality test

A total of 34 shy fish, 34 bold fish, and 23 fish of intermediate personality were identified. The median (interquartile range) time a shy fish spent in each compartment before moving to another one was 5 (2.5–5) min. In contrast, bold fish moved faster among compartments, with a residence time of 0.21 (0.15–0.34) min. In terms of the time spent in each specific compartment (from A–E; [Figure 3](#)), shy fish showed significant differences ($p < 0.001$); in particular, they spent more time in the first compartment (A) than in the rest of the compartments ($p < 0.001$). Bold fish moved more throughout the system and similarly showed significant differences in residence time among compartments ($p = 0.007$), where differences ($p = 0.021$) were observed between compartments A and E ([Figure 3](#)).

Regarding the total number of compartments visited during 5 min of the experiment, bold fish tended to visit more compartments than shy fish ($p < 0.001$). Whereas bold fish had a median of 12 (7–17) compartments visited, shy fish generally stayed in the initial compartment ([Figure 4](#)).

Group colonization test

The colonization success of different concentrations of sertraline was statistically different between shy and bold fish over time (see online [supplementary material Figure S1](#)). Bold fish colonized the highest sertraline concentration (10 µg/L) before the first minute, whereas shy fish took between 2–3 min (see online [supplementary material Figure S1](#)) to colonize the two highest concentrations (1 and 10 µg/L). In this test, most of the bold fish (a median of 3.5 individuals) succeeded in colonizing the highest concentration of sertraline. In contrast, only half (a median of 1.5 individual) from the shy fish population were able to colonize the last compartment (10 µg/L sertraline).

Regarding colonization efficiency, considering the fastest individual of the group of shy fish in each replicate, there was a greater capacity to explore the system in the presence of a sertraline gradient than in its absence (Table 1; Figure 5), particularly for concentrations of 0.01, 0.1 and 1 $\mu\text{g/L}$ ($p < 0.05$). For shy fish colonizing as a whole group, they showed a colonization efficiency of 0 for all treatments, because the four fish were not able to colonize all the different sertraline treatments in most replicates. On the contrary, colonization in group by bold fish increased in efficiency except in the last concentration, although these differences were not statistically significant.

With respect to the inhabited time of each sertraline concentration, statistically significant differences were found between shy and bold fish for all concentrations except 0.01 g/L (Figure 6). Within the inhabited time for shy fish, there were significant differences ($p = 0.001$) among the 0 $\mu\text{g/L}$ compartment (noncontaminated), where the fish stayed longer compared with compartments with 0.1, 1, and 10 $\mu\text{g/L}$ of sertraline (Figure 6). Bold fish tended to spend more time in the highest concentrations.

Individual colonization test

Colonization success was similar between personalities during the first 2 min of experimentation, when fish were individually exposed to a sertraline gradient. Differences between shy and bold fish were only observed at certain concentrations and times (see online supplementary material Figure S2). Shy fish did not increase colonization efficiency (Table 2; Figure 7) when tested individually. In contrast, bold fish decreased colonization

efficiency when exposed individually for all sertraline concentrations ($p < 0.001$), as the presence of sertraline increased the time taken to colonize each of the compartments with respect to the absence of the contaminant.

Finally, in terms of the time spent in each compartment, both shy and bold fish showed a tendency to spend more time in the uncontaminated compartment ($p < 0.001$; Figure 8).

EOG

Statistically significant differences were found in the olfactory sensitivity between shy and bold zebrafish populations for all the olfactory stimuli analyzed (two-way ANOVA, $p < 0.05$). Amino acids and acids tested in bold fish tended to evoke EOG of larger amplitude than those of shy fish (Figure 9). This difference was between 1.27 and 2.77 higher in bold fish, depending on the concentration and stimulus considered. Furthermore, bold fish tended to have lower detection thresholds to olfactory stimuli than shy fish (two-way ANOVA, $p = 0.005$), suggesting higher olfactory sensitivity (Table 3).

Significant differences were also observed between the different concentrations of each stimulus (two-way ANOVA, $p < 0.05$); in general, larger amplitude responses were observed at higher concentrations. The highest amplitude was observed for the most concentrated solution (10^{-3} M) L-methionine with a mean amplitude of 6.10 mV and 4.67 mV for bold and shy fish, respectively. The lowest olfactory amplitudes were observed for the most diluted solution (10^{-9} M) of TCA, obtaining a value of 0.35 mV for bold fish and 0.19 mV for shy fish. In addition, the detection thresholds for zebrafish were

