PET microplastics toxicity on marine key species is influenced by pH, particle size and food variations

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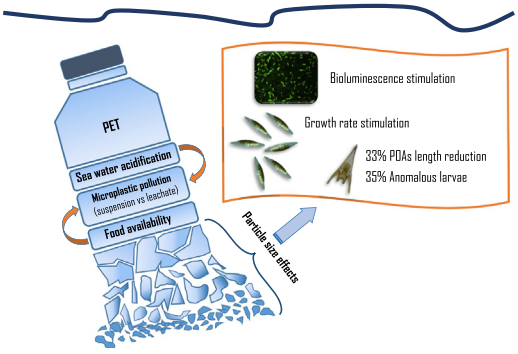
HIGHLIGHTS

- Impact of plastic-made packaging on key marine species is evaluated.
- Bacteria and algae were not affected but echinoderms showed severe effects.
- Particle-sizes of PET produce different effects not always related to the size.
- Acidified and standard pH conditions showed significant differences.
- Fasting and feeding conditions influenced effects recorded on *P. lividus*.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to evaluate effects induced by the exposure of key marine species to leachates and suspensions of different particle-size of PET microparticles, a plastic polymer that is actually considered safe for the environment. Leachates and suspensions of small (5–60 μm); medium (61–499 μm) and large (500–3000 μm) PET were tested on bacteria (*V. fischeri*; UNI EN ISO 11348-3:2009), algae (*P. tricornutum*; UNI EN ISO 10253:2016E), and echinoderms (*P. lividus*; EPA 600/R-95-136/Section 15) species both under standard (8.0) and acidified (7.5) pH conditions. Results obtained show that: *i*) conversely to larval stage of *P. lividus*, bacterial and algal tested species are not sensitive to PET pollution under all tested conditions; *ii*) different tested particle-sizes of PET produce effects that are not always related to their particle-size; *iii*) differences comparing acidified and standard pH conditions were recorded; *iv*) concerning echinoderms, food availability produce significant differences compared to fasting conditions; *v*) special attention on the possible interactions between MPs and other stressors (e.g., food and pH) is needed in order to give a better picture of natural occurring dynamics on marine ecosystems especially in the future frame of global changes.

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1. Introduction

An increase in plastic production resulted in an intensification in waste abandoned in the environment defacing the landscape, causing economic losses and threatening life. Legal or illegal dumps on coastal areas release waste polluting marine ecosystems (AEA, 2006). As consequence of the inadequate waste management performed, 6.2 items/linear meter of beach are recorded in Italy and plastic accounts for the 80% of the total amount collected (Carpentieri et al., 2018). Over than 40% of plastic production worldwide is used for packaging and largely for food packaging (UNEP, 2015) contributing to the marine litter (https://odims.ospar.org/maps/526/view). Marine environments are the ultimate stop for many pollutants including plastic litter. Without waste management infrastructure improvements, the cumulative amount of plastic waste available to enter the ocean is predicted to reach 250 million of metric tons by 2025 corresponding to an increase of an order of magnitude respect the level of 2015 (Jambeck et al., 2015). Plastic pollution represents a severe risk for marine ecosystems impacting marine species (Avio et al., 2015; Collard et al., 2017; Dehaut et al., 2016) by direct ingestion (Cole et al., 2011), by trapping and by translocation of smallest MPs fractions inside cells (Lusher et al., 2017) that could affect feeding habits and animals' reproductive success (Cole et al., 2014). Every year >12 million tons of plastic enter the oceans, of which >92% are particles generally smaller than 5 mm (Jambeck et al., 2015). These tiny particles, more commonly known as microplastics (MPs), may either result by the fragmentation of large items, or they can directly enter the environment as pellets, beads, and fibres (Li, 2018). Microplastics are ingested by a wide range of marine species (Dehaut et al., 2016), such as pelagic fish species (Renzi et al., 2018a), benthic species (Neves et al., 2015; Courtene-Jones et al., 2017; Pellini et al., 2018; Renzi et al., 2018b; Van Cauwenberghe et al., 2015; Karlsson et al., 2017; Renzi et al., 2018c), and marine mammals (Fossi et al., 2016). Microplastics are defined by GESAMP (2016) as "plastic pieces less than 5 mm in size" but any lower limits are defined. A more recent paper (Frias and Nash, 2019) defines the size-range interval 1–5000 μm as the most suitable to refer to microplastics. Lusher et al. (2017), in an extensive review, reported that particles larger than 150 μm are able to have low biological interactions while, particles smaller can migrate from the digestive apparatus of mammals towards their portal vein, their organs, and cells, also resulting able to overcome the brain barrier. Scientific interest towards microplastic in marine environment is quickly increased in the last five years; published researches on MPs related fields grew exponentially from 23 (2013) to 774 in 2019 (PubMed, searching "microplastic", publishing per year). Nevertheless, the largest number of papers is focused on toxicology and environmental chemistry and biological or ecological complex effects at the ecosystem level induced by the exposure to microplastic in marine environment represent yet a scarcely explored research field (Pauna et al., 2019).

In 2018, the global plastic production on Earth was estimated to be close to 359 millions of tonnes. In the same year, according to Plastic Europe (2019), PET (PolyEthylene Terephthalate) represented one of the most produced polymer in Europe (7.7% of the total amount produced). Since PET is light in weight, cheap and with very low production costs, it is proved to be one of the best candidates for single use products for mass consumption. PET is considered a safe polymer for human health and it is largely utilized for the production of sheets, films, and fibres, for the packaging of foods and beverages but, also, to produce parts of electronics, automotive parts, sports goods, and textiles (Sinha et al., 2010; Webb et al., 2013; Singh et al., 2018). Although PET is extremely resistant to weathering, it is not immune to fragmentation processes. The abiotic weathering of PET in marine environments is likely to occur principally by photo-oxidation and by hydrolysis (Gewert et al., 2015). Different marine and coastal compartments are contaminated by litter originated from PET and drinking bottles represent one of the items most frequently found in beach litter (Munari et al., 2016). Due to density driven mechanisms, PET can reach the seafloor (Mu et al., 2018). Nevertheless, in contrast to what expected on the polymer distribution based only on density features, PET-made microplastics are recorded in samples floating plastics collected in the Mediterranean Sea highlighting their contribution to the formation of the "soup" of polymers which affects the oceans worldwide (Suaria et al., 2016). Renzi et al. (in press) reported percentages of PET-made microplastics in sediments and holothurians respectively within 6.2–12.5% and 25.0–29.5%. Even if PET-made microplastics potentially affects both abiotic matrices and wild species, such records in natural water, sediments, and pelagic or benthic biota are scarce. Concerning water samples, the chemical composition of the fraction lower than 300 μm, which corresponds to the pore size of the manta net, is completely unknown. Concerning solid matrices, the European Technical Subgroup on Marine Litter (MSFD Technical Subgroup on Marine Litter, 2013) highlighted that this underestimation could be due to the low effective methods of extraction applied for microplastics. In fact, the density separation step, widely performed by literature using a hypersaline solution (NaCl, density of 1.2 g cm^{-3}), although cheap, is not suitable for denser polymers such as PET (density of 1.37–1.45 g cm^{-3}).

