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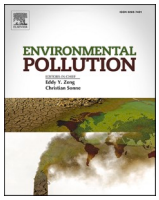

New insights into the impact of leachates from in-field collected plastics on aquatic invertebrates and vertebrates☆

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ABSTRACT

The impact of leachates from micronized beached plastics of the Mediterranean Sea and Atlantic Ocean on coastal marine ecosystems was investigated by using a multidisciplinary approach. Chemical analysis and ecotoxicological tests on phylogenetically distant species were performed on leachates from the following plastic categories: bottles, pellets, hard plastic (HP) containers, fishing nets (FN) and rapido trawling rubber (RTR). The bacteria *Alivibrio fischeri*, the nauplii of the crustaceans *Amphibalanus amphitrite* and *Acartia tonsa*, the rotifer *Brachionus plicatilis*, the embryos of the sea urchin *Paracentrotus lividus*, the ephyrae of the jellyfish *Aurelia* sp. and the larvae of the medaka *Oryzias latipes* were exposed to different concentrations of leachates to evaluate lethal and sub-lethal effects. Thirty-one additives were identified in the plastic leachates; benzophenone, benzyl butyl phthalate and ethylparaben were present in all leachates. Ecotoxicity of leachates varied among plastic categories and areas, being RTR, HP and FN more toxic than plastic bottles and pellets to several marine invertebrates. The ecotoxicological results based on 13 endpoints were elaborated within a quantitative weight of evidence (WOE) model, providing a synthetic hazard index for each data typology, before their integrations in an environmental risk index. The WOE assigned a moderate and slight hazard to organisms exposed to leachates of FN and HP collected in the Mediterranean Sea respectively, and a moderate hazard to leachates of HP from the Atlantic Ocean. No hazard was found for pellet, bottles and RTR. These findings suggest that an integrated approach based on WOE on a large set of bioassays is recommended to get a more reliable assessment of the ecotoxicity of beached-plastic leachates. In addition, the additives leached from FN and HP should be further investigated to reduce high concentrations and additive types that could impact marine ecosystem health.

1. Introduction

Every year around 9.5 million tons of plastic end up in the ocean (Lau et al., 2020). Once in the marine environment, plastics end up stranding on beaches, making these ecosystems a major final sink for plastic, including small fragments, known as microplastics (MPs; Pannetier et al., 2019; Lefebvre et al., 2021).

Plastics are formulated with additives (i.e. plasticizers, stabilizers, flame retardants) to impart beneficial properties (Bridson et al., 2021). Plastic fragmentation and degradation in MPs may facilitate the release of both additive and adsorbed chemicals (i.e. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), metals, etc.) in the surrounding environment and the transfer to organisms (Pannetier et al., 2019). Most of additives and adsorbed chemicals are hazardous to

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the marine biota (Oliviero et al., 2019; Gunaalan et al., 2020; Le Bihanic et al., 2020). Considering that global plastic pollution in the ocean is expected to increase (Jambeck et al., 2015), a load of tons of additives are estimated to be released into the marine ecosystems (Gallo et al., 2018). Therefore, there is an urgent need to assess the impact that substances leached from plastics may exert on marine ecosystems. Increasing our understanding of the chemical composition and effects of plastic leachates represents a significant step to evaluate plastic pollution consequences and to identify low-impact and safe plastic formulations, potentially useful to the plastic industry to replace harmful additives (Almeda et al., 2023).

Toxicity of leachates from plastics have been found across a wide range of organisms including bacteria, phytoplankton, zooplankton and fish (Bejgarn et al., 2015; Hamlin et al., 2015; Li et al., 2016; Oliviero et al., 2019; Lehtiniemi et al., 2021; Schiavo et al., 2021; Focardi et al., 2022; Paganos et al., 2023). Adverse effects on survival, growth, development, behaviour and DNA damage were correlated with dose, polymer type, particle size, additive composition and plastic weathering (Gunaalan et al., 2020; Schiavo et al., 2021; Bridson et al., 2021; Seuront et al., 2021; Delaeter et al., 2022). However, most of the aforementioned literature reports the ecotoxicity of leachates from pristine plastic - lacking environmental relevance - rather than in field collected plastics. The assessment of leachate effects using beached plastics is critically important, since the effects observed in aquatic biota exposed to leachates from virgin plastics can not be directly extrapolated to the environmental risks associated with plastic pollution (Pannetier et al., 2020; Alimi et al., 2022; Menicagli et al., 2022). For instance, high toxic effects were observed in mussel development after leachate exposure from beached versus virgin pellets, due to a more complex mixture of contaminants adsorbed on the beached-pellets (Gandara e Silva et al., 2016). Conversely, other studies reported reduced leachate toxicity after plastic weathering, likely due to the loss of additives (Sarker et al., 2020).

Plastic is the largest category within beached marine litter (Laglbauer et al., 2014), being commonly found in the Mediterranean Sea and the Atlantic Ocean (Cózar et al., 2015; Constant et al., 2019; Giovacchini et al., 2018; Monteiro et al., 2018). The highest plastic accumulation has been reported on Atlantic beaches in the industrial areas (Antunes et al., 2018) and Mediterranean beaches (Turner and Holmes, 2011; Laglbauer et al., 2014). Despite the high plastic abundance in these areas, the effects of beached plastic leachates are still poorly explored. Cormier et al. (2021) reported leachate toxicity from MPs stranded on Atlantic beaches towards early stages of aquatic organisms. In this study, we evaluated the potential ecotoxicity of leachates obtained from micronized plastics collected in Mediterranean and Atlantic beaches. Plastic micronization allows to obtain small size fractions (<250 μm) for aquatic bioassays (Oliviero et al., 2019; Beiras et al., 2019), comparability among materials, increasing particle surface, facilitating the release of additive and adsorbed chemicals, most of which are not covalently bound to the polymers and are prone to leach into the environment (Almeda et al., 2023).

In this study, we aimed at providing insights on the impact of leachates from in-field collected plastics on aquatic organisms, by mimicking an environmentally realistic mixture of plastic particles. With this aim, we performed a chemical characterization of the leachates obtained from different plastic categories (fishing ropes, hard plastics, pellets, fishing nets, plastic bottles) - commonly found on beaches and whose polymers are the most abundant in the marine litter (i.e. polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP); Iñiguez et al., 2017). The toxicity of micronized plastic leachates towards aquatic organisms belonging to different trophic levels was assessed. Specifically, the bacterium *Alivibrio fischeri*, the crustaceans *Amphibalanus amphitrite* and *Acartia tonsa*, the rotifer *Brachionus plicatilis*, the sea urchin *Paracentrotus lividus*, the jellyfish *Aurelia* sp. and the medaka *Oryzias latipes* were selected. Ecotoxicological results were analyzed within a quantitative Weight of Evidence (WOE) model to integrate and weight data from ecotoxicological line of evidence (LOE) based on 13 responses. This model provides synthetic hazard indices for each data (Regoli et al., 2014), before their integration in an environmental risk index for each plastic typology.

