



HAL
open science

**Combined exposure of the bivalve *Mytilus galloprovincialis* to polyethylene microplastics and two pharmaceuticals (citalopram and bezafibrate):
Bioaccumulation and metabolomic studies**

J.M. Castaño-Ortiz, F. Courant, E. Gomez, M.M. García-Pimentel, V.M. León, J.A. Campillo, L.H.M.L.M. Santos, D. Barceló, S. Rodríguez-Mozaz

► **To cite this version:**

J.M. Castaño-Ortiz, F. Courant, E. Gomez, M.M. García-Pimentel, V.M. León, et al.. Combined exposure of the bivalve *Mytilus galloprovincialis* to polyethylene microplastics and two pharmaceuticals (citalopram and bezafibrate): Bioaccumulation and metabolomic studies. *Journal of Hazardous Materials*, 2023, 458, pp.131904. 10.1016/j.jhazmat.2023.131904 . hal-04259698

HAL Id: hal-04259698

<https://hal.science/hal-04259698v1>

Submitted on 30 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Combined exposure of the bivalve *Mytilus galloprovincialis* to polyethylene microplastics and two pharmaceuticals (citalopram and bezafibrate): Bioaccumulation and metabolomic studies

J.M. Castaño-Ortiz^{a,b,*}, F. Courant^c, E. Gomez^c, M.M. García-Pimentel^d, V.M. León^d, J.A. Campillo^d, L.H.M.L.M. Santos^{a,b}, D. Barceló^{a,b,e}, S. Rodríguez-Mozaz^{a,b}

^a University of Girona, Girona, Spain

^b Catalan Institute for Water Research (ICRA-CERCA), C/ Emili Grahit 101, 17003 Girona, Spain

^c HydroSciences Montpellier, University of Montpellier, IRD, CNRS, Montpellier, France

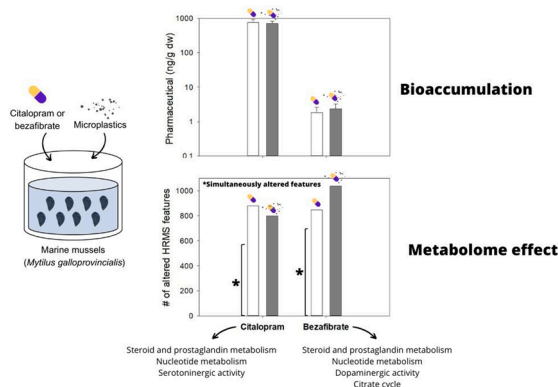
^d Instituto Español de Oceanografía (IEO-CSIC), Centro Oceanográfico de Murcia, C/Varadero 1, San Pedro del Pinatar, Murcia, Spain

^e Institute of Environmental Assessment and Water Research (IDAEA-CSIC) Severo Ochoa Excellence Centre, Department of Environmental Chemistry, C/ Jordi Girona 18-26, 08034 Barcelona, Spain

HIGHLIGHTS

- Citalopram bioaccumulates ~500 times more than bezafibrate in mussels.
- Magnitude of pharmaceutical effects not directly correlated with bioaccumulation.
- Possible alteration of reproduction, energy metabolism and immunity by the two pharmaceuticals.
- Bioaccumulation and metabolic effects generally independent of PE-MPLs co-exposure.
- PE-MPLs alone also dysregulated biological pathways (e.g., purine metabolism).

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Lingxin Chen

Keywords:

LC-HRMS
Antidepressant
Lipid regulator
Mixture
Depuration

ABSTRACT

Pharmaceuticals and microplastics constitute potential hazards in aquatic systems, but their combined effects and underlying toxicity mechanisms remain largely unknown. In this study, a simultaneous characterization of bioaccumulation, associated metabolomic alterations and potential recovery mechanisms was performed. Specifically, a bioassay on Mediterranean mussels (*Mytilus galloprovincialis*) was carried out with polyethylene microplastics (PE-MPLs, 1 mg/L) and citalopram or bezafibrate (500 ng/L). Single and co-exposure scenarios lasted 21 days, followed by a 7-day depuration period to assess their potential recovery. PE-MPLs delayed the bioaccumulation of citalopram (lower mean at 10 d: 447 compared to 770 ng/g dw under single exposure), although reaching similar tissue concentrations after 21 d. A more limited accumulation of bezafibrate was observed overall, regardless of PE-MPLs co-exposure (<MQL–3.2 ng/g dw). Metabolic profiles showed a strong

* Corresponding author at: University of Girona, Girona, Spain.

E-mail address: jcastano@icra.cat (J.M. Castaño-Ortiz).

<https://doi.org/10.1016/j.jhazmat.2023.131904>

Received 15 April 2023; Received in revised form 9 June 2023; Accepted 19 June 2023

Available online 21 June 2023

0304-3894/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

effect of pharmaceuticals, generally independent of PE-MPLs co-exposure. Alterations of the citrate cycle (bezafibrate exposure) and steroid and prostaglandin metabolism (citalopram and bezafibrate exposures) were highlighted. PE-MPLs alone also impacted metabolic pathways, such as neurotransmitters or purine metabolism. After depuration, relevant latent or long-lasting effects were demonstrated as, for instance, the effect of citalopram on neurotransmitters metabolism. Altogether, the observed molecular-level responses to pharmaceuticals and/or PE-MPLs may lead to a dysregulation of mussels' reproduction, energy metabolism, and/or immunity.

1. Introduction

Marine ecosystems are vulnerable to discharges of contaminants from various sources. For example, urban wastewater can be released through underwater emissaries into coastal waters, whereas nearby discharging rivers may also increase the input of wastewater-borne contaminants. Sewage discharges into the sea comprise treated and uncontrolled ones, such as runoffs during heavy rain episodes [1]. In coastal ecosystems subjected to sewage discharges, marine organisms are chronically exposed to a wide range of wastewater-borne contaminants, such as pharmaceuticals, pesticides, industrial chemicals, or plasticizers, typically at trace levels concentrations (ng to few $\mu\text{g/L}$) [2, 3]. Subsequently, pharmaceuticals have been detected in biotic and abiotic compartments from a diversity of marine environments, especially in estuarine and coastal ecosystems [4–7]. Although pharmaceutical pollution sources are mostly land-based, local marine activities may represent an additional pressure to the environment. For instance, mariculture (through medicated feed), or direct excretion by bathers [8] can also contribute to pharmaceutical occurrence in coastal areas.

Among pharmaceuticals, psychiatric drugs and lipid regulators constitute two relevant therapeutic groups given their increasing consumption [9], relatively hydrophobic properties (generally $\log K_{OW} > 3$) and tendency to accumulate in aquatic organisms [1,10,11]. The two selected pharmaceuticals (citalopram and bezafibrate) have been found in the range of 4–93 ng/L [12] and 1–67 ng/L [5,12,13], respectively, in estuarine and coastal waters. Different adverse effects have been described in response to laboratory exposures to antidepressants, generally at the sub-lethal level (e.g., behavioral and reproductive effects on mollusks) [14,15]. Comparatively little is known on the bioaccumulation, mode of action, and potential ecotoxicological effects of lipid regulators in marine invertebrates [16].

In addition, pharmaceuticals can co-occur with other contaminants, such as microplastics (MPLs), which have received increasing attention in the last decade. Monitoring studies have revealed a wide range of MPLs sizes, shapes, polymer types and concentrations in different marine habitats [17]. MPLs found in coastal ecosystems can derive from land-based sources (e.g., river and wind transport, wastewater effluents), marine activities (e.g., fisheries) or in-situ degradation of large plastic litter. Due to a higher bioavailability and capacity to interfere with physiological processes, the small plastics are those that most affect marine biota [18].

In addition to the direct effects of (micro)plastics on aquatic organisms, several studies have highlighted their possible role as vectors in the dispersion of macrofauna [19], microorganisms (i.e., biofouling) or antibiotic resistance genes (ARGs) [20] as well as of organic contaminants [21,22]. Following the sorption of contaminants, MPLs may act as carriers and modulate their bioaccumulation and ecotoxicological effects. Most studies have focused on the evaluation of MPLs interaction with persistent organic compounds (POPs) or heavy metals rather than emerging contaminants (e.g., pharmaceuticals, UV filters). Although the strongest sorption is expected for this type of hydrophobic contaminants ($\log K_{OW} > 4$), it is known that MPLs can adsorb more hydrophilic pharmaceuticals too [23]. The significance and magnitude of pharmaceutical adsorption on MPLs will depend on several factors, including polymer- and compound-specific properties, and environmental conditions (e.g., salinity) [24,25]. For example, the aging or weathering of

MPLs induces changes in surface properties (e.g., cracking, charge) that can affect contaminant sorption/desorption processes [24]. In this context, little is known about their interactive effects on aquatic organisms, especially marine species [26,27].

Bivalves have been described as suitable model organisms to address the impact of different pollutants in the marine environment [28], including the combined effects of MPLs and pharmaceuticals [23]. Among bivalves, mussels (*Mytilidae*) are extensively used in field and laboratory studies due to their wide geographical distribution, easy collection and maintenance, well-known physiology, stress tolerance, and capacity to accumulate a wide range of contaminants [29]. Whereas field studies with mussels may provide insight into effects under environmentally realistic scenarios (e.g., caging experiments), laboratory studies are necessary to overcome the difficulties of multiple interacting factors in the environment.

To evaluate the impact of environmental contaminants or their mixtures on aquatic organisms, omics approaches have recently emerged, including metabolomics, transcriptomics, proteomics, or their integration [10,30]. Metabolomics is based on the identification of low molecular weight metabolites (50–1500 Da), which are highly dependent on physiological, developmental or pathological conditions of organisms [31]. In fact, endogenous metabolites comprise final downstream products of genomic, transcriptomic, and/or proteomic alterations. For that reason, metabolomics has proved to be useful to screen biologically relevant alterations and gain insight into contaminants' mode of action (MOA) [32]. Such mechanistic knowledge is also important to better understand the possible adverse outcomes (at the individual level) of combined exposure to MPLs and co-occurring contaminants [33].

The aim of this study was to explore the combined effects of MPLs and organic pollutants such as pharmaceuticals on marine mussels. Mediterranean mussels (*Mytilus galloprovincialis*) were exposed to polyethylene MPLs in combination with either the antidepressant citalopram or the lipid regulator bezafibrate, which have been scarcely investigated in ecotoxicology studies. In addition, to the best of our knowledge, existing studies on mollusks have not addressed metabolomic alterations or their relationship with microplastics. Nevertheless, these compounds have been recently found in bivalves and fish from two coastal ecosystems in the Mediterranean (ND–3 ng/g dw) [1,34]. The differing citalopram and bezafibrate physical-chemical properties (e.g., pKa of 9.78 and 3.83 for citalopram and bezafibrate, respectively) might also lead to a different sorption behavior on microplastics. In addition, because of their specific modes of action, it was expected that these two compounds could trigger distinct pathways within mussels metabolome. The bioaccumulation of the two pharmaceuticals might have important implications for their monitoring and management. For example, bivalves can be suitable bioindicators of certain pharmaceuticals in coastal ecosystems, whereas other compounds may only accumulate to a much lower extent [35]. In addition, the impact of depuration (or “clean” periods) on the elimination of and organism recovery from such contaminants can be useful information for the planning of biomonitoring programs and the depuration of seafood for consumption [36].

In this context, a controlled laboratory experiment was conducted for 21 days at environmentally realistic concentrations to study i) the bioaccumulation of two selected pharmaceuticals in mussels, with and without MPLs (LC-MS/MS analyses in different tissues), ii) the possible

associated alterations of mussels' metabolome (untargeted metabolomics using LC-HRMS in digestive gland), and iii) their potential for recovery after a 7 days depuration period.

2. Materials and methods

2.1. Chemicals and reagents

Polyethylene MPLs (PE-MPLs) were purchased from Micro Powders Inc. (ref. MPP-635XF) in the form of a micronized powder of virgin HDPE yielding non-uniform particles with a maximum size of 22 μm (mean 4–6 μm), and density of 0.96 g/cm^3 (as indicated by the supplier). A short-term aging of PE-MPLs was conducted before exposure in order to favor the leaching of plastic additives, particularly relevant for virgin plastics, which can affect exposed organisms. Further details about chemicals, reagents, and particle aging are provided in [Supplementary Information \(SI-1\)](#).

2.2. Experimental design

Mediterranean mussels (*Mytilus galloprovincialis*) ($n = 1026$) were obtained from a local raft culture in Benalmádena (S Spain) and acclimated for seven days prior to the exposure experiment. During acclimation and exposure, mussels were kept under the following general conditions: i) aerated seawater (0.45 μm -filtered) at 37.5 psu and 16.5 °C; ii) natural photoperiod regime (12 h light:12 h dark), and iii) daily fed on the microalgae *Isochrysis galbana* (4–8 μm) in a proportion of 4% of mussels dry weight (high growth-promoting).

