Oyster reproduction is affected by exposure to polystyrene microplastics

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Plastics are persistent synthetic polymers that accumulate as waste in the marine environment. Microplastic (MP) particles are derived from the breakdown of larger debris or can enter the environment as microscopic fragments. Because filter-feeder organisms ingest MP while feeding, they are likely to be impacted by MP pollution. To assess the impact of polystyrene microspheres (micro-PS) on the physiology of the Pacific oyster, adult oysters were experimentally exposed to virgin micro-PS (2 and 6 μm in diameter; 0.023 mg·L−1) for 2 mo during a reproductive cycle. Effects were investigated on ecophysiological parameters: cellular, transcriptomic, and proteomic responses; fecundity; and offspring development. Oysters preferentially ingested the 6-μm micro-PS over the 2-μm-diameter particles. Consumption of microalgae and absorption efficiency were significantly higher in exposed oysters, suggesting compensatory and physical effects on both digestive parameters. After 2 mo, exposed oysters had significant decreases in oocyte number (−38%), diameter (−5%), and sperm velocity (−23%). The D-larval yield and larval development of offspring derived from exposed parents decreased by 41% and 18%, respectively, compared with control offspring. Dynamic energy budget modeling, supported by transcriptomic profiles, suggested a significant shift of energy allocation from reproduction to structural growth, and elevated maintenance costs in exposed oysters, which is thought to be caused by interference with energy uptake. Molecular signatures of endocrine disruption were also revealed, but no endocrine disruptors were found in the biological samples. This study provides evidence that micro-PS cause feeding modifications and reproductive disruption in oysters, with significant impacts on offspring.

Plastic production is continually increasing, with 299 million metric tons produced in 2013 and estimations of 33 billion tons for 2050 (1). Plastic waste entering the oceans was calculated for 2010 at 4–12 million tons per year (2). The consequences of macroplastic debris for wildlife are becoming well documented (3). Microplastic (MP) particles, defined as plastic particles smaller than 5 mm (4), derived from the fragmentation of larger debris (5, 6) or enter the environment directly as microscopic fragments (7). MP pollution in the world’s oceans has been recently estimated at over 5 trillion floating particles, corresponding to 250,000 tons (8).

Given the ubiquitous nature and small dimensions of MP (9), their ingestion and subsequent impact on marine life is a growing cause for concern, notably for suspension filter-feeding species, which filter large water volumes and may ingest large quantities of particles (10–13). Effects of MP ingestion have already been studied in several filter-feeding species, such as mussels (14–17), sea cucumbers (18), lugworms (13, 19), and some zooplankton (20–22). These studies mainly showed a reduction of feeding activity (19), reserve depletion (13), inflammatory responses (15, 17), and translocation of MP into the circulatory system (14, 17). Effects on fitness have been reported, with decreases in survival and fecundity in copepods (20, 22) and reproductive disruption in Daphnia (21). At cellular and molecular levels, alterations of immunological responses, neurotoxic effects, and the onset of genotoxicity have been observed in mussels exposed to polycyclic aromatic hydrocarbon-contaminated polystyrene particles (17). Additional impacts may arise from harmful plastic additives and persistent organic pollutants adsorbed on MP, which are known to be taken up and accumulated by living organisms (23).

In this study, the effects of MP exposure were assessed on reproductively active Crassostrea gigas adults and their offspring. The Pacific oyster was chosen because of its world-wide production, economic importance as seafood, and important role in estuarine and coastal habitats (24). A 2-mo exposure of adult oysters to microsized polystyrene spheres (micro-PS, 2 and 6 μm, 0.023 mg·L−1) was performed under controlled conditions suitable for germ-cell maturation. Polystyrene is one of the most commonly used plastic polymers worldwide, often found in microplastics sampled at sea (25, 26). In our study, toxic endpoints were investigated through an integrative approach, covering data from molecular and cellular parameters to ecophysiological behavior and energy budget modeling. Our results show that experimental microplastic | reproduction | energy allocation | oyster

