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Proceedings of the 2nd International Conference on Microplastic Pollution in the Mediterranean Sea

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Contents

The Impact of Microplastics on Filter-Feeding Megafauna

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1 Introduction

The Mediterranean basin, a worldwide biodiversity hotspot, as previously underlined, is one of the world seas most affected by marine litter, including microplastics [1–3]. Recent studies in the different regions of the basin suggest that some areas, including important MPAs and Specially Protected Areas of Mediterranean Importance (SPAMI) such as the Pelagos Sanctuary, are affected by important concentrations of microplastics and plastic additives, representing a potential risk for endangered species (baleen whales, sea turtles, filter feeder sharks) [4–10] living in this area and for the all Mediterranean biodiversity [11–14]. In this paper we reconstruct the scientific story of the invisible war between the charismatic megafauna (baleen whales, filter feeder sharks and manta rays) against the smallest marine debris (microplastics) and their potential toxicological effects.

2 The Impact of Microplastics on Filter-Feeding Megafauna

The first warning of this emergent threat in filter-feeding megafauna (baleen whales and filter feeder sharks) was reported by Fossi and collaborators for Mediterranean baleen whales (*Balaenoptera physalus*) in 2012, and few years later (2014 and 2017) confirmed also, by the same team, for filter feeder sharks such as basking shark (*Cetorhinus maximus*) and whale shark (*Rhincodon typus*). The authors report that filter-feeding megafauna are particularly susceptible to high levels of microplastic ingestion and exposure to associated toxins due to their feeding strategies, target prey, and for habitat overlap with micro-plastic pollution hot spots. Given the abundance of microplastics in some hot spot areas, such as the Mediterranean Sea, along with the high concentrations of Persistent Bioaccumulative and Toxic (PBT) chemicals, plastic additives and the detection of specific biomarker responses in the skin biopsies of these endangered species the authors suggest that the exposure to microplastics because of direct ingestion and consumption of contaminated prey poses a major threat to the health of this endangered marine species.

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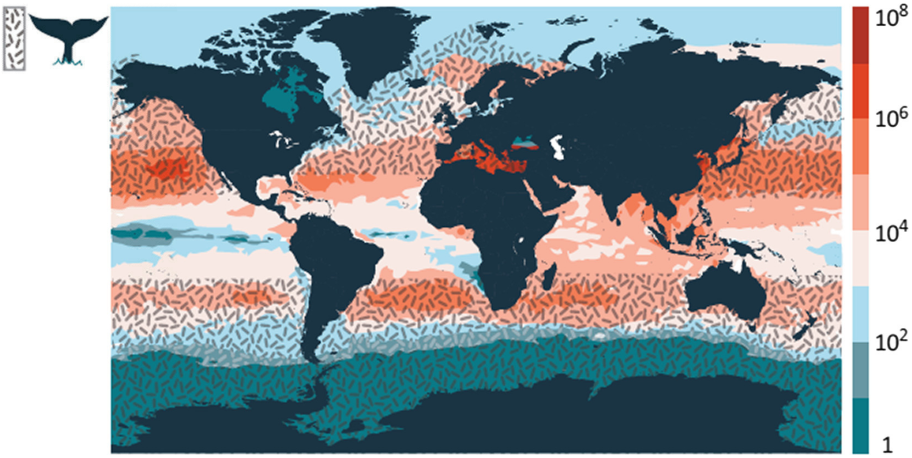

Fig. 1. Key Buoyant Microplastic Hotspots Overlap with Habitat Ranges of Filter-Feeding Marine Megafauna. The habitat ranges for *Balaenoptera physalus*, as indicated by thatched, lined, or dotted overlay, respectively, overlap with regions containing high levels of buoyant microplastic pollution. From Germanov et al. 2018 (Modified).

Recent studies suggest that debris, including micro-plastics and chemical additives (e.g., phthalates), tend to accumulate in pelagic areas in the Mediterranean, indicating a potential overlap between debris accumulation areas and endangered species' feeding grounds (*Balaenoptera physalus*) (Fig. 1). This fact highlights the potential risks posed to endangered, threatened and endemic species of Mediterranean biodiversity. In one of the most biodiverse area of the Mediterranean Sea, the Pelagos Sanctuary, cetaceans coexist with high human pressure and are subject to a considerable amount of plastic debris, including microplastics [4–10]. Therefore, filter-feeding megafauna resident in these area shave a high probability of ingesting microplastics, because they must filter hundreds to thousands of cubic meters of water daily to obtain adequate nutrition. They can ingest microplastics directly from polluted water or indirectly through contaminated planktonic prey. The high plastic: plankton weight ratios (0.5) in the Mediterranean might lead to a significant reduction in nutritional uptake for filter feeders, with animals feeding on the same quantities of particulate matter but receiving a lowered nutritional benefit. The estimated daily plastic ingestion rates for filter-feeding megafauna vary greatly, depending on location and feeding behavior, and range from as low as 100 pieces for whale sharks in the Gulf of California to as high as thousands of pieces for fin whales in the Pelagos Sanctuary (Fig. 1).

3 Conclusion

For these findings and because many megafauna species investigated by this research team are charismatic and iconic indicators that serve as flagship species for marine conservation, this research field became recently a new "trend topic". Currently the scientific community and the media are very attracted by this “story” despite this subject at the beginning has been treated with great suspicion. This scientific topic is also developed in the Plastic Busters MPAs project, recently financed by EU (Med-Interreg), focused on the study of the impact of microplastics on cetaceans inhabiting the Mediterranean SPAMI Pelagos Sanctuary. While umbrella species are useful for directing intervention strategies, flagship species could provide a global assessment of microplastics pollution and a mechanism for communicating awareness and stimulating action to tackle marine plastic pollution in all the marine ecosystems [10].

