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## Tire rubber chemicals reduce juvenile oyster (*Crassostrea gigas*) filtration and respiration under experimental conditions

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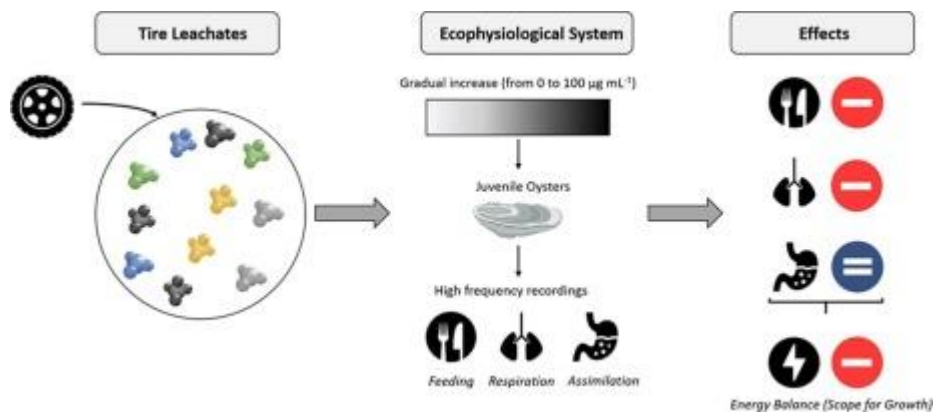
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### Abstract :

Tires can release a large number of chemical compounds that are potentially hazardous for aquatic organisms. An ecophysiological system was used to do high-frequency monitoring of individual clearance, respiration rates, and absorption efficiency of juvenile oysters (8 months old) gradually exposed to four concentrations of tire leachates (equivalent masses: 0, 1, 10, and 100  $\mu\text{g mL}^{-1}$ ). Leachates significantly reduced clearance (52 %) and respiration (16 %) rates from 1  $\mu\text{g mL}^{-1}$ , while no effect was observed on the absorption efficiency. These results suggest that tire leachates affect oyster gills, which are the organ of respiration and food retention as well as the first barrier against contaminants. Calculations of scope for growth suggested a disruption of the energy balance with a significant reduction of 57 %. Because energy balance directs whole-organism functions (e.g., growth, reproductive outputs), the present study calls for an investigation of the long-term consequences of chemicals released by tires.

## Graphical abstract



## Highlights

► Leachates of tires affected clearance and respirations rates of juvenile oysters. ► Decreases in Scope For Growth (SFG) calculations suggest energy balance impairment. ► Leachates did not affect the absorption efficiency suggesting no effect on the digestive functions.

**Keywords :** Oyster, Tire leachates, Ecophysiology, Scope for growth

## 24 1. Introduction

25 Exponential growth in the use of plastics, leading to continuous release of waste into  
26 environment, is a crucial issue (Borrelle et al., 2020; Lau et al., 2020). Each year, 50–70 % of  
27 annual rubber production (28.7 Mt in 2019) is used for the tire industry. Road traffic  
28 inevitably generates tire particles (TP) by mechanical abrasion. These TP display a large  
29 range of sizes, from a few nanometers to particles larger than 100  $\mu\text{m}$  (Kole et al., 2017;  
30 Wagner et al., 2018). Emissions of 0.23–4.7 (average = 0.81) kg TP year<sup>-1</sup> capita<sup>-1</sup> were  
31 estimated across the world (Kole et al., 2017). Annually, global TP emissions could represent  
32 2.9 million tons (Mt) (Evangelidou et al., 2020). Hence, they constitute a significant part of  
33 microplastic (MP; particles <5 mm) emissions, notably in the oceans, where TP represent  
34 emissions of 0.2–0.7 Mt year<sup>-1</sup> (Boucher and Friot, 2017). Overall, TP are expected to  
35 constitute 93% of the MP contamination in aquatic systems in terms of mass by 2040 if no  
36 major improvements are made in plastic waste management (Lau et al., 2020). In addition,  
37 tires and TP can release chemical compounds potentially hazardous for aquatic organisms  
38 (e.g., Halle et al., 2020). Indeed, tires contain chemical compounds that represent up to 50%  
39 of their mass, while this value is on average 7% for other conventional plastic products  
40 (Geyer et al., 2017; Wagner et al., 2018). Leachate from tires is made up of a wide diversity  
41 compounds of varying complexity. For instance, no less than 2,000 chemical signatures were  
42 found in leachates obtained from 250 mg TP L<sup>-1</sup> (Tian et al., 2020). They contain additives  
43 (e.g., antioxidants), transformation products, and other hazardous compounds such as metals  
44 (e.g., Zn) or extender oils and softeners (i.e., polycyclic aromatic hydrocarbons, PAHs)  
45 (Kolomijeca et al., 2020; Sadiktsis et al., 2012; Wagner et al., 2018). For instance, leachate  
46 analyses revealed the presence various compounds including zinc (519  $\mu\text{g L}^{-1}$ ), acenaphthene  
47 (24.1 ng L<sup>-1</sup>), acenaphthylene (222.6 ng L<sup>-1</sup>), benz[a]anthracene (2.1 ng L<sup>-1</sup>), naphthalene  
48 (438.5 ng L<sup>-1</sup>), phenanthrene (49.4 ng L<sup>-1</sup>), pyrene (5.1 ng L<sup>-1</sup>) or PCB101 (8.4 ng L<sup>-1</sup>) after 14  
49 days of leaching in seawater using a concentration of 100 g tires L<sup>-1</sup> (Tallec et al., 2022).

