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

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ABSTRACT

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.

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 (Jambeck 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 Cauwenberghe 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 (Franzellitti 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 Cauwenberghe et al., 2015; Watts et al., 2016; Rist et al., 2016), and assimilation efficiency, with consequences for

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energy balance (Blarer and Burkhardt-Holm, 2016; Gardon 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., 2018), specifically the antioxidant system, lysosomal compartment, and peroxisomal proliferation (Avio et al., 2015), as well as apoptotic processes in mussel (Détrée and Gallardo-Escárate, 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 Pörtner, 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 (Hõrak 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 during exposure to pollutants such as MP (Isaksson, 2010; Paul-Pont et al., 2016; Détrée and Gallardo-Escárate, 2018). Nevertheless, contrasting observations across experiments are often reported for these biomarkers (for instance). Thus, an integrative approach, based on the concept of energy-limited tolerance to stress (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 (Bernagout 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 biomineralization 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) biomineralization 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} (3.2×10^2, $\times 10^3$, $\times 10^4$ 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 2.10×10^8 (2.5% w/v, 5 ml) and 4.55×10^7 (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 metabarcoding 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 Bio-Analyzer 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/vecscreen/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 (Klopfenstein 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 19.21 ± 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 only nine

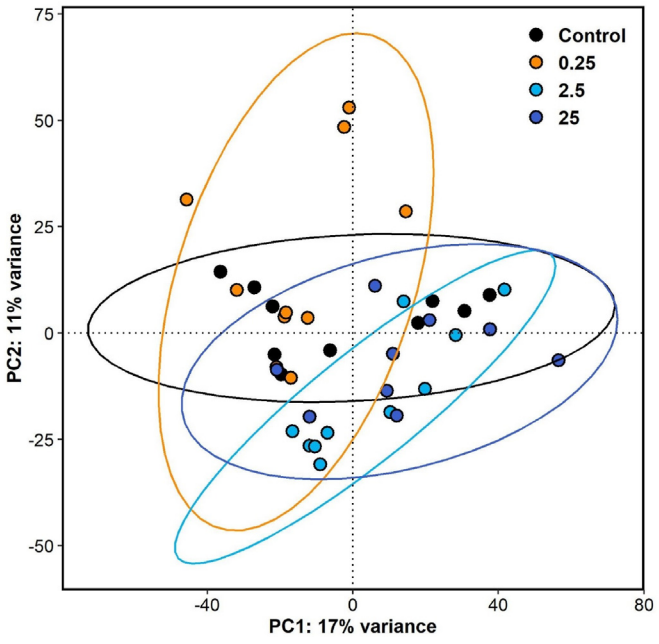

Fig. 1. PCA plot of individuals with 95% confident interval ellipses for each micro-PS concentration (0.25, 2.5 and 25 μg L^{-1}) and the control; n = 10 individuals per treatment, except for 25 μg L^{-1} where n = 9.

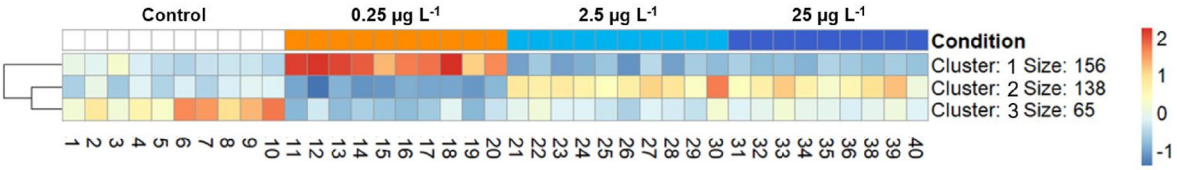

Fig. 2. Heatmap of differentially expressed genes (DEGs) over all micro-PS treatments. A common response between 2.5 and 25 µg L^{-1} is highlighted by clusters 1 and 2 showing a similar pattern of DEGs.