Concerning biological effects coming from the exposure to PET pollution, some recent studies demonstrated the bioavailability and toxicity of PET microplastics in different freshwater organisms such as amphipods, copepods, and fish (Heindler et al., 2017; Rochman et al., 2017; Weber et al., 2018). The main toxic effects recorded are the reduction in copepod egg production, the reduction of relative population size, and differences in feeding behaviour of sturgeon when exposed to prey fed with microplastics. Several other endpoints such as gene expression, development of the molting, metabolism, condition factor, and mortality seem to be unaffected. In addition, PET ecotoxicity on marine organisms remains a poorly explored field of investigation to date. Salinity represents the principal feature of marine ecosystem able to affect toxicity of chemicals on biota (Hall and Anderson, 1995; Hall et al., 1995). Even if pH of marine ecosystems is strictly buffered to 8.0, coastal areas could be affected by pH fluctuations (Hofmann et al., 2011). Surface ocean acidity increased by approximately 30% since preindustrial levels (Doney et al., 2009). Furthermore, a recent scenario models that, in 2100, as a result of global changes, ocean acidification will lead to a level of 7.5 pH units with loss of seawater buffer capacity (Raven, 2005). Recent papers suggested that changes of salinity (Lee et al., 2010; Fastelli and Renzi, 2019), and pH (Knutzen, 1981; Roleda et al., 2015) are able to determine, in marine species, unpredictable changes in ecotoxicological responses. Variation of pH of ocean water can potentially modifies the chemical equilibrium of microplastics increasing or decreasing the leaching rate of chemical substances that are on their surface as a result of the production process or adsorption from the water. Therefore, the PET that is currently considered relatively safe for the environment could potentially become dangerous in the near future with different environmental conditions. Another possible factor of confusion on the evaluations of the ecotoxicological effects is represented by the availability of nutrition under natural conditions. Laboratory tests are performed in fasting conditions while species in nature are exposed to stressors and have access to trophic resources at the same time (Renzi et al., 2019a). Furthermore, the relationship between the toxicity of microplastics and their average size is little explored by the literature.

This study aims to fill some of the exposed knowledge gaps testing the effects induced on bacteria (*Vibrio fischeri*), algae (*Phaeodactylum tricornutum*), and on larval stage of echinoderms (*Paracentrotus lividus*) following exposure to PET microplastics of three different particle-sizes. Recently Foley et al. (2018) reported that microplastics seem to exert a direct and indirect effects (i.e. chemical) threat to marine organisms. In this study, bacteria and algae species were exposed to leachates of PET MPs to evaluate chemical toxicity. Nevertheless, echinoderms were exposed both to leachates and suspensions of PET to evaluate also effects induced by the direct contact with MPs (i.e. ingestion for the smallest tested size; mechanical damages for the largest ones). In addition, all cases were tested in a standard (pH 8.0) or acidified scenario (pH 7.5),

to simulate the phenomenon of sea acidification as a consequence of global warming. Finally, effects were measured in presence and absence of trophic resources.

The tested species were selected due to their representativeness of different trophic levels in marine ecosystems (ISPRA, 2011). Furthermore, laboratory experiments are largely standardized on these species and experimental variability can be controlled. *Vibrio fischeri* bioluminescence inhibition bioassay is widely used for toxicity evaluations in water, soil, and sediment samples on account of multiple advantages encompassing shorter test duration, sensitive, cost-effective and ease of operation (Abbas et al., 2018). The genus *Vibrio* was previously used in other studies performed on polymers (Romeo et al., 2015; Gambardella et al., 2018). The algal growth inhibition test was used by previous literature to assess the MPs impact on primary producers (Zhang et al., 2017; Mao et al., 2018; Yi et al., 2019); nevertheless, PET has been never tested before. *P. lividus* has already been used in other similar studies. For instance, Oliviero et al. (2019) reported a drastic reduction of larval length in plutei exposed to PVC leachates probably due to the presence of phthalates. Messinetti et al. (2018) recorded significant differences in body and arm length of *P. lividus* plutei reared at 25 mg/L of 10 μm polystyrene beads. Hence, the embryo toxicity test with *P. lividus* aims to investigate the possible implications on the pelagic phase of a commercially relevant and very sensitive species.

2. Material and methods

2.1. Microplastics production and pre-treatment characterization

PET micrometric flakes were obtained by double trituration of 1 mm pellets using industrial mills. The resulting gross-sized powder was sifted in order to obtain the three desired particle-sizes: small (5–60 μm; S-PET), medium (61–499 μm; M-PET), and large (500–3000 μm; L-PET). S-PET was selected as it resulted widely lower than 150 μm, the upper limit referred to cut off for migration from the digestive system within mammal's portal vein (Lusher et al., 2017). M-PET (61–499 μm) represents the cut off size between the fraction able to penetrate organs and the upper limit for visual identification of microplastic. L-PET (500–3000 μm) is the largely documented fraction in environmental matrices and animal tissues by literature (Suaria et al., 2016); furthermore, it is considered to be relatively safer than other fractions as it cannot penetrate biological barriers. Particle-sizes obtained were collected separately in glass bottles, then quickly washed in ethyl alcohol 96%, drain off and dried in oven at 35 °C up to complete evaporation of residual traces of alcohol. This pre-treatment was effective to remove trace of external pollution on MPs surfaces without changing structural microplastic chemical composition (Renzi et al., 2019a; Supplementary materials). Furthermore, ethyl alcohol is highly volatile (5.95 kPa, vapour pressure at standard atmospheric condition) and according to OECD (2014) it is not toxic to aquatic organisms at residual levels (low or absence of toxicity at concentration < 100 mg/L). The generic shape of microplastics tested in this study can be approximated to very heterogeneous flakes, with jagged edges and surface irregularity (Fig. 1S – Supplementary materials). These shape features well simulate MPs particles present in environmental matrices (Lambert et al., 2017).

2.2. Preparation of solutions for the leaching tests

Natural Standard Water (**NSW**) pre-filtered to 0.45 μm (38.0 ± 0.8 PSU; 7.0 ± 0.2 mg/L DO_2; 20.0 ± 0.4 °C), stored in glass bottles was use as starting medium. Water pH was checked and correct to the desired level by the addition of opportune doses of HCl (0.1 M) to obtain both standard (**St**, pH = 8.0) and acidified (**Ac,** pH = 7.5) water starting medium stored separately (pH ± 0.1). MPs leachates of tested S-PET, M-PET, and L-PET particle-sizes were prepared by the addition of 0.1 mg/mL of single tested MPs particle-size to 500 mL of both standard and acidified starting medium in a glass beaker and put in the incubator at 18 °C 12:12 light/dark cycle for 72 h. The microplastic dose selected in this study was superior to that expected to be present in the ocean but is suitable for detecting response/no response effects. After 72 h, the experimental leachates were obtained by filtering the suspensions on 0.45 μm nitrate cellulose fibre filters in order to remove plastic particles.

