Effects of tire particles and associated-chemicals on the Pacific oyster (Magallana gigas) physiology, reproduction and next-generation

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Effects of tire particles and associated-chemicals on the Pacific oyster (*Magallana gigas*) physiology, reproduction and next-generation

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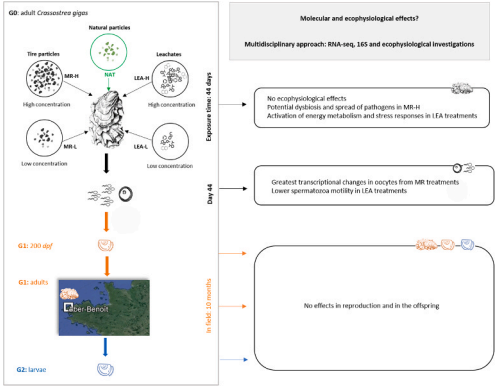
HIGHLIGHTS

- Leachate led to energy metabolism alteration and stress response.
- LEA and MR led to minor microbiota changes but spread of *Tenacibaculum spp*.
- Low concentrations of tire particles had no effects on adult oyster ecophysiology.
- Parental exposure to leachate and tire particles impacted gamete quality.
- Parental exposure did not lead to long-term effects on offspring.

GRAPHICAL ABSTRACT



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ABSTRACT

By 2040, tire particles (TP) are expected to dominate marine plastic contamination, raising concerns about their effects on marine animals. This study employed a multidisciplinary and multigenerational approach on the

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Microplastics
Leachates
Impacts
Toxicity
Offspring

Pacific oyster *Magallana gigas* to investigate the effects of TP and their leachates (LEA). Effects were analyzed at the individual scale, from cellular, molecular, and microbiota changes to reproductive outputs and offspring performance. Microbiota characterization revealed potential dysbiosis in oysters treated with high concentration of both TP and LEA. RNA-seq analyses highlighted the activation of energy metabolism and stress responses in the LEA treatment. Additionally, transcriptional changes in oocytes and the reduction of motile spermatozoa suggested potential effects on gamete quality. Notably, possible oyster resilience was pointed out by the lack of significant ecophysiological modifications in adults and impacts on the growth and reproductive outputs of the offspring. Overall, the implications of the observed oyster resilience under our experimental setting are discussed in relation to available toxicity data and within a comprehensive view of coastal ecosystems, where a higher diversity of plastic/rubber materials and harsher environmental conditions occur.

1. Introduction

The release of tire particles (TP) mainly originates from the abrasion of tires by the intense and widespread on-road traffic [1]. Global average emissions of TP were estimated of 0.81 kg year^{-1} per capita, i.e. 6.1 million tons [2]. Because of this massive spreading in our environment [3-6], TP represent one of the major contributors to microplastics (< 5 mm) pollution (e.g. [7-9]). Lau et al. [1] estimated that, in the worst scenario modelling, TP might largely increase their dominant role in the mass contribution of the aquatic microplastic contamination by 2040 (composing up to the 93 %) mainly because of the inefficiency of management technologies used for other microplastics' sources. Due to their small sizes, TP can be unintentionally ingested by a wide range of organisms [10-12].

While the literature shows contrasting biological effects of TP (reviewed in Rødland [13]), the greatest hazard of tires seems to be caused by the associated chemicals incorporated to improve tire properties (e.g. reviewed by Wik and Dave [14]). Tires-associated chemicals may represent up to the 50 % of the relative mass proportion compared to an average of 7 % in conventional plastics [15]. Aqueous leaching of 250 mg TP L^{-1} releases no less than 2000 chemical signatures [16], including potentially hazardous compounds such as sulfur, polycyclic aromatic hydrocarbons (PAHs), carbon black, trace metals, antioxidants, curative and protecting agents and their transformation products (e.g. [17-19,8,15]). More than a decade of environmental investigations has demonstrated that the release of a ubiquitous tire rubber-derived chemical (6-PPD-quinone) in roadway runoff stormwater induces mortality events in coho salmon in urban creeks of the US West Coast [16]. Laboratory-based studies have also demonstrated that tire leachates may induce detrimental effects on cellular activity [18,20], ecophysiological functions (feeding, respiration; [21]) and embryo-larval traits of marine organisms [22-24]. To date, most of the experiments performed were based on short-term experimental exposures while long-term chronic effects of low concentrations of TP and associated leachates are of concern in particular for the role of endocrine disruptor that tire chemicals could play.

In the present study, a multidisciplinary and multigenerational approach was implemented embracing over two generations of the Pacific oyster *Magallana gigas*, a marine bivalve mollusk of high economic value and ecological importance for the ecosystem functioning (e.g. regulation of water turbidity, creation of habitats, stabilizing the shoreline; [25]). As a species that lives in coastal and intertidal areas in numerous countries throughout the world, it is affected by marine plastic pollution, which includes TP from air deposition, wastewater effluents, and road surface runoff. The present study aims to test the hypothesis that exposure to microplastics, specifically TP and their associated chemicals, leads to long-term effects on the Pacific oyster (*M. gigas*). The study focuses on evaluating the impacts across multiple generations, examining both direct effects on the exposed generation (G0) and potential cross-generational (G1) and multi-generational (G2) effects. In detail, the study aims to: *i)* assess effects of tire particles, tire leachates and natural particles (diatomite as a particle control) at individual scale (generation G0) over a 6 weeks exposure during active gametogenesis by measuring transcriptomic profiles, gut microbiota, tissue alterations, feeding activity, growth, and reproductive outputs; *ii)* monitor cross-generational effects (*sensu* [26]) on the G1 offspring performance (development, survival, growth and reproduction of G1 larvae and juveniles) over one year; and *iii)* test multi-generational effects by looking at performances (i.e. development and survival) of the G2 larval progenies.

2. Materials and methods

2.1. Tire particles, leachates and natural particles

Fine rubber powder composed of irregular tire particles was purchased from Edge Rubber (Chambersburg, USA), which is obtained from the ambient grinding of passenger and truck tires. Laser granulometry (Beckman Coulter® LS 130) analyses performed on three subsamples of the stock powder in UV-treated 1-µm filtered seawater gave a size range of 176.4 ± 67.7 µm (min = 1.5 µm, max = 715 µm based on calculations from 0.375 µm to 2000 µm). Diatomite (mean size = 289.2 ± 677.7 µm, min = 0.01 µm, max = 3500 µm) was also used for an experimental group to provide a particulate control in addition to the particle-free control because of its natural origin, widespread presence in the ocean and size/form similarity to the selected TP [27].

Aqueous leachates stock solution was prepared at a concentration of 5.2 g TP L^{-1} in 1 µm-filtered seawater using the protocol described in Capolupo et al. [18] and detailed in Supplementary File S1. Previous chemical analyses performed using TP leachates 20 times more concentrated were not conclusive; metals, polychlorinated biphenyls (PCBs) and PAHs levels being below the detection and/or quantification limits [24]. Therefore, chemical analyses targeted in this study were only performed on the raw material (tire powder stock) through a multi-residue method to determine PAHs and PCBs by gas chromatography coupled with tandem mass spectrometry (GC-MSMS, Agilent 7890 GC system linked to an Agilent 7010 triple quadrupole MS). Identification of chemicals was performed by MRM (Multiple Reaction Monitoring) spectral mode that allows the selective and sensitive quantification of compounds in complex matrices. Detailed methodologies are reported in Supplementary File S1.

