

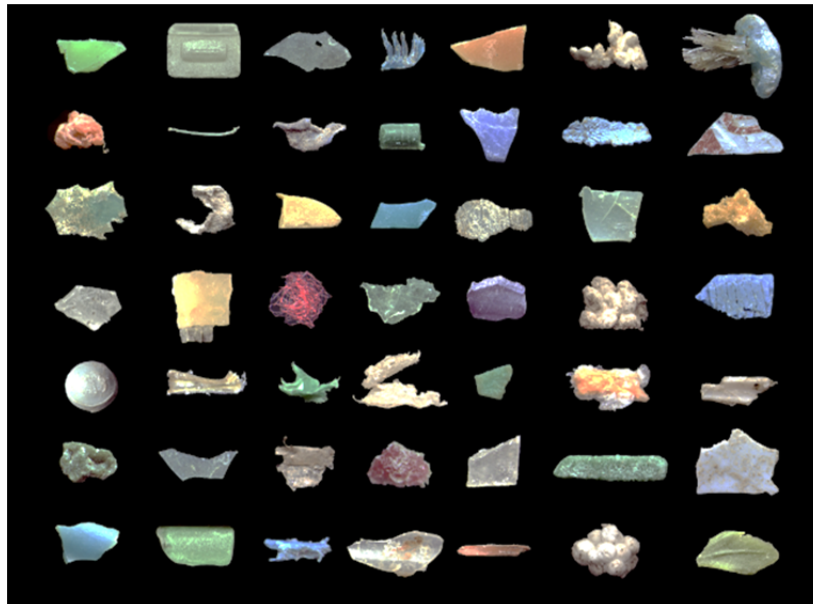
PROJECT FINAL REPORT

FULL PROJECT TITLE: ECOTOXICOLOGICAL EFFECTS OF MICROPLASTICS IN MARINE ECOSYSTEMS

Project acronym: EPHEMARE

Period covered: January 2016 – December 2018

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CONSORTIUM

A European Consortium of 14 partners from 9 European countries constitutes EPHEMARE

PARTNER 1 - University of Vigo, Spain

PARTNER 2 - Spanish Institute of Oceanography (IEO), Spain

PARTNER 3 - University of Murcia, Spain

PARTNER 4 - University of Bordeaux, France

PARTNER 5 - University of Heidelberg, Germany

PARTNER 6 - University of Antwerp, Belgium

PARTNER 7 - Marche Polytechnic University, Italy

PARTNER 8 – IAS (formerly ISMAR) National Research Council, Italy

PARTNER 9 - Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Portugal

PARTNER 10 - University of Algarve, Portugal

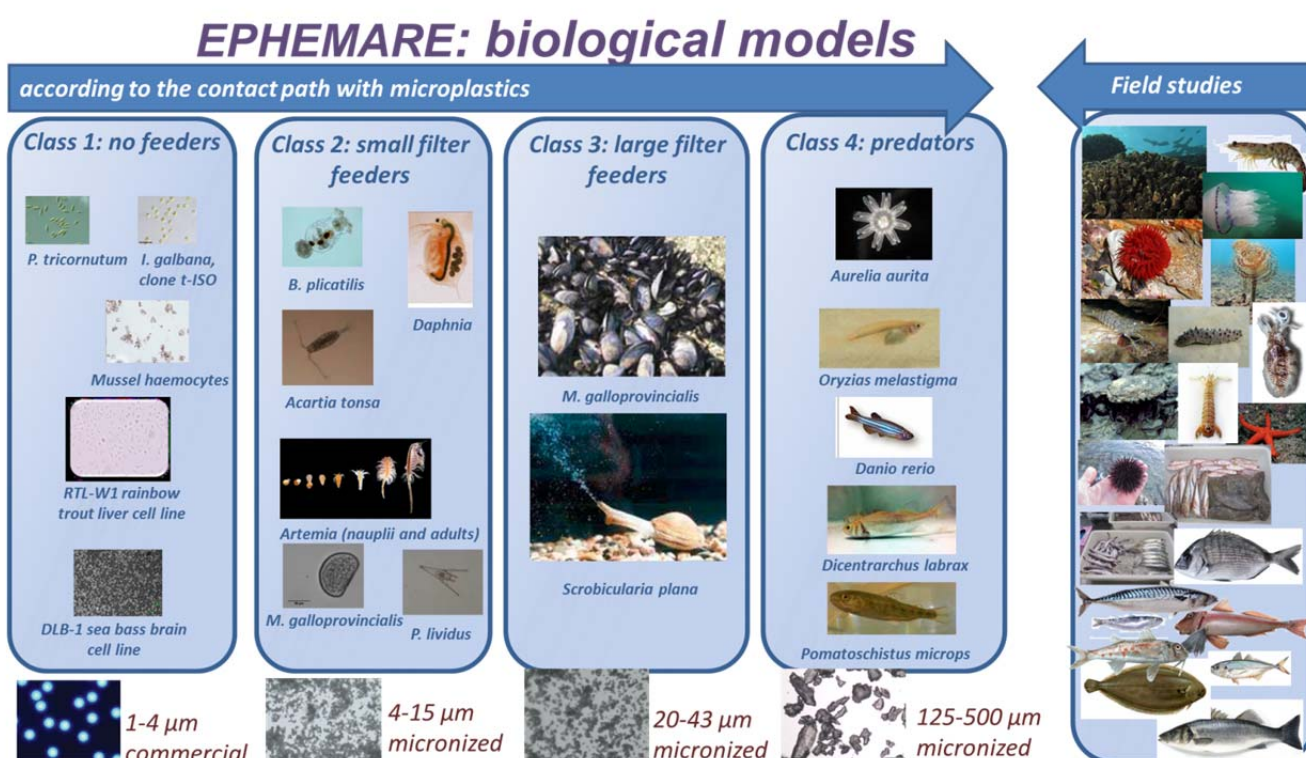
PARTNER 11 - University of Örebro, Sweden

PARTNER 12 - University of Oslo, Norway

PARTNER 13 - University College Cork, Ireland

PARTNER 14 - IFREMER, France

The EPHEMARE project is one of the four European initiatives approved by the Joint Programming Initiatives Healthy and Productive Seas and Oceans, JPI-Oceans, a strategic platform open to all members of the EU and associated countries that invest in marine and maritime research. EPHEMARE is a consortium of European dimension (14 partners from 9 countries) that deals with the ecotoxicological effects of microplastics (MP) in marine ecosystems. EPHEMARE implemented a **collaborative experimental effort** of unprecedented dimensions in marine ecotoxicology, with **18 of the 53 peer reviewed papers** so far produced **resulting from the joint effort of 2 or more JPI-Oceans funded partners**. The Project targeted, (1) the **uptake**, tissue **distribution**, and **effects** of microplastics in organisms representative of pelagic and benthic ecosystems, (2) the potential role of MP as **vectors** of model contaminants that readily adsorb to their surfaces, and (3) support **private and public stakeholders** with solid scientific grounds for environmentally sounded decisions, and raise public awareness on this issue.



With those aims, model MP of environmentally relevant shape, with or without spiked contaminants were made up as testing materials for all work packages (WP) (**WP1**). Subsequently, MP were used to expose marine species selected according to contact path and feeding mechanism (see Fig. above) to measure their uptake, elimination, accumulation rates (**WP2**), and potential trophic transfer (**WP5**). In these models, the project studied biological effects at molecular, cellular, physiological and organismic levels through the implementation of collaborative experiments among partner laboratories, to allow for detection of effects of microplastics across the main phyla of marine organisms covering most trophic levels, from bacteria to fish. A wide array of ecotoxicological effects (from transcriptomics to cell damage and organism responses), using different typologies of polymers both virgin and previously contaminated, and exposure conditions were assessed (**WP3** and **WP4**). Cell culture and slice tissues have allowed to better elucidate mechanistic relationships, cell expression and enzymatic response as mode of action of microplastics and associated chemical compounds. EPHEMARE's field validation studies (**WP6**) consisted of sampling campaigns in different areas of the Mediterranean and the NE Atlantic coasts of continental Europe, plus the Caribbean territories of the EU in the proximity of the oceanic gyre causing accumulation of marine debris. These data allowed assessing the extent of abundance, distribution (i.e. pelagic vs. benthic species), typology of polymers, and pollutants load, thus linking the ecotoxicological findings from laboratory studies to the environmental scale. A **BASEMAN-EPHEMARE** Partnership Agreement was signed for the standardization of MP monitoring techniques. EPHEMARE activities and findings have been documented (**WP7**) *via* the project social media outlets to communicate to diverse audiences, and project partners took part in several thematic outreach events to engage the public.

EPHEMARE raised awareness through factsheets, newsletters and campaigns such as the successful IMPACT 2017 - EPHEMARE photo competition.

The scientific conclusions reached by EPHEMARE can be summarized as follows:

- MP of environmentally relevant shape (Fig. 1a) are **easily ingested**, but also **easily egested** by filter-feeders and predators. In mussels (Fig. 1b), they are excreted through feces (large ones) or **translocated** from digestive gland into the gills (<2 μm), an excretory organ. Once experimental data fit uptake and distribution models, models predict that there is **no accumulation** of MP in mussels exposed to environmental concentrations. MP are **transferred** from preys to predators but they are egested and **do not bioaccumulate in predators**. This is confirmed by field samples.

Fig. 1a

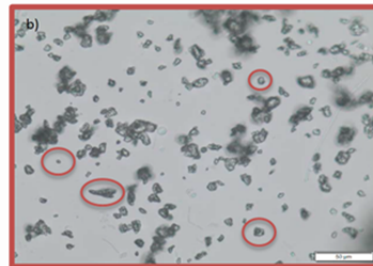
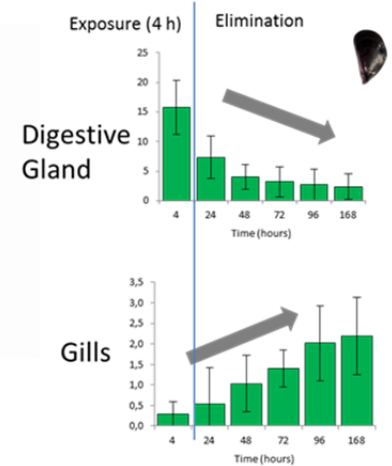


Fig. 1b



- MP may act as vectors of pollutants but they **do not increase bioavailability** and effects of model chemicals **compared to natural particulate matter**. Fig. 2 shows an example studying the accumulation in the mussel of the hydrophobic organic pesticide chlorpyrifos in absence of particles and in presence of microalgae and microplastics.

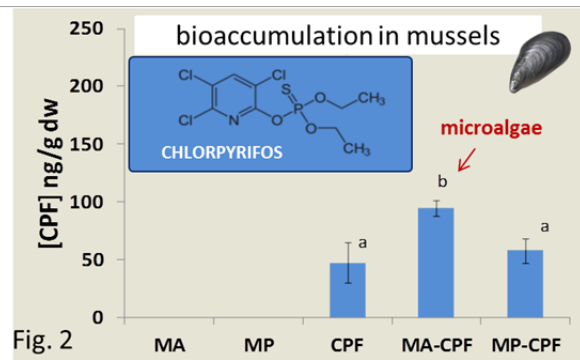


Fig. 2

- Conventional acute toxicity end-points are not sensitive enough and not appropriate for MP Risk Assessment, which should be based on long-term **reproductive, immune** and **behavioural** endpoints.

Microplastics may affect **oxidative stress** and **modulate the effects** of other chemical contaminants.

Although polymers are generally innocuous, **chemical additives** may pose ecological risk.

EPHEMARE developed **methods** (Fig. 3) based on chronic effects from early life stages of fish **suitable to assess the toxicity of microplastics**. Those methods can be useful for both **industry** (to develop environmentally friendly materials) and **regulatory agencies** (e.g. ECHA).

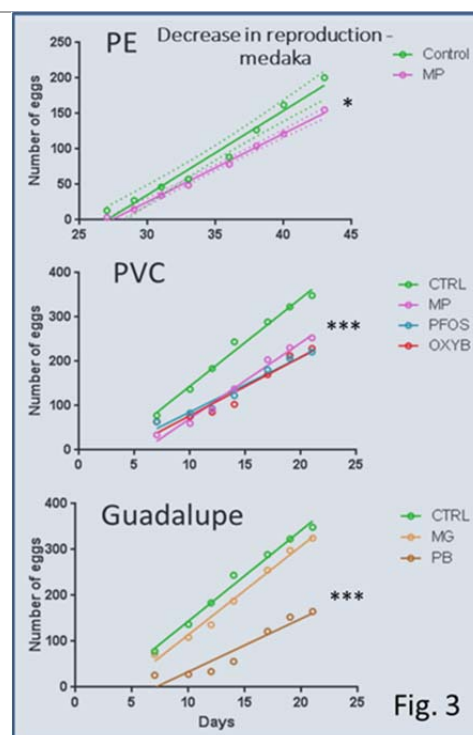


Fig. 3

INTRODUCTION AND AIMS OF THE PROJECT

Global plastic production continues to rise, and both solid waste mismanagement and direct disposal in the environment both contribute to between 4.8 and 12.7 million tons of plastic litter entering the global oceans annually (Jambeck et al. 2015). Conventional olefinic polymers are not biodegradable and bear the potential to persist in the sea for hundreds of years. However release of plasticizers combined to photooxidation and physical abrasion lead to fragmentation of plastic objects in pieces of increasingly small size (Barnes et al. 2009). This “invisible marine litter” is not monitored using the standard 300 microns nets commonly used in oceanography, but is readily ingested by zooplankton and filter-feeders, thus entering the marine trophic webs (Setälä et al. 2014).

MPs were originally defined as those plastic fragments that are difficult to see at naked eye (<5 mm). MPs originating from physical degradation of larger plastic objects are called secondary microplastics, whereas those used for certain applications, such as personal care products, already commercialised in small particles are termed primary microplastics. Plastics have the potential to sorb hydrophobic pollutants and concentrate them into particles ingestible by biota. Thus, the potential role of plastic particles as vectors of chemicals to organisms has been pointed out since the first studies on marine plastic pollution (Carpenter et al. 1972).

Although conventional polymers are non-reactive and with little toxicity, plastic objects bear in their composition a wide range of chemical additives intended to provide the commercial objects with the properties demanded by the market, including flexibility, physical robustness, resistance to oxidation and chemical insult, resistance to light and heat, non-flammability, color, lack of static electricity, etc. Unlike textiles or food, the composition of a commercial plastic in terms of chemical additives is not disclosed to the consumer. Some chemical additives have been associated with bioaccumulation, biomagnification and endocrine disruption and have been phased out (e.g. polybrominated diphenyl ethers) or restricted from uses such as toys or children feeding bottles (e.g. Bisphenol A, orthophthalates, chlorinated organophosphorus flame retardants). Anyhow, the toxicity of chemical additives to marine organisms remains largely unknown.