50 Although TP ingestion could induce physical damage (Halle et al., 2020), the toxicological  
51 risk posed by tires has been suggested to be mainly associated with the release of chemical  
52 compounds (McIntyre et al., 2021). Tire leachate has been reported to induce detrimental  
53 effects on survival, reproductive outputs, and developmental processes under experimental  
54 conditions (e.g., *Mytilus galloprovincialis*, Capolupo et al., 2020; *Crassostrea gigas*, Tallec et  
55 al., 2022; *Daphnia magna* and *Xenopus laevis*, Gualtieri et al., 2005; *Pimephales promelas*,  
56 Kolomijeca et al., 2020; *Ceriodaphnia dubia* and *Daphnia magna*, Wik et al., 2009). In

57 addition, it has recently been demonstrated that mortality events affecting coho salmon  
58 (*Oncorhynchus kisutch*) for decades in western North America are related to tire leachate  
59 released by road traffic (Tian et al., 2020). Commonly, the toxicity of tire leachates is  
60 associated to the release of metals (especially the zinc; e.g., Gualtieri et al., 2005), PAHs (e.g.,  
61 Kolomijeca et al., 2020) even though in some cases it is unknown products (e.g.,  
62 transformation products) that are responsible for the toxic effects (Tian et al., 2020).  
63 Investigating the “tire chemical risk”, therefore, appears to be an important issue that can  
64 provide additional data for risk assessments (McIntyre et al., 2021).

65 Monitoring the ecophysiological response of marine mollusks provides valuable data to  
66 estimate their bioenergetics and explore the effects of contaminants on their life history traits  
67 and population sustainability (Sokolova, 2013). Energy balance is based on controlled and  
68 integrated processes enabling the organism to regulate energy intake, expenditure, storage and  
69 excretion (Nadal et al., 2017). Alterations of energy budget can have long-term and  
70 irreversible consequences for individuals and populations. The automatic ecophysiological  
71 system used in the present study, based on flow-through chambers, is a powerful, non-  
72 invasive method to obtain reliable data on the individual physiological response of bivalves  
73 and to estimate their energy budget before, during, and after an exposure to environmental  
74 stressors. Here, we used the Pacific oyster (*Crassostrea gigas*) as a marine model organism.  
75 Oysters are engineer species regulating essential ecological functions (e.g., water quality,  
76 phytoplankton biomass, biodiversity) in coastal environments, and are commonly used as  
77 bioindicators of ecosystem health (e.g., Bayne, 2017). We assessed the effects of tire  
78 leachates on individual ecophysiological performances (clearance and respiration rates,  
79 absorption efficiency) of juvenile oysters using gradual concentration exposures (from 0 to  
80 100  $\mu\text{g}$  tires  $\text{mL}^{-1}$ ). A flow-through system was used and provided a constant flow of  
81 phytoplankton enriched seawater at controlled temperature, equipped with a tracking system  
82 consisting of continuous recorders of hydrobiological parameters taking high-frequency  
83 measurements. It provides a life-cycle approach as a complement to the standardized embryo-  
84 larval development tests available for marine bivalves and already shows to be disrupted in  
85 oyster when facing tires chemical compounds (Tallec et al., 2022).

## 86 **2. Materials and Methods**

### 87 **2.1. Animals**

88 Juvenile oysters (8 months old; length:  $31.6 \pm 2.9$  mm; dry mass:  $0.11 \pm 0.04$  g; produced as  
89 described in Petton et al., 2015) were transferred from the Ifremer nursery in Bouin, France,

90 to the experimental facility in Argenton, France, in September 2020. The oysters were  
 91 acclimatized for 10 days in a 50-L tank supplied with UV-treated 1- $\mu\text{m}$  filtered seawater  
 92 (17°C, pH 8.1) and fed *ad libitum* with a balanced mixture of two microalgae, *Tisochrysis*  
 93 *lutea* (T-iso CCAP927/14, cell volume = 40  $\mu\text{m}^3$ ) and *Chaetoceros* sp. (CCAP 1010/3, cell  
 94 volume = 80  $\mu\text{m}^3$ ), so that the algae concentration in the tank outflow was kept constant at  
 95 1500  $\mu\text{m}^3 \text{ mL}^{-1}$ . Between each trial, oysters were maintained under similar conditions to those  
 96 applied in the ecophysiological system (see section 2.3).

