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Intraspecific variations in oyster (*Magallana gigas*) ploidy does not affect physiological responses to microplastic pollution

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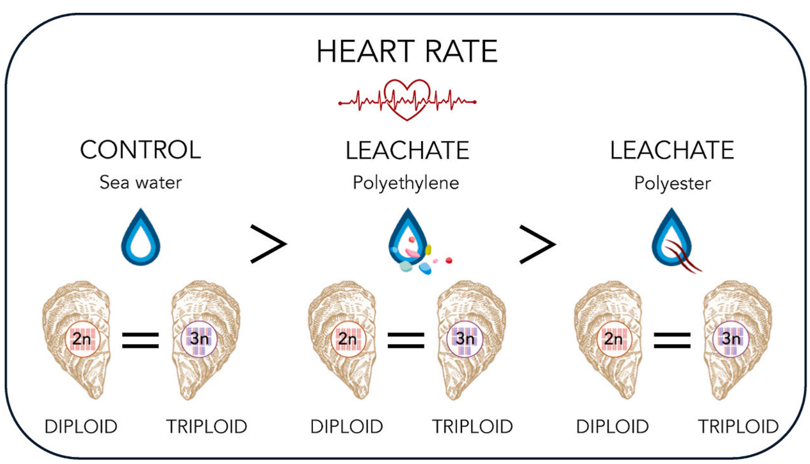
HIGHLIGHTS

- We tested whether ploidy affects oyster responses to microplastic pollution.
- We focused on diploid and triploid juvenile oyster, *Magallana gigas*.
- Diploid and triploid ingested similar amount of polyester microfibre.
- Diploid and triploid heart rate was similar but decreased after leachates exposure.
- Oyster ploidy did not affect physiological responses to microplastic pollution.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent advances in genetic manipulation such as triploid breeding and artificial selection, have rapidly emerged as valuable hatchery methodologies for enhancing seafood stocks. The Pacific oyster *Magallana gigas* is a leading aquaculture species worldwide and key ecosystem engineer that has received particular attention in this field of

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science. In light of the growing recognition of the ecological effects of intraspecific variation, oyster polyploids provide a valuable opportunity to assess whether intraspecific diversity affects physiological responses to environmental stressors. While the responses of diploid and triploid oysters to climate change have been extensively investigated, research on their sensitivity to environmental pollution remains scarce. Here, we assess whether genotypic (i.e., ploidy) variation within *Magallana gigas* affects physiological responses to microplastic pollution. We show that diploid and triploid *M. gigas* have similar clearance rates and ingest similar amounts of microplastics under laboratory-controlled condition. In addition, they exhibited similar heart rates after prolonged exposure to microplastic leachates. Our findings suggest that intraspecific variations within *M. gigas* ploidy does not affect oyster responses to microplastic pollution. However, regardless of ploidy, our work highlights significant adverse effects of microplastic leachates on the heart rate of *M. gigas* and provides evidence of microplastic ingestion in the laboratory.