References

1. Van Sebille, E., Wilcox, C., Lebreton, L., et al.: A global inventory of small floating plastic debris. Environ. Res. Lett. **10**(12), 124006 (2015)
2. Suaria, G., Avio, C.G., Mineo, A., et al.: The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. **6**, 37551 (2016)
3. Fossi, M.C., Romeo, T., Baini, M., et al.: Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos Sanctuary: a modeling approach. Front. Mar. Sci. (2017). Article no. 167. https://doi.org/10.3389/fmars.2017.00167
4. Fossi, M.C., Panti, C., Guerranti, C., et al.: Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). Mar. Pollut. Bull. **64**, 2374–2379 (2012)
5. Fossi, M.C., Coppola, D., Baini, M., et al.: Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. **100**, 17–24 (2014)
6. Fossi, M.C., Marsili, L., Baini, M., et al.: Fin whales and microplastics: the Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. **209**, 68–78 (2016)
7. Baini, M., Martellini, T., Cincinelli, A., et al.: First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal. Methods **9**(9), 1512–1520 (2017)
8. Fossi, M.C., Panti, C., Baini, M., et al.: A review of plastic-associated pressures: cetaceans of the Mediterranean Sea and Eastern Australian Shearwaters as case studies. Front. Mar. Sci. (2018). Article no. 173. https://doi.org/10.3389/fmars.2018.00173
9. Fossi, M.C., Pedà, C., Compa, M., et al.: Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. **237**, 1023–1040 (2018). https://doi.org/10.1016/j.envpol.2017.11.019
10. Germanov, E.S., Marshall, A.D., Bejder, L., et al.: Microplastics: no small problem for filter-feeding Megafauna. Trends Ecol. Evol. **33**(4), 227–232 (2018)
11. Galgani, F., Claro, F., Depledge, M., Fossi, M.C.: Monitoring the impact of litter in large vertebrates in the Mediterranean Sea within the European Marine Strategy Framework Directive (MSFD): constraints, specificities and recommendations. Mar. Environ. Res. **100**, 3–9 (2014)
12. Romeo, T., Pietro, B., Pedà, C., et al.: First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. **95**(1), 358–361 (2015)
13. Deudero, S., Alomar, C.: Mediterranean marine biodiversity under threat: reviewing influence of marine litter on species. Mar. Pollut. Bull. **98**, 58–68 (2015)
14. Compa, M., Alomar, C., Wilcox, C., et al.: Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea. Sci. Total Environ. **678**, 188–196 (2019)

Microplastic Contamination of Sediment and Water Column in the Seine River Estuary

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1 Introduction

Nowadays, microplastic (MPs) pollution is well documented in marine ecosystems since the first publication alarming about marine plastic pollution in 1972 [1]. Similarly, continental contamination is more and more investigated. More recently, interest for estuarine systems is growing. Estuaries are considered as a suspected predominant pathway for microplastic pollution from continent to oceans. The specific conditions of estuaries, like salinity gradient, tides and hydrodynamics, could affect the repartition, settling and transfer of microplastics to marine systems.

This study aims to quantify levels of microplastics in water column and intertidal sediments in the Seine river estuary to investigate the impact on estuary specific conditions on microplastic pollution.

2 Materials and Methods

2.1 Study Site

The Seine river watershed is equivalent to 80 000 km^2 and accounts of 40% of national economic activity. The catchment of the estuary represents 11 500 km^2 and concentrate 40% of national economic activity [2]. The Seine river is heavily anthropized. It is under very strong pressure mainly induced by the agglomerations of Paris and Rouen. The Seine river estuary represents the last 160 km of the river. This estuary is delimited from the dam of Poses to the mouth of the river at Le Havre. It is characterized by semi-

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diurnal tides, and a strong tidal range reaching 7 m. Current speed can reach 2 m.s^{-1} at the mouth of the river. Two petrochemical hubs are present in the estuary.

2.2 Samples Collection

Three sites were selected along the estuary: La Roque, Vieux-Port, and La Bouille (Fig. 1). Sampling trip was conducted in May 2017, during low flow period with a flow equal to 256 m^3.s^{-1}. Samples were collected during rising tide and ebb tide. At each location, two nets were towed, collecting surface (first 15 cm) and subsurface (50 cm) water. Both nets were plankton net 300 µm mesh, 50 cm diameter. The volume collected range from 10 to 90 m^3. All samples are transferred into glass bottles with aluminium cover.

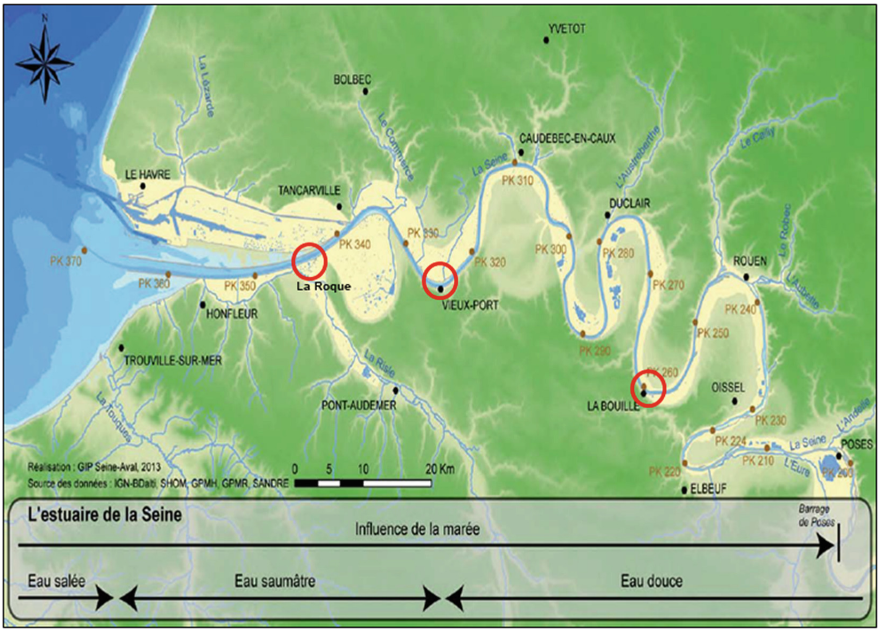

Fig. 1. Sampling sites location in the Sein river estuary

At each location, about 1.5 kg of sediment was sampled using a Van Veen grab. Sediment samples are also transferred in glass bottles with an aluminium cover.

