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Differential bioconcentration and sensitivity of *Dreissena polymorpha* and *Dreissena rostriformis bugensis* to the antidepressant sertraline

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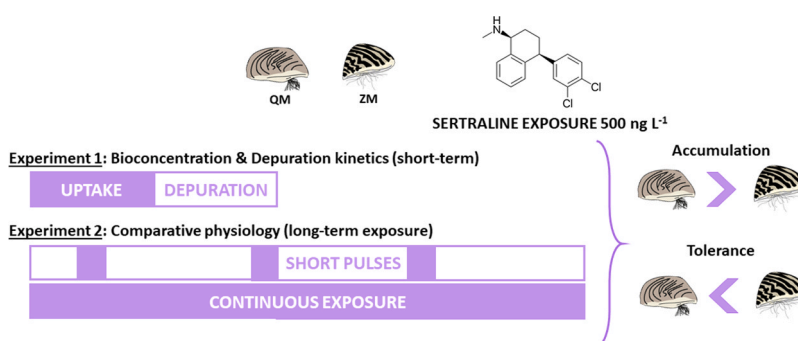
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HIGHLIGHTS

- Sertraline accumulates strongly in bivalve tissues over time, with very low elimination.
- Quagga mussels are more sensitive to sertraline than zebra mussels.
- Exposure leads to oxidative stress and lipid disorders.
- Quagga mussels could experience a discontinuation syndrome under pulsed-exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Sertraline is one of the most widely prescribed antidepressants, worldwide detected in rivers, thus raising concern about its ecotoxicology. However, there is knowledge gap on its pharmacokinetics and pharmacodynamics in freshwater bivalves. Comparative biology can help to gain in understanding and improve our ability to assess ecotoxicological risks in a wide range of species. This study investigated the kinetic-based bioconcentration and depuration of sertraline by two freshwater bivalve species, *Dreissena polymorpha* (zebra mussel, ZM) and *Dreissena rostriformis bugensis* (quagga mussel, QM), and (2) its biological effects depending on the exposure duration and frequency. Several biomarkers related to known sertraline side effects in human were followed. Results document a higher body burden in QM than in ZM. The steady-stage was not reached after 5 days of exposure. Bivalves were unable to depurate sertraline in 5 days in clean water. Findings provide evidence that environmentally relevant concentration of sertraline can disturb the physiology of *Dreissena* species, but not in the same way. QM was found to be more sensitive to sertraline than ZM, experiencing oxidative stress and lipid disorder. Intermittent exposure also led to biochemical changes in the two species, requiring further study.

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1. Introduction

The extent to which aquatic organisms accumulate chemicals is an essential knowledge for better environmental health protection and management, although this may be combined to toxicity assessments to quantify environmental risk. Most of industrial chemicals act through narcosis. This mode of toxic action suggests there would be variations in the sensitivities among aquatic species [1]. The traditional read-across approach relying on predicted toxicity values from a source chemical to predict the toxicity of a target chemical, may introduce potential uncertainty if differential species sensitivities are not considered [2]. Comparative research concerning the chemical toxicokinetics and toxicodynamics are thus needed to gain in understanding and improve our ability to assess ecotoxicological risks in a wide range of species.

Pharmaceutical contamination is found worldwide in the environment [3,4]. These compounds, which main source is household effluents [5], present a particular concern for aquatic organisms, due to their pseudo-persistence and bioaccumulative properties (e.g. [6,7]; Gomez-Regalado et al., 2023; [8-10]). However, available data are highly variable and primarily report accumulated concentrations for fish [11,12]. Studies on pharmaceutical uptake and depuration by bivalves are very limited (review in [6,13]), although they would make good biological models, as filter-feeders and sedentary organisms. They play an essential role in invertebrate environmental toxicological research and are recognized as good bioindicators of aquatic pollution (high capacities to bioaccumulate contaminants and sensitive to inorganic and organic contaminants) (reviewed by [14]). The few studies reporting pharmaceutical concentrations in tissues of both fish and bivalves showed that concentrations measured in bivalves collected from the field can reach at least an order of magnitude higher than those in fishes [7,15]. This also raises the question of their effects on aquatic organisms. For many pharmaceuticals, ecotoxicology information is lacking. Where ecotoxicity data are available, they are often limited to standard test species recommended by the European Commission (i.e. green algae like *Raphidocelis subcapitata*; crustacean like *Daphnia magna*; fish like *Danio rerio*), and short-term exposures [16-19]. Water concentrations leading to acute toxic effects (often in the mg L^{-1} range) are unlikely to occur in rivers, where pharmaceuticals are mostly quantified from low ng L^{-1} to low $\mu\text{g L}^{-1}$ [4]. In that case, the effects of pharmaceutical exposure are likely to be subtle, as they were designed to have low-dose effects in humans and/or domestic animals. Most of their molecular targets have been phylogenetically conserved, and even invertebrates may experience unintended, therapeutic-like and side effects from pharmaceutical exposure [20]. Studies aimed at highlighting these low-dose effects are therefore important.

In this context, psychiatric drugs constitute a relevant therapeutic family given their increasing consumption, hydrophobic properties (generally $\log K_{OW} > 3$) and tendency to accumulate in aquatic organisms [21-25]. Sertraline, one of the most commonly prescribed antidepressants from the selective serotonin reuptake inhibitor (SSRI) class, is regularly detected worldwide in surface waters from 1 to 120 ng L^{-1} , but can reach 600 ng L^{-1} in medium receiving wastewaters and $17 \mu\text{g L}^{-1}$ in untreated sewage [26,27]. However, its hydrophobic property ($\log K_{OW} \approx 5$) suggests that waters are not the best compartment to assess the extent of environmental impregnation. Sediments and organisms, more integrative of temporal variations in discharge, better showed the contamination by sertraline, the latter with often higher concentrations measured [12,28]. Sertraline, like other compounds, is highly detected in the liver of fish up to $14 \mu\text{g kg}^{-1}$ [6,12], but also in tissues from several bivalves up to $370 \mu\text{g kg}^{-1}$ [7]. Physiological and behavioral disorders of aquatic vertebrates and invertebrates have been reported in response to laboratory exposures [29-33]. However, knowledge on its accumulation in and/or effects on non-standard invertebrates, when exposed to low concentrations during long-term exposure, is scarce. By acting on the reuptake of serotonin, which regulates important physiological and behavioral processes in invertebrates (e.g. reproduction, development,

larval settlement and metamorphosis, locomotion or burrowing) [29, 34-36], sertraline may have strong long-term impacts all along life cycles from early-life stages to adults. Studies mostly focused on effects of fluoxetine, whereas sertraline is acutely more toxic [17,37]. Hence, research addressing the relationship between exposure, body burden and effects of sertraline at low concentrations are needed to provide empirical data for risk assessment [38].