## 97 **2.2. Tires and leachate preparation**

98 A mixture of three tires (one new and two secondhand; Table 1) was used to produce  
 99 leachates in order to incorporate the variability in tire chemical composition resulting from  
 100 usage (new/used) and brand (Redondo-Hasselerharm et al., 2018; Sadiktsis et al., 2012; Wik  
 101 et al., 2009). The outer rings of the tires (first 2 cm) were cut into 1–5 mm pieces using  
 102 stainless steel scissors, then mixed (taking equal weights of each tire) to produce leachates.  
 103 Leachates (concentrations detailed in section 2.3) were produced as described in Capolupo et  
 104 al. (2020), i.e., in sterile, 0.2- $\mu\text{m}$  filtered seawater for 14 days using an orbital shaker (240  
 105 rpm) in dark conditions at room temperature (~20°C). At the end of this incubation, stock  
 106 solutions were filtered on glass-fiber filters (GF/F; porosity 1.2  $\mu\text{m}$ ) to remove particles. To  
 107 avoid a degradation of chemicals in the leachate during the exposures, a new and fresh  
 108 leachate solution is used every day.

109 **Table 1.** Type of tires used in the present study.

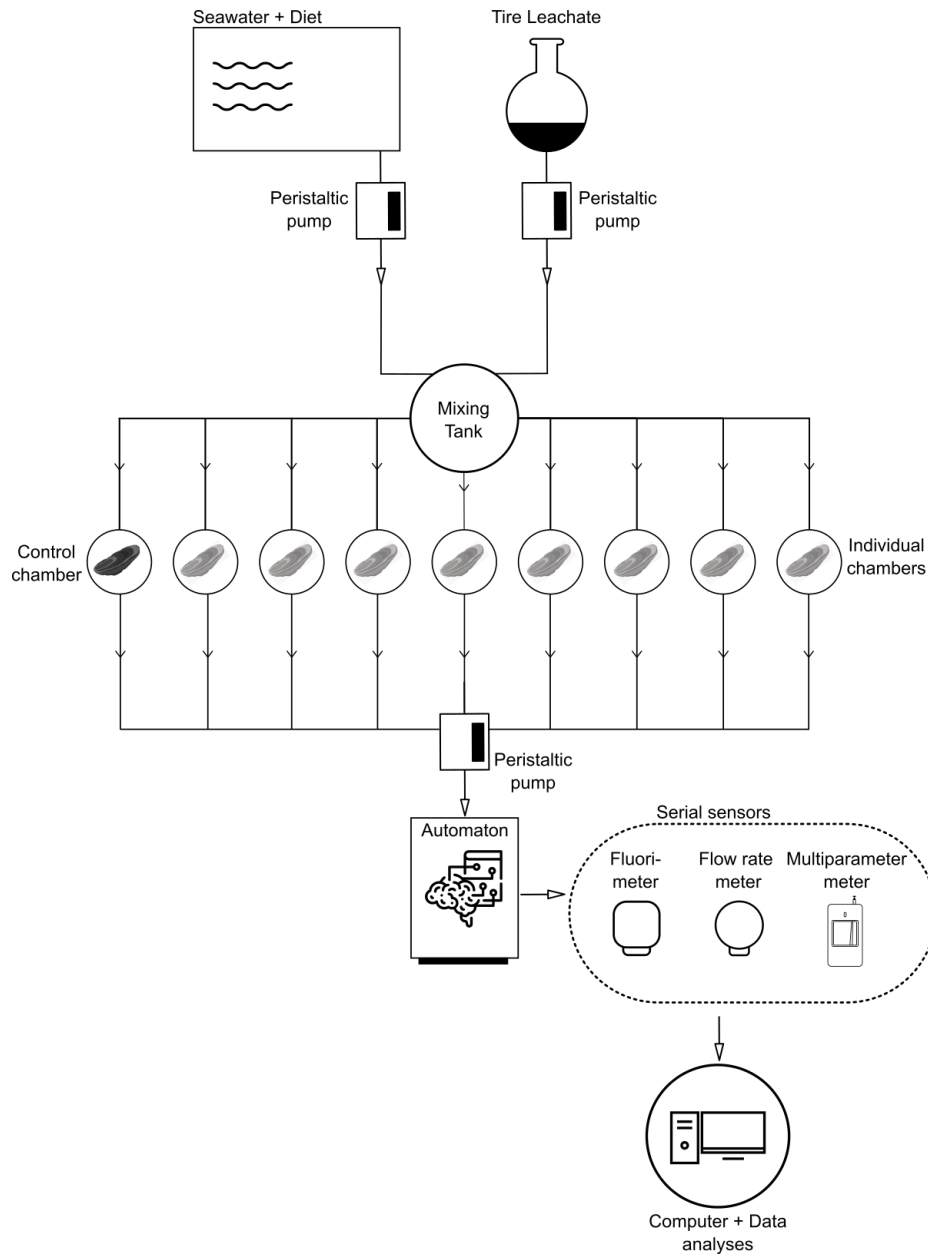
Tires	Sources
New Tire	Dunlop SP Sport Maxx (Dot code: DM75 JA2R 3916) purchased from an automobile repair garage located in Brest (France).
Used Tire 1	Dunlop SP Sport Maxx (Dot code: DM75 JA2R 3916) purchased from an automobile repair garage located in Brest (France).
Used Tire 2	Michelin Radial X (Dot code: HDWC KUBX 2013) obtained from personal usage.