1. Introduction

Understanding the responses to human-induced environmental change across all levels of biodiversity is a pressing concern that extends beyond ecological interest to encompass consequences for society, including ecosystem services, human well-being, and socio-economic systems (Díaz et al., 2019). Together with climate change, plastic pollution emerges as a serious anthropogenic threat to multiple levels of biodiversity. Macro and microplastic (<5 mm, MP) accumulate in ecosystems worldwide, negatively impacting habitats and organisms through entanglement, ingestion and leaching toxic chemicals (Bucci et al., 2020; Thushari and Senevirathna, 2020; Wang et al., 2021; Liu et al., 2024). The primary focus of initial research efforts examining the effects of plastic pollution on biodiversity has predominantly centred on studying its effects at the species level. However, insights gained over the past decade across a wide variety of taxa and systems, have stressed the importance of considering biodiversity below the species level (i.e., intraspecific variation; Mimura et al., 2017; Leigh et al., 2019; Exposito-Alonso et al., 2022). This is essential for a comprehensive understanding of the consequences of climate change and plastic pollution, to predict the likely consequences for broader ecosystems, and to devise effective management strategies (Jones et al., 2016; Carvalho et al., 2019; Cozzolino et al., 2022, 2024; Nicastro et al., 2023). For example, numerous species display variations in traits among populations, often following geographic clines aligned with environmental gradients (Zardi et al., 2015). Additionally, significant phenotypic differences can be observed among individuals within populations, as well as within specific categories such as sex, age, or size classes (Bolnick et al., 2003, 2011). Phenotypic variation within a species may be genetically based or stem from a single genotype in response to diverse interactions with other species (Nicastro et al., 2020, 2022; Zardi et al., 2015, 2023) or environmental conditions (Polyphenism *sensu* Mayr, 1963; Sommer, 2020). Overall, intraspecific diversity provides the foundation for species adaptation, survival and evolution (Fisher, 1999; Parmesan, 2006; Becks et al., 2010; Thompson et al., 2019). It regulates key ecological and evolutionary processes (Bolnick et al., 2011; Zardi et al., 2015; Des Roches et al., 2018), affecting community structure and species interaction (Crutsinger et al., 2006; Palkovacs and Post, 2009; Duffy, 2010; Siefert, 2012; Nicastro et al., 2020) as well as ecosystem functioning (Whitham et al., 2006; Raffard et al., 2019, 2021; Govaert et al., 2024), resilience and resistance (Hughes and Stachowicz, 2004; Reusch et al., 2005; Meyer et al., 2009; Des Roches et al., 2018). The effect of intraspecific diversity has been shown to be particularly relevant for ecosystem engineers (e.g., oyster reef, mussel beds, seagrasses, corals) that by modifying, maintaining and creating habitats, increase local biodiversity and provide ecosystem function and services (Jones et al., 1994, 1997; Wright and Jones, 2006).

The Pacific oyster *Magallana gigas* is an important coastal bioengineer (Smaal et al., 2019) native to the Pacific coast of Asia (Imai and Sakai, 1961; Molnar et al., 2008). It provides nurseries, refuge and feeding areas (Benaka, 1999; Peterson et al., 2003; Tolley and Volety, 2005; Lefcheck et al., 2019) as well as ecosystem services such as water filtration, coastal protection and carbon sequestration (Meyer et al., 1997; Borsje et al., 2011; Grabowski and Peterson, 2007; Gray et al., 2021). Importantly, due to its rapid growth and ability to tolerate a broad range of temperatures and salinities (Miossec et al., 2009), *M. gigas* has been introduced for aquaculture purposes in over 60 countries worldwide. Currently, it is the leading bivalve species in global aquaculture with a total production exceeding 600,000 tonnes in 2020 (FAO, 2022).

Recent advances in genetic manipulation have expanded the concept of intraspecific diversity, providing the opportunity to understand and to modulate genotypic and phenotypic variation. For example, advances in hatchery technology have enhanced oyster stocks through the production of triploids individuals (Stanley et al., 1981; Allen et al., 1989; Guo et al., 1996) and selective breeding that aims to create specimens that preserve commercially valuable traits such as faster growth, disease resistance, or an appealing shell colour (Robert and Gérard, 1999; Gutierrez et al., 2020; Jiang et al., 2024; Jourdan et al., 2023). Specifically, oyster growth and survival rates, as well as meat quality and resistance to disease have been significantly improved through triploid breeding (Allen et al., 1993; Gutierrez et al., 2018; Jiang et al., 2024). Triploids retain three sets of chromosomes instead of the usual two, thereby showing reduced reproductive potential (Yang, 2022). Consequently, the energy typically invested in reproduction is reallocated towards faster growth (Hawkins et al., 1994; Li et al., 2011; Wang et al., 2012; Zhang et al., 2019).

Understanding the trade-off between the aquacultural benefits of triploids and their physiological performance and tolerance of environmental stressors, as compared to diploids, becomes crucial in the context of human-driven environmental change. Indeed, recent laboratory experiments on the tolerance of the oyster *M. gigas* to environmental stressors (e.g., climate change), suggest that, though exceptions exist (Coxe et al., 2023), triploids have lower critical thermal maxima than diploids (Li et al., 2022), making them more vulnerable to extreme temperatures and desiccation (Meyers et al., 1991; George et al., 2023). Similar responses have been observed also for *Crassostrea virginica* under natural conditions (see Wadsworth et al., 2019; Bodenstein, 2019; Bodenstein et al., 2021, 2023). Despite the recent appreciation of the effects of intraspecific variation on species responses to plastic pollution (Nanninga et al., 2020; Nicastro et al., 2022; Cozzolino et al., 2022, 2023, 2024), effects of oyster ploidy on sensitivity to pollutants remains largely overlooked (with exceptions, see Miles et al., 2014; Prossner et al., 2023).

