Environmental Science & Technology
April 2018, Volume 52, Issue 9, Pages 5277-5286
http://dx.doi.org/10.1021/acs.est.8b00168
http://archimer.ifremer.fr/doc/00435/54684/

Microplastics Affect Energy Balance and Gametogenesis in the Pearl Oyster *Pinctada margaritifera*

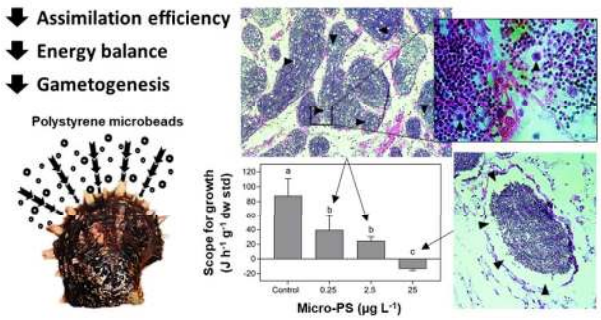
Gardon Tony [1], Reisser Celine [1], Soyez Claude [1], Quillien Virgile [1], Le Moullac Gilles [1]

[1] Ifremer, UMR Ecosystèmes Insulaires Océaniens (EIO), UMR 241 UPF/ILM/IRD/Ifremer, Centre du Pacifique, BP 49, 98719, Taravao, French Polynesia

Abstract :

Plastic pollution in the environment is increasing at global scale. Microplastics (MP) are derived from degradation of larger plastic items or directly produced in microparticles form (<5 mm). Plastics, widely used in structures and equipments of pearl farming, are a source of pollution to the detriment of the lagoon ecosystem. In order to evaluate the impact of MP on the physiology of *Pinctada margaritifera*, a species of ecological and commercial interests, adult oysters were exposed to polystyrene microbeads (micro-PS of 6 and 10 µm) for 2 months. Three concentrations were tested: 0.25, 2.5, 25 µg L-1 and a control. Ingestion and respiration rate and assimilation efficiency were monitored on a metabolic measurement system to determine the individual energy balance (Scope For Growth, SFG). Effects on reproduction were also assessed. The assimilation efficiency decreased significantly according to micro-PS concentration. The SFG was significantly impacted by a dose-dependent decrease from 0.25 µg L-1 ($p < 0.0001$). A negative SFG was measured in oysters exposed to 25 µg L-1. Gonads may have provided the missing energy to maintain animals' metabolism through the production of metabolites derived from germ cells phagocytosis. This study shows that micro-PS significantly impact the assimilation efficiency and more broadly the energy balance of *P. margaritifera*, with negative repercussions on reproduction.

TOC ART



INTRODUCTION

Through their immense potential and countless properties, plastics hold a highly prevalent place in contemporary society. Plastic production increased dramatically worldwide over the last 60 years, increasing from 0.5 million tons per year in 1960 to 322 million tons in 2015.[1] About 5-10% of this annual plastic production is rejected in the marine environment,[2] and their degradation can take several hundred years.[3] Microplastics (MP, plastic particles <5 mm),[4] can be defined as primary MP or secondary MP, depending on their origins.[3] The majority of MP found in the oceans are secondary MP produced through the fragmentation of larger items under the action of biotic (i.e., biodegradation) and abiotic factors (e.g., photolysis, hydrolysis, thermooxidative and thermal degradation).[5] By opposition, primary MP are directly released into the environment as microparticles, and come from industrial sources (e.g., toothpaste, manufactured products from cosmetic industry, or macroplastic manufacturing base).[5]

Given their ubiquitous nature and small dimensions, MP are likely to be ingested by many organisms[3,6–9] inducing various physical and biological effects,[10] especially in several filter-feeding species such as bivalves,[11–14] sea cucumbers,[15] lugworm,[16,17] and zooplankton.[18–21] MP ingestion substantially impacts key physiological functions such as nutrition,[18,22,23]

assimilation efficiency,[24] respiration,[25] reproduction,[14,26] growth,[23] and survival.[25,27] For example, in a chronic 4-weeks exposure to microfibers (1-5 mm in length, 1% plastic diet), the crab *Carcinus maenas* showed reduced food consumption (from 0.33 to 0.03 g d^{-1}) and a significant reduction in energy available for growth (scope for growth) from 0.59 to -0.31 kJ crab d^{-1}.[23] In addition to physical effects (e.g., induction of a false sense of satiety, alteration of feeding capacity, digestive tract obstruction),[18,19,22] ingested MP can also result in reserves depletion,[17] hepatic stress,[28] and reduced fecundity.[19,20] At the cellular and molecular level, MP can be translocated in the circulatory system,[11] induce oxidative stress,[21] neurotoxic effects, genotoxicity and alter immunological responses[13] after desorption of persistent organic pollutants.[29,30]

While the quantification of MP in seawater is very difficult, their potential ingestion by species of economical and commercial interest is a cause for concern. This is especially true for bivalves, since many are world-wide exploited species, and are suspension filter-feeding organisms, directly exposed to MP present in the water column.[12] A recent study examined the effects of MP on the Pacific oyster, *Crassostrea gigas*, by exposing the animals to fluorescent polystyrene microbeads (micro-PS of 2 and 6 μm diameter, 23 μg L^{-1}) over a 2-months period.[14] This study revealed a significant impact of MP on the reproduction of exposed oysters through a marked decrease in fertility (-41%), oocyte number (-38%) and size (-5%); as well as a decline in spermatozoa mobility (-23%). Furthermore, larvae produced by exposed oysters showed a delayed growth of about 20%.[14] This recent work highlights the reprotoxic effects of MP on *C. gigas*, and a transgenerational effect by altering the development of the next generation of individuals produced by breeders exposed to MP. The dose tested in this study (i.e., 23 μg L^{-1}) referring to a mass concentration in the range of the highest estimated field concentration >333 μm, from manta trawl sampling in North Western Mediterranean Sea.[31] Although this dose is likely to be higher than the mean concentrations

encountered in the natural environment (even if environmental doses are unknown at this range of sizes,[32] i.e. 2 and 6 μm), the long-term consequences of MP are of concern for oyster related economy.

In French Polynesia (FP), pearl farming occupies an important part in the economical, environmental and social landscape of the territory. The pearl oyster, *Pinctada margaritifera*, is a tropical species of economical interest for the trade of pearls and mother-of-pearl. It is also a species of ecological interest and serves as a biological model for lagoon ecosystems and environmental survey. Indeed, as most of filter-feeding organisms, oyster plays a predominant role in the benthic and pelagic ecosystems junction by exercising a local control on nutrient cycle and primary productivity.[33] In comparison with other bivalves, *P. margaritifera* has particularly high filtration rates, and can filter up to 26 L seawater per hour.[34] It can feed on a wide range of particle sizes (2 to 200 μm), with an optimal retention rate for particles of 5 μm and over.[34] As many filter-feeding species, the pearl oyster removes particulate matter from water column and transfers them to the sediment as biodeposits. Biodeposition is probably a key mechanism by which neutral buoyancy MPs are transported from water column to sediments.[35] The pearl oyster could therefore affect MP distribution and concentration, which could alter algal communities and benthic macrofauna,[36] and thus disrupt the biological balance of lagoon ecosystem.

In recent years, the economic decline of pearl farming led to concessions closures, which are sometimes abandoned with all the remaining structures still in the lagoon. In addition, operational concessions are also leaving their inoperative farming structures in the lagoon, by drowning them,[37] generating its own risk regarding the impact of MP on the local environment and economy. The inventory carried out by Andréfouët *et al.* in the pearl lagoon of Ahe atoll (FP) revealed large quantities of synthetic ropes, collectors, fencing, buoys and nylon cords.[37] These breeding structures, essentially made of plastics, could represent a

considerable source of MP, and more so considering the semi-closed environment of some cultured lagoons which could favour MP concentration.[37] The related proximity to the South Pacific subtropical gyre may also contribute to MP contamination in French Polynesia waters.[38] A recent study has estimated a mean MP concentration of 0.74 pieces m^{-2} surface area in Moorea (FP) water column.[39] Although MP contamination of Polynesian waters also likely comes from open sea and local industries, pearl farming might be generating its own risk regarding the impact of MP on the local environment and economy.

This study investigated the impacts of polystyrene microbeads (micro-PS 6 and 10 µm) on *P. margaritifera* by evaluating their effects on the individual energy budget, which gathers the ingestion, respiration and assimilation efficiency responses. Effects on reproduction were also discussed by the analysis of individual reproductive effort and the gametogenesis condition. Three main questions were posed: (1) what is the effect of microplastics on the physiology of the pearl oyster? (2) How is the energy managed and allocated between the basal metabolic rate and the reproduction? (3) Are the oyster's responses dose-dependent, and is there a threshold? To meet these objectives and target a response window with dose-effect relationship, a 2-month exposure of adult oysters to 3 micro-PS doses (0.25, 2.5 and 25 µg L^{-1}) was performed under controlled conditions. To our knowledge, this is one of the first studies investigating a specific dose-effect of microplastics on the energy available for growth (scope for growth) and reproduction in exposed marine organisms.