2. Materials and methods

2.1. Sampling collection, chemical characterization and grinding process

Stranded plastics were collected from six beaches of the Mediterranean Sea (Ligurian and Adriatic Sea, Italy) and Atlantic Ocean (Biscay Bay, France) in 2022 (Suppl. Fig. S1). The following plastic categories were collected: rapido trawling rubber (RTR), hard plastics (HP), pellets and fragments (pellets), fishing nets (FN), plastic bottles (PET; Supplementary Table S2). Specifically, FN, HP containers and RTR were collected in the Adriatic Sea, while plastic bottles in the Ligurian Sea. Pellets, HP, and a mixture of FN and oyster bags were collected in the Biscay Bay. Except for pellets, plastic items were cut by scissors into 1 cm^2 fragments. All plastic categories were characterized by using a Fourier Transform Infrared Spectroscopy (FT-IR) spectrometer. The spectra of the polymers were compared to reference spectra through libraries, with a >70% similarity threshold (Suppl. Table S2).

Plastics were then inserted into a rotatory mill filled with liquid nitrogen and ground to obtain MPs with a size <250 μm.

2.2. Leachate preparation

After grinding, 1 g of MPs was added to 1 L glass bottles with seawater (SW) to use 1 g/L solid/liquid ratio. Although this concentration exceeded the environmental ones (up to μg/L), it was selected on the basis of previous studies on aquatic bioassays, demonstrating that this ratio maximizes the sensitivity in detecting potential toxicity relased from the plastic (Beiras et al., 2019; Almeda et al., 2023).

The bottles were closed and incubated in a rotating wheel (2 rpm speed) for 24 h at 20 °C in darkness to obtain leachates (Beiras et al., 2019; Almeda et al., 2023). Plastic particles were removed from the bottles by using a vacuum filtration system equipped with microfiber filters (Whatman GF/F filters 0.8 μm). Undiluted leachates (1 g/L) were stored refrigerated in glass bottles before being used. As a blank, natural or artificial SW was used for chemical analyses and ecotoxicological bioassays (salinity = 35.1 ppt ± 0.2 for zooplankton and chemical analyses, 20 ppt for bacteria, 10 ppt for fish; Temperature = 20 °C, pH = 8.1 ± 0.1, O_2> 90% saturated air). All parameters were checked before and during the experiments. More details are reported in the Supporting information.

2.3. Chemical analysis

A total of 2000 mL of leachates (equivalent to 2 g plastics) was extracted. The Oasis HLB glass cartridges were first conditioned with 10 mL hexane, dichloromethane, methanol and 15 mL ultrapure water. The leachate for each plastic was extracted; the sorbents were then washed in ultrapure water and dried for 30 min. The cartridges were closed, put in 50 mL tubes and stored at −20 °C until the analyses. Detailed information is provided in the supporting information (Supplementary Tables S3–S6). Prior to elution internal standards of PAHs, organophosphorous Flame Retardants (OPFRs), benzophenone (BP), bisphenol A (BPA) and tetrabromobisphenol A (TBBPA) were added. The HLB cartridges were eluted with hexane:dichloromethane (1:1), hexane: acetone (1:1) and split into a liquid chromatography (LC) and a gas chromatography (GC) fraction. The solvents were evaporated, transferred to vials and evaporated to 100 μL of acetonitrile and toluene, respectively. For further characterization of the most polar compounds, the cartridges were also eluted with 8 mL of methanol. Batch standards with native PAHs, PCBs, 2,2′4,4′-tetrabromodiphenyl ether (PBDE-47), dichlorodiphenyldichloroethylene, OPFR, BP, dibutyl phthalate and 2,4-

Diisocyanatotoluene (2,4-TDI) were prepared in 100 μL toluene. Perylene-D_{12} was added as recovery standard to the toluene extracts prior to analysis on a GC Orbitrap® mass spectrometer. Helium was used as the carrier gas. Batch standards with native bisphenols, TBBPA, b-HBCD, triclosan, polyfluoroalkyl substances (PFAS), UV-328, 6 PPD quinone and 1-phenyl-4-(1-phenylethyl)tetralin were prepared in 100 μL of acetonitrile and 100 μL of methanol. Acetonitrile and methanol extracts were diluted in ultrapure water (1:1) and an internal standard mix was added before injection on an Acquity ultra-performance LC (UPLC) I-class coupled to a Xevo G2-XS quadrupole time of flight (QTOF) mass. Detailed information is provided in supporting information.

GC data analysis was conducted by using openly available software. Raw profile data were converted to centroided mzML format (Chambers et al., 2012); data were processed uing MS-DIAL software version 4.9 (Tsugawa et al., 2015) for high resolution mass spectrometry (HRMS) matching. HRMS spectral library (Price et al., 2021) combined with an in-house library were used for suspect screening. Representative spectra from the aligned peak list were also exported in msp format and matched against the NIST14 library. The area of the quantification ion was used to represent each detected compound. For identification NIST's suggested general guidelines for Match Factor scores were used by considering >900 an Excellent Match, 800–900 a Good Match, 700–800 a Fair Match, and <600 a poor match. Only match factors above 700 were considered (above 600 if the RI was within 1.5%).

The LC data was processed using UNIFI 1.9.4. Processing settings are detailed in supporting information. The library developed by Fries and Sühring (2023) and an in-house library were used for suspect screening. Information on compound fragmentation pattern was collected either by injection of the analytical standard, retrieved on the MassBank database (Horai et al., 2010), or predicted with the *in silico* fragmenter MetFrag. To assign confidence to the GC-orbitrap data by Koelmel et al. (2022) system was used. Here, Level 1 implies confirmed identification using in-house library, Level 2 the Probable structure or close isomer using external libraries, Level 3 Tentative using external library; alternatively, RI match with accurate mass fragment matches.

For the LC-HRMS suspect screening it was used the identification confidence levels suggested by Schymanski et al. (2014). Briefly, identification at level 2 gives a probable structure by a) library spectrum match or b) diagnostic evidence in contrast to level 1 that gives a confirmed structure by a reference standard.

2.4. Ecotoxicological analysis

Undiluted leachate (1 g/L) and dilutions in SW were directly used for ecotoxicological analysis (Table 1), according to those proposed by Beiras et al. (2019).

2.4.1. Aliivibrio fischeri

The toxicity of plastic leachates was analyzed with Microtox test, a standardized toxicity test, using the bioluminescent marine bacteria *A. fischeri* (ISO 11348-3). Bioluminescence emission was measured using the M500 Toxicity Analyzer device (Modern Water). A blank (NaCl, 2%) and a negative control (Instant Ocean, 20 PSU) were carried out. Negative control and leachates were tested undiluted, and diluted to 50, 25 and 12.5% in 2% NaCl. Four replicates were carried out on each sample.

