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Marine litter in the Mediterranean and Black Seas

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This collection offers a broad range of titles in the marine sciences, with a particular focus on emerging issues. The Monographs do not aim to present state-of-the-art reviews; they reflect the latest thinking of researchers gathered at CIESM invitation to assess existing knowledge, confront their hypotheses and perspectives, and to identify the most interesting paths for future action.

A collection founded and edited by Frédéric Briand.

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Marine Litter In The Mediterranean And Black Seas

Executive Summary [1]

This summary, outlined during the course of the workshop was developed and consolidated in the following weeks on the basis of further inputs provided by the participants under the coordination and synthesis of François Galgani. Frédéric Briand reviewed and edited the entire Monograph. The physical production was carried out by Valérie Gollino.

1. Introduction

Marine debris is now commonly observed everywhere in the oceans, drastically impacting the Mediterranean Sea which is now one of the most affected areas – if not the most affected area in the World Ocean – as noted by CIESM Director General, Frédéric Briand, in his opening remarks. In welcoming the group of 16 international guests invited on this occasion. He remarked that this was the first time that a CIESM Workshop was held in Albania, signaling the hope that this country would soon join the large family of CIESM Member States.

Dr Briand then introduced the Chair of CIESM Committee on Marine Biogeochemistry, Dr François Galgani, thanking him warmly for suggesting as a theme for this 46th CIESM Workshop an issue that was fast gaining world attention in the media but still presented vast gaps in knowledge, particularly regarding the impact of micro- and nano-plastics and their interactions with marine microbiota. As pointed out by the workshop moderator this would indeed constitute one of the central question addressed during the meeting.

Generally, litter enters seas from both land and water-based, diffuse and point sources and can travel long distances before depositing on shores and seabeds. While plastics typically do not constitute a high percentage of discarded waste, they are the most important part of marine litter, constituting up to 100% of floating items (Suaria and Aliani, 2014).

The Mediterranean Sea is the most affected area in the world with the highest amounts of municipal solid waste generated annually per person (208-760 kg/year, http://www.atlas.d-waste.com). It is mainly affected by land-based sources (Galgani, this volume). Debris densities may be enhanced by up to 40 % in the summer months due to the high numbers of tourists, who generate more than 75 % of the annual waste during the summer season (Galgani *et al.*, 2011). Input fluxes vary largely and are affected by factors such as proximity of urban activities, shore and coastal uses, wind and currents. Recently, a probable accumulation of 7-8 % of floating debris over the next 30 years in the Mediterranean Sea has been predicted (Lebreton *et al.,* 2012). However, available data do not indicate any clear, overall trends in the Mediterranean, with certain areas even showing decreases

[1] to be cited as :
Galgani F., Barnes D.K.A., Deudero S., Fossi M.C., Ghiglione J.F., Hema T., Jorissen F.J., Karapanagioti H.K., Katsanevakis S., Klasmeier J., von Moos N., Pedrotti M.L., Raddadi N., Sobral P., Zambianchi E. and F. Briand. 2014. Executive Summary pp. 7 - 20 *in* CIESM Workshop Monograph n°46 [F. Briand, ed.] Marine litter in the Mediterranean and Black Seas, 180 p., CIESM Publisher, Monaco.

in debris over the last 20 years, notably in the Gulf of Lion, where field data suggest neither change nor increase of marine plastic debris.

Microplastics, defined as synthetic polymer particles '<5 mm' (Arthur *et al.*, 2009), are ubiquitous in the marine environment, reaching mean densities of more than 100,000/ km^2 in the Mediterranean Sea (Collignon *et al.*, 2012). They constitute a highly heterogeneous assemblage of plastic pieces that vary in size, shape, color, specific density, chemical composition and origin (Hidalgo-Ruz *et al.*, 2012). Microplastics either enter the marine environment as preproduction pellets (primary microplastics) or emerge from the weathering and breakdown of larger items already present as marine litter in the oceans (secondary microplastics) through the combined action of mechanical, biological, photic and thermal abrasion, leading to their fragmentation into increasingly small pieces (Andrady, 2011; Cole *et al.*, 2011).

A correct estimate of global debris load cannot be provided until basic information on sources, inputs, degradation processes and fluxes is obtained. This will enable a better understanding of the processes of transport and the presence of accumulation areas, both at the surface and on the seafloor. Knowing the recent concentrations of plastic debris in the natural, aquatic environment will also improve our understanding of its impact and potential harm.