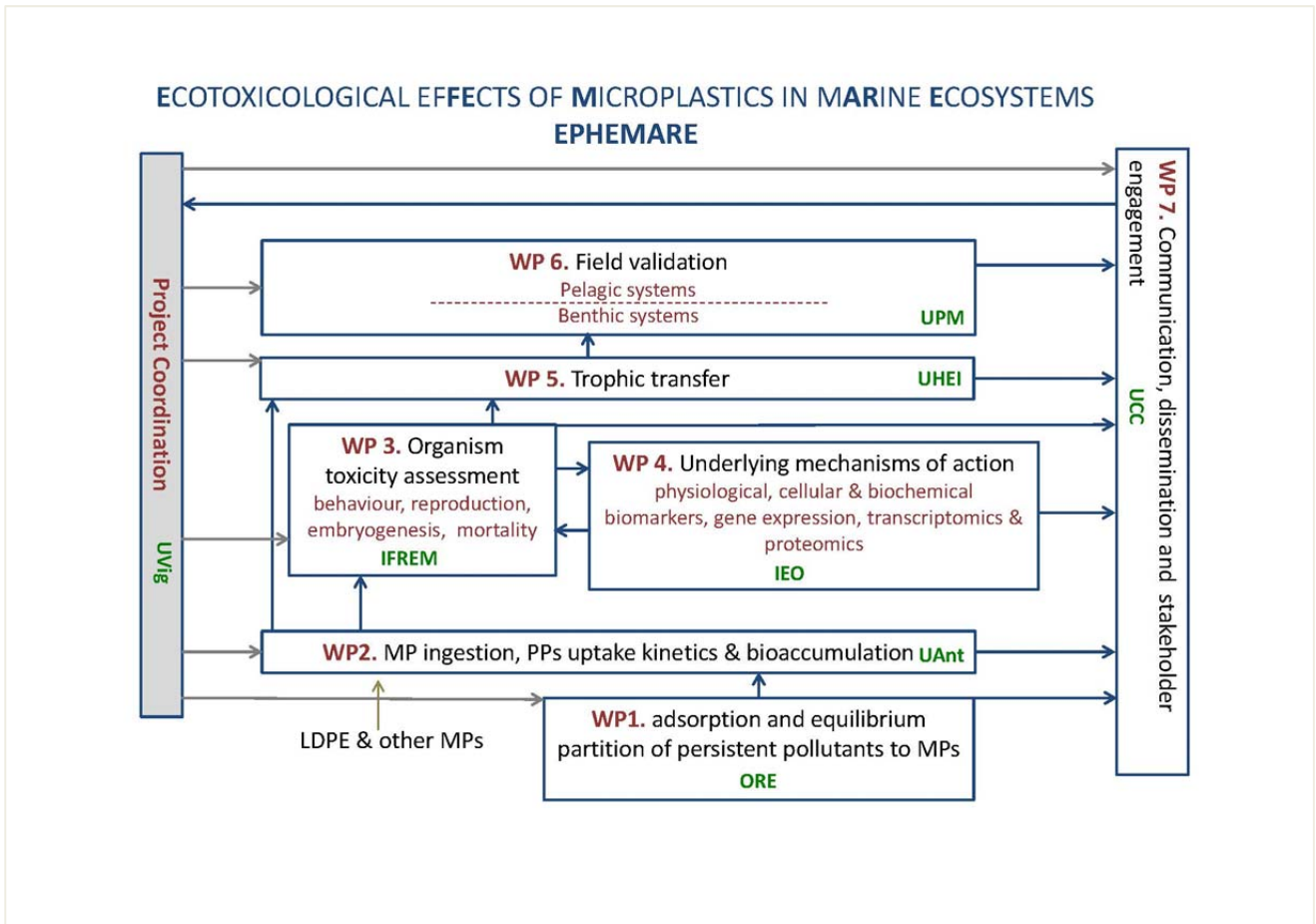
EPHEMARE, dealing with the ecotoxicological effects of microplastics in marine ecosystems, was an experimental project based on standard test species of recognized usefulness in aquatic ecotoxicology, combining internationally accepted standard methods with innovative end-points and test species in order to contribute cutting-edge experimentation specifically focused on the toxicity of plastic particles. EPHEMARE included a wide selection of biological models (from bacteria and primary producers to invertebrates and fish), experimental conditions (cell culture, in vivo laboratory exposure, field studies), and levels of biological organization (from molecular effects to cellular, physiological and organism responses) integrated in a holistic approach. The composition and capacities of the partnership allowed in-depth studies on fundamental mechanisms underlying these effects across the main phyla of marine organisms. Integration of investigated models, biological effect and exposure conditions makes the ecotoxicological data obtained useful for ecotoxicological risk assessment of environmental microplastics, derivation of environmental quality standards, environmental protection regulations and advise for a correct management of marine environments by national and international authorities.

Generally, the objective of EPHEMARE was to investigate the uptake, tissue distribution, final fate and effects of microplastics in organisms' representative of pelagic and benthic ecosystems by achieving the following main objectives:

- To examine the potential role of MPs as vectors of model pollutants that readily adsorb to their surfaces.
- To assess by means of internationally accepted standards and methods whether MP exposure leads to detrimental effects at molecular, cellular, physiological and organism levels.

- To test the suitability of exposure and effect biochemical, cellular and physiological biomarkers and cutting edge omics methods to trace MP exposure.
- To assist public and private stakeholders with the scientific basis for the development and compliance with general (risk assessment, environmental quality standards) and aquatic (WDF, MSFD) environmental regulations.
- To raise public awareness on the risk that the less visible plastic particles pose to marine ecosystems and, eventually, human health.

To achieve these general aims, EPHEMARE was structured in 7 tightly interconnected WPs:



The goals of EPHEMARE are specifically targeted through its WP structure:

- **WORKPACKAGE 1** aimed to investigate the **sorption equilibrium kinetics** of model pollutants to MPs, in order to test the potential role of MPs as vectors of marine pollutants. This WP **supplied the testing materials** used in the remaining WPs for the uptake, toxicity and trophic transfer studies. Particles of low-density polyethylene (LDPE) and other selected polymers with suitable size and shape were incubated in the presence of model contaminants, prioritizing hydrophobic chemicals posing environmental risk in coastal environments. The use of environmentally irrelevant microspheres was avoided. For each chemical adsorbed to the model particles, two concentrations were prepared and confirmed by up-to date chemical analyses of liquid and solid phases.
- **WORKPACKAGE 2** aimed at measuring the **uptake, elimination and accumulation** rates of small sized MPs (virgin and spiked) across the main phyla of marine organisms covering most trophic levels, from bacteria to fish. Model species included heterotrophic bacteria (*Vibrio fischeri*), unicellular algae (*Tetraselmis chuii*), rotifers, cnidarians (*Aurelia* sp.), copepods (*Acartia* sp.), bivalves (*Mytilus* spp, *Scrobicularia plana*), echinoderms (*Paracentrotus lividus*), and fish (the common goby *Pomatoschistus microps*, European Sea Bass

Dicentrarchus labrax, or zebrafish). Both acute and chronic experimental exposures sought to enhance sensitivity and to mimic ecologically relevant conditions. The experiments and results were directly linked to the work performed in WPs 3 to 6 which deal with effects at various levels of structural and functional organization including trophic transfer and field exposure.

- **WORKPACKAGE 3** aimed to evaluate the **effects** of MPs and sorbed contaminants on several key physiological functions for recruitment: **survival, growth, reproduction** (including sexual differentiation and maturation), **embryo-larval development**, and **behavior**, which allow assessing the abilities of individuals to explore their environment, to find food and partners. Exposures were performed according to procedures agreed by consensus among partners and for some models validated in intercomparison exercises. Data obtained were fit to dose-response models and toxicity thresholds (NOEC/LOEC, EC₁₀) were calculated using standard statistical procedures.
- **WORKPACKAGE 4** aimed to investigate the **underlying toxicity mechanisms** at molecular and cellular levels, using **biochemical, cellular and physiological biomarkers**, from gene expression to energy balance. In addition to the in vivo models, fish and mammalian cell lines already described above, were also used.
- **WORKPACKAGE 5** aimed to provide information as to the relevance of the public concerns about MPs bioaccumulation in marine food through **trophic transfer**. Experiments were designed to find out whether MPs accumulate along food chains and serve as vector for pollutants, thus leading to a **secondary risk** for the consumers. In a proof-of-concept approach, various simple model food chains were compared with respect to their suitability to mimic potential trophic transfer in marine ecosystems.
- **WORKPACKAGE 6** aimed to support results obtained in WPs 1 to 5 by means of validation with relevant **field data** describing the occurrence, distribution, typology and chemical loads of MPs in representative biota of different coastal areas from Mediterranean, North East Atlantic, a North Sea Fjord and a North Sea Estuary. The main species from benthic and pelagic systems were collected, including planktonic organisms, pelagic and benthonic fishes, and invertebrates. The analyzed species included most of the biological models that are characterized in laboratory conditions by WPs 2 to 5.
- **WORKPACKAGE 7** aimed to **communicate and disseminate projects findings** and outputs to relevant stakeholders and general public to increase **awareness** on MPs and associated concerns. Especial attention was paid to produce results useful for public authorities in charge of the implementation of **European Directives** (e.g. **MSFD**) and environmental regulations (e.g. **EU Plastic Strategy**), the private sector including **industry** seeking environment-friendly alternative polymers, and companies involved in selling and development of instruments for environmental monitoring of MPs.

RESULTS AND DISCUSSION

WORKPACKAGE 1 – ADSORPTION & EQUILIBRIUM PARTITION OF POLLUTANTS TO MPs

*The following partners are involved: **Partner 2, Partner 6, Partner 11** (Leader)*

- Biochemodynamic models of metal ion binding to micro- and nanoplastics predict high variability in the relevance of MP as vectors of metals depending on particle composition, size and concentration, but for filter feeders, e.g., mussels, metals released from ingested plastic particles may be significant as compared to metal exposure via the water phase. Considering a typical marine exposure scenario with 0.1 nM of **cadmium** (Cd) and MP in the range 1–100,000 particles per m³, a mussel with a filtration rate of order 2 L per hour per individual would be exposed to 0.2 nM of Cd per hour via the aqueous phase, and potentially 10⁻⁵ to 1 nM per hour via ingested MPs (**Town et al. 2018**).

- However, in experimental work conducted by **Partner 11 (on prep.)** it was not possible to reach sorption of Cd to MP after 7 days of exposure under any conditions tested. Rochman et al. (2013) showed that after 12-month exposure of MPs in San Diego Bay a complex mixture of metals, including Cd, can be found on LDPE and PVC. Thus, long-term exposure, different water conditions and weathering may facilitate sorption of Cd on MPs.

- **Mercury** (Hg) was readily sorbed onto LDPE particles, but 70% of the Hg incorporated through MPs were quickly eliminated in the biodeposits. After 7 days depuration, mussels exposed to Hg through MPs showed 235 µg Hg per individual, whereas those exposed to Hg via microalgae of similar size retained 701 µg Hg per individual. Thus, the potential of Hg to bioaccumulate was lower when it was sorbed onto MPs than when it was sorbed onto other seston particles as phytoplankton (Rivera-Hernández et al. 2019).

- Concerning the sorption of organics, the following chemicals were tested: **benzo-a-pyrene** (BaP), **oxybenzone** (BP3), **perfluorooctane sulfonate** (PFOS), and **chlorpyrifos** (CPF), and the results are summarized below.

- **PFOS** sorption was linear over 6 months, while BaP adsorbed onto particles decreased over the same period. Decrease of **BaP** levels might be due to its low persistency in water by photodegradation and biodegradation, and its potential sorption to glassware. The investigation of size effect of particles demonstrated an increase of PFOS sorption with a decrease of the size particle (increase of surface area). Sorption of **BP3** on LDPE showed that, unexpectedly, the smaller the particles the less BP3 was adsorbed on the LDPE. Sorption of BaP was not modified by the size of microparticles. Results demonstrated that sorption processes depend on the type of contaminant (e.g. functional groups) but may also depend on the size range of microparticles (Cormier et al., in prep.).

- **CPF** very rapidly sorbed to LDPE microparticles, consistently with the high Kow of this chemical. After just 2 h of incubation, more than 70% of CPF was adsorbed onto plastic surfaces. The sorption of CPF to the plastic particles reduced the bioavailability of this chemical to the microalgae. As a consequence, CPF toxicity decreased. For a given CPF dose, the presence of MP decreased by ca. 40% cell growth inhibition (Garrido et al. 2019).

Discussion

In our adsorption experiments, BaP in solution was above solubility, and we assume that sorption capacity and sites of the particles were rather quickly occupied, resulting in a decrease in the sorption efficiency of BaP to smaller MP; i.e., the effect of particle size could be suppressed by a quick sorption of available BaP at the surface of the particles (Abdullah et al. 2009). For sorption of BaP on PVC the double amount of BaP in solution was required. PE has a larger Brunauer-Emmett-Teller (BET) surface area than PVC, which may explain a higher sorption affinity for BaP on LDPE. Wang and Wang (2018) showed a similar sorption affinity of the PAH pyrene to PE and PVC. Long-term sorption experiment with BaP on LDPE showed that after a first increase of sorption the concentrations of BaP on LDPE declined until the end of exposure. However, the experiment was conducted under non-sterile conditions at room temperature, and degradation processes cannot be excluded. In contrast to BaP, PFOS sorption was like expected, and less PFOS in solution was necessary to reach the same concentrations on the different size classes of LDPE. In addition, our results suggest that PFOS has a higher sorption rate on PVC compared to LDPE; however, particle sizes were different. Wang et al. (2015) showed a higher sorption rate for PFOS on PVC as well after 7 days of exposure, which was explained by a lower electrostatic repulsion between anionic PFOS molecule and PVC surface. However, the results of the present long-term sorption experiment showed a great increase of PFOS sorption on LDPE after 90 days suggesting that surface properties of the tested plastic material changed over time.

Overall, the experimental results obtained in WP1 show that under the conditions tested sorption processes of environmental pollutants depend on the chemical properties of the compounds, the plastic material and particle size, and resulting sorption kinetics are very different (Llorca et al. 2018, Wang et al. 2015). In addition, sorption behavior under environmental relevant conditions will change as weathering, conductivity, pH and salinity of the surrounding water will change surface properties and sorption, respectively. Therefore, suggesting rather complex and dynamic sorption, desorption and degradation processes of pollutants on MPs and depending most likely on local environmental conditions (Andrady 2011, Brandon et al. 2016, Llorca et al. 2018, ter Halle et al. 2017).

WORKPACKAGE 2 – MP INGESTION, UPTAKE KINETICS & BIOACCUMULATION

The following partners are involved: Partner 2, Partner 3, Partner 6 (leader), Partner 7, Partner 9, Partner 10, Partner 14

NO FEEDERS

- No physical interaction or interiorization of MPs was observed for fish cell lines (Romero et al. 2018), or head-kidney leukocytes (HKL) (Espinosa et al. 2018).
- *T. chuii* exposed to MP (fluorescent red plastic microspheres 1-5 μm diameter) produced exudates, and heteroaggregates including microalgae exudates, MP, algae cells and cell fragments were formed in test medium. MP were observed on the external wall of the microalgae cells but the observations were inconclusive regarding the presence of MP inside the cells (Prata et al., 2018).

FILTER FEEDERS

- When mussels are exposed to MP and microalgae of similar size, there are no differences between the **uptake kinetics** for both types of particles, and a similar filtering rate was recorded. Mussels readily egest the ingested MP. After 120 h exposure, mussels had egested around 80% of the MP ingested. The highest volume of MP **egested** was quantified in faeces recovered within 24 h upon exposure. Subsequently (until 48 h and 120 h of the exposure), a lower volume of MP was recorded in faeces. The higher the average diameter of the MP particles the quicker they are egested, with the highest diameter tested (ca. 10 μm) being observed in MP particles egested after just 4 h of the exposure. After 120 h, from 2 to 6% of the MP ingested was **accumulated in the digestive gland**. The diameter of this particles (ca. 3 μm) was significantly lower than the mean size of the MP offered (8 μm), which suggested a specific removal through faeces of larger MP particles and the retention of smaller ones in the digestive system. Moreover, MPs are translocated from digestive gland into the gills (smaller ones) (Fernández & Albentosa, 2019a,b).
- When the dosed MP are previously spiked with CPF, mussels accumulated CPF in a different way depending exposure pathway, with a higher **bioaccumulation** when the chemical is offered **through microalgae**. Furthermore, mussels exposed to dissolved CPF accumulated also less CPF than when the pollutant is transported through the food. It might be concluded that microplastics may act as vectors of pollutants but in a similar way (or even less) as other particles of the seston (Albentosa et al. 2017).
- When mussels are exposed to Hg either dissolved or through previously spiked MP and microalgae, Hg is incorporated via digestive system when exposure is made through particles (both microalgae or MP). On the contrary, waterborne Hg is incorporated via epithelial tissues (gill and mantle). 70% of the **Hg incorporated through MPs is eliminated by the faeces**. However, when Hg is incorporated from microalgae or dissolved, it is mainly maintained in the mussel tissues. For small (<2 μm) MP, a **translocation** from the input tissue (gland and gill) to other tissues (rest and gland) has been observed (Rivera-Hernández et al. 2019).
- Partner 10 joint WP2 working with *Scrobicularia plana* clams. When clams with background BaP <1.6 ng g^{-1} dw were exposed to BaP-spiked MP for 14 days, a significant increase in clam tissue BaP compared to control and virgin MP treatment was obtained, reaching $7.3 \pm 2.0 \text{ ng g}^{-1}$ dw and proving the **transfer of BaP from the plastic to the organism**. This was not the case of BP3, where final body burdens in control and BP3-spiked MP treatments were 23.3 and 15.3 ng g^{-1} ww respectively (O'Donovan et al. 2018).
- E-SEM imaging of tissues of MP-exposed mussels was optimized (Partner 6, on prep.).
- *Daphnia magna* was found to take up MP (fluorescent red plastic microspheres 1-5 μm diameter) from test medium; MP were found in the gut, gills and also in the brood chamber. MP elimination from the gut occurred. In acute 96-h and 21-d bioassays (see WP3), *D. magna* specimens were exposed to MP (industrial plastic pellets, LDPE, 125 - 250 μm), virgin LDPE and LDPE contaminated with PFOS (LDPE-PFOS). The results indicated that LDPE-PFOS release PFOS to test medium and that PFOS was up taken from test medium by *D. magna*. MP size, MP concentration, particles aggregation, organism size, among other factors, influence uptake and elimination of MP by *D. magna* (Lopes 2016; Pacheco et al. 2018; Pacheco et al. in prep.).