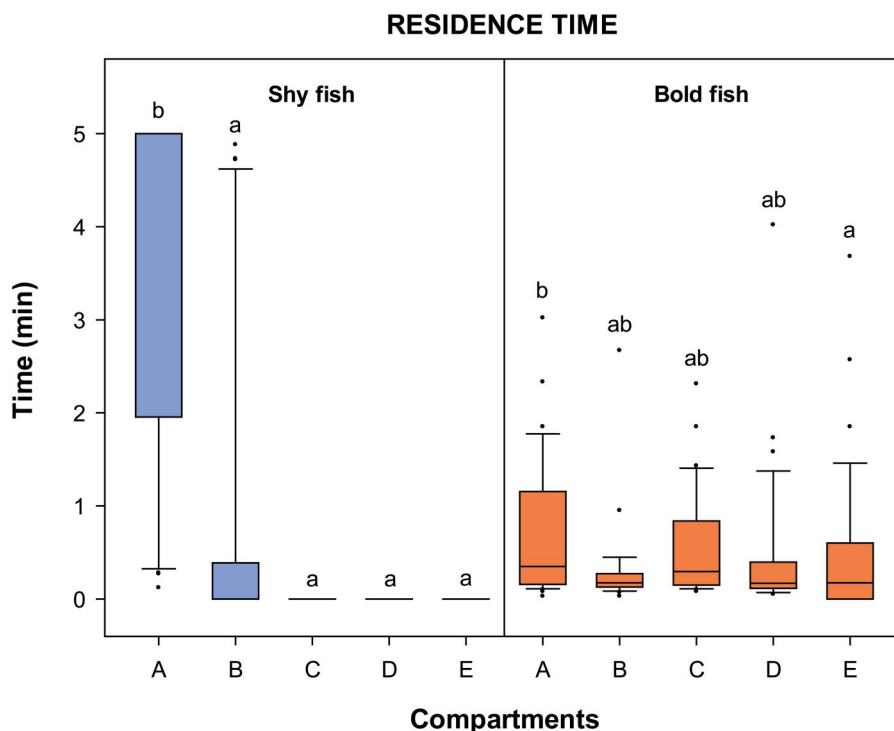


Figure 3 Residence time in each compartment (A–E) in min for both fish populations (blue box = shy fish population, orange box = bold fish population) after 5 min of the experiment. Data are presented as median \pm interquartile range. Different lowercase letters indicate significant differences among compartments within each personality group.

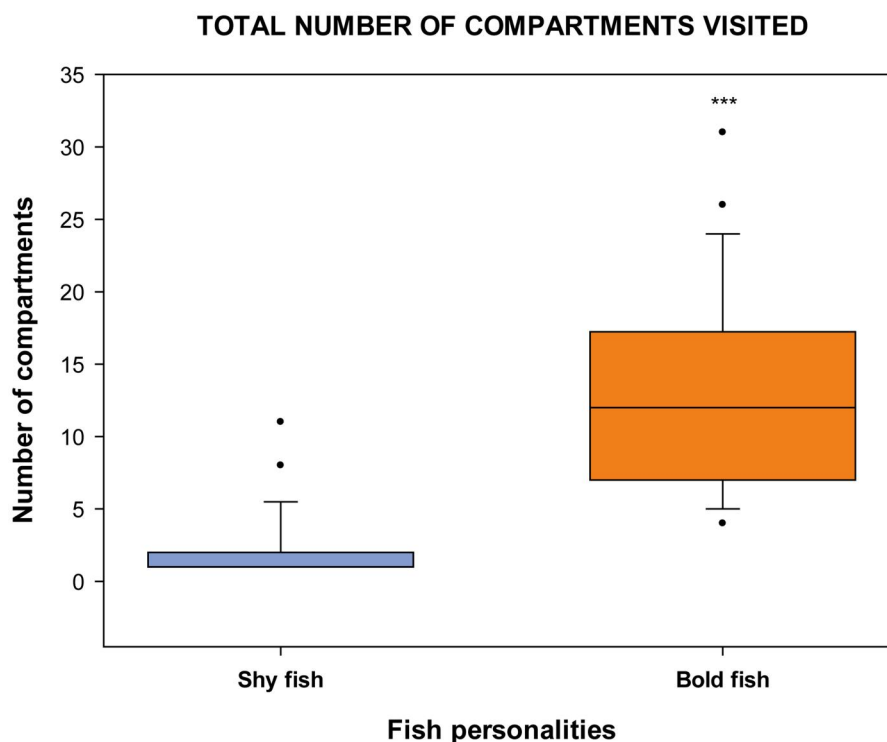


Figure 4 Total number of compartments visited by both fish populations (blue box = shy fish population; orange box = bold fish population) during the five-minute experiment. Data are presented as median \pm interquartile range. Asterisks represent significant differences between fish personalities (***) $p < 0.001$.