To fill in knowledge gaps, we used two freshwater bivalve species, *Dreissena polymorpha* (zebra mussel, ZM) and *Dreissena rostriformis bugensis* (quagga mussel, QM). Zebra mussels, well-established in French rivers, have become accepted bioindicators, commonly used in environmental monitoring. However, for quagga mussels, whose more recent invasion of Western European ecosystems resulted in their overgrowth within dreissenid populations, little is known on how they cope with environmental stressors [39,40]. Evaluation of the interspecies variability in bioaccumulation and stress response of these two species is particularly justified in this case where one species is progressively replacing the other, and biomonitoring is built on well-established sentinel species [39]. The objectives of our study were therefore to (1) investigate the kinetic-based bioconcentration and depuration of sertraline by the two dreissenid species, and (2) determine how these species may react to sertraline depending on the exposure duration and frequency. The impact of “clean periods” (i.e. exposure to not contaminated waters) on the elimination of sertraline and organism recovery from such contaminant can be useful information for the planning of biomonitoring programs using bivalves. This can also allow to identify potential discontinuation syndrome, as observed in about 20 % of depressive patients markedly reducing their antidepressant treatment. Chronic treatment with sertraline can lead some bodies to become dependent on the levels of serotonin, which trigger adverse reactions after discontinuation of the treatment [41].

2. Materials and methods

2.1. Chemical Preparation

Sertraline hydrochloride (CAS No. 79559-97-0) was purchased from Enzo Life Sciences (Villeurbanne, France) at analytical grade (purity $\geq 98\%$). A stock solution at 100 mg L^{-1} was prepared in ultrapure water.

2.2. *Dreissena* spp. sampling and maintenance

Bivalve sampling was performed in 2018 at the beginning of April for the first experiment, and in 2019 at the middle of June. All specimens were adults (shell length: $23.8 \pm 2.9 \text{ mm}$ for *D. polymorpha* and $23.9 \pm 3.0 \text{ mm}$ for *D. r. bugensis*), and hand-collected by byssus excision in the Meuse River for *D. polymorpha* (St-Mihiel, France) and in the Moselle River for *D. r. bugensis* (Jouy-aux-arches, France, E06°04'43" N49°03'58"). Both species were known to cohabit at St Mihiel [42], but at the time of the first experiment, only *D. polymorpha* were found. *D. r. bugensis* were sampled at Jouy-aux-arches, a site with similar physicochemical characteristics [42]. Mussels were sampled between 80 and 120 cm depth on the rocks in canalized sections of the rivers. Bivalves were transported to laboratory in coolers filled with river water. Shells were thoroughly cleaned for any surface deposits or epibionts, and organisms were acclimatized to laboratory conditions in experimental beakers (one individual per 100 mL) during ten days under natural photoperiod at 14°C , which corresponds to the mean temperature of the sampling sites. Water from the field was progressively replaced by Aurele spring water (Cristaline®, 1/3 per day; Ca^{2+} : 106; Mg^{2+} : 4.2; Na^+ : 3.5; K^+ : 1.5; HCO_3^- : 272; SO_4^{2-} : 50; Cl^- : 3.8; F^- : 0.9 mg L^{-1}), and let to maintain 7 days in 100 % spring water. Bivalves were fed once daily with *Chlorella vulgaris* (own culture in the laboratory) at constant density (2×10^4 cells/mL). Mortality of mussels were checked every day, and all dying or dead organisms were removed from the aquaria.

2.3. Experimental design

The study consisted of two experiments: (1) to investigate the kinetic-based bioconcentration and depuration of sertraline by the two dreissenid species and assess potential short-term effects; and (2) to evaluate long-term effects of sertraline according to the frequency of the exposure (Fig. 1). During each experiment, bivalves were fed in the same way as during the maintenance period, and we ensured that algae uptake was complete within 1 h. Mussels were kept in the same conditions of temperature (14 °C), light (natural photoperiod), dissolved oxygen (aerated water), and water mineralization as during the maintenance period.

2.3.1. Experiment 1: kinetics of sertraline accumulation and depuration and acute effects

The kinetics of sertraline accumulation and depuration was determined through a 120h-uptake phase followed by a 120h-depuration period. A single beaker contained two individuals of the same species in 200 mL of exposure media (spring water and sertraline). Bivalves were exposed to sertraline at a concentration of 500 ng L⁻¹ (water pH: 7.8). This concentration is slightly higher than the predicted no effect concentration from the NORMAN ecotoxicology database (460 ng L⁻¹; [43]). A total of 54 organisms were used per species and were sampled at 0, 6, 12, 24, 48, 120 h in the uptake phase followed by three time points at 24 h, 72 h and 120 h in the depuration phase (n = 3 pools of two individuals per time-point). When only bioaccumulation was assessed, three beakers with two mussels each were used (i.e. time points: 0, 6, 12, 24, 120 h in the uptake phase and 24 h, 72 h and 120 h in the depuration phase). For 48 h time-point, mussels were exposed by two in eight beakers either for sertraline accumulation or biomarker analyses (Fig. 1, experiment 1). Media was totally renewed every two days. Water (100 mL) was also sampled from experimental beakers for sertraline quantification. Additionally, three beakers without organisms were also used and sampled after 48 h to account for losses of sertraline in vessels. The light cycle followed natural photoperiod (12 h:12 h day:night).

The effects of a 48h-exposure to 500 ng L⁻¹ were also evaluated on both species using biomarkers measured in the digestive glands (n = 8 individuals per species exposed to the same conditions as detailed above) (see section “Biomarker analyses”). A portion of the digestive gland was excised from each organism, quickly frozen in liquid nitrogen and stored at -80° until analyses. All the remaining tissues were fixed in Bouin’s fixative (48 h) and processed to obtain Section (5 μm thick) through classic histology slide preparations (rinsing in water,

dehydration in graded series of ethanol-ROTI®Histo, and staining in Gill II hematoxylin/eosin) [44]. These slides were used to identify the stages of gonad maturation. Mussels were classified in one of six successive stages of gonad maturation, common to both sexes: an apparent sexual rest, gametogenesis initiation, early gametogenesis, advanced gametogenesis, sexual maturity and spawning [45]. On the same slides, 30–40 tissue sections were observed to identify parasites and/or tissue pathologies. The different parasites looked for, were those previously described in *D. polymorpha* [46].

