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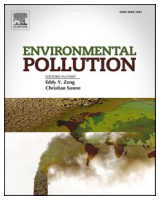

Effects of environmentally relevant concentrations of citalopram in freshwater mesocosms☆

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ABSTRACT

Increased pharmaceutical usage has led to their widespread presence in aquatic environments, resulting in concerns regarding their potential environmental impacts. Antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs) like citalopram, are frequently detected in European surface waters. Acute laboratory studies have demonstrated that citalopram can inhibit algal growth, immobilise *Daphnia magna,* and may result in foot detachment (i.e. the inability to adhere to a substrate) in snails. However, research on long-term citalopram exposure is scarce, and our understanding of its effects on aquatic community- and ecosystem-level is limited. Therefore, we investigated the impact of 13-week exposure to 0.01, 0.1, 1, 10 and 100 μg/L citalopram in outdoor freshwater mesocosms, focusing on water quality variables (i.e. pH, dissolved oxygen, electrical conductivity, temperature, algal chlorophyll-a, turbidity) and the structure of aquatic communities, with a special focus on mollusc foot detachment (*Lymnaea stagnalis, Planorbis* sp. and the total snail population).

We found that environmentally relevant citalopram concentrations did not affect water quality variables, bacterial composition, zooplankton and macroinvertebrate communities. In contrast to expectations based on literature, snail foot detachment was not observed while the tested concentrations overlapped with the reported effect concentrations. This is in line with the absence of indirect adverse effects of foot detachment, such as population changes that could be the result of an increased vulnerability to predation or the inability to feed or reproduce. Reported sublethal effects in the literature, as found in laboratory studies, do not appear to lead to population- or community-level impacts in a semi-field experiment within the concentration range tested in this study. The experimental outcomes suggest that environmentally relevant concentrations of citalopram might not pose a threat to water quality variables, bacterial composition, zooplankton and macroinvertebrate communities, and snail foot detachment.

1. Introduction

Increased usage of pharmaceuticals has resulted in their widespread occurrence in the aquatic environment (Aus der Beek et al., 2016; Wilkinson et al., 2022). The existence of pharmaceuticals in the environment and their potential environmental impact are emerging societal concerns (Carlsson et al., 2006; Wang et al., 2021). The use and prescription of antidepressant drugs have been increasing in the past decades (Bogowicz et al., 2021; NHS Business Services Authority, 2021). Selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed antidepressants in Europe (Bogowicz et al., 2021). The drug citalopram is a commonly used SSRI and frequently detected in European surface waters, with reported mean concentrations ranging from 0.0273 to 0.0641 μg/L (Davey et al., 2022; Wilkinson et al., 2022). In humans, citalopram is presumed to target the transporter of the neurotransmitter serotonin, inhibiting its reuptake into neurons (Sharbaf Shoar et al., 2024). Orthologs of the SSRI drug targets have been identified in various aquatic species (Gunnarsson et al., 2008).

High citalopram concentrations have been shown to inhibit algal growth (2 day, EC_{50}: 1.6 mg/L) (Christensen et al., 2007), immobilise

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Daphnia magna (2 day: EC_{50} 20–30.14 mg/L) (Christensen et al., 2007; Minguez et al., 2014) and cause neonate reduction and mortality in the water flea *Ceriodaphnia dubia* (7.2 day, LOEC = 4000 μg/L & LC_{50} = 3900 μg/L) (Henry et al., 2004). Lower citalopram concentrations have been reported to cause sublethal effects in molluscs, namely foot detachment, leaving the snails unable to adhere to a substrate and impairing their movement (4 h, LOEC: 0.000405–1030 μg/L) (Fong and Hoy, 2012; Fong and Molnar, 2013). Foot detachment is observed in laboratory experiments in the range of detected concentrations in European surface waters (Davey et al., 2022; Fong & Hoy, 2012; Wilkinson et al., 2022). Furthermore, a reduction in weight loss has also been observed in laboratory experiments (8 week, LOEC: 1000 μg/L) (Ziegler et al., 2021).

Movement is crucial for snails in order to feed, escape from predators, and reproduce. Impairing the snails' ability to move has an impact on their fitness and overall survival. The physiological mechanism behind this foot detachment remains unclear but is likely connected to the conserved serotonergic system (Gillette, 2006). This makes snails specifically sensitive to SSRIs targeting the serotonergic system. As the control of muscles and cilia in the sole of *Lymnaea stagnalis* are regulated by large serotonergic clusters (Syed and Winlow, 1989), these serotonergic systems may be affected by exposure to SSRIs (Fong and Hoy, 2012; Fong and Molnar, 2013). The reported effects of exposure to various SSRIs can be observed within a short period and at low concentrations (range of LOECs: 0.000405 μg/L – 4340 μg/L) (Fong and Hoy, 2012; Fong and Molnar, 2013; Ziegler et al., 2021).

Serotonergic mechanisms are also involved in the reproductive processes of molluscs. For example, the SSRI citalopram has the possibility to inhibit serotonin transporter (SERT) during embryo development of the pond snail *L. stagnalis* (Ivashkin et al., 2015). Several toxicity studies show how SSRIs can impact the reproduction of aquatic molluscs by reducing the number of embryos (Nentwig, 2007; Sánchez-Argüello et al., 2009), resulting in a reduced number of newborns (Péry et al., 2008; Gust et al., 2009). Opposite effects resulting in an increased number of eggs (Sánchez-Argüello et al., 2009) are also reported.

