Microplastics induce dose-specific transcriptomic disruptions in energy metabolism and immunity of the pearl oyster *Pinctada margaritifera*

Tony Gardona, 1, Lucie Morvana, 1, Arnaud Huvetb, Virgile Quilliena, b, Claude Soyeza, Gilles Le Moullaca, Jéremy Le Luyera, *

a Ifremer, Institut Louis-Malardé,IRD, Univ Polynésie française, EIO, F-98719, Taravao, Tahiti, Polynésie française, France
b Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280, Plouzané, France

**A B S T R A C T**

A combined approach integrating bioenergetics and major biological activities is essential to properly understand the impact of microplastics (MP) on marine organisms. Following experimental exposure of polystyrene microbeads (micro-PS of 6 and 10 μm) at 0.25, 2.5, and 25 μg L⁻¹, which demonstrated a dose-dependent decrease of energy balance in the pearl oyster *Pinctada margaritifera*, a transcriptomic study was conducted on mantle tissue. Transcriptomic data helped us to decipher the molecular mechanisms involved in *P. margaritifera* responses to micro-PS and search more broadly for effects on energetically expensive maintenance functions. Genes related to the detoxification process were impacted by long-term micro-PS exposure through a decrease in antioxidant response functioning, most likely leading to oxidative stress and damage, especially at higher micro-PS doses. The immune response was also found to be dose-specific, with a stress-related activity stimulated by the lowest dose present after a 2-month exposure period. This stress response was not observed following exposure to higher doses, reflecting an energy-limited capacity of pearl oysters to cope with prolonged stress and a dramatic shift to adjust to pessimum conditions, mostly limited and hampered by a lowered energetic budget. This preliminary experiment lays the foundation for exploring pathways and gene expression in *P. margaritifera*, and marine mollusks in general, under MP exposure. We also propose a conceptual framework to properly assess realistic MP effects on organisms and population resilience in future investigations.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Plastics represent the greatest proportion of marine litter (up to 60–80% of all marine debris) and quantities of marine plastic continue to increase due to both terrestrial sources and maritime activities (Jameck et al., 2015). Over the last decade, plastic debris, and more specifically “microplastics” (MP, defined as plastic particles < 5 mm) have emerged as a global issue (GESAMP, 2015), leading to increased concerns about their ecological impacts (Rochman et al., 2016). Indeed, owing to their ubiquitous nature and small size, the bioavailability of MP makes them easily ingestible by a wide range of organisms regardless of trophic position (Farrell and Nelson, 2013; Setälä et al., 2014). In filter-feeders, MP ingestion has been recorded in situ (Murray and Cowie, 2011; Mohsen et al., 2019), but has also been largely documented in controlled environment experiments conducted on species such as bivalves (Browne et al., 2008; Avio et al., 2015; Sussarellu et al., 2016; Gardon et al., 2018), zooplankton (Lee et al., 2013; Cole et al., 2013, 2015; Jeong et al., 2016), lugworms (Besseling et al., 2013; Wright et al., 2013a; Van Cauwenbergh et al., 2015), or sea cucumbers (Graham and Thompson, 2009; Mohsen et al., 2019). Experimental consumption of MP, mostly microbeads, can result in adverse health impacts (Frazzelli et al., 2019), including physical harm (Wright et al., 2013b), and physiological effects on feeding activity (Wright et al., 2013a; Cole et al., 2013, 2015), oxygen consumption (Van Cauwenbergh et al., 2015; Watts et al., 2016; Rist et al., 2016), and assimilation efficiency, with consequences for...
energy balance (Blarer and Burkhardt-Holm, 2016; Gordon et al., 2018), fecundity (Besseling et al., 2014; Cole et al., 2015; Sussarellu et al., 2016), growth (Besseling et al., 2014; Watts et al., 2015), and survival (Rist et al., 2016). At the cellular level, evidence of stress has been detected through an increase in reactive oxygen species (ROS) production in mussel haemocytes (Paul-Pont et al., 2016). At the molecular level, ingested MP affect several pathways involved in stress and immune response in coral (Tang et al., 2016; Dettre and Gallardo-Escaraté, 2018). Specifically, the antioxidant system, lysosomal compartment, and peroxisomal proliferation (Avio et al., 2015), as well as apoptotic processes in mussel (Dettre and Gallardo-Escaraté, 2018). At early developmental stages, exposure to polyethylene microbeads affects the nervous system and metabolic genes in zebrafish (LeMoine et al., 2018), and shell biogenesis, immunomodulation, and lysosomal enzymes in mussel (Capolupo et al., 2018).