Significance

Plastics are a contaminant of emerging concern accumulating in marine ecosystems. Plastics tend to break down into small particles, called microplastics, which also enter the marine environment directly as fragments from a variety of sources, including cosmetics, clothing, and industrial processes. Given their ubiquitous nature and small dimensions, the ingestion and impact of microplastics on marine life are a cause for concern, notably for filter feeders. Oysters were exposed to polystyrene microparticles, which were shown to interfere with energy uptake and allocation, reproduction, and offspring performance. A drop in energy allocation played a major role in this reproductive impairment. This study provides ground-breaking data on microplastic impacts in an invertebrate model, helping to predict ecological impact in marine ecosystems.


The authors declare no conflict of interest.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE71845).

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micro-PS exposure on adult oysters affects feeding, absorption efficiency, gamete quality, and fecundity, as well as impacting offspring growth.

Results

Ingestion and Fate of Micro-PS. Average daily ingestion of micro-PS particles was 14 ± 2% of the 2-μm particles and 69 ± 6% of the 6-μm particles supplied. From histological analysis, micro-PS particles were only detected in the stomach and intestine (Fig. 1) and did not reveal cellular inflammatory features in exposed animals.

Algal Consumption, Absorption Efficiency, and Growth. Over the whole experiment, algal consumption was 4.30 × 10^6 ± 9.05 × 10^5 μm³ of algae per oyster−1·h−1 with micro-PS and 4.26 × 10^6 ± 1.05 μm³ of algae per oyster−1·h−1 for the control. The two-way ANOVA revealed significantly higher algal consumption for exposed oysters (+3%, P < 0.01), a significant date effect, and a date-exposure interaction (P < 0.001). Absorption efficiency was 51.8 ± 7.2% and 46.6 ± 7.9% on average for micro-PS and control treatments, respectively. The two-way ANOVA revealed significantly higher absorption efficiency for exposed oysters (+11%, P < 0.01). A significant date effect was observed (P < 0.001). No significant difference in condition index was observed between exposed and control oysters (0.09 ± 0.01 and 0.10 ± 0.01, respectively).

Hemocyte Counts and Morphological and Functional Characteristics. Hyalinocytes and granulocytes were larger in exposed oysters (+6.7% and +16.1%, respectively) than in controls (P < 0.001) (Fig. S1). Significant interactions between date and exposure factors were found in oxidative activity for both hemocyte populations (P < 0.01). The post hoc test indicated that oxidative activity was higher in exposed oysters than in controls at T1 (+54% on average for both hemocyte types) and was lower at T2 (−31%) and T3 (−29.1%).

Reproduction, Gamete Quality, and Larval Development. Histological examination at T3 revealed that all control and exposed oysters were in stage 3, corresponding to ripeness.

For females, the total number of oocytes collected by stripping and oocyte diameter were significantly lower in exposed females than controls (−38%, P < 0.01 and −5%, P < 0.05, respectively). Total numbers of oocytes were 3.5 × 10^6 ± 1.2 for the exposed females and 8.3 × 10^6 ± 2.5 for controls. Oocyte diameter was 30.6 ± 0.9 μm for exposed females and 32.2 ± 1.1 μm for the control females. As an oocyte quality proxy, D-larval yield was estimated after making crosses by mixing oocytes collected from exposed and control females with control spermatozoa. A significant reduction in D-larval yield was observed in exposed females (29.6 ± 0.3%) compared with control females (49.8 ± 1.6%).

For males, significantly lower sperm velocity (−23%, P < 0.05) was observed in exposed individuals (59.5 ± 14.5 μm·s−1, P < 0.05) compared with controls (77.5 ± 9.3 μm·s−1). The percentage of motile sperm was similar between the two treatments, 40 ± 16% and 51 ± 11% for exposed and control males, respectively.