2.4.2. Amphibalanus amphitrite

Nauplii of the barnacle *A. amphitrite* were exposed to leachates. They were obtained from laboratory cultures at CNR (Italy) and maintained in beakers with FSW to a concentration of 10–15 larvae/mL (Piazza et al., 2016). Nauplii were transferred into each well containing 1 mL of undiluted and diluted leachates at 1/3 (0.33 g/L), 1/10 (0.1 g/L) and 1/30 (0.033 g/L) of each plastic category. Four replicates – including the control – were performed. They were incubated in the dark, for 48 h, at 20 °C. After exposure, immobility was checked under a stereomicroscope. Swimming Speed Alteration (SSA) percentage was also evaluated by using a Swimming Behavioural Recorder (SBR) system (Faimali et al., 2006). Swimming behaviour was monitored for 3 s in dark conditions. The resulting images were analyzed and the average swimming speed (mm/s) was measured for each test population. Data were expressed as SSA percentage normalized to controls' swimming speed, according to Faimali et al. (2006).

2.4.3. Acartia tonsa

A. tonsa was obtained from the laboratory stock cultures at the ULPGC (Spain). Early copepod nauplii were exposed to undiluted (1 g/L) and diluted leachates at 1/3, 1/10 and 1/30 of each plastic category and to FSW (negative control). Three replicates were carried out. To obtain nauplii, adults were separated from the stock culture using a 200 μm mesh sieve and incubated in a glass beaker with FSW and food for 48 h. Adults were removed and nauplii were collected with 40 μm-mesh sieve and placed in a 100 mL beaker with FSW. Nauplii were sorted with glass pipettes under a stereomicroscope and distributed in groups of 20 in petri dishes. They were transferred to bottles containing 5 mL of the exposure media and incubated for 48 h at 20 °C in the dark. After exposure, mortality was determined by using a stereomicroscope and the survival percentages were calculated in all replicates.

2.4.4. Brachionus plicatilis

Rotifer of *B. plicatilis* were obtained from dehydrated cysts (Micro-BioTests Inc., Belgium), following the Rotoxkit M protocol. About 15–20 organisms were placed in 24 well-plates containing 1 mL per well of undiluted and diluted leachates (1/3, 1/10, 1/30) of each plastic category. A negative control (FSW) was performed. Plates were incubated at 25 °C in dark conditions for 48 h. Each treatment was prepared in quadruplicates. After exposure, the percentages of immobility and SSA were evaluated, as reported for barnacle nauplii.

2.4.5. Paracentrotus lividus

Adults of *P. lividus* were collected in the Ligurian Sea (Italy) and brought to CNR laboratories (Italy). They were induced to spawn by oral administration of KCl (0.5 M). Dry sperm were collected from the genital pores, while the eggs were collected in SW at 18 °C and diluted to a final concentration of 1000 eggs/mL. Fertilization was carried out by adding 10 μL of pooled diluted sperm to egg suspension. Then, four sub-samples were observed under a stereomicroscope to check fertilization success. About 1000 fertilized eggs/mL were added to each well containing undiluted and diluted leachates (1/3, 1/10, 1/30) of each plastic category. Six replicates were performed for each treatment, including negative

Table 1
Summary of experimental set up used in the ecotoxicological bioassay.

Test organism	Exposure time	Endpoint	Reference
A. fischeri	30 min	Inhibition of bioluminescence	ISO 11348-3: 2019
A.amphitrite	48 h	Immobility, Behaviour (Swimming Speed Alteration)	Piazza et al., 2016
B.plicatilis	48 h	Immobility, Behaviour (Swimming Speed Alteration)	Garaventa et al., 2010
P. lividus	72 h	Developmental anomalies, Behaviour (Swimming Speed Alteration)	Gambardella et al., 2013; Morgana et al., 2016
Aurelia sp.	48 h	Immobility, Behaviour (Alteration of frequency of pulsations)	Costa et al., 2020
Oryzias latipes	96 h	Survival, malformations, cardiac activity	Bedrossiantz et al., 2023
Acartia tonsa	48 h	Survival	ISO 14669: 1999

controls (SW). Eggs were allowed to develop in darkness at 18 °C for 72 h. Then, larval SSA and developmental anomalies percentages were investigated. The SSA was recorded by the SBR system as described for barnacle nauplii. To assess development, larvae were fixed with 4% paraformaldehyde and observed under a stereomicroscope. to determine developmental anomalies percentage.

2.4.6. Aurelia sp

Colonies of polyps of *Aurelia* sp. were obtained from th Aquarium of Genoa (Italy), and transported to CNR laboratories (Italy). Strobilation was induced by thermic shock; the resulting ephyrae were used for the toxicity test. The ephyrae were placed into wells containing 2 mL of undiluted and diluted leachates (1/3, 1/10, 1/30) of each plastic category. Three replicates were prepared, each containing 8 ephyrae individually placed in each well. A negative control (SW) was also performed. Plates were kept at 20 °C in darkness for 48 h. Then, the immobility and alteration of the frequency of pulsations (AFp) were calculated for each dilution compared to controls, according to Faimali et al. (2006). The ephyrae ability to perform any kind of movement in 5 s was considered as immobility percentage, while the recording pulsations number made by the ephyrae in 1 min was used to calculate the AFp.

2.4.7. Oryzias latipes

The 24 h embryos of *O. latipes* were provided by INRAe (LPGP, France). They were placed in Petri dishes containing egg rearing solution 1X medium (ERS 1X) at 22 °C and 13L:11D photoperiod. Every 24 h until peak hatching, the embryos were checked using a microscope to remove the dead and half of the ERS 1X medium was renewed. Once hatched, the larvae were exposed to undiluted leachate (at 10 PSU) for 96 h at 22 °C and 13L:11D photoperiod. About 18–20 larvae were distributed in glass beakers containing 20 mL of leachate. Three replicates were carried out for each sample. Every 24 h, the dead larvae were counted, removed and half of the exposure medium was renewed. Larvae with spinal malformations were also recorded. Seventy hours after the start of the exposure, 8 larvae per replicate were collected, anesthetized and fixed dorsally in a 3% methylcellulose gel. Cardiac activity was recorded for each larva (24 larvae per condition) at 22 °C using a magnifying glass connected to a camera and the OBSstudio software (29.1.1). Heartbeats were measured using Ethovision software from a 60s video.

2.4.8. Statistical analysis and weight of evidence (WOE)

Ecotoxicological results reported the arithmetic mean ± standard error. Statistical analysis was conducted by using the program R 3.2.2 (R Development Core Team, 2015). Normality was tested with the Shapiro-Wilk test and homogeneity of variances with Levene's test. When the data were normally distributed and homogeneous, statistical differences between the leachate and corresponding controls were calculated by using one-way ANOVA. Mann-Whitney test was used for data with non-normal distribution and when homogeneity of variances was not achieved. Data were considered significantly different when $p < 0.05$. The Lowest Observed Effect Concentrations (LOEC) were also determined. The median Effective Concentrations (EC50: median effective dilution of leachate resulting in 50% immobility, mortality (as not survived copepod nauplii), developmental anomaly, SSA and AFp effect in the organisms and related 95% Confidence Limits (CL) were calculated using Trimmed Spearman–Karber analysis (Finney, 1978) after 48 h and 72 h exposure.