Individual physiological and metabolic responses to cope with stressful conditions have a high energetic cost that would be difficult to maintain in the long term (Guderley and Portner, 2010). Conceptually, the capacity to respond to a stress is intimately linked to the energy allocated for such maintenance of normal functioning in stressful conditions (Nevo, 2011); hence, the interpretation of snapshots of biomarker variations are only valid in the light of the bioenergetic budget (Hórak and Cohen, 2010). This idea is a focal point of energy-limited tolerance to stress (Sokolova, 2013). Interestingly, this concept should be transferable to experiments on the impact of stressors that are novel in some way and/or multiple stressors. Marine organisms have evolved numerous strategies to cope with environmental fluctuations such as temperature, salinity, hypoxia, and dessication (Hochachka and Somero, 2002), but less is known about their capacities of adaptation and acclimation to emerging pollutants. To date, several biomarkers have been commonly used to assess detoxification (e.g. glutathione-S-transferase [GST], super oxide dismutase [SOD], catalase [CAT]), stress (HSP), and immune (toll-like receptors [TLR]) responses. Observations and experiments performed in corals have adhered to this idea (Sokolova, 2013) and combining biomarkers and bioenergetics monitoring, would prove useful for understanding these variations and ultimately choosing an optimal framework for future experiments.

In French Polynesia, the pearl oyster is an economical interest species of which aquaculture (pearl-farming) is the second most important source of income through the trade of pearl and mother-of-pearl. Such as other filter-feeding organisms, it is also a species of ecological interest serving as a biological model for lagoon ecosystems and environmental survey (Barnagout et al., 2014). Pearl farming still relies almost exclusively on collection of wild spat; hence any biotic or abiotic changes on lagoons ecosystems might have dramatic effect on the sustainability of the activity. In order to grasp this emerging risk, we investigated here the molecular-level impacts of polystyrene microbeads (micro-PS) on the pearl oyster, taking advantage of a previous ecophysiological study that showed a dose-dependent energy decrease for individuals exposed to increased micro-PS concentration (Gardon et al., 2018). This molecular approach was conducted without a-priori effort to detect non-visible sublethal effects at the macroscopic level by exhaustive screening of gene expression or targeting potential biomarkers in response to micro-PS exposure. We used a genome-wide transcriptomic approach on the mantle tissue, a key organ in bivalves because it is the first sensor of the environment and responsible for the biominalization functions of shell and pearl formation in P. margaritifera (Joubert et al., 2010). We hypothesized that micro-PS exposure could have led to (1) a modulation of the expression of genes involved in energy metabolism and (2) biominalization functions, based on the energy deficiency observed at macroscopic level, and (3) an energy-dependent stress and immune response linked to exposition levels and a putative higher bacterial load associated with micro-PS. Thus, this transcriptomic approach was coupled with an additional metabarcoding experiment to trace possible rapid changes of microbiota communities associated with micro-PS. As a result of this study, potential biomarkers could ultimately be applied in future ecophysiological experiments testing relevant conditions with realistic MP levels encountered in lagoons of French Polynesia that had not yet been measured.

2. Material and methods

2.1. Experimental design

We used a random subset of mantle tissue samples collected as described in a study already published (Gardon et al., 2018). This earlier experiment made a two-month exposition of pearl oyster individuals to polystyrene microbeads (micro-PS of 6 and 10 μm). Briefly, adult pearl oysters were collected in a pearl farm located in Arutua atoll (French Polynesia) in October 2016 and transferred to Vairao lagoon (Ifremer marine concession, Tahiti, French Polynesia). These individuals were 1–1.5 years old with a mean height of 5.9 ± 0.41 cm and mean weight of 25.2 ± 4.9 g. They were conditioned in four experimental 20-L tanks per treatment (six oysters per tank, that is, 24 oysters per treatment) with a seawater supply filtered mechanically at 25 and 5 μm. After two weeks acclimation, the pearl oysters were exposed to micro-PS over a two-month period at 0.25, 2.5, and 25 μg L⁻¹ (3.2 × 10⁻⁷, 3.10⁻⁶, 3.10⁻⁵ particles L⁻¹, respectively) and compared with a control (0 μg L⁻¹). The maximum dose tested (25 μg L⁻¹) corresponded to a mass concentration in the range of the highest estimated field concentration of particles > 333 μm, from manta trawl sampling in the north western Mediterranean Sea (i.e. 23 μg L⁻¹; Collignon et al., 2012). This concentration was selected to serve as a benchmark for comparison with published exposures of Pacific oyster (Sussarellu et al., 2016), and was decreased twice, creating two lower treatments (2.5 and 0.25 μg L⁻¹), to target a response window with a dose-effect relationship. Meanwhile, although we know that the size range of particle retention in this species is between 2 and 200 μm (Pouvreau et al., 1999), quantitative data on the smallest environmental MPs is not yet available for the estuaries and lagoons where it grows. The use of dose—response experiments can therefore be useful in assessing toxicity thresholds for a given contaminant and organism (Paul-Pont et al., 2018) until such data becomes available, allowing greater environmental realism in subsequent work. For the present experiment, we used unlabeled micro-PS with diameters of 6 and 10 μm, purchased from Polyscience (Polybead, Washington, PA, U.S.) and packaged in aqueous solution (Milli-Q water) at a concentration of 2.10⁻³ (2.5% w/v, 5 ml) and 4.55 × 10⁻² (2.5% w/v, 5 ml) particles ml⁻¹, respectively. Micro-PS were mixed in 50-L reservoirs at equal weight (micro-PS ratio 6/10 μm = 4.615) with two microalgae (Tisochrysis lutea and Chaetoceros gracilis) at a daily ratio equal to 7–8% dry weight algae/ dry weight oyster (i.e., about 35–40 cells μl⁻¹ in the water surrounding the oysters). Micro-PS sizes (6 and 10 μm) were in the optimum retention range of pearl oysters (i.e., 5 μm; Pouvreau et al., 1999), and chosen to be as close as possible to the size of the microalgae (i.e., 4–8 μm). The microalgae/micro-PS mixtures were injected continuously into the experimental rearing tanks and renewed every 24 h in the reservoirs. Additional information on the
experimental design and the in vivo exposure are available in Gardon et al. (2018).