2.3.2. Experiment 2: long-term effects of sertraline

Sampled mussels (n = 180 individuals per species) were separated in three experimental groups after the acclimatization period: a control group in spring water and two groups exposed to sertraline at 500 ng L⁻¹, either continuously or by short pulses (mimicking occasional sertraline release into waters), during 23 days (Fig. 1). A single beaker contained five individuals of the same species in 500 mL of exposure media. For one species, a total of 12 beakers per experimental group were used. All media were prepared with Cristaline® and were totally renewed three times per week to ensure a semi-static exposure. For the continuous exposure, at each renewal old media was changed by a new solution of sertraline at 500 ng L⁻¹. In contrast, the exposure by pulse consisted in one pulse at the beginning of every week and after 48 h, mussels were placed in “clean” spring water. Each time the medium was changed, the water in the beakers was gently removed to minimize disturbance to the organisms. Sertraline concentrations were checked twice during the experiment in freshly prepared solutions. As during the first experiment, biomarker responses were analyzed in digestive glands (n = 8 individuals for biochemical biomarkers and 8 others for lysosome measurements), and the remaining tissues used for gonad maturity assessment, histopathology checking and parasite identification. Sertraline concentrations accumulated by the two species was also quantified (n = 3 pools of 2 individuals per species). For each set of analysis (i.e. biochemical biomarkers, lysosomes and bioaccumulation), the organisms were selected at random in different beakers.

2.4. Biomarker analyses

Mortality was checked every two days. Mussels were considered alive either when they were closed, or when they rapidly closed their valves after prodding, or when they exhibited filtration activity (siphons out). Mussels that were too slow, or not fully closed, were considered dying and were removed from the beakers. Before opening the mussels, the maximum length (L, maximum antero-posterior axis), height (h, maximum dorsoventral axis) and width (w, maximum lateral axis) of all shells were measured with a caliper rule to the nearest 0.01 mm. The soft tissues were weighted after removing of excess body fluids (m). A condition index (Cond.I) expressed in g mm⁻³ was calculated as follows: $Cond.I = m / (L \times h \times w)$ [47].

The procedure for biochemical biomarkers analyses using an automated spectrophotometer (Konelab 20-XTi, ThermoScientific) is described by Garaud et al. [48] and Potet et al. [49]. Briefly, digestive glands were homogenized in phosphate buffer 50 mM, pH 7.6 supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM L-serine borate mixture as protease inhibitors) at a 4/1 (v/w) ratio. Homogenates were centrifuged at 250g for 5 min at 4 °C and supernatants were divided in two fractions. One fraction was used to measure triglycerides (Trigly.), lipid hydroperoxide concentrations (LOOH), the electron transport system activity (ETS), and creatinine concentrations (Creat.), while the other part was used to obtain the cytosolic fraction after differential centrifugations (1000g for 20 min at 4 °C; the supernatant was recovered and centrifuged again at 20,000g for 50 min at 4 °C). Creatinine concentrations were measured using Thermo-Scientific Konelab™ ready-to use reagents. The cytosolic fraction was used to measure the total antioxidant capacity (TAC), glutathione-S-transferase

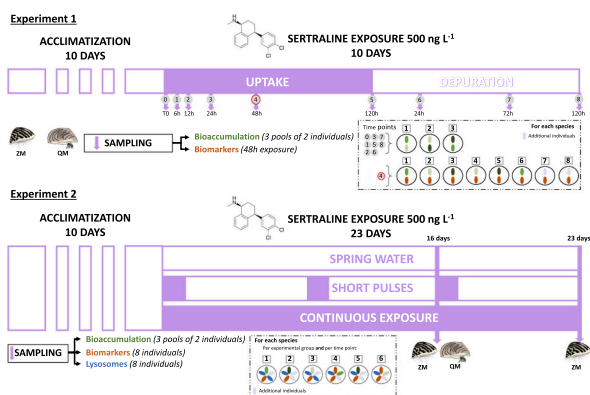


Fig. 1. Experimental design for the short and long-term experiments (Experiment 1 and 2, respectively). Beakers of 200 mL, each containing two mussels were used during Experiment 1, whereas it was 500-mL beakers with five mussels during Experiment 2. Mussels used for sertraline quantification in tissues are shown with 3 shades of green (3 pools of 2 mussels), for biomarker analyses in brown and for the study of digestive lysosomal system morphology in dark blue.

(GST), superoxide dismutase (SOD), and lactate dehydrogenase (LDH). The SOD activity was assessed using the WST-1 method [50] adapted to *D. polymorpha* and *D. r. bugensis*. Proteins were quantified in both fractions by the Pyrogallol red, using Scal bovine-based serum as a reference.

The potential structural changes of the digestive lysosomal system due to sertraline exposure were located by the revelation of β -glucuronidase activity in unfixed cryostat sections, according to Giambérini and Cajaraville [51]. Quantification was done on digestive tissue sections (8 μm thick) by image analysis (Analysis pro 3.2, Olympus, using a Sony DP50 color video camera connected to an Olympus BX41 microscope with a 100x objective) [44]. Briefly, five fields of view were randomly analyzed on one section per individual (total digestive area sampled per individual: 63172 μm^2). Four stereological parameters were calculated: volume density ($V_{V_L} = V_L/V_C$), surface density ($S_{V_L} = S_L/V_C$), surface to volume ratio ($S/V_L = S_L/V_L$, interpreted as the inverse of lysosome size) and numerical density ($N_{V_L} = N_L/V_C$), where C: cytoplasm of digestive cell, L: lysosomes, N: number, S: surface, V: volume.

2.5. Sertraline quantification

The stability of sertraline in waters during experiments was studied using ultra high-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry [52].

For each species, at each sampling time point, 3 beakers were randomly selected, the 6 mussels dissected, and soft tissues pooled by two, freeze-dried for 48 h and finely ground for the measurement of bioaccumulated sertraline concentrations. A method based on the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and LC-MS/MS was developed for the analysis of sertraline in mussels. The procedure involved the extraction of 0.1 g of dry sample with 10 mL 2 % NH_4OH in acetonitrile after mixing the sample with 5 mL of water containing internal standards. A liquid-liquid partitioning step was performed by adding a mixture of 4 g anhydrous MgSO_4 and 1.5 g NaCl. After centrifugation 4000 rpm min^{-1} for 5 min, 7 mL of supernatant was removed and evaporated to dryness. Then 0.5 mL water-acetonitrile (1:1) was used to reconstitute the sample. Detection and quantification were performed using UHPLC coupled with tandem mass spectrometry (Xevo TQS Waters) equipped with an electrospray ionization source (ESI). Chromatographic conditions were obtained using a column C18 BEH (1.7 μm 150 \times 2,1 mm, Waters) kept at 40 °C under gradient-elution conditions with eluent of water (A) and acetonitrile (B) both acidified with 0.1 % formic acid. The elution gradient program was performed as follows: 0–0.5 min, 50 % A; 0.5–3 min, 5 % A; 3–8 min, 5 % A, 8–8.10 min, 50 % A; and 8.1–13 min, 50 % A. The flow rate of the mobile phase was 0.4 mL min^{-1} . The total run time was 13 min. The acquisition by mass spectrometry was performed in MRM (multiple reaction monitoring) in ESI+ mode. Nitrogen gas was used as the nebulization, desolvation and collision gas. The optimized ESI conditions were as follows: capillary voltage, 3 kV; desolvation gas flow, 1000 L/Hr; desolvation gas temperature, 550 °C; source temperature, 150 °C; collision gas flow 0.2 mL min^{-1} . Optimum MRM transitions of the analytes are showed in Table 1. The analytical method has been validated according to the requirements of the NF V03–110 standard [53]. The QuEChERS approach achieved acceptable extraction recoveries

