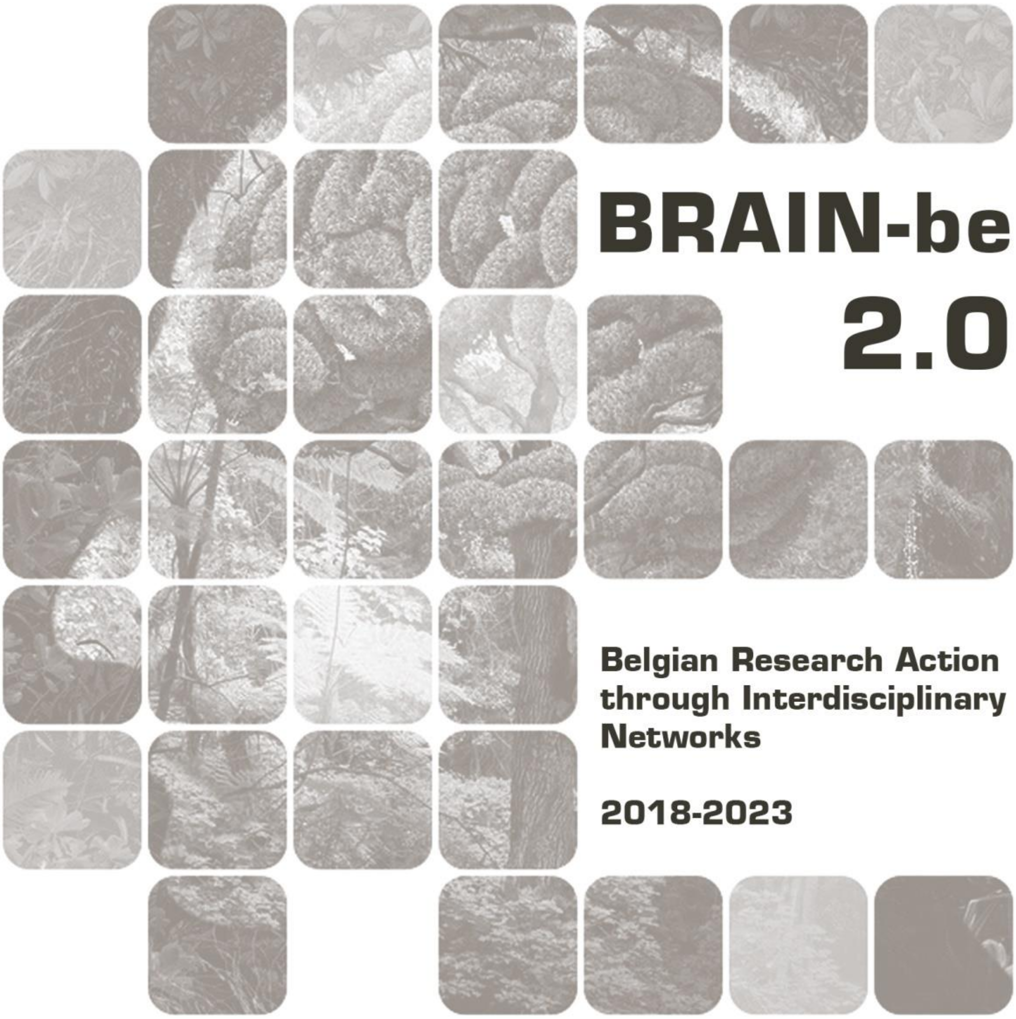
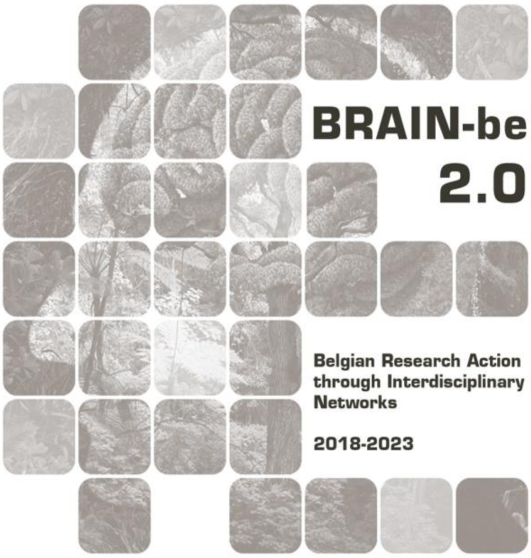


RESPONSE

Towards a risk-based assessment of microplastic pollution in marine ecosystems

Pillar 1: Challenges and knowledge of the living and non-living world



NETWORK PROJECT

RESPONSE

Towards a risk-based assessment of microplastic pollution in marine ecosystems

Contract - B2/20/P1/RESPONSE

FINAL REPORT

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ABSTRACT

Context

RESPONSE integrates expertise on oceanography, environmental chemistry, ecotoxicology, experimental ecology and modelling to answer key research questions on fate and biological effects of microplastics (MPs) and nanoplastics (NPs) in marine ecosystems.

Objectives

The overall aim of RESPONSE is to develop a quantitative Weight Of Evidence (WOE) model for MPs and NPs in the marine environment. The model integrates and differentially weights data from a suite of lines of evidence, including (1) the presence of MPs and NPs in water column and sediments, (2) their bioavailability and bioaccumulation in key indicator species from benthic and planktonic communities (3) sublethal effects measured *via* biomarkers, (3) the onset of chronic adverse effects at the organism level, and (4) ecological functioning.

Conclusions

The weight of evidence model, validated on RESPONSE data, supports a “site-oriented” process for monitoring the hazards associated with MPs/NPs and a straightforward and transparent comparison of different marine environments. The integrated approach is relevant for design of monitoring protocols and management strategies.

Keywords

weight of evidence, hazard, microplastic, nanoplastic, marine

1. INTRODUCTION

When not properly disposed or recycled, plastic poses a serious threat to the marine environment, where almost 10 million tons of these materials are released annually. Due to their non-degradability, the progressive accumulation has transformed plastic pollution into a large-scale phenomenon. Huge amounts of floating plastics have been widely documented, and the concept of "plastic islands" together with widespread graphic media images has certainly contributed to the recent rise of public awareness. However, large macroplastics are just the tip of the iceberg, the predominant component of marine debris being represented by invisible particles. Microplastics (MPs) with diameter in the range 100 nm to 5 mm, and nanoplastics (NPs), in the range 1 to 100 nm, derive from both a direct release of micro particles into the ocean and, in larger quantities, from continuous fragmentation of large plastic debris. In this respect, while trends of macroplastics occurrence are highly variable, the average size of these particles is continuously decreasing, with alarming estimates on the presence of microscopic MPs in the marine environment (Ryan et al., 2009).

Attempts to quantify the global abundance and weight of oceanic plastics are often controversial and difficult to compare due to the lack of standardized sampling methodologies, normalization units and expression of data (Ryan et al., 2009). In particular, the lowest particle sizes that are routinely sampled are in the order of 300 microns, thus neglecting a large portion of MPs and all NPs. High concentrations of plastic debris were first observed in the North Pacific central gyre, and "ocean garbage patches" have been progressively identified (Kaiser, 2010). According to results of 24 expeditions (2007 - 2013) across all five sub- tropical gyres, coastal Australia, Bay of Bengal and the Mediterranean Sea, a minimum of 5.25 trillion particles corresponding to almost 270.000 tons of plastic would currently be floating in the oceans (Eriksen, 2014). Also, the Mediterranean is recognized as being severely impacted by plastic pollution with values comparable to those detected in Oceanic gyres: the average estimate for floating particles < 500 µm is 1,700,000 per km^2 (500 g/km^2) with peaks up to 8,400,000 particles/km^2 (8 kg/km^2) (Suaria et al., 2016).

Such results have motivated the exponential growth of scientific activities towards evaluating the environmental impact of MPs in marine ecosystems. The evidence of plastic pollution in the marine environment has also prompted important normative and political actions. The European Marine Strategy Framework Directive (MSFD, 2008/56/EC) includes litter amongst the descriptors of good environmental status, and international expert committees (such as ICES and GESAMP) have worked on standardized protocols for monitoring environmental MPs. The "European Strategy for Plastics in a Circular Economy" was adopted in January 2018, to transform the way in which plastic products are designed, produced, used and recycled, thereby protecting the environment without limiting technological innovation and industrial growth.

Important financial efforts have recently been directed to better characterize the environmental fate and biological effects of MPs. Among these, JPI Oceans launched in 2015 the first call with a total budget of € 7.5 million Euros: four projects were funded (Baseman, Plastox, Ephemare and Weather-mic) and the combined network of 64 European Institutions aimed to provide new insights on the ecological problem of MPs, and to identify the most urgent knowledge gaps, including a better definition of baselines and of standardized protocols for monitoring environmental fate and impacts of MPs. Partners of the present proposal tightly collaborated in research activities of the Ephemare project investigating the adsorption and release of chemicals from MPs, their uptake and tissue distribution in key marine species, the modulation of biological effects ranging from molecular mechanisms to organism toxicity in species representative of different trophic levels, pelagic and benthic ecosystems (Town et al., 2018; Batel et al., 2016, 2018; Avio et al., 2015a,b, 2017; O'Donovan et al., 2018; Oliveiria et al., 2013; Barboza et al., 2018; Pittura et al., 2018; Gambardella et al., 2017, 2018; Beiras et al., 2019; Beiras and Tato, 2019; Ribeiro et al., 2017; Pannetier et al., 2019a,b; Barboza et al., 2018a,b,c,d; Fonte et al., 2016; Davarpanah and Guilhermino, 2015; Prata et al., 2018; Bour et al., 2018a,b; Kopke et al., 2017).

RESPONSE builds on, and goes beyond, the approaches used in previous projects. RESPONSE integrates expertise on oceanography, environmental chemistry, ecotoxicology, experimental ecology and modelling to answer key research questions on fate and biological effects of microplastics (MPs) and nanoplastics (NPs) in marine ecosystems.

2. STATE OF THE ART AND OBJECTIVES

The results obtained in Ephemare contributed to a significant advancement of the "state of the art" on the most relevant issues concerning the distribution and potential impacts of MPs in the marine environment. Among these, growing attention is being focused on the capability of plastics to offer a hydrophobic phase for sorption of many persistent organic pollutants (POPs), which have a very large water-polymer distribution coefficient, $K_{P/W}$ [L/kg], in favor of the plastic, particularly polyethylene PE (Rochman et al., 2013). In the Ephemare project, the sorption and equilibrium partition of various contaminants (PFOS, PAHs, Benzophenone-3, metals) were characterized toward MPs of different size (4 - 500 µm), shape and polymer typology, revealing the influence of both plastic and chemical concentrations, as well as of MPs' characteristics under short- and long-term experimental conditions.

Plastic polymers also **inherently contain metals and other additives**, which can be released into the aquatic environment. Contrasting results have been reported in the literature on the role of MPs and NPs in mediating the bioavailability and potential toxicity of associated contaminants. Such variability depends on a range of factors, including particle size, affinity constants, diffusion coefficients, as well as exposure time and local exposure conditions within the organism (Town et al., 2018). For environmental risk assessment, quantitative data are needed on the amount and physicochemical speciation of chemicals adsorbed on particles and their uptake/release kinetics. This information, together with knowledge on MPs and NPs ingestion rates and physiological factors such as gut transition times and modes of digestion, can be used to estimate the potential significance of plastic particles as vectors of pollutants as compared to the aqueous phase and diet. Most of the studies addressing uptake, translocation, excretion and effect of MPs and NPs use exposure concentrations far beyond what is environmentally relevant, and more realistic exposure scenarios are thus essential to evaluate the actual impact of NPs and MPs. MPs and NPs can be expected to alter the timescale of exposure, tissue distribution and the sub-cellular compartmentalization of organics and metal species within organisms, potentially resulting in subtle and long-term detrimental biological effects.

Mechanisms underlying uptake, transfer and toxicity of MPs, from unicellular organisms to vertebrates, remain largely unknown, but targeted experiments on a wide selection of biological models (including no-feeders, small filter feeders, large filter feeders and predators) have revealed the marked influence of "size" and "shape" of MPs in modulating ingestion, egestion, tissue distribution and translocation events (Batel et al., 2018; Gambardella et al., 2017; Beiras et al., 2018). When experimental data were used to fit uptake and distribution models, they predicted no long-term accumulation of MPs in marine organisms, evidence further confirmed by field investigations (Bour et al., 2018a). Similarly, the trophic transfer of MPs from preys to predators occurs, but it is also followed by egestion of these particles that, released in the environment, become again available for new ingestion processes. Most of the MPs (> 1 um) are removed *via* egestion in faeces but NPs can be accumulated in tissues, increasing the residence time in the organisms and their potential to transfer to other food levels: more research on NPs is still required.

A large selection of biological models, belonging to different trophic levels and life stages (from embryo to adults), including cell lines, bacteria, microalgae, zooplankton, crustaceans, mollusks, echinoderms and fish were used during the Ephemare project for a complex experimental design aimed to assess the onset and mechanisms of MPs toxicity at different levels of biological organization. At the organism level, batteries of ecotoxicological bioassays tested several endpoints such as survival, growth rates, behavior, reproduction, embryo development and energetic physiology, while responses analyzed at the molecular/cellular level were based on enzymatic biomarkers reflecting several pathways, immunology, genotoxicity, endocrine disruption, proteomic and gene expression. The overall results confirmed that MPs are ingested by all the biological models tested, but they did not affect toxicity in terms of short-term, acute responses, indicating that traditional ecotoxicological bioassays are not sensitive enough to assess the effects of MPs. Such experiments, however, enabled appropriate batteries of bioassays to be established, e.g., jellyfish behavioural responses and early life stages of fish, which might be useful for both industry and regulatory agencies to foster the development of environment-friendly materials and/or testing appropriate plastic additives (Gambardella et al., 2017, 2018; Beiras et al., 2018; Beiras and Tato, 2019).

The analyses of sublethal responses at the molecular/cellular level generally confirmed the lack of marked ecotoxicological effects in organisms exposed to MPs (alone) under short/medium-term conditions. Significant alterations were measured on the immune system, while more limited variations occurred on the oxidative status, neurotoxicity, and genotoxicity, with a different susceptibility of analyzed pathways, depending on the tissue, time, and typology of exposure (Pittura et al., 2018; Ribeiro et al., 2017; Pannetier et al., 2019a,b; Barboza et al., 2018a,b; Fonte et al., 2016). The overall results suggest that MPs induce weak cellular toxicity, but modulation of immune responses, still comprise an unexplored risk that these particles may provoke long-term, subtle effects on organisms' health status, under conditions of more chronic exposure (months to years) or interacting with other stressors. Importantly, Ephemare results clearly demonstrated that MPs can modulate (frequently enhancing) not only bioaccumulation, but also the toxicity of other pollutants, i.e. pharmaceuticals, nanomaterials, chlorpyrifos and PAHs (Pittura et al., 2018; Ribeiro et al., 2017; Pannetier et al., 2019a,b; Barboza et al., 2018a,b; Fonte et al., 2016; Davarpanah and Guilhermino, 2015; Prata et al., 2018).

A significant result obtained during Ephemare was the application of recently **validated weighted criteria** to summarize the overall biological significance of large datasets of heterogeneous toxicological results in **specific hazard indices** (Piva et al., 2011; Benedetti et al., 2014)**.** Such weighted criteria are based on toxicological relevance of measured endpoints, their specific threshold, sensitivity and magnitude of observed changes, providing a quantitative value assigned to one of five classes of hazard, from absent to severe (Piva et al., 2011; Benedetti et al., 2014). When these criteria were applied to biomarkers measured in mussels exposed to virgin or contaminated MPs, the elaborated hazard index ranged from slight to moderate (Pittura et al., 2018).

In addition to their utility in summarizing complex scientific results for non-expert stakeholders, such criteria are part of a more comprehensive **Weight-of-Evidence approach**, which could be used to elaborate and integrate data on MPs in environmental abiotic matrices, their presence in organisms, data on ecotoxicological effects from the molecular to organism level, and ecological effects. The overall results obtained in Ephemare on the toxicity of MPs to marine organisms strongly suggest that an environmentally relevant and risk- based monitoring of these particles will need to consider intrinsic characteristics of MPs (i.e. shape, age, size, polymer type) and their possibility to modulate the responsiveness of organisms simultaneously exposed to other stressors.

The bulk of knowledge obtained during the last years on mechanisms of MPs ingestion, trophic transfer and toxicity are of great value for going towards a **quantitative and holistic ecological risk assessment**. The intensive laboratory experiments carried out by the Ephemare team have been integrated with **field-oriented activities** aimed to characterize the presence and distribution of MPs in **marine food webs** from different European geographical areas, e.g. the Mediterranean Sea, the Atlantic Ocean and the North Sea. More than 1000 organisms were analyzed, among them key fish and invertebrate species representative of the main trophic levels and habitat preferences, highlighting that MPs can be readily taken up from zooplankton to top predators, being found in most of the investigated species, including those of commercial interest for human consumption (Avio et al., 2015, 2017; Bour et al., 2018a,b; Barboza et al., 2018). Ephemare results demonstrated that 50 % of MPs extracted from marine organisms are smaller than 100 µm, 70 % smaller than 500 µm and the remaining 30 % between 500 and 2000 µm, highlighting the current disconnect between MPs analyzed in the water column (typically larger than 300 µm) and those of biological significance. The overall results also revealed that MP ingestion is a widespread phenomenon with a frequency of 10 - 25 % of the organisms containing at least 1 particle. Geographical differences in size and typology of particles were observed in various sectors of the Adriatic Sea, possibly reflecting the influence of both local activities and hydrographic conditions such as runoff of northern rivers and waters circulation. At this time, however, the link between oceanographic conditions and biological distribution of MPs still remains to be explored. Invertebrates frequently show greater variability than fish, which are probably more influenced by local conditions. Generally, no clear relationships were observed between the number of ingested MPs and trophic position, feeding strategy or habitat preference, and field data confirmed that neither long-term accumulation nor biomagnification occurred for MPs in marine food webs.

The field activities carried out during Ephemare also provided new insights on general aspects which are often debated concerning the extraction and characterization of MPs in environmental matrices and organisms, such as the need for standardized analytical procedures. A specific protocol was developed, based on density separation of dried samples, possible use of potassium hydroxide and partial digestion in diluted hydrogen peroxide, and morphological and chemical characterization by µFTIR (28). Based on this protocol, two intercalibration and training courses were organized with partners of both the Ephemare and Baseman consortia. However, there are also other methodological approaches for extraction and/or quantification of MPs in biota, and the most appropriate procedure depends on the specific objective of the study.

The two main groups of analytical approaches comprise methods based on extraction and spectroscopic identification of polymers, and those based on chemical analyses with mass spectrometry. The first are non-destructive and allow characterization of MPs via spectroscopic techniques, ranging from FTIR to µFT-IR and µRAMAN. Upon lowering the size of the particles, the complexity of the approach increases, and many strategies cannot yet be used for the analysis of NPs. Chemical methods (i.e. pyrolysis-GC/MS; thermal desorption TDS-GC/MS) are much more rapid and provide the mass (the weight) of polymers within a tissue; however, they are also destructive and thus less informative on the particles (size or shape). From a biological perspective, methods based on extraction and spectroscopic characterization of particles are more widespread, while chemical ones could be made available for NPs. Again, a range of extraction protocols have been reported, each with specific advantages and disadvantages (Lusher et al., 2017). Whatever is the choice, appropriate Quality Assurance and Quality Control procedures should always be provided, including the validation with spiked tissues (different polymers and sizes), morphological and chemical check of particles' integrity, and rigorous control/blank procedures to check/correct for contamination.

Beside methodological aspects, there are other critical issues when **characterizing MPs in biota**, particularly in risk-based monitoring approaches combining the presence/abundance of MPs with ecotoxicological effects of these particles on marine organisms. The size definition of MPs (particles in the range 100 nm to 5 mm) is too broad to be meaningful in biological terms: 50% of the total MPs extracted from field-collected organisms during the Ephemare project were smaller than 100 µm. The size and shape of particles have implications for ingestion, translocation, retention and biological effects, while such characteristics are typically not adequately considered when interpreting the results of monitoring studies.

Recent data and Ephemare experimentation have revealed that expression units for «MPs in biota» are another critical issue. The numbers of particles extracted from biota are typically much lower than in other environmental compartments, and the units adopted for water and sediments (number or weight of plastic particles per unit volume or surface area) are difficult to interpret in biological terms, unless also the size classes and shape distribution are provided. Based on Ephemare results, the frequency of occurrence (% of specimens containing MPs) is a more sensitive parameter (compared to a number of particles) for interpreting monitoring results and differentiating between geographical risk areas. The expression of data as numbers of particles or mass of plastic per weight of tissues often confounds comparisons, because MPs are not homogeneously distributed among and within tissues, and tissues are subject to marked weight variations. The development of an "integrated value" combining number, frequency, shape, size, typology of MPs in biota has been recommended as a future target during conclusions of the Ephemare project.

Beside the strictly scientific objectives, Ephemare invested great effort in the dissemination of results to the key public and private stakeholders, and organized activities to raise public awareness on this topic. The importance of effective communication of scientific findings has been highlighted in the literature, specifically concerning the relationship between increasing public understanding of ocean- related issues and increasing public action.

Based on the preceding analysis of the state-of-the-art, the objectives of RESPONSE are:

- **to gain new knowledge on the spatial and temporal distribution of MPs and NPs** in marine systems investigating causal relationships between their occurrence along the water column, sediments and biota, covering a wide geographical area including the Mediterranean, Atlantic Ocean, North Sea and Baltic Sea, supported by modelling studies and *ad hoc* field, mesocosm and laboratory investigations. Such investigations have two main objectives: 1) **to characterize ecological thresholds** for specific characteristics of MPs that can modulate the ingestion and toxicity of these particles to marine organisms, and 2) **to investigate the ecotoxicological hazard of still unexplored particles** such as NPs and biodegradable polymers. This project will also give special attention to chronic effects of MPs, their interactions with other stressors, and their long-term consequences on different ecosystem descriptors or services, which are less understood aspects of the ecological impact of MPs.
- **to provide a quantitative "Weight Of Evidence" (WOE) approach and model** for assessing the potential impact of MPs in the marine environment, derived from a consolidated expertise in determining the environmental impact of multiple stressors. This model will be designed to integrate and assign weights to data from various investigations (or lines of evidence, LOEs), including the presence of MPs in abiotic matrices (seawater column and sediments), their bioavailability for key indicator species, and their impact at different levels of biological organization. Weights and ecological thresholds of specific characteristics of MPs (size, shape, polymer typology) will primarily be assessed in mesocosm experiments, testing the effects of MPs under environmentally and ecologically realistic chronic scenarios, alone or in combination with other stressors.
- **to set up a diffused analytical Smart Hub**, which will combine a comprehensive suite of complementary and advanced instrumental facilities available in different laboratories of some partners and of an external world-leading company.
- **to raise public awareness** on the ecological risk of MPs and NPs to guarantee not only the achievement of scientific objectives, but also sound advisory support to political and territorial agencies at both national and European levels

3. METHODOLOGY

RESPONSE adopts an integrated multidisciplinary approach, combining expertise on oceanography, environmental chemistry, ecological toxicology and experimental ecology, to answer key research questions on the fate and biological effects of microplastics (MPs) and nanoplastics (NPs) in the marine environment. To achieve the project's objectives, the tasks were divided into 8 highly complementary and interrelated Work Packages (WPs), the scope of which is described below.

