Science of the Total Environment 900 (2023) 165706

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Are early and young life stages of fish affected by paroxetine? A case study with *Danio rerio*

Carla S.S. Ferreira [a,*], Cátia Venâncio [a], Peter Kille [b], Miguel Oliveira [a]

[a] Centre for Marine and Environmental Studies (CESAM), Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal
[b] School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK

ARTICLE INFO

Editor: Damià Barceló

*Keywords:*
Selective serotonin reuptake inhibitors
Zebrafish
Lethal effects
Sub-lethal effects
Ontogenetic development
Behaviour modification

ABSTRACT

Paroxetine (PAR) is a selective serotonin reuptake inhibitor (SSRI) antidepressant increasingly detected in surface waters worldwide. Its environmental presence raises concerns about the potential detrimental effects on non-target organisms. Thus, this study aimed to increase knowledge on PAR's potential environmental impacts, assessing the effects of commercial formulation (PAR-c) and active ingredient (PAR-a) on fish. Therefore, the short-term exposure effects of PAR-c and PAR-a were assessed on zebrafish (*Danio rerio*) embryos/larvae to determine the most toxic formulation [through median lethal ($LC_{50}$) and effective concentrations ($EC_{50}$)]. PAR-c and PAR-a induced morphological abnormalities (scoliosis) in a dose-dependent manner from 96 hours post-fertilization onwards, suggesting the involvement of a fully functional biotransformation system. As PAR-c exhibited higher toxicity, it was selected to be tested in the subsequent stage (juvenile stage), which was more sensitive (lower $LC_{50}$). PAR-c significantly decreased fish swimming activity and disrupted fish stress response. Overall, the results highlight the ability of PAR-c to adversely affect fish swimming performance, an effect that persisted even after exposure ceases (21-day depuration), suggesting that PAR-c may impair individual fitness.

## 1. Introduction

The rise in depression and anxiety cases during the COVID-19 pandemic resulted in a notable increase in the utilization of selective serotonin reuptake inhibitors (SSRI) antidepressants (Diaz-Camal et al., 2022; Ferreira et al., 2023a). These antidepressants enhance the levels of the neurotransmitter serotonin, involved in a wide variety of physiological functions, and modulation of human mood and behaviour (Burns et al., 2018; Burkina et al., 2015), by acting on the serotonergic system, highly evolutionarily conserved across vertebrates (Silva et al., 2012; Nowicki et al., 2014). Paroxetine (PAR) is a second-generation SSRI, extensively prescribed for depression management, obsessive-compulsive disorder, panic disorder, social anxiety disorder, generalized anxiety disorder and post-traumatic stress disorder (Paroxetine, n. d.; Kowalska et al., 2021; Cunningham et al., 2004). Following oral intake, PAR is promptly assimilated and extensively metabolized in the liver, with an estimated body elimination half-life of 21 h. Approximately 64 % of the PAR ingested dose is eliminated in the urine, 2 % of which is the parent drug and 62 % as metabolites (pharmacologically inactive *in vivo* (Bourin et al., 2001) and easily cleared due to their polarity (Cunningham et al., 2004)). Thus, associated with the limited effectiveness of conventional wastewater treatment plants (WWTPs) in its elimination (Burns et al., 2018; Sumpter and Margiotta-Casaluci, 2022; Mole and Brooks, 2019), this SSRI has been widely found in surface waters, reaching levels as high as 90 ng/L (Burns et al., 2018; Mezzelani et al., 2018). In the effluents of pharmaceutical manufacturing industries that operate WWTPs, PAR levels as high as 3380 ng/L have been reported (Kleywegt et al., 2019), highlighting these facilities as significant sources of aquatic contamination (Burns et al., 2018). PAR has also been detected in fish tissues (e.g., *Salmo trutta, Ameiurus nebulosus, Dorosoma cepedianum, Morone americana*), especially in the liver and kidney, at concentrations up to 9.5 ± 7.7 ng/g, highlighting its bioaccumulation potential (Grabicova et al., 2017; Chu and Metcalfe, 2007).

While the lack of threshold values prevents a deterministic assessment of the environmental hazard posed by this compound, the probabilistic model for environmental hazard assessment reveals that PAR can be considered a contaminant of emerging concern (Sumpter and Margiotta-Casaluci, 2022). Reports of SSRI-induced effects on non-target organisms like fish are emerging, often associated with their

* Corresponding author.
*E-mail address:* csofia@ua.pt (C.S.S. Ferreira).

https://doi.org/10.1016/j.scitotenv.2023.165706
Received 20 May 2023; Received in revised form 8 July 2023; Accepted 20 July 2023
Available online 25 July 2023

potential to act on the fish serotonergic system, even at trace levels (McDonald, 2017; Silva et al., 2015). These effects include alterations in behaviour, physiology, and gene expression (Silva et al., 2015; Puckowski et al., 2016; Salahinejad et al., 2022; Kellner and Olsén, 2020; Huang et al., 2020). Based on the limited available information, the acute toxicity of PAR appears to be similar to that of fluoxetine, which is recognized as having the highest acute toxicity among SSRIs (Silva et al., 2015), inducing long-lasting effects in fish behaviour not easily reverted (Dzieweczynski et al., 2016).

Nonetheless, data on PAR effects on fish are scarce. In fact, PAR may be considered the least tested SSRI in terms of ecotoxicological effects.

Several factors associated with the organism (e.g., age/stage of development) and the biologically active substance (e.g., presence of other substances/excipients) may modulate the (eco)toxicological effects found. The assessment of effects on fish, at different life stages, especially at early and young life stages, can provide valuable data for regulatory purposes needed for establishing protective measures. In this sense, toxicity tests using the first life stages of zebrafish (*Danio rerio*), a model organism that shares high genetic homology and conservation of the monoaminergic systems with humans, are routinely used in toxicity screening (Nowakowska et al., 2020; Huang et al., 2019). Alterations in visual motor response and teratogenic effects causing scoliosis have been reported in zebrafish larvae after acute exposure to environmentally relevant concentrations of PAR (1, 10, and 100 μg/L), within five days post fertilization (Nowakowska et al., 2020; Huang et al., 2019). The mRNA gene expression of zebrafish larvae has also been reported sensitive to six days of exposure to 100 μg/L PAR (1518 genes were differentially expressed in the brain, 58 % of which were downregulated and 42 % upregulated) (Huang et al., 2020). However, to the authors' knowledge, the available studies with PAR have only used the active ingredient formulation but the environmental relevance of assessing PAR commercial formulation (PAR-c) should not be neglected as potential adverse effects related to its excipients may occur.

Therefore, in this study, the focus was given to PAR active ingredient formulation (PAR-a) and PAR commercial formulation (PAR-c) effects on survival (embryo-larval and juvenile stages), ontogenetic development (embryo-larval stage), and swimming behaviour (juvenile stage) of zebrafish. The inclusion of a battery of different biomarkers aimed to provide a more relevant and integrative assessment of PAR effects. The working hypothesis is that different formulations will cause distinct effects on fish and that the effects of the most toxic xenobiotic may not be easily reverted when transferred to clean media. In this sense, a comprehensive approach was adopted to assess PAR-induced changes in the juveniles' swimming behaviour pattern, through the inclusion of several swimming-associated parameters after exposure and a 21-day depuration period, which can provide relevant information on fish fitness.

## 2. Materials and methods

### 2.1. Fish maintenance and husbandry

Adult zebrafish (*Danio rerio*) AB wild type are kept in a ZebTEC (Tecniplast) recirculating system at the Department of Biology, University of Aveiro (Portugal) under a 14:10 h (light:dark) photoperiod. Culture water is obtained by reverse osmosis and activated-carbon filtered tap water, complemented with salt "Instant Ocean Synthetic Sea Salt" (Spectrum Brands, USA), and automatically adjusted for pH (7.5 ± 0.5) and conductivity (800 ± 50 μS/cm). Water temperature is maintained at 26.0 ± 1 °C and dissolved oxygen at 95 % saturation or higher. Nitrite and ammonia compounds are kept below 0.01 mg/L and nitrate below 0.1 mg/L. Fish are fed once a day with a commercially available artificial diet (GEMMA Micro 500, Skretting USA).

The embryos used in the embryo toxicity test were obtained within 30 min after the natural mating of adult fish. The eggs were washed in fish system water and screened for unfertilized eggs and unviable embryos using a stereomicroscope (Stereoscopic Zoom Microscope-SMZ 1500, Nikon Corporation). Only fertilized and normally developed eggs (in the blastula stage 3 hours post fertilization – hpf) were used in the assays.

The juvenile fish used in the acute toxicity test were obtained through crossbreeding and reared until the juvenile stage (2 months old). The feeding schedule and maintenance conditions followed those described above for breeding pairs' maintenance. Only fish with no observable signs or symptoms of compromised health (i.e., exhibiting normal morphology and behaviour) were selected for the test.

### 2.2. Experimental design

Considering the limited information regarding the effects of PAR on fish, this study included a two-tier approach, aimed to contribute to a better understanding of the potential effects of paroxetine on fish's different life stages, which included providing PAR benchmarks ($LC_x$ and $EC_x$).

In the first tier, the toxicity of both PAR types (commercial formulation – PAR-c and active ingredient – PAR-a) was assessed on zebrafish embryos/larvae (Fig. 1). The formulation considered most toxic to embryos/larvae in this first-tier approach - PAR-c - was then tested in the young life stage (*D. rerio* juveniles), to determine if the toxic effects were maintained or decreased (Fig. 2). This approach allowed a reduction in the number of organisms tested but still allowed the achievement of the proposed goal of the study.

As PAR seems to be the most photosensitive among SSRIs (Cunningham et al., 2004; Sumpter and Margiotta-Casaluci, 2022; Grabicova et al., 2017), the tests were initiated with and without medium renewal, to assess in which context more effects would be observed. Therefore, the effects of PAR - active ingredient (paroxetine hydrochloride hemihydrate; CAS 110429–35-1; TCI Europe), as well as commercial formulation (Paroxetina Aurovitas, Generis ®, pills), were first evaluated on *D. rerio* embryo-larval stage. Based on the data of these assays, the subsequent tests for estimation of lethal concentrations causing X% of effect ($LC_x$) and sublethal effective concentrations ($EC_x$) were performed with daily medium renewal. All experimental procedures were conducted according to the legal and ethical requirements of Portuguese (Decreto-Lei 113/2013) and European legislation (Directive 2010/63/EU) on the protection of animals used for scientific purposes and approved by the ethics committee and the Portuguese National Authority for Animal Health (009804). The experiments were performed with FELASA-certified researchers, following the ARRIVE guidelines (du Sert et al., 2020).

### 2.3. Toxicity assessment

#### 2.3.1. Embryo toxicity tests

All assays were carried out with reconstituted water (prepared by adding marine salts "Instant Ocean Synthetic Sea Salt" (Spectrum Brands, USA) to Milli-Q water and adjusting the conductivity to 800 ± 50 μS/cm). Stock solutions of PAR commercial formulation (PAR-c) and PAR active ingredient formulation (PAR-a) (80 and 40 mg/mL, respectively) were prepared in the reconstituted water and further diluted to the desired test solutions. The pills of the former were carefully grinded to allow easier dissolution in water. Each pill of 260 mg contained 20 mg of the active ingredient (PAR-a represents approximately 8 % of PAR-c constitution).