110

## 111 **2.3. Exposures and ecophysiological measurements**

112 The ecophysiological system consists of nine individual flow-through chambers (0.54 L)  
 113 supplied with seawater (flow rate =  $27 \pm 3 \text{ mL min}^{-1}$ ) containing a mixed diet of *T. lutea* /  
 114 *Chaetoceros* sp. (50/50, v/v). A schema of the system is shown in Figure 1. It is described in  
 115 detail in Pousse et al. (2018). This system has been designed to be as respectful as possible of  
 116 the rearing conditions and not to cause stress to the animals, which is shown by the absence of

117 a time effect in several series of experiments (*e.g.* Pousse et al., 2018; Tallec et al., 2021).  
118 Briefly, all chambers are managed by a programmable controller allowing high-frequency  
119 automatic recordings of fluorescence (used as a proxy for phytoplankton concentration),  
120 oxygen concentration, and water flow in the seawater outflow using a WETStar fluorimeter  
121 (WSCHL-1400 WETLABS; USA), a WTW multiparameter probe (WTW Multi 3430), and a  
122 SONOFLOW CO.55 ultrasonic flow meter (Sonotec; Germany), respectively. Temperature  
123 and pH were continuously recorded using the multiparameter probe (Supplementary Table 1  
124 to see the data of all trials). In each chamber, measures were performed for 12 minutes every  
125 3.5 h. Eight oysters were placed in the system, one animal per chamber, while a "fake oyster"  
126 (a clean empty shell) was put in the remaining chamber (control chamber – CC). Upstream of  
127 the nine chambers, a mixing tank (0.94 L) was used to inject leachates into running seawater,  
128 using a peristaltic pump to ensure a continuous exposure. Over one trial, oysters were exposed  
129 gradually to four concentrations of tire leachate corresponding to 0, 1, 10, and 100  $\mu\text{g}$  tire  $\text{mL}^{-1}$ .  
130 <sup>1</sup>. These gradual exposures were used to check the capacity of oysters to modify rapidly their  
131 responses to increasing concentrations of tire leachates based on tipping points approach very  
132 used in OA (*e.g.* Lutier et al., 2022). For each concentration, exposures were conducted for  
133 40.5 h. By targeting exposure duration of a mean of 2 days, a recording of 40.5 h was obtain  
134 taking into account the time required to set up and clean up the system plus the time needed  
135 for the oysters to start their breathing and feeding. One trial, therefore, lasted 7 days (40.5h  $\times$   
136 4 concentrations = 162h). Three successive trials were run leading to individual data for 22  
137 oysters (2 oysters remained completely closed during the trials and were, therefore, removed  
138 from the dataset).



139

140 **Figure 1.** Schema of the ecophysiological system.

141 *2.3.1. Clearance and respirations rates*

142 The individual clearance rate (CR), an indicator of feeding activity, of each oyster was  
 143 calculated as:  $CR = (fl \times (C_{CC} - C_N)/C_{CC})$ , where fl is the flow rate through the chamber ( $L h^{-1}$ ),  
 144  $C_{CC}$  is the fluorescence in the control chamber, and  $C_N$  is the fluorescence in a chamber  
 145 housing one oyster (e.g., Bayne, 2017). The individual respiration rate was calculated as:  $RR$   
 146  $= (fl \times (O_{CC} - O_N))$ , where  $O_{CC}$  and  $O_N$  are the  $O_2$  concentrations ( $mg O_2 L^{-1}$ ) in the control  
 147 chamber and in a chamber housing one oyster, respectively (Savina and Pouvreau, 2004). To  
 148 standardize CR and RR for 1 g of dry tissue, oysters were sacrificed at the end of the



149 experiment and stored at  $-20^{\circ}\text{C}$ , then used to estimate the individual dry mass after 24-h  
150 lyophilization of the soft tissues (Bayne et al., 1987).

### 151 2.3.2. Absorption efficiency

152 The absorption efficiency (AE) of organic matter from the diet was estimated using Conover's  
153 method, which consists of collecting feces over 24 hours and 50 mL of the diet. No pseudo-  
154 feces were produced by oysters in any of the chambers. Feces and microalgae were filtered  
155 and rinsed three times with isotonic ammonium formate. All filters were frozen at  $-20^{\circ}\text{C}$ . The  
156 total weight and organic fraction were calculated after measurement of the filter weight of  
157 each sample after 24 h at  $60^{\circ}\text{C}$  and then 4 h at  $450^{\circ}\text{C}$  (Pousse et al., 2020). Absorption  
158 efficiency was calculated as:  $\text{AE} = (f - e)/((1 - e) \times f)$ , where  $f$  corresponds to the organic  
159 fraction of the microalgae ration and  $e$  is the organic fraction of the feces (Conover, 1966).