The exposure experiment was conducted in methacrylate tanks of 30 cm (\varnothing) and maximum capacity of 20 L. A total of six treatment groups ($n = 3$ replicates, using three different tanks per case) were tested: mussels exposed to 1) clean seawater without contaminants (Control - CT), 2) polyethylene MPLs (PE-MPLs), 3) the antidepressant citalopram (CP), 4) a mixture of PE-MPLs and CP (PE-MPL/CP), 5) the lipid regulator bezafibrate (BZ), and 6) a mixture of PE-MPLs and BZ (PE-MPL/BZ). Mussels were exposed to the contaminants for 21 days. Then the spike of contaminants was stopped, and the mussels were kept for a period of 7 days more in clean seawater (depuration period). In total the experiment lasted 28 days. At the beginning of the experiment, each tank was filled with 15 L of seawater and 57 mussels were added. The water volume was adapted to maintain the same mussel/pharmaceutical ratio along the exposure: 10 L and 41 mussels (from day 10–21). During the depuration period 25 mussels were also kept in 10 L (from day 21–28). Mussels were sampled at days 10, 21, and 28. Exposure tanks were daily spiked to counterbalance the uptake of contaminants by mussels and maintain nearly constant concentrations of the different stressors: 1 mg/L of PE-MPLs, and 500 ng/L of both CP and BZ. Stock suspensions used for the spiking contained individual pharmaceuticals (at 37.6 $\mu\text{g}/\text{L}$ each) and/or PE-MPLs (75 mg/L) in filtered seawater and were left in contact for 24 h with agitation at 18°C.

The selected pharmaceuticals concentration (500 ng/L) was slightly higher than those reported in coastal waters (generally in 1–100 ng/L range) but around or below the established toxicity thresholds for citalopram and bezafibrate. For example, the predicted-predicted no-effect concentration (PNEC) values in marine waters are 230 and 1600 ng/L for bezafibrate and citalopram, respectively (according to NORMAN Ecotoxicology Database [37]). In the case of microplastics, the lack of standardized methods has challenged the quantitative understanding of their environmental occurrence [38], but the selected exposure level (1 mg/L) is comparable to reported concentrations in polluted estuaries (e.g., 0.8–2.2 mg/L in the Miri River Estuary) [39].

Seawater was renewed every three days throughout the experiment, in order to remove the suspended material (feces) which could affect the bioavailability of contaminants. All tanks received the same concentration of solvent (methanol, 0.0005% v/v). A negligible mortality rate (<3%) was observed during the experiment. Seawater aliquots (~100

mL) were taken from the tanks at different times after spiking (5', 60', 120', 300', 24 h) and at 48 h (before spike) and 72 h (before water renewal), and kept at –20°C, to determine pharmaceutical concentrations during the experiment. In addition, 200 mL of stock suspensions were filtered (GF/C 1.2 μm), and membrane filters were dried and stored for assessment of pharmaceutical loading on PE-MPLs. The concentration of PE-MPLs in seawater was also determined by assessing particle clearance after the spiking of tanks (via Coulter Counter, as shown in [Supplementary Information SI-7](#)).

2.3. Mussels' collection and dissection

Sixteen individuals were collected per tank at each sampling time (10, 21 and 28 days). Whole body of six individuals were pooled, freeze-dried and kept at –20°C until chemical analysis whereas ten individuals were dissected on ice for different purposes: chemical and biomarker analyses of hemolymph (3 inds), metabolomic analysis of digestive glands (3 inds) and MPL analysis of gills and digestive glands (4 inds). Biomarker and MPL analyses are not covered in this study. The collected tissues were individually frozen in liquid nitrogen (–196 °C) and stored at –80°C. Biometric information was recorded upon dissection, namely mussel length (with valve) and wet weight (without valve).

2.4. Pharmaceutical analyses

For the extraction of pharmaceuticals from water samples, a modification of the method based on solid phase extraction (SPE) described by Gros et al., 2012 [40] was implemented. The analysis of pharmaceuticals in whole mussel samples was carried out using the extraction protocol described by Santos et al., 2019 [41] using bead-beating (more details in [Supplementary Information SI-2](#)). In turn, hemolymph samples (200 μL) were mixed with 200 μL of methanol and centrifuged (10 min at 5000 rpm, 4°C). The supernatant was transferred into a glass vial, and the internal standard mixture was added (concentration in final extract = 20 $\mu\text{g}/\text{L}$ each). Finally, membrane filters (GF/C, 1.2 μm), theoretically containing PE-MPLs-loaded pharmaceuticals, were extracted by ultrasonication in methanol as described in León et al. (2019) [42], followed by filtration using syringe filters (RC 0.45 μm). The instrumental analysis of pharmaceuticals was carried out following the method described by Castaño-Trias et al. (submitted) [43] ([Supplementary Information SI-2](#)). Validation parameters (method detection (MDL) and quantification limits (MQL), recoveries) of the analytical methodologies used for the different matrices can be found in [Supplementary Information \(Table S1\)](#).

2.5. Data analysis of pharmaceuticals

For statistical calculations and graphical representation, concentration values below MQL were replaced by MQL/2. A two-way ANOVA was used to test the influence of PE-MPLs co-exposure (with/without) and time (10/21/28 d) on response variables (pharmaceutical concentration in mussels). Bonferroni's multiple comparison tests were used to determine the statistical differences between groups ($p < 0.05$). Bioaccumulation factors (BAFs) at day 21 were calculated using the following equation:

$$\text{BAF} \left(\frac{\text{L}}{\text{Kg}} \right) = \frac{C_m}{C_w}$$

Where C_m is the pharmaceutical concentration in mussels (ng/g dw) at the end of exposure (21 d), and C_w is the average concentration in water ($\mu\text{g}/\text{L}$). This equation was also adapted to calculate the partition between water and hemolymph (P_{H-W}), and between hemolymph and soft tissues (P_{T-H}).

2.6. Metabolomics LC-HRMS analyses

2.6.1. Tissue preparation

Tissue sample preparation was adapted from Bonnefille et al. (2018) [44]. Shortly, digestive gland samples ($20 \text{ mg} \pm 0.1 \text{ mg dw}$) were extracted in two steps with the biphasic methanol:dichloromethane:water (16:16:13, v-v:v). Further details about the extraction of digestive glands are provided in [Supplementary Information](#). Final extracts were filtered using a 500 μL centrifugal filter with a 10 kD modified polyethersulfone membrane (VWR, Fontenay-sous-Bois, France), and transferred into chromatographic vials. A quality control (QC) sample was obtained by pooling 35 μL of each sample extract ($n = 54$).

2.6.2. LC-HRMS data acquisition

The samples were injected using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) on an Exacto Orbitrap (Thermo Fischer Scientific), equipped with a heated electrospray ionization probe (HESI) source. A reverse phase PFP analytical column ($100 \times 2.1 \text{ mm}$; 3 μm particle size; Sigma Aldrich) was used for LC separation. The injection volume (10 μL) was loaded with a full loop injection. The Exacto HRMS was tuned to a mass resolution of 35,000 (FWHM, m/z 200) with a mass spectrum range of 50–750 m/z for full-scan acquisition. Further chromatographic and HRMS acquisition details are described in [Supplementary Information](#). Two batches of samples covering the total amount of samples were submitted: batch 1 (CT, PE-MPL, CP, PE-MPL/CP) and batch 2 (CT, PE-MPL, BZ, PE-MPL/BZ). Mussel samples were randomly distributed in the injection sequence to minimize the influence of confounding factors. A QC sample was injected every ten samples throughout the sequence to assess analytical repeatability and sensitivity. The relative standard deviation (RSD) was calculated for each feature in the QC sample. In addition, MS2 spectra were obtained from QC samples through data dependent acquisition (CE: 20 eV).

2.6.3. Data processing

Raw data files were converted into mzXML files with MSConvert freeware (ProteoWizard 3.0, [45]). A multi-step strategy was implemented for processing ESI+ and ESI- acquisitions, as detailed in Courant et al. (2009) [46] using the XCMS package [47] in the R environment. Optimized XCMS parameters were set: m/z intervals for peak peaking of 0.01, signal-to-noise ratio threshold of 3, group bandwidth of 8, and a minimum fraction of 0.5. After peak alignment, peak picking, and group comparisons, XCMS returned a results table containing feature characteristics (m/z and retention time), abundance (peak area), and a comparison of areas between groups (Welch t -test). Features with an RSD > 30% in QCs were excluded from statistical analysis.

2.6.4. Statistical analysis

A Welch t -test was performed using the XCMS package for the univariate statistical analysis of features between each exposed and control groups. Features with a p -value < 0.1 and an abundance ratio (fold-change) between groups > 1.3 were selected. The rationale for choosing such a p -value threshold was based on the interest of revealing trends in biological modulation due to exposure. A trend towards down- or up-modulation of several metabolites within the same pathway ($p < 0.1$) may be more biologically powerful than a single but statistically significant (at $p < 0.05$) marker metabolite. Multivariate statistical analyses (PCA) were performed on processed data using SIMCA 17 software (Umetrics, Sweden). Datasets were log transformed and Pareto scaled before analysis.

2.6.5. Metabolites annotation

The annotation of metabolites was achieved using the public databases Human Metabolome Database (<http://www.hmdb.ca>) and mzCloud. A mass precision was fixed at 0.002 Da and different annotation levels were used according to Sumner et al. (2007) [48] and

Blázenović et al., (2018) [49]. Level 1 annotations corresponded to unambiguous identification based on the comparison with m/z and retention time of a reference standard injected under the same analytical conditions (in-house database). Level 2 was characterized by putative annotation based on MS and/or MS/MS similarity with spectra from HMDB and mzCloud databases. Assignment of annotated metabolites to metabolic pathways was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.kegg.jp>) and HMDB.

3. Results

3.1. Bioaccumulation of pharmaceuticals

3.1.1. Concentrations in seawater

Acceptable relative recoveries (100 – 124%) and detection limits (< 0.5 ng/L) were obtained for citalopram and bezafibrate ([Table S1](#)). After the spiking of exposure tanks, measured concentrations of pharmaceuticals generally did not differ between groups (with or without PE-MPLs), suggesting that MPLs had a negligible effect on chemical availability in the water phase ([Fig. S1](#)). In the CT and PE-MPL tanks, pharmaceutical concentrations were below the analytical limits, hence ruling out cross-contamination problems. Also, based on the analysis of filtered PE-MPLs particles from stock solutions, the presence of adsorbed compounds could not be confirmed, as procedural blanks ('clean filters') showed a comparable loading (<1% of the total amount). Therefore, a negligible contribution of PE-MPLs to the variation in aqueous pharmaceutical concentrations was demonstrated.

Mean levels of citalopram ($n = 3$) per treatment group (CP and PE-MPL/CP) generally oscillated around 500 ng/L during each seawater spike-renewal cycle of 72 h (481 ± 62 and 303 ± 134 , respectively, after first spike), with some variation across individual tanks in the selected period (196 – 736 ng/L). In the first 24 h, mean bezafibrate concentrations were maintained around 500 ng/L (512 ± 87 and 550 ± 30 in BZ and PE-MPL/BZ, respectively, after first spike). However, in contrast with citalopram, with each spike (every 24 h), mean bezafibrate concentrations steadily increased regardless of MPL presence (1203 – 2704 ng/L at 72 h, before water renewal) ([Fig. S1](#)). Therefore, the expected exposure concentration (500 ng/L) laid near the observed range for citalopram, with a subtle decrease during the first 24 h, and a slight increase after the successive spiking of tanks (before water renewal at 72 h) (effect of 'Time': $F=18.0$, $p > 0.001$). The effect of PE-MPLs was generally negligible, except for the initially lower levels of citalopram in water (effect of 'Time x PE-MPLs': $F=3.0$, $p = 0.041$) ([Fig. S1](#)). A more pronounced increase was observed for bezafibrate, with up to ≈ 3 –4 times higher levels (mean) before water renewal and independently of PE-MPLs (effect of 'Time': $F=13.8$, $p < 0.001$). Altogether, considering the fluctuation of pharmaceuticals within each water spike-renewal cycle, we estimated that the actual exposure concentrations of citalopram (mean \pm SD) were 470 ± 144 (CP) and 505 ± 198 ng/L (PE-MPL/CP) in water throughout the whole exposure. Mean exposure levels of bezafibrate were higher and variable during the experiment: 1074 ± 505 (BZ) and 1232 ± 797 ng/L (PE-MPL/BZ).