The experimental assays were performed generally following OECD testing guideline 236 for fish embryo toxicity (FET) test to assess PAR-c and PAR-a effects on embryos/larvae development. In the first assay, conducted with and without medium PAR-c and PAR-a renewal, newly fertilized eggs (24 per experimental condition), were randomly distributed to the following concentrations: 0 (control), 400, 819, 1638, 2048, 2560, and 3200 μg/L PAR-a or PAR-c in 24-well plates under controlled conditions of temperature and light (27 ± 1 °C; 14:10 h light:dark

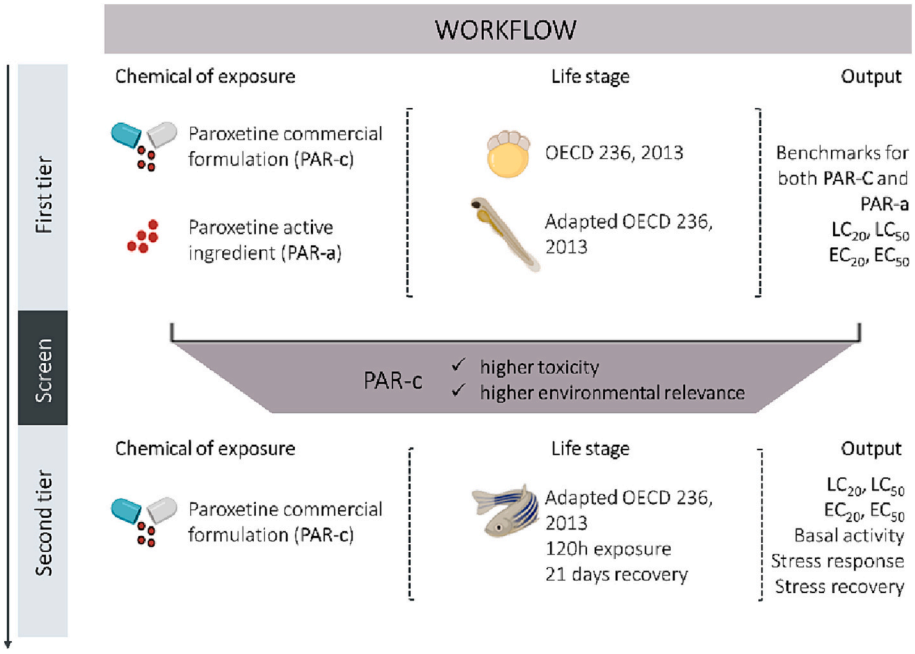


**Fig. 1.** Workflow of the two-tier approach carried out with *Danio rerio* embryos/larvae and juveniles exposed to paroxetine.

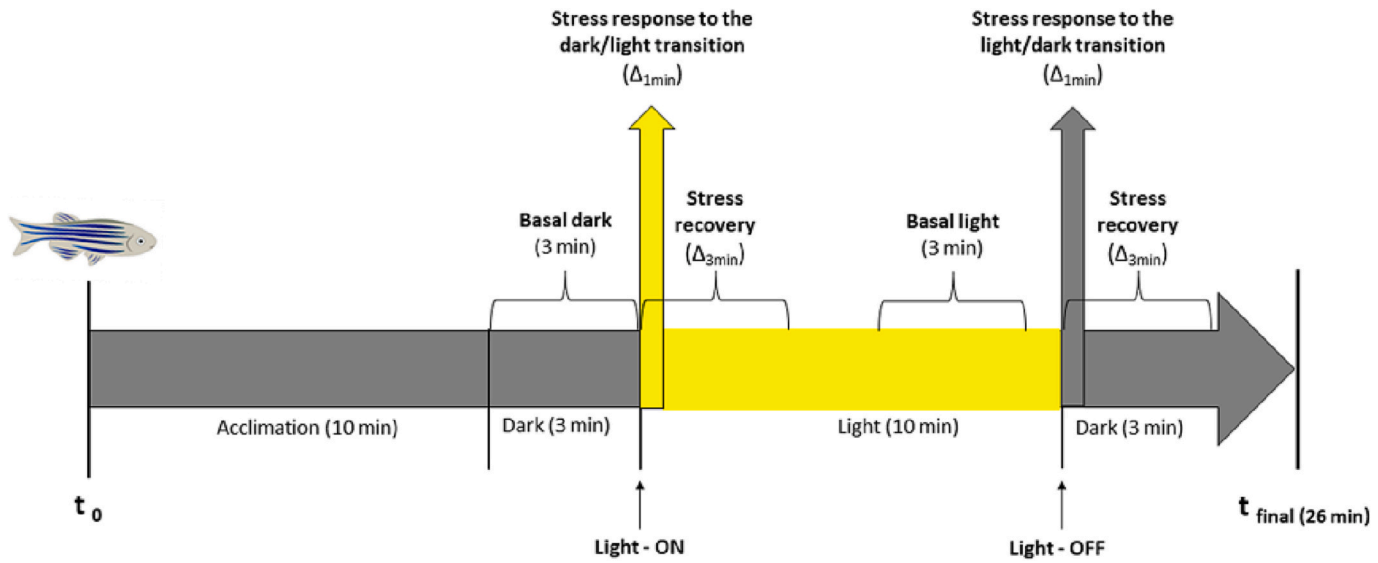


**Fig. 2.** Experimental setup carried out for behavioural analysis of *Danio rerio* juveniles following exposure to paroxetine.

photoperiod). A considerable increase of abnormalities was found between 72 and 96 h and, thus, the exposure period was extended up to 144 h to allow continued monitoring of effects. Zebrafish ontogenetic development was evaluated daily (24, 48, 72, 96, 120, and 144 hpf) and survival and larval morphological abnormalities were observed using a stereomicroscope (Stereoscopic Zoom Microscope-SMZ 1500, Nikon Corporation).

As data from the first assays revealed insufficient for LCx estimation, a second embryo test was performed for PAR-c and PAR-a under similar conditions and assessing the same endpoints but testing the following concentrations: 4000, 5000, 6250, 7813, 9000, and 13,500 μg/L. Throughout the aforementioned experimental trials, organisms remained unfed.

### 2.3.2. Juvenile toxicity tests

The assay was performed generally following OECD guideline 203 for fish acute toxicity test, with a slight modification. In this assay, the exposure period was 120 h, and in addition to lethal effects, assessed endpoints included alterations in fish swimming behaviour. The juvenile zebrafish were randomly allocated to nine experimental groups: 0 (control group without PAR-c), 40, 400, 819, 1638, 2048, 2560, 3200, and 4000 μg/L, at a density of 3 fish/0.75 L (5 replicates per treatment). Animals were daily checked for mortality and any signs of distress before renewing 70 % of the test media. Animals were not fed during the exposure period.

### 2.4. Behavioural assessment

The swimming behaviour of zebrafish juveniles showing no morphological alterations after PAR-c exposure was analysed after 120 h exposure and after a 21-day recovery period (to assess if PAR-induced effects were reversed in a clean medium). The behavioural trials were

performed in a rectangular tank (9.4 cm wide and 14.1 cm long) with 2.5 cm of the test media testing, individually, with a total of 15 fish per experimental group. The movement of each fish was continuously tracked and recorded using an automated video tracking system (Zebrabox; Viewpoint, Lyon, France), over a 16 min period (Fig. 2). An initial 10 min acclimation period in the dark was used, with analysis starting with a 3 min dark period, alternating with 10 min light and ending with 3 min dark (Fig. 2).

Basal swimming distance under dark conditions was obtained by measuring the total distance swam in the first 3 min after the acclimation period, to ensure the fish's full stabilization after handling. The same principle was applied to the calculation of basal swimming distance in light conditions, which was calculated by measuring the distance swam in 3 min after 5 min of the dark/light transition, to allow fish to recover from this light transition. The total time of inactivity and mean speed were also evaluated for a better characterization of fish swimming basal activity patterns in dark and light periods. The fish swimming trajectory was assessed through the analysis of swimming angles. Eight classes of angles were defined, generally following (Zhang et al., 2017) and calculated as described by (Almeida et al., 2019). These classes refer to high-amplitude angles (1 and 8; 2 and 7) that translate zig-zag movements with significant changes in direction (erratic/stress behaviour); medium amplitude angles (3 and 6) that indicate average turns; and low amplitude angles (4 and 5) which translate straightforward movements.

Behaviour alterations induced by light transitions (dark to light and light to dark) were used to study the effects on stress response and light sensitivity. For this purpose, four behavioural parameters were evaluated – swimming angles, total time of inactivity, total distance swam and mean speed – through the ratio of 1 and 3 min immediately before and after the light condition transition (to respectively collect information about the immediate stress response induced by this variation and recovery response).

### 2.5. Integrated biomarker response (IBR)

The behavioural endpoints were integrated into the IBRv2 index, according to Sanchez (Sanchez et al., 2013), allowing to reach a value representing the stress level for each treatment, based on the principle of reference deviation.

### 2.6. Statistical analysis

Median lethal and effective concentrations ($LC_{50}$ and $EC_{50}$) and threshold lethal and effective concentrations ($LC_{20}$ and $EC_{20}$), with corresponding 95 % confidence limits, were calculated by probit regression analysis using PriProbit software (Sakuma, 1998).

In the analysis of the behaviour endpoints, to assess significant differences between experimental groups, whenever normality and equality of variances assumptions were met, a one-way ANOVA analysis was performed followed by the post-hoc Holm-Sidak method (using the software SigmaPlot V.14.0). When assumptions were not met, a non-parametric ANOVA was performed (Dunn's test). Differences in behaviour between dark and light conditions were attested using the Student *t*-test. The level of significance was considered at $p < 0.05$.

## 3. Results

### 3.1. Zebrafish embryos

No significant mortality nor developmental abnormalities were observed in the control groups throughout the test. Embryos/larvae mortality was not observed, within the first 96 h of exposure, in organisms exposed to any of the tested PAR forms at concentrations up to 7813 μg/L. Considerable mortality was only found in organisms exposed to the highest tested concentrations (9000 and 13,500 μg/L) of PAR-c (50 % and 100 %, respectively). The estimated 96 h $LC_{50}$ and $LC_{20}$ values for PAR-c were 9000 (8789–9216) μg/L and 8649 (8448–8856) μg/L, respectively. For the same exposure period (96 h) a maximum of 4 % mortality was found in organisms exposed to 13,500 μg/L PAR-a.

In the assays with daily medium renewal for both PAR types, effects on development were only observed for concentrations higher than 819 μg/L (Table S3), allowing the calculation of PAR-c 96 h $EC_{50}$ as 3713 μg/L (3271–4241) and $EC_{20}$ as 2168 μg/L (1742–2524). The PAR-a 96 h $EC_{50}$ was 3348 μg/L (2239–5309) and $EC_{20}$ was 1827 μg/L (699.4–2627). The most frequently observed morphological abnormality was scoliosis. In the assays without medium renewal, no abnormalities were observed in the organisms exposed to PAR-a nor PAR-c (Table S1 and S2).

#### 3.1.1. Zebrafish juveniles

PAR-c induced lethality in juveniles at concentrations higher than 400 μg/L. The mortality data allowed the calculation of the 48 h and 96 h $LC_{50}$, corresponding to 1214 μg/L (895.0–1689) and 791.7 μg/L (747.7–838.9) respectively, as well as the 48 h and 96 h $LC_{20}$ values, 1164 μg/L (859.6–1616) and 740.9 μg/L (700.1–784.7) respectively.