In addition, we recreated a second experiment with similar algae/micro-PS mixtures to those used in Gardon et al. (2018), following identical doses and protocols, to assess bacterial colonization and dynamics over a 24-h period. This period corresponded to the maximum incubation time of mixtures prior their addition in the pearl oyster rearing media. Bacterial load and communities in tank systems were assessed by quantitative PCR. It should be noted that similar PS microbeads of 2 μm were previously reported to have no effects on microalgal status (Long et al., 2017). Bacterial community assessment was conducted with metabcoding of 16S rRNA (Supplementary Information).

2.2. Sampling, extraction, and sequencing

At the end of the two-month exposure period, a piece of mantle from each of 40 individuals (10 individuals per treatment) was placed in RNA later solution and stored at −80 °C. Total RNA were extracted with TRIzOL Reagent (Life Technologies, USA), at a ratio of 1 ml per 100 mg tissues, following manufacturer’s recommendations. We validated RNA quantity, integrity, and purity with a Nanodrop (NanoDrop Technologies Inc., USA) and a 2100 BioAnalyzer System (Agilent Technologies, USA). RNA was dried in RNA-stable solution (ThermoFisher Scientific, USA), following manufacturer’s recommendations, and shipped at ambient temperature to McGill sequencing platform services (Montreal, Canada). At this step, one individual from the 25 μg L⁻¹ treatment was excluded because its total RNA did not meet the quality threshold (i.e., only 9 individuals remained for this treatment). TruSeq RNA libraries were randomly multiplexed (n = 20–19 individuals per lane) and 100-bp paired-end (PE) sequenced on a HiSeq 4000 at the McGill Genome Quebec platform (Montreal, CA).

2.3. Transcriptomic data analysis

We first filtered raw reads with Trimmomatic v0.38 for a minimum length (60 bp) (Bolger et al., 2014), minimum quality (leading: 20; trailing: 20), and for the presence of putative contaminant and remaining adaptors (https://www.ncbi.nlm.nih.gov/tools/vectcreen/univec/). Read quality was assessed before and after trimming with FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and MultiQc v1.6 (Ewels et al., 2016). Only high-quality PE reads were retained and mapped against the reference genome (Le Luyer et al., 2019) using GSNAP v2018.07.04 (Wu et al., 2016) with default parameters, but allowing a minimum mismatch value of 2 and a minimum read coverage of 90%. We used only properly paired and uniquely mapped reads for the downstream analysis (Wu et al., 2016). The count of differentially expressed genes (DEGs) was conducted with HTSeq v0.11.2 (Anders et al., 2015). The DESeq2 v1.22.2 R package (Love et al., 2014) was used to examine differential expression between micro-PS treatments and the control using a series of pairwise contrasts and Wald’s tests. Genes were considered differentially expressed when absolute value of Log2Fold Change (Log2FC) > 2 and False Discovery Rate (FDR) < 0.01. Visualization of DEGs was conducted using an UpSet plot created with the UpSet and Pheatmap R package (Kolde and Kolde, 2015). For functional annotation, we tested for gene ontology (GO) enrichment using GOA-tools v0.7.11 (Klopstenen et al., 2018), implemented in the “go_enrichment” Github repository (https://github.com/enormandeau/go_enrichment) and the go-basic.obo database (release 2019-03-19) with Fisher’s exact tests. Our background gene list included the whole gene set used for differential expression after filtering for low coverage (n = 38,914 transcripts). Only GO terms with P < 0.05 and including a minimum of three differentially expressed genes were considered. Significant GO enriched terms were used for semantic-based clustering in REVIGO (http://revigo.irb.hr/). We used a principal component analysis (PCA) on DEG data to visually explore the individuals clustering per condition using the ggplot2 R package. Data are presented as mean ± standard deviation and all analyses were performed and graphics drawn in RStudio v3.5 statistics software.