PREDATORS

- In fish exposed to MP, histopathological and morphometric analysis of the intestine and liver resulted in certain levels of damage including liver disorganization and hepatocyte vacuolation as well as goblet cell number and villus alterations. Adsorption of pollutants to MPs did not increase the histopathological effects. No MPs were observed into the tissues (Partner 3, on prep.).

- A novel technique to investigate MP uptake and distribution throughout fish organs was developed. The **Nile Red** dyed MPs retain their fluorescence after ingestion by experimental organisms. In order to evaluate if this method was relevant to monitor labeled MPs in vivo, we have fed zebrafish and marine medaka larvae with PE, PVC and PS MPs labeled with Nile red. We were able to follow efficiently in vivo the ingestion and transit of all these MPs using fluorescence microscopy (**Batel et al. submitted**).

- Juveniles (2 cm length) of the fish *P. microps* **ingested** 1-5 μm and 420-500 μm MP (white, red and black microspheres, diameter). MP **elimination** occurred in ≥ 4 h. Red MP lost their red colour while in the gastrointestinal tract, indicating release of the dye from MP inside the fish gastrointestinal tract. 1-5 μm MP were found in the gastrointestinal tract where particles aggregates were formed. Large aggregates were retained for at least 96 h, whereas isolated particles and some small aggregates were eliminated more rapidly. MP were also found in gills, and evidences of particles presence in dorsal muscle and liver were obtained. MP exposure concentration, MP type and size of the fish influence MP elimination from the gut. *P. microps* juveniles exposed for 96 h to PFOS alone or to LDPE-PFOS were found to have PFOS in their body up to 2.4 ± 0.4 ng/g and 1.4 ± 0.2 ng/g, respectively (**Vieira et al. in prep.**).

Discussion

Marine animals, including filter feeders, deposit feeders and predators, actively ingest MP within the size range of their common food, but they also readily egest them through the faeces (for invertebrates Tosetto et al. 2016, **Fernández & Albentosa 2019a**; for fish Mazurais et al. 2015), and generally no bioaccumulation is observed. Biodynamic models also support this conclusion. For example, for the case of mussels, calculations performed starting from the EPHEMARE results, using a model which integrated parameters such as average filtration rate and average MP concentration in coastal waters, resulted in mussels having an average MP load of 2.4 and 3.6 MP ingested/individual/3 h for *M. edulis* and *M. galloprovincialis* respectively living in the water column. These values compare well to observed MP load per mussel (calculated from records present in literature), which amount to 2.9 MP/individual (excluding microfibrils, which frequently come from laboratory contamination of the samples). We must bear in mind though that standard field studies rarely detect small MP due to technical limitations and too time consuming procedures. Therefore, experimental evidence and field observations in general do not support accumulation of MPs in organisms exposed to present environmental concentrations. Nevertheless, measures must be taken to decrease MP input in the marine environment, in order to not increase the MP/plankton ratio – the predictions calculated within this study in fact are significant only with present environmental concentrations; if these will increase dramatically in the near future, the threat to filter-feeding biota will indeed become significant.

WORKPACKAGE 3 – ORGANISM TOXICITY ASSESSMENT

The following partners are involved: **Partner 1, Partner 2, Partner 4, Partner 5, Partner 8, Partner 9, Partner 10, Partner 11, Partner 12, Partner 14** (leader)

NO FEEDERS

- In an intercollaborative study conducted after methodological harmonization among 4 EPHEMARE partners, **no acute toxicity** of LDPE MP was found on *V. fisheri* bacteria or *P. tricornutum* microalgae at levels orders of magnitude above environmentally relevant concentrations. Therefore, no expected environmental risk of those MP on metabolism of heterotrophic marine bacterial or microalgal photosynthesis in the sea. These findings also highlight that the current approach for MP risk assessment in current toxicity tests used by environmental regulators should be revised, by providing alternative endpoints to be included in standardized protocols suitable to monitor the fate and biological effects of MPs (**Gambardella et al. 2019**).

- PE contaminated with PFOS has lower LOEC and NOEC when compared to the non-contaminated PE, which apparently has no negative effect in *V. fisheri*.

- Microalgae **growth was not affected** by the MP, until 25 mg l^{-1} . On the contrary, growth was clearly affected by the exposure to CPF from 2 mg l^{-1} onwards, with a total growth inhibition at concentrations upwards of 3 mg l^{-1} .

After incubation, 80% of CPF was sorbed onto MP surfaces. CPF bioassays showed two different dose-response curves depending on the presence of MP, with lower percentages of inhibition when CPF is offered through to MP. Thus, the adsorption of CPF onto **MP surfaces reduced its bioavailability** and toxicity to microalgae (Garrido et al. 2019).

- Using the microalga *T. chuii*, no significant differences in the algal growth rate were found up to 41.5 mg MP/l (LOEC > 41.5 mg/l). To investigate the potential effects of MP on the toxicity of chemical pollutants, bioassays were performed using two pharmaceuticals. Procainamide alone significantly reduced *T. chuii* growth rate with a 96h-EC₅₀ = 104 mg/l, and chlorophyll content with a 96h-EC₅₀ = 143 mg/l, 95 %. Doxycycline alone significantly reduced *T. chuii* average specific growth rate with a 96h-EC₅₀ = 22 mg/l, 95 %, and chlorophyll content with a 96h-EC₅₀ = 14 mg/l. In the presence of MP, the 96h-EC₅₀ values for chlorophyll content significantly decreased to 31 mg/l (95 % CI: 11.8 – 78.7) for procainamide, and 7 mg/l (95 % CI: 3.8 – 11.7 mg/l) for doxycycline. Overall, the results indicate that the MP tested had relatively **low toxicity** to *T. chuii* but when in mixture with procainamide or doxycycline **MP increased the toxicity of both pharmaceuticals** to *T. chuii* (Prata et al. 2018).

- Overall the results indicate that conventional acute toxicity end-points are not sensitive enough and not appropriate for MP Risk Assessment.

SMALL FILTER FEEDERS

- In a large collaborative effort among 6 EPHEMARE partners, toxicity of polyethylene (PE) MP of size ranges similar to their natural food to zooplanktonic organisms representative of the main taxa present in marine plankton, including rotifers, copepods, bivalves and echinoderms was evaluated. Early life stages (ELS) were prioritized as testing models in order to maximize sensitivity. Test species included the rotifer *Brachionus plicatilis*, the crustaceans *Tigriopus fulvus*, and *Acartia* sp., and larvae of the sea urchin *Paracentrotus lividus*. Planktonic ELS of the fish *Oryzias melastigma* were also used. Treatments included particles spiked with benzophenone-3 (BP-3), a hydrophobic organic chemical used in cosmetics with direct input in coastal areas. Despite documented ingestion of both virgin and BP-3 spiked microplastics **no acute toxicity** was found at loads orders of magnitude above environmentally relevant concentrations on any of the invertebrate models, although some developmental defects were observed in fish ELS. The results obtained do not support environmentally relevant risk of microplastics on marine zooplankton (Beiras et al. 2018a).

- In zooplankton (including not only filter feeders but also predators), the **swimming and feeding behavior** alteration endpoints seem to be particularly sensitive to low levels of MP (Gambardella et al. 2018). For example, **pulsation rate** of ephyra stage of two **jellyfish** species, *Aurelia* sp. and *Sanderia malayensis*, resulted to be affected by environmentally relevant concentrations of MP (Costa, 2018).

- Despite active ingestion of MP by *P. lividus* sea-urchin and *A. clausi* copepod larvae, the presence of MP did **not increase the toxicity** of a hydrophobic organic chemical, the 4-n-nonylphenol (NP) to these sensitive ELS (Beiras & Tato 2019).

- **Non-significant effects** of virgin MP were observed on the embryonic development of the Mediterranean mussel. Microplastics spiked with phenanthrene or with BP3 were not toxic at the tested concentrations (from 0.004 to 10 mg microplastics/L), with respect to water or microplastics controls. However, MP spiked with CPF (0.1-0.6 mg MP/L) or with Hg, caused a significant increase in the percentage of abnormal mussel embryos and larvae (LOEC: 0.2 mg MP/L and 1 mg MP/L, respectively). The **remarkable toxicity of MP spiked** with CPF and Hg could be either attributed to the desorption of the toxicant from plastics to water, or to the bioavailability of the toxicant adsorbed to the microplastics. Moreover, in the case of CPF, the presence of virgin MP in the experimental vials was found to significantly **increase the toxicity** of dissolved CPF to mussel embryos (LOEC = 200 µg/L). Further work is being carried out to complete chemical analyses of spiked MP in order to clarify the causal mechanism of the observed toxicity (Bellas et al. 2018).

- *D. magna* 21-day chronic bioassays were carried out to investigate the effects of MP (1 – 5 µm), gold nanoparticles (Au NP), and their mixtures. Mortality, mobility and reproductive fitness endpoints were investigated. MP induced reduction of reproductive fitness with LOEC = 0.02 mg/l. Au NP were **more toxic than MP**, and reduced the reproductive output with LOEC = 0.2 mg/l. In some parameters, the effects of mixtures were higher than the effects induced by MP or Au NP alone. Thus, MP increased the chronic toxic effects of Au NP (Pacheco et al. 2018).

- Another collaborative experiment was conducted among 3 EPHEMARE partners using *D. magna* to investigate the acute and chronic effects of LDPE (125 - 250 µm) virgin and spiked with PFOS (LDPE-PFOS). After 96 h of exposure, LDPE did not induce significant mortality (NOEC = 51.2 g/l; LOEC > 51.2 g/l), likely because juveniles did not intake MP due to their size. In the LDPE-PFOS bioassay, the PFOS concentrations measured by chemical analysis in test medium ranged from 1.5 to 146 µg/l. The results already obtained indicate **no acute toxicity of LDPE** to *D. magna*, and that LDPE-PFOS toxicity is mainly due to the **release of PFOS** from the LDPE to test medium followed by PFOS uptake from test medium by *D. magna* juveniles. After 21 days, LDPE did not induce reproductive or growth effects (NOEC = 3.2 g/l). **Waterborne PFOS impaired reproduction** of *D. magna* by significantly increasing the time of first brood release (LOEC = 0.138 µg/l), and decreasing the number of mobile juveniles (LOEC = 0.138 µg/l) at least partially due to the production of immobile juveniles. LDPE-PFOS caused several reproductive adverse effects, including the significant reduction of the number of mobile juveniles produced per female. Overall, these findings indicate that the **LDPE shows low toxicity** to *D. magna* but when contaminated with PFOS they are able to release PFOS to the water and this chemical caused reproductive adverse effects (**Pacheco et al. in prep**).

- Overall, the results obtained with phytoplankton and the main phyla composing the zooplankton challenge the hypothetical role of MP as vectors of hydrophobic contaminants to plankton, and point at a low environmental risk of current MP levels to the base of the marine food webs.

LARGE FILTER FEEDERS AND DEPOSIT FEEDERS

- Long-term effects of MP were investigated using the sediment-dwelling bivalves *Ennucula tenuis* and *Abra nitida*. MP extraction from the organisms showed that MPs were **ingested** at all concentrations tested. Despite MP ingestion, **no effects were observed on mortality, condition index or burrowing behaviour** for any treatment. However, significant changes in energy reserves were observed, and total **energy reserves decreased in bivalves exposed to the largest particles** (125 – 500 µm) (**Bour et al. 2018a**).

- For the long-term effects on *Arenicola marina*, no mortality was observed for any condition tested. There were significant differences in feeding behaviour between control and exposed organisms, and decreasing feeding activity over time. MP extraction from organisms and faecal casts show significant ingestion and excretion of MPs. Results on long-term effects on *A. marina* show no changes in energy reserves for any condition tested (Partner 12, unpublished).

PREDATORS

- Fish embryos of marine medaka were exposed from 1 to 13 day-post fertilization (dpf) under slight agitation (27 rpm). Exposure to BaP-spiked MPs **did not induce acute toxicity**. Exposure to PFOS-spiked MP significantly **reduced hatching success** at PFOS “high” 10 mg MPs/L concentration compared to the equivalent virgin MP or waterborne exposure to 556 ng PFOS/L (Fig. 1). Exposure to BP3-spiked MPs significantly reduced hatching success at BP3 “low” 1 mg MPs/L concentration compared to equivalent virgin MP or waterborne BP3 at 0.024 g/L. Waterborne BP3 exposure induced a non-linear dose-response. Exposure to BaP and PFOS coated MPs at 1 mg MP/L did not induce developmental toxicity at both concentrations. However, exposure to BaP-coated MP at 10 mg MP/L **reduced larvae head length**, total length and induced **morphological abnormalities** at hatching. Larvae exposed to 24 ng BP3/g of MP also displayed changes in head size at 1 and 10 mg/L and significant increase of deformities at 0.14 ng BP3 / g of MP. For BaP and BP3 exposure main deformities observed were yolk edemas and skeletal curvatures. Exposure to PFOS coated MPs did not alter swimming activity of fish larvae. However, exposure to BaP coated MPs induced hypoactivity of the fish in particular at the highest tested concentration. Exposure to BP3 coated MPs also altered fish behavior with hyperactivity reported both after OFFSET (dark period) and ONSET (light period) at 0.14 ng BP3 / g of MP as illustrated in figure 4B. No significant disruption of the swimming activity was observed at higher exposure concentration of BP3 (**Le Bihanic et al. submitted**).

- Studies with zebrafish ELS were used to test different types of polymers and associated pollutants, to reveal accumulation patterns, and to test the potential role of MP as vectors to zebrafish. An **interlaboratory study** was performed (involving Partners 4, 5, 11 and 14) to test suitability of acute test procedure (OECD TG 236). In all

experiments, no morphological effects were visible in fish embryos, indicating that there was **no acute toxicity**. CYP 1A induction, neurotoxicity and behavioral changes were observed; however, these changes were only minor, if compared to positive controls or water-borne exposure routes. MP exposure does not seem to induce strong acute toxicities in these sensitive models; yet, **chronic** long-term analyses such as **fish early life-stage tests** (OECD TG 210) supplemented by additional endpoints including monitoring of EROD activity, cyp1a gene transcription and swimming behavior of larvae are more promising as tools to test MP effects (Cormier et al. submitted).

