




Environmental concentrations of fluoxetine antidepressant affect early development of sea urchin *Paracentrotus lividus*

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ABSTRACT

Fluoxetine (FLX), one of the most widely prescribed selective serotonin reuptake inhibitors, is frequently detected in the aquatic environment. In this study we assessed the ecotoxicological effects of FLX on the early life-stages of the sea urchin *Paracentrotus lividus*, a key species in the Mediterranean Sea. Fertilization rate, developmental anomalies and behavioural alterations were evaluated up to 72 h by exposing gametes, zygotes, and embryos (gastrula) to environmental (0.001, 0.01 mg/L) and high concentrations (0.1, 1, 10 mg/L). Further, the different types and frequency of morphological anomalies at larval level were classified to estimate the Index of Contaminant Impact (ICI) at relevant and high concentrations. The ICI was applied to predict which FLX concentrations may pose a risk to sea urchins. Although FLX did not affect fertilization, significant skeletal anomalies and behavioural alterations were found in plutei from each exposed stage. Based on EC50 values, the sensitivity level ranks as follows: zygote > gastrula > sperm. The ICI values indicated high and moderate impacts only at high concentrations. However, a slight impact was also found in plutei from zygote exposure at relevant environmental concentrations, highlighting a potential risk for sea urchin early development. Considering increasing FLX consumption, we suggest to include this PC in monitoring plans, to not exceed levels that may impair and severely affect the early developmental stages of echinoderms. In addition, our findings promote the use of ICI as a novel tool for FLX impact assessment.

1. Introduction

Pharmaceuticals (PCs) are one of the most common groups of emerging contaminants. Their increasing consumption and frequent detection in the marine environment have recently raised the attention of the scientific community (Čelić et al., 2019; Lu et al., 2024; Ramírez-Morales et al., 2024).

To date, antidepressants consumption has increased worldwide, mainly after the COVID-19 pandemic that has led to many psychological disorders such as insomnia, anxiety, post-traumatic stress disorders, and depression (Castillo-Zacarias et al., 2021; Souza et al., 2022; Pinto et al., 2024).

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants. They are frequently detected in surface waters of estuarine and coastal areas at concentrations of up to 1 µg/L (Minguez et al., 2016; Mezzelani et al., 2018; Duarte et al., 2019; Souza et al., 2022; Pinto et al., 2024). Among them, fluoxetine (FLX) – the active ingredient of Prozac® – is one of the most prescribed psychoactive drugs in the market (Fong and Ford, 2014). It is primarily used

to treat various psychiatric disorders being recommended by the World Health Organization (WHO, 2017) as safe and effective for the treatment of depression in humans. In addition, it is also used in the treatment of obsessive-compulsive disorder, anxiety, panic disorder and anorexia. It acts as a selective inhibitor of neuronal serotonin (5-hydroxytryptamine, 5-HT) reuptake, and thereby affecting neuronal signal transmission (Franzellitti et al., 2014; Nielsen et al. 2018; Cortez et al., 2019; Duarte et al., 2019; Yamindago et al., 2021).

After administration, FLX can undergo metabolic biotransformation. However, approximately 10–30 % is excreted in urine as unchanged compound. This incomplete metabolization, coupled with insufficient removal by sewage treatment plants (Vasskog et al., 2006) results in FLX entering the aquatic environment without being completely degraded (Bachour et al., 2020; Diaz-Camal et al., 2022; Moreira et al., 2022). Thus, it has been detected in surface waters worldwide at concentrations ranging from < 1 to 100 ng/L to up to 40 µg/L (Duarte et al., 2022).

Once in the ocean, the unchanged FLX provides for compound stability and lipophilicity, becoming resistant to hydrolysis, photolysis, and microbial degradation processes (Gonzalez-Rey and Bebianno, 2013).