3. Results

3.1. RNA sequencing results

Sequencing yielded a mean of 19,219 ± 1,99 M raw reads per individual. After trimming, 92.23 ± 0.37% of the reads were recovered and then used for downstream analyses. Mapping rate reached 62.90 ± 1.39% (Table S1), with no significant differences among treatments (ANOVA, df = 3, F = 1.47, P = 0.24). The PCA (Fig. 1) shows that axes 1 and 2 explain 17 and 11% of the total variance, respectively, and that treatments 2.5 and 25 seemed more closely related compared with the 0.25 treatment. This was verified by hierarchical K-means clustering, as shown in Fig. 2, where individuals from treatments 2.5 and 25 can be seen gathered in the same cluster compared to those from treatment 0.25 and the control. The correlation matrix (Fig. S1) shows all individuals to be tightly correlated to each other, ranging from r = 0.69 to r = 0.91 (Pearson’s correlation). The maximum mean Pearson’s correlation across all individuals is 0.87 ± 0.03 (N = 38,913 genes total), suggesting that overall intra-group variation is limited (Fig. S1).

3.2. Common transcriptomic response shown across micro-PS doses

We identified a total of 359 DEGs (|Log2FC| > 2; FDR < 0.01) between micro-PS (all concentrations included) and control treatments: with 54.32% of the genes annotated according to Uniprot entries (Fig. 3). We found a total of 19 common DEGs across all comparisons (regardless of the expression profiles) with nine

---

**Fig. 1.** PCA plot of individuals with 95% confidence interval ellipses for each micro-PS concentration (0.25, 2.5 and 25 μg L⁻¹) and the control; n = 10 individuals per treatment, except for 25 μg L⁻¹ where n = 9.
having a significant match on Uniprot (Table S2). Specifically, we found pervasive down-regulation of the cytochrome P450 2D11 (cyp2d11), glutathione-S-transferase 1 (gst1, Fig. 4-A), sulfotransferase 1C4 (sult1c4) and ATP-binding cassette B1 (abcb1) coding genes in all micro-PS treatments compared with the control. These genes are all involved in the xenobiotic detoxification pathway. Similarly, the bile salt-activated lipase (CEL) and actin genes were also down-regulated in all MP treatments compared with the control. In addition, we found dose-specific effects on gene expression that mostly differentiated the 0.25 treatment from the 2.5 and 25 treatments. For instance, the organic cation transporter (orct) and solute carrier family 22 member 21 (slc22a21) genes, which have a fundamental role in xenobiotic excretion and energy metabolism, were down-regulated in the 0.25 treatment but up-regulated in the 2.5 and 25 treatments compared with the non-exposed control treatment. Inversely, the myeloid differentiation primary response 88 (myd88), a specific gene of the immune process, was up-regulated in the 0.25 treatment but down-regulated in the two other micro-PS treatments compared with the control (Fig. 4-C). We note that all the 19 common genes responded in a similar fashion (up- or down-regulated) in the 2.5 and 25 treatments (Table S2). The complete list of DEGs with associated log2FC values are reported in Table S3.

3.3. Differential abundance of transcripts with the lowest MP dose exposure

We found a total of 133 and 112 genes up and down-regulated, respectively, in the 0.25 treatment compared with the control (Fig. 3). Enriched GO encompassed several biological processes related to oxidoreductase activity and glutathione metabolism, including the glutathione derivative biosynthetic process (GO:1901685; log10P: −7.07, GO:1901687; log10P: −7.07, GO:0015038; log10P: −5.68), but also toxin catabolic process (GO:0009407; log10P: −5.39). Interestingly, DEGs up-regulated in the 0.25 μg L$^{-1}$ treatment only included gsto1, up-regulated, as well as two toll-like genes.

