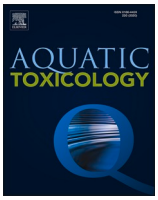
Aquatic Toxicology 277 (2024) 107160

Contents lists available at ScienceDirect

Aquatic Toxicology

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



Trophic transfer effects of PS nanoplastics and field-derived nanoplastics in the freshwater clam *Corbicula fluminea*

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ARTICLE INFO

Keywords:
Nanoplastics
Bivalve
Trophic transfer
Biomarker
Behavior
Gene expression

ABSTRACT

Plastic pollution is of global concern. Many studies investigated the effect of micro and nanoplatics towards aquatic organisms. However, relatively few studies were assessed on freshwater organisms. Another aspect of this pollution is the impact of trophic transfer on plastic distribution and on food chain in order to evaluate its potential risk towards environmental and human health. In this context, the objective of this study was to assess the ecotoxicological impacts of different types of nanoplastics (NPs) on freshwater organisms exposed through trophic transfer. Freshwater microalgae *Scenedesmus subspicatus* were contaminated for 48 h with realistic concentrations of NPs (0.008, 10 and 100 µg/L). Two types of NPs were tested: commercial PS NPs and NPs generated from macro-sized plastics collected in the field (ENV NPs). Freshwater *Corbicula fluminea* bivalves were then fed with the contaminated algae every 48 h for 21 days. Results showed that trophic exposure led to the induction of oxidative stress (CAT activity). Overall, NPs trophic exposure caused downregulations of genes implicated in many cellular processes (immunity, oxidative stress, neurotoxicity, endocytosis, apoptosis).

This present study allowed to demonstrate the relevance of investigating the trophic transfer effects of NPs on a freshwater trophic chain. Further studies should focus more on larger levels of the food chain.

1. Introduction

Continental waters represent the main route for transporting plastic to the oceans, in particular through wastewater discharges containing many types of plastic particles, mainly coming from synthetic fibers from washing clothes (Acharya et al., 2021) and cosmetic products (Browne et al., 2007). These waters can also carry plastic macro-waste or macroplastic dispersed accidentally, or even intentionally, in the environment during their transport to recycling centers (packaging and construction materials). The plastic debris found daily in the environment will then undergo strong physicochemical constraints inducing transformations inherent to the environment and to the material such as photodegradation, thermal degradation, biodegradation resulting in fragmentation (Zhang et al., 2021). This fragmentation leads to the formation of micrometric-sized debris, called "microplastics" (MPs), whose size is less than 5 mm (Gigault et al., 2016), and also of "nanoplastics" (NPs), whose size ranges from 1 to 1000 nm (Gigault et al., 2018; Suhrhoff and Scholz-Böttcher, 2016).

Numerous studies have investigated the effects of MPs and NPs on aquatic organisms (see review on MPs: (Chouchene et al., 2023); on NPs: (Mattsson et al., 2018; Shi et al., 2024). However, NPs concentrations in the environment and in organisms are difficult to estimate, mainly due to technical limitations (da Costa et al., 2016; Hernandez et al., 2017; Koelmans et al., 2015). Both MPs and NPs can easily cross biological barriers and accumulate in tissues and organs (Chae and An, 2017; Mattsson et al., 2018). NPs have been demonstrated to have a longer retention time than MPs in two species of suspension-feeding bivalves: the mussel *Mytilus edulis* and the oyster *Crassostrea virginica* (Ward and

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https://doi.org/10.1016/j.aquatox.2024.107160
Received 26 June 2024; Received in revised form 18 October 2024; Accepted 9 November 2024
Available online 10 November 2024

Kach, 2009). Furthermore, due to their nanoscale properties and especially their high surface area, NPs possess stronger sorption affinities for chemicals than MPs (da Costa et al., 2016; Koelmans et al., 2015; Velzeboer et al., 2014), representing a significant risk of contamination for aquatic life. Additives added during the plastic production process have also been largely demonstrated to influence NPs toxicity (Besseling et al., 2019; Koelmans et al., 2022).

Another aspect to consider when assessing the risk associated with plastic pollution is the potential trophic transfer of these particles throughout trophic chains (Gong et al., 2023; Latchere et al., 2021). The first producers or consumers exposed to NPs ingest and bioaccumulate these compounds and transfer them to higher trophic level organisms (Chae et al., 2018; Qu et al., 2020; Stienbarger et al., 2021; Wang et al., 2021). Indeed, as organisms are exposed to MPs or NPs through trophic transfer, these particles can continuously accumulate in upper-trophic-level organisms until eventually reaching humans (Bergmann et al., 2015). Wang et al. (2021) showed that the Asian paddle crab *(Charybdis japonica*) fed with mussels (*Mytilus coruscus*) previously exposed to high-dose of polystyrene MPs (diameter: 5 μm; 1000 particles.mL^{-1}) accumulated these MPs in its digestive tract and gills. They also depicted an increase in detoxification capacity (EROD - Ethoxyresorufin-O-demethylase) and in the antioxidant defense enzyme GST (Glutathione S-transferase) as well as a decrease in AChE (acetylcholinesterase) as biomarker of neural activity. Santana et al. (2017) also demonstrated a transfer of MPs (PVC - with a size range of 0.1 to 1.0 μm in diameter) between the mussel *Perna perna* towards two of its predators: the crab *Callinectes ornatus* and the fish *Spheoeroides greeleyi*. These particles were found in the feces of both predators and did not remain in the gut.

Kim et al. (2022) evaluated the trophic transfer of 190-nm-sized amine-modified polystyrene NPs in an algae-crustacean-fish food chain (*Dunaliella salina-Artemia franciscana-Larimichthys polyactis*). They demonstrated that NPs adsorbed on the algae cell walls were gradually transferred via the food chain to fish, whose digestive enzyme α-amylase was inhibited. The equivalent fresh water three-step food chain (*Chlorella pyrenoidosa-Daphnia magna-Micropterus salmoides*) was explored by Liao et al. (2023) after exposure of the algae and crustacean to polystyrene NPs (80 nm). This resulted in amplification of the NPs through the food chain leading to an antioxidant response, histopathological damage and disruption of lipid metabolism in fish, the upper trophic level of the chain.

Among aquatic organisms, filter-feeders are particularly exposed to MPs and NPs because they filter large quantities of water (Wesch et al., 2016). Among them, *Corbicula fluminea* is an endobenthic bivalve, which has been largely used as sentinel species for freshwater ecosystem quality risk assessment (Arini et al., 2019; Guo and Feng, 2018; Zhou et al., 2008). Indeed, it can assimilate small particles from both sediment and water column and hence represent relevant model for investigating contamination in these compartments (Guo and Feng, 2018). Previous ecotoxicological studies have been conducted on this species to evaluate the effects of plastic particles (Guo et al., 2021; Latchere et al., 2023; Oliveira et al., 2018; Vidal et al., 2023). The microalgae *Scenedesmus subspicatus* is frequently found in freshwaters and has a key role of primary producer for numerous aquatic food chains. It has been widely used to evaluate the effects of various pollutants (Bucková et al., 2023; Sackey et al., 2021). However, very few studies have investigated the trophic transfer of NPs from microalgae to bivalve (Baudrimont et al., 2019). Arini et al. (2023) demonstrated inflammatory effects after trophic transfer of NPs coming from natural sources and polystyrene NPs in *C. fluminea* fed with previously contaminated microalgae *D. subspicatus*. They notably highlighted the need to pursue studies with NPs that are close to field conditions (Arini et al., 2023).

The objective of this study is to evaluate the ecotoxicological impacts of environmental and polystyrene NPs on organisms of a freshwater trophic chain: the algae *Scenedesmus subspicatus* and the bivalve *Corbicula fluminea*.

2. Material and methods

2.1. Preparation of the experiment

2.1.1. Collection, preparation and characterization of environmentally derived NPs

Plastic waste was collected by hand with pliers on the right bank of the Garonne River at low tide, near the Langoiran bridge (44°42′14.56″N, 0°24′3.91″W). The most oxidized plastic debris were sampled, rinsed in the laboratory with ultra-pure water, dried at 45 °C for 48 h before preparation of the nanoplastic suspensions.