2. Marine litter in the Med

According to UNEP (2011), marine litter on Mediterranean beaches is mainly composed of plastics (bottles, bags, caps/lids, etc.), aluminium (cans, pull tabs) and glass (bottles) making up 52% of total litter based on item counts. Smoking-related items account for another 40% (of total collected items), which is considerably higher than the global average. Most studies conducted on Mediterranean beaches have reported plastic densities in the range of 1/m^2 with very high concentrations resulting from specific local conditions or after flooding events (e.g. 5,058 items/m^2, reported by Topçu *et al.*, 2013. For comparison, in the Black Sea plastics account for a large part of litter on various beaches with up to 91 % in the southern basin (Topçu *et al.*, 2011).

Floating debris in the Mediterranean can generally reach densities in the range of 1-5 items/km^2 (Galgani, this volume). A recent large scale study reported densities between 0 and 194.6 items/km^2, of which 95.6% were polymers, with maxima in the Adriatic Sea and Algerian Basin (Suaria and Aliani, 2014). There are no long-term accumulation areas of floating debris in the Mediterranean, aside from meso-scale structures in the timescale of months (Zambianchi *et al.*, this volume). However, Mediterranean submarine canyons are important accumulation sites. Continental shelves are narrow and the coastal location of many heads of canyons is responsible for a transfer to deep sea environments. The abundance of plastic debris on the sea floor highly depends on a location's specificities, with mean values ranging from 0 to more than 100,000 items per km^2.

2.1 Water circulation and litter

Circulation is the primary driver of marine litter transport. Currents are responsible for the advection of items of every size at all depths, as a function of their composition and specific weight. This is also true for litter that is less dense than seawater and floats at the surface and thus easily accumulates in convergent regions. The role of currents, however, may be complex: they may act as conveyors, as is typically the case for jets, and more generally when they are characterized by open streamlines. On the other hand, recirculation may well induce retention of particles, enhancing dispersion within closed streamlines and thus acting as blending mechanisms. The possible chaotic characteristics, even of two-dimensional time-dependent flow, makes transport difficult to predict. In practice, a number of non-trivial Lagrangian behaviour expressions exist, which predict the formation of attractive and repulsive features in coastal and offshore flow fields.

2.2 Biodegradation of plastics in the marine environment

Although synthetic polymers are largely considered biologically and chemically inert, physical, chemical or biological degradation can theoretically occur in sediments and in the water column (Raddadi *et al.*, this volume), albeit extremely slow and over centuries in some cases.

Microbial degradation of synthetic plastics/polymers, such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) has been reported for pure microbial isolates as well as by mixed microbial consortia. Generally, a decrease in molecular weight and/or in crystallinity results in a higher biodegradability of the polymer. Photolytic, thermal and chemical pre-treatments can induce changes in the mechanical, electrical and chemical properties due to bond scissions, cross linking, chemical transformation and the formation of new functional groups, which in turn can remarkably increase the final biodegradability of a given polymer. Physical (UV exposure, thermal treatment and gamma radiation) and/or chemical (ozonation) treatments improve the hydrophilic character of the material (either by surface modification or by controlled oxidation), reduce the crystal/amorphous ratio and decrease the molecular weight, thereby making a polymer more amenable to microbial attack. Factors influencing biodegradation include the chemical properties of the polymer, the time it stays in the system and chemical/physical pretreatments it may have undergone.

Some biodegradation pathways are known for model organisms and have been mainly described for natural and degradable polymers (cellulose, polyesters, etc.) and to a lesser extent for some plastic components such as Polyethlene (Raddadi *et al.*, this volume). The process involves several steps, some of them being more critical than others for biodegradation to occur (Dussud and Ghiglione, this volume). The formation of a biofilm is the first step for microorganisms to colonise the hydrophobic surface of plastic, but very little data is actually available on the colonization of plastics compared to the large amount of data on biofilm formation on other physical supports. The "ecology" of microorganisms colonising polymers is largely unknown. Major knowledge gaps include such questions as which species preferably colonize what types of plastics, what mechanisms support bacterial attachment onto hydrophobic plastic surfaces, what activities they perform to do so and what factors control their stability (resource or predation controls). Very few studies have addressed the role of biofilms for bio-deterioration processes, i.e. the biotic mechanism responsible for the fragmentation of plastics that generally acts together with other physical mechanisms. By the excretion of exoenzymes, biofilms contribute to the bio-fragmentation of the plastic, i.e. the lytic catalysis of reactions principally at the edge of the plastic polymer. This critical step is supported by oxygenase enzymes that destabilise the highly balanced charges of the plastic and make it more available for biological processes. Some cultured organisms were able to perform this step under laboratory conditions, but pre-treatment (thermal, light, chemical, mechanical) of the plastic has been shown to greatly enhance this process. The integration of water soluble intermediates into microbial cells, called (3) bio-assimilation and (4) bio-mineralisation to completely oxidised CO_2 are performed by various microorganisms in seawater.