2.2. Experimental design

Acclimated adult oysters ("G0") were placed in air bubbling and pressurized water inflowed (sensu [28]) 50-L experimental PMMA tanks (40 oysters tank^{-1}) supplied continuously with UV-treated 1-µm filtered seawater (12 L h^{-1}; 17.7 ± 0.2 °C; 34.5 ± 0.4 PSU; pH 8.2 ± 0.1) containing a balanced mixture (50/50, v/v) of two microalgae (*Tisochrysis lutea* and *Chaetoceros* sp) at a daily ratio equal to 8 % dry weight algae/dry weight oyster. The algal concentration was measured then adjusted every morning using an electronic particle counter (Multisizer 3; Beckman Coulter, USA). This conditioning period lasted 44 days to ensure complete gametogenesis. Photoperiod was 12 h light:12 h dark. More details of oyster origin and acclimation set up are reported in Supplementary File S1.

Throughout the entire 44 days conditioning period, a total of six treatments were applied (3 replicate tanks per treatment): (1) Control

(no particle exposure; CTL); (2) Exposure to TP at low concentration of microplastics in ocean (10 TP mL^{-1}; 5.2 μg L^{-1}, namely MR-L micro-rubber low); (3) Exposure to TP at high concentration (100 TP mL^{-1}; 52 μg L^{-1}, namely MR-H micro-rubber High); (4) Exposure to leachate corresponding to a low concentration of TP (5.2 μg L^{-1}; LEA-L); (5) Exposure to leachate corresponding to the high concentration of TP (52 μg L^{-1}; LEA-H); (6) Exposure to natural particles (diatomite; 52 μg L^{-1}; NAT). Useful and more complete information on TP and our choices are reported in Supplementary File S1.

Stock solution of TP, leachates and natural particles in sterile 0.2-μm filtered seawater were administered using peristaltic pumps from concentrated TP, leachates or natural particle solutions in order to conduct chronic and continuous exposures. Tween-20 was distributed in all tanks to avoid particles aggregation and confounding effects. Further information is provided in Supplementary File S1. Every 48 h, tanks were emptied and cleaned; the first wash water was put into the effluent treatment system.

At the end of the exposure (44 days of treatment), oocytes and spermatozoa were collected from mature G0 adults in each condition to assess gametes quality and fertilization success, as well as next generation (generation 1, "G1") embryo-larval development and settlement as described below. Three months-old G1-oyster spats were then placed in a local oyster farm and monitored (growth, survival) for 10 months (see details below in Section 2.5), before being retrieved on month 13 and used for assessing gametes quality and fertilization success through a second controlled reproduction to produce G2-larvae (employing the same procedure used to produce G1-larvae; details below). The whole experimental plan is summarized in Fig. S1 (Supplementary File S1). Analyses performed on G0, G1 and G2 oysters are detailed in the sections below.

2.3. Biometrics, ecophysiological, and cellular parameters in G0 adult oysters

Adult oyster growth (length and dry weight), survival and ecophysiological parameters (algal consumption, absorption efficiency) were monitored during the time-course of the exposure (44 days). Algal counts measured every morning allowed to estimate the algal consumption of G0 oysters (AC; μm^3 day^{-1} $oyster^{-1}$) and 24-h faeces were collected from each tank alongside with a sub-sample of the diet (500 mL) at 12-, 23-, 32- and 40-days of exposure to estimate the absorption efficiency (AE; %) of organic matter from the diet using the Conover's method [29]. Details of ecophysiological parameters methods are reported in Tallec et al. [21] and in Supplementary Information 1.

At the end of the exposure, hemolymph from adult oysters (n = 5 for CTL, NAT, and MR-H; n = 6 for the other conditions) was extracted from the posterior adductor muscle using a hypodermic syringe (23-gauge needle). Hemocyte viability measured using both SYBR Green I (Molecular Probes) and propidium iodide (PI, Sigma), reactive oxygen species (ROS) production through 2',7'-dichlorofluorescein diacetate DCFH-DA (Sigma) and activity of the MXR pumps assessed by staining hemocytes with calcein-AM (Sigma-Aldrich; 0.25 μM) were analyzed using flow cytometry (BD FACSverse flow cytometer, BD Biosciences, France) as described in Détrée et al. [30] and Marques-Santos et al. [31], respectively. Ten oysters were used for histology to evaluate sex ratio, gonadic development and tissue alteration following Fabioux et al. [32] and Steele and Mulcahy [33].

2.4. Molecular analyses – transcriptomics and microbiome in G0 adult oysters

Gills and digestive gland were collected from 6 individuals at the end of the acclimation period just before the start of the exposure (T0) and then after 44 days of exposure (T44; 6 oysters/condition) and stored in RNA later at − 80 °C until the total RNA extraction performed using RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA from each sample of both tissues was addressed both to gene expression profiling (RNA-sequencing) and for microbiota characterization (16S rRNA Amplicon sequencing). In parallel, 20,000 oocytes collected at T44 from 4 pools of 3 mature females per condition were homogenized in 1.5 mL of Extract-all (Eurobio) and stored at − 80 °C for further transcriptomic analyses. Oocytes' total RNA was extracted by Extract-All kit (Eurobio Scientific; Les Ulis, France) and addressed to RNA-sequencing for transcriptomic analysis. Furthermore, seawater samples (250 mL, 3 samples per condition collected at T44) were first filtered to 20 μm (discarded) and then through a 1 μm pore size 47 mm diameter polycarbonate membrane (Whatman, Inc., Buckinghamshire, United Kingdom) using a vacuum pump. Half of the membrane was used for RNA extraction using RNeasy PowerWater Kit (Qiagen) for microbiota characterization (16S).

Detailed methods for gene expression profiling (RNA-sequencing) and for microbiota characterization (16S rRNA Amplicon sequencing) are provided in Supplementary File S1. Sequences are available in NCBI SRA; https://www.ncbi.nlm.nih.gov/sra; BioProject PRJNA856813.

2.5. Gamete quality (G0, G1)

The quality of spermatozoa and oocyte of G0 and G1 oysters were assessed by flow cytometry after the separated incubation in SW for 45 min. Oocyte diameter was estimated in micrographs from optical microscopy using the ImageJ software, and spermatozoa behavior (percentage of motile spermatozoa and Velocity of the Average Path (VAP; $\mu m\ s^{-1}$)) was measured by video analyses using the Computer-Assisted Sperm Analyzer (CASA) plug-in developed in ImageJ [34] as described in Tallec et al. [35].

Additionally, mitochondrial function was assessed on G1 oocytes following the analysis of ATP levels and mitochondrial content through the luminescence measuring by using a PerkinElmer EnSpire plate reader. More details of gamete quality analyses are reported in Supplementary File S1.

2.6. Fertilization, embryo-larval development (G1, G2), larval rearing and larval behavior (G1)

Gametes obtained from G0 and G1 oysters were pooled in 2 L of SW (21 °C) with a spermatozoa-to-oocyte ratio of 100:1 (avoiding polyspermy) and a final concentration of 1000 oocytes mL^{-1}. After 1 h30 of contact, the fertilization success (F; %) was estimated as: F = (number of fertilized oocytes ÷ number of oocytes) × 100. Then, 500,000 embryos (2-cells or 4-cells stage) per replicates were collected and placed in 5 L of FSW (21 °C), i.e., 100 embryos mL^{-1}. After 36 h of incubation, all beakers were sieved at 40 μm to measure the D-Larval yield (%; (number of normal d-Larvae ÷ number of larvae) × 100) in G1 and G2 embryos. Abnormal D-larvae referred to larvae displaying morphological malformations (hinge, shell, mantle) or embryos exhibiting developmental arrests [36].