Table 1
Precursor and product ions m/z values and mass spectrometer parameters.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	
		Quantitative Ion (Collision energy in V)	Qualitative Ion (Collision energy in V)
Sertraline	306.1	158.9 (25)	274.9 (14)
Sertraline-d3	309.1	159.0 (25)	274.9 (15)

(78.0–119.9 %) for sertraline. The validation of the method was carried out by the assessment of the following parameters: concentration range, linearity study, the limit of quantification (LOQ), and accuracy (using recovery assays). The LOQ value was validated of 2.14 ng g^{-1} dry weight. Linearity was assessed using the coefficient of determination (R^2), obtained after constructing eleven-point calibration curves from 0.2 to 100 $\mu\text{g L}^{-1}$. Accuracy was evaluated by the recovery of target analyte at low, medium, and high concentrations (2.15, 10.7, and 771.4 ng g^{-1} dry weight) in bivalves (at least 4 series of 2 replicates, minimum $n = 8$). Sertraline concentrations in mussels are expressed as ng g^{-1} dry weight (dw).

2.6. Data analyses

All data analyses were performed with R version 3.6.3 [54], using the RStudio interface (version 1.2.5033).

Kinetic modelling and parameter estimation. No growth of the organisms was observed during the experiment. The estimation of sertraline accumulation followed a first-order kinetic

$$\text{model} : \frac{dC_{org}}{dt} = k_{in} \times C_w - k_{out} \times C_{sertraline} \quad (1)$$

where $C_{sertraline}$ is the concentration of sertraline accumulated in mussels ($\mu\text{g g}^{-1}$ dw), C_w is the dissolved concentration of sertraline in the water ($\mu\text{g mL}^{-1}$) which was constant during the experiment in beakers without mussels, k_{in} and k_{out} are, respectively, the uptake rate constant ($\text{L g}^{-1} \text{d}^{-1}$) and the elimination rate constant (d^{-1}). The integration of Eq. (1) defined sertraline accumulation as follows:

$$C_{org}(t) = \frac{k_{in} \times C_w}{k_{out}} (1 - e^{-k_{out}t}) \quad (2)$$

At the end of the uptake phase, mussels are introduced in a clean media to start the depuration phase. Under this condition, Eq. 1 is reduced to: $\frac{dC_{org}}{dt} = -k_{out} \times C_{sertraline}$ (equation 3), and integrated as follows: $C_{org}(t) = C_{org}(0) \times e^{-k_{out}t}$ (equation 4), where $C_{org}(0)$ is the concentration of sertraline in mussels at the beginning of the depuration phase ($\mu\text{g g}^{-1}$ dw).

The kinetic models were fitted to sertraline concentrations in mussels for both uptake and depuration phases, since they share the parameter k_{out} (Eq. 2 and 4). Bioconcentration factors (BCF) was calculated as $C_{org}/C_{sertraline}$.

Biological responses of exposed bivalves. An integrated biomarker response (IBR) index was calculated to summarize a series of biological responses into one index [55]. As this index will define the biochemical status of the two species at the beginning of the experiments, no a priori on the potential activation or inhibition of enzymatic systems was done (i.e. Z parameter in the IBR calculation set at +1). Principal component analysis (PCA) was used to facilitate the comparison between biomarker responses of the bivalve species after a 2d-exposure to sertraline. PCA analysis was performed with the FactoMineR package [56] and data were visualized using the factoextra package [57]. Effect sizes (Hedges' g) were calculated to determine how much the exposed groups differ from the untreated groups. An effect size is considered significant when its confidence interval does not include 0. A given effect size is considered as small if $0.2 \leq g < 0.5$; moderate if $0.5 \leq g < 0.8$; large if $0.8 \leq g < 1.3$; and very large if $1.3 \leq g$ [58]. Data did not satisfy the assumptions of normality and variance homogeneity. The significant differences were tested using the Kruskal-Wallis ANOVA and the non-parametric Mann-Whitney test for pairwise comparisons.

3. Results

3.1. Experiment 1. Kinetics of sertraline accumulation and depuration and short-term effects

The two species differed significantly in their sertraline accumulation patterns (Fig. 2). Quagga mussels (QM) accumulated more quickly in the first days of exposure, reaching an average of $634 \pm 132 \text{ ng g}^{-1} \text{ dw}$ of sertraline, whereas the accumulation kinetics of zebra mussels (ZM) are slower, with three times less sertraline accumulated in their tissue than QM (ZM maximum = $219 \pm 22 \text{ ng g}^{-1} \text{ dw}$). The resulting uptake rate constant was $223 \text{ L kg}^{-1} \text{ day}^{-1}$ (CI₉₅ %: 167–366) for QM and $94 \text{ L kg}^{-1} \text{ day}^{-1}$ (CI₉₅ %: 80–121) for ZM. At the end of the exposure phase, mean BCF (\pm sd) for the two species were thus significantly different ($\text{BCF}_{\text{ZM}} = 435 \pm 44$ and $\text{BCF}_{\text{QM}} = 1173 \pm 265 \text{ L kg}^{-1}$). It should be noted that the steady state was not reached after 5 days of exposure for the two species. During the depuration phase, slow and no elimination at all were observed for QM and ZM, respectively, preventing to calculate accurate elimination rate constants.

Despite a similar condition index between the two species at the beginning of the experiment ($\text{Cond.}I_{\text{QM}} = 124.9 \pm 32.8$ and $\text{Cond.}I_{\text{ZM}} = 137.1 \pm 28.7$), QM and ZM differed significantly by their physiological attributes (Fig. 3 A). QM showed higher basal levels of cellular damages (LOOH), creatinine and triglycerides, as well as lower antioxidant capacity (TAC) and ETS activity than ZM. This biological difference between the two species was still observed after the short-term experiment, with a clear discrimination on the F1-axis of QM (negative loads) and ZM (positive loads (Fig. 3B)). This factor 1 of the PCA explains over 42 % of the total variance in the data matrix, and is mainly defined by TAC, ETS, GST (to positive coordinates) and cellular damages (LOOH), creatinine and triglyceride levels (to negative coordinates). The early response of the two bivalve species to sertraline after a 48h-exposure led to a shift of cluster centers towards negative loads on F1-axis (Fig. 3B), explained mainly by slight increases of creatine levels for QM and triglyceride contents for ZM, as well as a decrease in TAC for both species (Table S1). The exposure also led to a decrease in inter-

interindividual variability, showed by the reduced size of the ellipses on the PCA (Fig. 3B). No signs of tissue pathology or parasites were observed in this experiment.