### 3.2. Swimming behaviour after PAR-c acute exposure

#### 3.2.1. Basal swimming activity

In general, 5 days of exposure to PAR-c decreased fish basal locomotor activity and increased their inactivity time during both light and dark periods (Figs. 3A and B). Under light conditions, fish exposed to 40 μg/L PAR-c exhibited significantly lower basal locomotor activity than control and 400 μg/L PAR-c exposed fish. In the control fish, the basal swimming activity was significantly higher in the light period, a trend also observed in organisms exposed to 400 μg/L PAR-c, whereas fish exposed to 40 μg/L PAR-c displayed a non-significant trend to swim more in the dark (Fig. 3A). Controls and fish exposed to 400 μg/L PAR-c, spent significantly less time swimming in the dark than in the light conditions. However, this response pattern was not observed in 40 μg/L PAR-c, which displayed no significant differences between light conditions (Fig. 3B). In terms of swimming speed, no notable impact of exposure to PAR-c was found (under both dark and light conditions). Significant differences between light and dark conditions were only observed in fish exposed to 400 μg/L PAR-c, which displayed significantly higher swimming speed in the dark (Fig. 3C).

The analysis of the swimming angles displayed by fish in basal activity revealed that control fish exhibit a significantly higher proportion of high-amplitude angles in light conditions (Figs. 3D and E) and of low-amplitude angles in the dark period (Fig. 3F). This trend was also observed in PAR-c-exposed fish but with no statistical significance. No effects of PAR-c were found in light conditions. However, under dark conditions, fish exposed to PAR-c displayed a higher frequency of class 1, 2, 7 and 8 angles (high-amplitude) and a lower frequency of classes 4 and 5 (low-amplitude). The decrease in the frequency of low-amplitude angles was more pronounced in organisms exposed to 400 μg/L PAR-c (Fig. 3F).

#### 3.2.2. Response to light transitions

The fish response to light transitions is depicted in Figs. 4 and 5. Significant differences were found between exposed and non-exposed fish for the assessed endpoints [(total distance swam, total time of inactivity, mean swimming speed and swimming angles of high (classes 1, 2, 7, and 8) and low amplitude (classes 4 and 5)], following 1-min and 3 min of the light transitions.

*3.2.2.1. Dark/light transition.* Within the first min after the sudden shift from dark to light, 40 and 400 μg/L PAR-c exposed fish remained inactive for a longer period and swam faster than control fish (Figs. 4B and C). The 40 μg/L PAR-c exposed fish swam significantly lower

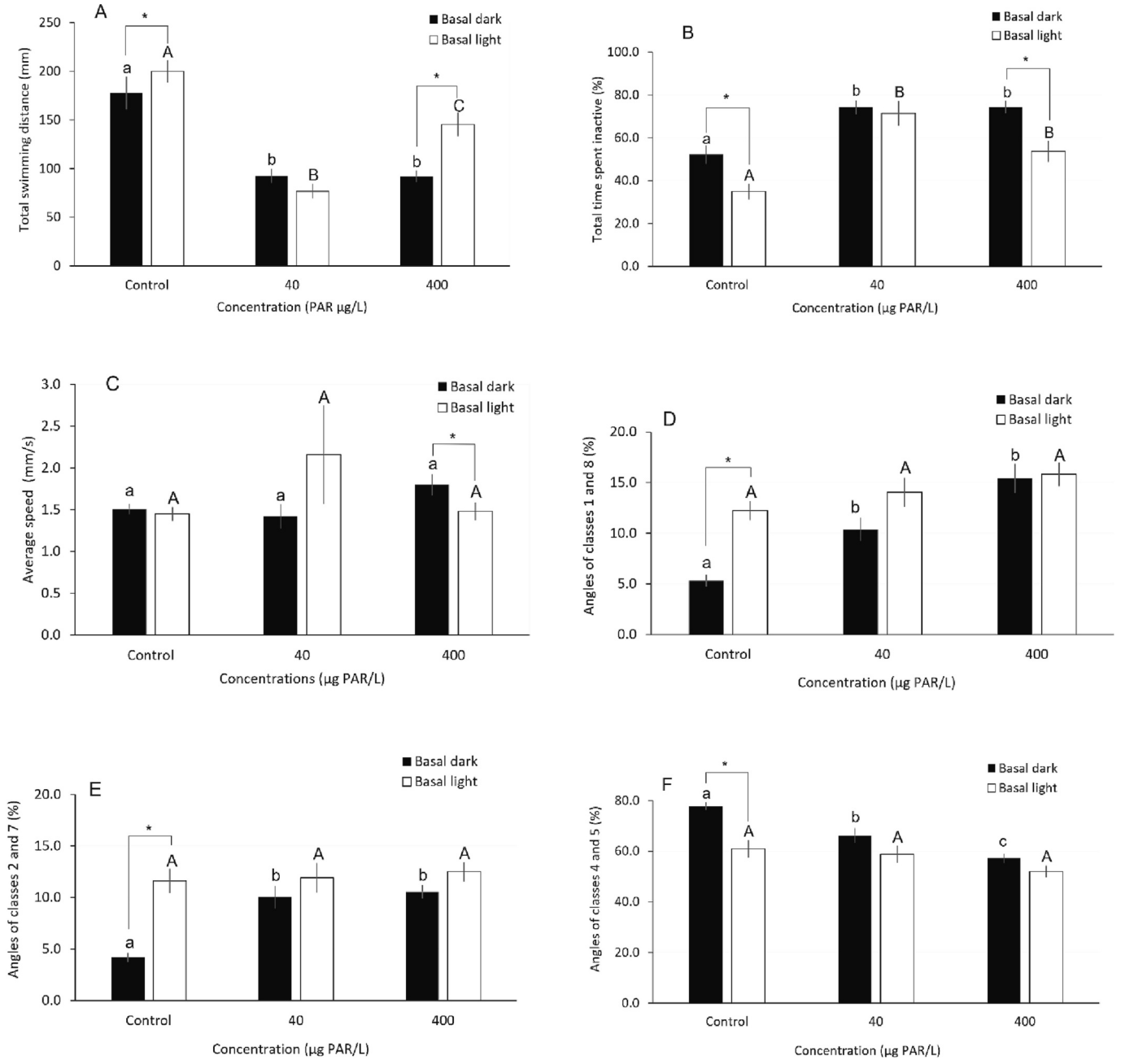


**Fig. 3.** Effects of paroxetine concentrations on basal swimming activity of zebrafish in dark and light conditions after 5 days of exposure. A – Total swimming distance (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. Black bars represent the dark period and white bars the light one. "*" indicates differences between dark and light periods for each tested condition; Different letters indicate significant differences across treatments for dark (a,b) and light conditions (A,B,C). One-way ANOVA followed by the Holm-Sidak method, $p < 0.05$).

distance and displayed a higher proportion of class 1 and 8 angles (high-amplitude) when compared to the control and 400 µg/L PAR-c exposed fish (Figs. 4A and D). The effects of PAR-c exposure on fish trajectory were also reflected by a decreased frequency of class 2 and 7 angles during this light transition (Fig. 4E). PAR-c exposure also had a significant effect on low-amplitude angles, with exposed fish displaying a lower frequency of class 4 and 5 angles, after stress induced by the dark/light transition (Fig. 4F).

After 3 min of light transition, no differences were found between exposed and non exposed fish in terms of total swimming distance, total time spent in inactivity and in the frequency of low-amplitude angles (classes 4 and 5) (Figs. 5A, B and F). However, PAR-c exposed animals' swimming pattern revealed a lower frequency of angles of classes 1, 2, 7 and 8 during this light transition (Figs. 5D and E). Fish exposed to 400 µg/L PAR-c appeared to recover slower from stress induced by the dark/light transition, as lower mean swimming speed was found within this period (Fig. 5C).

*3.2.2.2. Light/dark transition.* In response to a sudden change from light to dark (1 min immediately following the transition), fish exposed to 40 µg/L PAR-c were significantly more active (higher distance moved) than control and 400 µg/L PAR-c exposed fish (Fig. 4A). However, no differences in swimming speed were observed between treatments (Fig. 4C). PAR-c exposed fish spent less time inactive and swam in a

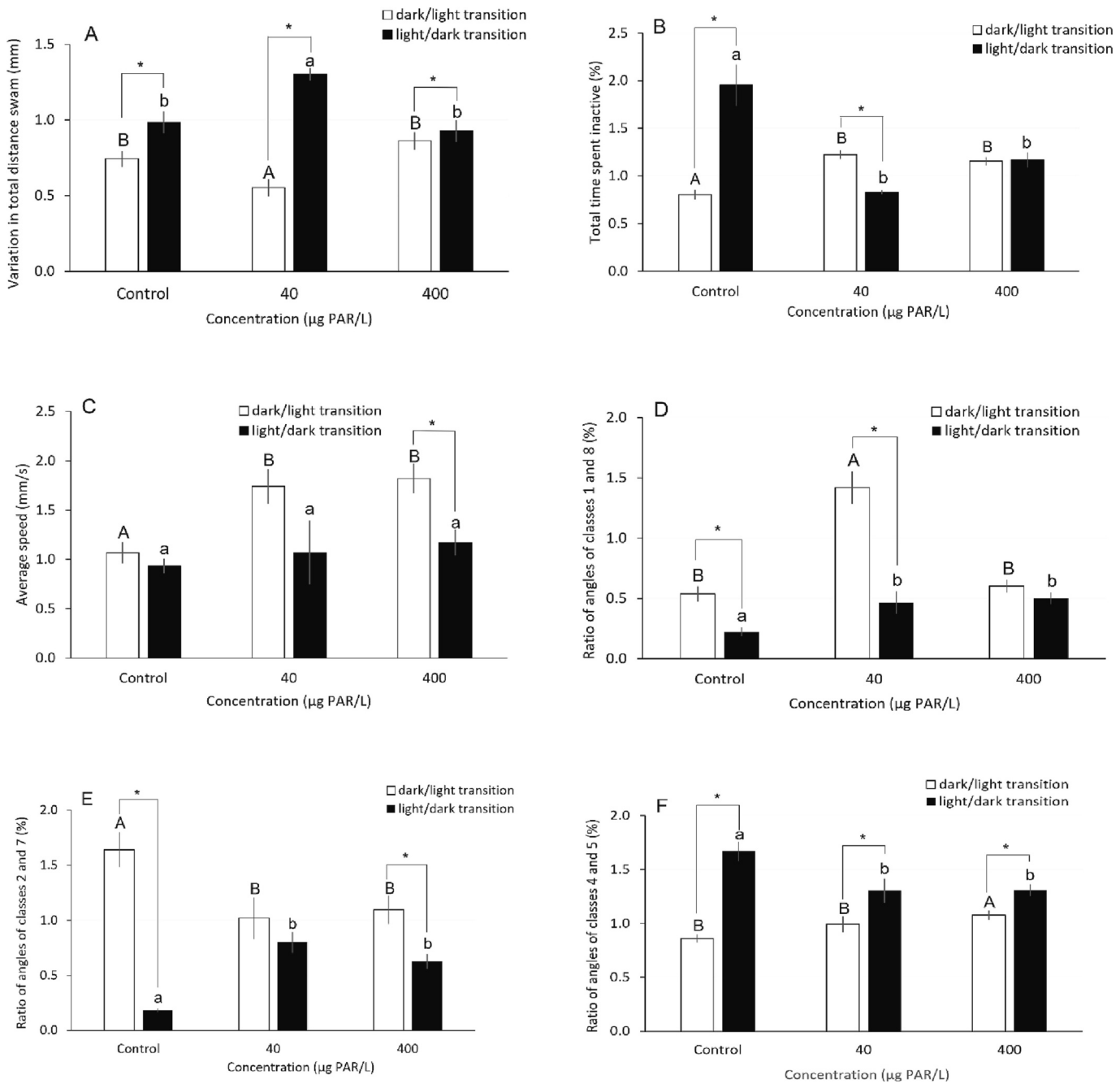


**Fig. 4.** Effects of paroxetine on zebrafish response to stress induced by sudden light transitions (dark to light and light to dark) after 5 days of exposure. A – Variation in total distance swam (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. White bars represent the dark/light transition and the black bars the light/dark transition. "*" indicates differences between dark/light and light/dark transition for each tested condition; Different letters indicate significant differences across treatments for dark/light (a,b,c) and light/dark transition (A). One-way ANOVA followed by the Holm-Sidak method, $p < 0.05$).

trajectory with an increased proportion of classes 1, 2, 7 and 8 (high-amplitude angles) and decreased proportion of classes 4 and 5 (low-amplitude angles) (Figs. 4B, D, E and F).