These studies demonstrate that citalopram can affect different aquatic species both in a lethal and non-lethal way. However, the focus has been on single species evaluated in laboratory experiments. In order to understand how citalopram impacts entire aquatic ecosystems, we need to investigate its effects on the population, community and ecosystem levels. Long-term exposure scenarios will allow the reveal of the non-lethal effects on higher levels of ecological organisation. Previous experiments evaluating the effects of a long exposure duration demonstrated that citalopram can affect the reproduction of *Ceriodaphnia dubia* (Henry et al., 2004) and the fitness of *Planorbarius corneus* (Ziegler et al., 2021). Within ecosystems, these effects could cause changes in the community and ecosystem structure. Mesocosms are controlled and complex experimental systems that allow to study the effects of citalopram on different levels of biological organisation and have been previously used to study the effects of other SSRIs on aquatic ecosystems (Richmond et al., 2016; Schuijt et al., 2024). In a mesocosm study with citalopram, Richmond et al. (2016) did not find any population-level effects. The exposure period of 14 days, however, might have been too short to discover population-level effects as a result of changes in reproduction and organism behaviour. Therefore, there is a need for more experimental work investigating the effects of long-term exposure to citalopram.

In this study, we investigated the impact of a 13-week exposure to 0.01–100 μg/L citalopram on aquatic pond ecosystems using mesocosms. These shallow ponds contain organisms ranging from primary producers to small predators. For a description of the various functional groups within the mesocosms, see Traas et al. (2004). The studied concentrations overlap with those detected in surface waters but also include higher ones to have more overlap with laboratory-based effect concentrations for snail foot detachment. Impacts were quantified by assessing water quality properties and the structure of aquatic communities. As snails are expected to be sensitive to the SSRI citalopram (Fong and Ford, 2014), additional efforts were put into studying the indirect effects of snail foot detachment. Via different sampling methods, the snail populations on the bottom and sides of the mesocosm were monitored. The innovative aspects of this study lie in its integration of long-term exposure to environmentally realistic concentrations of citalopram with efforts to link sublethal effects to implications at higher levels of biological organisation, all within a single experiment.Overall, this study will contribute to our knowledge of the consequences of long-term citalopram exposure on populations of different aquatic species and the freshwater ecosystem as a whole.

2. Material and methods

2.1. Experimental design

An outdoor mesocosm experiment was conducted from the end of July to the end of October 2022 at the Sinderhoeve experimental station in Renkum, the Netherlands (www.sinderhoeve.org). The experiment was carried out using 28 outdoor artificial ponds or mesocosms (diameter 180 cm; depth 60 cm; water depth 48 cm) containing circa 1200 L water and a 10 cm layer of sediment. These mesocosms aimed to mimic the Dutch drainage ditches. Two months before the start of the experiment, all mesocosms received zooplankton and macroinvertebrate aliquots, and several shoots of macrophytes (*Elodea nuttallii* and *Myriophyllum spicatum*) collected from uncontaminated basins at the Sinderhoeve. During the pre-treatment period, the mesocosms were connected via PVC tubing and water was circulated to obtain similar water conditions in all test systems. The mesocosms were randomly divided into seven experimental citalopram treatment groups (negative control, solvent control, 0.01, 0.1, 1, 10 and 100 μg/L) with four replicates for each treatment, which cover environmentally detected concentrations of citalopram (Davey et al., 2022; Wilkinson et al., 2022) as well as higher concentrations that overlap with snail foot detachment effects found in laboratory studies (Fong and Hoy, 2012). The experimental methods described in the subsequent sections followed methods used by (Schuijt et al. (2024).

2.2. Citalopram application, sampling and analysis

Intended citalopram concentrations were maintained by thirteen weekly applications of citalopram and the solvent using prepared dosing solutions. An initial stock solution was prepared by dissolving citalopram hydrobromide (Thermo Fisher Scientific, Lot# A0434753, 99.9% purity) in methanol. The use of methanol as an organic solvent was needed to assist in properly dissolving citalopram in the dosing solutions. The dosing solutions (1200 times the target concentration in the mesocosms) were prepared in a dilution series one day before each dosing by dissolving a higher citalopram hydrobromide dosing solution in Milli-Q water with 120 ml/L methanol, resulting in a final concentration of 0.002 ml/L methanol in all mesocosms (with the exception of the negative controls). The final solvent concentrations were below the maximum recommended solvent concentration (OECD, 2019). The dosing solutions were poured over the water surface of the mesocosm, after which the water was stirred with a rod to mix in the compound well. The water level was monitored during the experiment. When it dropped more than 5 cm, groundwater was added to avoid the effects of evaporation on the citalopram concentrations in the water.

To analyse the citalopram concentrations in water, water samples were taken 1–24 h before dosing and 1 h after dosing (for exact days, see Table 1). Additional samples were taken after the last application on day 83 for a more accurate determination of the dissipation time of citalopram. Depth-integrated water samples with a total volume of 2 L were collected evenly from the mesocosms and mixed to ensure representative citalopram concentrations. A subsample of c.a. 100 mL was stored in an HDPE sample bottle, of which a 1-mL subsample was

Table 1
Overview of the investigated endpoints and their sampling days.

Endpoint	Unit	Experimental day samplings
Citalopram water concentration	µg/L	0, 1, 8, 14, 15, 22, 29, 36, 42, 43, 50, 64, 70, 71, 76, 78, 80, 83, 85, 87, 91, 93
Abiotic variables		
pH, dissolved oxygen, electrical conductivity, temperature	-, mg/L, µS/cm, °C	−1, 13, 28, 41, 55, 69, 76, 78, 83, 85, 90, 92
Algal chlorophyll-a, turbidity	µg/L, FTU	−1, 13, 41, 69
Nutrients		
Total phosphate, ortho phosphate, total nitrogen, ammonia, nitrite + nitrate	mg P/L, µg P/L, mg N/L, mg N/L, mg N/L	−15, 13, 41, 69
Organic matter decomposition	g/day	20, 48, 68
Macrophytes		
Bottom coverage	%	−1, 13, 28, 41, 55, 69
Dry weight	g	96
Community		
Macroinvertebrates	#	−21, 7, 35, 63
Zooplankton	#/5L	−15, 13, 41, 69
Microbial		−8, 20, 48, 68
Snail plates		−21, 7, 35, 63

transferred to an amber glass HPLC vial. Both the sample and subsample were stored at −20 °C pending analysis, see **SI 1**. The limit of quantification in the samples was 0.0102 µg/L, and the limit of detection was 0.003 µg/L.