After depuration, and considering a daily renewal of water, remaining levels of citalopram in water ranged between 213 ± 36 ng/L (24 h) and 77 ± 25 ng/L (72 h). For bezafibrate, only trace concentrations could be detected during depuration: 8.0 ± 0.3 ng/L (24 h) ([Fig. S2](#)). Therefore, the highest concentrations of both compounds were reported at the first stage of depuration (24 h). In addition, PE-MPLs seemed to diminish the excretion of citalopram by mussels, as shown by consistently lower concentrations in tank water. On average, after 72 h of depuration, the amount of excreted citalopram was 4.7 μg (CP) and 3.0 μg (PE-MPL/CP). In addition to pharmaceuticals, a rapid clearance of daily MPL doses, completely cleared from seawater within 15 min and accumulated in mussels, is shown in García-Pimentel et al., (in prep) and [Supplementary Information \(SI-6\)](#).

3.1.2. Concentrations in mussels' tissues

At the end of the exposure period (21 d), citalopram reached mean concentrations of 757 ± 155 and 701 ± 123 ng/g dw (whole body) in CP and PE-MPL/CP exposed mussels, respectively. Bezafibrate concentrations (21 d) were considerably lower, with mean levels below the MQL (<2.7 ng/g dw) in both treatments. Hemolymph results also showed higher concentrations of citalopram (4.5 ± 0.9 in CP and 6.1 ± 2.9 ng/mL in PE-MPL/CP exposed mussels) than bezafibrate (0.5 ± 0.2 in BZ and 0.4 ± 0.1 ng/mL in PE-MPL/BZ exposed mussels) (Fig. S3). Accordingly, the partition coefficient from water-to-hemolymph (P_{H-W}) and the overall bioaccumulation factor (BAF) were between one and three orders of magnitude higher, respectively, for citalopram than bezafibrate (Fig. 1). Partition from hemolymph to soft tissues was also higher for the antidepressant (Table S2. Partition tissues), indicating that not only transfer to hemolymph, but also subsequent retention in soft tissues, was weaker for the lipid regulator.

Two-way ANOVA showed a significant effect of both MPLs and time in the bioaccumulation of citalopram (whole body) ($F=25.5$, $p < 0.001$): whereas MPLs mainly delayed the internalization of citalopram (effect of 'PE-MPLs', $p = 0.034$), depuration decreased concentrations by 5-fold within seven days (effect of 'Time', $p < 0.001$) (Fig. 2). Following depuration, the residual whole body burden did not differ significantly between groups, as comparable losses were observed in CP (78%) and PE-MPL/CP groups (81%). The partial depuration of citalopram was also reflected in hemolymph. In turn, PE-MPLs did not modulate bezafibrate concentrations in whole body, where depuration was the only significant predictor ($F=13.4$, $p < 0.001$). Bezafibrate showed an increasing tendency in hemolymph from day 10–21 in both single and combined groups ($p = 0.053$), and it was not detected at all after depuration.

A complementary mass balance analysis, based on water and mussel results, demonstrated a moderate retention of citalopram in mussels (9.0–9.6% of the total amount added to the system), in comparison to bezafibrate ($<0.1\%$), which remained almost completely in the water phase (Fig. S4).

A complementary mass balance analysis, based on water and mussel results, demonstrated a significant retention of citalopram in mussels (9–9.6% of the total amount added to the system), in comparison to bezafibrate ($<0.1\%$), which remained almost completely in the water phase (Fig. S4. Mass balance).

3.2. LC-HRMS metabolome analysis

The LC-HRMS analysis of digestive gland samples after 21 d of exposure to the contaminants under ESI+ and ESI- modes led to metabolic fingerprints consisting of 3793 and 4540 features (batch 1: CT, PE-MPL, CP, PE-MPL/CP groups), and 3198 and 4491 features (batch 2: CT, PE-MPL, BZ, PE-MPL/BZ groups), respectively. A comparable number of features were acquired for these batches at 28 d (post-depuration) (Supplementary Information, SI-4). On average, 68% and 69% of QC

features in ESI+ and ESI- had a RSD $< 30\%$. An acceptable repeatability was obtained in all cases, as confirmed by the QC samples injected at regular intervals throughout the analytical run, which clustered together in the PCA. PCA score plots generally showed a clustering of two main groups in each case, i.e., without pharmaceutical and pharmaceutical-exposed. Principal components (7) explained between 51% and 54% of the total variance in ESI+ and ESI- datasets (batches 1 and 2). The co-exposure to PE-MPLs did not lead to any clear split into sub-groups in the PCA (Fig. S5-6).

Univariate analysis between treatment groups was performed for the prioritization of features. Based on these filtered datasets ($p < 0.1$; RSD QC $< 30\%$; fold-change $> 1.3x$), Venn diagrams were built to highlight the number of relevant features across treatment groups (Fig. 3). For instance, 348 differential features (ESI- and ESI+) were simultaneously altered in CP and PE-MPL/CP groups, but not in PE-MPL mussels. In turn, 477 features were differential and simultaneously modulated in BZ and PE-MPL/BZ groups. Many of the features that were significantly altered in pharmaceutical-exposed mussels showed very high fold-changes (15–23% with fold-change > 5), whereas features modified by PE-MPL alone led to more moderate values (7–10% with fold-change > 5) (data not shown). This probably explains the clustering of samples according to pharmaceutical exposure in the PCAs. Nevertheless, a non-negligible number of features were modulated in the presence of PE-MPLs, though many alterations were non-specific (60–67%), as they were also responsive to the other treatments.

3.2.1. Modulation of metabolites and pathways

Metabolites identified by confirmation with analytical standards ($n = 39$) or putative annotation ($n = 25$) are listed in a heatmap that shows their modulation amplitude and p -values for the different comparisons (against the control) at 21 d (Fig. 4). In addition, corresponding metabolite responses after depuration (28 d) are also provided to elucidate potential recovery mechanisms. Citrate (TCA) cycle, purine metabolism, or tyrosine metabolism were among the most impacted pathways after 21 d. An elevated number of metabolites were simultaneously altered by exposure to the different stressors, encompassing pharmaceuticals, PE-MPLs and their mixtures (e.g., Inosine monophosphate (IMP), 4-Methylpentanal). On the other hand, other metabolites were specifically altered in response to a given pharmaceutical compound, e.g., TCA cycle intermediates by bezafibrate (up-regulation).

Complementarily, a Venn diagram of annotated metabolites was built to explore common and specific responses to the two pharmaceuticals (CP and BZ groups) and PE-MPLs (Fig. S7). Among them, six metabolites were commonly altered by all stressors (4-Methylpentanal, 5,6-dihydroxyindol, IMP, lactaldehyde, lysophosphatidylcholine (LPC) (18:1(9Z)/0:0), uridine monophosphate (UMP)), and four were altered by both CP and BZ (e.g., 3,4-dihydroxyphenylacetaldehyde, hydroxyypregnenolone, prostaglandin D2/E2 (PGD2/PGE2), suberic acid), constituting responses to general and PhAC stress, respectively. In turn,

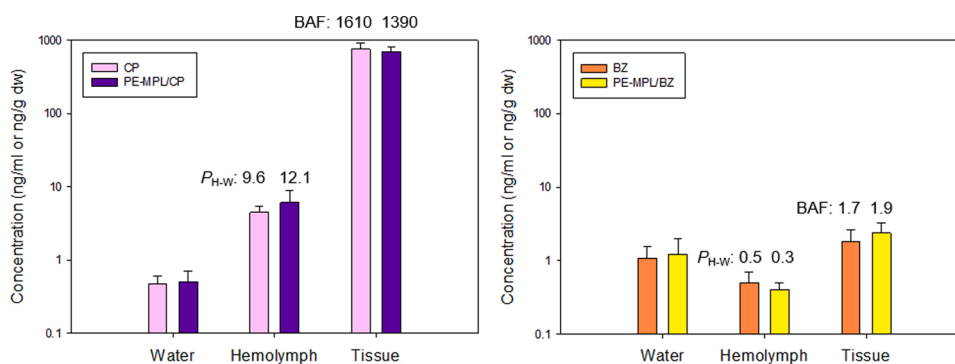


Fig. 1. Mean measured concentrations of citalopram or bezafibrate in water (ng/L), mussel hemolymph (ng/mL) and whole mussel (ng/g dw). The partition coefficient from water to mussel hemolymph (P_{H-W}) and the bioaccumulation factor (BAF in L/kg) are also shown.

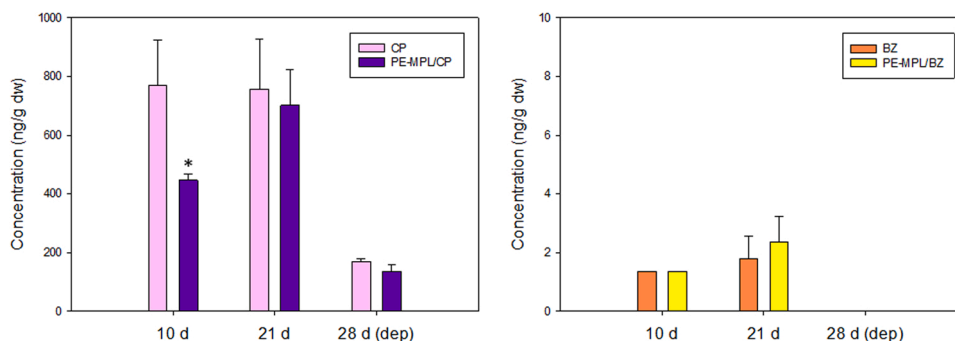


Fig. 2. Mean concentration of citalopram (left) and bezafibrate (right) in whole mussel (ng/g dw) at days 10, 21 and 28 (post-depuration). Note the difference in the Y-axis scale between compounds. SD is not shown in B (10 d) because all samples were <MQL (MQL/2 used). *: significant difference ($p < 0.05$) between treatment groups.

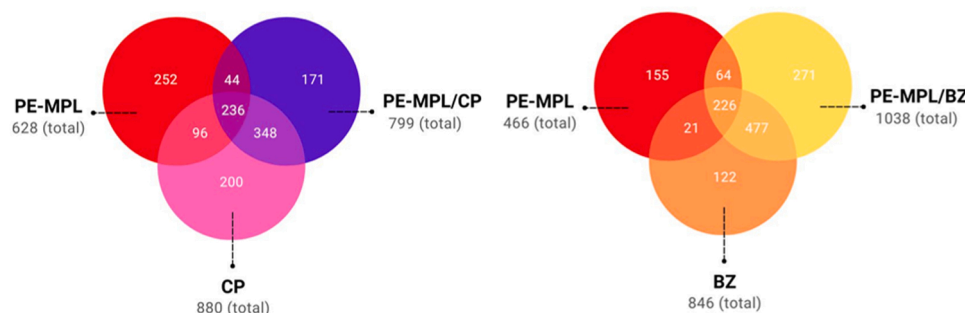


Fig. 3. Venn diagrams highlighting shared and uniquely altered metabolites in response to PE-MPLs and citalopram (A) or bezafibrate (B) groups, relative to the control (CT). Results for the selected signals ($p < 0.1$ and fold-change > 1.3).

among specific biomarkers, 14 metabolites were observed in BZ and PE-MPL, and two were associated with CP. For example, in BZ exposed mussels, four out of the 14 compounds were connected to the TCA cycle (i.e., cis-aconitic acid, isocitric acid, itaconic acid, and oxoglutaric acid).

In mussels exposed to pharmaceuticals, dysregulated metabolites tended to fluctuate in the same direction regardless of PE-MPLs co-exposure (e.g., TCA cycle intermediates, purine metabolism). In fact, none of the impacted pathways showed an opposite response when comparing exposures to a pharmaceutical with or without the PE-MPLs. However, despite the weaker effect suggested by the PCA, PE-MPLs alone triggered relevant modulations, such as purine metabolism (down-modulation) or glycerophospholipid metabolism (up-regulation).

In addition, several metabolites remained altered after the depuration period (28 d), such as purine metabolites, PGD2/PGE2, or a LPC. Conversely, only a few metabolites changed in a different direction after depuration or returned to CT levels (e.g., TCA cycle intermediates, nicotinic acid). Finally, other metabolites were exclusively altered after depuration, such as serotonin in mussels exposed to citalopram.

Besides endogenous metabolites, a retrospective analysis of LC-HRMS data allowed the annotation of the pharmaceutical metabolite N-desmethyl citalopram. This compound, later confirmed with its analytical standard, could be studied qualitatively in digestive glands. Despite the lack of significant differences between treatment groups (i.e., CP vs PE-MPL/CP), a higher mean concentration of N-desmethyl citalopram, along with a lower concentration of the parent compound ($p < 0.1$), was found in PE-MPL/CP exposed mussels (Fig. S8).