MATERIALS AND METHODS

Experimental animals and acclimation. Pearl oysters were sampled on October 17, 2016 in a pearl farm located in the Arutua atoll (15°14′43″S; 146°36′43″O), in the Tuamotu archipelago (French Polynesia, FP). A stock of approximately 350 adult oysters (1-1.5 years old; height, 5.9 ± 0.41 cm; weight, 25.2 ± 4.9 g, mean ± SD) were transferred (transfer

119 authorization No. 3761 issued by the Ministry of Marine Resources of French Polynesia) to
120 the lagoon of Vairao (Ifremer marine concession No. 8120/MLD: 17°48′26.0″S,
121 149°18′14.4″W, Tahiti, FP) on October 19, 2016. Before being packaged and transferred, all
122 oysters were carefully inspected, cleaned and treated with saline water to remove parasites.

123 **Polystyrene microbeads.** Polystyrene is one of the most commonly used plastic polymers
124 worldwide, often found in MP sampled in marine ecosystems.[3] Dependent on the optimum
125 retention rate of pearl oysters, we used unlabeled polystyrene microbeads (micro-PS) with
126 diameters of 6 and 10 µm, purchased from Polyscience (Polybead®, Washington, PA, U.S.).
127 Micro-PS of 6 and 10 µm were packaged in aqueous solution (Milli-Q® water) at a
128 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}
129 respectively. The two solutions were mixed in order to obtain a stock solution (10 mL)
130 containing the two microbead diameters at equal weight. Polymer type of virgin micro-PS
131 was confirmed using a Raman micro-spectroscopy analysis (Figure SI.1 in the Supporting
132 Information). A fragment of additive-free reference polystyrene supplied by GoodFellow
133 Cambridge Ltd (Lille, France) was used as spikes for the spectra comparison and
134 measurements were carried out using a LabRAM HR800 Raman micro-spectrometer (Horiba
135 Scientific), equipped with a Horiba Scientific ParticleFinder module for LabSpec6.

136 ***In vivo* exposure.** To assess the dose-effect of MP on the physiology and reproduction of
137 *P. margaritifera*, oysters were exposed to 3 doses of micro-PS over a 2-month period: 0.25,
138 2.5, 25 µg L^{-1} (3.2×10^{2}, $\times10^{3}$, $\times10^{4}$ particles L^{-1}, respectively) and were compared to a control
139 (0 µg L^{-1}) (Figure 1). After acclimation (2 weeks of depuration), oysters were conditioned in 4
140 experimental 20-L tanks per treatment (6 oysters per tank, i.e. 24 oysters per treatment). The
141 tanks seawater supply (natural seawater pumped from the lagoon) was mechanically filtered
142 on 25 and 5 µm sock filters. In each tank, a system of 2 air-lifts connected to the pressurized
143 air circuit maintained the homogeneity of the medium. Micro-PS of 6 and 10 µm were

incorporated at equal weights according to the different tested doses (micro-PS ratio 6/10 µm: 4.614), and were injected continuously in the experimental design associated with a mixed diet of two microalgae (*Tisochrysis lutea*, formerly *Isochrysis galbana*, Tahitian strain: *T-Iso,* and *Chaetoceros gracilis*) at a daily ratio equal to 7-8% dry-weight-algae/dry-weight-oyster. The ration was determined according to the threshold for triggering pseudo-faeces production, so as to avoid an overestimation of ingestion measurements. This resulted in a mean concentration of 35 to 40 cells μL^{-1} in the water surrounding the oysters. The micro-PS/microalgae mixture was made every 24 hours in four 50-L cylindro-conical tank (1 per treatment). To avoid agglutination, micro-PS particles were preliminary mixed with Tween-20 at 10% of the micro-PS stock solution volume before being supplied to tanks. Tween-20 was also supplied to the control tank at the same rate (7 µL) as for the lowest micro-PS concentration (i.e., 0.25 $\mu g\ L^{-1}$). For each treatment, the microbeads and algae mixture was adjusted to 40 L with filtered (25 and 5 µm) seawater. The mixture was distributed by a peristaltic pump (16-way head, Ismatec®) set at 75 turns min^{-1}, i.e., 1.5 L of injected mixture per hour into the seawater supply of each 4 treatments for a total flow of 28 L h^{-1} (i.e., 7 L h^{-1} $tank^{-1}$). Oysters were maintained at 28.9 ± 0.3 °C under a 12 h light: 12 h dark cycle throughout the duration of the exposure. The pH, dissolved oxygen and salinity of the seawater were 8.2, 6.6 ± 0.7 mg O_2 L^{-1} and 35 psu, respectively. In order to avoid contamination of the natural environment with microbeads, discharges were filtered successively on 5 and 1 µm sock filters.

Ecophysiological measurement system. After 1-month exposure, four oysters per treatment were placed in the ecophysiological measurement system (EMS) to monitor clearance rate and oxygen consumption. The EMS consisted of five hemispheric open-flow chambers in transparent Altuglas®. One oyster was placed in each chamber and the fifth chamber was occupied by an empty oyster shell to be used as control.[40] Experimental

conditions during *in vivo* exposure were replicated in the EMS during measurements. For this, the chambers contained water at the same temperature (mean of 28.5 ± 0.3 °C) and concentration of algae as in the treatments tanks. Flow rates in the chambers were constant at 12 L h^{-1}. Each chamber was equipped with a two-way electromagnetic valve activated by an automaton. When the valve of one measuring chamber was opened, the released water was analysed for 3 min using a fluorometer (10-AU™, Turner Designs, Sunnyvale, CA) to measure microalgae fluorescence, then an oximeter (OXI 538/CellOX® 325, WTW, Weilheim, Germany) to measure dissolved oxygen. Data on clearance rate and oxygen consumption were stored on a computer with an acquisition software (computer programming by National Instruments™) and each cycle was completed within 3 min and another cycle started in the control chamber for 3 min (sequence: chamber 1, control, chamber 2, control, chamber 3, etc.). Oysters remained in the chambers for at least 48 h; measurements of each oyster were taken every 24 min until 120 measurements of clearance rate and oxygen consumption had been recorded.[40] A total of 32 oysters were individually monitored in the EMS (8 oysters per treatment). Assimilation efficiency was measured after collecting biodeposits (i.e., faeces) in each hemispheric chamber and 50 mL of microalgae mixture administered during ecophysiological measurements.

Ingestion Rate (IR). IR is an indicator of feeding activity, and is defined as the quantity of microalgae cleared per unit of time. IR was estimated using fluorescence measurements and calculated as: $IR = V \times (C_1 - C_2)$, where C_1 is the fluorescence level of the control chamber, C_2 is the fluorescence of the experimental chamber containing one oyster, and V is the constant water flow rate (12 L h^{-1}).[40]

Oxygen Consumption rate (OC). OC was measured (mg O_2 h^{-1}) by calculating the differences in OC between the control and experimental chambers: $OC = V \times (O_1 - O_2)$,

where O_1 is the oxygen level in the control chamber, O_2 is the oxygen level in the experimental chamber, and V is the water flow rate.[40]

Ingestion and oxygen consumption rates were estimated and an average calculated for each oyster, taking into account all values recorded after the measurements to stabilize. To compare IR and OC, it was necessary to correct for differences in specimen weight. Values of the ecophysiological activities were converted to a standard animal basis (1 g, dry weight), using the formula: $Ys = (Ws/We)^b \times Ye$, where *Ys* is the physiological activity of a standard oyster, *Ws* is the dry weight of a standard oyster (1 g), *We* is the dry weight of the specimen, *Ye* is the measured physiological activity, and *b* is the allometric coefficient of a given activity. The average *b* allometric coefficients were 0.66 for ingestion rate and 0.75 for oxygen consumption rate.[41]

Assimilation Efficiency (AE). AE of organic matter was assessed by analysing microalgae and faeces according to the method of Conover (1966) and described by Chávez-Villalba *et al.* (2013).[40] Biodeposits of each individual were treated individually by filtration on a GF/C filter (1.2 µm of porosity, Ø 47 mm, Whatman®) previously burned at 450 °C and weighed. Filters were then dried at 60 °C for 24 h in order to obtain the dry weight of biodeposits (DW) and then burned (Thermolyne™ Type 47900 & 48000 Furnaces, Thermo Scientific™) at 450 °C for 4 h to obtain the weight of the mineral matter (W_{MM}). The weight of organic matter (W_{OM}) biodeposits was obtained by difference between DW and W_{MM}. The W_{OM} of the food ration was calculated in the same way from the mixture of microalgae. Microalgae organic matter was obtained by filtering 50 mL of the microalgae mixture and followed by treatment of biodeposits according to the same procedure as for organic waste.