As QM and ZM showed great physiological differences that could hide the potential subtler responses to low concentrations of sertraline, the following results after the long-term exposure will be presented by separating the two species.

3.2. Experiment 2. Sertraline long-term effects

Quagga mussels appear to be sensitive to sertraline as exposed organisms experienced higher mortality than unexposed congeners, especially when the exposure was intermittent (observed mortality after 16 days in the unexposed group: 9 %, SER_{c} : 11 %, SER_{int} : 25 %) (Fig. 4 A). This result forces us to stop the exposure of QM after only 16 days to still have enough organisms for the assessment of their physiological status. These different mortality rates observed between exposure groups cannot be explained with the analyzed biomarkers as no significant differences were highlighted between SER_{c} and SER_{int} groups (Fig. 4B, Table S1). Overall, sertraline exposure seems to affect QM through a significant decrease in TAC ($\text{g-SER}_{\text{c}} = -3.09 \pm 1.56$ and $\text{g-SER}_{\text{int}} = -1.79 \pm 1.4$) associated with increased lipoperoxidation ($\text{g-SER}_{\text{c}} = 1.64 \pm 1.22$ and $\text{g-SER}_{\text{int}} = 1.36 \pm 1.32$), a decreased ETS activity ($\text{g-SER}_{\text{c}} = -1.52 \pm 1.2$ and $\text{g-SER}_{\text{int}} = -1.34 \pm 1.31$), and increased triglyceride ($\text{g-SER}_{\text{c}} = 4.13 \pm 1.86$ and $\text{g-SER}_{\text{int}} = 1.69 \pm 1.38$) and creatinine levels ($\text{g-SER}_{\text{c}} = 1.19 \pm 1.15$ and $\text{g-SER}_{\text{int}} = 1.44 \pm 1.33$). For all those biomarkers, the effect is considered as large to very large.

The mean concentrations of sertraline in QM tissues after 16 days were $39 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 17$, $n = 3$), $2973 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 549$, $n = 3$), and $708 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 203$, $n = 3$) for the unexposed, SER_{c} and SER_{int} groups, respectively. Similar concentrations were found in ZM tissues after 16 days of experiment: $3 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 0.7$, $n = 3$), $3608 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 465$, $n = 3$), and $1538 \text{ ng g}^{-1} \text{ dw}$ ($\text{sd} = 203421$, $n = 3$) for the unexposed, SER_{c} and SER_{int} groups, respectively.

Zebra mussels appear to be less impacted by sertraline exposure than

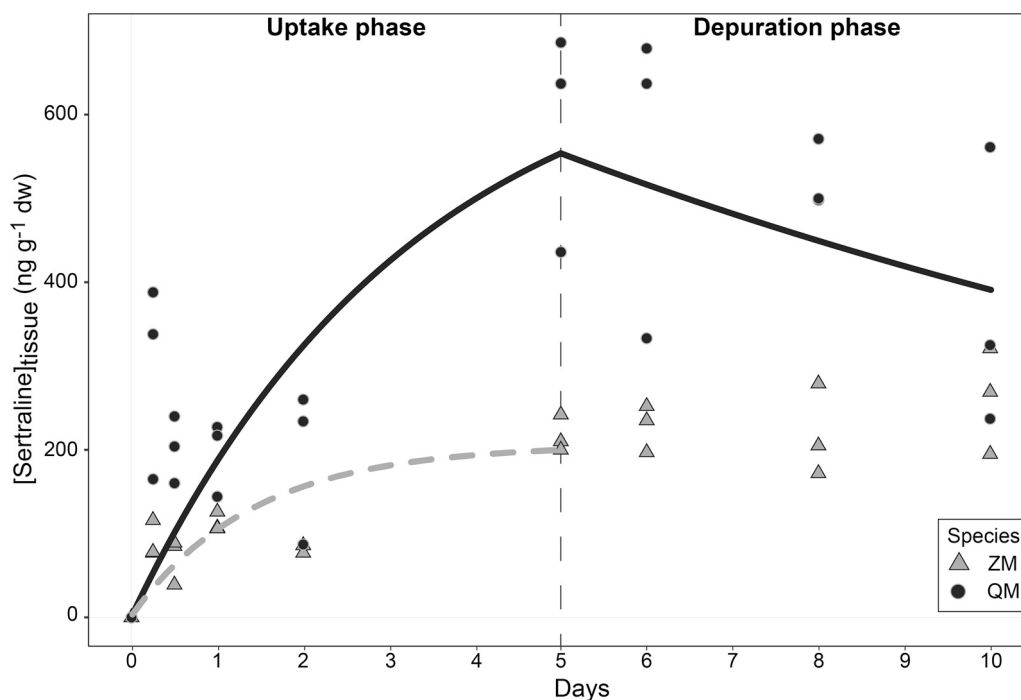


Fig. 2. Bioconcentration kinetics of sertraline by zebra mussels (ZM) and quagga mussels (QM). Organisms were exposed to $500 \text{ ng sertraline L}^{-1}$ the first 5 days followed by a depuration phase in clean water ($n = 3$ pools of 2 mussels/time point for each species). The lines represent the best model fit on the net bioaccumulation.