2.3 Analytical Techniques

In the lab, water samples are subjected to a purification protocol. They are first sieved through 5 mm mesh sieve to remove all macroplastics and vegetal waste. Then, sodium dodecyl sulfate (SDS), and biozymes are successively added to denature all proteins, lipids and carbohydrates in the samples for 24 h at 40 °C each. Next, hydrogen peroxide 30% (H_2O_2) is added to remove remaining organic fraction for 24 h at 40 °C. After this, sample is transferred in a separating funnel with sodium iodide (NaI, d = 1.65 $g.cm^{-3}$), and after a night of settling, MPs are recovered in the supernatant. Finally, supernatants are filtered through glass fiber filters 47 mm. Each filter is observed under a stereomicroscope and MPs-like particles are enumerated measured. MPs-like particles shape is noted as well.

Finally, about 25% of MPs-like particles is characterized using Raman spectroscopy to assess polymers proportions in each sample.

Sediment samples are also subjected to a purification protocol. First, 4 × 25 g of the samples are transferred in four separation funnels with NaI (d = 1.65 $g.cm^{-3}$). After a night of settling, supernatant is recovered and SDS is added for 24 h at 40 °C. Next, H_2O_2 30% is added also for 24 h at 40 °C. Between each step, samples are filtered on metallic filters (10 μm pore size) to remove all the solutions. As well as for water samples, filters are observed under stereomicroscope. Thanks to an image processing software, MPs-like particles were defined by length and shape.

Characterization FTIR micro spectroscopy is the final step to assess polymer proportions in each sample. As for column water samples, only 25% of MPs-like particle by sample will be analysed in the interest of time and efficiency.

3 Results and Discussion

3.1 Water Column

First results show that concentrations in MPs-like particles in the water column range from 1.7 $particles.m^{-3}$ to 7.1 $particles.m^{-3}$ (Fig. 2.). The lower concentrations are found at the upstream location, La Bouille. Levels of contamination in the Seine river estuary are higher than other concentrations found in France, 0.24 ± 0.35 $particle.m^{-3}$ in the Bay of Brest [3]. Compared to the literature, these levels are higher than levels reported for other estuaries in the world, like Goiana river, Brazil, with 0.26 $particle.m^{-3}$ [4] or for the Tamar river estuary with 0.74 $particles.m^{-3}$ [5] Europe. There is a hot spot contamination at Vieux-Port, with 37.7 $particles.m^{-3}$ and 8.6 $particles.m^{-3}$ due to a point-source pollution of translucent microbeads. Indeed, these microbeads represented half of both samples.

Considering the samples in La Roque, most particles are lower than 1 mm; they represent respectively 81% and 87% of surface and subsurface water. A small part of particles was between 1 mm and 2 mm, and there were almost none between 2 mm and 5 mm. At La Bouille, most of particles were also smaller than 1 mm. They represent respectively 87% and 71% of surface and subsurface water.

During observation, particles were divided into four categories: fragment, sphere, film and foam. Particles were mostly fragment in shape. There were no differences in shape distribution between surface and subsurface water whatever the sample. Fragment shapes represent between 59% and 73% percent of the distribution, and films represent between 16% and 29%. However, the largest proportion of fragments were found in surface water.

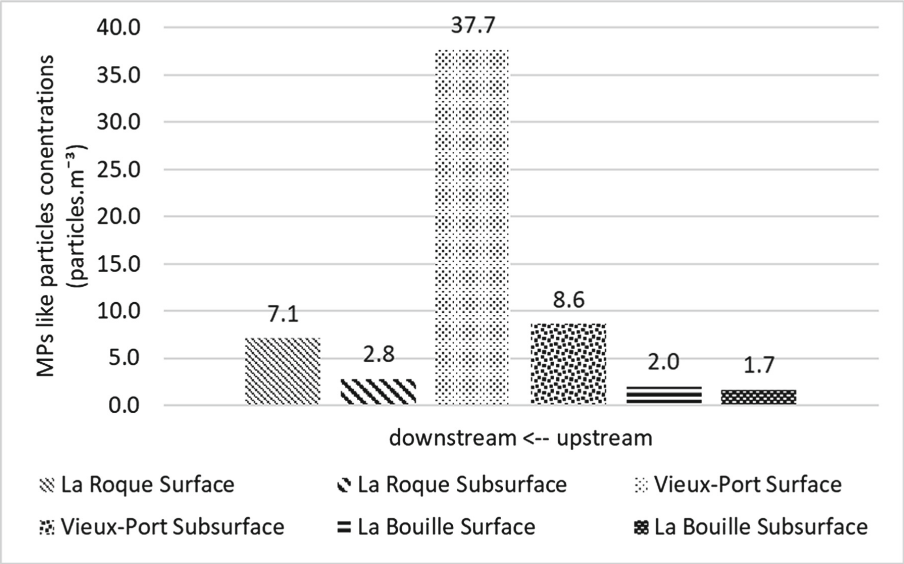

Fig. 2. MPs-like particles concentrations in the Seine river estuary

Characterization step using Raman spectroscopy of 25% of MPs-like particles in each sample showed a majority of polyethylene representing 28% of analyzed particles, then polystyrene with 22%, and polypropylene with 13%. After this, polyamide and polyethylene terephthalate represented 4% of analyzed particles. However, 51% of particles did not respond of spectrum was impossible to identify. Consequently, transformed Fourier infrared microspectroscopy (µFTIR) will be used in addition of Raman spectroscopy to identify the rest of refractory particles.

3.2 Sediments

First results on sediment samples show MPs-like particle contamination about 300 particles.kg^{-1} of dry sediment in Vieux-Port. Fiber contamination was about 360 fibers. kg^{-1} of dry sediment. Most of the particles were films. Particles size range from 38 to 1 200 µm (Fig. 3). Some particles are found in both sediment and water column. This result involves the settling of particles from the water column to the river bed. Fibers size range from 126 to 4 260 µm (Fig. 4). Globally, most of fibers are longer than particles. Compared to the literature, concentrations in the Seine river are higher than concentrations found in South Africa, 20.0 ± 7.5 particles.m^{-3} to 46 particles.m^{-3} [6]. These high concentrations suggest that the Seine river estuary could be a sink for microplastics, but more results are required to go further. Analysis in the sediment are still ongoing.