Finally, the larval growth was significantly slower (P < 0.001) (Fig. 2) in progeny issued from exposed genitors than in progeny issued from control genitors. A mean reduction in size of 18.6% was observed at 17 d postfertilization: mean shell length was 279.8 ± 12.5 μm for control progeny and 227.5 ± 8.5 μm for progeny issued from exposed genitors, for which a 6-d lag in time to metamorphosis was observed.

Transcriptomic and Proteomic Analyses. In the digestive gland, 76 transcripts were differentially expressed between exposed and control oysters (P < 1.10^−8, false-discovery rate (FDR) < 5%) (Dataset S1) and 1,206 transcripts were differentially expressed between sampling times T1 and T3 (P < 0.01, FDR < 5%). Two clusters of transcripts with similar expression patterns, down-regulated (cluster 1, n = 51) and up-regulated (cluster 2, n = 25), were revealed in exposed digestive glands compared with controls (Fig. S2). Response to gluocorticoid stimulus, fatty acid catabolic processes, respiratory burst, and cellular response to mechanical stimulus were the main significantly enriched Gene Ontology (GO) biological processes.

In gonads, 46 transcripts were differentially expressed between exposed and control oysters (P < 0.01, FDR < 5%) (Dataset S2), and 8,136 between the sampling time T1 and T3 (P < 1.10^−8, FDR < 5%). Two distinct clusters with similar expression patterns were found, with transcripts down-regulated (cluster 1, n = 31) and up-regulated (cluster 2, n = 15) in exposed gonads compared with controls (Fig. S2). Glutamine biosynthetic processes, positive regulation of insulin secretion, positive regulation of epithelial cell proliferation, and ovarian follicle cell-cell adhesion were among the significantly enriched GO biological processes.

In oocytes, 81 transcripts were differentially expressed between the two treatments (P < 0.01, FDR < 5%) (Dataset S3); 41 transcripts appeared to be down-regulated (cluster 1, n = 41) and 40 up-regulated (cluster 2, n = 40) in oocytes collected from exposed females compared with controls (Fig. S2). Proteolysis, embryo development, and ion binding were some of the enriched GO biological processes. Finally, the proteome of oocytes revealed two abundant protein spots that showed a marked difference in expression levels between exposed and control oysters (P < 0.001, FDR < 5%) (Dataset S3); 1.10 ± 0.10 μm/s was observed in exposed oocytes compared with 0.7 ± 0.05 μm/s in control oocytes.
between exposed and control samples. These two spots were identified as arginine kinase, characterized by a lower amount in oocytes collected from exposed females, and the protein sevenerin, which was present in a higher amount in oocytes collected from exposed females than in oocytes collected from controls.

**Dynamic Energy Budget Model Simulations.** Control oysters were simulated with standard dynamic energy budget (DEB)-model parameters (action of energy used for growth plus somatic maintenance, \( \kappa = 0.45 \), and volume-specific cost of maintenance \( [p_M] = 44 \text{ J cm}^{-3} \text{d}^{-1} \)) and with the absorption efficiency measured in the control (Fig. 3, “control”). Exposed oysters were simulated with standard DEB model parameters and the absorption efficiency measured for this condition (Fig. 3, “micro-PS,std”). Simulated relative differences in final dry flesh mass (DFM) and oocyte production were overestimated compared with values observed at T3. To make the model parameters fit with observed DFM and oocyte production, numerous simulations were performed with a set of parameter values (\( \kappa \) from 0 to 1 and \([p_M]\) from 0 to 200 \text{ J cm}^{-3} \text{d}^{-1} \)). The best fit between observations and simulations (Fig. 3, “micro-PS,cal”) was reached with a single set of the two parameters \( \kappa = 0.77 \) and \([p_M] = 84 \text{ J cm}^{-3} \text{d}^{-1} \), which corresponds to increases of 71% and 90% beyond standard values, respectively.