![Heatmap of differentially expressed genes (DEGs) over all micro-PS treatments. A common response between 2.5 and 25 μg L$^{-1}$ is highlighted by clusters 1 and 2 showing a similar pattern of DEGs.](image1)

![Venn diagram of differentially expressed genes (DEGs) in each condition compared to the control ([Log2FC] > 2; FDR < 0.01) and shared between up- and down-regulated genes. The total number of DEGs in micro-PS conditions amount to 245, 112 and 96 in 0.25, 2.5 and 25 μg L$^{-1}$, respectively.](image2)
receptors (tlr2 and tlr13), myd88, hsp70, and hsp71 (Fig. 4). The subset of common DEGs between the 0.25 and other micro-PS treatments provides further information on biological functions likely to be involved in the dose-specific response to micro-PS exposition. DEGs significantly enriched in treatments 0.25 and 2.5 \( \mu \text{g L}^{-1} \) included genes related to biological functions such as catalytic activity (GO: 0003824; \( \log_{10}P \): -3.64), iron ion binding (GO: 0005506; \( \log_{10}P \): -4.62), lipid metabolic process (GO: 0006629; \( \log_{10}P \): -3.63), and steroid metabolic process (GO: 0008202; \( \log_{10}P \): -3.48). The subset of common DEGs between 0.25 and 25 \( \mu \text{g L}^{-1} \) vs. the control, revealed enrichment for GOs such as transport of components like anions (GO: 0006820; \( \log_{10}P \): -3.99), ions (GO: 0006811; \( \log_{10}P \): -3.41), and nitrogen compounds (GO: 0044271; \( \log_{10}P \): -3.13). Generally, DEGs from the lowest micro-PS treatment at 0.25 \( \mu \text{g L}^{-1} \) were outliers compared to 2.5 and 25 \( \mu \text{g L}^{-1} \) (Fig. 4). Details of all GO enrichments are provided in Supplementary Table S4.

3.4. Response to maximum micro-PS concentration exposure

In 2.5 \( \mu \text{g L}^{-1} \), 59 vs. 53 genes were up- and down-regulated, respectively, compared with the control (Fig. 3). Lactase activity (GO:0000016; \( \log_{10}P \): -7.71) and glycosylceramidase activity (GO:0017042; \( \log_{10}P \): -7.71), which take part in energy metabolism, were highlighted by down-regulation of the lactase-phlorizin hydrolase (LCT) coding gene. We again found enrichment for oxidoreductase activity (GO:0016712; \( \log_{10}P \): -5.89), and heme binding (GO:0020037; \( \log_{10}P \): -5.65), which was associated with a dramatic down-regulation of several cytochrome P450-related genes such as cyp3a56, cyp17a1, cyp2f6, cyp1a1, cyp2d11, and cyp2c8. We also found significantly enriched GOs involved in cell death (GO:008219; \( \log_{10}P \): -2.30), including down-regulation.
of birc2, and myd88.

In 25 μg L⁻¹, we observed 51 vs. 45 genes up- and down-regulated, respectively, compared with the control (Fig. 3), again including cytochrome P450-related genes (cyp3a56, cyp2d11, cyp2c23, and cyp2c8) involved in oxidoreductase activity (GO:0016712; log₁₀P: -3.66). All cytochromes P450 were up-regulated, while the genes of organic anion transmembrane transporter activity (GO:0008514; log₁₀P: -3.29) (SCL family) were down-regulated. These transporter genes, together with the dbcb1 genes up-regulated in 25 μg L⁻¹ treatment, are also included in drug transmembrane transporter activity (GO:00015238; log₁₀P: -3.10). Details of all GOs enrichments are provided in Table S5 and Table S6. Finally, we noted that treatments 2.5 and 25 shared 31 common DEGs (18 up- and 13 down-regulated) with similar patterns and levels of mRNA expression. The GO enrichment analysis revealed that most of enriched functions for these common genes are linked to oxygen metabolism, including mono-oxygenase activity, oxidoreductase activity (acting on paired donors with incorporation or reduction of molecular oxygen) and heme binding.

A complementary experimentation served to detect putative changes in bacterial load and microbe's community assemblies in the mix of water and algae with or without addition of micro-PS is available in the SI file. We observed no significant difference in total bacterial load or in bacterial community across conditions (P > 0.05).

4. Discussion

The transcriptomic approach shows that energy, stress, and immune-related genes appear profoundly impacted by micro-PS exposure but that the main biominalerization-related genes (Joubert et al., 2010; Yarra et al., 2016) are not affected. However, we can not exclude the hypothesis that the overall energy deficiency induced by micro-PS exposure (Gardon et al., 2018) may ultimately lead to a lower energy investment in the biominalerization process, as already demonstrated in previous experiments manipulating temperature and food availability (Joubert et al., 2014; Le Moullac et al., 2018). How reduced energy, and the associated growth of oysters would ultimately affect pearl quality (mostly total pearl nacreous deposit) still needs to be assessed in depth since it is a major criterion for the economic sector that depends on this species.