WP1: Monitoring of MPs in European coastal seas

Field sampling was conducted across a wide European geographical area to provide novel insights into the vertical distribution of MPs along the water column down to sediments focusing on sizes and shapes of biological relevance, their correlation with MPs bioavailability and oceanographic dynamics in the investigated areas. The main aim is validation of ecologically relevant strategies for assessing the distribution pathway of MPs in marine ecosystems and their biological impact. Field data, combined with those from *ad hoc* experiments and the body of literature data, provides the basis to extrapolate weights and ecological thresholds for different typologies of MPs in the environment, and their elaboration in the WOE model. Geographical areas include the Adriatic Sea, Western Mediterranean Sea, North East Atlantic Ocean (from Southern Portugal to the Bay of Biscay), North Sea (from the Belgian coast to Skagerrak), Baltic Sea (Danish Fjords, Gulf of Finland and Gulf of Bothnia). In each subarea, at least 3 sites are selected, representative of wild and urbanized coasts, and estuarine sites to better estimate the range of natural exposure conditions occurring in the field. In each of the selected sites, seawater, sediments and biota (including plankton, benthos and fish) are sampled in different periods to evaluate seasonal differences according to variations of both environmental factors and human pressures. A state-of-the-art SMART sampling approach is applied to cover the range from micro- (starting at 5 mm according to the most common definition) down to nanoplastics. MPs distribution in the water column is monitored as a function of depth in the different water masses, from the surface layer to the sediments, characterizing their number, shapes, size classes and polymer typology. Sampling strategies include plankton nets, Niskin bottles and the adaptation of a new filtration system developed for sampling MPs within the EU CleanSea project 2012-2014: a stainless-steel pump will be connected to a combination of filters of different sizes (from 300 µm to at least 20 µm), allowing the filtration of large volumes of water at different depths. Particular attention is paid to the use of smaller filters and ultrafiltration protocols to adapt this system for a reliable monitoring strategy also for NPs. Results on MPs vertical distribution is integrated into the oceanographic models and parametrization of vertical processes, as an effort toward standardisation of new tools to overcome the limits of actual sampling procedures with traditional Manta net. Sediments are collected through sediment cores or quantitative samplers (e.g. box corer), and MPs are characterized in terms of numbers, shapes, size-classes (emphasizing those smaller than 100 µm), and polymer typology. Since the actual expression of MPs in seawater and sediments as number (or mass) of particles has a limited biological value, a new classification system will be developed and validated using weights and ecological thresholds for specific characteristics of MPs, derived from field results on organisms and from the *ad hoc* field, mesocosm and laboratory investigations.

The characterization of MPs in biota from various geographical areas goes beyond the scope of monitoring. Based on the bulk of knowledge obtained during the Ephemare project, RESPONSE focuses on 3 to 4 species that differ in their structural and functional organisation and are thus exposed to MPs in different ways (e.g. copepods, worms, mussels, benthic fish). Such species will include those indicated within the MSFD as bioindicators for the presence and effects of pollutants (descriptor 8). The characterization of MPs ingestion in at least two seasonal periods, and in sites differently influenced by fluvial inputs or anthropogenic pressure during the tourist season, will provide novel insights on the role that biological factors (i.e. reproductive cycle, food availability, feeding strategy and habitat) or environmental variations (i.e. human impact, estuarine loads, hydrodynamic conditions) may have in modulating the bioavailability of MPs and their ingestion by marine organisms at small geographical and temporal scales. In strict connection with experimental WPs, activities will be mainly oriented to define an integrated value for expressing MPs in biota considering the number of particles and the relative biological importance of their size, shape and polymer typology, together with their (trans)localization in tissues. Such an integrated value is expected to have practical relevance in orienting monitoring protocols and management strategies within European Directives such as the MSFD.

WP2: Biological fate of MPs and NPs

Tasks in this WP are designed to investigate, under controlled ecologically relevant experimental conditions, how specific characteristics of MPs influence key biological mechanisms such as ingestion, residence time, depuration, and translocation from digestive tracts to other organs. Organisms of the same or comparable species characterized in the field study (e.g. copepods, jellyfish, mussels and a benthic fish) and organisms with particular ecological relevance for transfer of MPs in the food webs (e.g. zooplankton) will be exposed to a range of MPs differing in terms of size-classes, shapes and polymer typology. The ecological relevance is corroborated by mesocosm experiments carried out on field-collected (micronized) plastic particles, in addition to biodegradable polymers and NPs as examples of future challenges. For the latter, also tissue slices, cell cultures, *in vivo* and *ex vivo* assays will provide novel insights on the biological reactivity of NPs. The overall results of this WP contribute to defining weights and ecological thresholds for different plastics, sizes and shapes, useful for the calibration of the WOE model and for elaborating the biological significance of data obtained in WP1.

WP3: Biomarkers in the ecological risk of MPs

WP3 develops a biomarker-based approach and an integrated elaboration procedure that, using weights and ecological thresholds for a combination of exposure and effects biomarkers, summarizes whether MPs contribute, directly or indirectly, to environmental risk. The ecological relevance of this experimental approach is ensured via appropriate selection of experimental conditions, typology of tested MPs, choice of analyzed biomarkers and inclusion of exposures to multiple stressors. Traditional species (mussels and fish) and innovative model organisms (jellyfish) among those tested in WP2 will be exposed in mesocosms for long-term periods (> 2 months); MPs of different size-classes and shapes are obtained from field-micronized plastic particles and biodegradable polymers (in analogy with WP2). Considering the lack of acute effects of MPs, this WP mostly investigates the onset of chronic and subtle effects, assessing the importance of size and shape of MPs in modulating cellular responsiveness and induced sensitivity to secondary stressors including those related to global change or anthropogenic pollution.

Since it is not possible to define a set of biomarkers specific for MPs, emphasis is given to responses related to homeostatic physiological processes and involved in responsiveness to other environmental stressors. Among these, biomarkers of the immune system, antioxidant and oxidative stress pathway, cellular damage and energy metabolism were shown to be modulated by MPs, and they may provide useful insights on the long-term consequences of MPs on food intake and digestion, weakened immunity and increased susceptibility to additional stressors including viral and bacterial-induced diseases. The overall significance of biomarker responses will be summarized in a hazard index using weighted criteria based on the toxicological relevance of measured endpoints and their variations compared to thresholds specific for each biomarker. This approach is of particular relevance for assessing the ecological risk of MP in the marine environment, defining chronic and indirect effects of MPs on the sensitivity of organisms toward additional multiple stressors.

WP4: Bioassays in the ecological risk assessment of MPs

WP4 is mostly based on microcosm or dedicated-laboratory studies specifically oriented to investigate chronic effects of MPs at the organismal level**,** and validating the correct use of a battery of bioassays. During the Ephemare project, a large selection of ecotoxicological bioassays was tested, spanning from non-feeding organisms up to predators. The overall results showed that individual assays are not sensitive enough to reveal acute, direct effects of MPs, while it remains to be established whether MPs can indirectly modulate the adverse effects of other stressors. In this respect, WP4 employs a limited number of bioassays (with different species and biological endpoints), and ecologically relevant exposure conditions. Typology of tested MPs includes leachates of field- collected plastics (to extrapolate potential long-term hazards), biodegradable polymers and NPs as an emerging challenge which remain poorly characterized. Effect-directed analyses (EDA) is also performed to obtain important information regarding biological pathways and characterization as well as identification of the substances causing the effects. As a novel aspect of this WP, results of bioassays are not evaluated as individual tests, but rather elaborated as “integrated batteries”, considering the toxicological relevance of all tested endpoints, responsiveness of the species and experimental conditions. The weighting procedures and elaboration structure of this WP will be derived from flow-chart and mathematical algorithms previously validated in several field studies and recently included in the Italian law for characterization of the ecotoxicological potential of sediments.

WP5: Effects of MPs on ecological functioning

WP5 explores the effects of MPs on selected indicators of ecological structure and functioning.

Mesocosm scenarios and field manipulative experiments are used to identify critical environmental thresholds at which MPs (alone or in combination with other stressors) affect physiological processes, such as ingestion/egestion rates, energy allocation and the related consequences on depositional processes of MPs in sediments. The influence of zooplankton on the transfer of MPs in food webs through vertical migrations, and on benthic-pelagic coupling through fecal pellets is investigated. Likewise, bivalves, by excreting MPs as nondigested feces and pseudofeces might, in turn, affect local bio-deposition rates and concentrations of MPs in sediments. Mesocosm and field experiments will be conducted to investigate whether such bio-deposits, rich in carbon and nitrogen, can provide a food resource thereby potentially increasing ingestion of MPs in benthic organisms**.** The knowledge gained establishes a robust basis for functional mapping of hot-spots for potential MP impacts in the marine environment, which simultaneously consider the link between exposure conditions and predicted biological effects across levels of biological organization. The main indices to describe ecological functioning are converted into a mathematical tool for the development of an additional line of evidence within the WOE model.

WP6: Weight of Evidence (WOE) model for MPs

A quantitative Weight Of Evidence (WOE) model is applied to integrate various typologies of data (lines of evidence, LOEs), obtained in previous WPs and to validate this approach for risk-based monitoring of MPs in the marine environment. While multidisciplinary studies are highly encouraged by European Directives, the lack of standardized procedures for the integration of complex datasets of heterogeneous results often prevents the adoption of decision-supporting procedures. Following the structure of a WOE model recently developed for traditional chemical pollutants, results on MPs in environmental matrices, their bioavailability and bioaccumulation, biomarkers, ecotoxicological bioassays and ecological descriptors are individually elaborated through logical flowcharts and mathematical algorithms. Such elaborations is based on criteria specific for various typologies of data, including the number and characteristics of MPs (size, shape, polymer typology) exceeding the ecological thresholds defined for seawater, sediments and biota or, for biological responses, the ecotoxicological relevance of measured endpoints and their variations compared to specific thresholds. The overall WOE integration of hazard indices elaborated from the field and mesocosm LOEs allows a better assessment of biological effects and ecological risk of MPs in the marine environment. The development of a dedicated and software-assisted tool provides a sound support tool for monitoring guidelines and policy makers ensuring both scientific reliability and synthetic indices for stakeholders.

WP7: "Smart Hub" of analytical facilities

A diffused **Smart Hub of analytical facilities** is organized to overcome methodological problems in particles' characterization for all the RESPONSE partners. A state-of-the-art analytical tool box is assembled to cover particle dimensions from small MPs (< 100 µm) to NPs. Previously standardized methods are used for MPs larger than 20 µm, while some new analytical approaches will be tested for particles in the size classes of 2 - 20 µm and NPs. No validated techniques are available for the latter particles, which represent a critical and challenging issue, particularly in terms of biological effects and environmental risk assessment. Instrumental facilities and expertise of 4 partners (University of Antwerpen, University of Heidelberg, Örebro University and Polytechnic University of Marche) and one external leading company (Perkin Elmer) are shared for analytical needs, harmonization and standardization of common procedures and for training activities of less experienced participants. The Smart Hub provides a comprehensive combination of analytical techniques to characterize polymer typology, particle size and shapes, surface charge, and sorbed compounds. Instruments available include attenuated total reflectance-FTIR, µFTIR and µRAMAN spectroscopy, pyrolytic and thermal desorption Gas Chromatography/Mass Spectrometry (Pyr-GC-MS, TD-GC-MS), atomic force microscopy (AFM), nanoparticle tracking analysis (NTA), Dynamic Light Scattering (DLS), Environmental Scanning Electron Microscopy with EDAX microprobe; ICP-MS, LC-MS and GC-MS further contribute to the analytical suite for chemical characterization of plastic leachates, chemical additives, and sorption of trace metals or organic chemical compounds.

WP8: Communication, Dissemination and Stakeholder Engagement

Project outcomes are communicated and disseminated to a range of audiences, such as public authorities relevant to European Directives, Regional Sea Conventions, private sector organisations and industries whose operational practices may result in MPs contamination, academia in related fields of expertise to support associated research, and the general public to increase awareness of MPs and associated concerns. WP8 employs several tools and targeted dissemination approaches through the project website, fact sheets and e-newsletters, ensuring project information is circulated to and assimilated by project audiences across disciplines and sectors. WP8 utilises provided JPI Oceans resources where applicable and appropriate, and implements the JPI Oceans best practices guidelines to ensure scientific outcomes, policy-related content and societal impact of the project is communicated within the framework of JPI Oceans. The use of social media outlets (Facebook and Twitter) facilitates complimentary information exchange with relevant audiences and activities, and stakeholder engagement is undertaken throughout the whole project, including *via* dedicated interactive workshops. The strict cooperation with NGOs involved in awareness campaigns on MPs as well as citizen science activities are also included.

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4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

This section is presented by work package.

WP1: Monitoring of MPs in European coastal areas

Task 1.1. Procedures for sampling /extraction MPs

Common and previously agreed methods were applied by the consortium during the sampling activities. Concerning surface waters (Sw), Manta trawl (330-335 µm) was used in all the sampling areas. It was usually deployed from the side of the vessel and trawled for a period that could vary between 10 and 30 minutes due to different in- situ factors (e.g local productivity, intense boat traffic or weather conditions), with a maximum tow speed limit of 3 knots. During the trawling, the filtered volume was measured with a flow meter to normalize the concentration of MPs per volume unit. After each sampling event, the whole net was rinsed thoroughly from the outside to concentrate all materials to the cod-end, which was removed, and the collected samples were carefully transferred into a plastic jar and stored at room temperature and in dark condition to prevent the photodegradation until the laboratory analysis. Niskin Bottles were used as a traditional method to collect water at desired depths. At the end of sampling, the water from the Niskin Bottles was transferred to a jar and the inside of the device was rinsed with decontaminated water (microfiltered water) to collect MP particles that remain attached. The sample was preserved until laboratory analysis.

Innovative filtration pump systems were also applied and, among these, the UFO system (Universal Filtration Objects system), a plastic-free pump-filter system, was applied to collect particles down to 10 µm (Figure 1). It was composed of a metal hose deployed in the water, a pump controlled by an inverter, and a modular filtering device capable of filtering large volumes of water (approximately 1m3 of water). The water was pumped at 5 m depth, through a flexible metal hose, the mouth of which was equipped with a stainless- steel metal cage of 5 mm mesh to protect the system against large debris. The water first passed through a filter of 300 mm mesh to retain bigger items with the purpose of protecting the finer filtering mesh from clogging. The water was then divided onto two parallel units with filters of 10 mm. The outlets were re-combined and connected to a mechanical flow meter to quantify the filtered volume. After sampling, the system was disassembled, and the single filter cartridges were opened inside a clean fume hood to prevent plastic contamination.

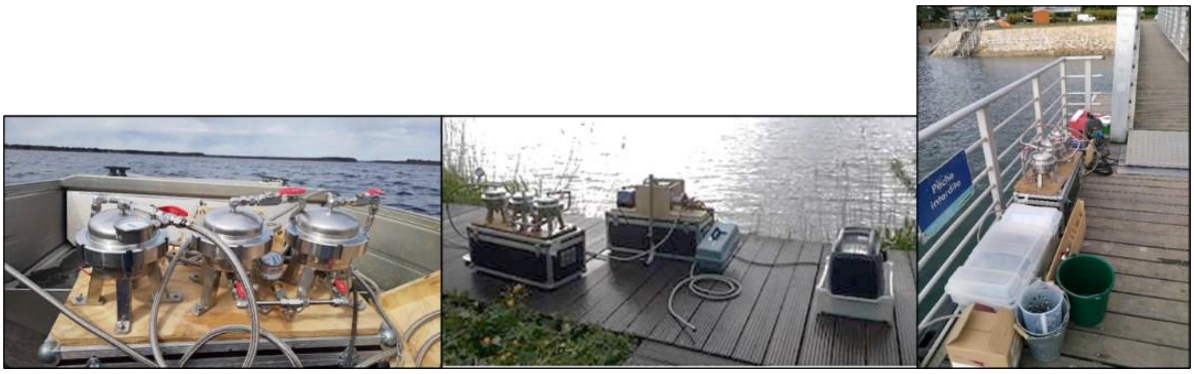

Figure 1. UFO system used during the sampling activities of RESPONSE project

Sequential Filtering Devices (SFD), were used to collect water from the surface and at 10 m depth. It consists of a stainless-steel apparatus with three levels for stainless-steel filters with different meshes mounted in series (300-100-20 µm) to allow to retain the selected size fraction and to avoid the possible clogging of the device. During the sampling, two SFD were used to work simultaneously: one sampling in surface (5 to 10 cm from the surface) and one to sample in depth (approximately 10 m). To allow continuous flow of water within the SFD, the devices were attached each to a deck pump with a high sampling capacity, capable of bringing water from the sea level to a higher height (where the pump itself is located), with a sufficient outlet pressure to allow the discharge of water. At the start of the sampling a GPS- tracker was used to register the coordinates and a flowmeter to measure the volume of water filtered (about 500L). Another submergible pump system developed by KC Denmark, was used to collect water, ca. 2000 L potentially up to 40 meters, through a flexible range of stainless-steel filters sizes (ranging from 1000 µm down to 20 µm).

Among the partners involved in WP1, the most common devices used to collect sediments for MPs analysis were grabs and corers. The van Veen grab was used to collect the first 10 cm of seabed surface. Conversely, core samplers were applied to collect cylindrical sections, thus maintaining the sediment integrity, allowing to determine historical profiles and inputs of MPs as indicators of anthropogenic activity. Within the framework of RESPONSE Project, the GEMAX gravity corer was applied to collect marine sediment. It is an improvement of the hydrodynamically balanced corer, is quick and easy to use from any vessel equipped with a light-weight winch with a lifting capability of a few hundred kilograms and with a wire speed of around 1 m/s. The corer is ideally suited for undisturbed sampling of soft muds for environmental monitoring purposes. The extruder unit permits very precise slicing of the sediment core for detailed analysis either directly on the wet sample using various probe technologies or for later analysis with more conventional methods.

Concerning biota samples, a multispecies approach was performed, covering several species with different trophic positions, habitat and feeding strategies. Overall, the selected species were collected according to their high abundance and geographic distribution and ecological relevance (i.e. key species in maintaining ecosystem functions) and commercial value. Organisms were collected by using grasps, traps, creels, or bottom trawling (benthic species), or provided by local fishermen.

Water, sediments and biota samples collected from different geographical areas, were subjected to MPs extraction following specific protocols, validated and directly experienced by the partners of RESPONSE consortium and other JPI Oceans Projects (EPHEMARE, BASEMAN). Overall, the procedures were based on pre-treatment approaches including visual inspection, density separation, filtration, screening and organic matter digestion, followed by chemical FT- characterization. For water samples a digestion step was usually applied by adding hydrogen peroxide (H2O2; 15% solution) at the sample in order to degrade and digest all biological material without altering the polymeric nature of the plastic particles. After that, a density separation approach was followed by adding a saturated salt solution (usually NaCl) with a known density at the sample; different filtration steps to concentrate and collect the putative plastic particles on filters (e.g. nitrate cellulose filter) were also performed. Specific protocols were developed to analyze the samples collected by the UFO and SFD system, by following additional digestion processes with various enzyme solutions, Fenton or H2O2 oxidation to remove any remaining organic matter on the steel filters. Further filtration steps were usually applied, and the samples were treated according to the specific chemical characterization adopted to identify the polymer type.

Concerning sediments, CNR (P10) applied a density separation method, validated also by UPM (P1), by adding a higher-density salt (NaI) at the sample in a Sediment-Microplastic Isolation (SMI) device. It consists in a transparent PVC tube with ball valve to allow the density separation of the sample and the collection of the supernatant with the putative plastic particles inside. After separation of the supernatant, it was collected and transferred on Nitrocellulose filter through the filtration of the sample by a vacuum pump.

The procedures for MPs extraction from the organisms were tested with several species of invertebrates and fish and previously inter-calibrated in different joint exercises between several partners of the JPI EPHEMARE and BASEMAN projects. Overall, the organisms were dissected to obtain the gastrointestinal tract. A digestion method with 10% KOH solution added to the gastrointestinal tract was performed and then a saturated NaCl salt solution was used to carry out twice density separation steps to allow a better extraction performance. The supernatant, obtained from sequential extractions described before, was then filtered through a nitrate cellulose filter. Conversely, when the tissues to be analyzed were particularly fatty, samples were dried in an oven at a maximum temperature of 50 °C, before the density separation and the filtration steps. At the end of filtration, an additional digestion step of the nitrate cellulose filter was performed, by adding H2O2 solution (15%,) and then storing at 50°C for 24 hours. Dried filters were observed under a stereomicroscope for the manual isolation of all putative plastic particles.