**Chemical Analysis.** Following methods described in the Supporting Information, analyses on extracted micro-PS particles detected bibenzyl and 1(2H)napthalenone,3,4-dihydro4phenyl with >90% correspondences (Fig. S3). Analyses in the aqueous phase or digestive styles did not show any molecules leaching from micro-PS particles compared with the controls, with a detection limit at 0.1 ng L\(^{-1}\) for compounds with a log \( K_{ow} \) less than 3.

**Discussion**

**Ingestion and Fate of Micro-PS in Oyster.** Micro-PS were efficiently ingested by filtration in oysters, presumably because of their similarity in size and shape to phytoplankton. Oysters preferentially ingested the 6-\( \mu \)m micro-PS over the 2-\( \mu \)m-diameter particles. This result may be explained by the oyster particle selection mechanism, which is 100% efficient for 5- to 6-\( \mu \)m particles (27). Ingested micro-PS particles were visually observed in feces (under microscope) and no accumulation in the gut was observed on histological slides, suggesting a high potential of egestion of micro-PS. However, smooth and spherical micro-PS beads differ greatly from plastic debris, such as the fibers and fragments of varying form and roughness present in the marine environment. Therefore, caution must be taken when extrapolating the rapid egestion rate observed here (28). Despite evidence of MP translocation in bivalves from some other studies (14, 15, 17), here no evidence of micro-PS transfer from the digestive tract to the circulatory system and other tissues was detected on the histological slides. Future studies on marine bivalves should address translocation processes by testing nonspherical fragments down to nano-sized particles, the size class most prone to this phenomenon via transcellular uptake in the gastrointestinal epithelium in mammals (29).

**Impacts of Micro-PS on Energy Uptake and Allocation.** Consumption of microalgae and absorption efficiency appeared significantly higher in exposed oysters, suggesting a compensatory effect on food intake and absorption efficiency and an enhancement of mechanicanal digestion. Indeed, an improvement of mechanical disruption in the stomach of mussels was demonstrated in response to moderate silt ingestion, which enhances clearance rate and absorption efficiency (30). Nevertheless, increased food consumption can be viewed as compensation to adjust energy intake in response to digestive interference caused by micro-PS in the gut. The variations in mRNA levels of lipid-related proteins, such as enzymes involved in fatty acid oxidation, also suggest impairment of fatty acid metabolism and reduced energy intake from food (31). In any case, this compensation is insufficient to counterbalance the energy-flow disruption induced by micro-PS uptake as demonstrated by DEB modeling. Energy flows seem to shift toward organism maintenance and structural growth at the expense of reproduction. A recent study on mussels revealed increased energy consumption measured by respiration in MP-exposed animals, suggesting increased stress and energy demand to maintain homeostasis (16). Furthermore, in our data, there are signs of disturbance of homeostasis reflected by changes in hemocyte size and oxidative activity (32), and enrichment of transcripts involved in the response to glucocorticoid stimulus GO process. Glucocorticoids are hormonal corticostereoids involved in stress response, able to inhibit the expression of enzymes involved in fatty acid oxidation (33, 34).