4.1. Long-term exposure to micro-PS impairs detoxification and oxidative response in pearl oyster

Detoxification and oxidative responses became activated after 2 months of exposure to micro-PS, regardless of the concentration. We found several genes involved in xenobiotic detoxification pathways to be down-regulated in all micro-PS treatments compared with the control. Among these genes, gst, a widely-used marker of oxidative stress, was down-regulated by a micro-PS concentration as low as 0.25 μg L⁻¹. GST is usually involved in xenobiotic detoxification during phase II biotransformation, which is characterized by an oxidation by CYP450 (phase I mono-oxygenase reaction) followed by a conjugation to an anionic group (Sokolova, 2013). Similarly, several biomarker genes and biological functions affected here correspond to different physiological stages of the individuals among the MP doses tested; hence supporting the energy budget results previously obtained on the same individuals (Gardon et al., 2018). In the concept of energy-limited tolerance to stress proposed by Sokolova (2013), a gradient of conditions, ranging from optimal to lethal, are characterized by specific biomarker activities coupled to physiological status (Sokolova, 2013). We showed that, after a 2-month exposure to 0.25 μg L⁻¹ of micro-PS, pearl oysters display a significant reduction in their total energetic budget together (−48.4 J h⁻¹ g⁻¹ dw) with up-regulation of stress-related genes, including hsp70 and hsp71. HSPs are molecular chaperones responsible for protecting protein structure and functioning from oxidative stress (Portner, 2012). Elevated expression of HSPs and high energetic cost associated with protein synthesis are hallmarks of the pejus condition, characterized by a reduced fitness but positive growth and reproduction (Sokolova, 2013). Individuals exposed at 0.25 μg L⁻¹ indeed showed no significant reduction in...
gonadic status, but had lower fitness in terms of scope for growth (39.7 ± 19.9 J h⁻¹ g⁻¹ dw), which was significantly lower than controls (88.1 ± 23.1 J h⁻¹ g⁻¹ dw; Gardon et al., 2018). Hsp expression was low to absent in higher MP dose treatments. Together with the overall lower number of DEGs (mostly down-regulated), strong reduction of the overall energy budget, and certainly the oxidative stress response (through inactivation of normal detoxification response), individuals exposed to higher doses showed typical physiological and molecular signs of pessimum (or lethal) environmental conditions, representing a high or extreme degree of stress (Sokolova, 2013). Ultimately, it would not be possible to sustain such dramatic effects on individual fitness in the long term, mostly likely leading to lower survival. It is relevant to note that such results were observed in experimental conditions of abundant (ad libitum) food and oxygen supply, and constant and optimal temperature conditions. Such parameters are variable in environmental conditions, leading to a multiple stressor context with combined effects that could have a more profound impact on marine organisms (Ferreira et al., 2016; Wen et al., 2018). Thus, there is a vital need to investigate the interactions of multiple stressors (exposome) with MP exposure in the context of climate change to predict population resilience and improve conservation plans, keeping in mind that MP exposure has to be as realistic as possible, considering such aspects as shape, size, ruggedness, interaction with organic matter, and biological and chemical MP loads.

4.3. Sources of stress induced by micro-PS

Exposure to micro-PS causes common molecular stress responses, which are also observed when marine mollusks are exposed to chemical pollutants or bacterial challenges (Green et al., 2015). Because, at the higher doses, overall immune response was supposedly constrained by low energy budget, we focused on the 0.25 μg L⁻¹ treatment with the goal of disentangling the possible sources of micro-PS-related stress. We found that the signaling adaptor protein-coding gene (myd88), a central actor in the Toll Like Receptors (TLRs) pathways, was significantly up-regulated in the 0.25 μg L⁻¹ treatment. The myd88 gene is well recognized as having a crucial role in inducing activation of hemocytes and various inflammatory cytokine genes within the NF-kB pathway (Lee et al., 2011; Zhang et al., 2013; Ning et al., 2015; Xin et al., 2016). Inversely, we showed that two TLRs, including TLR13, which specifically recognizes bacterial 23S rRNA, were down-regulated in the 0.25 μg L⁻¹ micro-PS treatment. Pathogen-specific pattern recognition (e.g. lipopolysaccharide, lipoprotein, peptidoglycan, and lipoteichoic acid) by TLRs leads to pro- and anti-inflammatory cytokine gene activation, with downstream effects on inflammation response, cell proliferation and communication, and defense against bacterial and viral infections (Wang et al., 2018; Toubiana et al., 2013; Gerdol et al., 2018). Here, we detected no significant difference in total bacterial load nor diversity or variation in bacterial communities across treatments. Thus, inactivation of the TLR receptor, together with the absence of difference in bacterial load or bacterial community suggest that specific MyD88 pathways were most likely not triggered by bacterial pathogens, which therefore does not support the hypothesis of micro-PS as an aggregating structure in the present study. However, activation of myd88 indicates activity of the immune system function and is relevant in the context of the “stress-memory” immune response, as discussed by Détrière and Gallardo-Escárate (2018), who observed MyD88 only during a second exposure to polyethylene microbeads in mussel. Admittedly, our experimental design did not allow us to explore the possibility that micro-PS would impact individuals’ microbiota communities (Wu et al., 2019), or other “non-self” organisms such as virus or fungi, or the presence of a “danger-signal”, all of which would be of interest for further specific study. Furthermore, our experiment was limited to a 24-h period, but biofilms are very dynamic during first hours/days and bacterial communities might therefore require more time to stabilize (Dang and Lovell, 2000; Harrison et al., 2014). Potentially, the inactivation of detoxification process would favor the production of a damage-associated molecular pattern (DAMP) according to the model proposed by Matzinger (2002), but further in-depth mechanistic studies need to be conducted to assess the origin of MP-induced stress.