4. Discussion

4.1. Effect of MPLs on pharmaceutical bioavailability

Mussels accumulated a much larger amount of citalopram than bezafibrate, especially in whole body but also in hemolymph samples.

The final concentration of these two pharmaceuticals in mussel tissues was independent of microplastic co-exposure, but PE-MPLs affected the early assimilation of citalopram (at 10 d). Considering the negligible effect of PE-MPLs on water levels of the two pharmaceuticals, such effect might be related to other processes taking place within the mussels. Additionally, whereas the low-level accumulation of bezafibrate was completely cleared after the depuration period, a significant proportion of citalopram remained in the mussels. The amount of excreted citalopram upon depuration was lower in mussels co-exposed to PE-MPLs. In the next sub-sections, these major findings will be thoroughly discussed.

4.1.1. Bioaccumulation and tissue distribution of pharmaceuticals

Few studies have addressed the uptake and bioaccumulation of citalopram in aquatic organisms, but some have also documented a moderate bioaccumulation in exposed bivalves [35] or fish [50]. For example, Serra-Compte et al. (2018) reported mussel concentrations (\pm SD) of $12.9 \pm 2.1 \mu\text{g/g dw}$ after a 20-day exposure to $5 \mu\text{g/L}$ (BAF ≈ 2500 , compared to 1390–1610 L/Kg in this study) [35]. According to its $\log K_{OW}$ (3.74 [37]), as moderately hydrophobic pharmaceutical in its neutral form, citalopram represents a medium concern for bioaccumulation [51].

Nevertheless, after converting our data to ww (i.e., dividing by a factor of 5.2), neither citalopram nor bezafibrate could be classified as ‘bioaccumulative’ in this study according to the threshold value established by the EPA ($>1000 \text{ L/Kg ww}$) [52]. Pharmaceutical uptake by organisms is pH-dependent, since uncharged molecules can pass biomembranes more readily than their ions [53]. Selective serotonin reuptake inhibitors (SSRIs) are ionizable compounds (weak bases) with pKa values ranging between 9.05 and 10.5, meaning that their cationic form typically predominates under typical marine seawater pH (8.1–8.3). Significant proportions of both neutral and ionized citalopram could be expected to exist in seawater from exposure tanks. It has been suggested that, besides tested concentration, exposure time can also

Metabolic pathway	Metabolite	21 d					28 d (deuration)				
		PE-MPL	CP	PE-MPL/CP	BZ	PE-MPL/BZ	PE-MPL	CP	PE-MPL/CP	BZ	PE-MPL/BZ
Alanine, aspartate and glutamate metabolism	N-Acetyl-L-aspartic acid*		0.0828					0.0212	0.0246		
Arachidonic acid metabolism	PGD2/PGE2		0.0346	0.0703	0.0337	0.005		0.0009	0.0071	0.0003	0.002
Arginine and proline metabolism	4-Guanidinobutyric acid					0.0428					
	4-Hydroxy-L-glutamic acid*	0.0377									
	Creatinine*				0.0197						
	L-Arginine*										
	Ophthalmic acid*				0.0422				0.0891		
Proline betaine*					0.0333						
Biotin (vitamin B7) metabolism	Pimelic acid*						0.0128	0.0197			
Carnitine metabolism	L-Carnitine*	0.0803				0.0416					
Citrate (TCA) cycle	cis-Aconitic acid				0	0.0011					
	Citric acid*					0.0042		0.0703	0.0609		
	Isocitric acid				0.0283	0.0071		0.0857			
	Itaconic acid				0.0413	0.0066		0.0449	0.0097	0.0178	
	Oxoglutaric acid*			0.0924	0.0451	0.0601					
Fatty acid degradation	Glutaric acid*					0.0142	0.0062	0.0079	0.0091	0.0183	
	Suberic acid		0.0375	0.0373	0.0486	0.0164				0.0255	0.0196
	Tetradecanedioic acid*						0.0001	0.0008			
Glutathione metabolism	5-L-glutamyl-L-alanine*	0.0422	0.0237	0.0878							
	Pyroglutamic acid*				0.0542	0.0058			0.0242		
Glycerophospholipid metabolism	Choline*					0.0266	0.0058	0.0001	0	0.0195	0.0021
	Glycerol 3-phosphate	0.0219				0.0346					
	LysoPC(18:1(9Z)/0:0)	0.0443	0.0053		0.0005	0.0024	0.0341	0.0028	0	0.0315	0.0023
	sn-Glycero-3-phosphocholine (GPC)*	0.0624							0.0052	0.0371	0.0178
Glycine, serine and threonine metabolism	L-Homoserine*		0.0285				0.0027	0.0006	0.0006	0.0114	0.0036
Nicotinate and nicotinamide metabolism	N1-Methyl-4-pyridone-carboxamide	0.0823						0.0262			
	Niacinamide*								0.0747		
	Nicotinic acid*	0.0309	0.0298	0.048		0.0557	0.007	0.007	0	0.0663	0.034
Pentose phosphate pathway	Glyceric acid*					0.0099					
Phenylalanine metabolism	ortho-Hydroxyphenylacetic acid	0.0663				0.0287					
	Phenylethylamine*	0.0371					0.0221		0.0091	0.0492	
Polycyclic aromatic hydrocarbon degradation	Phthalic acid	0.096									0.0324
Purine metabolism	2'-Deoxyguanosine			0.0995					0.0091	0.0103	
	2'-Deoxyguanosine 5'-monophosphate (dGMP)	0.0325							0.013		
	3'-Adenosine monophosphate (AMP)*	0.0152							0.004		
	Adenine*	0.0285		0.0233	0.0069	0.0102					
	Adenosine*				0.0061	0.0103			0.0199	0.0012	0.0002
	Adenosine diphosphate (ADP)	0.0733	0.019	0.0433					0.0387		
	Guanine*				0.0055	0.0006					0.023

Fig. 4. Heatmap of metabolites modulated by PE-MPLs, CP, BZ or their selected mixtures (PE-MPL/CP and PE-MPL/BZ) in mussel digestive gland (21 d), as well as their corresponding fold-change (colours) and p -value ($p < 0.1$). The response of selected metabolites after depuration is also reported. The colour code indicates a fold-change increase (green) or decrease (red) relative to the Control (CT): increasing intensities represent fold-changes between 1.3x and 1.5x (light), 1.5x and 2x, or higher than 2x (dark). Level 1 annotations are marked with an asterisk (*).

influence the tissue distribution of citalopram in exposed organisms. In fish, while short-term exposures led to negligible levels of citalopram in fish muscle (relative to liver/brain) [54], longer exposures resulted in high concentrations [50].

Comparatively, mean bezafibrate concentrations in whole mussels (<2.7 ng/g dw) were approximately 300–400-fold lower than citalopram ones, in agreement with a more limited bioaccumulation capacity previously suggested [55]. This would be surprising given the relatively low hydrophilicity ($\log K_{OW}=4.25 > 3.74$ for citalopram).

However, as an acidic pharmaceutical ($pK_a=3.2$), bezafibrate remains predominantly in its deprotonated form at marine seawater pH values. The electrostatic repulsion of acidic drugs limits their transport through biomembranes and retention in tissues, because they are also negatively charged [56]. For the same reason, bezafibrate shows a limited sorption on suspended matter (e.g., microalgae), which can decrease their uptake with food [57].

Regarding tissue distribution, besides P_{H-W} and overall BAF (Fig. 1), partition from hemolymph into tissues (P_{T-H}) was lower also for

	Guanosine*	0.0527						0.0063		0.0006		
	Hypoxanthine				0.0674	0.0204	0.0437		0.0117			
	Inosine*						0.0086		0.0001	0.0663	0.0149	
	Inosinic acid (IMP)	0.0222	0.0427	0.04	0.0733	0.0786	0.0206		0.0005	0.002	0	
	Uric acid			0.0123	0.0052	0.0005						
Pyrimidine metabolism	2'-Deoxycytidine diphosphate (dCDP)	0.0614										
	Cytidine*	0.0873										
	Deoxycytidine*				0.079							
	Uridine 5'-monophosphate (UMP)	0.0811	0.031		0.0449	0.0457	0.0003	0.0535	0.0014		0.0056	
Pyruvate metabolism	Lactaldehyde*	0.0306	0	0	0	0						
	Pyruvaldehyde				0.003	0.0003			0.0379			
	S-Lactoylglutathione						0.0076		0	0.0002	0.0054	
Steroid hormone biosynthesis	4-Methylpentanal*	0.0001	0	0	0	0		0.0482				
	Hydroxypregnenolone		0.0217	0.0352	0.0078	0.0006	0.0152	0	0	0.0004	0.0115	
Taurine and hypotaurine metabolism	5-L-Glutamyl-taurine*	0.072	0.0211	0.0112		0.0822	0.0431	0.001	0.0006	0.0216	0.0016	
Tryptophan Metabolism	4-Indolecarbaldehyde		0.0423									
	Formylanthranilic acid*											
	L-Tryptophan*					0.0386		0.0896				
	Serotonin*	0.053						0.0404	0.0039			
Tyrosine metabolism	3,4-Dihydroxyphenylacetaldehyde		0.0001	0.001	0.0092	0.0003						
	5,6-Dihydroxyindol	0.0629	0	0	0.0006	0						
	Dopamine*	0.0927			0	0.0001				0.0005	0	
	Dopamine 4-sulfate*				0.0265	0.0012	0.026	0.0014	0.0018	0.0009	0.02	
	Homogentisic acid*						0.1	0.0139	0.0016		0.0711	
Valine, leucine and isoleucine degradation	L-Isoleucine*	0.0606	0.0212				0.0006	0.0024	0.0001	0.0462		

Fig. 4. (continued).

bezafibrate than citalopram (Table S2. Partition), meaning that, compared to whole mussel levels, a higher relative proportion of bezafibrate remained in hemolymph. Other factors could also affect the bioavailability and/or bioaccumulation of pharmaceuticals. Yang et al. (2020) [58] suggested that the bioaccumulation of bezafibrate and other compounds cannot be predicted by log K_{OW} and/or log D_{OW} (pH-dependent), because biodegradation mechanisms are involved, challenging an obvious correlation with the physico-chemical properties of these compounds (e.g., log K_{OW} and/or log D_{OW} (pH-dependent)). Actually, in a study on mussels where bezafibrate was not detected after exposure to concentrations of up to 180.5 $\mu\text{g/L}$, fast metabolism was hypothesized to occur [55]. The digestive gland is a key metabolic organ in bivalves, which may actively contribute to the elimination of this pharmaceutical [59]. However, in this study, the limited uptake, bioaccumulation/biodegradation and dissipation processes of bezafibrate likely explain its progressive increase in seawater following the spiking of contaminants (Fig. S1).

4.1.2. The effect of MPLs

A recent meta-analysis suggested a general tendency of MPLs to increase the bioaccumulation of pharmaceuticals and personal care products (PPCPs) in aquatic organisms [27]. In our study, although a clear effect of PE-MPLs on pharmaceutical bioavailability in the water column could not be depicted, two remarkable trends were observed. Firstly, under co-exposure to PE-MPLs, the accumulation of citalopram in mussels' tissues during the first stage (10 d) was diminished (Fig. 2). The accumulation of organic contaminants in marine bivalves derives from the direct uptake of compounds from the water phase (via gills) or those adsorbed onto particles (via digestive tract) [60]. Under a co-exposure scenario, MPLs might affect the bioavailability of pharmaceuticals in the water phase [23]. However, this was not demonstrated in our study, since the concentrations of citalopram in water were similar regardless of PE-MPLs presence. Liu et al. (2020) suggested that salinity is negatively linked with the adsorption of hydrophilic organic chemicals on MPLs [24], which could explain the limited loading of

pharmaceuticals on PE-MPLs in this study. Therefore, given the negligible effect of PE-MPLs on citalopram availability in water, a possible explanation to the observed differences in bioaccumulation at 10 d might be due to the MPLs interference with key surfaces such as the gills, affecting the regulation of contaminant uptake [61]. Therefore, the PE-MPLs accumulated in both gills and digestive gland tissue (data not shown) might have slowed down the absorption of citalopram in mussels.