**Micro-PS Impaired Gametogenesis, Gamete Quality, and Fecundity.** Strong negative effects were observed on reproductive health indices, which significantly impacted fecundity and offspring performance during larval stages. The 23% reduction in sperm velocity in exposed oysters may lower their ability to fertilize oocytes. Indeed, in sea urchin a decrease in sperm motility was linked to an increase in the number of sperm required for fertilization success (35). Oyster oocyte number and size in micro-PS-exposed oysters were...
also significantly reduced over the same period (−38% and −5%, respectively). As oocyte quality predictors, mean oocyte diameter has been identified as a direct consequence of nutrition (36), supporting the hypothesis of energetic disruption in exposed oysters. Moreover, egg size and shape have been found to vary significantly over the same period (20), and in vertebrates for ATP buffering on phosphagens, which are essential for cell proliferation, and the arginine kinase protein, responsible in invertebrates for larval yield, offspring growth, and settlement, was observed for larvae produced from gametes collected after dichloromethane extraction. Bibenzyl-diol core molecules may potentially act as endocrine disruptors. The micro-PS concentration tested in the present study was below the one estimated in Besseling et al. (21) that may occur at the sediment–water interface, where wild oysters live (Table S1). The exposed mass concentration (0.023 mg L−1) was also in the range of the highest estimated field concentration >333 μm, from manta trawl sampling (Table S1), based on the assumption of a steady fragmentation of plastic debris (9, 51). It should, nonetheless, be noted that there is a lack of consistent field evaluations of the presence of microplastics as compared to those used in the present study. This is mainly because of methodological limitations: current methods exclude the possibility of quantifying small size domains [reviewed by Filella (51)]. Moreover, assuming no waste management infrastructure available to enter the marine environment from land is predicted to increase by an order-of-magnitude by 2025 (2), especially in estuaries and coastal waters where oysters live and where waters are greatly influenced by increased human expansion. Therefore, our study also contributes to an early warning system and provides stakeholders with the necessary data to limit the impact of the microplastics legacy in decades to come.

To conclude, this study highlighted microplastic impacts on energy uptake and allocation and on reproductive health indices (i.e., quantity and quality of gametes produced), when oysters were exposed to micro-PS during gametogenesis. Strong negative effects were shown on broodstock fecundity and offspring growth at larval stages. The two explanatory hypotheses discussed in the present paper, a fall in energy allocated to reproduction may potentially affect recruitment of wild and farmed populations of Pacific oysters, with consequences for both ecology and aquaculture.

We hypothesize that the energetic disruption allocated to reproduction seemed to shift toward structural growth and high maintenance costs. Disruption of energy balance may result from the down-regulation of several transcripts coding for proteins involved in the insulin pathway, with GO terms corresponding to cell proliferation and differentiation processes, in both digestive gland and gonads. The insulin pathway plays a crucial role in mobilizing reserves during gametogenesis, and has an essential role in germinal cell proliferation and maturation (43). We thus hypothesize that micro-PS exposure negatively impacts cell proliferation and differentiation processes in gonads through the down-regulation of transcripts coding for proteins involved in the insulin signaling pathway in exposure to micro-PS.

Methods

Experimental Exposure of Adult Oysters to Micro-PS. The experimental procedures comply with French law and with institutional guidelines. Adult oysters purchased from a commercial hatchery (18 mo, 16.9 ± 5.3 g) were transferred to Institute Français de Recherche pour l’Exploitation de la Mer’s experimental facilities in March 2013. Histological visual inspection showed they were at reproductive stage 0 to early stage 1, corresponding to an average weight oyster. Control and micro-PS exposed treatments were set up with tanks per condition. For each treatment, a fourth tank was deployed without oysters to evaluate algal and micro-PS sinking or sticking to the tank walls. To prevent micro-PS sinking, the water inflow was pressurized to a concentration >333 μm, from manta trawl sampling (Table S1), based on the assumption of a steady fragmentation of plastic debris (9, 51). It should, nonetheless, be noted that there is a lack of consistent field evaluations of the presence of microplastics as compared to those used in the present study. This is mainly because of methodological limitations: current methods exclude the possibility of quantifying small size domains [reviewed by Filella (51)]. Moreover, assuming no waste management infrastructure available to enter the marine environment from land is predicted to increase by an order-of-magnitude by 2025 (2), especially in estuaries and coastal waters where oysters live and where waters are greatly influenced by increased human expansion. Therefore, our study also contributes to an early warning system and provides stakeholders with the necessary data to limit the impact of the microplastics legacy in decades to come.