Evaluation of polymer biodegradation efficiency is based on a combination of different analytical methods including surface changes (FTIR), thermal stability (TGA) as well as reduction in molecular weight (GPC). To date, the lack of standardized procedures does not allow an effective comparison of different research results. This especially concerns important aspects such as thickness of the plastic film and molecular weight of the polymer under investigation.

3. Impact

The resulting "harm" of marine litter is multidimensional and hence can be divided into three general categories including (i) ecological, (ii) social, and (iii) economic impacts. Ecological harm includes mortality or sub-lethal effects on organisms through entanglement, unintentional captures from ghost nets, physical damage and ingestion. Uptake of microparticles may be connected with the release of associated chemicals, the facilitation of invasion by alien species, and the alteration of benthic community structure. Social harm includes the reduction of recreational, aesthetic or educational values of areas such as beaches, as well as risks to human health and threat to navigation. Economic harm includes direct cost and loss of income due to marine litter affecting a range of maritime sectors including aquaculture, fishery, shipping, tourism and leisure boating.

3.1 Litter as an important vector for the transport of species

The upcoming regulation on the prevention and management of the introduction and spread of invasive alien species provides the following definitions:

1) 'Alien species' are defined as any live specimens of species, subspecies or any lower taxon of animals, plants, fungi or micro-organisms introduced outside its natural past or present distribution; it includes any part, gametes, seeds, eggs, or propagules of such species, as well as any hybrids, varieties or breeds that might survive and subsequently reproduce.

2) An 'Invasive alien species' is an alien species whose introduction or spread has been found, through risk assessment, to threaten biodiversity and ecosystem services, and that may also have a negative impact on human health or the economy.

Many studies around the world suggest that the large availability of floating litter can greatly assist the transport of species beyond their natural boundaries and their introduction to environments where they were previously absent (Winston, 1982; Barnes, 2002; Barnes and Milner, 2005). Barnes (2002) estimated that human litter more than doubles the rafting opportunities for biota, assisting the dispersal of alien species. However, very few studies on the role of marine litter in the introduction and spread of alien species exist in European Seas. Marine litter has not been included as a potential vector of introduction of alien species in any of the recent assessments of pathways in Europe focusing on primary pathways of introduction (Zenetos *et al*., 2012; Katsanevakis *et al*., 2013; Galil *et al*., 2014). In these assessments, shipping, corridors (Suez Canal and inland corridors), aquaculture and aquarium trade have been identified as the most important pathways.

However, thirteen species that are alien to the Mediterranean have been found to colonize floating litter elsewhere in the world. In many cases, plastic can be colonized more easily than metals, especially metals coated with anti-fouling paints (i.e. vessel hauls). Thus, species that have been reported to foul the hauls of vessels can, quite probably, colonize floating plastic as well. Furthermore, more than 80% of the known alien species in the Mediterranean might have been introduced by colonizing marine litter or could potentially use litter for expanding their range further (estimation based on the life cycle and traits of species, and whether they have been reported to foul the hauls of vessels). Moreover, large amounts of litter arrive in the Mediterranean through the Suez Canal and the potential of Red Sea organisms gaining access to the Mediterranean by rafting on marine litter is not negligible (Galil *et al.*, 1995). For all these reasons, the role of litter as a vector for the introduction and dispersal of alien species could be important in the Mediterranean.

3.2 Ecological harm

Interactions of marine fauna with plastics can lead to both physical and chemical harm, the latter encompassing i) exposure to persistent, bioaccumulating and toxic (PBT) substances concentrated on plastics; ii) leaching of plastic additives, such as phthalates (Wright *et al.*, 2013), which may lead to their biomagnification.

Primary impacts of marine litter are ingestion (which can cause internal blockage and abrasion) and entanglement, with more than 660 marine species known to be impacted (GEF, 2012).

So far, 79 studies have investigated the interactions of marine biota with marine litter (mainly plastics) in the Mediterranean basin (Deudero and Alomar, this volume). These studies cover a wide range of depths (0 m to 850 m) and a large temporal scale (1986 to 2014), unravelling a vast array of species affected by litter ranging from invertebrates (polychaetes, ascidians, bryozoans, sponges, etc.), fish and reptiles to cetaceans, including species found in IUCN categories. Effects described in these studies can be classified in various categories, such as ingestion, entanglement and colonization and rafting. However, there is still little monitoring data on the occurrence of macro- and microplastics in marine organisms in the Mediterranean Sea. In particular, the potential impact of macro and especially microplastics (not to mention nanoplastics) on large filter feeding marine organisms such as baleen whales or sharks is unknown.

Impacts on fish have been found to vary greatly as a function of their ecological compartments. Highly affected species include *Boops boops*, myctophids, *Coryphaena hippurus, Seriola dumerilii, Schedophilus ovali* and *Naucrates ductor* (Deudero and Alomar, this volume).