The present study explored the effects of genotypic diversity (i.e., ploidy) on the physiological responses of the Pacific oyster *Magallana gigas* to microplastic exposure. Specifically, we assessed intraspecific differences between diploid and triploid *M. gigas* (i) clearance rates (CR), (ii) microfibres (MF) ingestion in laboratory experiments, and (iii) the sublethal effects of MF and microplastic leachates (MPLs) on heartbeat (as a proxy for physiological effects). Building upon previous research on the metabolic efficiency and feeding behaviour of oyster diploid and triploid (see Hawkins et al., 2000; Kesarcodi-Watson et al., 2001; Bodenstein et al., 2023), we first assumed that the CR of triploids would be greater than for diploids. Secondly, we hypothesised that triploid *M. gigas* would ingest more microfibres than diploid. Finally, based on recent findings demonstrating the potential for genotypic diversity to affect physiological responses to MP in another bivalve species (Cozzolino et al., 2024), we hypothesised that diploid and triploid *M. gigas* would exhibit different heart rates following exposure to microplastic pollution (i.e., MF or MPL).

Given the crucial role of *Magallana gigas* as ecosystem engineer, understanding the physiological effects of microplastics, particularly among genotypically diverse individuals, is of great ecological and economic significance. Potential alterations in oyster physiology could disrupt the ecosystem functions and services they provide as well as the sustainability of aquaculture activities with consequences for food safety.

2. Material and methods

2.1. Study organism

Diploid and triploid juveniles *Magallana gigas* (8 months old; shell length: 35.9 ± 2.7 mm; shell width: 27.2 ± 2.4 mm; shell height: 13.4 ± 1.8 mm; mean ± SD) were transferred in September 2023 from an oyster hatchery in Normandy (France), to the experimental location at the Marine Station in Wimereux (France). Prior to experiments, the ploidy level of oysters was measured using a flow cytometer (Sysmex, CyFlow Ploidy Analyser). Before each experiment, oysters were acclimatised for 10 days in glass aquaria under continuous seawater flow directly pumped from the experimental location and thus representative of *in situ* conditions (*i.e.,* S = 32.5 PSU, T = 17 °C).

2.2. Clearance rate

The clearance rate (CR) of diploid and triploid oysters of similar size (mean shell length = 36.85 mm; width = 28.21 mm; height = 13.98 mm; dw = 0.08 g; n = 20) was measured. Oysters (n = 10 individuals per genotype) were placed in individual 1 L glass jars containing 0.5 L of pre-filtered seawater (Whatman GF/C 47 mm diameter 1.2 µm pore size) at room temperature (T = 17 °C). Oysters were then allowed to rest for 12 h in continuously aerated seawater (100% O_2) before adding a fixed concentration of algal solution (*Nannochloropsis* spp; ca 50,000 cells mL^{-1}). Immediately afterwards, 1 mL of seawater was collected (t_0) and a second sample was taken after 90 min (t_{90}). The number of algal cells in the water samples was quantified using a haemocytometer (Bright-Line™; see additional information in Supplementary Material).

2.3. Microfibre (MF) ingestion – laboratory assessment

A stock solution of synthetic (polyester; PES) microfibres (MFs; Gütermann; 23 324; grey colour) was prepared by sectioning PES filaments with a microtome (Cole, 2016) and suspending them in ultrapure water mixed with Tween-20 (0.01%) to avoid clumping (Sussarellu et al., 2016). MFs were quantified (C = 3.5×10^5 MF mL^{-1}) using a Malassez haemocytometer and measured (length = 103 µm ± 45 µm; diameter = 15 µm ± 4 µm; mean ± SD) using ImageJ software, then stored in dark conditions at 4 °C. The choice of polyester microfibre was based on its environmental abundance and frequency of ingestion in oysters (De Falco et al., 2018; Lozano-Hernández et al., 2021).