Task 1.2 Sampling in Adriatic Sea

UPM (P1) and CNR-IAS (P10) performed sampling activities in the Adriatic Sea (September 2020, and June – July 2021). A multi-matrix sampling approach was carried out with a specific focus along the Ancona coast (Conero Riviera) in 2020 and 2021, collecting Surface waters (Sw), Sub-surface water (Ssw), Sediments (S) and Biota (B) samples in 3 sub-areas subjected to different anthropogenic pressures (“Passetto”, “Trave” and “Portonovo”). Common sampling strategies were adopted, including devices for the Sw (i.e. Manta nets of 300 µm mesh size) and sediment samples (i.e. Van Veen Grab). In addition, the innovative Sequential Filtering Device (SFD), based on a stainless-steel pump, with a set of filters (from 300 µm to 20 µm) was used to collect Ssw samples (10 meters depth). Concerning Sw, the final results highlighted a plastic-pollution gradient from the most impacted northen sub-area “Passetto” (mean value of MPs abundance 0.40 ± 0.16 items/m^3) up to less impacted at the southern “Portonovo” (mean value of MPs abundance 0.06 ± 0.04 items/m^3); such results are probably related to the close proximity to point sources of MPs like the Ancona harbor located in the “Passetto” sub-area. The same trend was confirmed also from Ssw and S samples, although different patterns of MPs distribution with significantly different concentrations of MPs compared to Sw, were reported (10 items/m^3 for Ssw 300 µm and 74.49 ± 54.67 items/km^2). The parallel use of Manta net (Sw) and SFD (Ssw) showed a significantly higher number of particles with fibers (MFs) as the dominant shape, in the samples collected at 10 meters depth, compared to Sw (mean value of abundance: MPs 0.08 ±0.01 items/m^3 and MFs 0.01 ±0.01 items/m^3 for Sw; MPs 2.6 ± 3.06 items/m^3 and MFs 5.3 ± 1.15 items/m^3 for Ssw), suggesting overall that probably the Manta net underestimate both MPs and MFs in the water compartment. This assumption was further investigated in 2021, where the use in parallel of the Manta net and the SFD system to collect Sw (Manta net) and the latter also to collect Ssw samples (SFD) along the Ancona Coast, from Marine Protected Areas (MPAs) and in an industrialized area of South Adriatic Sea was applied. Overall, the results observed in Adriatic Sea (Conero Riviera and South Adriatic Sea) confirmed that the SFD system allowed to collect a higher number of particles of smaller sizes and a more significant sampling of MFs compared to Manta net (mean value of abundance: MPs 0.32 ±0.47 items/m^3 and MFs 0.04 ±0.07 items/m^3 for Sw; MPs 4 ± 3.46 items/m^3 and MFs 10.6 ± 13.31 items/m^3 for Ssw 300 µm at meters depth). In addition, the parallel use of SFD system to collect Sw and the water from 10 meters depth (Ssw), highlighted a patchy pattern of particles accumulation along the water column both along Ancona Coast and South Adriatic Sea. Overall, the SFD system highlighted that the multi mesh approach (300-100-20 µm) increases sampling relevance allowing to highlight the presence along the water column, of particles smaller than 0.1 mm, not collected by using the Manta net, that were also those preferentially found in biota. The multi-matrix approach collecting Sw, Ssw, S adopted in the Adriatic Sea with a special focus on the Conero Riviera (2020-2021) pointed out as the fibers dominate in the water column, while their contribution decreases at 10 m depth in favor of fragments which are the dominant type of MPs in the sediment. The pattern of distribution of the size classes highlighted that Sw and Ssw samples were characterized by a similar composition but at 10 m depth the contribution of particles in the range 0.1-1 mm increases and that of those between 1-5 mm in size decreases; in sediments only MPs bigger than 0.3 mm were found. In line with the chemical composition of particles extracted from the different matrices sampled in Adriatic Sea, the most abundant polymers found in the samples collected in Conero Riviera were, PP and PE predominant in water and PET predominant in sediment samples.

Task 1.3 Sampling in Western Mediterranean (6-16 months).

CNR-IAS (P10) and UPM (P1) carried out a first sampling campaign in the Western Mediterranean Sea during the Summer 2020. Sw samples were collected by using the Manta net in 21 different stations located along the Tyrrhenian and Ligurian Coasts as far as Corsica channel. Among these, the SFD system (300, 100 and 20 µm mesh size) was used in 9 stations to pump Ssw (at 10 m depth) and the van Veen Grab to collect S samples at different depths. The results for Sw, Ssw and S highlighted different patterns of MPs distribution with significantly different concentrations in Sw (highest MPs abundance 4.13 items/m^3 in Corsica channel), Ssw (highest MPs abundance 253,33 items/m^3 for 300 µm filter in Arno river) and S (Highest MP abundance 53, 59 items/km^2 in Giglio Porto). Overall, MPs abundance in terms of items/m^3 found in the Ssw samples collected by using the SFD resulted to be higher than those found in the Sw (Manta net). In addition, the same pattern of MPs/MFs observed between Sw (Manta net) and Ssw (SFD), previously described for the Adriatic Sea, was reported also in the samples collected in Tyrrhenian and Ligurian Sea (mean value MPs abundance Manta net: 0.61 ± 1.07 items/m^3 and MFs abundance 0.05 ± 0.03 items/m^3; SFD 300µm: 1.70 ± 1.74 items/m^3 and MFs abundance 72.12 ± 85.06 items/m^3). Overall in the Mediterranean Sea (2020-2021), the polymers distribution among matrices pointed out that high density synthetic-polymers as PVC were predominant in sediments, while low density polymers as PP and PE are predominant in Sw. Ssw samples showed heterogeneous polymer composition, with a relevant contribution of cellulose-based

MFs that were the most detected also in sediments, suggesting the seafloor as sink of this component of the microlitter. CNR-IAS (P10) carried out the second sampling campaign in the Western Mediterranean Sea (October 2021) collecting Sw and Ssw samples (i.e. Manta net and filtration system) at 0 and 10 m depth from different sites located in the North West of Sardinia (Olbia). The results showed a plastic-pollution gradient from the internal to the external side of the Olbia Harbour (the range of MPs abundance between 1.1 items/m^3 and 0.1 items/m^3). Overall, the same trend was reported for the samples collected by using the SFD, although at 0 meters depth it allowed to collect a higher number of particles and more significant sampling of MFs compared to Manta net (mean value of abundance: MPs 0.54 items/m^3 and MFs 0.02 ± 0.03 items/m^3 for Sw Manta net; MPs 22.8 ± 13.6 items/m^3 and MFs 10.4 ± 6.21 items/m^3 for Ssw 300 SFD µm at 0 m depth). Considering the results from the samples collected by using the SFD (Sw and Ssw), in four out of eleven stations sampled the abundance of MPs at 0 m depth was higher compared to that of the samples collected at 10 m depth (mean value of MPs abundance 50.4 ± 14.9 items/m^3 at 0 meters and 26.4 ± 22 items/m^3 at 10 m); conversely in five out of eleven stations, the opposite results were reported (mean value of MPs abundance 58.6 ± 21.6 items/m^3 at 0 m and 69.16 ± 26.1 items/m^3 at 10 m). Thus, as previously found for the Adriatic Sea, the parallel use of SFD system to collect Sw (0 m depth) and the water from 10 m depth (Ssw), highlighted a patchy pattern of particles accumulation along the water column also in the Western Mediterranean Sea.

Task 1.4 Sampling in NE Atlantic, South Portugal (6-16 months)

CIMA-UAlg (P6) carried out two sampling campaigns (in Spring and Autumn 2021) in the South Coast of Portugal and collected Sw, B (mussels) and sediments in areas affected by different anthropogenic pressures (i.e. Guadiana (3) and Arade Rivers (2), and Sagres (1)). Results on SW and Biota collected by CIMA-UALg (P6) from the first campaign, indicate that there is a trend between Guadiana (no microplastics were detected) and Sagres. 15 microplastics of different sizes and shapes were detected which corresponds to a mean of 1.5±0.77 items/m^3 ranging from 25 µm and 2.75 mm. In what concerns the shape of MPs, 89% were fragments and 11% fibers. Regarding the color of the microplastics at the most contaminated site 1 (Sagres), 47% were blue, 27% red and there was a similar percentage of green and black (13%). At Arade River significantly less microplastics were detected (0.5 ± items/m^3). Regarding MPs levels in bivalves the trend was similar with MPs detected ranging from 93% (at Sagres) and 7% in the Guadiana. Fragments were the dominant shape at Sagres (83%) and the highest amount of fibers were at Arade river (53%). During the second campaign, site 3 remained the site with fewer microplastics in water and microplastics were only detected in August. Moreover, the highest number of microplastics was at site 1. Regarding microplastics present in mussels, again site 3 was the site where mussels had less microplastics and at this site microplastics were only detected in February. At the other sites, the number of microplastics present in mussels was similar. In August the microplastics detected in mussels at site 1 had the smallest size while at site 2 the microplastics were bigger. Regarding color, black was the color more common in water with the highest percentage at site 2 (>40%) while at site 1 it ranged from 4 to 30%. Black was also the dominant color in mussels. In both compartments (water and mussels) blue, white and red were also detected. Other colors such as orange, yellow, pink, green and clear were detected, but, in less expressive amounts.

Task 1.5 Sampling in NE Atlantic waters, NW Portugal

CIIMAR (P9) performed three campaigns along the North-West coast of Portugal (NW Portuguese coast): autumn 2020; summer 2021 and winter 2021/2022. In 7 sampling sites with different levels of anthropogenic impact, mussels (*Mytillus galloprovincialis*) and gastropods (*Phorcus lineatus*) were handpicked, and superficial shore water samples were collected (bottle). Animals were used for analyses of microplastics (MPs), other contaminants and biomarkers. Water samples were used for the determination of MPs and other contaminants. Fish from Portuguese waters of the NE Atlantic Ocean (several species) obtained from the commercial fleet and aimed at human consumption as seafood were also sampled for analyses of MPs, other contaminants and biomarkers. In collaboration with other projects of the CIIMAR team, additional samples of fish (Platichthys flesus, Mugil cephalus, Cyprinus carpio) from the Minho River estuary (winter and summer), other fish species (collected in the spring, summer, autumn and winter), and mussels (spring, summer, autumn and winter) from the Douro River estuary were also analyzed. The recovery of particles from the biotic and abiotic samples was done according to the protocols developed in the scope of RESPONSE.

Characterization of MPs is described in following tasks. MPs recovered from CIIMAR's (P9) samples were chemically characterized by FTIR, µFTIR or µRaman spectroscopy. MPs were found in all the analyzed species, sampling sites/areas, and sampling periods. Among the plastic particles recovered from animal samples collected in the NW coast, estuaries and NE Atlantic waters, in general, fibers predominated over other shapes (e.g. fragments, pellets, foams), and a diversity of polymers, colors and sizes were observed. The MP contamination varied with the sampling site/area, sampling period, species, analyzed tissue, and other factors. For example, 1289 particles were recovered from Minho estuary fish (41 carps, 43 mullets and 44 flounders), 883 of which were plastics, with MPs accounting for 97% of the plastics were fibers (84%), and 35 polymers were identified by µFTIR analyses. The percentage of fish with MPs (< 0.5 mm) was 98%, 100% and 79% for carps, mullets and flounders, respectively. The mean ± standard deviation (SD) of the number of MPs per individual fish was 8 ± 6 MPs/ind for carps, 10 ± 9 MPs/ind for mullets and 2 ± 2 MPs/ind for flounders. These results indicate a high contamination of Minho estuary fish with MPs. From mussels collected (spring, summer, autumn, winter) at the mouth of the Douro estuary but at a certain distance of the main navigation channel, 129 particles were recovered, 63 were natural and 65 were MPs, including synthetic/modified microfibers Most were fibers (72%) and eight polymers were identified by FT-IR. The seasonal percentage of mussels with MPs ranged from 20% (summer) to 40% (spring). The mean ± standard error of the mean (SEM) of MPs/ind varied from 0.164 ± 0.061 (summer) to 0.728 ± 0.202. In addition to the experimental work, a compilation data on the global contamination of wild fish species with MPs and its implications to conservation and sustainability of wild fish stocks, and future directions was done and published. In fish, MPs were found in the gastrointestinal tract, gills, liver, brain and heart.

Biota (mussel, several fish species) from the NW Portuguese coast and water samples collected simultaneously to those used for MP analysis, were analysed for the presence of other contaminants (P9). In general, relatively low levels of metals (e.g. Cr, Cd, Cu, Zn), PAHs, and contaminants of emerging concern, including pharmaceuticals, and several chemicals associated with MPs (e.g., bisphenols) were found. Nevertheless, the concentrations of individual substances varied with the sampling site, sampling period, type of sample (water, biota) and species. In addition, plastic debris were collected in the Portuguese coast, chemical characterized (FTIR), fragmented and sieved to produce MPs of different sizes for use in mesocosms and bioassays (WP2, WP3, WP4).

Task 1.6 Sampling in NE Atlantic, Bay of Biscay

Sampling activities were performed in the NE Atlantic (SW coastline, France) during 4 seasonal (April, July, October and January) campaigns between 2019 and 2020 by University of Bordeaux (P3). Sw by using 300 µm Manta net, Wc by using a stainless-steel pump with 5 mm, 250, 125 and 50 µm mesh filters (UFO System), S from beaches (100m transect line) and B samples (oyster, spider crab, common sole and sea bass) were collected in three sites inside the Arcachon bay (Branne, Ile aux Oiseaux and Bélisaire) and two sites outside (Truc vert and La Salie). A distinction was made between all anthropogenic particles (AP, i.e. visually sorted) and MP (i.e. plastic polymer confirmed by ATR-FTIR spectroscopy). Results from samples collected by the University of Bordeaux (P3) reported APs in all abiotic compartments (Sw, Wc and S) and in 62% to 76% of B samples. MPs were found in 75% to 100% of abiotic samples and in 7% to 33% of B samples. Sw samples showed a greater diversity of shapes compared to other sample types. Moreover, they showed different shape compositions over seasons, in particular in summer (rubbery fragment) and winter (fibers made of PP). These particular contaminations could come from anthropogenic activities such as road and boat traffic or fishery activities. Polymer compositions were stable over seasons in all studied sample types. Overall, the main polymer at Sw was PE while it was cellulose and PET in Wc samples. Cellulose was prevailing in S and B. At outside stations, the Sw and Wc presented a blended composition regarding shapes and polymers and low to high concentrations (e.g. 0.16 ± 0.08 MP m^{-3} and 561.7 ± 68.5 MP m^{-3}, respectively for Sw and Wc), possibly be due to coastal processes and nearby input sources. The inlet station displayed a well-marked pattern only at the sea surface. High AP and MP concentrations were recorded, and fragments along with polyethylene were overwhelmed (respectively 76.0% and 73.2%). Higher surface currents could explain this pattern. At the back of the bay, AP and MP concentrations were lower and fibers were mainly recorded. Weaker hydrodynamics in this area were suspected to drive this contamination profile. Overall, fragments and buoyant particles were mainly detected at the sea surface while fibers and negatively buoyant particles prevailed in other compartments.

Task 1.7 Sampling in North Sea, Scheldt Estuary

Sampling activities were performed along the Scheldt estuary by the ECOSPHERE (P14) in collaboration with field campaigns conducted by the Research Institute for Nature and Forest (Instituut voor Natuur- en Bosonderzoek - INBO). Specifically, sampling was conducted along the Scheldt estuary in Belgium and in the Netherlands in fall 2020. Biota (bivalves, crabs, periwinkles, shrimps and fishes) were collected at 8 sites along the Scheldt estuary. Surface water was sampled using a pump with 300 µm and 10 µm stainless steel filters (UFO MPs system) at 3 sites where the biota was collected. Furthermore, water and sediment samples were collected along the Scheldt estuary (tidal influence) and two of its tributaries (no tidal influence) in collaboration with the Flemish waterways (De Vlaamse Waterweg). Water samples were collected at three locations on a seasonal basis in 2022, and at two locations in the Demer valley at two seasons (winter and spring 2022). Sediment samples were collected at fourteen locations along the Scheldt estuary and at nine locations in the Demer River in Spring 2022. Overall results from samples collected along the Scheldt estuary estimate higher concentrations of MPs (35 µm - 5000 µm) in sediment than in the water column. The main plastic types found in the samples were polyethylene, polypropylene, polystyrene, polyethylene terephthalate, polyvinyl chloride, polyacrylamide and polyurethane.

Task 1.8 Sampling in North Sea, Skagerrak (6-24 months)

UiO (P11) performed sampling activities in the North Sea (Norwegian region). Ws at 600 m depth were collected in Skagerrak and filtered down to 10 and 0.1 µm. Samples were collected at different depths (0-2 cm; 2-4 cm and 4-10 cm) in the anoxic Drammen fjord. Due to a technical problem with the cold storage at the Department, the sediment samples were unfortunately lost. An innovative large-volume (50 L) water sampler was designed to avoid any potential contact with plastic during sampling. The water sampler could be connected directly to the UFO MP filtration system (the work was done in collaboration with the University of Aalborg), reducing contamination risk. Samples were collected at two depths in the Glomma estuary (Norway's largest river) – the pycnocline and bottom – as well as at 430 m depth in the Skagerrak, close to Torbjørnskjær. Samples were filtered sequentially by 300 then 10 m steel filters. There were low amounts of plastic collected on the 300 m filters and the overall counts were within the same area for all samples. The deep sample had a density of 140 particles m^{-2}.

Task 1.9 Sampling in Baltic Sea, Danish Fjords

DTU (P5) carried out sampling of fourteen stations during a research cruise in the Kattegat/Skagerrak Sea (Denmark). Surface water samples were obtained using the plastic- free pump-filter "UFO" system. A pump filter sampler with multi 'UFO' units ("Kraken") was employed to collect subsurface MPs from different depths. A multinet was used to collect the zooplankton. Fecal pellets and marine snow samples were collected using stainless-steel sediment traps. The concentrations of MPs (> 10 µm) in surface Danish marine waters ranged from 11 to 87 MPs m^{-3}. The most abundant synthetic polymers were polyester, polypropylene, and polyethylene. Approximately 88% of the MPs were < 300 µm and 56% were fragments. The concentration of MPs (>10 µm) in the subsurface samples ranged from 13 to 156 MPs m^{-3}. The dominating polymers across all depths were polyester, polypropylene, polyethylene, and polystyrene, with approximately 94% of MPs being <300 µm. High-density polymers accounted for two-thirds of the total plastic polymers. The concentration of MPs inside zooplankton and their fecal pellets was relatively low compared to the concentration of MPs in the water. However, large quantities of MPs associated with marine snow were found in samples collected at the beginning of the pycnocline in the Kattegat (median: 883 MPs per cm^3 of marine snow).

Task 1.10 Sampling in Baltic Sea, Gulf of Finland

TalTech (P2) performed sampling activities in the Gulf of Finland, (Baltic Sea) in August and September 2021. Sw and Wc samples were collected by using Manta net (330 µm) and UFO system (P5) from 3 different stations, at 1 and 5 m depth. Sediment samples were collected by using GEMAX corer, biota samples (mussels Limecola balthica and Mytilus trossulus) by using Van Veen grab. Pelagic commercial fishes were also collected.

Task 1.11 Sampling in Baltic Sea, Gulf of Bothhnia

Sampling in the Gulf of Bothnia (Baltic Sea) was carried out by Örebro University (P11) collecting Sea water samples using pump and trawl, sediment, bivalves (*Macoma Baltica*) and perch (*Perca Fluviatilis*). The samples were collected in two urban areas (Umeå and Sundsvall surroundings) and one area that is used as a reference area in the national monitoring program (Örefjärden, sites B3 and B7). The methods used for analysis were microscopy and ATR-FTIR for MP > 0.3 mm and micro-FTIR Imaging for MP < 0.3 mm. Based on the results of 12 trawl samples average levels of MP > 0.3 mm were 0.1 MP/m3 in the reference area (n=4), 0.4 MP m^{-3} in Umeå (n=6) and 0.6 MP m^{-3} in Sundsvall (n=2). In Umeå samples were collected during two seasons (spring and late summer) and the levels were higher in late summer. However, the samples were few, two in spring and four in summer, hence the finding in weak. MP content on filters from pump filtration down to 20 µm was evaluated in samples from Umeå and Sundsvall. In Umeå concentrations ranged between 19 and 1100 MP m^{-3} (n=12) and in Sundsvall between 91 and 730 MP m^{-3} (n=2). The majority of MPs were found in the size fraction 20-100 um. Fragments were dominating over fibers making up 50-95% of total MP in the individual samples. The bivalves living this far north in the Baltic are very small, approximately 1 cm in width. Pooled samples of 15-20 mussels each were analyzed. Triplicate samples from two geographical areas were analyzed using micro FTIR imaging but no MP count was above the level of detection. In Umeå and the reference area 80-100 g of dry sediment was analyzed. On the 0.3 mm filters 2-8 fibers were found in each sample. The content on the 0.02 um and 0.1 mm filters were analyzed using imaging micro FTIR and here a clear difference was seen between the urban area (Umeå) and the reference area. In the reference area, no MPs were detected (n=2) whereas in Umeå (n=3) 16-200 MPs were found. The identified polymers were PS, PP, PUR and PVC. Perch from all three study areas were dissected and 20 from each area were selected for analysis. Visual examinations under microscopy reveal low frequency of ingested MP, less than 5% of the fish had ingested fibers.

Task 1.12 Characterization of MPs

Harmonized protocols for the characterization of particles extracted from environmental samples (Sw, Ssw, S and B) were validated and shared between UPM (P1) and CNR (P10) partners. The putative plastic particles were categorized according with shape (fragment, fibre, line, pellet, foam/beads and film) and size (<0.1 mm; 0.1- 0.3 mm; 0.3-1 mm; 1-3 mm; 3-5 mm and < 5 mm only for Manta net). The chemical approach applied for chemical characterization was based on FT-IR; µFT-IR and µ-Raman spectroscopy (particles < 100 µm) (Task 7.3).

Task 1.13 Chemical analyses, field macroplastics

The leachates obtained from beach materials, collected from the Adriatic coast for micronization purposes (Task 1.14), according to a standardized protocol, were characterized in terms of organic contaminants and trace metals, through HPLC and GC-MS, to determine the role of plastic as vectors of pollutants (P1). Results showed that all categories of plastics sorbed both polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons (IAs) with particularly high values in polystyrene, probably due to its “spongy” nature: one kg of this material is able to release about 2 mg of PAHs and nearly 1 g of aliphatic hydrocarbons. Polystyrene is also the polymer capable of sorbing the highest concentrations of some metals, with quantities released from one kg of material equal to about 200 mg of Al, 650 mg of Fe, 25 mg of Mn, 1.5 mg of Zn and 0.75 mg of Ni. Other metals, on the other hand, are more sorbed on rubbers (0.15 mg of Cd and 2 mg of Pb per kg of polymer) or in mussel nets, which release about 15 mg of V for every kg of material. A wide variety of the leachates were also analyzed using high resolution (HR) GC- and LCMS and a suspect screening workflow (P12). Additives from the major compound groups plasticizers, OPFRs, antimicrobial substances, polymer intermediates and UV stabilizers were identified. An interesting finding was that bio-based polymers leached more additives compared to conventional polymers like PE and PP.