- Bioassays with *P. microps* juveniles were carried out testing the effects of MP (0.184 mg/l), cephalixin (1.3, 2.5, 5 and 10 mg/l), their mixtures, and temperature rise ($20 \pm 1^\circ\text{C}$ and $25 \pm 1^\circ\text{C}$). MP induced mortality was 8 % at 20°C and 33 % at 25°C . Therefore, **temperature rise increased the toxicity of MP**. The EC_{50} of cephalixin alone was 3.8 mg/L (95% CI 3.00 – 4.74), increasing to 5.2 mg/L (CI 5.11 – 6.96) in the presence of MP. This indicates a slight **reduction in the toxicity of the antibiotic due to the MP particles** (Fonte et al. 2016).

- *P. microps* juveniles from two populations (Minho and Lima estuaries) were used to investigate the acute effects of 1-5 μm PE MP (0.14 mg/l), cadmium (3 to 50 mg/l), and their mixtures. Regarding mortality, no significant differences between populations or between the mortality induced by cadmium alone and in mixture with MP were found (overall 96h-LC50 = 8 mg/l). Cd alone and in mixtures with MP significantly decreased the predatory performance of juveniles from both populations (Minho estuary fish LOEC = 6 mg/l; Lima estuary fish LOEC = 3 mg/l). Evidences of antagonism (i.e. **reduction of Cd toxicity in the presence of MP**) were found (Miranda et al. submit).

- *P. microps* juveniles were also used (in collaboration with the Örebro University, the Antwerp University and the University of Vigo), to test the effects of LDPE (125 - 250 μm diameter), LDPE-PFOS and waterborne PFOS. **No effects on mortality** were found within the range of concentrations tested after 96 h of exposure to LDPE (NOEC = 80 mg/l), LDPE-PFOS (NOEC = 80 mg/l LDPE + 4.5 $\mu\text{g/l}$ PFOS) or to PFOS (NOEC = 4.5 $\mu\text{g/l}$). Concerning swimming performance, toxicity thresholds were the following. LDPE: LOEC = 40 mg/l; PFOS: LOEC = 1.1 $\mu\text{g/l}$; LDPE-PFOS: LOEC = 40 mg/l LDPE + 2.2 $\mu\text{g/l}$ PFOS. Therefore, **LDPE did not modify PFOS toxicity** (Vieira et al. in prep).

- Overall, the findings of the studies with *P. microps* juveniles indicated that different types of MP disrupt the behaviour of juveniles, with inhibition of the predatory performance and swimming velocity that are crucial to individual fitness and individual contribution to population growth rate. MP modulate the toxicity of other contaminants, whereas temperature and other biotic and abiotic factors influence MP toxicity and the toxicological interactions of MP with other environmental contaminants.

- Long-term dietary exposures to MP were realized both in marine medaka and in zebrafish and resulted in **reduction of female growth at 4.5 month** (decrease of 10 to 18% depending on MPs compared to control and on species) and a significant **decrease of the reproductive output** was observed when fish were exposed to MPs alone (PVC) or MPs spiked with either PFOS or BP3 (PE and PVC). Survival of offspring of marine medaka was not significantly modified whatever parental exposure but PE-BP3 larvae were smaller at 12 dpf. For zebrafish, a higher larval mortality was observed as well as behavioural defects in PVC-BP3 and Guadalupe collected MPs.

Discussion

Lethal or acute effects of MP at environmentally relevant concentrations have not been observed. MP can though modulate the effects of chemical pollutants in a way similar to natural particulate matter. Modeling these effects in the environment is complicated by the different properties of polymers, additives, sorbed contaminants, biofouling, weathering, etc.

Although testing was conducted also with PVC, PS and some other materials, EPHEMARE focused mainly on the most common polymer in the marine environment, PE. Lithner and co-workers (2011) ranked polymers based on monomer hazard classification, and PE was classified as one of the less hazardous polymers both for humans and the environment. However, whereas the present Project failed to show acute toxicity of PE particles to early life stages of zebrafish, slight alterations of the hatching rate were observed after exposure to MPs contaminated with BP3. A similar experiment using embryonic stage of the marine medaka (*Oryzias melastigma*) exposed to 10 mg/L microplastics spiked with the same concentration of BP3, revealed premature hatching and a **significant decrease in hatching rate** (Beiras et al. 2018). The same authors also demonstrated a direct contact of microplastics to villi at the surface of the medaka chorion, which may facilitate the uptake of toxicants. Although villi or similar structures do not

exist on the chorion of zebrafish embryos, **Batel et al. (2018)** were successful in demonstrating the transfer of benzo[a]pyrene from externally adhering MP particles to zebrafish embryos across the chorion. Regardless, the exposure time might represent another factor for the different responses between both species as it was shown for Japanese medaka and zebrafish (Perrichon et al. 2014). For marine medaka, the embryonic stage lasts until days 10 to 11 dpf (Inoue and Takei, 2002), whereas this period is restricted to 48 to 72 hpf in zebrafish, depending on the temperature (Kimmel et al. 1995). **Short term dietary exposures produced no modification** of any of the measured endpoints (survival, growth, larval photomotor response, EROD, cyp1a induction) in marine medaka. Conversely, **long-term dietary exposures** were realized both in marine medaka and in zebrafish and **resulted in reduction of female growth** at 4.5 month (decrease of 10 to 18% depending on MPs compared to control and on species) and a significant **decrease of the reproductive output** was observed when fish were exposed to MPs alone (PVC) or MPs spiked with either PFOS or BP3 (PE and PVC). Survival of offspring of marine medaka was not significantly modified whatever parental exposure but PE-BP3 larvae were smaller at 12 dpf.

Therefore, **future investigations on MP effects in fish starting from ELS should consider subchronic or even chronic exposures**. Long term growth from embryo to juveniles seemed to be a sensitive endpoint suitable to assess MP risk using fish.

WORKPACKAGE 4 – UNDERLYING MECHANISMS OF ACTION

*The following partners are involved: **Partner 1, Partner 2 (leader), Partner 3, Partner 4, Partner 5, Partner 7, Partner 10, Partner 9, Partner 11, Partner 12, Partner 14***

CELL LINES AND BACTERIA

- Sea bass brain cells (DLB-1) supplied by the University of Murcia and rainbow trout liver cells (RTL-W1) were exposed to DMSO extract of virgin PE 20-25 µm at 1 and 10 mg/L MPs and DMSO extract of the same MPs coated with B(a)P 15.07 µg/g, PFOS 46.06 µg/g and BP-3 0.062 µg/g at 1 and 10 mg/L. Cytotoxicity, EROD activity, ROS production and DNA damage were investigated. Three replicates of each exposure were performed. **No cytotoxicity** was observed on DLB-1 and RTLW1 cells exposed to different amount of virgin or coated MPs extracts. Exposure to DLB-1 to 1 and 10 mg/L of virgin or spiked microplastics induced a low ROS production. Only a significant **ROS production** was observed after exposure to 10 mg/L of **B(a)P coated microplastics**. A significant increase in **DNA damage** was detected in DLB-1 for both DMSO extract of virgin and spiked MPs at 1 and 10 mg/L. (**Partner 4, unpub.**).

- In RTL-W1 cell line, a significant increase of CYP1A1 was reported for virgin microplastics at 1 mg/L and 100 mg/L. No significant CYP1A1 induction was observed for DMSO extract of MP-PFOS or MP-BP-3. A dose-dependent **increase of EROD activity** was reported for **B(a)P-MP** for RTL-W1 cells as already reported in a recent publication (**Pannetier et al. 2019a**). Concerning the transfer of sorbed chemicals, the most hydrophobic EROD inducers such as TCDD or BkF did not seem to desorb from PE MP, which argues **against the role of MP to hypothetically increase pollutant uptake** into aquatic organisms. However, the role of environmentally relevant **surfactants** should not be overlooked (**Heinrich & Braunbeck, 2019a, b**). Investigation of other *in vitro* endpoints (genotoxicity, cytotoxicity, endocrine effects) proved non-feasible due to insufficient sensitivity of established assays.

- When pollutant-free MPs were added to cells exposed to pollutants *via* the medium, the particles reduced toxic effects, presumably *via* competition for the binding of pollutants to MPs; this effect was dependent on the pollutants' lipophilicities (**Heinrich & Braunbeck, 2019c**). Overall, results mostly agreed with integrative models on sorption of pollutants from and to MPs, the foundations of which – among other technical details – were summarized and discussed in a review article (**Heinrich & Braunbeck, 2019c**).

- Viability of sea bream or sea bass primary cultures of leucocytes (HKL), erythrocytes or cell lines (SAF-1 and DLB-1) was slightly affected by MP exposure *in vitro*. MP of virgin or oxidized PE larger than 20-25 µm were toxic for sea bass HKL only, but not to sea bream HKL, and never for cell lines or erythrocytes. Immunity of HKLs was not affected by either MP or MP with adsorbed PFOS. At gene level, MP failed to show inflammation but results suggest some level of cellular and **oxidative stress**. The effects of MP with PFOS were quite similar to those for only PFOS indicating **no additive toxicological effects for MP** (**Espinosa et al. 2018**).

- The *Salmonella typhimurium* strains TA98 and TA100 were used with or without exogenous metabolic activation (S9 induction by β -naphthoflavone/phenobarbital) to measure frameshift mutations and base pair substitutions, respectively. The extracts were diluted with DMSO on each plate to reach a concentration range between 62.5 $\mu\text{g/L}$ and 2 mg/L MPs equivalents per test medium. Each concentration was tested in triplicate. The bacteria were diluted in exposure medium and exposed to extracts, 2% DMSO serving as solvent control, and a positive control. Mixture sample was composed of a mixture of BaP and PFOS at a concentration range from 2.19 $\mu\text{g/mL}$ to 70 $\mu\text{g/mL}$ for BaP, and from 9.38 $\mu\text{g/mL}$ to 300 $\mu\text{g/mL}$ for PFOS. LDPE and PVC without pollutants did not induce mutagenicity in the Ames fluctuation assay. Test with the BaP in solution induced significant mutagenicity at 35 to 70 $\mu\text{g/mL}$, using TA100 with exogenous metabolic activation. Similar mutagenic effects were found testing the 1:1 mixture of PFOS and BaP after exposure to the same concentrations; however, using TA98 with exogenous metabolic activation. BaP is known to induce mutagenicity after exogenous metabolic activation as S9, and it is used as positive control in the OECD 471 guideline to investigate mutagenicity of chemicals. For all other samples, **no significant mutagenic effect was detected under any conditions tested**. Therefore, the Ames test seems to be inappropriate to determine genotoxicity of contaminated MPs and further investigations may consider other bioreporter systems to detect genotoxic effects (Partner 11, unpub.).

FILTER FEEDERS

- Filtration rates and the functionality of digestive gland were affected in any of CPF exposures, and consequently the physiological biomarker **scope for growth (SFG) was reduced** (Partner 2, in prep).

- Results on long-term effects on sediment-dwelling bivalves shows significant **decrease in energy fractions** in organisms exposed to MPs, with different patterns depending on the species, MP size and concentration (see WP3) (Bour et al. 2018a).

- Concerning enzymatic biomarkers, **Acetylcholinesterase (AChE) activity** in digestive gland and gills was significantly **inhibited** at all CPF treatments, disregarding exposure time. Levels of **GST** activity in the digestive gland in the three CPF treatments (CPF, MA+CPF and MP+CPF) after 7 days exposure were significantly **higher** than levels in treatments without CPF. However, after 21 days exposure, GST activity in the controls significantly increased, and differences with controls disappeared. For GST in gills, a significant increase in activity was observed in the MP, CPF and MA-CPF treatments after 7 days, compared to the MA control. When the nine biomarkers recorded are combined using the Integrated Biomarker Response (IBR) index a similar response in the three CPF treatments is initially observed (7 d), but after 21 d an enhanced response is observed in the MA+CPF and MP+CPF treatments only. In conclusion, AChE inhibition was similar in all CPF treatments disregarding the presence of particles. However, both MP and MA particles in CPF-exposed mussels produced in the long term an increase in biomarker response compared to waterborne exposure. Therefore, **MP seem to play a similar role than natural organic particles** as vectors of organics to marine organisms (Vidal Liñán et al. in prep.).

- Concerning immune and cytotoxicity, we found that most of the immune activities studied (protease, peroxidase and bactericide activities) increase upon exposure to MP, disregarding the presence of CPF. The effect went on upon 21 of exposure, probably due to the fact that MP kept in the tissues inducing these effects. High levels of immune activities impair the ability to trigger a proper immune response upon a pathogen. Moreover, high levels of oxidative enzymes, activities need to induce pathogen death, also establish a high oxidative status in the tissues, as shown with the biochemical biomarkers. These data point to **immune functions as good biomarker** of biological deregulation upon **MP exposures** (Espinosa et al. 2016).

- Exposure of mussels to MPs did not significantly affect lysosomal membrane stability (LMS) or phagocytosis responses. Significant differences were demonstrated after in vitro co-exposure to Hg (<250 $\mu\text{g}\cdot\text{L}^{-1}$ FSW) and MPs on LMS and phagocytosis for some cases, suggesting the existence of an enhanced toxicity when MPs and Hg are concurrent in the extracellular medium (Partner 2, in prep).

- Concerning endocrine disruption, in vertebrates vitellogenin (Vtg), a precursor of the egg yolk synthesized in the liver of oviparous females, is commonly used as a biomarker of effects. The presence of Vtg in male or juvenile individuals is interpreted as indicative of estrogenic endocrine disruption, such as that induced by certain plastic additives (e.g. bisphenol A). In molluscs Vtg was traditionally quantified using the colorimetric ALP technique. Partner 1 demonstrated that this method is unreliable and showed that **shotgun proteomics**, unlike the classical ALP method, **was suitable for the identification and quantification of Vtg** in marine mussels (Sánchez Marín et al. 2017).

- Results of BaP bioavailability and biomarkers analyses were further elaborated within a quantitative and software-assisted **weight of evidence (WOE)** model which, using weighted criteria, provided synthetic hazard indices, for both chemical and cellular results, before their final integration in a combined index. Elaboration of data summarized as Severe the hazard level for bioavailability in mussels exposed to BaP or BaP contaminated LDPE at all exposure periods. On the other hand, considering the magnitude of variations observed for various biomarkers, their statistical significance and the toxicological relevance of each biological endpoint, the model summarized the **hazard** for cellular responses as Slight for organisms exposed to BaP, virgin and contaminated LDPE, and Moderate only for organisms exposed to BaP after 14 days. The integration of hazard indices elaborated for bioavailability and biomarker data resulted in a combined WOE effect classified as Slight for mussels exposed to virgin LDPE and Major for those treated with both contaminated LDPE and BaP alone, without variations at different times of exposure; it is quite obvious that the final evaluation of the risk caused by virgin and contaminated LDPE was greatly influenced by the marked accumulation of BaP. The overall results suggest that **microplastics induce a slight cellular toxicity** under short-term (28 days) exposure conditions. However, **modulation of immune responses**, along with bioaccumulation of BaP, pose the still unexplored risk that these particles, under conditions of more chronic exposure (months to years) or **interacting with other stressors**, may provoke long-term, subtle effects on organisms' health status. (Pittura et al. 2018)

- Effects of virgin and BaP-spiked PE MPs on the infaunal bivalve *S. plana* were assessed. A battery of biomarkers was recorded in the gills and digestive gland of the clams upon exposure. Results indicate that the accumulation of smaller size MP (4-5 μm) was higher than on MPs with 20-25 μm , without or with BaP. Oxidative stress was size and tissue specific and antagonistic effects were detected for BaP contaminated MPs. Integrated biomarker approach (IBR version 2) and the Health Index revealed that the effect was more important for smaller MPs with higher IBR levels for the gills exposed to MPs contaminated with BaP and the opposite occur in the digestive gland. In addition, effects of 14 d exposure to 20 μm PS MP were also tested. **PS MP induced neurotoxicity (AChE inhibition) and oxidative stress** in *S. plana* (Ribeiro et al. 2017).