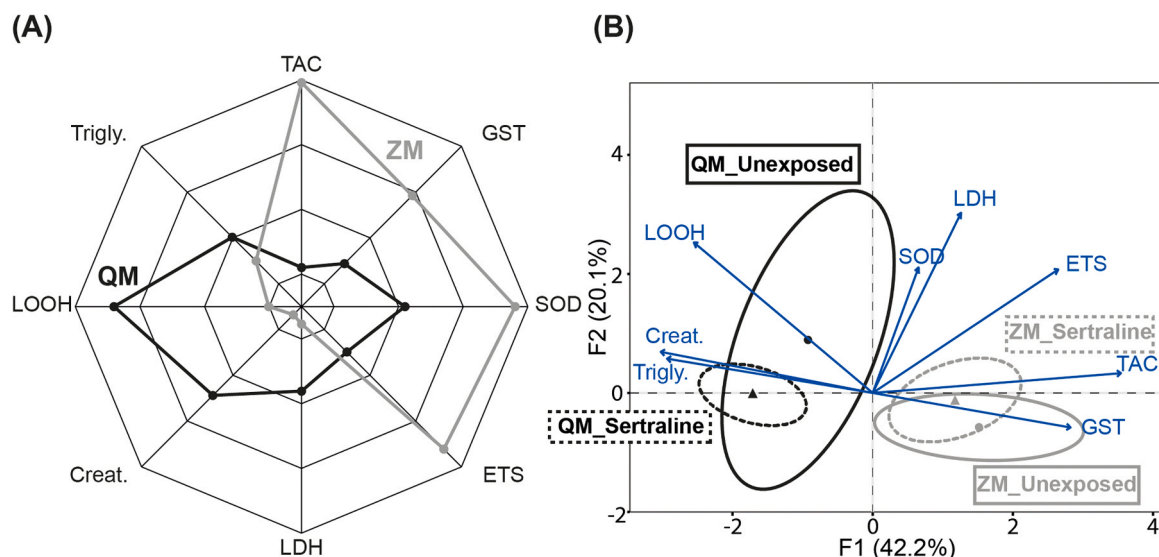


Fig. 3. (A) Star plot of the integrated biomarker response (IBR) of zebra mussels (ZM) and quagga mussels (QM) at t0 (i.e. basal physiological attributes) ($n = 8$ individuals per species); (B) Principal component analysis (PCA) of biomarker analyses in both species exposed or not to sertraline during two days ($n = 8$ individuals per species). Creat.: creatinine, ETS: electron transport system, GST: glutathione-S-transferase, LDH: lactate dehydrogenase, LOOH: lipid hydroperoxide, SOD: superoxide dismutase, Trigly.: triglycerides, TAC: total antioxidant capacity.

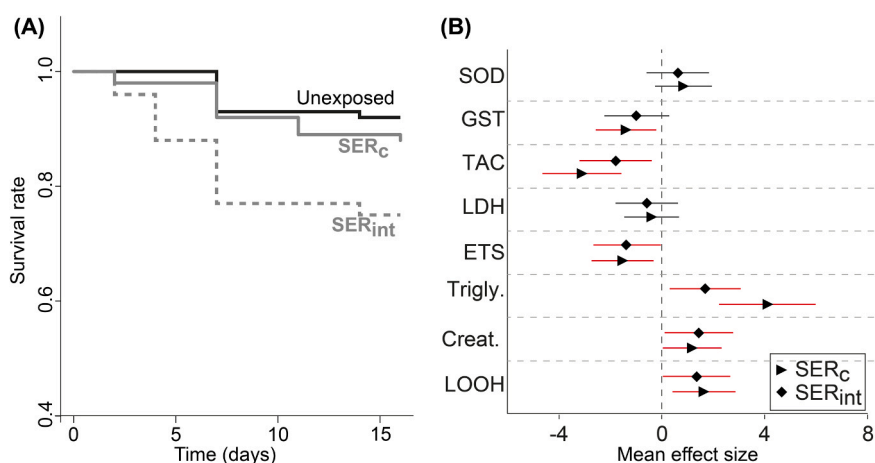


Fig. 4. (A) Kaplan-Meier survival curves of quagga mussels after exposure to 500 ng L^{-1} sertraline through continuous (SER_c , solid grey line) or intermittent exposure (SER_{int} , dashed grey line); (B) Effect size estimates in Hedge's g (with 95 % CI) of biomarkers between sertraline-exposed and unexposed quagga mussels, after 16 days of exposure. Red lines: significant effects of sertraline exposure compared to unexposed group.

QM. Almost no mortality was observed during the experiment ($<4\%$ of mortality) (Fig. 5 A). The low exposure concentration used resulted in very few significant biological effects on this species, but differences depending on the mode of exposure seem to emerge after 23 days of exposure (Fig. 5B, Table S1). After 16 days of exposure, no significant effect of sertraline on the markers studied were observed. After 23 days, sertraline exposure resulted in a significant decrease in the activity of some antioxidant enzymes (SOD in the SER_c group and GST in the SER_{int} group) ($g\text{-SER}_c = -2.66 \pm 1.94$ and $g\text{-SER}_{int} = -5.31 \pm 3.41$) but without changing significantly the total antioxidant capacity ($g\text{-SER}_c = 0.02 \pm 1.43$ and $g\text{-SER}_{int} = -1.77 \pm 1.65$). A significant decrease in ETS activity for the two sertraline-exposed groups ($g\text{-SER}_c = -2.1 \pm 1.76$ and $g\text{-SER}_{int} = -2.39 \pm 2.09$), and a decrease in LDH activity only in the SER_{int} group ($g\text{-SER}_{int} = -1.98 \pm 1.95$) were also observed. A slight increase in creatinine content compared to the non-exposed groups can be noted but remains non-significant ($g\text{-SER}_{int} = 0.76 \pm 1.66$). Mean effect sizes of sertraline treatments are large to very large. The mean concentrations of sertraline in ZM tissues after 23 days were $3 \text{ ng g}^{-1} \text{ dw}$ ($sd=0.5$, $n = 3$), $4269 \text{ ng g}^{-1} \text{ dw}$ ($sd= 1231$, $n = 3$), and 1025 ng g^{-1}

dw ($sd= 577$, $n = 3$) for the unexposed, SER_c and SER_{int} groups, respectively.

The analysis of the remaining tissues of the two species revealed no pathology or parasites.

The structure of the digestive lysosomal system changed with the sertraline exposure (Fig. 6). A shift in the response of this marker to exposure between the two species can be seen. The increase in the volume of the digestive lysosomal system in continuously exposed organisms (SER_c group) was observed at 16 days of exposure for QM, while no change in the lysosomal system was seen until 23 days of exposure for ZM. Similarly, this global increase in the volume of the system was not linked to the same structural changes for the two species, with an increase in the size of the lysosomes for $\text{SER}_c\text{-QM}$ but an increase in the number of lysosomes for $\text{SER}_c\text{-ZM}$. The SER_{int} group in both species tends to have a smaller digestive lysosomal system, due to smaller (higher surface to volume ratio) and more or less numerous lysosomes.

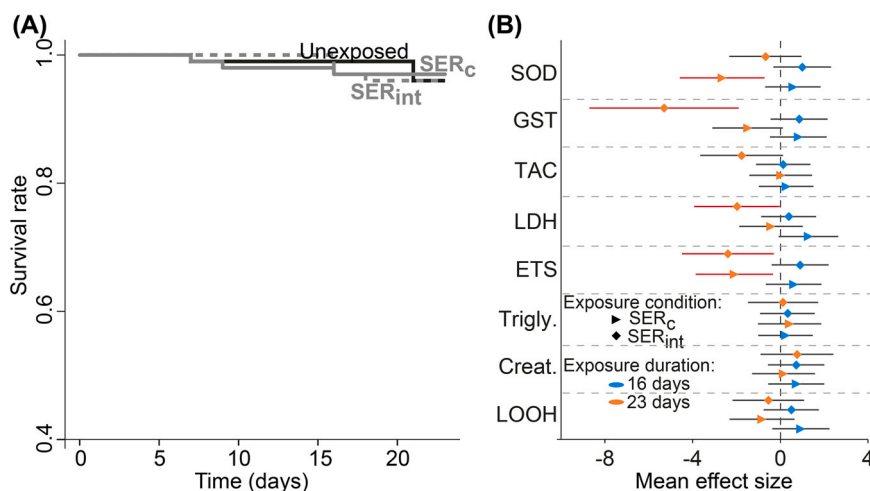


Fig. 5. (A) Kaplan-Meier survival curves of zebra mussels after exposure to 500 ng L⁻¹ sertraline through continuous (SER_c, solid grey line) or intermittent exposure (SER_{int}, dashed grey line); (B) Effect size estimates in Hedge's *g* (with 95 % CI) of biomarkers between sertraline-exposed and unexposed quagga mussels, after 16 days (blue symbols) and 23 days (orange symbols) of exposure. Red lines: significant effects of sertraline exposure.