2.1.2. Environmental nanoplastics production

Environmental nanoplastics (ENV NPs) were generated from macro-sized plastic debris according to the protocol described by Blancho et al. (2021). Briefly, NP were produced using a process coupling agitation and sonication in an aqueous medium. The size range of ENV NPs was 235 ± 70 nm. They were characterized in terms of composition, size, shape and surface properties by Pyrolysis (Pyrolyzer PY-3030 Frontier Lab) coupled to gas chromatography-mass spectrometry (Py-GC–MS) (5977B, Agilent Technologies). Plastic analysis showed that ENV NPs and ENV MPs were mainly composed of polyethylene (PE) (95%) (Supplementary Information Fig. 1). They were anisotropic, polydisperse in size (Supplementary Information Fig. 2) and possessed high levels of carboxylic groups on their surface. Zeta potential of the ENV NPs was −36.2 mV. In addition to ENV NPs, carboxylated polystyrene nanobeads (PS NPs) with 200 nm of size, were used as reference material (Polysciences).

2.1.3. Acid digestion and ICP-MS measurements

To optimize total digestion, 100 mg of NP powder were acid-digested (12 N HNO_3 sub grade) using a multi-step procedure in a microwave oven (MW7000 system from Anton-Paar) with an increasing temperature ramp of 6.6 °C per minute until reaching 250 °C, then left for 25 min at 250 °C under 140 bars of pressure. Solutions from three tubes were mixed, evaporated at 90 °C and solubilized in 0.37 N HNO_3 prior to ICP-MS measurements. Metal concentrations were measured by Inductively Coupled Plasma-Mass Spectrometry 5 (ICP-MS) from Agilent Technologies (7700x Model, Agilent) (Supplementary Information Table A). The digestion and analysis process was validated using reference materials (ERM-EC 680 and ERM-EC 681) from the Joint Research Center of the European Commission (JRC, Ispra, Italy).

2.1.4. Bivalve collection and laboratory trophic exposure assay with microalgae

Individuals of *Corbicula fluminea* were collected in lakes of Biscarrosse and Parentis (South-West of France). Clams were transported to the laboratory in boxes filled with sediment and water from the collection site. The bivalves were then transferred to aquaria (30 L) containing 27 L of aerated tap water in a thermoregulated laboratory at 15 °C for an acclimation period of 7 days. The photoperiod was maintained at 12:12h The water in the aquaria was completely renewed every three days. Microalgae *Scenedesmus subspicatus* was contaminated with 0.008, 10 or 100 μg.L^{-1} PS NPs or ENV NPs under agitation during 48 h before being distributed to the clams. These concentrations are close to the ones measured for MPs (no data is available for NPs) from coastal regions to oceanic gyres (Desforges et al., 2014; Goldstein et al., 2013). The experimental conditions are abbreviated in the results section: for example, ENV NPs 10 is used for environmental microplastics at 10 μg L^{-1}. Clams were fed every 48 h with control or contaminated microalgae at a concentration of 70 000 cells per individual per liter. The use of plastic material was avoided during all the experiment.

After 7 days and at the end of the experiment at 21 days, 5 individuals per condition were sampled for gene expression and 5 individuals for biochemical biomarkers analysis. At 21 days, 3 individuals were sampled to determine the clearance rate, 10 individuals to perform

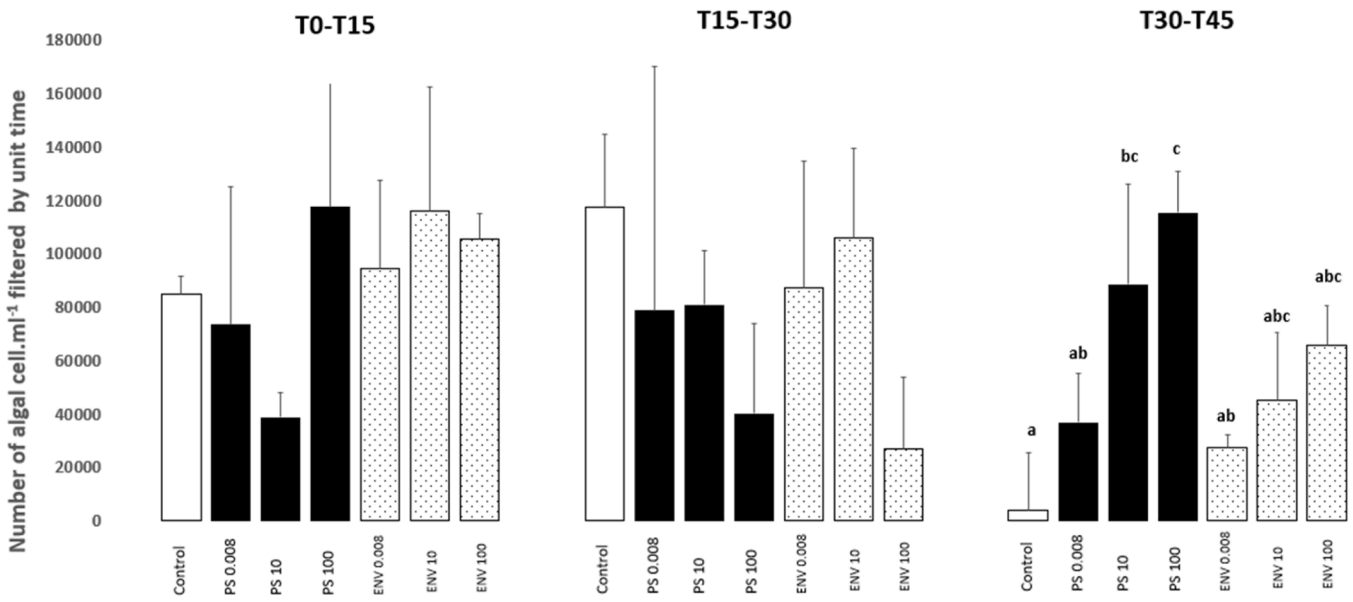

Fig. 1. Clearance rate of *Corbicularia fluminea* after 21 days exposure to standard polystyrene nanoplastics (PS) and environmental nanoplastics (ENV) at 0.008, 10 and 100 µg.L-1 (mean ± SD, $N = 5$).