Task 1.14 Micronization of field collected plastics

Virtual meetings (on 01/04/2021 and 17/06/2021) were organized to define the partners available to perform the micronization process, the plastic size classes and amount needed for the bioassays and mesocosms experiments (WP2, WP3, WP4, WP5). A specific “Micronization Protocol” has been developed and shared among partners. Beach macroplastics collection has been performed by partners from the Baltic Sea (P2) – Mediterranean Sea (P1-P10) – Atlantic Ocean (P3) (geographical effect). Collected items were divided into different Objects Categories (O.C.): O.C. 1 Hard plastic containers fragments (MAIN), O.C. 2 Plastic bottle caps, O.C. 3 Plastic bottles (PET), O.C. 4 Fishing nets (difficult to grind) etc. Three partners (CNR (P10); UPM (P1) and U Bordeaux (P3) were involved in the micronization process (mechanical sieving and separation) and produced the following different size ranges of MPs: 1000-500µm; 500- 200µm; 200-100µm; 100-50µm; 50-20µm.

WP1 partners defined the amount (g) of plastic particles needed for the ecotoxicological tests according to different size ranges (1000-500µm, 500-200µm, 200-100µm, 100-50µm, 50- 20µm) and defined the objective categories (O.C.) collected in the field to micronize or to be used for leachate experiments (Task 1.13). These included: O.C.1 Hard plastic fragments, O.C. 2 Plastic bottle caps, O.C. 3 Plastic bottles, O.C. 4 Fishing nets. Some partners involved in field- plastic collection determined the polymer within each O.C. by means of FTIR to define polymer proportion of stranded O.C. Moreover, each partner cut the different objects into approximately 0.5 cm (max 1 cm) size pieces, keeping each O.C. separate before sending them to CNR (P10) for micronization. CNR (P10) completed the micronization of plastics collected in the field by WP1 partners, sent all micronized plastics to UPM (P1) for mechanical sieving and separation. Micronized samples with size < 250 µm were used for leachate experiments (WP4), while the different plastic size ranges (1000-500µm; 500-200µm; 200-100µm; 100-50µm; 50-20µm) were sent to each JPI Response partner.

Task 1.15 Hydrography and modelling circulation

Taking into account terrestrial sources from rivers and cities, and marine inputs from major shipping lanes, long-term drift of floating debris in the Adriatic basin microplastic dispersion, path and final faith have been simulated by P1 using a 3-D Lagrangian model TrackMPD. The model uses a 4th order Runge-Kutta scheme to calculate particle displacement due to advection and a random-walk model to simulate diffusivity. The current was obtained from an internally developed hindcasting system, made of coupled atmospheric-circulation-wave models, with the advantage of obtaining fields at a higher resolution namely 1 km. The model is an implementation of Coupled Ocean-Atmosphere-Wave-Sediment Transport modeling system (COAWST) for the whole Adriatic Sea, where the Regional Ocean Modeling System (ROMS), the wave driver Simulating Waves Nearshore (SWAN) and the atmospheric model Weather Research and Forecasting (WRF) are coupled together.

Modelling MPs behaviour and fate in the Gulf of Finland was conducted by P2. Current fields are calculated using a GETM hydrodynamic model with a horizontal grid spacing of 250m and 60 vertically adaptive layers. Initial T, S and current fields and open boundary conditions are taken from the 1 km Baltic Sea model. Atmospheric forcing is taken from ERA5 re-analysis. The simulation period is 2015-2019. A Lagrangian particle transport model is developed and applied to follow the dispersion and accumulation of MPs for the sources in the region (rivers and point sources). Sensitivity analysis of the transport model has been conducted to consider horizontal diffusion, beaching, resuspension and biofouling effects. Maps for dispersion and accumulation of MPs in the Gulf of Finland using realistic load scenarios are derived. Simulation of the MP pathways and potential accumulation zones in the Gulf of Finland over a span of three model years using a realistic load scenario highlights the dominant role of riverine sources in introducing MP into the Gulf, surpassing contributions from WWTPs. The results provide particle concentrations for surface, water column and sediments. The lower mean probability of PET and PP/PE particles in the surface layer suggests limited dispersal over longer distances. Additionally, based on model calculation, sedimentation rates are higher near the coastal regions than in the central gulf. It appears that these systems serve as effective sink and retention units for MP, safeguarding the open gulf from pollution. As a result, future MP monitoring efforts should not solely concentrate on the surface layer but also extend to the water column and the seabed. Multipoint sampling across various layers should be conducted in the vicinity of emission sources to comprehensively assess MP pollution.

Task 1.16 Weighted elaboration of MPs – NPs data

Data of MPs in abiotic matrices from the Adriatic Sea (P1) and from Tyrrhenian basin (P10), including surface water and sediments, as well as microplastic ingestion, were firstly elaborated using the LOE1 module (Microplastics quantification and characterization in abiotic matrices) and the LOE2 module (Microplastics ingestion) respectively; they were finally processed according to WOE procedures and assigned to one of five classes of hazard, from Absent to Severe. Concerning Adriatic Sea, MPs data on the water surface elaborated with LOE1 revealed some differences among sampling sites (task 1.2) with a Slight hazard level in “Trave” independently from the sampling period, Slight and Moderate in “Portonovo” in autumn 2020 and summer 2021, and Moderate and Major hazard level in “Passetto” the most polluted site in autumn 2020 and summer 2021 respectively. On the contrary, the hazard levels for all collected sediment samples were Absent or Slight regardless of seasonality, indicating a generally low risk of microplastic contamination in this compartment. Data on MPs ingestion elaborated with LOE2 showed a hazard level ranging between Major to Severe independently from sites, sampling seasons and species (the Mediterranean mussel *Mytilus galloprovincialis*, the sea urchin *Paracentrotus lividus* and the Snakelocks anemone *Anemonia viridis*). The integration of heterogeneous data from different LOEs revealed a class of risk “Moderate” in all sampling sites collected both during the Autumn 2020 and Summer 2021, with the only exception for "Portonovo" site in the Summer 2021 which showed “Major” risk.

Concerning the North Tyrrhenian Sea, the elaboration of LOE1 revealed a Slight hazard in all investigated sites for surface water, except the "Rio Marina" site, in which the hazard level was Major, and the Ligurian Sea sites in which the hazard level ranged from Slight to Severe. In the sediments the model elaboration showed Slight hazard levels in all sampling sites. Data on microplastic ingestion (LOE2) in the Tyrrhenian Sea organisms (North Tyrrhenian and Ligurian Seas), showed a hazard level ranging between Moderate to Severe. The integration of various LOEs into WOE and class of Risk revealed a Moderate risk class for North Tyrrhenian Sea area and Major for Ligurian Sea.

WP 2: Biological Fate of MPs and NPs

Task 2.1 Mesocosm exposure

UPM (P1) carried out two experiments using the Mediterranean mussel *Mytilus galloprovincialis* as model species. The first experiment aimed to evaluate: (i) interactions of mussels with microfibers (both of synthetic and of natural origin), in terms of bioaccumulation, egestion, organ translocation (Task 2.2) and onset of sub-lethal effects in organisms through a biomarker- based approach (neuroendocrine-immune and antioxidant system alterations, onset of oxidative stress and damages, lipid metabolism) (WP3), (ii) the differences between natural and synthetic polymers fate and effects (Task 2.7). In the second experiment, mussels were exposed to polyethylene microplastics of different size classes (PE-MPs) to define weights and ecological thresholds for calibration of the Weight Of Evidence model and future elaboration of field data (Task 2.7).

Another experiment with exposure of *M. galloprovincialis* to mixture of micronized MPs derived from field-collected plastic items (task 1.14) and commercial particles was performed by UPM (P1). Due to COVID-19 restrictions, P9 carried out a preliminary bioassay with the freshwater bivalve *Corbicula fluminea* exposed to model nanoplastics (NPs), namely polystyrene fluorescent NPs (50 nm diameter) mainly for WP3. Wild bivalves were collected in the estuary of the Minho River, acclimated to laboratory conditions, and exposed to different treatments (control and three concentrations of NPs). At the end of the exposure period, several biomarkers were determined. Bioassays were also conducted using Daphnia magna as model to investigate ingestion (Task 2.2), localization and/or effects of fluorescent MPs and NPs (WP4), and toxicological interactions with other stressors (temperature, light intensity, lithium). Bioassays with marine microalgae (*Tetraselmis chuii*) and freshwater microalgae (*Chlorella vulgaris*) exposed to NPs were also performed (WP4). An experiment to investigate the ingestion and effects of field-MPs (produced from selected plastic pieces collected in the NW Portuguese coast) and post-exposure exposure recovery was performed using the Mediterranean mussel (*M. galloprovincialis*) as model. Briefly, wild mussels were collected in a low contaminated site of the NW Portuguese coast and were acclimated to laboratory conditions. Then, they were individually exposed to a mixture of MPs (≥ 63 and < 500 µm) for 8 days, followed by a recovery period of 6 days in the absence of MPs. After 8 days and 14 days, animals were sampled for determination of MP ingestion (Task 2.2) and several biomarkers (WP3). Moreover, a mesocosm experiment to investigate the effects of field-MPs on different trophic levels of a simple marine food chain (*Tetraselmis chui, M. galloprovincialis, Porchus lineatus* and *Nucella lapillus*) has been carried out.

CNR (P10) carried out several experiments using the brine shrimp nauplii and jellyfish ephyrae to assess the biological fate of MPs and NPs. Within this aim, ingestion (Task 2.2) was measured by means of traditional and novel detection methods (Task 2.5), using commercial fluorescent plastic particles or fluorescent dyes. Mesocosm exposure was carried out at laboratory level, by also evaluating ecotoxicological effects in marine crustaceans and cnidarians (Task 2.3).

A comprehensive benthic mesocosm exposure was performed by UiO (P11). Pristine sediment collected from the outer Oslofjord was dosed with 1 and 6 µm fluorescent polystyrene particles as well as two concentrations of ground beach plastic from the Adriatic, resulting in four treatments: control, only polystyrene, polystyrene and 5 g m^{-2} beach plastic, polystyrene and 33 g m^{-2} beach plastic. The beach plastic particles were 100-200 m. The mesocosms were maintained with flow-through seawater from 50 m depth for two months before termination.

In the summer 2023, the whole consortium was involved in a mesocosm exposure at the "Mesocosm Facility at Umeå Marine Science Center, MF-UMSC". In this experiment, two local species *Gastrosteus aculeatus* and *Macoma balthica* were exposed to conventional and biobased polymers, namely polypropylene (PP) as petroleum-based polymer, and polylactic acid (PLA) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBv) as biobased polymers. These polymers were micronized and tested at size <250 µm in a nominal concentration of 0.2 g L^{-1}. Collected samples have been used to analyze a panel of biological responses at biochemical and cellular level, including oxidative metabolism, biotransformation pathway and neurotoxicity, induced by microplastics from either conventional or biobased polymers.

Task 2.2- MPs/NPs ingestion in mesocosm species

As described above (Task 2.1) the UPM (P1) partners provided laboratory exposure on Mediterranean mussel Mytilus galloprovincialis as model species to microfibers (MFs), both of synthetic and of natural origin (polyamide, polyester and cotton). One of the aims of this study was to evaluate the interactions of mussels with different MFs in terms of bioaccumulation, egestion, organ translocation. Results on digestive gland showed the presence of polyamide- MFs in tissues, while no accumulation was observed for polyester and cotton ones.

Another experiment carried out by UMP was aimed to investigate the ingestion and the biological effects of PE-MPs at different sizes (20-50 µm, 50-100 µm, 100-250 µm, 250-500 µm, 500-1000 µm) on Mediterranean mussels *M. galloprovincialis*. Results confirmed that ingestion occurred for all the tested size classes, from 20 µm to 1000 µm, with a number of items varying from 1 to a maximum of 31 per individual. The highest number of fragments was found in mussels exposed to 100 – 250 µm PE-MPs, significantly higher than in organisms exposed to PE-MPs 500 – 1000 µm. Statistically comparable MPs levels were observed in the other treatments, while PE-fragments were not detected in control organisms, as expected.

P9 conducted an experiment to investigate the ingestion, effects and post-exposure recovery of field-MPs in mussels. After 8-day exposure, mussels had MPs in the digestive tract indicating that they ingested the particles, as well as in gills. After the post- exposure period, no MPs were observed in animals. Short-term exposure of D. magna to fluorescent model MPs showed the presence of the particles in the gut, gills, brood chamber and other body locations.

Experiments were carried out by CNR (P10) exposing jellyfish ephyrae and brine shrimps' larvae to biocompatible polymers namely polylactic acid (PLA) and polyvinylidene difluoride (PVDF) (polyvinylidene difluoride - PVDF). Both polymers were ground in fragments < 500µm in size to obtain nanofibers and MPs, that were stained with Nile Red. Larval stages of jellyfish and crustaceans were separately exposed to growing concentrations (0,1,10,100 mg L^{-1}) of PVDF and PLA fibres and MPs for 24 h, in dark conditions. Ingestion of MPs was evaluated in *A. franciscana* using epi-fluorescence microscopy, that allowed to detect PVDF and PLA MPs in the gut only at high concentrations (100 mg L^{-1}). Since this traditional technique did not allow detection of MP uptake in jellyfish ephyrae, MP internalization was assessed by using Tomocube's holotomography (HT), able to create 3D representations on the basis of the specific refractive index which characterizes different components of the sample. Thus, MPs were observed in the gelatinous tissue of jellyfish ephyrae, among nematocysts only at high concentrations (100 mg L^{-1}).

In the case of benthic mesocosm exposures performed by UiO (P11), macrofauna was sieved from sediment, comprehensively rinsed, and then each individual counted for fluorescent particles. Wherever needed, the organisms were dissected to facilitate counting. Almost all the organisms had taken up particles in the mesocosms with added fluorescent particles, including meiofauna such as foraminifera. Crustaceans and some tube dwelling polychaetes had somewhat lower concentrations. The presence of ground beach plastic did not appear to affect accumulation in any group of organisms.

Task 2.3- Zooplankton behaviour/MPs in food webs

All experiments related to zooplankton behavior and the ingestion of microplastics were completed by DTU (P5). how the behavior of planktonic copepods influences the risk of MPs entering marine food webs was investigated by applying a trait-based approach and by combining bottle incubations, video observations and trait biogeography analysis. We used different species as models of the main foraging behaviors in planktonic copepods. All behaviors showed a similarly low risk of MP ingestion, up to 1 order of magnitude lower than for similar- sized microalgae.

The same experimental set up described in the Task 2.2. by CNR (P10) was used to detect the behaviour of two different zooplankton species exposed to MPs of biocompatible polymers (PLA, PVDF). Thus, brine shrimp larvae and jellyfish ephyrae were exposed to several concentrations of MPs from biocompatible polymers to assess behaviour, including crustacean swimming speed alteration and ephyrae frequency of pulsations, by means of an automatic recording system developed by P10. Behaviour was not affected in brine shrimp larvae after MP exposure, while a significant alteration in terms of pulsation mode and a toxic effect in terms of EC50 were found in jellyfish ephyrae after exposure to both PVDF and PLA MPs (EC50: 77.43 mg L^{-1}).

Task 2.4- Modulation of zooplankton MPs ingestion

DTU (P5) focused the studies on planktonic copepods. Results indicated that (1) there is a size threshold for chemical decimation of MPs in planktonic copepods, (2) copepods can efficiently reject MPs (20 um). High rejection rates of MPs (80%) were independent of polymer type, shape, presence of biofilms, or sorbed pollutant (pyrene), indicating that MPs are unpalatable for feeding-current feeding copepods and that post-capture taste discrimination is a main sensorial mechanism in the rejection of MPs.

IFREMER (P8) has performed exposures of medaka (several stages) to biodegradable MPs and checked that MPs are indeed ingested using Nile red labelling. In these samples the ingestion and egestion, and larval growth upon different exposure conditions were monitored. Some MPs were collected after ingestion and have been sent for analyses of structural changes to other colleagues in Montpellier.

ECOSPHERE (P14) exposed blue mussels (*Mytilus edulis*) to micro- and nanoplastic particles (aged polyethylene terephthalate, APET) as outlined in Task 2.5 (*in-vitro*) and Task 2.8 (*in-vivo*), including particle characteristics detailed in Task 7.2. The in-vivo experiment involved the assessment of different cellular and tissue-level effect endpoints at environmentally relevant microplastic particle concentrations in various time points during the exposure period (day 1, 3, 7, and 14) and the subsequent depuration period (day 17 and 24). The timescale and extent of responses of the hemocytes subpopulations, specifically the granulocytes and hyalinocytes, were assessed. These assessments included functional assays such as cellular viability/mortality, lysosomal stability, and oxidative activity using flow cytometry. Additionally, histopathological assessments were conducted to examine alterations in gills and digestive gland tissues. The in-vitro exposure experiment (outlined in Task 2.5) is focused on understanding the interactions of nanoplastic particles (aged polyethylene terephthalate, APET) within cellular environments and their influence on the functionality of hemocytes (Mytilus edulis). Specific work mechanistically investigated the extent to which internalized nanoplastic particles are concentrated and distributed within the cellular compartments, and the timescale over which this occurs, using a 3D label-free live-cell imaging technique.

The same experimental setup described in Task 2.3. by CNR (P10) was used to detect the MPs ingestion in two different zooplankton species, the shrimp larvae and jellyfish ephyrae, exposed to MPs of biocompatible polymers (PLA, PVDF). The internalization of PVDF and PLA MPs was observed in the larval stage (nauplii) of the crustacean *A. franciscana* and in the cnidarian *Aurelia sp*. within 24 h of exposure. For both polymers, the uptake occurred only at the highest concentration (100 mg L^{-1}). Fluorescently labeled PVDF and PLA MPs were localized in the crustacean gut and jellyfish gelatinous tissue by means of epifluorescence microscope and Tomocube's HT.

Task 2.5- Methods for tracking smallest MPs/NPs

In order to get insight about fate of MPs during ingestion, IFREMER (P8) evaluated the feasibility of MPs identification in fish feces and showed that MPs stained with Red Nile could be identified and recovered in feces after filtration. These preliminary experiments pave the way for later analysis of MPs after ingestion by fish.

CNR-IAS (P10) performed experiments by exposing Aurelia sp. jellyfish ephyrae to environmental and high commercial fluorescent polystyrene particles (100 nm). By using traditional methods (epi-fluorescence microscopy), NPs could not have been localized in jellyfish body, thus innovative methods (i.e. holotomographic microscope) were employed for tracking NPs in jellyfish. After exposure of jellyfish to NPs for 24 hours, the novel method able to acquire a fluorescence signal due to the polymer and the sample refractive index at the same time. Thanks to the different refractive index range values of biological structures of jellyfish ephyrae and polystyrene, it was possible to localize NPs inside jellyfish gelatinous body and specifically among the nematocysts, cells able to capture prey and to protect jellyfish from predators.

ECOSPHERE (P14), in collaboration with the Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC, Université de Lorraine, France), conducted a mechanistic assessment of the interaction of nanoplastic particles at the cellular level. An advanced 3D imaging technique was employed to (1) detect and assess the spatial distribution of internalized nanoplastic particles (aged polyethylene terephthalate, with D50 = 0.57 µm and D90 = 1.1 µm) within hemocytes of the blue mussel (Mytilus edulis), and (2) investigate whether nanoplastic particle exposure induces changes in morphological and biophysical parameters which are integral to assessing the functional integrity of hemocytes.

The in-vitro exposure experiment comprised the following conditions: (1) negative control treatment (cells incubated in culture media); (2) positive control treatments (cells incubated in culture media containing cadmium chloride ($CdCl_2$) at concentrations reported to induce morphological changes (100 µM) and cellular death (800 µM)); and (3) nanoplastic exposure treatments (cells incubated in culture media with a range of concentrations of nanoplastic particles: 10 particles/mL, 103 particles/mL, 105 particles/mL; and 106 particles/mL). Cell samples were incubated under stable culture conditions up to the desired time points (24 h and 48 h).

The label-free, live cell imaging was performed using a holotomographic microscope (HT-2H, Tomocube). The detection and localization of internalized nanoplastic particles within the cell membrane and organelles is being performed through the measurement of three dimensional (3D) refractive index (RI) tomograms using the plugin MorpholibJ in ImageJ-FIJI. Additionally, acquired 3D images are being processed to obtain quantitative information relevant to the assessment of morphological and biochemical parameters, such as such the surface area, sphericity, volume, dry mass, protein density, and RI values of the cellular structures and organelles. The experimental results will provide essential quantitative information for analyzing dose-response relationship analysis and elucidating the physical processes of internalization and subsequent localization within the cell.

Task 2.6- Chemical desorption by intestinal fluids

In order to evaluate a chemical desorption of contaminants associated to MPs, P1 carried out preliminary experimental plan obtaining leachates from stranded MPs. The leachates were obtained from different objects categories and polymers by shacking MPs in artificial sea water for 72 hours. After that, to mimic the effects of gastrointestinal fluids, an aliquot of extract was acidified in nitric acid and another in methanol solution. Trace metals and organic compounds (i.e. PAHs, PCB and others) were measured. Results showed a specific contaminant desorption for each of the object categories with a general concentration increase after acid and organic extraction (P1).

A method was developed by UiO (P11) to clarify whether pyrene would leach from plastic particles under physiologically relevant conditions. Stomach and intestinal content from six different individual cod and whiting were incubated with polystyrene particles coated with the contaminant with gentle movement for 24 h, then pyrene analyzed following separation of a liquid phase from particles. The concentration of pyrene in all samples was below the detection limit for the method. Future work will try to decrease the detection limit of the method (extraction).

Task 2.7- Species sensitivity distributions (SSDs)

Since field data showed that fibers are dominant in the environment and are ingested by biota regardless of their natural or synthetic origin, UMP (P1) performed two laboratory experiments on *M. galloprovincialis* (as described above in Task 2.1). These studies evaluated the differences between accumulation, distribution, effects of natural and synthetic polymers, their interaction with a secondary stressor (thermal stress), and the accumulation and effects of different size classes of polyethylene microplastics (PE MPs) mostly oriented to define weights and ecological thresholds for MPs characteristics, focusing on size, for calibration of the Weight Of Evidence model and elaboration of field data. For the first experiment, preliminary results on digestive glands showed the presence of polyamide-MFs in tissues, while no accumulation was observed for polyester and cotton ones.

The experiment carried out by P9 with mussels indicated biological responses to field MPs exposure (e.g. energy and oxidative stress) and post-exposure recovery of several biological parameters. Bioassays with *M. gallopovincialis*, *C. fluminea*, *Daphnia magna* and microalgae showed differences of sensitivity to fluorescent polystyrene NPs among species, with microalgae (*Tetraselmis chuii* and *Chlorella vulgaris*) showing lower sensitivity than animals, and *D. magna* higher sensitivity than bivalves (as detailed in WP3 and WP4). In *C. fluminea*, fluorescent polystyrene NPs caused adverse effects (as detailed in WP3). Long-term bioassays with D. magna showed adverse effects of MPs and NPs, and toxicological interactions among stressors (MPs, temperature, light intensity, lithium) as detailed in WP4.

