

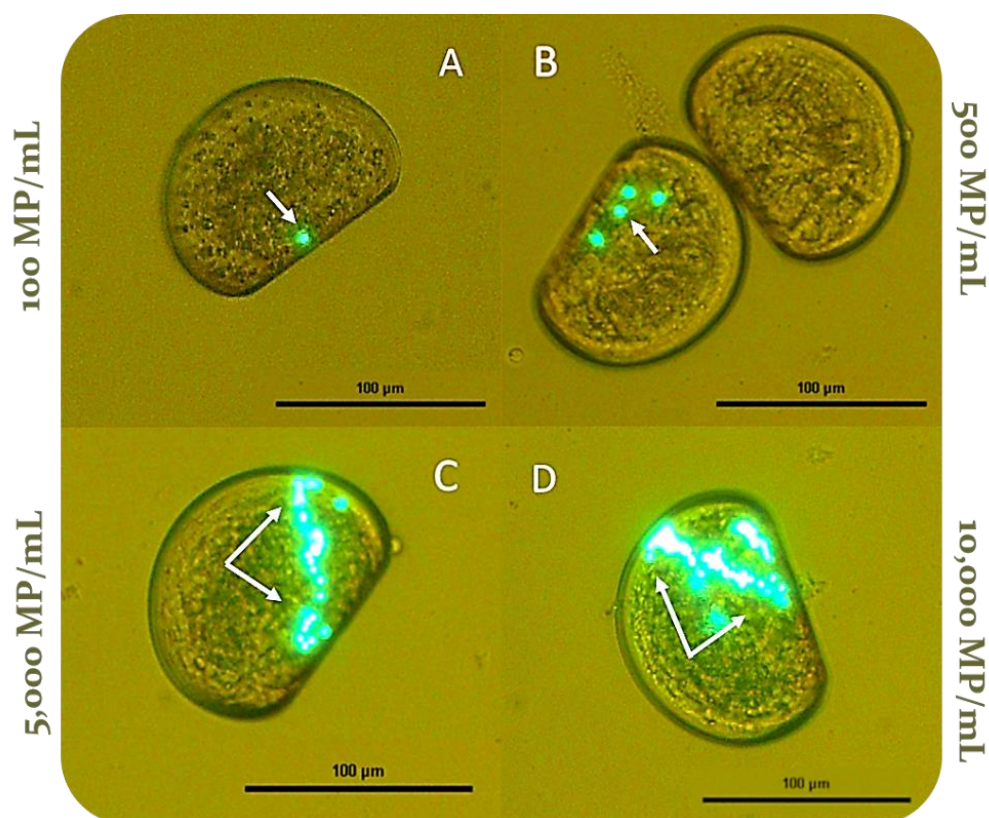
FINAL PROJECT REPORT

Direct and indirect ecotoxicological impacts of microplastics on marine organisms

Project acronym: PLASTOX

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Picture: Marco Capolupo (University of Bologna)

Coordinating institute: SINTEF Ocean

Project coordinator: Dr Andy Booth, Senior Researcher, Environmental Technology Dept., SINTEF Ocean

Tel: +47 93089510

E-mail : andy.booth@sintef.no

PLASTOX website address: <https://www.sintef.no/projectweb/plastox/>

CONSORTIUM

Names	Organisation	Country
Dr Andy Booth	SINTEF Ocean, Environmental Technology Department, Trondheim	Norway
Dr Kaori Sakaguchi-Söder	Technische Universität Darmstadt (TUDA), Darmstadt	Germany
Prof. Paula Sobral	NOVA.ID FCT (NOVA-FCT), Caparica	Portugal
Prof. Laura Airoidi, Prof. Elena Fabbri, Prof. Giulio Zanaroli	Alma Mater Studiorum - University of Bologna (UNIBO)	Italy
Dr Richard Sempéré	Aix-Marseille Université-Mediterranean Institute of Oceanography (AMU-MIO), Marseille	France
Dr Jan Andries van Franeker	Wageningen Marine Research (Wageningen University and Research) (WUR), Den Helder	Netherlands
Dr Tom Doyle, Dr Liam Morrison	National University of Ireland Galway (NUIG), Galway	Ireland
Dr Iurgi Salaberria	Norwegian University of Science and Technology (NTNU) Trondheim	Norway
Dr Carl van Colen	Ghent University (UGhent), Ghent	Belgium
Dr Dorte Herzke	Norwegian Institute of Air Research (NILU), Tromsø	Norway
Dr Kerstin Magnusson	IVL Swedish Environmental Research Institute (IVL), Göteborg	Sweden
Prof. Geir Gabrielsen	The Norwegian Polar Institute (NPI), Tromsø	Norway
Associated partners		
Dr Amaia Orbea, Prof. Miren P Cajaraville	Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV-EHU), Basque Country	Spain
Prof. Tamara Galloway	University of Exeter (UoE), Exeter	United Kingdom
Dr Berit Brockmeyer	Federal Maritime and Hydrographic Agency (BSH), Hamburg	Germany
Albert van Oyen	CARAT (Control and Research Analysis Thermoplastics), Bocholt	Germany

The PLASTOX project had the overall aim of understanding the ecotoxicological impact of microplastics (MP) and persistent organic pollutants (POPs) and metals associated with them on key European marine species and food webs. To achieve this goal, the project used a combination of fieldwork and laboratory studies that tried to simulate realistic environmental conditions where possible. Using novel approaches with a combination of commercial MPs and marine litter-derived MPs, the project attempted to study the interaction of MPs with a wide range of key marine species representing most trophic levels, from planktonic species through to seabirds. To control MP exposure in ecotoxicity studies and understand uptake and accumulation pathways more clearly, PLASTOX attempted to employ a range of state of the art analytical techniques for MP characterisation and quantification.

In an attempt to move away from using the pristine, spherical MP test materials of a single polymer type that are employed in most studies, PLASTOX generated a more environmentally relevant MP reference material derived from macroplastic litter collected from the marine environment. The resulting reference material comprised a mixture of irregular shaped MP of different polymer types and varying degrees of degradation, which is more comparable to MP found in the environment. To increase our understanding of the processes occurring in the natural environment, a number of the PLASTOX studies were conducted with this marine litter-derived MP mixture where feasible.

Uptake, accumulation and excretion of MP particles by marine organisms was studied through integrated field and laboratory studies. MP was readily ingested, either directly or indirectly, by a wide range of species collected from different trophic levels (primary producers, herbivores, detritivores and deposit feeders, and carnivores) and ecosystem types (water column and bottom sediments) from across Europe. MP levels measured at a single time point and location were generally low and extremely variable among individuals. Despite the variability, there were some emerging trends where sediment species typically ingested more MP than species relying on the water column for their diet. Furthermore, larger organisms tended to retain more MP in the gastrointestinal tracts, while predators and omnivores did not contain significantly more MP than herbivores from lower trophic levels. There was generally a selection for smaller sized particles (<50 µm), particularly in low trophic level organisms, and we observed that fibres were more frequently found in gastrointestinal tracts, possibly as their shape could lead to slower transit rates.

Using the single species accumulation studies as a basis, experiments were conducted to determine if true MP accumulation occurs in the tissues of organisms and whether MP can transfer from one species to another through the food web. Our studies showed MP >20 µm is predominantly limited to the digestive systems of these organisms and eventually excreted, indicating most MP (1 µm – 1 mm) is not truly accumulated in organism tissues and that trophic transfer could be quantitatively limited through predator-prey interactions. However, field and laboratory experiments showed that filter-feeding organisms such as bivalves can act as “aggregators” of the smallest MP (<50 µm size), transferring them from the water column to sediments via faeces. This process can facilitate the subsequent uptake of MP by sediment detritus-feeders such as polychaetes. As a result, transfer of MP and associated contaminants to higher trophic levels may be more likely via detrital pathways than via predator-prey interactions.

Lethal and sublethal effects of MP exposure were studied using a combination of whole organism and cellular level toxicity studies and employing a variety of different MP materials and a wide range of marine organisms representing different trophic levels and marine environments from across Europe. Nanoplastic (NP) and MP were readily taken up by all test species from plankton to fish larvae, resulting in a range of sublethal effects (e.g. reduced reproduction, inflammation, genotoxicity, oxidative stress, development), but no significant toxicity. NP and MP up to 10 µm were readily ingested by mussels in a concentration dependent manner and were able to enter cells and penetrate connective tissues and the stomach walls. Importantly, these effects appear to be species and life-stage dependent, and strongly influenced by factors such as exposure concentration and MP size and shape. The studies indicate that the smallest MP and NP cause the largest biological responses, possibly due to their ability to pass through biological membranes. Isolated cells, embryos and larvae appear to be the most sensitive to MP exposure. However, effects seem not to be severe enough to cause mortality or impair key biological functions in most cases. Long-term experiments with relevant MP concentrations followed by longer depuration periods are needed to assess the reversibility of some of the responses.

The PLASTOX project conducted a series of studies investigating the adsorption/desorption of POPs and metals to MP and the desorption of plastic additive compounds, including development of innovative methods based on long-term field experiments using a range of different MPs: polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP) and polyethylene (PE). In both laboratory and field experiments, polymer sorption capacity for POPs typically follows the order of $PET \leq PS \leq PP \ll PE$, which correlates well with polymer surface energy, hydrophobicity and density. The sorption of POPs to MP particles in the marine environment is complex and influenced by parameters such as polymer type and size, POP physicochemical properties and seawater temperature. For example, there is a transition from absorption (into the polymer) to adsorption (on the surface) as MP size decreases, suggesting POP bioavailability may be higher on smaller MP particles. Partially degraded MP can accumulate a wider range of POPs and at concentrations significantly higher than pristine MP. UV degradation by sunlight causes the MP surface to crack, increasing the surface area for adsorption to take place. Accumulation of POPs on a weathered polymer surface may also be driven by a longer residence time in the ocean, increased wettability due to the increase of surface energy and migration of hydrophobic additives from the polymer core to the surface.

Many plastic materials contain additive chemicals that give them specific properties (e.g. flexibility, protection from UV, flame retardance, colour). PLASTOX conducted studies to establish if some of these additive chemicals can leak out into environment and whether they are bioavailable and toxic to marine organisms. We have been able to show that a range of organic additives are able to leak into both seawater and biological fluids (seabird stomach oil). The release of additive chemicals from plastic was not found to be significantly influenced by pressure, despite higher concentrations of such additives and plastic debris being found in seawater close to the sediment surface. Importantly, when additive chemicals leak into seawater they are bioavailable to marine organisms and we have shown that they can cause acute and sublethal toxic effects in marine organisms, including algae and mussels.

Using the knowledge generated in the uptake, accumulation, ecotoxicity and POP adsorption studies, the bioavailability to marine organisms of POPs and metals adsorbed to MP was investigated. Our results show that MP-sorbed POPs and metals are bioavailable under certain conditions, with reduced or no bioavailability under others. We found that species-dependent factors such as lipid store and gut residence time of MPs play a crucial role in the potential for desorption and transfer of MP-sorbed organic contaminants organisms. Exposure conditions are also important, with MP-sorbed POPs not readily accumulated from MP when they are also present as dissolved pollutants in the surrounding environment. Furthermore, there needs to be a greater focus on understanding and measuring the true exposure conditions in such studies, ensuring bioavailability can truly be linked to pollutants present on an ingested particle surface and not that they have simply dissolved into the surrounding exposure media.

Finally, studies were conducted within PLASTOX to gain an improved understanding of the role of MP as surfaces for microbial colonisation in the marine environment and the role of microbes in biotransformation of POPs associated with MP. Results showed that colonisation of MP occurs rapidly (<2 weeks) and that community structure varies with polymer type, possibly influenced by MP surface properties and chemical additives. Polychlorinated biphenyl (PCB)-contaminated MPs are also rapidly colonised by microbes, and that reductive dechlorination of PCBs can take place more rapidly on MP than in the sediment. Colonisation of MP and microbial transformation of MP-associated PCBs may thus markedly affect the bioavailability of MP-associated PCBs and the toxicity of PCB-contaminated MPs.

While our understanding of MP in the marine environment has increased in the recent years, knowledge gaps still remain. Evidence suggests that the current levels of MP (>10 μm) to which marine biota is exposed are substantially lower than those expected to cause a significant risk, although hotspots of MP pollution and long-term exposure may represent concerns. Furthermore, a new set of research questions related to MP and plastic litter in the environment have been identified. The highest risks may be associated with NP and MP <10 μm due to their greater potential for passing biological barriers and accumulating in organisms, although knowledge of their environmental concentrations is virtually unknown. The role of NP and MP as vectors of POP and metal exposure needs more detailed study, while the release of additive chemicals into the marine environment from plastic waste and MP is emerging as a concern.

INTRODUCTION

The PLASTOX project was conceived and developed in 2015 in response to the Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) call for projects on 'Ecological aspects of microplastics in the marine environment'. At the time of development, the project was designed to address what were identified as some of the most pressing knowledge gaps and research challenges related to microplastic (MP) pollution in the marine environment. One of the distinctive characteristics of PLASTOX was to study and answer these questions by using a combination of fieldwork and laboratory studies, trying to simulate realistic environmental conditions where possible. The PLASTOX project addressed the following research challenges:

- i. A greater understanding of the potential for MP uptake, accumulation and excretion by marine organisms from different trophic levels and marine ecosystems.
- ii. Determining if there was evidence for true MP accumulation by organisms and subsequent trophic transfer through the food web.
- iii. Improved knowledge regarding the potential for acute and sublethal ecotoxicological responses in a broad range of marine organisms from different trophic levels.
- iv. A clearer overview of adsorption and absorption of organic and inorganic pollutants to different types of MP, their role as vectors for pollutant exposure and the bioavailability of MP-sorbed pollutants to marine organisms.
- v. Establishing what types of plastic additive chemicals have the potential to leach into environmental matrices and whether these are sufficiently bioavailable to elicit effects in marine organisms.
- vi. Understanding the role of MP as surfaces for microbial colonisation in the marine environment and how this is influenced by the physicochemical properties of different MP particles.

In the period 2010-2016, the number of peer-reviewed scientific publications related to MP research quantity of research related to MP pollution in the environment increased from 3-4 published manuscripts per year to over 80 manuscripts per year². Although data is not currently available for 2017-2019, it is clear that this trend continued. Importantly, this focus on MP in the environment has resulted in significant progress being made to address some of the identified research challenges, including many outside the scope of the current project (e.g. analytical approaches for MP identification and characterisation, plastic degradation, MP transport). Through a close collaboration, the PLASTOX project and the three other projects funded through JPI Oceans (BASEMAN, EPHEMARE and WEATHER-MIC) have provided a meaningful contribution to this body of research. However, some knowledge gaps still remain unclosed or unsolved due to a variety of factors, including use of different methodologies across individual studies, analytical identification and characterisation limitations, and simply the time required to generate a sufficient data and knowledge base. This is perhaps best exemplified by the fact that the first ever attempt at a risk assessment of MP in the ocean was only conducted in 2018³.

As our understanding of MP in the marine environment has grown through the body of research conducted globally in the last few years, these same research activities have also raised a new set of questions and knowledge gaps related to MP and plastic litter in the environment. The PLASTOX project readily acknowledged that the research field of MP is fast moving and has attempted to maintain a relevant research profile, responding to new knowledge and developments in the field by modifying the research activities and methodologies accordingly, yet without deviating significantly from the original project goals. As a consortium, we hope that the work presented in this report for JPI Oceans showcases the contribution we have made to improving the scientific community's and society's understanding of the fate and effects of MP in the marine environment.

AIMS OF THE PROJECT

The PLASTOX project aimed to understand the ecotoxicological impact of microplastics and persistent organic pollutants (POPs) and metals associated with them on key European marine species and food webs.

To achieve this aim, the following objectives were established:

- Characterise and quantify MP uptake, accumulation and excretion at the cell, fluid, tissue and organism levels.
- Determine acute and a broad range of sublethal ecotoxicological effects and modes of action of MP exposure on key marine species.
- Quantify the adsorption of toxic persistent organic pollutants (POPs) and metals to MP and the desorption of plastic additive compounds.
- Assess the role of POP and metal adsorption/desorption on MP ecotoxicity.
- Follow the trophic transfer and accumulation of MP in the marine food web and identify subsequent potential for impacts.
- Use current knowledge from nanoparticle ecotoxicity research to influence experimental design and to evaluate data generated.

The PLASTOX project had the goal of significantly advancing our current knowledge regarding the effects of MPs and MP-associated chemicals on marine organisms. Using novel approaches with a combination of commercial MPs and marine litter-derived MPs, the project attempted to study the interaction of MPs with a wide range of key marine species representing most trophic levels (from planktonic species through to seabirds). To control MP exposure in ecotoxicity studies and understand uptake and accumulation pathways more clearly, PLASTOX attempted to employ a range of state-of-the-art analytical techniques for MP characterisation and quantification. As little was known about the transfer of MPs between species and between trophic levels and whether true trophic transfer was possible for micron-sized particles, this process was evaluated as part of the general MP ingestion, uptake and accumulation studies conducted within the framework of the project.

In an attempt to move away from using the pristine, spherical MP test materials of a single polymer type that are employed in most studies, PLASTOX had the aim of generating a more environmentally relevant MP reference material derived from macroplastic litter collected from the marine environment. This field sampled and laboratory generated MP reference material would represent an MP mixture derived from plastic consumer waste exhibiting various degrees of degradation. To increase our understanding of the processes occurring in the natural environment, a number of the PLASTOX studies were to be conducted with the marine litter-derived MP mixture where feasible.

Increasing our understanding of how MPs enter food webs and investigating their subsequent accumulation and transfer from basal to higher trophic levels was another key aim of the PLASTOX project. As ingestion and transfer of MP particles by organisms has been previously studied in the laboratory, the project sought to integrate field studies and laboratory experiments in pelagic (planktonic) and benthic (mostly shallow sedimentary) habitats in an attempt to extend our knowledge into natural ecosystems. To do this the accumulation of MPs was to be quantified in field sampled key species at different trophic levels (primary producers, herbivores, detritivores and deposit feeders, and carnivores) in a variety of locations around Europe. These studies aimed to provide knowledge on ingestion rates using realistic densities of MPs and to identify which MP characteristics (composition, size, shape and colour) facilitate their direct uptake by target species. Ultimately, PLASTOX had the goal of attempting to assess the transfer of MPs from lower to higher trophic levels in the food webs (primary producers to herbivores and/or deposit feeders; and herbivores and/or deposit feeders to carnivores). During the project it also became clear that MP have complex and variable dynamics across different systems, and progress was made to attempt to describe some of these patterns and small-scale variability.

PLASTOX also aimed to assess acute ecotoxicological responses of MP exposure in species representing different marine environments across Europe and organisms from a broad range of trophic levels. Furthermore, the project

planned to investigate sublethal responses (genotoxicity, cytotoxicity, oxidative stress, scope for growth, development and reproduction) of MP exposure and propose modes of action. Key model species (adults and early-life stages) should be selected for each ecosystem on the basis of their ecological and commercial relevance, and sensitivity to accumulation of MPs at the different trophic levels. Specific studies aimed to investigate the mutagenic potential of MP exposure, and its relation to oxidative stress, histopathology and detoxification processes in model species. A key goal was to study and identify the role of MP physicochemical properties on the type and degree of effects in key model species.

While there is a growing body of research and knowledge concerning the adsorption and desorption of POPs to MPs and the desorption of plastic additives, few studies had sought to extend this into the potential impacts on marine organisms. No controlled long-term field studies were available that had simultaneously investigated desorption of additive-derived chemicals from MPs and adsorption of POPs onto MP in the marine environment. The PLASTOX project therefore had a key focus on studying the process of POP adsorption and desorption to MPs and understanding how this influences POP bioavailability to marine organisms through alternative uptake and exposure routes (e.g. ingestion on MPs vs exposure to POPs in the water column). The project sought to develop innovative methods to understand the fate and transport of selected pollutants adsorbed to the surface of MP using a long-term field experiment on a range of different MPs (various polymers, sizes, shapes). To account for environmental factors, studies were to be conducted in a range of marine environments across Europe (in Mediterranean, Adriatic, North-eastern Atlantic, North, and Baltic Seas, and Arctic conditions in northern Norway). The goal was to try and link pollutant adsorption/desorption to specific physicochemical properties of MPs.

In an attempt to combine the knowledge generated in the uptake, accumulation, ecotoxicity and POP adsorption/desorption studies, the PLASTOX project aimed to assess the ingestion, accumulation and effects of POPs, metals and additive chemicals associated with MPs to individual species. These studies were expected to permit the quantification of POP, metal and additive chemical uptake and accumulation in individual species exposed to contaminated MP and determine if such chemicals and metals are sufficiently bioavailable to organisms to elicit toxicological effects in individual species. Importantly, the project aimed to determine if POPs, metals and additive chemicals associated with MPs exert greater or reduced effects in species compared to the effects of these toxicants in the absence of MPs.

Finally, the PLASTOX project aimed to describe the dynamics of MP colonisation by natural microbial biofouling and the biotransformation of POPs associated to MPs particles by marine microbial communities, which might in turn affect their bioavailability and toxicity. These studies expected to determine if polymer composition played a key role in microbial community structure formed on the surface of MP, and how this varies over time. In a further step, PLASTOX attempted to investigate the biotransformation of POPs associated to MPs particles by marine microbial communities and whether community structure was altered in the presence of POPs. The studies planned to focus in particular on polychlorinated biphenyls (PCBs), which are commonly found as sediment contaminants in impacted marine sites. The project planned to expose PCB-sorbed MP and biotransformed PCB-sorbed MPs to selected marine organisms to determine the influence on bioavailability and toxicity.

RESULTS

Environmentally relevant MP reference material

Marine litter-derived MP reference material (WUR, CARAT, SINTEF).

The cryomilling process generates MP that is irregular in shape, which is more realistic than uniform spherules often used in exposure experiments (Figure 1)⁴. The polymer composition was selected to comprise mainly polyethylene (PE) and polypropylene (PP) to ensure comparability to the main polymers produced on a global scale and found in seawater and marine organisms. Chemical analyses confirmed the abundance of various elements, including the heavy metals such as cadmium, copper and lead. Well-known organic chemical plastic additives used as plasticisers, UV stabilisers, flame retardants and colourants were also detected. Several analysis techniques were applied for most

characterisations, ensuring that the mixture is homogeneous in its composition. The reproducibility of such material is challenging, as each litter item differs in origin, initial purpose and weathering conditions at sea. The use of this material can help closing the gap between pristine and environmentally relevant experiments, whereby crucial insights in the fate of plastic uptake and their consequences on marine organisms can be gained. One successful example of the use of this material in the PLASTOX project is an ongoing experiment, whereby the leakage of chemical substances from the plastic mixture to gastrointestinal fluids of marine organisms has been demonstrated.

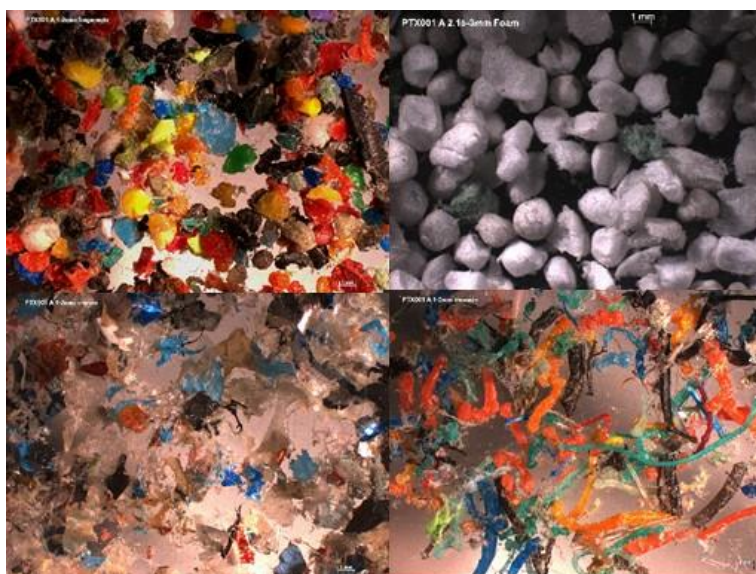


Figure 1. Compilation of different beached litter items milled to microplastic. After milling all types were combined to create an environmentally realistic mixture.

Microplastic uptake, accumulation and food web transfer

Quantifying MP presence and variable distribution in key species (UNIBO, UGhent, IVL, FCT, WUR, NUIG). Studies were conducted across multiple partners using a range of key species at different trophic levels in a variety of marine ecosystems around Europe. The work was field-based with all organisms collected from the natural environment. Several localities around Europe were sampled using a common approach at replicated sites, covering 5-6 trophically-related taxa at each site (Figure 2) and using rigorous anti-contamination protocols and cost-effective digestion techniques⁵.

We found a generally low and variable occurrence of MP between individuals, species, & locations (Table 1). As a general trend MP tended to be more frequently found in macrobenthic deposit feeders, epibenthic predators⁵ and demersal fish⁶, while it was rarer at lower trophic levels. This low content of MP did not seem to reflect low local environmental contamination, and we hypothesise that MP >20µm could transit relatively quickly through the digestive tracts of organisms and be egested relatively quickly. This could limit transfer via predator-prey interactions but enhance alternative detrital pathways via uptake by detritus feeders and their predators of MP concentrated into organic-rich faecal pellets.