At the end of the G1 embryo-larval development, 90 G1 D-larvae per replicate were placed in 96-well plates filled with SW (21 °C). Videos were acquired under a microscope (Keyence VHX6000, Japan; × 40) for 1 min at 30 frames s^{-1} then analyzed using the tracking software Ethovision XT 13.0 (Noldus, The Netherlands) to estimate the percent of active larvae (Activity; %), the activity duration (seconds), the distance traveled (mm) and the velocity (mm sec^{-1}). At 15 days post fertilization (dpf), eyed-larvae were sampled to compare the settlement behavior among treatments following the procedure detailed in Supplementary File S1.

For the first generation G1, 100,000 normal G1 D-larvae per replicate were placed in 5-L cylinder (4 per condition) in a flow-through rearing system [37]. Larvae were continuously supplied with SW (24 °C) containing a mixed diet of *T. lutea/C. neogracile* (50/50, v/v) at a mean concentration of 1500 μm^3 mL^{-1}. At 2-, 7-, 10-, 12- and 15-dpf, 20–30 larvae were sampled from each replicate and fixed in a formaldehyde-seawater solution (0.1 % final) then pictured under a

microscope to estimate the larval growth rate (μm day^{-1}) using the ImageJ software. Larvae were considered as "ready to settle" when more than 50 % of larvae in each replicate from a treatment reached 50 % of eye d-larvae (i.e. the metamorphosis competent stage), indicating the end of the pelagic phase. For G1 larvae, the larval rearing was sieved on 80-mm mesh and 20,000 larvae were transferred to 30-L tanks at 25 °C to settle on cultch using a down welling system (n = 3 batches per treatment) as described in Petton et al. [38]. After 3 months of G1 larvae conditioning, a subsample of them were transferred in a local oyster farm as described in the following Section 2.7.

For G2 D-larvae, the mitochondrial metabolism was assessed through the analysis of the ATP levels described above for oocytes and through the evaluation of mitochondrial contents. Mitochondrial content was determined as the fluorescence values normalized to the control group. Details are in Supplementary File S1.

2.7. Monitoring for growth, survival and reproduction of the offspring (G1, G2)

300 G1 oyster seeds (200-days-old) per condition were deployed on an oyster farming site in the Aber Benoit (Latitude: 48.575244 | Longitude: − 4.60758) in November 2021, where they were reared in onshore oyster farm facilities for 10 months (3 oyster bags per condition, 100 oysters per bag). Survival (counting all the live oysters and removing the dead ones), individual growth (total weight, drained meat weight, shell weight, shell length) and reproductive status (see Histology sub-section in Supplementary File S1) were monitored by sampling 30 oysters per treatment at the final sampling in September 2022. The day after, gametes of G1 oysters (3 males and 3 females per oyster's bag) were collected for gamete quality measurements, fertilization and embryo-larval development up to 48 h D-larvae. The procedure to obtain gametes and cross them was identical for G0 and G1 generations and is described in Sections 2.6, 2.7 and Supplementary File S1.

2.8. Statistical analysis and graphical representation

Statistical analyses and graphical representations were conducted using either Statgraphics or the R software [39]. Percentages were analyzed after angular transformations. Normality and variance homogeneity were screened using the Shapiro and Levene tests, respectively. Data from algal consumption were analyzed using repeated measures ANOVA. One-way ANOVA were used to analyze effects of treatments on the growth, the absorption efficiency, the gamete quality, larval development, and ATP content. Post-hoc Tukey HSD test was employed for multiple comparisons. Comparisons of sex distributions between conditions were made using Fisher exact test. Differences were considered significant when p-values < 0.05. Data is reported as mean ± standard deviation.

3. Results

3.1. Chemical analyses on the raw material

Targeted chemical analyses detected 27 compounds (16 PAHs, 11 PCBs). Of these, 7 were below limits of quantification (Table S1 in Supplementary File S1). Pyrene, phenanthrene, fluoranthene, benzo(ghi) perylene, and chrysene had the highest concentrations ranging between 3175 and 13,865 μg kg-1 for chrysene and pyrene, respectively. In addition, the SCAN-mode detected six additional compounds: 1,2-Dihydro-2,2,4-trimethylquinoline (antioxidant), dicyclohexylamine (vulcanization), 2-(tert-Butyl)-1H-indole (indole), 2-phenylbenzothiazole (vulcanization), 10,18-Bisnorabieta-8,11,13-triene (antioxidant), N(1,3-dimethyl-butyl)-N'-phenyl-p-phenylenediamine (6-PPD; antioxidant).

3.2. Ecophysiological parameters, growth monitoring and hemocytes features (G0 adult oysters)

The 44-days exposure did not result in significant differences (*p*-values > 0.05) among all treatment regarding the total hemocyte count (mean THC value $= 1.54 \times 10^5 \pm 6.74 \times 10^4$ cell mL^{-1}), the proportion of live hemocytes (mean value = 93.2 ± 5.2 %), their ROS production (mean value = 194.5 ± 121.3 A.U.) and the activity of the hemocyte MXR transmembrane pumps (mean value = 4538.8 ± 2575.5 A.U.) (Fig. S2; Supplementary File S1).

3.3. Gene expression analysis (G0 adult oysters)

Principal Component Analysis, performed separately for digestive gland, gills and oocytes are reported in Fig. 2. After discarding the unwanted variance based on RUVs function, gene expression profiles of the digestive gland (Fig. 2a) indicated a slight separation of CTL and NAT treatments from other groups along the first (6.57 %) and second (4.98 %) components of variation. In gills (Fig. 2b), a slight separation of oysters exposed to LEA-H and to MR-H from other treatments was observed along the x-axis (5.95 %). These low values for variance suggest quite low to moderate biological differences in both tissues among treatments. A clearer distinction, but still based on low variance, among treatments was found for oocytes (10.44 % and 8.92 % variation, respectively).

Differentially expressed genes (DEGs) identified through pairwise comparisons between each treatment and the control group (CTL) are listed in Supplementary File 2. Both gills and digestive gland tissues showed a lower amount of DEGs in response to MR and LEA (from 2 to 26 DEGs) compared to oocytes for which pointed out more than 400 DEGs in all treatments in comparison to the CTL group. The number of DEGs in each tissue/treatment are reported in Supplementary File S1-Table S3.

In digestive gland, the highest number of DEGs and significant pathways observed in the Gene Set Enrichment Analysis (GSEA) were found following MR-H and LEA-H exposures, while no significant DEGs were found following NAT exposure compared to the CTL. In detail, GSEA (summarized in Table 1 and reported in Supplementary File 3) showed the activation of detoxification process as point out by the common up-regulation of "xenobiotic metabolism" following exposures to MR-H and both LEA exposures, the up-regulation of *cytochrome p450* (CYP450) in LEA-H and by the KEGG pathway "drug metabolism cytochrome p450" in MR-H exposed oysters. While the up-regulation of "oxidative phosphorylation" and "fatty acid metabolism" were observed in all treatments including NAT, disruption of energy metabolism was mainly observed in oysters exposed to LEA-H, with the up-regulation of "glycolysis" (also in LEA-L), "citrate cycle" (also in MR-H) and "energy derivation by oxidation of organic compounds". Alterations of stress and signaling responses were specifically detected in LEA-H oysters, showing the up-regulation of pathways involved in "apoptosis", "protein secretion", "reactive oxygen species pathway", "mTORC1 signaling", "TNF signaling via NFKB" and "NOTCH signaling pathway". Exposure to leachate led also to changes in immune pathways, such as "complement", "interferon response" and "defense response to virus". Specific transcriptional changes were also observed following MR-H exposures, representing the unique treatment showing the up-regulation of several pathways involved in neurotransmission/synapses ("excitatory synapse", "GABAergic synapse", "glutamatergic synapse", "regulation of neurotransmitter levels") and of the KEGG "pathways in cancer".