If entanglement is of concern for all species with individuals exposed to ghost driftnets and other fishing gear, cetaceans are also affected by ingestion at a global level. Based on studies on stranded

individuals, it has been found that large mysticetes may ingest large plastics sheets generally at a low rate. Most odontocetes (toothed whales) are marginally and accidentally affected by plastic ingestion, with the exception of *Grampsus griseus*, which easily mistakes plastics for squid, and *Physeter macrocephalus*, which consumes benthic marine litter incidentally together with bottom-dwelling prey.

All evaluated sea turtle species are affected by ingestion and entanglement with preferential ingestion of white or uncoloured plastics, due to their resemblance with jellyfish. Though *Caretta caretta* is the only Mediterranean turtle species which has been extensively investigated so far, it seems probable that all turtle species are affected (Gramentz, 1988; Tomás *et al.*, 2002; Casale *et al.*, 2008).

Filter feeders are highly affected by ingestion, while predators do not exhibit a clear pattern, which may be explained by the wide variety of trophic traits (piscivorous, mesograzers, invertebrate feeders, etc.). Overall, endangered species, as defined by IUCN categories, are highly affected by plastic (41%).

3.3 Concentration and release of pollutants by marine litter

There is an increased concern regarding persistent, bioaccumulative (PBT), and toxic chemicals such as polycyclic aromatic hydrocarbons (PAH) and pesticides adsorbed onto plastics, which may become vectors for the bioaccumulation of these highly toxic pollutants in fatty tissues (Mato *et al.*, 2001; Ogata *et al.*, 2009; Rios *et al.*, 2007; Rochman *et al.*, 2013), posing a long term risk to the environment.

Based on data from beaches on both the Greek and Portuguese coasts (Karapanagioti *et al.*, 2011; Antunes *et al.*, 2013), pellets near port facilities may reach PAH concentrations as high as $\mu g\ g^{-1}$ exhibiting congener patterns from petrogenic sources (Sobral *et al.*, this volume). PCB contamination was higher in aged pellets than in any of the other types and the more chlorinated congeners recorded higher concentrations in the proximity of urban areas. The highest total DDT was found near industrial sites and port facilities. Though there are no defined levels of toxicity for persistent organic pollutants adsorbed to plastic particles, it is probable that effects may exist as these pollutants are known to desorb in certain conditions (Endo *et al.*, 2013).

The most common polymers in beach samples from Portugal were found to be polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyurethane (PU). Beaches located downstream from industries and/or port facilities presented higher quantities of plastic debris and microplastics as well as higher concentrations of POPs (PAH, PCB and DDT). Colorless PE pellets showed lower contaminant values than aged, yellow-brown ones, reflecting their residence time in the water. Black pellets were mainly composed of PS and PU and showed high values of adsorbed POPs (Frias *et al.*, 2010; Antunes *et al.*, 2013), near urban areas and port facilities. Nevertheless, modelling studies by Koelmans *et al.* (2013) showed that ingestion of contaminated plastics does not necessarily lead to increased bioaccumulation in the organisms. One of the reasons may be the limited retention time of the material which prevents complete desorption of co-transported contaminants during gut passage. Finally, relationships between harm (at a specific endpoint) and particle size are still to be determined, especially for nanoparticles below 30 - 100 nm in size due to a possible uptake (von Moos, this volume).

4. Legal instruments

The UNEP/MAP Regional Plan on Marine Litter (Mediterranean countries) adopted in the framework of Land based Sources and Activities Protocol of the Barcelona Convention and the Marine Strategy Framework Directive (MSFD) for European countries are the two main frameworks for marine litter in both the Mediterranean and Black Seas.

The 18th Meeting of the contracting Parties of the Barcelona Convention and its 'land based sources and activities' protocol held in Istanbul, Turkey in December 2013, adopted a Regional Plan for the Management of Marine Litter in the Mediterranean. This was the first regional sea for which legally binding commitments were made through measures, programmes and related implementation of timetables at regional and national levels, thus contributing to the Honolulu

Commitment and the Rio + 20 marine litter target. The major objectives of the Regional Plan are to achieve good environmental status through the prevention and reduction to a minimum of marine litter and its environmental, health and socio economic impacts. Most of the measures aim at improving solid waste management, implementing innovative tools related to a sustainable production and consumption and the use of economic incentives, the removal of existing marine litter and the elimination of hot spots, etc. The Regional Plan intends to create a sound framework for knowledge enhancement, monitoring and assessment, research, awareness, cooperation and partnerships among different stakeholders at regional and national levels including the scientific community and the large public. In this respect, the MEDPOL programme of UNEP/MAP is mandated to undertake the assessment of marine litter on a six-year basis at the Mediterranean level as well as to coordinate the formulation and implementation of a marine litter monitoring programme based on an ecosystem approach by all Mediterranean countries. The Regional Plan (http://www.unepmap.org/index.php?module=news&action=detail&id=158) indicates a list of 30 priority research topics on marine litter and invites the research community to actively contribute to filling these knowledge gaps, facilitating the efficient implementation of measures and assessing their effectiveness.