Diploid and triploid oysters (n = 10 each genotype) were placed in separate glass aquaria (n = 4 per genotype) filled with 5 L pre-filtered (Whatman GF/C 47 mm diameter 1.2 µm pore size) aerated seawater under standardized conditions of salinity (S = 32.5 PSU) and temperature (T = 17 °C). Microfibres (at a concentration of 100 MF L^{-1}) and Tween-20 (0.0002%) were directly added to each tank. Oysters were exposed to MFs treatment for 72 h. Every 24 h, each aquarium was cleaned and refilled with newly prepared treatment seawater. One procedural control for each genotype, consisting of oysters in control seawater plus Tween-20 (0.0002%), was run simultaneously with the MFs ingestion experiment. At the end of the experiment, five oysters were randomly selected from each aquarium for MFs ingestion assessment.

2.3.1. MF extraction

In the laboratory, individuals of each genotype were rinsed with pre-filtered ultrapure water (purified by an Elix equipment and filtered through a GF/C Whatman 1.2 µm pore size) to remove potential external contaminants adhered to the shells. Morphometric measurements of shell length, width and height were made using digital callipers (0.01 mm precision) and the soft tissue was extracted and weighed (g WW) using a Sartorius microbalance (0.001 g precision), then rinsed again with pre-filtered ultrapure water. Soft tissue digestion and MPs extraction were conducted following Cozzolino et al. (2023) with a modified version of the protocol of Dehaut et al. (2016). Specifically, each individual was placed in a 250 mL flask and 1.8 M KOH solution was added to digest the organic matter. The solution was covered with aluminium foil and placed in the oven at 60 °C for 24 h. After incubation, the warm solution was vacuum filtered through a Whatman GF/C glass-fibre filter (diameter 47 mm, 1.2 µm pore size). The resulting filters were placed in glass Petri dishes with lids, dried in the oven at 40 °C for 24 h and thereafter examined for the presence of MFs under a stereomicroscope (Olympus, SZX16) operated at 100× magnification.

Rigorous contamination control measures were implemented throughout the laboratory procedures. The entire laboratory analysis for MP extraction was conducted within a laminar flow cabinet. To minimise post-sampling contamination, gloves and 100% cotton laboratory coats were worn during the process. All equipment used was non-plastic (i.e., glass), and was rinsed twice with pre-filtered ultrapure water between each sample extraction. In addition, to account for possible contamination, a procedural (blank) control (containing KOH solution only) was performed in parallel to each digestion batch, yielding no contamination. Filters were kept sealed when not in use to avoid airborne contamination.

2.4. Heart rate (HR) assessment

2.4.1. Virgin microplastic leachate (MPL) treatment

Diploid and triploid oysters were separately placed (n = 10 for each genotype) in glass aquaria (n = 2 for each genotype) filled with 5 L of pre-filtered (Whatman GF/C 47 mm diameter 1.2 µm pore size) aerated seawater under standardized conditions of salinity (S = 32.5 PSU) and temperature (T = 17 °C). Two treatments were set up (i) control seawater (consisting of seawater used for acclimatisation) and (ii) seawater treated with virgin (factory-fresh) microplastic pellets. Virgin MPLs seawater was prepared from commercially available virgin polyethylene pellets (white colour, 3 ± 0.5 mm longest dimension measured on a sample of 50 particles; Materialix, Jaslo, Poland) mixed with control seawater at a concentration of 20 mL of plastic pellets per litre of seawater (equivalent to 405 ± 40 MPs per L) and aerated for 24 h before being use for the experiment (Luo et al., 2019; Seuront, 2018). The MP concentration used here has occasionally been recorded in nature at the study site and thus represents a realistically high level of plastic pollution (Seuront, 2018). MPL seawater was sieved from the pellets and stored in cool, dark conditions until use. Oysters were exposed to virgin MPL treatment and to control seawater for 72 h. Every 24 h, each aquarium was cleaned and refilled with newly prepared treatment seawater. At the end of the experiment, five oysters were randomly selected from each aquarium for heart rate measurements.