ECOSPHERE (P14) developed a comprehensive database for construction of biological species sensitivity distributions (SSDs). Thus far the collated data comprises a range of endpoints for 49 biological species extracted from 64 published experimental studies. Different databases were utilized for this search: Elsevier, Google Scholar, Scopus, and Web of Science. The following information was extracted: biological species, biological classification, life stage, feeding strategy, environment, polymer type, shape, size, concentrations, exposure pathways, and exposure duration. All reported test endpoints were identified and attributed to a main endpoint category based on the biological significance, such as (1) fitness; (2) behavioral, sensory, and neuromuscular function; (3) metabolism; (4) immune system; (5) alimentary and excretory systems; and (6) circulatory and respiratory systems. The specific dose descriptors, such as the lowest observed effect concentration (LOEC), no observed effect concentration (NOEC), half maximal lethal concentration (LC50), and half maximal effect concentration (EC50) were extracted.

Task 2.8- Modeling dynamic exposure conditions

ECOSPHERE (P14) conducted a mechanistic assessment of the effects of aged microplastic particle exposure on blue mussel (*Mytilus edulis*), with a specific focus on cellular and tissue level effect endpoints. The exposure experiments were carried out using environmentally relevant concentrations of aged polyethylene terephthalate (PET, D50 = 1.4 µm and D90 = 3.1 µm): low concentration (10 particles L^{-1}), mid concentration (103 particles L^{-1}), and high concentration (105 particles L^{-1}). Test organisms were subjected to a 14 day exposure period, followed by a 10-day depuration period in artificial seawater (ASW) under controlled laboratory conditions. Before the exposure experiments, a secondary characterization of the aged PET was conducted to ensure stability under the specified exposure condition using Dynamic Light Scattering (outlined in Task 7.2). The responses were evaluated using various histopathological and immunological biomarkers, employing a combination of flow cytometry and advanced microscopy techniques.

Histopathological alterations were assessed in the gills (including lipofuscin aggregates, hemocyte infiltration, central vessel enlargement, and lamellar fusion) and digestive gland (such as lipofuscin aggregation, hemocyte infiltration, atrophy, and necrosis) in both the control group and the group exposed to the highest concentration of APET-MPs (105 particles L^{-1}). Although not statistically significant, the group exposed to the highest concentration of APET-MPs exhibited more pronounced occurrences of atrophic and necrotic digestive gland tubules, as well as increased hemocyte infiltrations compared with the control. Additionally, more pronounced instances of lamellar fusion, central vessel enlargement, and hemocyte infiltration, which are prominent markers of inflammatory response in gill tissue, were evident in the exposed group.

To further evaluate the underlying mechanisms induced by plastic exposure at the cellular level, an in-depth assessment was carried out using a series of functional assays using flow cytometry. The responses of the hemocytes subpopulations, specifically granulocytes and hyalinocytes were assessed at various time points during the exposure period (day 1, 3, 7, and 14) and the subsequent depuration period (day 17 and 24), such as the cellular viability/mortality, oxidative activity, and lysosome stability. Differential responses between immune cell types were observed, in particular, lysosome destabilization was evident during the exposure period, followed by subsequent stabilization during the depuration period.

WP 3: Biomarkers in ecological risk of MPs

Task 3.1. Task 3.1- Exposures to MPs and other stressors (estimation end month 20)

Three laboratory experiments have been carried out by P1, aiming to evaluate the biological effects of microfibers, MFs (both of synthetic and of natural origin, experiment 1); of different classes of size of polyethylene MPs (experiment 2); and of different micronized environmental and commercial MPs (experiment 3). In experiment 1, at the end of the exposure phase organisms were subjected to thermal stress to evaluate the influence of MF exposure on the susceptibility toward a second stress. All the experiments were conducted using *Mytilus galloprovincialis* as model species. Mediterranean mussel (*M. galloprovincialis*) was used as a model species also for a laboratory experiment performed by P6 (experiment 4); in this experiment, organisms were exposed to 10 µg L^{-1} of fluorescently labeled polystyrene (PS) nanoparticles (0.05 µm). After an acclimation period of 96 h, mussels were exposed and sampled at 0, 3, 7, 14 and 21 days. Exposures were also carried out with other bivalves (*Ruditapes decussatus*) and include a co-exposure of MPs leachate and a mixture of micro and nanoplastics, as well as the interaction of a cytostatic anticancer drug with nanoplastics.

Experiments were carried out with *C. fluminea* by P9. Wild bivalves were collected in the Minho estuary, acclimated to laboratory conditions, and exposed for 14 days to drinking water with low chlorine concentration without NPs (control) or with fluorescent polystyrene NPs, 50 nm diameter

(0.2 – 5 mg L^{-1}) in the presence of food (*Chlorella vulgaris*). At the end of the exposure period, the post-exposure water filtration of each animal was determined, and samples were collected for several biomarkers. Another experiment was carried out with mussels (*M. galloprovincialis*) exposed to field-MPs (≥63 and < 500 µm). Wild mussels were collected in a lowly contaminated site of the NW Portuguese coast and acclimated to laboratory conditions, including the artificial salt water that was used a test medium. Then, mussels were exposed to different concentrations of field-MPs (low ppm and ppb ranges) or artificial seawater only (control). Mussels were individually exposed in glass flasks, test medium was renewed every 24 h, and commercially available food was provided. After 8 days of exposure, half of the animals from each treatment were used to collect samples for biomarkers. The other animals were transferred in a clean test medium to investigate post-exposure recovery (8 days). During this period, test medium was renewed and food was provided as in the MP-exposure period. At the end, samples for biomarkers were collected (experiment 5).

CNR-IAS- (P10) carried out several experiments to localize and quantify biomarkers in the ephyrae of the marine jellyfish Aurelia sp. exposed to leachates of micronized plastics collected in the WP1 in the Mediterranean Sea. Specifically, *Aurelia sp*. ephyrae were exposed for 24 h to leachates of hard plastics and rapido trawling nets collected in the Adriatic Sea to evaluate biomarkers. Since no literature data were available on biomarkers in jellyfish, but the latter have been shown to have a cholinergic-like system, acetylcholinesterase (AChE) activity was selected as biomarker, as described in the Task 3.2 (experiment 6).

P13 partner carried out laboratory exposures with zebrafish (*Danio rerio*) embryos which were exposed to 1 mg L^{-1} polystyrene (PS) nanoparticles (0.05 µm) with or without sorbed pyrene or triclosan (experiment 7). In this context, a micro-method to separate beads from solutions that can be useful for a wide range of applications was presented.

The whole consortium was involved in mesocosm exposure (experiment 8) of *Gastrosteus aculeatus* and *Macoma balthica* to conventional and biobased polymers as described in Task 2.1.

Task 3.2- Analyses of cellular biomarkers and Task 3.3- Analyses of molecular biomarkers

Experiment 1 (P1): among the selected panel of biological responses, the results showed immune system alterations (as ratio between granulocytes and hyalinocyte populations, phagocytosis efficiency of granulocytes, lysosomal membrane stability of haemocytes and acetylcholinesterase activity in haemolymph), limited perturbation of antioxidant system (as total oxyradical scavenging capacity), onset of lipid peroxidation (lipofuscin content), altered lipid accumulation (neutral lipids content) and lack of oxidative damages to DNA (micronuclei frequency). Biological samples for qPCR of selected genes have been prepared and are ready to be analyzed (Task 3.3).

Experiment 2 (P1): results highlighted negative effects on haemocyte lysosomal membrane stability, subpopulations ratio (granulocytes vs. hyalinocytes) and functional activity (phagocytosis), mainly caused by smaller classes of size (20-50µm and 50-100 µm PE MPs). Limited onset of genotoxic damage emerged as well as a moderate induction of acetylcholinesterase activity in gills of organisms exposed to 100-250 µm PE MPs.

Experiment 3 (P1): Haemocytes sub-populations ratio, lysosomal integrity, functionality and DNA damage, Granulocytes on hyalinocytes ratio were significantly reduced in organisms exposed to environmental plastics mixture (EPM), and micronized “Rapido” trawling rubber (RTR). Similarly, a lower phagocytosis rate was observed in the same treatments compared to control organisms. Cholinergic function and neurotoxic effects (acetylcholinesterase activity) in haemolymph were significantly increased in organisms exposed to EPM and to micronized (RTR), while scarce effects were detected in the gills. Limited alterations of antioxidant enzymes were observed in the digestive gland of exposed organisms: the only statistical difference detected regarded glutathione S-transferase (GST) activity in organisms exposed to RTR compared to organisms exposed to Virgin Plastic Mixture. On the other hand, significant increase compared to control organisms were detected on total glutathione peroxidases and GST activities in the gills of organisms exposed to RTR and EPM, respectively. Despite the limited variations on the investigated antioxidant enzymes in the digestive gland, total oxyradical scavenging capacity toward was significantly affected by the exposure to the different micronized plastic typologies collected from the environment; by contrast, less evident alterations were observed in the gills.

Experiment 4 (P6): Biological responses included genotoxicity, antioxidant and increase of genotoxic damage (DNA strand breaks) after 3 and 14 days of exposure. A moderate modulation of antioxidants enzymes was observed both in gills and digestive gland during all the exposure time which is reflected in the increase of oxidative stress damage in both tissues. No alteration was observed for biotransformation pathway enzymes. Obtained results showed a high sensitivity of gills compared to digestive glands.

Experiment 5 (P9): The biomarkers investigated in the experiment with C. fluminea were: post-exposure filtration rate (FR); lipid peroxidation levels (LPO); and the activity of the enzymes acetylcholinesterase (AChE), octopine dehydrogenase (ODH), isocitrate dehydrogenase (IDH), glutathione S-transferases (GST), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx). After 14 days of exposure to NPs, bivalves showed significantly reduced water filtration, elevated GST and CAT activities, IDH inhibition and increased LPO levels, as well as slight changes in other biomarkers. These results indicate reduced decreased performance of bivalves induced by NPs. The biomarkers investigated in mussels (*M. galloprovincialis*) exposed to field-MPs included LPO levels, and the activity of the enzymes AChE, IDH, ODH, GST, CAT, GR and GPx. After 8 days of exposure, mussels showed alterations in oxidative stress and energy-related enzymes, with post-exposure recovery observed in some of them.

Experiment 6 (P10): CNR-IAS carried out several experiments to localize and quantify biomarkers in the ephyrae of the marine jellyfish *Aurelia sp*. exposed to leachates of micronized plastics collected in the WP1 in the Mediterranean Sea. Specifically, *Aurelia sp*. ephyrae were exposed to leachates of hard plastics and rapido trawling nets collected in the Adriatic Sea for 24 hours to evaluate biomarkers. Since no literature data are available on biomarkers in jellyfish, but the latter have been shown to have a cholinergic-like system, acetylcholinesterase (AChE) activity was selected as biomarker. AChe activity in jellyfish ephyrae was confirmed by exposing the organism to referent neurotoxic compound like eserine. After that, the ChE inhibition was reported in Aurelia sp. ephyrae jellyfish exposed to leachates of hard plastic and rapido collected in the WP1 (Task 3.1). An inhibition of AChE activity was observed in jellyfish exposed to 1 g L^{-1} of hard plastics and rapido trawling nets collected in the Adriatic Sea.In addition, the same trend was observed in the ephyrae exposed to 0.1 g

L^{-1} of rapido trawling nets, suggesting an impairment of the biomarker at low and high percentage of the leachate from plastics collected in the field.

Experiment 7 (P13): A micro-method to separate beads from solution that can be useful for a wide range of applications was presented. Obtained results showed a clear effect from water- borne pyrene or triclosan to zebra fish embryo. Co-exposure with nanoplastics reduced effects to control levels.

Experiment 8 (P1): Among the selected panel of biological responses, analyses showed both for *Gastrosteus aculeatus* and *Macoma balthica* a general induction in acetylcholinesterase activity and slight perturbation of antioxidant system including single antioxidants and total oxyradical scavenging capacity. A certain modulation of AcylCOA oxidase enzyme was observed in fish highlighting an effect of biopolymers on fatty acid β oxidation.

Task 3.4 Elaboration of toxicity hazard indices

Weighted elaboration of results has been performed with all the available results of the experiments. The approach is shown schematically in Figure 2. The possibility to summarize a large data set of scientifically complex data in a synthetic hazard index was confirmed to be of great utility to better assess the overall biological significance of the obtained results.

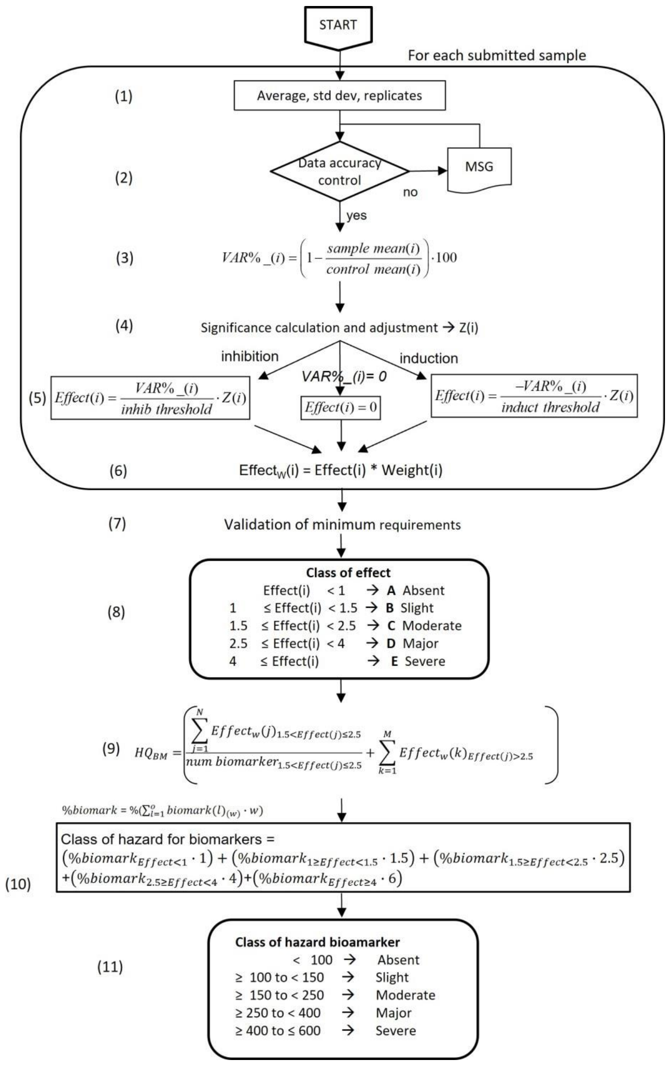

Figure 2. Flow chart for WoE model on biomarker sublethal effects

WP 4: Bioassays in ecological risk of MPs/NPs

Task 4.1. Tested materials and leachates

MPs of biodegradable commercial plastics and field collected plastics were tested with different model organisms to assess the potential risks for the environment. Some experiments show some remarkable results (P4). The sea urchin (*Pacentrotus lividus*) embryo bioassay on biodegradable commercial plastic (10 g L^{-1}) was done at different conditions: contact time at 24 h, 72 h, 7 days and size at <250 µm and <1 mm. The same experiments were done for field collected plastics. In this batch of bioassays, the toxicity is between absent and relevant. There were no significant differences for the various contact times or plastic size for toxicity. To standardize the bioassays protocol future toxicity tests will be done with particles <250 µm and 24 h of contact time to facilitate the development of the assay. Mussel embryo tests were performed on two different commercial polymers: PLA and PHBv. The results showed no-growth of the larvae at a 50% of MP concentration for the PHBV and in 100% for the PLA.

Leachates of commercial (PP and PE), biodegradable (PHBv and PLA) and field collected plastics (Pellets, hard containers, PET bottles, Fishing nets and mussel bags from three studied areas) were prepared by Univ. of Bordeaux (P3) according to the protocol developed previously using micronized particles of less than 250 µm. The toxicity of the different leachate samples was analysed on the marine bacteria *Aliivibrio fischeri* (Microtox assay) and on larvae of Japanese medaka *Oryzias latipes* (larval toxicity assay).

Plastic of different types and colours were collected manually, weighted and classified according to the colour and type. More than 60% was fishing nets debris. A leachate from each colour of the fishing net debris and a mixture of them (using the same percentage of the colour found in the marine environment) was prepared according to the protocol provided by the WP leaders.

P9 tested the following materials and leachates: fluorescent model MPs, 1-5 µm diameter (D. magna bioassays) and polystyrene NPs 50 nm diameter (*T. chuii, C. vulgaris, D. magna*). Potential toxicological interactions among stressors (temperature, light intensity, and other contaminants) were investigated in some of the bioassays.

Task 4.2 Bioassays on Early life-stages and Task 4.3. Physiological and behavioural bioassays

Bioassays were performed with microalgae, jellyfish, fish, shrimps and sea urchin for different MPs. Sea urchin behaviour (swimming) in the first developmental stages revealed a decrease in swimming speed on the first days after fertilization with NPs-MPs. The mixture leachate (obtained as described in Task 4.1) was analysed to determine the acute toxicity using a Microtox test. The leachate mixture was used in a long- term chronic exposure experiment using mussels *Mytilus galloprovincialis* collected at 0, 3, 7, 14, 21 and 28 days. The chemical analysis of the leachate, revealed levels of metals and organic compounds. The morphometrics, biochemical and genotoxic effects indicated DNA damage, protein carbonylation, antioxidant enzymes and oxidative damage as well as histopathological alterations in different tissues.

Marine medaka bioassays ELS were performed by (P8) in collaboration with DTU (P5) with tire leachates. Exposures were performed during 12 days and revealed no change in development, survival or any other endpoint (including behaviour) compared to the control.

P10 performed bioassays with marine crustaceans, jellyfish, rotifers and sea urchin larvae exposed to leachates of micronized plastics collected in WP1 (and micronized in Task 1.14), by analysing traditional ecotoxicological responses (i.e. mortality, immobility; Task 4.2) and innovative ones, including behavioural ones (Task 4.3: swimming speed alteration, pulsation mode alterations). Leachates were prepared for both bioassays by following a protocol provided in the first year by Response partners (P4, P5). A stock solution of 1 g L^{-1} of leachate was prepared from micronized plastic particles of each plastic typology; in addition, several leachate dilutions were also tested (0.33, 0.1, 0.033g L^{-1}) to calculate a toxicity index. Traditional ecotoxicological responses showed toxicity only in sea urchin larvae exposed to leaching products obtained from fishing “rapido” trawl, fishing nets and hard plastics collected in the Atlantic Ocean and Adriatic Sea. Regarding behavioural assays, the swimming speed of marine rotifers, crustacean and sea urchin larvae was evaluated after exposure to MP leachates, while the alteration of the frequency of pulsations was selected as behavioural endpoint in jellyfish ephyrae. Results showed toxic effects (jellyfish and crustacean behaviour alterations) for leaching products obtained from “rapido” trawl and fishing nets sampled in the Adriatic Sea. All the results were then integrated into the Weight of Evidence (WoE) model to estimate the potential environmental hazard (WP6). In addition, the leachates were extracted and sent to ORU (P12) to perform the chemical analyses, to be further integrated into the WoE model by P1. PE ground caps at 0.5g L^{-1} were already tested producing 50% of effects. Similar experiments were done with NPs (100 nm PS particles) and PVDF, a biocompatible polymer. Results show no immobility in jellyfish and brine shrimps, no alteration of frequency of pulsations in jellyfish and no alteration of swimming behaviour in brine shrimps for the PVDF. For the NPs at day 0 the frequency of pulsations was less than 50% of the control.

Leachates of commercial petroleum-based plastics (PP and PE) and field collected plastics (pellets, hard containers, PET bottles, fishing nets and mussel bags) were not toxic or slightly toxic (less than 20% of bioluminescence inhibition) to the marine bacteria Aliivibrio fischeri (Microtox assay). The same leachate samples did not increase mortality and malformations of Japanese medaka larvae but two of them (fishing nets from the Biscay Bay and PET bottles from the Ligurian Sea) significantly increased heartbeat. In addition, leachates of the biodegradable plastics PHBv and to a lesser extent PLA appeared moderately to highly toxic to A. fischeri (between 20 and 75% of bioluminescence inhibition) (P3).

Additional, early life stage tests were conducted on various organisms (the sea urchin *Paracentrotus lividus*, the mussel *Mytilus galloprovincialis* and the copepod *Acartia tonsa*) with environmental plastics collected along the European coastal line and new plastic bio-based polymers, and bags. The 10g L^{-1} leachates from environmental plastics showed no short-term impact on sea urchin embryo-larval growth. In a separate experiment involving plastic pellets collected in the environment, and with various stages of weathering, insights were gained into the correlation between increased weathering and elevated pellet toxicity. Finally, the comparative assessments of commercial bio-based polymers (PLA and PHBv) against conventional plastic (PP) revealed variable toxicity levels across marine species. Notably, PHBv leachates exhibited elevated toxicity, underscoring the necessity for thorough toxicological risk assessments associated with bio-based polymers in marine environments (P4).

Task 4.4 Life-cycle of microalgae/invertebrates

A life cycle experiment (from egg to adults, 3 weeks) with the cosmopolitan copepod Acartia tonsa was conducted (P5) using two different concentrations of leachates from field-collected micronized plastics. Also experiments to evaluate the effects of leachates from MPs on the growth rates of microalgae were performed. P9 found that NPs (fluorescent polystyrene spheres, 50 nm diameter) did not cause significant effects on the average specific growth rate per day of marine microalgae (*Tetraselmis chuii*) and freshwater microalgae (*Chlorella vulgaris*) after 96 h of exposure to NP concentrations up to 100 mg L^{-1}. However, these NPs significantly reduced the reproduction and the population growth rate of *Daphnia magna* (freshwater species used as model to assess long-term effects) after 21 days of exposure to environmental relevant concentrations (low ppb range). Long-term (21-days) bioassays with D. magna were carried out to investigate the potential influence of water temperature increase (20ºC to 25ºC) and light intensity (10830 lx to 26000 lx, low UV radiation in both cases) on the toxicity of fluorescent model MPs (1-5 µm diameter), lithium and their mixtures. MPs, lithium and their mixtures, caused parental mortality, and significantly reduced the somatic growth, reproduction and population growth rate of D. magna. The adverse effects were higher at 25ºC than at 20 °C, and at high light intensity than at moderate light intensity. High light intensity interacted synergistically with MPs in all the exposure scenarios (low, medium and high MP concentrations), whereas temperature interacted with MPs antagonistically (low MP concentration) or synergistically (medium and high MP concentrations). At 20 °C, lithium and MPs interacted antagonistically at low and high concentrations, and synergistically at medium concentrations of both contaminants in the mixture. High light intensity interacted synergistically with lithium and lithium-MPs mixtures in most of the tested scenarios, whereas warmer water interacted with chemical stress (lithium and lithium-MPs mixtures) always synergistically. The microtox bioassay was carried out with collected field plastics at different sizes without significant differences.