A micro-PS quantification in faeces was realised in order to refine assimilation values. The estimation of the micro-PS share in the W_{OM} of biodeposits was obtained during a specific experiment. The method used in this study is based on a digestion protocol of the OM

218 with potassium hydroxide (KOH),[42] adapted by Dehaut *et al.* (2016).[43] Oysters were isolated
219 at the end of the exposure (J60) in order to avoid the contamination of faeces by micro-PS
220 present in the media. Oysters were regrouped according to their treatment (18 per treatment)
221 in 5 L Pyrex crystallizing dishes filled with filtered sea water. After 12 hours, faeces were
222 taken using a plastic pipette (Deutscher) and poured into glass tubes. Biodeposits were
223 homogenized and fractionated into two equal volumes: fraction 1 (F1) and fraction 2 (F2). F1
224 was directly filtered on a GF/C filter as described previously, while F2 underwent 24 h
225 digestion with potassium hydroxide (10% KOH) at 60 °C, in order to digest the OM and only
226 conserve the micro-PS.[43] F2 was then filtered on a polycarbonate filter (3 µm of porosity, Ø
227 47 mm, ipPORE^{TM} Track Etched Membrane, itip$^{®}$). These filters were rinsed with acetic acid
228 (90%) to clean any residual substances resulting from saponification due to KOH treatment.
229 The two filters were dried, and the filter F1 was burned and then weighed in order to obtain
230 the W_{OM} of F1. The filter F2 was analyzed under a microscope and 6 areas were selected
231 randomly and photographed to count the micro-PS. The average of the counts obtained was
232 then scaled to the entire filter surface in order to estimate the total number of micro-PS in the
233 sample and their contribution to the W_{OM} of F1 for each treatment (micro-PS weight 6 and 10
234 µm = 1.19×10^{-4} and 5.48×10^{-4} µg). This proportion of micro-PS was then subtracted in
235 proportion to the individual DW biodeposits for each treatment. After conversion of the W_{OM}
236 biodeposits and W_{OM} microalgae to relative values, the assimilation efficiency (AE, %) was
237 calculated by the relation (Conover, 1966): $AE = (\%OM_m - \%OM_w) / ((100 - \%OM_w) \times$
238 $\%OM_m)$, where AE is the assimilation efficiency, OM_m is the microalgae organic matter (0.87
239 for *Tisochrysis lutea* and 0.6 for *Chaetoceros gracilis*) and OM_w is the waste organic matter
240 (biodeposits).

241 **Energy budget.** Ecophysiology data were converted into energy values to define the
242 scope for growth (SFG) for each oyster: SFG = (IR × AE) − OC, where IR is the ingestion

243 rate, AE is the assimilation efficiency, and OC is the oxygen consumption. We used 20.3 J for
244 1 mg of particulate organic matter and 14.1 J for 1 mg O_2.[44–46]

245 **Shell growth rate.** To investigate shell growth, the shells were sawn with a 'Swap Top'
246 Trim Saw machine (Inland, Middlesex, UK), which included a diamond Trim Saw Blade
247 (Thin Cut) IC-40961. Shell edges were then polished for 5 s with various grades of water
248 sandpaper sheets. The shell sections were examined under a Leitz Dialux 22 compound
249 fluorescence microscope equipped with an I3-filter block and an optical micrometer. Shell
250 growth was measured by evaluating the thickness of deposits at the ventral side of the shell,
251 from the surface to the calcein marks, with an optical micrometer.[47] Shell deposit rate (SDR)
252 was calculated by dividing the thickness of deposits by the time which had elapsed since the
253 marking. SDR is expressed in $\mu m.d^{-1}$.[47,48]

254 **Measurement of the reproductive effort.** After flesh dissection, the visceral mass (VM)
255 was drained on absorbent paper, weighed and put in 10% formalin seawater for 72 h before
256 being transferred into 70% ethanol. VM were cut along the sagittal plane and digital images
257 (600 dpi) were obtained using a desktop scanner. The digital pictures were then analysed
258 using ImageJ software (v. 1.6.0). Gonad size was characterized using a gonad development
259 index (GDI) which is equal to the ratio of the gonad surface (G) to the VM area of a sagittal
260 section: GDI = G/VM.[49,50]

261 **Gametogenesis analysis by histology.** For histological analysis, the fixed gonads were
262 dehydrated through a graded series of ethanol, embedded in paraffin, sectioned into 3 µm
263 slices on a rotary microtome, stained using haematoxylin and eosin and finally mounted on
264 glass microscope slides. Gametogenesis condition was evaluated through the identification of
265 regression signs (i.e., epithelial detachment, advanced regression stage). The presence or
266 absence of regression signs was assessed for each individual.

Statistical analysis. Data are presented as mean ± standard deviation. Normality of data distribution and homogeneity of variance were tested with the Shapiro-Wilk test and the Bartlett test, respectively. Means of IR, OC, AE, SFG and GDI were compared using one-way ANOVA for each treatment ($\alpha = 0.05$). Data expressed in proportion (AE and GDI) were previously transformed by the arcsine square root function. Tukey's post hoc test was used to determine the significant differences between the averages of each group. Since the assumptions of normality and homogeneity of variance were not met for SDR data, we used the non-parametric Kruskall-Wallis test to compare means of each treatment. Fisher's exact test was used to analyse gametogenesis condition (i.e., presence or absence of regression signs). For this test, individuals at T0 were first compared to those placed under control condition (T0 *vs* control), in order to rule out potential laboratory or time effect on the gametogenesis condition, and be able to pool T0 and control conditions to increase the power of the statistical test. This "new control" group could thus be compared to the pooled individuals exposed to micro-PS, as to perform T0/control *vs* micro-PS comparison. Results were considered significant at $p < 0.05$.

RESULTS

Metabolic rates: ingestion and respiration. Fluorescence measurements revealed an average ingestion rate of 20.7 ± 5.4 × 10^7 cells h^{-1} g^{-1} dry weight (dw) under control conditions; 18.7 ± 7.7 × 10^7 cells h^{-1} g^{-1} dw at 0.25 µg micro-PS L^{-1}; 20.9 ± 3.2 × 10^7 cells h^{-1} g^{-1} dw at 2.5 µg L^{-1} and 20.3 ± 4.5 × 10^7 cells h^{-1} g^{-1} dw at 25 µg L^{-1}. The one-way ANOVA revealed no significant difference between conditions ($F_{3, 32} = 0.279$, $p = 0.840$) (Figure 2a).

The average of oxygen consumption was of 1.34 ± 0.37 mg O_2 h^{-1} g^{-1} dw under control conditions; 1.47 ± 0.37 mg O_2 h^{-1} g^{-1} dw at 0.25 µg L^{-1}; 1.45 ± 0.20 mg O_2 h^{-1} g^{-1} dw at 2.5 µg

290 L^{-1} and 1.40 ± 0.19 mg O_2 h^{-1} g^{-1} dw at 25 µg L^{-1}. The one-way ANOVA revealed no
291 significant difference between conditions ($F_{3, 32} = 0.216$, $p = 0.885$) (Figure 2b).

292 **Digestion: assimilation efficiency.** The counts carried out on polycarbonate filter allowed
293 to estimate the weight of micro-PS in the dry weight of biodeposits. This estimate amounts to
294 approximately 2.8 × 10^{-3} µg micro-PS $µg^{-1}$ dw at 0.25 µg L^{-1}; 4.3 × 10^{-3} µg micro-PS $µg^{-1}$ dw
295 at 2.5 µg L^{-1} and 28.0 × 10^{-3} µg micro-PS $µg^{-1}$ dw at 25 µg L^{-1}. The corrected OM
296 measurements obtained in oysters placed under control conditions were 73.6 ± 7.4% OM
297 biodeposits (OM_w) for 82.4% OM microalgae (OM_m). For oysters exposed to 0.25; 2.5 and 25
298 µg micro-PS L^{-1}, OM measurements were respectively 69.0 ± 8.5% OM_w for 75.1% OM_m;
299 79.8 ± 3.4% OM_w for 81.9% OM_m and 78.3 ± 6.6% OM_w for 75.9% OM_m.

300 The assimilation data obtained from the OM data were used to obtain for each oyster an
301 average assimilation efficiency of 48.9 ± 18.4% for individuals placed under control
302 conditions; 30.1 ± 24.7% at 0.25 µg micro-PS L^{-1}; 20.4 ± 6.7% at 2.5 µg L^{-1} and 3.0 ± 13.4%
303 at 25 µg L^{-1}. The one-way ANOVA revealed a significant difference between conditions ($F_{3,}$
304 $_{13} = 5.576$, $p = 0.011$). The Tukey post-hoc test revealed a significant difference between the
305 control condition and the 25 µg L^{-1} treatment (mean diff. 45.8%, $p = 0.009$) (Figure 2c).