Ecotoxicological results were elaborated within a quantitative WOE model that provides a synthetic hazard index based on a specific algorithm and mathematical procedures (d'Errico et al., 2021). The assessment criteria were set on specific thresholds (15%) and weights (survival 3, bioluminescence inhibition 2.4, immobility/development/cardiac activity 1.9, behaviour 1), assigned to each bioassay. The criteria depends on the ecotoxicological endpoint, tested matrix and exposure time (Regoli et al., 2019; d'Errico et al., 2021). In this LOE, the cumulative hazard quotient ($HQ_{Battery}$) was obtained by the sum of all weighted effects. Five classes of hazard (Absent, Slight, Moderate, Major, Severe) were assigned depending on $HQ_{Battery}$ result.

Multivariate principal component analysis (PCA) of ecotoxicological (in term of HQ specific) and chemical results was applied to visualize the relationships among micronized plastics collected in different geographical areas.

3. Results

3.1. Chemical analysis

A broad range of additive organic chemicals were identified in the leachates of the different plastic categories. A list of compounds (n = 31) identified at confidence level 1 (confirmed structure) and level 2 (probable structure) using literature and in-house LC- and GC-HRMS libraries is compiled in Table 2. Of these 31 compounds, 16 were identified by GC-HRMS data, 9 by LC- HRMS data and 6 by both GC and LC-HRMS data. Quality control parameters, compounds identified at confidence level 3 and detailed information of identified compound data are presented in the supporting information. Benzothiazole, 2,2,4-trimethyl-1H-quinoline, 2-(methylthio)benzothiazole, 1,2-dyphenylguanidine and 1,2-benzisothiazolin-3-one were only identified in the RTR leachate, while diphenylamine and N,N-dimethylaniline only in FN and RTR. The additives identified in all categories were benzophenone (BP), benzyl butyl phthalate (BBP) and ethylparaben. Tinuvin 770, dimethyl phthalate, tributyl phosphate and 4-methylbenzophenone were identified in almost all plastic leachates. Acetyl tributyl citrate was identified in all plastic leachates except RTR and triclosan in all except PET. Tris (nonylphenyl)phosphite was identified in all plastic leachates except for HP from the Adriatic Sea and dimethyl phthalate in all except for the FN. BPA was identified in FN, HP and Pellet, whereas bisphenol S was detected only in FN. Substances identified infrequently were 2,4-TDI, triphenyl phosphate, 6:2 diPAP (HP), UV-327 (HP), dinoseb (PET, Pellet) 4-tert-Octylphenol (FN, PET), and oxybenzone (Pellet). Analytical standards were available for several of the identified compounds, allowing quantification and semi-quantification. The concentrations ranged from 8 ng/g plastic to 2196 ng/g, lowest levels for benzyl butyl phthalate and highest levels for tris(2-chloroethyl) phosphate (Suppl. Table S6).

3.2. Leachate toxicity

The ecotoxicological results on the exposure of marine vertebrates and invertebrates to undiluted (1 g/L) leachates of each plastic category are reported in Fig. 1.

Exposure to leachates from different plastic categories never affected bacteria bioluminescence, with the exception of pellets, and immobility of early stages of marine organisms. Conversely, survival, development, behaviour and cardiac activity were impaired in many species exposed to leachates from different plastic categories. The toxicity was calculated in terms of EC50 values in the marine invertebrates after exposure to RTR, HP and FN collected in both Mediterranean Sea and Atlantic Ocean (Table 3). Specifically, exposure to RTR was toxic for sea urchin development, barnacle nauplii and jellyfish ephyrae behaviour (Suppl. Fig. S7). The leachates of FN sampled in the Adriatic Sea induced a toxic effect measurable by means of EC50 values on copepod survival, jellyfish behaviour in term of AFp and sea urchin development; the latter was also observed after exposure to HP (Suppl. Fig. S8). Regarding plastic categories collected in the Atlantic Ocean, the leachates of FN and HP induced toxic effects in sea urchin development and copepod survival (Suppl. Fig. S9). Although severe toxic effects were not observed for all leachates of beached plastics in the different geographic areas, LOEC values were reported for several endpoints in the marine organisms (Table 3).

Table 2
Compounds identified at confidence level 1 (confirmed structure) and level 2 (probable structure) using litterature and in-house libraries on GC and LC-HRMS data.

Compound	Cas-nr	Uses	Apparatus	Leachates
1,2-Benzisothiazolin-3-one	2634-33-5	Preservative	LC	RTR
1,2-diphenylguanidine	102-06-7	Vulcanizing agent of rubber	LC	RTR
2-(Methylthio)benzothiazole	615-22-5	Fungicide	GC	RTR
2,2,4-trimethyl-1H-quinoline	147-47-7	Rubber antioxidant	GC	RTR
2,4,7,9-Tetramethyl-5-decyn-4,7-diol	126-86-3	Paints, adhesives, and dyes	GC	HP Adriatic & Biscay/Pellet
2,4-Diisocyanatotoluene[a]	584-84-9	Chemical intermediate	GC	FN Adriatic/HP Biscay/Pellet
2,6-Diisocyanatotoluene[a]	91-08-7	Chemical intermediate	GC	FN Adriatic/HP Biscay/Pellet
4-Methylbenzophenone	134-84-9	UV absorber	GC/LC	FN Biscay/HP Adriatic & Biscay/Pellet/RTR
4-tert-Octylphenol	140-66-9	Chemical intermediate	GC	FN Adriatic/PET
6:2 diPAP[a]	57677-95-9	PFAS, food packaging materials	LC	HP Biscay
Acetyl tributyl citrate	77-90-7	Plasticizer	GC	FN Adriatic & Biscay/HP Adriatic & Biscay/PET/Pellet
Benzophenone[a]	119-61-9	UV absorber	GC	All
Benzothiazole	95-16-9	Antimicrobial, rubber accelerator	GC	RTR
Benzyl butyl phthalate	85-68-7	Plasticizer	GC/LC	All
Bisphenol A[a]	80-05-7	Chemical intermediate	LC	FN Adriatic/HP Adriatic & Biscay/Pellet
Bisphenol S[1]	80-09-1	Chemical intermediate	LC	FN Adriatic & Biscay
Dibutyl phthalate[a]	84-74-2	Plasticizer	GC	PET
Dimethyl phtalate	131-11-3	Plasticizer	GC	HP Adriatic & Biscay/PET/pellet/RTR
Dinoseb	88-85-7	Herbicide	LC	PET/Pellet
Diphenylamine	122-39-4	Antioxidant, stabilizer, fungicide	GC/LC	FN Biscay/RTR
Ethylparaben	120-47-8	Preservative	LC	All
N,N-Dimethylaniline	121-69-7	Paints, adhesives, and dyes	LC	FN Adriatic & Biscay/RTR
Oxybenzone	131-57-7	UV absorber	GC	Pellet
Phthalic anhydride	85-44-9	Chemical intermediate	GC	HP Adriatic & Biscay
Tinuvin 770	52829-07-9	UV stabilisers	LC	FN Adriatic & Biscay/HP Adriatic & Biscay/PET/Pellet
Tributyl phosphate[a]	126-73-8	Flame-retardant, plasticizer	GC	FN Biscay/HP Adriatic & Biscay/PET/Pellet/RTR
Triclosan[a]	3380-34-5	Antimicrobial, fungicide	GC/LC	FN Adriatic & Biscay/HP Adriatic & Biscay/Pellet
Triphenyl phosphate[a]	115-86-6	Flame-retardant, plasticizer	GC	FN Adriatic/HP Biscay/Pellet
Tris(2-chloroethyl) phosphate[a]	115-96-8	Flame-retardant, plasticizer	GC/LC	FN Adriatic/HP Biscay/Pellet
Tris(nonylphenyl)phosphite	16784-72-8	Stabilizer	GC	FN Adriatic & Biscay/HP Biscay/PET/Pellet
UV-327	3864-99-1	UV absorber	GC/LC	HP Adriatic & Biscay