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The purchased micro-PS were yellow-green fluorescent polystyrene beads (2 and 6 μm; Polysciences). These were supplied continuously to the tanks by in-glass flask on a magnetic stirrer. Micro-PS concentrations were daily counted on an EasyCyte Plus flow cytometer (Guava-Merck-Millipore) giving an in-flow concentration of $2,062 \pm 170$ and $118 \pm 15$ beads per mL$^{-1}$ for 2- and 6-μm particles, respectively (namely a mass concentration of 0.023 mg·L$^{-1}$) corresponding to an inflow daily flow of 9.6 mg micro-PS·L$^{-1}$. The mass concentration in the surrounding water was of 0.01 mg L$^{-1}$ (i.e., 1.86 ± 0.76 and 21 ± 6 beads per mL$^{-1}$ for 2- and 6-μm particles, respectively), which is far lower than most to which marine invertebrates have been exposed (from 0.8 to 2,500 mg·L$^{-1}$) (10, 15, 17, 21) (Table S1). Microparticle concentration corresponded to 0.21% of the volume (μm$^3$) of algae supplied.

Ecophysiological Measurements. Once a day, inflow and outflow seawater was sampled from each tank. Phytoplankton counts were made using an electronic particle counter (Multiziser 3 equipped with a 100-μm aperture tube) to provide 50 of continuous data. Algal consumption (C) was expressed in algal cell volume per oyster per day (μm$^3$ oyster$^{-1}$·day$^{-1}$); as in Savina and Pavon (53). Polysyrte particle ingestion (I) was measured in percentage micro-PS ingested: $I = \frac{[I - I_o]}{I_o} \times 100$, I being number of beads at the inlet, $I_o$ number of beads at the outlet, $I_o$ being number of beads remaining in the tank without oysters by subtracting inlet from outlet. Once a week, three samples were collected from each tank to calculate particle consumption efficiency (absorption efficiency, percent) of organic matter from ingested food (53).

Sampling. At the beginning and the end of the experiment, 12 oysters per condition were killed to measure bimetric parameters (total, shell, and dry weight). Condition index was calculated as: dry weight/total weight-shell weight. At 2, 5, and 8 wk after the beginning of exposure (corresponding to T1, T2, and T3, respectively), eight animals per tank were sampled for fresh weight, hemolymph [taken as described by Haberkorn et al. (54)], and a transversal section of the gonadic area for histological examination. The remainder of the gonad and digestive gland were immediately frozen in liquid nitrogen for subsequent analyses. Oocytes were collected from five females per treatment, filtered in a 40-μm sieve, counted and transferred into 1.5 mL Extract-all reagent (Eurobio) (20,000 oocytes) and 5 mL lysis buffer (55) (200,000 oocytes) for RNA isolation and protein analyses, respectively. For gamete quality measurements and larval rearing, gametes were collected at T3 in nine control and nine exposed animals of each sex by stripping the gonads.

Dynamic Energy Budget Design. The DEB model simulations were performed as in Bernard et al. (63) to evaluate how physiological changes induced by micro-PS exposure affect energy fluxes and could explain observed phenotypic changes. The DEB model describes dynamics of four state variables: (i) energy allocated to development and reproduction, ER; and (ii) energy for maintenance, $\kappa$; (iii) energy stored in reserves, $E_{so}$; and (iv) energy used in the construction of gametes, $E_{G}$ (see ref. 63 for a full description). Initial state was obtained from the initial bimetrics measurements and maturity observations. Oocyte production was calculated according to an energy content of 9.3 × 10$^{-1}$ J·oocyte$^{-1}$ (18). Oocyte size was calculated using the allocation fraction to structural growth and structural maintenance from reserves (the remaining being allocated to development/production and maturity maintenance, $\kappa$) and the volume specific cost for maintenance rate ($\kappa$ cm$^{-3}$·day$^{-1}$), which were fitted to evaluate the disturbance level in terms of micro-PS exposure that would lead to the observed growth and reproductive traits.