In gills, the highest number of DEGs and molecular pathways were observed following LEA-H exposures, showing the down-regulation of three pathways involved in synapses, the up-regulation of pathways involved in immune response ("interferon response" and "defense to virus"), energy metabolism ("oxidative phosphorylation"), "UV response", and in signaling ("Notch signaling pathway" and "WNT signaling pathway"). Both MR and LEA exposed oysters showed also

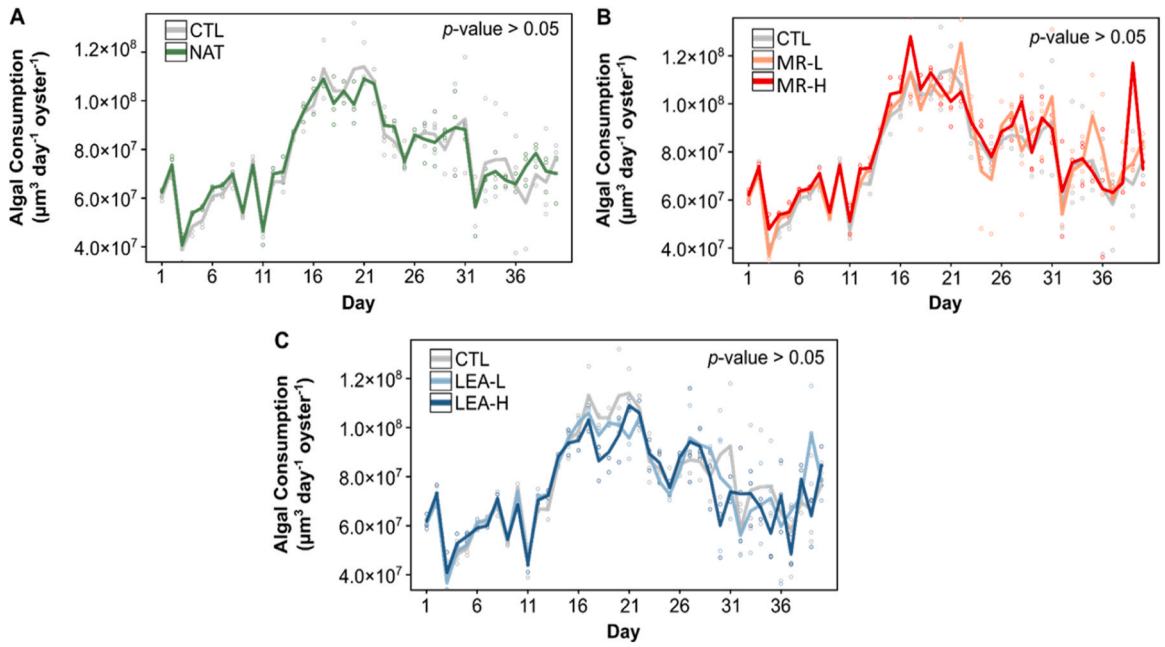

Fig. 1. Algal consumption (μm^3 day^{-1} $oyster^{-1}$) in adult oysters. (A) Adult oysters from the control treatment (grey line – CTL) vs. the natural treatment (green line – NAT); (B) Adult oysters from the control treatment (grey line – CTL) vs. the MR (micro-rubber) treatments (High: Red – MR-H; Low: Light red – MR-L); (C) Adult oysters from the control treatment (grey line – CTL) vs. the leachate treatments (High concentration: Blue – LEA-H; Low Concentration: Light blue – LEA-L). Repeated measures ANOVA were conducted to compare treatments at the 5 % level; n = 3 per treatment.

transcriptional changes in pathways involved in cell cycle regulation and proliferation. Among them, the down-regulation of "G2M checkpoint", "mitotic spindle" (in MR-H), "E2F targets" (MR-H and LEA-L), both "MYC targets" pathways (LEA-L) and "pathways in cancer" (LEA-H). Up-regulation of "drug metabolism cytochrome p450" was also found following LEA and MR-H treatments.

In oocytes, almost all significant pathways were down-regulated in exposed oysters, with major effects detected in oocytes of MR-L exposed oysters. Among them, "Notch signaling pathway" (MR-L, MR-H, LEA-L), "regulation of reproductive process" (MR-L, LEA-L), "apoptosis", "pathways in cancer" and "TNF signaling via NFKB" (MR-L). Major effects in MR-L treatment were also suggested by the up-regulation of "PI3K/AKT/mTOR pathway" and by the down-regulation of several pathways involved in immune response and inflammation, including "immune system process", "immune response", "NOD-like receptor signaling" and "inflammatory response". Concerning the immune response, down-regulation of "response to bacterium" and "interferon response" were found in LEA-H and MR-H, respectively. Noteworthy, in oocytes 34 genes were commonly down-regulated in NAT and both MR treatments including *Lysozyme 1,* while *Neuropeptide Y receptor (NPYr)* was down-regulated in both NAT and MR-H oocytes.

3.4. Microbiota analyses in gills and digestive glands (G0 adult oysters)

PCoA did not show a clear separation between treatments in both tissues, except for a weak separation in gills of LEA-H exposed oysters (Fig. S3-B; Supplementary File S1), similar to the results obtained for gene expression profiles. Furthermore, no significant changes in microbial diversity (i.e. Chao1, Shannon's and Simpson's Index) between treatments and the control group were observed (Fig. S4, Supplementary File S1). Few changes in microbiota composition following exposures to NAT, MR and LEA were confirmed by pairwise comparisons with CTL group, highlighting a small amount of differently represented taxa in both tissues (Table S4, Supplementary File S1). The full lists of significant taxa for each comparison are reported in Supplementary file 4. Noteworthy, the genus *Tenacibaculum* was over-represented in oyster gills (log_2FC = 8.7), digestive gland (log_2FC = 23.8) and in filtered seawater of the tank of the MR-H treatment (log_2FC = 24.6). This genus was also detected in digestive gland and gills of MR-L and LEA-H experimental groups, respectively.

3.5. Reproductive outputs (G0 adult oysters) and G1 larval growth and behavior

Measurements performed on gametes from exposed-adults showed that exposures to leachates (LEA-L and LEA-H) reduced significantly the percentage of motile spermatozoa in G0 oysters compared to the control treatment (i.e. mean reduction of 31 % in both leachates conditions compared to control; p-value < 0.05; Table 2); other treatments had no effect (p-value > 0.05). Other proxies of gametes quality (i.e. VAP and oocyte diameter) were statistically similar among all treatments (p-value > 0.05; Table 2). The D-larval yield (average value = 82.1 ± 8.9 %) was not affected in comparison to the control treatment (p-values > 0.05; Table 2).