Secondly, the amount of excreted citalopram during depuration (in seawater) was higher in mussels exposed to citalopram alone (CP, compared to PE-MPL/CP) (Fig. S2), which may be explained by different factors. For instance, it has been suggested that MPLs can affect the excretion of other contaminants, generally through a "cleaning effect". Sun et al. (2022) [62] proposed a model that highlights a major influence of water and food routes in the uptake of pharmaceuticals in marine fish, while aged MPLs (polystyrene) contributed to the cleaning effect, decreasing their bioaccumulation. This would be in agreement with the observed results in mussels. Another possibility could be an enhanced biotransformation into and excretion of citalopram's main metabolite in the presence of PE-MPLs. Different studies have shown that the uptake of MPLs enhances xenobiotic biotransformation [63]. In aquatic organisms, biotransformation of citalopram into N-desmethylcitalopram has been described in experimental studies [64]. In this study, based on the retrospective LC-HRMS analysis of digestive glands (Fig. S8), an increased metabolism of citalopram into N-desmethylcitalopram was suggested in PE-MPL/CP exposed mussels. Therefore, the biotransformation of citalopram in mussels, which appears to be enhanced in the presence of PE-MPLs, could partially account for the observed differences in depuration water (higher levels of citalopram in CP than PE-MPL/CP tanks) (Fig. S2).

4.2. Effects on the metabolome

The effects assessment of pharmaceuticals and polyethylene MPLs (PE-MPLs) was based on the evaluation of associated metabolomic

profiles on Mediterranean mussels. This approach allowed us to highlight various impacted pathways, uniquely (e.g., TCA cycle in response to bezafibrate) or more generally modulated by the selected stressors (e.g., nucleotide metabolism). According to the PCAs, the two pharmaceuticals (citalopram or bezafibrate), with or without PE-MPLs, induced the strongest modulation of mussels' metabolomic profile compared to the modulation caused by exposure to PE-MPLs alone (Fig. S5-6). This is particularly relevant for bezafibrate, which showed a very limited bioaccumulation but provoked similar adverse effects on the organisms. The pharmaceutical-driven clustering may be explained by the contribution of features with large fold-changes (data not shown) that, unfortunately, could not be annotated in many cases. The metabolic pathways most consistently impacted by citalopram and/or bezafibrate exposures were: neurotransmitters metabolism, i.e., serotonergic activity (citalopram) and dopaminergic activity (bezafibrate), energy metabolism (bezafibrate), nucleotide metabolism (citalopram and bezafibrate) and steroid and prostaglandin metabolism (citalopram and bezafibrate).

In the co-occurrence of PE-MPLs, the magnitude and direction of feature modulation were comparable to those under single pharmaceutical exposure. Nevertheless, PE-MPLs alone also triggered significant metabolomic responses, which were generally common to other stressors (i.e., non-specific, such as the effects on purine metabolism) (Fig. 3). In the following sub-sections, the main impacted pathways will be separately discussed, as well as their drivers and, finally, their possible implications at the individual level.

4.2.1. Metabolism of neurotransmitters

Two neurotransmitters (dopamine and serotonin) were impacted in mussels following exposure (21 d) and/or depuration (28 d). The serotonergic activity was mainly down-regulated by citalopram and/or PE-MPLs, whereas dopaminergic activity was modulated by bezafibrate (up-regulation) or PE-MPLs (down-regulation).

The effect of SSRI antidepressants on bivalve metabolism has been previously addressed [15,65]. The mechanism-of-action (MOA) of these antidepressants consists in increasing the serotonergic activity by preventing serotonin (5-hydroxytryptamine [5-HT]) reuptake from the synaptic cleft. Therefore, according to the human MOA of citalopram, an increase in 5-HT levels would be expected in exposed mussels. The blocking of serotonin reuptake transporters on the cell membrane has been documented in fluoxetine-exposed mussels [66]. This MOA is shared by the different SSRIs, including citalopram, although with varying specificity and sensitivity. In this study, a statistically significant effect of citalopram on 5-HT levels in digestive gland (down-regulation) was only observed after the depuration period (28 d) (Fig. 4). A similar result was observed in fish exposed to another SSRI (fluoxetine), which experienced a decrease in the levels of brain serotonin during exposure or after a recovery period (depending on exposure level) [67]. In general, the higher availability of 5-HT in the nervous system upon reuptake inhibition leads to a decrease in its synthesis and release [68], which may be reflected in other tissues. Therefore, the decreasing content of 5-HT in mussels' digestive gland may arise from an altered metabolism following exposure to citalopram (during the depuration phase). Additionally, the lower levels of citalopram in mussels after depuration (28 d) may have contributed to increasing the reuptake of serotonin (compared to the exposed period) and therefore decreasing its levels. In addition, 5-HT levels are generally lower in bivalves' digestive gland [69] compared to the nervous system, which may hamper the elucidation of SSRI effects.

In addition, serotonergic activity was also down-regulated with PE-MPLs (Fig. 4), which may point out towards a general effect of different stressors on 5-HT metabolism. For example, a depletion of 5-HT levels was also observed in mussels exposed to heavy metals and, because 5-HT and dopamine (DOP) cause a relaxation of muscles, the effect was linked with muscle contraction and shell closure during stress [69]. Various contaminants, including MPLs [70], can decrease the activity of

tryptophan hydroxylase (TPH), a limiting enzyme in 5-HT biosynthesis. In this study, the conversion of tryptophan into 5-HT in mussels exposed to PE-MPLs was probably impaired, shifting towards the production of other metabolites (e.g., DL-Kynurenine, $p < 0.1$ during depuration) (Fig. S9).

On the other hand, the synthesis of the neurotransmitter DOP is associated with tyrosine metabolism, which was markedly modulated by bezafibrate, leading to an increased production of DOP and its metabolites DOP 4-sulfate and 3,4-dihydroxyphenylacetaldehyde (Fig. S10). The mitochondrial enzyme complex monoamine oxidase (MAO) mediates the oxidation of DOP, and its modulation by environmental stressors could affect its elimination [71]. Alternatively, bezafibrate could enhance the conversion to DOP mediated by tyrosine hydroxylase (TH), which catalysed the rate-limiting step in the biosynthesis of DOP. This effect can be accompanied by depletion of tyrosine as precursor [72], as seen in this study (not significant). In this sense, it has been observed that another fibrate (gemfibrozil), through peroxisome proliferator-activated receptor (PPAR)- α activation, facilitates TH activity in a mouse model of Parkinson's disease [73]. In bivalves, TH activity and/or DOP also increased after exposure to pharmaceuticals [71] and other contaminants.

In contrast to bezafibrate, PE-MPLs alone down-regulated the levels of DOP in exposed mussels. Nevertheless, most of the studies on MPLs have reported the opposite response in bivalves and fish. For instance, an increase in DOP in freshwater mussels exposed to PS-MPLs (1 and 10 μm) was described as part of defence mechanisms to either reduce MPLs uptake or enhance their elimination [74]. However, the mechanisms involved in the interference of MPLs with neurotransmitters metabolism are largely unknown [75]. A possible explanation to our findings would be a time-dependency of PE-MPLs effects on the dopaminergic system. While short exposures could manifest early defence responses involving DOP [76], these may be compensated for by chronic exposures.

4.2.2. Energy metabolism

In this study, a clear disturbance in the TCA cycle in bezafibrate-exposed mussels was shown (Fig. 5). Given the regulatory role of PPARs on energy metabolism (β -oxidation of fatty acids, TCA cycle and glycolysis), the observed effect in mussels may correspond to the MOA of bezafibrate. Whereas an alteration of "upstream" fatty acid β -oxidation could not be identified and the end-product acetyl-CoA was not detected, an increased abundance of different TCA metabolites was highlighted (i.e., citrate, cis-aconitate, isocitrate, and α -ketoglutarate), which may be indicative of an activated energy metabolism (Fig. 5). It is worth mentioning that sample preparation may have hampered the elucidation of fatty acids, as the analysis was based on the polar phase of the extracts. The exposure to different fibrate drugs has previously been related to an increased TCA cycle activity in rats [77]. However, the accumulation of certain TCA cycle intermediates may also be indicative of TCA cycle obstruction and consequent shortages of ATP (not detected) [78]. In addition, the TCA intermediate that increased more significantly in this study (cis-aconitate, 21-fold), up-regulated its alternative metabolite itaconic acid, suggesting that associated effects may expand beyond the TCA cycle (e.g., C₅-branched dibasic acid metabolism).

Interestingly, the effect observed on TCA cycle intermediates disappeared after the depuration period (28 d) (Fig. 4). In fact, none of these metabolites were consistently up- or down-modulated, meaning that they returned to baseline levels (reversible effect of bezafibrate on energy metabolism). On the other hand, depuration triggered an alteration of nicotinic acid and nicotinamide metabolism (Fig. 4), related to the biosynthesis of the redox cofactor NAD⁺. An increase in nicotinic acid (NAD⁺ precursor), which occurred in all treatment groups after depuration, has been associated with an activation of energetic metabolism in snails [79].

Despite the activation of energy metabolism, clear signs of oxidative stress were not observed. The fact that carnitines and taurine, which

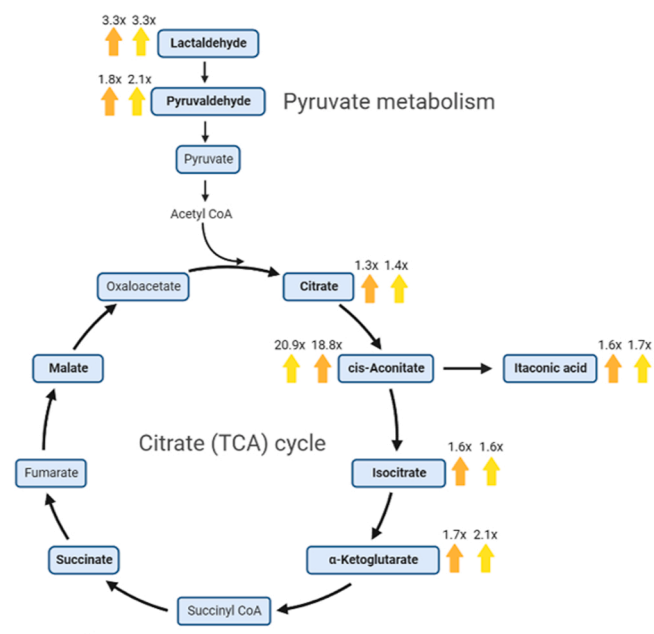


Fig. 5. Partial map of pyruvate metabolism and citrate (TCA) cycle in the response of mussels (*Mytilus galloprovincialis*) to the pharmaceutical bezafibrate, with or without microplastics (BZ in yellow, PE-MPL/BZ in orange). Detected metabolites are marked in bold, and only the fold-change of significant alterations ($p < 0.1$) is indicated.

constitute important markers of oxidative stress in bivalves [80], were not consistently modulated in this study (Spreadsheet S1. All annotations) despite being widely detected in mussels, may point out the lack of oxidative and osmotic stress. However, another oxidative stress indicator (the amino acid phenylalanine and its metabolites) was disrupted in PE-MPLs exposed mussels (increase in both ortho-Hydroxyphenylacetic acid and phenylethylamine) (Fig. 4). A disruption of phenylalanine metabolism has been previously reported in marine mussels (*Mytilus coruscus*) treated with polystyrene MPLs, which might lead to oxidative stress and neurotoxicity [81].

4.2.3. Nucleotide metabolism

Nucleotide metabolism was broadly altered by all stressors in this study, as well as their mixtures. An elevated number of metabolites were consistently modulated, especially those belonging to the metabolism of purines (Fig. S11). Purine and pyrimidine metabolism are among the most impacted pathways in fish and mussels exposed to pharmaceuticals, MPLs, and other pollutants [30,72], besides other stressors (e.g., captivity, hypoxia, food limitation). Therefore, dysregulation of nucleotide metabolism may constitute a general marker of health status and stress in mussels.

In this study, a general increase in IMP (ca. 2.6–3.6 -fold) was accompanied by a down-regulation of AMP and related metabolites (adenosine, adenine, and/or ADP) (Fig. S11). In this scenario, AMP deaminase, the enzyme responsible for the conversion of AMP into IMP, could be overexpressed by exposure to contaminants. An increased conversion into IMP, and/or a decrease in AMP, was observed in bivalves subjected to hypoxia [82] or food limitation [83]. Therefore, it could be that IMP metabolism shifted towards the production of uric acid, which was up-regulated as end product of purine catabolism. It has been suggested that peroxisome proliferation in mussels, mediated by PPAR agonists (like bezafibrate), may be connected with alterations of nucleotide metabolism [84]. Although alterations on nucleotide metabolism have been widely reported in different models, Colás-Ruiz et al. (2022) highlighted their tissue specificity: whereas purine metabolites were mainly up-regulated in fish plasma, they presented an opposite behaviour in liver [10].