The ecotoxicological effects of commercial fluorescent NPs were investigated by P10 by performing bioassays on marine jellyfish ephyrae of two different life stages, to test the sensitivity of NPs in the first early developmental stages of jellyfish life-cycle. Two different life stages (0 and 7 days) of ephyrae were exposed to increasing concentrations (0-0.1-1-10 mg L^{-1}) of commercial polystyrene NPs suspension for 24 h in dark conditions, at 20 °C. After exposure, MPs uptake was assessed by using optical microscope and 3D holotomographic microscopy. Ingestion occurred in 10%, 30% and 100% of ephyrae of both ages respectively exposed to the lowest (0.1 mg L^{-1}), medium (1 mg L^{-1}) and highest NP concentration (10 mg L^{-1}). Holotomographic analysis allowed NP internalization in ephyra body and specifically into the gelatinous matrix and cnidocytes containing nematocysts. However, internalization did not affect survival, but it impaired jellyfish behaviour only in 0 day old ephyrae.

Task 4.5 Chemistry and EDA of plastic leachates

For EDA, beach plastic was sampled, cleaned, and polymer type characterization was done by using a Fourier transform infrared (FTIR) spectroscopy. Polymers were ground and particle size characterization was done by using Multisizer Coulter counter III (MSIII). Particles were extracted and extracts were applied on various cell-based bioassays. Preliminary results indicate high biological potencies in various extracts, which were selected for effect-directed analysis (EDA). Analysis of polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and pesticides from marine Mussels were providing (P12). Briefly mussels from various locations were freeze-dried and extraction is being done for the measurement of above-mentioned chemical classes.

Field-collected particles were provided within the framework of task 1.14. To give an impression of both the worst-case hazard potential as well as give bioanalytical information on the degree to which contaminants were present in the samples, a batch of the particles were subjected to organic extraction with hexane, while additional tests were conducted with particles introduced into the test system in their native state, representing more realistic conditions by considering bioavailability of MP-adsorbed pollutants (P13). To give an impression of toxicity by the actual particles, additional experiments were performed with commercial MP & NP particle suspensions both in their integral state (particles & liquid phase) as well as the solid and liquid phases separately after fractionation. To investigate sorption of substances to and from MPs under laboratory conditions, the extracted field particles as well as commercial particles were (co)exposed to relevant model pollutants and subsequently analyzed in appropriate test systems.

Task 4.6 Weighted elaboration of bioassays data

The multitude of bioassays allowed several combinations of batteries to be elaborated simultaneously. The results established ecologically relevant batteries of bioassays as those including species of various phylogenetic groups and different sensitivity, measurements of biological endpoints of variable relevance, and different experimental conditions.

Ecotoxicological results on the bioassays performed by P10 on sea urchin larvae, barnacle nauplii, rotifers and jellyfish ephyrae exposed to MP leachates of in field collected plastics (WP1) were sent to P1 to calculate HQ for organism effects. By integrating these values for each plastic category collected in the Mediterranean and Atlantic areas, it was possible to estimate the potential environmental hazards. A slight level of hazard was found in hard plastics, rapidi rubbers fishing nets and mussel nets collected in the Adriatic Sea. A moderate hazard level was only found in hard plastic containers derived from Biscay Bay, while no hazard was observed in plastic bottles collected in the Ligurian Sea, in mussel nets and beads collected in Biscay Bay.

WP 5: Effects of MPs on ecological functioning

Task 5.1 Microbial community interaction with MPs

P10 performed several activities, including (i) the ecotoxicity of water collected from UMEA mesocosm; (ii) the successional dynamics of biofilm growth on UMEA facility; (iii) the palatability and impact of fouled and not-fouled MPs from in field collected plastics on ephyrae jellyfish. (i) During the mesocosm experiments at UMEA facility, P10 checked the ecotoxicity of leachates from conventional plastics (PP) and biodegradable ones (PLA, PHBv) present in mesocosm tanks. After 1 month exposure, 100 ml of water from each tank were collected to evaluate the ecotoxicological effects of conventional and biodegradable plastics leachates in a battery of freshwater organisms, due to the low salinity of seawater (3‰) measured in the mesocosms. The bacteria *Aliivibrio fischeri*, the green alga *Selenastrum* *capricornutum* and the freshwater crustacean *Daphnia magna* were used to evaluate bacteria bioluminescence inhibition, algal growth inhibition, crustacean mortality and immobility following standardized protocols. A lack of toxicity was observed in organisms exposed to weathered biodegradable (PLA, PHBv) plastics. Conversely, conventional plastics (PP) induced toxicity, affecting bacteria bioluminescence and algal growth after 30 minutes and 72 h of exposure, respectively. In conclusion, no toxic effects were observed in freshwater food chain organisms exposed to biocompatible polymers. (ii) Biofilm growth successional dynamics were investigated by P10 through experiments performed at UMEA facility, by introducing 2x2 cm sheet samples of conventional (PP) and biodegradable (PLA, PHBv) plastics in each mesocosm tank for 1 month. Biofilm colonization was monitored each week, through several approaches (i.e. macroscopic observation and SEM analysis for biofilm presence, histochemical staining for microbial abundance). Samples observation showed biofilm growth increase according to the exposure time. The samples collected after 1-week exposure were mainly colonized by prokaryotic-sized assemblages, while eukaryotic microorganisms (i.e. diatoms) appeared after the second exposure week, as revealed by SEM analyses. These data were confirmed by Acridine orange staining, showing a great amount of photosynthetic organisms in the 3rd week. The microbial community was assessed in both conventional and biodegradable plastics after 1-month mesocosm exposure though a metabarcoding approach. The latter showed that biodegradable plastics (PLA, PHBv) are grouped separately from conventional (PP) plastics, as highlighted from PCA analysis. (iii) Regarding the palatability, the hypothesis that bacteria composition/growth on plastics could be able to make the MPs more palatable to jellyfish ephyrae was tested by P10. A plastic mixture was collected in the field in the frame of the WP1 and then micronized and sieved to obtain plastics of different size. The 50-200 µm size class was selected to be maintained for 1 month in natural environment in the Experimental Marine Station (EMS) of CNR-IAS at Ligurian harbor, to further investigate the palatability of fouled-MPs on jellyfish. Thus, MPs were left in the field environment for one month (June-July 2023): then, the samples were used to assess biofilm growth by means of chemical characterization and to perform ecotoxicological bioassays. Scanning Electron Microscopy (SEM) analyses showed microbial community and biofilm growth in fouled-MPs, indicating that biofilm growth occur after 1 month of exposure in natural environmental. To assess the palatability of fouled-MPs in jellyfish, ecotoxicological bioassays were performed by exposing both fouled- and not fouled MPs to ephyrae for 24 hours and evaluating immobility and behavioural alterations. The results showed an impairment of jellyfish behaviour – in terms of frequency of pulsations – that caused a toxic effect. This effect was not related to a higher ingestion of fouled-MPs rather than not fouled MP, as demonstrated by µFT IR analysis in jellyfish ephyrae. No differences in terms of polymer type by the ephyrae exposed to fouled and not-fouled MPs were observed in the ephyrae: such results suggest that ephyrae do not discriminate between micronized fouled and not-fouled MPs in terms of ingestion and polymer type.

Task 5.2 Zooplancton and vertical flux of MPs

P5 evaluated the role of zooplankton fecal pellets in the vertical exportation of MPs by field and mesocosms studies. Zooplankton fecal pellets were collected from Danish waters, and their analyses showed a median concentration of 3×10^{-3} MPs per fecal pellet. This suggests a quantitatively low, but ecologically relevant, contribution of fecal pellets to the vertical exportation of MPs in the ocean. The presence of MPs in fecal pellets and their fluxes was also investigated in a mesocosm in the Baltic Sea at the community level.

Task 5.3 Effects of NPs/MPs on plankton food web

P5 investigated the effects of weathered MPs on the structure and dynamics of a Baltic Sea planktonic community during ca. 5 weeks of exposure using a mesocosm approach (2 m^3) mimicking natural conditions. MPs were obtained from micronized commercial materials of polyvinyl chloride, polypropylene, polystyrene, and nylon previously weathered by immersion in the environment for 90 days and UV radiation exposure. The plankton community was exposed to 2 µg L^{-1} and 2 mg L^{-1} of MPs < 120 µm corresponding to measured particle concentrations (10-120 µm) of 680 MPs L^{-1} and 680 MPs mL^{-1}, respectively. The abundance and composition of all size classes and groups of plankton and chlorophyll concentration were periodically analyzed throughout the experiment. Our results show that weathered MPs of the studied conventional plastic materials have minimal or negligible impact on plankton communities after long-term exposure at environmentally relevant concentrations.

Task 5.4 Effects of MPs on meroplanktonic larvae

P5 investigated the acute toxicity of leachates from tire wear particles, beach MPs and micronized cigarette butts in the early life stages of echinoderm species.

Task 5.5 Effects of mussels on MPs in sediments

An initial experiment on the effects of mussels on MPs in sediments was conducted in the Baltic Sea to evaluate the bioaccumulation rate of plastic, microparticles and their associated organic compounds. Cages with *Mytilus trossulus* mussels were put into two coastal areas with different anthropogenic impacts (Eru Bay and Narva Bay) (P2). Samples are processed and analyzed, although one cage was lost during the experiment. Microplastic content in mussels reflects the similar pattern of microplastic concentration in the sea surface assessed earlier by in-situ observations and numerical modeling: much higher in areas with high anthropogenic pressure (Narva Bay). The application of caging compensated the absence of suitable organisms for biological effect study. It became possible to conduct analyses to reveal the exposure consequences by translocating organisms from supposedly unpolluted sites to the areas where contamination monitoring is necessary.

A pilot study has been carried out by P1 to evaluate the role of mussel beds on MPs enrichment and fluxes in water column and sediments: samples of mussels, water and sediments have been collected and processed. The obtained results didn't confirm the role of mussel beds in sediment MPs enrichment. A second pilot study performed by UPM (P1) included the role of macroalgal forest in MPs distribution and bioavailability in the Conero riviera (Central Adriatic Sea); in this context algal surface complexity might influence surface adhesiveness of MPs and macroalgal forests were considered a sink for MPs particles. The ability of macrophyte to attenuate wave and reduce current velocity is potentially facilitating MPs deposition and due to their morphologies (filamentous and non-filamentous) they might work as important factors to govern MPs retention. Results highlighted that all algal considered species (*Cystoseira compressa, Gongolaria barbata* and *Ulva lactuca*) trapped MPs with levels varying depending on the sampling period, reaching the highest concentration of 3.8 MP g^{-1} w.w (*Gongolaria barbata*, April). In general, fibers were the dominant shape (98%), polyester was the most frequent polymer and most MPs fell in the 1-3 mm size class.

Task 5.6 Data analyses and weighted elaboration

Unfortunately, no data obtained from various tasks could be used to define new weighted criteria and ecological thresholds.

WP6: Weight Of Evidence (WOE) model for MPs

A quantitative Weight Of Evidence (WOE) model was developed to integrate various typologies of data (lines of evidence, LOEs). The considered LOEs allow to evaluate the characteristics of MPs, and possibly NPs, extracted in water, sediments and biota, including residence/egestion times, bioavailability of (de)sorbed contaminants, synergy with other stressors, modulation of chronic effects at cellular, organism or community/ecological levels. Logical flow charts and algorithms have been included in a conceptual and software-assisted tool, to elaborate results on the basis of assigned thresholds and weights. For each LOE, data can be summarized in specific HQs, and integrated into an overall WOE evaluation. The model validation on RESPONSE data supports a “site-oriented” process for monitoring the hazards associated with MPs/NPs and a straightforward and transparent comparison of different marine environments.

Task 6.1 HQ for MPs/NPs in seawater/sediments

For the quantification and characterization of microplastics in abiotic matrices including surface water, water column, and sediments (LOE 1), the model adopts weighted criteria based on number of items, size, shape, and polymer. In addition to presenting results in both tabular and graphical formats, detailing the number of items (MPs), percentage chemical composition, distribution of shapes, and sizes classes of microplastics, the model elaborate a specific Hazard Quotient for microplastic characterization (HQI). The Hazard Quotient (HQI) is calculated based on the number of isolated items, corrected for weight assigned to their shape, size, and polymer typology:

HQI = number Items · (Wshape) · (Wsize) · (Wpolymer)

The weighted values of HQ are assigned to one of five classes of hazard (Absent, Slight, Moderate, Major and Severe). Thresholds for classes of hazard were elaborated from data on Mediterranean surface water, net tows, mesh size 200-400 µm and worldwide sediments using the data available on the Online Portal for Marine Litter (LitterBase, awi.de) and different data reported in scientific literature. Weights associated with the size, shape, and polymer typology of MPs were determined on expert judgment based on the following criteria: size effects relationships on biota to assign weights to the size classes, for assigning weights to the shape was considered percentages of predominant shape in marine organisms to assign weights to shape and density and status of degradation of polymers for weights of polymers typology.

Task 6.2 HQ for MPs/NPs in biota

The Hazard Quotient calculation (HQMI) for microplastics ingestion is based on microplastic ingestion frequency (MI), which was adjusted for a weight assigned to specific microplastic characteristics (size, shape, and polymer) considering literature data on effects and accumulation of different typologies of microplastics and based on expert judgment.

HQMI = MI · (Wshape) · (Wsize) · (Wpolymer)

The values of HQ are assigned to one of five classes of hazard: Absent, Slight, Moderate, Major and Severe. Results of MI related to mussels, sea urchins and anemones were elaborated using the WOE model in order to obtain a Level of Hazard for each species.

Task 6.3 HQ for subcellular effects of MPs/NPs

The module for the elaboration of biomarkers (LOE 3) includes a wide battery of biological responses selected from those widely used by the scientific community. Each biological response is associated to a weight and thresholds; weights are assigned according to the relevance of the endpoint and the mechanistic knowledge on this in an “organism health” perspective, while thresholds are selected to reflect the biological relevance of measured changes compared to “control” conditions and considering the possibility of biphasic responses. Besides weights, the model accounts also for the magnitude of variation: thresholds for changes of biological significance were extrapolated from the scientific literature, considering differences among both species and tissues, as well as the possibility of both induction and/or inhibition. For each biomarker, the values measured in organisms from the experimental treatment are compared with those of the control treatment and corrected for the statistical significance of the difference, according to the function z(i). Then, the ‘effect’ (E) is calculated as the ratio between the measured percentage variation and the relative threshold of the response and the ‘weighed effect’ (Ew) is finally obtained by multiplying E by the weight (w) assigned to the specific response. Regarding how each biological response is assigned to one of the five classes of hazard, following, classification of the effect was conducted based on expert judgment. The HQBI is calculated, following, by summing i) the summation of weighed effects of each biomarker in class C normalized on the number of biomarkers with this classification and ii) the summation of weighted effects of each biomarker in class D and E. The class of hazard for biomarkers is assigned, followed, by summing the percentage of biomarkers in each class multiplied by the weight assigned to each class, 1 for biomarkers in class A, 1.5 for biomarkers in class B, 2.5 for biomarkers in class C, 4 for biomarkers in class D and 6 for biomarkers in class E. The resulting value is then used to assign the treatment to one of the five classes.

Task 6.4 HQ for organism effects of MPs/NPs

Weighted criteria to elaborate results from ecotoxicological bioassays (LOE 4) are based on specific thresholds and weights assigned to each bioassay depending on the biological endpoint, tested matrix, time of exposure, and the possibility of hormetic responses. For this LOE the calculation of HQ for bioassays (HQBattery) requires that a minimum of two species and three biological end points are tested. For each bioassay, the percentage of variation compared to the control conditions was calculated and corrected for the statistical significance according to function Z(i); the Effectw was then obtained from the ratio between the percentage of variation and the threshold of the assay, and further weighted for its specific weighting (Ew). The cumulative hazard quotient (HQBattery) is obtained by the summation (Σ) of the weighted effects (Ew), i.e., the variations measured for each test compared with specific thresholds, corrected for the statistical significance of the difference (w), and biological importance of the endpoint and exposure conditions (w2).

The HQBattery is normalized to a scale ranging from 0 to 10, where 1 is the battery threshold (when all the measured bioassays exhibit an effect equal to the threshold, 10 when all the assays exhibit 100% of effect). The HQBattery is then assigned to one of the five classes of hazard, from absent to severe.

Task 6.5 HQ for ecological effects of MPs/NPs

No data from WP5 could be used to define new weighted criteria or thresholds preventing the development of a new LOE on ecological effects specific for MPs or NPs.

Task 6.6 WOE elaboration of environmental MPs/NPs

The results obtained from the single modules were integrated with a classical WOE approach that assigns different weightings to the various Lines Of Evidence (LOEs). Scales used within the different LOEs to calculate the class of HQ were normalized to a common scale; the values were multiplied by 1.0 (for HQI and HQBI) and by 1.2 (for HQMI and HQBattery), thus giving different importance to the results of these LOEs. An overall WOE level was thus calculated and assigned to one of five classes of risk, from Absent to Severe. The approach is shown schematically in Figure 3.

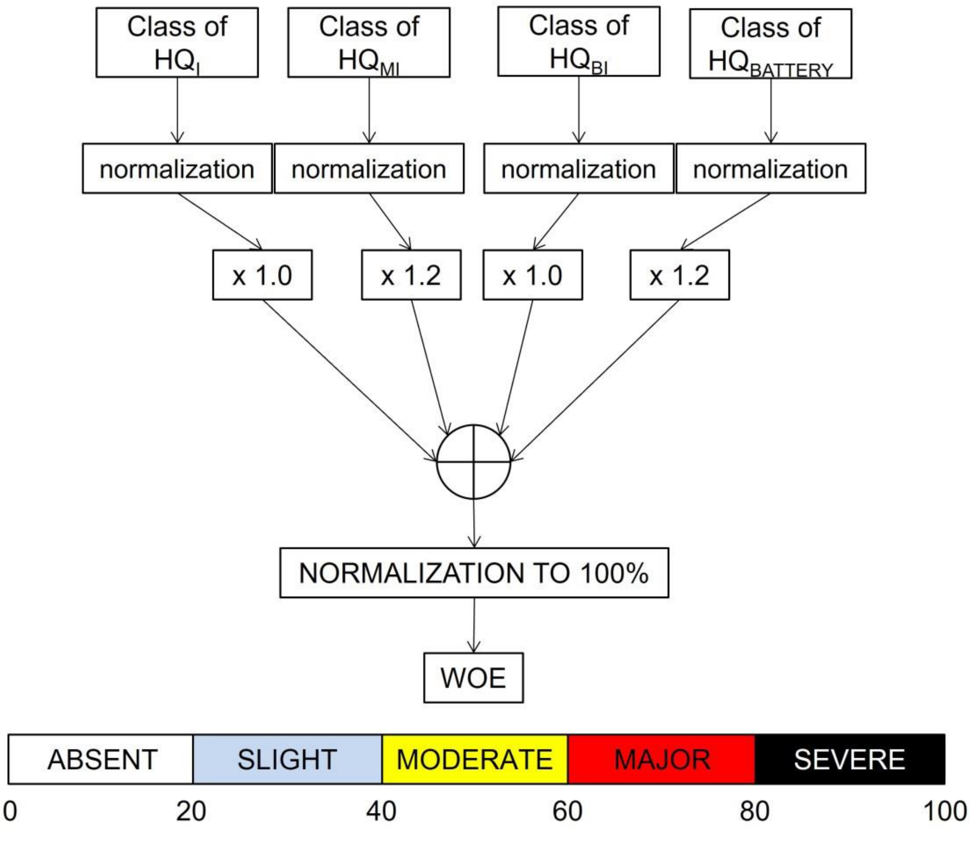

Figure 3. WoE integration flow chart

WP7: “Smart Hub” of analytical facilities

Task 7.1 Compilation of literature database

A literature database was compiled by P14 on sampling and characterization of MPs and NPs.

Task 7.2 Determination of size and shape

P14 assessed the utility of a range of characterization methods for analysis of MPs and NPs as a function of particle size and polymer type. The samples included commercial MPs, aged micro-and-nanoplastic particles (obtained in collaboration with the European Commission’s Joint Research Centre, JRC, Geel, Belgium), and field collected plastics in simple matrices (Milli-Q water and sea water) and complex matrices (sediment and fauna samples). Methods used to date and relevant for the determination of size and shape of MPs and NPs in environmental matrices include fluorescence staining, confocal microscopy, holotomographic microscopy, environmental SEM, scanning electron microscopy - Energy Dispersive X-ray spectroscopy (SEM-EDX), dynamic light scattering, single particle extinction and scattering (SPES), coulter counting, flow cytometry, ATR-FTIR and µFTIR spectroscopy, Raman spectroscopy and Raman spectroscopy coupled to confocal microscopy (µRaman). Some measurements were done in collaboration with other laboratories at Universiteit Antwerpen (Sense lab research group and Electron Microscopy for Materials Science), Universiteit Gent (Belgium), Université of Lorraine (Laboratoire Interdisciplinaire Des Environnements Continentaux, LIEC, France) and the JRC.

In addition, secondary characterization of the micro-and-nanoplastic particles (aged polyethylene terephthalate) was conducted to ensure the stability of the particles under conditions adopted for the in-vivo (outlined in Task 2.8) and in-vitro exposure experiments (outlined in Task 2.5). The hydrodynamic size, zeta potential, and electrophoretic mobility of the nanoplastics (D50 = 0.57 µm and D90 = 1.1 µm) and microplastics (D50 = 1.4 µm and D90 = 3.1 µm) were assessed at various time points (1 h, 24 h and 48 h) in the various aqueous exposure media (cell culture media: Basal Medium Eagle with supplements; live-cell imaging solution; phosphate-buffered saline; and artificial seawater). Measurements were also conducted to assess the electrophoretic mobility of nanoplastic particles as a function of solution ionic strength (0.1 mM to 250 mM NaCl) using Dynamic Light Scattering (DLS, Zetasizer NanoSeries, Malvern Instruments).

Task 7.3 Determination of polymer type

UPM (P1) activated a collaboration with Politecnico di Milano_ Department of Physics to investigate the possibility to use innovative techniques as hyperspectral microscopy and Raman signal to detect microplastics and microfibers in different organisms’ tissues or in abiotic matrices (Task 7.4). The advantage of this innovative technique is the capability to analyze MPs with dimensions smaller than 1 µm (impossible to detect and characterize with µFTIR or Raman) with a relatively easy and cheap approach compared to classical instrumentation used for MPs characterization.