No effects of the experimental treatments were detected on the G1 larval development (p-value > 0.05; Fig. 3). The growth rates varied from 19.9 ± 1.0 µm day^{-1} to 21.6 ± 0.7 µm day^{-1} for LEA-H and MR-H, respectively. D-Larvae (2 dpf) displayed similar activity, active duration, distance traveled, and velocity among all treatment (p-values > 0.05; Table S5 Supplementary File S1). The level of exploring larvae at 15 dpf was similar among treatments, regardless of the tested chemical cues with average values of 39.6 ± 3.5 %, 71.3 ± 7.4 % and 64.4 ± 5.8 %, in the "Without fragrance", "Predator fragrance", and "Oyster fragrance" conditions, respectively (p-value > 0.05, Table S6 in Supplementary File S1). Overall, eyed-larvae exhibited a higher exploring behavior in the presence of chemicals cues (predator and oyster fragrances) compared to the condition "without fragrance" (+ 71 %; p-value < 0.001).

3.6. Long term effects on G1 and G2 generations

Growth (i.e. oyster length and weight) of G1 offspring in an oyster farming area appeared similar among treatments (Table S7 in Supplementary File S1). Survival ranged from 36 ± 10 % for MR-L to 20

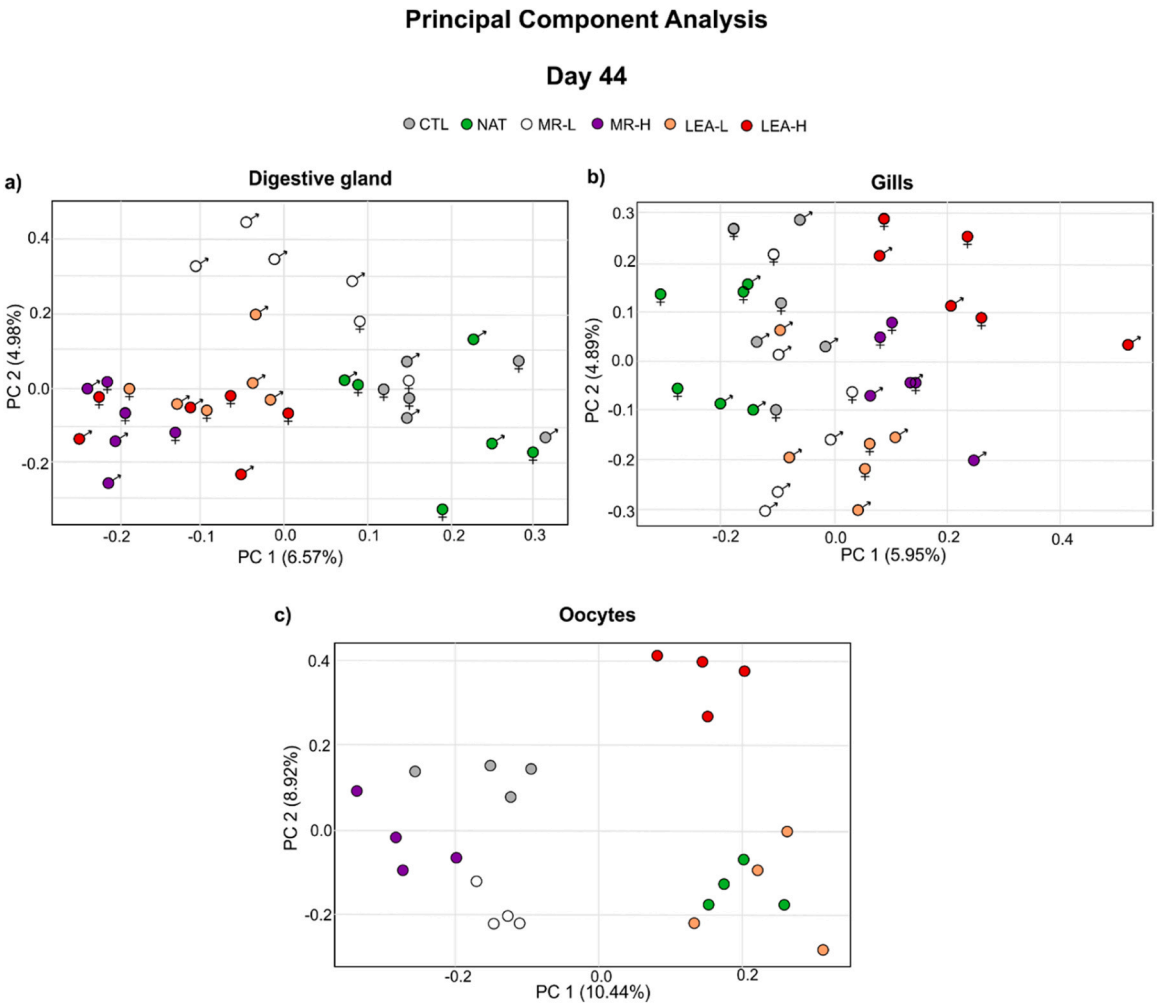

Fig. 2. Principal component analysis on the whole digestive gland, gills and oocytes RNAseq dataset after normalization using the RUVs function. Different colors indicate control treatment (grey dot – CTL), natural treatment (green dot – NAT); MR high concentration purple - MR-H); MR low Concentration (white – MR-L); leachate high concentration (Red – LEA-H); Leachate low Concentration: orange – LEA-L). For digestive gland and gills, male and female oysters are also indicated with ♂ and ♀, respectively.

± 16 % for LEA-H, with no significant differences among treatments. For gametogenesis, all the oysters were in stage 3 at the final sampling. The sex-ratio (male/female) varied from 7/86 in NAT to 53/47 in MR-H (Table S8 in Supplementary File S1). The proportion of females was high in most of the conditions (5 out of 6 conditions) and only MR-H had a high number of males, which is only significantly different from the NAT condition ($p < 0.05$; Table S9 in Supplementary File S1). Mean gonadic tissue surface percentages varied from 50.6 ± 16.0 to 58.4 ± 9.8 % for the conditions control and LEA-L, respectively, with no significant differences among treatment (Table S7 in Supplementary File S1). No effects was observed on G1 spermatozoa motility and velocity of the Average Path (VAP) (Table S10 in Supplementary File S1). For G1 oocytes, two shapes were recorded, pear-shaped (circularity < 0.8) and circular (circularity > 0.8) and their respective proportions significantly differed between conditions ($\chi^2 = 171$, $p = 0$, $df = 5$) with pear-shaped oocytes ranging from 64.8 (LEA-L), 81.7 (NAT), 84.7 (MR-L), 87.2 (MR-H), 89.5 (CTL) to 90.1 % (LEA-H). The fertilization rate in the NAT condition (86.2 ± 3.0 %) was found significantly lower compared to all other conditions (Kruskal-Wallis test = 13.02; p-value = 0.023, Table S10 in Supplementary File S1). Finally, ATP energy measurements showed no difference among treatments for G1 oocytes with mean ATP value ranging from 316 ± 11 (MR-L) to 446 ± 116 fmol/oocyte (LEA- L) (Table S11 in Supplementary File S1). The G2 D-larval yield, ranging from 65.2 ± 3.0 % to 80.9 ± 15.6 % for the NAT and LEA-H conditions, respectively, was similar among groups (except for NAT; Table S10 in Supplementary File S1). No difference in D-larvae size was observed at 48 h among conditions (Table S10). As observed for G1 oocytes, ATP energy measurements showed no difference among treatments for G2 D-larvae with mean values ranging from 22 ± 14 (LEA-H) to 43 ± 8 fmol/larvae (MR-H) (Table S11 in Supplementary File S1). The mitochondrial content remained similar in the G2 D-larvae regardless of the treatment (MR-L: 0.85 ± 0.38, LEA-H: *0.93 ± 0.51, LEA-L: 0.99 ± 0.72, CTL: 1.0 ± 0.46, MR-H: 1.59 ± 0.91, NAT: 2.04 + 0.57; data represented as relative fluorescence values compared to the CTL group).*

4. Discussion

4.1. Molecular analyses reveal the alteration of energy metabolism and stress response following leachate exposure

Gene expression analyses of oyster's digestive gland revealed oxidative stress, increased energy metabolism as well as the modification of several signaling pathways following exposure to tire leachate. Alterations of molecular mechanisms were already observed in bivalves (e.g. Pacific oyster, [28]; pearl oyster, [40]; blue mussel, [41]; reviewed in Khanjani et al. [42]) after exposure to plastic particles under laboratory conditions with effects that lead to profound changes in molecular functioning within cells. For instance, Gardon et al. [40] reported

Table 1
Summary of significant pathways obtained through GSEA for digestive gland, gills and oocytes. Green and red colors indicated down- or up-regulation, respectively, for each significant pathway.