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 processed related to oxidoreductase activity and glutathione metabolism, including the glutathione derivative biosynthetic process (GO: 1901685; $log_{10}P$: −7.07, GO:1901687; $log_{10}P$: −7.07, GO:0015038; $log_{10}P$: −5.68), but also toxin catabolic process (GO:0009407; $log_{10}P$: −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

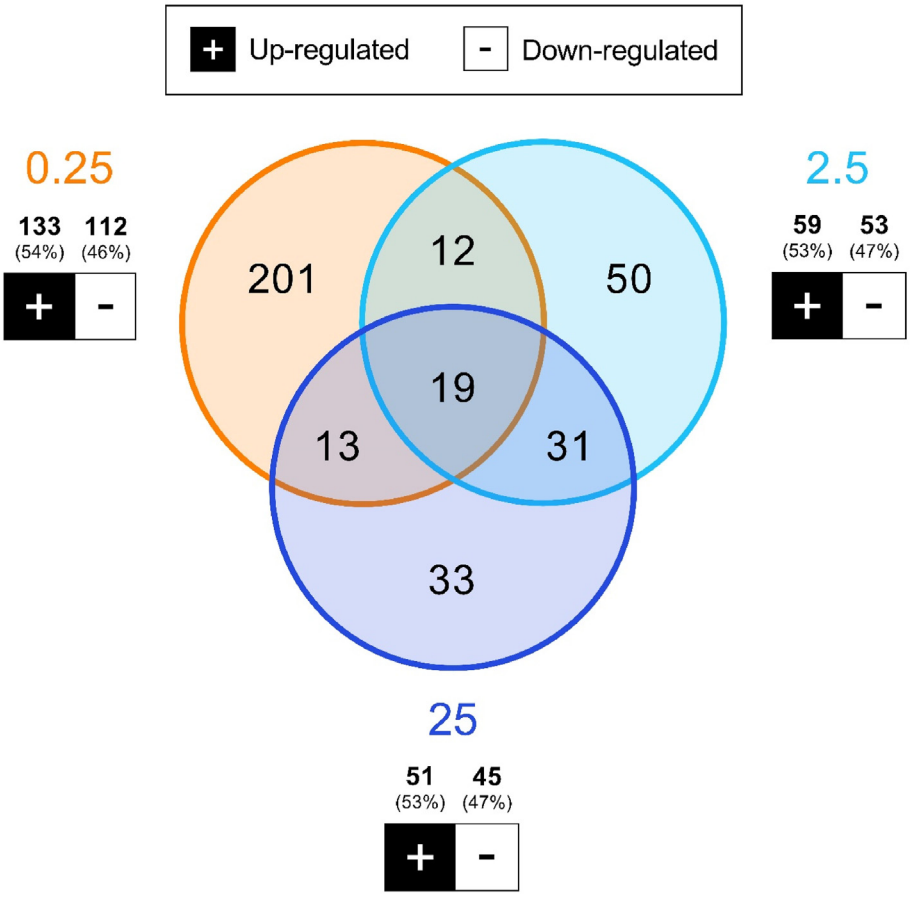

Fig. 3. Venn diagram of differentially expressed genes (DEGs) in each condition compared to the control (|Log2FC| > 2; FDR < 0.01) and shared between up- and down-regulated genes. The total number of DEGs in micro-PS conditions amount to 245, 112 and 96 in 0.25, 2.5 and 25 µg L^{-1}, respectively.