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Acting as SSRI, FLX causes 5-HT increase in post-synaptic receptor sites, by disrupting the endocrine systems and many biological functions of marine invertebrates and vertebrates (Ribeiro et al., 2013; Lopes et al., 2020; Pauly et al., 2021). Thus, acute FLX effects have been observed in marine algae (Minguez et al., 2014; Feijão et al., 2020; Duarte et al., 2022; Li and Gaitan-Espitia, 2024), crustaceans (Minguez et al., 2014), echinoderms (Lo et al., 2021; Pauly et al., 2021), bivalves (Cortez et al., 2019), and gastropods (Fong and Molnar, 2013). At relevant environmental concentrations, FLX causes genotoxicity, immunotoxicity, oxidative stress, and acetylcholinesterase activity inhibition, leading to development, reproduction, and behaviour alterations (Bossus et al., 2014; Di Poi et al., 2013; Franzellitti et al., 2014; Munari et al., 2014; Lacaze et al., 2015; Peters et al., 2017; Duarte et al., 2019; Cortez et al., 2019; Byeon et al., 2020; Lo et al., 2021). Indeed, 5-HT has been reported in oocytes, early embryos and larvae of marine invertebrates (i.e. cnidarian, molluscs, and echinoderms), highlighting its importance in the regulation of oogenesis, embryonic development, metamorphosis, growth, reproduction, and behaviour (Brown and Shaver, 1987; McCauley, 1997; Satterlie et al., 2000; Mayorova et al., 2014; Byeon et al., 2020). For instance, in the sea urchin early development, 5-HT regulates cleavage division, gastrulation, morphogenesis and swimming behaviour (Gustafson and Toneby, 1970; Brown and Shaver, 1987; Xu et al., 2012). Therefore, due to its serotonergic action, FLX exposure can interfere with the regular embryonic development of sea urchins (Lo et al., 2021; Pauly et al., 2021). To date, information on FLX toxicity towards echinoderms is scant and fragmented and it is only available for Atlantic and Pacific Ocean sea urchin species (i.e. *Echinometra lucunter*, *Heliocidaris crassispina*; Lo et al., 2021; Pauly et al., 2021). However, it is important to carry out multi-species studies, even within the same taxonomic group, to improve risk assessment, as species may differ in their susceptibility to pollutants (Martino et al., 2017; Trifuoggi et al., 2017; Di Giglio et al., 2020; Rist et al., 2023).

Within this aim, the ecotoxicological effects of environmental and high concentrations of FLX were evaluated on the early life-stages of the Mediterranean sea urchin *Paracentrotus lividus*, selected for its ecological role and economic importance (Pereira et al., 2020). Through grazing, *P. lividus* regulates the dynamics of seaweed and seagrass (Barrier et al., 2024), including *Posidonia oceanica*, the most important seagrass species for the Mediterranean Sea's ecological coastal balance (Gobert et al., 2006). Moreover, *P. lividus* is an edible species of significant economic importance, with a high market demand for its roes, especially in the Mediterranean and Atlantic (Régis et al., 1986; Barrier et al., 2024), conversely to the co-occurring non-edible and non-harvested sea urchin *Arbacia lixula* (Pais et al., 2007). Its abundance is currently threatened by intense harvesting; therefore, it is crucial to assess whether additional factors, such as exposure to pharmaceuticals, may impact *P. lividus* survival, particularly during its early life stages, which are critical for population dynamics.

Furthermore, due to its sensitivity and cost-effectiveness, the sea urchin development test is frequently adopted to screen the toxicity of environmental matrices and contaminants of emerging concern (Ministry Decree 173/2016; Beiras et al., 2018; Gambardella et al., 2024). Therefore, we assessed FLX toxicity by exposing sperm, fertilized eggs, and embryos. This novel approach based on the exposure of three different biological systems with a different sensitivity towards pollutants (Manzo et al., 2013; Chiarelli et al., 2019) has been proposed for the first time to assess FLX toxicity. Fertilization rate, developmental anomalies and behavioural alterations were then evaluated up to 72 h. Further, we ranked the different types of larval morphological anomalies and their frequency, in order to determine FLX impact at environmental and high concentrations, by estimating an Index of Contaminant Impact (ICI, Gambardella et al., 2021).

2. Materials and methods

2.1. Chemicals

A stock solution of FLX Hydrochloride (CAS:56296-78-7; Sigma-Aldrich, USA) was prepared in dimethyl sulfoxide (DMSO, 100 %). Then, the stock solution was diluted in filtered natural seawater (FNSW; 0.22 µm) to reach desired test concentrations. In order to avoid negative effects on *P. lividus* fertilization and development, the final DMSO concentration never exceeded 0.02 % v/v (Semenova et al., 2024). However, a DMSO control was run in parallel to the PC treatments to confirm the absence of solvent toxicity.

2.2. Sea urchin sampling and gamete emission

Adults of *P. lividus* were sampled in the Ligurian Sea and brought to CNR-IAS laboratory in a refrigerated bag according to Amemiya (1996). An injection of KCl (0.5 M) was administered into the coelomic cavity to induce spawning (Morgana et al., 2016). Eggs and sperm were then taken from three females and three males as reported by Morroni et al. (2016). Specifically, the eggs were collected in FNSW (0.22 µm) at room temperature and washed three times, while the sperm was sampled "dry" - without dilution in seawater - directly from the genital pores and stored in a sterile tube at 4 °C to preserve its activation. Before fertilization, gamete quality (mobile sperm and spherical eggs without damaged or lacking jelly coat) was checked under Leica DMi1 microscope (Carballeira et al., 2011). Then, sperm from three different males was mixed and the same was done with the eggs.