		DIGESTIVE GLAND					GILLS					OOCYTES				
		Digestive gland					Gills					Oocytes				
Process	Pathways	NAT	LEA-L	LEA-H	MR-L	MR-H	NAT	LEA-L	LEA-H	MR-L	MR-H	NAT	LEA-L	LEA-H	MR-L	MR-H
ENERGY METABOLISM/METABOLIC PROCESSES	HALLMARK_OXIDATIVE_PHOSPHORYLATION	x	x	x	x	x			x							
	HALLMARK_FATTY_ACID_METABOLISM	x	x	x	x	x										
	HALLMARK_GLYCOLYSIS		x	x												
	GO_ENERGY_DERIVATION_BY_OXIDATION_OF_ORGANIC_COMPOUNDS			x												
	KEGG_CITRATE_CYCLE_TCA_CYCLE			x		x										
CELLULAR COMPONENT	HALLMARK_PEROXISOME	x	x		x	x										
	HALLMARK_APICAL_JUNCTION	x			x											
	HALLMARK_APICAL_SURFACE	x	x		x						x					
STRESS RESPONSE	HALLMARK_XENOBIOTIC_METABOLISM		x	x		x						x				
	KEGG_DRUG_METABOLISM_CYTOCHROME_P450					x		x	x		x					
	HALLMARK_DNA_REPAIR							x			x					
	HALLMARK_UV_RESPONSE_DN							x	x							
	HALLMARK_PROTEIN_SECRETION			x												
	HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY			x												
IMMUNE RESPONSE	HALLMARK_COMPLEMENT		x									x				
	HALLMARK_INTERFERON_RESPONSE		x						x							x
	IMMUNE SYSTEM PROCESS													x	x	
	KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY														x	
	IMMUNE RESPONSE													x	x	
	GO_INNATE_IMMUNE_RESPONSE					x										
	RESPONSE_TO_BACTERIUM													x		
	HALLMARK_INFLAMMATORY_RESPONSE														x	
	GO_DEFENSE_RESPONSE_TO_VIRUS		x						x							
NEUROTRANSMISSION/SYNAPSE	GO_EXCITATORY_SYNAPSE					x			x							
	GO_GABA_ERGIC_SYNAPSE					x			x					x		
	GO_GLUTAMATERGIC_SYNAPSE					x			x							
	GO_REGULATION_OF_NEUROTRANSMITTER_LEVELS					x										
REPRODUCTION/DEVELOPMENT	GO_REGULATION_OF_REPRODUCTIVE_PROCESS	x									x		x		x	
	GO_CANONICAL_WNT_SIGNALING_PATHWAY				x				x							
	KEGG_NOTCH_SIGNALING_PATHWAY			x					x				x		x	x
	HALLMARK_MYOGENESIS					x										
	HALLMARK_ADIPOGENESIS	x	x		x	x										
CELL CYCLE/PROLIFERATION	HALLMARK_APOPTOSIS			x											x	
	KEGG_PATHWAYS_IN_CANCER					x			x						x	
	HALLMARK_G2M_CHECKPOINT										x					
	HALLMARK_MITOTIC_SPINDLE										x					
	HALLMARK_MYC_TARGETS_V2							x								
	HALLMARK_E2F_TARGETS							x			x					
	HALLMARK_MYC_TARGETS_V1							x								
	HALLMARK_MTORC1_SIGNALING			x												
	HALLMARK_PI3K_AKT_MTOR_SIGNALING														x	
	HALLMARK_TNFA_SIGNALING_VIA_NFKB			x											x	

Table 2
Percentage of G0 motile spermatozoa (%), sperm velocity (VAP; μm s^{-1}), oocyte diameter (μm), and fertilization yield (%), D-larval yield (%) of obtained G1-larvae after 44 days of parental exposure to: (1) CTL; (2) NAT; (3) MR-L; (4) MR-H; (5) LEA-L; (6) LEA-H. One-way ANOVA was conducted to compare treatments at the 5 % level with Tukey-HSD when necessary; * = p-value < 0.05; n = 3.

Treatment	Sperm motility (%)	VAP (μm s^{-1})	Oocyte diameter (μm)	Fertilization yield (%)	D-larval yield (%)
CTL	55.4 ± 7.7	91.6 ± 15.3	30.9 ± 0.7	87.8 ± 1.3	91.3 ± 3.9
NAT	48.7 ± 10.6	82.5 ± 10.0	30.2 ± 0.6	82.5 ± 6.8	65.5 ± 38.9
MR-L	47.9 ± 9.3	77.8 ± 9.6	31.4 ± 0.6	90.5 ± 5.9	81.0 ± 17.8
MR-H	43.1 ± 8.9	77.5 ± 6.4	30.5 ± 0.6	87.5 ± 9.2	87.0 ± 5.3
LEA-L	37.4 ± 5.8 *	78.4 ± 4.3	30.7 ± 0.3	82.8 ± 6.6	85.3 ± 7.5
LEA-H	38.8 ± 5.3 *	72.8 ± 6.1	30.6 ± 0.2	83.8 ± 4.4	82.7 ± 14.6

dose-specific transcriptomic responses of pearl oysters in response to polystyrene microspheres exposure alongside with alteration of the oysters' energetic capacities that demonstrated a significant reduction of the energetic budget. Specifically, the expression of genes involved in "transport and metabolism of carbohydrates" was found to be higher in pearl oysters exposed to micro-nanoplastics from pearl farming gears compared with unexposed oysters, seen in parallel with an early depletion of glycogen, consistent with its role as a rapidly mobilizable metabolic fuel to meet the energy demands of bivalves [43].