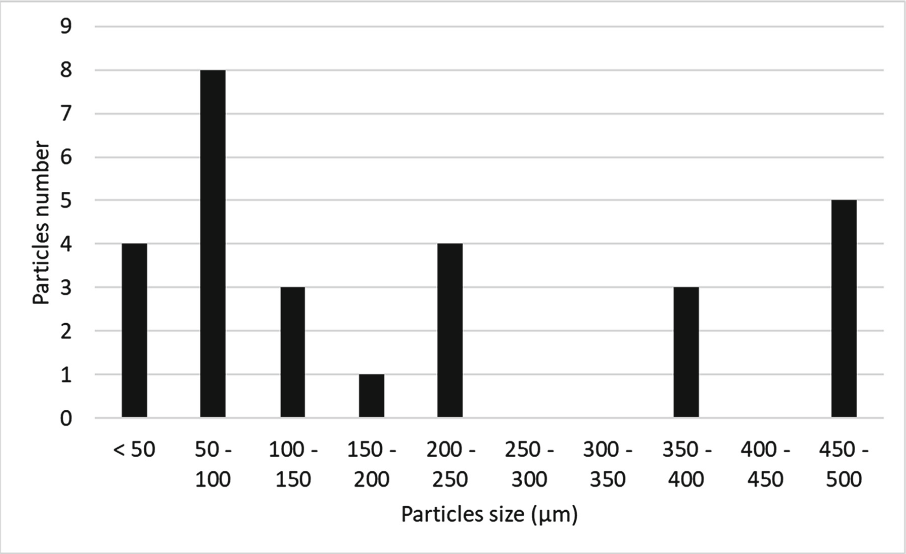

Fig. 3. Size distribution of MPs-like particles in sediment at Vieux-Port

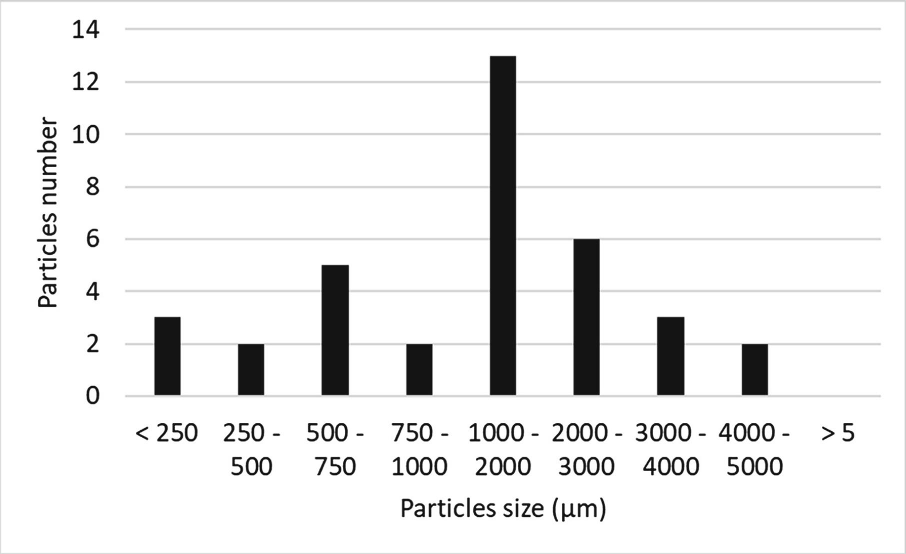

Fig. 4. Size distribution of fibers in sediment at Vieux-Port

4 Conclusions

Concentrations in MPs are high in the Seine river estuary water column, ranging from 1.7 particles.m^{-3} to 37.7 particles.m^{-3}. Same trend is found for sediment with 300 particles.m^{-3} and 360 fibers.m^{-3}. These concentrations show important contamination of the seine river estuary in MPs. These strong concentrations are not surprising as the estuary is subjected to a very strong anthropic pressure and important accumulation of plastic litter. Predominance of fragments indicates fragmentation of larger plastics as the major source of MPs in the estuary. Considering sizes of MPs, results showing most of MPs lower than 1 mm is consistent with the literature. Besides, since some results highlight the sink of particles from the water column to the sediment, consequently, this estuary is suspected to be a sink area for microplastics.

Nevertheless, because estuaries are not well documented, it is difficult to compare levels of contamination in MPs with other studies. Moreover, the lack of standardised protocols makes difficult the comparison of levels of contamination in MPs.

Moreover, characterization step is planned to identify polymer types in the sediment. Both Raman spectroscopy and µFTIR will be used to achieve this goal.

References

1. Carpenter, E.J., Smith, K.L.: Plastics on the Sargasso sea surface. Science **175**, 1240–1241 (1972)
2. Fisson, C., Leboulenger, F., Lecarpentier, T., Moussard, S., Ranvier, G.: L'estuaire de la Seine : état de santé et évolution. Fascicule Seine-Aval **3**(1) (2014). 48 p.
3. Frère, L., Paul-Pont, I., Rinnert, E., Petton, S., Jaffré, J., Bihannic, I., et al.: Influence of environmental and anthropogenic factors on the composition, concentration and spatial distribution of microplastics: a case study of the Bay of Brest (Brittany, France). Environ. Pollut. **225**, 211–222 (2017). https://doi.org/10.1016/j.envpol.2017.03.023
4. Lima, A.R.A., Costa, M.F., Barletta, M.: Distribution patterns of microplastics within the plankton of a tropical estuary. Environ. Res. **132**, 146–155 (2014). https://doi.org/10.1016/j.envres.2014.03.031
5. Sadri, S.S., Thompson, R.C.: On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull. **81**, 55–60 (2014). https://doi.org/10.1016/j.marpolbul.2014.02.020
6. Naidoo, T., Glassom, D., Smit, A.J.: Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. Mar. Pollut. Bull. **101**, 473–480 (2015). https://doi.org/10.1016/j.marpolbul.2015.09.044

Plastic Debris in Urban Water and in Freshwater: Lessons Learned from Research Projects Launched in the Seine Basin Catchment

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Since 2014, several research projects were launched or in progress on plastic debris issue at the scale of the Seine Bassin catchment, combining a high population density and a strong anthropogenic pressure. These projects, illustrated in Fig. 1, are investigating both macro- and micro-plastics (<5 µm) in urban water and in freshwater upstream and downstream of Paris Megacity (France). The keynote will provide a global overview on the knowledge gained from these projects and draw the major learned lessons.