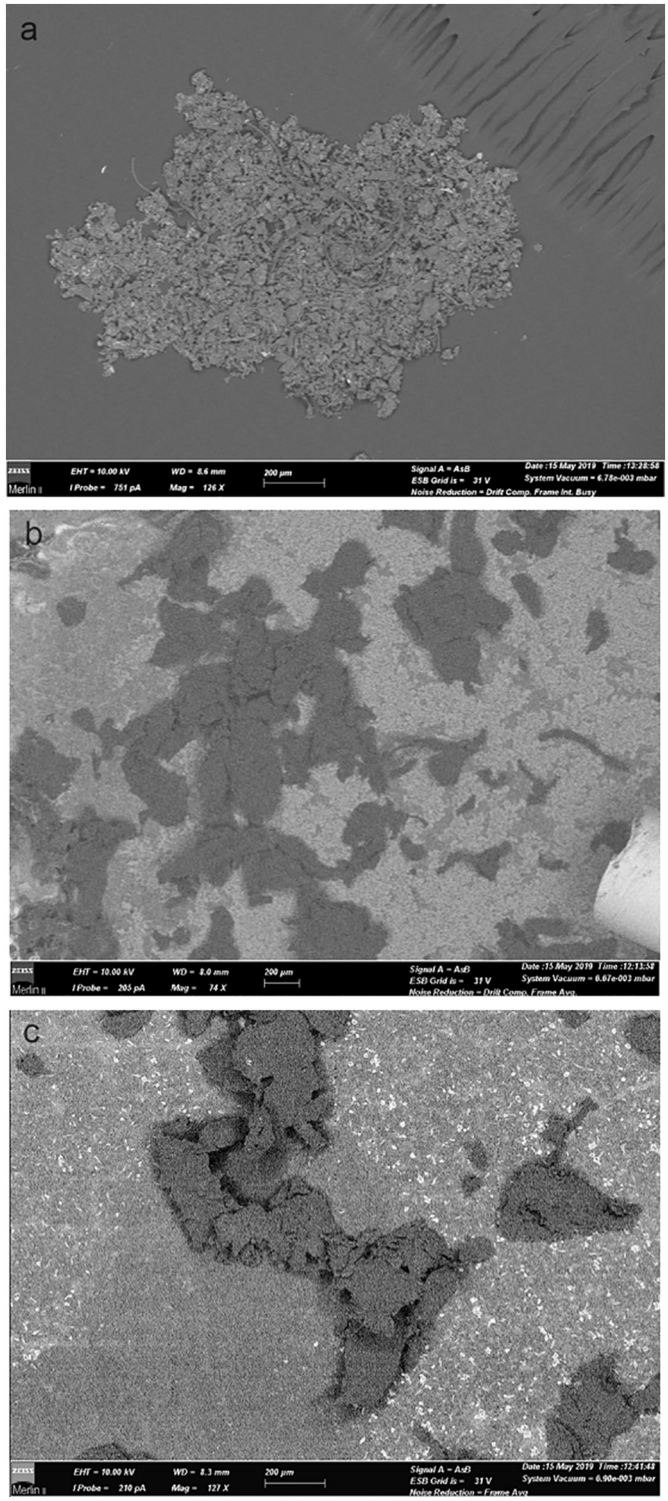

Fig. 1. FESEM topographic details and alterations occurring on MPs surfaces. Effects due to the exposure for 72 h to natural marine water at different pH conditions are reported: a) pH = 4.0; b) pH = 7.5; c) pH = 8.0. Images are referred to ethyl alcohol 96% quickly rinsed MPs particles.

A parallel filtration was carried on with Anodisc® filters (Whatman, lot n. A21184266; aluminium oxide membrane; 0.2 μm porosity) for μFT-IR analysis of the particle-size (see paragraph 3.1). Finally, filtered leachates solutions were aliquot to run ecotoxicological tests on *V.* fischeri, *P. tricornutum*, and *P. lividus*. Tests were performed immediately and residual aliquots were stored at −20 °C.

2.3. μFT-IR analysis and post-treatment characterization

A methodology based on microscopy associated to Fourier Transform Infrared Spectroscopy technique (μFT-IR; Nicolet, iN10 MX; Thermo Fisher Scientific) was run on Anodisc® filters for the identification of plastic features. The μFT-IR was equipped with liquid nitrogen cooled MCT-A operating within the spectral range 7800–650 cm^{-1} and with OMNIC Picta (Thermo Scientific, Waltham, MA, USA) users' interface. Filters were analysed by the Wizard-operating in transmission mode to determine particles mean and median sizes according to the 72 h of exposure to tested pH. PET particles thicker than 35 μm were analysed by ATR (Attenuated Total Reflection) using a germanium crystal (spectral range 3000–1300 cm^{-1}). Chemical spectra of both 8.0 and 7.5 pH exposed microplastics were collected to determine the occurrence of possible spectral differences. The threshold for IR spectra back-recognition was fixed over 65% of match. Limit of detection of chemical composition of targeted particles was 10 μm. The carbonyl index (CI) was used by literature to evaluate plastic degradation by acid exposure (Prata et al., 2019). CI was calculated by dividing the intensity of the carbonyl peak (1715–1735 cm^{-1}) by the intensity of the reference peak. Reference peak for PET was read at 1504 cm^{-1} (Pires et al., 2015).

Microplastics were, also, analysed by the Field Emission Scanning Electron Microscopy technique (FESEM, mod. Merlin II, Zeiss®) coupled with Wavelength Dispersive and Energy Dispersive spectrometer combined micro analyser (WD/ED mod. X-Max 50, Oxford Instruments®). FESEM was applied to check on targeted particles the occurrence of nano-changes on surface. This technique provides topographical and elemental information at magnifications within 10× - 300,000×, with virtually unlimited depth of field.

2.4. Ecotoxicity tests

2.4.1. Test on bacteria

Biological responses on bacteria were checked on the species *Vibrio fischeri* according to UNI EN ISO 11348-3:2009 using Microtox® (Ecotox) photometer and lyophilized bacteria purchased by Microbiotests Inc. The inhibition percentage of natural bioluminescence was measured after 5, 15, and 30 min of exposure to 90% of the concentration of the leachate, using two experimental replicates. Tests were performed both under standard and acidified pH conditions. Filtered Natural Standard Water (NSW) was used as control of the test. An experimental control was run also on acidified NSW.

2.4.2. Test on algae

Phaeodactylum tricornutum was selected as representative of effects on algal species. Growth inhibition percentage (I%) after 72 h of exposure was run on leachates under both standard and acidified conditions. An algal lot purchased by Ecotox® was tested after pre-enrichment in an ASPM culture medium. Illumination, temperature, salinity, and dark/light photo-cycles were set as reported in ISO 10253:2016 (E). Cell density measures were performed by spectrophotometer (Onda, mod. UV-30 scan; optical length 10 cm) and calculated by light absorbance at the wavelength 670 nm. The spectrophotometer response was calibrated using a cell density versus absorbance curve developed on tested algal stock by Burker's chamber counts at each of the 10 points scalar dilution of 10^6 cell/mL stock. Effects compared to controls were measured after 24 h, 48 h, and 72 h of exposure on three experimental replicates and calculated as reported by literature (Renzi et al., 2014).

2.4.3. Test on echinoderms

Paracentrotus lividus was selected as representative of effects on embryos. Tests were performed following EPA 600/R-95-136/Section 15; adapted according to ISPRA (2017) method. Percentage of abnormal larvae was measured after 72 h of exposure to both leachates and suspensions. The effects were assessed using sea urchin embryo-toxicity test and assessing both regular development and biometric impairment. The direct exposure test was performed using the single concentration of MPs (0.1 mg/mL) used to produce leachates. The exposure did not foresee the preparation of a stock solution to avoid both the influence in terms of toxicity of surfactants required to stabilize the suspension and possible variations in particle bioavailability. Thus, the desired mass of MPs was directly added to testing plates without the use of any surfactants. In this case the experimental design was developed both under standard and acidified conditions and, also, under fasting and feeding conditions to evaluate effects due to either ocean acidification and presence of feeding resources on toxicity of microplastics. In details, fertilized eggs were exposed to four microplastic-enriched solutions (Ac NSW + food, St NSW + food, Ac NSW – food, and St NSW – food) and associated negative controls. Three replicates and corresponding controls for each of the tested size (S-PET, M-PET, and L-PET) were carried out. Mature specimens of sea urchin were caught in a natural marine area (Tuscany) and maintained in captivity until the starting of the experiment. In vitro fertilization was performed according to ISPRA protocol (ISPRA, 2011). Fertilized eggs were allowed to develop in incubator at 18 °C under darkness conditions. Daily, the experimental group under feeding conditions were fed with few drops of algal (*P. tricornutum*) suspension at 3×10^5 cell/mL density. The same correction of dilution was performed for fasting population by the addition of NSW drops. At 72 h post fertilization (hpf), samples were fixed with two drops of filtered Lugol solution, and 100 embryos for each plate were classified under the stereomicroscope (Nikon, mod. SMZ-800 N equipped with a digital camera interfaced to Nikon ACT-1 software) to perform counts of abnormal larvae. Larvae were considered abnormal if, at the developmental stage of 72 hour-old, plutei showed developmental arrest, all arms missing or with different length, additional arms cross lateral rods, asymmetrical body width and other anomalies listed by literature (ISPRA, 2017). Additionally, on 15 normal larvae per plate (including negative controls), length reductions of both anterior-oral arms (AOAs) and post-oral arms (POAs) were measured to evaluate effects on body size. This further endpoint was added as it resulted sensitive to highlight early stress on exposed larvae during pre-screening tests than percentage of anomalies. The measures were carried out on larvae in the same spatial position in order to avoid perspective offsets. Micrometric measurements were performed by stereomicroscopy (Nikon, mod. SMZ-800 N equipped with a digital camera interfaced to Nikon ACT-1 software) and statistically analysed to evaluate solid differences compared to controls.