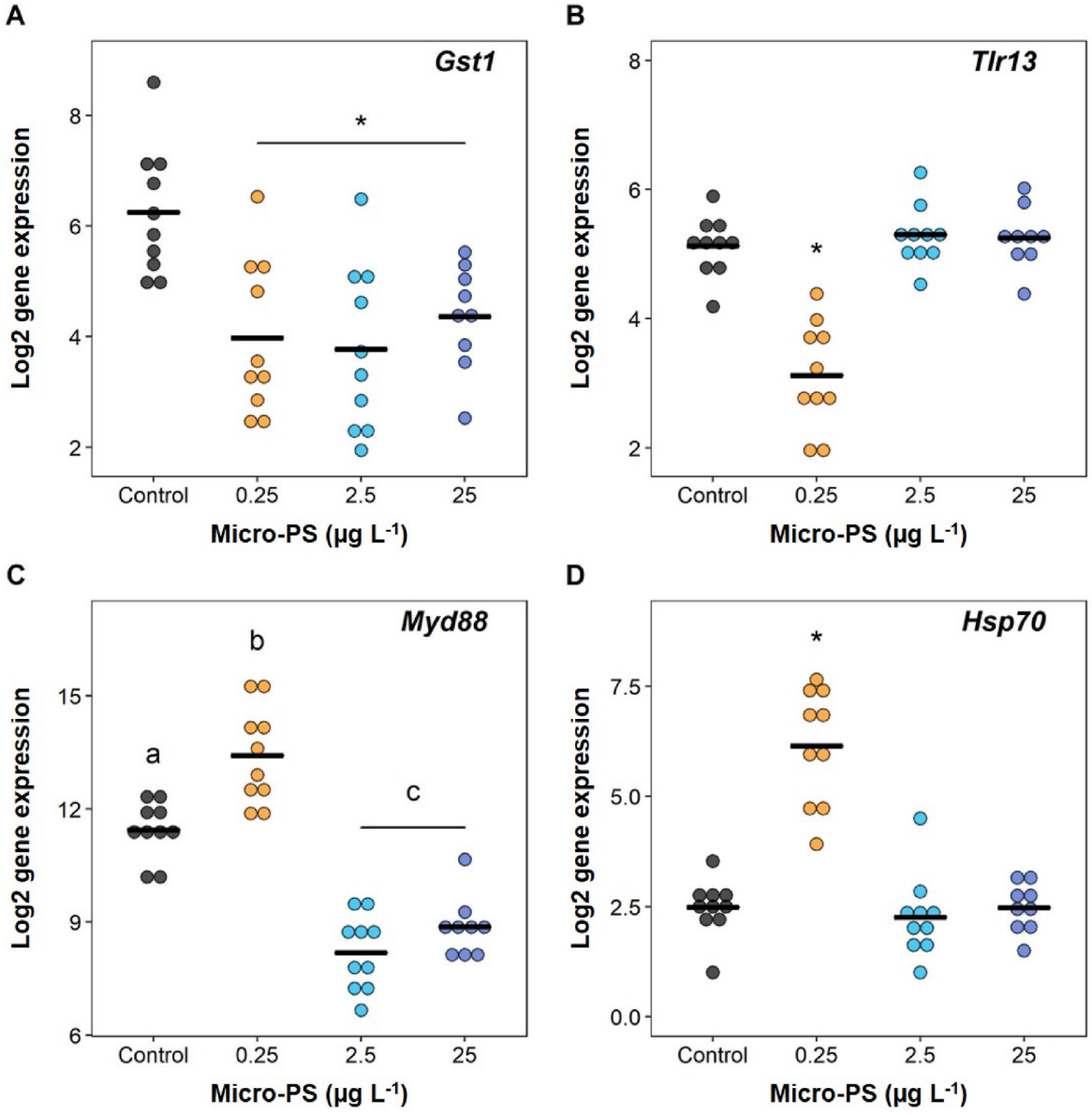

Fig. 4. Dot plots of the expression of genes involved in stress and immune response in the pearl oyster *Pinctada margaritifera* after a 2-month exposure to micro-PS. (A) glutathione-S-transferase 1 (*gst1*), (B) toll-like receptor 13 (*tlr13*), (C) myeloid differentiation primary response 88 (*myd88*), and (D) heat shock protein 70 (*hsp70*). Significant differences between control and treatments (|Log2FC| > 2 and FDR < 0.01) are represented by an asterisk ("*") or with letters when several inter-groups differences occurred.