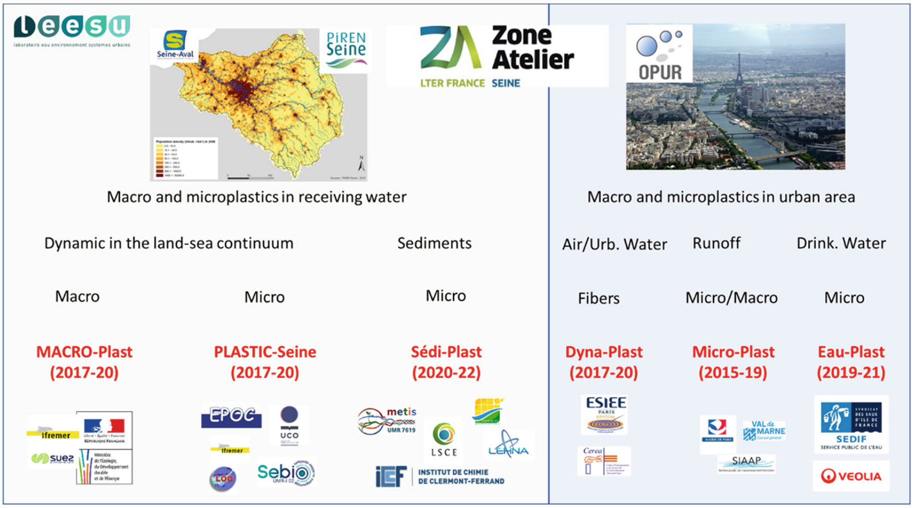

Fig. 1. Research projects launched and in progress on plastic debris issue at the scale of the Seine Bassin catchment

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A first part of this keynote was dedicated to macroplastic pollution and will present first levels found in urban water (Micro-Plast project). Based on tagged plastic litters and GPS-trackers, the fluxes of plastic litter in the Seine River were estimated (MACRO-Plast project). Our results suggest that for countries having a high GDP per capita as France, the assumption of 2% of mismanaged waste proposed by Jambeck et al. should be revisited.

The second part was focused on the microplastic contamination, by reviewing the levels of microplastics in urban water, in total atmospheric fallout, as well as in freshwater from Paris megacity to the Seine River estuary. Both water column and sediments will be considered. To give perspective, the main scientific barriers and issues related to microplastics in freshwater will be also discussed.

Insights on Ecotoxicological Effects of Microplastics in Marine Ecosystems: The EPHEMARE Project

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1 Introduction

The Ephemare project was supported in the period 2015–2018 by JPI Oceans, as one of 4 sister projects in the joint action on ecological aspects of microplastics. Ephemare investigated several issues concerning the ecotoxicological effects of microplastics (MPs) in marine organisms. Ephemare included 16 European Institutions from 10 Countries and was organized into seven, highly complementary Work Packages (WPs) with the aim to elucidate adsorption and release of chemicals to/from MPs, coupled with MP ingestion rates, translocation in different tissues, trophic transfer and egestion, potential toxicological effects and mechanisms of action, as well as real distributions of MPs in marine organisms from several European areas. The project was also designed to raise public awareness through scientifically-sound and research driven results.

Ephemare tested several biological model organisms in laboratory experiments grouped according to their contact/ingestion pathway, comprising no feeders (algae, isolated cells, haemocytes or cell cultures), small and large filter feeders, and predators; these organisms were exposed under laboratory conditions to MPs of different sizes, shapes, polymer type, origins (commercially available vs field micronized) and contaminated with chemical pollutants. Likewise, a wide array of biological species was also collected in the field at coastal locations throughout Europe and analyzed for their content of MPs; as far as feasible, the sampled organisms included representatives from different trophic positions, feeding strategies and habitat preferences.

A suite of biological effects was evaluated at the individual, cellular, and molecular level to elucidate the potential toxicity of MPs and their mechanisms of action. At the individual organism level, the toxicity endpoints ranged from survival, growth rate, behavior, reproduction success, embryo and larval development to energetic physiology and performance. At the cellular and molecular levels, the main investigated pathways included immune responses, oxidative stress, neurotoxicity, biotransformation (particularly for MP-bound chemicals), genotoxicity and endocrine effects. The experimental conditions were designed to evaluate the direct effects of MPs, as well as their capability to modulate bioavailability and toxicity of sorbed chemical pollutants, in comparison with other particles in marine ecosystems.

A detailed description of the experimental set-ups and the obtained results have been published in a series of papers [1]. Herein we summarize the most relevant scientific “take home messages”. Among these, the first is that all the investigated species, from plankton to top predators, did ingest MPs both under laboratory and field conditions. Dynamic modeling confirmed the experimental observations that rate of MP uptake in different tissues, as well as the potential translocation between different tissues, and the egestion kinetics cannot be generalized in terms of “MPs” alone. Rather the involved processes are strongly influenced by the MPs’ size and shape, as well as composition.

Ingestion of MP is not only a direct phenomenon, since these particles can also be easily transferred through trophic chains. In this respect, the uptake of MPs in the jellyfish *Aurelia* can occur directly from water but also via feeding on nauplii of the copepod *Tigriopus fulvus* previously loaded with polyethylene fluorescent MPs (1–

4 μm in diameter, 10 mg/L) [2]. Other examples of simple artificial food chains confirmed that crustaceans and protozoa can efficiently transfer very small MPs (1–20 μm) to both adults and larvae of fish [3, 4]. In many cases, egestion of ingested MPs is rapid (4–6 h), although some particles can be retained within the mucus of intestinal villi and be taken up by epithelial gut cells. The uptake of chemical contaminants, however, is not necessarily increased by adsorption to MPs [5].

Moving the analysis of MPs from the laboratory scale to the field conditions places different demands on the protocols for the extraction and characterization of MPs in marine organisms from natural habitats. Two practical international training courses were held in Ancona to share different experiences among participants of both the Ephemare and Baseman consortia, resulting in a common JPI-Oceans deliverable on a harmonized protocol for monitoring MP in biota, including all the methodological details [6, 7].