Within MSFD, EU Member States are requested to determine a set of characteristics that define Good Environmental Status (GES) of their relevant waters, based on a list of 11 qualitative descriptors that include descriptor 10 defined as "Properties and quantities of marine litter do not cause harm to the coastal and marine environment" (2010/477/EU). Four indicators are associated with this descriptor and according to the MSFD definition, GES can be regarded as achieved when litter and its degradation products present in and entering EU marine waters (i) do not cause harm to marine life and habitats, (ii) do not pose direct or indirect risks to human health, and (iii) do not lead to negative socio-economic impacts. The directive represents an important step ahead by acknowledging marine litter as a serious ecological issue, but it remains limited of course to European countries.

5. Knowledge gaps and recommendations for future research

5.1 Definition of size classes

The characterization of the environmental status with respect to litter contamination requires reliable and comparable quantitative data in various compartments. A wealth of data on plastics in marine waters and beach sediments has been collected and published by researchers worldwide. However, the general evaluation of these data poses a number of problems. Due to a lack of standardized protocols for sampling, extraction and detection of plastic debris, the comparability of available data remains highly limited, especially with respect to different size class categories, sampling procedures, analytical methods and reference values (weight, volume or area). As a first step towards the necessary harmonization, the Technical Group for Marine Litter (TG-ML) recently suggested to differentiate between macroparticles (> 25 mm), mesoparticles (5 – 25 mm) and microparticles (< 5 mm) with a further subdivision into large microparticles (1 – 5 mm) and small microparticles below 1 mm (Galgani *et al.*, 2013). However, this categorization probably needs to be amended by a further subdivision of the smallest size class of microplastics to include nanoplastics. The European commission has recently recommended the following definition for nanomaterials (2011, revised 2014):

'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

Nonetheless, a definition of nanomaterials that is both practical and unambiguous to scientists, legislators, decision makers and consumers alike is still lacking (Kreyling, 2010).

5.2 Harmonization of methods

Standardized methods exist for the sampling of macro- and meso- marine litter on beaches, at sea or on the seafloor and for ingested litter (Galgani *et al.*, 2013). For monitoring, we currently lack the information to determine the required number of replicates in time and space. This is an even bigger problem for microplastics, for which in addition there is uncertainty about the optimal sampling depth in sediments and water. Since the study of microplastics in the Mediterranean Sea is still in an early stage of development, an harmonization of sampling protocols for the water surface is highly recommended.

In addition to the (stereomicroscopic) counting of sorted microplastics, parameters such as length, area occupied by plastics and particle roundness could also be routinely assessed, which would enable an estimation of the total area occupied by plastics on the ocean surface. This would also allow the simultaneous study of the respective area occupied by microplastics and planktonic communities. Recent findings suggest that digital and visual analyses (by eye) are comparable, except for the very small pieces where the digital scanner was more adequate (Goldstein *et al.*, 2012) and for the characterization of particles. Digital analysis tools can be easily developed by image analysis systems.

The analytical determination of plastic particles from environmental samples is another challenge, which becomes increasingly more difficult for smaller particles. Extraction methods for microplastics from sediments are generally based on the principles of fluidization or flotation (Imhof *et al.*, 2012; Claessens *et al.*, 2013; Nuelle *et al.*, 2013), while water samples are filtrated *in situ*. Since the mesh size used for filtration provides a lower boundary for the size of detected particles, this is another critical issue with respect to comparison of data from different monitoring programs. Currently, various net types (manta, WPII, bongo net) with different mesh sizes and surface apertures (500, 330, 280, 200, 180µm) are being used for the collection of microplastics, which are then separated and counted by visual identification. The commonly used mesh size of 333 µm has basically been selected for practical rather than for scientific reasons. Since sediment extraction does not even include such an inherent lower size boundary, it will be very difficult to draw parallels between microplastic numbers determined in water and beach samples. After the particles have been separated from the matrix, quantification requires weighing or counting, which is a difficult task especially for small particles barely visible to the naked eye. Microscopic inspection of sample extracts has been shown to bear a high risk of overestimation due to large amounts of natural substances (animal parts, minerals) still present in the extracts (Dekiff *et al.*, 2014). Hence chemical identification of particles suspected to be plastics is indispensable after visual pre-sorting. Recommended techniques for the proper identification of plastics are Raman or FTIR spectroscopy (Imhof *et al.*, 2012; Claessens *et al.*, 2013) or pyrolysis-GC/MS (Nuelle *et al.*, 2013).