306 **Standardized scope for growth.** Analysis of metabolic rates and assimilation efficiency
307 indicated a mean energy balance (SFG) decreasing from the control condition to the micro-PS
308 treatments at 0.25 and 2.5 µg L^{-1} (control, 88.1 ± 23.1 J h^{-1} g^{-1} dw; 0.25 µg L^{-1}, 39.7 ± 19.9 J
309 h^{-1} g^{-1} dw; 2.5 µg L^{-1}, 24.8 ± 5.6 J h^{-1} g^{-1} dw) and becoming negative at 25 µg L^{-1} (-13.2 ± 2.5
310 J h^{-1} g^{-1} dw). The one-way ANOVA revealed a significant difference between conditions ($F_{3,}$
311 $_{32} = 62.47$, $p < 0.0001$). The Tukey post-hoc test showed a significant difference ($p < 0.0001$)
312 between the means of the control condition and the 3 treatments at 0.25; 2.5 and 25 µg L^{-1}. A
313 significant difference was also observed between the mean of treatments at 0.25 and 25 µg L^{-1}

(mean diff. 52.8 J h^{-1} g^{-1} dw, $p < 0.0001$) and those of treatments at 2.5 and 25 µg L^{-1} (mean diff. 37.9 J h^{-1} g^{-1} dw, $p = 0.0004$) (Figure 3).

Shell growth. Shell deposition rate (SDR) measurement was obtained through calcein marking in order to analyse the effect of a 2-month micro-PS exposure at different doses on shell growth. The Kruskall-Wallis test showed no significant effect of micro-PS on SDR ($p = 0.252$) (Figure SI.2, SI).

Reproductive effort and gametogenesis. Means of gonad development index (GDI) measured in individuals at T0 (before micro-PS exposure) and those placed under control conditions were respectively of 9.2 ± 3.2% and 7.2 ± 7.3%. For individuals treated with 0.25; 2.5 and 25 µg micro-PS L^{-1}, means of GDI were respectively of 7.6 ± 8.2%, 8.3 ± 5.1% and 8.0 ± 6.4% (Figure SI.3, SI). One-way ANOVA revealed no significant differences between conditions ($F_{4, 45} = 0.156$, $p = 0.959$).

Gonads observations revealed that 100% of samples were male, which can be expected for pearl oysters at this age. Histological analysis identified "normal" gametogenesis with the presence of gonadal tubules containing germ cells ranging from spermatogonia (localized in the periphery of tubules) to spermatids and spermatozoa (more central) (Figure 4a-b). Gametogenesis was also marked by a high proliferation activity in the epithelium of gonadal tubules. Small "holes" in gonadal tubules were also observed in some individuals exposed to 0.25 and 2.5 µg L^{-1} (Figure 4c) but not seen in the T0, control and 25 µg L^{-1} samples. Larger magnification analysis revealed the presence of large cells within or near to these breakthroughs (Figure 4d). These cells were composed of an eccentric nucleus (Figure 4e) and a cytoplasm that sometimes contains many granules. These cells could be assimilated to haemocytes. Epithelial detachments were also observed in gonadal tubules of individuals exposed to 25 µg L^{-1}, with little or no proliferation activity (Figure 4f). Indeed, the remaining germ cells were essentially spermatids that were centred within tubules. Although advanced

regression stages were observed in oysters for every micro-PS doses, they were especially visible at 25 µg L^{-1}. This stage leads to haemocytes infiltrations in gonadal tubules completely or partially emptied of their germ cells whose degradation products appear in pink (Figure 4g).

The exact Fisher test performed on the presence or absence of regression signs revealed no significant difference between T0 and the control condition (p = 0.211). However, a significant higher proportion of pearl oysters exposed to micro-PS showed signs of regression of gametogenesis (63%, p = 0.0012) when compared to unexposed individuals (T0 and control condition, 15%).

DISCUSSION

The aim of this study was to investigate the impact of micro-PS on the physiology of *P. margaritifera* by evaluating the effects of different exposition levels micro-PS on the energy balance and reproduction. We found that there was a significant dose-dependent negative response of oysters exposed to micro-PS, with unpredictable detrimental consequences for the sustainability of pearl oyster populations, even at low concentrations of micro-PS (i.e., 0.25 µg L^{-1}).

Energy budget is the amount of energy expended for an individual's maintenance (i.e., general metabolism, somatic tissue growth, and gametes production). The scope for growth (SFG) defines the surplus of energy available for growth beyond that required for maintenance, and can thus be seen as a measure of an individual's potential to thrive in its environment. The metabolic responses of a stressed organism can result in an increase in energy costs of an individual's maintenance, which, if it is not compensated by an equivalent increase in energy intake, forces the organism to sacrifice some of the energy normally allocated to functions such as growth and/or reproduction to mitigate this increase in energy

363 cost. Hence, a SFG of zero would reflect a cessation of growth for an individual, while a
364 negative SFG would indicate that the metabolic costs associated with the individual's
365 maintenance (respiration, metabolism and reproduction) outweigh the energy absorbed by
366 food ingestion, endangering the survival of the individual. In our study, pearl oysters
367 ingesting plastics had a significant dose-dependent decrease in SFG, observed from doses of
368 0.25 μg L^{-1} onward. However, the shell deposit rate (i.e., actual physical growth) was not
369 significant different among conditions after 2-months exposure, and was thus somehow
370 maintained, despite the decrease in SFG. In addition, SFG became negative at the dose of 25
371 μg L^{-1}. These results indicate that exposed oysters had to draw their energy from reserves, or
372 had to gain it from another biological compartment (not growth).

373 Ingestion rate and assimilation efficiency are considered to be the main processes
374 responsible for energy gain in bivalves,[51] and the physiological parameters that most
375 influence SFG.[52] Ingestion rate of microalgae by *P. margaritifera* was not affected by the
376 presence of micro-PS at the tested doses. These results are in agreement with those obtained
377 for the flat oyster *Ostrea edulis*, whose filtration remained unchanged after mesocosm
378 exposure to PLA (polylactic acid) and HDPE (high density polyethylene) MP type at low (0.8
379 μg L^{-1}) and high (80 μg L^{-1}) concentrations.[4] However, these ingestion results are apparently
380 in contradiction with those obtained in *C. gigas*, which showed an increase in microalgal
381 consumption in individuals exposed to micro-PS (23 μg L^{-1}).[14] This divergence may be
382 related to individual measurements carried out in our study and population measurements in
383 the case of work on *C. gigas*. It is also suggested that micro-PS exposure could induce a
384 compensatory effect on food intake in the Pacific oyster.[14] In the crab *C. maenas*, a decrease
385 in the energy allocated to the animal's growth has been shown after 4-weeks exposure to PP
386 fibers (polypropylene, 1-5 mm).[23] In this study, the most important factor influencing SFG
387 was ingestion rate, which resulted in a clear decrease of 90%.[23]

Although ingestion rate measurements did not demonstrate any alteration due to micro-PS exposure, assimilation measurements revealed a dose-dependent decrease in AE in exposed oysters. This means that for an equal volume of water filtered by the oysters, the assimilation of microalgae was disrupted by the presence of micro-PS, and the energy provided by food intake was lower for exposed oysters. This inhibitory effect of micro-PS on food assimilation could occur either directly, through interference with the assimilation processes within the digestive system, or indirectly, through competition with microalgae present in the acclimation medium. The impact of micro-PS on assimilation efficiency has also been reported in other studies: the reduction in body weight and assimilation efficiency for the deposit-feeder *Arenicola marina* was correlated with a reduction in the sediment organic matter content due to the presence of plastics;[16] Wright *et al.* (2013) found that inflammatory responses associated to MP, combined with a reduction in feeding activity and a longer residence time in the intestine, could be responsible of an energy reserves depletion in *A. Marina*;[17] also, the presence of polyamide (PA) fibers in the digestive tract of the amphipod *G. fossarum* was shown to inhibit assimilation efficiency.[24] However, in contrast to *P. margaritifera*, an increase in absorption efficiency was observed and an enhancement of mechanical digestion was suggested in *C. gigas* after micro-PS exposure.[14]

With assimilation efficiency decreasing in correlation with micro-PS concentrations, and an ingestion rate being similar for all concentrations, the metabolic rate (indirectly measured by respiration through oxygen consumption) could have been reduced in order to compensate for the energy deficit observed with the SFG. However, our results indicate that respiration, and thus metabolic rate, were not impacted by micro-PS. Therefore, the only possibility for exposed oysters to compensate the decrease in energy balance seems to be to feed back on reproduction, by altering reproductive effort and/or gametogenesis.