4.2.4. Steroid and prostaglandin biosynthesis

Steroid hormone and prostaglandin metabolism were mainly disturbed by exposure to citalopram or bezafibrate, regardless of PE-MPLs co-exposure (Fig. 4). Specifically, exposure triggered an up-regulation of the steroid hydroxypregnenolone, which derives from the metabolism of cholesterol. In this study, along with the increase in hydroxypregnenolone, the alternative metabolite 4-methylpentanal was down-regulated. Therefore, the metabolism of cholesterol seemed to shift towards the production of hydroxypregnenolone, which could have physiological implications since it acts as precursor (prohormone) of sex steroids (e.g., testosterone, estradiol). In agreement with our results, freshwater mussels exposed to 200 ng/L of another SSRI antidepressant (fluoxetine) showed an increase in the levels of esterified estradiol [85]. Dysregulation of steroidogenesis is among the side effects of SSRIs in humans, which affect CYP-mediated reactions in steroid hormone biosynthesis [86].

Along with steroid hormone biosynthesis, the metabolism of arachidonic acid leading to prostaglandins was also impacted. Specifically, higher concentrations of PGD₂/PGE₂ were observed in pharmaceutical-exposed mussels. Cyclooxygenases (COX) are the enzymes responsible for the formation of different prostanoids from arachidonic acid, including prostaglandins and thromboxane. Whereas nonsteroidal anti-inflammatory drugs (NSAIDs) are already known to inhibit COX enzymes to exert their effects [87], it is less clear how other pharmaceuticals could affect arachidonic acid metabolism. Nonetheless, this effect has been suggested for citalopram in human patients [88], or fluoxetine, which enhanced the expression of COX enzymes in the gastric tissue of administered rats, probably through an indirect effect on plasma adrenaline [89]. Altogether, these and our study reinforce the idea that pharmaceuticals other than NSAIDs could exert effects on prostaglandin biosynthesis, probably through modulation of COX enzymes, also on non-target organisms.

4.3. Possible individual-level implications

4.3.1. Pharmaceuticals

In this experiment, the bioaccumulation of pharmaceuticals (citalopram and bezafibrate) in mussels was confirmed. Tissue concentrations of citalopram were higher overall, regardless of PE-MPLs. However, the magnitude of pharmaceutical effects seemed comparable between the two compounds, despite the lower tendency for bioaccumulation of bezafibrate. Compound-specific properties can influence internal effect concentrations, which may aid in the characterization of the relationship between bioaccumulation and toxicity [90]. Therefore, such threshold levels may contribute to explaining a similar toxicity between compounds that are retained by organisms to a different degree (e.g., pharmaceuticals in this study). Although several studies have simultaneously reported contaminant bioaccumulation and measured their effects on biota, only a few have tried to correlate both. In fact, it was suggested that the link between pharmaceutical bioaccumulation and harmful biological effects is very much unknown and remains a knowledge gap [91,92].

It is known that pharmaceutical effects are preceded by an effective uptake, a relevant part of the accumulation process, but different factors can complicate a prediction of ecotoxicity based on bioaccumulation models [90]. For example, physiological mechanisms of contaminant detoxification (e.g., sequestration in intracellular granules) can contribute to preventing a direct relationship between bioaccumulation and toxicity [93]. Based on the human therapeutic levels (C_{max} plasma, ng/mL) of bezafibrate (15,000 ng/mL) and citalopram (50 ng/mL) [94], one could expect a higher potency of citalopram in non-target species. However, uncertainty factors are commonly applied to account for inter-species variability in their internal effect concentration, in order to estimate the potential hazards of pharmaceuticals in biota [95]. The results of this study highlight an increased sensitivity of mussels to bezafibrate given that although a very low bioaccumulation was

observed, the related effects were significant.

Regarding effects assessment, a concordance in the sensitivity of metabolomics and classical toxicity studies has been noted for a diversity of compounds [96]. Thus, most of the studies combining omics with classical ecotoxicology parameters observed simultaneous alterations of the two (e.g., enzymatic activity and metabolome in mussels) [78]. However, advances in omics have improved the detection of subtle changes not detected by traditional biological endpoints, and have shown potential as part of biological early warning systems [97]. For instance, metabolomic approaches turned out to be more sensitive in mussels exposed to sulfamethoxazole [98].

Following pharmaceutical-driven alterations at lower levels of biological organization, a series of individual-level effects may arise. In this scenario, the Adverse Outcome Pathway (AOP) framework emerged as a tool to represent associated biological events and to connect them with plausible adverse outcomes at individual- and population- levels [99] (More detailed information can be found in [Supplementary Information SI-7](#)). For example, SSRI could affect CYP enzymes involved in human steroidogenesis [86], which might cause impaired reproduction in non-target species too, as suggested in this study by the modulation of cholesterol metabolism in mussels. Additionally, the possible interaction of citalopram with COX enzymes, typically inhibited by NSAID drugs, but also suggested in this study, might influence the functions regulated by prostaglandins in invertebrates (e.g., immunity, gametogenesis).

In the case of bezafibrate, it has been hypothesized that evolutionarily conserved sequences related to PPAR in invertebrates may contribute to explaining similar effects on lipid and energy metabolism (mediated by PPAR-like receptors) to those in vertebrates [100], as suggested by this study. An alteration of energy metabolism can result in increased energy expenditure and loss of reserves, which can ultimately affect growth and reproduction in bivalves. A down-regulation of the TCA cycle has been connected to a loss of body weight in oysters [101], which was however not observed in exposed mussels from our study ([Table S4](#)). Nevertheless, downstream effects of PPAR activation by bezafibrate may extend beyond the stimulation of fatty acid metabolism. For example, PPAR α -mediated up-regulation of NURR1 (transcription factor) is critical to the expression of enzymes involved in the synthesis of DOP, such as tyrosine hydroxylase (TH) [102]. DOP has been enhanced in bivalves from contaminated sites [72] and in bezafibrate-exposed mussels from this study too. Increased TH activity and levels of DOP are involved in byssus formation [44] and in the inhibition of gamete release [103], counteracting the effect of 5-HT in mussels [123]. Additionally, both DOP and 5-HT play an important role in feeding behaviour and digestion [104,105], so the observed changes could affect the function of the digestive gland.

4.3.2. MPLs

In general, the construction of AOPs for MPLs remains challenging, because there are no described specific targets for their action. However, relevant metabolic pathways can be disturbed by MPLs too [106]. According to this study, the metabolism of neurotransmitters, or purine metabolism, were markedly impaired following PE-MPLs exposure. Differences in exposure time, or activation of compensatory mechanisms to MPLs, could lead to contrasting findings in the metabolome across studies. In addition to neuroendocrine disruption, PE-MPLs may result in the disturbance of building blocks for DNA and RNA turnover and repair, or the production of energy cofactors (e.g., ATP) that are necessary for cell survival and proliferation [107].

Only tentative AOPs have been suggested for micro- and nanoparticles, based on the matching of key events with those in existing AOPs for other contaminants [75,106,108]. Commonly altered endpoints include ROS generation and related antioxidant and apoptotic pathways, as well as neurotoxicity via inhibition of acetylcholinesterase (AChE), but mechanistic aspects are generally undetermined [75,106]. Additionally, indirect metabolic effects may play a role in the toxicity of MPLs, e.g., neurotoxicity through oxidative stress-mediated inhibition of

AChE, which can complicate the identification of mechanisms and AOPs [75,81]. For example, lipid peroxidation caused by MPLs might induce the rupture of acetylcholine-containing vesicles, releasing neurotransmitters into the synaptic clefts, which activate AChE production as a compensatory mechanism [109]. Also, the diversity of MPLs (e.g., size, shape, surface, polymer, additives) and the lack of standardized methodologies add further difficulties to hazard assessment, which often leads to contradictory outcomes in different studies [75].

In this study, expected individual-level effects comprise a disturbance of mussels' energy metabolism, reproduction, behaviour, or immunity, in response to one or more of the selected stressors. The possible combined effects of PE-MPLs and pharmaceuticals were not further discussed, since no significant interactions on the mussels' metabolome could be identified.

4.3.3. Depuration effects

Another interesting aspect of this study was the assessment of depuration effects. This approach allowed us to characterize potential recovery mechanisms, as well as long-lasting or latent effects on the mussels. For example, energy metabolism was recovered in bezafibrate exposed mussels after depuration. In turn, the effects on purine metabolism, common to all stressors, were long-lasting and not compensated by depuration. Finally, different metabolites were exclusively altered during the depuration period, the so-called latent effects, such as the effect of citalopram on 5-HT metabolism. This is environmentally relevant because bivalves from freshwater and coastal areas may be subjected to pulses of contamination separated by "clean" periods [110]. This effect has been assessed by few other metabolomic studies. For instance, in oysters previously exposed to fluoxetine (1 $\mu\text{g/L}$), only a partial recovery was observed after the depuration period, with several long-lasting alterations [65]. In fish from treated wastewater ponds, more than two months of depuration were needed to stabilise their metabolism [111]. On the other hand, in mussels exposed to the antibiotic sulfamethoxazole, the antioxidant system showed a delayed activation, not previously triggered during exposure [112]. Therefore, biomarkers also constitute useful tools to assess the possible latent effects of contaminants on organisms. Finally, the evaluation of depuration of xenobiotics in commercial bivalves is also relevant from the point of view of risk assessment for consumers [98], as commercialization and consumption are commonly preceded by a depuration process. For example, in this study, around 20% of the accumulated citalopram, remained in the mussels after a 7-d depuration ([Fig. S3](#)).

5. Conclusions

By combining non-target LC-HRMS metabolomics and target chemical analyses, the effect of MPLs (PE-MPLs) on the bioaccumulation and metabolomic effects of two common pharmaceuticals (citalopram and bezafibrate) was examined in marine mussels (*Mytilus galloprovincialis*). The bioaccumulation of the SSRI antidepressant citalopram exceeded that of the lipid regulator bezafibrate by almost two orders of magnitude. PE-MPLs delayed the accumulation of citalopram, prolonging the time needed to reach the concentrations observed in singly exposed mussels. In addition, PE-MPLs seemed to enhance the biotransformation of citalopram, limiting the excretion of the parent pharmaceutical into depuration water.

The assessment of effects through metabolomics data highlighted the alteration of mussels' endogenous metabolites in response to PE-MPLs, pharmaceuticals, or their mixtures. The strongest effects on the metabolome were driven by the two pharmaceuticals, mostly regardless of PE-MPLs co-exposure, but PE-MPLs alone had an impact on different metabolic pathways too. The metabolism of neurotransmitters, steroid and prostaglandin hormones, purines, or the TCA cycle, were among the most impacted pathways after exposure to pharmaceuticals and/or PE-MPLs. Toxicity mechanisms remain largely unknown, but certain effects were linked to the pharmaceuticals' MOA. In this sense,

metabolomics can aid in the characterization of mechanisms leading to adverse outcomes at the individual level, which constitutes a powerful tool for the improvement of ecological risk assessment. The observed molecular alterations may lead to individual-level implications for the mussels, such as disrupted energy metabolism, reproduction, DNA and RNA synthesis, or immunity.

Environmental implication

The effect of pharmaceuticals on non-target organisms commonly relates to the pharmaceuticals' mode of action, but their interaction with physical hazards such as microplastics, as well as their aquatic toxicity at environmentally relevant concentrations (bezafibrate), and that microplastics can affect the bioaccumulation of citalopram. In addition, it was demonstrated that effects on the metabolome may persist or appear after a recovery period of 7 days.

CRediT authorship contribution statement

J.M. Castaño-Ortiz: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original draft; **F. Courant:** Formal analysis, Investigation, Data Curation, Writing – review & editing; **E. Gómez:** Formal analysis, Investigation, Writing – review & editing; **M.M. García-Pimentel:** Methodology, Formal analysis, Investigation, Writing – review & editing; **V.M. León:** Conceptualization, Methodology, Funding acquisition, Writing – review & editing; **J.A. Campillo:** Methodology, Investigation, Writing – review & editing; **L.H.M.L.M. Santos:** Investigation, Supervision, Writing – review & editing; **D. Barceló:** Conceptualization, Supervision, Writing – review & editing; **S. Rodríguez-Mozaz:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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

This work was supported by the Spanish Inter-Ministerial Science and Technology Commission through the 'PLAS-MED' (CICYT, CTM2017–89701-C3) and PHARMASEA (PCI2021-121933) projects and by the European Union through the European Regional Development Fund (ERDF). JM Castaño Ortiz acknowledges the predoctoral grant from AGAUR (2019 FI_B 00881REF). M. García Pimentel acknowledges the Spanish Ministerial Science, Innovation and University for her predoctoral fellowship (PRE2018-085502). The authors are grateful for funding from the CERCA Programme, Generalitat de Catalunya. The Ultra-Performance Liquid Chromatograph coupled to a triple Quadrupole hybrid Linear Ion Trap Detector (Acquity UPLC-MS QTRAP 5500, Waters-SCIEX) received support from the CERCAGINYS programme, funded by the Spanish Ministry of Science and Innovation. The authors thank the Platform of Non-Target Environmental Metabolomics (PONTEM) of the consortium facilities Montpellier Alliance for Metabolomics and Metabolism Analysis (MAMMA). The authors acknowledge the support of the Economy and Knowledge Department of the Catalan Government through a Consolidated Research Group (ICRA-ENV – 2021 SGR 01282).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131904.