5.3 Circulation

Models simulating the three-dimensional circulation in the Mediterranean Sea are presently available to the scientific community even in an operational (predictive) mode. They are becoming more and more accurate thanks to the ever increasing abundance of *in situ* data and the development of sophisticated assimilation techniques for such data. An effort in the direction of marine litter transport modelling is feasible and should be seriously taken into consideration by the Mediterranean oceanographic community. This should include a correct representation of local wind-induced effects on floating material, windage and Stokes' drift, and dedicate investigations on the possible functioning, role, and parametrization of submesoscale effects, both in a two-dimensional and a three-dimensional perspective. Coastal related input and stranding processes also need to be investigated with great care, as they are crucial for assessing budgets of marine litter present in Mediterranean waters. Coastal studies may require the development or the refinement and focusing of regional models, characterized by higher spatial and temporal resolutions.

While the role of currents in this regard is presently being studied in the world oceans, with particular focus on large scale accumulation areas in the middle of oceanic gyres (so called "garbage patches"), and with some regional applications concentrated in the South East Asian seas and the Hawaii region, investigations in the Mediterranean are still in their infancy.

The Mediterranean situation appears particularly delicate for the possible accumulation of floating plastics, since the Basin is characterized by a net inflow of surface waters of Atlantic origin through the Strait of Gibraltar, with no outflow possibility for items less dense than seawater anywhere. In addition to this, floating item inflow through the Suez Canal may not be overlooked, in particular for the possibility of litter representing a vector for invasive species.

5.4 Biodegradability of plastics

Persistence is a key characteristic of plastics at sea. Improving our knowledge of the ecology of microbial life on the 'plastisphere' is of great importance to better understand (i) the potential risk of pathogen dispersion with plastic transport all around the Mediterranean Sea, (ii) the fate of toxic molecules attached to plastics that can be degraded by microorganisms, and (iii) the potential for microbial degradation of synthetic plastics. With respect to the last point, it is important to not only consider the metabolic pathways involved in plastic degradation but the entire biodegradation process (attachment and biofilm formation, bio-deterioration, bio-fragmentation, bio-assimilation and bio-mineralisation). Classical bioremediation strategies (biostimulation, bioaugmentation) with or without pre-treatments of the synthetic polymers (mechanical, light, thermal, chemical) should also be evaluated to find ways of improving plastic biodegradation.

We need a better understanding of degradation of litter in the marine environment and this should include consideration of "biodegradable" materials with enhanced degradation properties as there is concern they may break down into non degradable fragments.

To date, the few data available on the evaluation of synthetic plastics biodegradation are related to the use of pure culturable strains. Studies applying enzymes for this purpose are very scarce. First, it would be interesting to know the size of the molecules (number of carbon units) that microbial ectoenzymes can bind and better characterize their ability to transform polymers into oligomer or monomer more easily biodegradable. Standardized tests for the demonstration of bio-assimilation and further bio-mineralisation are also needed, not only for micro- but also nano-plastics.

Moreover, most of the knowledge on plastic biodegradation processes used culture-based approaches which consider less than 0.1% of the total bacterial diversity. Future studies, should take advantage of the so-called "-omics" approaches (metagenomics, metatranscriptomics, metaproteomics, metabolomics, etc.) that consider the entire microbial community as a whole. Complementary labelling with stable-isotope (DNA-stable isotope probing) or radioactive isotope (microautoradioactivity in combination with fluorescence in situ hybridization, MAR-FISH) should also be considered. This does not mean that culture approaches should be avoided, but rather used as complementary approaches for breaking-down general processes into metabolism pathways. Finally, the relative importance of bacteria/archaea/fungi in plastic biodegradation in the environment is still unknown.

Bacteria have the ability to degrade additives and/or also the polymer chains creating microniches that could allow them to get inside the polymer. Better knowledge of the environmental parameters driving plastic biodegradation efficiency may further support biostimulation strategies that aim to improve plastic biodegradation. Several solutions may be taken into consideration such as (i) improving the accessibility of hydrophobic plastic surfaces by adding surfactants, (ii) removing nutrient limitation (bottom-up control) by the addition of fertilizers, (iii) supporting biodegradation of recalcitrant components by the addition of other sources of carbon to improve co-metabolism and priming-effects, (iv) evaluating the importance of light, chemical or physical plastic pre-treatment in each steps of plastic biodegradation, as well as the positive or negative effect of other molecules attached to plastics.