Table 1 Colonization efficiency (%) of the shy and bold fish populations tested in groups at different concentrations of sertraline.

Fish population		Sertraline concentrations ($\mu\text{g/L}$)				
		0	0.01	0.1	1	10
Shy fish	Fastest fish	n.c.	93.99	90.94	83.53	87.31
	Group	n.c.	0.00	0.00	0.00	0.00
Bold fish	Fastest fish	n.c.	63.84	43.27	37.14	34.17
	Group	n.c.	24.51	13.71	28.38	-431.47

Note. Percentages were calculated using the median values of the data. The efficiency for the first fish to arrive (fastest fish of the group) and the efficiency for the four fish (group) are shown. n.c. = not calculated; values of colonization efficiency were not calculated for the control compartment (0 $\mu\text{g/L}$ of sertraline) because it is the compartment where fish are initially introduced; therefore, no colonization is expected. Negative values indicate lower colonization efficiency.

significantly lower for amino acids (around 100 nM) than for bile acids (around 1 nM).

In the case of sertraline, no response was seen at any of the concentrations tested (10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1 mg/L). Therefore, it is considered that this compound did not elicit an olfactory response in zebrafish.

Discussion

The study of personality traits in fish has recently been included in behavioral studies to explain differences both within and

among populations. Numerous methodologies have been established for this differentiation in fish, such as predator detection behavior (Dugatkin & Alfieri, 2003), time to emerge from a dark to an illuminated area (Araujo-Silva et al., 2023), time leaving from or staying in a shelter (Al Shuraiqi et al., 2024; Daniel & Bhat, 2020), and different poststress reactions (Yuan et al., 2018). In our case, the differentiation between shy and bold fish was based on the percentage of the HeMHAS system explored during a set time (5 min). This differentiation, dependent on the exploratory behavior of fish, has also been considered in previous studies using different methods (Baker et al., 2018; Beigloo et al., 2024; Bellot et al., 2022; Dos Santos et al., 2023; Rajput et al., 2022; Thomson et al., 2020).

During the personality tests, the hypothesis that bold fish would colonize more and travel greater distances, visiting each compartment multiple times, was supported. Moreover, the movement from one compartment to another took a short time, 0.21 min, evidencing a continuous movement during the time of the experiment. These results show that bold zebrafish colonize new environments more easily as they usually take more risks (Daniel & Bhat, 2020). In contrast, most of the shy fish were unwilling to leave the first compartment, remaining there much longer. This is because shy fish are more cautious in unfamiliar situations (Budaev & Brown, 2011; Toms et al., 2010). These behavioral responses, as well as other characteristics of shy fish previously studied by exposing them to a new environment, such as spending more time on the bottom or against the aquarium wall (Dos Santos et al., 2023; Rajput et al., 2022), are indicative of increased anxiety in unknown environments (Blaser et al., 2010; Bownik & Włodkovic, 2021).

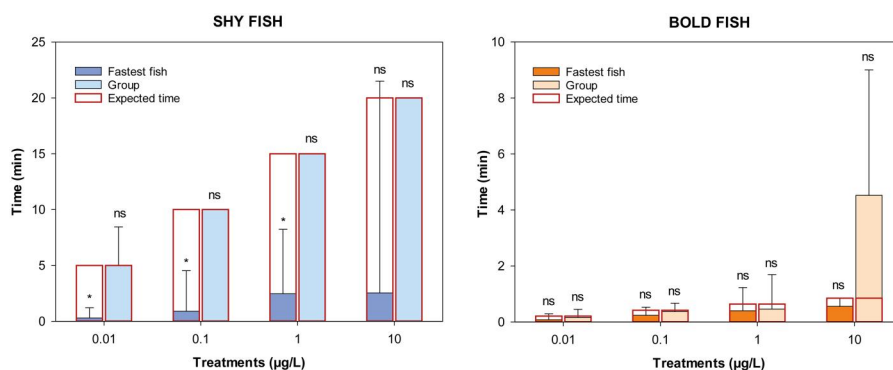


Figure 5 Colonization efficiency along the sertraline gradient for both fish populations (blue box = shy fish population; orange box = bold fish population) tested in groups. Data are presented as median \pm interquartile range. Red lines represent the expected colonization time. Asterisks indicate significant differences between observed and expected colonization times. (* $p < 0.05$); ns = not significant.