2.3. Sperm exposure

Ten µL of "dry" sperm were exposed for 1 h to the FLX solution at serial concentrations by adding 1 mL of test solution to the Eppendorf vials (Volpi Ghirardini et al., 2005; Gambardella et al., 2013; ISPRA, 2017). The concentrations of 0.001 and 0.01 mg/L were selected to evaluate FLX effects at environmental levels, according to FLX levels measured in surface waters, ranging from 0.3×10^{-6} to 0.04 mg/L (Duarte et al., 2022). High FLX levels - including 0.1, 1 and 10 mg/L - were also tested, based on previous ecotoxicological studies on marine species (Lemaire et al., 2024; Pauli et al. 2021; Duarte et al., 2022) and considering the growing FLX increase in the next future. Negative and solvent (DMSO 0.02 % v/v) controls were run in parallel. Specifically, negative controls were performed by adding 1 mL of standard FNSW.

After 1h, 10 mL of approximately 300 eggs/mL were placed in multi-well plates and 10 µL of sperm from each vial were added (Gambardella et al., 2013). Under normal conditions, egg activation occurs within 60 s from sperm addition. Twenty minutes after sperm addition, 2 mL of water containing ~600 zygotes were collected from each well and observed under a stereomicroscope (Zeiss, Germany) to check the fertilization success (Amaroli et al., 2017).

The remaining zygotes (~2.400 per replica) were left to develop at 18 ± 1 °C in a dark thermostatic room and were monitored for 72 h, corresponding to the late pluteus stage. Then, to assess developmental anomalies, plutei were fixed in 4 % paraformaldehyde, observed under a Leica DMi1 microscope and photographed with a digital camera. To this end, 100 plutei were counted for each replicate and classified as either 'developed' or 'non-developed', depending on their synchronous development and morphology (Gambardella et al., 2024). In detail, the first category included well-structured larvae, while the second one showed delayed or malformed development. Before fixation, a Behavioural Alteration Recorder (BAR) system was used to measure the larval Swimming Speed Alteration (SSA) rate (Faimali et al., 2006). To reach a steady speed and uniform spatial distribution, sea urchin larvae were dark-adapted for 2 min before videorecording. After this acclimation period, swimming behaviour was then recorded for 5 s under dark conditions (Morgana et al., 2016). The images were finally analysed

with an advance image analysis software (Biomonitor), determining the average swimming speed (mm/s). Data were expressed as SSA percentage normalized to controls' swimming speed (Morgana et al., 2016).

2.4. Zygote and embryo exposure

A 1000 eggs/mL egg suspension was mixed with 10 μ L of sperm diluted 1:1000 in FNSW (Sartori et al., 2017). After 20 min, four sub-samples were observed under a stereomicroscope to check fertilization success. The acceptability criteria were fixed at > 90 % fertilization rate, according to Carballeira et al. (2011). Zygote exposure was then carried out by adding 1 mL of fertilized egg suspension into each well, previously filled with 9 mL of test solution (0.001, 0.01, 0.1, 1, 10 mg/L). A total of 1000 fertilized eggs were obtained for each replicate. The plates were finally incubated at 18 ± 1 °C in the dark, for 72 h (Sartori et al., 2017). Developmental anomalies and SSA were monitored as reported above.

The same procedure was used for exposing 24-h embryos (gastrula stage), whose development and SSA were monitored for 48h, corresponding to the late pluteus stage.

2.5. Index of Contaminant Impact (ICI)

'Non-developed' plutei, obtained from sperm, zygote and gastrula exposure, were ranked based on 18 types of reversible and irreversible anomalies, according to Gambardella et al. (2021). For each FLX concentration, the frequency of each type of anomaly, expressed as percentages, was used to calculate the Index of Contaminant Impact (ICI; Gambardella et al., 2021).

To assess the degree of FLX toxicity and its environmental impact, the following formula was applied:

$$ICI = [0 \times \% \text{ level } 0 + 1 \times \% \text{ level } 1 + 2 \times \% \text{ level } 2 + 3 \times \% \text{ level } 3] / 100$$

The ICI index ranged from 0 (no impact) to 3 (high impact), also including the levels 1 (slight impact) and 2 (moderate impact).

2.6. Statistical data analysis

All experiments were carried out in triplicate. All data are expressed as means \pm standard error (SE) of the triplicates. EC50s (Effective Concentration resulting into 50 % unfertilized eggs, developmental anomalies or behavioural alterations in the exposed organisms) and related 95 % confidence limits were calculated using Trimmed Spearman Karber analysis (Finney, 1978). Normality and homogeneity of variances were tested by groups with the Shapiro-Wilk and Levene's tests, respectively, by using a R statistical software (R version 4.0.2). ANOVA (One-way analysis of variance) and Dunnett's multiple comparison tests were then used to determine any significant differences between controls and treated samples (* $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$).