The distribution of MPs in marine food webs was investigated in more than 1,200 specimens representative of almost 50 biological species, with different ecological and biological characteristics, sampled in different European areas, from the Mediterranean, the Atlantic Ocean and the North Sea [8, 9]. Just to give a snapshot on the Adriatic food webs, almost 500 organisms from 26 commercial species were sampled from the 3 main sectors, Northern, Central and Southern Adriatic. The overall results did not reveal marked differences in the number of particles extracted in different species and areas; however, the frequency of ingestion was significantly higher in organisms from Central and Southern compared to Northern Adriatic. This work also provided the first extensive characterization of textile microfibers (MFs) which documented more elevated numbers compared to MPs and confirmed the higher percentage of organisms ingesting MPs in Central and Southern Adriatic. Geographical differences were also observed in terms of size, shape and chemical typology of ingested MPs. Specifically, on the basis of frequency and characteristics of particles extracted in marine organisms, principal component analysis distinguished between the 3 Adriatic regions (North, Central, South) which correspond to 3 sectors of the Adriatic basin highly differentiated in terms of bathymetry, morphology and main currents circulation [9].

These field studies enabled several overall conclusions to be drawn. MPs ingestion is a widespread phenomenon, and the frequency of ingestion is a more appropriate index to highlight differences in exposure, rather than the number of particles. Textile MFs are more abundant than MPs, both in terms of numbers and frequency of ingestion. Frequency of ingestion typically ranged between 15 and 35% for MPs and between 50 and 90% for textile MFs. More than 32% of ingested MPs were smaller than 100 μm, 55% smaller than 300 μm, and 70% smaller than 500 μm. In contrast, widely used sampling methods for MPs in seawater typically only collect particles with dimensions greater than 300 μm; our findings highlight the need to quantify and characterize the smaller size fractions to properly evaluate potential biological effects. An unclear influence was found for trophic position, feeding strategy and habitat preference on MPs ingestion and, although local relationships could be observed, they were not easily generalized. Regional activities and hydrographic characteristics might influence the dynamics of the local exposure conditions and thereby the frequency of MPs ingestion and the differences in terms of size and typology of ingested particles. Finally, we found that biological species already used as indicators for biomonitoring programs (such as those indicated by MSFD or national guidelines) should be also considered for MPs monitoring [9].

Concerning the toxicological effects caused by MPs at the organism level, standard ecotoxicological bioassays typically showed no effects, indicating that MPs *per se* are not acutely toxic under short term conditions [10, 11]. Lowest observed effect concentrations LOEC were typically greater than 30 mg/L, irrespective of particle size, shape, or polymer type. In general, concentrations of MPs causing acute toxicological effects in laboratory conditions were 5 orders of magnitude higher than typical environmental levels [12]. However, lack of acute toxicity does not necessarily mean lack of hazard: long-term and/or less acute MP exposure scenarios revealed significant biological effects in some cases. Virgin particles, those previously loaded with chemical pollutants, or field-collected and micronized MPs caused biometry abnormality and behavioral effects in medaka larvae [13, 14]; growth defects and decreased number of eggs appeared in adults of marine medaka after long exposures (3–4 months), and spawning success was decreased in zebrafish [15]. Some of these effects were more evident in organisms exposed to MPs previously loaded with different chemicals or to environmental MPs, compared to organisms exposed to virgin particles of commercial origin. These observations point to a non-negligible role of environmentally acquired contaminants on the overall toxicity exerted by MPs in aquatic ecosystems A significant decrease in predatory performance was observed in sandy goby juveniles [16], while two species of sediment-dwelling bivalves exhibited, with a different sensitivity, some changes in energy metabolism when MPs were present in sediments [17]. Overall, these data highlight that long-term and sub-lethal responses are needed to assess the effects of MPs in marine organisms, coupled with understanding of the uptake/release kinetics of associated contaminants [18, 19].

One of the major take-home messages from Ephemare is that the blanket definition of «microplastics» in biota an inadequate and too generic concept. Ingestion of these particles, excretion rate or potential translocation to different tissues, cellular compartmentalization and biological effects are strongly modulated by size and shape of MPs. Although they are still defined as particles smaller than 5 mm, the size classes of biological relevance are much lower, typically below 200 μm for ingestion, and below 20 μm for cellular compartmentalization. Likewise, shape modulates such phenomena, with spherules, fibres or fragments having different effects. Standard methods have been developed to test the toxicity of those "small microplastics" to zooplankton [12].

We also need to better address indirect effects that MPs might have in combination with other environmental stressors. Among these, chemical pollutants have received particular attention, for the capability of MPs to bind and release these compounds after ingestion, i.e. the so-called Trojan-horse effect. The sorption behaviour of pollutants to MPs has been evaluated during Ephemare under various experimental conditions: it appears to be a rather dynamic process, which depends on exposure conditions, typology of chemical, time of contact and characteristics of particles (size, shape, polymer type, virgin vs weathered). It is even more complex to generalize the release of contaminants from MPs, which is modulated by the particle radius, diffusion coefficient within the polymer matrix, polymer crystallinity, lipophilicity and gut conditions [19]. For amorphous polymers such as PE, affinity to hydrophobic chemicals is higher than that of biological tissues, and thermodynamic models point at diffusion within the polymer as the rate-limiting process. Appreciable desorption dies occur for chemicals with relatively low lipophilicity, the accumulation of which on MPs is negligible in the environment. However, the role of surfactants should be considered further since these compounds appear to facilitate desorption of chemicals and are present in the digestive tract of many organisms [5].

Bioavailability of chemicals bound to MPs could be demonstrated in several experiments after ingestion but also merely by external surface contact. Larvae of *Artemia* could transfer very small MPs (1–20 μm) loaded with benzo[a]pyrene to zebrafish (BaP) [4]. Fluorescence tracking of BaP indicated that even a lipophilic chemical may be desorbed in the intestine of fish and be transferred to the intestinal epithelium and liver. Similar results were observed for the transfer of contaminants from MPs to fish larvae via *Paramecium* previously fed with BaP-loaded MPs. Although the majority of studies have investigated the transfer of chemicals after oral ingestion of MPs, transfer of BaP could also be shown after only superficial contact of MPs with gills of adult zebrafish [3]. Yet, there was no accumulation of particles on or inside the gills; most MPs remained trapped on the superficial mucus layer of the gill filaments and were thus excreted. However, BaP-borne fluorescence indicated the transfer of BaP to the cells of the gill filaments and arches after 6 and 24 h incubation, a phenomenon confirmed by gill EROD induction.