INHABITED TIME

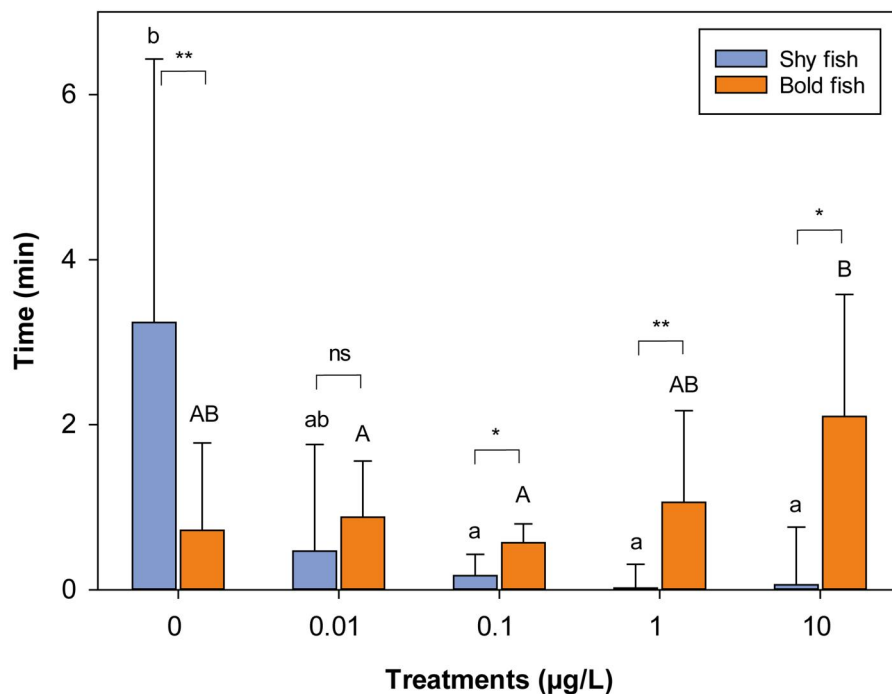


Figure 6 Inhabited time of each compartment for both fish populations (blue box = shy fish population, orange box = bold fish population) tested in groups. Data are presented as median \pm interquartile range. Lowercase letters indicate significant differences between concentrations within the shy population, whereas uppercase letters indicate differences within the bold population. Asterisks represent significant differences between personality groups at each concentration (* $p < 0.05$; ** $p < 0.01$; ns, not significant).

Risk-taking behavior for different personality traits (shy and bold) in zebrafish is closely related to serotonin metabolism (Beigloo et al., 2024; Winberg & Thörnqvist, 2016) and dopamine receptor expression (Thörnqvist et al., 2019). These are higher in bold fish than shy fish, so administration of pharmaceuticals that modulate the presence and metabolism of these neurotransmitters, such as SSRIs, may influence this phenotypic plasticity in personality traits. Specifically, sertraline exposure increases the levels of serotonin and dopamine, including their metabolites, in *D. rerio* (Faria et al., 2022). In fact, according to our results, when fish were exposed as a group, some shy fish

had a higher colonization efficiency at different concentrations of sertraline and the colonization behavior was faster than in the absence of contamination; therefore, sertraline seems to make them less shy.

This increase in the colonization behavior of a shy fish population exposed to a sertraline gradient was not observed when the group as a whole was considered. This could be attributed to the disaggregation of the group provoked by the presence of this pharmaceutical. Al Shuraiqi et al. (2024) observed a reduction in social behavior in zebrafish exposed to sertraline (from 5 ng/L to 5 μ g/L). This could be due to a decrease in stress levels when

exposed to sertraline, as exposure to other antidepressants such as fluoxetine reduces the level of cortisol in adult zebrafish (Vera-Chang et al., 2019). Furthermore, this decrease in *D. rerio* social activity has been observed with other widely used antidepressants, for example, citalopram at 100 and 1,000 µg/L (Hong et al., 2021) and 10, 50, and 100 µg/L concentrations of fluoxetine (Giacomini et al., 2016; Hong et al., 2021). However, long-term exposure could reverse these effects, as Correia et al. (2022) observed no differences in social behavior relative to the control after a 21-day exposure. Zebrafish naturally have strong social behavior, as it benefits food foraging and avoiding predation (Engeszer et al., 2007; Miller & Gerlai, 2012). Although fish can exhibit several coping style strategies regarding stress in nature (Koolhaas et al., 2007), this alteration induced by antidepressants in some shy fish could pose a high risk to natural populations. On the contrary, group disaggregation was not observed in bold fish,

as they all colonized the different sertraline compartments, thereby maintaining the group character.