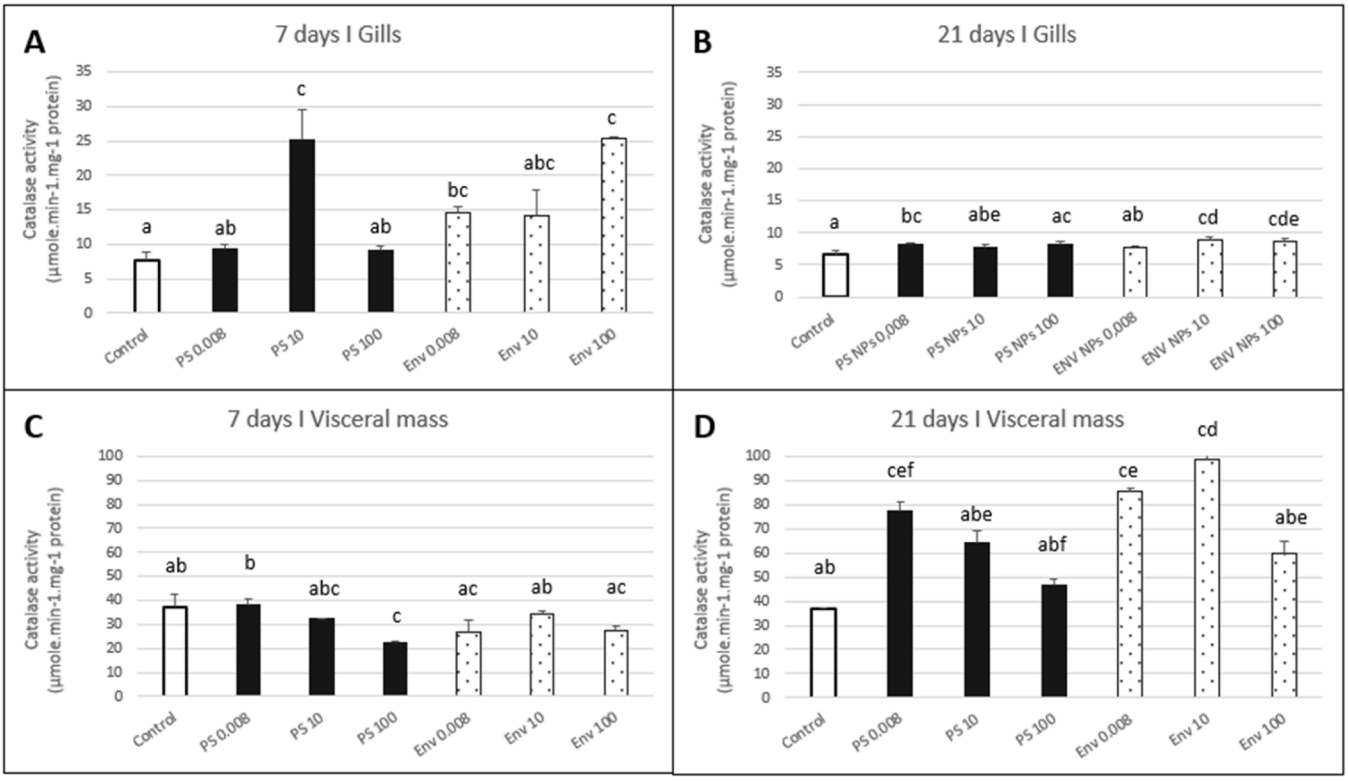

Fig. 2. Enzymatic activity of catalase in gills (A, B) and visceral mass (C, D) of *Corbicularia fluminea* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L-1 (mean ± SD, $N = 5$).

the burrowing test and 10 to determine the condition index. Gills and visceral mass were dissected, immediately flash frozen in liquid nitrogen and stored at −80 °C for further gene expression and biochemical biomarkers measurements.

2.2. Analyses

2.2.1. Condition index

The total weight and wet weight of soft tissues were measured on 10 individuals per condition. The condition index was calculated according to the following formula (Kanduč et al., 2018):

$$CI = \frac{Wet\ weight\ of\ soft\ tissue\ (g)}{Whole\ bivalve\ weight\ (g)} \times\ 100$$

2.2.2. Clearance rate

Clearance rate, defined as the volume of water cleared of suspended particles per unit of time, was estimated for clams previously exposed for 21 days to clean freshwater (negative control) or fed with microalgae exposed to PS or ENV NPs (0.008, 10 and 100 µg L^{-1}). Clams were placed in 600 mL glass beaker for 1 h before measurements started, then supplied with *S. subspicatus* at an initial concentration of 300,000 cells mL^{-1}. Mixing was promoted by fine bubble aeration and the clams were placed on a metal grid to minimize resuspension of feces. The clearance rate was measured by an indirect method assessing the concentration of the algae solution every 15 min for 45 min, according to the standard algae cell counting technique (Utermöhl, 1958). Briefly, algal cell fixation was realized by adding one drop of Lugol's solution to 10 ml of sample. Cells were counted under a light microscope using a Malassez counting chamber.

2.2.3. Burrowing test

Burrowing tests were performed as described in Bonnard et al. (2009) after 21 days of exposure (Bonnard et al., 2009). The burrowing experiments were conducted in glass crystallizers containing 5 cm of artificial sediments (90% sand, 9% kaolinite, 1% calcium carbonate) covered with tap water (2 cm above the sediment). The sediment was hand prepared and placed in crystallizers 5 days before the experiment. Burrowing behavior was evaluated by placing 10 individuals per condition on the sediment surface, in the dark, and observing how many of them had burrowed at frequent intervals. The interval was every 5 min during the first hour, every 10 min during the second hour, and every 20 min from the third to the sixth hour. Percentages of unburrowed individuals over time were determined for each condition.

2.2.4. Biochemical biomarkers

Enzyme activities (catalase - CAT, Glutathione-S-transferase - GST, and phosphatase acid - AcP) were quantified in gills and digestive gland tissues. First, the tissues were thawed, weighed, and homogenized with a FastPrep® 24 homogenizer (3 times for 40 s) in Tris buffer at pH 7.4 (Tris 0.8 M (Trizma-base Sigma® T6791); NaCl 2 M (Sigma® S9888); DTT 100 mM (Sigma® 8349–12–3); CIP according to supplier protocol (Protease Inhibitor Cocktail, Sigma® P8340)). The homogenates were centrifuged at 12,000 g for 15 min. The amount of total protein was quantified using the Bradford method (Bradford, 1976). Protein concentrations were expressed in $mg.mL^{-1}$. Catalase (CAT) activity was measured spectrophotometrically at 240 nm ($\varepsilon = 0.04\ mM^{-1}.cm^{-1}$; Uvi-Line® 9600), following the dismutation of hydrogen peroxide (H_2O_2) (Claiborne, 1985). Specific activity was expressed in $\mu moles.min^{-1}.mg^{-1}$ of protein. Glutathione-S-Transferase (GST) activity was measured spectrophotometrically at 340 nm ($\varepsilon = 9.6\ mM^{-1}.cm^{-1}$, BioTek EPOCH) by observing the conjugation of the 1-chloro-2,4-dinitrobenzene (CDNB) and the l-glutathione reduced (GSH). Specific activity was expressed in $nmoles.min^{-1}.mg^{-1}$ of protein. Phosphatase acid (AcP) activity was determined spectrophotometrically at 340 nm ($\varepsilon = 18.3\ mM^{-1}.cm^{-1}$; BioTek EPOCH; Phosphatase acid Assay kit Sigma® CS0740), measuring the appearance of p-nitrophenol (pNP) by the hydrolysis of p-nitrophenylphosphate (4-NPP) and expressed in $nmoles.min^{-1}.mg^{-1}$ of protein.

2.2.5. Analysis of gene expression by quantitative PCR

Total RNA was purified from gills and visceral mass of clams, using the SV Total RNA Isolation System kit (Promega) according to the manufacturer's recommendations. RNA concentration ($\mu g.\mu L^{-1}$) was quantified using a Take3 plate with a microplate spectrometer (BioTek EPOCH). First-strand cDNA was synthesized from RNA using a RT system kit (GoScript, Promega) and performed in an Eppendorf Mastercycler™. cDNA samples were stored at −20 °C until qPCR amplifications. The expression levels of twenty genes involved in endocytosis, oxidative stress, detoxication, respiratory chain, immunity, neurotoxicity and apoptosis were measured. They were analyzed using a set of forward and reverse primers by quantitative RT-PCR. Three genes were used as housekeeping genes, including *β-actin, elongation factor 1 α* (ef1α) and *ribosomal protein 7* (rpl7) (Table 1). Specific primers for acetylcholinesterase *AchE* and phosphatase acid *ACP* genes were designed using Primer 3 V 4.0 software. Quantitative PCR (qPCR) amplifications were carried out in 384-well microplates (LightCycler 480, Roche) using GoTaq® qPCR Master Mix kit (Promega). A mix of primer pair was prepared for each gene (5 µM). For each reaction, 1 µL of the pair mix, 12.5 µL of the qPCR Master Mix mixed with 6.5 µL of RNA-free water, and 5 µL of cDNA were added to each well. The qPCR reactions consisted of a first step of 10 min at 95 °C (enzyme activation) followed by 40 cycles (95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s). The samples were gradually heated from 60 °C to 95 °C to get a dissociation curve and check for amplification specificity. The relative gene expression ratio of each target gene was calculated following the delta-delta method normalized with reference genes (Livak and Schmittgen, 2001) and defined as follows:

$$ratio = \frac{2 - \Delta\Delta Ct\ (exposed)}{2 - \Delta\Delta Ct\ (control)}$$

2.3. Data analysis

The statistical analyzes were performed using the software XLSTAT 2019 (21.4.63762 version). Normality of data distribution and homogeneity of variance were tested using the Shapiro-Wilk test and Bartlett test, respectively. As the assumptions for parametric tests were not met for the condition index, the clearance rate, the gene expression and biochemical biomarkers measurements, we used the Kruskal-Wallis test to test for differences between the treatments. As the overall test was significant, a Dunn procedure was performed to determine which means were significantly different. Concerning the burrowing test, percentages of unburrowed individuals were first ln-transformed for linearization, then the regression coefficients of the least-square regression lines were compared using an analysis of covariance (ANCOVA). In all cases, p values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Condition index (CI)

No significant differences were observed between exposure conditions with a mean CI of *C. fluminea* ranging from 144.02 to 155.90 (Table 2).