3. Results

3.1. Fertilization rate

Fertilizing ability was not affected by any FLX concentrations and solvent control (data not shown) if compared to controls (0 mg/L; Fig. 1), since >90 % of fertilized eggs were observed after 20 min of sperm addition.

3.2. Development

The percentage of developed and non-developed larvae, sampled at 72 h from sperm, zygote and at 48h from gastrula exposure to FLX is shown in Fig. 2. No effect was recorded for the solvent control (<4 %,

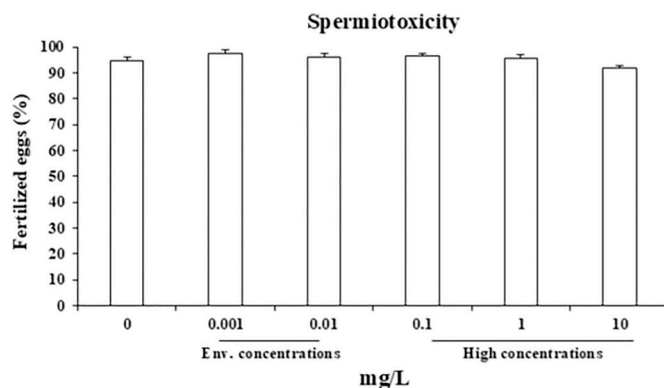


Fig. 1. Percentage of fertilized eggs after sperm exposure to environmental and high concentrations (0.001, 0.01, 0.1, 1, 10 mg/L) of FLX. Data are mean \pm standard error ($M \pm SE$) of three replicates. No significant differences were observed (One-Way ANOVA). Env: environmental.

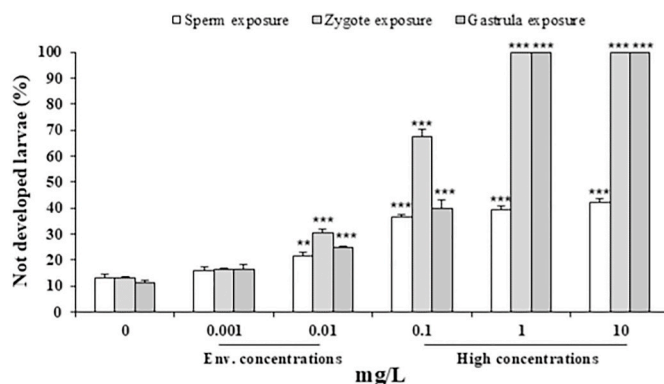


Fig. 2. Percentage of not developed larvae (72 h) from sperm (white bars), zygote (light grey bars) and gastrula (grey bars) exposure to environmental and high concentrations (0.001, 0.01, 0.1, 1, 10 mg/L) of FLX. Data are mean \pm standard error ($M \pm SE$) of three replicates. ** $p < 0.001$; *** $p < 0.0001$. Env: environmental.

data not shown), however, plutei obtained from each treatment showed significant percentages of delayed or malformed development starting from environmental concentrations (0.01 mg/L).

Sperm exposure had a significant 42 % increase of not developed larvae at the highest tested concentration (10 mg/L), while zygote and gastrula exposure showed 100 % of anomalous plutei already at 1 mg/L.

Thus, EC50 values in terms of anomalous development were calculated only after zygote and gastrula exposure (Fig. S1, Table 1), ranging from 0.05 mg/L to 0.12 mg/L.

The different anomaly types found in plutei by exposing different early stages (i.e. sperm, zygote, gastrula) are reported in Fig. 3 and Fig. S2.

Sperm exposure to FLX mainly induced slight anomalies typically occurring in controls, such as asymmetrical or truncated plutei (Type 12

Table 1

EC50 values and their confidence limits (C.L) of sea urchin larvae (72h) obtained from the exposure of sperm, zygote and gastrula to FLX. Values are expressed in mg/L. n.c.: not calculable.