2.4.2. Chemical assessment of virgin microplastic pellets

The identification of the additive content associated to virgin microplastic pellets used in the present study (Table 1SM adapted from Cozzolino et al., 2024) was conducted in a separate study (Cozzolino et al., 2024) using a CDS Pyroprobe 6150 pyrolyzer (CDS Analytical) in conjunction with a GC-HRMS instrument (GC Trace 1310-MS Orbitrap Q Exactive, Thermo Fisher Scientific). Thermal desorption was performed

(350 °C) to remove the potential additives from the samples. The samples were then separated using a Restek Rxi-5-MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness) with a cross-linked poly 5% diphenyl-95% dimethyl siloxane stationary phase (slip ratio: 1:5). The acquisition was conducted on full-scan (FS) mode (m/z = 30.00000–600.00000) and the resulting chromatograms were analysed using Xcalibur and TraceFinder software for the identification of organic plastic additives among a selection of 57 additives (i.e., plasticisers, flame retardant, antioxidants and UVs stabilizers; see full list in Supplementary Material). Identification of the respective additives was based on their retention times, m/z values, and specific ions, which were compared with the chromatograms obtained from standard solutions of each additive.

2.4.3. Microfibre (MF) treatment

Diploid and triploid oysters were separately placed (n = 10 each genotype) in glass aquaria (n = 2 for each genotype) filled with 5 L of pre-filtered (Whatman GF/C 47 mm diameter 1.2 μm pore size) aerated seawater under standardized conditions of salinity (S = 32.5 PSU) and temperature (T = 17 °C). Two treatments were set up (i) control seawater and (ii) seawater treated with PES MFs as described in section 2.3. Oysters were exposed to the treatments for 72 h. Every 24 h, each aquarium was cleaned and refilled with newly prepared treatment seawater. At the end of the experiment, five oysters were randomly selected from each aquarium for heart rate measurements.

2.4.4. Chemical assessment of polyester microfibre

The identification of the additives and organic micropollutants (e.g. polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs)) associated to the polyester microfibres used in the present study (Table 2SM adapted from Détrée et al., 2023) was conducted in a previous study (see Détrée et al., 2023; same PES MF batch) using gas chromatography and mass spectrometry (GC-MSMS, Agilent 7890 GC system linked to an Agilent 7010 triple quadrupole MS). Chemical compounds were identified by multiple reaction monitoring (MRM) including spectral mode for organic micropollutants and scan mode for additives (see Supplementary Material for additional details).

2.4.5. HR measurement

The heart rate (heartbeat min^{-1}) of diploid and triploid oysters was measured after 72 h of exposure to either microplastic leachates (MPLs), polyester microfibres (MFs) or control seawater (Control), using infrared (IR) sensors. At the end of each treatment, five oysters were randomly selected from each aquarium and individually transferred to glass arenas filled with 2 L of clean seawater as in control conditions. Oysters were left acclimating for 5 min before heart rate measurement after which cardiac activity was continuously recorded at 10 Hz for 5 min (n = 5 individuals per genotype and treatment) using IR sensors (Vishay CNY70) placed on the shell directly above the heart while each oyster was submerged (see Supplementary Materials for details).

2.5. Statistical analysis

Statistical analyses and graphical representations were conducted using R software (R Studio). Normality and homogeneity of variance were checked using Shapiro–Wilk and Levene tests, respectively and transformed (sqrt) when data did not meet these assumptions. A series of one-way ANOVAs with genotype as a fixed factor were used to test for differences in oyster morphology (i.e., shell length, width, height and body weight). Data on clearance rates, although homoscedastic, did not follow a normal distribution ($p < 0.05$). Thus, a non-parametric Kruskal-Wallis test with genotype (diploid; triploid) as fixed factor, was applied. Data on microplastic ingestion were expressed as number of microfibres per gram of wet weight biomass (n MFs.g^{-1} WW) and analysed using one-way ANOVA with genotype as a fixed factor. Data on the heart rate were analysed using two-way ANCOVAs with genotype and treatment as fixed factors and dry weight as the covariate.