2.5. Quality assurance & quality control

Tests were performed in a certified laboratory (UNI EN ISO 9001:2015; UNI EN ISO 17025:2005) to ensure the quality of produced data. QA/QC tests were performed as described by the reference methods previously cited. Positive controls were performed by the direct exposure of tested species to standard toxicants: *V. fischeri* was exposed to 3,5′-dichlorophenol (I% 30 min = 21.7 ± 0.5); *P. tricornutum* was exposed to $K_2Cr_2O_7$ (EC_{50} = 37.2, range 24.6–56.3 mg/L); *P. lividus* was exposed to $Cu(NO_3)_2{}^*3H_2O$ (EC_{50} = 25.9, range 21.6–30.3 μg/L) resulting within the acceptability criteria defined by standard methods. Negative controls were performed on natural standard water under experimental conditions. Recorded data were within the acceptability of tests under standard conditions (pH = 8.00).

2.6. Statistical analyses

Data were statistically analysed by GraphPad Prism (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Routines related to column statistics (mean, standard deviation, min-max ranges), one-way repeated measure ANOVA were run on *V. fischeri* dataset, 1-way and 2-way ANOVA (significance of observed differences between exposure and controls and within treatments) were performed on *P. tricornutum* and *P. lividus* dataset. Differences were considered significant at p-level < 0.05. Tukey's multiple comparison test was used to highlight significant differences within treatments and Dunnet's test between treatments and controls. Sidak's multiple comparisons test for disentangles differences between each size level of leachate with the other of suspension was performed. Multivariate analyses were performed by Primer v7.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) following methods reported by Clarke and Warwick (1998). Euclidean matrix of distance was calculated on normalized data of effect expressed as percentage. Tested factors on mean effects were: particle-size (four levels, fixed, 0-PET, S-PET, M-PET, L-PET), pH condition (two levels, fixed, 8.0 and 7.5), and food availability (two levels, fixed, feeding and fasting conditions).

3. Results

3.1. Microplastic characterization

Spectra collected by μFT-IR analyses confirmed the PET nature of the tested particles. Collected PETs spectra matched over than 80% with the spectra for PET from the reference library (Thermo Scientific®). In particular, matches recorded were: 82.8% for S-PET, 87.8% for M-PET, and 87.9% for L-PET. Microplastic polymer may change due to degradation process including the exposure to acids (Da Costa et al., 2018). To evaluate polymer degradation, spectra of MPs collected under St pH conditions were saved as reference library and matched against spectra collected on Ac treated PETs. In this case, a significant reduction of matches was recorded as follow: -12.2% (S-PET), −1.8% (M-PET), and − 5.0% (L-PET) as resulted by Fig. 2S (Supplementary materials). CI index calculated for each tested PET particle-size are reported in Table 1. Values highlights a general reduction of levels after Ac exposure with the following intensity M-PET ≫ L-PET. Concerning levels in S-PET particles under Ac conditions, CI index showed an increase. Concerning PET characteristic peaks (1715 cm^{-1}, C=O; 1245 & 1100 cm^{-1}C-O) a significant reduction of intensity following acidification was recorded. S-PET and L-PET also highlight significant reduction of not characteristic peaks (3427, 2963, 2365, 1958, 1408, 1337, 1246 cm^{-1}).

The μFT-IR analyses of particle-size of tested PETs confirmed ranges theorized at the beginning of the experiment (Table 1). After 72 h of incubation, S-PET showed a mean length (±standard dev.) of 18 ± 14 μm (Ac) and of 15 ± 12 μm (St). Mean lengths of M-PET were: 168 ± 120 μm (Ac) and 151 ± 126 μm (St). L-PET under St conditions showed a longer mean length (928 ± 450 μm) compared to Ac exposed ones (738 ± 185 μm).

FESEM analyses showed topographic details and alterations occurring on MPs superficies when exposed to Ac pH conditions compared to control (St) as reported in Fig. 1. Jagged edges, surface irregularities and heterogeneity in size, that represent the main features of PET microplastics under standard pH conditions (**1c**), are progressively modified under acidified condition. In particular, surface irregularities (**1b**) are notably increased after the exposure under strong acid pH = 4.0 (**1a**).

3.2. Ecotoxicity of PET leaching

3.2.1. Tests on bacteria (Vibrio fischeri)

Results on inhibition percentages recorded after 5, 15, and 30 min of exposure of bacteria to leachates of S-PET, M-PET, and L-PET are represented in Fig. 2. Results obtained under St and Ac conditions are compared. Concerning St, mean values of effects ranged between 0% and

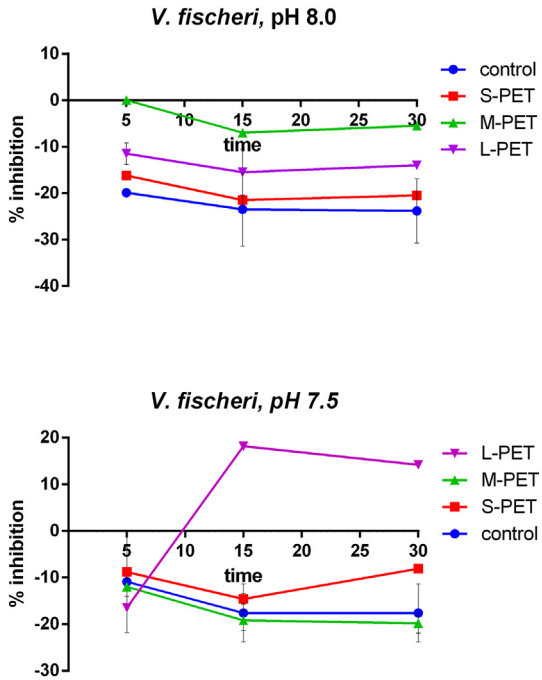

Fig. 2. Ecotoxicological effects on bacteria recorded after the exposure under different pH conditions to leachates of three particle-sizes of PET. Percentages of inhibitions of the natural biolominescence are reported after 5, 15, 30 min of exposure to leachates. Results are reported grouping data according to the pH of the leachate (pH = 8.0, standard condition; pH = 7.5, acidified conditions). Each point represent mean value of two replicates (±SEM). Negative values mean biostimulation.

−21.5% (biostimulation). Biostimulation was recorded in particular for the smallest particle-size tested (S-PET). The occurrence of biostimulation was confirmed under Ac conditions for S-PET (−19.8%) and M-PET (−8.1%). However, concerning L-PET, after 15 min a reversal of trend occurred causing a maximum inhibition of +18.2%. ANOVA (one-way) analysis revealed significant differences between M-PET and L-PET respect to control (p < .01) and between PET-size, under standard pH conditions.

3.2.2. Tests on algal species (Phaeodactylum tricornutum)

There were no significant differences of growth rates between treatments and control, or within treatments after 72 h of exposure under both pH conditions. However, the ANOVA two-way calculated on growth rate inhibition (%) highlighted significant differences in all treatments respect to control at both pH conditions and differences between S-PET and both M-PET and L-PET at pH 7.5 (Fig. 3).

3.2.3. Tests on echinoderms (Paracentrotus lividus)

A particle-size dependent response was recorded under St condition, where abnormal larvae (%) increased proportionally with the

Table 1
Results of μFT-IR particle-size analyses. Mean size, standard deviation (SD), median, minimum and maximum (expressed in μm) of the three tested typologies of PET (S-PET; M-PET; L-PET) microplastics after 72 h of exposure to natural standard water both under pH 8.0 and pH 7.5. Statistics do not highlight significant differences between acidified and standard conditions. CI = Carbonyl Index.