3.2. Clearance rate

NPs contamination had no significant impact on the clearance rate of clams between 0 and 15 min and between 15 and 30 min (Fig. 1). The clearance rate was significantly higher for PS NPs 10 and PS NPs 100 compared to the control condition between 30 and 45 min.

3.3. Burrowing test

Results of burrowing test of *C. fluminea* after 21 days of exposure to PS and ENV NPs are reported in Table 3. As the higher the slope value, the higher the burial speed, the burrowing kinetic was significantly higher for individuals exposed to PS 100 (slope = −0.0023) compared to control (slope = - 0.0004). Comparison between exposure to PS NPs and ENV NPs showed that there was no difference for the low concentrations (0.008), while *C. fluminea* exposed to ENV NPs showed a significantly more impaired burrowing capacity compared to PS NPs when exposed at

Table 1
List of genes, protein names, functions and sequences of specific primers used in this study. [a]Forward primer, [b]Reverse primer.

Gene	Protein name	Function	Accession number	Primers (5′–3′)	Source
β-actin	Beta Actine	Reference	A4L694	GATGGATGGTCCAGACTCGT[a] GGTCTGGATCGGTGGTTCTA[b]	Arini et al. 2019
rpl7	Ribosomal protein L7		P18124	CACCATCGTTGAAGTGGTTG[a] CTTCAAACAGGCTGCCAACT[b]	Arini et al. 2019
ef1α	Elongation factor 1 alpha		J9Z6R5	CTTCGTGCCAATCTCTGGAT[a] TTCCTCTCTACAGCCCAACC[b]	Arini et al. 2019
cltl	Clathrin heavy chain 1	Endocytosis	Q00610	GCAAACATCACACAGGTTGC[a] CGTGCGTAGTTGGACACATT[b]	Arini et al. 2019
cav	Caveolin		P51636	GTCCAGCACCATACGTGGTT[a] AACTGTTGACCACCCTCTGTG[b]	Arini et al. 2019
mt	Metallothionein	Detoxication	EF185126	CGGCTATCTCCCGCGA[a] AGCTTTTACCAGAACCAAACAGT[b]	Arini et al. 2019
mdr	ATP Binding Cassette Subfamily		KJ001772	ATCCTGGTTGATGGCACTGA[a] AGGTTCCTGGCTCACAATACC[b]	Chen et al., 2015
gst	Glutathione transferase, GST class-pi		AY885667	ATGACTTCATCAAGAGTTTACCAG[a] GCATGTTCTTGAACTCTTCTGA[b]	Bigot et al., 2009
cox1	Cytochrome C oxidase	Respiratory	Q9G2B4	CCTGTTTGGAGAAAGGGTCA[a] CCGTGGCATTCCACTTATTC[b]	Arini et al. 2019
12 s	Mitochondrial 12 s rRNA	chain	EF446612	AGCATTACTATGTTACGACTTACCTCA[a] AGTTCAGGTAGACGTGTAGGG[b]	Arini et al. 2019
sod2	Superoxide dismutase 2	Oxidative stress	P04179	CCAGGCTAATGGCAGACTTC[a] GTAGGCATGCTCCCAAACAT[b]	Arini et al. 2019
sod1	Superoxide dismutase 1		U3PWG5	CCAGCAGCCAGACCAGTTAT[a] AGGGAGACGCTAATGTGTCG[b]	Arini et al. 2019
cat	Catalase		A4L695	CACCAGGTGTCCTTCCTGTT[a] CTCAGCATTCACCAGCTTGA[b]	Arini et al. 2019
gpx7	Glutathione peroxidase 7		Q96SL4	AGGATGCATCTGAAGCTTGG[a] CGTTCACCACATGTCCATTC[b]	Arini et al. 2019
ache	Acetylcholinesterase	Neurotoxicity		CTTGCTCTGGATCCTGCTCC[a] TGCAAAAACCGGGACTCCAA[b]	Designed from the transcriptome
atg13	Autophagy-related protein 13	Immunity	O75143	CTCAGCTGTCCGTAACGAGAT[a] AAGTGCATTCTGAGGCGAAG[b]	Arini et al. 2019
atg12	Ubiquitin-like protein ATG12		O94817	CCTCTCAACTGCCCATTTCT[a] GTACACCAACGAGTCCTTTGC[b]	Arini et al. 2019
gal	Galectin-3		P17931	TACTGACTTCCCGCTCTTCG[a] CACTTTGATGTGCGCTTCC[b]	Arini et al. 2019
acp	Phosphatase acid			TGGCTTGTTGGCTTCCTTGA[a] AAGAGGAGGTTCTGCATGCC[b]	Designed from the transcriptome
bcl2	Apoptosis regulator Bcl-2	Apoptosis	P10415	AAGGAACAGGTCCATTCACG[a] GGGACGGATTGTTGGTGTT[b]	Arini et al. 2019
p53	Tumor protein p53		P04637	TCCTGCCACAGTCACAAATG[a] GTCGAGATTTTCCCTCCTTAGC[b]	Arini et al. 2019
bax	BCL2 associated X		Q07812	AAAGGGGAGGATGGGAGAT[a] GCTATAACTGCCCCTGCTGT[b]	Arini et al. 2019
gadd45	Growth Arrest and DNA Damage		P24522	GGAGCAGGTGATGCTGTGTA[a] CCAGCAGTGTGCCTCAATAA[b]	Arini et al. 2019

Table 2
Condition Index (mean and standard deviation).

	Concentrations (μg l^{-1})	CI means	CI SD
Control	0	155.59	22.04
PS NPs	0.008	155.56	21.40
	10	148.53	17.56
	100	144.51	18.60
ENV NPs	0.008	155.90	12.87
	10	144.02	28.16
	100	150.03	20.08

Table 3
Slopes of burrowing tests for *C.fluminea* in control condition and after exposure to standard polystyrene nanoplastics (PS NPs) and environmental nanoplastics (ENV NPs) at 0.008, 10 and 100 μg l^{-1}, ($N = 10$). *(Superscripts letters a, b, c, d for significant differences between conditions and in bold for significant differences between control and conditions $p < 0.005$).*

	Concentrations (μg l^{-1})	Slopes
Control	–	−0.0004 [a, b]
PS NPs	**0.008**	−0.0007 [a]
	10	- 0.0012 [b, c]
	100	**−0.0023 [c]**
ENV NPs	**0.008**	−0.0002 [a]
	10	−0.0010 [a]
	100	−0.0013 [a, b]

10 and 100 μg. l^{-1}. Regarding concentration effect for both PS NPS and ENV NPs, only PS NPs at the low concentration (0.008) showed a significant impact on burrowing behavior compared the higher concentrations (10 and 100 μg. l^{-1}).

3.3.1. Oxidative stress and detoxification

In the gills, after 7 days of exposure, a higher catalase (CAT) activity was measured for the organisms exposed to PS 10, ENV NP 0.008 and ENV NP 100 compared to the control (Fig. 2A). After 21 days of exposure, organisms exposed to PS 0.008, ENV 10 and ENV 100 have a slightly higher CAT activity than the control (Fig. 2B). In the visceral mass, after 7 days of exposure, a decrease in CAT activity was measured for organisms exposed to PS 100 compared to the control (Fig. 2C). After 21 days, the exposure to PS 0.008, ENV NP 0.008 and ENV NP 10 induced an increase in CAT activity compared to control (Fig. 2D). No difference of the glutathion-S-transferase (GST) activities was observed in both gills and visceral mass after 7 and 21 days of exposure (Fig. 3).

3.4. Immunity

Clam's exposure to PS and ENV NP had no significant impact on the phosphatase acid (AcP) activities irrespective to tissues (gills or visceral mass) and exposure time (Fig. 4).