The analysis of fish stress recovery response (3-min after light condition transition) revealed that PAR-c exposed fish spent significantly less time inactive than control organisms (Fig. 5B). Changes in the proportion of high and low-amplitude angles were also observed in exposed fish, which displayed a higher frequency of class 1, 2, 7 and 8 angles and a lower frequency of class 4 and 5 (Figs. 5D, E and F). No significant differences between control and exposed fish were found in terms of swimming distance and mean speed (Figs. 5A and C).

*3.2.2.3. Comparison of fish response pattern between both light transitions (dark/light versus light/dark).* Within the first min immediately following light transitions, a disruption of the normal response pattern was found for 400 µg/L PAR-c exposed fish which did not respond differently to the two light transitions in terms of inactivity time and proportion of class 1 and 8 angles, unlike the other treatments (Figs. 4B and D). Although fish displayed a general trend to swim faster after dark/light transition, only 400 µg/L PAR-c displayed significantly higher speed (Fig. 4C). The effects of 40 µg/L PAR-c exposure were noticeable on high-amplitude swimming angles (classes 2 and 7) as no differences between light transition conditions were found in the

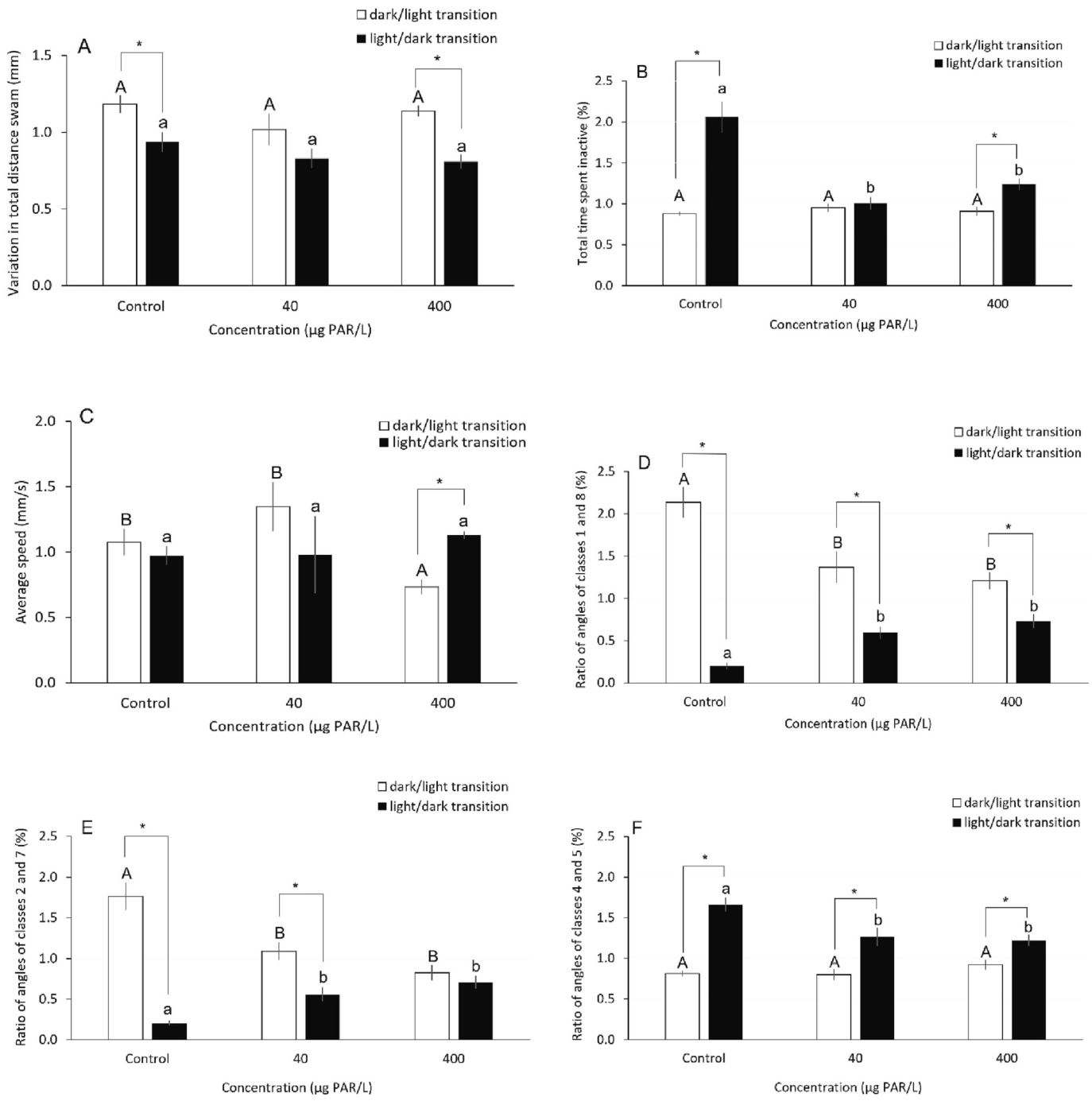


**Fig. 5.** Effects of paroxetine on zebrafish adaptation response to stress induced by sudden light transitions (dark to light and light to dark) after 5 days of exposure. A – Variation in total distance swam (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. White bars represent the dark/light transition and the black bars the light/dark transition. "*" indicates differences between dark/light and light/dark transition for each tested condition; Different letters indicate significant differences across treatments for dark/light (a,b,c) and light/dark transition (A). One-way ANOVA followed by the Holm-Sidak method, $p < 0.05$).

proportion of these classes of angles, unlike the other treatments (Fig. 4E). Furthermore, these organisms, opposite to the control, displayed a significant trend to spend more time inactive 1-min following the dark/light transition (Fig. 4B).

When analysing fish swimming distance within the first 3-min after light transitions, all experimental groups displayed an overall similar pattern, swimming more after the dark/light transition, although not significantly in 40 µg/L PAR-c exposed fish (Fig. 5A). The lack of a differentiated response to the two light transitions was also found for these organisms in terms of total inactivity time, whereas control and 400 µg/L PAR-c exposed fish spent significantly more time inactive after the light/dark transition (Fig. 5B). Moreover, in control and 40 µg/L PAR-c exposed fish, the mean speed of the fish, was higher after the dark/light transition, although not significantly, whereas 400 µg/L fish displayed an opposite significant trend to swim faster after the light/dark transition (Fig. 5C). Additionally, whereas control and 40 µg/L PAR-c exposed fish showed a significantly higher proportion of class 1, 8, 2 and 7 swimming angles during stress adaptation response to the dark/light transition, 400 µg/L fish revealed a disruption of this normal response pattern as they did not respond differently to the two light

transitions.

### 3.3. Swimming behaviour after recovery

Potential long-lasting effects arising from PAR-c acute exposure were also analysed (Figs. 6, 7 and 8). After a 21-day recovery period, the effects of acute PAR-c exposure on fish basal swimming activity and stress response were still observed.

#### 3.3.1. Basal swimming activity

The basal locomotor activity of fish exposed to 40 μg/L PAR-c, was significantly higher than controls and 400 μg/L in both light and dark periods (Fig. 6A). The basal locomotor activity was significantly different in dark and light conditions in all tested conditions, with fish swimming a higher distance during the light period. Effects from acute exposure to PAR-c were also observed in the total time of inactivity, in mean speed and in fish swimming trajectory after the depuration period (Fig. 6B). PAR-c exposed fish displayed a decreased time of inactivity under dark and light conditions. This effect was higher on 40 μg/L

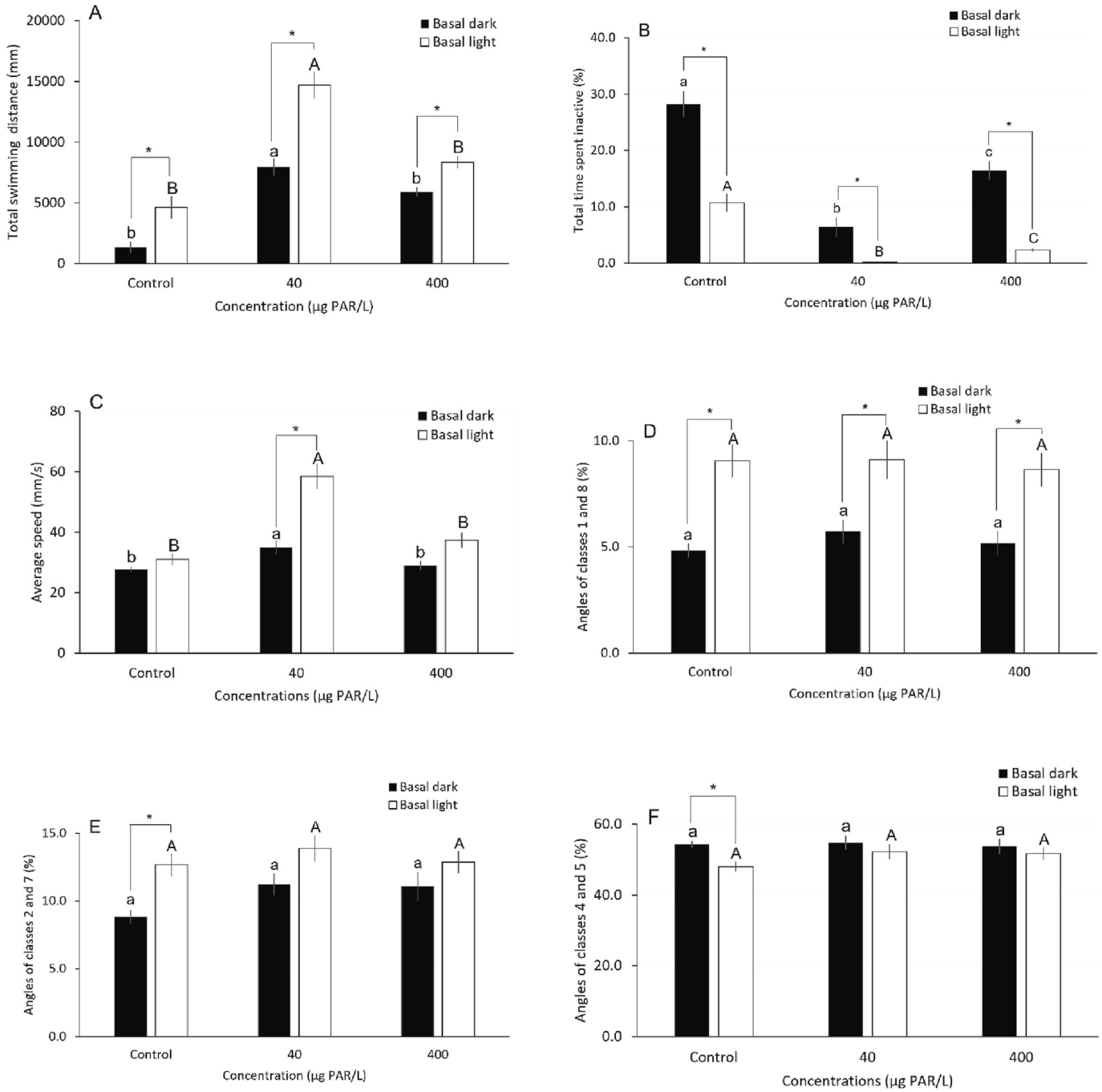


**Fig. 6.** Effects of paroxetine on basal locomotor activity of zebrafish in dark and light conditions after a 21-day depuration period. A – Total swimming distance (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. Black bars represent the dark period and white bars the light one. "*" indicates differences between dark and light periods for each tested condition; Different letters indicate significant differences across treatments for dark (a,b) and light conditions (A,B). One-way ANOVA followed by Dunn's and Holm-Sidak's method, $p < 0.05$).