- A proteomic approach was carried out in the gills of *S. plana*. In virgin LDPE MPs 28 proteins were up-regulated and 12 down-regulated compared to controls. In BaP treated MPs, 46 proteins were up-regulated and 21 down-regulated in clam gills compared to uncontaminated clam gills indicating that **LDPE contaminated with BaP induce more protein changes than virgin LDPE**. The statistical analysis (Kruskal-Wallis) indicates that a significant statistical difference exists for 20 proteins between unexposed, virgin and BaP contaminated MPs conditions. (Bebiano et al. in prep).

- The ecotoxicological effects of exposure to LDPE microplastics with and without adsorbed BP-3 were investigated in the *Scrobicularia plana* clam. The clams were exposed for 14 days to 1 mg L^{-1} MP with a size range of 11–13 μm spiked with an environmentally relevant concentration of BP-3 (82 ng g^{-1}). A tissue specific response in **oxidative stress** was observed in clams in both virgin and BP-3 contaminated MP exposures. The integrated biomarkers index (IBR) and health index indicated that **gills were more affected than digestive gland**, and biomarkers alterations are apparently more related to the **toxicity of adsorbed chemicals** than LDPE itself. Synergistic effects of LDPE and adsorbed chemicals are not clear. (O'Donovan et al. submitted).

PREDATORS

- In the 96 h bioassays testing the effects of **MP, cephalixin**, their mixtures and temperature rise on *P. microps* juveniles (see WP3), the activity of the enzyme acetylcholinesterase (AChE), as neurotoxicity biomarker, and the levels of lipid peroxidation (LPO) as biomarker of lipid oxidative stress and damage, were determined. At 20°C, juveniles exposed to MP had 21 % of **AChE inhibition** but with no significant differences in relation to the control group and no significant differences in LPO levels. At 25°C, no significant differences in AChE or LPO levels among treatments were found. However, **increases of AChE activity (14 %) and LPO (72 %) in fish exposed to the MP-cephalexin mixture** containing the highest cephalixin concentration were found. The integrated data analysis (of the two bioassays) indicated significant interactions between MP and cephalixin for both biomarkers, and **significant interactions** among all stressors (i.e. temperature, MP and cephalixin) for LPO (Fonte et al. 2016).

- In the 96 h bioassays testing the effects of **MP and cadmium (Cd)** in *P. microps* juveniles from two populations (Minho and Lima estuaries), the activity of the enzymes AChE and glutathione S-transferases (GST) (involved in biotransformation), and LPO levels were determined. GST activity or LPO levels were not affected, but in fish from the Lima estuary **AChE was inhibited by MP** alone (LOEC = 0.14 mg/l) and by **MP-Cd mixtures** (LOEC = 6 $\text{mg/l Cd} + 0.14 \text{ mg/l MP}$) (Miranda et al. submitted).

- In the 96 h bioassay carried out to assess the effects of **LDPE**, **PFOS** and **LDPE-PFOS** on *P. microps* juveniles (in collaboration with Partners 1, 6 and 11), the activity of AChE, several antioxidant enzymes, and LPO levels were determined. **LDPE alone and LDPE-PFOS** caused significant **AChE inhibition** in fish brain (LDPE: LOEC = 80 mg/l; LDPE-PFOS: LOEC = 40 mg/l LDPE + 2.2 µg/l PFOS) but not PFOS alone (NOEC = 4.5 µg/l). Juveniles exposed to the **LDPE-PFOS** also showed alterations in antioxidant enzymes and significant increase of liver LPO levels (LOEC = 40 mg/l + 2.2 µg/l PFOS) indicating lipid **oxidative damage** (Vieira et al. in prep).

- *Dicentrarchus labrax* brain AChE activity and muscle cholinesterases (ChE) from bioassays carried out by the University of Murcia to test the effects of LDPE, PFOS, benzo[a]pyrene (BaP), and LDPE contaminated with PFOS or BaP. The results finding was that LDPE inhibited AChE of this species, thus causing neurotoxicity.

- Dietary intake of PVC or PE by gilthead sea bream or European sea bass resulted in histopathological alterations in the gut and liver. **No translocation** of MPs was observed. No important changes were observed in the immune response after exposure to MPs. Biochemical markers showed little alterations and suggested some liver damage and **chronic stress**. Gene expression failed to show inflammation but also suggested some cellular and **oxidative stress**. When PFOS or BP-3 were adsorbed to PE-MPs the effects were very similar to those found with MPs or the contaminants alone at histopathological, immunological and transcription levels (Espinosa et al. 2017).

- In zebrafish and marine medaka larvae acutely exposed to virgin or spiked (BaP-, PFOS- or BP3) MP or DMSO extracts, **no significant CYP1A1 induction** was reported. Exposure to 1 and 10 mg/L of virgin or spiked MP (particles and DMSO extracts) did not induce significantly DNA damage (LeBihanic et al. in prep).

- In marine medaka and zebrafish, brain, liver, gonad and blood samples have been collected at the end of long-term exposures. We observed **no evidence of genotoxicity** or oxidative stress, and **no induction of cyp1a** in the liver in marine medaka. In the case of MP-PFOS, we observed an **increase in circulating estradiol** concentration in both females and males. These results suggest that life-long exposure to MPs can trigger physiological disruptions in fish which may impair recruitment and population sustainability.

Discussion

Recent publications have documented low or little acute toxicity of virgin microplastics on marine organisms (Beiras et al., 2018; Jovanovic et al., 2018, Pannetier et al. 2019b) and especially on fish cells (Espinosa et al., 2018) but those studies mainly investigated the direct contact with virgin MP particles and not with the MP extracts. It has been documented that concentration levels of additive chemicals are relatively high in new plastics. Oliviero et al. (2019) showed that while **virgin PVC polymer did not result toxic** on *P. lividus* embryos, commercial PVC MP did exert an evident **toxic effect** due to leaching of **chemical additives** used to manufacture the plastic in the medium.

Since the release of pollutants from MPs is primarily driven by distribution equilibria (see WP1), the particular circumstances chosen in tests are crucial for the environmental relevance of the results obtained. Employing a cell culture with a medium supplemented with serum may reflect conditions similar to many organismal fluids such as blood or interstitial fluid, but the harsh conditions in the gut cannot be readily reproduced due to cytotoxic effects of low pH and high detergent concentrations. Desorption of pollutants potentially due to natural surfactants is relevant only for compounds with an intermediate lipophilicity ($\log K_{OW} < 6$), while such with higher lipophilicity are barely or not at all released, which agrees with thermodynamic models of the sorption mechanism. However, bringing these results into context with the findings on binding of pollutants, it has been shown that – under environmentally relevant conditions – only pollutants with high lipophilicity ($\log K_{OW} > 5.5$) have the potential for significant accumulation on MP, which indicates that **increased uptake of pollutants via MP is highly unlikely**. However, the **role of natural surfactants** present in the digestive tract remains to be assessed. In our model, detergents increased bioavailability of pollutants less than two-fold, but detergent concentrations were below those expected in organisms.

Inhibition of AChE activity, increased **oxidative stress**, and **behavioural disruption**, but no marked effect on bioaccumulation of sorbed chemicals, are common findings observed in fish exposed to MP (e.g. Barboza et al. 2018a,b). Therefore, rather than a risk by themselves, MP may contribute to general stress and interact with other environmental challenges. Thus, **effects of MP should be assessed within a holistic approach** encompassing the main anthropogenic pressures on the marine ecosystems.

WORKPACKAGE 5 – TROPHIC TRANSFER

The following partners are involved: **Partner 5 (leader), Partner 6, Partner 8, Partner 9, Partner 14**

- Trophic transfer was documented in various organisms indicating complex MP accumulation and transfer patterns in environmental food webs. Very small MPs are yet ingested by unicellular organisms, indicating an accumulation at the first level of food webs. Transfer was shown to be very sensitive in early life-stages of fish, and trophic transfer might represent a dominant MP exposure pathway throughout a whole life-cycle. Furthermore, it was shown **in adult fish stages that MPs can be taken up at very high amounts via food chains**; however, in all experiments, **no acutely toxic effects** were seen in fish. MPs were rapidly excreted with no further indications of stress or negative effects on fish health.

- Both in fish larvae and adult fish, pollutants associated with MPs were transferred to organisms, however, only at lower amounts compared to waterborne exposure routes. Likewise, long-term chronic feeding experiments with MPs and adult fish also showed no effect on fish health, and **histological analyses revealed no tissue transfer of any size of MPs**, despite reports in corresponding literature.

- As a conclusion, MPs were shown to be transferred in food chains at high rates; MPs were ingested by even unicellular organisms, filtered by zooplankton and transferred to higher trophic organisms, leading to a potential whole-life exposure beginning from the first feeding. However, **a negative apical impact was not detectable** in fish. Using these methods, a fish full life-cycle test with additional sensitive endpoints such as reproduction similar to that made in WP3 should be conducted to further test on potential MP effects transferred from preys in fish.

- The development and validation of protocols aimed to investigate MP trophic transfer, using larvae of the crustacean *Artemia* sp. and *Tigriopus fulvus* as preys, and the ephyrae stage of the jellyfish *Aurelia* sp. as predator, were assessed. Trophic transfer of MPs could only be demonstrated in the simplified two-level chain formed by copepod nauplii and jellyfish ephyrae. Therefore, it is important to study different preys to clarify MP trophic transfer. **Although MP transfer occurred from copepods to ephyrae**, no ecotoxicological effects were detected in terms of immobility and behavioural responses (frequency of pulsations), indicating that **a negative impact was not detectable** in this trophic chain.

- *Paramecium* efficiently transferred at very high amounts 1 - 5 µm particles only, whereas *Artemia* transferred both 1 - 5 and 10 - 20 µm particles. Zebrafish and marine medaka larvae were feeding from the very first day of active food intake (2/3 days post hatching) on *Paramecium* with ingested MPs and from 6/7 days post-hatch on *Artemia* nauplii with ingested MPs. Following MP ingestion, neither transfer to tissues nor accumulation of MPs could be detected with MPs egestion within hours following ingestion. Yet, BaP was efficiently transferred *via* trophic transfer, with higher exposure rates for small 1 - 5 µm MPs when ingested *via Paramecium*. Induction of cyp1a transcription revealed that BaP transferred from MPs was bioavailable in fish larvae. Given the dependence of the transfer rates on MP size and also re-desorption rates of chemicals depending of plastic type, **very small (1-20 µm) MP** and – most likely – nanoplastic particles might thus **pose a significant threat for both early life-stages and juveniles** of fish until adulthood with ongoing exposure *via* trophic transfer (**Batel et al. 2016**).

WORKPACKAGE 6 – FIELD VALIDATION

The following partners are involved: **Partner 1, Partner 6, Partner 7 (leader), Partner 10, Partner 12**

- In three years of the EPHEMARE project, Partner 7 has conducted sampling activities in the areas included in the project (North, Central, and South **Adriatic Sea**). Respect to the original EPHEMARE schedule, additional sampling activities were included. In summer 2017 UPM researchers participated to the international tour “LESS PLASTIC MORE MEDITERRANEAN” carried out by GREENPEACE in order to gather direct data on pollution from plastic in our seas and to inform public opinion about this environmental emerging issue. During the Italian tour of the campaign (started on June 24th from Genoa and finished in Ancona on 9th of July) different marine organisms were collected in collaboration with local fishermen, with the main aim to provide useful information on the microplastics distribution in the **Tyrrhenian Sea** biota: organisms were collected in the areas of Genoa, Talamone, Giglio and Ventotene Island, Naples.

- The organisms collected from 3 different sites of Adriatic Sea (**North, Central and South Adriatic**) have been processed, with a total of 259 fish representative of thirteen commercial species, and 216 invertebrate organisms belonging to twelve species. Concerning the vertebrates, 216 organisms were analysed and the 27% were positive to MP ingestion, 71% showed 1 particle and 29% 2 particles. The shape of isolated particles was largely dominated by **fragments** (43%), followed by films (33%), lines (17%) and pellets (7%); 26% of extracted microplastics were of the smaller size class between 0.01 and 0.1mm, 34% was between 0.1 and 0.5 mm, 7% between 0.5 and 1 mm, and 31% between 1 and 5 mm. The extraction of microplastics from invertebrate species highlighted their presence in 24.3% of the 259 examined specimens. The majority of the positive organisms (71%) showed **1 particle in the tissues**, while 21% contained **2 particles**, 5% contained 3 particles and 3% had 4 particles. The shape of the plastic particles was mostly characterized by fragments (59%), followed by lines (28%), films (9%) and pellets (3%); 34% of extracted microplastics exhibited the smaller size class (lower than 0.1mm), 30% was between 0.1 and 0.5 mm, 14% between 0.5 and 1 mm, and 21% between 1 and 5mm. μ FT-IR analyses on microplastics isolated from fish showed that the 48% of extracted polymers were composed by **PE**, followed by polypropylene (PP) (15%), polyamide (PA) (10%), polyester (7%); a lower frequency was observed for other 7 polymers (polyurethane (PU), ethylenevinyl acetate (EVA), PS, PVC, PET, Polyacrylic, polyisoprene). Regarding invertebrates, results indicate that PE, PP, polystyrene (PS) and polyester were the main extracted polymers with a frequency of 24, 21, 13 and 10% respectively. The remaining percentage was covered by other 13 plastic polymers including PVC, PET, PA, EVA, Polyisoprene, PU, epoxydic resin, polybutylene terephthalate, polyterpenic rubber, polyvinyl acetate (PVA), silicone and copolymers (Avio et al. 2018).

- Clustering the species in relation to the habitat in pelagic, benthopelagic and benthonic species, there is not a significant difference in terms of number of items per individual, but considering the frequency, results suggest that **pelagic and benthopelagic** species are more subject to ingestion respect to benthonic ones, in all the investigated areas. There is no a clear trend in terms of shape, while benthonic organisms ingest in general smaller particles of high density polymers (PVC, PA, polyesters) (Avio et al. 2018).

- The study on the Adriatic Sea shows that MPs ingestion is a widespread phenomenon: in the Center and South, microplastics ingestion is more frequent but not higher than in North, suggesting that **frequency can be a more appropriate index than number of ingested particles to monitor** the presence of microplastics in organisms. Particles in organisms from Central and Southern Adriatic are smaller than those ingested by the Northern organisms; hydrographic characteristics, such as runoff of northern rivers and waters circulation, might cause a higher accumulation of plastics debris in Central and South sector. Overall no differences were related to feeding strategies or trophic level.

- Concerning the **Tyrrhenian Sea**, on a total of 157 fish 46 specimens contained at least **1 particle**, thus revealing an overall frequency of approximately 30% of organisms positive to microplastics ingestion. Similar percentages (on a lower number of specimens) were obtained for invertebrates, with 13 organisms containing microplastics on a total of 44 analysed (29.5%) (Fig. 8). Overall these values of frequency are comparable to those described for the central and southern sectors of Adriatic Sea, with 25-30% of marine organisms containing microplastics. The shape was quite homogeneous being largely dominated by fragments (typically >55%), followed by lines and films which (together) prevailed only in organisms from Napoli. The most common size classes were those smaller than 0.5 mm which generally accounted for at least 60% of all ingested microplastics: once again, the only exception was for fish from Napoli, where 50% of extracted particles were between 1 and 5 mm and related to the elevated abundance of lines and films. The μ FT-IR results indicated a certain heterogeneity of the polymer composition, especially in those sites represented by a large number of analysed organisms (Genoa and Naples); **PE** is the most represented chemical typology followed by polyester, EVA, PA and PP, which might reflect the influence of both specific local activities, or hydrographic conditions.