412 Gametogenesis in bivalves represents a particularly high energy demand period. In *P.*
413 *margaritifera*, reproduction is continuous. This "opportunistic strategy" consists in investing
414 any excess energy into the gametes production.[53] The reproductive effort measured by the
415 GDI allows for the evaluation of the amount of energy allocated specifically to reproduction.
416 The method used in this work was previously validated in the pearl oyster and is suited to
417 study the relationship between the trophic level and the reproductive effort.[50] Our results
418 showed that micro-PS did not influence the development of gonadal tissue, and thus had no
419 effect on the reproductive effort of *P. margaritifera*. In all conditions, GDI measurements
420 were particularly low in our experiment (between 7.2 and 8.3%) compared to the data found
421 in the literature (15 to 18% and 18 to 34%).[49,50] This difference may be explained by the age
422 of oysters used here (i.e., 1-1.5 year old), for whom the reproductive effort is 5 times lower
423 than individuals aged of 3 to 4 years.[53] Nonetheless, despite having a similar reproductive
424 effort, exposed oysters' gametogenesis was strongly impacted by the presence of micro-PS.
425 Abnormal phenomena were observed with increasing concentrations of micro-PS and could
426 be referred to as regression induced by energy deficiency. The presence of "small holes" in
427 the gonadal tubules seems to be the premises of a regression phase. The presence of
428 haemocytes within these breakthroughs supports this hypothesis. This could be areas where
429 phagocytosis of germ cells is initiated. It is conceivable that these phenomena of gonadal
430 resorption encountered in exposed oysters to micro-PS are linked to the metabolites
431 production. These observations were made in several exposed individuals, and corroborate the
432 bioenergy results. Indeed, a negative energy balance indicates that the animal must draw from
433 its reserves to satisfy its metabolism and provide its maintenance, to the detriment of its
434 growth and reproduction. In *P. margaritifera*, the excess of energy being directly intended for
435 the gametes production, it is the gonads that also constitute the reserves (while we
436 demonstrated that growth was not impacted). Once the gonads are exhausted, and if adverse

environmental conditions persist, the animal's energy impairment is likely to become lethal. These results agreed with those derived from the DEB (Dynamic Energy Budget) analysis by Sussarellu *et al.* (2016) in *C. gigas*, suggesting that the energy fraction allocated to reproduction seemed to shift toward structural growth and high maintenance costs following exposure to micro-PS. Transcriptome approaches have also revealed a decrease in the transcripts regulation coding for proteins involved in the insulin pathway.[14] Hence, micro-PS exposure may affect gene regulation in response to insulin signalling which is responsible for germ cell proliferation, differentiation and maturation.[54] This hypothesis would explain the interruption of germ cell proliferation in the gonadal tubules of exposed pearl oysters which result in epithelial detachments. This phenomenon was also reported in the pearl oyster conditioned in a medium weakly enriched in microalgae (1.5 cells μL^{-1}).[50] That provide a second explanation to the divergence in food behaviour between *C. gigas* and *P. margaritifera* which could be related to the energetic support to gametogenesis, partly based on glycogen reserves in *C. gigas,*[55] and completely dependent on food for *P. margaritifera*; the gonadic tissue serving as energy buffer explaining resorption figures observed in histology. In any case, these results provide further knowledge about the impact of MP on marine bivalves. At a different reproduction level, the impact of micro-PS on the gametogenesis of *P. margaritifera* complements results on gametes quality/quantity and larval development in *C. gigas*,[14] with upstream new data.

Note that virgin micro-PS used in this study contain a slight anionic charge from sulfate ester and are cross-linked with divinylbenzene (DVB). These non-functionalized microbeads are packaged in an aqueous suspension with minimal surfactant in the final preparation. Thus, we cannot distinguish between the possible mechanisms explaining the toxicity of micro-PS, to which direct particle toxicity and effects of micro-PS associated-chemicals such as DVB,

461 may contribute. Although we cannot establish an impact of these chemicals in our
462 experiment, background effects should be consider.[56]

463 In conclusion, this study highlights the impact of ingesting a diet containing polystyrene
464 microbeads on the assimilation efficiency of the pearl oyster which directly influences its
465 energy balance. The dose-dependent decrease in AE and SFG supports these results and
466 demonstrates an immutable effect of micro-PS on the oyster physiology. Given the bioenergy
467 results and the strategy reserves management of *P. margaritifera*, the decrease in energy gain,
468 which in some cases (i.e., 25 μg L^{-1}) results in an energy deficit, is reflected on its
469 reproduction. Gonads appear to provide the missing energy to maintain animals' metabolism
470 through the production of metabolites derived from germ cells phagocytosis. The pearl oyster
471 exposed to micro-PS during a 2-month period thus maintains its metabolism and its vital
472 functions at the expense of its reproduction, and thus of its future population sustainability.
473 Taken together, our study and future work on the topic should promote decision-making on
474 plastic waste management measures in Polynesian lagoons, especially in atolls that have 30 to
475 40 years of pearl farming history without waste regulations nor clean up. Our work aims to
476 provide information to local authorities in order to regulate the flow of pearl farm equipments
477 in direction to pearl farming atolls and implement a waste management policy such as
478 collection and recycling of these equipments.

479 **ASSOCIATED CONTENT**

480 ** Supporting information*

481 The Supporting Information is available free of charge on the ACS Publications website at
482 DOI:

The SI contains supplementary figures about the polymer type confirmation of micro-PS 6 and 10 µm by Raman micro-spectroscopy analysis; the effect of micro-PS on the SDR and the GDI of *P. margaritifera*. Figure SI.1, SI.2 and SI.3 (PDF)

AUTHOR INFORMATION

Corresponding Author

* Phone: +689 40 54 60 47; fax: +689 40 54 60 99; e-mails: tony.gardon@ifremer.fr and gilles.le.moullac@ifremer.fr

Present Address

Ifremer, UMR Ecosystèmes Insulaires Océaniens (EIO), UMR 241 UPF/ILM/IRD/Ifremer, Centre du Pacifique, BP 49, 98719, Taravao, French Polynesia.

ORCID

Tony Gardon: 0000-0002-5761-0526

Gilles Le Moullac: 0000-0003-3749-8239

Notes

The authors declare no competing financial interest.

ACKNOLEDGMENTS

We would like to thank all the RMPF technicians and personnel who helped during the experimental stages of this work. Our thanks extend to Arnaud Huvet (Ifremer Centre de Brest, LPI, UMR LEMAR) and Ika Paul-Pont (CNRS, Université de Bretagne Occidentale, UMR LEMAR) for fruitful discussions. This study was a part of the MerLIN microplastics project, funded by Ifremer. Tony Gardon was funded by a grant of the Labex Corail.

REFERENCES

(1) PlasticsEurope. Plastics – the Facts 2016: An analysis of European Plastics Production, Demand and Waste Data (14.02.17); PlasticsEurope: Brussels, Belgium, 2017; http://www.plasticseurope.org/.

(2) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic Waste Inputs from Land into the Ocean. *Science* **2015**, *347* (6223), 768–771.

(3) Barnes, D. K. A.; Galgani, F.; Thompson, R. C.; Barlaz, M. Accumulation and Fragmentation of Plastic Debris in Global Environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364* (1526), 1985–1998.

(4) Arthur, C.; Baker, J.; Bamford, H. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. NOAA Technical Memorandum NOS-OR&R-30 2009.

(5) Andrady, A. L. Microplastics in the Marine Environment. *Mar. Pollut. Bull.* **2011**, *62* (8), 1596–1605.

(6) Derraik, J. G. B. The Pollution of the Marine Environment by Plastic Debris: A Review. *Mar. Pollut. Bull.* **2002**, *44* (9), 842–852.

(7) Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E. Lost at Sea: Where Is All the Plastic? *Science* **2004**, *304* (5672), 838–838.

(8) Ng, K. L.; Obbard, J. P. Prevalence of Microplastics in Singapore's Coastal Marine Environment. *Mar. Pollut. Bull.* **2006**, *52* (7), 761–767.

(9) Fendall, L. S.; Sewell, M. A. Contributing to Marine Pollution by Washing Your Face: Microplastics in Facial Cleansers. *Mar. Pollut. Bull.* **2009**, *58* (8), 1225–1228.