3.5. Gene expression

Exposures to PS NPs and ENV NPs generally had a greater impact on gene expression levels in the gills than in the visceral mass (Tables 4 and 5).

3.5.1. Gills

As shown in Table 4, after 7 days of exposure, no gene expression was impacted after exposure to PS 0.008 condition. The genes studied were generally more impacted after 21 days of exposure than after 7 days, except for PS 100 condition. Among the studied genes involved in endocytosis, *calveolin* was repressed after 21 days of exposure only for ENV PS 10 and 100 conditions compared to the control. *Clathrin* gene

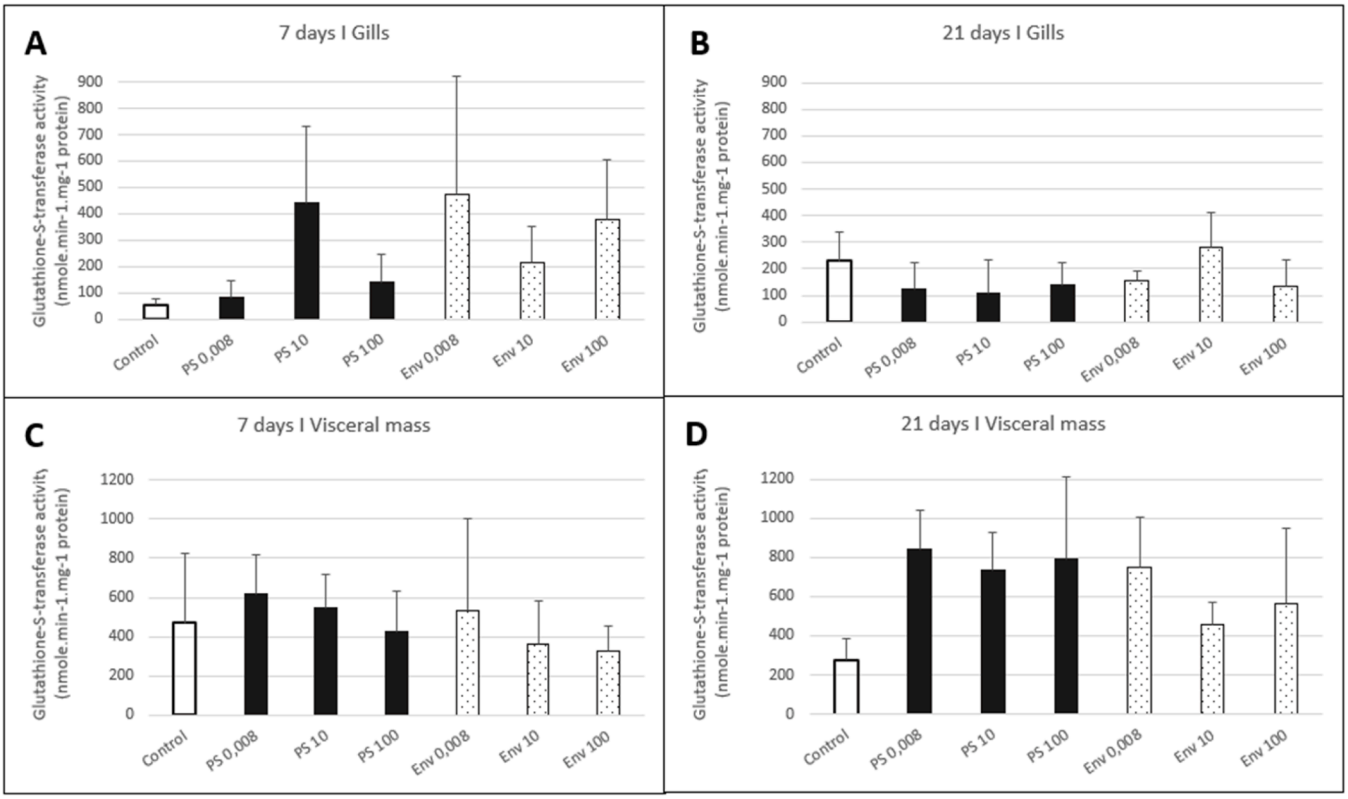

Fig. 3. Enzymatic activity of GST in gills (A, B) and visceral mass (C, D) of *Corbicularia fluminea* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS) and environmental nanoplastics (ENV) at 0.008, 10 and 100 µg.L-1 (mean ± SD, $N = 5$).

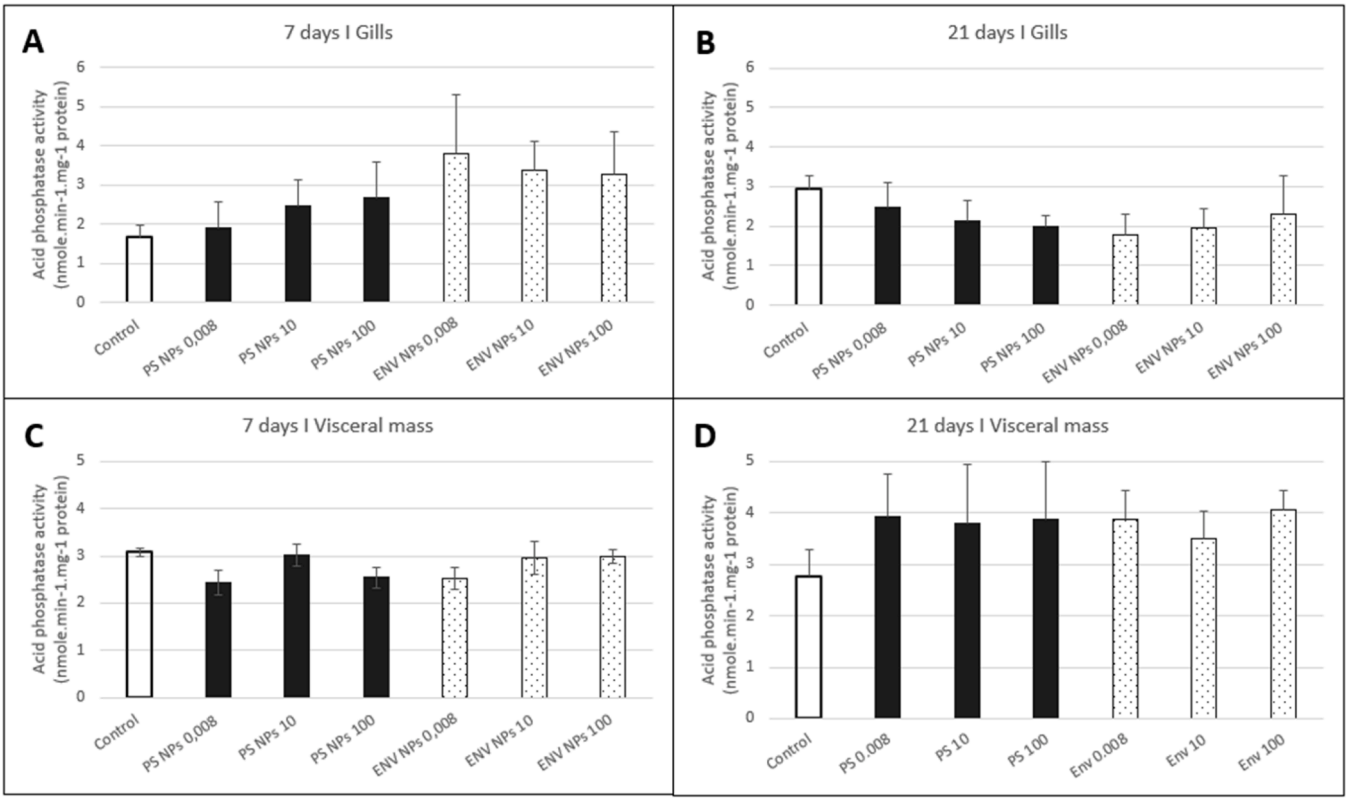

Fig. 4. Enzymatic activity of AcP in gills (A, B) and visceral mass (C, D) of *Corbicularia fluminea* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L-1 (mean ± SD, $N = 5$).