### 160 2.3.3. Scope for growth (SFG)

161 The scope for growth ( $\text{J h}^{-1} \text{g}^{-1}$ ) was estimated as:  $\text{SFG} = (\text{C} \times \text{AE}) - \text{R}$ , where C is the rate of  
162 energy ingestion ( $\text{J h}^{-1} \text{g}^{-1}$ ), AE the absorption efficiency, and R the rate of energy catabolized  
163 through respiration ( $\text{J h}^{-1} \text{g}^{-1}$ ). The rate of energy ingestion was calculated as:  $\text{C} = \text{CR} \times \text{POM}$   
164 where POM corresponds to the particular organic matter measured in the water ( $\text{mg L}^{-1}$ ). For  
165 energy conversion, values of 20.3 J and 14.1 J were used for 1 mg of particulate organic  
166 matter and 1 mg of  $\text{O}_2$ , respectively (Bayne et al., 1987; Bayne and Newell, 1983; Gnaiger,  
167 1983).

### 168 2.3.4. Histology

169 In addition to the ecophysiological parameters, 10 control oysters were sampled in the oyster  
170 batch (see section 2.1) just before the beginning of the exposure ( $\text{T}_0$ ) and 6 oysters (2 oysters  
171 randomly selected per trial) were sampled at the end of the exposure to the highest leachate  
172 concentrations to conduct histology analyses. A 3-mm cross-section was cut and fixed in  
173 modified Davidson's solution ( $4^{\circ}\text{C}$  for 48 h) and further processed as described in Fabioux et  
174 al. (2005). Finally, sections were observed under a microscope to identify potential tissue  
175 damage (Zeiss AxioObserver Z1).

## 176 2.4. Chemical analyses

177 Chemical analyses were conducted on: (1) the seawater not supplemented with leachate, i.e.  
178 the control treatment, (2) the tire leachate at concentration 1 ( $1 \mu\text{g mL}^{-1}$ ), (3) the tire leachate  
179 at concentration 2 ( $10 \mu\text{g mL}^{-1}$ ), and (4) the tire leachate at concentration 3 ( $100 \mu\text{g mL}^{-1}$ ).  
180 The samples (1L per treatment) were stored at  $-20^{\circ}\text{C}$  until chemical analyses. Targeted

181 chemicals were selected according to the data available in the literature (e.g., Aatmeeyata and  
182 Sharma, 2010; Kolomijeca et al., 2020; Tallec et al., 2022). Metal concentrations (Zn, Al, Cu,  
183 Fe; LOQ and LOD described in the Supplementary Table 2) were quantified by inductively  
184 coupled plasma optical emission spectrometry (ICP-OES) according to the standardized  
185 protocol NF ISO 11885. Analyses were performed using an Agilent ICP-OES 5110 coupled  
186 with an Agilent SPS 4 autosampler. To reduce matrix effects, samples were diluted (1:10)  
187 with acidified water and HNO<sub>3</sub> (0.5%). An internal standard (Yttrium) was added to all  
188 samples. To verify results, control solutions were injected every 10 samples and compared  
189 with control charts. Organic micropollutant concentrations were measured by gas  
190 chromatography–mass spectrometry (GC-MS/MS). Analyses were performed using a 6890 N  
191 Network GC System coupled with an autosampler 7683 B Series Injector and a 5973 Network  
192 Mass Selective Detector. Internal standards (PCB 29 D5, PCB 103, pyrene D10,  
193 benzo(*a*)pyrene D12, naphthalene D8, benzanthracene D12, dibenzanthracene D14,  
194 benzo(*ghi*)perylene D12, and fluorene D10) were added to the sample. We performed a first  
195 extraction with CH<sub>2</sub>Cl<sub>2</sub>. For the second extraction, the leachate was adjusted to pH 2 using  
196 H<sub>2</sub>SO<sub>4</sub> 50/50. Then, we added ethyl acetate, glacial acetic acid and CH<sub>2</sub>Cl<sub>2</sub> and the sample  
197 was agitated for 20 min. The organic phase was added to the first fraction. Finally, a last  
198 extraction was performed using CH<sub>2</sub>Cl<sub>2</sub>. The extracts were purified by passing them through a  
199 fluorisil column. Before injection into the GC-MS, we completed an evaporation step at 35°C  
200 in an evaporator (Zymark). For the four treatments, 20 polycyclic aromatic hydrocarbons  
201 (PAHs; acenaphthene, acenaphthylene, anthracene, anthraquinone, benz(*a*)anthracene,  
202 benzo(*1,2*)fluorene, benzo(*a*)pyrene, benz(*e*)acephenanthrylene, benzo(*ghi*)perylene,  
203 benzo(*k*)fluoranthene, chrysene, dibenzanthracene, fluoranthene, fluorene, indenopyrene,  
204 methyl(2)fluoranthene, methyl-2-naphthalene, naphthalene, phenanthrene, pyrene; LOQ and  
205 LOD described in the Supplementary Table 2) and 9 polychlorinated biphenyls (PCBs; PCB  
206 101, PCB 105, PCB 118, PCB 138, PCB 153, PCB 156, PCB 180, PCB 28, PCB 52; LOQ  
207 and LOD described in the Supplementary Table 2) were screened using the selected ion  
208 monitoring (SIM) method.