2.3. Water quality variables and primary producers

Dissolved oxygen, water temperature, pH and electrical conductivity were measured using a multimeter (Hach HQ40d) at 8 a.m. (for exact days, see Table 1).

To take the nutrient samples, depth-integrated water samples with a total volume of 5 L were mixed and a sample of c.a. 100 mL was stored in an HDPE sample bottle on experimental day −15, 13, 41 and 69. An aliquot of 50 mL was filtered on a 1.2 µm filter (MCE Membrane Filter, Millipore) to analyse ortho-phosphate (PO_4^{3-}), ammonia (NH_3) and nitrite + nitrate ($NO_2^- + NO_3^{2-}$). The remaining unfiltered 50 mL aliquot was analysed for the total phosphorous (P) and total nitrogen (N) concentrations. Nutrient concentrations were measured from a 1 ml subsample using a segmented flow analyser (Skalar 5100 Autoanalyzer, Breda, Netherlands).

Chlorophyll-a concentrations and turbidity were measured on experimental days −1, 13, 41 and 69, using the ALGAETORCH (bbe Moldaenke GmbH, Germany) at three different locations per mesocosm. The macrophyte coverage was classified as the percentage of the sediment surface covered by plants on experimental days: 1, 13, 28, 41, 55 and 69. At the end of the experimental period (experimental day 96), the macrophytes were harvested, washed and dried at 70 °C for 48 h to determine the dry weight of the plants.

2.4. Organic matter decomposition

The decomposition of organic material was investigated using litter bags filled with 2 g of dried P*opulus* leaves. Three mesh bags (500 µm mesh size, closed off so that macroinvertebrates could not enter) were introduced to the mesocosm 8 days before the first dosing and retrieved from the mesocosms on days 20, 48 and 68. The mesh bags were gently rinsed and the *Populus* leaves were transferred to pre-weighted aluminium foil and dried at 60 °C for 48h to determine their dry weight. The weight difference was used to determine the decomposition rate of the organic material.

2.5. Macroinvertebrates and zooplankton

The macroinvertebrate abundance and diversity were assessed on experimental days −21, 7, 35 and 63. Three sampling traps were used: sediment trays and pebble baskets to collect macroinvertebrates in general, while vertically placed snail plates were introduced to the mesocosm to assess the ability of aquatic snails to crawl up these plates. Two weeks prior to the first sampling, ten sediment trays (diameter 26 cm, depth 3 cm) were placed on the sediment in each mesocosm. Two pebble baskets (LxWxH = 17x17 × 11 cm) on concrete tiles and two black untreated PVC snail plates (LxWxH = 25x0.3 × 50 cm) were placed on opposite sides of the mesocosms two weeks before the sampling days, allowing the macroinvertebrates to colonise the pebble baskets and snail plates. On sampling days, the two pebble baskets, the two snail plates and two of the sediment trays were carefully collected with the help of a net (mesh size 0.3 mm). After live identification and counting of the macroinvertebrates, all invertebrates were returned to their original mesocosm. The abundance of the two sediment trays and pebble baskets was summed for each sampling date and mesocosm to represent the macroinvertebrate community.

Snail foot detachment was assessed by comparing the snails on the snail plates with those in the pebble baskets and sediment trays, the latter representing the baseline snail presence in the mesocosm. At each macroinvertebrate sampling, the two snail plates were also retrieved, and snail taxa were identified and returned to the respective mesocosm. The relation between the snail abundance on the snail plates and the monitoring traps was determined using data from the macroinvertebrate sampling before the first citalopram application. Only snail species with an abundance of >5 individuals on the snail plates before the first dosing (i.e. *Lymnaea* sp., *Planorbis* sp., and total mollusc count) were further investigated for foot detachment.

To assess citalopram effects on different developmental stages of *L. stagnalis*, the size of the individuals present on the snail plates was recorded. The L. *stagnalis* size was defined as the length from the tip of the apex of the shell to the tip of the aperture. All individuals were put in four groups depending on their shell length: young juveniles (shell length <4 mm), juveniles (4–16 mm), young adults (16–25 mm), and adults (>25 mm). Individuals with a shell length >16 mm were considered mature and able to reproduce (Ivashkin et al., 2015; Lombardo and Miccoli, 2017), yet a cutoff of >25 mm is frequently used for mature organisms to be sure that they are able to reproduce (OECD, 2016). The additional shell length grouping at 4 mm was used to better distinguish between the neural development stages of *L. stagnalis* described by Croll & Chiasson (1989).

Zooplankton samples were collected on experimental days −15, 13, 41 and 69. Depth-integrated samples taken around the mesocosms were mixed to a total volume of 5 L. The water sample was concentrated by filtering over a 55 µm zooplankton net. Zooplankton samples were preserved with Lugol solution and stored in the dark at 7 °C pending identification. Both macrozooplankton (Cladocerea, Copepoda and Ostracoda) and microzooplankton (Rotifera and nauplii) were identified to the lowest achievable practical taxonomic level. Macrozooplankton were identified and counted using an Olympus SZX10 stereomicroscope at 25× magnification. A 1-mL subsample was used for microzooplankton identification using a Olympus CK40 inverted microscope with 100-200× magnification. Zooplankton counts were recalculated to numbers per liter.

2.6. Microbial DNA

The microbial community in the mesocosm water was assessed on four sampling days (day −8, 20, 48 and 68). For detailed protocols on DNA sampling, library preparation, and sequencing, see Supplementary Methods 2. After sequencing, raw sequencing data were processed using the NG-Tax 2.0 semantic framework (Ramiro-Garcia et al., 2016; Poncheewin et al., 2020). Barcode and primer sequences were removed

from the reads, and amplicon sequencing variants (ASVs) below a 0.1% relative abundance threshold per sample were discarded. Taxonomic assignment was performed via BLASTN searches against the Silva 132 SSU Ref database (Edgar, 2010; Yilmaz et al., 2014). The resulting BIOM file and tree files were further processed in R to generate phyloseq objects (McMurdie and Holmes, 2013).