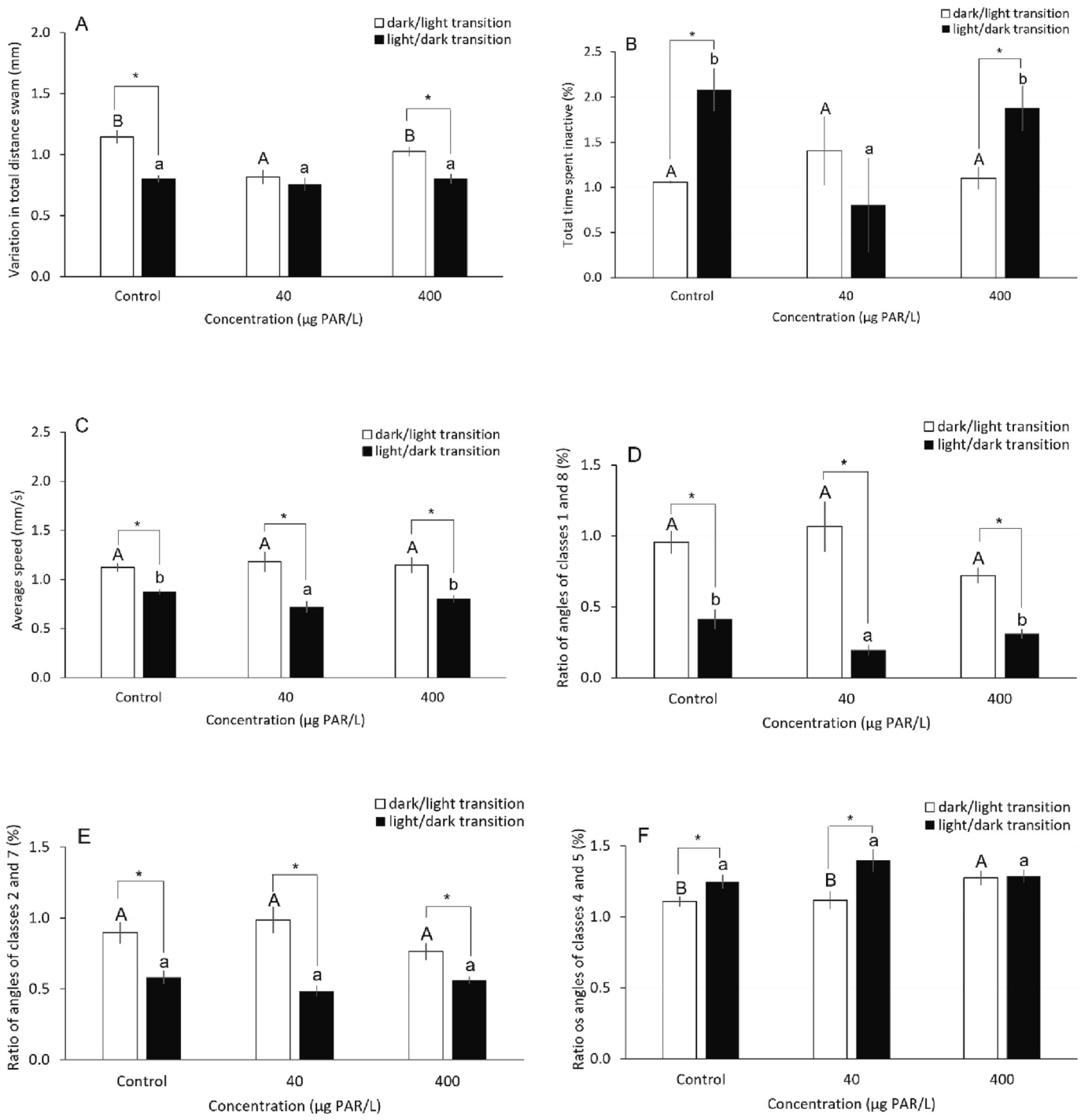


**Fig. 7.** Effects of paroxetine on zebrafish response to stress induced by sudden light transitions (dark to light and light to dark) after a 21-day depuration period. A – Variation in total distance swam (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. White bars represent the dark/light transition and the black bars the light/dark transition. "*" indicates differences between dark/light and light/dark transition for each tested condition; Different letters indicate significant differences across treatments for dark/light (a,b) and light/dark transition (A,B). One-way ANOVA followed by the Holm-Sidak method, $p < 0.05$).

exposed fish that also spent significantly less time in inactivity than 400 µg/L. Moreover, 40 µg/L PAR-c exposed organisms displayed a significantly higher mean speed than control and 400 µg/L PAR-c exposed organisms, in both light and dark periods (Fig. 6C). The analysis of fish swimming angles showed no significant differences, between exposed and non-exposed fish, in the proportion of class 1 and 8 angles (Fig. 6D). However, a disruption of the normal response pattern was observed for exposed fish in terms of the frequency of angles of classes 2 and 7 and classes 4 and 5. These organisms, unlike controls, did not respond differently to dark and light conditions (Figs. 6E and F).

### 3.3.2. *Response to light transitions*

PAR-c exposed fish displayed significant differences to light transitions in total distance swam, total time of inactivity, swimming mean speed and swimming angles, immediately following 1-min and 3-min (Figs. 7 and 8).

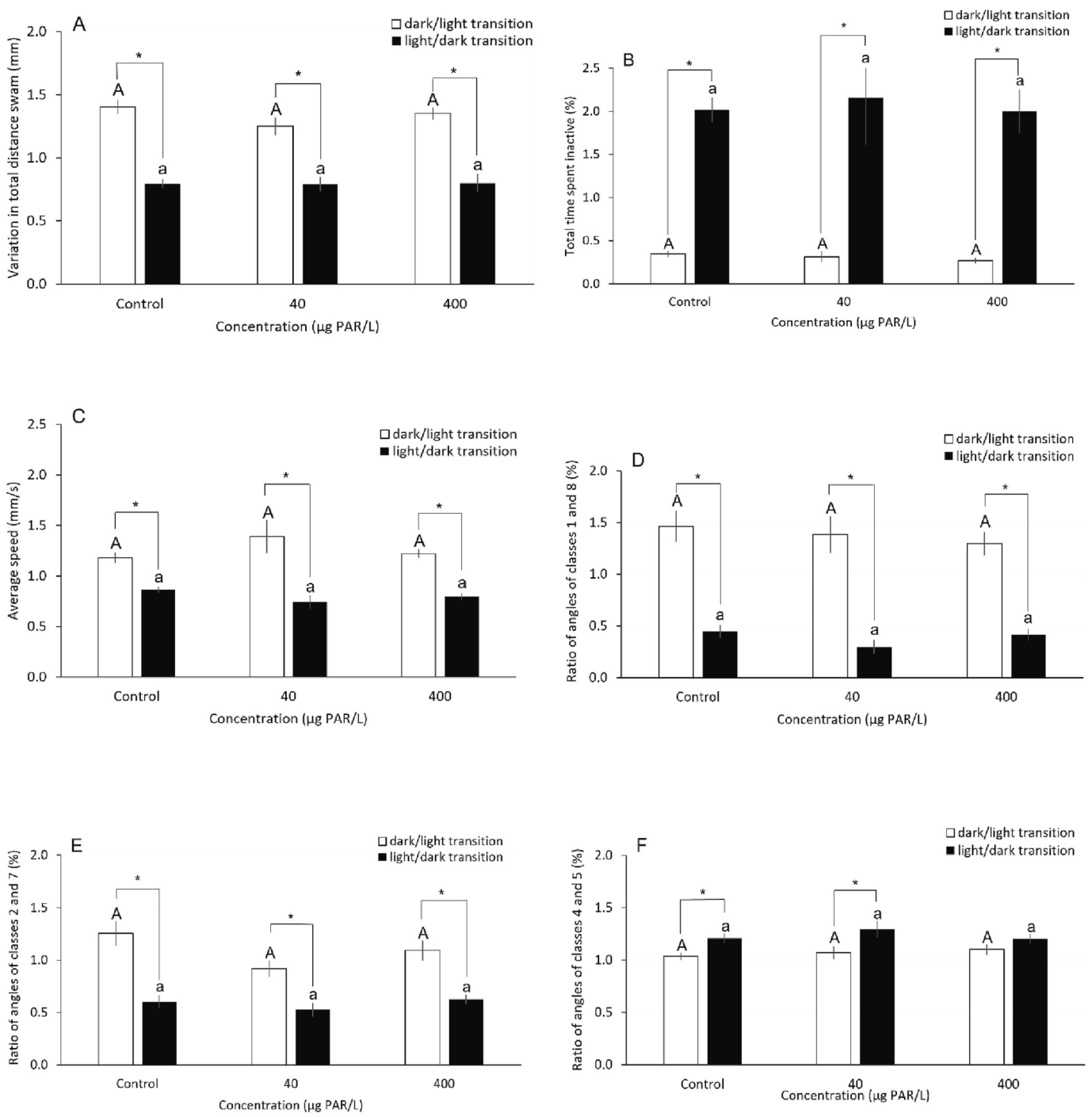


**Fig. 8.** Effects of paroxetine on zebrafish adaptation response to stress induced by sudden light transitions (dark to light and light to dark) after a 21-day depuration period. A – Variation in total distance swam (mm); B – Total time spent in inactivity (%); C- Mean speed (mm/s); D – Ratio of angles of classes 1 and 8 (%); E – Ratio of angles of classes 2 and 7 (%); F- Ratio of angles of classes 4 and 5 (%). Results are expressed as mean values ± standard error. White bars represent the dark/light transition and the black bars the light/dark transition. "*" indicates differences between dark/light and light/dark transition for each tested condition; Different letters indicate significant differences across treatments for dark/light (a,b) and light/dark transition (A,B). One-way ANOVA followed by the Holm-Sidak method, $p < 0.05$).