3. Results

3.1. Clearance rate (CR)

Data on oyster shell length, width, height and dry weight showed a normal distribution (Shapiro-Wilk, $P > 0.5$) and homoscedasticity (Levene's test, $P > 0.5$). There were no significant differences in the morphology of diploid and triploid oysters (ANOVAs, $P > 0.05$). Diploid and triploid oysters displayed no statistically significant differences in clearance rates ($P = 0.74$; see Fig. 1). Specifically, the CR of diploid and triploid *M. gigas* was 1.62 ± 1.36 L gDW^{-1} hr^{-1} (mean ± SD) and 1.42 ± 1.32 L gDW^{-1} hr^{-1}, respectively.

3.2. MF ingestion – laboratory assessment

Oyster shell length, width, height and fresh weight were normally distributed (Shapiro-Wilk, $P > 0.5$) and homoscedastic (Levene's test, $P > 0.5$). Diploid and triploid oysters showed similar morphological features except for length (ANOVA; $P < 0.05$), which was significantly larger for triploids. To meet a normal distribution, data on MFs ingestion were square root transformed. There was no effect of intraspecific diversity on MF ingestion (one-way ANOVA, $P = 0.56$), suggesting that diploid and triploid oysters ingested similar amounts of MFs (Fig. 2). Specifically, diploid and triploid *M. gigas* ingested on average 10.3 ± 7.8 MFs gWW^{-1} (mean ± SD) and 10.6 ± 4.2 MFs gWW^{-1}, respectively.

3.3. Heart rate (HR)

3.3.1. MPL treatment

Diploid and triploid oysters did not show significant morphological difference ($P > 0.05$). There was no significant effect of the covariate "DW" or of intraspecific diversity on oyster heart rates (two ways ANCOVA; Genotype, $P = 0.98$). Under control condition, both diploid and triploid *M. gigas* showed a mean heart rate of 32 ± 0.8 HB min $^{-1}$ and 32 ± 1.6 HB min $^{-1}$ respectively. However, after exposure to MPLs, the heart rates of both diploid and triploid oysters decreased significantly to 30 ± 1.6 HB min $^{-1}$ and 29 ± 2 HB min $^{-1}$, respectively (Fig. 3A; Treatment, $P = 0.003$).

3.3.2. MF treatment

Diploid and triploid oysters showed similar biomass (hereafter referred as DW). There was a significant effect of the interaction term (Genotype:Treatment) on oyster heart rates (Two-ways ANCOVA; $P = 0.03$). Tukey HSD was run on the interaction term without significant

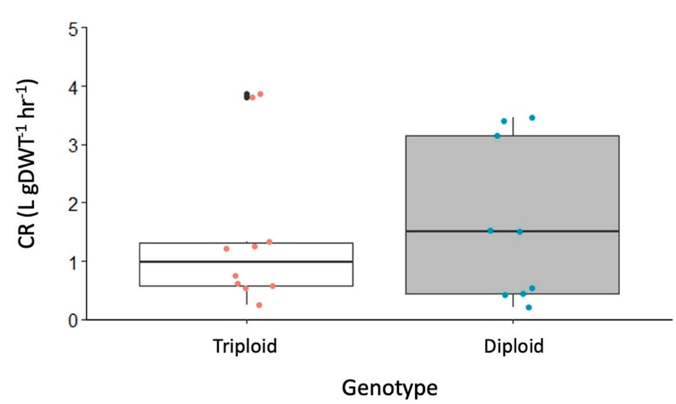

Fig. 1. Intraspecific comparison of clearance rate (n = 10). Clearance rate refers to the dry weight corrected (DW) volume of water cleared of particles (litre) per unit of time (hour).

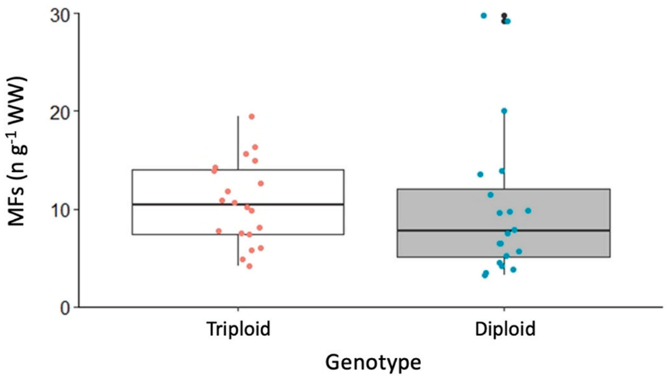

Fig. 2. Intraspecific comparison on microfibres ingestion (n = 20). Microfibre abundance is expressed as number of MFs per gram of wet weight biomass.