In terms of colonization success, bold fish were able to colonize the different concentrations of sertraline more than the shy fish, because shy fish take longer to establish site preferences (Bellot et al., 2022). Despite this, bold fish as a group took longer than expected to colonize the maximum concentration of sertraline (10 µg/L), although this increase in time was not significant. In addition, based on the inhabited time of each sertraline concentration, bold fish had a preference to stay at that concentration during the experimental period. Attraction response to sertraline was also observed in the crayfish *Orconectes virilis* (Woodman et al., 2016) and in other aquatic organisms exposed to other antidepressants, such as fluoxetine (Abreu et al., 2016; Stremmel et al., 2023). The tendency to move towards areas contaminated with this type of pharmaceutical may represent a maladaptive behavior and pose a long-term threat to the population in the ecological context, masking the toxic effects they cause (see review by Jacquin et al., 2020; Silva et al., 2015). However, this attraction was not observed when shy or bold fish were exposed individually in the system; they spent less time in the contaminated compartments and took much longer to colonize them (negative colonization efficiency). This may have occurred because the sertraline concentrations used in this study have been observed to cause hypolocomotion, decreased swimming speed, and increased resting time in zebrafish (Faria et al., 2022; Hong et al., 2021; Richendrer & Creton, 2018; Yang et al., 2021). Furthermore, Al Shuraiqi et al. (2024) observed that males previously categorized as “high bold” became less bold following a 5 µg/L sertraline exposure. In addition, another aspect to consider is the time of exposure, as some behavioral alterations

Table 2 Individual colonization efficiency (%) of the shy and bold fish populations at different concentrations of sertraline.

Fish population	Sertraline concentrations (µg/L)				
	0	0.01	0.1	1	10
Shy fish	n.c.	0.00	0.00	0.00	0.00
Bold fish	n.c.	-1821.57 ^a	-1076.47 ^a	-684.31 ^a	-488.24 ^a

Note. Percentages were calculated using the median values of the data. n.c. = not calculated; values of colonization efficiency were not calculated for the control compartment (0 µg/L of sertraline) because it is the compartment where fish are initially introduced; therefore, no colonization is expected.

^aNegative values indicate lower colonization efficiency.

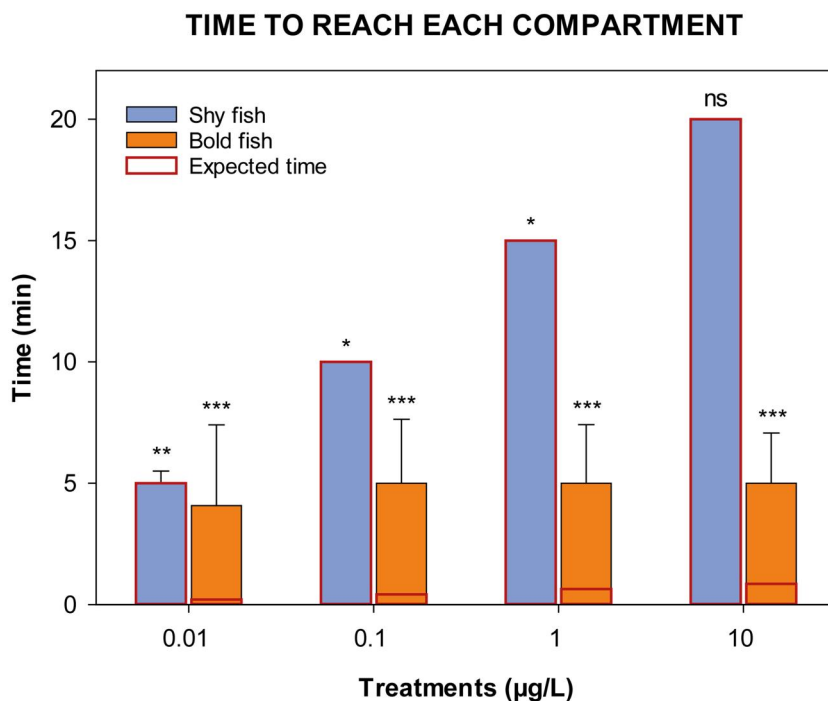


Figure 7 Colonization efficiency along the sertraline gradient for both fish populations (blue box = shy fish population, orange box = bold fish population) tested individually. Data are presented as median ± interquartile range. Red lines represent the expected colonization time. Asterisks indicate significant differences between the observed and expected colonization times. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) ns = not significant.