*3.3.2.1. Dark/light transition.* After depuration, in response to stress induced by the dark/light transition (1 min immediately following the light transition), fish exposed to 40 µg/L PAR-c swam significantly less whereas 400 µg/L PAR-c fish swam in a trajectory with an increased proportion of low-amplitude angles (classes 4 and 5) (Fig. 7, A, F). For the remaining endpoints assessed – total time of inactivity, swimming means speed, and swimming angles of high amplitude – no PAR-c-induced effects were observed (Figs. 7B, C, D and E).

After 3-min of light transition, fish from all experimental groups exhibited a similar stress adaptation response as no significant differences were found between exposed and non-exposed fish (Figs. 8A, B, C, D, E and F).

*3.3.2.2. Light/dark transition.* Within the first min after light transition, fish exposed to 40 µg/L PAR-c reacted to this stressor with decreased inactivity time, mean speed, and class 1 and 8 swimming angles (high

amplitude), when compared to control and 400 μg/L PAR-c exposed organisms (Figs. 7B, C and D). In terms of total swimming distance and proportion of class 2 and 7 swimming angles as well as classes 4 and 5, no PAR-c-related effects were detected (Figs. 7A, E and F).

The analysis of fish stress recovery response (3-min following light transition) revealed that, after 21 days of recovery, fish from all experimental groups respond similarly to this stimulus (Figs. 8A, B, C, D, E and F).

*3.3.2.3. Comparison of fish response pattern between both light transitions (dark/light versus light/dark).* After 21 days of recovery, fish previously exposed to PAR-c displayed a similar pattern of response after 1 min of light condition variation, swimming more after the dark/light transition in comparison with the light/dark transition (Fig. 7A). However, fish exposed to 40 μg/L displayed no significant differences in terms of distance swam, between the two light transitions (dark/light versus light/dark), unlike control and fish previously exposed to 400 μg/L. The analysis of the total time that organisms spent inactive in response to the same stressor (abrupt light variations) showed that control and 400 μg/L PAR-c organisms were more active in dark/light transition, spending more time in inactivity in light/dark transition (Fig. 7B). The 40 μg/L PAR-c exposed fish also showed a disruption of the normal response pattern to light transition for this endpoint, not responding differently to the two light transitions (dark/light versus light/dark).

Fish from all experimental groups displayed a higher proportion of low-amplitude angles (class 4 and 5) 1 min after the dark/light transition (Fig. 7F). However, 400 μg/L PAR-c exposed fish, unlike control and 40 μg/L PAR-c fish, did not react differently to both light transitions, displaying a similar proportion of class 4 and 5 angles, (1-min following light transitions) (Fig. 7F).

During stress response recovery (3-min after light variation), upon dark/light transition, fish from all experimental groups were more active (swimming more and faster), in a trajectory with a higher proportion of high-amplitude angles (class 1, 8, 2 and 7) and a lower proportion of low-amplitude angles (class 4 and 5) (Figs. 8A, B, C, D, E and F). The only noticeable exception was found in terms of class 4 and 5 angles for fish exposed to 400 μg/L that, although exhibiting the same trend, was not significant as for the other treatments (8F).

### 3.4. Integrated biomarker response

The IBR values for the assessed behavioural parameters after 5 days of exposure to 40 and 400 μg/L PAR-c were respectively 46.11 and 44.25, revealing, altered patterns of responses after PAR-c exposure. The values estimated after the depuration period, however, reveal that the degree of alterations was considerably reduced, with IBR values decreasing to 7.68 and 7.38, respectively.

## 4. Discussion

The available information on the effects of PAR to fish may be considered limited, particularly for early and young life stages. This is a critical issue as crucial developmental processes occur during these early stages (Guo et al., 2018), and effects at these stages may have potential negative outcomes on fish development and survival ability.

The data obtained in this study reveal that early and young life stages of fish can be affected by short-term exposure to PAR, in terms of development and behaviour. The initial tests revealed that PAR commercial formulation (PAR-c) was more toxic to embryos/larvae than the active ingredient (PAR-a), suggesting that the pharmaceuticals taken by patients may have a higher impact on biota, associated with the presence of excipients. The observed higher toxicity of PAR-c may be associated with increased bioavailability and time of interaction/resilience in the tissues, promoted by the excipients, often designed to achieve such effects (Martins-Gomes et al., 2022). The results obtained in the present study are in accordance with other studies addressing the toxicity of other bioactive substances (e.g., several insecticides, herbicides, fungicides, and pharmaceuticals), which also reported higher toxicity of the commercial formulations when compared to their pure active ingredients (Beggel et al., 2010; Nagy et al., 2020; Jacob et al., 2016). In one of these studies (Jacob et al., 2016), which assessed the toxicity potential of different formulations of several pharmaceuticals in *Aliivibrio fischeri*, the authors reported that the higher toxicity of drugs is related to the excipients rather than solely to the active ingredients, suggesting that excipients may play a key role in the absorption of the active ingredient in organisms, potentially augmenting their overall toxicity. However, there is insufficient data to explain the underlying mechanisms of such responses.

The data from the embryo/larvae tests showed a limited effect of the tested concentrations, until 96 h of exposure. However, from 96 h onwards, the abnormalities rate increased in a dose-dependent manner at concentrations higher than 819 μg/L. Thus, these data suggest a higher sensitivity of larvae to PAR (PAR-a and PAR-c) than embryos, as reported in previous studies (Kristofco et al., 2018; Oliveira et al., 2016; Qian et al., 2019; Domingues et al., 2010). This life stage-dependent sensitivity may be associated with the protection provided by the chorion, which acts as a physical-chemical barrier, particularly to lipophilic compounds like PAR (Braunbeck et al., 2005), protecting the embryo from contact with xenobiotics (Domingues et al., 2010), but also with the development stage of fish tissues (Qian et al., 2019). This last hypothesis is supported by the fact that the zebrafish biotransformation system is not fully developed until around 3–5 days post-fertilization (Katoch and Patial, 2021). In this sense, the toxic effects observed in fish exposed to PAR-c may be associated with its metabolization to more reactive metabolites as corroborated by the lethal sensitivity estimated for PAR-c for the juvenile stage (96 h $LC_{50}$–791.7 μg/L) > embryonic stage (96 h $LC_{50}$–9000 μg/L). Nonetheless, the hypothesis that the observed effects at the larval stage may also be associated with a sequence of events starting at the embryo stage, which may lead to a higher sensitivity at the larval stage, must also be considered.

Fish swimming activity can be considered an environmentally relevant endpoint, playing a key role in many fitness-related behaviours, such as feeding, predator avoidance, social interaction, and reproduction (Faimali et al., 2017; Ferreira et al., 2023b; Ford et al., 2021). It is considered sensitive to environmental contaminants and has been increasingly used to assess behavioural toxicity (Faimali et al., 2017; Ferreira et al., 2023b; Kluver et al., 2015; Nusser et al., 2016; Velki et al., 2017). In this study, PAR-c-exposed fish were significantly less active than control fish, both in light and dark conditions (swam lower distances and spent more time inactive). These PAR-c-induced swimming behavioural effects were more evident under dark conditions (e.g., erratic behaviour was only found in this condition). The findings of the present study are in line with a previous study that reported decreased locomotor activity in zebrafish adults after a 10-min acute exposure through immersion in 300 and 3000 μg/L PAR (Fontes et al., 2015). Nonetheless, Hong et al. (2021) observed no effects on the locomotor activity of zebrafish juveniles after 21-day exposure to 0.1, 1, 10 and 100 μg/L PAR. Other studies have found that short-term exposure (from 30 min to 14 days) to the SSRI fluoxetine (0.3–3457.9 μg/L) led to behavioural alterations (e.g., decreased feeding behaviour, reduced exploratory and locomotor behaviour, antipredator behaviour disruption) in adults and juveniles of various fish species (e.g., *D. rerio*, *Betta splendens, Cyprinodon variegatus, Cichlasoma dimmers, Poecilia reticulata*) (Eisenreich et al., 2017; Eisenreich and Szalda-Petree, 2015; Vera-Chang et al., 2018; Vera-Chang et al., 2019; Winder et al., 2012; Correia et al., 2022; Dorelle et al., 2020; Saaristo et al., 2017; Meijide et al., 2018). The hypoactivity observed in the present study was more pronounced in fish exposed to 40 μg/L (under light conditions). These organisms also displayed a disruption of the normal response pattern to the light shift stimulus (light versus dark conditions) for all the assessed endpoints (distance moved, time spent in inactivity and swimming angles), except

for mean speed. These results suggest a non-monotonic dose response, that has already been reported in SSRIs.

Anxiety and fear responses are part of a set of coping strategies that ensure fitness-enhancing processes, such as adaptive antipredator responses, foraging efficiency, mating opportunities, and exploration (Ferreira et al., 2023a; Salahinejad et al., 2022). It is known that, when subjected to sudden light transitions, fish may display behaviour alterations in activity (e.g., zebrafish larvae display hyperactivity when subjected to an abrupt transition from light to dark) (Egan et al., 2009; Kalueff et al., 2013; Burton et al., 2017). Nonetheless, to the authors' knowledge, there is no available data on the ability of PAR to interfere with fish stress response. The results obtained in the present study demonstrate that PAR-c acute exposure can affect not only fish's immediate response to stress but also their adaptation response. The analysis of control fish swimming behaviour suggests that the sudden switch from dark to light works as a startle resulting in fish hyperactivity and erratic behaviour. Upon this light transition, the PAR-c exposed fish displayed higher freezing and swimming speed than the control fish. Although this effect was observed in organisms at both PAR-c tested concentrations, the changes in the stress response were more pronounced at the lowest tested concentration (40 μg/L PAR-c) that, in addition to the higher freezing and swimming speed, also exhibited higher erratic behaviour, suggesting a higher stress response to this PAR-c concentration.

The alteration of swimming behaviour of control fish, upon light to dark shift, suggests that this transition may be perceived by fish as a potential threat (e.g., the shadow of a predator, to which fish respond with freezing to go unnoticed followed by an escape attempt to minimize the risk of predation), as fish increased freezing, swimming distance, and straight motion. PAR-c-exposed fish (40 μg/L and 400 μg/L) presented a different response pattern, reducing freezing and straight motion and increasing erratic behaviour. These PAR-induced modifications in the stress response were more evident in fish exposed to 40 μg/L PAR-c, which also displayed an increased swimming distance, suggesting higher stress. In this light transition, data also suggest a different perception of the stimulus from control and PAR-c exposed fish, which although responding to a stimulus, adopted a different behavioural strategy that might be related to fish becoming bolder. Given that boldness is a fish behavioural trait known to correlate with serotonin levels, it is expectable that drugs that influence the expression of the serotonergic system, such as SSRIs, could potentially induce phenotypic behavioural modulation (Ferreira et al., 2023a; Fior et al., 2018).

Effects of PAR-c exposure were also noticeable in the ability of fish to adapt and recover from the light transitions. PAR-c exposed fish (40 μg/L and 400 μg/L) appeared to recover faster (decreased erratic swimming) from the stressful stimulus induced by the dark/light transition when compared to controls, which is consistent with a PAR-c anxiolytic effect. However, on the recovery to the light/dark transition, PAR-c-exposed fish (40 μg/L and 400 μg/L) displayed higher stress behaviour (reduced freezing and straight motion; increased erratic movements). These behavioural modifications were more evident in 40 μg/L PAR-c exposed fish as these organisms also showed hyperactivity associated with increased swimming distance. The impact of PAR-c seems to be greater on the capacity to respond to the light/dark transition, supporting the idea that there may be an altered perception of the type of stimulus.