[a] Confirmed structure with analytical standard.

3.3. Weight of evidence and relationship between chemical and ecotoxicological analyses

All ecotoxicological data were used in a LOE able to assess the probability and magnitude of environmental hazard due to each of the plastic categories. According to the magnitude of variations observed for ecotoxicological responses, the statistical significance of such differences and the ecotoxicological relevance of each biological endpoint, the WOE mode was used. The hazard indices elaborated for ecotoxicological data depending on $HQ_{Battery}$ outcome (Suppl. Fig. S10) resulted in a WOE effect classified as "absent" for marine vertebrates and invertebrates exposed to leachates of pellets, PET, RTR and FN from the Biscay Bay. A "Slight" effect was found for the ecotoxicological battery exposed to HP from the Adriatic Sea, while a "Moderate" effect was observed for HP collected in Biscay Bay and FN collected in Adriatic Sea (Fig. 2).

PCA analysis (Supplementary Fig. S11) provided two-dimensional patterns of separation among micronized plastics, always explaining 60% of the total variance, confirming the sampling site separation.

4. Discussion

The potential toxicity related to leachates from beached-plastics was investigated in several aquatic organisms belonging to different trophic levels. To our knowledge, this is the first study reporting leachate ecotoxicity from beached plastics in the Mediterranean and Atlantic on decomposers, primary and secondary consumers. Among them, the decomposers were the less sensitive organisms to all plastic categories, since *A. fischeri* was only affected by the exposure to one plastic leachate (pellet) out of seven. The low sensitivity of marine bacteria towards plastic leachates is confirmed by literature based on virgin plastics. Indeed, PET leachate did not significantly affect the bioluminescence of *A. fischeri* (Piccardo et al., 2020) and the growth of marine heterotrophic bacteria (Romera-Castillo et al., 2018). Regarding pellets, bacteria bioluminescence inhibition was observed after exposure to virgin pellets of several polymers (PP, PE, PS; Schiavo et al., 2018) and to beached-plastics as reported in the present study, showing a sensitivity of bacteria for this plastic category.

Primary consumers were affected by the leachates of beached-plastics in a different manner, depending on zooplankton species. Thus, the rotifers were less sensitive to all beached-plastic leachates in comparison to crustaceans, echinoderms and cnidarians, since no toxicity was found for any plastic category. Rotifers are one of the most dominant zooplankton, occupying an important ecological niche in the food web (Jurgens et al., 1999). Only recently the toxicity of the leachates from virgin plastics (i.e. tyre-wear particle) was demonstrated in *B. plicatilis*, affecting the survival (Shin et al., 2022). Conversely, we did not observe any effect on immobility and behaviour by exposing rotifers to a wide range of beached-plastic leachates, including the RTR with a polymer composition similar to tyre-wear particles. Such findings suggest that leachate origin (i.e. virgin *versus* beached-plastics) of similar plastic categories may induce different ecotoxicological responses in the marine rotifers.

Crustaceans play a key role in the aquatic food webs, being an important link in the pelagic food web (Szaniawska, 2018). Among them, *A. tonsa* and *A. amphitrite* are ecologically relevant species in the marine environment and the impact of plastic leachates has been demonstrated so far (Li et al., 2016; Koski et al., 2021; Bournaka et al., 2023). In the present study, the larval stages of these species showed a different sensitivity according to the plastic category. Thus, FN from the Adriatic Sea and HP collected in the Biscay Bay induced a high toxicity in term of LC50 values on copepod survival, while RTR significantly impaired barnacle nauplii swimming. Lethal and sub-lethal effects were also observed in several copepod species and in *A. amphitrite* nauplii exposed to leachates of different plastics (i.e. PE bags, rubber from recycled car tires, PP storage containers; Li et al., 2016; Lehtiniemi et al., 2021), suggesting that the impact of plastics on crustaceans may depend on the polymer type and species.