528 (10) Rochman, C. M.; Browne, M. A.; Underwood, A. J.; van Franeker, J. A.; Thompson, R.
529 C.; Amaral-Zettler, L. A. The Ecological Impacts of Marine Debris: Unraveling the
530 Demonstrated Evidence from What Is Perceived. *Ecology* **2016**, *97* (2), 302–312.
531 (11) Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C.
532 Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel,
533 *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2008**, *42* (13), 5026–5031.
534 (12) Van Cauwenberghe, L.; Janssen, C. R. Microplastics in Bivalves Cultured for Human
535 Consumption. *Environ. Pollut.* **2014**, *193*, 65–70.
536 (13) Avio, C. G.; Gorbi, S.; Milan, M.; Benedetti, M.; Fattorini, D.; d'Errico, G.; Pauletto,
537 M.; Bargelloni, L.; Regoli, F. Pollutants Bioavailability and Toxicological Risk from
538 Microplastics to Marine Mussels. *Environ. Pollut.* **2015**, *198*, 211–222.
539 (14) Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M. E. J.; Le
540 Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; et al. Oyster Reproduction Is
541 Affected by Exposure to Polystyrene Microplastics. *Proc. Natl. Acad. Sci.* **2016**, *113*
542 (9), 2430–2435.
543 (15) Graham, E. R.; Thompson, J. T. Deposit- and Suspension-Feeding Sea Cucumbers
544 (Echinodermata) Ingest Plastic Fragments. *J. Exp. Mar. Biol. Ecol.* **2009**, *368* (1), 22–
545 29.
546 (16) Besseling, E.; Wegner, A.; Foekema, E. M.; van den Heuvel-Greve, M. J.; Koelmans,
547 A. A. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm
548 *Arenicola marina* (L.). *Environ. Sci. Technol.* **2013**, *47* (1), 593–600.
549 (17) Wright, S. L.; Rowe, D.; Thompson, R. C.; Galloway, T. S. Microplastic Ingestion
550 Decreases Energy Reserves in Marine Worms. *Curr. Biol. CB* **2013**, *23* (23), R1031-
551 1033.

552 (18) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway,
553 T. S. Microplastic Ingestion by Zooplankton. *Environ. Sci. Technol.* **2013**, *47* (12),
554 6646–6655.
555 (19) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Galloway, T. S. The Impact of
556 Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod
557 *Calanus helgolandicus*. *Environ. Sci. Technol.* **2015**, *49* (2), 1130–1137.
558 (20) Lee, K.-W.; Shim, W. J.; Kwon, O. Y.; Kang, J.-H. Size-Dependent Effects of Micro
559 Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. *Environ. Sci.*
560 *Technol.* **2013**, *47* (19), 11278–11283.
561 (21) Jeong, C.-B.; Won, E.-J.; Kang, H.-M.; Lee, M.-C.; Hwang, D.-S.; Hwang, U.-K.;
562 Zhou, B.; Souissi, S.; Lee, S.-J.; Lee, J.-S. Microplastic Size-Dependent Toxicity,
563 Oxidative Stress Induction, and p-JNK and p-P38 Activation in the Monogonont
564 Rotifer (*Brachionus koreanus*). *Environ. Sci. Technol.* **2016**, *50* (16), 8849–8857.
565 (22) Murray, F.; Cowie, P. R. Plastic Contamination in the Decapod Crustacean *Nephrops*
566 *norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* **2011**, *62* (6), 1207–1217.
567 (23) Watts, A. J. R.; Urbina, M. A.; Corr, S.; Lewis, C.; Galloway, T. S. Ingestion of Plastic
568 Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and
569 Energy Balance. *Environ. Sci. Technol.* **2015**, *49* (24), 14597–14604.
570 (24) Blarer, P.; Burkhardt-Holm, P. Microplastics Affect Assimilation Efficiency in the
571 Freshwater Amphipod *Gammarus fossarum*. *Environ. Sci. Pollut. Res.* **2016**, *23* (23),
572 23522–23532.
573 (25) Rist, S. E.; Assidqi, K.; Zamani, N. P.; Appel, D.; Perschke, M.; Huhn, M.; Lenz, M.
574 Suspended Micro-Sized PVC Particles Impair the Performance and Decrease Survival
575 in the Asian Green Mussel *Perna viridis*. *Mar. Pollut. Bull.* **2016**, *111* (1–2), 213–220.

576 (26) Besseling, E.; Wang, B.; Lürling, M.; Koelmans, A. A. Nanoplastic Affects Growth of
577 *S. obliquus* and Reproduction of *D. magna*. *Environ. Sci. Technol.* **2014**, *48* (20),
578 12336–12343.
579 (27) Wright, S. L.; Thompson, R. C.; Galloway, T. S. The Physical Impacts of Microplastics
580 on Marine Organisms: A Review. *Environ. Pollut.* **2013**, *178*, 483–492.
581 (28) Rochman, C. M.; Hoh, E.; Kurobe, T.; Teh, S. J. Ingested Plastic Transfers Hazardous
582 Chemicals to Fish and Induces Hepatic Stress. *Sci. Rep.* **2013**, *3* (1), 3263.
583 (29) Bakir, A.; Rowland, S. J.; Thompson, R. C. Enhanced Desorption of Persistent Organic
584 Pollutants from Microplastics under Simulated Physiological Conditions. *Environ.*
585 *Pollut.* **2014**, *185*, 16–23.
586 (30) Paul-Pont, I.; Lacroix, C.; González Fernández, C.; Hégaret, H.; Lambert, C.; Le Goïc,
587 N.; Frère, L.; Cassone, A.-L.; Sussarellu, R.; Fabioux, C.; et al. Exposure of Marine
588 Mussels *Mytilus spp.* to Polystyrene Microplastics: Toxicity and Influence on
589 Fluoranthene Bioaccumulation. *Environ. Pollut.* **2016**, *216*, 724–737.
590 (31) Collignon, A.; Hecq, J.-H.; Galgani, F.; Voisin, P.; Collard, F.; Goffart, A. Neustonic
591 Microplastic and Zooplankton in the North Western Mediterranean Sea. *Mar. Pollut.*
592 *Bull.* **2012**, *64* (4), 861–864.
593 (32) Huvet, A.; Paul-Pont, I.; Fabioux, C.; Lambert, C.; Suquet, M.; Thomas, Y.; Robbens,
594 J.; Soudant, P.; Sussarellu, R. Reply to Lenz et Al.: Quantifying the Smallest
595 Microplastics Is the Challenge for a Comprehensive View of Their Environmental
596 Impacts. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (29), E4123-4124.
597 (33) Barbier, E. B.; Hacker, S. D.; Kennedy, C.; Koch, E. W.; Stier, A. C.; Silliman, B. R.
598 The Value of Estuarine and Coastal Ecosystem Services. *Ecol. Monogr.* **2011**, *81* (2),
599 169–193.

(34) Pouvreau, S.; Jonquières, G.; Buestel, D. Filtration by the Pearl Oyster, *Pinctada margaritifera*, under Conditions of Low Seston Load and Small Particle Size in a Tropical Lagoon Habitat. *Aquaculture* **1999**, *176* (3–4), 295–314.

(35) Cole, M.; Lindeque, P. K.; Fileman, E.; Clark, J.; Lewis, C.; Halsband, C.; Galloway, T. S. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environ. Sci. Technol.* **2016**, *50* (6), 3239–3246.

(36) Green, D. S. Effects of Microplastics on European Flat Oysters, *Ostrea edulis* and Their Associated Benthic Communities. *Environ. Pollut.* **2016**, *216*, 95–103.

(37) Andréfouët, S.; Thomas, Y.; Lo, C. Amount and Type of Derelict Gear from the Declining Black Pearl Oyster Aquaculture in Ahe Atoll Lagoon, French Polynesia. *Mar. Pollut. Bull.* **2014**, *83* (1), 224–230.

(38) Eriksen, M.; Maximenko, N.; Thiel, M.; Cummins, A.; Lattin, G.; Wilson, S.; Hafner, J.; Zellers, A.; Rifman, S. Plastic Pollution in the South Pacific Subtropical Gyre. *Mar. Pollut. Bull.* **2013**, *68* (1), 71–76.

(39) Connors, E. J. Distribution and Biological Implications of Plastic Pollution on the Fringing Reef of Mo'orea, French Polynesia. *PeerJ* **2017**, *5*, e3733.

(40) Chávez-Villalba, J.; Soyez, C.; Aurentz, H.; Le Moullac, G. Physiological Responses of Female and Male Black-Lip Pearl Oysters *(Pinctada margaritifera)* to Different Temperatures and Concentrations of Food. *Aquat. Living Resour.* **2013**, *26* (3), 263–271.

(41) Savina, M.; Pouvreau, S. A Comparative Ecophysiological Study of Two Infaunal Filter-Feeding Bivalves: *Paphia rhomboïdes* and *Glycymeris glycymeris*. *Aquaculture* **2004**, *239* (1–4), 289–306.