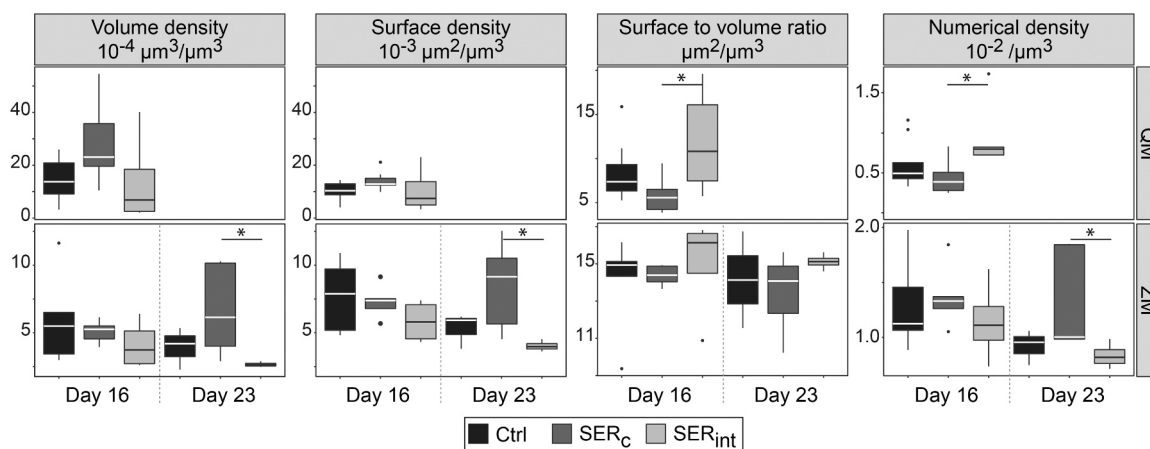


Fig. 6. Structural changes of lysosomal digestive system in quagga mussels (QM) and zebra mussels (ZM) unexposed (Ctrl) or exposed continuously (SER_c) or intermittently (SER_{int}) to 0.5 µg L⁻¹ sertraline during 16 or 23 days. Surface to volume ratio is interpreted as the inverse of lysosome size. Box plots show median values, quartiles (hinges), whiskers (1.5 × interquartile range), and outliers (dots). Stars indicate significant differences between groups (Wilcoxon's test, *p* < 0.05).

4. Discussion

Antidepressants, like all pharmaceuticals, are designed to resist chemical degradation during therapeutic treatments [59], leading to half-lives ranging from few hours to few days once consumed by patients [60]. However, values reported in aquatic environment are much longer, questioning the interactions between these compounds and aquatic organisms, from both a pharmacokinetic and pharmacodynamic point of view. As sessile organisms able to accumulate large amounts of contaminants in their tissues, that may alter several biomarkers, *D. polymorpha* proved to be an efficient sentinel species informing on the contamination status of the habitat where they live in an integrated way [39]. Although several extrinsic (environment) and intrinsic factors (e.g. age, gender, parasitism) are already known to influence the health status of *D. polymorpha* (e.g. [44,47]), such data on *D. r. bugensis* are scarce. Because of their invasive nature, they have distinct biological characteristics and ecological preferences [61], suggesting that they may not have the same strategies for dealing with pharmaceuticals.

Our results revealed that the two species shows different uptake pattern for sertraline. Ionizable pharmaceuticals, such as sertraline, are commonly reported to be rapidly accumulated by aquatic organisms. Invertebrates and especially bivalves showed considerable abilities to

accumulate such compounds [6,62]. For example, similar sertraline tissue levels as those of the present study were reported in the freshwater bivalve *Corbicula fluminea* after a 7-to-41d in situ caged study [63]. Despite a common general rapid accumulation pattern of sertraline, differences between species were observed, which may be explained by the physiology and metabolic needs of the species. *In natura*, QM of same selected size can filter up to 37 % faster than ZM [64]. Uptake rates measured in the present study ($ku_{QM} = 223 \text{ L kg}^{-1} \text{ day}^{-1}$ vs $ku_{ZM} = 94 \text{ L kg}^{-1} \text{ day}^{-1}$) are in line with known differences in filtration abilities of the two species. Distinct lipid contents between the two species (as illustrated here by different triglycerides contents) may also contribute for different accumulation patterns of lipophilic compounds between species [65,66]. Calculated BCF for the two species after 5 days were below 2000 L kg⁻¹ (REACH B criterion; [67]) meaning that sertraline displayed low bioaccumulation potential in *Dreissena* spp. However, we can note that 5 days of exposure were not enough to reach the steady-state. The few kinetic studies on bivalves start to observe this equilibrium between uptake and elimination only after 15 days [6,68]. Concentrations measured in ZM tissues during our long-term experiment seem to support this, with no significant differences in sertraline body burden after 16 days and 23 days of exposure. Accumulation data obtained during the experiment 2 led this time to BCF well above the

REACH B criteria (BCF after 16 days: ZM-SER_c = 7216 ± 930 L kg⁻¹ and QM-SER_c = 5947 ± 1098 L kg⁻¹). Longer-term accumulation data for such compound are therefore required to conclude on sertraline bioaccumulation potential by these bivalves. BCFs higher than this REACH threshold have already been reported for other aquatic organisms [11, 63]. After transfer to clean water, only a slight sertraline depuration was observed for QM and no elimination at all for ZM, suggesting that both species did not readily metabolize and/or eliminate the compound over a 5d-period. In aquatic invertebrates, the levels of total cytochrome P450 enzymes, which play a key role in drug metabolism, were found to be lower than those found in vertebrates [69]. A low metabolism of sertraline cannot be ruled out. Further studies are needed to gain knowledge on ADME (i.e. Absorption, Distribution, Metabolism, and Excretion) processes in bivalves.