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 µg 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 µg 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 µg L^{-1} were outliers compared to 2.5 and 25 µg 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 µg 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*, *cyp2j6*, *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; $log_{10}P$: −3.66). All cytochromes P450 were up-regulated, while the genes of organic anion transmembrane transporter activity (GO:0008514; $log_{10}P$: −3.29) (SLC family) were down-regulated. These transporter genes, together with the *abcb1* genes up-regulated in 25 μg L^{-1} treatment, are also included in drug transmembrane transporter activity (GO:0015238; $log_{10}P$: −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 biomineralization-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 biomineralization 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 inactivated 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 like GST, or SULT which usually creates a more water-soluble compound (Wang et al., 2018). Some GSTs are central actors in the elimination of reactive oxygen species (ROS), which show an increased production during aerobic cell activity following environmental stress and that have detrimental consequences for host homeostasis and metabolic balance (Donaghy et al., 2015; Manduzio et al., 2015). Our results contrast in some ways with recent findings in *Mytilus galloprovincialis*, where up-regulation of *gst* was shown in mantle tissue only after a second 18-day exposure to micro-PS at 30 μg L^{-1} (Détrée and Gallardo-Escárate, 2018). This difference should not come as such a surprise since *gst* expression and GST enzyme activities show large variations (including seasonal variation; Chainy et al., 2016) and major discrepancies of response across experiments (Paul-Pont et al., 2016; Wang et al., 2016; Ribeiro et al., 2017). A recent meta-analysis on the impact of pollution on oxidative stress in marine organisms showed that GST activity tends to be reduced in response to pollutants (Isaksson, 2010). Hence, while short-term exposure to MP and other pollutants might trigger GST gene expression or activity (Lüchmann et al., 2015; Milan et al., 2016; Pessatti et al., 2016), their inactivation here (compared with the control) should lead to the incapacity of individuals to maintain proper detoxification pathways in the long run, most likely resulting in chronic oxidative stress and oxidative damage (Araújo et al., 2016). One reasonable hypothesis for such impairment is the progressive dose-dependent energy budget decline (ranging from −48.4 to −101.3 J h^{-1} g^{-1} dw compared to control) in pearl oysters exposed to micro-PS (Gardon et al., 2018) leading to a transition towards stress conditions in the pessimum range (Sokolova, 2013). Ideally, further data on downstream genes as well as protein activity would confirm our observations. However, even lower doses ultimately reduce individual fitness, as no compensatory mechanisms are satisfactory with regard to total energy budget (39.7 ± 19.9 J h^{-1} g^{-1} dw), which prompts questions concerning population resilience. Our results also highlight the importance of interpreting biomarkers such as detoxification activity in the context of individual energetic potential, enabling conclusions to be drawn about the biological effect of stressful conditions. Similarly, to properly quantify the effect of any stressor, at both individual and population levels, we stress the need for long-term experiments coupled with non-a priori transcriptomic approaches for the integration of these techniques in ecotoxicology. This would allow the detection of potential acclimation mechanisms or simple accumulative effects, enabling their interpretation to be disentangled from limited short-term stress responses.

4.2. Response to micro-PS considering the energy tolerance to stress model

Overall, transcriptomic activity and breakdowns in stress-specific response were observed between lower and higher micro-PS doses (0.25 μg L^{-1} *vs.* 2.5 and 25 μg L^{-1}). We noticed that the overall number of differentially expressed genes relative to the control was more than double in the 0.25 μg L^{-1} treatment (n = 245) compared with the higher micro-PS treatments (2.5 μg L^{-1}, n = 112 and 25 μg L^{-1}, n = 96). 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 (Pörtner, 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 mostly 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 Détrée 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. **Jérémy 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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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/btu638.

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., Madrigal-Santillan, E.O. (Eds.), A Master Regulator of Oxidative Stress - the Transcription Factor Nrf2. InTech. https://doi.org/10.5772/65366.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

Bernagout, S., Bouisset, P., Leclerc, G., Rua, J., Nohl, M., Garen, P., Levy, P., Tetaura, N.-L., Poroi, D., Sham Koua, M., Liao, V., Bosserelle, P., Planes, S., Espiau, B., 2014. Réseau de surveillance des polluants anthropiques dans les lagons de Polynésie française. Rapport final - Septembre 2014. Contrat de projet Etat-Pays. https://archimer.ifremer.fr/doc/00249/35975/.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343. https://doi.org/10.1021/es503001d.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600. https://doi.org/10.1021/es302763x.

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. 23, 23522–23532. https://doi.org/10.1007/s11356-016-7584-2.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42, 5026–5031. https://doi.org/10.1021/es800249a.

Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C.S., Fabbri, E., 2018. Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. Environ. Pollut. 241, 1038–1047. https://doi.org/10.1016/j.envpol.2018.06.035.

Chainy, G.B.N., Paital, B., Dandapat, J., 2016. An overview of seasonal changes in oxidative stress and antioxidant defence parameters in some invertebrate and vertebrate species. Scientifica 2016 (1–8). https://doi.org/10.1155/2016/6126570.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.

Collignon, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864. https://doi.org/10.1016/j.marpolbul.2012.01.011.