Knowledge on the presence of additives to enhance plastic biodegradation processes needs more detailed and independent (academic) researches. In fact, presence of pro-oxidant additives for example (oxo-biodegradable plastics) looks very promising but lacks evidence for their biodegradability in marine waters. Oxo-biodegradable plastics are made of classical polymers such as polyethylene (PE), polypropylene (PP) and polystyrene (PS) containing additives that facilitate (i) the abiotic fragmentation of the plastic and (ii) its oxidation to reach a sufficient oxidation level for further biodegradation. The abiotic phase of oxo-biodegradation can be as short as few months

depending on the temperature, UV light and other mechanic stresses (wind, waves, etc.). However, the time for their complete bio-assimilation is unknown and the toxicity of the additives and intermediate compounds remains to be tested. The same questions subsist with hydro-biodegradable (vegetable-based) plastics that were tested to biodegrade in the special conditions found in industry composting but not under marine conditions.

5.5 Understanding the interactions between species and plastic

The formation of biofilm significantly alters the surface properties of plastic items. The electric charge of the biofilm plays an important role in its interaction with dissolved pollutants and the formation of anaerobic and aerobic zones is important for the transport and fate of many pollutants. However, the development of biofilms on plastic surfaces in the marine environment is not extensively studied in relation to their interaction with pollutants, and properties such as behaviour, sorption/desorption and ability to degrade organic pollutants must be better understood.

Biofilm formation depends on orientation to light (and UV), temperature and roughness of plastic surface (amongst other factors). Its development can take a few days, and then a variety of protists, algae and animals can colonise, depending mostly on which species are nearby or have dispersal stages in the water at that particular time. Usually, the first animals colonizing plastic surfaces are suspension feeders (foraminifera, polychaetes, bryozoans, hydroids and barnacles). Mobile scavengers and predators, such as peracarid crustaceans and crabs, gradually join and ultimately there can be a wide variety of other animals, largely depending on chance meetings. The plastic may be entirely covered in just a few months. Most if not all of the colonisers grow to adult and, under proper conditions, can reproduce – so the raft becomes a source of larvae (e.g. which may colonise other nearby plastics). This can drastically change the directions, spread and chance of success for aliens to spread and establish. However, these processes have never been studied in the Mediterranean. Although there are many studies on the colonization of fixed plastic panels, the colonization process of floating marine litter and the relevant ecological succession needs further research, as it is inherently different when compared to fixed submersed plastic panels (interaction with the atmosphere, effects of weather conditions, direct sunlight, etc.).

5.6 Risk assessment

Risk assessment of plastics can obviously not be performed using classical approaches developed for chemical contaminants. Such risk assessment has not yet been developed for plastics at sea, which would involve the definition of "predicted no effect concentrations (PNEC)" and "predicted environmental concentrations (PEC)" for various litter types, which is not practical. However, to date, no alternative approaches exist for risk assessment of plastics at sea. No available thresholds for harm have been given for plastic components such as additives (BPA, Phtalates). The development of models to predict the degradation and subsequent harm (release of contaminants, toxicity) will be an important step in this respect. Also the evaluation of spatial extension of litter (mapping), subsequent harm, release of contaminants and toxicity, the distribution of harm targeted species (atlas of sensitivity) and the possible extension of related species may support a better understanding of how litter impacts the Mediterranean Sea. From a management point of view, this approach will allow to better determine the sources and support reduction measures.

5.7 Harm to biodiversity

As marine litter affects different ecological compartments, the study of its impact on marine biota of all trophic levels on the same temporal and spatial scale is of increasing importance. With regard to biodiversity, it is essential to focus research on ingestion by turtles, marine mammals, seabirds, invertebrates and fish. There is substantial ingestion by epipelagic and mesopelagic fish, thus bioaccumulation and transfer through the food web need to be investigated. Moreover, the existence of a possible 'biological pump' enhancing particle transport from surface to deeper waters through ingestion at the surface and faecal pellets released at deeper depths through nictameral migration deserve investigation. Protocols have also to be developed to assess early warning effects on key species and key habitats (Deudero and Alomar, this volume).

Further the identification of interactions between litter and fauna strongly depends on data collection methods. For example, most data on fish, turtles and cetaceans are provided by stomach contents analyses, stranded individuals or bycatches, reflecting only a small snapshot of actual

interactions. The effect of marine litter on marine populations is difficult to quantify as unknown numbers of marine animals die at sea because of entanglement or ingestion of litter, and may quickly sink or be consumed by predators, eliminating them from potential detection. New methods for the unbiased estimation of mortality rates and the effects on the population dynamics of many affected species are urgently needed. Combined studies including telemetry, dynamics of currents, biological traits, migration patterns, species spatial distribution have to be integrated in a holistic approach to tackle marine litter effects.