Overall, the data show the ability of PAR-c to alter the recognition of the light stimulus and induce more effects at the lowest tested concentration (40 μg/L). All the assessed behaviour parameters were responsive to light condition variations, demonstrating their suitability and sensitivity for SSRIs toxicity screening. The mechanisms involved in the observed response patterns should be studied in more detail, as different PAR-c effects were observed in fish behaviour depending on the light conditions and tested concentrations. The role played by excipients in the effects of pharmaceuticals' active ingredients should also be further explored. To the authors' knowledge, this is the first report on the effects of PAR-c on the stress response. The obtained results suggest that PAR-c may interfere with light perception and ultimately fish optomotor response (OMR). It is known that light perception affects the production of glucocorticoids via functional connections of the retina to the hypothalamus-pituitary-adrenal (HPI) axis, therefore modulating stress response (Sakamoto and Sakamoto, 2019; Muto et al., 2013). On the other hand, it is well established that SSRIs antidepressants like PAR, interfere with the expression of the serotonergic system which in turn interacts with both the adrenergic system and the HPI axis, also modulating stress response (Sumpter and Margiotta-Casaluci, 2022; Kreke and Dietrich, 2008). The ability of SSRIs to induce anxiolytic-like effects has already been reported after exposure (from 15 min to 35 days) to fluoxetine (0.025–100 μg/L) on a variety of fish species (e.g., *D. rerio, Gasterosteus aculeatus, Oryzias latipes, Gambusia holbrooki*, *Poecilia reticulata*, *Pimephales promelas*) (Saaristo et al., 2017; Meijide et al., 2018; de Farias et al., 2020; Martin et al., 2017; Pelli and Connaughton, 2015; Martin et al., 2020; Ansai et al., 2019). However, in these studies, the behavioural assessment was strictly done under light conditions (during daytime), using different behavioural protocols/tests. The results obtained in this study also suggest a PAR-c anxiolytic effect in the light and during recovery to the dark/light transition.

An important aspect to be considered when assessing the potential effects of contaminants is the ability of animals to revert from the potentially pernicious effects of exposure. Data show that acute exposure to PAR-c is still reflected in fish basal swimming activity and stress response, after 21 days of depuration. Fish previously exposed to PAR-c still showed signals of hyperactivity under both dark and light conditions that were more noteworthy in 40 μg/L exposed fish. Furthermore, when subjected to stress induced by both light transitions (dark/light and light/dark), exposed fish still exhibited significant changes in stress response, also more evident in fish exposed to 40 μg/L PAR-c. These depuration data suggest a delay in the stress response, probably due to an anxiolytic effect of 40 μg/L. The fish previously exposed to 400 μg/L PAR-c displayed a higher number of straightforward movements upon dark/light transition after the depuration period than the control, and their ability to differentiate both light transitions (dark/light versus light/dark) was affected, suggesting potential alterations in light perception.

The observed PAR-c-induced alterations in the response to stress and stress adaptation may adversely impact fish's ability to optimize their response to challenges they may find in nature (e.g., predator escape/avoidance). The impairment of swimming efficiency will directly interfere with the predator-prey interactions (e.g., to eat or to be eaten), potentially compromising individual growth and survival and, ultimately, population persistence.

Overall, behavioural data suggest neuronal disruption by PAR. These outcomes highlight the need for further studies to increase knowledge of PAR's single effects on behaviour at environmentally relevant exposure scenarios but also on the role of PAR in modulating the behavioural response of other environmental contaminants.

## 5. Conclusions

This study demonstrated that the early and young life stages of fish may be affected by PAR presence, highlighting its value as a useful and sensitive model for contaminant toxicity assessment.

Data allowed the estimation of PAR benchmarks, providing $LC_x$ and $EC_x$ liable to contribute to improved toxicity databases and support the idea that the toxic effects in embryos/larvae may be associated with fully developed fish (e.g., fully functional biotransformation system). The commercial formulation (PAR-c) was more toxic than the active ingredient (PAR-a), highlighting the relevance of the interaction of the active drug/excipients in the observed effects.

The analysis of behaviour modifications induced by light transitions (dark/light and light/dark) proved a sensitive and useful screening tool for the assessment of impaired adaptative ability, and, therefore, should

be considered in future studies regarding this and other SSRIs. Behavioural data provided evidence of a clear non-monotonic response pattern in PAR-c exposed fish and that PAR-c exposure can have lasting effects on behaviour, impairing normal fish responses (e.g., behavioural strategies) to stress-inducing conditions, and compromising their sensitivity and perception of stress.

Thus, data highlights the need for more studies regarding the effects of PAR to improve the knowledge regarding potential ecological consequences of PAR in the environment, namely long-term vigilance of effects even after drug exposure suppression.

## CRediT authorship contribution statement

**Carla S.S. Ferreira:** Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Cátia Venâncio:** Investigation, Data curation, Writing – review & editing. **Peter Kille:** Writing – review & editing, Supervision. **Miguel Oliveira:** Conceptualization, Resources, Methodology, Formal analysis, Visualization, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

## Acknowledgements

Thanks are due to the financial support to CESAM by FCT - Foundation for Science and Technology (UIDB/50017/2020 + UIDP/50017/2020+LA/P/0094/2020) and to FCT/MCTES for the financial support to the project NanoPlanet (2022.02340.PTDC) through national funds (OE). Carla Melo was supported by FCT through a PhD Grant (2021.04580.BD).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165706.

## References

Almeida, A.R., Tacão, M., Machado, A.L., Golovko, O., Zlabek, V., Domingues, I., Henriques, I., 2019. Long-term effects of oxytetracycline exposure in zebrafish: A multi-level perspective. Chemosphere 222, 333–344.

Ansai, S., Hosokawa, H., Maegawa, S., Kinoshita, M., 2019. Chronic fluoxetine treatment induces anxiolytic responses and altered social behaviors in medaka Oryzias latipes. Behavioural Brain Research. 303, 126–136.

Beggel, S., Werner, I., Connon, R.E., Geist, J.P., 2010. Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (Pimephales promelas). Sci. Tot. Environ. 408 (16), 3169–3175.

Bourin, M., Chue, P., Guillon, Y., 2001. Paroxetine: a review. CNS Drug Reviews 7, 25–47.

Braunbeck, T., Bottcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., Rudolf, M., Seitz, N., 2005. Towards an alternative for the acute fish LC50 test in chemical assessment: the fish embryo toxicity test goes multi-species — an update. Altex-Altern. Tierexp. 22, 87–102.

Burkina, V., Zlabeka, V., Zamaratskaia, G., 2015. Effects of pharmaceuticals present in an aquatic environment on Phase I metabolism in fish (Review). Environmental Toxicology and Pharmacology 40, 430–444.

Burns, E.E., Carter, L.J., Snape, J., Thomas-Oates, J., Boxall, A.B.A., 2018. Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals. Journal of Toxicology and Environmental Health, Part B. Critical Reviews 1 (3), 115–141.

Burton, C.E., Zhou, Y., Bai, Q., Burton, E.A., 2017. Spectral properties of the zebrafish visual motor response. Neuroscience Letters. 646, 62–67.

Chu, S., Metcalfe, C.D., 2007. Analysis of paroxetine, fuoxetine and norfuoxetine in fsh tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1163, 112–118.

Correia, D., Domingues, I., Faria, M., Oliveira, M., 2022. Chronic effects of fluoxetine on Danio rerio: A biochemical and behavioural perspective. Applied Sciences. 12, 2256.

Cunningham, V.I., Constable, D.J.C., Hannah, R.E., 2004. Environmental Risk Assessment of Paroxetine. Environmental Science and Technology 38, 3351–3359.

Diaz-Camal, N., Cardoso-Vera, J.D., Islas-Flores, H., Gómez-Oliván, L.M., Mejía-García, A., 2022. Consumption and occurrence of antidepressants (SSRIs) in pre- and post-COVID-19 pandemic, their environmental impact and innovative removal methods: A review. Science of The Total Environment 829, 154656.

Domingues, R., Oliveira, J., Lourenço, C., Koppe, S., Mendo, A.M.V.M. Soares, 2010. Biomarkers as a tool to assess effects of chromium (VI): comparison of responses in zebrafish early life stages and adults. Comp. Biochem. Physiol. C 152, 338–345.

Dorelle, L.S., Da Cuña, R.H., Sganga, D.E., Rey Vázquez, G., López Greco, L., Lo Nostro, F. L., 2020. Fluoxetine exposure disrupts food intake and energy storage in the cichlid fish Cichlasoma dimerus (Teleostei, Cichliformes). Chemosphere 238.

Dzieweczynski, T.L., Kane, J.L., Campbell, B.A., Lavin, L.E., 2016. Fluoxetine exposure impacts boldness in female Siamese fighting fish. Betta splendens. Ecotoxicology 25, 69–79.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhavat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural Brain Research 205 (1), 38–44.

Eisenreich, B.R., Szalda-Petree, A., 2015. Behavioral effects of fluoxetine on aggression and associative learning in Siamese fighting fish (Betta splendens). Behavioural Processes 121, 37–42.

Eisenreich, B.R., Greene, S., Szalda-Petree, A., 2017. Of fish and mirrors: Fluoxetine disrupts aggression and learning for social rewards. Physiology and Behavior. 173, 258–262.

Faimali, M., Gambardella, C., Costa, E., Piazza, V., Morgana, S., Estevez-Calvar, N., Garaventa, F., 2017. Old model organisms and new behavioural endpoints: Swimming alteration as an ecotoxicological response. Marine Environmental Research 128, 36–45.

de Farias, N.O., Oliveira, R., Moretti, P.N.S., e Pinto, J.M., Oliveira, A.C., Santos, V.L., Rocha, P.S., Andrade, T.S., Grisolia, C.K., 2020. Fluoxetine chronic exposure affects growth, behaviour and tissue structure of zebrafish. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 237, 108836.

Ferreira, C.S.S., Soares, S.C., Kille, P., Oliveira, M., 2023a. Identifying knowledge gaps in understanding the effects of selective serotonin reuptake inhibitors (SSRIs) on fish behaviour. Chemosphere 335, 139124.

Ferreira, C.S.S., Venâncio, C., Oliveira, M., 2023b. Nanoplastics and biota behaviour: Known effects, environmental relevance, and research needs. Trends in Analytical Chemistry 165, 117129.

Fior, D., Dametto, F., Fagundes, M., Santos da Rosa, J.G., Sander de Abreu, M., Koakoski, G., Idalencio, R., de Alcântara Barcellos, H.H., Piato, A., Gil Barcellos, L.J., 2018. Divergent action of fluoxetine in zebrafish according to responsivity to novelty. Sci. Rep. 8, 13908.

Magno, L.D.P, Fontes, A., B.M.N. Gonçalves, Gouveia Jr., A. Pharmacological study of the light/dark preference test in zebrafish (Danio rerio): Waterborne administration. Pharm. Biochem. Behav. 2015, 135, 169-176.