Dang, H., Lovell, C.R., 2000. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. Appl. Environ. Microbiol. 66, 467–475. https://doi.org/10.1128/AEM.66.2.467-475.2000.

Détrée, C., Gallardo-Escárate, C., 2018. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel *Mytilus galloprovincialis*. Fish Shellfish Immunol. 83, 52–60. https://doi.org/10.1016/j.fsi.2018.09.018.

Donaghy, L., Hong, H.-K., Jauzein, C., Choi, K.-S., 2015. The known and unknown sources of reactive oxygen and nitrogen species in haemocytes of marine bivalve molluscs. Fish Shellfish Immunol. 42, 91–97. https://doi.org/10.1016/j.fsi.2014.10.030.

Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32, 3047–3048. https://doi.org/10.1093/bioinformatics/btw354.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.

Ferreira, P., Fonte, E., Soares, M.E., Carvalho, F., Guilhermino, L., 2016. Effects of multi-stressors on juveniles of the marine fish Pomatoschistus microps: gold nanoparticles, microplastics and temperature. Aquat. Toxicol. 170, 89–103. https://doi.org/10.1016/j.aquatox.2015.11.011.

Franzellitti, S., Capolupo, M., Wathsala, R.H.G.R., Valbonesi, P., Fabbri, E., 2019. The multixenobiotic resistance system as a possible protective response triggered by microplastic ingestion in Mediterranean mussels (*Mytilus galloprovincialis*): larvae and adult stages. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 219, 50–58. https://doi.org/10.1016/j.cbpc.2019.02.005.

Gardon, T., Reisser, C., Soyez, C., Quillien, V., Le Moullac, G., 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. Environ. Sci. Technol. 52, 5277–5286. https://doi.org/10.1021/acs.est.8b00168.

Gerdol, M., Gomez-Chiarri, M., Castillo, M.G., Figueras, A., Fiorito, G., Moreira, R., Novoa, B., Pallavicini, A., Ponte, G., Roumbedakis, K., Venier, P., Vasta, G.R., 2018. Immunity in molluscs: 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/10.1007/978-3-319-76768-0_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_microplastics%20full%20study.pdf (accessed 15.04.19.).

Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 368, 22–29. https://doi.org/10.1016/j.jembe.2008.09.007.

Green, T.J., Raftos, D., Speck, P., Montagnani, C., 2015. Antiviral immunity in marine molluscs. 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/Z10-039. 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/s12866-014-0232-4.

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

Hõrak, P., Cohen, A., 2010. How to measure oxidative stress in an ecological context: methodological and statistical issues. Funct. Ecol. 24, 960–970. 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/s10393-010-0345-7.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. 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-p38 activation in the monogonont rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441.

Joubert, C., Linard, C., Le Moullac, G., Soyez, C., Saulnier, D., Teaniniuraitemoana, V., Ky, C.L., Gueguen, Y., 2014. Temperature and food influence shell growth and mantle gene expression of shell matrix proteins in the pearl oyster *Pinctada margaritifera*. PloS One 9, e103944. https://doi.org/10.1371/journal.pone.0103944.

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

Klopfenstein, D.V., Zhang, L., Pedersen, B.S., Ramírez, F., Warwick Vesztrocy, A., Naldi, A., Mungall, C.J., Yunes, J.M., Botvinnik, O., Weigel, M., Dampier, W., Dessimoz, C., Flick, P., Tang, H., 2018. GOATOOLS: a Python library for Gene Ontology analyses. Sci. Rep. 8, 10872. https://doi.org/10.1038/s41598-018-28948-z.

Kolde, R., Kolde, M., 2015. Package Pheatmap.

Le Luyer, J., Auffret, P., Quillien, V., Leclerc, N., Reisser, C., Vidal-Dupiol, J., Ky, C.-L., 2019. Whole transcriptome sequencing 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.

Le Moullac, G., Schuck, L., Chabrier, S., Belliard, C., Lyonnard, P., Broustal, F., Soyez, C., Saulnier, D., Brahmi, C., Ky, C.-L., Beliaeff, B., 2018. Influence of temperature and pearl rotation on biomineralization in the pearl oyster, *Pinctada margaritifera*. J. Exp. Biol. 221, jeb186858. https://doi.org/10.1242/jeb.186858.