Table 4
Differential gene expression observed in the gills of *Corbicula fluminea* after 21 days of trophic exposure to the different treatments : PS (polystyrene) NPs and ENV NPs from plastics collected in the Garonne River. Results are presented as fold-change factors between gene expression in controls and gene expression in exposed organisms (> 1: induction; < 1: repression). Only significant differences are represented (p values ≤ 0.05) and only factors <0.5 and >1.5 are considered as significant.

Function	Gene	Gills											
		PS 0.008		PS 10		PS 100		Env 0.008		Env 10		Env 100	
		J7	J21	J7	J21	J7	J21	J7	J21	J7	J21	J7	J21
Endocytosis	cltl	–	0.24	2.16	–	3.14	–	–	–	0.24	0.23	–	0.20
	cav	–	–	–	–	–	–	–	–	–	0.42	–	0.46
Detoxification	mt	–	–	0.28	–	–	0.15	–	–	–	–	–	–
	mdr	–	–	–	0.30	–	–	–	0.06	–	0.19	–	0.07
	pi GST	–	–	–	1.67	–	–	–	–	–	–	2.76	–
	ACP	–	0.31	–	0.19	0.39	–	–	0.11	–	0.14	–	0.20
Respiratory chain	cox1	–	–	–	0.34	0.11	–	0.13	0.35	0.11	0.38	4.10^{-3}	0.37
	12 s	–	–	–	0.29	0.49	–	0.44	0.21	0.37	0.25	0.62	0.22
Oxydative stress	sod2	–	–	–	0.43	0.87	–	–	–	0.66	0.24	–	0.31
	sod1	–	–	–	0.38	–	–	–	–	–	0.22	–	0.18
	cat	–	0.22	–	–	–	–	0.10	0.17	–	0.09	–	0.09
	gpx7	–	0.14	–	0.31	–	–	–	0.19	–	0.10	–	0.13
Neurotoxicity	AchE	–	0.12	–	0.08	–	0.06	0.21	0.02	0.12	0.02	0.22	0.03
Immunity	atg13	–	–	–	0.14	–	–	0.19	0.14	–	0.13	–	0.10
	atg12	–	0.20	–	0.12	–	0.14	0.12	0.11	0.26	0.05	0.37	0.06
	gal	–	–	–	0.35	–	–	–	0.30	0.41	0.19	–	0.11
	bcl2	–	0.28	–	0.32	–	–	0.15	0.25	0.17	0.14	0.29	0.09
Apoptosis	p53	–	0.06	–	0.09	–	0.10	–	0.08	–	0.02	0.22	0.01
	bax	–	–	–	–	–	–	0.15	0.18	0.22	0.04	0.31	0.03
	gadd45	–	0.21	–	0.22	–	–	–	0.23	0.28	0.06	–	0.05

Table 5
Differential gene expression observed in the visceral mass of *Corbicula fluminea* after 21 days of trophic exposure to the different treatments : PS (polystyrene) NPs and ENV NPs from plastics collected in the Garonne River. Results are presented as fold-change factors between gene expression in controls and gene expression in exposed organisms (> 1: induction; < 1: repression). Only significant differences are represented (p values ≤ 0.05) and only factors <0.5 and >1.5 are considered as significant.

Function	Gene	Visceral mass											
		PS 0.008		PS 10		PS 100		Env 0.008		Env 10		Env 100	
		J7	J21	J7	J21	J7	J21	J7	J21	J7	J21	J7	J21
Endocytosis	cltl	–	–	–	–	–	–	–	–	–	–	–	–
	cav	–	–	–	–	–	–	–	–	–	–	–	–
Detoxification	mt	–	–	–	–	–	–	0.40	–	–	–	–	2.38
	mdr	–	–	–	0.40	–	0.38	0.33	0.29	–	0.21	–	0.29
	pi GST	2.33	–	2.37	–	–	–	–	–	–	–	–	–
	ACP	–	–	–	–	–	–	0.45	–	–	–	–	–
Respiratory chain	cox1	–	–	–	0.46	–	0.42	0.33	–	–	1.61	0.32	1.92
	12 s	–	–	–	0.22	–	0.28	–	–	–	–	–	–
Oxydative stress	sod2	–	–	–	–	–	–	–	–	–	–	–	–
	sod1	–	–	–	–	0.35	–	–	–	–	–	–	–
	cat	–	–	–	–	–	–	0.31	–	–	–	–	–
	gpx7	–	–	–	–	–	–	–	–	–	–	0.36	–
Neurotoxicity	AchE	–	–	–	–	–	–	0.15	–	–	–	–	–
Immunity	atg13	–	–	–	–	–	–	–	–	–	–	–	–
	atg12	–	–	–	–	0.23	–	0.13	–	–	–	0.19	–
	gal	–	–	–	–	–	–	–	–	–	–	–	–
	bcl2	–	–	–	–	–	–	–	–	–	–	–	–
Apoptosis	p53	–	–	–	–	–	–	–	–	–	–	–	–
	bax	–	–	–	0.34	–	0.35	–	–	–	–	–	–
	gadd45	0.39	–	–	–	–	–	–	–	–	–	–	–

was downregulated for ENV 10 at 7 days of exposure and for PS 0.008, ENV 10 and ENV 100 at 21 days of exposure. Only the PS NP 10 and 100 conditions induced an over-expression of *clathrin* at 7 days of exposure. Concerning detoxification, exposure to ENV NP led to a repression of the *mdr* and *ACP* genes compared to the control for all the concentrations tested at 21 days of exposure.

In addition, the studied genes involved in the respiratory chain (*cox 1* and *12S*) and in neurotoxicity (*AchE*) were downregulated compared to the control for all the ENV NP concentrations at both sampling times, except for PS 100 after 7 days of exposure. Genes involved in apoptosis (*p53, bax, gadd45*) and immunity (*atg13, atg12, ga*l, *bcl2*) were repressed compared to the control for individuals exposed to ENV NPs at 7 days and/or 21 days of exposure for all concentrations.

It should be noted that most of the genes studied were repressed compared to the control for individuals exposed to PS 10, ENV NP10 and ENV NP 100 after 21 days of exposure.

3.5.2. Visceral mass

In the visceral mass, only few effects of the exposure conditions were noted on gene expression related to endocytosis: the *clathrin* gene was under-expressed for the ENV NP 0.008 condition at 7 days of exposure (Table 5). The *mt* gene was downregulated after 7 days of exposure to ENV NP0.008 and up-regulated after 21 days of exposure to ENV 100. Exposure to PS NP 10 and 100 at 21 days induced downregulation of the *mdr* gene. This gene was also downregulated for ENV NP 0.008 (7 and 21 days) and ENV NP 10 and 100 at 21 days of exposure. The *pi gst* gene was

upregulated after 7 days of exposure to PS 0.008 and 10 conditions. The *ACP* gene was downregulated only in organisms exposed to ENV NP 0.008 at 7 days of exposure. Regarding respiratory chain, *cox 1* was repressed compared to the control for PS10 and PS 100 at 21 days of exposure and for ENV NP 0.008 and ENV NP 100 at 7 days of exposure. At the opposite, the expression of this gene was induced for ENV NP 10 and 100 at 21 days of exposure compared to the control. The *12S* gene was only downregulated for PS 10 and PS 100 compared to the control at 21 days of exposure. Among the oxidative stress genes, *sod1, cat* and *gpx7* were downregulated compared to the control for the conditions PS 100 at 7 days, ENV NP 0.008 at 7 days and ENV NP 100 at 7 days respectively. The *AchE* gene related to neurotoxicity was only downregulated for ENV NP 0.008 compared to the control. Exposure to both types of NP induced relatively few effects on the immunity-related genes studied. There was no dose response, nor effect of the type of NP on these genes. Exposure to PS NPs led to a repression of two genes involved in apoptosis compared to control: *bax* for PS 10 and PS 100 conditions and *gadd45* for PS 0.008.