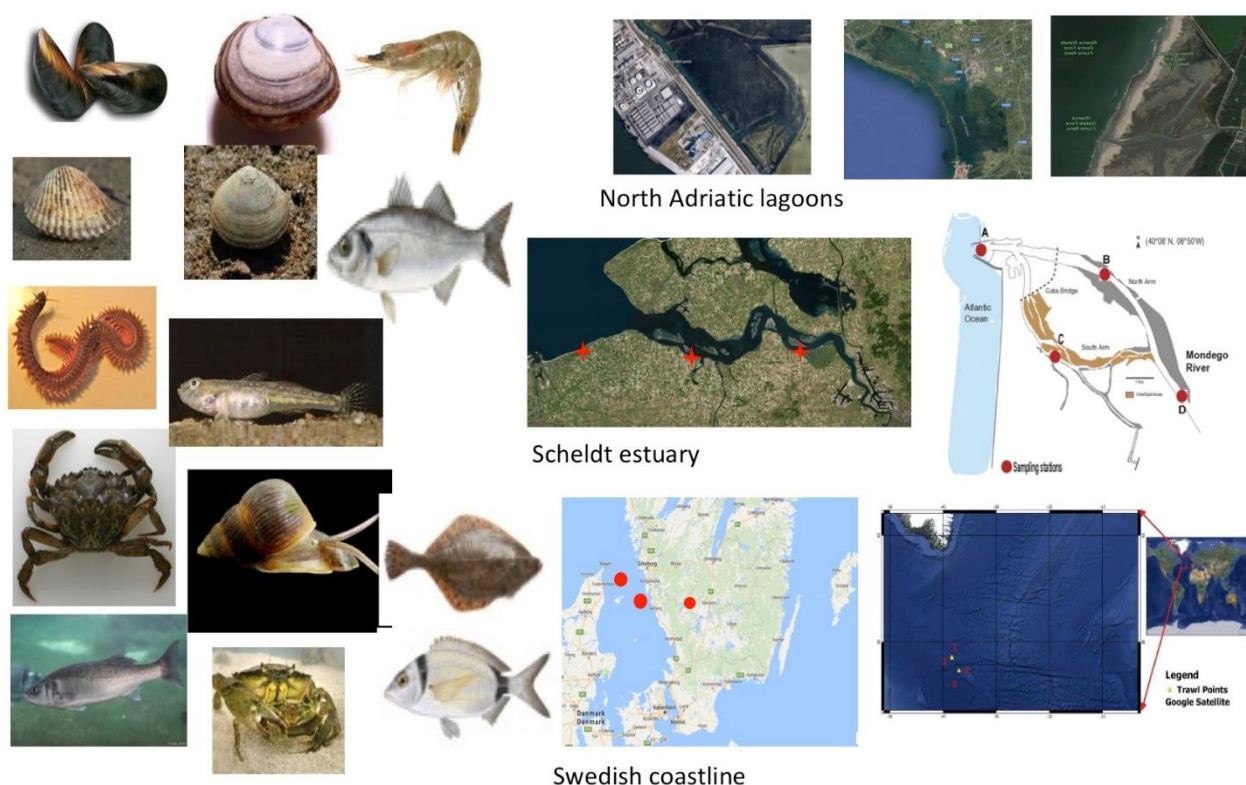


Figure 2. Some of the sites and species sampled to quantify the distribution of MP in key taxa at different trophic levels around Europe.

Table 1. Sampled species, their life habit, feeding mode, sampling area and the number of analysed specimens. MP contamination is given as the percentage of positive individuals (i.e. containing at least one MP) and the average number of MP (\pm SE) per species. Feeding modes are taken from www.marlin.ac.uk/Data are from UNIBO and UGhent.

Species	Life habit	Feeding mode	Region	n of analysed individuals	Positive individuals (%)	Number of MP ingested
Bivalve						
<i>Cerastoderma glaucum</i>	Infrauna	Suspension feeder	N Adriatic	70	1.4%	0,04 (\pm 0,01)
<i>Mytilus galloprovincialis</i>	Infrauna	Suspension feeder	N Adriatic	30	3.3%	0,03 (\pm 0,03)
<i>Limecola balthica</i>	Infrauna	Surface deposit feeder/suspension feeder	Schelde estuary	20	20%	0,25 (\pm 0,12)
<i>Scrobicularia plana</i>	Infrauna	Surface deposit feeder/suspension feeder	Schelde estuary	20	5%	0,05 (\pm 0,05)
Polychaete						
<i>Hediste diversicolor</i>	Infrauna	Omnivore	N Adriatic	100	1%	0,01 (\pm 0,01)
<i>Hediste diversicolor</i>	Infrauna	Omnivore	Schelde estuary	10	10%	0,1 (\pm 0,1)
Crabs						
<i>Cardinus aestuarii</i>	Epifauna	Omnivore	N Adriatic	90	6.7%	2,19 (\pm 1,54)

Identifying the effect of MP characteristics on uptake and egestion rates (UNIBO, UGhent, NUIG, NTNU, SINTEF, IVL). The work included both laboratory and field based analyses, and covered a range of benthic and planktonic species (Figure 3) as well as a variety of MP characteristics (size, shapes, materials and densities) trying to mimic as much as possible realistic field conditions and concentrations.

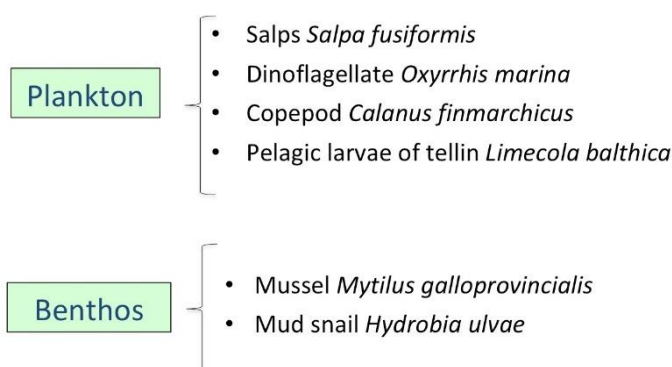


Figure 3. Summary of the main target taxa used in laboratory and field-based work by various partners (UNIBO, UGhent, NUIG, NTNU, SINTEF, IVL) for assessing MP uptake and egestion rates.

NUI Galway found that salps are a key player in the biological pump, ingest MP particles and incorporate them into their faecal pellets⁶. Experiments showed that only a few salps ingested MP at environmentally relevant MP concentrations. Up to 46 % of salps ingested MP at higher concentrations, simulating conditions potentially found in convergent zones or at expected future concentrations. MP incorporated into salp faecal pellets decreased their sinking velocity (1.35 times for PE to 1.47 times for PS, Figure 4). These results suggest that today MP ingestion by salps has little impact on the biological pump, however under potential future MP concentrations (or in current convergent zones) MP could have the potential to lower the efficiency of the biological pump.

UGhent demonstrated that the planktonic larvae of the widely distributed tellin *Limecola balthica* ingested MP (PS fluorescent microbeads 5 μm), but only at an exposure concentration of 100 particles mL^{-1} upwards. Furthermore, the number of ingested particles increased with exposure concentration. Organisms contained an average of >1 particle only when the exposure concentration was 1000 particles mL^{-1} and above. This suggests caution at interpreting the outcomes of experiments that make use of unrealistically high concentrations of MP.

Results from laboratory experiments by IVL on the marine snail *Hydrobia ulvae* indicated no differences in ingestion/excretion rates between PET and PS, but control snails produced significantly fewer faecal pellets. A majority of the MPs were excreted relatively quickly (i.e. within the first few hours), indicating that the gut retention time for MP may not be longer than for natural particulate material in *H. ulvae*. Furthermore, no differences in retention time between MP of different sizes was observed.

NTNU and SINTEF found that phytoplankton species such as the dinoflagellate *Oxyrrhis marina* engulf MP beads and accumulate them up to a certain degree, thereby reducing their microalgae prey intake and potentially contributing to the trophic transfer of MP to grazers preying on *O. marina* (Figure 5). MP composition and/or MP size appear to have a larger impact on MP uptake than MP concentration. Conversely, in the marine copepod *C. finmarchicus* retention but not accumulation of polystyrene microbeads was observed as these beads were rapidly excreted, thereby potentially accelerating their transfer to sediments. Excretion dynamics were affected by the presence of microalgae.

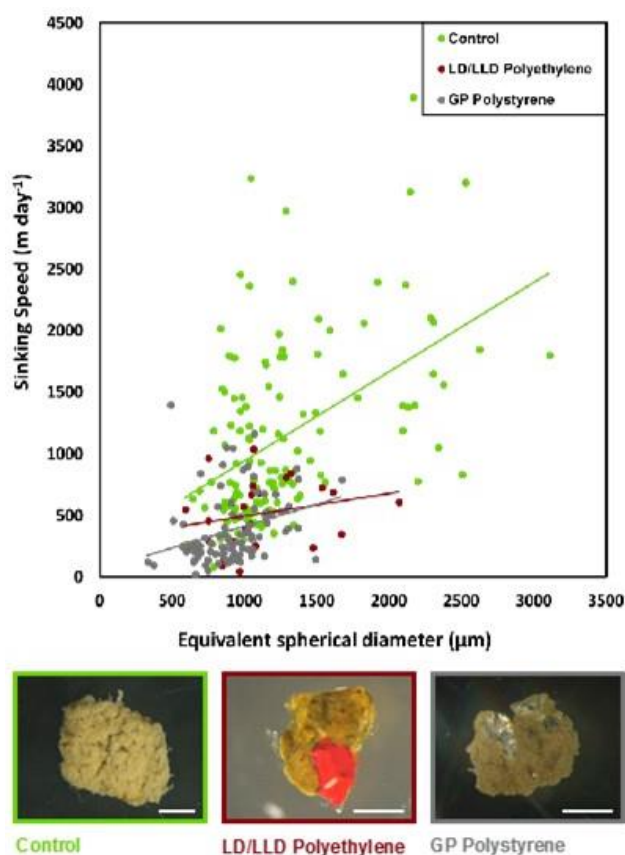


Figure 4. Sinking speed (m day^{-1}) as a function of Equivalent Spherical Diameter (μm) for the faecal pellets from Control, LD/LLD polyethylene and GP polystyrene incubations together with example images of microplastic free pellets and pellets containing LD/LLD polyethylene and GP polystyrene; white bars in images scale 500 μm (adapted from Wieczorek et al., 2019)¹.

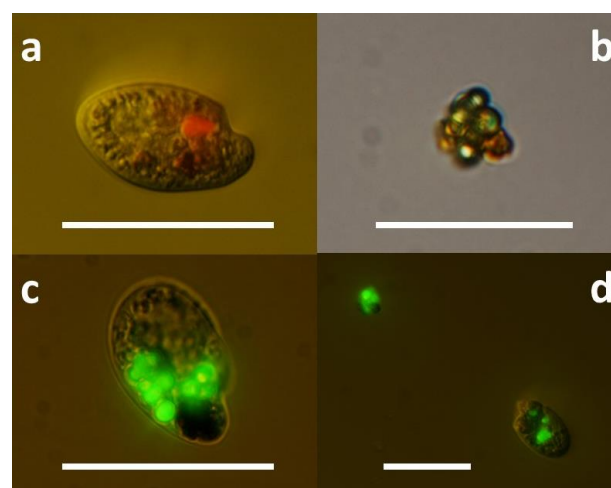


Figure 5. Visualisation of 1-5 μm green fluorescent (GF) MP internalised by *Oxyrrhis marina* cells using fluorescence microscopy. (a) *O. marina* cells with only microalgae prey (*Rhodomonas baltica*); (b) free GF MP aggregating with brown material assumed to be a bolus of excretes from *O. marina*; (c) *O. marina* cell containing several GF MP; and (d) a GF MP containing *O. marina* cell together with free GF MP suspended in the culture medium. Scale bars: 20 μm . Images by Dionis J. Lyakurwa and Dag Altin from NTNU, BioTriX and SINTEF.

Field experiments using biodeposit traps custom made by UNIBO and jointly used in experiments by UNIBO and UGhent showed that filter-feeding mussels (*Mytilus galloprovincialis*, *Mytilus edulis*) act as “aggregators” of the smallest MP (*M. galloprovincialis* <50 µm size; *M. edulis*: 100-300 µm) filtered out the water column to the sediments via faeces and pseudofaeces. This process significantly accelerates the vertical flux of small MP from the water column to the bottom. A large spatial variability in MP distribution and bioavailability was also measured among nearby coastal sites, as well as among mussels located at different water depths. However, there was no evidence of relevant differences between wild and farmed mussels.

Pathways for MP transfer along the trophic chain (UNIBO, UGhent). This study focused on the pathways of MP transfer along the trophic chain and between different marine compartments. UNIBO tested experimentally whether the uptake of MP by mussels (*M. galloprovincialis*) and their incorporation into faecal pellets can facilitate their subsequent uptake by benthic detritus feeders, such as polychaetes. Furthermore, UNIBO quantified the effects and fate of MP deposited on the seafloor on the functioning and diversity of soft-sediment communities. UGhent also tested the hypothesis that MP ingestion could alter predator-prey interactions due to changes in prey behaviour and changes in filtration rates of predators.

UNIBO confirmed that MP are transported to the seabed via filtration by mussels (Figure 6). Over a short temporal scale, this process did not seem to significantly influence sediment community diversity or metabolism (total sediment community oxygen uptake). However, the incorporation of MP into bivalves’ faeces and pseudo-faeces more than doubled their subsequent uptake by benthic detritus-feeder polychaetes, playing a relevant role in the dynamic of MP between different trophic levels and marine compartments.

The predation experiment by UGhent demonstrates trophic transfer of MP from zooplankton to benthic filter feeders through consumption of contaminated prey (i.e. prey with ingested MP). Predation rates of contaminated prey were ~30 % lower as compared to predation rates of prey that had no MP ingested (Figure 7). As predator clearance rates were not affected by consumption of MP, the lower predation rates of MP by larvae could primarily be explained by disruption in larval swimming behaviour that reduces their filtration risk.

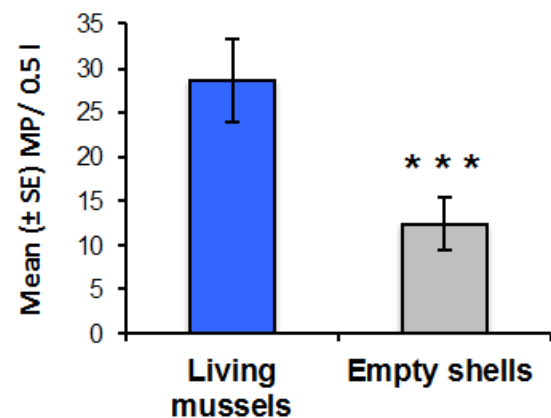


Figure 6. Concentration (mean ± 1SE) of MP 41 µm in the biodeposit of aquaria containing either living mussels (blue bars) or empty shells (orange bars) used as controls. Data are from UNIBO.

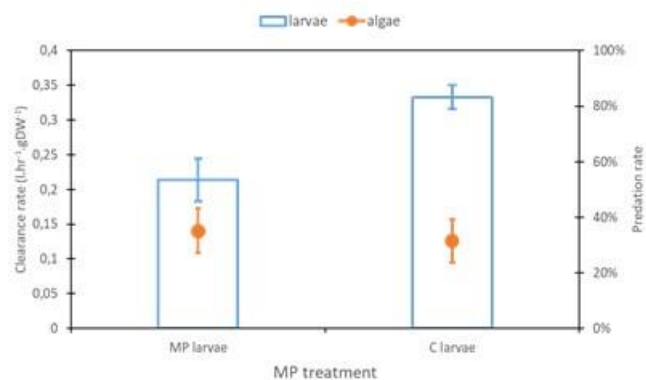


Figure 7. Algal clearance rate (primary y axis) and larval consumption rates (secondary y-axis) by predators (adult *Cerastoderma edule*) in the presence and absence of MP in the prey (*Limecola balthica* veliger larvae). Data are from UGhent.

Evaluating the toxic effects of ingested and accumulated microplastic

Effects of polystyrene (PS) MP exposure in phytoplankton species (NTNU, SINTEF).

Reduced reproduction and chlorophyll production was observed in *Rhodomonas baltica* (Cryptophyceae) at the highest PS MP concentration (7500 PS mL⁻¹) (Figure 8), possibly due to a shading effect. The lowest PS MP concentration (75 PS mL⁻¹) caused no effects whereas the medium concentration (750 PS mL⁻¹) had a stimulatory effect on reproduction. Exposure of the dinoflagellate *Oxyrrhis marina* to the same concentration range of PS and green fluorescent MP reduced intake of natural prey, *R. baltica*, and caused a mortality of approximately 20%.

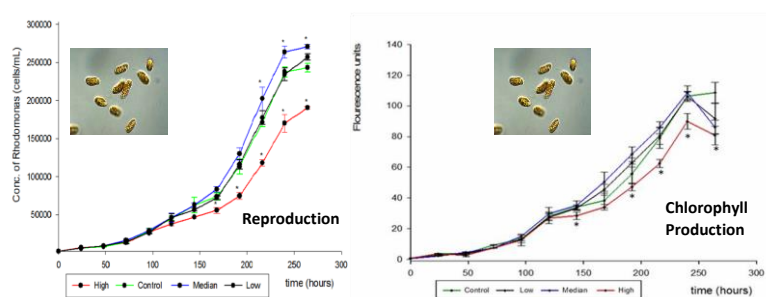


Figure 8. Reproduction and chlorophyll production curves for *R. baltica* exposed to PS microplastics (11 d): 75 (low), 750 (medium), 7500 (high) PS mL⁻¹.

Effects of PS MP in a selected zooplankton species, *Calanus finmarchicus* (NTNU, SINTEF).

Quantitative determination of uptake and excretion rates of 10 µm PS MP (750 PS mL⁻¹) show unconventional uptake kinetics in the marine copepod *C. finmarchicus* when compared to dissolved organic chemicals (Figure 9). Excretion of MP was rapid, although there was indication that retention may occur. PS MP exposure had no effect on survival. To investigate the impact of long-term exposure to MP, *C. finmarchicus* was exposed for a full life cycle to 3 different concentrations of 10 µm PS MP: 7.5, 75 and 750 PS mL⁻¹. Effects on growth, development and reproduction were studied. Analysis of results is in progress.

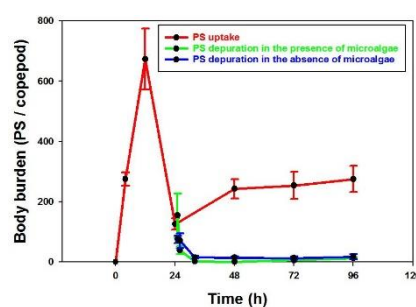


Figure 9. PS MP body burden in *C. finmarchicus* exposed to 750 PS mL⁻¹ (red) or transferred to clean water after PS exposure in the presence (green) or absence (blue) of microalgae prey.

Effects of nylon MP on feeding, lipid accumulation and moulting in copepods (UoE, SINTEF, NTNU).

The effects of fibre (10x30 µm) and granular (10-30 µm) nylon MP on feeding, lipid accumulation, growth and moulting in *C. finmarchicus* was investigated⁷. Copepods showed a shift in prey selectively and exposure to fibres caused an average 40% reduction in algal ingestion. No difference was observed for copepods exposed to nylon granules. No significant difference in the prosome length of juvenile, female or male copepods was observed with either type of MP. There was no significant difference in the total lipid mass of juvenile, female or male copepods. However, a significantly greater proportion of copepods had moulted in the fibre (36.1±8.6%) and granule treatments (34.4±10.7%) relative to controls (9.0±3.2%) after 5 days (Figure 10).

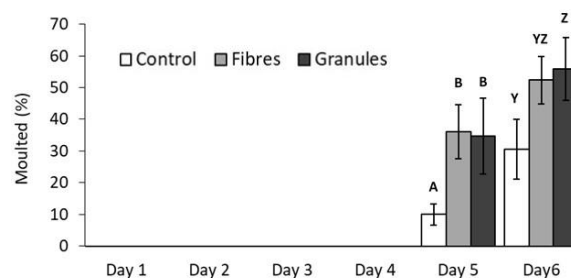


Figure 10. Percentage of *C. finmarchicus* moulting on each day of the exposure period. Treatments: control (white), nylon fibres (light grey) and nylon granules (dark grey). Letters denote significant difference between treatments (P<0.05).

Internalisation of PS NP and MP by haemocytes (UPV-EHU).

Internalisation of fluorescently labelled 0.05 µm PS nanoplastics (NP) into mussel haemocytes was detected only at the highest exposure concentration tested: 10¹² particles mL⁻¹ (Figure 11). Unlabelled 0.5 µm PS MP were

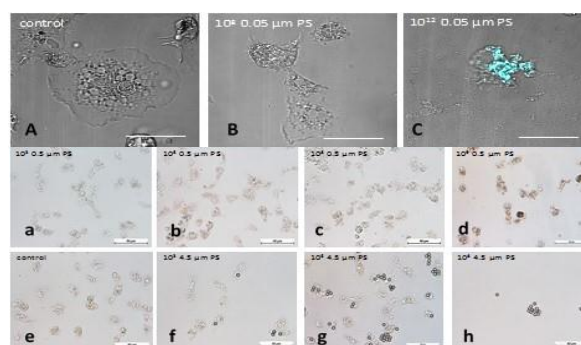


Figure 11. Mussel haemocytes exposed for 24 h to fluorescently labelled 0.05 µm PS NP. A: control; B: exposed to 10⁹ part.mL⁻¹ and C: exposed to 10¹² particles.mL⁻¹ (scale bars = 20 µm); and to unlabelled 0.5 and 4.5 µm PS MP. a-d: exposed to 10³–10⁹ part.mL⁻¹ of 0.5 µm PS MPs; e: control cells; and f-h: cells exposed to 10³–10⁸ part.mL⁻¹ of 4.5 µm PS MPs. Scale bars = 50 µm.

not detected in haemocytes, but at 10^9 particles mL^{-1} cells showed increased endolysosomal vesicles, possibly related to a higher uptake of MP (Figure 11, a-d). Unlabelled $4.5 \mu\text{m}$ PS MP were detected inside haemocytes at low and high exposure concentrations (Figure 11, f-h).

NP and MP cytotoxicity towards mussel haemocytes (UPV-EHU). NP and MP were cytotoxic to mussel haemocytes only at high tested concentrations (Figure 12). Haemocytes viability decreased at 10^{12} part. mL^{-1} of $0.05 \mu\text{m}$ NP (Figure 12A), 10^9 particles mL^{-1} of $0.5 \mu\text{m}$ MPs (Figure 12B) and at 10^6 and 10^8 particles mL^{-1} of $4.5 \mu\text{m}$ MP (Figure 12C). Based on these results, cytotoxicity seems to be related with the mass of plastic particles.

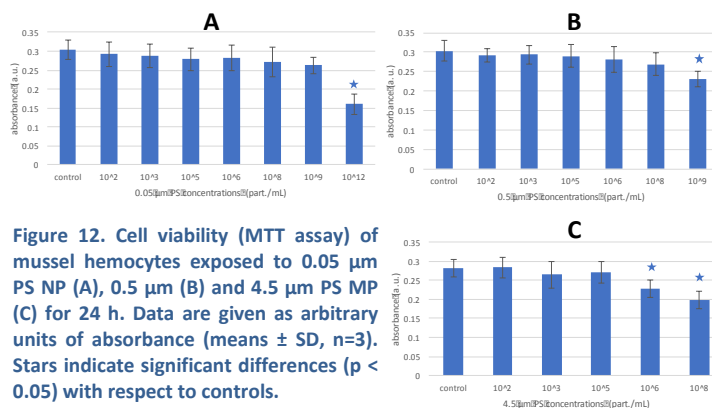


Figure 12. Cell viability (MTT assay) of mussel hemocytes exposed to $0.05 \mu\text{m}$ PS NP (A), $0.5 \mu\text{m}$ PS MP (B) and $4.5 \mu\text{m}$ PS MP (C) for 24 h. Data are given as arbitrary units of absorbance (means \pm SD, $n=3$). Stars indicate significant differences ($p < 0.05$) with respect to controls.

Effects of PS microplastics exposure in mussel early life stages (UNIBO). Uptake of $3 \mu\text{m}$ MP by mussel D-shaped veligers tended to increase with increasing concentrations (Figure 13A-D). The analysis in epifluorescence microscopy did not highlight MP aggregation or plate wall adherence after 24 h of suspension. At all tested post-fertilisation (pf) periods, the exposure to 50–10,000 MP mL^{-1} resulted in significant increases of both the percentage of larvae showing MP ingestion⁸ (Figure 14A-D) and the number of ingested particles (Figure 14E-H). However, only at 48 h, 3 and 6 d pf were the levels of both parameters significantly and positively correlated to the nominal MP concentrations, while no significant relationship was noted at 9 d pf. The lowest uptake levels and variations among treatments were exhibited by larvae at 48 h pf, where significant increases of both parameters were detected only at the highest tested concentration (Figure 14A, E). Major changes in the number of ingested MP were detected at 6 d pf, with levels reaching 14.2 MP larvae⁻¹ at 10,000 MP mL^{-1} (Figure 14G). The only significant decrease in ingested MP with increasing concentration was detected in larvae at 9 d pf exposed to 10,000 MP mL^{-1} compared to 5,000 MP mL^{-1} (Figure 14H).

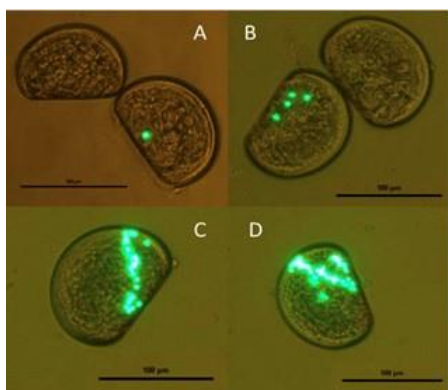


Figure 13. Ingestion of MP by mussel larvae (6 d pf) - (A) 100 MP mL^{-1} , (B) 1000 MP mL^{-1} , (C) 5,000 MP mL^{-1} , and (D) 10,000 MP mL^{-1} .

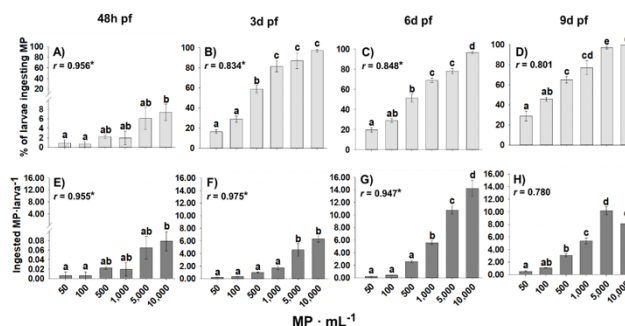


Figure 14. MP uptake by mussel larvae (% of D-shaped veligers showing uptake of MP (A-D) and average number of MP ingested per single larva (E-H) as a function of larvae age and MP concentration (mean \pm SE; $n=4$). Letters indicate statistically significant variations between groups ($p < 0.05$). Pearson's correlation coefficients (r) between assessed parameters and MP concentrations are also reported (* $p < 0.05$).