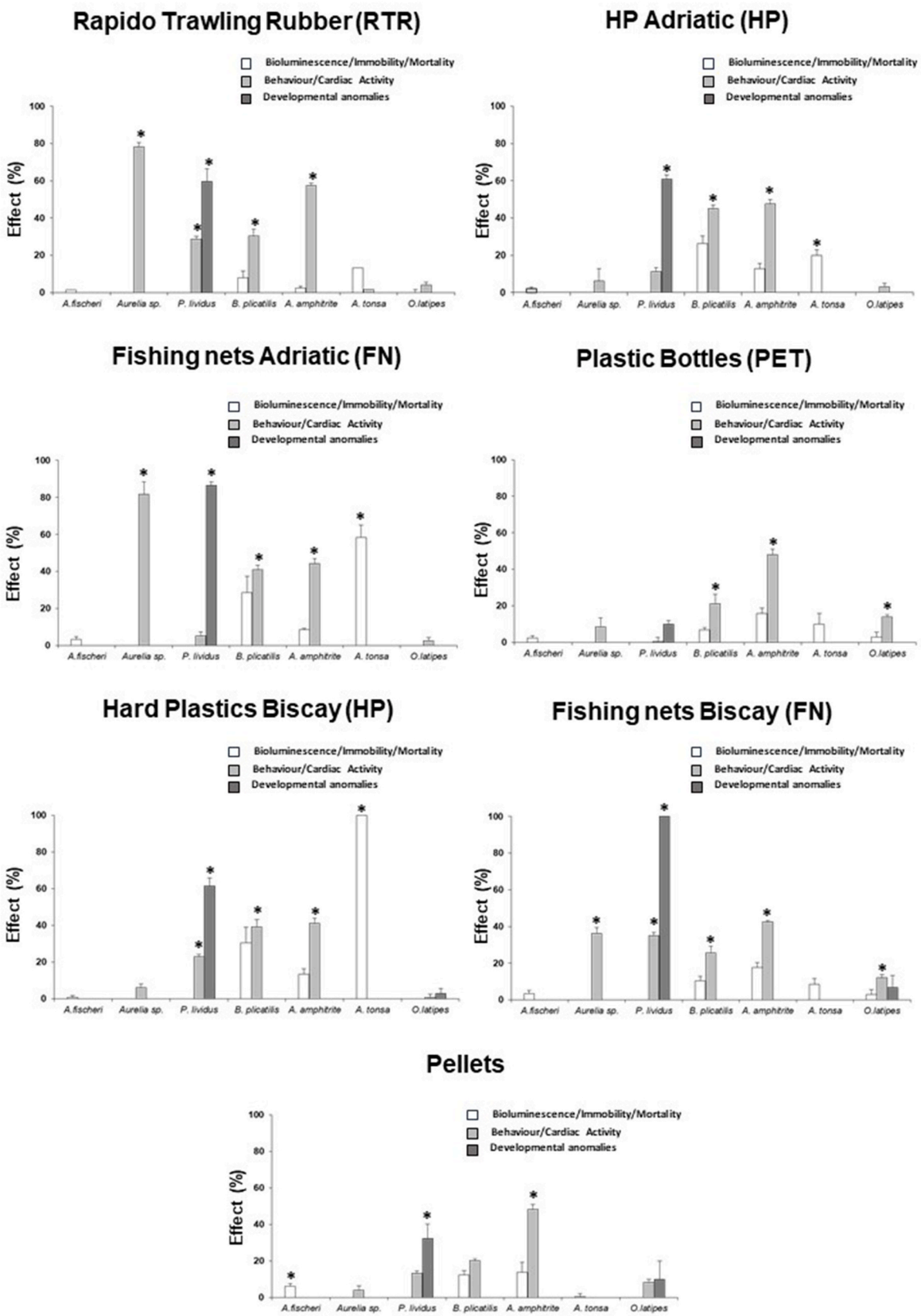

Fig. 1. Percentage of effect of the ecotoxicological responses of each biological species (*Aliivibrio fischeri*, *Aurelia* sp., *Paracentrotus lividus*, *Brachionus plicatilis*, *Amphibalanus amphitrite*, *Acartia tonsa*, *Oryzias latipes*) exposed to undiluted (1 g/L) leachates of the following plastic category: Rapido Trawling Rubber (RTR), hard plastics (HP) and Fishing Nets (FN) collected in the Adriatic Sea, bottles (PET) from the Ligurian Sea, HP, FN and pellets from the Biscay Bay. Asterisks indicate differences between treated samples and controls ($*p < 0.05$).

Table 3
Leachate toxicity obtained from <250 μm beached-plastics on aquatic organisms. Lowest observed effect concentration (LOEC) refers to leachate dilutions obtained using a solid/liquid ratio of 1 g/L. EC50: leachate dilution producing a 50% effect compared to the control. CL confidence limits. EC50 values are expressed in g/L. Dev. Anomalies: developmental anomalies.

Geographic area	Plastic typology	Organism	Endpoint	LOEC	EC50 and CL
Mediterranean Sea	Rapido Trawling Rubber (RTR)	*A.fischeri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility	>1	>1
			Behaviour	0.1	0.37 (0.29–0.47)
		P. lividus	Dev Anomalies	1	0.82 (0.69–0.92)
			Behaviour	1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	1	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.033	0.66 (0.45–0.96)
		A.tonsa	Survival	>1	>1
		O. latipes	Survival/Dev. Anomalies/Cardiac activity	>1	>1
	Hard plastics (HP)	*A.fischeri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility/Behaviour	>1	>1
		P. lividus	Dev. Anomalies	0.33	0.64 (0.48–0.85)
			Behaviour	1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	0.33	>1
		A.amphitrite	Immobility/Behaviour	>1	>1
				0.033	>1
		A.tonsa	Survival	1	>1
		O. latipes	Survival/Dev. Anomalies/Cardiac activity	>1	>1
	Fishing nets (FN)	*A.fischeri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility	>1	>1
			Behaviour	1	0.38 (0.31–0.47)
		P. lividus	Dev. Anomalies	0.1	0.23 (0.16–0.32)
			Behaviour	>1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	0.33	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.1	>1
		A.tonsa	Survival	1	0.90(0.80–0.99)
		O. latipes	Survival/Dev. Anomalies/Cardiac activity	>1	>1
	Plastic bottles	*A.fischeri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility/Behaviour	>1	>1
		P. lividus	Dev. Anomalies/Behaviour	>1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	1	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.1	>1
		A.tonsa	Survival	>1	>1
		O. latipes	Survival/Dev. Anomalies	>1	>1
			Cardiac activity	≤1	>1
Atlantic Ocean	Hard plastics (HP)	*A.fisheri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility/Behaviour	>1	>1
		P. lividus	Dev. Anomalies	0.33	0.51 (0.24–1)
			Behaviour	1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	0.1	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.033	>1
		A.tonsa	Survival	0.033	0.44 (0.36–0.52)
		O. latipes	Survival/Dev. Anomalies/Cardiac activity	>1	>1
	Fishing nets + Mussel nets (FN)	*A.fischeri*	Bioluminescence inhibition	>1	>1
		Aurelia sp.	Immobility	>1	>1
			Behaviour	1	>1
		P. lividus	Dev. Anomalies	1	0.43 (0.37–0.54)
			Behaviour	1	>1
		B.plicatilis	Immobility	>1	>1
			Behaviour	1	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.1	>1
		A.tonsa	Survival	>1	>1
		O. latipes	Survival/Dev. Anomalies	>1	>1
			Cardiac activity	<1	>1
	Pellets	*A.fischeri*	Bioluminescence inhibition	1	>1
		Aurelia sp.	Immobility/Behaviour	>1	>1
		P. lividus	Dev. anomalies	1	>1
			Behaviour	>1	>1
		B.plicatilis	Immobility/Behaviour	>1	>1
		A.amphitrite	Immobility	>1	>1
			Behaviour	0.033	>1
		A.tonsa	Survival	>1	>1
		O. latipes	Survival/Dev. Anomalies/Cardiac activity	>1	>1

Area	Sample code	$HQ_{Battery}$	Level of ecotoxicological hazard
Adriatic Sea	FN Adriatic	1,51	MODERATE
	RTR	0,78	ABSENT
	HP Adriatic	1,05	SLIGHT
Biscay Bay	HP Biscay	1,72	MODERATE
	Pellet	0,4	ABSENT
	FN Biscay	0,92	ABSENT
Ligurian Sea	PET	0,47	ABSENT

Fig. 2. Summary of the ecotoxicological hazard level of the following plastic category: Rapido Trawling Rubber (RTR), hard plastics (HP) and Fishing Nets (FN) collected in the Adriatic Sea, bottles (PET) from the Ligurian Sea, HP, FN and pellets from the Biscay Bay. The level of ecotoxicological was estimated by the value of the Hazard Quotient (HQ) of the ecotoxicological battery.