Another investigation carried out by P1 aimed to produce nanofibers starting from synthetic and non-synthetic MFs (obtained by cutting pristine cigarette filters) with artificially sunlight photodegradation processes. The NFs concentrations and structure were measured in a sample obtained from laboratory experiment using dynamic slight scattering, Scanning electron microscopy and Small Angle X-ray Scattering technique at Synchrotron of Trieste). Results showed the capability to produce NFs by photodegradation process.

CNR IAS (P10) determined the polymer type of MPs and NPs ingested in marine zooplankton (experimental set-up described in WP2) by using the innovative and emerging system namely Tomocube's holotomography, thanks to the collaboration with a private company (Schaefer SEE srl, Italy). This system consists in a laser interferometric technique providing the 3D distribution of refractive indices (RI) characteristic of fixed and live cells as well as tissues. Conversely to conventional methods – i.e. phase contrast microscopy, differential interference contrast microscopy - HT does not need preparation steps, such as fixation, transfection, and staining, for environmental monitoring and in situ diagnostics. By using this technique, it was possible to detect the uptake of polystyrene NPs in jellyfish ephyrae (according to WP2) and the uptake of innovative and biocompatible polymers (PLA, PVDF) in crustacean larvae and jellyfish ephyrae.

CNR IAS (P10) determined the polymer type of putative plastic particles extracted from the water samples collected by using the SFD system (20 µm) by means of the Lumus II (Bruker Optics Gmbh, Germany) micro-FTIR equipped with OPUS software (Bruker Optics Gmbh, Germany)). A Mid band MCT-Detector (Liquid nitrogen cooled) was used in transmission mode. For this analysis the samples were filtered on Anodisc filters (Al2O3, Ø 25 mm) and the polymers were identified comparing acquired spectra to a dedicated library (provided by Bruker) in the range 4000-1200 cm-1 (because of the strong absorption below 1250 cm-1 of Anodisc filters).

Task 7.4 Measurement of associated contaminants

A set of methodology approaches to characterize MP associated chemicals have been evaluated and utilized to provide data on associated chemicals (organic compounds, metals) and their effects, including mixture effect, using bioreporter gene assays. Within the project, a scheme for characterization of leachable and desorbed organic chemicals using liquid chromatography- mass spectrometry (LC-MS), gas chromatography (GC)-MS, and bioreporter gene assays was developed. A protocol for extraction of organic contaminants in leachate water from lixiviation, using solid-phase extraction was developed and shared with all partners, including suitable sample volumes based on the solid to liquid ratio. Desorption of organic chemicals from plastic particles was demonstrated, together with suitable clean-up methods to reduce matrix effects and facilitate chemical and effect-based analysis (P12).

Task 7.5 Consortium needs, harmonization-training

Due to the COVID pandemic, the harmonization and training activities were readjusted in a different schedule and realized only in the third year of the project on the use of the WOE model and associated software.

WP8: Communication and dissemination

Task 8.1 Dissemination Plan and Communications Strategy

This iterative document designed to support partners to communicate project outputs was delivered in March 2021 and was updated on an ongoing basis. It includes key information on copyright, publication guidelines, social media and website dissemination activities and acts as a record of all actions delivered within WP8. As part of this task, project logo was delivered in month 1 of the project to ensure that RESPONSE had a tangible brand that would be identified throughout Europe.

Task 8.2 Website and Social Media

The RESPONSE project's online presence was established in month November 2020 (https://www.response-jpioceans.eu/) to make it immediately visible, and to provide easily accessible information to both experts and non-experts. The website comprises several static pages (detailing project aims, consortium details, funding acknowledgements etc.), and a news page that is updated regularly based on partner activities and outputs. Additional pages were created as necessary (i.e. multimedia, publications, etc.). Website communication is supported through social media for fast and effective correspondence on project outreach. The RESPONSE twitter account was delivered in February 2021, being monitored and utilized by the project team on an ongoing basis. The RESPONSE Twitter account was used to compliment the activities that are promoted through the project website.

Task 8.3 Review of Science Based Knowledge in the Public Domain

Work on this task was presented at the Marine Institute in Ireland during the final meeting of the JPI Oceans Microplastics projects in September 2023. The associated report is accessible from the project website and was widely disseminated via the project social media and shared via research gate DOI: 10.13140/RG.2.2.15212.33928.

Task 8.4 Stakeholder Engagement

The RESPONSE stakeholder workshops facilitated discussion around the experiences of scientists, and decision and policymakers concerning inaccuracies relating to microplastics in mainstream and social media; identified experienced challenges and barriers that hinder effective science communication about marine microplastics; and encouraged participants to make targeted recommendations on how effective science communication about microplastics can be supported.

Task 8.5 Factsheets and Newsletters

Five project fact sheets, including a project brochure, were developed to cater to different target audiences throughout the project lifecycle. The project factsheets and the three project newsletters are available from the project website.

5. DISSEMINATION AND VALORISATION

An overview of the dissemination and valorisation activities is given in the Table below. As appropriate, all such activities were further communicated via the project website, social media accounts, and newsletters. Peer-reviewed publications are listed separately in the following section.

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
Aug 2020	Project Logo	To communicate the unique project identity and brand	Zip file with high resolution images	A. Dozier	Completed and utilised for project branding to all audiences
Nov 2020	Project Website	Website account set up and launched to present RESPONSE research goals and deliverables	https://www.response-jpioceans.eu/	A. Dozier, O.-P. Power	Ongoing
23-27 Nov 2020	MICRO2020	Conference presentation as part of JPI-Oceans panel in Micro2020	https://www.micro.infini.fr/ article21.html#23.5 Me Oral Presentation	F. Regoli	> 2000 participants
		Monthly survey of microplastic beaching in the Arcachon Bay (France) and relationship with environmental factors.	Oral Presentation	C. Lefebvre, C. Clerandeau, F. Le Bihanic, I Jalon Rojas, B . Morin, S. Lecomte J. Cachot	Scientific Community
Jan – May 2021	Visiting Researcher	MSc Maximilan Rinderknecht (University of Landau, Germany) visited UHEI for MSc work (endocrine disruption)		T. Braunbeck	General Public, Scientific Community
Jan – July 2021	Visiting Researcher	MSc Nadine Kämmer (University of Landau, Germany) visited UHEI for PhD work (neurotoxicity)		T. Braunbeck	General Public, Scientific Community
Jan - Dec 2021	Visiting Researcher	MSc Thallita Monteiro Teixieira (University of Goiás, Brazil) visited UHEI for PhD work (histology, embryo toxicology)		T. Braunbeck	General Public, Scientific Community
Feb 2021	Project Twitter Account	Twitter account set up to promote RESPONSE work.	@RESPONSE_JPIO	O.-P. Power, A. Dozier	

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
March 2021	SAREBIO Virtual Conference	Are Biopolymers the Remedy for the Plastics Disease? Remarks from Marine Ecotoxicological Science	Online presentation link.	R. Beiras	Scientific Community
21 March 2021	Field Sampling – Microplastic Hunters Project	Microplastic monitoring activities undertaken in the waters of the Marine Protected Area of Portofino as part of the **Microplastic Hunters Project** collaboration with the **CRABS Team from Outdoor Portofino.**	**Press Release** https://www.cnr.it/it/nota-stampa/n-10117/al-via-le-attivita-di-monitoraggio-del-progetto-micro-plastic-hunters **Video** • https://www.cnrweb.tv/cacciatori-di- micro-plastiche/	F. Garaventa	General Public, Scientific Community
22 March 2021	Collaboration with professional documentary makers	Partners from UNIVPM participated in video produced by Lac Studio Films as part of the annual World Water Day	**Video Link** • https://www.youtube.com/watch?v=R_ItlATdiuY	S. Gorbi, A. Nardi, L. Pittura	General Public

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
March - Dec 2021	Visiting Researcher	J. Koegst (University of Landau, Germany) visited UHEI MSc work (endocrine disruption)		T. Braunbeck	General Public, Scientific Community
May 2021	Report	Plastic Litter in the Adriatic Basin.	**Link** • 98c3eab4-report- plastic-litter-in-the-adriatic-basin-2021.pdf (greenpeace.org)	C. Mazzoli, F. Gorbi	Scientific Community, General Public, Policy and Decision Makers
5 May 2021	ETV Interview	Researcher I. Kuprijanov joined **ETV** to discuss the United Nation's second global analysis of the state of the oceans, under a segment entitled 'Scientist: Humans Accelerated the Extinction of Species in the Ocean'	**Interview** • https://etvpluss.err.ee/1608202210/uc henyj-ljudi-uskorili-vymiranie-vidov-v-mirovom-ookeane	I. Kuprijanov	General Public
3 – 7 May 2020	SETAC Europe	'Distribution of microplastics in abiotic and biotic compartments of Arcachon Bay' during spring and summer'	Virtual meeting (CO) Oral presentation	C. Lefebvre, F. Le Bihanic, C. Clérandeau, S. Villette, B. Morin, S. Lecomte, J. Cachot	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
6 May 2021	Public Talk Galeria da Biodiversidad e, Centro de Ciência Viva e Museu de História Natural e da Ciência, Universidade do Porto, Porto, Portugal.	A public talk entitled 'E foram plástico para sempre! Pode o plástico ter um final feliz? Diversidades – Ciclo de Conversas sobre Biodiversidade e Sustentabilidade Ambienta'	Oral presentation	H. Souza, L. Guilhermin, J. Teixeira	General Public
18 May 2021	Public Talk Centro de Monitorização e Interpretação Ambiental (CMIA) Matosinhos, Portugal	'Contaminação e efeitos de microplásticos em organismos marinhos'	Oral Presentation	L. Guilhermino	General Public
31 May - 4 June 2021	EcoBIM Conference 2021	'Contamination des ressources halieutiques par les microplastiques et étude de l'exposition : cas de la Baie d'Arcachon (côte l'Atlantique Nord française)'	Virtual Meeting Oral Presentation	F. Le Bihanic, B. Cormier, C. Lefebvre, S. Lecomte, J. Cachot, B. Morin	Scientific Community
May-Aug 2021	Visiting Researcher	Bozidar Raskovic (University of Belgrade) visited UHEI for sabbatical (histology, embryo toxicology).		T. Braunbeck	General Public, Scientific Community
1 – 4 June 2021	Isobay 17 Online Conference	'Microplastic contamination of the ecosystem and sea products. Arplastic: a case study of the Arcachon Bay'	Virtual event Oral presentation	B. Morin, J. Cachot, C. Clérandeau, B. Cormier, G. Detandt, I. Jalón Rojas, F. LeBihanic, S. Lecomte, C. Lefebvre, A. Sottolichio, S. Villette	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
June 2021	Report	Difendiamo il Mare Campaign 2020 Promossa da Greenpeace Italia: Microplastiche e microfibre in pesci e invertebrati campionati nel Tirreno centro-settentrionale. Report Finale.	**Report:** • 31e5102a-univpm-microplastiche-e- microfibre-in-pesci- e-invertebrati-campionati-nel- tirreno-centro-settentrionale.pdf (greenpeace.org)	S. Gorbi, L. Pittura, A. Nardi, L. Ventura, F. Regoli	Scientific Community, General Public, Policy and Decision Makers
June 2021	Mirpuri Foundation Ocean Award 2021	*J. M. Gonçalves* (CIMA- UALg) was awarded the MFOA2021 for her poster presentation on 'EMERGING: Environmental Mixtures of emerging Contaminants Repercussions on Gonads and Impact of Next Generations.'	Poster presentation	J. M. Gonçalves	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
June – Aug 2021	Keep the Planet Videos and Campaign	Collaboration with non-profit organization and participation in video documentaries and awareness campaigns on plastic and microplastic pollution	**Youtube Videos** • https://www.youtube.com/watch?v=AYJF33J41d0 • https://www.youtube.com/watch?v=lx zvV8Iq7QE • https://www.youtube.com/watch?v=vF_oKgEfoKQ	S. Gorbi, A. Nardi, F. Regoli	General Public, Scientific Community
June 2020	Greenpeace Video	Collaboration with non-profit organisation Greenpeace through participation in video and awareness campaigns on plastic and microplastic pollution. Undertaken as part of 'Defendiamo il Mare' 2021 campaign	**Link** • https://www.youtube.com/watch?app=desktop&v=fjsn8C HRBY0	S. Gorbi	General Public

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
6 – 18 June 2021	Field Sampling – Microplastic Hunters Project	Microplastic monitoring activities undertaken in the waters of the Marine Protected Area of Portofino as part of the **Microplastic Hunters Project** collaboration with the **CRABS Team from Outdoor Portofino.**	**Press Release** • https://www.cnr.it/ it/nota-stampa/n-10117/al-via-le- attivita-di-monitoraggio-del- progetto-micro- plastic-hunters **Video** • https://www.cnrweb.tv/cacciatori-di- micro-plastiche/	F. Garaventa	General Public, Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
21 June – 10 July 2021	Field activities in the Mediterranean Sea (Adriatic basin)	Participation to "Difendiamo il Mare 2021": a research and awareness campaign on plastic and microplastic pollution. Promoted by Greenpeace Italy with the scientific support of UPM and CNR-IAS.	Launch of Tour on June 21 as part of the Moby Litter A Year On conference. **Moby Litter Programme:** • https://www.cnr.it/ en/eventi/allegato/ 12501 **UNIVOM Announcement**: • https://www.disva. univpm.it/content/ moby-litter-un- anno-dopo-impatti-minacce-ed-opportunit%C3%A0 -un-mare-pericolo **CNR-IAS Press Release:** • https://www.cnr.it/ it/evento/17359/m oby-litter-un-anno- dopo-impatti-minacce-e-opportunita-per-un-mare-in-pericolo	S. Gorbi, F. Garaventa	Scientific Community, General Public, Policy and Decision Makers

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
28 – 30 June 2021	Encontro Ciência 2021 poster presentation	'EMERGING: Environmental Mixtures of Emerging contaminants Repercussions on Gonads and Impact on Next Generations'	Poster Presentation	J. M. Ribeiro Gonçalves	Scientific Community
30 June 2021	Blue Panda Week	CNR-IAS researchers were present on board the WWF "Blue Panda" boat as part of Blue Panda Week 2021, analysing microplastic samples collected as part of 'Microplastic Hunters Project' activities.	Images, videos, and press release. **Link** • https://www.cnr.it/en/event/17377/blue-panda-week-2021	CNR-IAS	General Public, Scientific Community, Policy and Decision Makers
13 July 2021	Wise and Shine Podcast	K. Lind, of the Institute of Marine Systems of Tallinn University of Technology participated in an episode of the **Wise and Shine** podcast, that was about plastic pollution and microplastics.	**Podcast Episode #4** • http://www.wiseandshine.ee/podcast-kati-lind/?fbclid=IwAR2lcVUO3KCTrPGlNuiHbm2E4mjEt9NoGXxnTHjm1OdWDA4vbcQ-g-aIntc	K. Lind	General Public

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Aug – Dec 2021	Visiting Researcher	Imen Ben Chabchoubi (University of Monastir, Tunisia) visited UHEI for PhD work (histology, embryo toxicology).		T. Braunbeck	General Public, Scientific Community
Aug – Dec 2021	Visiting Researcher	BSc Thomke Ehrlich (University of Düsseldorf, Germany) visited UHEI for MSc work (endocrine disruption)		T. Braunbeck	General Public, Scientific Community
Sept 2021	Collaboration with non- profit organization Marevivo	UNIVPM participation in 'Adopt a Beach' awareness campaign with non-profit Marevivo, on plastic and microplastic pollution and Francesco's position on the scientific committee.	https://marevivo.it/sub- attivita/adotta-una- spiaggia-marche/ https://marevivo.it/en/abo ut-us/#organizzazione	F. Regoli	
Sept 2021	Factsheets (Brochure)	At-a-Glance document that can be used to promote the key objectives of the project at front facing events. **Multimedia** page added to **Resources** header tab to disseminate project brochure.	Pdf/png files provided to consortium and website structure update.	O.-P. Power, A. Dozier	General Public, Scientific Community
Sept 2021	Microplastic Podcast	Podcast with Estonian Museum of Natural History about microplastics in the Baltic Sea	**Podcast** https://www.loodusmuuseum.ee/ru/mikromusor	N. Bukhalko	General Public,

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
11 Sept 2021	Blu Di Genova Sampling Activities	Field sampling activities	**Press Release** • https://www.cnr.it/ it/nota-stampa/n-10572/blu-di-genova-un-nuovo-modo-di-scopteggio-studiare-e-proteggere-la-sc%20greatly-%20small%20town **Video** https://www.cnrwe b.tv/blu-di-genova/	F. Garaventa, CNR-IAS	General Public, Scientific Community
16 – 17 Sept 2021	Consortium Meeting, I	General presentation and discussion on WP1, WP2, WP7, WP8 and cross-cutting activities carried out during 1st year	Online meeting with oral presentations from relevant WP leads.	Coordinated by F. Regoli, M. Benedetti.	Consortium Partners

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23-24 Sept 2021	Consortium Meeting II	General presentation and discussion on WP3, WP4, WP5, and cross-cutting activities carried out during 1st year.	Online meeting with oral presentations from relevant WP leads.	Coordinated by F. Regoli, M. Benedetti	Consortium Partners
25 Sept 2021	European Research Night	*A. Nardi* participated in outreach/dissemination activities as part of SHARPER Night 2021 / European Researchers Night in Ancona in their UPM unit. This included a poster for the RESPONSE project highlighting the aims and objectives, along with practical demonstrations and activities about plastic pollution including MPs collected from the environment being available to view under a microscope.	Stakeholder Engagement, Education, Outreach, Poster, Practical Activities	A. Nardi	General Public
Oct – Dec 2021	Visiting Researcher	W. Henderson (University of Wageningen, NL) visited UHEI for Internship		T. Braunbeck	General Public, Scientific Community
1-10 Oct 2021	Stranded Beach Macroplastic s Campaign	A sampling campaign of stranded beach macroplastics was organized from 1st to 10th of October 2021 and was participated in by the microplastics team from Tallinn University of Technology	Text and images	N. Buhhalko	General Public, Scientific Community

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14 – 16 Oct 2021	XXI SPB – National Congress of Biochemistry Conference, Évora	Are micro and nanoplastics toxic?' (Invited Speaker)	**Conference Website** • http://www.xxispbcongress2020.uevor a.pt/	M. J. Bebianno	Scientific Community
25 Nov 2021	RE-MAR II Event 'Seminar on the transfer of results and good practices of the Re-MAR II project'	The ECOTOX team at Vigo participated in a dissemination event organised to promote the results of the RE-MAR II project that is coordinated by the non-profit organisation Amicos.org. As part of this event the team gave an overview of their ongoing projects including RESPONSE.	**RE-MAR II Website** • https://amicos.org/remar/#socio • Project Details **Tweets** • https://twitter.com/ECOTOX2/status/1460940926558289921?s=20 oral presentation	O. Alonso, B. Noya, R. Beiras	•From Amicos.org: -'Agents of change' (, people part of the association and which possess different capacities), -therapists and technicians on staff, -communication team. •National park staff •Scientists •Science journalists, science commentators / science communicators •Public administration •General public
29-30 Nov 2021	Gulf of Finland Science Days	Implementation of microplastic, chemical and biochemical analyses and caging approach to monitor the level of pollution across the southern coast of the Gulf of Finland.	Poster presentation	I. Kuprijanov, N. Buhhalko, N. Kolesova, K. Künnis-Beres, M. Lipp, F. Buschmann	Scientific Community

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29 Nov – 2 Dec 2021	CICTA2021, Blumenenau, SC, Brasil	'In Vivo Exposure Effects of Polystyrene Nanoplastics In The Marine Mussel *Mytilus galloprovincialis*'	Oral Presentation / Slide Presentation	J.M. Ribeiro Gonçalves, M.J. Bebianno	Scientific Community
29 Nov – 2 Dec 2021	CICTA2021, Blumenenau, SC, Brasil	'Contamination and effects of microplastics in marine organisms: an ´One Health´ approach'	Oral Presentation	L. Guilhermino	Scientific Community
29 Nov – 2 Dec 2021	CICTA2021, Blumenenau, SC, Brasil	'Assessing the effects of the cytostatic drug 5- Fluorouracil and a mixture of emerging contaminants on the marine mussel *Mytilus galloprovincialis'.*	Oral Presentation	Beckmann, C., Gonçalves J. M., Bebianno M.J.,	Scientific Community
1 – 4 Dec 2021	20º Encontro Nacional de Ecologia, Portugal	'Microplásticos e zooplâncton: riscos acrescidos num mundo mais quente?'	Oral Presentation	Martins, A.; Guilhermino, L.	Scientific Community
		'Avaliação do estado fisiológico da espécie Mytilus galloprovincialis (Lamark, 1819) através da análise de biomarcadores do sistema antioxidante'	Poster Presentation	Manuel, V.Y.L.L., Barboza, G., Guilhermino, L.	Scientific Community

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19 Jan 2022	Marine Institute Presentation	WP5 team attended a meeting organised by Irish National Funding bodies (Marine Institute and the Department of Housing, Planning and Local Government) to discuss the RESPONSE project and it's progress to date along with JPIO funded ANDROMEDA and MicroPlastix projects.	Oral presentation with PPT given online.	O.-P. Power	Funders, Policy, Local Government, Scientific Community
7 - 21 Jan 2022	Workshop and Beach Clea	Researchers from ECOTOX (UVigo) accompanied staff from the Spanish Ministry for the Ecological Transition and the Demographic Challenge to the magnificent beach of A Lanzada to learn from them and do our own marine litter collection, mostly plastics	Text and images	B. Noya- Mariño	General Public, Scientific Community
2 March 2022	II JEPA 2022, Jornadas de Estudo e Proteção do Ambiente. Universidade de Trás-os-Montes e Alto Douro, Portugal.	'Poluição por plásticos em estuários: será que estamos também a comer microplásticos?'	Oral Presentation	L. Guilhermino	Scientific Community