623 (42) Foekema, E. M.; De Gruijter, C.; Mergia, M. T.; van Franeker, J. A.; Murk, A. J.;
624 Koelmans, A. A. Plastic in North Sea Fish. *Environ. Sci. Technol.* **2013**, *47* (15), 8818–
625 8824.
626 (43) Dehaut, A.; Cassone, A.-L.; Frère, L.; Hermabessiere, L.; Himber, C.; Rinnert, E.;
627 Rivière, G.; Lambert, C.; Soudant, P.; Huvet, A.; et al. Microplastics in Seafood:
628 Benchmark Protocol for Their Extraction and Characterization. *Environ. Pollut.* **2016**,
629 *215*, 223–233.
630 (44) Bayne, B. L.; Hawkins, A. J. S.; Navarro, E. Feeding and Digestion by the Mussel
631 *Mytilus edulis* L. (Bivalvia: Mollusca) in Mixtures of Silt and Algal Cells at Low
632 Concentrations. *J. Exp. Mar. Biol. Ecol.* **1987**, *111* (1), 1–22.
633 (45) Gnaiger, E. Heat Dissipation and Energetic Efficiency in Animal Anoxibiosis:
634 Economy Contra Power. *J. Exp. Zool.* **1983**, *228* (3), 471–490.
635 (46) Bayne, B. L.; Newell, R. C. Physiological Energetics of Marine Molluscs; 1983; Vol. 4,
636 pp 407–515.
637 (47) Linard, C.; Gueguen, Y.; Moriceau, J.; Soyez, C.; Hui, B.; Raoux, A.; Cuif, J. P.;
638 Cochard, J.-C.; Le Pennec, M.; Le Moullac, G. Calcein Staining of Calcified Structures
639 in Pearl Oyster *Pinctada margaritifera* and the Effect of Food Resource Level on Shell
640 Growth. *Aquaculture* **2011**, *313* (1), 149–155.
641 (48) Joubert, C.; Linard, C.; Le Moullac, G.; Soyez, C.; Saulnier, D.; Teaniniuraitemoana,
642 V.; Ky, C. L.; Gueguen, Y. Temperature and Food Influence Shell Growth and Mantle
643 Gene Expression of Shell Matrix Proteins in the Pearl Oyster *Pinctada margaritifera*.
644 *PloS One* **2014**, *9* (8), e103944.
645 (49) Fournier, J.; Levesque, E.; Pouvreau, S.; Le Pennec, M.; Le Moullac, G. Influence of
646 Plankton Concentration on Gametogenesis and Spawning of the Black Lip Pearl Oyster

Pinctada margaritifera in Ahe Atoll Lagoon (Tuamotu Archipelago, French Polynesia). *Mar. Pollut. Bull.* **2012**, *65* (10–12), 463–470.

(50) Le Moullac, G.; Soyez, C.; Sham-Koua, M.; Levy, P.; Moriceau, J.; Vonau, V.; Maihota, M.; Cochard, J. C. Feeding the Pearl Oyster *Pinctada margaritifera* during Reproductive Conditioning. *Aquac. Res.* **2013**, *44* (3), 404–411.

(51) Hawkins, A. J. S.; James, M. R.; Hickman, R. W.; Hatton, S.; Weatherhead, M. Modelling of Suspension-Feeding and Growth in the Green-Lipped Mussel *Perna canaliculus* Exposed to Natural and Experimental Variations of Seston Availability in the Marlborough Sounds, New Zealand. *Mar. Ecol. Prog. Ser.* **1999**, *191*, 217–232.

(52) Albentosa, M.; Viñas, L.; Besada, V.; Franco, A.; González-Quijano, A. First Measurements of the Scope for Growth (SFG) in Mussels from a Large Scale Survey in the North-Atlantic Spanish Coast. *Sci. Total Environ.* **2012**, *435–436*, 430–445.

(53) Pouvreau, S.; Gangnery, A.; Tiapari, J.; Lagarde, F.; Garnier, M.; Bodoy, A. Gametogenic Cycle and Reproductive Effort of the Tropical Blacklip Pearl Oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), Cultivated in Takapoto Atoll (French Polynesia). *Aquat. Living Resour.* **2000**, *13* (1), 37–48.

(54) Gricourt, L.; Mathieu, M.; Kellner, K. An Insulin-like System Involved in the Control of Pacific Oyster *Crassostrea gigas* Reproduction: HrIGF-1 Effect on Germinal Cell Proliferation and Maturation Associated with Expression of an Homologous Insulin Receptor-Related Receptor. *Aquaculture* **2006**, *251* (1), 85–98.

(55) Jouaux, A.; Blin, J. L.; Adeline, B.; Heude-Berthelin, C.; Sourdaine, P.; Mathieu, M.; Kellner, K. Impact of Energy Storage Strategies on Gametogenesis and Reproductive Effort in Diploid and Triploid Pacific Oysters *Crassostrea gigas* — Involvement of Insulin Signaling. *Aquaculture* **2013**, *388–391* (Supplement C), 173–181.

671 (56) Karami, A. Gaps in Aquatic Toxicological Studies of Microplastics. *Chemosphere*
672 **2017**, *184*, 841–848.
673

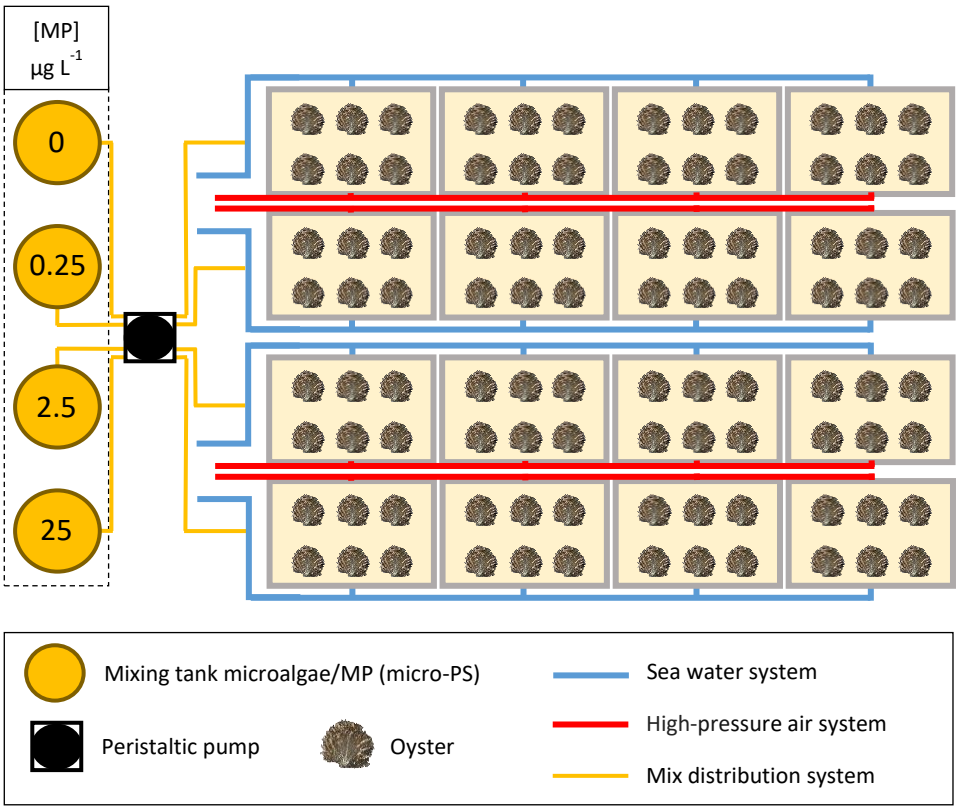


Figure 1. Experimental exposure device of oysters to microplastics.