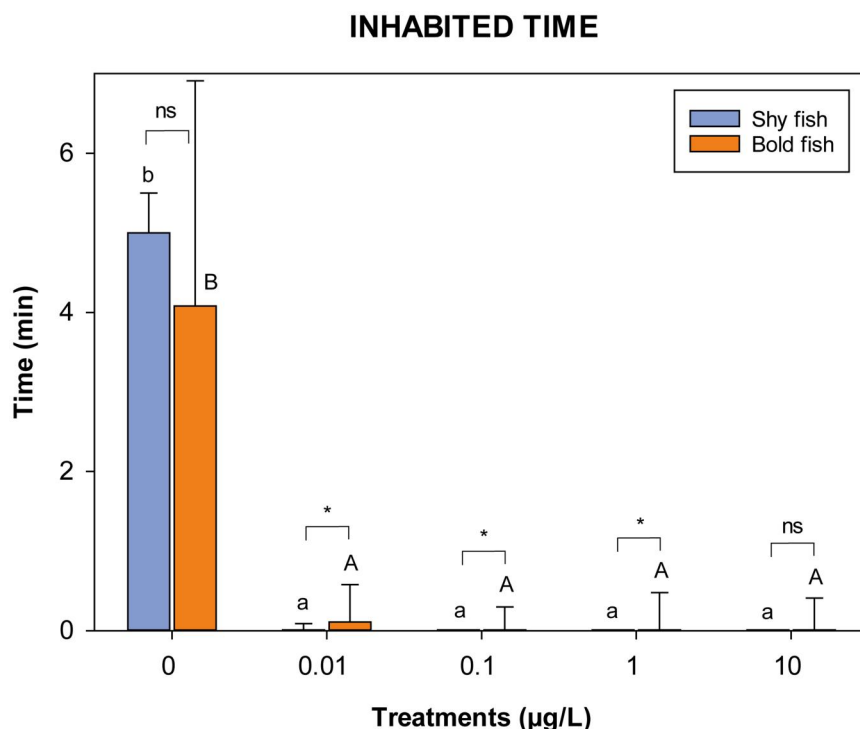


Figure 8 Inhabited time of each compartment for both fish populations (blue box = shy fish population, orange box = bold fish population) tested individually. Data are presented as median \pm interquartile range. Lowercase letters indicate significant differences between concentrations within the shy population, whereas uppercase letters indicate differences within the bold population. Asterisks represent significant differences between personality groups at each concentration (* $p < 0.05$); ns = not significant.

can be a result of short-term exposure. However, it is also important to consider that antidepressants may require longer exposure periods to determine whether these behavioral patterns persist, intensify, or provoke different responses. For example, [Takai et al. \(2023\)](#) reported no significant behavioral effects in medaka (*Oryzias latipes*) after 96 hr of exposure to sertraline. Given our finding that zebrafish do not detect sertraline via the olfactory system, any observed behavioral shifts in this species are likely mediated by direct effects on the central nervous system. These responses are also altered in zebrafish when exposed to other antidepressants ([Bachour et al., 2020](#); [Demin et al., 2017](#); [Hong et al., 2021](#)).

Fish behavior is the result of physiologically processed environmental stimuli ([Derby & Sorensen, 2008](#); [Scott & Sloman, 2004](#)). One of the mechanisms for perceiving such stimuli is through their olfactory system, which uses specific olfactory receptors to detect different chemical cues present in the water and sends the information to the brain ([Hubbard, 2024b](#)). Some of the important odorants, such as amino acids, are highly relevant to behavior ([Hara, 2006](#); [Hubbard, 2024a](#); [Valdés et al., 2015](#)). By using an EOG system, it is possible to identify whether fish are responding to the stimulus and how intense that response is. It has been shown that fish are able to detect and discriminate different odorants from the aquatic environment ([Valentincic et al., 2005](#)) even at very low concentrations (10^{-9} M- 10^{-12} M; [Michel & Lubomudrov, 1995](#); [Tierney et al., 2010](#); [Valdés et al., 2015](#)). According to our results, all concentrations of the acids and amino acids studied (range 10^{-3} M- 10^{-9} M) were detected by the zebrafish. In addition, a difference in

sensitivity was observed between the shy and bold fish populations, as shy fish tended to show a lower and more variable olfactory sensitivity among individuals than the bold fish population. Amino acids, such as alanine, arginine, histidine, and methionine, are ubiquitous chemical cues associated with food availability and prey odors ([Hubbard, 2024a](#)). This increased sensitivity in bold individuals is consistent with their proactive lifestyle, characterized by higher metabolic rates and consequently greater energy demands and more efficient foraging strategies ([Yuan et al., 2018](#)). Conversely, the reduced olfactory sensitivity of shy fish could limit their success in long-distance foraging, supporting their risk-aversion strategy and their tendency to stay in familiar or sheltered areas ([Sneddon, 2003](#)). In addition, lower sensitivity to bile salts such as TCA and TCDC in shy individuals suggests an alteration in the perception of signals important for shoal cohesion and conspecific recognition ([Quinn & Hara, 1986](#); [Tierney et al., 2010](#)). These variations in the responses to these odorants between shy and bold zebrafish provide evidence that olfactory sensitivity to the odorants studied could be influenced by differences associated with personality traits, and these sensory differences could explain the differences observed in the behavior of both populations. However, the mechanism(s) responsible for this difference remain unclear. In contrast, sertraline did not elicit a clear response from the fish's olfactory system. This suggests that the perception of these pharmaceuticals in fish occurs via alternative pathways, whether through direct detection of the compound or as a response to its metabolic processing. Such compounds could alter behavioral traits through internal