Sample	Size-class (declared)	Treatment	CI	Mean	SD	Median	Min	Max
S-PET	5–60	7.5 pH	2.72	18.0	14.3	12.1	4.9	61.5
		8.0 pH	1.92	14.8	11.8	10.9	4.9	61.3
M-PET	61–499	7.5 pH	3.12	167.5	119.3	116.5	58.1	626.5
		8.0 pH	4.02	151.2	126.4	95.0	58.4	586.5
L-PET	>500	7.5 pH	2.46	737.6	185.1	719.5	494.6	1192.4
		8.0 pH	2.68	927.7	449.9	747.8	494.5	2345.6

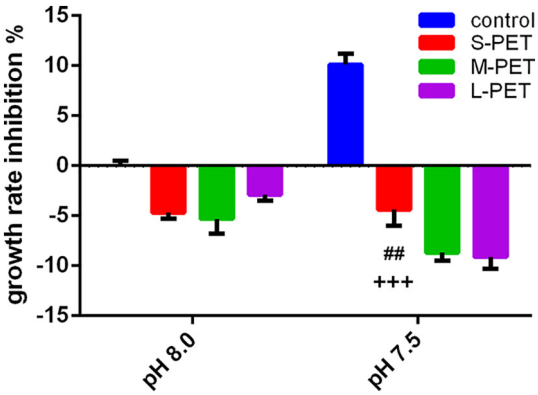

Fig. 3. Ecotoxicological effects on algae recorded after the exposure under different pH conditions to leachates of three particle-sizes of PET. Percentages of inhibitions of the natural growth rates recorded after 72 h of exposure to leachates are reported. Results are reported grouping data according to the pH of the leachate (pH = 8.0, standard condition; pH = 7.5, acidified conditions). Each bar represents mean value of three replicates (standard deviation). Negative values mean biostimulation. Although not graphically reported, all treatments differ from control (Dunnett test, $p < .05$). In addition, S-PET significantly differs from other treatments (Turkey test, $p < .05$). # means differences from M-PET; + means differences from L-PET.

particle-size of MPs (Fig. 4). Under Ac conditions, except for L-PET, a similar trend was reported and significant differences were found between controls, S-PET, and M-PET ($p < .01$). Under St conditions, L-PET ($p < .001$) and M-PET ($p < .05$) differ from controls. Tukey's multiple comparison tests highlighted significant differences between L-PET-S-PET ($p < .05$) and L-PET-M-PET ($p < .01$) couples under Ac conditions. Under St, Tukey's multiple comparison tests evidenced significant differences between L-PET and S-PET ($p < .001$). Significant differences between controls and PET leachates were recorded for both AOAs and POAs of plutei under Ac and St conditions ($p < .0001$) (Fig. 5). As AOAs and POAs are strongly positive correlated ($p < .001$) only POAs correlations are reported. In general, treated plutei reported shorter POAs than control (up to −33.4%) and AOAs (up to −32.3%) when exposed to both pH scenarios (Table 2). Finally, the Ac scenario induced higher length reduction compared to St conditions, especially for M-PET (+18% of reduction) as represented in Fig. 6.

3.3. Ecotoxicity of suspensions on echinoderms

3.3.1. Fasting conditions

The direct exposure to the different particle-size of PET under St conditions seems not to affect the larval growth. However, under Ac conditions, L-PET treated larvae significantly differ both from control ($p < .05$) and S-PET and M-PET ($p < .01$) as represented in Fig. 7.

3.3.2. Feeding conditions

The direct exposure to PETs suspensions under Ac was not effective on larval growth if feeding conditions occurred ($p > .05$; Fig. 8). On the contrary, under St, M-PET significantly differs from control ($p < .01$). Concerning leachates, greater consequences have been recorded. Under Ac, S-PET ($p < .05$) and M-PET ($p < .001$) treated larvae significantly differed from controls. Also under St, plutei exposed to leachates differed from controls (M-PET, $p < .01$; L-PET, $p < .0001$). In the case of leachates, S-PET differed from others MPs under both pH conditions. Under Ac + feeding conditions, ANOVA (two-way) revealed significant differences respect to control both to suspension and leachate concerning POAs ($p < .0001$; Fig. 9). Under St + feeding conditions, strong significant differences were detected for all tested particle-size among each other and compared to controls.

3.4. Overview by multivariate analyses

Non-metric multidimensional scaling (*nm*-MDS) performed on the whole database is represented in Fig. 10. The ANOSIM test performed on the factor "food availability" highlight significant segregations (Global $R = 0.255$, $p < .01$; Table 3) In this case, factors "particle-size", "treatments", and "pH conditions" should be tested separately respectively on feeding and fasting conditions datasets. Nevertheless, the amount of records did not allow testing factors of interest on two separate feeding resources databases. A global overview on significant relation recorded in this study is summarized in Table 1S (Supplementary materials).

4. Discussions

4.1. Microplastic changes due to pH variation

The reduction of particle-size is associated to increasing toxicity as documented by studies performed on nanoparticles (Jeong et al., 2016; Lu et al., 2016; Karami et al., 2017; Cui et al., 2017). Materials that are safe in their bulk form become active on biota if reduced in size to their nanoforms (Jeevanandam et al., 2018). This is due to the increase of the surface/volume ratio following the size reduction that, consequently, increases the surface that particles are able to expose to water. Microplastic is an "umbrella" term including any plastic-made particle with a dimensional range included within 1–5000 μm (Frias and Nash, 2019). Bioactivity within this wide size range could be very different as focused by this study on tested species exposed to three different particle sizes. The chemical equilibrium of microplastics could be affected by variations of ocean pH, as effect of global changes or human activities, which could exacerbate the release of chemicals modifying the toxicity of the polymers. Results obtained by analyses performed in this study support an ultrastructural change of PET MPs exposed to acidified conditions at the nanoscale-level. These results enhance the

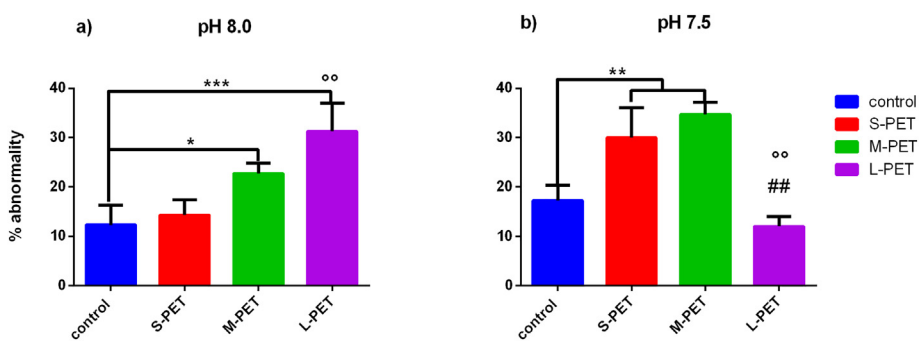

Fig. 4. Percentage of abnormalities (mean ± st. dev.; $n = 3$) of the sea urchin larvae after 72 h of incubation in different leaching solutions of PET microplastics (S-PET; M-PET; L-PET) at pH 8.0 (a) and 7.5 (b). Results were not normalized according to Abbott (1987), controls are represented. * = significant differences from control; ° = differences from S-PET; # = differences from M-PET. The repetition of * indicates the level of significance according to the following code: $p < .05$ *; $p < .01$ **; $p < .001$ ***; $p < .0001$ ****. Results from 1way-ANOVA (Tukey test for disentangle differences between treatments; Dunnett test for differences with control).

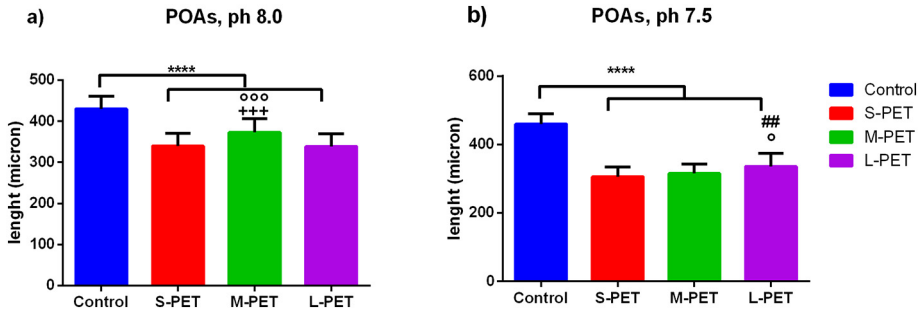

Fig. 5. Post oral arms (POAs) analysis of plutei exposed to different leaching solutions of PET MPs (S-PET; M-PET; L-PET) at pH 8.0 (a) and 7.5 (b). Results were not normalized according to Abbott (1987), controls are represented. * = significant differences from control; ° = differences from S-PET; # = differences from M-PET; + = differences from L-PET. The repetition of * indicates the level of significance according to the following code: $p < .05$ *; $p < .01$ **; $p < .001$ ***; $p < .0001$ ****. Results from 1way-ANOVA (Tukey test for disentangle differences between treatments; Dunnett test for differences with control).