## 209 **2.5. Statistical analyses**

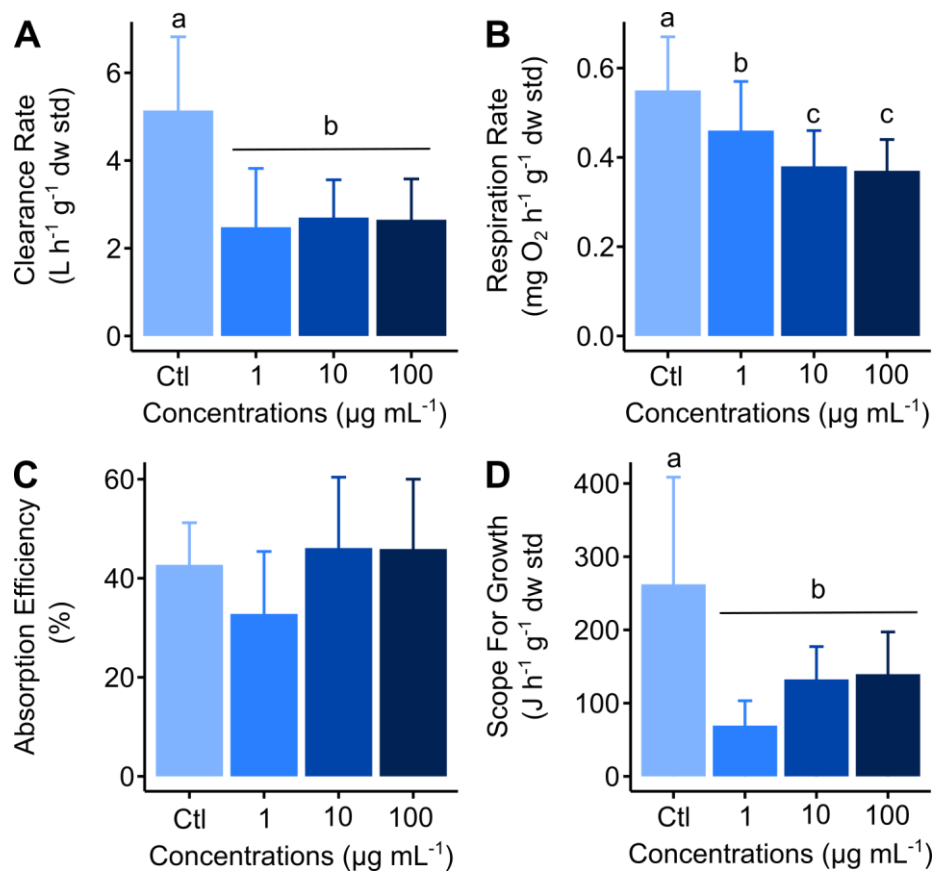
210 Statistical analyses and graphical representations were conducted using R software (R version  
211 4.0.2). Normality and homogeneity of variance were screened for using Shapiro–Wilk and  
212 Levene tests, respectively. Clearance and respiration rates estimated from data recorded by  
213 the ecophysiological system were analyzed using repeated measures ANOVAs with pairwise

214 comparisons using Tukey's method when necessary. Absorption efficiency and SFG were  
215 compared using one-way ANOVAs and Tukey tests. Differences were considered significant  
216 when  $p$ -values  $< 0.05$ . Data are reported as mean  $\pm$  standard deviation.

### 217 3. Results and discussion

218 **Tire leachates impair juvenile oyster physiology with repercussions for the energy**  
219 **budget.** Investigating changes in ecophysiological parameters of mollusks in response to  
220 plastic debris, specifically tire leachates, is a novel approach. From the lowest leachate  
221 concentration ( $1 \mu\text{g mL}^{-1}$ ), both clearance rate (CR) and respiration rate (RR) were  
222 significantly altered compared with the control (Figures 2A & 2B). At  $1 \mu\text{g mL}^{-1}$ , the CR  
223 decreased significantly by 52% ( $p$ -value  $< 0.001$ ), but no differences ( $p$ -value  $> 0.05$ ) were  
224 observed among leachate concentrations. The RR was significantly reduced by 16% at  $1 \mu\text{g}$   
225  $\text{mL}^{-1}$  and by 32% at the two higher concentrations, which remained statistically similar ( $10$   
226 and  $100 \mu\text{g mL}^{-1}$ ) ( $p$ -values  $< 0.001$ ). No effects ( $p$ -value  $> 0.05$ ) were recorded on the  
227 absorption efficiency whatever the leachate concentration (average AE =  $41.8 \pm 6.2\%$ ; Figure  
228 2C). These energetic and metabolic losses lead to a direct incidence on the oyster  
229 bioenergetics, reflected by significant decreases in SFG for all leachate concentrations ( $-56\%$ ;  
230  $p$ -value  $< 0.001$ ; Figure 2D). No significant differences were observed among leachate  
231 concentrations, suggesting no cumulative impacts despite the use of gradual exposures ( $p$ -  
232 value  $> 0.05$ ; Figure 2D). The identification of tipping points beyond which adverse effects  
233 are observed would be a broadly generalizable approximation of the susceptibility of tire  
234 leachates, but this proved impossible as effects surprisingly appeared at the lowest  
235 concentration. Scope for growth is defined as the remaining energy after the maintenance  
236 costs necessary for individual survival, i.e., it is the energy available for growth, reproduction,  
237 and survival (Bayne and Newell, 1983; Stickle and Bayne, 1987). Overall, a positive SFG  
238 means that animals have enough energy for growth and reproduction, while a negative SFG  
239 means the animal loses energy, with drastic consequences potentially implying death (Stickle  
240 and Bayne, 1987). The SFG reduction from  $1 \mu\text{g mL}^{-1}$  is noteworthy but may be  
241 inconsequential for oysters in such experimental conditions based on the concept of energy-  
242 limited tolerance to stress (Sokolova, 2013). Our lab-controlled "environmental" conditions,  
243 i.e., seawater temperature and oxygen concentration, trophic conditions, and absence of  
244 environmental stressors of any sort except tire leachates, can be considered to be optimal for  
245 *C. gigas* (e.g., Bayne 2017). The aerobic scope decreased upon leachate exposure but we  
246 cannot detect any potential shift from the optimum range to the pejus (moderate stress that