References

- [1] Castaño-Ortiz, J., et al., 2023. Bioaccumulation and fate of pharmaceuticals in a Mediterranean coastal lagoon: temporal variation and impact of a flash flood event. *Environ Res* vol. 228.
- [2] Nödler, K., Voutsas, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. *Mar Pollut Bull* vol. 85 (1), 50–59.
- [3] Arpin-Pont, L., Bueno, M.J.M., Gomez, E., Fenet, H., 2016. Occurrence of PPCPs in the marine environment: a review. *Environ Sci Pollut Res* vol. 23 (6), 4978–4991.
- [4] Meador, J.P., Yeh, A., Gallagher, E.P., 2017. Determining potential adverse effects in marine fish exposed to pharmaceuticals and personal care products with the fish plasma model and whole-body tissue concentrations. *Environ Pollut* vol. 230, 1018–1029.
- [5] Sousa, J.C.G., Barbosa, M.O., Ribeiro, A.R.L., Ratola, N., Pereira, M.F.R., Silva, A. M.T., 2020. Distribution of micropollutants in estuarine and sea water along the Portuguese coast. *Mar Pollut Bull* vol. 154, 111120.
- [6] Rodríguez-Mozaz, S., Álvarez-Muñoz, D., Barceló, D., 2017. Pharmaceuticals in the marine environment: analytical techniques and applications. *Environmental Problems in Marine Biology*. CRC Press, pp. 268–316.
- [7] Castaño-Ortiz, J., Serra-Compte, A., Álvarez-Muñoz, D., Barceló, D., Rodríguez-Mozaz, S., 2023. Chapter 3: Antibiotics in the marine environment: occurrence and impact. *Contaminants of Emerging Concern in the Marine Environment: Current Challenges in Marine Pollution*. Elsevier, p. 390.
- [8] Moreno-González, R., Rodríguez-Mozaz, S., Gros, M., Barceló, D., León, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). *Environ Res* vol. 138, 326–344.
- [9] Forns, J., et al., 2019. Antidepressant use in Denmark, Germany, Spain, and Sweden between 2009 and 2014: Incidence and comorbidities of antidepressant initiators. *J Affect Disord* vol. 249, 242–252.
- [10] Colás-Ruiz, N.R., Ramirez, G., Courant, F., Gomez, E., Hampel, M., Lara-Martín, P.A., 2022. Multi-omic approach to evaluate the response of gilt-head sea bream (*Sparus aurata*) exposed to the UV filter sulisobenzone. *Sci Total Environ* vol. 803, 150080.
- [11] Martínez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C., Gomez, E., 2014. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program. *Anal Bioanal Chem* vol. 406, 601–610.
- [12] Madikizela, L.M., et al., 2020. Pharmaceuticals and their metabolites in the marine environment: Sources, analytical methods and occurrence. *Trends Environ Anal Chem* vol. 28, e00104.
- [13] Mijangos, L., et al., 2018. Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental risk. *Water Res* vol. 147, 152–163.
- [14] Fong, P.P., Molnar, N., 2013. Antidepressants cause foot detachment from substrate in five species of marine snail. *Mar Environ Res* vol. 84, 24–30.
- [15] Ramirez, G., et al., 2022. Early biological modulations resulting from 1-week venlafaxine exposure of marine mussels *mytilus galloprovincialis* determined by a metabolomic approach. *Metabolites* vol. 12 (3), 197.
- [16] Canesi, L., et al., 2007. Effects of Triclosan on *Mytilus galloprovincialis* hemocyte function and digestive gland enzyme activities: Possible modes of action on non target organisms. *Comp Biochem Physiol Part C Toxicol Pharmacol* vol. 145 (3), 464–472.
- [17] Cincinelli, A., Martellini, T., Guerranti, C., Scopetani, C., Chelazzi, D., Giarrizzo, T., 2018. A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea. *TrAC Trends Anal Chem* vol. 110, 321–326.
- [18] Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: Source, distribution and threats — a review. *Sci Total Environ* vol. 698, 134254.
- [19] Gregory, M.R., 2009. Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos Trans R Soc B Biol Sci* vol. 364 (1526), 2013–2025.
- [20] Sathicq, M.B., Sabatino, R., Corno, G., Di Cesare, A., 2021. Are microplastic particles a hotspot for the spread and the persistence of antibiotic resistance in aquatic systems? *Environ Pollut*, 116896.
- [21] Ruiz-Fernandez, J.M., et al., 2019. Informe de síntesis sobre el estado actual del Mar Menor y sus causas en relación a los contenidos de nutrientes. *Proj Sustain Conserv Mar Menor Lagoon Its Basin; Univ Alicante Spain*.
- [22] Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the ocean. *Environ Sci Technol* vol. 46 (22), 12302–12315.
- [23] Santos, L.H., Rodríguez-Mozaz, S., Barcelo, D., 2021. Microplastics as vectors of pharmaceuticals in aquatic organisms – An overview of their environmental implications. *December 2020 Case Stud Chem Environ Eng* vol. 3. December 2020.
- [24] Liu, P., Wu, X., Liu, H., Wang, H., Lu, K., Gao, S., 2020. Desorption of pharmaceuticals from pristine and aged polystyrene microplastics under simulated gastrointestinal conditions. *J Hazard Mater* vol. 392, 122346.

- [25] Santos, L.H., Rodríguez-Mozaz, S., Barceló, D., 2020. Sorption of pharmaceuticals on microplastics. *Handb Micro Environ* 1–36.
- [26] Zhou, W., et al., 2021. Fine polystyrene microplastics render immune responses more vulnerable to two veterinary antibiotics in a bivalve species. *Mar Pollut Bull* vol. 164, 111995.
- [27] Sun, T., Wang, S., Ji, C., Li, F., Wu, H., 2022. Microplastics aggravate the bioaccumulation and toxicity of coexisting contaminants in aquatic organisms: a synergistic health hazard. *J Hazard Mater* vol. 424, 127533.
- [28] Świacka, K., Maculewicz, J., Smolarz, K., Szaniawska, A., Caban, M., 2019. Mytilidae as model organisms in the marine ecotoxicology of pharmaceuticals - a review. *Environ Pollut* vol. 254, 113082.
- [29] Faggio, C., Tsarpali, V., Dailianis, S., 2018. Mussel digestive gland as a model tissue for assessing xenobiotics: an overview. *Sci Total Environ* vol. 636, 220–229.
- [30] Dumas, T., et al., 2022. An integrated metabolomics and proteogenomics approach reveals molecular alterations following carbamazepine exposure in the male mussel *Mytilus galloprovincialis*. *Chemosphere* vol. 286, 131793.
- [31] Courant, F., Antignac, J., Dervilly-Pinel, G., Le Bizec, B., 2014. Basics of mass spectrometry based metabolomics. *Proteomics* vol. 14 (21–22), 2369–2388.
- [32] Dumas, T., et al., 2020. Metabolomics approach reveals disruption of metabolic pathways in the marine bivalve *Mytilus galloprovincialis* exposed to a WWTP effluent extract. *Sci Total Environ* vol. 712.
- [33] Menéndez-Pedriz, A., Jaumot, J., 2020. Interaction of environmental pollutants with microplastics: a critical review of sorption factors, bioaccumulation and ecotoxicological effects. *Toxics* vol. 8 (2).
- [34] Castaño-Ortiz, J., et al., 2023. Fate of pharmaceuticals in the Ebro River: The combined use of biomonitoring, water, sediment and plastic litter sampling. *Prep. Serra-Compte, A., et al., 2018. Effects of water warming and acidification on bioconcentration, metabolism and depuration of pharmaceuticals and endocrine disrupting compounds in marine mussels (Mytilus galloprovincialis). Environ Pollut* vol. 236, 824–834.
- [35] Fehrenbach, G.W., Pogue, R., Carter, F., Clifford, E., Rowan, N., 2022. Implications for the seafood industry, consumers and the environment arising from contamination of shellfish with pharmaceuticals, plastics and potentially toxic elements: A case study from Irish waters with a global orientation. *Sci Total Environ* vol. 844, 157067.
- [36] NORMAN, "NORMAN Ecotoxicology Database. (<https://www.norman-network.com/nds/ecotox>). Accessed May 2023."
- [37] Wang, W., Wang, J., 2018. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC - Trends Anal Chem* vol. 108, 195–202.
- [38] Liong, R.M.Y., Hadibarata, T., Yuniarto, A., Tang, K.H.D., Khamidun, M.H., 2021. Microplastic occurrence in the water and sediment of Miri river estuary, Borneo Island. *Water, Air, Soil Pollut* vol. 232 (8), 342.
- [39] Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. *J Chromatogr A* vol. 1248, 104–121.
- [40] Santos, L.H., et al., 2019. Impact of fullerenes in the bioaccumulation and biotransformation of venlafaxine, diuron and triclosan in river biofilms. *Environ Res* vol. 169, 377–386.
- [41] León, V.M., et al., 2019. PAHs, pesticides, personal care products and plastic additives in plastic debris from Spanish Mediterranean beaches. *Sci Total Environ* vol. 670, 672–684.
- [42] Castaño-Ortiz, M., Rodríguez-Mozaz, S., Buttiglieri, G., 2023. A Decade Water Monit A Mediterr Reg: Pharm prioritisation Upgrad Anal Methodol.
- [43] Bonnefille, B., Gomez, E., Alali, M., Rosain, D., Fenet, H., Courant, F., 2018. Metabolomics assessment of the effects of diclofenac exposure on *Mytilus galloprovincialis*: Potential effects on osmoregulation and reproduction. *Sci Total Environ* vol. 613, 611–618.
- [44] Chambers, M.C., et al., 2012. A cross-platform toolkit for mass spectrometry and proteomics (Oct-). In: *Nature Biotechnology*, vol. 30. Nature Publishing Group, pp. 918–920.
- [45] Courant, F., Pinel, G., Bichon, E., Monteau, F., Antignac, J.-P., Le Bizec, B., 2009. Development of a metabolomic approach based on liquid chromatography-high resolution mass spectrometry to screen for clenbuterol abuse in calves. *Analyst* vol. 134 (8), 1637–1646.
- [46] Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R., Siuzdak, G., 2006. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem* vol. 78 (3), 779–787.
- [47] Sumner, L.W., et al., 2007. Proposed minimum reporting standards for chemical analysis. *Metabolomics* vol. 3 (3), 211–221.
- [48] Blaženović, I., Kind, T., Ji, J., Fiehn, O., 2018. Software tools and approaches for compound identification of LC-MS/MS data in metabolomics. *Metabolites* vol. 8 (2), 31.
- [49] Ziegler, M., et al., 2020. Impact of the antidepressant citalopram on the behaviour of two different life stages of brown trout. *PeerJ* vol. 8, e8765.
- [50] Howard, P.H., Muir, D.C.G., 2011. Identifying new persistent and bioaccumulative organics among chemicals in commerce II: pharmaceuticals. *Environ Sci Technol* vol. 45 (16), 6938–6946.
- [51] EPA, "Environment Protection Agency, 2012. Office of pollution prevention and toxics. TSCA work plan chemicals: methods document. *Off. Pollut. Prev. Toxics*.
- [52] Boström, M.L., 2019. Uptake and bioaccumulation of ionizable pharmaceuticals in aquatic organisms. *Lund Univ.*
- [53] Grabicova, K., et al., 2014. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. *Sci Total Environ* vol. 488–489, 46–50.
- [54] Contardo-Jara, V., Lorenz, C., Pflugmacher, S., Nützmann, G., Kloas, W., Wiegand, C., 2011. Exposure to human pharmaceuticals Carbamazepine, Ibuprofen and Bezafibrate causes molecular effects in *Dreissena polymorpha*. *Aquat Toxicol* vol. 105 (3–4), 428–437.
- [55] Cravo, A., et al., 2022. Understanding the bioaccumulation of pharmaceutical active compounds by clams *Ruditapes decussatus* exposed to a UWWTP discharge. *Environ Res* vol. 208, 112632.
- [56] Bhatt, P., Bhandari, G., Turco, R.F., Aminikhoie, Z., Bhatt, K., Simsek, H., 2022. Algae in wastewater treatment, mechanism, and application of biomass for production of value-added product. *Environ Pollut* vol. 309, 119688.
- [57] Yang, H., et al., 2020. Residues, bioaccumulation, and trophic transfer of pharmaceuticals and personal care products in highly urbanized rivers affected by farm diversion. *J Hazard Mater* vol. 391, 122245.
- [58] Canesi, L., et al., 2007. Effects of blood lipid lowering pharmaceuticals (bezafibrate and gemfibrozil) on immune and digestive gland functions of the bivalve mollusc, *Mytilus galloprovincialis*. *Chemosphere* vol. 69 (6), 994–1002.
- [59] Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. *Environ Toxicol Chem* vol. 35 (4), 799–812.
- [60] Barboza, L.G.A., Vieira, L.R., Branco, V., Carvalho, C., Guilhermino, L., 2018. Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in *Dicentrarchus labrax* juveniles. *Sci Rep* vol. 8 (1), 15655.
- [61] Liu, P., et al., 2022. Contribution of aged polystyrene microplastics to the bioaccumulation of pharmaceuticals in marine organisms using experimental and model analysis. *Chemosphere* vol. 287, 132412.
- [62] Zhang, H., Pap, S., Taggart, M.A., Boyd, K.G., James, N.A., Gibb, S.W., 2020. A review of the potential utilisation of plastic waste as adsorbent for removal of hazardous priority contaminants from aqueous environments. *Environ Pollut* vol. 258, 113698.
- [63] Hubená, P., et al., 2021. Prescribed aggression of fishes: pharmaceuticals modify aggression in environmentally relevant concentrations. *Ecotoxicol Environ Saf* vol. 227, 112944.
- [64] Brew, D.W., Black, M.C., Santos, M., Rodgers, J., Henderson, W.M., 2020. Metabolomic investigations of the temporal effects of exposure to pharmaceuticals and personal care products and their mixture in the eastern oyster (*Crassostrea virginica*). *Environ Toxicol Chem* vol. 39 (2), 419–436.
- [65] Franzellitti, S., Buratti, S., Valbonesi, P., Fabbri, E., 2013. The mode of action (MOA) approach reveals interactive effects of environmental pharmaceuticals on *Mytilus galloprovincialis*. *Aquat Toxicol* vol. 140–141, 249–256.
- [66] Gaworecki, K.M., Klaine, S.J., 2008. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquat Toxicol* vol. 88 (4), 207–213.
- [67] Wong, D.T., Bymaster, F.P., Engleman, E.A., 1995. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci* vol. 57 (5), 411–441.
- [68] Almeida, E.A., Bains, A.C.D., Medeiros, M.H.G., Di Mascio, P., 2003. Effects of trace metal and exposure to air on serotonin and dopamine levels in tissues of the mussel *Perna perna*. *Mar Pollut Bull* vol. 46 (11), 1485–1490.
- [69] Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). *Environ Res* vol. 151, 58–70.
- [70] Aguirre-Martínez, G.V., André, C., Gagné, F., Martín-Díaz, L.M., 2018. The effects of human drugs in *Corbicula fluminea*. Assessment of neurotoxicity, inflammation, gametogenic activity, and energy status. *Ecotoxicol Environ Saf* vol. 148, 652–663.
- [71] Cappello, T., Maisano, M., Giannetto, A., Parrino, V., Maueri, A., Fasulo, S., 2015. Neurotoxicological effects on marine mussel *Mytilus galloprovincialis* caged at petrochemical contaminated areas (eastern Sicily, Italy): ¹H NMR and immunohistochemical assays. *Comp Biochem Physiol Part - C Toxicol Pharmacol* vol. 169, 7–15.
- [72] Gottschalk, C.G., Jana, M., Roy, A., Patel, D.R., Pahan, K., 2021. Gemfibrozil protects dopaminergic neurons in a mouse model of Parkinson Disease via PPAR α -dependent astrocytic GDNF pathway. *LP - 2300 J Neurosci* vol. 41 (10), 2287. LP - 2300.
- [73] Magni, S., et al., 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). *Sci Total Environ* vol. 631–632, 778–788.
- [74] Hu, M., Palić, D., 2020. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biol* vol. 37, 101620.
- [75] Jeong, S., et al., 2022. Size-dependent seizurogenic effect of polystyrene microplastics in zebrafish embryos. *J Hazard Mater* vol. 439, 129616.
- [76] Cornwell, P.D., De Souza, A.T., Ulrich, R.G., 2004. Profiling of hepatic gene expression in rats treated with fibric acid analogs. *Mutat Res Mol Mech Mutagen* vol. 549 (1), 131–145.
- [77] Gu, Y.-Y., et al., 2021. A comprehensive study of the effects of phthalates on marine mussels: Bioconcentration, enzymatic activities and metabolomics. *Mar Pollut Bull* vol. 168, 112393.
- [78] Della Torre, C., et al., 2021. Alginate coating modifies the biological effects of cerium oxide nanoparticles to the freshwater bivalve *Dreissena polymorpha*. *Sci Total Environ* vol. 773, 145612.
- [79] Leonard, J.A., Cope, W.G., Barnhart, M.C., Bringolf, R.B., 2014. Metabolomic, behavioral, and reproductive effects of the aromatase inhibitor fadrozole