- The campaign “Less Plastic more Mediterranean” gave us the possibility to investigate the variations of MP pollution in a well-defined case study related to the removal of the **wreck of the Costa Concordia** from the Giglio Island. Results obtained during the campaign in 2017 were compared with those already obtained in a previous work when fish had been collected in a reference site and near to the wreck in 2014 after the end of rescue operations, when the ship was finally towed away to Genova (Avio et al., 2017a). At that moment, benthic fish sampled in the wreck site exhibited a frequency of 95% of organisms with ingested microplastics and an average content of 4 ± 1.8 particles. Overall, these results suggest that, compared to the previous monitoring study performed in 2014, a

significant **improvement** has occurred in 2017 in the site impacted by the operations for the wreck removal, with a **lower number of organisms positive to the MPs ingestion** (35% versus 95%) and a lower number of ingested particles (1.16 ± 0.4 versus 4 ± 1.8 particles). More comparable, but still higher numbers have been obtained in 2014 also in the Giglio reference site, which had previously shown a frequency of 77% positive fish compared to 60% in 2017 and a content of 2.5 ± 2 particles compared to 1.66 ± 1.1 in 2017. In this case, the difference could be related to the different sampling period which, in 2014 occurred in mid-September, at the end of summer touristic season.

- Another extra activity has been carried in collaboration with the Race for Water Foundation when on board of the RACE FOR WATER trimaran. Personnel from Partners 1, 4 and 7 collected marine organisms from the **Caribbean Sea** around Guadalupe and Bermuda Islands. In addition to water and sediment samples, MP extraction has been performed in 30 specimens of *Holocentrus adscensionis* (Squirrel fish) collected at two different locations: Petit Bourg and Marie Galante. **More than 90%** in mass of the plastics found in beaches were **PE** (Pannetier et al. 2019a). Results suggest that the level of ingested MPs in those organisms was in line with evidence obtained from other investigated geographical areas, with a frequency of **33% of organisms ingested 1 MP**.

- The South Portugal survey sampled specimens of *Carcinus maenas*, *Mytilus galloprovincialis* and *Scrobicularia plana* from 2 locations, Praia de Faro and Tavira, in Ria Formosa lagoon, Portugal. In addition, the fish *Mullus surmuletus* was obtained from local fisherman. Around 150 samples were shipped to Partner 7 for microplastics identification and quantification analysis. No microplastics were detected in the crabs *Carcinus maenas* nor in the clams *S. plana*. Results indicate that in mussels percentage of MPs ranged from 7 to 17% with 1.5 to 2 items per mussel. Size ranged from 290 μm and the MP polymers found were PVA, EVA and PE. In *Mullus barbatus* fish MPs of 250 μm of PE were detected. In the gills of one crab a 280 μm of PP blue was detected but not in the hepatopancreas. An additional survey was carried out where only mussels *Mytilus galloprovincialis* were collected from seven sites of in the Southern Coast of Portugal. Around 40 mussels were collected. The percentage of MPs detected in mussels ranged from 60 to 100% with a range of 0.6 to 2.2 items per mussel. Types of plastic detected were PA and PP. Results are on process (Partner 10, in prep.).

- The Oslofjord survey sampled ten sediment-dwelling and epibenthic species representative of different habitat, feeding modes and trophic levels within the inner Oslofjord (Oslo, Norway). Analysed species included fish, bivalves, echinoderms, crustaceans and polychaetes. MPs were present in all the species with a frequency up to 65% of positive individuals for some species. In most cases, 1 or 2 MPs were found per individual, but some organisms contained up to 7 particles. A total of 8 polymer typologies were identified, with PE and PP being the most common ones. Occurrence of MPs in analysed biota is not influenced by organism habitat or trophic level, while characteristics and typology of polymers might be significantly affected by feeding mode of organisms (Bour et al. 2018b).

- On 1st June 2018, a Partnership Agreement between JPI-Oceans funded projects **BASEMAN** and **EPHEMARE** was signed for the standardization of MP monitoring techniques, where EPHEMARE took in charge the **standardization** of methods for **MP analysis in biota**. Thus, both consortia benefited from the **Training courses** organized by Partner 7 in Ancona in 2017 and 2018 on “Extraction and characterization of microplastics from marine organisms”.

WORKPACKAGE 7 – COMMUNICATION, DISEMINATION & STAKEHOLDER ENGAGEMENT

The following partners are involved: **Partner 1, Partner 2, Partner 3, Partner 4, Partner 5, Partner 6, Partner 7, Partner 8, Partner 9, Partner 10, Partner 11, Partner 12, Partner 13 (leader), Partner 14**

- EPHEMARE operational project website from January 2016 till November 2018: provided visitors general information about the project in addition to generating over 56 news items featuring the wide range of partner activities such as conferences, published papers, outreach events, public appearances, TV interviews)

- EPHEMARE outreach material such as four factsheets, project brochure and three yearly newsletters are available to download from the project website

- EPHEMARE social media outlets included dedicated Facebook and twitter accounts to effectively support the project science communication, project information distribution and stakeholder engagement

- IMPACT 2017 – International Microplastics Photo Contest, offered 1000 € in prizes, invited submissions in 2017 from January till end of May, and received 41 Entries from 5 countries, which subsequently featured in

exhibitions and outreach events about microplastics in our oceans e.g. Cork Science Festival, Ireland's Science Week, Dingle Oceanworld Aquarium, the Italian Festival of Science in Genoa and selected entries featured in the Ocean Plastics Lab travelling exhibition.

- EPHEMARE Stakeholder workshop took place on the 21st of February in 2018 in Antwerp, Belgium and involved 7 Industry representatives, 6 Representatives for Policy/Regulation, in addition to representations from JPIO, PLASTOX, WEATHER-MIC, R4W and the Ocean Plastics Lab. 11 EPHEMARE representatives actively engaged with workshop participants on current research findings, relevant existing and emerging EU regulation, policy and legal requirements, the direction future research should take and exploring of effective and implementable solution that can address adverse impacts of micro-plastics on marine life.

- A briefing note post EPHEMARE stakeholder workshop 'Preliminary Outcomes of the EPHEMARE Stakeholder Workshop Concerning the Direction of Future Microplastic Research', was disseminated to project stakeholders including the JPIO and national funding bodies.

- Dissemination of projects findings and outputs to a range of audiences such as public authorities relevant to European Directives, relevant private sector organizations and Industry, academia in related fields of expertise to support associated research as well as the general public to increase awareness of micro plastics and associated concerns.

CONCLUSIONS

- **Microplastic ingestion is widespread in marine organisms but they are also egested**

MPs of suitable size are **easily ingested**, but also **easily egested** by filter-feeders and fish. In mussels, they are excreted through feces (large ones) or **translocated** from digestive gland into the gills (< 2µm). Once experimental data fit uptake and distribution models, models predict that there is **no accumulation** of MPs in mussels exposed to environmental concentrations. This has been confirmed by field samples.

- **Microplastics can be transferred from preys to predators but do not biomagnify across aquatic food webs**

In experimental food chains MPs **are transferred** from preys to predators, but they are egested and **do not bioaccumulate in predators**.

- **Microplastics can vehicle contaminants but they are not a major source of exposure**

MPs of conventional polymers such as polyethylene **do not increase bioavailability** and effects of model chemicals **compared to natural particulate matter**. In fact accumulation in the mussel of a hydrophobic organic pesticide (chlorpyrifos) is higher in presence of microalgae than with microplastics.

- **Microplastics may cause oxidative stress, behavior and reproductive disruption, and modulate effects of other chemicals**

Despite lack of acute effects in standard tests, MPs can cause **oxidative stress, behavior** disruption and **reproductive effects** upon chronic exposure, elicit subtle **immune responses, modulate the cellular effects** of chemicals, and cause interaction between chemical and physical challenge.

- **EPHEMARE developed toxicity test methods specifically suitable for microplastics.**

EPHEMARE developed dedicated methods based on chronic effects and early life stages suitable to assess the toxicity of microplastics (e.g. **Beiras et al. 2018b**). Those methods can be useful for both **industry** to develop **new polymers** based on environmentally friendly materials, and **regulatory agencies** to set **environmental standards** for plastics.

- **“Microplastic” is a too generic term for environmental risk assessment**

The term “microplastic” is too broad, encompassing a variety of particles in terms of size, shape and chemical composition with very different ecotoxicological properties and interactions with biota. Considering size, for zooplankton and filter feeders the fraction more effectively ingested ranges between 2 and ca. 100 µm. Generally, **the smaller the microplastics the more ecotoxicological risk they may pose.**

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LIST OF EPHEMARE CONFERENCE PRESENTATIONS

Albentosa, M. Una nueva amenaza al medio marino: Las Basuras Marinas. Presentation of the Project to the students of the Institute Manuel Tárrega of San Pedro the Pinatar, March 27, 2017.

Albentosa, M. EPHEMARE Project: effects of microplastics in marine ecosystems. Presentation of the project to a delegation of Ministry of the Environment from Turkey, 16 participants. 7-10 November 2016

Albentosa, M., León-León, V.M., Bellas, J., Santos, J., Campillo, J.A. 2017. Comparative exposure of mussels to chlorpyrifos through microplastic and microalgal particles. 16th International Conference on Chemistry and the Environment (ICCE), Oslo (Norway) from the 18th to the 21st of June 2017.

Albentosa M., C.G. Avio, A. Batel, M. Bebianno, M.L. Begout, R. Beiras, J. Bellas, R. Blust, T. Braunbeck, J. Cachot, C.C. Carteny, B. Cormier, X. Cousin, A. Cuesta, M.A. Esteban, M. Faimali, F. Garaventa, S. Gorbi, L. Guilhermino, K. Hylland, S. Keiter, K. Kopke, B. Morin, L. Pittura, F. Regoli, R.M. Town. 2019. Ecotoxicological effects of microplastics in marine ecosystems: insights from the EPHEMARE project. 20th International Symposium on Pollutant Responses In Marine Organisms (PRIMO20). Charleston, SC USA, May 19 – 22 May. (oral presentation)

Avio C.G., Fattorini D., Di Carlo M., Gorbi S., Regoli F. Distribution and ecotoxicological effects of microplastics in marine organisms. Sixth International Marine Debris Conference, 12-16 March. San Diego, California, USA 2018. (oral presentation)

Avio C.G., Pittura L., Fattorini D., Di Carlo M., Gorbi S., Regoli F. Levels and distribution of microplastics along Mediterranean marine species. Euromarine workshop “Modelling ocean plastics litter in a changing climate: challenges and mitigations. 26-27 April, Oporto, Portugal. 2018. (oral presentation)

Avio C.G., Pittura L., Gorbi S., Abel S.M., Amorello S., Regoli F. Distribution of microplastics in Adriatic food webs. International Conference on Microplastic Pollution in the Mediterranean Sea (μ MED), Capri, Italy, 26-29 September 2017. (oral presentation)

Avio C.G., Cardelli L., Berlino M., Gorbi S., Pittura L., Benedetti M., Regoli F. Presence, distribution and characterization of microplastics in marine organisms. 30th ESCPB congress, New European Society of Comparative Physiology and Biochemistry, 4-7 September, Barcelona, Spain. 2016. (oral presentation)

Avio C.G., Gorbi S., Berlino M., Regoli F. Presence, distribution and characterization of microplastics in commercial organisms from Adriatic Sea. International Conference: "Fate and Impact of Microplastics in Marine Ecosystems From the Coastline to the Open Sea". 25-27 May, Lanzarote, Canary Islands, Spain. 2016. (Oral presentation)

Avio C.G., Pittura L, Gorbi S., Marino G., Keiter S., Cormier B, Regoli F. Distribution and ecotoxicological effects of microplastics in marine organisms from the Adriatic Sea. MICRO2018 Fate and Impact of Microplastics: Knowledge, action and solutions, 19-23 November, Lanzarote, Spain. 2018. (oral presentation)

Avio C.G., Gorbi S., Cardelli L., Pellegrini D., Regoli F. Extraction and characterization of microplastics in marine organisms sampled at the Giglio island after the costa Concordia Wreck. International Conference: "Fate and Impact of Microplastics in Marine Ecosystems From the Coastline to the Open Sea". 25-27 May Lanzarote, Canary Islands, Spain. 2016. (oral presentation).

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Batel, A., Borchert, F., Reinwald, Braunbeck, T. Alternative pathways of microplastic exposure – microplastics and associated POPs on zebrafish gills (*Danio rerio*) and zebrafish eggs. SETAC Europe 26th Annual Meeting, Nantes. (Poster presentation).

Batel, A., Scherer, M., Linti, F., Braunbeck, T., Trophic transfer of microplastics and associated POPs. SETAC Europe 26th Annual Meeting, Nantes. (Oral presentation).

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Bebiano, M. J., 2018. WP 3 & WP 4 - Assess the ecotoxicological effects of exposure to LDPE, with and without adsorbed chemicals BaP, BP3 and PFOS in the clam *Scrobicularia plana*, 4th EPHEMARE Meeting Lanzarote, Spain, 19th November (Oral presentation)

Bebiano, M. J., O'Donovan, S., Ribeiro, F., Rodrigues, A. R., Abel, S., Fonseca, T. G., Carteny, C. C., Blust, R., Cormier, B., Keiter, S. S., Mestre, N. C. 2018. Are microplastics facilitating the accumulation of organic pollutants in benthic bivalves? Micro2018 International Conference, Fate and Impacts of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain, 19-23 November (Oral presentation).

Beiras R., Sara Pérez, Tania Tato, Noelia Salgueiro-González, E. Concha-Graña, S. Muniategui-Lorenzo . Do microplastics really act as vectors of organic pollutants to marine zooplankton? Experiments with pyrene and nonylphenol spiked polyethylene using *Paracentrotus lividus* larvae. (Oral presentation). SETAC Europe 27th annual meeting. Brussels. 7-11 May 2017.

Beiras R., L. Mantilla-Aldana, T. Tato, P. Campoy, L. Vidal-Liñán. Bioassays stress the ecotoxicological differences between polymers and plastics additives in the marine environment. (Poster) SETAC Europe 28th annual meeting. Rome. 13-17 May 2018.

Beiras R., T. Tato, S. González, L. Vidal-Liñán, P. Sánchez-Marin. Toxicity of the biocide and plastic additive Triclosan to marine organisms: environmental risk assessment (Poster) SETAC Europe 26th annual meeting. Nantes, France. 22-26 may 2016.

Beiras, R., S. Keiter, R. Blust, X. Cousin, M. Albentosa, T. Braunbeck, F. Regoli, K. Kopke. 2018. Ecotoxicological effects of microplastics in marine ecosystems (EPHEMARE); Synthesis of results. MICRO 2018. Fate and impact of microplastics: Knowledge, actions and solutions, Lanzarote, Spain, 19-23 November 2018. (Oral presentation).

Beiras, R., M. Oliviero, T. Tato. A standard protocol for the assessment of microplastics toxicity using planktonic marine organisms. MICRO 2018, Arrecife (Canary Islands). 19-23 November 2018. Cabildo de Lanzarote, UVSQ, Plymouth University, AWI, Cornell University, Università di Siena, Marine Sciences for Society. (Oral presentation).