Statistical Analysis. All analysis data were processed and analyzed using the language RBioConductor (60), R Development Core Team (2008) by ANOVA. The fixed factors for the two-way ANOVA were treatment (MP exposure vs. control) and sampling time (T1 or T3). For oocytes, differentially expressed transcripts were detected by t-test. The FDR associated with the selected transcripts was determined by [total number of analyzed transcripts (31,918) × P-value/number of differentially expressed transcripts] × 100; the FDR cut-off value was 5%. Hierarchical clustering was performed using the Ward method, and 1-correlation as dissimilarity matrix. Putative annotations of transcripts were identified using ngKlast software (KL Korilog Bioinformatics Solutions) against a protein database (E-value 1.0 × 10$^{-4}$) obtained from the C. gigas sequenced genome and the GO database (Gene Ontology). GO terms were obtained from the Swissprot database (E-value 1.0 × 10$^{-3}$). GO terms enrichment analysis was performed using the Fisher’s Exact test on Blast2Go (62).

Statistical Analysis. All analysis data were processed and analyzed using the language RBioConductor (60), R Development Core Team (2008) by ANOVA (fixed factors were condition and sampling date) or t test. Normality was screened on residuals and further tested using the Shapiro-Wilk test. When necessary, data were log-transformed, and angular transformation was used for percentage data. Homogeneity of variance matrices was assessed with a Fligner test. Least-significant difference post hoc tests were performed to discriminate groups. Data are expressed as mean ± confidence intervals (α = 5%). Analyses of microarray data are detailed above in the microarray data analysis section.

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Methods of Micro-PS Chemical Analysis

Organic molecules leaching from micro-PS were measured either by: (i) dichloromethane extraction, (ii) in seawater, or (iii) in digestive styles by Stir Bar Sorptive Extraction (SBSE) (64). Micro-PS particles were extracted for 30 min using dichloromethane (Carlo Erba), then filtered with 0.7 μm GF/F WHATMAN filters. Extracted particles were analyzed by liquid injection in pulsed splitless mode at 300 °C, as described in Lacroix et al. (64), operating the mass spectrometer in scan mode. For the seawater samples, micro-PS were put in 100 mL seawater for 24 h at a similar experimental concentration, and SBSE were stirred in solution at 700 rpm for 2 h. For digestive styles, two pools of five styles collected in adult oysters were analyzed (control sample containing 10 μL Milli-Q water 0.1% Tween-20 and a sample containing 6,107 mL⁻¹ of micro-PS particles in addition). Samples were incubated at room temperature for 24 h on an oscillating mixer, then 5 mL of reverse-osmosis water were added to the sample and SBSE were introduced for a 16 h period of stirring at 700 rpm. For both the aqueous phase and digestive styles, SBSE were removed from the solution, rinsed with reverse osmosis water, dried and placed on the automatic sampler to thermally desorb the compounds before GC/MS analysis following Lacroix et al. (64), using an Agilent 5975 Mass Selective Detector in scan mode.