Further filtering steps excluded ASVs assigned to the Archaea domain and mitochondrial sequences. ASVs with fewer than 10 reads were also removed. We performed rarefaction analysis using the vegan v2.6-6 package (Oksanen, 2024) to ensure sample completeness, we normalised the samples to 3100 reads to keep maximum samples for our analysis, which resulted in the exclusion of 9 out of 112 samples. Data visualisation and inspection were performed using the microbiome (v1.24.0, Lahti & Shetty (2017)) and microbiomeutilities (v1.00.17, Shetty & Lahti (2022)) R packages. All data handling was performed in R (v4.4.1., R Core Team (2020)). The final dataset consisted of 1225 unique ASVs belonging to 123 families.

2.7. Data analysis

The dissipation rates (k) and half dissipation times (DT_{50}) of citalopram in water were calculated via linear regression of ln-transformed concentrations against time. This was done for all applications that had one water sample after application and one sample 5–7 days later. Time-weighted average citalopram water concentrations in the mesocosms were calculated according to the method described in OECD guideline 211 (OECD, 2012).

Treatment-related changes in the bacterial, macroinvertebrate and zooplankton communities were assessed by univariate and multivariate analyses. Microbial sequencing data was processed as described in **SI 2**. Before analysis, macroinvertebrate and zooplankton abundance data were respectively transformed $\ln(2x+1)$ and $\ln(10x+1)$, for rational see Van den Brink et al. (2000). The multivariate Principal Response Curves (PRC) method was used to assess changes in community compositions (Van den Brink and Ter Braak, 1999). The PRC method reduces the variation in community composition due to the treatment of a small set of curves. These principal response curves show the deviation of the treatments from the control over time. The taxon-specific weight (b_k) represents the affinity of each taxon with the effect response indicated by these curves. The significance of the treatment effects on the community was tested with 499 Monte Carlo permutations by permuting whole times series (Van den Brink and Ter Braak, 1999). To assess the significance of each citalopram treatment on communities for each sampling date separately, a Monte Carlo permutation test under the Redundancy Analysis (RDA) option was performed by testing each treatment against the control for each sampling date. All multivariate analyses were performed using CANOCO v.5.15 program (Ter Braak and Smilauer, 2012)

Effect concentrations (concentrations significantly deviating from the control) were calculated for the transformed macroinvertebrate (including the four *L. stagnalis* life stages on the snail plates) and zooplankton population counts and for all ecosystem properties: abiotic properties, chlorophyll-a concentration, turbidity, macrophyte coverage, macrophyte dry weight, organic matter decomposition rate and nutrient concentrations. Effect concentrations were calculated using Dunnett's test ($p \leq 0.05$), which does not assume an increasing effect with increasing citalopram concentrations. This is chosen to allow for the detection of non-monotonic responses frequently reported for SSRIs (De Lange et al., 2006; Guler and Ford, 2010; Fong et al., 2017). For each endpoint, the effect of the solvent was evaluated using Dunnett's test. Since they did not differ significantly from the negative control for each investigated endpoint, the controls were grouped for further analysis. Dunnett's tests were performed using the DunnettTest function in the "DescTools" R-package, using R version 4.3.3. (R Core Team, 2020). To avoid the false identification of citalopram-related effects (Type I error), effects were only considered reliable when statistically significant deviations were found on at least two consecutive sampling days. Significant treatment-related effects before the first dosing indicate deviations unrelated to citalopram application; effects at this and further time points were not interpreted as treatment-related effects. Additionally, population-level effects were only accepted when, for a taxon, more than three macroinvertebrate individuals were observed in controls on that sampling day. Zooplankton effects were only accepted when 1.5 macrozooplankton (Cladocerea, Copepoda and Ostracoda) individuals per Liter or 0.6 microzooplankton (Rotifera and nauplii) individuals per liter of a taxon were observed in controls. These criteria are based on 3 times the minimum abundance that theoretically could be assessed.

A regression analysis was applied to investigate the effects of citalopram exposure on the total mollusc count and the counts of snail species with an abundance >5 individuals (*Lymnaea* sp. and *Planorbis* sp.) at the four sampling days. Since the snail count data was overdispersed, a quasi-Poisson regression was applied (Wedderburn, 1974). For each sampling day, the response variable was the count of snails on the snail plates. The citalopram treatment and snail count in the traps were taken as explanatory variables. The quasi-Poisson distributed generalised linear models were fitted in R (R Core Team, 2020).

3. Results

3.1. Citalopram water concentrations

The citalopram concentration before dosing was below the limit of detection for all sampled mesocosms. Over time, the measured citalopram concentrations in the control mesocosm extracts exceeded the limit of detection (LOD) and limit of quantification (LOQ). To reach contamination levels of this magnitude, it would require the addition of several liters of the highest treatment concentration to the control mesocosms, which is highly improbable. Therefore, the observed concentrations in the negative control are likely attributable to a measurement error, potentially resulting from a post-sampling contamination event (e.g. during the solid phase extraction). If the contamination were at the level of the mesocosms and not in the samples, the subsequent interpretation of effects at the lowest citalopram concentrations would not have been valid. Weekly citalopram dosing restored the water concentrations to their intended target levels (Fig. 1). Time-weighted average concentrations in the water were 5.9 and 81 μg/L for the 10 and 100 μg/L citalopram treatments. For clarity, we will refer to nominal concentrations in the results. On average, the DT_{50} value (time in which the first 50% of the initial dose dissipates from the water phase) of citalopram in water comprised 1.9 ± 1.2 and 6.1 ± 3.6 days for the 10 and 100 μg/L citalopram treatments, respectively. The individual DT_{50} values after each application increase over time (Table A.2).