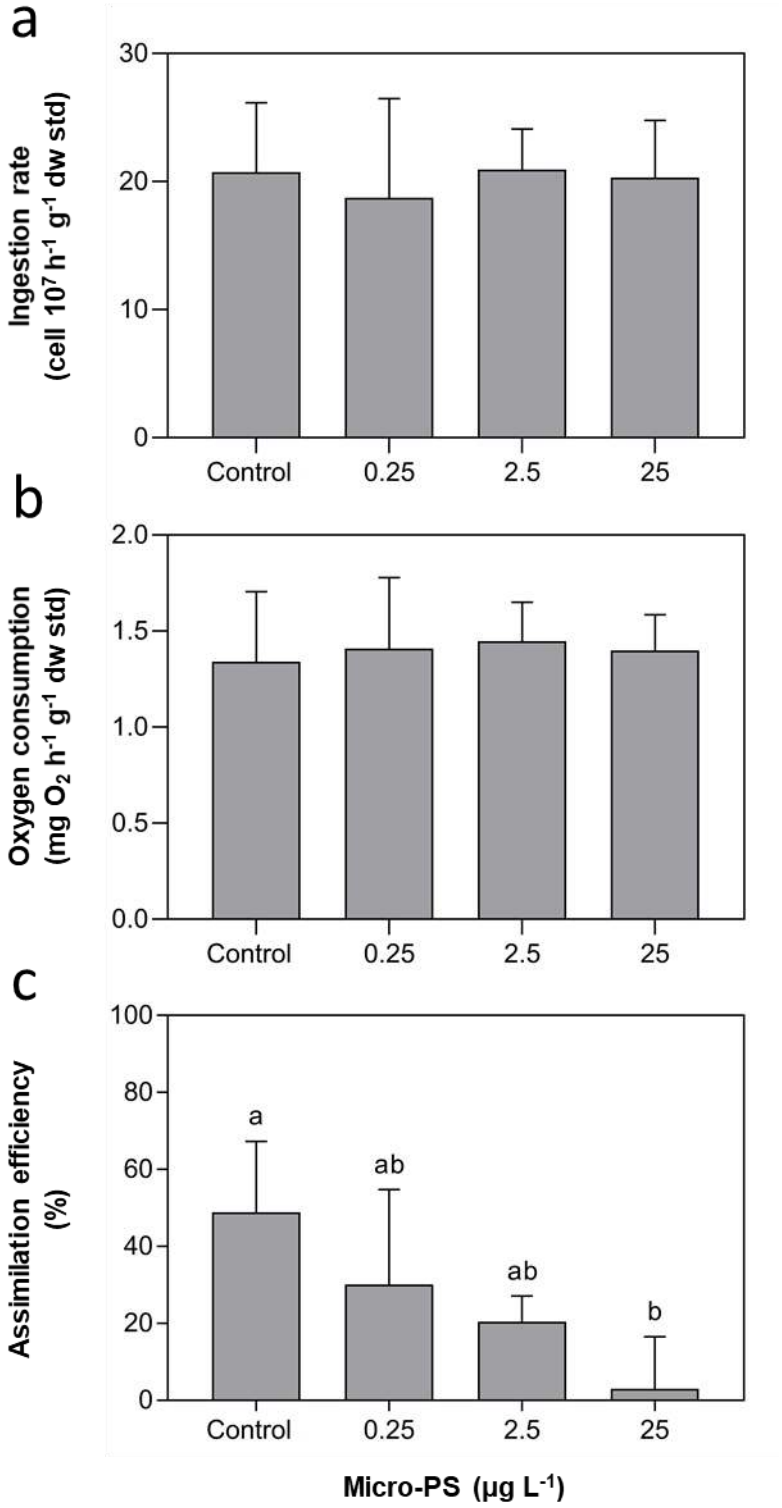


Figure 2. Effect of microplastics on bioenergetic parameters of *P. margaritifera*. Impact of micro-PS on: ingestion rate (a), oxygen consumption (b) and assimilation efficiency (c). Mean ± standard deviation ($8 \leq n \leq 12$).

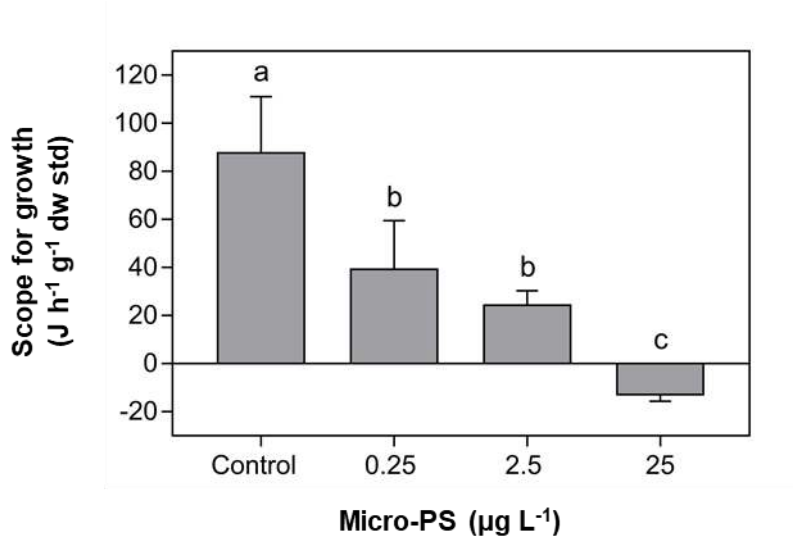


Figure 3. Energy balance of oysters exposed to micro-PS. Mean ± standard deviation ($8 \leq n \leq 12$).

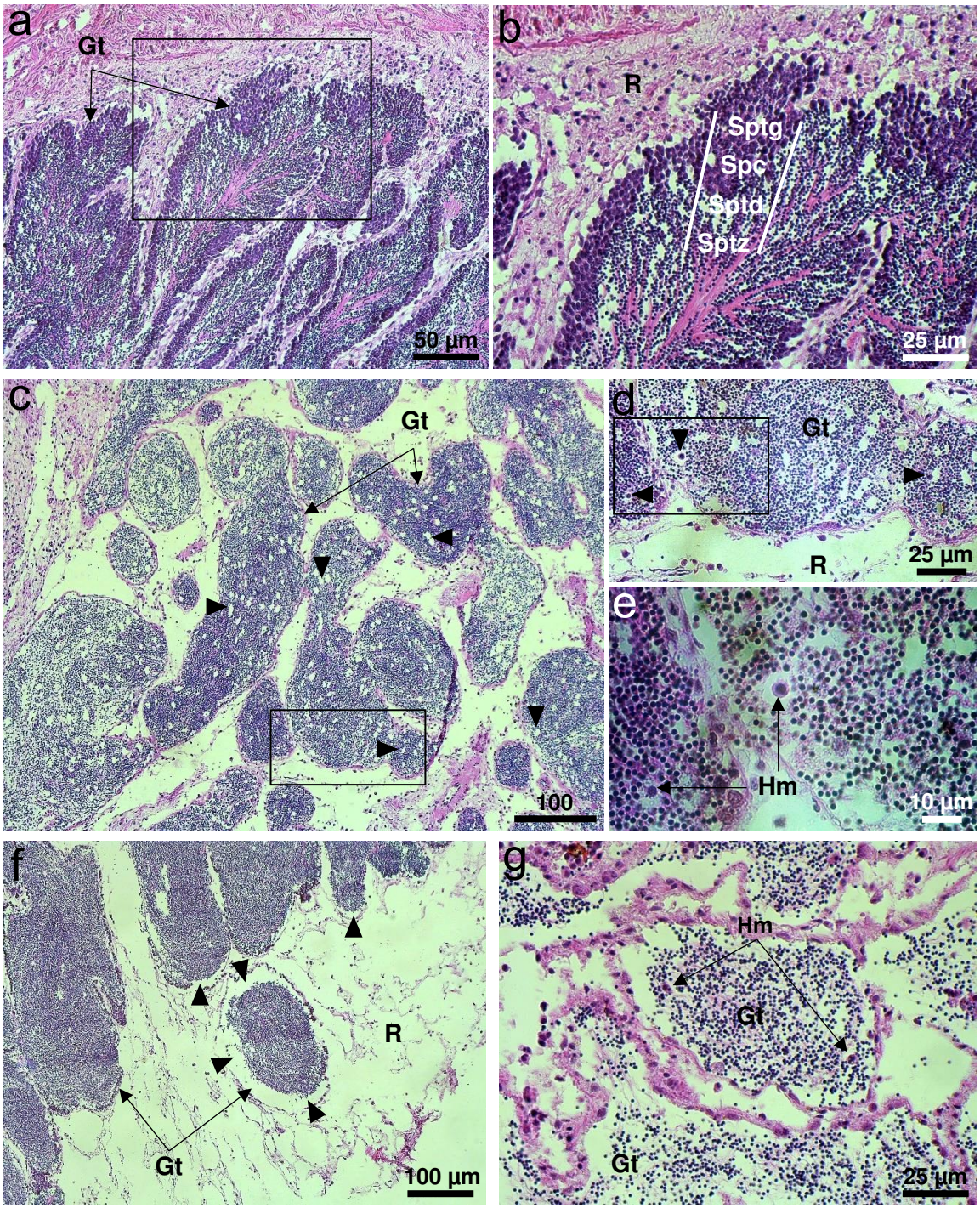


Figure 4. Histology panels from the gonads analysis of oysters exposed to micro-PS. (a, b): intermediate stage gametogenesis in a control sample; (c, d, e): gametogenesis at an intermediate stage in a sample exposed to 2.5 µg L^{-1}; (f): gametogenesis at an intermediate stage in a sample exposed to 25 µg L^{-1}; (g): regression of gametogenesis in a sample exposed to 25 µg L^{-1}. Hm: haemocytes; Sptg: spermatogonia; Sptc: spermatocytes; Sptd: spermatids; Sptz: spermatozoa; Gt: gonadal tubules; R: reserve tissues. Arrowhead: regression areas. (b) and (d) are expanded images from (a) and (c), respectively, and (e) is expanded image from (d).