Bellas, J., D. Rial, L. Vidal-Liñán, J.A. Campillo, V. M. León, M. Albentosa. 2018. Are microplastics a real environmental threat? Impact on marine invertebrate embryos and larvae, ISMS, International Symposium on Marine Sciences. Vigo, 20-22 junio 2018

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- Blust, R. 2018. Plastic in de oceaan - Wat is het probleem en wat kunnen we doen. Ocean Plastics Lab public debate, Brussels, Belgium, 17 April 2018 (Oral presentation)
- Cachot, J.), UHei (Batel, A.; Heinrich, T.; Braunbeck, T.), UMU (Espinosa, C.; Esteban, M.A.; Cuesta, A.), UiO (Bour, A.; Hylland, K.), UVI (González, S., Beiras, R.). 2017. Toxicity assessment, WP3 Organism level, WP4 Cellular and Molecular levels – No feeders, planktonic and benthic organisms. 2nd JPI Oceans Conference, Lisbon 26th October.
- Campoy-López P, Messinetti S, Mantilla-Aldana L, Ricardo Beiras. Possible estrogenic effects of ethynil estradiol on gonadal maturation in edible sea-urchin *Paracentrotus lividus* L. (Poster) VI International Symposium on Marine Sciences. Vigo. 20-22 June 2018. Universidade de Vigo.
- Campoy López P., E. Pereira-Pinto, L. Mantilla-Aldana, R. Beiras. Estrogenic effects of an organophosphorous Flame Retardant (TCPP) on Edible Sea Urchin "*Paracentrotus lividus*". (Poster) SETAC Europe 28th annual meeting. Rome. 13-17 May 2018.
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- Catarci Carteny C, Town RM, Blust R. 2018. Furthering the dynamic uptake model for microplastics in blue and Mediterranean mussels. MICRO 2018 International Conference, Fate and Impacts of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain, 19-23 November 2018. (Oral presentation).
- Catarci Carteny, C., Blust R. 2018. EPHEMARE's activities, Aquimer CONFERENCE on OCEAN MICROPLASTICS ISSUE AND THE BIO-BASED PLASTICS SOLUTION, Brussels, Belgium, 27 June 2018. (Oral presentation)
- CIIMAR (Vieira, L; Guilhermino, L.), IFREMER (Crebassa, J-C.; Bégout, M.-L; Cousin, X.), ORU (Cormier, B.; Keiter, S.), UBor (Le Bihanic, F.; Cachot, J.), UHei (Batel, A.; reinwald, H.; Braunbeck, T.), UMur (Espinosa, Esteban, M.A.; Cuesta, A.) 2017. Toxicity assessment, WP3 Organism level, WP4 Cellular and Molecular levels – Fish. 2nd JPI Oceans Conference, Lisbon 26th October.
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- Cormier B, Keiter SH, Engwall M, Larsson M, Yeung L, Cachot J (2018). Effets toxiques de microplastiques industriels primaires, en tant que vecteur de polluants modèles, utilisant le poisson zèbre (*Danio rerio*). Polymères et Océans, Montpellier, France.
- Cormier B, Le Bihanic F, Keiter SH, Engwall M, Larsson M, Yeung L, Clérandeau C, Crebassa JC, Morin B, Bégout ML, Cousin X, Cachot J (2018). EPHEMARE : Méthodologie pour étudier les effets toxiques des microplastiques sur les téléostéens. ECOBIM, 22-25 May 2018, Bordeaux, France. Poster.
- Cormier B, Le Bihanic F, Joassard L, Keiter S, Cachot J, Bégout ML, Cousin X (2019) Conséquences physiologiques d'une exposition chronique de poissons à des microplastiques vierges ou contaminés. Polymères et Océans, June24-26, 2019, Créteil, France.
- Cormier B., Larsson M., Yeung L., Clérandeau C., Kärrman A., Morin B., Magnus E., Bégout M-L., Cousin X., Cachot J., Keiter S., 2018. Sorption of model pollutants on microplastics and toxicity assessment using early life stage of zebrafish (*Danio rerio*). SETAC Europe, 28th annual meeting, Rome, May 14-17, 2018 (oral)

- Cormier B., Larsson Maria, Yeung Leo, Clérandeau Christelle, Kärrman Anna, Morin Bénédicte, Engwall Magnus, Bégout Marie-Laure, Cousin Xavier, Cachot Jérôme and Keiter Steffen H., 2018. Sorption of model pollutants on microplastics and toxicity assessment using early life stage of zebrafish (*Danio rerio*). SETAC, Rome, Italy.
- Cormier B, Le Bihanic F, Keiter SH, Engwall M, Larsson M, Yeung L, Clérandeau C, Crebassa JC, Morin B, Bégout ML, Cousin X, Cachot J (2018). Toxicité comparée des microplastiques pour les premiers stades de développement de deux poissons modèles. SEFA, Montpellier, France.
- Cormier B., Larsson M., Yeung L., Clérandeau C., Kärrman A., Morin B., Engwall M., Bégout M.-L., Cousin X., Keiter S., Cachot J. Toxic effects of industrial primary microplastics, spiked with model pollutants, using zebrafish (*Danio rerio*). Polymères et Océans, Montpellier, 15-17 January 2018, France (oral)
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Ephemare. Technical report of microtox test inter-laboratory comparison between the University of Algarve team, CNR and the University of Vigo.

Ephemare. Preliminary Results and Impacts of EPHEMARE For workshop Handout: <http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/About/Antwerp%20Factsheet%20Final.pdf>

EPHEMARE Educational Factsheet: <http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/About/EducationalFactsheet%2020180403.pdf>

EPHEMARE Microplastic Poster for public events: http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/About/MicroplasticsPoster_General.pdf

EPHEMARE contributed entries from the IMPACT 2017 Photo Competition to the Ocean Plastics Lab which exhibited in Turin (Italy), Paris (France), Brussels (Belgium), Washington D.C (USA), Ottawa (Canada) and Berlin (Germany)

EPHEMARE researcher Steffen Keiter of Örebro University presented to King Carl XVI Gustav of Sweden on microplastics and the results of the EPHEMARE project.

EPHEMARE scientists took part in the 'Less Plastic more Mediterranean' mission with Greenpeace in 2017.

MaREI UCC researchers exhibited the EPHEMARE IMPACT 2017 International Microplastics Photo Competition as part of the Science of Sustainability event during the Cork Science Festival, Ireland in 2017.

Vidal Touza, V., Beiras, R. 2018. Efectos dos microplásticos nos ecossistemas mariños. Cerna 78:20-22.

LIST OF EPHEMARE WORKSHOPS, STAKEHOLDER MEETINGS (ETC) (BY APPROX CHRONOLOGICAL ORDER)

Organization of **Ephemare Kick-off Meeting** in Murcia (Spain), 19-20 January 2016, including talks with stakeholders.

The laboratory of Ecotoxicology of Marche Polytechnic University (UPM) participated in the event "**TIPICITÀ IN BLU**", which was held in Ancona in 2016 and included several teaching laboratories were organised for students, to disseminate information about the Adriatic Sea (ADRIATICOLAB).

EPHEMARE researchers from CNR ISMAR and Marche Polytechnic University participated in the **Italian Festival of Science** (October 27th to November 6th) in **2016**, in Genoa and organised an educational laboratory titled: "Can you tell me where plastics end up?" and a public conference "An ocean of microplastics: invisible signs into sea future".

MICROPLÁSTICOS 2016 - 1^o **Congresso Português sobre Microplásticos**: Contaminação Ambiental por Microplásticos e suas Implicações para a Saúde dos Ecossistemas, animal e Humana. Porto, 19 and 20 December 2016. (also in the scope of two other projects) <https://www4.icbas.up.pt/microplasticos16/>

1st **training course** within the European project EPHEMARE, entitled "Extraction and characterization of microplastics from marine organisms" 27-29 March 2017, Ancona, Italy.

Avio CG, Pittura L, Gorbi S, Regoli F. Technical Document: Training course on "Extraction and characterization of microplastics from marine organisms" Ancona, 27-29 March 2017, 25 pp.

Organization of **2nd Ephemare Annual Meeting** (2017) in Genoa (Italy), 30-31 March 2017

EPHEMARE scientists from the University of Heidelberg gave a **public talk** about microplastics on board the MS Wissenschaft in 2017.

At the PRIMO meeting "Pollutant Responses in Marine Organisms" in Japan, 30 June – 3 July 2017 Lucia Pittura PhD Student at Polytechnic University of Marche, received the "**Student Presentation Award**" sponsored by Society for Environmental Toxicology & Chemistry (SETAC) for the oral presentation "PRESENCE AND ECOTOXICOLOGICAL RISKS OF MICROPLASTICS IN MEDITERRANEAN ORGANISMS". Lucia presented data derived from the EPHEMARE Project.

EPHEMARE researchers participated in the '**Race for Water**' **Odyssey** in 2017, harvesting, sampling and analysing microplastics in waters around Bermuda (July) and Guadeloupe (October) and interacted with local stakeholders and residents of both Bermuda and Guadeloupe.

Kathrin Kopke (MaREI UCC) presented EPHEMARE at the **Ocean Teacher Global Academy** (OTGA) course: Ocean Literacy and Story Telling Maps Training Course in Santa Marta, Columbia in September 2017.

Keiter SH, Albentosa M, Avio CG, Batel A, Bebianno M, Begout ML, Beiras R, Bellas J, Blust R, Braunbeck T, Cachot J, Carteny CC, Cormier B, Cousin X, Cuesta A, Faimali M, Garaventa F, Gorbi S, Guilhermino L, Hylland K, Kopke K, Morin B, Pittura L, Regoli F,

Town R (2017): EU-projektet EPHEMARE- ekotoxikologiska effekter av mikroplaster i marina ekosystem. **Renare Mark Symposium – Framtida problem**, Östersund, Sweden.

EPHEMARE researchers from CNR ISMAR and Marche Polytechnic University participated in the **Italian Festival of Science** in 2017, in Genoa which included an educational laboratory 'Plastoc contacts' and organized a public conference about marine litter and microplastics 'Aliens are among us'.

Dr Ricardo Beiras (Ephemare coordinator) was invited by the EC (**DG Research**) to the **Blue Growth Research and Innovation - Horizon 2020 Societal Challenge 2 Information Week**. He presented the project and took part in the discussions. Brussels, Nov 2017.

Organization of the **3rd Ephemare Annual Meeting** and the **EPHEMARE Stakeholder workshop**, February 2018, Antwerp Belgium, facilitated and implemented by MaREI UCC.

EPHEMARE researchers Camilla Carteny (University of Antwerp), Marco Faimali (ISMAR), Kathrin Kopke and Sophie Power (University College Cork) visited the **Ocean Plastic Lab** exhibition in Brussels, and directly engage with visitors at Container 3, which exhibits the EPHEMARE contributions in April 2018.

Carteny, C.C. 2018. Plastic pollution in our ocean - why is it a problem and what be done about it. **Ocean Plastics Lab public debate**, Brussels, Belgium, 11 April 2018 (Oral presentation)

Carteny, C.C. 2018. Plastique dans l'océan - quel est le problème et que pouvons-nous faire. **Ocean Plastics Lab public debate**, Brussels, Belgium, 18 April 2018 (Oral presentation)

Dr Ricardo Beiras (UVigo), Dr Lucia Guilhermino (CIIMAR) and Dr Francesco Regoli (Marche Polytechnic University) were presenting EPHEMARE at the **EuroMarine Foresight Workshop**: 'Modelling Ocean Plastics Litter in a Changing Climate: Challenges and Mitigations', providing a forum to discuss the issue of plastic pollution in the marine environment in April 2018

EPHEMARE researchers Camilla Carteny (University of Antwerp), Marco Faimali (ISMAR), Kathrin Kopke and Sophie Power (University College Cork) attended the **SEARICA conference Mission Ocean: Science and Innovation for a Healthy Ocean** on the 10th of April 2018 at the **European Parliament**. The conference focused on discussing the issue of marine plastic pollution with international and cross-sectorial guests and coincided with the Ocean Plastics Lab visit to the European Parliament as part of its travelling exhibition.

Participation to **Poly Talk 2018** (Malta, 26-27 April 2018) organized by Plastics Europe to engage with stakeholders to prevent marine litter in the world. Marco Faimali (IAS-CNR) participated to a panel discussion on "Improving efficiency and accelerating innovation to increase circularity-research and innovation"

Dr Marco Faimali, EPHEMARE researcher at CNR-ISMAR attended **PolyTalk 2018** hosted in St. Julians, Malta and was part of a panel discussion in the session 'Improving resource efficiency and accelerating innovation to increase circularity - research and innovation'.

EPHEMARE researchers Jerome Cachot and Xavier Cousin joined artist Roman Kroke to hosted a **student workshop** titled "Microplastics and Medusae: expeditions into H2O." in May 2018.

S. Keiter was invited on behalf of the EPHEMARE consortium to the **ECHA Stakeholder Workshop on Microplastic**, held in Helsinki (Finland) on 30-31 May 2018, where he presented: Keiter SH, Albentosa M, Avio CG, Batel A, Bebianno M, Begout ML, Beiras R, Bellas J, Blust R, Braunbeck T, Cachot J, Carteny CC, Cormier B, Cousin X, Cuesta A, Faimali M, Garaventa F, Gorbi S, Guilhermino L, Hylland K, Kopke K, Morin B, Pittura L, Regoli F, Town R (2018): Knowledge gaps for the ecotoxicological assessment and modelling of microplastics in the sea – Conclusions of the EPHEMARE project.

EPHEMARE researchers from the Spanish Oceanographic Institute (Oceanographic Centre of Murcia) gave a **public talk** about the problem of plastics in the Calblanque Region Park on the 30th of June, 2018 that included a practical session held at a local beach including collection and the study of the plastics found in the sand of the beach.

EPHEMARE representative Camilla C. Carteny gave a **presentation to middle and high-schoolers** demonstrating what microplastics are and how they can effect the marine environment at the by UNESCO, the IOC, and the Surfrider Foundation organized stellar event for World Oceans Day on 8 June where over 200 students attended.

EPHEMARE has given several informative and entertaining seminars about the project at The Oceanographic Center of Murcia (COMU). The presentations took the format of a gastro-scientific seminar to go along with COMU's Friday 'tapas' series. These events are part of the current "**Pint of Science**" initiative, designed to disseminate scientific work to science and beer lovers. June and July 2018

2nd **training course** within the EPHEMARE-BASEMAN European projects, entitled "Extraction and characterization of microplastics from marine organisms" 24-26 July 2018, Ancona, Italy. The event was coordinated by C. G. Avio, L. Pittura, S. Gorbi, and F. Regoli, and with the technical support of Perkin Elmer Italia.

EPHEMARE researchers from CNR-ISMAR attended **La Festa del Mare** in Rapallo, Italy August, 2018 with an educational laboratory on the life cycle of plastic.

EPHEMARE researchers from CNR-ISMAR participated in an **educational laboratory** (title: "Plastic Changes") at FdS2018 in Genova, Italy, from 25 October - 5 November.

EPHEMARE researchers from CNR-ISMAR were present at **Maker Faire 2018** in Rome, Italy with a stand dedicated to explain the problem of marine litter in the environment and the Ephemare project.

EPHEMARE researchers from University of Antwerp were invited as speakers and panelists at the consecutive 'Plastic Pollution in our Oceans' series of talks at the **Brussels Royal Museum for Natural Sciences**. The three talks were delivered in English, French and Dutch, with researchers Camilla Carteny and Professor Ronny Blust (both University of Antwerp) representing the EPHEMARE project and their research to the public.

R. Beiras, EPHEMARE coordinator, was invited to the MICRO 2018 side event: **ECHA meeting on REACH restriction on intentional use of microplastics** in the EU, organized by P. Simpson (ECHA) in Lanzarote, Nov 2018.

Putting the 'Work' back into Workshop – the **EPHEMARE stakeholder workshop** on microplastics in the marine environment. Kopke, K., Power, S., and Carteny, C.C. (In preparation)

LIST OF EPHEMARE PUBLIC ACTIVITIES - FLYERS, BROCHURE, VIDEO/FILM

Bellas, J. *El IEO y la Universidad de Vigo estudian el efecto de los 'microplásticos' en la ría*. Newspaper: El Faro de Vigo, 18 January 2016.

Bellas, J. *Vivir o mar*. TV: program, Televisión de Galicia, 7 February 2016.

Bellas, J. *Micropartículas de plástico: cuando tu pasta de dientes es un peligro para el medio ambiente*. Newspaper: El Mundo, 15 February 2016.

Bellas, J. *España Directo*. TV: program. Televisión Española. 8 June 2016.

Bellas, J. *Los microplásticos son ya parte del ecosistema*. Newspaper: ABC 14 October 2016.

Bellas, J. *Plástico, La nueva especie invasora*. Newspaper: Faro de Vigo, 21 May 2017.

Bellas, J. *La Era de Plástico*. TV: program, 'Equipo Investigación', La Sexta, 27 May 2017.

Bellas, J. *La sexta noticias*. TV: News program. La Sexta, 22 June 2017.