Cnidarians were used to assess the toxicity of beached-plastics, since jellyfish was proposed as innovative bioindicator in ecotoxicology due to its key role in the trophic chain and high sensitivity to pollutants, including plastics and MPs (Faimali et al., 2006; Costa et al., 2020; Macali and Bergami, 2020). In this study, jellyfish were never affected by plastic leachates in term of immobility, while the behaviour was impaired by FN and RTR leachates collected in the Adriatic Sea. These data confirm those reported by Cormier et al. (2021), showing AFp but not acute effects after exposure to beached-plastic fragments and fibers leachates.

Echinoderms have been deeply investigated in plastic pollution. The embryonic development and the growth of different sea urchin species (i.e. *P. lividus, Lytechinus variegatus*) were affected after exposure to the leachates of virgin and beached-plastics (i.e. Nobre et al., 2015; Martínez-Gómez et al., 2017; Piccardo et al., 2020; Cormier et al., 2021). In this study, sea urchin development was the most affected endpoint if compared to other aquatic organisms' responses. Thus, sea urchin developmental impairment was found in five plastic leachates out of seven (i.e. RTR, HP and FN from Biscay Bay and Adriatic Sea). Similar findings were reported in sea urchins exposed to leachates from beached-plastics composed by PE and PP mixture (Cormier et al., 2021), a similar polymer composition to that in the HP from both geographic areas. Likewise, the exposure to car tyres leachates has been found to affect the development of three sea urchin species, including *P. lividus* (Rist et al., 2023).

In the present study the ecotoxicological effects of leachates from beached-plastics were investigated in the fish *O. latipes* as secondary consumer. None of the studied plastic leachates affected the survival and the development; however, leachates of FN from the Biscay Bay and PET bottles from the Ligurian Sea induced a significant increase of heartbeats. Recently the exposure to aquaculture-derived plastic leachates (i.e. FN, fishing ropes) was found to affect the endocrine system, growth, reproduction and immunity process of several fish, including medaka (Lin et al., 2023). Regarding *O. latipes,* both aqualculture-derived plastic leachates and virgin/aged plastic leachates altered the reproductive system, inducing vitellogenin gene transcription, influencing oocytes development, spermatogenesis and offspring sex ratio (Knorr and Braunbeck, 2002; Na et al., 2002; Horie et al., 2020; Qiu et al., 2023). We did not observe any toxicity in terms of survival and development, suggesting that such endpoints were not sensitive enough to the five studied plastic categories (FN, PET bottles, HP, RTR, pellets) to cause an ecotoxicological effect. Nevertheless, more studies should be addressed on fish cardiac activity, since a significant hearbeat increase was observed after PET and FN exposure.

In this study, micronization was used to prepare leachates, since it is a useful tool to obtain particles that mimic environmental MP for relevant ecotoxicity tests (Almeda et al., 2023). However, micronization process may also produce nanoparticles (Gardon et al., 2022). The latter may be responsible for the toxicity due to several plastic leachates on zooplankton, such as echinoderm larvae and crustaceans (Li et al., 2022; Manzo and Schiavo, 2022). Therefore, future research addressing the potential presence of these small particles is needed to confirm this hypothesis.

The WOE approach is used to integrate information to estimate the probability and magnitude of hazard in the environment (Environment Canada, 2012). Although the WOE has been widely applied for sediment quality assessment (Chapman, 2007; Regoli et al., 2014), its use has been only recently proposed for plastic contamination assessment in the aquatic environment. Thus, Teng et al. (2021) used the biomarker LOE on the oysters exposed to virgin PE and PET to provide MP hazard in the marine environment. To date, no studies are available on the WOE approach to beached-plastic leachates. In the present study, the WOE integration applied on 13 ecotoxicological responses as LOE displayed a "moderate" environmental hazard for leachates from FN and HP and an "absence" of environmental hazard for the other leachates (PET, RTR, Pellet). The index obtained for PET and Pellet is consistent with the ecotoxicological results, where no toxicity was observed for the different endpoints. Regarding RTR, no hazard was estimated by the WOE, although high HQ specific values (i.e. crustacean, cnidarian, echinoderm behaviour and development) were highlighted by the WOE model. The latter did not consider the worst result but it integrated the effects on all bioassays; conversely, by following the "worst result" approach based on single bioassays, the ecotoxicological classification of RTR leachates would have been conditioned, likely overestimating the ecotoxicological hazard.

A slight environmental hazard of HP from Adriatic beached plastics was observed while a moderate effect was found in HP from Biscay Bay and FN from the Adriatic Sea. By considering each single bioassay, the toxic effects can only be measured in zooplankton species (crustaceans, echinoderms, cnidarians) by evaluating different responses (survival, behaviour, development). These responses, measured at the organism level and quantified by means of toxicity indices, seem to mostly contribute to the environmental hazard, as indicated by the high specific HQ value (Suppl. Fig. S10). Thus, the biological relevance of such endpoints together with the threshold derived from the sensitivity of these species are responsible for the slight and moderate hazards. Noticeably, zooplankton seem to mainly affect the WOE rather than decomposers and secondary consumers, as previously observed in the contaminated marine sediments assessment (Manfra et al., 2021). Moreover, all the investigated endpoints were impaired aside from the selected species, suggesting that primary consumers may have an important role in estimating the environmental hazards due to plastic leachates.

The impact of plastic leachates on aquatic organisms may vary depending on the tested materials and species. Moreover, the toxicity of certain plastic categories may be related to the presence of specific compounds released after leaching, that were not found in control samples. Thus, RTR exposure induced a high toxicity – in term of EC50s - towards many invertebrate species (i.e. jellyfish, crustaceans, sea urchins), probably due to the release of several toxic compounds and, among them, benzothiazole and its derivatives, 1,2-dyphenylguanidine and 2,2,4-trimethyl-1H-quinoline. These compounds were the only ones found in the leachate of RTR but not in other plastic categories. Benzothiazoles and their derivates are heterocyclic compounds; together

with dyphenylguanidine are used as accelerators in rubber production (Fishbein, 1991; De Wever and Verachtert, 1997). These compounds were detected in car tyre rubber leachates (Unice et al., 2015; Capolupo et al., 2020, 2021), inducing toxicity in fish and crustaceans (Sheftel, 2000; Chibwe et al., 2021; Halle et al., 2021; Bournaka et al., 2023). The 2,2,4-trimethyl-1H-quinoline is a common tyre antioxidant, responsible for a high toxicity in aquatic organisms (Wang et al., 2023). The presence of benzothiazoles and other organic antioxidants associated with rubber pollution may pose a risk to the marine ecosystem, as suggested by zooplankton ecotoxicological results. However, despite the presence of antimicrobials, rubber accelerators and antioxidants in RTR leachates that are likely to affect sub-lethal effects in primary consumers, the WOE based on 13 responses did not estimate any environmental hazard for RTR. Changes in the structure of biological communities are indicators of natural and anthropic impacts, affecting environmental quality assessment (Mouillot et al., 2011). Single ecotoxicological bioassays may overestimate the ecological effects of plastic leachates that occur in the field, conversely to the elaboration procedure using WOE model. The latter is based on different weighted criteria and may offer a more realistic scenario, by providing simple interpretation of complex data.