Although ZM and QM are seemingly alike, we can wonder whether they have similar physiology. All the studied biomarkers could be affected by sertraline exposure. Oxidative stress, mitochondria and lysosome impairments have been previously reported to be potentially involved in sertraline mode of action [70-72]. We showed in digestive glands higher lipidic peroxidation and triglyceride levels for QM, and greater TAC, GST and ETS activities for ZM. Similar results were reported by Nowicki and Kashian [73] and Potet et al. [74]. Digestive gland is the key organ of bivalve metabolism for storing nutrition and supplying energy. During gametogenesis and ovulation, mussel's digestive gland contains higher amounts of lipids (mainly triglycerides), which will be transferred to the gonad [75,76]. Quagga mussels start their spawning season earlier than ZM [77]. The temporal shift in the reproductive cycle could explain the observed differences in the digestive gland status between the two species, the two organs being closely linked. But might these different basal physiological attributes also imply differential susceptibility to stressors?

Both species reacted in the same way to the short exposure (2d) to sertraline. Due to the high intragroup variability for unexposed organisms and the low chosen concentration, sertraline induced only a slight increase of triglyceride contents and a decrease of total antioxidant capacity. This is consistent with the slow kinetics of accumulation and metabolism of the compound, described above. To highlight potential subcellular and individual effects of sertraline on ZM and QM, longer exposure is thus needed.

No consensus on a general pattern of sensitivity between the two species facing stressors during long period can be found in the literature. For example, Farkas et al. [39] do not observed any interspecies differences in the field; Potet et al. [74] reported higher tolerance of ZM populations to nickel exposure than QM populations, whereas Nowicki and Kashian [73] suggest that QM are more tolerant to stressors than ZM. All these studies have been performed on adults, but the critical phase is often early-life stages (eggs, embryos and larvae) [34]. However, those stages in *Dreissena* spp. are difficult to obtain and maintain under laboratory [78], making studies on their sensitivities to contaminants difficult. In our study, after two weeks, the effects of continuous exposure of sertraline were different between the two species, QM being more sensitive than ZM. Our results suggest that continuously-exposed QM experienced oxidative stress, related to a decrease in antioxidant and detoxification activities, mitochondrial dysfunction (decrease of ETS activity) enhancing production of reactive oxygen species (ROS), thus leading to damages (increase lipidic peroxidation and creatinine-like content). This imbalance between the ability of body defenses to neutralize the harmful effect of reactive species, which in turn causes cellular damages is a hazardous side effect of sertraline already reported for other species [79-81]. The lysosomal system is also a known target of antidepressants. Disruptions to this system have been highlighted in several aquatic organisms [71,81]. As cationic amphiphilic compounds (CADs), they have a particular affinity for acid organelles, a phenomenon known as lysosomotropism [82,83]. Lysosomes by trapping CADs will increase in size. The digestive lysosomal system of QM continuously exposed to sertraline displayed this pattern after 16

days. This drug-induced disturbance of the lysosomal system is also often associated to lipid metabolic disorders, like excessive lipid accumulation (e.g. phospholipids and triglycerides) [82,84]. This can result from a promotion of lipogenesis and/or a decrease in lipolysis [81]. Herein, continuously exposed QM displayed higher triglyceride contents compared to unexposed mussels. This kind of lipid alteration was identified in marine mussels exposed to organic pollutants, both in field conditions [85] and in experimental settings [81,86]. All these sertraline-induced effects on QM could contribute to the higher mortality observed in this group compared to unexposed group. In comparison, significant biological effects of sertraline only appeared after 23 days of exposure for ZM. Decreased SOD activity was compensated by other cellular antioxidants since sertraline did not affect total antioxidant capacity compared to unexposed group and no increased cellular damages were observed. Similar results were obtained by Magni et al. [87] in ZM exposed to 500 ng L⁻¹ of fluoxetine, another antidepressant. Exposed ZM also experienced a decrease in ETS activity, like QM, but whose suggested consequences (increase of ROS production) would be offset by more efficient cellular antioxidant mechanisms.

Lysosomal storage and lipid metabolic disorders, suggested phenomena occurring in SER_c exposed QM, are reversible. Therefore, once drug treatment ends, the drug is eventually expelled and lipid levels should return to normal (unexposed levels) [88]. According to mean effect sizes, our data on SER_{int} QM tend to go this way (with means approaching 0), with also lysosomes being smaller and more numerous. However, the higher mortality observed in this group suggests that something happened but which cannot be explained by the selected biomarkers. Further studies are thus needed to determine what could be the key events leading to mortality outcome, when QM are not continuously exposed to sertraline. Glutathione *S*-transferase plays a critical role in cellular detoxification against xenobiotics as well as oxidative stress. Sertraline (but also serotonin, its precursors and metabolites) by binding to GST inhibits its activity [89,90], inhibition observed in the two species. However, by regularly removing the drug, we expected to find GST activity similar to unexposed levels. This was not the case for SER_{int}-ZM, which showed a significant decrease in GST activity compared with the unexposed group. This result suggests that sertraline might have biological consequences that persist even during intermittent exposure, which might be explained by its low metabolism and/or elimination by the mussels. In environments with multiple stressors, this mechanism has also the potential of enhancing the toxicity of other concurrently administered drugs which normally require glutathione *S*-transferase for detoxication. In the same way, even if lower LDH activity is not a cause of concern, the lower LDH activity observed in SER_{int}-ZM should be considered. The exposure to chemical stressors, like sertraline, leads mostly to increased LDH activity (e.g. [31, 91]). Low activity may be a result of digestive gland disease, where enough LDH cannot be produced.

5. Conclusion

This study attempted to investigate interspecies differences in bioconcentration and depuration abilities, and physiological attributes between two phylogenetically-close *Dreissena* species (*D. polymorpha* or ZM and *D. r. bugensis* or QM) facing sertraline stress. Our results suggest a slow but great accumulation of sertraline in bivalve tissues after exposure to an environmental concentration. The two species differed by their basal physiological attributes influencing their response to sertraline, which were stronger for QM than for ZM. The antidepressant caused evident damages on exposed organisms (significant alteration of oxidative status and mitochondria properties). Intermittent exposure also led to biochemical changes in the two species, requiring further study.

Environmental implication

Despite a breakthrough in knowledge of antidepressant environmental hazards, gaps remain on their pharmacokinetics and pharmacodynamics in freshwater bivalves. The tested sertraline hydrochloride concentration was detected in waters receiving wastewaters and is the predicted no effect concentration (PNEC, NORMAN database). Bivalves were exposed continuously or intermittently (mimicking potential pulsed discharge) to assess bioconcentration, depuration kinetics and long-term physiological effects. They accumulate sertraline and metabolize it very slowly, with long-term consequences. The PNEC notion is thus questioned. Greater physiological disturbances were observed during intermittent exposure. Such studies are necessary to refine the risk assessment of pharmaceuticals.

CRedit authorship contribution statement

Justine Flayac: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Céline Passignat:** Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Laetitia Minguez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Romane Lerebourg:** Methodology, Formal analysis, Data curation.

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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2024.136628](https://doi.org/10.1016/j.jhazmat.2024.136628).

Data availability

Data will be made available on request.

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