Microplastic retention time and effects on food consumption by mussel larvae (UNIBO). Larvae exposed to a concentration of 1,000 MP mL^{-1} ($3 \mu\text{m}$) showed an average accumulation lower than 10 MP larvae⁻¹ (based on uptake information reported in Figure 14-H), thus avoiding the possible effect of a high gut load on the subsequent elimination of ingested particles. At the end of the exposure period, 92% of exposed larvae showed ingested MP. However, egestion was marked after 24 h, when only 23% of D-veligers showed ingested MP and further dropped to 9.1% in the following 24 h. After 8.5 d in clean water, all examined larvae showed no MP. No

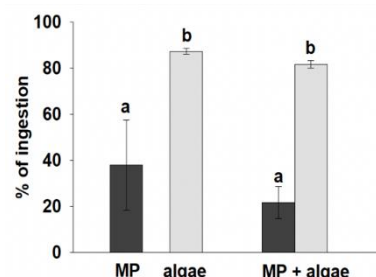


Figure 15. Effects of PS MP ($3 \mu\text{m}$) on microalgae consumption by mussel larvae (mean \pm SE; $n = 4$). Single exposure treatments consisted of 24-h exposure to 2,000 MP mL^{-1} or 2,000 algae mL^{-1} . Co-exposure treatment consisted of 24-h exposure to a mixture of cells and MP. Letters show statistically significant differences ($p < 0.05$).

mortality of larvae was registered. To assess effects of MP on food consumption, mussel larvae were exposed to a mixture of 2,000 MP mL⁻¹ (3 µm) and 2,000 cell mL⁻¹ of the microalgae *Nannochloropsis oculata*. A significant preference for nutritious items (algae) was registered, 80% of algal cells vs 20% of MP ingested (Figure 15). Similar results were obtained in single exposures, where algal consumption was significantly higher (87 %) compared to MP (about 40 %) after 24 h of exposure. The consumption of both MP and *N. oculata* did not change significantly between single and mixed treatments.

Embryotoxicity and mRNA expression in mussels (UNIBO). No statistically significant differences were observed after 48 h of embryo-larval development of *M. galloprovincialis* when exposed to 50-10,000 MP mL⁻¹ (3 µm). Figure 16 reports mRNA expression changes (full symbols representing mean values) and the average trend of variations (lines) within each functional response group. Only transcripts with statistically significant changes in at least one experimental condition (vs control) were considered (p < 0.05; randomisation analysis with REST).

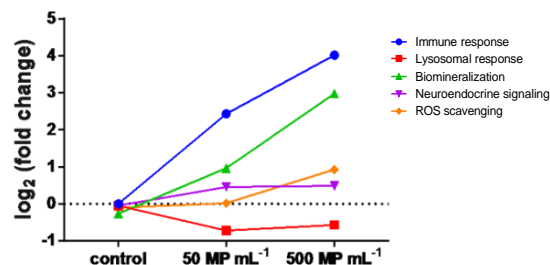


Figure 16. Transcriptional effects induced by 3 µm MP on *M. galloprovincialis* larvae achieving the normal development after 48 h post fertilisation. Data points represent the mean of the log₂-transformed fold changes (vs control).

Effects of MP on MXR-related parameters (UNIBO). Results on the effects of 50-500 MP (3 µm) mL⁻¹ on MXR [Multi Xenobiotical Resistance] related parameters are shown in Figure 17. Embryos exposed to 500 MP mL⁻¹ displayed a significant accumulation of Rho 123, indicating a down-regulation of the MXR transport activity with respect to controls (Figure 17A). The transcription of genes *ABCB* and *ABCC*, encoding for the MXR transporters P-gp and Mrp, were significantly down-regulated (-94% and -32%, p < 0.05) in embryos exposed to the same concentration (Figure 17B).

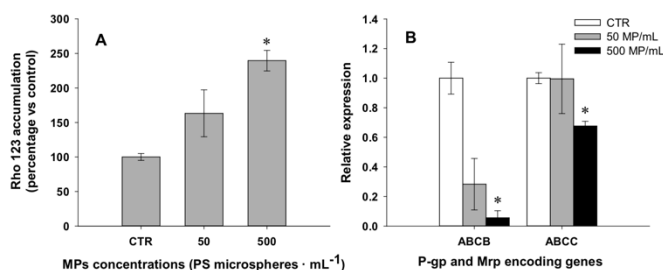


Figure 17. Effects of 3µm MP ingestion on the MXR system in embryos of Mediterranean mussels after a 48h exposure. (A) Rho123 accumulation, (B) ABCB (encoding the P-glycoprotein, P-gp) and ABCC (encoding the Multidrug resistance related protein, Mrp) expression changes. Data are expressed as mean ± SEM (N= 4). *, p < 0.05 vs CTR.

Effects of 50 nm and 45 µm polystyrene particles on mussel fertilisation and embryonic development (UNIBO). The results of the fertilisation and embryotoxicity tests performed on marine mussels exposed to increasing concentrations of 50 nm NP and 45 µm MP are shown in Figure 18. No effect was induced by performed treatments on the fertilisation of marine mussels (Figure 18A). Conversely, a significant reduction in the percentage of normal larvae was observed after exposure to 50 µg mL⁻¹ of both 50 nm and 45 µm PS particles (Figure 18B).

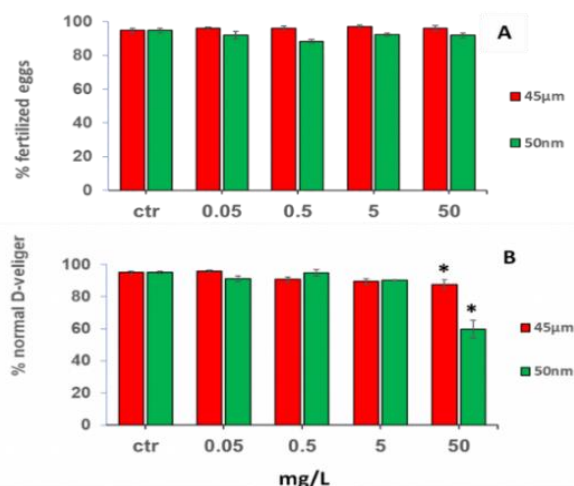


Figure 18. Effects induced by 50 nm and 45 µm PS particles on the gamete fertilisation (A) and embryo-larval development (B) of the Mediterranean mussel *M. galloprovincialis* (mean ± SE, n=4)*, p<0.05 vs control.

Ingestion and distribution of MP in adult mussel tissues (UPV-EHU, NOVA-FCT). Short-term exposure (1 d) of mussels to 4.5 and 45 µm PS MP resulted in rapid ingestion and distribution of MP in the digestive gland in a concentration dependent manner. Smaller MP were found in the stomach lumen inside the digestive tubules and the gut epithelium⁹ (Figure 19: A-C) as well as in the tissue surrounding the digestive tubules and gonads; the same was found for 10 µm MP in a 28 d exposure. After 7 days depuration, MP were still present.

Long-term exposures (26 d) to 0.5 and 4.5 μm MP resulted in genotoxic effects (Comet assay) after 26 d exposure to both sizes¹⁰ (Figure 20). However, there were no effects in haemocyte viability and oxidative stress (measured as neutral red uptake and catalase activity, respectively). A significant change in basophilic cell volume density (VvBas) and absorption efficiency was detected, though other physiological parameters (clearance rate, respiratory rate), were not significantly different from the control.

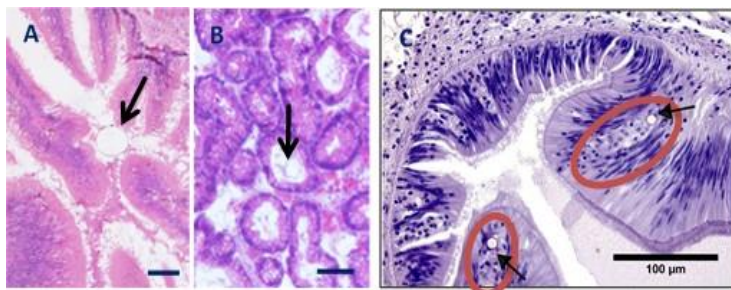


Figure 19. Distribution of ingested MP in adult mussels. A: 45 μm MP in the stomach lumen B: 4.5 μm MP in the lumen of a digestive tubule. Arrows: MPs. Bars: 50 μm ; C: 10 μm MP in inflammatory foci in the digestive gland epithelium (28 d exposure).

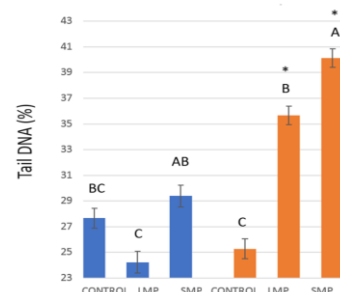


Figure 20. Comet Assay total DNA (%) in adult mussels after 26d exposure to 0.5 and 4.5 μm MP. Letters indicate significant differences between treatments within the same day (Kruskal-Wallis test, $p < 0.05$). 7 d exposure in blue and 26 d exposure in orange.

Short-term MP exposure in larvae of the Senegalese sole, *Solea senegalensis* (NOVA-FCT). Ingestion of MP by sole larvae aged 5 d (5dph) was confirmed in a short term exposure (3 h) to a range of concentrations from 1- 10000 MP mL^{-1} of commercial 1-10 μm MP and mixtures (Figure 21). Particles were readily visible in the digestive tract. No mortality occurred.

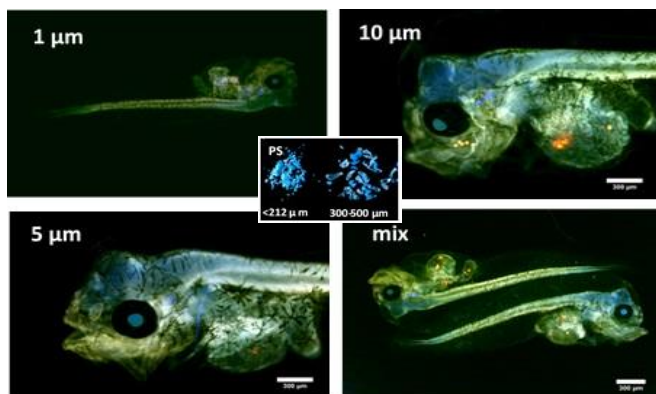


Figure 21. *S. senegalensis*. Ingestion of fluorescent MP by 5 dph sole larvae in a 3 h exposure.

Long term exposure of *S. senegalensis* juveniles to post-consumer PS MP (NOVA-FCT). Ingestion of MP by sole juveniles was confirmed by the presence of MP in the gastrointestinal tract during 56 d exposure to <212 μm and 300-500 μm MP in concentrations of 2.8 and 28 MP $\text{ind}^{-1} \text{d}^{-1}$. Particles were obtained from post-consumer single use plastics and incorporated into customised food pellets. MP were eliminated in the faeces and no accumulation was registered. No significant histopathological alterations were found in the digestive tract. In general, exposures to MP <212 μm yielded higher responses (total GSH and LPO) than 300-500 μm at the same concentration, but the pattern is not clear. Increased levels of total glutathione in liver exposed to MP <212 μm indicates oxidative stress (Figure 22).

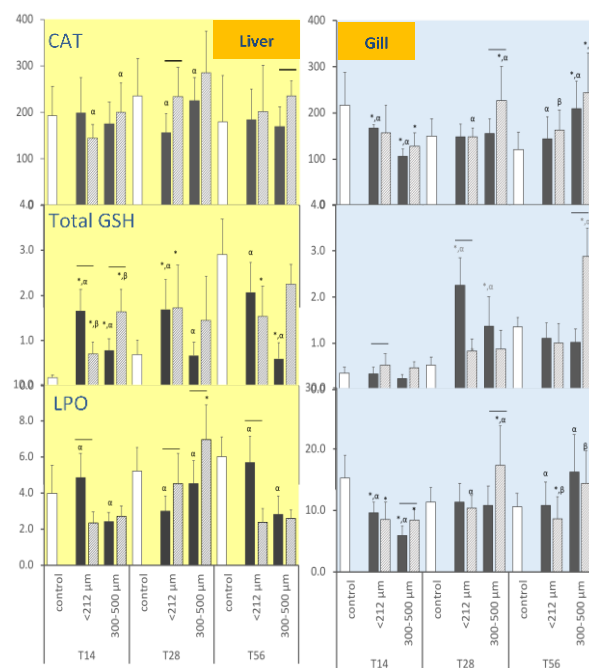


Figure 22. Biomarkers in liver and gill of *S. senegalensis* juveniles (catalase activity, total glutathione and lipoperoxidase). Significant differences ($p < 0.05$): to the respective control (*), between MP concentrations (horizontal bars), between MP size (Greek letters).

Adsorption and desorption of persistent organic pollutants to microplastic

Adsorption and desorption of organic additives (AMU-MIO, NILU). A modelling exercise showed that only 1% of the estimated plastic waste is actually floating at the ocean surface¹¹, leading to the hypothesis that plastic debris sinks to the seafloor. This hypothesis was later verified by measured concentrations of plastic debris in sediment samples and supported by the highest plastic additive chemical concentrations being found in seawater at the bottom of the Yellow Sea and the Mediterranean Sea^{11,12}, which are considered two of the marine areas most affected by MP pollution at the global scale¹³. Studies in PLASTOX attempted to fill the knowledge gap on the effect of hyperbaric conditions (case study at 1000 m depth) on release of common additives. Using seven phthalates (PAEs) and their primary monoester metabolites (MPAEs), nine organophosphate flame retardants (OPFRs) and six bisphenols (BPs) as representative plastic additive chemicals, their release to seawater from plastic materials under varying laboratory-controlled conditions of pressure and bacterial content was studied. Two MP materials of 3-5 mm, PTX131 (recycled PE) and PTX500 (soft PVC) selected for PLASTOX studies, were incubated in natural seawater (5 g of polymer in 130 mL of seawater) for 30 days under varying exposure conditions. Additives released from the plastic materials in the dissolved fraction were enriched using solid phase extraction and analysed by GC-MS and LC-MS¹⁴. Bacteria content was measured by flow cytometry and pressure was controlled using hyperbaric devices. The four major findings were: (1) dissolved organic carbon and phosphorous were released from PVC (30 and 0.14 $\mu\text{mol/g}$ respectively), (2) we found that PVC reduced natural bacteria abundance in seawater, implying potential toxicity of this polymer, possibly via carbon and phosphorous release, (3) the sum of target additives concentration never exceeded μg per gram (ppm) of polymer incubated, whatever the exposure condition applied, after 30 days of exposure, and (4) bacteria increased the total amount of additive released, whereas pressure (0.1 and 10 MPa, i.e. difference between 0 and 1000 m depth) decreased additive release (Figure 23).

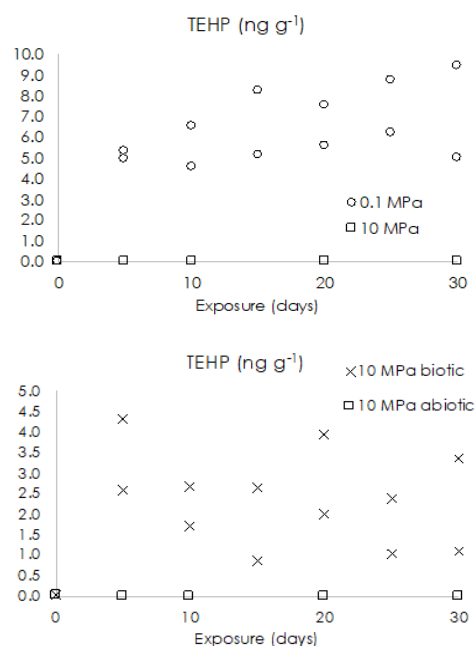


Figure 23. TEHP release from PE upon a 30-day exposure at 0.1 and 10 MPa (left), under biotic and abiotic conditions (right).

As an additional example for organic plastic additive chemicals, the kinetics of brominated flame retardants, in the form of polybrominated diphenyl ethers (PBDEs), was determined in four types of post-industry plastics PTX131 (PE), PTX331 (PS), PTX450 (PET) and PTX500 (PVC) placed in seawater. In addition, adsorption kinetics of PBDEs on MP particles produced by cryomilling a naturally weathered fish box (PTX171) and four types of virgin pellet (PTX102.00 (PE), PTX200 (PP), PTX300 (PS), PTX401 (PET)) were investigated. Both experiments were implemented as part of long-term field experiments at the Sportboat harbour, Tromsø, Norway. Results showed that PBDEs adsorbed to all plastic types with varying concentrations over time, but that concentrations were mostly low and not due to use of PBDEs as an additive. This suggests there was no significant loss of PBDEs through leaching, but rather that adsorption of PBDEs to the plastic surface from the surrounding water was the dominant process. The only exception was for sample PTX331 (PS), where PBDEs seemed to be used as an additive, showing concentrations 1.53 $\mu\text{g/g}$ of total PBDE prior to deployment. During deployment, concentrations of total PBDE associated with PTX331 increased to 1.92 $\mu\text{g/g}$, before levelling off at 6 months, mostly due to high concentrations of PBDE 153 in the material. This does not indicate the release of the PBDEs, but rather a migration of PBDEs from the core to the surface of the material driven by weathering processes. Increased additive concentrations on the surface may increase the bioavailability and the potential for harmful impacts on organisms and ecosystems.

Adsorption and desorption of inorganic metal additives (NUIG). To assess the sorption behaviour of metals (Sb, Ge, Mo, Nb, Te, Sn, Ti, W, Al, As, Ba, Be, Bi, B, Cd, Cr, Co, Cu, Ga, Fe, Pb, Mn, Ni, Sc, Se, Ag, Tl, U, V, Zn) on different MP polymer types in the marine environment, a series of laboratory and field based experiments were conducted in Galway Bay. Owing to the lack of a standardised extraction procedure, direct and meaningful comparisons on metal contaminants associated with MP from marine ecosystems around the world are confounded. Therefore, initial laboratory studies focused on assessing the most appropriate extraction procedure for the determination of metals attached to MPs. In terms of establishing the best extraction method for metals, the study is not yet completed. However, observations to date suggest that the most efficient extraction is both metal and polymer specific. Preliminary observations from adsorption experiments on LDPE and PVC pellets indicate that PVC has a greater affinity for metals, which is likely due to a function of surface porosity and the chemical nature of the polymer itself. Desorption studies indicate that PVC has a higher desorption rate for metals compared to LDPE. It is suggested this is due to a combination of PVCs affinity for metals and its own chemical composition, which differs from that of LDPE. The field component of the deployment study indicated that the novel deployment structure designed within the project and deployed in Galway Bay was a success. The standard operating procedure (SOP) developed in PLASTOX has the potential to be employed in monitoring programs (Figure 24). Samples of the biomonitoring seaweed species *Ascophyllum nodosum* were analysed for metal content and a gradient of contamination from the inner harbour to the outer coast was observed. It is expected that a similar trend will be observed for the pellets from the deployment study, once the optimisation of the metal extraction is completed (expected in August 2019).

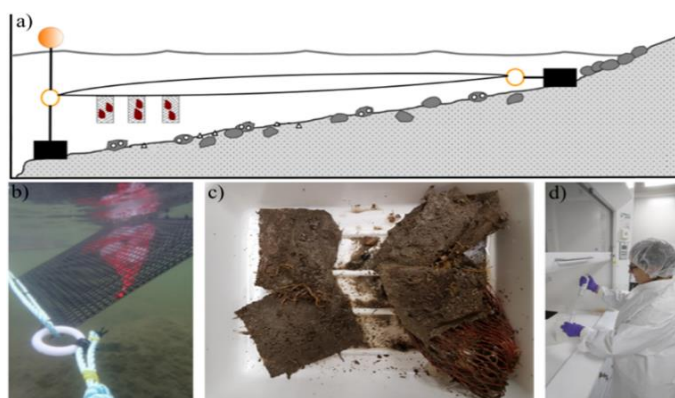


Figure 24. Field based experiments for the adsorption / desorption of metals in the marine environment; a) scheme of the deployment structured used in all sites, b) picture of the structure with bags in the marine environment, c) samples collect after one year in the water, d) extraction procedure in a class 1000 (ISO class 6) clean room.

Adsorption kinetics of organic additives (TUDA, SINTEF). To investigate sorption behaviour of hydrophobic organic compounds (HOPs) on MP, a series of sorption kinetics experiments were conducted. The sorption capacity and mechanisms of the PAHs fluoranthene (FLA) and phenanthrene (PHE) to model MP (PE and PS) were determined as a function of MP size (surface area), seawater temperature (10 °C and 20 °C) and pollutant hydrophobicity. By mass and estimated surface area, the sorption of PAHs was higher in PE than in PS MP of the same size, while sorption capacity was generally higher at 20 °C than at 10 °C (Figure 25). While isotherm model fit varied with temperature and MP polymer type and size, the same best fit model was found for both PHE and

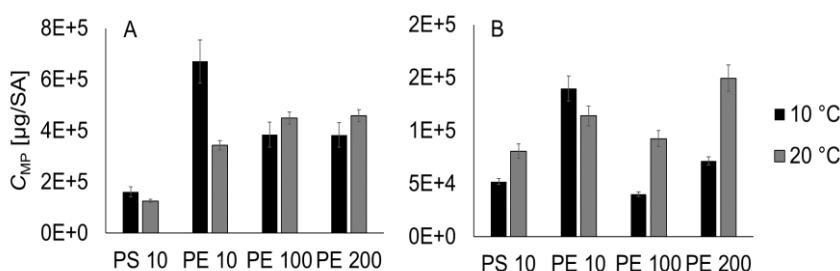


Figure 25. Surface area normalised sorption (µg PAH per m² of MP) of A) fluoranthene (FLA) and B) phenanthrene (PHE). Nominal C_{free} was 20 µg/L PAH.

FLA where conditions were otherwise similar. Larger (100 µm) PE MP had PAH sorption isotherms best described by linear regression, suggesting absorption is the main process. For smaller particles (10 µm PE and PS), sorption was generally best explained by the Redlich-Peterson model, with the exception of 10 µm PE at 20 °C. On a surface area basis, both PE and PS types showed higher sorption capacity for FLA than PHE, reflecting differences in their hydrophobicity. The best fit of Redlich-Peterson suggests a combined monolayer and multilayer adsorption process, with monolayer sorption dominating at lower PAH concentrations and multilayer sorption at higher concentrations. As the concentrations used are well above expected environmental concentrations, PAHs are expected to be found

adsorbed in monolayers to MP in the environment. Furthermore, a good fit of the Dubinin-Astakhov model for PAH sorption to 10 μm PE particles at 20 $^{\circ}\text{C}$ indicates that PAHs are increasingly able to transition into micropores of PE particles with increasing temperature¹⁵.

For larger MP (median 300-600 μm , <1 mm), MP-water partition coefficients ($K_{\text{MP-W}}$) for PAHs with different hydrophobicity was determined. First, influence of the origin (virgin, with lubricant, recycled, weathered) and size of polyethylene (PE)-based MP on $K_{\text{MP-W}}$ for naphthalene (NAP; $\log K_{\text{ow}} = 3.36$) was investigated. In addition, sorption of PHE ($\log K_{\text{ow}} = 4.56$) to the same PE with and without UV stabiliser was examined. The results are summarised in Figure 26. The $K_{\text{PE-W}}$ determined for NAP derived using cryo-milled LDPE pellets (PTX102.00; median 368 μm) was 2.80 ± 0.04 , which is comparable to the two available literature values by Smedes¹⁶ 2.81 ± 0.14 (70 μm) and Cornelissen¹⁷ 3.04 ± 0.14 (100 μm), indicating that the size of the LDPE does not significantly influence the $K_{\text{PE-W}}$ of NAP. The $K_{\text{PE-W}}$

values of NAP determined using PE with lubricant, post-industrial recycled LDPE with colorants and HDPE pellets generated from a beached yellow fish box were determined as 2.68 ± 0.05 , 2.97 ± 0.05 and 2.83 ± 0.04 , respectively. To some extent the origin and additives of PE have an influence on $K_{\text{PE-W}}$, yet to confirm and quantify the effect, a wider range of polymers with different additives and origin

needs to be investigated. The $K_{\text{PE-W}}$ values for PHE using LDPE pellets with (PTX102.00) and without UV-stabiliser (PTX102.50) were determined as 4.00 ± 0.06 and 3.96 ± 0.05 , respectively, indicating the UV stabiliser has no significant influence on the $K_{\text{PE-W}}$ values of PHE. Conversely, HDPE with and without UV-stabiliser (PTX103.00 and PTX103.50, respectively), showed a significant difference in $K_{\text{PE-W}}$ values of PHE. The UV-stabilisers in the LDPE and HDPE materials are unknown. The $K_{\text{PE-W}}$ values derived for PHE are systematically lower than literature available values (4.1-4.3)¹⁸. The use of larger sorbent particles (e.g. pellets) requires a longer period to reach sorption equilibrium, which may increase the loss of PHE through sorption to the walls of the exposure system. This may lead to an underestimation in the PHE concentration sorbed to the pellets (C_s), where C_s is determined by comparing the PHE concentration in the aqueous media of exposure systems with and without pellets.

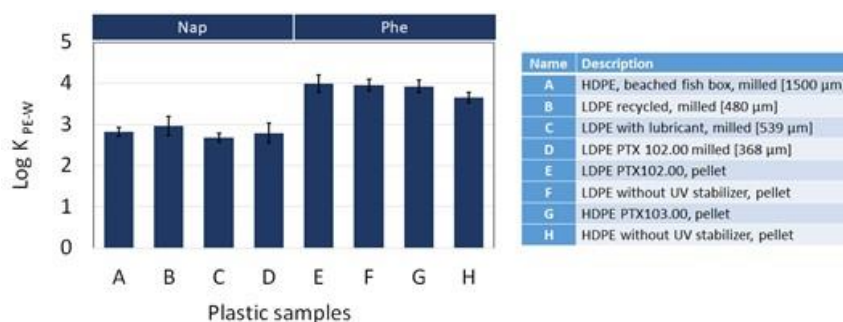


Figure 26. MP-Water Partition coefficients of naphthalene and phenanthrene: PE from different origins, additives and the sizes.