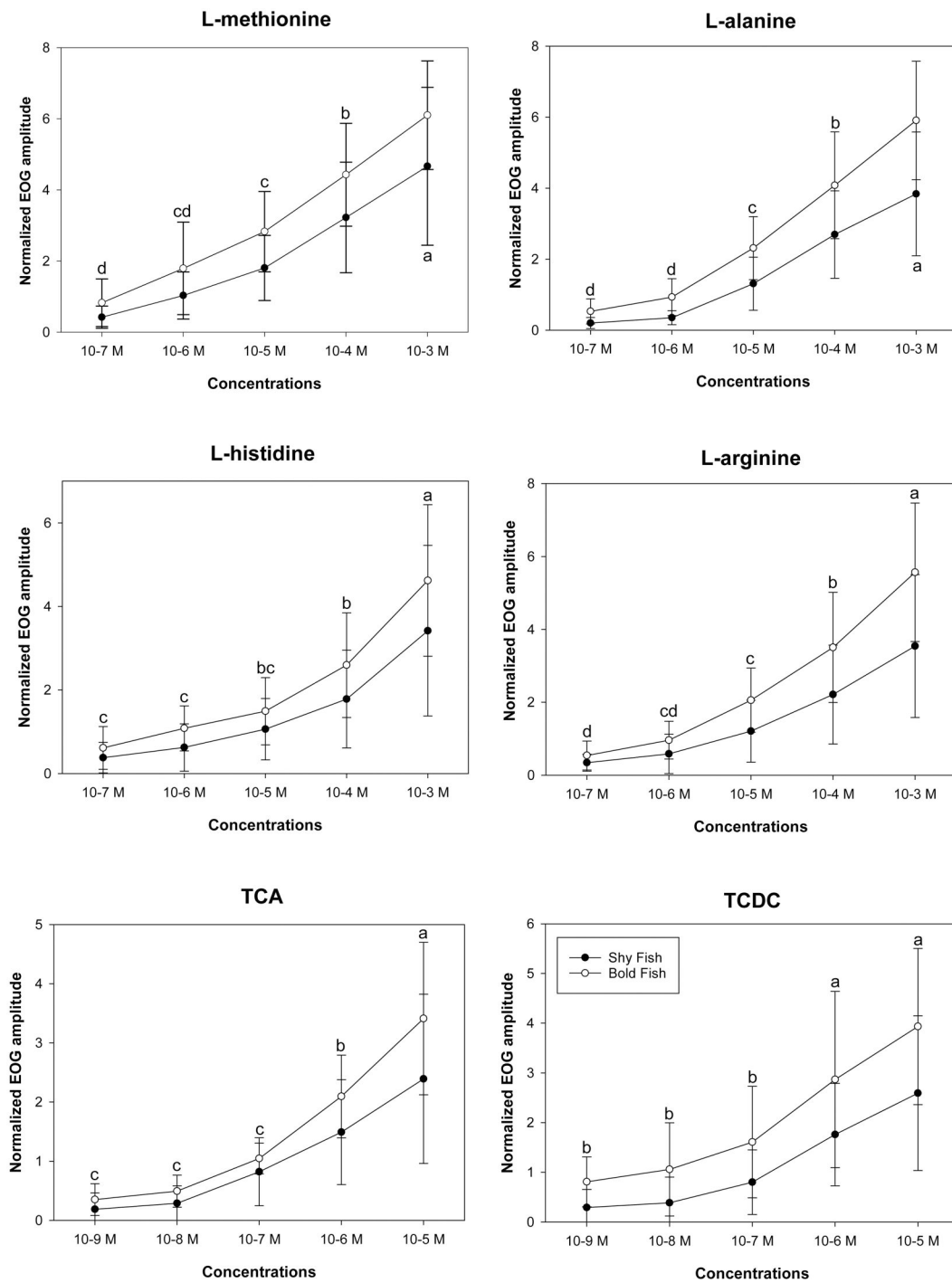


Figure 9 Olfactory sensitivity to different concentrations of L-methionine, L-alanine, L-histidine, L-arginine, taurocholic acid (TCA), and taurochenodeoxycholic acid (TCDC) for shy (black circles) and bold fish (white circles). Data are presented as $M \pm SD$. Different letters indicate significant differences across treatments.