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*. Environ. Sci. Technol. 47, 11278–11283. https://doi.org/10.1021/es401932b.

Lee, Y., Whang, I., Umasuthan, N., De Zoysa, M., Oh, C., Kang, D.-H., Choi, C.Y., Park, C.-J., Lee, J., 2011. Characterization of a novel molluscan MyD88 family protein from manila clam, *Ruditapes philippinarum*. Fish Shellfish Immunol. 31, 887–893. https://doi.org/10.1016/j.fsi.2011.08.003.

LeMoine, C.M.R., Kelleher, B.M., Lagarde, R., Northam, C., Elebute, O.O., Cassone, B.J., 2018. Transcriptional effects of polyethylene microplastics ingestion in developing zebrafish (*Danio rerio*). Environ. Pollut. 243, 591–600. https://doi.org/10.1016/j.envpol.2018.08.084.

Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., Soudant, P., 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. 228, 454–463. https://doi.org/10.1016/j.envpol.2017.05.047.

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/s13059-014-0550-8.

Lüchmann, K.H., Clark, M.S., Bainy, A.C.D., Gilbert, J.A., Craft, J.A., Chipman, J.K.,

Thorne, M.A.S., Mattos, J.J., Siebert, M.N., Schroeder, D.C., 2015. Key metabolic pathways involved in xenobiotic biotransformation and stress responses revealed by transcriptomics of the mangrove oyster *Crassostrea brasiliana*. Aquat. Toxicol. 166, 10–20. https://doi.org/10.1016/j.aquatox.2015.06.012.

Manduzio, H., Rocher, B., Durand, F., Galap, C., Leboulenger, F., 2015. The point about oxidative stress in molluscs. Invertebr. Surviv. J. hal 32.

Matzinger, P., 2002. The danger model: a renewed sense of self. Science 296, 301–305. https://doi.org/10.1126/science.1071059.

Milan, M., Matozzo, V., Pauletto, M., Di Camillo, B., Giacomazzo, M., Boffo, L., Binato, G., Marin, M.G., Patarnello, T., Bargelloni, L., 2016. Can ecological history influence response to pollutants? Transcriptomic analysis of Manila clam collected in different Venice lagoon areas and exposed to heavy metal. Aquat. Toxicol. 174, 123–133. https://doi.org/10.1016/j.aquatox.2016.02.024.

Mohsen, M., Wang, Q., Zhang, L., Sun, L., Lin, C., Yang, H., 2019. Microplastic ingestion by the farmed sea cucumber *Apostichopus japonicus* in China. Environ. Pollut. 245, 1071–1078. https://doi.org/10.1016/j.envpol.2018.11.083.

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032.

Nevo, E., 2011. Evolution under environmental stress at macro- and microscales. Genome Biol. Evol. 3, 1039–1052. https://doi.org/10.1093/gbe/evr052.

Ning, X., Wang, R., Li, X., Wang, S., Zhang, M., Xing, Q., Sun, Y., Wang, S., Zhang, L., Hu, X., Bao, Z., 2015. Genome-wide identification and characterization of five MyD88 duplication genes in Yesso scallop (*Patinopecten yessoensis*) and expression changes in response to bacterial challenge. Fish Shellfish Immunol. 46, 181–191. https://doi.org/10.1016/j.fsi.2015.06.028.

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.-L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus spp.* to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737. https://doi.org/10.1016/j.envpol.2016.06.039.

Paul-Pont, I., Tallec, K., González-Fernández, C., Lambert, C., Vincent, D., Mazurais, D., Zambonino-Infante, J.-L., Brotons, G., Lagarde, F., Fabioux, C., Soudant, P., Huvet, A., 2018. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Front. Mar. Sci. https://doi.org/10.3389/fmars.2018.00252.

Pessatti, T.B., Lüchmann, K.H., Flores-Nunes, F., Mattos, J.J., Sasaki, S.T., Taniguchi, S., Bícego, M.C., Dias Bainy, A.C., 2016. Upregulation of biotransformation genes in gills of oyster *Crassostrea brasiliana* exposed *in-situ* to urban effluents, Florianópolis Bay, Southern Brazil. Ecotoxicol. Environ. Saf. 131, 172–180. https://doi.org/10.1016/j.ecoenv.2016.04.003.