247 still allows the animal to sustain itself) or the pessimum range (high stress implying whole-  
 248 organism effects, e.g., decreases in growth and reproductive output) (Sokolova, 2013; Nadal  
 249 et al., 2017). Based on the present ecophysiological data, we consider that more investigation  
 250 is needed on the effects of long-term exposures on key oyster functions (e.g., growth,  
 251 reproduction) in a more realistic context (harsher environmental conditions such as low food  
 252 and water quality). In addition, because some chemicals commonly added to tires during their  
 253 manufacture (e.g., bisphenol A; PCBs, PAHs) are considered as “metabolism-disrupting  
 254 chemicals” (Nadal et al., 2017), assessing their impact on oyster maturity over generations is  
 255 of interest to investigate the long-term toxicity to aquatic life of chemicals released by tires.



256

257 **Figure 2.** Individual clearance rate (A;  $\text{L h}^{-1} \text{g}^{-1} \text{dw std}$ ), respiration rate (B;  $\text{mg O}_2 \text{h}^{-1} \text{g}^{-1} \text{dw}$   
 258  $\text{std}$ ), absorption efficiency (C; %) and scope for growth (D;  $\text{J h}^{-1} \text{g}^{-1} \text{dw std}$ ) in juvenile oysters  
 259 exposed to different concentrations of tire leachates (from 0 – Ctl to  $100 \mu\text{g mL}^{-1}$ ). Results are  
 260 expressed as mean  $\pm$  SD ( $n = 22$  oysters). Repeated measures ANOVAs were conducted to  
 261 compare treatments for the clearance and respiration rates while one-way ANOVAs were  
 262 used for the absorption efficiency and SFG at the 5% level; homogeneous groups are  
 263 indicated by the same letter.

264 **Potential toxic pathways.** The decreases in clearance and respiration rates with exposure to  
 265 tire leachates suggest detrimental effects on the gills, which are a major barrier oysters have  
 266 against any physical particles and chemical molecules they come into contact with in the

267 surrounding environment, including contaminants. Indeed, gills have several crucial roles in  
268 bivalves as they are responsible for oxygen and food intake. Initially, we thought that, in an  
269 extreme case, tire leachates could induce gill tissue damage, but histology revealed no effect  
270 of this kind on any of the analyzed oysters (Supplementary Figure 1). The effect must,  
271 therefore, operate in another manner. As bivalves possess chemoreceptors on pallial organs  
272 that react to chemical cues (Newell and Jordan, 1983; Ward et al., 1992), the gills may act as  
273 sensors of chemical signals from the tire leachates. This could affect valve activity (e.g., valve  
274 gap, Jørgensen, 1996) as previously suggested in response to various contaminants (e.g., MP,  
275 Bringer et al., 2021; algal toxins, Mat et al., 2013, Castrec et al., 2018). Valve activity  
276 modifications can lead to changes in energy budget that result in whole-organism effects. For  
277 instance, in juvenile oysters, MP alter valve activities, reducing the daily growth, a sign of  
278 change in food intake (Bringer et al., 2021). Furthermore, the “chemicals × gills” interaction  
279 could also affect the production and quality of mucus. In oysters, mucus on pallial organs  
280 participates in many functions, including particle capture, defenses, and depuration processes  
281 (Pales Espinosa et al., 2016; Sze and Lee, 1995). Chemical modifications in the surrounding  
282 environment lead to modifications in mucus secretion in marine bivalves and affect nutrition,  
283 notably particle ingestion and rejection processes (Meseck et al., 2020; Pales Espinosa et al.,  
284 2016). It would therefore be interesting to study the mucus to test the relationship between  
285 mucus production and gill functioning during exposure to tire leachates. At the cellular level,  
286 the reduction in oxygen uptake could be linked to a disruption of mitochondria functioning in  
287 gills following an oxidative stress, i.e., a modified balance between reactive oxygen species  
288 (ROS) and antioxidant production in the presence of stressors (Sokolova and Lannig, 2008;  
289 Bhatti et al., 2017). Overproduction of ROS in gills can result in them attacking mitochondria,  
290 altering membrane permeability, and inducing mitochondrial DNA damage and homeostasis  
291 disruption, resulting in overall mitochondria damage, notably to oxidative phosphorylation  
292 (the mechanism that uses O<sub>2</sub> to generate ATP) (Bhatti et al., 2017). In line with this  
293 hypothesis, recent studies have suggested that oxidative stress resulting from tire leachate  
294 exposures can lead to increased ROS production in oyster spermatozoa (Tallec et al., 2022),  
295 increased lipid peroxidation in mussels (Capolupo et al., 2021), and disruption of the  
296 antioxidant system in rainbow trout (Stephensen et al., 2005).