EC50 values (C.L), mg/L		
Stage exposure	Development	Swimming behaviour
sperm	>10 (n.c.)	>10 (n.c.)
zygote	0.05 (0.03–0.06)	0.17 (0.13–0.22)
gastrula	0.12 (0.09–0.16)	2.29 (1.94–2.71)

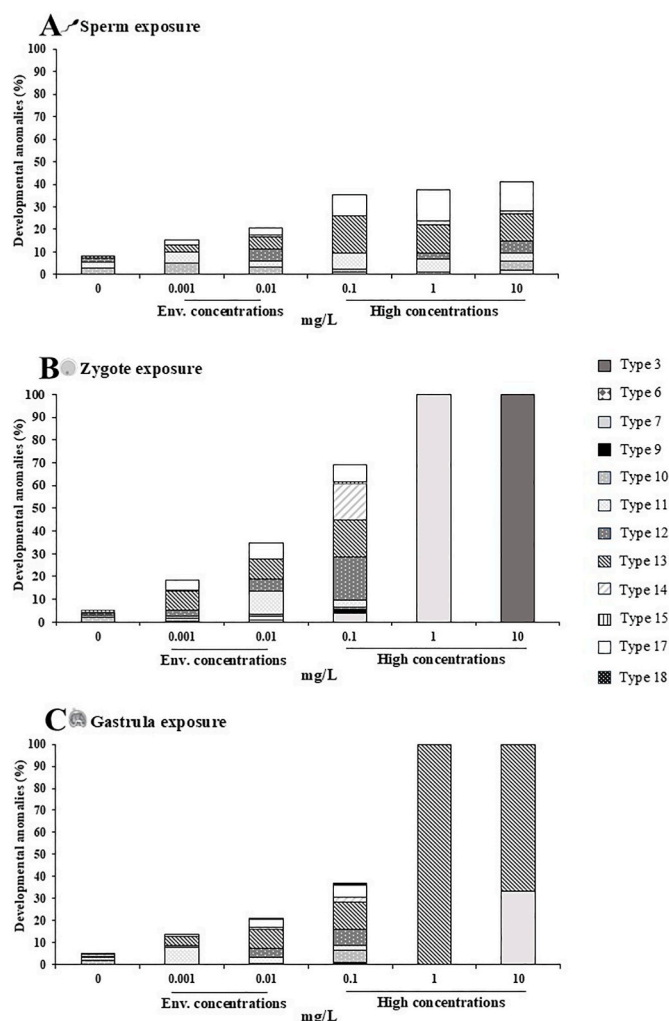


Fig. 3. *P. lividus* main type of developmental anomalies at 72 h pluteus stage from sperm, zygote and gastrula exposure to environmental and high concentrations (0.001, 0.01, 0.1, 1, 10 mg/L) of FLX. Env: environmental.

and Type 10), and plutei with crossed tips (Type 11). Moreover, larvae with supernumerary skeletal rods (Type 13) and larvae with swollen intestine and anus (Type 17) were observed at high concentrations. Zygote and gastrula exposure to FLX induced moderate and severe anomalies, featuring larvae with skeletal regressions (Type 14), besides those previously described and observed after FLX sperm exposure. The most severe anomalies were recorded at the highest concentrations after zygote exposure (Fig. 4): sea urchin development was completely prevented at blastomere (Type 3) and gastrula stage (Type 7) at 10 mg/L and 1 mg/L, respectively.

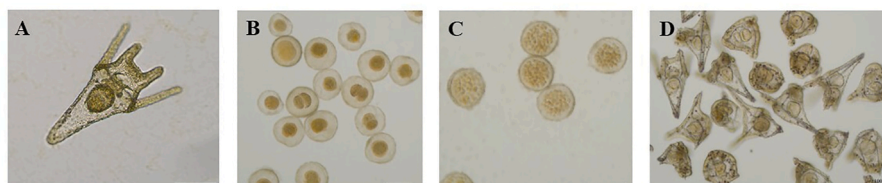


Fig. 4. *P. lividus* main anomalies found in 72 h plutei after zygote exposure to high concentrations (0.1, 1, 10 mg/L) of FLX. A) Control pluteus; B) anomalous cleavages arrested at blastomere stage (Type 3); C) arrested gastrulae (Type 7); D) example of skeletal anomalies. Bar equals 100 μ m.

3.3. Index of Contaminant Impact (ICI)

Anomalies were classified according to larval alteration degree, in order to establish FLX impact severity. No impact was found after sperm exposure to environmental concentrations (Table 2), while a slight impact was observed at high concentrations. Regarding zygote exposure, a slight impact (ICI = 0.60–1.33) was induced by FLX at environmental concentrations, from 0.01 mg/L up to 0.1 mg/L, and a high impact (ICI = 3.0) at the highest concentrations (1, 10 mg/L). Gastrulae exposure to FLX did not induce any impact at environmental concentrations, while a slight impact (ICI = 0.60) and a moderate impact (ICI = 2.0–2.33) were observed at 0.1 mg/L and at the highest concentrations tested (1–10 mg/L), respectively.