5. Conclusions

Homeostasis, stress, and detoxification showed no sign of dose-dependent responses to micro-PS concentration, but were impacted by the long-term polystyrene microbead exposure. Immune response and detoxification capacities are mostly limited and hampered by lower energetic budget in pearl oysters exposed to micro-PS. The reduction of energetic budget should increase basal metabolic rate to the detriment of other energy demanding compartments (mainly reproduction), significantly lowering Darwinian fitness. Certainly, at higher doses, physiological maintenance would become difficult. This study supports the use of the energy-limited tolerance to stress model in the context of micro-PS exposure and refine treatment-specific associated markers at molecular levels. Our work lays the foundation for exploring pathways and gene expression under experimental MP exposure in ecologically and economically important marine bivalves such as P. margaritifera to allow them to be tested in complex in situ systems. Finally, this study also highlights possible divergence in markers response across studies focusing on physiological impacts of micro-plastics; ideally, a comprehensive approach, including genes expression, protein activities and deeper knowledge on bivalve’s immunity would serve our understanding of MP-associated risk in marine ecosystems.

CRediT authorship contribution statement

Tony Gardon: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization. Lucie Morvan: Formal analysis, Writing - original draft, Visualization. Arnaud Huvet: Writing - original draft. Virgile Quillien: Methodology, Resources. Claude Soyez: Methodology, Resources. Gilles Le Moullac: Conceptualization, Methodology, Validation, Project administration, Funding acquisition. Jeremy Le Luyer: Conceptualization, Software, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Supervision.

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.

Acknowledgments

This study was a part of the MICROLAG project funded by Ifremer and the Direction des Ressources Marines (DRM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at
https://doi.org/10.1016/j.envpol.2020.115180.

References

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq—a python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169. https://doi.org/10.1093/bioinformatics/btv341.

Araújo, R.F.F.de, Martins, D.B.G., Borba, M.A.C.S.M., 2016. Oxidative stress and disease. In: Morales-Gonzalez, J.A., Morales-Gonzalez, A., Madrild-Santillan, E.O. (Eds.), A Master Regulator of Oxidative Stress - the Stress Response Factor Nrf2. Intech. https://doi.org/10.5772/65366.

Avio, C.G., Gorbi, S., Milan, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics in mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics in mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

Bersolbatch, B.C., Paital, B., Dandapat, J., 2016. An overview of seasonal changes in D. magna. Environ. Sci. Technol. 52, 5277–5286. https://doi.org/10.1021/acs.est.6b00168.

Gerdol, M., Gomez-Chiarri, M., Castillo, M.C., Figueras, A., Fiorito, G., Moreira, R., Neves, M.D., Pallavicino, A., Neves, P., Rossi, E., 2014. Microplastics in mussel (Mytilus galloprovincialis) biomass: recognition and effector mechanisms, with a focus on Bivalvia. In: Cooper, E.L. (Ed.), Advances in Comparative Immunology. Springer International Publishing, Cham, pp. 225–341. https://doi.org/10.1007/978-3-319-15601-4_11.

GESAMP, 2015. Sources, Fate and Effects of Microplastics in the Marine Environment: a Global Assessment [WWW Document]. URL: http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/GESAMP_Marine_environmental_sources.pdf accessed 15 October 2014.

Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinoidea) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 368, 22–29. https://doi.org/10.1016/j.jembe.2008.05.007.

Green, T.J., Rafato, D., Speck, T.S., Thistle, D., 2015. Antiviral immunity in marine mussels. J. Gen. Virol. 96, 2471–2482. https://doi.org/10.1099/jgv.0.000244.