need for specific evaluation of ecotoxicological and ecological effects to better model risks for marine environments associated to packaging pollution under a global changing scenario. Performed analyses highlighted significant effects on PET material following exposure to Ac solution (size-trend M-PET ≫ L-PET). The reverse trend to increase CI index, which was recorded for S-PET particles, could be explaining as technical artefact due to the interference of size effect on spectra signal for smallest particles. In fact, the same occurrence was reported on virgin microplastics by Raman spectroscopy and attributed to the impact point of the laser on the surface of the polymer (Dehaut et al., 2016). Following acidification, a significant reduction of intensity of PET characteristic peaks (1715 cm^{-1}, C=O; 1245 & 1100 cm^{-1}C-O) was recorded and explained by C=O and C—O degradation (Ioakeimidis et al., 2016). The reduction of intensity that resulted associated to the non-characteristic PET zone of spectra for all of the exposed particle-size, could be due to the release of chemical additives and structural modifiers. Information on such chemical additives requires additional chemical analysis, such as GC–MS (not performed in this context). Finally, the pre-treatment washing of PET-MPs with ethyl alcohol 96% is a widely used step in ecotoxicological study with microplastic (e.g. Jemec et al. 2016), showing effectiveness in reducing microparticles impurities (i.e. talc) absorbed on microplastic surfaces during their production without significantly alterations of μFT-IR spectra collected on both washed and unwashed microplastic (Renzi et al., 2019a; Supplementary materials). In spite of that, the extraction of some plastic additives during the preliminary rinsing performed, could not be a priori excluded and for this reason obtained results could underweight toxicity due to additive contribution. This specific aspect will be subject of further research aimed at achieving this goal.

4.2. Effects induced by leachates

4.2.1. Standard conditions (St, pH = 8.0)

Leaching of plastic packaging and microplastics represents an indirect source of impact for marine ecosystems, as leaching could determine significant releases of chemicals in water (Rochman et al., 2013; Pedà et al., 2016). Our results reported that, under St condition, the exposure of marine species to leachates of the three different particle-sizes of PET tested, are not sufficient to highlight significant acute effects on bacteria and algae species that pointed out, on the contrary, biostimulation. Results obtained in this study on bacteria are in agreement with literature. A handful of studies deployed the marine bacterium for ecotoxicity test of microplastics (Booth et al., 2015; Gambardella et al., 2018) and *V. fischeri* often reported no effect or a scarce sensitivity. This study supported the idea that this species is not a sensitive model for plastic toxicity. Due to a major chemical affinity, microplastics are able to absorb more organic compounds, but *V. fischeri*, is more sensitive to trace elements rather than to organic-due pollution and this could partially explain the weak sensitivity reported in this study. Control algae exposed to the acidified scenario highlight a slight increase (although not biologically significant) in growth rate inhibition, suggesting a sensitivity of such species to pH water variations. However, this early stress is totally buffered by the leachates of PET-MPs, which instead trigger biostimulation responses. Sjollema et al. (2016) tested effects following the exposure to nanoplastics (tested range within 0.05, 0.5 and 6 μm) and, reported adverse effects, were demonstrated to increase with decreasing particle size. Effects observed in this study concerning biostimulation on algal species (*P. tricornutum*), could represents an early sign of chronic stress (Renzi et al., 2014). A similar phenomenon was recorded by Mao et al. (2018) who tested the toxicity of polystyrene microplastics on the growth inhibition of *Clorella pyrenoidosa* at the same concentration (100 mg/L). This study evidenced how the effects of plastic can impair or enhance the algal growth depending on the developmental stage of the algae. The algal growth is characterized by four stages: lag, logarithmic, stationary, and death (Tsai et al., 2017). When the algae reach the logarithmic phase, it generally shows the strongest ability to maintain its activities under biotic and abiotic stress. For this reason, the algal growth was stimulated from the end of the logarithmic stage to the stationary phase. In our study, the stimulation of algal growth occurred during logarithmic phase, suggesting *P. tricornutum* is a "plastic-resistant" species.

Tests performed on larval stage of *Paracentrotus lividus* highlight significant effect both on the two tested endpoints: % of anomalies after 72 h of exposure and body-size reduction in normal larvae. In particular, particle-size is directly related to the percentage of abnormal larvae recorded (toxicity of L-PET ≫ S-PET; M-PET). In our study, all leachates from different PET particle-size tested, evidenced significant reduction

Table 2
Length reduction (%; standard deviation) of anterior and posterior oral arms of plutei exposed for 72 h to different leaching solutions (S-PET; M.PET; L-PET). Differences between acidified and standard values highlight the pH-effect.

		S-PET		M-PET		L-PET	
		pH 7.5	pH 8.0	pH 7.5	pH 8.0	pH 7.5	pH 8.0
Post Oral Arms POA	Length reduction (%)	33.4 (3.5)	20.9 (4.3)	31.4 (7.9)	13.3 (5.4)	26.9 (3.2)	21.3 (2.4)
	Difference (Ac-St) %	12.5		18.1		5.6	
Anterior Oral Arms AOA	Length reduction (%)	32.3 (4.2)	19.6 (2.8)	31.4 (5.2)	15.1 (6.3)	25.0 (3.3)	23.6 (2.9)
	Difference (Ac-St) %	12.7		16.2		1.4	

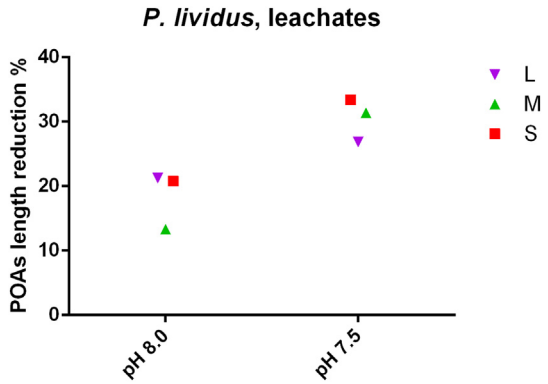

Fig. 6. Percentage of length reduction of POAs in different pH scenarios and PET particle-size. The acidified scenario seems to exert a stronger effect.

of POAs in normal larvae suggesting that all tested leachates are effective to determine a significant body-size reduction in exposed embryos also in normal phenotypes. These results are in agreement with literature. Oliviero et al. (2019) reported a drastic reduction of larval length (33%) in plutei exposed to PVC leachates probably due to the presence of phthalates. Our results support studies indicating *P. lividus* as a good bioindicator of marine pollution (Cunha et al., 2005; Alvarez et al., 2010; Sanchez-Marın et al., 2010). To evaluate more complex endpoint in ecotoxicological responses, embryo toxicity on sea urchin is reported to be more sensitive than acute tests on other species (Losso et al., 2004) and, also, than fertilization tests on the same species (Lera and Pellegrini, 2006). The exposure to low doses of chemicals acting as endocrine disruptors (i.e. *nonyl*- and *octyl*-phenols, bisphenol-A) resulted in this species is able to affect the embryological development (Roepke et al., 2005; Arslan and Parlak, 2007; Ozlem and Hatice, 2007). These chemicals are common additives in plastic packaging to increase plastic performance. In conclusion, sea urchin larvae resulted to be the most sensitive organism and POAs length resulted to be a sensitive endpoint to detect early sub-lethal effects at lower exposure doses.