4. Discussion

The objective of the present study was to evaluate the effects of NPs on the freshwater bivalve *C. fluminea* exposed through trophic transfer. This study was based on a two-level trophic transfer experiment, from freshwater algae to bivalves, using PS NPs and NPs derived from macroplastics sampled in the environment. The invasive Asian clam *Corbicula fluminea* is well recognized as a sentinel species cumulating several advantages as it has spread in many freshwaters, where it is indeed ubiquitous, and can be found and collected in abundance. Its large filtration capacity leads to a potential accumulation of contaminants and has made it an interesting model for ecotoxicological studies in freshwater (Castro et al., 2018). Studies on the trophic transfer of NPs are relatively recent and scarce. Knowledge gaps persist in understanding the mechanisms of bioaccumulation, transfer and toxicity of these plastic particles along aquatic trophic chains (Holzer et al., 2022; Latchere et al., 2021). It therefore appears crucial to develop studies on the trophic transfer of NPs to better assess the risk represented by these particles within aquatic ecosystems.

Once released into the aquatic environment, nanoparticles and NPs behavior is dependent upon their own properties such as shape, size, chemical composition, surface charge, coating and particle state. These particles can complex with ligands and then agglomerate, react with natural materials like dissolved organic matter (DOC) and natural particulates, release ions or still be present as nanoparticles (Châtel and Mouneyrac, 2017). These changes in chemical composition modify their physico-chemical properties and reactivity towards biological systems and hence their toxicity (Bergami et al., 2017; Corsi et al., 2020). Nanoplastics have been demonstrated to have the ability to first adsorbed algae cell walls (Nolte et al., 2017) and then to be transported through biological membranes and to enter cells, resulting in decreasing growth and photosynthesis and in modulating oxidative stress response, conducting sometimes to lethality (Corsi et al., 2020).

Primary producers are the first exposed to those polymers and bioaccumulation have been demonstrated to occur in organisms from the highest levels of the food chain. Micro and nano plastics have been detected in the stomach, hepatopancreas, ovary, and gills of the crab *Carcinus maenas* that was fed with mussels (*Mytilus edulis*) previously contaminated to 0.5 µm PS microspheres (Farrell and Nelson, 2013). Mattsson et al. (2017) investigated NP trophic transfer by exposing the algae *Scenedesmus* sp. to NPs (53-nm and 180-nm) which were given to *Daphnia magna* and then to carp (*Carassius carassius*): NP were found in the brains of the fish.

In the present study, the effects of NP on *C. fluminea* exposed through trophic pathway were investigated at multiple levels of biological organization.

4.1. Individual markers

4.1.1. Condition index

Bivalve physiology and growth can be evaluated using the condition index (Filgueira et al., 2013; Zeng and Yang, 2021). This index is a commonly used metric to assess bivalve health and physiological status as well as effects of environmental stresses (Zeng and Yang, 2021). In our study, no effect of the different NPs tested on the CI was depicted. This is consistent with previous studies showing no effect of different types of plastic particles in bivalves (Bour et al., 2018; Métais et al., 2023; Ribeiro et al., 2017). As pointed out by Bour et al. (2018), CI might not be a sensitive biomarker to study the toxicity of plastic particles. Other markers could be more relevant to study plastic particles impacts such as energy reserves which decreased in *C. fluminea* after exposure to a mixture of MPs for 21 days (Vidal et al., 2023). In addition, a disruption of energy balance has been reported in oysters exposed to PS MPs for 2 months using dynamic energy budget modeling (Sussarellu et al., 2016).

4.1.2. Clearance rate

Clearance rate in bivalves is function of gill type, body size, body condition, temperature, current speed, particle size, particle type and their concentration (Gatenby et al., 2013; Rosa et al., 2020). The clearance rate was only significantly higher for PS NP 10 and PS NP 100 compared to the control condition. Such an increase of the clearance rate has also been demonstrated in *C. fluminea* fed with microalgae *S. subspicatus* previously contaminated with environmental NPs at 1 and 10 µg.L^{-1} or directly exposed to PS NPs at 1 and 10 µg.L^{-1} for 21 days (Arini et al., 2023). After a 2 month-exposition to PS MPs, oyster *C. gigas* had a higher algal consumption and absorption efficiency than control oysters (Sussarellu et al., 2016). The authors suggested that this increased food consumption could be a compensation to adjust energy intake in response to the PS MPs (Sussarellu et al., 2016).

4.1.3. Burrowing

It is increasingly recognized that behavioural endpoint is a sensitive screening tool for detecting the effects of environmental stress on organisms as behavioral changes represent adaptive responses to environmental stimuli (Burghardt, 2020; Peterson et al., 2017). Several studies reported the behavioral effects of xenobiotics, even at very low concentrations (Amiard-Triquet, 2009; Guimarães et al., 2021; Hellou, 2011). In this study, no impairment of the burrowing activity was recorded between treatments (PS NPs and ENV NPs) and control except for the NPs PS 100 µg. l^{-1} group exhibiting rather a significant faster burrowing activity when in clean sediment compared to control. However, in Latchere et al. (2023) study, after a 21-days exposure to waterborne PS NPs at the same concentrations of the current study (0.008 µg. l^{-1}; 10 µg. l^{-1}; 100 µg. l^{-1}), *Corbicula fluminea* specimens showed the opposite results, with individuals of NPs PS 10 µg. l^{-1} group taking longer to burrow than those under control conditions. Differences observed between the current data and the previous study can be likely explained by the exposure strategies (direct or trophic) and enables ruling out aspects such as NPs size, concentrations, exposure periods, biological models and behavioural parameters. This finding can reinforce the undermentioned perspective for more NPs trophic transfer studies. Chronic exposure examining the transfer and accumulation of PS-NPs-NH2 (100 µg. l^{-1}) in *D. magna* through waterborne routes significantly disrupted the growth, molting, and reproduction of *Daphnia* compared to the foodborne group (Wang and Wang, 2023). This implies that *D. magna* exposed to NPs through waterborne pathways being more susceptible to hazards. In the marine bivalve *Scobicularia plana,* exposed to NPs PS through dietborne, the burrowing activity was significantly impaired in clams exposed to 10 and 100 µg. l^{-1} PS NPs and to 0.008 µg. l^{-1} environmental NPs (Métais et al., 2023). As for *C. fluminea* in this study, it has been reported that *H. diversicolor* exposed to PMMA NPs (0.5 – 500 µg. l^{-1}) burrowed faster into sediments than

control organisms (Silva et al., 2023). However, the same species displayed opposite results, worms exposed between 5 and 500 μg. l^{-1} of PS NPs took longer to burrow than those in control conditions (Silva et al., 2020). The absence of behavioural impairments may be due to an increase in particle size due to agglomeration especially at high PS NPs concentrations, which decreases the bioavailability and thus the potential incorporation of the NPs into cells (Silva et al., 2023, 2020). In addition, the characteristics of the plastic particles interact with the mucus on the gills resulting in particle rejection or ingestion (Rosa et al., 2018; Ward et al., 2019).

4.2. Molecular and cellular effects

In our study, the expression of twenty genes targeting seven different metabolic functions were studied both in gills and visceral mass. Almost no effect was depicted in visceral mass whatever the type of NP and the concentration: 12.5% of the tested genes were significantly impacted at J7 and 11.66% at J21, while in gills 28% of the tested genes were impacted at J7 and 65% at J21, mostly downregulated compared to control group. For the environmental NP, 25 genes mRNA expressions impacted in gills at J7 were also impacted at J21. All the target functions (18 of 20 genes) were impacted by the highest concentrations of Env NP (10 and 100 μg.L^{-1}). Gene expression analysis showed globally a downregulation of the selected genes which is consistent with the study of Arini et al. (2023) who also showed similar downregulation of gene expression on the same species *C. fluminea* fed with environmental and PS NPs contaminated microalgae at the same conditions of exposure (concentration and duration). Lebordais et al. (2021) also showed molecular impacts of dietary exposure of NPs in oyster *Isognomon alatus*, with differential gene expressions between environmental and PS NPs. NP could cause impairment of promoting factors that have an influence on the expression of genes implicated in the main cellular processes investigated: endocytosis, detoxification, oxidative stress, immune response, apoptosis, neurotoxicity. Qi et al. (2023) also studied the impact of NPs in mussels. They explained that the downregulation of gene expression also observed after NPs exposure may suggest different stages of an inflammatory response and hence demonstrate that NPs exposure may exert more toxicity than MPs exposure. ENV NPs may be more oxidized than PS NPs and hence present more negatively charged groups on the surface (Blancho et al., 2021) which could lead to higher toxicity as demonstrated by Arini et al. (2023). NPs have been shown to be absorbed by phagocytes that cause a different interaction within the cells than MPs (Al-Thawadi, 2020; Kögel et al., 2020). Indeed, expressions of genes implicated in detoxification (*cyp*32, *gst,*), DNA repair (*p53*), cell stress-response (*hsp*70) and innate immunity (*lyso, tlr-i, MytB, Mytc*) depicted significant alterations in mussels *Mytilus galloprovincialis* exposed to NPs (Auguste et al., 2020; Brandts et al., 2018), as demonstrated in the present study. In gills, endocytosis was not impacted by the lowest concentration of Env NP (0.008 μg.L^{-1}), nevertheless more downregulations of genes were observed in this tissue. (Qi et al., 2023) suggested that NPs could more preferably penetrate through the mucosal barrier, triggering a more severe inflammatory response, which in turn leads to tissue destruction that promotes the ingestion of plastic particles. This could explain the main effects observed in gills as compared to digestive mass.