Development of a fast approach for assessing organic chemical sorption (TUDA). A broader suite of polymer types containing a range of different additive chemicals needs to be investigated to confirm the influence of MP physicochemical properties on $K_{\text{MP-W}}$. A cost-effective way to achieve this would be to conduct sorption experiments using commercially available industrial resin pellets, as these are known to be present in the environment with a range of hydrophobic organic chemicals (HOCs) sorbed to their surface¹⁹⁻²³. The use of resin pellets for the sorption experiments has several advantages, including 1) a wide range in selection, 2) reasonable in price, 3) consistent in properties and 4) easy to handle. However, implementation of HOC sorption experiments on pellet-sized polymer particles is challenging due to the low water solubility of HOCs, and their high affinity for organic carbon and other surfaces (e.g. inner walls of exposure systems). A quick and reproducible method for investigating HOC sorption to plastic pellets was developed by TUDA. The use of a water-soluble solvent like alcohol at a ratio of 30 vol-% increases the solubility of HOCs, while at the same time decreasing HOC affinity for pellets and minimising HOC loss to walls of the exposure system (<1 %). If the amount of co-solvent is appropriate, the change of HOC concentration in the solution is fully traceable. Under these “artificial” conditions, a HOC sorption experiment can be completed within 14 d, even when using sorbate polymers of pellet size (i.e. 3-5 mm). Purely for comparison purposes, sorption capacity of a range of widely used plastic resin pellets, LDPE (PTX102.00), HDPE (PTX103.00), PP (PTX200), PS (PTX300) and PET (PTX401) for FLA was investigated using the quick sorption method. The results show that sorption of FLA increases in the order of PET < PS < PP << HDPE = LDPE. In addition, a very good correlation between $\log K_d$ derived through this

quick sorption experiment and the melting temperature of the polymer was found ($\log K_d = -0.0213 \cdot \text{melting point} + 4.5384$, $R^2=0.966$).

Adsorption of HOCs to MP in the natural marine environment (TUDA, AMU-MIO, NUIG, UGhent, NOVA-FCT, IVL, UNIBO, NILU, BSH, WUR). Adsorption of HOCs to virgin LDPE, PP, PS, and PET, selected for the quick sorption study, as well as marine litter derived HDPE (ML; PTX171) was also investigated under “real” environmental conditions. The pellets were deployed at 12 locations around Europe representing a variety of marine environments. To extract groups of chemicals with a wide range of physicochemical properties from the different polymers, extraction methods were optimised for each polymer type. More than 400 samples were handled and analysed by GC-MS for a total of 57 different chemical compounds per sample. To date, the data evaluation has only been completed for selected chemicals (16 PAHs, 13 PCBs, and 6 DDTs) on a limited number of pellets, and the fully data set will be completed in the future. The available data are summarised in Figure 27. Among the virgin pellets, LDPE has a larger capacity to accumulate PAHs compared to the virgin pellets made of PP, PS and PET. However, the amount of PAH extracted from ML (PTX171) was significantly higher than LDPE deployed at the same locations. The accumulation of PCBs, HCHs and DDTs on the selected pellets was not significant over the two-year duration of the exposure experiment in the environment.

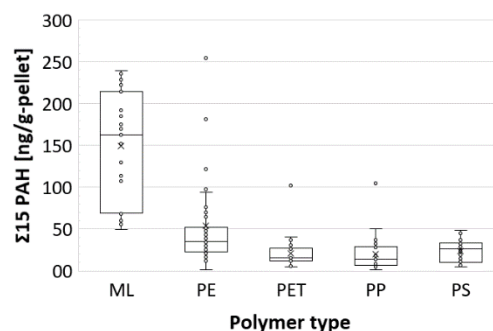


Figure 27. Σ15 PAH accumulation on different polymer pellets in the long-term field experiment.

The PLASTOX project also sought to gain an insight into the sorption of pollutants to items of macroplastic that appeared to have been in the environment for longer than 2 years. This study utilised the large number of macroplastic litter items collected from beaches on the Northern coast of the Netherlands for production of the environmentally relevant reference MP material (PTX001)⁴. From the >180 different PE plastic items, 15 items were selected and analysed for HOC accumulation using the same analytical procedural developed for LDPE pellets. For a comparison, the sum of 15 PAHs on LDPE pellets deployed in three different locations for longer than 6 months and on PE marine litter are presented in Figure 28. The median of Σ15PAHs on PE

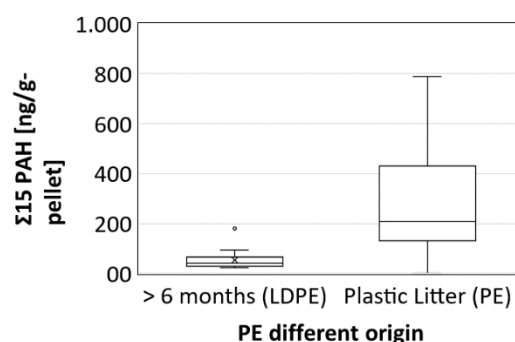


Figure 28. PAH accumulation on LDPE deployed more than 6 months and PE beached plastic litter.

marine litter and LDPE pellets deployed more than 6 months in the European marine environment are 207.2 ng/g and 42.4 ng/g, respectively. In addition to PAHs, the PE marine litter items carry a number of PCBs and DDTs. PCBs are detected in 8 of 15 PE marine litter items at levels of 14 to 337 ng/g (Σ13 PCBs). The DDTs 4,4'-DDE, 4,4'-DDD, 4,4'-DDT and 2,4'-DDT were detected in four marine litter items at concentrations ranging from 58 to 280 ng/g (Σ4 DDTs).

Stable carbon isotope method development (TUDA). To investigate the fate and transport of common organic pollutants found on marine plastic debris, a stable carbon isotope method was developed. The minimum on-column mass per compound required to generate reproducible data (precision < ± 0.1 ‰) using the current system was determined to be 1.4 nmol-C. However, kilogram quantities of plastic are required to achieve this mass of carbon as the actual concentration of a single PAH such as PHE on LDPE from the field experiment was approximately 14 ng/g.

Bioavailability of microplastic-associated POPs and additive chemicals

Plastic additive leaching to Seabird stomach oil (WUR, SINTEF).

Experiments were conducted where the marine litter-derived MP reference material (PTX001)⁴ and cryomilled PS were exposed to the stomach oil isolated from Northern Fulmars (*Fulmarus glacialis*) (Figure 29). GC-MS analysis of the extracted stomach oil shows that transfer of above-mentioned substances from plastic to the stomach oil takes place within two weeks of exposure and does not increase significantly up to 3 months of exposure. A broad range of organic chemicals were tentatively identified using a >90 % match to NIST 2017 library mass spectra. These included known plasticisers, UV stabilisers, antioxidants, flame retardants, polymer impurities and precursors to resins/copolymers used in coatings, inks and adhesives. This study represents a successful example of the use of the PTX001 reference material in the PLASTOX project



Figure 29. Beach-derived MP mixture (PTX001) suspended in stomach oil of Northern Fulmars and after filtration.

and is an ongoing experiment investigating the leaching of organic chemical substances from the plastic mixture to the gastrointestinal fluids of marine organisms. The results indicate that leaching of additive chemicals occurs rapidly and that in a closed system equilibrium between the particles and the stomach oil is established within 2 weeks. This suggests that additive chemicals associated with plastic can readily transfer to biological fluids in the digestive system of fulmars and are potentially bioavailable which may lead to accumulation and toxicity.

Characterisation and toxicity of MP leachates to algae and mussels (UNIBO, SINTEF).

Leachates from car tyre rubber (CTR), polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) showed polymer-specific chemical composition and toxicity on the microalgae *Raphidocelis subcapitata* (freshwater) and *Skeletonema costatum* (seawater), and the mussel *Mytilus galloprovincialis*. Following leaching (14 d), chemical enrichments of water matrices were in the order of µg/L and included organic additives such as acetophenone (CTR, PP, PS and PVC), Benzothiazole (CTR), n-cyclohexylformamide (CTR, PP) and bisphenol A (CTR, PP and PS), and inorganic elements,

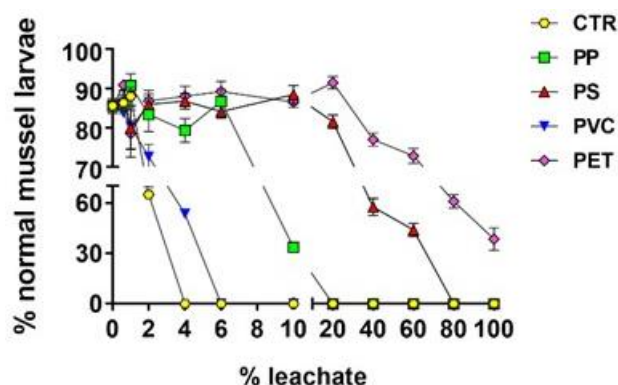


Figure 30. Effects of MP leachates on the embryonic development of the mussel *M. galloprovincialis*. Data expressed as mean \pm SEM of the percentage of normal D-shaped veligers after 48h of exposure.

notably Al (PP, PS, PVC), Cr, (PET), Co (CTR, PET), Cu (PET, PP) and Zn (CTR, PET, PP, PVC) and Sb (CTR, PET, PP, PS). All leachates, except those of PET, inhibited the algae growth after 72 h exposure, with IC₅₀ between 0.5 % (CTR) and 64% (PP) of total leachates (Figure 30). Leachates also affected endpoints measured in mussels, including the lysosomal membrane stability in haemocytes, and early life stage endpoints such as gamete fertilisation, embryonic development and larvae motility and survival. Embryonic development was the most sensitive endpoint in mussels, with EC₅₀ values ranging from 0.81% (CTR) to 65% (PET) of total leachate. Lowest impacts were induced on the survival of D-shell larvae, likely reflecting their ability to down-regulate motility and filtration in the presence of chemical stressors. Overall, this study provides clear lines of evidence on the relationships between chemical composition and toxicity of plastic leachates. In line with general contamination by organic and inorganic additives, leachates were found to be slightly-highly toxic to mussel and algae, urging the need for a better understanding of their overall impact on freshwater and marine ecosystems.

Bioavailability and ecotoxicity of MP-sorbed benzo[a]pyrene and cadmium to mussels (UPV-EHU). Results of a series of studies are summarised in Figure 31. A first short-term (3 d) *in vivo* experiment aimed to elucidate the ability of PS MP of 4.5 µm to carry Cd and benzo(a)pyrene (BaP) and to compare the biological effects provoked by pristine and

contaminated MP (1000 MP/mL), as well as by dissolved Cd and BaP (1 μ M). Increased BaP concentration was measured in mussels exposed to BaP-MP, but BaP levels were highest in organisms exposed to free BaP. Mussels exposed to Cd-MP exhibited similar concentrations to controls, while organisms exposed to dissolved Cd showed much higher levels. No changes were measured in the activity of antioxidant and peroxisomal enzymes nor in lysosomal membrane stability. Histology showed a significant increase in basophilic cells and a reduction in

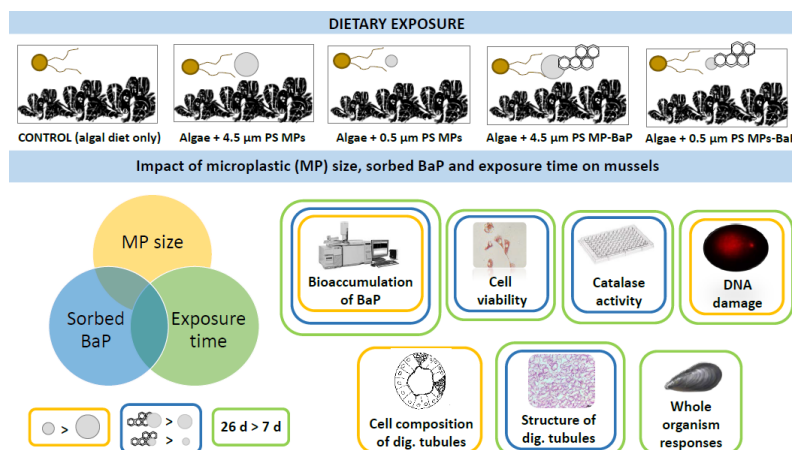


Figure 31. Summary of the effects caused by different sized MPs with sorbed BaP on dietarily exposed mussels.

digestive epithelium thickness in mussels exposed to BaP-MP, but not Cd-MP. This suggests that BaP taken up through MP ingestion exerts biological effects. Mussels exposed to free Cd showed a significant increase in the volume density of basophilic cells and a reduction in digestive epithelium thickness compared to mussels exposed to Cd-MP. A second *in vivo* experiment (7 and 26 d) aimed to investigate the role of MP size on the carrier capacity of MP dosed through the diet. Mussels were fed with a mixture of microalgae and 0.058 mg MP/L (1000 MP/mL/d of 4.5 μ m MP or 7.44 $\times 10^5$ MP/mL/d of 0.5 μ m MP) previously incubated in 1 μ M BaP. BaP levels increased with time in mussels exposed to BaP-MP of both sizes, but was more notable in mussels exposed to 0.5 μ m BaP-MP, indicating smaller MP pose a higher risk. Increased effects of BaP-MP were demonstrated on viability and catalase activity of haemocytes, as well as on the quantitative structure of the epithelium of digestive tubules, but there was no effect of sorbed BaP on DNA damage. At whole organism level a compensatory effect was observed on absorption efficiency across MP treatments at day 26, resulting in increased SFG in mussels exposed to small MP with sorbed BaP. This could be related to an increased energy demand to deal with stress observed in biomarkers. The third *in vivo* experiment (7 and 21 d) aimed to determine the toxicity of 4.5 μ m MP (1000 MP/mL/d) incubated in a complex mixture of PAHs, such as the 100% or 25% water accommodated fraction (WAF) of a Naphthenic North Sea crude oil. Due to the low PAH concentration in the WAF used, adsorption to MP was very low for the most abundant PAHs and not measurable for the rest of PAHs. Accordingly, PAHs were not bioaccumulated in mussels and no effects were recorded on viability, catalase activity and DNA damage of haemocytes of mussels exposed to contaminated MP. Waterborne exposure to WAF alone resulted in accumulation of PAHs, reduction of cell viability in haemocytes and decreased scope for growth. Finally, a series of *in vitro* exposures (24 h) of mussel haemocytes to 0.05 μ m MP (10^2 - 10^{12} MP/mL) in combination with 1 μ M BaP (co-exposure) and to 0.5 μ m (10^2 - 10^9 MP/mL) and 4.5 μ m MP (10^2 - 10^8 MP/mL) with sorbed BaP were conducted to investigate effects on cell viability. Results showed that MP were not highly cytotoxic to mussel haemocytes, but in combination with BaP cytotoxicity increased. 1 μ M BaP alone was not toxic to mussel haemocytes.

Copepod accumulation, ecotoxicity and exposure routes for MP-sorbed PAH (SINTEF, NTNU). Ingestible (PE 10 μ m) and non-ingestible (PE 100 μ m) MP were used to elucidate the mechanisms of MP-sorbed PAH (FLA and PHE) exposure and bioavailability towards two marine copepods (*Calanus finmarchicus* and *Acartia tonsa*) under environmental conditions representing existing PAH contamination. In

control samples (no MP), *A. tonsa* mortality remained above 50 % for both PAHs as expected (Figure 32A). MP addition reduced dissolved FLA by 66 \pm 22 % (PE-10) and 67 \pm 18 % (PE-100), and dissolved PHE by 37 \pm 6 % (PE-10) and 41 \pm 6 % (PE-100), which reduced mortality to 0% in all cases (Figure 32A). In *C. finmarchicus*, dissolved PAH reduction due to MP sorption caused a comparable reduction in accumulation of both FLA and PHE (Figure 32B). Previous

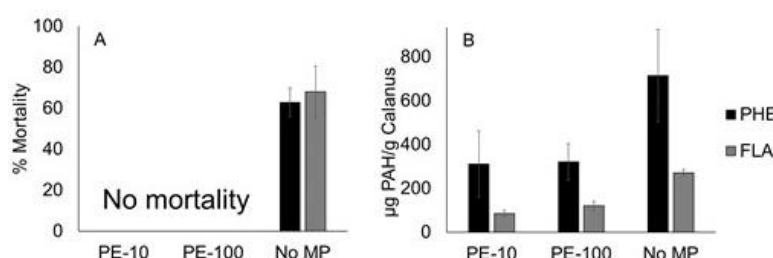


Figure 32. Modulation effect of MP presence on PAH toxicity and bioaccumulation in copepods. A) Reduction in mortality in *Acartia tonsa*, and B) Reduction in PAH body burden in *C. finmarchicus*. Error bars represent standard deviation.

studies have applied pre-loaded MP particles, but without accounting for contaminant leaching into the (then) pristine exposure media, it is not possible to directly link effects to the MP-sorbed contaminants. While the addition of MP caused a reduction in dissolved PAHs, the total concentration of each PAH in the exposure system remained above the LC₅₀ value for *A. tonsa*. In the case of the non-ingestible 100 µm PE particles, the result indicates that the sorbed PAHs are no longer bioavailable given the time-scale of exposure. This indicates that PAHs sorbed to ingestible MP are not a significant contributor to toxicity under typical gut residence times in copepods. Calculated bioaccumulation factors for dissolved PAH concentrations at the end of exposure in MP-PAH exposures were comparable to those calculated for PAH-only solutions. This indicates that only the dissolved fraction of PAHs has contributed to the accumulated fraction of PAHs in *C. finmarchicus* and that MP-sorbed contaminants are not expected to contribute significantly to body burden and detrimental effects in marine copepods.

Sublethal toxicity and embryotoxicity of MP-sorbed chrysene to mussels (UNIBO).

Sorption of the PAH chrysene (CHR) on 3-µm PS MP was studied for its potential to induce physiological and embryo-toxicological effects in the mussel *M. galloprovincialis*. Exposure to uncontaminated MP (20,000 particles/mL for embryos, 5,000 particles/L for adults) and the same concentration of CHR-loaded MP (4 µg CHR/mg MP according to GC-MS analysis) did not affect mussel embryonic development but did alter lysosomal endpoints of general stress. This was indicated by changes in the lysosomal membrane stability and the neutral lipid content after 7-d *in vivo* exposure. Pristine and CHR-loaded MP differentially altered oxidative stress parameters: the former led to an increased content of lipofuscin, which represents the final product of lipid peroxidation of biological membranes²⁴. Conversely, a downregulation of the catalase activity was induced by MP alone, suggesting an inhibiting effect on antioxidant mechanisms. Toxicity data were integrated using the Mussel Expert System²⁵, which assigns an A-E scaled health Status Index (HSI) to performed treatments based on the observed biological responses. Both the exposure to CHR-loaded and uncontaminated MP produced health status alterations representative of a low stress condition (HSI = B) (Figure 33). This study shows that CHR-loaded MP alter lysosomal parameters and induce pro-oxidant effects in *M. galloprovincialis*. Based on assessed endpoints, however, CHR-loaded MP were not found to induce greater effects than uncontaminated MP on marine mussels.

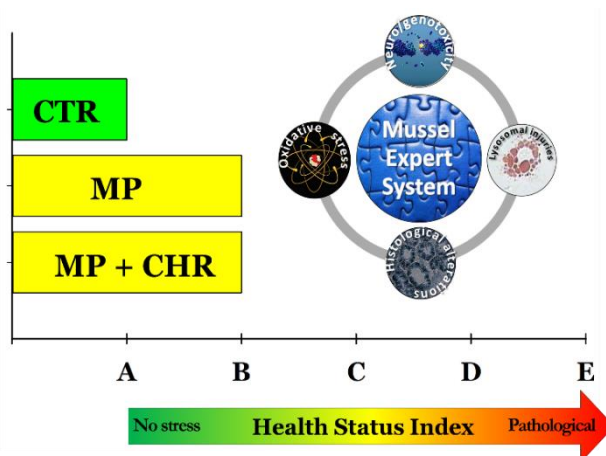


Figure 33. Output of the mussel expert system (MES) integrating toxicity data from endpoints measured after mussel exposure to chrysene-loaded MP (MP + CHR) and uncontaminated MP (MP).

Ecotoxicity and sublethal toxicity of an MP-sorbed PCB mixture to larval Senegalese sole and juvenile turbot (NOVA-FCT, UNIBO).

The potential toxicity of PS post-consumer MP (250-400 µm size) with sorbed Aroclor 1254 PCB was compared with uncontaminated MP, at 1000 MP/mL, in *S. senegalensis* larvae (8 dph), following 7 and 14 d exposure. MP were detected in the digestive tract of larvae after 14 d, but no impairment of metamorphosis was observed. However, stress caused by MP seems to have a slight impact on ocular migration. At 14 d, Aroclor-sorbed MP yielded an increase in lipid peroxides and glutathione-S-transferase (GST) activity compared with control and uncontaminated-MP treatments (Figure 34). Results indicate that 250-400 µm post-consumer MP sorbed with Aroclor 1254 PCB are ingestible by 22 dph fish larvae and induce oxidative damage and phase II-conjugation enzymes. A comparative study into the potential toxicity of 200-250 µm PS MP with sorbed Aroclor 1254 PCBs and MP with biotransformed Aroclor 1254 PCBs (after incubation

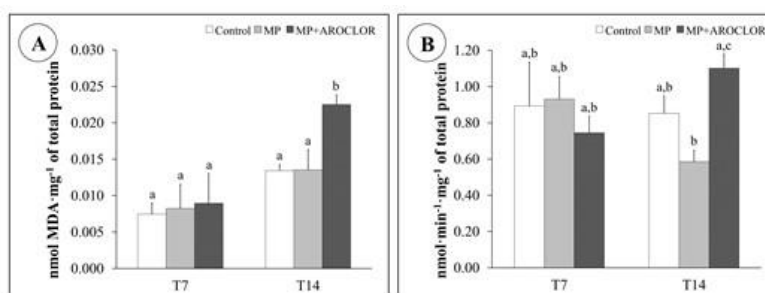


Figure 34. Mean results of biomarkers response in *S. senegalensis* larvae exposed to Control, MP and MP+AROCLOR, for 7 and 14 days. Error bars indicate 95% confidence intervals. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$). A) Lipid peroxides (given by TBARS). B) Glutathione S-transferase activity (as $\mu\text{mol conjugated CDNB min}^{-1}$).

with a marine microbial community) was undertaken using juvenile turbot (*Scophthalmus maximus*). Fish diets were prepared with contaminated MP (30 mg PCBs/Kg MP) and uncontaminated MP. No differences were observed in erythrocyte nuclear abnormalities in fish blood. There were no clear differences between hepatic parenchyma of fish fed without MP and those fed with MP (all-treatments). Increased ethoxyresorufin-O-deethylase activity (EROD) and GST activity were observed in fish liver exposed to diets with contaminated MP compared to MP and control treatments. This indicates increased phase-I bioactivation and phase II-conjugation mechanisms. These findings suggest that Aroclor-sorbed MP trigger biochemical responses in juvenile turbot, without a clear difference between parent and biotransformed PCBs.

Microbial colonisation of microplastic and influence on adsorbed pollutants

Successional dynamics of colonisation of different MP types (UNIBO).

These studies investigated successional dynamics of colonisation in anoxic sediments. Five types of MP provided by PLASTOX partner Carat GmbH were selected for the study: pristine low density polyethylene pellets (PE; 3 mm); pristine crystalline polyethylene terephthalate pellets (PET; 2.5 mm); pristine general purpose polystyrene pellets (PS; 3 mm); pristine homo-polypropylene pellets (PP; 2.6 mm); and pristine polyvinyl chloride soft pellets (PVC; 3 mm).

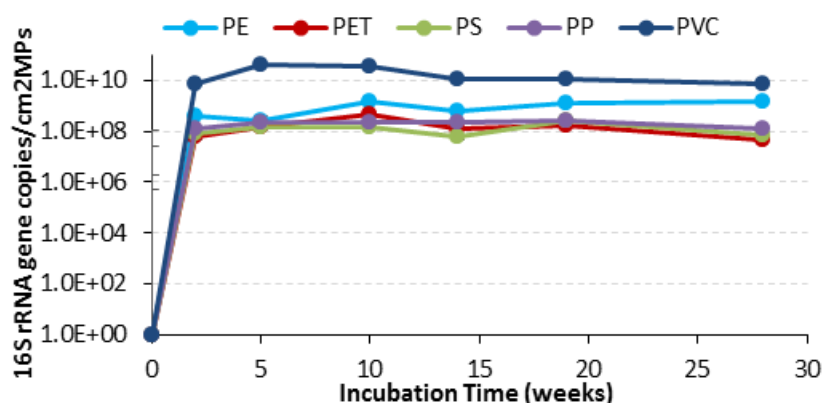


Figure 35. Cell abundances on bacteria biofilm expressed as bacterial 16S rRNA genes per cm² of MP (PE, PET, PS, PP and PVC) over time. Error bars represent one standard error (n = 3).

Bacterial 16S rRNA genes extracted from MP pellets during the 28-day experiment were quantified through qPCR analysis as an approximation of cell density on the MPs surface, thereby obtaining information on the colonisation rate (Figure 35). Colonisation took place very rapidly (within 2 weeks) on all MPs, followed by a biofilm maturation step involving further production of extracellular polymeric substances (EPS) and cyclic detachment and regrowth of biofilm portions. The significant difference observed between the biofilm community composition and the original inoculated culture, as well as between the biofilm communities present on the different MP types, indicates that colonisation of MPs is a selective process.

Biotransformation of MP-sorbed persistent organic pollutants (POPs) by MP-associated marine microbial biofilms (UNIBO).

The biotransformation of PCBs sorbed on MP pellets was investigated in marine environments. No PCB reductive dechlorination was observed for MP incubated in the field, nor in the water column or buried into the sediment. This was probably due to the presence of non-favourable redox conditions in the water column and the lack of specialised PCB dehalorespiring bacteria in the sediment community. However, when incubated in anoxic sediment microcosms in the presence of a marine PCB dehalorespiring community, MP-sorbed PCBs were rapidly and extensively dehalogenated to low-chlorinated congeners. In particular, the PCBs sorbed on different MP types were dehalogenated much faster than those sorbed on sediments, suggesting that they are markedly more bioavailable (Figure 36A&B). In addition, MP-sorbed PCBs are susceptible to bioconversion, which makes them even more bioavailable, although less toxic, low-chlorinated PCBs. This increased bioavailability indicates that microbial biofilms growing on contaminated MP might significantly affect the uptake, bioaccumulation and toxicity of contaminated MP in the marine environment by changing the congener composition of the sorbed PCB mixture. The microbial colonisation took place very rapidly on contaminated MP, where the biofilm composition was influenced mainly by the MP type (Figure 36). One of the factors driving this selective colonisation process may be the presence of sorbed contaminants, although this appears limited to the few members of the community involved in the pollutant degradation/biotransformation.