In addition to an impaired energy metabolism [44], microplastics were reported to affect DNA integrity, oxidative stress and antioxidants enzymes [40,42,45,46]. Considering that oxidative stress is a key mechanism for metal and PAH toxicity in aquatic organisms [47,48], the up-regulation of "reactive oxygen species pathway" in LEA-H exposed oysters could be explained by leachate's additives, as already proposed by Shin et al. [44]. The increased oxidative stress may lead to excessive energy consumption required to maintain cellular homeostasis, as suggested by the up-regulation of several functional categories involved in

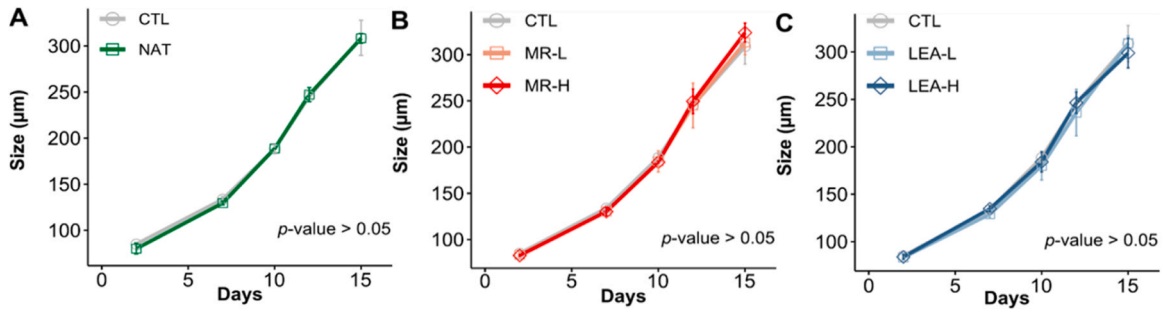

Fig. 3. Size of larvae (G1) issued from parental exposures. (A) Control treatment (grey line – CTL) vs. the natural treatment (green line – NAT); (B) Control treatment (grey line – CTL) vs. the micro-rubber treatments (High concentration: Red – MR-H; Low Concentration: Light red – MR-L); (C) Control treatment (grey line – CTL) vs. the leachate treatments (High concentration: Blue – LEA-H; Low Concentration: Light blue – LEA-L).

energy metabolism, from glycolysis to oxidative cycle and citrate cycle. Additional evidence of oyster's attempts to maintain homeostasis was provided by the up-regulation of pathways involved in drug metabolism and "mTORC 1 signaling"; in fact the most important role of mTORC1 is to monitor the energy status of the cell and, consequently, activate protein translation [49], thus favouring cell and tissue growth [50] suggested by the up-regulation of "protein secretion" category in the same treatment (LEA-H). Changes of the expression of genes involved in detoxification processes and protein synthesis were detected in oysters (*S. glomerata*) following the long-term treatment with few PAHs [51], corroborating the potential effect of leachate chemicals of our results.

Moreover, oxidative stress due to leachate exposure may have also interfered with other key signaling pathways. Among them, the up-regulation of the NOTCH signaling pathway, a highly conserved cell signaling system, in both gills and digestive gland of LEA-H exposed oysters was detected suggesting potential consequences in an enormous diversity of developmental processes [52]. The up-regulation of NOTCH signaling pathway was already described in liver of rats exposed to benzo[*a*]pyrene [53], as well as in the gut of planarians exposed to microplastics [54]. Disruption of key signaling pathways following leachate exposures is also suggested by the up-regulation of "apoptosis", "TNF signaling via NFKB" and "WNT canonical signaling pathway". The latter is a highly evolutionarily conserved signal transduction pathway playing pivotal roles in cell proliferation and tissue homeostasis; its up-regulation recorded in gills could be triggered by oxidative stress as already described in previous studies following exposures to nano- and microplastics [55-58].

The up-regulation of pathways involved in synapse and neurotransmission was observed in digestive gland following the exposure to the highest tire particle concentration (MR-H). Conversely, the same pathways were down-regulated in gills following leachate exposure (LEA-L, LEA-H). To date, most of the available studies that pointed out the potential neurotoxicity caused by microplastics were focused on changes occurring mainly in the neurotransmitter acetylcholinesterase (AchE) activity [59-63]. To our knowledge, disruption of synapses and neurotransmitter regulation has never been described in the digestive gland following TP and LEA exposure. In accordance with Yurchenko et al. [64], we speculate that these findings may be related to the three pairs of ganglia directly connected to the digestive tissues that form the nervous system in oysters. Despite increasing evidence suggesting the involvement of bivalve neurotransmitters in a variety of behaviors and biological functions, their diversity and distribution in the nervous system remain somewhat unclear, making further analyzes necessary to correctly interpret the tissue- and treatment-specific responses highlighted here at the transcriptional level.

Overall, our data showed the putative toxicity of leachates exercised through molecular modifications leading to the alteration of several key signaling pathways and biological processes. The increasing of energy metabolism has both costs and benefits: on one side, it may help to maintain cell homeostasis, while, on the other side, the allocation of extra energy required to face chemical stress might drive to a lower availability of energy for several traits such as reproduction. Potential effects on reproductive outputs were therefore sought as described below.

4.2. Oyster's microbiota characterization reveals minor changes in microbial communities but over-representation of Tenacibaculum spp.

Despite minor changes in microbial communities of gills and digestive glands upon a 44-days exposure to tire particles and leachates, an over-representation of *Tenacibaculum spp*. was observed in digestive gland of oysters exposed to both tire particles concentrations (MR-L and H) and in gills of oysters exposed to MR-H and LEA-H, suggesting the onset of dysbiosis following exposure to high concentration of both tire particles and leachates [65]. *Tenacibaculum* spp is a gram-negative and motile bacterial genus from the family of Flavobacteriaceae that includes opportunistic species often associated to mortality events in fish and shellfish species [66,67]. Recently, *Tenacibaculum* has been also associated to mortality events occurring in Italy in adult Pacific oysters, where it induced necrotic lesions [67]. The dominance of *Tenacibaculum* spp. in exposed oysters as compared to controls suggested a potential perturbation of the gut microbiome that could have consequences on the oyster physiology and resistance to stress [68]. Indeed, the oyster microbiota and its stability are closely linked to host resistance to stress and pathogens [69,70].

4.3. Low concentrations of tire particles and leachates had no effects on adult oyster ecophysiology

No effects were observed in adult oysters on growth, feeding rate and absorption efficiency, as well as on hemocyte oxidative and detoxification processes upon exposure to tire particles or their leachates. This suggest that (i) oysters exposed to tire particles reasonably managed to cope with the presence of various chemical compounds (e.g. pyrene, phenanthrene, fluoranthene, benzo(ghi)perylene) in the tire powder and/or (ii) the leaching of such compounds was somewhat moderate as it did not exacerbate toxicity in exposed oysters. Regarding the first point, the most elevated levels of PAHs detected in tire powder used in the present study (pyrene 13,865, phenanthrene 7025, fluoranthene 5481, benzo(ghi)perylene 5582 µg kg^{-1}), are clearly much higher than the toxicity threshold previously observed in aquatic species without knowing the exact quantity desorbed in water and potentially accumulated in animals, although the exact quantity desorbed in water and potentially accumulated in animals is unknown. For example, for pyrene, the measured EC50 is equal to 1024 µg L^{-1} in short-term exposure in *Daphnia magna* and to 9.3 µg L^{-1} in 7 days test in *Ceriodaphnia dubia* [71]. Interesting, consequences at molecular level were detected in oyster (*Saccostrea glomerata*) after the treatment for prolonged period (7 days) to pyrene and fluoranthene by using rice flour (particle sizes up to 50 µm) as chemicals carrier, such as the alteration of genes related to PAH detoxification (e.g. *cytochrome*

P450), innate immune responses (e.g. pathogen recognition, phagocytosis) and protein synthesis [51]. In previous studies in *M. gigas*, sub-lethal effects of tire particles leachates were reported both on early life stages [24] and juveniles of oyster, reflected by significant reductions in algal and oxygen consumption by a mean of 52 % and 16 %, respectively [21]. The difference of effects with the present study may be explained by several non-mutually exclusive explanations: the concentration of tested tire particles chemical-associated leachates in the present study was 20 times less concentrated than the lowest dose tested in Tallec et al. [21]. In addition, the brands and life-history of the tires used to produce the leachates were different between both studies, which may lead to differences in their chemical composition, thus influencing the intrinsic toxicity of the material.