3.4. Swimming behaviour

Swimming speed alteration (SSA) of 72h plutei from early life-stage exposure to FLX is shown in Fig. 5. Differently from sperm exposure, with zygote and gastrula exposure it was possible to calculate EC50 values (0.17 (0.13–0.22) mg/L and 2.29 (1.94–2.71) mg/L, respectively). Significant dose-dependent SSA inhibition was observed only after gastrula and zygote exposure. Specifically, a significant SSA inhibition (54 %) was observed at 10 mg/L in plutei from gastrula exposure, although high percentages of anomalous larvae were recorded since 0.01 mg/L. Conversely, the highest toxic effect (100 %) leading to severe anomalies was reported after zygote exposure to 1 and 10 mg/L.

4. Discussion

Among available SSRIs, FLX is frequently detected in the aquatic environment at concentrations ranging from 0.3×10^{-6} to 0.04 mg/L (Minguez et al., 2016; Duarte et al., 2022). Such concentrations represent a potential threat to the marine biota due to FLX ability to disrupt physiological pathways of non-target organisms (Lopes et al., 2020; Pauly et al., 2021). To date, only limited studies on FLX toxicity towards echinoderms are available, and they mainly focus on Atlantic and Pacific sea urchin species (i.e. *E. lucunter*, *H. crassispina*; Lo et al., 2021; Pauly et al., 2021). Given the potential differences in susceptibility within the same taxonomic group, we selected the Mediterranean sea urchin *P. lividus* based on its ecological role and high economic value to

Table 2

Index of Contaminant Impact (ICI) for fluoxetine (FLX) classified in moderate, slight and no impact (Gambardella et al., 2021). Bold values indicate environmental concentrations.

FLX concentration (mg/L)	Stage exposure		
	Sperm	Zygote	Embryo
0.001	No impact (0.20)	No impact (0.33)	No impact (0.19)
0.01	No impact (0.31)	Slight (0.60)	No impact (0.37)
0.1	Slight (0.64)	Slight (1.33)	Slight (0.60)
1	Slight (0.68)	High (3.0)	Moderate (2.0)
10	Slight (0.73)	High (3.0)	Moderate (2.33)

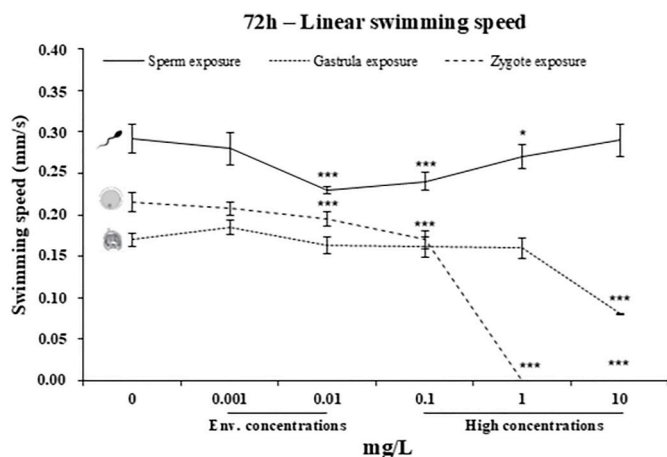


Fig. 5. Linear swimming speed of larvae (72 h) from sperm (solid line), zygote (dashed line) and gastrula (dotted line) exposure to environmental and high concentrations (0.001, 0.01, 0.1, 1, 10 mg/L) of FLX. Data are mean \pm standard error (M \pm SE) of three replicates. *p < 0.05; **p < 0.001; ***p < 0.0001. Env: environmental.

improve FLX hazard risk assessment (McClay, 2011; Bernardo and Carlo, 2017; Farina et al., 2020). Therefore, for the first time, we investigated fertilization, development, and behavioural alterations occurring in the *P. lividus* larvae from sperm, fertilized eggs and embryos exposed to environmental and high FLX concentrations. The early developmental stages of sea urchins have been identified as one of the most sensitive life phases to stressors in *P. lividus* (Gambardella et al., 2021) and in other echinoderm species (i.e. *Lytechinus variegatus*, Collin and Chan, 2016), and any environmental perturbations could have serious detrimental effects on populations and ecosystems (i.e. decreasing recruitment; Ringwood, 1992). By applying for the first time spermotoxicity assays in sea urchins exposed to FLX, we found no effect on fertilization. FLX acts as SSRI, leading to an increase in 5-HT levels. Although 5-HT and its receptors are present in sea urchin sperm, contributing to sperm motility (Parisi et al., 1984), low levels are expressed in gametes up to 31 h post fertilization (Brown and Shaver, 1989; Katow et al., 2004). Hence, FLX may bind to 5-HT receptors during sperm exposure without inhibiting fertilization but affecting the early cleavages. This may explain the fertilization success and FLX impact from the first developmental stages, where the number of 5-HT receptors increases exponentially (Brown and Shaver, 1989). In this regard, we found a significant effect (42 %) in 72 h plutei from sperm exposure to the highest FLX concentration. Notably, 5-HT content and receptor numbers doubled in plutei of 72 h if compared to those found in gametes (Brown and Shaver, 1989), suggesting that FLX impact can be observed from the first developmental stages onwards.