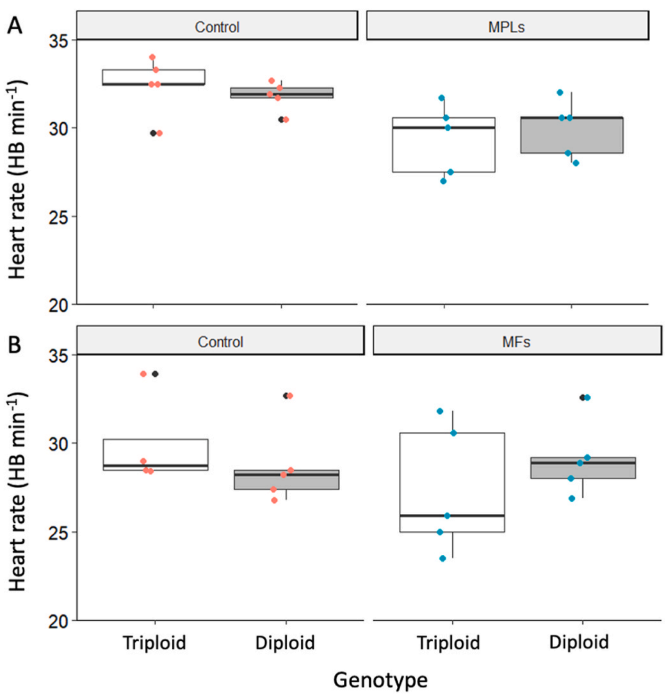

Fig. 3. Intraspecific comparison of heart rate after 72 h of exposure to (A) virgin microplastic leachates (MPLs) and (B) polyester microfibres (MFs). Heart rate is expressed as mean heartbeat (HB) per minute.

results. Thus, to further explore the nature of the interaction, an interaction plot was created using the R "ggplot" function (Fig. 2SM). It suggests that the heart rate did not vary in the same way across all levels of Genotype and Treatment. Specifically, under control condition, diploid and triploid *M. gigas* showed mean heart rates of 29 ± 2.3 HB min $^{-1}$ and 31 ± 3.3 HB min $^{-1}$, respectively. However, after exposure to MFs, the mean heart rate of diploid oysters increased to 31 ± 2.1 HB min ^{1} while that of triploids decreased to 27 ± 3.6 HB min ^{1} (Fig. 3B). The covariate DW did not significantly affect mean heart rate.

4. Discussion

Here, we assessed for the first time, the potential effects of genotypic (i.e., ploidy) variation within the physiological responses of *M. gigas* to microplastic pollution. Our work revealed no effects of intraspecific variation between diploid and triploid oysters.

Our results led to reject our first hypothesis, i.e. that diploid and triploid *Magallana gigas* exhibit different clearance rates CR (i.e. dry weight corrected volume of water cleared of particles per unit of time). These results are in line with what previously reported on diploid, triploid, and tetraploid oysters (Mizuta et al., 2021). We also reject our second hypothesis by showing that diploid and triploid *M. gigas* ingested similar amounts of MFs under laboratory-controlled conditions. These observations are again consistent with the fact that diploid and triploid oysters often show similar physiological performances (e.g., clearance, filtration, respiration; Shpigel et al., 1992; Kesarcodi-Watson et al., 2001) and feeding behaviour (e.g., particle selection and absorption efficiency, diet, valve-opening behaviour; McCarthy et al., 2016; Payton et al., 2017; Mizuta et al., 2021). These similarities would potentially trigger similar sensitivity to MF ingestion. Our work complements a recent study on the brown mussel *Perna perna* showing MP ingestion within intraspecific genetic lineages that coexist under natural conditions (Cozzolino et al., 2023). Taken together, these recent investigations suggest that intraspecific variation may not affect microplastic ingestion in bivalve species under either laboratory or natural conditions.