Albentosa, M. *Microesferas presentes en la pasta de dientes, detergentes, cremas...un peligro para el medio ambiente y para las personas*. TV: La Sexta News, 6 September 2017. https://www.lasexta.com/noticias/ciencia-tecnologia/pasta-de-dientes-detergentes-cremas-todos-tienen-microesferas-de-plastico-un-peligro-para-elmedioambiente_2017090359ac04f00cf27a5b1bd0ac0d.html

Campillo, J.A., Santos-Echeandía, J., Albentosa, M. *Los microplásticos y su interacción con metales pesados en el medio ambiente*. Newspaper: La Verdad, 23 January, 2018.

Beiras R. Interview and documentary Video for Galicia webcite (GCIencia) "A Sea of plastic": 2016. <http://www.ecotox.gal/es/un-mar-de-pl%C3%A1stico-0>

Beiras R. Interview for Spanish Radio (RNE-Radio 5) 2016: "Españoles en la Mar" Programme – "EPHEMARE Project"

Beiras R. Interview and Documentary Film for Spanish TV (La Sexta): "La Era del Plástico". 2018. https://www.lasexta.com/programas/equipo-investigacion/avances/podriamos-vivir-sin-plastico-este-viernes-en-equipo-de-investigacion_201705235924b0410cf20292e7da75f2.html

Beiras R. Interview and Documentary Film for Spanish TV (La Cuatro): "En el punto de mira". 2018. https://www.cuatro.com/enelpuntodemira/punto-mira-completo-HD_2_2625030190.htm

Beiras R. Interview for Spanish Radio (RNE-Radio 5) 2018: "Españoles en la Mar" Programme – "Lanzarote MICRO Congress". <http://www.rtve.es/alacarta/audios/espanoles-en-la-mar/espanoles-mar-micro-2018-23-11-18/4854099/>

Beiras R. Interview for Spanish Radio and Documentary (Cadena COPE) 2019. <https://www.cope.es/ultimo-grito-contra-el-plastico>

Beiras R. Report in the newspaper La Voz de Galicia, Especial día del medio ambiente, 5th June 2019, p. 3. https://www.lavozdeg Galicia.es/noticia/biodiversa/2019/06/05/impacto-poco-treinta-anos-habra-plastico-oceanos-peces/0003_201906G5E2992.htm

Bellas, J. *Herrera en COPE*. Radio: program, COPE, 29 May 2018.

Bellas, J. *El gran cambio global de los océanos*. Newspaper: La Voz de Galicia, 8 June 2018.

Bellas, J. *Los microplásticos colonizan las Cíes*. Newspaper: Faro de Vigo, 9 December 2018.

Martínez-Gómez, C. *Científicos de la Región avanzan en la batalla contra los microplásticos*. Newspaper: La Verdad, 21 January 2019.

Bellas, J. *Basura en España: un problema al cubo*. TV: program, 'La Sexta columna', La Sexta, 29 January 2019.

Blust, R. Drijvende vuilniskar gaat strijd aan met plasticsoep, *Gazet van Antwerpen*, 9 September 2018

Carteny, C.C. Microplastics zijn overal: tegen 2050 zal er wellicht meer plastic dan vis in de oceanen zitten, *vrt NWS*, 22 May 2018

We moeten onze oceanen nú beschermen. *UAntwerp magazine*, December 2017.

<https://www.uantwerpen.be/images/online/magazine/MUA26/files/assets/basic-html/index.html#42>

Detailed Project Information: http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/News/EPHEMARE_SummaryFactsheets_edited.pdf

Dissemination activity at SHARPER 2018, European Researchers' Night, Ancona, 28 settembre 2018.

Dissemination activity at Inauguration of the Academic Year 2018/19, Università Politecnica delle Marche: inaugural address by Prof.ssa Stefania Gorbi entitled "Pericoli emergenti e pericoli emersi, nuove minacce per la salute del mare". <https://www.youtube.com/watch?v=sNgNiIS21qU&index=3&list=PL0fKordpLTjLsYU0A4pph6ZiHdW8FukOE>

Dissemination activity at the CNR Workshop "FATTI DI PLASTICA" titled "Microplastiche negli organismi marini: distribuzione, effetti e rischi lungo le reti trofiche", Roma, 5 June 2018

Dissemination activity at the workshop "Allarme blu. Oceani: il futuro da salvare" entitled: "Plastiche e microplastiche negli oceani: da contaminanti emergenti ad emergenza ambientale", Padova University, 8 June 2018.

Dissemination activity at SHARPER 2017, European Researchers' Night, Ancona, 29 settembre 2017.

Dissemination activity at SHARPER 2016, European Researchers' Night, Ancona, Italy, 30 September 2016.

Dissemination activity at Italian Festival of Science, Genova, Italy, 27th October.

EPHEMARE researcher Marina Albentosa from the Spanish Institute of Oceanography featured on local TV channel TV7 on the programme 'Country Journal', on how plastics in the sea affect marine fauna. <http://webtv.7tvregiondemurcia.es/divulgativos/diario-del-campo/2018/martes-10-de-abril/>

EPHEMARE's Camilla C. Carteny, University of Antwerp was interviewed by Belgian newscast (RTL) highlighting EPHEMARE and explaining the science of the OPL exhibitions which exhibited the pictures from the EPHEMARE organised contest in April 2018.

EPHEMARE partners at UPM attended the annual academic inauguration 2018/2019 at Polytechnic University Ancona on 26 March. The discussion was framed around the topic "Pericoli emergenti e pericoli emersi... nuove minacce per la salute del mare."
<https://www.youtube.com/watch?v=sNgNiIS21qU>

EPHEMARE featured in an article in the regional newspaper La Verdad, published on January 23, 2018. EPHEMARE scientists Juan Santos-Echeandía and Juan A. Campillo spoke about metal pollution in the Mediterranean, while Marina Albentosa comments on the studies that are being carried out under the EPHEMARE project on the role of microplastics as heavy metal transporters.

Italian Festival of Science 2018 - <http://festival2018.festivalscienza.it/site/home.html>

Italian Festival of Science 2017 - <http://festival2017.festivalscienza.it/site/home.html>

Italian Festival of Science 2016 - <http://festival2016.festivalscienza.it/site/home.html>

Results on Greenpeace campaign 2018 <https://www.youtube.com/watch?v=pSD3IRIjkPs>

Italian Festival of, Genova, Italy, seminar entitled "Meno Plastica più Mediterraneo", 27 October 2018.
<http://www.festivalscienza.it/site/home/programma/meno-plastica-piu-mediterraneo.html>

InTOUR 2017. "Plastiche e microplastiche negli oceani: da contaminanti emergenti ad emergenza ambientale" Milan 23 May, Rome 25 May 2017. file:///C:/Users/ufficio/Downloads/INTour_Flyer_-2017_IT_MILANO.pdf;
http://www.chimiciroma.it/files/Corsi_altri_enti/Locandina_25_05_2017_PerkinElmer_Ordine.pdf

Kärman A, Keiter SH, Rotander A (2017): Microplastic in the Baltic Sea – a small problem with big consequences. Seminar for the Swedish King Carl XVI Gustaf at Örebro University, Sweden. <https://www.oru.se/nyheter/nyhetsarkiv/nyhetsarkiv-2017/kunskap-fran-fyra-forskare-ar-kungens-70-arspresent/>

Newsletter 2016: [http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/News/EPHEMARE%20E-Newsletter%20Issue%201%20\(December%202016\).pdf](http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/News/EPHEMARE%20E-Newsletter%20Issue%201%20(December%202016).pdf)

Newsletter 2017: http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/News/Newsletter%202017_A4.pdf

Newsletter 2018: http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Ephemare_Newsletter_2018_Final.pdf

Participation to the oceanographic cruise in the Mediterranean organized by Greenpeace "Less Plastic more Mediterranean" and organization of dissemination activity during the OPEN BOAT DAY in Genova, Pozzuoli and Ancona. July 2017:
<https://www.cnr.it/it/nota-stampa/n-8073/nel-mediterraneo-livelli-di-microplastiche-paragonabili-a-quelli-dei-vortici-di-plastica-del-pacifico>; <http://www.greenpeace.org/italy/it/ufficiostampa/rapporti/Microplastic-investigation-in-water-and-trophic-chain-along-the-Italian-coast-/>; <https://tg24.sky.it/ambiente/2018/04/23/greenpeace-microplastiche-mediterraneo.html>;
<http://www.repubblica.it/ambiente/2018/04/23/news/greenpeace-cnr-in-italia-microplastiche-in-mare-come-negli-oceani-siamo-messi-male-perfino-alle-tremite-194610995/>;
<http://www.rinnovabili.it/ambiente/microplastiche-nel-mediterraneo-picchi/>;
<http://www.repubblica.it/ambiente/2018/04/23/foto/dalle-cinque-terre-alle-tremite-a-caccia-di-microplastiche-194615405/1/#1>; <http://www.today.it/ambiente/mare-plastica-italia-2018.html>;
<http://www.greenpeace.org/italy/it/ufficiostampa/comunicati/Microplastica-anche-in-pesci-e-invertebrati-lo-conferma-una-ricerca-GreenpeaceUNIVPMISMAR-CNR->;
<http://www.greenpeace.org/italy/it/ufficiostampa/rapporti/Microplastic-investigation-in-water-and-trophic-chain-along-the-Italian-coast-/>;
<http://www.repubblica.it/ambiente/2018/06/21/news/greenpeace-microplastiche-in-un-quarto-di-pesci-e-invertebrati-199626226/>;
<http://www.lasicilia.it/news/sostenibilita/169511/microplastiche-anche-in-pesci-e-invertebrati.html>;
<https://tg24.sky.it/ambiente/photogallery/2018/06/21/greenpeace-microplastiche-mar-tirreno.html>; <https://www.ilfoglio.it/adnkronos/2018/06/21/news/microplastiche-anche-in-pesci-e-invertebrati-201687/>;
<http://247.libero.it/focus/44654135/1/microplastiche-anche-in-pesci-e-invertebrati/>;
<http://www.rinnovabili.it/ambiente/inquinamento-da-microplastiche/>

Participation to the research activities in collaboration with Race 4 Water Foundation, to evaluate distribution of microplastics in marine organisms from Guadalupe, October 2017: <http://www.greenreport.it/news/rifiuti-e-bonifiche/race-for-water-catamarano-solare-caccia-microplastiche-nel-mare-guadalupe-video/>; <http://www.vivereancona.it/2017/10/13/guadalupe-proseguono-i-campionamenti-dellunivpm-sul-race-for-water/656719/>; <https://www.rivieraoggi.it/2017/11/26/254597/race-for-water-in-corsa->

[per-salvare-i-mari-dall'inquinamento/](https://www.facebook.com/UNIVPM/videos/738235416364010/); <https://www.facebook.com/UNIVPM/videos/738235416364010/>;
https://www.youtube.com/watch?v=9_zp8q2qnLI.

Participation at the national television broadcast "Mi manda RAI 3" (from minute 02:24:36)
<http://www.raiplay.it/video/2016/12/Mi-Manda-Raitre-3c344b99-6b65-4419-86d7-5a40cb881f55.html>

Project Overview Brochure: <http://jpi-oceans.eu/sites/jpi-oceans.eu/files/public/EPHEMARE/Images/About/EPHEMARE%20Brochure%2023102017.pdf>

Seminar by invitation: "Plastiche e Microplastiche negli Oceani, una nuova minaccia per la salute del mare" (Relatore Prof. Stefania Gorbi), organized by Rotary Club Ancona e Rotary Club Ancona-Conero, Teatro delle Muse 2 October 2018, Ancona. <https://www.disva.univpm.it/sites/www.disva.univpm.it/files/disva/files/news/plastiche%20e%20microplastiche.jpg>

LIST OF EPHEMARE SOCIAL MEDIA ACTIVITIES

2017 - Participation to the campaign "Less Plastic, More Mediterranean" promoted by Greenpeace, aimed at monitoring the status of plastic contamination in sea water and arise public opinion attention about this problem.

2017 - Participation to the campaign "Odyssey" promoted by "Race for Water", aimed at monitoring the status of plastic contamination in sea water and arise public opinion attention about this problem.

2017- Participation of Marco Faimali to the Italian TV program "Rai News 24" on April 2017
<https://www.youtube.com/watch?v=EKMD4SD5ZLQ>

2017- Interview of Francesca Garaventa published in the magazine "The Medi Telegraph" on June the 2017
<http://www.themeditelegraph.com/it/green-and-tech/pollution/2017/06/22/beware-microfragments-poisoning-the-sea-Fyr9FAjNY8iqpzu7Oj0aP/index.html>

2018- Participation of Francesca Garaventa to the Italian TV program "Geo&Geo" on May the 3rd 2018 and Radio 24 (<http://www.ismar.cnr.it/eventi-e-notizie/notizie/le-plastiche-nel-mediterraneo>, from minute 1:41:30;
http://www.ismar.cnr.it/file/divulgazione/radio-e-tv/GARAVENTA%20EDIT%20Kiss%20kiss%20radio%2024%2004%2018.mp3/at_download/file)

Ana Luísa Ribeiro; Alexandre Pacheco and Lúcia Guilhermino, L. organized in collaboration with the "Núcleo do Porto dos Estudantes de Bioquímica" the Workshop "Ferramentas bioquímicas em estudos ambientais: avaliação de efeitos neurotóxicos de poluentes em moluscos" that included na introduction talk and a laboratorial session where the methods and results of the EPHEMARE project (and other projects) were disseminated. XI ENEBIQ – Encontro Nacional de Estudantes de Bioquímica. ICBAS-UP, Porto, Portugal 26th March 2018.

Bebiano, M. J. Plásticos menores do que 0,5 mm encontrados em bivalves. TVI Portuguese televisão channel, 30th December 2018.

Bebiano, M.J. Combater o plástico na UALG, 16th November 2018.

Bebiano, M. J. UALG estuda o impacto dos microplásticos em espécies marinhas. Dia Mundial dos Oceanos, TVI Portuguese television channel, 12 de Junho 2018

Bebiano, M. J. Efeitos de microplásticos em seres marinhos, 24th May 2018, RTP1 Television Channel.

Bebiano, M. J., UALg elimina plásticos de cantinas e bares. 15th of November 2018 RTP1 Television Channel

[Bebiano, M. J Microplásticos encontrados em mexilhões e ostras no Algarve - Revista Sábado](#) 19th November 2018

"EPHEMARE - Ecotoxicological effects of microplastics in marine ecosystems" on Research Gate.
<https://www.researchgate.net/project/EPHEMARE-Ecotoxicological-effects-of-microplastics-in-marine-ecosystems>. 106 followers and 987 reads (latest checked February 15th, 2019).

EPHEMARE Twitter: 454 followers since its launch in 2017, on average 24 new followers per month, on average 44 tweets per month, reached over 145 000 people since its launch of the EPHEMARE twitter account

EPHEMARE Facebook: 493 followers since its launch in 2016, on average 24 new likes per month, on average 15 posts per month and reached over 105 000 people

Gastro-scientific seminars intends to combine social activities we carry out at the IEO, the snack on Fridays, with scientific activities. In the relaxed environment provided by the snack, we showed the advances that EPHEMARE has produced. This initiative is part of the current 'Pint of Science' started in England in 2013 whose objective is to disseminate scientific work to science and beer lovers. Among the most characteristic pinchos of our region is the marinera (Russian salad mounted on a bread donut and topped with an anchovy), hence the title of this cycle of seminars: 'A round of EPHEMARineras'

Guilhermino, L. 2018. "Ferramentas bioquímicas em estudos ambientais: avaliação de efeitos neurotóxicos de poluentes em moluscos - Introdução". Workshop "Ferramentas Bioquímicas em estudos ambientais: avaliação de efeitos neurotóxicos de poluentes em moluscos." XI ENEBIQ – Encontro Nacional de Estudantes de Bioquímica. ICBAS-UP, Porto, Portugal 26th March 2018. (Talk)

Guilhermino, L. 2017. Efeitos de microplásticos em organismos aquáticos – projeto EPHEMARE. # EU in My Region, An Open Sea for Innovation, Talks with Science. CIIMAR, Matosinhos, Portugal, 13th May 2017.

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