3.2. Water quality variables and primary producers

Throughout the experimental exposure period, water temperatures decreased from 20.2 to 9.6 °C (Figure A.1). Oxygen concentrations fluctuated over time, with a drop to circa 6 mg/L at the start of the experiment but an increase to circa 9 mg/L in all treatments later (Figure A.1). A significant 1.5 mg/L decrease in dissolved oxygen concentration compared to the control treatment was found for the 0.01 μg/L treatment halfway through the exposure period (Table A.3), but this effect disappeared over time. A non-consistent change in pH was observed on two separate occasions and before the first application (Table A.3). We found that citalopram exposure did not affect the coverage of macrophytes throughout the experiment, nor the macrophyte biomass at the end of the experiment (Figure A.2; Table A.3). The 1 μg/L citalopram treatment had a consistently lower macrophyte coverage and final biomass, however, this difference is not significant and already present before the first dosing (SI Figure A2). The

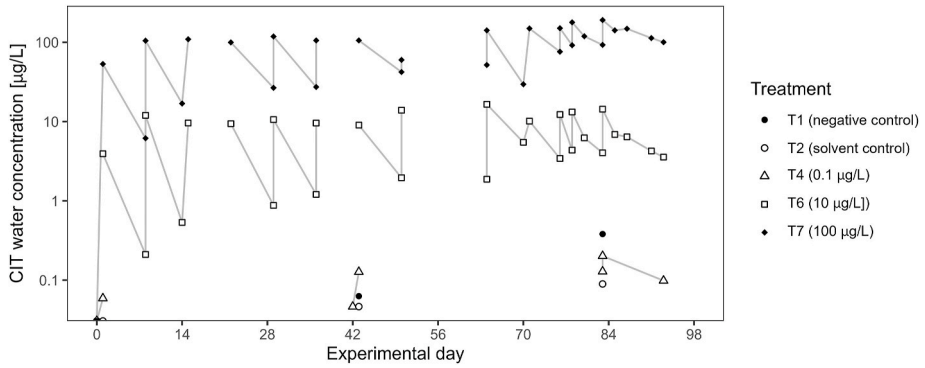

Fig. 1. Geometric mean measured citalopram water concentrations of the treatments throughout the experiment.

degradation rates of *Populus* leaves in the mesocosms were comparable in all treatments (SI Figure A3). The pH, electrical conductivity, chlorophyll-α concentrations, turbidity, nutrient concentrations and organic matter decomposition were not significantly affected by citalopram for two or more consecutive sampling days. (Figure A.1, **A.3**, **A.4**, Table A.3).

3.3. Macroinvertebrate, zooplankton and microbial community

The macroinvertebrate and zooplankton communities were sampled once before the initial citalopram application and continued to be monitored throughout the exposure period. Over the whole duration of the study, 56 macroinvertebrate taxa, 41 zooplankton taxa and 123 microbial families were identified. A PRC analysis with data from only the negative control and solvent control mesocosms demonstrated that the macroinvertebrate, zooplankton and microbial community composition did not differ between the negative control and the solvent control (Monte-Carlo permutations: macroinvertebrates: p-value: 0.352; zooplankton: p-value = 0.134; microbial Monte-Carlo permutations were not reliable due to missing samples, observations based on PRC plot, Figure A.5C). An additional visual inspection of the top 10 most abundant bacterial families does not indicate treatment-related effects in these families (Figure A.6). For further community analysis, the negative control and solvent control were grouped. PRC analyses of the communities followed by Monte Carlo permutations under RDA found that neither the macroinvertebrate, the zooplankton, nor the microbial communities were significantly affected by citalopram exposure at any sampling date (Figure A.5; Table A.4). Univariate testing did not identify any consistent significant differences in the macroinvertebrate and zooplankton populations as a result of citalopram exposure (Table A.5, A.6).

3.4. Snail plates and foot detachment

In total, 14 different mollusc taxa were identified in the monitoring traps (pebble baskets and sediment trays), of which 9 were present at all four sampling dates. All taxa were also found on the snail plates. Before the first citalopram application, 12 taxa were found in the pebble basket and sediment trays, of which 11 taxa also occurred on the snail plates. On the first sampling day, the snail counts on the snail plates were positively correlated to the snail counts in the monitoring traps (Figure A.7). Only the operculate snails *Valvata* sp., *Potamopyrgus* sp. and *Viviparus* sp. were less abundant on the snail plates than in the monitoring traps (Figure A.7). This remained throughout the whole experiment.

Further investigation into the abundances of *Lymnaea* sp., *Planorbis* sp. and total snail count after citalopram exposure showed that their abundance on the snail plates stayed positively correlated to their abundance in the monitoring traps at the following sampling dates (Fig. 2). Throughout the experiment, snail abundance in the monitoring traps increased (Fig. 2). The abundance of the total number of snails and the *Planorbis* sp. on the snail plates also increased over time, whereas the abundance of *Lymnaea* sp. showed a decrease. With the exception of the first sampling day (day −21), snail abundance of *Lymnaea* sp., *Planorbis* sp. and the total count on the snail plate was significantly correlated with snail abundance in the monitoring traps (Table A.7). Citalopram exposure did not significantly affect the abundance of *Lymnaea* sp. and the total snail count on the snail plates (Table A.7). However, on day 35, the count of *Planorbis* sp. on the snail plates was significantly higher in all citalopram treatments. Notably, the control and solvent control groups diverged significantly from the other data points in this instance (Fig. 2). While this effect was deemed significant on day 35, it was not observed on the next sampling day, indicating a statistical artefact or a type I error.

The life-stage composition of *L. stagnalis* differed throughout the exposure period (Figure A.8). The amount of *L. stagnalis* young-juveniles (<4 mm) and young-adults (16–25 mm) increased at the second sampling occasion (day 7) and slowly decreased again throughout the experiment while juveniles (4–16 mm) steadily decreased over time. Adult (>25 mm) populations were generally small but constant. These patterns were observed for all citalopram treatments (Figure A.8), and the different life stage counts were not significantly affected by the citalopram treatment (Table A.5).