4.2.2. Acidified conditions (Ac, pH = 7.5)

Results obtained in this study on FESEM and infrared spectral microanalyses performed on PET MPs exposed to acidified marine water highlighted, at the nano- scale, the occurrence of disaggregation, structural changes (i.e. reduction of acute angles), and dissolution of plastics with losses of infrared peaks that are in the PET characteristics region of the infrared spectra. Results from dimensional analyses performed, supported the occurrence of a general fragmentation supported by the recorded reduction of mean dimension that was more significant in Ac L-PET that other particle-size tested. Literature reported microplastic degradation occurring during extraction process of microplastic by environmental matrices with acid solutions (i.e. 22.5 M of nitric acid; Avio et al., 2015) supporting the effectiveness of the exposure to acid solutions even for short times (20 min) in producing structural damages. Our results suggested that also the exposure to weak acids (Ac) for longer exposure times (72 h) could be effective to produce significant structural changes on MPs. Bacteria and algae exposed to MPs leachates under Ac conditions compared to their Ac controls showed any acute effects as also observed under St conditions. Concerning the algal species, in this case, a greater mean stimulation (+7.4%) was recorded than under St conditions (+4.5%). Results recorded by literature (Besseling et al., 2014) at exposure doses similar to ours, highlighted a maximum growth inhibition of 2.5% in the algal species *Scenedesmus obliquus* following to the exposure to nano-polystyrene. This suggests that ecotoxicological responses are strictly species dependent, particle-size dependent, and that generalization on the basis of models based on are scarcely realistic. Also, under Ac conditions, the larval stage of echinoderms represents a sensitive stage able to detect the tested stress. Concerning particle-size, Ac conditions produced a significant effect in S-PET and M-PET while L-PET resulted not effective compared to Ac controls. This result is strictly linked to release in leachates of plastic and associated chemicals as reported by infrared spectra results. Normal larvae exposed to leachates resulted in a significantly lower POAs mean compared to Ac controls in all tested particle-size supporting results obtained under St conditions on this endpoint. Literature evidenced that early developmental stages of echinoderms are relatively robust to predicted ocean acidification resulted not effectively impacted by water acidification if pH > 7.7 (Martin et al., 2011). Nevertheless, our study suggests that particle-size represents a key aspect to be considered concerning ecological and ecotoxicological effects under acidified scenarios. On the contrary, endpoints based on body-size (i.e. AOAs and POAs) resulted both very sensitive and effective under acidified conditions. Under changing scenarios concerning water acidification, POAs reductions in normal larvae could mean an increase of energy consumption during 72 h of development induced by the pH stress and consequently enhance the ecotoxicological response. This could be explained by the synergic effect of an increased energy consumption and gene transcription associated to chemical damages. Literature reported a temporal delay of the natural development of sea urchin exposed under acidified conditions that is due to 20-fold increase in gene up regulation and consequently an increase of energy consumption occurring at pH within 7.5–7.0 (Martin et al., 2011).

4.3. Effects induced by suspensions on echinoderms

It is known that exotrophy in sea urchin larvae starts around at 48 hpf (Giudice, 1986). The mouth of plutei that opens at this developmental stage is around 20 μm (Messinetti et al., 2018). As reported by Beiras

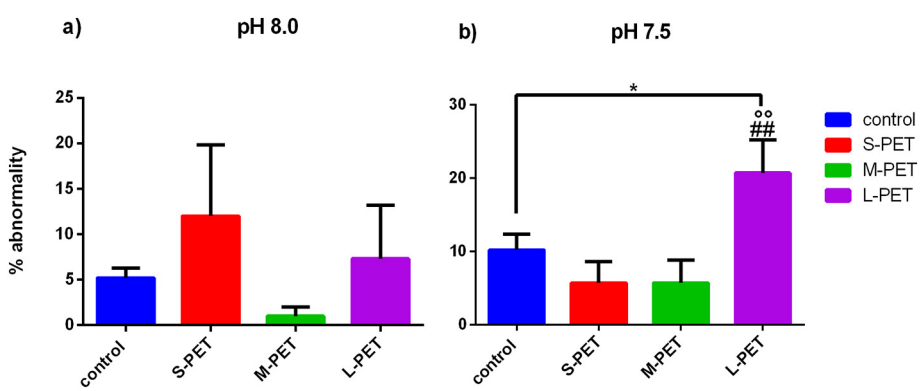

Fig. 7. Percentage of abnormalities (mean ± st. dev.; n = 3) of the sea urchin larvae after 72 h of incubation in different PET microplastics suspensions (S-PET; M-PET; L-PET) at pH 8.0 (a) and 7.5 (b). Results were not normalized according to Abbott (1987), controls are represented. * = significant differences from control; ° = differences from S-PET; # = differences from M-PET. The repetition of * indicates the level of significance according to the following code: p < .05 *; p < .01 **; p < .001 ***; p < .0001 ****. Results from 1way-ANOVA (Tukey test for disentangle differences between treatments; Dunnett test for differences with control).

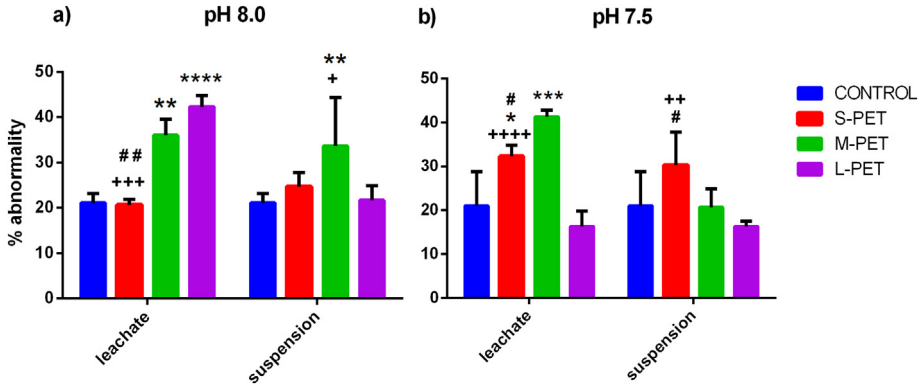

Fig. 8. Percentage of abnormalities (mean ± st. dev.; n = 3) of the sea urchin larvae after 72 h of incubation in different leachates and suspensions of PET microplastics (S-PET; M-PET; L-PET) under feeding conditions at pH 8.0 (a) and 7.5 (b). Results were not normalized according to Abbott (1987), controls are represented. * = significant differences from control; ° = differences from S-PET; # = differences from M-PET; + = differences from L-PET. The repetition of * indicates the level of significance according to the following code: $p < .05$ *; $p < .01$ **; $p < .001$ ***; $p < .0001$ ****. Results from 2way-ANOVA (Tukey test for disentangle differences between treatments; Dunnett test for differences with control). Results from 2way-ANOVA (Sidak's multiple comparisons test for disentangle differences between each size levels of leachate with the other of suspension) highlighted significant differences among leachate and suspension tested for L-PET ($p < .001$; St pH condition; data not represented) and for M-PET ($p < .001$; St pH condition; data not represented).

and Tato (2019) and Messinetti et al. (2018) plutei of sea urchin are able to ingest microplastic smaller than 10 μm, size compatible with the largest mass fraction of the smallest class (S-PET) used in this study. In this study, starved larvae reported an increased percentage of abnormalities when exposed to larger microplastics (L-PET, >500 μm) and under feeding condition, M-PET exerts toxicity. M-PET and L-PET are not compatible with the buccal apparatus of the animals, so the statistically difference reported is more likely due to the greater concentration of compounds leached during the 72 h of exposure. This result is in agreement with leachate test results discussed above. Even in this test, POAs length reduction says the same information of % of abnormalities and is in agreement with results reported by Messinetti et al. (2018) who recorded significant differences in body and arm length of *P. lividus* plutei reared at 25 mg/L of 10 μm polystyrene beads. In conclusion, the adverse effects were not due to the polymer but, more probably, to the cocktail of contaminants presents in the PET flakes. Ingestion of the smallest PET particle-size did not determine significant effects compared to those recorded by the exposure to "not ingestible" PET fraction. This may also be due to the fact that the fraction that can be ingested (S-PET) is numerically lower than mean size of the S-PET fraction as determined in this study by microscopic analyses. Further studies will need to better clarify these aspects.