297 Absorption efficiency (AE), i.e., the capability of oysters to convert the organic content of  
298 food into energy to supply key functions, represents a robust proxy of the overall functioning  
299 of the digestive system (concept developed by Conover, 1966; see Huvet et al., 2015 for an

300 example). In the past, it was demonstrated that exposures to toxins or chemicals can  
301 potentially affect energy intake from food by alterations of the gut cells and microbiota  
302 (Nadal et al., 2017; Widdows et al., 1979). In our control treatment, the level is in line with  
303 the AE described in the literature for similar conditions (Hall et al., 2020; Sussarellu et al.,  
304 2016; Tallec et al., 2021). During the exposures, the AE remained unaltered, suggesting that  
305 our short-term exposures to tire leachates did not affect food assimilation or general digestive  
306 functions.

307 **Chemical compounds beyond the scope of quantification.** Our targeted chemical analyses  
308 performed on all leachate concentrations did not permit us to detect or quantify chemical  
309 compounds (metals, PAHs and PCBs) released into the seawater during the 14-day leaching  
310 period. Detection of chemicals in tire leachates are usually performed on much higher  
311 concentrations ( $> 1 \text{ g L}^{-1}$ ; e.g., Capolupo et al., 2020, Kolomijeca et al., 2020) than those of  
312 the present study (the highest concentration =  $0.1 \text{ g L}^{-1}$ ). In a previous study using the same  
313 tires but a leachate concentration of  $100 \text{ g L}^{-1}$ , a wide range of hazardous chemicals were  
314 identified, including zinc, acenaphthene, acenaphthylene, benz(*a*)anthracene, naphthalene,  
315 phenanthrene, and pyrene (Tallec et al., 2022). However, as seen in our study, chemicals  
316 present in quantities below the detection limit, acting alone or in combination with others  
317 (cocktail effect), can induce deleterious effects (Escher et al., 2020). Using *in situ* tire  
318 fragments, smaller than those used in the present study, would be interesting to carry out, in  
319 particular testing whether smaller particles with a higher surface/volume ratio lead to higher  
320 leaching and toxicity. A first study has suggested a low incidence of particles size in the  
321 leaching potential (Halsband et al., 2020).

322 While the numerous compounds listed above can be responsible for toxicity (Dey et al., 2021;  
323 Sun et al., 2020), we cannot demonstrate their implication in the observed impacts. Yet  
324 understanding the modes of action and decision support need to know which compound alone  
325 or in a mixture is responsible for the toxicity even for low or ultra-low chronic exposures. The  
326 SIM mode was used to target specific compounds commonly identified in tire leachates (e.g.,  
327 Kolomijeca et al., 2020). However, it is known that tires can harbor hundreds of chemicals,  
328 including unknown compounds (e.g., transformation products) in a similar way to NIAS (non-  
329 intentionally added substances (such as solvent, reaction, and degradation by-products) in the  
330 context of food or article contact materials (FCM or FCA) (Halle et al., 2020; Tian et al.,  
331 2020; Wiesinger et al., 2021; Zimmermann et al., 2021). Transformation products are hardly  
332 detectable or measurable without specific analytical developments (Tian et al., 2020)

333 characterized by low detection limits, which requires significant methodological work that is  
334 time and budget intensive. High-throughput nontarget chemical analyses to identify chemicals  
335 released by tires (and plastics more generally), such as UPLC-QTOF-MS (Zimmermann et al.  
336 2021), are critical in the context of regulation as well as for developing methods to spotlight  
337 potential tracers of tire debris (Klöckner et al., 2021; Tian et al., 2020; Wiesinger et al., 2021;  
338 Zimmermann et al., 2021). Because toxic effects may be related to a cocktail effect rather  
339 than to individual compounds, chemical analyses are not always enough for risk assessment  
340 and decision support (Svingen and Vinggaard, 2016; D’Almeida et al., 2020; Escher et al.,  
341 2020). Furthermore, not all chemicals can be detected and quantified owing to current  
342 methodological limits (Escher et al., 2020). Toxicological tools are, therefore, an alternative  
343 to gain an overall view of chemical risks (Kristiansson et al., 2021). Considering that bio-  
344 assays are sometimes more powerful than chemical quantification methods to detect presence  
345 and effect of compounds often present below detection limit (Kiyama and Wada-Kiyama,  
346 2015), the ecophysiological system constitute a usefool tool to assess the whole risks related  
347 to tire (and other plastic products) leachates pending fine characterization of the unknown  
348 toxic compounds in order to set up specific regulations.

349

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362 The authors report no conflicts of interest.

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