Early development changes may impair their swimming behaviour (Morgana et al., 2016), where biochemical and physiological responses are integrated. Significant developmental anomalies and behavioural alterations were therefore observed in plutei from each early stage exposed to environmental and high concentrations. These findings confirm the ecotoxicological effects of FLX on several marine invertebrates, including echinoderms, as reported in literature (Table S1; Bi et al., 2018; Lo et al., 2021; Pauly et al., 2021; Li and Gaitan-Espitia, 2024). Our findings show a dose-dependent toxic effect for both development and behaviour due to FLX exposure, confirming previous reports on the embryonic development and swimming activity of aquatic organisms (Lo et al., 2021; Pauly et al., 2021; Yamindago et al., 2021). With regards to echinoderms, developmental toxicity was demonstrated in the Atlantic *E. lucunter* sea urchin by exposing zygotes to FLX (Pauly et al., 2021; Table S1). These authors report EC50 values after a 36 h exposure similar to those measured in *P. lividus* after 72 h. Specifically, EC50s of 0.025 and 0.05 mg/L were found for *E. lucunter* and *P. lividus*,

respectively. Taken together, these findings suggest that FLX toxicity does not increase with time and is similar between the Mediterranean and Atlantic echinoderm species. However, by exposing different *P. lividus* stages, we found that FLX toxicity depends on stage exposure. In this study we found higher toxicity on plutei development and swimming behaviour after zygote exposure rather than sperm and gastrula exposure in terms of EC50 values. Thus, according to the sea urchin early stages exposed, the following sensitivity level to FLX can be proposed:

zygote > gastrula > sperm

This sensitivity may be ascribed to FLX action on sea urchin serotonergic system soon after fertilization, that may explain the low toxicity found in sperm. Indeed, FLX is a selective uptake inhibitor of 5-HT, a neurotransmitter found in oocytes, embryos and larvae of several organisms. It plays a crucial role in the development and muscular activity of marine invertebrates, including sea urchins (Buznikov et al., 2001). Serotonin concentration and the distribution of binding sites (5-HT receptors and 5-HT transporters (SERT)) are stage-dependent, changing regularly since the first minutes after fertilization, as demonstrated by immunocytochemical and genomic studies on several sea urchin species (i.e. *P. lividus*, *Lytechinus variegatus*, *Strongylocentrotus droebachiensis*; Buznikov, 1967; Katow et al., 2004; Buznikov et al., 2005; Burke et al., 2006; Falugi et al., 2008). Thus, an increase in 5-HT has been reported in the sea urchin *Strongylocentrotus droebachiensis* soon after fertilization up to the first four cleavage divisions, while very low concentrations were found in late embryos (Deeb, 1972). Therefore, 5-HT signaling is involved in cleavage division regulation and interactions among blastomeres in *P. lividus* early embryos (Shmukler and Tosti, 2002). By binding and inhibiting SERT, FLX may lead to an increase in 5-HT levels, to which *P. lividus* embryos are extremely sensitive during the first cleavage divisions. At the zygote stage, high FLX concentrations may increase 5-HT levels, which can delay or even block cleavage divisions (Deeb, 1972; Brown and Shaver, 1987), causing developmental malformations (Buznikov et al., 2005), as demonstrated in the present study where high developmental anomalies percentages were found.

At gastrula stage, 5-HT and SERT are functionally active, regulating key morphogenic events through post-gastrulation stages (Buznikov et al., 2005). The exposure to several drugs - including 5-HT agonists/antagonists and SERT inhibitors - altered 5-HT levels, by inducing developmental malformations (reviewed in Buznikov et al., 2005). Since FLX is a SERT antagonist, it may act as re-uptake inhibitor during gastrulation, by impairing the development and affecting serotonergic system (Fent et al., 2006).

Independently of life-stage exposed to FLX, we were able to measure toxicity after 72 h from fertilization, by following embryonic development. Even by exposing sperm to FLX, we found a high percentage of anomalous larvae, which however never exceeded 50 %. These results suggest that FLX may affect the early life-stages of sea urchins, whose effects in terms of development can be observed only after fertilization, when 5-HT expression may change (Buznikov, 1967). Notably, zygote exposure to FLX strongly affected both development and behaviour, if compared to the other exposed early stages, reaching 100 % of anomalous and non-swimming larvae already from 1 mg/L upwards (Figs. 3 and 5). Swimming behaviour was affected from 0.01 mg/L onwards, while no significant impairment was found at lower concentrations (i.e. 0.001 mg/L). These findings confirm those reported by Lo et al. (2021), where FLX did not affect the gross speed of 7 days post fertilization larvae of the sea urchin *H. crassispina* up to 0.0001 mg/L. Although these studies focus on plutei collected at different times from fertilization, the larvae share the same morphology, being characterized by 4-arms (Formerly et al., 2022). Taken together, these studies suggest that the swimming behaviour is not impaired by FLX up to 0.001 mg/L in 4-arms plutei, conversely to higher concentrations.