5. Conclusions and future work

PET is a material actually considered safer than others plastic polymers and consequently widely used in single-to-use products. Its occurrence in different marine compartments has been proven (Munari et al., 2016; Mu et al., 2019; Suaria et al., 2016; Ter Halle et al., 2017). Nevertheless, especially for particles smaller than 150 μm (which are considered the more bioactive), its environmentally concentration is highly probable underestimated for the reasons presented in the introduction of this paper. In addition, PET eco-toxicological studies on marine species are poorly investigated by literature. For these reasons, in order to compare our results with those performed on other polymers at similar concentrations (Della Torre et al., 2014; Mao et al., 2018) and to highlight biological responses in the frame of global changes characterized by an increase of plastic pollution (Jambeck et al., 2015) and water acidification, we selected the concentration of 100 mg/L. However, it is important to point out that the concentration levels tested in this study are undoubtedly orders of magnitude higher than actual recorded levels in marine water (1.25 ± 1.62 particles/m^2; Suaria et al., 2016; SAPEA, 2019). Consequently, the authors suggest caution in the interpretation of the results here presented, inviting a reading in view of the future scenario of global changes.

Using this key to reading, from an ecological point of view, even if bacteria (detritivore) and algal species (primary producers) resulted not impacted by acute effects, larval stages of primary consumers (echinoderms) show increase of embryo toxicity under tested conditions. Consequently, consumers-linked trophic levels of marine trophic web could be significantly affected by PET MPs, directly and indirectly, by impacting the species fitness due to a significant reduction of larvae recruitment by the reproductive pool of the natural population. Furthermore, the significant reduction of body-size recorded on normal larvae species exposed to PET MPs, could represent a signal of subtle chronic

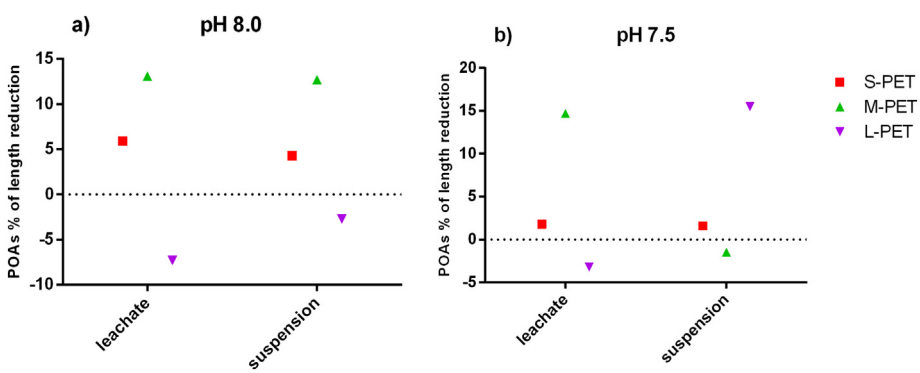

Fig. 9. Percentage of length reduction of posterior oral arms (POAs) of plutei exposed under feeding conditions to leachates and suspensions of PET MPs of different particle-size (S-PET; M-PET; L-PET). One graph for each pH scenario: a) pH 8.0; b) pH 7.5. Negative values mean increase in length. A general reduction of POAs length is detectable at both pH conditions, except for L-PET at pH standard which seems to increase the length (up to +7.3%) and L-PET and M-PET in acidified conditions (+3.2%, +1.5%, respectively).

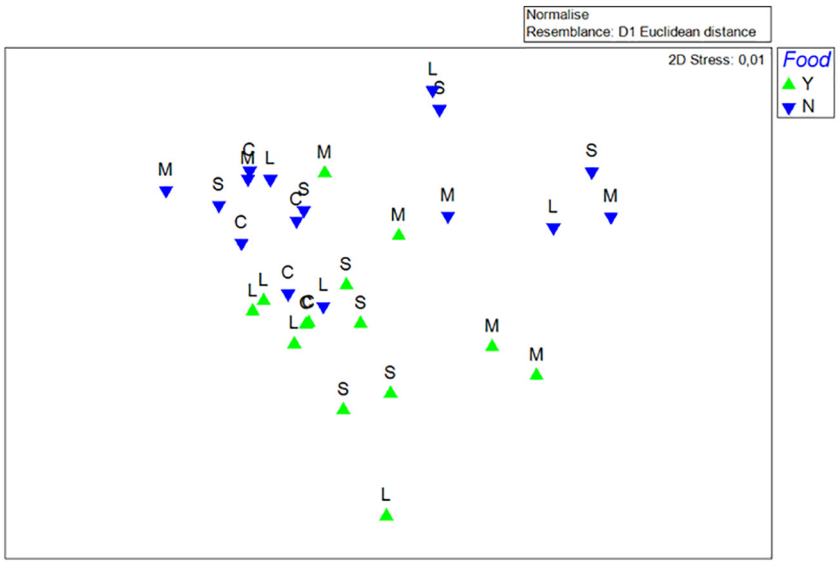

Fig. 10. Non-metric multidimensional scaling (*nm*-MDS) performed on normalized data. POA and % of anomalies data collected on echinoderms are represented.

stress that could affect significantly the survival rates on a longer temporal scale. Total energy transferred from zooplankton towards upper trophic levels could be also significantly affected. Concerning biostimulation recorded on exposed algal species, effects on primary producers on a longer temporal scale should also be better explored by future researches focusing on ecological possible consequences.

This study investigated if other stressors (i.e., food and pH variations) can influence the microplastic toxicity. Multivariate statistics performed highlighted a significant effect related to the factor food availability on ecotoxicological responses (% of anomalies and body-size length) of echinoderms. Food supply induces energy additional input compared to fasting treated species and could reduce larval stress induced by PET impacts. Furthermore, algal addition could actively affect chemical bioavailability by absorption with a significant lower impact. Otherwise, algal absorption on microplastic surfaces could affect palatability, buoyancy of MPs increasing or decreasing ingestion rates. For example, the availability of food influenced the ingestion of PE microplastic in *Tripneustes gratilla* larvae reducing the occurrence of MPs by a factor of 4–10 (Kaposi et al., 2014). Finally, algal absorption on MPs surfaces could reduce MPs/water chemical exchanges reducing chemical releases from MPs towards water.

Plastic pollution could represent a significant threat for the conservation of marine ecosystems and for the associated losses of ecosystems service value following marine impacts by plastic packaging. Furthermore, such effects are not possible to be modelled on a theoretical basis without specific experimental data due to their specie-specificity response behaviour. Tested species resulted not all sensitive to PET leachates and data obtained in this study confirm the complexity of the microplastic toxicity mechanism, able to be influenced by different stressors suggesting the needing to take them into account when an environmental risk assessment is carried out.

To sum up, results obtained in our study show that, in the context of a realistic future scenario characterized by ocean acidification and increase in plastic pollution: i) *V. fischeri* and *P. tricornutum* are not responsive to PET induced stress under standard and acidified condition; ii) *P. lividus* larval stage are able to highlight effects following the exposure to leachate and suspension of PET MPs; iii) AOAs and POAs lengths on normal larvae resulted a suitable and more sensitive than anomalies endpoint to evaluate effects under both standard and acidified conditions; iv) different particle-size of PET produce different effects not always related to the particle size itself, suggesting the occurrence of more complex interactions than theorized ones; v) effects under acidified conditions are significantly different compared to standard pH suggesting changing ecotoxicity of plastic materials under global changing scenarios; vi) food availability produce significant different responses compared to fasting conditions; vii) ecological consequences following PET exposure could produce significant and not attended impacts on marine trophic webs; viii) special attention on the possible interactions between MPs and other stressors (e.g., food and pH) is needed in order to give a better picture of natural occurring dynamics.

Declaration of competing interest

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

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Table 3
ANOSIM test one-way performed on factors affecting ecotoxicological responses on echinoderms. Notes: Pairwise test performed between couples of considered levels of tested factors is reported (Signif. Couples). Only significant relationships are reported. In bold are highlight significant relations, ** = high significant; * = slight significant.

Part a	Factors	Levels	Pairwise Couples	Global R	Signif. level (%)	Signif. couples
Global database	Size	4	S-M; S-L; M-L; S-C; M-C; L-C	0.057	11.6	M-C; S-C*
	Feeding	2	Y-N	**0.225**	**0.01****	–
	pH	2	Ac-St	−0.032	81.0	–

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.136947.

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