4. Discussion

The presence of selective serotonin reuptake inhibitors, like citalopram, in aquatic environments has raised concerns about their potential ecological impacts. Previous studies have demonstrated various effects of citalopram on aquatic organisms, including significant impacts on molluscs foot detachment in laboratory settings (Fong and Hoy, 2012; Fong and Molnar, 2013). While mollusc foot detachment is initially not a lethal effect, subsequent lethality may occur as snails are prone to predation or may starve and are not able to reproduce anymore. As a consequence, foot detachment in an individual may result in cascading effects on the population level. This study aimed to investigate the effects of 13 weekly applications of citalopram in mesocosms, focusing on water quality variables, macroinvertebrate and zooplankton community composition, bacterial community, and effects on mollusc populations. Our findings indicate no significant relevant effects of citalopram on water quality, primary producers or macroinvertebrate, zooplankton or microbial communities, suggesting that these ecosystem components are not sensitive to the tested treatment levels and exposure profiles. Furthermore, the number of *Lymnaea* sp. and the total number of snails on the snail plates were not affected by citalopram. Isolated differences were observed for populations of the snail *Planorbis* sp. on the snail plates, but these differences had no long-term ecological implications.

The calculated half dissipation times of citalopram in water (DT_{50}: 1.3–11.5 days) were lower compared to degradation tests in natural lake

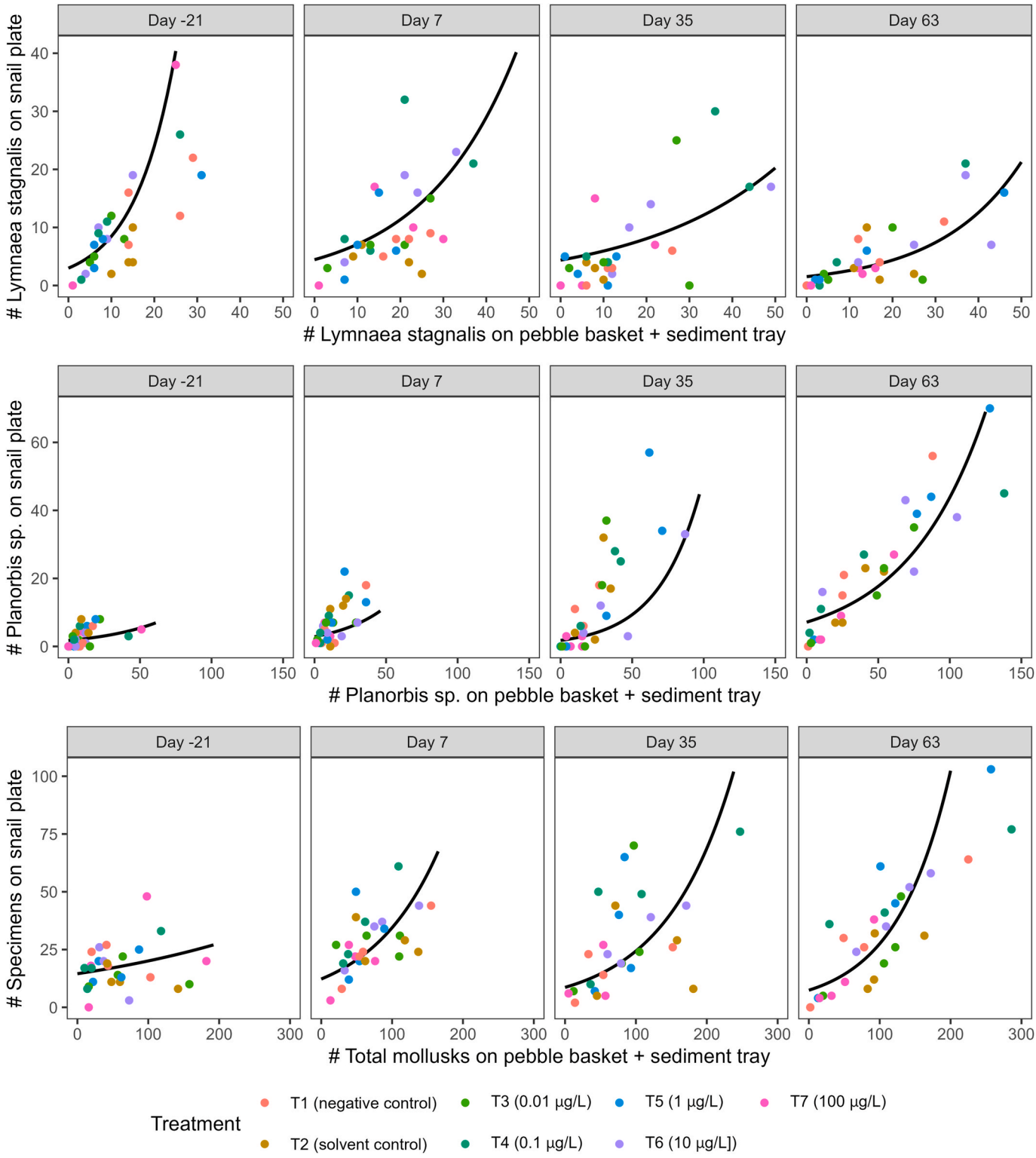

Fig. 2. Abundance of snails on the pebble basket and sediment tray (x-axis) and snails on the snail plates (y-axis) at the four sampling days. The quasi-Poisson regression for the grouped solvent and control treatments is plotted over the data of each mesocosm.

water, where values of 14–43 days were reported (Kwon and Armbrust, 2005; Black and Armbrust, 2007). By comparing degradation under light and dark conditions, Black & Armbrust (2007) found that citalopram hardly degrades due to photolysis. With a relatively low log K_{OW} of 1.39, the compound was expected to be hydrophilic and to dissolve well in water (Black and Armbrust, 2007). However, with a higher log K_{OC} value of 5.64, citalopram has an affinity to adsorb to sediments (Black and Armbrust, 2007), decreasing the half dissipation time of this compound in aquatic environments by building up in sediments. The half-life of citalopram in the mesocosms increased with time after each application (Table A.2). A possible explanation for this could be the effect of compound building up in the sediment, decreasing the sorption potential of the sediment over time and with that increasing the half-life of citalopram in the water phase.