Pörtner, H., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar. Ecol. Prog. Ser. 470, 273–290. https://doi.org/10.3354/meps10123.

Pouvreau, S., Jonquières, G., Buestel, D., 1999. Filtration by the pearl oyster, *Pinctada margaritifera*, under conditions of low seston load and small particle size in a tropical lagoon habitat. Aquaculture 176, 295–314. https://doi.org/10.1016/S0044-8486(99)00102-7.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122, 379–391. https://doi.org/10.1016/j.marpolbul.2017.06.078.

Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. Mar. Pollut. Bull. 111, 213–220. https://doi.org/10.1016/j.marpolbul.2016.07.006.

Rochman, C.M., Browne, M.A., Underwood, A.J., van Franeker, J.A., Thompson, R.C., Amaral-Zettler, L.A., 2016. The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. Ecology 97, 302–312. https://doi.org/10.1890/14-2070.1.

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013.

Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. 53, 597–608. https://doi.org/10.1093/icb/ict028.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Tang, J., Ni, X., Zhou, Z., Wang, L., Lin, S., 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. Environ. Pollut. 243, 66–74. https://doi.org/10.1016/j.envpol.2018.08.045.

Toubiana, M., Gerdol, M., Rosani, U., Pallavicini, A., Venier, P., Roch, P., 2013. Toll-like receptors and MyD88 adaptors in *Mytilus*: complete cds and gene expression levels. Dev. Comp. Immunol. 40, 158–166. https://doi.org/10.1016/j.dci.2013.02.006.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008.

Wang, L., Peng, Y., Nie, X., Pan, B., Ku, P., Bao, S., 2016. Gene response of CYP360A, CYP314, and GST and whole-organism changes in *Daphnia magna* exposed to ibuprofen. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 179, 49–56. https://doi.org/10.1016/j.cbpc.2015.08.010.

Wang, L., Song, X., Song, L., 2018. The oyster immunity. Dev. Comp. Immunol. 80, 99–118. https://doi.org/10.1016/j.dci.2017.05.025. Aqua species immunity.

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026.

Watts, A.J.R., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016. Effect of microplastic on the gills of the shore crab *Carcinus maenas*. Environ. Sci. Technol. 50, 5364–5369. https://doi.org/10.1021/acs.est.6b01187.

Wen, B., Zhang, N., Jin, S.-R., Chen, Z.-Z., Gao, J.-Z., Liu, Y., Liu, H.-P., Xu, Z., 2018. Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. Aquat. Toxicol. 195, 67–76. https://doi.org/10.1016/j.aquatox.2017.12.010.

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013a. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013b. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Wu, T.D., Reeder, J., Lawrence, M., Becker, G., Brauer, M.J., 2016. GMAP and GSNAP for genomic sequence alignment: enhancements to speed, accuracy, and functionality. In: Mathé, E., Davis, S. (Eds.), Statistical Genomics: Methods and Protocols, Methods in Molecular Biology. Springer New York, New York, NY, pp. 283–334. https://doi.org/10.1007/978-1-4939-3578-9_15.

Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., Wang, Y., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res. 165, 114979. https://doi.org/10.1016/j.watres.2019.114979.

Xin, L., Wang, M., Zhang, H., Li, M., Wang, H., Wang, L., Song, L., 2016. The categorization and mutual modulation of expanded MyD88s in *Crassostrea gigas*. Fish Shellfish Immunol. 54, 118–127. https://doi.org/10.1016/j.fsi.2016.04.014.

Yarra, T., Gharbi, K., Blaxter, M., Peck, L.S., Clark, M.S., 2016. Characterization of the mantle transcriptome in bivalves: *Pecten maximus, Mytilus edulis* and *Crassostrea gigas*. Mar. Genomics 27, 9–15. https://doi.org/10.1016/j.margen.2016.04.003. Cells to Shells: The genomics of mollusc exoskeletons.

Zhang, Y., He, X., Yu, F., Xiang, Z., Li, J., Thorpe, K.L., Yu, Z., 2013. Characteristic and functional analysis of toll-like receptors (TLRs) in the lophotrocozoan, *Crassostrea gigas*, reveals ancient origin of TLR-mediated innate immunity. PloS One 8, e76464. https://doi.org/10.1371/journal.pone.0076464.