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22 – 24 April 2022	VII edição das Jornadas da Bioquímica, ICBAS, University of Porto, Portugal.	'Contaminação por microplásticos e seus efeitos em peixes selvagens: uma ameaça para a 'One Health'?'	Oral Presentation	L. Guilhermino	Scientific Community
4-6 May, 2022	15.º Encontro de Investigação Jovem da U.Porto. Universidade do Porto, Portugal.	'Selected environmental biomarkers in wild mussels (Mytillus galloprovincialis) from different sampling sites of the NW Atlantic coast of Portugal'	Oral Presentation **Video of Event** • https://www.youtube.com/watch?v=9YU34HPFZNs	Samara, M.I.; Barboza, L.G.; Guilhermino, L.	
11 – 13 May, 2022	17th annual conference of the Ecobim network Presentation	Spatial distribution of contamination by microplastics and anthropogenic particles in the Arcachon Bay (Atlantic coast).	Oral Presentation	Morin B., Lefebvre C., Le Bihanic F., Clérandeau C., Lecomte S., Cachot J.,	Scientific Community
16 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Plastic Contamination of Edible Parts of Marine Organism in the North Atlantic French Coast'	Poster Presentation	B. Cormier, F. Le Bihanic, S. Lecomte, C. Lefebvre, J. Cachot, B. Morin	Scientific Community
16 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Effect- Directed Analysis (EDA) of Beach Plastic (PlastEDA) From Baltic Sea'	Poster Presentation	A. Hashmi	Scientific Community

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16 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'In-Vitro Assessment of the Sensitivity of Bivalve Hemocytes to Nanoplastic Exposure'	Poster Presentation	J. Hara, M. Vercauteren, S. Schoenaers, C. Janssen, R. Blust, J. Asselman, R.M. Town	Scientific Community
17 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	Impact of Tyre Wear Particle Leachates on Marine Phytoplankton	Poster presentation	T. S. Page, R. Almeda, E. Bournaka, R. Mejlholm, M. Koski, and T. G. Nielsen	Scientific Community
17 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Effects of Leachates from Tyre Wear Particles on Marine Copepods'	Poster presentation	E. Bournaka, R. Almeda, T. S. Page, R. Mejlholm, M. Koski, T. G. Nielsen	Scientific Community
17 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Analysis of Small Microplastics and NanoPlastics in Complex Matrices: Towards a Strategic Toolbox'	Oral presentation	M. Falcou-Préfol, M. Vercauteren, C. Janssen, R. Blust, J. Asselman, R.M. Town	
18 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Temporal variability of microplastic and anthropogenic particles uptake by four commercial species from Arcachon Bay (North-East Atlantic, France)'	Poster presentation	C. Lefebvre, B. Cormier, F. Le Bihanic, G. Rampazzo, B. Morin, S. Lecomte, J. Cachot	Scientific Community

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18 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Where Does the Marine Plastic Go? Abundance and Distribution of MPs in the Kattegat Sea'	Oral presentation	G. Kuddithamby, R. Almeda, T. G. Nielsen, C. Laurance, A. Vianello, R. Rodriguez- Torres, L. Iordachescu, K. Papacharalampos, J. Vollertson	Scientific Community
18 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Commercial Plastic Degradation Under Seawater Conditions'	Oral presentation	S. López Ibáñez	Scientific Community
18 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	'Risk Assessment of Microplastics in Marine Ecosystems; From detectioN of Presence to Quantitative Assessment of Effects'	Oral presentation	R. Beiras	Scientific Community
18 May 2022	SETAC Europe 32nd Annual Conference, Copenhagen, Denmark	Seasonal distribution of microplastics and anthropogenic particles uptake by four commercial species from the Arcachon Bay (North East Atlantic, France).	Poster Presentation	**Lefebvre C.,** Cormier B., Le Bihanic F., Rampazzo Magalhães G., Lecomte S., Cachot J., Morin B.,	Scientific Community

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23 May 2022	PRIMO 21, Goteborg, Sweden	'From Sea Surface to Sediment and Biota: Microplastics are Ubiquitous in the Marine Ecosystem'	Oral Presentation	F. Garaventa, E. Costa, R. Minetti, F. Castelli, A. Montarsolo, V. Piazza, G. Ungherese, L. Pittura, L. Ventura, A. Nardi, M. Benedetti, F. Regoli, S. Gorbi	Scientific Community
24 May 2022	PRIMO 21 Goteborg, Sweden	'Microplastics Occurrence in the Edible Tissues of Seafood of Atlantic French Coast and its Impact on Food Safety'	Poster Presentation	B. Morin, B. Cormier, F. Le Bihanic, S. Lecomte, C. Lefebvre, J. Cachot	Scientific Community
24 May 2022	PRIMO 21, Goteborg, Sweden	'Leachates from beached and new plastic items: assessment of chemical composition and ecotoxicity to marine organisms'	Poster Presentation	C Mazzoli, D Fattorini, M Di Carlo, G d'Errico, L Pittura, A Nardi, S Gorbi, F Regoli	Scientific Community
	Best Poster Award	PhD researcher *C Mazzoli* received an award for her poster presentation.	Poster Presentation	C Mazzoli,	Scientific Community
25 May 2022	PRIMO 21, Goteborg, Sweden	'Biological Effects of Cigarette Butts on Marine Sentinel Species'	Poster Presentation	G Lucia, ME Giuliani, S Gorbi, G d'Errico, M Di Carlo, D Fattorini, F Regoli	Scientific Community

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25 May 2022	PRIMO 21, Goteborg, Sweden	'Cellular disturbance and effects on thermal stress response in mussels exposed to synthetic and natural microfibers'	Oral Presentation	A Nardi, L Pittura, C Mazzoli, F Mongera, G d'Errico, M Benedetti, S Gorbi, F De Falco, M Cocca, M Avella, F Regoli	Scientific Community
25 May 2022	PRIMO 21, Goteborg, Sweden	'Ecotoxicity of emerging contaminants individually and as a mixture in the reproductive organ of marine mussels *Mytilus galloprovincialis'*	Oral Presentation	Gonçalves J. M. & Bebianno M.J.,	Scientific Community
20 May - 31 Aug 2022	Omero Museum Exhibition	Our research partners at Università Politecnica delle Marche have collaborated on a special exhibition in the Omero Museum located in Ancona, entitled "Plastic culture: art, design, the environment" which will run until August 31st.	Text and images from UNIVPM **Link** • https://www.museoomero.it/en/exhibitions/exhibition-plastic-culture-art-design-the-environment/		General Public

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3 – 8 July 2022	Iberian Ecological Society (SIBECOL) AIL Meeting 2022	'Long-term combined effects of lithium and microplastics on Daphnia magna growth, reproduction and population growth rate'	Oral Presentation	Martins, A.; Guilhermino, L.	Scientific Community
6 - 8 July 2022	VIII International Symposium on Marine Sciences	"Marine Plastic Litter Risk Assessment: A Proposal Based on Chemical and Physical Properties of Plastic"	Oral Presentation	B. Noya- Mariño, O. Alonso-López, F. Laranjeiro, R. Beiras	Scientific Community
6 - 8 July 2022	VIII International Symposium on Marine Sciences	"How car tire leachates affect sea urchin larvae"	Oral Presentation	S. Rist, R. Almeda	Scientific Community
		"Comparison of effective methods for quantification of plankton- sized microplastics: the case of the Gulf of Bothnia, Sweden"	Poster Presentation	K. Ugwu, A. Rotander, A. Vianello, R. Almeda	Scientific Community
6 - 8 July 2022	VIII International Symposium on Marine Sciences	"Impact of leachate from car tire microplastics on the marine heterotrophic dinoflagellate, *Oxyrrhis marina*"	Oral Presentation	J. Le Du-Carrée, R. Almeda.	Scientific Community
6 - 8 July 2022	VIII	"Effect of tire wear leachates on phytoplankton communities of the Canary Islands"	Poster Presentation	M. Sampalo, Jessy le Du-Carrée, I. Martinez, M. Gomez, R. Almeda	

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	International Symposium on Marine Sciences	"Copepods Behaviour Reduces the Risk of Entry of Microplastics in Marine Food Webs"	Poster Presentation	R. Rodríguez–Torres, R. Almeda, J. Xu, N. Hartmann, S. Rist, P. Brun, T. Gissel Nielsen	
6 - 8 July 2022	VIII International Symposium on Marine Sciences	"Is zooplankton an entry point of microplastics in the marine food web? A case study in the Kattegat Sea"	Poster presentation	G. Kuddithamby, R. Almeda, T. Nielsen, C. Lorenz, A. Vianello, R. Rodriguez- Torres, L. Iordachescu, K. Papacharalamps, J. Vollertsen	
6 - 8 July 2022	VIII International Symposium on Marine Sciences	"Plastic doesn´t taste good! Efficient taste-discrimination of microplastics in planktonic copepods"	Poster Presentation	J. Xu, R. Rodríguez-Torres, S. Rist, T. Nielsen, N. Hartmann, P. Brun, D. Li, R. Almeda	
14 Sept 2022	EUROQCHAR M 2022, Amsterdam, the Netherlands	'Comparison of effective methods for quantification of plankton-sized microplastics: the case of the Gulf of Bothnia, Sweden"		K. Ugwu, A. Rotander, A. Vianello, R. Almeda	Scientific Community
18 – 21 Sept 2022	Interfaces Against Pollution: Chemical and Biological Perspectives, Antwerpen, Belgium	Chair of the conference 'Interfaces Against Pollution: Chemical and Biological Perspectives conference	Event organisation	R. M. Town and R. Blust	Scientific Community

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18 – 21 Sept 2022	Interfaces Against Pollution: Chemical and Biological Perspectives, Antwerpen, Belgium	'Plastic Pellet Flux in the Harbour of Antwerp'	Poster Presentation	Diels H., Blust R., Town R.M.,	Scientific Community
18 – 21 Sept 2022	Interfaces Against Pollution: Chemical and Biological Perspectives, Antwerpen, Belgium	'Automated quantification of apoptosis signals in bivalve hemocytes as a proxy for micro- and nanoplastic risk assessment'	Poster Presentation	Hara J., Schoenaers S., Vercauteren M., Janssen C., Blust R., Asselman J. and Town R.M	Scientific Community
		"Analysis of small microplastics and nanoplastics in complex matrices"	Poster Presentation	Falcou-Préfol M., Vercauteren M., Janssen C., Blust R., Asselman J., Town R.M	Scientific Community
27 Sept 2022	µMED 2022 conference	'Microplastics are ubiquitous in the marine ecosystem: an integrated assessment in water, sediments, and biota'	Oral presentation	F. Garaventa, E. Costa, R. Minetti, F. Castelli, A. Montarsolo, V. Piazza, G. Ungherese, L. Pittura, L. Ventura, A. Nardi, M. Benedetti, F. Regoli, S. Gorbi	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
		'A citizen science project to validate a new sampling method for microplastic monitoring in coastal marine environment' as part of µMED 2022 under Session 3: Monitoring and Detection of Micro and Nanoplastic.	Oral Presentation	R. Minetti, E. Costa, A. Liconti, L. Tixi, M. Lategola, U. Verna, C. di Penta, S. Genocchio, M. Catta, M. Faimali, F. Garaventa	Scientific Community
		'Uptake and ecotoxicological effects of microplastics and nanoplastics on gelatinous zooplankton' as part of µMED 2022 under the poster session Source, Fate and Effects of Micro and Nanoplastics.'	Poster Presentation	E. Costa, C. Gambardella, M. di Giannantonio, R. Miroglio, R. Minetti, V. Piazza, S. Lavorano, M. Smerieria, S. Passaglia, G. Carraro, M. Faimali, F. Sbrana, F. Garaventa	Scientific Community
		'New Insights into plastic degradation: Ecotoxicological effects of plastic leachates in marine invertebrates'	Poster Presentation	C. Gambardella, R. Miroglio, M. di Giannantonio, **E.** Costa, R. Minetti, V. Piazza, L. Castellano, N. Perez, V. Piazza, M. Faimali, F. Garaventa	Scientific Community
		"A Matter of Size: Toward an Effect-based Approach for Microplastics Risk Assessment"	Oral Presentation	L. Pittura , A. Nardi, G. d'Errico, M. Benedetti, S. Gorbi, F. Regoli.	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
		"Towards a Risk-ased Assessment of Microplastic Pollution in Marine Ecosystems"	Oral Presentation	F. Regoli.	Scientific Community
28 – 30 Sept 2022	Italian Ecotoxicology Workshop	"Toxicity of beached plastics compared to virgin items" and "Preliminary investigation on the toxicity of recycled plastic materials and potential risks for the marine environment"	Oral and Poster Presentation	C. Mazzoli , G. D'errico , F. Iezzi , M. Orsini , L. Pittura , M. Benedetti , S. Gorbi , F. Regoli	Scientific Audience
29 Sept 2022	Docufilm 'PlasticWater ' launched	Premiere of the docufilm "PlasticWater", 29th September 9pm @ Teatro Le Muse, Ancona - a documentary on plastic pollution, in which also UPM researchers and scientists were interviewed and followed in sampling and laboratory activities	Film	Università Politecnica delle Marche	Scientific Audience, Public
30 Sept 2022	European Researchers Night	Dissemination actions and practical activities dedicated to increase awareness and knowledge on plastics and microplastics pollution and impacts in the general public, within the **European Research Night**, 30th September, Ancona			

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12 Oct 2022	JPI-Oceans Communicat ors Event	Presentation of RESPONSE comms activities, emphasising the potential for utilising the upcoming survey and workshop tasks as joint actions for JPIO funded projects.	Oral presentation.	K. Kopke, O.-P. Power	Scientific Community
29 – 30 Oct 2022	Microplastic Hunters Project, Festival of Science, 29-30th October, Genova	Research partners from CNR-IAS participated in practical activities dedicated to increase awareness and knowledge on plastics and microplastics pollution and impacts in the general public by a citizen approach.	Workshops and Video **Project Link** • https://mphp.it/ • http://www.festival scienza.eu/site/en/ home.html	CNR-IAS, E. Costa	Scientific Community, General Public
28 Oct 2022	RTP 1 Interview	Interview on the paradigm of microplastics and its implications to environmental, animal and human health with images of other researchers, students and lab activities to RTP 1 (public TV channel),	**TV Interview Link** • https://www.rtp.pt/noticias/pais/detetad os-microplasticos-em-sangue-placenta-e-leite-materno_v1443353?fbclid=IwAR3mA8lksBK5x9JohneaclW hgiDvdAbr_JxKqAJ 0d_Dw_6M6hGdJZr KecYk	L. Guilhermino	General Public

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14-17 Nov 2022	MICRO 2022 International Conference (online Atlas Edition). Plastic Pollution from Macro to Nano	“Microplastic distribution, abundance and composition in the water column, sediments and biota samples collected along the Tyrrhenian Coasts (Mediterranean Sea)”	Oral Presentation	F. Garaventa, E. Costa, R. Minetti, F. Castelli, A. Montarsolo, V. Piazza, G. Ungherese, L. Pittura, L. Ventura, A. Nardi, M. Benedetti, M. Faimali, F. Regoli, S. Gorbi	Scientific Community
		“Plankton Sized Microplastics Dominate in Coastal Waters of the Gulf of Bothnia”	Oral Presentation	K. Ugwu Hernandez, A. Rotander, A. Vianello, R. Almeda	Scientific Community
		“Sensitivity of Sea Urchin Larvae to Car Tire Leachates”	Poster Presentation	S. Rist, J. Le Du-Carrée, K. Ugwu, C. Intermite, R. Almeda	
		“Ecotoxicological Effects of Micro- and Nanoplastics in Gelatinous Zooplankton”	Poster Presentation	E. Costa, C. Gambardella, M. Di Giannantonio, R. Miroglio, R. Minetti, V. Piazza, S. Lavorano, M. Faimali, F. Sbrana, F. Garavent	Scientific Community

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		“First Insights Into Leachate Toxicity of Field Collected Plastics towards Marine Zooplankton”	Poster Presentation	C. Gambardella, R. Miroglio, E. Costa, R. Minetti, V. Piazza, L. Castellano, N. Perez, L. Pittura, M. Orsini, V. Vivani, B. Morin, J. Cachot, M. Faimali, F. Regoli, F. Garaventa	Scientific Community
		“Impact of Tire Wear Leachates on Phytoplankton Communities”	Poster Presentation	M. Sampalo, J. le Du, I. Martinez, M. Gomez, R. Almeda	
		“Distribution and dynamic of anthropogenic microparticles and microplastics: A multi-compartment approach”	Oral Presentation	C. Lefebvre, F. Le Bihanic, C. Clérandeau, B. Morin, S. Lecomte and J. Cachot,	
		“Growth Response of the Marine Heterotrophic Dinoflagellate, *Oxyrrhis marina*, Exposed to Biodegradable Plastic Bag Leachates”	Poster presentation	J. Le Du-Carrée and R. Almeda	
		“Biological and chemical analysis of micronized beach plastic from Baltic Sea”	Poster presentation	A. Hashmi, A. Kärrman, M. Larsson, A. Rotander, M. Albentosa, M. Engwall, S. Keiter	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
		"Neurotoxicity of Polystyrene Nanoplastics and Their Ingestion in the Marine Mussel *Mytilus galloprovincialis*"	Poster presentation	J. Gonçalves, M. J. Bebianno	Scientific Community
17 Nov 2022	ASLO ASM 2023	Session at the ASLO ASM 2023 has been officially accepted. This session entitled 'JPI Oceans Joint Action: Ecological Aspects of Microplastics – From Scientific Findings to Political Actions' includes speakers from all 6 projects funded under JPIO call	**Event Link** • https://www.aslo.org/palma-2023/	ALL JPIO Funded Projects	Scientific Community,
18 Feb 2023	Science for Environment Policy Future Brief 27	The RESPONSE project is included in a comprehensive list of EU funded projects that are advancing understanding of nanoplastics and their potential future remediation. • European Commission, 2023. Science for Environment Policy Future Brief 27: Nanoplastics: State of Knowledge and Environmental and Human Health Impacts	**Link to Document** • https://op.europa.eu/en/publication-detail/-/publication/a9088790-ace5-11ed-8912-01aa75ed71a1/language-en/format-PDF/source-280658643	European Commission	Scientific Community, Policy and Decision Makers, General Public

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
8 March 2023	Educational Laboratory, Celebration of the Centenary of the CNR, Genova, Italy	CNR-IAS participated in an Educational Laboratory: A plastic Sea: presence and effects of microplastics in the marine environment.	In person event and demonstrations Photographs taken	CNR-IAS	Scientific Community, General Public
29 – 31 March 2023	Final Consortium Meeting	Final consortium meeting held in Ancona with project partners.	Oral Presentations	All partners	Consortium, Advisory Board, Funders.
16 May 2023	MARLIPO Workshop on Marine Litter Pollution', Lecce, Italy.	'Advancements in plastics and microplastics: from integrated risk characterization to novel approaches and possible solutions'	Oral Presentation	A. Nardi	Scientific Community
24 May 2023	Newsletter	RESPONSE newsletter Issue No.2 published online.	Text and image-based document	O.-P. Power	Scientific Community, General Public
May 2023	The Water Code Project	CNR-IAS participated in editorial board to develop a Digital Teaching Kit to promote sustainable development as part of 'The Water Code' project	Project participation	CNR-IAS	General Public, Policy and Decision Makers,
25 May 2023	Workshop 1 on Scientific misinformation	First workshop addressing scientific misinformation in the public domain was held with scientists and specialists in the field of research.	Oral Presentations, videos, guided discussion.	K. Kopke, O.-P. Power, S. Agnew, A. Dozier, E. Fitzgerald	Scientific Community

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
6 June 2023	ASLO ASM Conference	'Development of a Weight-of-Evidence Model for Risk Assessment of Microplastics Pollution Within RESPONSE Project'	Oral Presentation	F. Regoli	Scientific Community
21 June 2023	Workshop 2 on Scientific misinformati on	First workshop addressing scientific misinformation in the public domain was held with policy makers.	Oral Presentations, videos, guided discussion.	K. Kopke, O.-P. Power, S. Agnew, A. Dozier, E. Fitzgerald	Scientific Community
June 2023	Plastic Pirates, Rome, Italy	CNR-IAS attended the Plastic Pirates global citizen project general assembly	In person event participation.	CNR-IAS	Scientific Community,
June 2023	Educational laboratory/ Workshop, Genova, Italy	CNR-IAS participated in an Educational laboratory entitled 'Micro-plastics in the sea: a big problem' which formed part of a Call for Innovation within The Ocean Race: The Grand Finale	In person event and demonstrations Photographs taken	CNR-IAS	Scientific Community, General Public
25 July 2023	Workshop	CNR-IAS participated in the Moby Litter workshop on 25th July 2023 which was organized by the Polytechnic University of Marche	In person event and demonstrations Photographs taken	CNR-IAS	Scientific Community, General Public

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
31 Aug 2023	Workshop Report	Science Communication of Marine Plastic Pollution. JPI Oceans-funded RESPONSE project.	**Publication Link** https://www.researchgate.net/publication/373649661_Science_Communication_and_Marine_Plastic_Pollution_Perspectives_on_the_mis_communication_of_microplastics	K. Kopke, O.-P. Power, S. Agnew, A. Dozier, E. Fitzgerald, F. Regoli, A. Mateos-Cárdenas	Scientific Community, General Public
14-15 Sept 2023	JPIO Closing Event	A JPI Oceans closing event was held to showcase the results of all 6 projects funded under the joint action call. F. Regoli spoke on behalf on the RESPONSE consortium and gave an overview of the project and its key outputs.	Oral Presentation and PowerPoint.	F. Regoli, and all project partners	Scientific Community, General Public, Policy and Decision Makers, National funders,
		Process-based sensitivity analysis of a Lagrangian particle tracking model for microplastics	Poster Presentation	E. Siht, A. Mishra, G. Väli, T. Liblik, N. Buhhalko, U. Lips	Scientific Community, General Public, Policy and Decision Makers, National funders,
		Modelling the pathways of Microplastics in the Gulf of Finland, Baltic Sea	Poster Presentation	A. Mishra, E. Siht, G. Väli, T. Liblik, N. Buhhalko, U. Lips	Scientific Community, General Public, Policy and Decision Makers, National funders,

Date	Activity Type	Details	Activity/ Materials	Responsible	Impact/ Audience
		Seasonal distribution of microplastics and anthropogenic particles uptake by four commercial species from the Arcachon Bay (North East Atlantic, France).	Poster Presentation	C. Lefebvre, B. Cormier, F. Le Bihanic, G. Rampazzo Magalhães, S. Lecomte, J. Cachot, B. Morin	Scientific Community, General Public, Policy and Decision Makers, National funders,
Early 2024	Journal Environment al Pollution - Special Issue	Special issue of pollution accepted which will focus specifically on research outputs from the 6 projects funded under the JPI Oceans joint action call. Project coordinator F. Regoli is a co-guest editor	Peer reviewed open access journal with 5 contributions from RESPONSE project partners.	All	Scientific Community, Policy and Decision Makers, National funders,

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