Some isolated, significant differences were found for the pH and dissolved oxygen concentrations in the mesocosm (Table A.3). These differences were small and not consistent (Figure A.1). The significantly lower oxygen concentration in the 0.01 µg/L treatment at two consecutive timepoints was not considered of environmental relevance as the difference is very small, and the effect disappeared over time.

While macroinvertebrate, zooplankton and microbial communities showed some variability, statistical analyses did not reveal treatment-related deviations from control community composition (Figure A.5; Table A.4, A.5, **A.6**). Previous studies with individual aquatic organisms have reported citalopram effects on algae and crustaceans at concentrations higher than those we tested (Fig. 3).

Other studies researching the effects of citalopram on aquatic organisms primarily focused on fish and molluscs. Previous studies demonstrate that citalopram can cause foot detachment of the freshwater snails: *Lymnaea elodes* (4-h, LOEC: 4.05 µg/L) and *Leptoxis carinata* (4-h, LOEC: 405 pg/L) (Fong and Hoy, 2012), and in the marine snails: *Chlorostoma funebralis* (4-h, LOEC: 1030 µg/L), *Tegula fasciatus* (4-h, LOEC: 4.05 µg/L) and *Lithopoma americanum* (4-h, LOEC: 4.05 µg/L) (Fong and Molnar, 2013) (Fig. 3). In these studies, snails were recorded showing foot detachment if, after initial detachment, the snail was placed back and unable to reattach during a 20-min observation period. This mollusc foot detachment has been observed at concentrations within our tested range (Fig. 3). If such foot detachment had occurred in our mesocosms and the snails were unable to adhere to any substrate, their presence would only have been observed in the monitoring traps (pebble baskets and sediment trays) on which the detached snails remained present. This effect was, however, not observed (Fig. 2).

Our mesocosm study differs from previous snail foot detachment studies in several ways. For instance, the duration of our exposure was much longer, and the observations on snail abundance occurred at a much later stage after initial exposure than in the foot detachment studies performed in the laboratory (Fong and Hoy, 2012; Fong and Molnar, 2013). If the snails were only temporarily affected and were able to regain their muscle control throughout the exposure period, the effects would not have been visible on the sampling days used in our mesocosm experiment. Another difference between the laboratory studies and our mesocosm study was the method of defining foot detachment. In those laboratory studies, individual organisms were continuously observed for 4 h to determine whether they were able to stick to the surfaces of their experimental unit. In our mesocosms, the foot detachment observations were based on population counts on a vertical surface and compared to population counts in traps on the sediment of the mesocosm. Unfortunately, due to their large sizes, our