Previous research has showed variation in oyster HR following environmental stress such as variable temperature and salinity or hypoxia (Lowe, 1974; Feng and Van Winkle, 1975; Domnik et al., 2016; Davis et al., 2023). Significant variation in HR has also been reported after exposure to contaminants (e.g. metals) in various shellfish (Marchan et al., 1999; Bakhmet et al., 2012), including oysters (Liu and Wang, 2016). Here, we assessed the HR of diploid and triploid of *M. gigas* exposed to microplastic leachates (MPL) from polyester (PES) MF and polyethylene (PE) pellets. Our findings show similar HR between *M. gigas* individuals with different ploidy under both control conditions and microplastic treatments (i.e., MF and MPL), rejecting our third hypothesis. Regardless of intraspecific variation, PES MF and PE MPL showed distinct effects on *M. gigas*. While polyester MF exposure did not affect oyster HR, a significant decrease in heartbeat was observed after exposure to polyethylene MPL. This differential response highlights the importance of polymer composition to the physiological effects of microplastics on organisms. Although both are synthetic materials, PE and PES show different properties, chemical structures and potentially, different leachates composition. For example, our analysis of additive content on PE microplastic revealed the presence of numerous plasticisers including nonylphenols, bisphenols, and phthalates (see Supplementary Material for a detailed list). These compounds have raised worldwide concerns due to their potential adverse health effects, particularly as endocrine disruptors (Xing et al., 2022). Although using a different analysis, plasticisers were also detected in lower abundance on PES microfibers, along with other harmful pollutants such as phenanthrene (Bhuyan and Giri, 2020). Taken together, such differences between polymers possibly explain the distinct physiological responses observed on the HR of *M. gigas*. This interpretation is supported by recent findings showing minimal effects of PES MFs and their leachates on the physiological responses of *M. gigas* (Détrée et al., 2023), and significant effects of PE MP on both, oyster physiological and behavioural responses (Bringer et al., 2020a,b, 2021). Similar results have been found in other bivalve species (e.g., mussels) after short-term exposure to PE MPL (Cozzolino et al., 2024; Zardi et al., 2024). The decrease in HR observed in this study can be interpreted as a sign of physiological stress or reduced metabolic activity underlining potential effects on oyster growth, reproduction and survival rates. Such physiological effects may compromise the overall well-being of oyster reefs, consequently affecting the ecosystem functions and services they provide. In coastal habitats, oysters ensure water clearance, nutrient turnover as well as coastal protection and carbon sequestration (Meyer et al., 1997; Borsje et al., 2011; Grabowski and Peterson, 2007; Gray et al., 2021). Because oysters are key ecosystem engineers, the effects of microplastics may not be limited to the organism itself but escalate as cascading effects at the ecosystem level, with consequences for biodiversity and human well-being. *M. gigas* is a fundamental component of the aquaculture industry generating a global annual revenue of USD 1.4

billion (FAO, 2022). Given its relevance as worldwide delicacy, MP ingestion raises concern for food safety. This can affect consumer confidence and demand, potentially leading to additional economic losses for the seafood industry. Understanding the impact of microplastics on oysters is therefore crucial to ensure the sustainability of the ecosystem and to support global economy.

5. Conclusion

Our findings provide novel insights on the sensitivity of *Magallana gigas* to microplastic ingestion and microplastic leachates, further strengthening previous knowledge on the physiological responses of oyster ploidy to environmental stressors. We show that diploid and triploid *M. gigas* have similar clearance rates, heart rates and ingest similar amounts of microplastics under laboratory-controlled conditions. Importantly, the heart rate of *M. gigas* was significantly reduced following exposure to microplastic leachates. The implications of our findings extend from the immediate effects of MPs on oysters to the broader consequences at the ecosystem level for the numerous functions and services that oysters support. Our work provide valuable initial insights and baseline data, which are crucial for understanding early effects and serve as a foundation for designing and interpreting more extensive, long-term research.

CRediT authorship contribution statement

Lorenzo Cozzolino: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Katy R. Nicastro:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Camille Detree:** Writing – review & editing. **Laura Gribouval:** Writing – review & editing. **Laurent Seuront:** Writing – review & editing. **Fernando P. Lima:** Writing – review & editing, Software. **Christopher D. McQuaid:** Writing – review & editing, Supervision. **Gerardo I. Zardi:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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