Fig. S1. Boxplots of oyster hemocyte parameters showing significant condition effect or condition–time interaction in the two-way ANOVA. T1, sampling time 1 (2 wk of micro-PS exposure); T2, sampling time 2 (5 wk); T3, sampling time 3 (8 wk); MP, micropolystyrene exposed oysters; C, control oysters (n = 24). Letters represent statistically different groups calculated by the least significant difference post hoc test.
Fig. S2. Heatmaps of differentially expressed transcripts in female oyster digestive glands (A), gonads (B), and oocytes (C). For A and B, columns represent the averaged mRNA levels for each group (n = 5–8; T1 = sampling time 1, 2 wk of exposure; T3 = sampling time 3, 8 wk of exposure; MP = oysters exposed to polystyrene microbeads, T = control oysters). For C, individual samples are presented corresponding to oocytes collected in three exposed and five control females. Expression levels are shown with a color scale in which shades of red represent higher expression and shades of green represent lower expression.
Fig. S3. Micro-PS chemical analysis. Scan chromatogram of micro-PS extracted with dichloromethane (A). Superimposed scan chromatograms of control digestive styles (black) and digestive styles with micro-PS particles (red) (B).
<table>
<thead>
<tr>
<th>Source</th>
<th>In situ location or experiment*</th>
<th>Size</th>
<th>Plastic type and polymer†</th>
<th>Mass concentration in standard unit mg L(^{-1})</th>
<th>Mass concentration in original unit‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>(65)</td>
<td>California current system</td>
<td>&gt;333 (\mu)m</td>
<td>Fragments</td>
<td>5.33</td>
<td>732 g km(^{-2})</td>
</tr>
<tr>
<td>(66)</td>
<td>South Pacific Gyre</td>
<td>&gt;333 (\mu)m</td>
<td>Fragments, lines, films spheres,</td>
<td>0.073(^3)</td>
<td>30,169 g km(^{-2})</td>
</tr>
<tr>
<td>(67)</td>
<td>North Pacific Central Gyre</td>
<td>&gt;333 (\mu)m</td>
<td>Fragments, lines, films</td>
<td>3.02(^3)</td>
<td></td>
</tr>
<tr>
<td>(68)</td>
<td>Danube(^*)</td>
<td>&gt;500 (\mu)m</td>
<td>Pellets, flakes, spherules</td>
<td>0.697</td>
<td></td>
</tr>
<tr>
<td>(69)</td>
<td>India, Sediment</td>
<td>&gt;0.45 (\mu)m</td>
<td>Fragments, PU, PA, PS, PES</td>
<td>162(^{ll})</td>
<td>89 mg kg(^{-1}) dry weight</td>
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<td>(10)</td>
<td>Exp, mussel</td>
<td>30 nm</td>
<td>Spheres, PS</td>
<td>100–200–300</td>
<td></td>
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<tr>
<td>(15)</td>
<td>Exp, mussel</td>
<td>1–80 (\mu)m</td>
<td>Particles, HDPE</td>
<td>2,500</td>
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<tr>
<td>(17)</td>
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<td>Particles, PS, PE</td>
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<td>(21)</td>
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<td>(\sim)70 nm</td>
<td>Spheres, PS</td>
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<tr>
<td>Present study</td>
<td>Exp, oyster</td>
<td>2 and 6 (\mu)m</td>
<td>Spheres, PS</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

*Exp indicates experimental study, for which targeted species was added.
†HDPE: high-density polyethylene particle; PA: polyamides (nylon); PE: polyethylene; PES: polyester; PS: polystyrene; PU: polyurethane.
‡When estimates in mg L\(^{-1}\) were calculated, original published data are given.
§For these two manta trawl samplings, mass concentration was estimated on reported concentrations in g km\(^{-2}\), with an estimated trawling depth of 0.01 m, as done by Besseling et al. (21).
\(^{ll}\)Estimated to directly enter the Black Sea, considered as a proxy of the concentration in the Danube estuary, where there are populations of wild oysters. 
\(^{ll}A\) concentration in pore water of 162 mg L\(^{-1}\) was estimated by Besseling et al. (21), based on a mean concentration of 89 mg kg\(^{-1}\) dry sediment (69) and with a sediment density of 2 kg L\(^{-1}\) and a water content of 50% on mass basis. This estimate can go up to 780 mg L\(^{-1}\) for the highest reported concentration in sediment (i.e., 391 mg kg\(^{-1}\) dry sediment) (70).

Dataset S1. Differentially expressed transcripts in female oyster digestive glands between micro-PS and control treatments: GenBank accession, best hit, fold-changes, heatmap clusters, and GO enriched terms

Dataset S2. Differentially expressed transcripts in female oyster gonads between micro-PS and control treatments: GenBank accession, best hit, fold-changes, heatmap clusters, and GO enriched terms

Dataset S3. Differentially expressed transcripts in oocytes between micro-PS and control treatments: GenBank accession, best hit, fold-changes, heatmap clusters, and GO enriched terms