Overall, swimming performance of planktonic larvae may be related to larval morphology and development (Chan, 2012). In this study, the

swimming inhibition observed in sea urchins from 0.01 mg/L is due to severe and irreversible anomalies, such as arrested development at gastrula stage (Type 3), odd blastomere cleavages, and nuclear division without complete cytoplasmic furrow (Type 7). Severe anomalies may occur after zygote exposure to FLX, due to changes in 5-HT distribution, usually found in the cytoplasm (Buznikov et al. 1979, 2003; Markova et al., 1985; Amireault and Dubé, 2005). Further investigations aimed at localizing 5-HT distribution in sea urchin larvae after zygote exposure may confirm these assumptions.

The skeletal anomalies found in this study have been already reported in sea urchin development after zygote exposure to several contaminants, including PCs (Gambardella et al., 2021). They could be lethal or impair larval ability to feed and swim, caused by stunted skeleton growth (Byrne et al., 2013; Gambardella et al., 2021). Such anomalies affecting swimming behaviour may reflect changes in the serotonergic system. In sea urchins, the latter regulates several pathways, including cleavage divisions, protein synthesis, ciliary activity, membrane potential changes, and morphogenesis (Brown and Shaver, 1987; Buznikov et al., 2005; Falugi et al., 2008). Sea urchin embryos and larvae swim in the water column by using ciliary beating (Formerly et al., 2022). Thus, any related dysregulation, together with reversible and irreversible developmental anomalies – including arm asymmetry, reduced larval size, arrested gastrula – may inhibit swimming speed. For instance, small sea urchin larvae observed after exposure to high FLX concentrations may have shorter ciliated bands than controls to generate lift, thus reducing their speed (Strathmann and Grünbaum, 2006). Conversely, the skeletal anomalies (i.e. truncated larvae – Type 10, crossed tips – type 11, asymmetric arms - Type 12) mainly found by exposing all early stages (sperm, zygote, gastrula) to environmental FLX levels, do not affect sea urchin swimming after 72 h. Notably, such anomalies are reversible (Gambardella et al., 2021, 2023, 2024) and responsible for a low ICI toxicity index (equals to 1), thus confirming previous data on the sea urchin *H. crassispina* (Lo et al., 2021).

Environmental and high concentration of FLX induced slight and severe/moderate impacts, respectively. Such findings based on ICI values were based on morphological alterations for each life-stage exposed to all FLX concentrations. The slight estimated impact of zygote exposure to environmental FLX levels confirmed the results of ecotoxicological responses (EC50). However, with the ICI approach, it is possible to estimate toxic effects that may occur in future scenarios and predict which FLX concentration may pose a risk to the coastal marine environment. Considering that FLX consumption is expected to further increase in the coming years due to the global incidence of anxiety disorders (Zhao et al., 2024), our findings based on the novel use of ICI for impact assessment suggest that FLX levels released into the aquatic environment may pose a moderate risk to the early developmental stages of marine invertebrates. However, at significantly increased concentrations, FLX could induce irreversible, high impact anomalies (starting from 1 mg/L). Therefore, FLX levels in the marine environment should not be underestimated, since, as reported for other emerging contaminants (i.e. phthalates, Gambardella et al., 2024), high concentrations could affect sea urchin biological mechanisms.

5. Conclusions

Our findings suggest that early life-stages of echinoderms are sensitive to PCs and in particular to FLX. Relevant environmental concentrations of this antidepressant lead to developmental and behavioural alterations in sea urchins, without affecting fertilization. When comparing the sensitivity of sea urchin early stages to FLX exposure, sensitivity levels rank as follows (from the most to the least sensitive): zygote > gastrula > sperm. Considering the increasing consumption of FLX and its high and moderate impact at high concentrations on sea urchin early stages, we suggest to include this PC in monitoring plans, to avoid exceeding levels (i.e. 0.1 mg/L) that may impair and severely affect the early developmental stages of these echinoderms, which are a

key species in the coastal ecosystem.

CRediT authorship contribution statement

Roberta Miroglio: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roberta Nugnes:** Writing – original draft, Methodology, Investigation, Data curation. **Lisa Zanetti:** Methodology, Investigation. **Marco Faimali:** Resources, Funding acquisition. **Chiara Gambardella:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107080>.

Data availability

No data was used for the research described in the article.

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