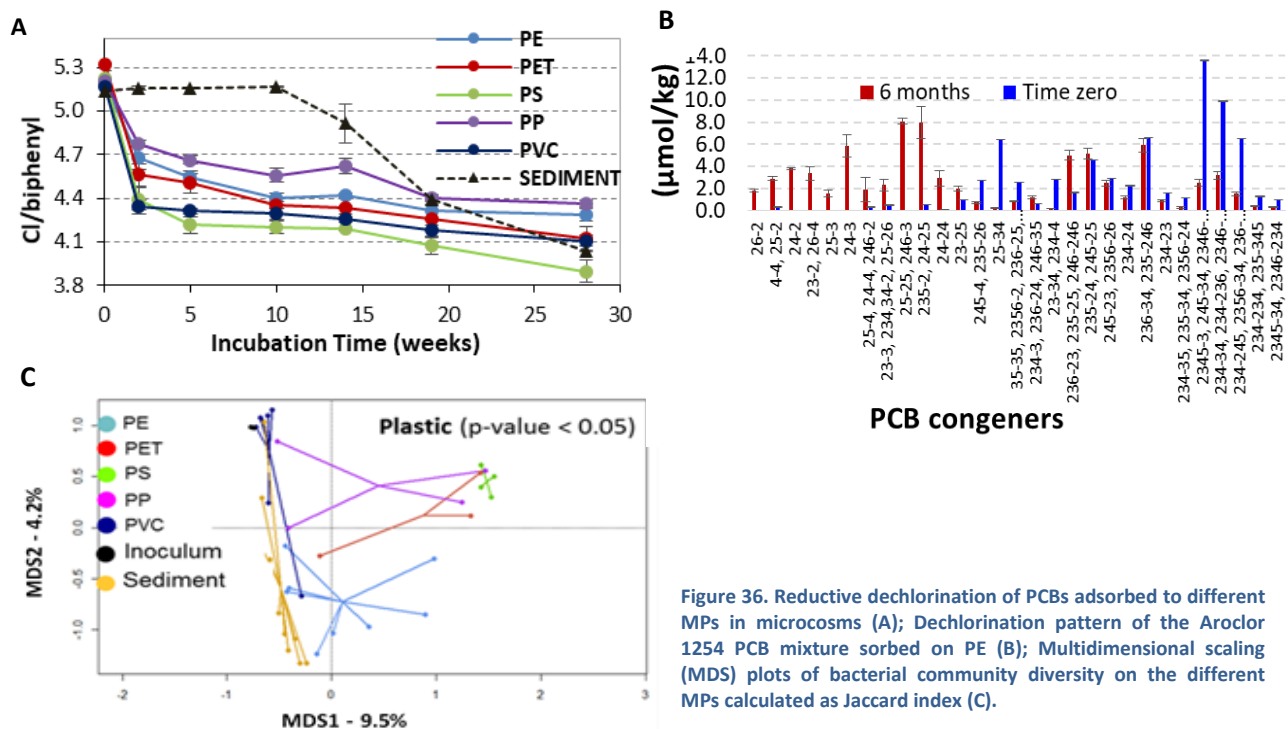


Figure 36. Reductive dechlorination of PCBs adsorbed to different MPs in microcosms (A); Dechlorination pattern of the Aroclor 1254 PCB mixture sorbed on PE (B); Multidimensional scaling (MDS) plots of bacterial community diversity on the different MPs calculated as Jaccard index (C).

DISCUSSION

The extensive field studies in the PLASTOX project showed that MP is readily ingested by a wide range of organisms representing all trophic levels and ecosystem types (benthic and pelagic). However, MP contamination measured at a single time point and location is generally low and extremely variable among individuals of the same species, probably reflecting differences in how recently uptake and egestion of MP particles has occurred in specific individuals. Despite the variability there were some emerging trends, where species that feed in or on the sediment had generally ingested more MP than those species relying on the water column for their diet. Furthermore, larger organisms tended to retain more MP in the gastrointestinal tracts, while predators and omnivores did not contain significantly more MP than herbivores from lower trophic levels. As expected, there was often a selection for smaller sized particles ($<50 \mu\text{m}$), particularly in low trophic level organisms, and we observed that fibres were more frequently found in gastrointestinal tracts, possibly as their shape could lead to slower transit rates. The relatively low number of MP found at any single time in each organism suggests that transfer to higher trophic levels is more likely to occur via detrital pathways than via predator-prey interactions (Figure 37) as generally hypothesised. This was confirmed by our field measurements and laboratory experiments, which showed that filter-feeding organisms, such as bivalves, can act as “aggregators” of the smallest MP ($<50 \mu\text{m}$ size) filtered out the water column to the sediments via faeces and pseudofaeces, facilitating their subsequent uptake by benthic detritus-feeders. The small

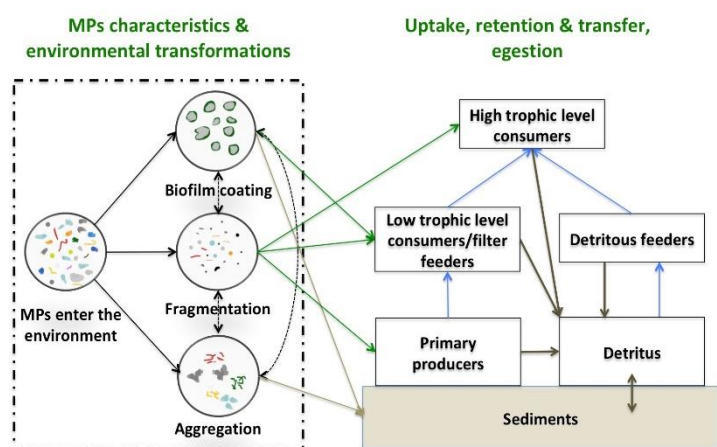


Figure 37. Mechanisms and pathways of the transfer of MP particles along the trophic chain and between different marine compartments (drawing L. Airolidi).

amount of MP found in each organism, combined with the high small-scale variability in MP distribution, properties and bioavailability is operationally challenging, requiring cost-effective methods to quantify the real integrated impact over time of MP on organisms and ecosystems at environmental concentrations.

The toxicity studies conducted within PLASTOX suggest that exposure to MP can result in a range of effects, but this appears to be species dependent and strongly influenced by factors such as exposure concentration and particle size and shape. PS MP can affect microalgae reproduction and photosynthetic activity, albeit at relatively high concentrations. MP are readily taken up by the dinoflagellate *O. marina*, where they can accumulate to a certain extent and increase mortality. In contrast, the copepod *C. finmarchicus* manages to excrete MP effectively without the induction of acute effects. When exposed to fibrous MP, juvenile *C. finmarchicus* shifted feeding, suggesting they avoid microalgae of similar shape and size to fibres. Even with a 40% reduction in ingested biomass, a lack of change in copepod total lipid mass suggests that juvenile *C. finmarchicus* still consumed sufficient energy to continue laying down lipid reserves. Reduced feeding and stymied lipid accumulation may both have contributed to earlier moulting⁷. NP and MP were readily taken up by mussels (*M. galloprovincialis*) in a concentration dependent manner and were able to enter isolated cells (up to 4.5 µm in mussel haemocytes) and penetrate connective tissues and the stomach epithelium, causing inflammation⁹. Genotoxicity was registered in mussel haemocytes in long term assays¹⁰. In early life stages, the ingestion rate is related to larval development and results suggest no selectivity between food and non-food items⁸. Abnormalities observed in mussel embryos suggest that MP particle size might play a key role in the onset of MP-related embryo-toxicological alterations, which may in turn impact viability. Exposure of sole larvae (*S. senegalensis*) to MP resulted in rapid ingestion. Long-term exposure of juvenile sole to post-consumer PS MP caused oxidative stress. However, the response patterns were inconsistent, and no significant histopathological changes were observed. Our findings indicate that the smallest MP cause the largest responses of sub-cellular biomarkers, and that isolated cells, embryos and larvae are the most sensitive to MP exposure. However, effects seem not to be severe enough to cause death or function impairment in most cases. Long-term experiments with relevant MP concentrations followed by longer depuration periods are needed to assess the reversibility of some of the responses. While the mechanisms of toxicity of NP and MP are still not completely elucidated, the effects observed suggest the relevance of sub-cellular endpoints towards a full understanding of their harmful effects.

In both laboratory and field experiments, the sorption capacity of polymers for HOCs typically follows the order of PET ≤ PS ≤ PP << PE. This correlates well with polymer surface energy and density, which decreases in the order PET > PS > PP ≥ PE²⁶. PE has the lowest surface energy, the lowest wettability and the highest hydrophobicity, indicating a preferable matrix for HOC adsorption. When comparing the same polymer, those with higher densities tend to have a higher degree of crystallinity, lower free volume and a shorter distance between polymeric chains, making it more difficult for pollutants to diffuse through it and giving a lower HOC sorption capacity^{18,27-31}. The sorption of HOCs to MP particles in the marine environment is complex and influenced by parameters such as polymer type and size, HOC physicochemical properties and seawater temperature. Importantly, there is a transition from absorption to adsorption as MP size decreases, suggesting HOC bioavailability may be higher on smaller MP particles. In warmer marine waters, MP particles can be expected to carry increased levels of HOCs, with more hydrophobic compounds partitioning to a greater level. Although sorption can potentially be in the form of monolayers and multilayers, under environmentally relevant HOC concentration, monolayer adsorption to MP is expected to predominate. Under warmer seawater conditions, transition of HOCs into MP micropores may occur, possibly reducing their bioavailability when organisms interact with MP.

Weathered PE macroplastic litter can accumulate a wider range of HOCs and at concentrations up to one order of magnitude higher than observed for virgin PE pellets. Exposure to UV radiation causes the surface of polymers to crack and craze, resulting in an increased surface area for adsorption/desorption to take place. Weathering can also increase polymer density and make the surface properties more hydrophilic due to the formation of hydroxyl, carboxyl and carbonyl groups, which may promote desorption rather than adsorption of HOC. The release of hydrophobic additives as well as adsorbed HOC from polymers in the environment, therefore, does make sense. Accumulation of a high degree of HOCs on a weathered polymer surface may also be driven by other factors, including biofilm development, increased wettability and migration of hydrophobic additives from the polymer core to the

surface. Despite the fact that plastic debris accumulates in marine sediment and high concentrations of additive chemicals have been measured in waters close to the seafloor, the influence of high pressure on additive chemical release appears to be limited. Therefore, the mechanisms of additive enrichment in seawater at the bottom of the sea still need to be investigated. Our studies show that weathered PE carrying HOCs may represent the highest exposure risk to marine organisms. Irrespective of the polymer type, increased additive concentrations on the surface may increase their bioavailability and potential for harmful impacts on organisms and ecosystems.

While some laboratory studies have demonstrated the potential for transfer of organic contaminants to organisms via ingestion of contaminated MP, others indicate that MP-sorbed organic contaminants may not be readily bioavailable (reviewed by Ziccardi, et al. ³²). A comprehensive study modelling the transfer of POPs from PVC and PE MP particles to a benthic invertebrate, a fish and a seabird has suggested that this exposure route was negligible with respect to the combined intake from food and water³³. From the available literature, it appears the experimental conditions employed in such studies play a crucial role in the bioavailability MP-sorbed POPs. The results across the studies conducted within the PLASTOX project also show that MP-sorbed organic contaminants are bioavailable under certain conditions, with reduced or no bioavailability under others. From the current study and available literature, it is evident that species-dependent factors, such as lipid store and gut residence time of MPs play a crucial role in the potential for desorption and transfer of MP-sorbed organic contaminants to organismal tissue, which again determines an eventual toxicological influence of these chemicals. Furthermore, there needs to be a much greater focus on understanding and measuring the true exposure conditions in such studies and ensuring bioavailability can truly be linked to pollutants present on an ingested particle surface and not that they have simply dissolved into the surrounding exposure media.

Despite recent reports of thousands of different organic and inorganic chemicals being used as additives in plastic materials, our understanding of their potential leaching and desorption into the marine environment remains poorly understood³⁴. Although the PLASTOX project only had a relatively small focus on this issue, we have been able to show that a range of organic additives are able to leach into both seawater and biological fluids (seabird stomach oil). Furthermore, when present as a complex mixture of chemicals in seawater, additives are bioavailable and have the potential to cause effects in marine organisms. Although several of the early plastic additive compounds have since been banned, they are still found in old plastic fragments at concentrations as high as 1 mg/g³⁵, suggesting they may become legacy pollutants due to the slow degradation of plastic.

Recent studies reported that MPs are readily colonised by environmental microorganisms in few hours or days, and the bacterial assemblages colonising MPs are significantly different in taxonomic composition compared to those present in the surrounding water and/or sediment³⁶. The PLASTOX project showed that colonisation of MP occurs rapidly (<2 weeks) and that community structure varies with polymer type. These differences may be attributed to factors such as polymer chemistry, MP surface properties and the presence of chemical additives. Under the anoxic conditions occurring in sediments, PCBs can undergo microbial reductive dechlorination, a process converting highly chlorinated congeners into less chlorinated products³⁷, which are often less toxic and less prone to bioaccumulation. We also showed that PCB-contaminated MPs are rapidly colonised by microbial communities, and that the presence of MP-sorbed PCBs can enrich the biofilm with reductive dechlorinating bacteria. It also showed that reductive dechlorination can take place more rapidly when PCBs are associated to MP than to the sediment, indicating a higher PCB bioavailability on MP. Colonisation of MP and microbial transformation of MP-associated PCBs may thus markedly affect the bioavailability of MP-associated PCBs and the toxicity of PCB-contaminated MPs.

Future research focuses.

Nanoplastics. The concentrations of small MP (<10 µm) and nanoplastic (NP) particles in the environment are virtually unknown at present. This means that we are potentially missing data and knowledge about the bulk of the particles in the marine environment (in terms of particle number). There is currently a lack of available methods for identifying and quantifying NP in environmental samples which needs to be addressed. As the behaviour of NP is likely to differ significantly from that of MP or larger plastic particles, an understanding of NP environmental fate and behaviour is needed to identify environmental matrices most at risk from exposure and accumulation. With increasing evidence

that MP are unable to transfer across biological barriers, NP are small enough to undergo this process. This mobility within organisms increases the risk of NP accumulation and, consequently, adverse effects at cellular and molecular levels. Knowledge is urgently needed on the exposure, uptake, accumulation and hazards associated with NP.

Additives. Most research to date has been specifically focused on MP that are differentiated primarily by size and polymer composition. However, additive chemicals in plastic products and materials may represent up to 60% of the bulk material. The term additive chemical is used to describe the 100s-1000s of different organic compounds and metals that can be added to plastic to give it specific physical and chemical properties (e.g. UV stabilisers, plasticisers, antioxidants, flame retardants). Little is known about additive leaching from MP in the environment or when passing through marine organisms and their subsequent role as potential environmental pollutants and toxicants. Importantly, many of these additive chemicals are used in plastic products to increase their resistance to degradative processes, thus making the same polymer types more or less susceptible to natural degradation processes. Therefore, a clearer understanding of their role on the degradation of plastics in the environment is needed.

Degradation. Knowledge of particulate and chemical products formed from environmental degradation of plastic and the potential transport, behaviour, fate and impacts of these products in the marine environment is limited. Plastic degradation mechanisms in the marine environment are extremely slow and influenced by polymer type, the presence of additive chemicals, environmental conditions, seasonal variation. There is a need to understand which degradation processes are most relevant in different environmental matrices (e.g. seafloor, beaches, water column, surface waters, inside organisms etc). The complexity and duration of plastic degradation makes it difficult to design environmentally relevant and reliable laboratory studies and improved experimental design and accelerated methods are needed. There is also very little information regarding the influence of degradation process on the impacts of plastic exposure and ingestion by marine organisms, especially resulting from chemical changes to the plastic.

Characterisation and quantification methods. Current methods for detecting and quantifying MP in laboratory can be significantly costly, time consuming and resource heavy. In addition, the cost of purchasing the instrumentation required for such analyses is, in many cases, prohibitive for routine monitoring or analysis (e.g. μ FTIR spectroscopy and Raman spectroscopy). The development of alternative technologies for analysing and characterising MP should be considered a research priority and a platform for 'blue sky thinking' to address this may be beneficial as solutions may well come from outside the existing research community working in this field. Furthermore, it is suggested that the development of methods and technologies to detect, characterise and quantify MP remotely in the field should have a focus in future research activities.

Specific test cases, monitoring and approaches. There are now sufficient studies reporting on the types of MP litter for us to have a good indication about what the main sources of this pollution are (e.g. car tyres, microfibres from textiles). However, a number of these MP materials are not amenable to the techniques developed for more 'conventional' MP. It is therefore relevant for future research to defined cases studies (e.g. car tyres or textiles) where the necessary methods are developed (or existing methods are modified) to facilitate an investigation of their transport routes, environmental sinks and environmental behaviour. This information should be used as a basis for defining those environmental compartments and organisms most likely to be exposed to MP and therefore most in need of effects studies. For example, many studies highlight that sediments are the main environmental sink for MP, yet most effects studies focus on pelagic species rather than sediment species.

IMPACT - OUTCOME

The main outcome of the PLASTOX project has been a significant contribution to the body of research concerning the environmental fate and effects of MPs on marine organisms. The project also had the ambition to create impact outside of the core research activities. Through the public outreach and media-focused dissemination activities conducted within the project, PLASTOX has communicated key findings and general knowledge about the environmental fate and effects of MP to the public (see Part B of report). A summary of the main project outcomes from an impact perspective are present below.

Contributions to regulations, policies and management practices

- Andy Booth (SINTEF) was appointed as a member of the EU MSFD Technical Group on Marine Litter to facilitate direct knowledge transfer from PLASTOX to an EU MSFD Position Paper on "Considerations for MSFD implementation and in preparation of TG Marine Litter monitoring guidance update" (still under development).
- NUI Galway partners answered technical questions in relation to mesopelagic fish study from EU MSFD TG ML.
- Andy Booth (SINTEF) was appointed co-chair of the International Council for Exploration of the Seas (ICES) Working Group on Marine Litter to facilitate direct knowledge transfer from PLASTOX and other JPI Oceans projects to the development of MP monitoring.
- NUIG partners were invited to meet the Irish Minister for Climate Action, Communications and Environment (Mr. Denis Naughten) to present the PLASTOX research activities carried by NUIG and discuss how this can be used to inform national policy.
- MIO colleagues were invited (July, 2nd 2019) to the French national assembly for discussion regarding endocrine disruptors released by plastic material in the Ocean. These discussions may play a role in French policy on regulations concerning plastic and endocrine disrupters.
- Richard Sempere (MIO) was invited by the German Embassy in Canada at Dalhousie University in Halifax and by the French Embassy in Ottawa for discussion regarding plastic in the Ocean (December, 2018).
- Dorte Herzke (NILU) and Andy Booth (SINTEF) were invited into the AMAP Litter and Microplastics Expert Group.

Innovation

During the project, members of the PLASTOX consortium have been responsible for the development of a number of novel exposure systems, items of scientific equipment and methodologies, including:

- SOPs for monitoring POP/metal desorption from MP, as well as additive release from MP, in long-term field studies applicable for a wide range of European marine environment (TUDA, NILU, UGhent, NUIG).
- Experimental apparatus to quantify uptake and egestion rates of MP by filter feeders in the marine environment by collecting faecal matter produced by the organisms (UNIBO).
- A simplified enzymatic digestion protocol for use on the gastrointestinal tract of marine invertebrates such as crabs (IVL, UNIBO).
- Novel exposure methods for studying the bioavailability and biological effects of MP-sorbed POPs under conditions representing multiple exposure routes (SINTEF, NTNU, UPV/EHU).
- Production of a marine litter-derived MP plastic reference material and a method for achieving this (WUR; CARAT, SINTEF).
- An online method for compound-specific stable carbon isotope analysis (CSIA) for selected PAHs has been developed (TUDA).
- New protocols were developed for extraction and analysis of phthalate, bisphenol and organophosphate ester additives¹⁴, and for studying their release from plastics in laboratory controlled experiments³⁸ (MIO).
- Development of a method for the detection of PS MP in paraffin-embedded histological sections³⁹ (NOVA-FCT).
- Novel type of plankton wheel including water cooling bath for studying ingestion and excretion of MP in planktonic organisms as well as short- and long term effects (NTNU).

Newly developed and optimised analytical chemical methods have been compiled into a 'PLASTOX Project Method Catalogue'. An overview of these methods are presented in Appendix 1.

Exhibitions

PLASTOX partner have contributed to a number of public exhibitions where key findings and general knowledge about MP environmental fate and effects have been exhibited at national and international levels. A full overview of these dissemination measures is presented in Part B of the report, but a key example is:

Ocean Plastics Lab (<https://oceanplasticslab.net/>). PLASTOX contributed to the displays in this international travelling exhibition, which is designed as a hands-on science lab, inviting visitors to assume the role of scientists explore the extent and impact of plastics in the ocean. To date the exhibition has visited Turin, Italy; Paris, France; Brussels, Belgium; Washing D.C., USA; Ottawa, Canada; Berlin, Germany; Lisbon, Portugal. The Ocean Plastics Lab has been initiated by the German Federal Ministry of Education and Research together with the German Marine Research Consortium, supported by the European Commission and international partners from politics and science.

Education and career development

The PLASTOX project has funded or co-funded 8 PhD students and 5 Post-Doctoral researcher positions. In addition, the project has supported the studies of ~40 BSc and MSc students. In additional, the findings from PLASTOX and general information about MP pollution have been communicated to school-aged children on a number of occasions.

New cooperation and collaboration

The multinational PLASTOX consortium has established a strong network of European research centres studying the environmental fate and effects of MPs on marine ecosystems. The consortium has sought to use the competences and knowledge gained within PLASTOX to collaborate more broadly. This has included direct links being established with ongoing research and social science projects funded at the national and international level (e.g. SINTEF collaboration with India). PLASTOX partners formed particularly strong links formed with the BASEMAN, EPHEMARE and WEATHER-MIC projects funded by JPI Oceans. For example, Stefania Piarulli (UNIBO) spent 8 months working at with Dr Gunnar Gerdt (coordinator of BASEMAN) and colleagues at the Alfred Wegener Institute, Helgoland, Germany.

Follow-up projects and activities

The PLASTOX project has represented an important basis for partners to secure new research projects that have been developed using the results and new research questions identified. Examples of some of these new projects include:

- Microplastics: Long-term Effects of plastics and Additive Chemicals on marine organisms (MicroLEACH). Funded by Norwegian Research Council, 2019-2021 (SINTEF).
- Factors influencing the formation, fate and transport of microplastic in marine coastal ecosystems (FORTRAN). Funded by Norwegian Research Council, 2019-2022 (SINTEF).
- Evaluating the fate, effects and mitigation measures for microplastic fibre pollution in aquatic environments (MICROFIBRE). Funded by Norwegian Research Council, 2017-2020 (SINTEF, NTNU).
- Distribution of additives and plastics in Marseille Bay in northwestern Mediterranean Sea. Blue Polut and Caremed projects funded by the French 'Agence de l'Eau' (MIO).
- POPs adsorbing to Marine plastic litter in the Arctic marine environment acting as a new vector of exposure; expanding PLASTOX to the North (PLASTOX-NORTH). Funded by FRAM Centre flagship, 2018-2019 (NILU).
- Plastic pollution; a global Challenge; Towards harmonised understanding, education and methodology in Europe, USA and China (PlastPoll). Funded by Norwegian Research Council INTPART program, 2018-2021 (NILU).
- Strategies for the reduction of urban plastic emissions into limnic systems (PLASTRAT). Funded by Federal Ministry of Education and Research in Germany (BMBF) "Plastik in der Umwelt", 2017-2020 (TUDA).
- Development of a characterisation method for microplastics in composts and application on the examination of compost samples (co(MP)ost). Funded by Fritz und Margot Faudi Stiftung, 2018-2020 (TUDA).
- Exploring the fate of Mediterranean microplastics: from distribution pathways to biological effects (EMME). Funded by Italian Ministry of University and Research, 2019-2022 (UNIBO).
- Land-based sources of marine litter and microplastics: Evaluation and modelling transport in rivers and estuaries, and implementation of strategies for prevention and reduction at source (RIVERSEA). Funded by National Science and Technology Foundation, Portugal, 2018-2021 (NOVA-FCT).
- Microplastics in intertidal and subtidal sediments from the Irish marine environment. Funded by the Irish Government Department of Housing Planning and Local Government (DHPLG), 2019-2020 (NUIG).

CONCLUSION

The PLASTOX project had the overarching goal of contributing to an improved understanding of the ecotoxicological impact of MP and the POPs, metals and additive chemicals associated with them. The laboratory and field studies conducted within PLASTOX highlighted that MP is readily ingested, either directly or indirectly, by a wide range of organisms from different trophic levels and ecosystem types. The studies showed that MP >20 µm is predominantly limited to the digestive systems of these organisms and is eventually excreted. We can conclude that most MP is not truly accumulated and therefore trophic transfer through predator-prey interactions may be less relevant than previously thought, but more important via detrital pathways. Furthermore, measuring MP in the digestive systems of organisms may not represent a good approach for monitoring the contamination of biota by MP, as the wide variation in MP content of individuals collected from the same site indicates that 'snapshot' measurements are influenced by recent ingestion and excretion events.

The *in vivo* and *in vitro* toxicity studies conducted within PLASTOX show that exposure to MP can result in a range of sublethal effects in multiple species representing different trophic levels. Importantly, these effects appear to be species and life-stage dependent, and strongly influenced by factors such as exposure concentration and MP size and shape. Our studies indicate the smallest MP and NP cause the largest responses of sub-cellular biomarkers, possibly due to their ability to penetrate tissues and pass through biological membranes. Isolated cells, embryos and larvae appear the most sensitive to MP exposure. However, effects seem not to be severe enough to cause death or impair key biological functions in most cases. While the mechanisms of toxicity of NP and MP are still not fully elucidated, the effects observed suggest the relevance of sub-cellular endpoints towards a full understanding of their harmful effects.