Table 3 Detection thresholds (log₁₀ M) of the olfactory stimuli L-methionine, L-alanine, L-histidine, L-arginine, taurocholic acid (TCA), and taurochenodeoxycholic acid (TCDC) for shy and bold fish.

Fish population	L-methionine	L-alanine	L-histidine	L-arginine	TCA	TCDC
Shy fish	-6.91 ± 0.83	-6.72 ± 0.20	-6.91 ± 0.33	-6.83 ± 0.48	-8.59 ± 0.53	-8.38 ± 0.81
Bold fish	-7.39 ± 0.65	-6.97 ± 0.29	-6.98 ± 0.19	-6.99 ± 0.27	-8.78 ± 0.27	-9.13 ± 0.66

Note. Data are presented as $M \pm SD$ ($n = 8$). More negative values indicate higher olfactory sensitivity.

neurochemical effects, without requiring prior chemosensory detection. For example, Brand et al. (2025) reported that wild salmon exposed to environmental levels of anxiolytics exhibited bolder behaviors, likely due to neurochemical alterations rather than sensory detection. In our case, sertraline may have been absorbed through the gills and/or skin, causing an effect on neural pathways, despite the absence of olfactory detection. This highlights the importance of considering both behavioral and physiological pathways of action when assessing the ecological impact of this type of contaminant. Nevertheless, the presence of these chemicals in the environment has been shown to produce a toxic effect on the olfactory system. Huang et al. (2022) observed that 28-day exposure to fluoxetine caused an effect on the initiation, transmission, and processing of olfactory signals in the fish *Carassius auratus*, with decreased EOG responses to olfactory stimuli.

Important ecological consequences may result because of the differences in colonization responses and in the olfactory sensitivity found between the zebrafish personality traits. The greater ability to colonize sertraline-contaminated environments in terms of success, efficiency, and group preference of bold fish could make them more vulnerable if this behavior persists under long-term exposure in sertraline-contaminated areas and fish are not able to avoid it. In addition, for shy fish in a group context, sertraline was observed to modulate their personality so that they tended to take more risks, which is ecologically associated with leaving shelters earlier, more active foraging, and faster task learning. This all represents a change in how organisms may deal with different types of stress factors present in the environment as well as a change in resource exploitation. Furthermore, differences in olfactory sensitivity between shy and bold fish determine individual differences in specific responses, such as foraging, reproduction, predator detection, or social interactions.

Conclusion

Our results revealed significant differences in colonization patterns between shy and bold zebrafish in sertraline-contaminated environments in terms of colonization success, efficiency, and inhabited time for each concentration. Bold zebrafish tended to colonize higher sertraline concentrations compared with shy fish; conversely, shy fish increased their exploration and risk-taking behavior compared with their baseline responses in the absence of sertraline. Furthermore, olfactory sensitivity varied by personality trait, as the EOG responses of shy and bold individuals diverged when exposed to common olfactory stimuli. The shy fish exhibited lower olfactory sensitivity than the bold fish. In contrast, sertraline was not detected by the fish's olfactory system under the test conditions. In summary, this study highlights the necessity of considering individual variation in physiological and behavioral traits, as this could provide a more comprehensive understanding of how animals interact with their environment and how contaminants differentially impact fish populations based on their personality.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Data available on request.

Author contributions

María Pilar González (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft), David Salvatierra (Conceptualization, Methodology, Writing—review & editing), Elvira Fatsini (Conceptualization, Methodology, Writing—review & editing), Peter Hubbard (Conceptualization, Data curation, Methodology, Writing—review & editing), and Cristiano V. M. Araujo (Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing—review & editing)

Funding

This study is part of the BeingHavior project (PID2022-137402OB-I00) funded by the Spanish Ministry of Science, Innovation and Universities (MCIN/AEI/10.13039/501100011033/FEDER, EU). It also received Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020 (DOI : 10.54499/UIDB/04326/2020), UIDP/04326/2020 (DOI : 10.54499/UIDP/04326/2020, LA/P/0101/2020 (DOI : 10.54499/LA/P/0101/2020) and 2020.04181.CEECIND (to E. Fatsini).

Conflicts 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 article.

Acknowledgments

M.P. Gonzalez thanks the Spanish State Investigation Agency for the predoctoral contract (PREP2022-000459). D. Salvatierra is grateful to the Andalusian Regional Government for the predoctoral contract under the Andalusian Research, Development and Innovation Plan (PAIDI 2020). The authors thank P. Gavaia and B. Ferreira from the Center of Marine Sciences—CCMAR (Universidade do Algarve) for the supply and care of the zebrafish and M. Pintado from the University of Cadiz for the chemical analysis of the samples. In addition, they thank J. Nesbit for his English revision of the text.

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