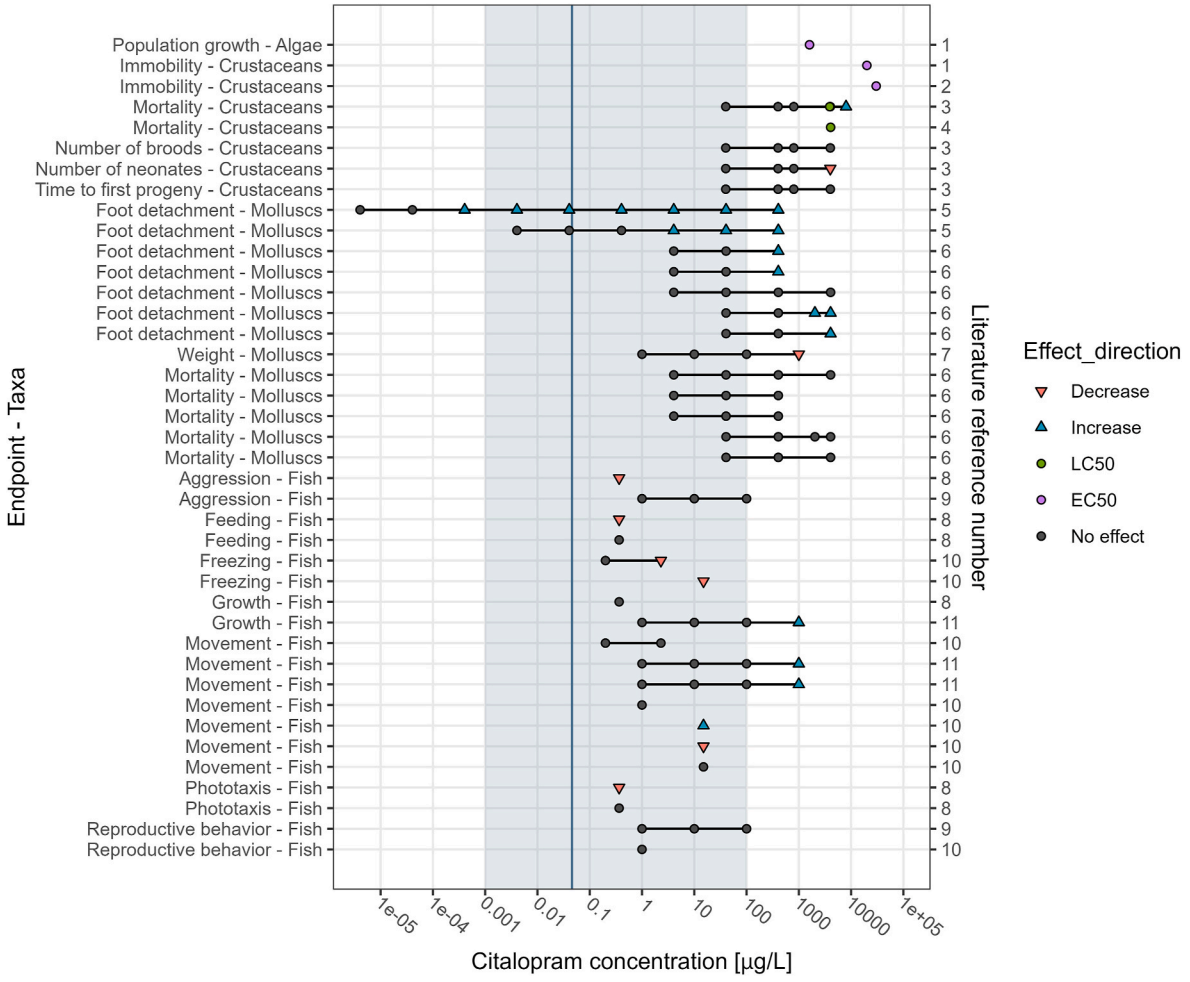

Fig. 3. Overview of citalopram effects on individual aquatic organisms as reported by various laboratory studies. Each dot represents a tested concentration, coloured by the found type of effect or an effect concentration (EC_{50} or LC_{50}). The horizontal lines represent the concentration range of the corresponding study. The grey marked area represents the concentration range tested in this study. The vertical blue line represents the average citalopram concentration detected in European surface waters (Davey et al., 2022; Wilkinson et al., 2022). The numbers on the right side of the figure correspond with the following studies: 1. Christensen et al. (2007); 2. Minguez et al. (2014); 3. Henry et al. (2004); 4. Henry & Black (2007); 5. Fong & Hoy (2012); 6. Fong & Molnar (2013); 7. Ziegler et al. (2021); 8. Kellner & Olsén (2020); 9. Holmberg et al. (2011); 10. Olsén et al. (2014); 11. Ziegler et al. (2020); 12. this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mesocosm systems do not allow for detailed observation of individuals. The benefit of our method is, however, that many mollusc taxa could be observed at the same time for a long exposure duration and that the endpoint was assessed in a realistic habitat setting. In this way, we were able to collect data on multiple snail species that are more applicable to real-world scenarios.

Our experiment took place in the reproductive time window of *L. stagnalis* (Nakadera et al., 2015), as confirmed by the presence of many juveniles throughout the experiment (Figure A.8). Furthermore, *L. stagnalis* reaches maturity between 30 and 60 days post-hatching (Fodor et al., 2020), and this also felt within the observational period of this study. Consequently, as there are no differences in the life stage abundances of *L. stagnalis* between the treatment groups, it is most likely that the citalopram treatments did not affect *L. stagnalis* reproduction.

Populations are not only influenced by reproduction but also by mortality. Snails are predated by, among others, leaches and fish and their susceptibility to predation increases when they are unable to shelter off the aperture of their shell (Ahlgren and Brönmark, 2012). In natural environments, detached snails can also be extra prone to predation because they might be transported to unfavourable environments where they cannot escape their predators. Furthermore, they might be unable to feed after detachment because they cannot reach their food sources. All of this may result in increased mortality of individuals and impact the population as a whole. Although fish were absent in our mesocosms, other predators of small snails were present, like leeches such as *Alboglossiphona* sp., *Erpobdella* sp., *Glossiphonia* sp., and *Helobdella stagnalis*, as well as the flatworm Tricladida. The populations of these leeches and flatworm did not show any changes as a result of citalopram treatment. Although these small predators are known to prey on small snails (Brönmark, 1992), the number of juveniles (<16 mm) on the snail plates did not differ from the control treatments at any sampling moment (Table A.5; Figure A.8) and therefore mortality, as well as reproduction, is unlikely to be indirectly affected by citalopram treatment.

A possible, yet unlikely, contamination of the lowest citalopram treatments (i.e. 0.01 and 0.1 μg/L) might affect the subsequent interpretation of effects at the lowest citalopram concentrations. There were, however, no effects at these or higher treatment concentrations. Previous studies did not detect any non-monotonic dose-response effects for citalopram (Figure A.8); we therefore do not expect any of such effects in this study either and do not expect to have missed them.

However, it is important to interpret the results with caution, as the absence of larger predators, such as fish or crayfish, could have affected the dynamics within the mesocosms compared to natural environments where predators are present. Fish are a crucial component of many aquatic ecosystems and their predation pressure on snail populations can differ markedly from that exerted by invertebrates. Therefore, the absence of larger predators might have led to an underestimation of the overall predation pressure on the snails in our experimental setup. This should be considered when using these results to inform decisions on drug prescription practices and regulatory controls based on environmental criteria.

5. Conclusions

We found that citalopram exposure did not affect any of the tested endpoints on water quality and bacterial, zooplankton and macroinvertebrate communities. With the exception of literature on molluscs, previous studies reporting the effects of citalopram on aquatic organisms differ from our mesocosm study by using higher citalopram exposure concentrations and shorter exposure durations. This study indicates that long-term exposure with weekly applications of citalopram does not affect the aquatic ecosystem without larger predators at environmentally relevant concentrations.

Foot detachment was investigated as literature reported effects within the same concentration range as our tested environmentally relevant concentrations. The snail plates were effective observational tools for determining populations of most snail species present in the mesocosms. This allowed us to gather detailed data on the snail populations and assess the potential impact of citalopram exposure. While our method for foot detachment differed from the methods used in laboratory settings concerning observation time and period, we did not detect lasting foot detachment of snails. Additionally, we did not observe any citalopram-related changes in snail populations.

CRediT authorship contribution statement

Elien Versteegen: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tong Mou:** Writing – review & editing, Methodology, Investigation. **Dailing Wu:** Writing – review & editing, Methodology, Investigation. **Ineke Heikamp-de Jong:** Writing – review & editing, Methodology, Investigation. **Ivo Roessink:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Edwin T.H.M. Peeters:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Paul J. van den Brink:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Paul van den Brink reports financial support was provided by Dutch Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125570.

Data availability

Data will be made available on request.

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