Using a combination of field and laboratory studies, PLASTOX has shown that the sorption capacity of MP for HOCs is closely correlated to the physical and chemical properties of specific polymers, in particular surface energy, hydrophobicity and density. PE, which is the most abundant polymer type found in the marine environment, was found to have the highest sorption capacity for HOCs. Sorption process in the natural marine environment are influenced by a complex set of intrinsic and extrinsic parameters that affect whether adsorption (monolayer or multilayer) or absorption occurs and subsequently bioavailability of HOCs. The weathering of plastic appears to increase its capacity to sorb HOCs through increases in available surface area and changes in surface chemistry.

Studies have indicated the bioavailability of MP-sorbed POPs and metals to organisms is complex and influenced by a number of factors. Results from PLASTOX and other studies show contradictory findings, suggesting bioavailability is strongly dependent upon exposure conditions and species-dependent factors (lipid content, gut residence time). Differences in test systems and approaches in different studies, combined with a limited control of the true POP exposure conditions (dissolved, sorbed) contribute to this uncertainty. The release of additive chemicals from plastic was not significantly influenced by pressure, despite higher concentrations of additive chemicals and plastic debris in seawater close to sediments. However, we have shown that a range of organic additives can leach into both seawater and biological fluids (seabird stomach oil), and exposure by marine organisms can result in toxicological effects.

PLASTOX has shown that microbial colonisation of MP occurs rapidly (<2 weeks) and that community structure varies with polymer type, possibly further influenced by MP surface properties and additive chemicals. PCB-contaminated MPs are also rapidly colonised, where MP-sorbed PCBs can enrich the biofilm with reductive dechlorinating bacteria. Microbial transformation of MP-associated PCBs can occur which may influence their bioavailability and toxicity.

The evidence from PLASTOX and other published studies suggests that current levels of MP in the environment are substantially lower than those expected to result in a significant risk, although hotspots of MP pollution may represent a concern. PLASTOX and other recent studies have also indicated that the risks associated with very small plastic particles (<10 µm and in the nanoscale) may be greater due to their increased potential for traversing biological barriers and undergoing true accumulation within the tissues of organisms. Furthermore, the role of additive chemicals and metals in plastic materials remains poorly understood, especially in weathered plastic. At least some of these chemicals leach from plastic materials and particles when present in the aquatic environment and biological matrices, and their subsequent bioavailability can lead to toxic effects.

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PART B - STATISTIC REPORT

LIST OF SCIENTIFIC QUALIFICATIONS (PHD, MSC AND BSC THESES)

PhD Students fully or partially funded by PLASTOX:

- Marco Capolupo (UNIBO) - New perspectives in the use of sentinel organisms in emerging contaminants ecotoxicological assessments for coastal waters. Erasmus Mundus Joint Doctorate in Marine and Coastal Management, MACOMA (2018).
- Michael Gottschling (TUDA) - How can a model for the estimation of distribution coefficients be developed from the context of substance-specific parameters of pollutants and microplastics (MP)?
- Susanne Kühn (WUR) - Foodweb transfer and ecotoxicological impacts of plastics on marine organisms, in particular the northern fulmar.
- Ana Rita Mendes (NUIG) - Microplastics, metals and the marine environment.
- Stefania Piarulli (UNIBO) - Ecological effects of microplastics in coastal marine environments.
- Brecht Vanhove (UGhent) - Ecological aspects of microplastics in marine benthic communities.
- Alina Wieczorek (NUIG) - Microplastic pathways in marine pelagic systems.

PhD students associated with PLASTOX through other funding sources:

- Rachel Coppock (UoE and Plymouth Marine Laboratory) - Microplastics in the marine environment; From top to bottom.

Post Doc positions fully or partially funded through PLASTOX:

- Vincent Fauvelle (MIO)
- Joanne Wong (UNIBO)
- Antonella Rosato (UNIBO)
- Francesco Paolo Mancuso (UNIBO)
- Marco Capolupo (UNIBO)

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- Dr Marta Martins (NOVA-FCT) has contributed to the PLASTOX project through funding provided by the Portuguese Foundation for Science and Technology.
- Dr Alberto Katsumity (UPV-EHU) has contributed to the PLASTOX project through funding provided by the Basque Government (Consolidated research group).
- Nagore Gonzalez-Soto (UPV-EHU) has contributed to the PLASTOX project through a predoctoral contract funded by the University of the Basque Country.
- Silvia Franzellitti (UNIBO) has contributed to the PLASTOX project as part of her Post Doc position.

MSc & BSc theses defended:

- Michael Gottschling (2016). MSc Thesis. Microplastics – Investigation of sorption behaviour of selected polycyclic aromatic hydrocarbons (PAHs) onto different plastic materials (TU Darmstadt, Germany).
- Felicitas Vieweg (2016). BSc. Thesis. Microplastics – Investigation of adsorption behaviour of fluoranthene on the surface of polyethylene fragments in different sizes (TU Darmstadt, Germany).
- Natascha Schmidt (2016). MSs Thesis. Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea) (MIO, France).
- Rebecca von Helfeld (2016) MSc Thesis. Effects of microplastics and associated contaminants in marine mussels (University of the Basque Country).

- Bernike van Werven (2016). BSc Thesis. Microplastic Ingestion by North Sea Sprat. A pilot study towards a better research methodology (Wageningen Marine Research / University of Utrecht, The Netherlands).
- Claudia Sanz-Lanzas (2016). BSc Thesis. An evaluation of the effects of polystyrene microplastics on embryonic stages of the Mediterranean mussel *M. galloprovincialis* (Catholic University of Valencia, Spain).
- Paolo Comandini (2017). MSc Thesis. Microplastics quantification in *Carcinus aestuarii* from salt marsh habitats (University of Bologna, Italy).
- Sara Scapinello (2017). MSc Thesis. Characterisation of microplastics ingested by marine benthos: a methodological and field-based study (University of Bologna, Italy).
- Gunhild Rogne Halland (2017). MSc Thesis. Uptake and excretion of polystyrene microplastics in the marine copepod *Calanus finmarchicus* (NTNU, Norway).
- Dionis Joachim Lyakurwa (2017). MSc Thesis. Uptake and effects of microplastic particles in selected marine microalgae species; *Oxyrrhis marina* and *Rhodomonas baltica* (NTNU, Norway).
- Julia Heide (2017). MSc Thesis. Microplastics – Investigation of the effect of different virgin polyethylene pellets of various producers and lots on the polyethylene-water partitioning (TU Darmstadt, Germany).
- Dinh-An Tran (2017). BSc. Thesis. Evaluation of cost-effectiveness of selected Solid Phase Extraction (SPE) methods for the separation and enrichment of microplastic related persistence organic pollutants (POPs) (TU Darmstadt, Germany).
- Simon Schultheis (2017). BSc. Thesis. Microplastics – Investigation of the effect of different plastic additives on the polyethylene-water partitioning coefficient for naphthalene in a batch system in the lab (TU Darmstadt, Germany).
- Marius Kobilke (2017). BSc. Thesis. Development of an elution scheme to maximize the recovery of persistence organic pollutants (POPs) from silica-based Solid Phase Extraction (SPE) cartridges (TU Darmstadt, Germany).
- Arijan Goharnia (2017). BSc. Thesis. Evaluation of chemical stability of different polymers during the solvent extraction of persistent organic pollutants (POPs) on microplastics (TU Darmstadt, Germany).
- Laura Sandoval (2017). BSc Thesis. Optimization of protocols to isolate and culture mussel haemocytes for *in vitro* toxicity testing (UPV-EHU, Spain).
- Joseph Hatfield (2017). MSc Thesis. Impacts of exposure to microplastics alone and with adsorbed benzo(a)pyrene on scope for growth and biomarker responses in the marine mussel *Mytilus galloprovincialis* (L.) (UPV-EHU, Spain).
- Morena Scaglione (2017). MSc Thesis. Contaminants associated to the occurrence of microplastics in marine environment: Physiological effects on the mussel *Mytilus galloprovincialis* (UNIBO, Italy).
- Sofia Giampreti (2017). MSc Thesis. Experimental evaluation of the effects induced by microplastics in the Mediterranean mussel *Mytilus galloprovincialis* (UNIBO, Italy).
- Viren Dhimmer (2017). MSc Thesis. Microplastics in gastrointestinal tracts of *Trachurus* and *Scomber colias* from the Portuguese coastal waters (NOVA-FCT, Portugal).
- Benoit Carlez (2017). Development of an analytical method for the multi-class determination of endocrine disruptors and additives in microplastics present in environmental matrices and plastics (MIO, France).
- Anastasia O'Donoghue (2017/2018). MSc Thesis. Microplastic ingestion in North Sea prey fish (Wageningen Marine Research / University of Utrecht, The Netherlands).
- Sadia Sharmin (2018). MSc Thesis. Embryo-larval Toxicity and Sub-lethal Effects of Chrysene-Loaded Microplastics on Mediterranean Mussel *Mytilus galloprovincialis* (UNIBO, Italy).
- Elena Tondo (2018). MSc Thesis. Ontogenesis of the environmental stress response in marine invertebrates (UNIBO, Italy).
- Sara Albarello (2018). MSc Thesis. Analysis of Oxidative stress parameters in the Mediterranean mussel (*Mytilus galloprovincialis*) exposed to nano- and microplastics (2018).
- Emilie Rogers (2018). MSc Thesis. Investigation of polycyclic aromatic hydrocarbons (PAH) absorption from seawater to model microplastic particles (SINTEF & NTNU, Norway).
- Erica Parenti (2018). BSc Thesis. Uptake of microplastics by the filter-feeder *Mytilus galloprovincialis* at different water depths (UNIBO, Italy).
- Ailynn Swiers & Marrit Starkenburg (2018/2019). Plastic in commercial fish species (Wageningen Marine Research / University of Applied Science Van Hall Larenstein, The Netherlands).

- Yacine Ryckebush (2018). MSc Thesis. Microplastics in the Belgian part of the North Sea: spatial variability and ingestion by benthic biota (UGhent, Belgium).
- Anna Diem (2018). MSc Thesis. Microplastic exposure to early life stages of *Limecola balthica*: effects on hatching and filtration risk (UGhent, Belgium).
- Darya Kurdyukova (2018). BSc. Thesis. Microplastics - Development of quick methods for the charging of selected persistent organic pollutants (POPs) on microplastic surface (TU Darmstadt, Germany).
- Dominik Dörder (2018). MSc Thesis. Microplastics - Optimization of GC/MS analytical conditions for the determination of persistent organic pollutants (POPs) on microplastics (MPs) in the environment (TU Darmstadt, Germany).
- Typhaine Neuvéglise (2018). MSc Thesis. Microplastics - Development of an optimal analytical protocol to extract persistent organic pollutants (POPs) from different microplastics (TU Darmstadt, Germany).
- Yanyue Zhou (2018). MSc Thesis. Microplastics - Development of an experimental protocol to determine the sorption capacity of plastic resin pellets for hydrophobic organic pollutants (TU Darmstadt, Germany).
- Franziska Kirchen (2018). BSc. Thesis. Microplastics - Comparing the sorption capacity of fluoranthene on the surface of different plastic materials using a quick sorption method (TU Darmstadt, Germany).
- Ines Schwabe (2018). BSc. Thesis. Microplastics – Determination of polyethylene-water partition coefficients of different polycyclic aromatic hydrocarbons (PAHs) in batch tests in the laboratory (TU Darmstadt, Germany).
- Joana Filipa Marujo Neves (2018). MSc Thesis. Effects and biochemical responses to microplastic exposure in *Solea* sp. (NOVA-FCT).
- Pedro Miguel Pereira (2019). MSc Thesis. Microplastic transfer between two trophic levels: the brine shrimp *Artemia salina* and the turbot *Psetta maxima*. (NOVA-FCT).
- Thu Quynh Bui (2019). MSc Thesis. Microplastics – Determination of polymer-water partition coefficients of phenanthrene using different polymer types in batch tests in the laboratory (TU Darmstadt, Germany).
- Lieke Moereels (2019). Mussel-mediated transport of microplastics to the seafloor: implications for benthic ecosystem functioning (UGhent, Belgium).
- María Zarzuelo (2019) Microplastic accumulation and depuration in mussels. (UPV-EHU, Spain). To be defended in 2019.

LIST OF PUBLICATIONS

To date, the PLASTOX project has published 23 peer-reviewed manuscripts and a number of other manuscripts reporting studies from the project are expected to be published in the near future. The following is a summary of the published peer-review manuscripts:

1. Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., van Franeker, J. A. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, 2017, **115**, (1), 86-90 [doi: [10.1016/j.marpolbul.2016.11.034](https://doi.org/10.1016/j.marpolbul.2016.11.034)].
2. Baztan, J., Bergmann, M., Booth, A. M., Broglio, E., Carrasco, A., Chouinard, O., Clüsener-Godt, M., Cordier, M., Cozar, A., Devrieses, L., Enevoldsen, H., Ernsteins, R., Ferreira-da-Costa, M., Fossi, M. C., Gago, J., Galgani, F., Garrabou, J., Gerdt, G., Gomez, M., Gómez-Parra, A., Gutow, L., Herrera, A., Herring, C., Huck, T., Huvet, A., Ivar do Sul, J. A., Jorgensen, B., Krzan, A., Lagarde, F., Liria, A., Lusher, A., Miguelez, A., Packard, T., Pahl, S., Paul-Pont, I., Peeters, D., Robbens, J., Ruiz-Fernández, A. C., Runge, J., Sánchez-Arcilla, A., Soudant, P., Surette, C., Thompson, R. C., Valdés, L., Vanderlinden, J. P., Wallace, N., Breaking Down the Plastic Age. In *Fate and Impact of Microplastics in Marine Ecosystems*, Elsevier: 2017, pp 177-181 [doi: [10.1016/B978-0-12-812271-6.00170-8](https://doi.org/10.1016/B978-0-12-812271-6.00170-8)].
3. Gonçalves, C., Martins, M., Costa, M. H., Costa, P. M. Development of a method for the detection of polystyrene microplastics in paraffin-embedded histological sections. *Histochemistry and Cell Biology*, 2017, **149**(2), 187–191 [doi: [10.1007/s00418-017-1613-1](https://doi.org/10.1007/s00418-017-1613-1)].
4. Paluselli, A., Fauvelle, V., Galgani, F., S. Net-David, R. Sempéré. Distribution of phthalates in Marseille Bay (NW Mediterranean Sea). *Science of the Total Environment*, 2018, **621**, 578-587 [doi: [10.1016/j.scitotenv.2017.11.306](https://doi.org/10.1016/j.scitotenv.2017.11.306)].

5. Wieczorek, A.M., Morrison, L., Croot, P.L., Allcock, A.L., MacLoughlin, E., Savard, O., Brownlow, H. and Doyle, T.K. Frequency of Microplastics in Mesopelagic Fishes from the Northwest Atlantic. *Frontiers in Marine Science*, 2018, **5**, Article 39 [doi: [10.3389/fmars.2018.00039](https://doi.org/10.3389/fmars.2018.00039)].
6. Schmidt, N., Thibault, D., Galgani, F., Paluselli, A., and R. Sempéré. Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea). *Mermex special issue, Progress in Oceanography*, 2018, **163**, 214-220 [doi: [10.1016/j.pocean.2017.11.010](https://doi.org/10.1016/j.pocean.2017.11.010)].
7. Kühn, S., Schaafsma, F.L., van Werven, B., Flores, H., Bergmann, M., Egelkraut-Holtus, M., Tekman, M.B., van Franeker, J.A. Plastic Ingestion by Juvenile Polar Cod (*Boreogadus saida*) in the Arctic Ocean. *Polar Biology*, 2018, **41**(6), 1269–1278 [doi: [10.1007/s00300-018-2283-8](https://doi.org/10.1007/s00300-018-2283-8)].
8. van Franeker, J.A., Rebolledo, E.L.B., Hesse, E., Ijsseldijk, L.L., Kühn, S., Leopold, M., Mielke, L. Plastic Ingestion by Harbour Porpoises *Phocoena phocoena* in the Netherlands: Establishing a Standardised Method. *Ambio*, 2018, **47**, 387–397 [doi: [10.1007/s13280-017-1002-y](https://doi.org/10.1007/s13280-017-1002-y)].
9. Capolupo, M., Franzellitti, S., Valbonesi, P., Sanz Lanzas, C., Fabbri, E. Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. *Environmental Pollution*, 2018, **241**, 1038 – 1047 [doi: [10.1016/j.envpol.2018.06.035](https://doi.org/10.1016/j.envpol.2018.06.035)].
10. Fauvelle, V., Castro-Jiménez, J., Schmidt, N., Carlez, B., Panagiotopoulos, C., Sempéré, R. One-Single Extraction Procedure for the Simultaneous Determination of a Wide Range of Polar and Nonpolar Organic Contaminants in Seawater. *Frontiers in Marine Science*, 2018, **5**, 1-10 [doi: [10.3389/fmars.2018.00295](https://doi.org/10.3389/fmars.2018.00295)].
11. Wathsala, R.H.G.R., Franzellitti, S., Scaglione, M., Fabbri, E. Styrene impairs normal embryo development in the Mediterranean mussel (*Mytilus galloprovincialis*). *Aquatic Toxicology*, 2018, **201**, 58-65 [doi: [10.1016/j.aquatox.2018.05.026](https://doi.org/10.1016/j.aquatox.2018.05.026)].
12. Kühn, S., van Oyen, A., Booth, A.M., Meijboom, A., van Franeker, J.A. Marine Microplastic: Preparation of Relevant Test Materials for Laboratory Assessment of Ecosystem Impacts. *Chemosphere*, 2018, **213**: 103-113 [doi: [10.1016/j.chemosphere.2018.09.032](https://doi.org/10.1016/j.chemosphere.2018.09.032)].
13. Gonçalves, C., Martins, M., Sobral, P., Costa, P.M. & Costa, M.H. An assessment of the ability to ingest and excrete microplastics by filter-feeders: A case study with the Mediterranean mussel. *Environmental Pollution*, 2019, **245**, 600-606. [doi: [10.1016/j.envpol.2018.11.038](https://doi.org/10.1016/j.envpol.2018.11.038)].
14. Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R. Phthalates release and biodegradation from plastic fragments in seawater. *Environmental Science & Technology*, 2019, **53** (1), 166–175 [DOI: [10.1021/acs.est.8b05083](https://doi.org/10.1021/acs.est.8b05083)].
15. Franzellitti, S., Canesi, L., Auguste, M., Wathsala, R.H.G.R., Fabbri, E. Microplastic exposure and effects in aquatic organisms: A physiological perspective. *Environmental Toxicology and Pharmacology*, 2019, **68**, 37-51 [doi: [10.1016/j.etap.2019.03.009](https://doi.org/10.1016/j.etap.2019.03.009)].
16. Piarulli, S., Scapinello, S., Comandini, P., Wong, J.X.W., Magnusson, K., Granberg, M., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Airolidi, L. Microplastic in wild populations of the omnivorous crab *Carcinus aestuarii*: A review and a regional-scale test of extraction methods, including microfibrils. *Environmental Pollution*, 2019, **251**, 117-127 [doi: [10.1016/j.envpol.2019.04.092](https://doi.org/10.1016/j.envpol.2019.04.092)].
17. González-Soto, N., Hatfield, J., Katsumiti, A., Duroudier, N., Lacave, J.M., Bilbao, E., Orbea, A., Navarro, E., Cajaraville, M.P. Impacts of dietary exposure to different sized polystyrene microplastics alone and with sorbed benzo(a)pyrene on biomarkers and whole organism responses in mussels *Mytilus galloprovincialis*. *Science of the Total Environment*, 2019, **684**, 548–566 [doi: [10.1016/j.scitotenv.2019.05.161](https://doi.org/10.1016/j.scitotenv.2019.05.161)].
18. Wieczorek, A.M., Croot, P.L., Lombard, F., Sheahan, J., Doyle, K. Microplastic ingestion by gelatinous zooplankton may lower efficiency of the biological pump. *Environmental Science & Technology*, 2019, **53** (9), 5387-5395 [doi: [10.1021/acs.est.8b07174](https://doi.org/10.1021/acs.est.8b07174)].
19. Provencher, J.F., Borrelle, S.B., Bond, A.L., Lavers, J.L., van Franeker, J.A., Kühn, S., Hammer, S., Avery-Gomm, S., Mallory, M.L. Recommended best practices for plastic and litter ingestion studies in marine birds: collection, processing, reporting. *FACETS*, 2019, **4** (1), 111-130 [doi: [10.1139/facets-2018-0043](https://doi.org/10.1139/facets-2018-0043)].
20. Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S., Booth, A.M. Effects of nylon microplastic on feeding, lipid accumulation and moulting in a coldwater copepod. *Environmental Science & Technology*, 2019, **53** (12), 7075-7082 [doi: [10.1021/acs.est.9b01853](https://doi.org/10.1021/acs.est.9b01853)].

21. Franzellitti, S., Capolupo, M., Wathsala, R.H.G.R., Valbonesi, P., Fabbri, E. The Multixenobiotic resistance system as a possible protective response triggered by microplastic ingestion in Mediterranean mussels (*Mytilus galloprovincialis*): Larvae and adult stages. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 2019, **219**: 50-58. [doi: [10.1016/j.cbpc.2019.02.005](https://doi.org/10.1016/j.cbpc.2019.02.005)].
22. Schmidt, N., Fauvelle, V., Ody, A., Castro-Jiménez, J., Jouanno, J., Changeux, T., Thibaut, T., Sempéré, R. (2019). The Amazon River – a major source of organic plastic additives to the tropical North Atlantic? *Environmental Science & Technology* (**In press**) [doi: [10.1021/acs.est.9b01585](https://doi.org/10.1021/acs.est.9b01585)].
23. Patterson, J., Jeyasanta, K.I., Sathish, N., Booth, A.M., Patterson Edward, J.K. (2019). Profiling microplastics in the Indian edible oyster, *Magallana bilineata* collected from the Tuticorin coast, Gulf of Mannar, Southeastern India. *Science of the Total Environment* (**In Press**).

The following is a summary the manuscripts that have been submitted and are currently under peer-review:

- Van Colen, C., Vanhove, B., Diem, A., Moens, T. (2019). Does microplastic ingestion by zooplankton disrupt the marine food web? An experimental study on larviphagy. *Environmental Pollution* (**Submitted**).
- Sørensen, S., Rogers, E., Altin, D., Salaberria, I., Booth, A.M. (2019). Microplastic properties and environmental conditions influence the sorption and bioavailability of PAHs to *Acartia tonsa* and *Calanus finmarchicus*. *Environmental Pollution* (**Submitted**).

LIST OF CONFERENCE PRESENTATIONS

The results generated within the PLASTOX project have been disseminated in the form of posters and oral presentations at a large number of scientific conferences. The following is a summary (by year) of the presentations from PLASTOX given at national and international conferences:

2016

- Eine optimierte Extraktionsmethode für Fluoranthen mittels ASE aus Mikroplastik. Poster Presentation. FH-DGGV-Tagung. Karlsruhe, Germany, 13-17th April 2016. Kandziora, J., Schiedek, T., Sakaguchi-Söder, K.
- Investigation of adsorption behaviour of selected hydrophobic pollutants on polyethylene from different origin in different sizes. Sakaguchi-Söder, K., Gottschling, M., Vieweg, F., Schebek, L. Poster Presentation. SETAC Europe 26th Annual Meeting. Nantes, France, 22-26th May 2016.
- An Optimised Extraction Method of Fluoranthene from Microplastics using Accelerated Solvent Extraction. Kandziora, J., Schiedek, T., Sakaguchi-Söder, K. Poster Presentation. SETAC Europe 26th Annual Meeting. Nantes, France, 22-26th May 2016.
- Microplastic Prey? An Assay to Investigate Microplastic Uptake by Heterotrophic Nanoflagellates. A.M. Wicczorek, T.K. Doyle, P.L. Croot (NUIG). Oral Presentation. MICRO2016 Conference - Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open Sea. Lanzarote, Spain 25-27th May 2016.
- Occurrence of potential microplastics in Commercial Fish from an Estuarine Environment: Preliminary Results. Bessa F, Barria P, Neto J and Sobral P (NOVA-FCT). Oral Presentation. MICRO2016 Conference - Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open Sea, Lanzarote, Spain 25-27th May 2016.
- PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Booth, A.M., Sakaguchi-Söder, K., Sobral, P., Airolidi, L., Sempéré, R., van Franeker, J., Magnusson, K., Doyle, T., Morrison, L., Salaberria, I., Van Colen, C., Herzke, D., Orbea, A., Gabrielsen, G.W., Nies, H., Galloway, Tamara., van Oyen., A. Poster Presentation. MICRO2016 Conference - Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open Sea. Lanzarote, Spain 25-27th May 2016.

- Characteristics of Plastic in stomachs of Northern Fulmars (*Fulmarus glacialis*). Kühn, S., Van Franeker, J.A. Poster Presentation. MICRO2016 Conference - Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open Sea. Lanzarote, Spain 25-27th May 2016.
- Ingestion of microplastics by Mediterranean mussel (*Mytilus galloprovincialis*): Influence of particle size on histopathological and oxidative stress responses. Gonçalves, C., Martins, M.G., Sobral, P. Costa, M. (NOVA-FCT). Poster Presentation. International Meeting on Marine Research (IMMR) 2016. Peniche, Portugal, 14-15th July, 2016.
- Ecotoxicological impacts of microplastics on marine organisms. Booth, A.M. (SINTEF). Oral Presentation. International Council for the Exploration of the Sea (ICES) Annual Scientific Conference. Riga, Latvia, 16-23rd Sept 2016.
- PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Van Colen, C., Booth, A.M., Sakaguchi-Söder, K., Sobral, P., Airoidi, L., Sempéré, R., van Franeker, J., Magnusson, K., Doyle, T., Morrison, L., Salaberria, I., Herzke, D., Orbea, A., Gabrielsen, G.W., Nies, H., Galloway, Tamara., van Oyen., A. Poster Presentation. North Sea Open Science Conference, Oostende, Belgium, 7-10th November 2016.
- Microplastics Disrupting the Biological Pump? Wieczorek, A.M., Lombard, F., Croot, P.L., Doyle, T.K. Oral Presentation. IEA - 1st Ecology and Evolution Ireland Conference, Ireland, Institute of Technology Sligo, 24-26th November 2016.
- Microplastics and associated contaminants in the Ocean, a major issue: The PLASTOX JPI European project. Poster Presentation. Sempéré, R., Booth, A.M., Sakaguchi-Söder, K., Sobral, P., Airoidi, L., van Franeker, J., Magnusson, K., Doyle, T., Morrison, L., Salaberria, I., Van Colen, C., Herzke, D., Orbea, A., Gabrielsen, G.W., Nies, H., Galloway, Tamara., van Oyen., A. Future Earth Oceans Knowledge-Action Network Workshop. Kiel, Germany 4-5th December 2016.