Exposure to HP leachates from both the Mediterranean Sea and Atlantic Ocean did not affect behaviour, cardiac activity and bacteria bioluminescence, conversely to copepod survival and sea urchin development. In this case, it was possible to estimate a similar effect in sea urchins after exposure to HP from both geographic areas, mainly formed by PE and PP. Likewise, abnormal development has been reported in *P. lividus* and in the oysters exposed to PE and a mixture of PE and PP leachates of virgin and beached-plastics (Martínez-Gómez et al., 2017; Gardon et al., 2020; Cormier et al., 2021). In addition, the similar toxicity level estimated by means of EC50s on sea urchin development after HP exposure from the Atlantic and Mediterranean areas are likely to depend on the release of similar compounds (i.e. phthalic anhydride, BPA, triphenyl phosphate, UV-327; Table 2), that impact echinoderm early stages. Although similar results were obtained in sea urchin development in terms of toxicity index and HQ specific, the survival of *A. tonsa* was only affected by leachates of HP collected in Biscay Bay. Marine copepods are sensitive to leachates from PP and PE weathered products (Bejgarn et al., 2015). The difference of toxicity observed in the leachates of both geographic areas could be ascribed to copepod sensitivity to the additives released during the leaching process and to their potential cocktail effects. For instance, PFAS and some flame-retardants, plasticizers and stabilizers were the only compounds measured in HP from Biscay Bay, not present in the same plastic category of the Adriatic Sea. In addition, the levels of 2,4 and 2,6-TDI, tris(2-chloroethyl) phosphate and 4-methylbenzophenone were higher in HP from Biscay Bay than those found in the Adriatic. Although no literature is available on the toxicity of these compounds towards copepods, their presence and concentrations may be responsible for the difference of toxicity in the leachates from HP collected in the two geographic areas.

Leachate toxicity might differ from beached and/or virgin plastics due to differences in composition, polymer type, aging conditions and additive presence and quantity (Gunaalan et al., 2020). Leachate toxicity may have strong differences between the virgin and beached plastics, caused by fugacity diffusion gradients due to the concentration of additives and the surrounding environment. Thus, additives have a higher fugacity in the plastic phase rather than in the surroundings which promotes their release (Kwon et al., 2017). Most environmental plastic are highly weathered and the 90% is estimated to have been in the environment for more than 2 years (Koelmans et al., 2016). In the present study, plastics collected in the beaches of Mediterranean and Atlantic areas may have been subjected to different aging times, leading to different additives that may be responsible for inducing different ecotoxicological effects.

Leachate toxicity is likely to depend on the cocktail of additives and pollutants released during plastic leaching process (Nobre et al., 2015; Halsband et al., 2020). Such cocktail at different levels or with different composition may be responsible for the absent or moderate impact of FN leachates. Chemical analyses revealed that BPA and its derivates (bisphenol S, BPS) were only measured in FN from the Adriatic Sea and not present in Biscay Bay FN. Bisphenols are plasticizers present in the natural environment with an endocrine disrupting activity. Many aquatic organisms - including zooplankton and fish - are affected by bisphenols (Gunaalan et al., 2020). In the present study most of the ecotoxicological effects on the marine organisms were similar in term of HQ specific after FN exposure from both geographic areas, conversely to copepod survival. Copepod life-cycle is affected by BPA in term of survival and reproduction (Dahms et al., 2017; Tato et al., 2018). The presence of both bisphenols (BPA, BPS) and chemical compounds only detected in FN of the Adriatic Sea may explain the different hazard for this plastic category in the two geographic areas.

Moreover, other additives and compounds not detectable or not analyzed with the extraction method reported in the present study could have affected FN, as previously demonstrated (Oliviero et al., 2019). In non-target analysis (NTA) workflow sample processing and data analysis steps influence chemicals types that can be detected and identified. Therefore, it is difficult to assess whether the analyte non-detection in an NTA method is due to a true absence in a sample or to a false negative driven by limitations of the workflow (Black et al., 2023). Although the current method covered a wide range of substances by using both GC and LC, undetected substances may have had an impact on the observed toxicities. Considering that about 90% of plastic is estimated to have been in the environment for more than 2 years (Koelmans et al., 2016) and that environmental factors (i.e. UV irradiation, pH, salinity, temperature) play an important role on plastic leachability (Kedzierski et al., 2018; Chen et al., 2019; Gunaalan et al., 2020; Dhavamani et al., 2022), it is difficult to distinguish between additives from the polymer itself and those adsorbed from the environmental contamination (Delaeter et al., 2022, Rani and Shanker, 2023). Therefore, we cannot exclude that the ecotoxicological effects observed in this study likely related to additives may be due to polymer fabrication rather than environmental contamination.

5. Conclusions

We report the ecotoxicity of leachates from beached-plastics on aquatic species of different trophic levels: decomposers, primary and secondary consumers. Our results show that certain leachates are toxic to marine biota, with hazard indices ranging from slight to moderate, depending on plastic category and sampling location. The latter indicates that the additive composition and processes experienced in the environment may notably influence the toxicity of plastics debris. An integrated approach based on WOE on a large set of bioassays is recommended to get a more reliable assessment of the ecotoxicity of beached-plastic leachates. In addition, some additives leached from plastics (i.e. FN, HP) should be further monitored to reduce high concentrations that could impact marine ecosystem health.

CRediT authorship contribution statement

Chiara Gambardella: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Roberta Miroglio:** Writing – original draft, Methodology, Investigation. **Elisa Costa:** Writing – review & editing, Investigation. **Jérôme Cachot:** Writing – review & editing, Funding acquisition, Data curation. **Bénédicte Morin:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Christelle Clérandeau:** Writing – review & editing, Writing – original draft, Methodology. **Anna Rotander:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation. **Kevin Rocco:** Writing – review & editing, Investigation. **Giuseppe d'Errico:** Writing – review & editing, Software, Data curation. **Rodrigo Almeda:** Writing – review & editing, Funding acquisition, Data curation. **Olalla Alonso:** Writing –

review & editing, Writing – original draft, Methodology, Investigation. **Etienne Grau:** Writing – review & editing, Writing – original draft, Investigation. **Veronica Piazza:** Writing – review & editing, Investigation. **Lucia Pittura:** Writing – review & editing, Methodology. **Maura Benedetti:** Writing – review & editing, Methodology. **Francesco Regoli:** Writing – review & editing, Funding acquisition. **Marco Faimali:** Writing – review & editing, Supervision. **Francesca Garaventa:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124233.

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