SUMMARY

TOWARD A RISK-BASED ASSESSMENT OF MICROPLASTIC POLLUTION IN MARINE ECOSYSTEMS (RESPONSE)

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 will be designed to integrate and differentially weight 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. The overall aim is achieved by the constituent objectives:

- **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, supported by modelling and *ad hoc* field, mesocosm and laboratory studies.
- **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.
- **to set up a diffused analytical Smart Hub**, which combines a suite of instrumental facilities.
- **to raise public awareness** on the ecological risk of MPs and NPs.

Methodology

Links between oceanographic conditions, environmental distribution of MPs and NPs, trophic transfer and impact on pelagic food webs and benthic communities is addressed by analysing their abundance and typologies in representative marine species, as well as relevant ecosystem functions and services. Innovative mesocosm and laboratory studies enable validation of weighting factors and toxicological thresholds for MPs and NPs. The approach assesses the role of size, shape and other polymer characteristics in modulating biological effects of particles, both alone and in combination with other environmental stressors.

Results, decisions, and recommendations, by workpackage

<u>WP1: Monitoring MPs in European coastal areas.</u>

Sampling activities were performed in the Adriatic Sea, Tyrrenian and Ligurian coasts, Western Mediterranean Sea, South and NW Portugal areas, NE Atlantic Ocean, North Sea and Baltic Sea. As an

example, results from organisms collected along the Adriatic coast showed that the 23% of the analyzed specimens ingested a maximum of 2 MP items. Almost all the organisms were positive to microfibers ingestion, mainly of natural origin.

WP2: Biological fate of MPs and NPs.

To evaluate ingestion, compartmentalization and effects of MPs in marine organisms, investigations with larvae and adult mussels, jellyfish ephyrae, adult copepods, brine shrimps' larvae and medaka were performed with MPs, NPs and microfibers (MFs) of synthetic, natural and biocompatible origin. Results showed a different accumulation rate of different polymers in various marine organisms: polyamide-MFs were accumulated in the digestive gland of adult mussels, while no accumulation was observed for polyester and cotton fibres. Brine shrimp larvae ingested MPs derived from both caps and biocompatible polymer (PVDF), while jellyfish and copepods did not internalize such particles. Copepods rejected 80% of the MPs after touching them with their mouth parts. Biodegradable polymers were accumulated in medaka tissues. Exposure to NPs induced cytotoxicity in the different hemocyte subpopulations of mussels.

WP3: Biomarkers in the ecological risk of MPs.

The effects of MPs, NPs and MFs in marine organisms were evaluated using a battery of biological responses including molecular, biochemical and cellular parameters. Laboratory experiments on MF exposure to mediterranean mussels showed immune system alterations, some perturbation of the antioxidant system, onset of cellular alterations, and lack of oxidative damages to DNA. Exposure of mediterranean mussel, fresh-water clams and zebrafish embryos to polystyrene (PS) NPs resulted in negative effects on membranes of subcellular organelles, some modulation of the antioxidant system, alterations in filtration rate and energy production, and onset of genotoxic damage.

WP4: Bioassays in ecological risk of MPs/NPs

Moderate toxicity of biodegradable commercial plastics and field-collected plastics leachates were observed using bioassays with sea urchin embryos and mussels. Alterations in organisms' behavior, and swimming speed were observed in sea urchin embryos exposed in long-term experiments with NPs-MPs, while no developmental alterations were observed in medaka exposed to tire wear particle (TWP) leachates. TWP leachates were toxic to five species of marine copepods and three phytoplankton species. Leachates of field-collected and micronized MPs affected the frequency of jellyfish pulsations, while no immobility, alteration in frequency of pulsation or swimming behaviour were observed in brine shrimps and in jellyfish exposed to a biocompatible polymer. The toxicity of MPs towards copepods was increased at elevated light intensity and high temperature.

WP5: Effects of MPs on ecological functioning.

The effects of weathered microplastics on micro- and meso-zooplankton communities of the Baltic Sea were investigated in mesocosms. The role of mussel beds on MPs enrichment and fluxes in water column and sediments was studied by sampling from a natural rocky reef mussels, water and sediments in the Baltic Sea. A study on the role of algae in MPs coastal distribution and bioavailability was performed in

the Conero Riviera (Central Adriatic Sea); all investigated algal species (*Cystoseira compressa, Gongolaria barbata and Ulva lactuca)* trapped MPs with depending on the sampling period. Fibers were the dominant shape (98%), and polyester was the most frequent polymer. The highest concentration of 3.8 MP/g w.w was measured in *Gongolaria barbata*, in April and most MPs fell in the 1-3 mm size class.

WP6: Weight Of Evidence (WOE) model for MPs.

A quantitative Weight Of Evidence (WOE) model was developed to integrate various types of data (lines of evidence, LOEs). The LOEs evaluate the characteristics of MPs and 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. Development of mathematical algorithms and logical flowcharts was validated with data produced by all partners.

WP7: "Smart Hub" of analytical facilities.

Conventional and innovative methods were compared for characterization of MPs and NPs as a function of particle size, shape, and polymer type. Conventional methods included fluorescence staining, confocal microscopy, environmental SEM, dynamic light scattering, coulter counting, flow cytometry, FTIR, and Raman spectroscopy. The more innovative techniques included scanning electron microscopy, small Angle X-ray scattering, and hyperspectral microscopy. These techniques were tested in collaboration with other Universities and Research Centers namely Universiteit Gent (Belgium); Politecnico di Milano, and the Synchrotron of Trieste.

WP8: Communication and dissemination.

RESPONSE's online presence was established in November 2020 through the creation of the website (**https://www.response-jpioceans.eu/)** and social media accounts. A brochure was released and made available to download via the project website. Three newsletters were published through the project website and social media channels. The newsletters provided an overview of project progress, focusing on project activities and results obtained in the RESPONSE project. Two workshops were organized and facts sheets created. The project results have been disseminated by conferences, workshop, scientific and popular publications, exhibitions and video.

Overall, 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