In the present study, effects of enzymatic markers were evaluated to potentially link molecular events to cellular functions. Overall, among the studied enzymes, only the catalase activity varied significantly, increasing in gills and visceral mass of clams after 21 days feeding with algae exposed to ENV NP (10 and 100 μg. l^{-1} in gills and 0.008 and 10 μg. l^{-1} in visceral mass). Reactive Oxygen Species (ROS) constitute a pool of oxidative species including superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) that adversely react with cellular macromolecules including DNA, proteins and lipids. Under physiological conditions, animals maintain a balance between the generation and neutralization of ROS. The oxidative stress occurs when there is an imbalance between the production of ROS and the antioxidant capacity of the cell (Châtel and Mouneyrac, 2017). Excessive ROS production has been demonstrated to be the main toxic effect due to direct NP exposure in bivalves (Zhou et al., 2023), depending on the exposure concentration. In their study, Zhou et al. (2023) showed a similar trend between CAT activity and the total antioxidant capacity in *Ruditapes philippinarum*: no difference with the control for the tested concentration of 0.02 and 0.2 mg.L^{-1} and a decrease for higher concentrations (2 and 20 mg.L^{-1}). However, the concentrations tested are higher than those used in our study. Li et al. (2020) reported oxidative stress in the three organs studied after a direct short exposure to PS NPs (80 nm; 0.1 mg.L^{-1}, 1 mg.L^{-1} and 5 mg.L^{-1} for 96 h) in *C. fluminea*: visceral mass, gill and mantle. In our study, the exposure via the trophic chain did not show such a clear oxidative stress response induction perhaps due to the low concentrations tested. At the same concentration of PS NPs (100 μg.L^{-1}) than Li et al. (2020), no CAT induction was depicted nor in gill, nor in visceral mass whereas their study showed an induction in gills. The PS NP used in our study were 200 nm in size and carboxylated. Greven et al. (2016) reported that both polycarbonate (158.7 nm) and polystyrene (41 nm) nanoplastics activated the neutrophil function of the fish fathead minnow (*Pimephales promelas*) as evidenced by phagocytosis, degranulation, neutrophil extracellular trap release and oxidative burst. In a similar way, Canesi et al. (2015) described an increase in lysozyme activity, ROS and nitric oxide production in Mediterranean mussel (*Mytilus galloprovincialis*) hemocytes exposed *in vitro* to PS nanoplastics. Likewise, (Bergami et al., 2019) reported that PS nanoplastics affected *in vitro* cellular phagocytosis of coelomocytes in the Antartic sea urchin (*Sterechinus neumayeri*), as well as modulated genes involved in inflammatory responses, oxidative stress and apoptotic pathways.

4.3. Comparison of trophic and direct clam exposures

The originality of the present study is that the results obtained allow to establish a comparison of this trophic exposure to a previous study of the laboratory that investigated effects of similar NP and concentration on the same species (*C. fluminea*) through direct exposure (waterborne exposure). Plastic particles can be ingested through direct exposure by filtration or be consumed with preys already contaminated with plastics (Setälä et al., 2018). Gills are the first organ in relation with suspended particles, the water flow entangling them in gill filaments then leading them towards the mouth. Their ingestion or rejection depends on the density, the size and the agglomeration of particles (Castro et al., 2018). Another uptake way considers the transfer of ingested particles via endocytosis to the oral cavity then to the digestive mass. MPs are uptaken by both ways but there are too few studies on the ingestion of NPs to know the real route of NPs uptake. Li et al. (2020) demonstrated in *C. fluminea* that the visceral mass and gills could accumulate NPs through ingestion and respiration respectively.

The exposure strategy greatly influences the effects of MPs and NPs on the species. Few studies investigated the trophic transfer of MPs on aquatic food chain. However, no studies evaluate a comparison between direct and trophic exposures measuring toxicity effects on bivalves from molecular to cellular levels. Having a parallel between these two types of exposure, allow to depict main similarity and differences. Kim et al. (2022) showed a significant decrease of PS-NH_2 NP uptake when comparing direct and trophic exposure (*D. salina*) of *A. franciscana.*

First, whatever the tested organ and exposure duration, gene expression was in general the only parameter demonstrating significant changes in NPs exposed animals compared to control group in both exposure scenarii. In general, overexpressions and downregulations of genes were observed after direct bivalve exposure whereas only downregulations of genes were measured after trophic exposure (Arini et al., 2023). The comparison with studies on gene expression modulation by NPs is difficult as they focused on some genes, on specific metabolic pathways, on target organ or on species (Auguste et al., 2020; Deng

et al., 2023; Li et al., 2023). Most of them showed differential expression in presence of NP compared to control condition. Epigenetic modulations or alterations could also be a response for the organisms to face NPs (and MPs) exposure (Poma et al., 2023). Additional investigations need to be addressed to fully characterize the mechanisms implicated in NPs ingestion and toxicity, taking into account additives that strongly influence NPs toxicity as demonstrated by Arini et al. (2023). RNA seq analysis could be of great interest to get an overview of all transcriptomic pattern of trophic exposed animals without any selection of measured genes.

5. Conclusion

The main conclusions of the present study demonstrate the importance of considering trophic transfer as regards to MPs and NPs to fully investigate the effects of this ubiquitous pollution towards the environment. Even though additional work needs to be performed to get better understanding on the behavior, fate and effects of NPs on organisms, it is also important to consider larger food chain scenarii for their representativeness of environmental conditions. The absence of clear link between biochemical biomarkers and gene expression highlights a possible post-transcriptional regulation of the tested genes and the weak response of the tested enzymatic biomarkers following NPs exposure. For the large number of publications of this field and results obtained in this study, it appears that transcriptomic approach could represent a sensitive tool to depict effects of NPs and slight differences between types and concentrations of NPs. In addition, it would be interesting to use transmission electron microscopy to characterize the effects of trophic exposure to plastic particles on the ultrastructure of the gills and visceral mass of bivalves.

CRediT authorship contribution statement

Oihana Latchere: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Isabelle Métais:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Hanane Perrein-Ettajani:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Magalie Lemoing:** Formal analysis. **Agnès Feurtet-Mazel:** Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Patrice Gonzalez:** Methodology, Investigation. **Guillemine Daffe:** Methodology, Investigation. **Julien Gigault:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Charlotte Catrouillet:** Writing – original draft, Formal analysis. **Amélie Châtel:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Magalie Baudrimont:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Amelie Chatel reports financial support was provided by National Agency for Food Environmental and Occupational Health and Safety. 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.

Acknowledgement and funding

This work was supported by the project TROPHIPLAST, funded by the ANSES 558 (French Agency for Food, Environmental and Occupational Health & Safety, grant agreement 2018/1/017). Through the support of the GeOHeLiS analytical platform of Rennes University, this publication is also supported by the European Union through the European Regional Development Fund (FEDER), the French Ministry of Higher Education and Research, the French Region of Brittany and Rennes Metropole. The authors are grateful to M. Bouhnik Le Coz and M. Pattier for their assistance in the ICP-MS measurements. We also thank Mélanie Bruneau for technical support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2024.107160.

Data availability

Data will be made available on request.

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