The transfer of a chemical from MPs to tissues does not necessarily mean that these particles should be considered as a major source of exposure for marine organisms, and BaP visualized by fluorescence microscopy under experimental conditions did not reach sufficiently high concentrations to induce toxic effects in the fish embryo [3, 4]. Compared to waterborne exposure, MPs certainly influence the tissues a chemical might be released to, and the timescale thereof. Mussels exposed to Hg2+, dissolved or sorbed onto particles (including MP and microalgae) accumulated the same amount of Hg independently of the exposure route but in different tissues, namely the digestive gland for particle exposure, and the gills for waterborne exposure. Approximately 70% of the Hg incorporated through MPs was quickly eliminated through biodeposits, while Hg2+ uptake via microalgae or water was translocated to other tissues [20, 21]. A different organotropism for chemicals released from MPs compared to waterborne exposure has been observed also for other compounds, such as Chlorpyrifos [22].

MPs do not appear to increase the load of bioaccumulated pollutants [23], nonetheless several lines of evidence showed that these particles can modulate the biological effects of chemicals [24, 25]. At the organism level, virgin MPs were shown to increase toxicity of chlorpyriphos to mussel larvae, while chlorpyriphos-spiked MPs were less toxic than the combination of MPs and dissolved chlorpyriphos. Synergistic effects of PFOS and MPs were observed on the 21d chronic assay with *Daphnia magna*, while both synergistic and antagonistic effects were caused by gold nanoparticles (5 nm) and MPs (1–5 μm) on mortality and reproduction success of *Daphnia magna* [26].

Molecular and cellular mechanisms by which MPs can modulate biological effects of chemicals have been further addressed in experiments with invertebrates and fish, exposed to various combinations and typologies of MPs dosed alone and in combination with chemicals. Beside the ingestion of particles, translocation and possible bioaccumulation of chemicals, a wide array of biomarkers including immune responses, oxidative stress, neurotoxicity, lipid metabolism, peroxisomal proliferation and genotoxicity have been investigated at the functional cellular level, proteomic profile and gene expression [27–30]. The main overall results confirmed the ingestion of MPs both *via* both water and diet, an uncertain translocation of MPs to different tissues depending on their size, some typical inflammatory responses at histological and gene expression levels, accompanied by the confirmation that MPs can also act as vehicles of associated contaminants which are desorbed and accumulated by organisms, even though concentrations were not particularly elevated [31, 32]. The analyses of several biomarkers confirmed a certain involvement of oxidative pathways and cholinesterase inhibition, but immunological parameters were generally those revealing more frequent and rapid variations. When the overall biological significance of cellular variations was summarized using weighted criteria based on toxicological relevance and magnitude of observed variations, the elaborated level of hazard generally ranged between slight and moderate, confirming a general lack of acute effects in the medium-term. At the same time, however, the overall results highlighted a clear shift from a physical to a chemical toxicity in mussels exposed to BaP-contaminated MPs [30]. At the beginning of the exposure, the main effects were induced by MPs (possibly reflecting a physical challenge), followed by effects ascribed to a combination of MPs and BaP; only after prolonged exposure, effects of BaP prevailed over those induced by MPs (chemical impacts dominant).

The main conclusions on biological effects of MP ingestion in marine organisms can be summarized as follows: standard ecotoxicological assays do not reveal acute toxic effects after short-term exposure, whereas sublethal effects may appear at longer exposure times; MPs can bind and release pollutants to organisms in a way that depends on the physicochemical features of the MPs and the physiological features of the organisms, and they do not represent a major source of chemical exposure in absolute terms; MPs can modulate the effects of chemicals and may cause interaction between chemical and physical challenges; effects at the cellular level were moderate, but the observed susceptibility of the immune system points to potential subtle effects on organisms' health status under chronic exposure conditions; the possibility of MPs to modulate organismal responsiveness towards other stressors including climate change variables deserves attention.

References

1. Ephemare Project Final Report (2020). http://www.jpi-oceans.eu/sites/jpi-oceans.eu/files/public/Microplastics/JPI%20Oceans%20Final%20Project%20Report%20-%20EPHEMARE.pdf
2. Gambardella, C., Morgana, S., Bramini, M., Rotini, A., Manfra, L., Migliore, L., Piazza, V., Garaventa, F., Faimali, M.: Ecotoxicological effects of polystyrene microbeads in a battery of marine organisms belonging to different trophic levels. Mar. Environ. Res. **141**, 313–321 (2018)
3. Batel, A., Borchert, F., Reinwald, H., Erdinger, L., Braunbeck, T.: Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (Danio rerio) gills and zebrafish embryos. Environ. Pollut. **235**, 918–930 (2018)

4. Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T.: Transfer of benzo[a]pyrene from microplastics to *Artemia* nauplii and further to zebrafish *via* a trophic food web experiment: Cyp1a induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. **35**, 1656–1666 (2016)
5. Heinrich, P., Braunbeck, T.: Bioavailability of microplastic-bound pollutants in vitro: the role of adsorbate lipophilicity and surfactants. Comp. Biochem. Physiol. **221C**, 59–67 (2019)
6. Avio, C.G., Gorbi, S., Regoli, F.: Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Mar. Environ. Res. **111**, 18–26 (2015)
7. Bessa, F., Frias, J.P.G.L., Knögel, T., Lusher, A., Andrade, J.M., Antunes, J., Sobral, P., Pagter, E., Nash, R., O'Connor, I., Pedrotti, M.L., Kerros, M.E., León, V., Tirelli, V., Suaria, G., Lopes, C., Raimundo, J., Caetano, M., Gago, J., Viñas, L., Carretero, O., Magnusson, K., Granberg, M., Dris, R., Fischer, M., Scholz-Böttcher, B., Muniategui, S., Grueiro, G., Fernández, V., Palazzo, L., de Lucia, A., Camedda, A., Avio, C.G., Gorbi, S., Pittura, L., Regoli, F., Gerdts, G.: Harmonized protocol for monitoring microplastics in biota. Technical report D4.3 BASEMAN project (2019). https://doi.org/10.13140/rg.2.2.28588.72321/1
8. Bour, A., Avio, C.G., Gorbi, S., Regoli, F., Hylland, K.: Presence of microplastics in benthic and epibenthic organisms: influence of habitat, feeding mode and trophic level. Environ. Pollut. **243**(Part B), 1217–1225 (2018)
9. Avio, C.G., Pittura, L., d'Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F.: Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: general insights for biomonitoring strategies. Environ. Pollut. **258**, 113766 (2020)
10. Gambardella, C., Piazza, V., Albentosa, M., Bebianno, M.J., Cardoso, C., Faimali, M., Garaventa, F., Garrido, S., González, S., Pérez, S., Sendra, M., Beiras, R.: Microplastics do not affect standard ecotoxicological endpoints in marine unicellular organisms. Mar. Pollut. Bull. **143**, 140–143 (2019)
11. Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., Gambardella, C., Garaventa, F., Keiter, S., Le Bihanic, F., López-Ibáñez, S., Piazza, V., Rial, D., Tato, T., Vidal-Liñán, L.: Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. J. Hazard. Mater. **360**, 452–460 (2018)
12. Beiras, R., Tato, T., López-Ibáñez, S.: A 2-Tier standard method to test the toxicity of microplastics in marine water using Paracentrotus lividus and Acartia clausi larvae. Environ. Toxicol. Chem. **38**, 630–637 (2019)
13. Pannetier, P., Morin, B., Clerandeau, C., Laurent, J., Chapelle, C., Cachot, J.: Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: part II-adverse effects on Japanese medaka first life stage. Environ. Pollut. **248**, 1098–1107 (2019)
14. Pannetier, P., Morin, B., LeBihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J.: Environmental samples of microplastics induce significant toxic effects in fish larvae. Environ. Intern. **134**, 105047–105057 (2020)
15. Cormier, B., Batel, A., Cachot, J., Bégout, M.-L., Braunbeck, T., Cousin, X., Keiter, S.: Multi-laboratory hazard assessment of contaminated microplastic particles by means of enhanced fish embryo test with the zebrafish (*Danio rerio*). Front. Environ. Sci. **7**, 135 (2019)
16. Fonte, E., Ferreira, P., Guilhermino, L.: Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (Pomatoschistus microps): post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. Aquat. Toxicol. **180**, 173–185 (2016)

17. Bour, A., Haarr, A., Keiter, S., Hylland, K.: Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environ. Pollut. **236**, 652–660 (2018)
18. Town, R.M., van Leeuwen, H.P., Blust, R.: Biochemodynamic features of metal ions bound by micro- and nano-plastics in aquatic media. Front. Chem. **6**, 627 (2018)
19. van Leeuwen, H.P., Duval, J.F.L., Pinheiro, J.P., Blust, R., Town, R.M.: Chemodynamics and bioavailability of metal ion complexes with nanoparticles in aqueous media. Environ. Sci. Nano **4**, 2108–2133 (2017)
20. Fernández, B., Albentosa, M.: Insights into the uptake, elimination and accumulation of microplastics in mussel. Environ. Pollut. **249**, 321–329 (2019)
21. Rivera-Hernández, J.R., Fernández, B., Santos-Echeandía, J., Garrido, S., Morante, M., Santos, P., Albentosa, M.: Biodynamics of mercury in mussel tissues as a function of exposure pathway: natural vs microplastic routes. Sci. Total Environ. **674**, 412–423 (2019)
22. Garrido, S., Linares, M., Campillo, J.A., Albentosa, M.: Effect of microplastics on the toxicity of chlorpyrifos to the microalgae Isochrysis galbana, clone t-ISO. Ecotoxicol. Environ. Saf. **173**, 103–109 (2019)
23. Beiras, R., Muniategui-Lorenzo, S., Rodil, R., Tato, T., Montes, R., López-Ibáñez, S., Concha-Graña, E., Salgueiro-González, N., Quintana, J.B.: Polyethylene microplastics do not act as vectors of two hydrophobic organic pollutants to marine zooplankton. Sci. Total Environ. **692**, 1–9 (2019)
24. Pannetier, P., Cachot, J., Clerandeau, C., Faure, F., van Arkel, K., de Alencastro, L.F., Levasseur, C., Sciacca, F., Bourgeois, J.P., Morin, B.: Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: part I-adverse effects on fish cell line. Environ. Pollut. **248**, 1088–1097 (2019)
25. Prata, J.C., Lavorante, B.R.B.O., Montenegro, M.C.B.S.M., Guilhermino, L.: Influence of microplatics on the toxicity of the pharmaceuticals procainamide and doxycycline on the marine microalgae Tetraselmis chuii. Aquat. Toxicol. **197**, 143–152 (2018)
26. Pacheco, A., Martins, A., Guilhermino, L.: Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in Daphnia magna. Sci. Total Environ. **628–629**, 474–483 (2018)
27. Fernández, B., Albentosa, M.: Dynamic of small polyethylene microplastics (≤ 10 µm) in mussel's tissues. Mar. Pollut. Bull. **146**, 493–501 (2019)
28. Espinosa, C., Cuesta, A., Esteban, M.A.: Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol. **68**, 251–259 (2017)
29. Espinosa, C., García Beltrán, J.M., Esteban, M.A., Cuesta, A.: In vitro effects of virgin microplastics on fish head-kidney leucocyte activities. Environ. Pollut. **235**, 30–38 (2018)
30. Pittura, L., Avio, C.G., Giuliani, M. E., d'Errico, G., Keiter, S., Cormier, B., Gorbi, S., Regoli, F.: Microplastics as vehicles of environmental PAHs to marine organisms: combined chemical and physical hazards to the Mediterranean mussels, Mytilus galloprovincialis. Front. Mar. Sci. (2018). https://doi.org/10.3389/fmars.2018.00103
31. O' Donovan, S., Mestre, N.C., Abel, S., Fonseca, T.G., Carteny, C.C., Cormier, B., Keiter, S.H., Bebianno, M.J.: Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the clam Scrobicularia plana. Front. Mar. Sci. (2018). https://doi.org/10.3389/fmars.2018.00143
32. Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L. M., Bebianno, M.J.: Microplastics effects in Scrobicularia plana. Mar. Pollut. Bull. **122**(1–2), 379–391 (2017)