4.4. Parental exposure impacted gamete quality without consequences on the fertilization success

A significant incidence of the parental exposure was mainly observed on the percentage of motile G0 spermatozoa with a 22 % reduction in the two leachates conditions (LEA-L and LEA-H, p-value < 0.05) as compared to the other treatments. Such effects of parental exposure on their reproductive outputs (reviewed in [72]) were suspected to be due to (i) endocrine-like toxicity of MP and/or their associated chemical compounds (e.g. [73]), as well as to (ii) a disruption of the energy metabolism (e.g. [28]) as mentioned above or to (iii) transfer of particles in the offspring (as observed for NP; [74]). A reduction in the proportion of motile spermatozoa may have consequences on the overall fertilization success [75]. This was not the case here for both G1 and G2 early life stages supposedly due to the optimal fertilization conditions providing sufficient amount of spermatozoa to fertilize the oocytes (initial concentration spz:oocyte set at 100:1). In natural coastal environments where high dilution process occurs the probability of encounter is much lower, thus any significant alterations of the gamete's quality could have consequences on the fertilization success.

When considering the molecular pattern in oocytes, the high number of DEGs (more than 400) in comparison with other tissues (no more than 26 in pairwise comparisons in gills or digestive glands) demonstrates that oocytes are significantly targeted in cases of prolonged exposures of the broodstock during gametogenesis as suggested in the zebrafish exposed to NP PS [74]. Overall, molecular changes observed in oocytes, mainly following tire particles exposures, could be explained by the presence of microparticles in follicular fluid, able to influence the regulation of genes involved in cell cycle and apoptosis with possible consequent disruption of gamete functioning in oocytes [76]. Among DEGs, some support the hypothesis of endocrine disruption. The NOTCH signaling pathway was down-regulated in oysters treated with both tire particles (MR-H and MR-L) and LEA-L. Such impairment could lead to subsequent effects in embryonic development, as demonstrated in metazoan organisms [52], but was not observed here in G1 nor in G2.

Conversely to adult digestive gland and gills, most important effects at molecular level in oocytes were found in oysters exposed to low tire particles concentration (MR-L). Tire particles immunotoxicity is repeatedly noted by the impairment of molecular pathways playing key role in *i)* cell cycling ("TNF signaling via NFKB" and "PI3K/AKT/mTOR pathway"); *ii)* immune system and changes in apoptosis regulation (down-regulation of several pathways/genes involved in immune response and apoptosis as *BIRC7*, *BIRC3 and IAP1*) as already observed in oocytes of zebrafish exposed to NP PS [77]; *iii)* down-regulation of *NPY receptor* and *Lysozyme 1 (both down-regulated)*, already observed in fertilized *M. galloprovincialis* eggs exposed to NP [78]. While NPY plays a major role in the coordination of energy balance and reproduction, a potential immunomodulatory role was also suggested in invertebrates, including *M. gigas*, in which NPYr down-regulation was reported in larvae exposed to heat and bacterial stress [79].

Sub-lethal effects of tire particles and leachates exposures on oocytes are also demonstrated by the up-regulation of *Growth arrest and DNA-damage-inducible protein GADD45*, playing a key role in cell cycle arrest, DNA repair, cell survival, and apoptosis. GADD45 was described up-regulated in several bivalve species following exposure to different stressors including microplastics [80-83]. Indeed, GADD45 appeared significantly over-expressed in response to low tire particles and leachates concentrations, questioning its loss of function at higher concentrations as already reported in oocytes of zebrafish exposed to different microplastic concentrations [77]. A negative correlation between GADD45 and D-larvae yields was recently highlighted in *Pecten maximus* [84] suggesting potential detrimental effects in reproductive efficiency following contaminant exposures, not observed here.

4.5. Parental exposure did not lead to long-term effects on offspring growth and reproductive outputs

No long-term consequences of parental exposure (G0) on the consecutive generation (G1) were observed by monitoring growth, survival and reproductive success over 13 months, despite sub-lethal effects on molecular functioning, gamete quality and microbiota observed in exposed broodstock. Even during early development, a life stage characterized by fine-tuning of the cellular metabolic program and by a high metabolic rate [85], mitochondrial function showed no changes in oocyte and larvae in terms of ATP levels and mitochondrial contents. This suggests the ability for oysters to cope with moderate contamination levels of tire particles and their associated leachates in our experimental conditions.

5. Conclusions

While our results bring a positive note on the apparent resilience of Pacific oysters to tire particles and associated leachates exposure by activating transcriptional changes to cope with chemical stressors, caution should be taken when extrapolating these results to other rubber materials or to natural – harsher – conditions occurring in coastal ecosystems. As previously demonstrated, the toxicity of plastic and rubber materials are highly dependent on their chemical formulations and life history [24,16,86] leading to end-less combinations of toxicity potential. Another aspect to bear in mind is that, in the natural environment, food deprivation and/or environmental stressors (e.g. heatwaves) may occur, leading to a limited energy budget to withstand the consequences of contaminants such as tire particles and their leachates, resulting in largely different outcomes from the ones observed here under optimal experimental conditions compared to unfavorable environmental conditions (e.g. global change, ocean acidification, pollutions).

Environmental Implications

Tire particles and associated chemicals led to the disruption of oysters' energy metabolism and the activation of molecular mechanisms involved in stress response. Possible impacts on gamete quality and the spread of opportunistic pathogens have also been observed. Fortunately, the lack of significant long-term consequences regarding fertilization success, growth and reproductive outputs of the offspring suggests possible oyster resilience. However, the increase of tire particles concentrations and the harsher environmental conditions compared to our laboratory experimental setting expected in the coming years are of particular concern for the conservation of species chronically exposed to tire particles and associated chemicals.

CRediT authorship contribution statement

Christophe Lambert: Writing – original draft, Investigation. **Kevin Tallec:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabienne Lagarde:** Writing – original draft, Investigation. **Ika Paul-Pont:** Writing – original draft, Visualization, Validation, Supervision, Resources,

Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luca Peruzza:** Writing – original draft, Software, Data curation. **Camille Détrée:** Writing – original draft, Investigation. **Arnaud Huvet:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Claudie Quéré:** Writing – original draft, Investigation. **Virgile Quillien:** Writing – original draft, Investigation. **Jacqueline Le Grand:** Writing – original draft, Investigation. **Nelly Le Goïc:** Writing – original draft, Investigation. **Ilaria Bernardini:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Rafael Trevisan:** Writing – original draft, Investigation. **Giulia Dalla Rovere:** Writing – original draft, Investigation. **Charlotte Corporeau:** Writing – original draft, Investigation. **Matthias Huber:** Writing – original draft, Investigation. **Tomaso Patarnello:** Supervision. **Carole Di Poi:** Formal analysis, Data curation. **Massimo Milan:** Writing – original draft, Visualization, Supervision, Conceptualization. **Hugo Koechlin:** Writing – original draft, Investigation.

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Data availability

16S Sequence data and RNAseq data sequencing files areavailable in NCBI Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra; BioProject PRJNA856813).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Arnaud Huvet reports financial support was provided by European INTERREG France (Channel) England project Preventing Plastic Pollution. Massimo Milan reports financial support was provided by National Recovery and Resilience Plan CUP C93C22002810006 Project title National Biodiversity Future Center - NBFC. Kevin Tallec reports financial support was provided by INTERREG Preventing Plastic Pollution. Rafael Trevisan reports financial support was provided by Marie Sklodowska-Curie grant agreement number 899546. 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.

Data availability

Data will be made available on request.

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Disclosure Statement

The authors report no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135742.

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