2017

- Uptake and effects of microplastic particles in the marine copepod *Calanus finmarchicus*. Halland, G.R., Altin, D., Hansen, B.H., Booth, A.M., Olsen, A.J., Salaberria, I. (NTNU and SINTEF). Poster Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Characterising physical- and photodegradation of polystyrene and polyethylene microplastic particles. Hepsø, M.O., Schmid, R., Salaberria, I., Altin, D., van Oyen, A., Booth, A.M. (SINTEF and NTNU). Poster presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Uptake and effects of microplastic particles in selected marine microalgae species. Lyakurwa, D.J., Altin, D., Hansen, B.H., Booth, A.M., Olsen, A.J., Salaberria, I. (NTNU and SINTEF). Poster Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Investigation of the effect of plastic additives on the plastic-water partition coefficient of selected persistent organic pollutants (POPs) in batch tests. Gottschling, M., Sakaguchi-Söder, K., van Oyen, A., Schebek, L. Poster Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Rapid filtering and ingestion of microplastics by marine mussels under ecologically-relevant scenarios. Gonçalves, C., Martins, M., Sobral, P., Costa, P.M., Costa, M.H. Oral Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Accumulation and biomarker responses of mussels *Mytilus galloprovincialis* to polystyrene microplastics and adsorbed organic and metallic contaminants. Orbea, A., von Hellfeld, R.S., Cajaraville, M.P., Zaldibar, B. Poster Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Uptake and toxicity of polystyrene nanoplastics compared to microplastics, alone and with adsorbed benzo(a)pyrene, in haemocytes of mussels *Mytilus galloprovincialis*. Madinabeitia, A., Katsumiti, A., Orbea, A., Cajaraville, M.P. Poster Presentation. SETAC Europe 27th Annual Meeting. Brussels, Belgium, 7-11th May, 2017.
- Microplastics occurrence in the gastrointestinal tract of key marine invertebrates at different trophic levels from the North Adriatic coast. Piarulli, S., Wong, J.X.W., Scapinello, S., Comandini, P., Catelli, E., Sciutto, G.,

Mazzeo, R., Airoidi, L. Oral Presentation. International Conference of Microplastics Pollution in the Mediterranean Sea, Capri, Italy 26-29th September 2017.

- Zooplankton and plastic additives – insights into the chemical pollution of the low-trophic level of the Mediterranean marine food web. Schmidt, N., Castro-Jimenez, J., Fauvelle, V., Sempéré, R. Oral Presentation. International Conference of Microplastics Pollution in the Mediterranean Sea, Capri, Italy 26-29th September 2017.
- WP1: Adsorption and Desorption of Pollutants on Microplastics - PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Sakaguchi-Söder, K., Gottschling, M., Booth, A., Sobral, P., Martins, M., Airoidi, L., Piarulli, S., Sempéré, R., Fauvelle, V., Kühn, S., Magnusson, K., Morrison, L., Mendes, A., Van Colen, C., Vanhove, B., Herzke, D., Kirchgeorg, T., van Oyen, A. Poster Presentation. Auftaktveranstaltung BMBF-Fördermaßnahme "Plastik in der Umwelt - Quellen, Senken, Lösungsansätze", 17-18th October 2017. Berlin, Germany.
- PLASTOX WP1: Adsorption and Desorption of Pollutants on Microplastics. Sakaguchi-Söder, K., Gottschling, M., Booth, A., Sobral, P., Martins, M., Airoidi, L., Piarulli, S. et al. Poster Presentation. 2nd JPI Oceans Conference, Pavilhão do Conhecimentos – Ciência Viva, Lisbon, Portugal 26th October 2017.
- PLASTOX WP2: Evaluation of Toxic Effects of Ingested and Accumulated MPs. Sobral, P., Martins, M., Costa, M. H., Booth, A., Salaberria, I., Fabbri, E., Capolupo, M., Franzellitti S., Orbea, A. et al. Poster Presentation. 2nd JPI Oceans Conference, Pavilhão do Conhecimentos – Ciência Viva, Lisbon, Portugal 26th October 2017.
- PLASTOX WP3: MPs uptake and food web transfer. Piarulli, S., Airoidi, L., Bessa, F., Comandini, P., Doyle, T., Capolupo, M., Fabbri, E., van Franeker, J., Granberg, M., Kühn, S., Magnusson, K., et al. Poster Presentation. 2nd JPI Oceans Conference, Pavilhão do Conhecimentos – Ciência Viva, Lisbon, Portugal 26th October 2017.
- PLASTOX WP4: Microplastic-associated POPs: Effects and Food Web Transfer. Poster Presentation. Booth, A., Salaberria, I., Sobral, P., Airoidi, P., Zanaroli, G., Sempéré, R., Magnusson, K., Morrison, L., Herzke, D., Orbea, A., et al. 2nd JPI Oceans Conference, Pavilhão do Conhecimentos – Ciência Viva, Lisbon, Portugal 26th October 2017.
- PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Booth, A. Oral Presentation. 2nd JPI Oceans Conference, Pavilhão do Conhecimentos – Ciência Viva, Lisbon, Portugal 26th October 2017.
- POPs on MPs – challenges of understanding adsorption of POPs to MP in laboratory and in the environment. Sakaguchi-Söder, K. Oral Presentation. JPI Oceans Pilot Action "Ecological Aspects of Microplastics" Mid-Term Review Meeting. 27th October 2017, Lisbon, Portugal.
- Plastic Organic Additives in Surface Seawater and Zooplankton from the Gulf of Lion (NW Mediterranean Sea). Schmidt N., Castro-Jimenez J., Fauvelle V., Sempéré R. Poster Presentation. 18th European Meeting on Environmental Chemistry, Porto, Portugal 26-29th November 2017.

2018

- Plastiques et additifs en Méditerranée. Sempéré, R., Castro-Jimenez, J., Galgani, F., Garnier, C., Fauvelle, V., Paluselli, A., Panagiotopoulos, C., Ourgaud, M., Schmidt, N., Thyssen, M. Oral Presentation. Polymères et Océans, Montpellier, France, 15-17th January 2018.
- The fate of microplastics ingested by the Mediterranean mussel: biochemical biomarkers and histopathology. Gonçalves C, Martins M, Sobral P, Costa PM, Costa MH. Oral presentation. Sixth International Marine Debris Conference, San Diego, USA, 12-16th March 2018.
- Exploring the effects of nylon microplastic on the development and energy reserves in coldwater copepods. Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Booth, A.M., Galloway, T.S. Oral Presentation. Sixth International Marine Debris Conference, San Diego, USA, 12-16th March 2018.
- Marine Microplastic: Realistic test materials for laboratory studies of ecosystem impacts. Kühn, S., Booth, A.M. van Oyen, A., Meijboom, A., van Franeker, J. Poster Presentation. Norwegian Environmental Toxicology Symposium, Svalbard, Norway, 14-16th March 2018.

- Seabirds in politics: The Northern Fulmar in EU marine litter policies. van Franeker, J., Kühn, S., Bravo Rebolledo, E.L. & the SNS Fulmar study Group. Poster Presentation. 6th International Marine Debris Conference (IMDC), San Diego, USA. 12-16th March 2018.
- Hanging by a fibre: The risk of sample contamination by airborne plastic fibres. Kühn, S., O'Donoghue, A., van Franeker, J. Poster Presentation. 6th International Marine Debris Conference (IMDC), San Diego, USA. 12-16th March 2018.
- An optimised extraction method of fluoranthene from micro-plastics using accelerated solvent extraction (ASE). Kandziora, J., Schiedek, T., Sakaguchi-Söder, K. Poster Presentation. 6th International Marine Debris Conference (IMDC), San Diego, USA. 12-16th March 2018.
- Benchmarking the uptake and excretion dynamics of microplastics in the boreal marine copepod *Calanus finmarchicus*. Salaberria, I., Halland, G.R., Glomstad, B., Alkiza, M., Altin, D., Booth, A.M., Olsen, A.J. Oral Presentation. Norwegian Environmental Toxicology Symposium, Svalbard, Norway, 14-16th March 2018.
- Exploring the effects of nylon microplastic on the development and energy reserves in coldwater copepods. Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Booth, A.M., Galloway, T.S. Poster Presentation. Norwegian Environmental Toxicology Symposium, Svalbard, Norway, 14-16th March 2018.
- Ingestion, accumulation and trophic transfer of microplastics in the benthos of the Belgian part of the North Sea and Westerschelde estuary. Vanhove, B., Moens, T., Vrielinck, H., Van Colen, C. Oral Presentation. VLIZ Marine Science Day. Bredene, Belgium, 21st March 2018.
- Microplastics: Impacts and challenges. Sobral P. Oral presentation. EUROMARINE Workshop Model Plastic. Modelling Ocean Plastic Litter in a Changing Climate: Challenges and Mitigations. Porto, Portugal, 26-27th April 2018.
- Nanoplastic: Does Size Matter? Booth, A.M. Oral Presentation. Ocean Week 2018: Oceans in Change, Trondheim, Norway, 7-9th May 2018.
- Kinetics of POPs adsorbing to a variety of plastic polymers under Arctic conditions. Herzke, D., Nikiforov, V., Sakaguchi-Söder, K., Booth, A. Oral Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- The sub-lethal impact of polystyrene microplastics and nanoplastics on the Mediterranean mussel *M. galloprovincialis*. Capolupo, M., Valbonesi, P., Franzellitti, S., Fabbri, E. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- Kinetics of POPs sorption and plastic additives release to a variety of polymers under Arctic conditions. Herzke, D., Sakaguchi-Söder, K., Sempéré, R., Fauvelle, V., Booth, A.M. Oral Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- Marine microplastic: Preparation of relevant test materials for ecosystem impact studies in laboratory assessments. Kühn, S., Booth, A.M. van Oyen, A., Meijboom, A., van Franeker, J. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- Using pyrolysis GC-MS in combination with multivariate tools to identify and differentiate polymer type and weathering of microplastics. Størseth, T.R., Sørensen, L., Almås, I.K., Hepsø, M.O., Brakstad, O.G., Booth, A.M. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- The role of microplastics size and type on PAH sorption and bioavailability to copepods. Sørensen, L., Rogers, E., Altin, D., Rønsberg, M.U., Booth, A.M. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- Biochemical responses and histological effects resulting from foodborne exposure to post-consumer microplastics in juvenile *S. senegalensis*. Martins, M., Neves, J., Gonçalves, C., Sobral, P., Costa, M.H. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17 May 2018.
- Comparison of spiking and dialysis tubing methods for the determination of sorption capacity and plastic-water partition coefficient of three different polycyclic hydrocarbons on microplastics. Gottschling, M., Zhou, Y., Van Oyen, A., Schebek, L., Sakaguchi-Söder, K. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17 May 2018.

- Development of an optimal analytical protocol for the extraction of persistent organic pollutants adsorbed on plastic debris in the environment. Sakaguchi-Söder, K., Neuveglise, T., Goharnia, A., Gottschling, M., van Oyen, A. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17 May 2018.
- Impacts of exposure to microplastics alone and with adsorbed benzo(a)pyrene on scope for growth and biomarker responses in the marine mussel *Mytilus galloprovincialis* (L.). Hatfield, J., González-Soto, N., Katsumiti, A., Duroudier, N., Lacave, J.M., Orbea, A., Navarro, E., Cajaraville, M.P. Poster Presentation. SETAC Europe 28th Annual Meeting, Rome, Italy, 13-17th May 2018.
- Determination of Polystyrene accumulation in mussels (*Mytilus galloprovincialis*) by pyrolysis and GC-MS. Rombolà, A.G., Fabbri, D., Torri, C., Vassura, I., Franzellitti, S., Fabbri, E. Poster Presentation. 22nd International Symposium on Analytical and Applied Pyrolysis (PYRO2018), Kyoto, Japan, 3rd - 8th June 2018.
- Colonization dynamics of micro plastics by a marine microbial community and transformation of sorbed PCBs. Rosato, A., Negroni, A., Fava, F., Zanaroli, G. Oral Presentation. 7th European Bioremediation Conference (EBC-VII) & 11th International Society for Environmental Biotechnology Conference (ISEB 2018), Chania, Crete, Greece, 25-28th June 2018.
- Nanomaterials and microplastics as carriers of persistent organic pollutants in the aquatic environment: development of tools for risk assessment based on alternative methods and model organisms. Cajaraville, M.P., Martínez-Álvarez, I., Hatfield, J., Nicolussi, G., González-Soto, N., Katsumiti, A., Bilbao, E., Navarro, E., Tomovska, R., Budzinski, H., Orbea, Amaia. Oral Presentation. 11th Iberian conference and 8th Ibero-American conference of Contamination and Environmental Toxicology: "Environmental protection in a global and changing world: technological, scientific and societal challenges". Madrid, Spain, 11-13th July 2018.
- Kinetics of POPs adsorbing to a variety of plastic polymers under Arctic conditions. Herzke, D., Nikiforov, V., Sakaguchi-Söder, K., Booth, A. Oral Presentation. FRAM Dagen, Tromsø, Norway, 22nd August 2018.
- Ingestion, accumulation and trophic transfer of microplastics in the benthos of the Belgian part of the North Sea and Westerschelde estuary. Van Colen, C., Vanhove, B., Ryckebusch, Y., Vrielinck, H., Moens, T. Oral Presentation. ECSA 57: Changing estuaries, coasts and shelf systems - Diverse threats and opportunities, Perth, Australia. 3-6th September 2018.
- A procedure to evaluate different techniques for the extraction of pollutants from plastic debris in the environment. Sakaguchi-Söder, K., Neuveglise, T., Dörder, D., Kurdyukova, D., Gottschling, M., van Oyen, A., Schebek, L. Oral Presentation. SETAC GLB: Umwelt 2018, Münster, Germany, 9-12th September 2018.
- Wie lassen sich aus einem Zusammenhang stoffspezifischer Parameter ausgewählter Schadstoffe und MP Verteilungskoeffizienten ableiten? Gottschling, M., Zhou, Y., Schwabe, I., van Oyen, A., Heide, J., Schultheis, S., Sakaguchi-Söder, K., Schebek, L. Poster Presentation. SETAC GLB: Umwelt 2018, Münster, Germany, 09-12th September 2018.
- Response of the MXR system to microplastic accumulation in Mediterranean mussels (*Mytilus galloprovincialis*): larvae vs adult stages. Franzellitti, S., Capolupo, M., Rombolà, A.G., Fabbri, D., Fabbri, E. Poster Presentation. 31st Congress of the New European Society of Environmental Physiology and Biochemistry (ESCPB), Porto (Portugal), 9-12th September 2018.
- Paraffin in Dutch Waters: Preliminary insights into paraffin-like substances (PLS) on beaches and in Northern Fulmars. Kühn, S., Kwadijk, C., Kotterman, M., van Franeker, J. Poster Presentation. Noordzeedagen. Texel, The Netherlands. 4-5th October 2018.
- Exposure of juvenile turbot (*Scophthalmus maximus*) to contaminated microplastic diet: Oxidative stress biomarkers, erythrocytic nuclear abnormalities and histopathological effects. D'ambrosio, M., Martins, M., Gonçalves, C., Rosato, A., Zanaroli, G., Costa, P.M., Sobral, P., Costa, M.H. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Sub-lethal and embryo-toxicological effects of chrysene-loaded microplastics on the Mediterranean mussel *Mytilus galloprovincialis*. Capolupo, M., Sharmin, S., Rombolà, A.G., Fabbri, D., Fabbri, E. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November 2018.

- Assessment of polystyrene uptake by analytical pyrolysis and relationships with physiological responses in the Mediterranean mussel, *Mytilus galloprovincialis*. Rombolà, A.G., Fabbri, D., Torri, C., Vassura, Capolupo, M., Fabbri, E., Franzellitti S. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November 2018.
- Toxicological effects of *Solea senegalensis* larvae after exposure to PCB contaminated microplastics. Gonçalves, C., D'Ambrosio, M., Martins, M., Costa, P.M., Sobral, P., Costa, M.H. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- PLASTOX: A big assessment of small particles? Booth, A.M., Airoidi, L., Sobral, P., Sakaguchi-Söder, K. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November 2018.
- Microplastics in the Belgian Part of the North Sea: spatial variability and ingestion by benthic biota. Van Colen, C., Vanhove, B., Ryckebusch, Y., Vrielinck, H., Moens, T. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Exploring the effects of nylon microplastic on development and energy reserves in a coldwater copepod. Cole, M., Coppock, R., Lindeque, P.K., Galloway, T.S., Pond, D., Altin, D., Booth, A.M. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- PLASTOX: A big assessment of small particles? Booth, A.M., Airoidi, L., Sobral, P., Sakaguchi-Söder, K. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Marine microplastic: Preparation of relevant test materials for ecosystem impact studies in laboratory assessments. Kühn, S., Booth, A., van Oyen, A., Meijboom, A., van Franeker, J. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Bioavailability of microplastic in laboratory exposure studies: What are we exposing to? Nadvornik-Vincent, C., Glomstad, B., Altin, D., Booth, A.M., Salaberria, I. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Benchmarking the uptake and excretion dynamics of microplastics in the marine copepod *Calanus finmarchicus*. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018. Salaberria, I., Halland, G.R., Glomstad, B., Alkiza, M., Hepsø, M.O., Altin, D., Booth, A.M. Olsen, A.J.
- Microplastic properties and environmental conditions influence PAH sorption and bioavailability to copepods. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018. Sørensen, L., Rogers, E., Altin, D., Schmid, R., Salaberria, I., Booth, A.M.
- WP1: Adsorption and Desorption of Pollutants on Microplastics - PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Sakaguchi-Söder, K., Gottschling, M., Booth, A., Sobral, P., Martins, M., Airoidi, L., Piarulli, S., Sempéré, R., Fauvelle, V., Kühn, S., Magnusson, K., Morrison, L., Mendes, A., Van Colen, C., Vanhove, B., Herzke, D., Kirchgeorg, T., van Oyen, A. Poster Presentation. JPI Oceans Microplastics Projects Joint Final Meeting, Lanzarote, Spain, 20th November 2018.
- PLASTOX-WP1: Ad-/Desorption of pollutants on MPs - Project results. Sakaguchi-Söder, K. Oral Presentation. JPI Oceans Microplastics Projects Joint Final Meeting, Lanzarote, Spain, 20th November 2018.
- Lessons learned in the laboratory for the determination of POPs on MPs in the field. Sakaguchi-Söder, K. Oral Presentation. JPI Oceans Microplastics Projects Joint Final Meeting, Lanzarote, Spain, 20th November 2018.
- Comparison of sorption behaviour of persistent organic pollutants (POPs) on plastic resin pellets from different origins using a quick sorption method. Oral Presentation. Sakaguchi-Söder, K., Kurdyukova, D., Kirchen, F., Gottschling, M., van Oyen, A., Schebek, L. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.

- Comparison of one-time spiking, multiple spiking and dialysis tubing methods for the determination of plastic-water partition coefficient of one polycyclic hydrocarbons on microplastic. Gottschling, M., Zhou, Y., Schwabe, I., Bui, T. Q.: Van Oyen, A., Schebek, L., Sakaguchi-Söder, K. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Microplastics Disrupting the Biological Pump? Wieczorek, A.M., Croot, P.L., Lombard, F., Sheahan, J.N., Doyle, T.K. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Impact of microplastics alone or with adsorbed compounds from the water accommodated fraction of a North Sea crude oil on marine mussels *Mytilus galloprovincialis*. González-Soto, N., Katsumiti, A., Duroudier, N., Orbea, A., Bilbao, E., Navarro, E., Cajaraville, M.P. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Effects of exposure to microplastics alone and with adsorbed benzo(a)pyrene on marine mussels *M. galloprovincialis* at cell, tissue and physiological levels. Hatfield, J., González-Soto, N., Katsumiti, A., Duroudier, N., Lacave, J.M., Orbea, A., Navarro, E., Cajaraville M.P. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Transfer of microplastics from the water column to sediments by the suspension-feeder *Mytilus galloprovincialis*. Piarulli, S., Zanni, E., Airoidi, L. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- WP 3: MPs uptake and food-web transfer. Airoidi, L., Piarulli, S., Diem, A., Doyle, T., Granberg, M., Krång, A.S., Magnusson, K., Moens, T., Rosato, A., Salaberria, I., Van Colen, C., Vanhove, B., Wieczorek, A.M., Zanaroli, G., Zanni, E., Booth, A.M. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- Colonization of microplastics and transformation of sorbed PCBs by a marine microbial community. Rosato, A., Negroni, A., Fava, F., Zanaroli, G. Oral Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November, 2018.
- PLASTOX WP4: Microplastic-associated pollutants: Effects and Food Web Transfer. Booth, A.M., Sørensen, L., Rogers, E., Salaberria, I., Sobral, P., Airoidi, L., Zanaroli, G., Orbea, A., González-Soto, N., Katsumiti, A., Cajaraville, M.P., Altin, D. Poster Presentation. MICRO2018 Conference - Fate and Impact of Microplastics: Knowledge, Actions and Solutions, Lanzarote, Spain 18-23rd November 2018.

2019

- Microplastic properties and environmental conditions influence PAH sorption and bioavailability to copepods. Booth, A.M., Sørensen, L., Rogers, E., Altin, D., Salaberria, I. Oral Presentation. Arctic Frontiers, Tromsø, Norway, 20-24th January 2019.
- Kinetics of POP sorption and additive release of a variety of polymers under Arctic conditions. Herzke, D., Sakaguchi-Söder, K., Booth, A.M. Oral Presentation. Arctic Frontiers, Tromsø, Norway, 20-24th January 2019.
- Microplastics in the oceans: Dangerous liaisons. Sobral, P. Oral presentation. Congress of Ornithology - Portuguese Society for the Study of Birds. Peniche, Portugal, 2-5th March 2019.
- Microplastics Disrupting the Biological Pump? Wieczorek, A.M., Croot, P.L., Lombard, F., Sheahan, J.N., Doyle, T.K. Oral Presentation. IMBeR Future Oceans2 -Open Science Conference, Brest (France), 17th-21st June 2019.