Guderley, H., Pörtner, H.O., 2010. Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. Can. J. Zool. 88, 753–763. https://doi.org/10.1139/cjz2009-035. The present review is one of a series of occasional review articles that have been invited by the Editors and will feature the broad range of disciplines and expertise represented in our Editorial Advisory Board.

Harrison, J.P., Schratzberger, M., Sapp, M., Osborn, A.M., 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. BMC Microbiol. 14, 232. https://doi.org/10.1186/1471-2180-14-232.

Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press.

Hörak, P., Cohen, A., 2010. How to measure oxidative stress in an ecological context: methodologies and statistical issues. Funct. Ecol. 24, 968–970. https://doi.org/10.1111/j.1365-2435.2010.01755.x.

Isaksson, C., 2010. Pollution and its impact on wild animals: a meta-analysis on oxidative stress. EcoHealth 7, 342–350. https://doi.org/10.1007/s10393-010-0100-2.

Jambek, R.J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2013. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/science.1260352.

Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S., Hwang, U.-K., Zhou, B., Souissi, S., Lee, S.-J., Lee, J.-S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-ASK activation in the monogonont rotifer (Bruchionus plicatilis). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01410.

Joubert, C., Linard, C., Le Moullac, G., Soyez, C., Saulnier, D., Teanuiaritamaoana, V., Ky, C.L., Gueguen, Y., 2014. Temperature and food influence shell growth and mantel gene expression of shell matrix proteins in the pearl oyster Pinctada margaritifera. PLoS One 9, e309344. https://doi.org/10.1371/journal.pone.0109344.

Joubert, C., Piquemal, D., Marie, B., Manchon, L., Piret, F., Zanella-Cléon, I., Cocherelle-Laureau, N., Gueguen, Y., Montagnani, C., 2010. Transcriptome and proteome analysis of Pinctada margaritifera calcifying mantle and shell: focus on biominalization. BMC Genom. 11, 613. https://doi.org/10.1186/1471-2164-11-613.

Klenow, D.V., Zhang, L., Pedersen, B.S., Ramírez, F., Warwick Vesztrocy, A., 2015. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). Environ. Sci. Technol. 49, 5026–5031. https://doi.org/10.1021/acs.est.5b02648.

Klopfenstein, D.V., Zhang, L., Pedersen, B.S., Ramírez, F., Warwick Vesztrocy, A., 2015. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). Environ. Sci. Technol. 49, 5026–5031. https://doi.org/10.1021/acs.est.5b02648.

Kolde, R., Kolde, M., 2015. Pigeon Plasmatic. Le Loyer, J., Auffret, P., Quillivic, Y., Reiser, C., Vital-Dupiol, J., Ky, C.-L., 2019. Whole transcriptome sequencing and biominalization gene architecture associated with cultured pearl quality traits in the pearl oyster, Pinctada margaritifera. BMC Genom. 20. https://doi.org/10.1186/s12864-019-5443-5.

Le Moullac, G., Chabuck, S., Chabard, S., Belliard, C., Lyonard, P., Broustal, F., Soyez, C., Saulnier, D., Braham, C., Ky, C.-L., Bellafi, B., 2018. Influence of temperature and pearl ontogeny on biominalization in the pearl oyster, Pinctada margaritifera. J. Exp. Biol. 221, jeb186858. https://doi.org/10.1242/jeb.186858.

Linard, C., Gueguen, Y., 2015. Whole transcriptome sequencing and biominalization gene architecture associated with cultured pearl quality traits in the pearl oyster, Pinctada margaritifera. BMC Genom. 20. https://doi.org/10.1186/s12864-019-5443-5.

Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus. J. Exp. Mar. Biol. Ecol. 47, 11278–11285. https://doi.org/10.1016/j.jembe.2013.06.015.

Love, E., Whang, I., Umasuthan, N., De Zoya, M., Oh, C., Kang, D.-H., Choi, C.Y., 2010. Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. Can. J. Zool. 88, 753–763. https://doi.org/10.1139/cjz2009-035. The present review is one of a series of occasional review articles that have been invited by the Editors and will feature the broad range of disciplines and expertise represented in our Editorial Advisory Board.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with Deseq2. Genome Biol. 15, 550. https://doi.org/10.1186/1471-2105-15-550.

Luchian, K.H., Clark, M.S., Rainy, A.C.D., Gilbert, J.A., Craft, J.A., Chipman, J.K., 2018. Transcriptome and biomineralization gene architecture associated with cultured pearl quality traits in the pearl oyster, Pinctada margaritifera. BMC Genom. 20. https://doi.org/10.1186/s12864-019-5443-5.