Ford, A.T., Ågerstrand, M., Brooks, B.W., Allen, J., Bertram, M.G., Brodin, T., Dang, Z., Duquesne, S., Sahm, R., Hoffmann, F., Hollert, H., Jacob, S., Klüver, N., Lazorchak, J., Ledesma, M., Melvin, S.D., Mohr, S., Padilla, S., Pyle, G., Scholz, S., Saaristo, M., Smit, E., Steevens, J.A., van den Berg, S., Kloas, W., Wong, B.B.M., Ziegler, M., Maack, G., 2021. The role of behavioral ecotoxicology in environmental protection. Environ. Sci. Technol. 55, 5620–5628.

Grabicova, K., Grabic, R., Fedorova, G., Fick, J., Cerveny, D., Kolarova, J., Turek, J., Zlabek, V., Randak, T., 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res 124, 654–662.

Guo, X., Zhang, S., Lu, S., Zheng, B., Xie, P., Chen, J., Li, G., Liu, C., Wu, Q., Cheng, H., Sang, N., 2018. Perfluorododecanoic acid exposure induced developmental neurotoxicity in zebrafish embryos. Environ. Pollut. 241, 1018e1026.

Hong, X., Zhao, G., Zhou, Y., Chen, R., Li, J., Zha, J., 2021. Risks to aquatic environments posed by 14 pharmaceuticals as illustrated by their effects on zebrafish behaviour. Sci. Tot. Environ. 771, 145450.

Huang, I.J., Sirotkin, H.I., McElroy, A.E., 2019. Varying the exposure period and duration of neuroactive pharmaceuticals and their metabolites modulates effects on the visual motor response in zebrafish (Danio rerio) larvae. Neurotoxicology and Teratology 72, 39–48.

Huang, I.J., Dheilly, N.M., Sirotkin, H.I., McElroy, A.E., 2020. Comparative transcriptomics implicate mitochondrial and neurodevelopmental impairments in larval zebrafish (Danio rerio) exposed to two selective serotonin reuptake inhibitors (SSRIs). Ecotoxicology and Environmental Safety 203, 110934.

Jacob, R.S., Santos, L.V.S., Souza, A.F.R., Lange, L.C., 2016. A toxicity assessment of 30 pharmaceuticals using Aliivibrio fischeri: a comparison of the acute effects of different formulations. Environ. Tech. 37 (21), 2760–2767.

Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C.P., Pittman, J., Rosemberg, D.B., Gerlai, R., Echevarria, D., Lamb, E., Neuhauss, S.C.F., Weng, W., Bally-Ciuf, L., Schneider, H., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish. 10 (1), 70–86.

Katoch, S., Patial, V., 2021. Zebrafish: An emerging model system to study liver diseases and related drug discovery. J. Appl. Toxicol. 41, 33–51.
Kellner, M., Olsén, K.H., 2020. Divergent Response to the SSRI Citalopram in Male and Female Three Spine Sticklebacks (Gasterosteus aculeatus). Archives of Environmental Contamination and Toxicology 79, 478–487.
Kleywegt, S., Payne, M., Ng, F., Fletcher, T., 2019. Environmental loadings of Active Pharmaceutical Ingredients from manufacturing facilities in Canada. Science of The Total Environment 646, 257–264.
Kluver, N., Konig, M., Ortmann, J., Massei, R., Paschke, A., Kuhne, R., Scholz, S., 2015. Fish embryo toxicity test: identification of compounds with weak toxicity and analysis of behavioural effects to improve prediction of acute toxicity for neurotoxic compounds. Environ. Sci. Technol. 49, 7002–7011.
Kowalska, M., Nowaczyk, J., Fijałkowski, Ł., Nowaczyk, A., 2021. Paroxetine - Overview of the Molecular Mechanisms of Action (Review). International Journal of Molecular Sciences 22, 1662.
Kreke, N., Dietrich, D.R., 2008. Physiological Endpoints for Potential SSRI Interactions in Fish. Critical Reviews in Toxicology. 37 (3), 215–247.
Kristofco, L.A., Haddad, S.P., Chambliss, C.K., Brooks, B.W., 2018. Differential uptake of and sensitivity to diphenhydramine in embryonic and larval zebrafish. Environ. Toxicol. Chem. 37, 1175–1181.
Martin, J.M., Saaristo, M., Bertram, M.G., Lewis, P.J., Coggan, T.L., Clarke, B.O., Wong, B.B., 2017. The psychoactive pollutant fluoxetine compromises antipredator behaviour in fish. Environ. Pollut. 222, 592–599.
Martin, J.M., Nagarajan-Radha, V., Tan, H., Bertram, M.G., Brand, J.A., Saaristo, M., Dowling, D.K., Wong, B.B., 2020. Antidepressant exposure causes a nonmonotonic reduction in anxiety-related behaviour in female mosquitofish. J. Hazard. Mater. Lett. 1, 100004.
Martins-Gomes, C., Silva, T.L., Andreani, T., Silva, A.M., 2022. Glyphosate vs. glyphosate-based herbicides exposure: A review on their toxicity. Journal of Xenobiotics 12 (1), 21–40.
McDonald, M.D., 2017. An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish (Review). Comparative Biochemistry and Physiology 197, 19–31. Part C.
Meijide, F.J., Da Cuña, R.H., Prieto, J.P., Dorelle, L.S., Babay, P.A., Nostro, F.L.L., 2018. 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.
Mezzelani, M., Gorbi, S., Regoli, F., 2018. Pharmaceuticals in the aquatic environments: Evidence of emerged threat and future challenges for marine organisms. Marine Environmental Research 140, 41–60.
Mole, R.A., Brooks, B.W., 2019. Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. Environmental Pollution 250, 1019–1031.
Muto, A., Taylor, M.R., Suzawa, M., Korenbrot, J.I., Baier, H., 2013. Glucocorticoid receptor activity regulates light adaptation in the zebrafish retina. Frontiers in Neural Circuits 7, 145.
Nagy, K., Duca, R.C., Lovas, S., Creta, M., Scheepers, P.T.J., Godderis, L., Ádám, B., 2020. Systematic review of comparative studies assessing the toxicity of pesticide active ingredients and their product formulations. Environ. Res. 181, 108926.
Nowakowska, K., Giebułtowicz, J., Kamaszewski, M., Adamskic, A., Szudrowicz, H., Ostaszewska, T., Solarska-Dzięciołowskaa, U., Nałęcz-Jaweckib, G., Wroczyński, P., Drobniewska, A., 2020. Acute exposure of zebrafish (Danio rerio) larvae to environmental concentrations of selected antidepressants: Bioaccumulation, physiological and histological changes. Comparative Biochemistry and Physiology, Part C 229, 108670.
Nowicki, M., Tran, S., Muraleetharan, A., Markovic, S., Gerlai, R., 2014. Serotonin antagonists induce anxiolytic and anxiogenic like behavior in zebrafish in a receptor-subtype dependent manner. Pharmacology Biochemistry and Behavior 126, 170–180.
Nusser, L., Skulovich, O., Hartmann, S., Seiler, T., Cofalla, C., Schuettrumpf, H., Hollert, H., Salomons, E., A., 2016. A sensitive biomarker for the detection of aquatic contamination based on behavioral assays using zebrafish larvae. Ecotoxicol. Environ. Saf. 133, 271–280.
Oliveira, R., Grisolia, C.K., Monteiro, M.S., Soares, A.M., Domingues, I., 2016. Multilevel assessment of ivermectin effects using different zebrafish life stages. Comp. Biochem. Physiol. C 187, 50–61.
Paroxetine FDA Label [Link].
Pelli, M., Connaughton, V.P., 2015. Chronic exposure to environmentally relevant concentrations of fluoxetine (Prozac) decreases survival, increases abnormal behaviours, and delays predator escape responses in guppies. Chemosphere. 139, 202–209.
Puckowski, A., Mioduszewska, K., Łukaszewicz, P., Borecka, M., Caban, M., Maszkowska, J., Stepnowski, P., 2016. Bioaccumulation and analytics of pharmaceutical residues in the environment: A review. Journal of Pharmaceutical and Biomedical Analysis 127, 232–255.
Qian, L., Qi, S., Cao, F., Zhang, J., Li, C., Song, M., Wang, C., 2019. Effects of penthiopyrad on the development and behaviour of zebrafish in early-life stages. Chemosphere. 214, 184–194.
Saaristo, M., McLennan, A., Johnstone, C.P., Clarke, O., B., Wong, B. B. M., 2017. Impacts of the antidepressant fluoxetine on the anti-predator behaviours of wild guppies (Poecilia reticulata). Aq. Toxicol. 183, 38–45.
Sakamoto, T., Sakamoto, H., 2019. 'Central' actions of corticosteroid signaling suggested by constitutive knockout of corticosteroid receptors in small fish. Nutrients 11 (3), 611.
Sakuma, M., 1998. Probit analysis of preference data. Appl. Entomol. Zool. 33, 339–347.
Salahinejad, A., Attaran, A., Meuthen, D., Chivers, D.P., Niyogi, S. Review, 2022. Proximate causes and ultimate effects of common antidepressants, fluoxetine and venlafaxine, on fish behavior. Science of the Total Environment 807, 150846.
Sanchez, Wilfried, Burgeot, Thierry, Porcher, Marc, Jean., 2013. A novel 'integrated biomarker response' calculation based on reference deviation concept. Environ. Sci. Pollut. Control Ser. 20 (5), 2721–2725.
du Sert, N.P., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E. J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 14;18(7):e3000411.
Silva, L.J.G., Lino, C.M., Meisel, L.M., Pena, A., 2012. Review - Selective serotonin reuptake inhibitors (SSRIs) in the aquatic environment: An ecopharmacovigilance approach. Science of the Total Environment 437, 185–195.
Silva, L.J.G., Pereira, A.M.P.T., Meisel, L.M., Lino, C.M., 2015. Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. Environmental Pollution 197, 127–143.
Sumpter, J.P., Margiotta-Casaluci, L., 2022. Environmental Occurrence and Predicted Pharmacological Risk to Freshwater Fish of over 200 Neuroactive Pharmaceuticals in Widespread Use. Toxics 10, 233.
Velki, M., Di Paolo, C., Nelles, J., Seiler, T., Hollert, H., 2017. Diuron and diazinon alter the behavior of zebrafish embryos and larvae in the absence of acute toxicity. Chemosphere 180, 65–76.
Vera-Chang, M.N., St-Jacques, A.D., Gagné, R., Martyniuk, C.J., Yauk, C.L., Moon, T.W., Trudeau, V.L., 2018. Transgenerational hypocortisolism and behavioral disruption are induced by the antidepressant fluoxetine in male zebrafish Danio rerio. Proc. Natl. Acad. Sci. 115 (52), E12435–E12442.
Vera-Chang, M.N., St-Jacques, A.D., Lu, C., Moon, T.W., Trudeau, V.L., 2019. Fluoxetine Exposure During Sexual Development Disrupts the Stress Axis and Results in Sex- and Time- Dependent Effects on the Exploratory Behavior in Adult Zebrafish Danio rerio. Frontiers in Neuroscience. 13, 1015.
Winder, V.L., Pennington, P.L., Hurd, M.W., Wirth, E.F., 2012. Fluoxetine effects on sheepshead minnow (Cyprinodon variegatus) locomotor activity. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes. 47 (1), 51–58.
Zhang, B., Chen, X., Pan, R., et al., 2017. Effects of three different embryonic exposure modes of 2, 2', 4, 4'-tetrabromodiphenyl ether on the path angle and social activity of zebrafish larvae. Chemosphere 169, 542–549.