LIST OF NON-SCIENTIFIC, POPULARIZED PUBLICATIONS

The results and knowledge generated within the PLASTOX project have been communicated and disseminated through a large number of non-scientific, popular science reports and stories across a range of different national and international media platforms. The following is a summary (by year) of the popular science publications related to activities conducted within the framework of PLASTOX:

2016

No.	Article Title	Date Published	Media Type	Publication Name, Country	PLASTOX Partner(s)
1	Der Kreislauf des Plastiks	01/03/2016	Newspaper	Darmstädter Echo	TUDA
2	Forsker på mikroplastens mørke sider	09/2016	Magazine	Gemini, Norway	SINTEF
3	Las microesferas de plástico de cientos de productos contaminan el mar.	12.11.2016	Regional News Show	EITB (Basque TV), Spain,	UPV-EHU
4	Forsker på mikroplastens mørke sider. Gemini	21.09.2016	Magazine	Gemini, Norway	SINTEF and NTNU
5	Macro Problems with Micro Plastics	03.01.2016	Radio interview	Radio Adelaide Digital, Australia	NOVA-FCT
6	Planeta Mar	09.09.2016	Radio interview	Antena 2, Portugal	NOVA-FCT

2017

No.	Article Title	Date Published	Media Type	Publication Name, Country	PLASTOX Partner(s)
1	Sjømat-elskere får i seg tusenvis av plastbiter i året	02/2017	Newspaper	Bergens Tidende, Norway	SINTEF
2	Skal finne ut hva mikroplasten gjør med kroppen vår	03/2017	Newspaper	Bergens Tidende, Norway	SINTEF
3	Bekymret for mikroplast i havet	01/2017	Regional News webpage	NRK Hordaland	SINTEF
4	Kan mikroplasten være skadelig for mennesker?.	29.09.2017	Regional Newspaper	Lokalavisa Hitra-Frøya, Norway	SINTEF
5	Mikroplast funnet i springvann i hele verden.	08.09.2017	National Newspaper	Aftenposten, Norway	SINTEF
6	Mikroplast fra treningstøy kan havne i fisken.	22.06.2017	Regional Newspaper	Adresseavisen, Norway	SINTEF and NTNU
7	Dette er en av de største utfordringene mennesket har	07.12.2017	National Newspaper/	A-magasinet, Norway.	SINTEF

	stått overfor.		magazine		
8	Nanoplast kan gi fisk hjerneskade.	23.12.2017	National news website	NRK.no, Norway.	SINTEF
9	Viten og vilje: Plasthavet.	aired on 09.11.2017	TV show	NRK 1, Norway.	SINTEF, UoE, BioTrix
10	Risikerer å spise vårt eget søppel.	09.11.2017	National news website	NRK.no, Norway.	SINTEF, UoE, BioTrix
11	Der Müll und das Meer.	Oct 5th / Oct 18th, 2017	TV documentary	SRF1/SWR, Swiss/German.	WUR
12	We eten plastic, we ademen plastic en het regent plastic.	1st November 2017	Newspaper article	Algemeen Dagblad and several local newspapers, Netherlands	WUR
13	Kostaldean ikusten dugun zaborra izebergaren punta besterik ez da. Amarauna Euskadi Irratia.	26th March 2017	News website	http://www.eitb.eus/eu/irratia/euskadi-irratia/programak/amarau-na/oso/4729674/euskal-kostaldea-garbitzen-aritundira-boluntarioak-bilbotik-pasaia/	UPV-EHU
14	“Conversas QB’ – Mar e plástico,	19th December 2017	Radio interview	Radio Renascença, Portugal	NOVA-FCT

2018

No.	Article Title	Date Published	Media Type	Publication Name, Country	PLASTOX Partner(s)
1	A plastic tide: combatting the scourge of microplastics in our seas and rivers.	10/02/2018	Newspaper	Irish Independent, Ireland.	NUIG
2	Let's turn the tide on plastic.	24/02/2018	Newspaper	Daily Mail, Ireland.	NUIG
3	NUIG reveal deep sea fish in Atlantic ingest our plastic.	26/02/2018	Newspaper	Western People, Ireland.	NUIG
4	Plastic in most fish.	20/02/2018	Newspaper	The Sun (Eire), Ireland.	NUIG
5	Urgent call for microbeads ban after fish study.	20/02/2018	Newspaper	The Times, Ireland.	NUIG
6	73% of fish in Irish waters contain plastic.	19/02/2018	Newspaper	The Daily Mirror, Ireland.	NUIG
7	Even deepwater fish are not safe from toxic microplastics.	19/02/2018	Newspaper	The Times, Ireland.	NUIG

8	Microplastics found in deep-water fish.	19/02/2018	Newspaper	The Irish Examiner, Ireland.	NUIG
9	Over 70% of fish had ingested plastic, NUIG research finds.	19/02/2018	Newspaper	The Irish Times, Ireland.	NUIG
10	Not strictly fish: 73% of deepwater fish contain microplastics.	19/02/2018	News website	RTE Online (Web), Ireland.	NUIG
11	Forsker på mikroplast.	31/01/2018	News website	VA.forum	SINTEF
12	9 ting du bør vite om mikroplast.	2018	Science Magazine	Gemini	NTNU
13	Plastikk, ikke så fantastisk?	22/11/2018	Online newspaper	Under Dusken, Norway	SINTEF
14	Plasten kan komme inn i maten du spiser.	20/08/2018	Online news	NRK nettside, Norway	NTNU
15	RTE Six ONE News: Microplastics in Deep Sea Fish	19/02/2018	National news	RTE ONE, IE	NUIG
16	L'impatto della plastica sugli ambienti marini: un convegno alla capitaneria di porto di Ravenna	07/03/2018	Online news	Ravennanotizie.it (Italy)	UNIBO
17	Mare, nel 2050 più rifiuti che pesci	07/03/2018	Online news	La Pressa (Italy)	UNIBO
18	Mare, cotton fioc e cannucce: nel 2050 più rifiuti che pesci	07/03/2018	Online news	DIRE (Italy)	UNIBO
19	Microplastic in de Noordzee	07/06/2018	TV news show	VRT, Belgium	UGhent
20	Kleine deeltjes plastic in de Noordzee	07/06/2018	TV Youth news show	VRT, Belgium	UGhent
21	World ocean's day 2018	08/06/2018	Radio interview	Studio Brussel, Belgium	UGhent
22	Ponto de partida: Plásticos e microplásticos	23.05.2018	Radio interview	Antena 1, Portugal	NOVA-FCT
23	Lixo marinho e microplásticos	03-04.2019	Magazine article	Indústria e ambiente Magazine	NOVA-FCT
24	Viver sem Plástico - Microsféras em produtos de higiene pessoal	13.10.2018	Interview for magazine	Sábado Magazine	NOVA-FCT

2019

No.	Article Title	Date Published	Media Type	Publication Name, Country	PLASTOX Partner(s)
1	Vehicle tyre particles a major marine polluter.	31.05.2019	Newspaper	Mail & Guardian, South Africa	SINTEF
2	Forskar fortel: Plastfri bursdag.	19.02.2019	Newspaper (for kids)	Framtida Junior, Norway	SINTEF
3	Glitrande miljøskade.	18.02.2019	Newspaper (for kids)	Framtida Junior, Norway	SINTEF
4	Microplastics in ocean 'disrupt natural carbon storage'	30.04.2019	Newspaper	The Times, UK	NUIG
5	Plastic eaten by plankton may impair oceans' ability to trap CO2	29.04.2019	Newspaper	The Irish Times, IE	NUIG
6	Microplastics in squishy creature's poo hurt ocean's ability to absorb CO2	29.04.2019	Online Newspaper	Silicon Republic, IE	NUIG
7	Microplastics in faeces of jellyfish-like animals 'could affect CO2 in atmosphere'	30.04.2019	Newspaper	Irish Independent, IE	NUIG
8	Marine litter may have wider impact on environment than previously thought, study shows	29.04.2019	Newspaper	The Irish Examiner, IE	NUIG
9	Study Finds Microplastic Ingestion by Zooplankton May Alter the Amount of Carbon Dioxide Reaching the Sea Floor	30.04.2019	Online Newspaper	Irish Tech News, IE	NUIG
10	Microplastic ingestion by zooplankton may alter amount of carbon dioxide reaching sea floor – study	29.04.2019	Online Newspaper	Irish Engineers Journal, IE	NUIG
11	Vehicle tyre particles a major marine polluter.	31.05.2019	Newspaper	Mail & Guardian, South Africa	SINTEF
12	Plastic pollution in our oceans even worse for the environment than believed	30.04.2019	Online Newspaper	Galway Daily	NUIG
13	Microplastic Consumed by	30.04.2019	Online	Afloat, IE	NUIG

	Plankton May Interrupt Oceans' Carbon-Capture Abilities, Says NUI Galway Study		Newspaper		
14	Microplastics continue to threaten our oceans	30.04.2019	Radio	RTE 1 – Good Morning Ireland, IE	NUIG
15	Plastic eaten by planktons may impair ocean's ability to trap carbon dioxide	30.04.2019	Radio	Galway Bay FM, IE	NUIG
16	Untitled	02.05.2019	Radio	Kildare FM, IE	NUIG
17	Untitled	03.05.2019	Radio	Dublin City FM – This Island Nation, IE	NUIG
18	Untitled	09.05.2019	Radio	Connemara Community Radio, IE	NUIG
19	Luftballons und ihre Auswirkungen für die Umwelt	15.05.2019	Radio	WDR 5: Quarks. Germany	WUR
20	Mikroplastikoak Pirinioetaraino iritsi dira	8.05.2019	Radio Interview	Euskadi Irratia (Basque public radio)	UPV-EHU
21	"A vivir que son dos días Euskadi". Plástico, prevenir y reciclar	08.06.2019	Radio Interview	Cadena SER	UPV-EHU
22	5 spørsmål om mikroplast	28.05.2019	Science Magazine	Teknisk Ukeblad	NTNU
23	A ameaça invisível	25.03.2019	P'ros & Contrás TV program	RTP1 Channel	NOVA-FCT
24	Plástico	26.03.2019	Sociedade Civil TV program	RTP2 Channel	NOVA-FCT
25	Plástico nosso de cada dia	20.06.2019	Grande reportage TV program	SIC Channel	NOVA-FCT

LIST OF WORKSHOPS, STAKEHOLDER MEETINGS

The results generated within the PLASTOX project have been communicated and disseminated at a number of workshops and seminars, and to a range of different stakeholder groups. The following is a summary (by year) of the communication and dissemination activities where partners from PLASTOX have been invited to present aspects of the PLASTOX project:

2016

- Ecotoxicological impacts of microplastics on marine organisms, including species providing a source of food. Andy Booth, Amy Lusher, Chelsea Rochman. Oral Presentation. United Nations Open-ended Informal Consultative Process, New York, USA, 13-17th June 2016.
- Mikroplastik in der marinen Umwelt. Oral Presentation. Michael Gottschling & Kaori Sakaguchi-Söder. Ökoprofit Klub Runde (UEBZ) graduation ceremony, Ingelheim am Rhein, Germany, 30th August 2016.
- Ecotoxicological Impacts of Microplastics on Marine Organisms. Andy Booth. Oral Presentation. European Parliament - Marine Litter "European Answers to a Global Challenge", Brussels, Belgium, 6th September 2016.
- Ecotoxicological Impacts of Microplastics on Marine Organisms. Oral Presentation. Andy Booth. International Council for Exploration of the Seas (ICES) Annual Scientific Conference, Open Session on "Microplastics in the oceans", Riga, Latvia, 21st September 2016.
- New concerns in environmental pollution: microplastics (MPs) and nanomaterials (NMs) in interaction with emerging and legacy contaminants. Amaia Orbea. Oral Presentation. Invited lecture in the Master's in Environmental Contamination and Toxicology. University of the Basque Country, 13th October 2016.

2017

- The small problem with tyres. Andy Booth and Susie Jahren. Oral Presentation. Nordisk Råd (Nordic Council of Ministers) Seminar, Oslo, Norway, 25th January 2017.
- PLASTOX and MICROFIBRE: Microplastic research projects. Andy Booth. Oral Presentation. Holde Norge rent Samarbeidsarenaen for aktører som arbeider med marin forsøpling, Stavanger, Norway 8-9th February 2017.
- Microplastiche: dall'ambiente agli organismi. Piarulli S., Capolupo M., Fabbri E., Sciutto G., Mazzeo R., Airolidi L. Oral Presentation. Mare Mostro. Un mare di plastica, Ravenna 7th March 2018.
- Plastic degradation. Andy Booth. Oral Presentation. Miljødirektoratet Workshop om marin forsøpling og mikroplast, Oslo, Norway, 18th September 2017.
- Microplastic and marine litter. Iurgi Salaberria. Oral Presentation. Researchers' Night. Trondheim, Norway, 29th September 2017.
- WP1: Adsorption and Desorption of Pollutants on Microplastics - PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Poster Presentation. Sakaguchi-Söder, K., Gottschling, M., Booth, A., Sobral, P., Martins, M., Airolidi, L., Piarulli, S., Sempéré, R., Fauvelle, V., Kühn, S., Magnusson, K., Morrison, L., Mendes, A., Van Colen, C., Vanhove, B., Herzke, D., Kirchgeorg, T., van Oyen, A. Auftaktveranstaltung BMBF-Fördermaßnahme "Plastik in der Umwelt - Quellen, Senken, Lösungsansätze", Berlin, Germany, 17-18th October 2017.
- Toxic effects studied in PLASTOX. Oral Presentation. Marta Martins. JPI Oceans Pilot Action "Ecological Aspects of Microplastics" Mid-Term Review Meeting. Lisbon, Portugal, 27th October 2017.
- POPs on MPs – challenges of understanding adsorption of POPs to MP in laboratory and in the environment. Kaori Sakaguchi-Söder. Oral Presentation. JPI Oceans Pilot Action "Ecological Aspects of Microplastics" Mid-Term Review Meeting. Lisbon, Portugal, 27th October 2017.
- Plastic degradation. Andy Booth. Oral Presentation. JPI Oceans Pilot Action "Ecological Aspects of Microplastics" Mid-Term Review Meeting. Lisbon, Portugal, 27th October 2017.
- Are we using appropriate methods and approaches in MP-POP toxicity assessment? Andy Booth. Oral Presentation. JPI Oceans Pilot Action "Ecological Aspects of Microplastics" Mid-Term Review Meeting. Lisbon, Portugal, 27th October 2017.

- New emerging challenges: effects caused by microplastics in the marine environment. Marta Martins. Oral Presentation. Seminar on Nanotoxicology e Micropollution, NOVA-FCT, Caparica, Portugal, 22nd November 2017.

2018

- Caballos de Troya en el Mar. Amaia Orbea. Oral Presentation. Workshop “Mar de plástico”. International Ocean Day, Bilbao, Spain, 8th March 2018.
- PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Andy Booth. Oral Presentation. European Parliament Intergroup Seas, Rivers, Islands and Coastal Areas (SEArice). Mission: Plastic Free Ocean. European Parliament, Brussels, Belgium, 10th April 2018.
- Microplastics: Chemical Trojan Horses in the Ocean. Amaia Orbea. Oral Presentation. Invited lecture in the European Master Erasmus Mundus in Marine Environment and Resources. University of the Basque Country, Bilbao, Spain, 20th June 2018.
- Overview of the JPI Oceans Projects BASEMAN, EPHEMERE, PLASTOX, WEATHER-MIC. Sobral, P. Oral presentation. 12th Meeting of the MFSD Technical Group on Marine Litter and workshops on litter baselines and thresholds, Larnaca, Cyprus 25-28th June 2018.
- Microplastics - why are we concerned? Sobral, P., Antunes, J., Bessa, A.F., Clode, J.C., Costa, M.H., Domingos, I., Gonçalves, A.M., Marques, J.C., Martins, M. Oral presentation. Portuguese Science and Technology Meeting. Lisbon, Portugal. 2-4th July 2018.
- Microplastic Impacts on Marine Organisms. Andy Booth. Oral Presentation. Norsk vannforening: Plastforurensing fra land til vassdrag og kystvann. Oslo, Norway, 23rd October 2018.
- PLASTOX WP1 - Determination of POPs accumulated on MP in the environment: lesson learned. Sakaguchi-Söder, K. Oral Presentation. Plastik in der Umwelt Workshop „Bewertungsmethoden möglicher Auswirkungen von Plastik in der Umwelt (inkl. Toxikologie)“, Frankfurt am Main, Germany, 26th October 2018.
- Quantifying trophic transfer. Airolti L., Piarulli S., Gerdt G., Booth A.M. Oral Presentation. MICRO2018 International conference - Fate and impacts of microplastics: knowledge, actions and solutions, Lanzarote, Spain 19th-23rd November 2018.

2019

- Final results and impact of the JPI Oceans microplastics projects. Booth, Andy; Gerdt, Gunnar; Jahnke, Annika; Beiras, Ricardo. Oral Presentation. Seminar JPI Oceans seminar: Increasing the impact of European investments in marine and maritime research, Brussels, Belgium, 17th January 2019.
- PLASTOX: Direct and indirect ecotoxicological impacts of microplastics on marine organisms. Sørensen, Lisbet; Booth, Andy. Oral Presentation. Seminar: Samordning av arbeid mot marin forurensning på Vestlandet, Bergen, Norway, 2nd April 2019.
- Microplastics for Research and Risk assessment: First Results. van Oyen, Albert, Booth, Andy, Kühn, Susanne, van Franeker, Jan, Sakaguchi-Söder, Kaori, Bouwmeester, Andre, Jansen, Erwin E. Oral Presentation. Workshop: State-of-the-art of analytical methods for reliable detection of micro- and nanoplastics, Ispra, Italy, 13th May 2019.
- Using marine organisms to monitor plastic litter at sea. Kühn, Susanne; van Franeker Jan A. Oral Presentation. Workshop: North Sea meets Baltic workshop at Leibniz Institute for Baltic Sea Research. Special guest: HRM The King and HRM The Queen of The Netherlands Warnemünde, Germany, 20th May 2019.
- Microplásticos: cócteles químicos en el mar. Amaia Orbea. Oral Presentation. Conference on Marine Litter. 24th May 2019.
- Microplastics: Chemical Trojan Horses in the Ocean. Amaia Orbea. Oral Presentation. Invited lecture in the European Master Erasmus Mundus in Marine Environment and Resources. University of the Basque Country, Bilbao, Spain, 20th June 2019.

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

The results generated within the PLASTOX project have been communicated to the public through a variety of different events. The following is a summary of these events:

- Ocean Plastics Lab. Booth, Andy; Salaberria, Iurgi; Altin, Dag. International Travelling Exhibition. Turin, Italy; Paris, France; Brussels, Belgium; Washing D.C., USA; Ottawa, Canada; Berlin, Germany; Lisbon, Portugal (2017-2019) (Figure 38).
- Mikroplast og hvordan det påvirker vår hverdag. Iurgi Salaberria. Oral Presentation. Kystmuseet Presentation Series, Trondheim, Norway, 25th March 2017.
- Impacto ecotoxicológico de los microplásticos en los organismos marinos. EkoFish campaign. Amaia Orbea. Oral Presentation. San Sebastian Aquarium, Spain. 25th March 2017.
- Plastikoak eta mikroplastikoak ingurunean. Amaia Orbea. Oral Presentation. Conference Series within Local Agenda 21 action plan to 16-year old students. March 2019.
- Why is plastic pollution a problem for the marine environment? Carl Van Colen. Oral Presentation. Plastic Pollution in our Ocean – Tackling a Mammoth Task. A public debate. Royal Belgian Institute for Natural Sciences, Brussels, Belgium, 11th April 2018.
- Mikroplast i havet. Stand Barnas Store Fjæredag, Salaberria, Iurgi; Monticelli, Giovanna; Rogstad, Dina Tevik; Sathananthan, Dhiya; L'Abée-Lund, Sanne Marie Green. Trondheim, Norway, 28th April 2019.
- Saving our Seas from Plastic Pollution: Microplastics. Paula Sobral. Oral Presentation. National Geographic Summit. Porto, Portugal. 29th April 2019.
- Launch of Mandy Barker's "Beyond Drifting: Imperfectly Known Animals" (Art exhibition supported by PLASTOX; 28th May – 9th July 2017). Tom Doyle. Oral Presentation. Galway, Ireland, 7th May 2017.
- Océanos, microplásticos y contaminantes persistentes. Amaia Orbea. Oral Presentation. International Ocean Day, Bilbao, Spain, 8th June 2017.
- Mikroplast: hvordan kan du bli påvirket? Iurgi Salaberria. Oral Presentation. Klimafestivalen, Trondheim, Norway 28th August 2017.
- Microplastic and marine litter. Iurgi Salaberria. Oral Presentation. Forskningstorget. Trondheim, Norway 22-23rd September 2017.
- Hvor farlig er mikroplast? Iurgi Salaberria. Oral Presentation. Forskningskafé, Trondheim, Norway, 25th September 2017.

In addition to the public dissemination events, PLASTOX has also produced a number of publicly available media and information resources, including:

- Project factsheet
- Project infographic
- Project summary slide (ppt)
- Project websites:
 - <http://www.jpi-oceans.eu/plastox>
 - <https://www.sintef.no/projectweb/plastox/>

Copies of these resources are presented in Appendix 2 of this report.



Figure 38. Ocean Plastics Lab (OPL) international travelling exhibition, with a contribution from the PLASTOX project. The OPL showcases the contribution of science to understand and tackle the problem of plastic in the ocean. To date, the exhibition has visited Turin, Italy; Paris, France; Brussels, Belgium; Washing D.C., USA; Ottawa, Canada; Berlin, Germany; Lisbon, Portugal (2017-2019).

LIST OF SOCIAL MEDIA ACTIVITIES

The PLASTOX project has its own Facebook page (<https://www.facebook.com/plastox/>) and project partners have used their private and institute Twitter accounts to post updates and information from the PLASTOX project.

Appendix 1: PLASTOX Project Method Catalogue

The newly developed and optimised analytical chemical methods and approaches from the project have been compiled into a 'PLASTOX Project Method Catalogue'. A overview of the methods contained in the catalogue is provided here:

- Implementation of a quick sorption test for the comparison of plastics from different origin (TUDA).
Contact: Dr. Kaori Sakaguchi-Söder (K.Sakaguchi@iwar.tu-darmstadt.de).
- An online method for compound-specific stable carbon isotope analysis (C-CSIA) for selected PAHs (TUDA).
Contact: Dr. Kaori Sakaguchi-Söder (K.Sakaguchi@iwar.tu-darmstadt.de).
- A single extraction procedure for the simultaneous determination of a wide range of polar and nonpolar organic contaminants in seawater (AMU-MIO).
Contact: Prof. Richard Sempere (richard.sempere@mio.osupytheas.fr).
- A method for determining phthalate release from plastic fragments and degradation in seawater (AMU-MIO).
Contact: Prof. Richard Sempere (richard.sempere@mio.osupytheas.fr).
- Organic additive release from microplastic under simulated deep seawater conditions (AMU-MIO).
Contact: Prof. Richard Sempere (richard.sempere@mio.osupytheas.fr).
- Method for the extracting organic additive chemicals from polymers for chemical analysis (SINTEF).
Contact: Dr. Andy Booth (andy.booth@sintef.no).
- Method for the desorbing metals from plastic pellets and their quantification (NUIG).
Contact: Dr. Liam Morrison (liam.morrison@nuigalway.ie).

Appendix 2: Publicly available media resources

Project Factsheet

PLASTOX

Direct and indirect ecotoxicological impacts of microplastics on marine organisms

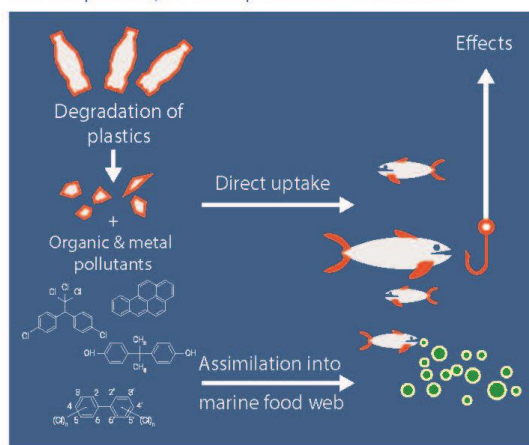


Project Description

Project Coordinator: Dr. Andy Booth, SINTEF Ocean, Environmental Technology Department, Trondheim, Norway

Project period: January 1, 2016 - December 31, 2018

The PLASTOX project investigates the ingestion, food-web transfer, and ecotoxicological impact of microplastics (MPs), together with the persistent organic pollutants (POPs), metals and plastic additive chemicals associated with them, on key European marine species and ecosystems. It also studies the temporal dynamics of MP colonisation by microbial communities in the field and the influence of microbial biofilms on ingestion rates and POP toxicity. The influence of MP physicochemical properties (e.g. size, shape, surface area and composition) on these processes are evaluated.



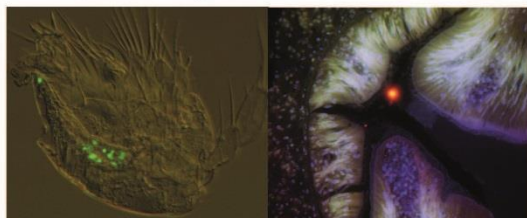
PLASTOX employs a combination of commercially available feedstock MP and multi-component marine litter-derived MP mixture that has been cryo-milled from litter collected along the northern Dutch North Sea coast and characterized in detail. Two additional single-component MP reference materials have been prepared from beached fish-boxes and

characterized. The use of common reference materials allows a meaningful comparison of data generated by different partners and across the different activities of PLASTOX.

Adsorption and desorption of organic and inorganic pollutants to MPs are being investigated using a range of common POP and metal contaminants. Ongoing activities include optimised laboratory studies and long-term field experiments (up to 2 years) at 13 locations representing a range of European marine environments. A method for extracting a broad range of POPs and additives from seawater has been developed. Studies have highlighted how adsorption/desorption behaviour varies between different POPs, identifying which physicochemical properties are most influential, including size, production method and additive content (e.g. plasticisers and colourants). Metal adsorption tests under laboratory conditions have also been performed using PVC pellets.

PLASTOX investigates uptake through ingestion and other routes following controlled exposures and field samples. Alkaline and enzyme-based digestion methods have been developed and evaluated for MP extraction from a broad range of marine organisms. The methods have been used to study the MP content in a wide range of wild-caught species from across Europe. Data suggest that the MP content in wild-caught organisms varies significantly between species, but fibres appear to comprise a high proportion of ingested MPs in marine organisms. Laboratory studies have shown that MPs are rapidly ingested and excreted by a range of species, but that there is also evidence of retention/accumulation in some species (e.g. planktonic organisms and mussels). Such organisms are a potential vehicle for MPs along the trophic

food chain. POPs adsorbed to MPs also appear bioavailable to mussels, but the heavy metals assessed were not.



Fluorescent (right) MPs (~10 µm) in the digestive tracts of *C. finmarchicus* naupilii (left) and the mussel *M. galloprovincialis* (right). Picture credits: NTNU, SINTEF and NOVA-FCT.

The acute and sublethal ecotoxicological effects of MPs are being assessed on marine organisms from phyto- and zooplankton to (shell)fish. Data generated so far indicate ecotoxicological effects on mussels and copepods appear to be limited for pristine MPs in the sizes and concentrations

evaluated in the current studies. Although no significant histopathological effects were observed in mussels, inflammatory responses and a decrease in cell membrane stability were detected. MPs also produced alterations on molecular and cellular parameters involved in mussel embryonal development, although there was no observable impact on the development of individuals.

Using new data and competence generated in these studies, a more detailed understanding of the potential for MP transfer between trophic levels, and the subsequent impacts this may have, will be obtained. Finally, PLASTOX will culminate in a series of experiments bringing together the knowledge generated about MPs and POPs/metals to study the combined fate and effects of these marine contaminants in food web studies. The knowledge generated will be summarized in a guidance document for development of future legislation and remedial efforts.

Consortium

Name	Organisation	Country
Dr. Andy Booth	SINTEF Materials and Chemistry, Environmental Technology Department, Trondheim	Norway
Dr. Kaori Sakaguchi-Söder	Technische Universität Darmstadt, Darmstadt	Germany
Dr. Richard Sempere	AIX-MARSEILLE UNIVERSITE-M.I.O. (Mediterranean Institute of Oceanography), Marseille	France
Dr. Jan Andries van Franeker	Wageningen Marine Research (Wageningen University and Research), Den Helder, The Netherlands	The Netherlands
Dr. Kerstin Magnusson	IVL Swedish Environmental Research Institute, Göteborg	Sweden
Prof. Laura Airoidi	Alma Mater Studiorum - University of Bologna	Italy
Prof. Geir Gabrielsen	The Norwegian Polar Institute, Tromsø	Norway
Prof. Paula Sobral	NOVA.ID FCT, Caparica	Portugal
Dr. Thomas Doyle	National University of Ireland Galway, Galway	Ireland
Dr. Iurgi Salaverria	Norwegian University of Science and Technology (NTNU) Trondheim	Norway
Dr. Dorte Herzke	Norwegian Institute of Air Research (NILU), Tromsø	Norway
Dr. Carl van Colen	Ghent University, Ghent	Belgium

Associated partners

Dr. Amaia Orbea	Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Basque Country	Spain
Prof. Tamara Galloway	University of Exeter, Exeter	United Kingdom
Dr. Berit Brockmeyer	BSH / Federal Maritime and Hydrographic Agency, Hamburg	Germany