- hydrochloride on the unionid mussel *Lampsilis fasciola*. *Gen Comp Endocrinol* vol. 206, 213–226.
- [81] Huang, W., et al., 2021. Toxicity mechanisms of polystyrene microplastics in marine mussels revealed by high-coverage quantitative metabolomics using chemical isotope labeling liquid chromatography mass spectrometry. *J Hazard Mater* vol. 417, 126003.
- [82] Haider, F., Falfushynska, H.L., Timm, S., Sokolova, I.M., 2020. Effects of hypoxia and reoxygenation on intermediary metabolite homeostasis of marine bivalves *Mytilus edulis* and *Crassostrea gigas*. *Comp Biochem Physiol Part A Mol Integr Physiol* vol. 242, 110657.
- [83] Roznere, I., Watters, G.T., Wolfe, B.A., Daly, M., 2014. Nontargeted metabolomics reveals biochemical pathways altered in response to captivity and food limitation in the freshwater mussel *Amblema plicata*. *Comp Biochem Physiol Part D Genom Proteom* vol. 12, 53–60.
- [84] Watanabe, M., Meyer, K.A., Jackson, T.M., Schock, T.B., Johnson, W.E., Bearden, D.W., 2015. Application of NMR-based metabolomics for environmental assessment in the Great Lakes using zebra mussel (*Dreissena polymorpha*). *Metabolomics* vol. 11 (5), 1302–1315.
- [85] Lazzara, R., Blázquez, M., Porte, C., Barata, C., 2012. Low environmental levels of fluoxetine induce spawning and changes in endogenous estradiol levels in the zebra mussel *Dreissena polymorpha*. *Aquat Toxicol* vol. 106–107, 123–130.
- [86] Hansen, C.H., Larsen, L.W., Sørensen, A.M., Halling-Sørensen, B., Styrisshave, B., 2017. The six most widely used selective serotonin reuptake inhibitors decrease androgens and increase estrogens in the H295R cell line. *Toxicol Vitro* vol. 41, 1–11.
- [87] Courant, F., et al., 2018. Exposure of marine mussels to diclofenac: modulation of prostaglandin biosynthesis. *Environ Sci Pollut Res* vol. 25 (7), 6087–6094.
- [88] Richter, T., Alusik, S., Paluch, Z., Burianova, I., Cybulja, A., Sadilkova, L., 2015. Suppressive effect of citalopram on plasma concentrations of thromboxane B2. *Scand J Clin Lab Invest* vol. 75 (7), 615–620.
- [89] Salem Sokar, S., Elsayed Elsayad, M., Sabri Ali, H., 2016. Serotonin and histamine mediate gastroprotective effect of fluoxetine against experimentally-induced ulcers in rats. *J Immunotoxicol* vol. 13 (5), 638–651.
- [90] Sijm, D.T.H.M., Hermens, J.L.M., 2000. Internal effect concentration: link between bioaccumulation and ecotoxicity for organic chemicals. *Bioaccumulation—New Asp Dev* 167–199.
- [91] Huerta Buitrago, B., 2016. Pharmaceuticals Endocr disruptors: Accumul Aquat biota *Environ Eff* (PhD Thesis).
- [92] Boxall, A.B.A., et al., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? *Environ Health Perspect* vol. 120 (9), 1221–1229.
- [93] Vaufléury, A.G., Pihan, F., 2002. Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: Metal bioavailability and bioaccumulation. *Environ Toxicol Chem* vol. 21 (4), 820–827.
- [94] Schulz, M., Iwersen-Bergmann, S., Andresen, H., Schmoldt, A., 2012. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Crit Care* vol. 16 (4), R136.
- [95] Tanoue, R., et al., 2015. Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-impacted streams. *Environ Sci Technol* vol. 49 (19), 11649–11658.
- [96] van Ravenzwaay, B., et al., 2014. The sensitivity of metabolomics versus classical regulatory toxicology from a NOAEL perspective. *Toxicol Lett* vol. 227 (1), 20–28.
- [97] Jeong, T.-Y., Simpson, M.J., 2019. *Daphnia magna* metabolic profiling as a promising water quality parameter for the biological early warning system. *Water Res.* vol. 166, 115033.
- [98] Serra-Compte, A., Álvarez-Muñoz, D., Solé, M., Cáceres, N., Barceló, D., Rodríguez-Mozaz, S., 2019. Comprehensive study of sulfamethoxazole effects in marine mussels: bioconcentration, enzymatic activities and metabolomics. *Environ Res* vol. 173, 12–22.
- [99] Ankley, G.T., et al., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem Int J* vol. 29 (3), 730–741.
- [100] Raingard, D., Bilbao, E., Cancio, I., Cajaraville, M.P., 2013. Retinoid X receptor (RXR), estrogen receptor (ER) and other nuclear receptors in tissues of the mussel *Mytilus galloprovincialis*: cloning and transcription pattern. *Comp Biochem Physiol Part A Mol Integr Physiol* vol. 165 (2), 178–190.
- [101] Islam, R., et al., 2021. Exposure to estrogenic mixtures results in tissue-specific alterations to the metabolome of oysters. *Aquat Toxicol* vol. 231, 105722.
- [102] Gottschalk, C.G., Roy, A., Jana, M., Kundu, M., Pahan, K., 2019. Activation of peroxisome proliferator-activated receptor- α increases the expression of nuclear receptor related 1 protein (Nurr1) in dopaminergic neurons. *Mol Neurobiol* vol. 56 (11), 7872–7887.
- [103] Fong, P.P., Noordhuis, R., Ram, J.L., 1993. Dopamine reduces intensity of serotonin-induced spawning in the zebra mussel *Dreissena polymorpha* (Pallas). *J Exp Zool* vol. 266 (1), 79–83.
- [104] Giard, W., Favrel, P., Boucaud-Camou, E., 1995. In vitro investigation of α -amylase release from the digestive cells of the bivalve mollusc *Pecten maximus*: effect of second messengers and biogenic amines. *J Comp Physiol B* vol. 164, 518–523.
- [105] Kemenes, G., 1997. In vivo neuropharmacological and in vitro laser ablation techniques as tools in the analysis of neuronal circuits underlying behavior in a molluscan model system. *Gen Pharmacol Vasc Syst* vol. 29 (1), 7–15.
- [106] Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. *Chemosphere* vol. 231, 249–255.
- [107] Wei, Q., et al., 2021. Comparative evaluation of high-density polyethylene and polystyrene microplastics pollutants: Uptake, elimination and effects in mussel. *Mar Environ Res* vol. 169, 105329.
- [108] Kim, Y., Jeong, J., Lee, S., Choi, I., Choi, J., 2020. Identification of adverse outcome pathway related to high-density polyethylene microplastics exposure: *Caenorhabditis elegans* transcription factor RNAi screening and zebrafish study. *J Hazard Mater* vol. 388, 121725.
- [109] Barboza, L.G.A., et al., 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Sci Total Environ* vol. 717, 134625.
- [110] Olds, H.T., Corsi, S.R., Dila, D.K., Halmo, K.M., Bootsma, M.J., McLellan, S.L., 2018. High levels of sewage contamination released from urban areas after storm events: A quantitative survey with sewage specific bacterial indicators. *PLoS Med* vol. 15 (7), e1002614.
- [111] Koubová, A., et al., 2022. Metabolome adaptation and oxidative stress response of common carp (*Cyprinus carpio*) to altered water pollution levels. *Environ Pollut* vol. 303, 119117.
- [112] Chen, S., et al., 2021. Sulfamethoxazole induced systematic and tissue-specific antioxidant defense in marine mussels (*Mytilus galloprovincialis*): Implication of antibiotic's ecotoxicity. *Chemosphere* vol. 279, 130634.