5.8 Harm to indicator species

Sentinel organisms need to be selected for the monitoring of content (including detection of phthalate concentrations and POPs) and effects (biomarker responses) of marine litter (in particular plastic) in different ecological compartments (water column, sea bottom, coastal shore) and with different sized biotopes (wide-, medium and spot). Several sentinel species can be proposed as bioindicators for marine litter (macro- and microplastic) and for the implementation of both the UNEP/MAP Regional Action Plan and the EU Marine Strategy Framework Directive:

Large filtrating marine organisms, such as baleen whales and sharks, which ingest microplastics by filter feeding, can be selected as wide-scale indicators for the whole Mediterranean pelagic environment. The fin whale (*Balaenoptera physalus*), the second largest filter feeder in the world, primarily feeds on planktonic *euphausiid* species. The fin whale, the only resident mysticete in the Mediterranean Sea, forms aggregations on feeding grounds. With each mouthful, the whales trap approximately 70,000 L of water, and they also feed at the surface. The basking shark (*Cetorhinus maximus*) is a large filter-feeding pelagic species. Both species could face risks caused by the ingestion and degradation of microplastics. Monitoring activities on these species can be implemented through the detection of plastic additives (e.g. phthalates) and PBT (OCs, PAHs, PBDEs) in tissues from stranded animals and from skin biopsies from live individuals, including biomarkers for the latter.

Several epipelagic fishes (*Trachurus* spp. *Naucrates ductor, Seriola dumerilii, Coryphaena hippurus*) have exhibited plastic ingestion in the Western Mediterranean (cited in Deudero and Alomar, this volume). Medium pelagic fishes such as *Boops boops*, *Sardina* spp may be good indicators of presence of microplastics in the environment if sedentary. Already, methodological achievements may include development of sorting and quantification techniques of plastic items in stomach contents altogether with new derived indices. Biomarkers of oxidative stress might be applied to test for species responses to plastic and contaminants ingestion.

The loggerhead sea turtle (*Caretta caretta*), which is known to feed on macro-plastic, can be proposed as large-scale indicator of plastic presence and impact in the Mediterranean sub-basins. In the worst case, it may lead to death by entanglement or by occlusion of the gastro-intestinal tract. Their monitoring can be implemented through two steps: 1) detection of macro- and micro-plastic in stomach contents, detection of plastic additives (e.g. phthalates) and PBT (OCs, PAHs, PBDEs), in Mediterranean loggerhead turtles stranded along the Mediterranean coast; 2) blood and skin biopsies samples from loggerhead turtles, collected in several rescue centers located around the Mediterranean. For the evaluation of impact and effects, the analysis of the levels of contamination (phthalates and POPs) as well as of the responses of a set of biomarkers (e.g. Vitellogenin, Zona Radiata Proteins, Estrogen Receptors, Aromatase, porphyrins in feces) are recommended.

Finally, spot-scale bioindicators of micro-plastics in Mediterranean Sea bottom (*Mullus barbatus, Solea* sp.) and coastal shores *(Mytilus galloprovincialis, Arenicola marina)* need further exploration of ingestion rates, including field studies, in order to provide quantitative data on plastic availability in coastal sandy and seagrass bottoms and a better understanding of harm.

5.9 Harm: physical stress

If larger litter items bear the risk of entanglement for many marine organisms, while smaller particles may be ingested and induce physical stress.

Except in the case of occlusions (sea turtles, mammals, etc.) or storage by some species (procellariforms), excretion of ingested indigestible particles with feces is very common for most species. Nevertheless, a number of harmful effects of ingested litter have been reported; the most

serious effects are the blockage of the digestive tract and internal injuries by sharp objects, which may be a cause of mortality (Katsanevakis, 2008).

Sub-lethal effects caused by marine litter ingestion may greatly affect populations on longer time-scales. One potential sub-lethal effect is diminished feeding stimulus and nutrient dilution, i.e. reduced nutrient gains from diets diluted by consumption of debris. This may have serious implications on the population level, because of possible reduced growth rates, longer developmental periods at sizes most vulnerable to predation, reduced reproductive output, and decreased survivorship (McCauley and Bjorndal, 1999). Such sub-lethal effects of marine litter and their impacts on the population level need to be further investigated.

5.10 Harm: bioaccumulation and toxicity

In conjunction with plastic ingestion by organisms often comes the question of whether transfer or enhanced bioaccumulation of persistent organic pollutants (POPs) may occur as a consequence of the high sorption capacity of many plastics for lipophilic compounds (Rochman *et al.*, 2013). Using a model, Koelmans *et al.* (2013) showed that for the lugworm *A. marina* ingestion of plastics will lead to decreasing bioaccumulation due to 'dilution' of the sediment contamination and 'cleaning' mechanisms that outweigh the carrier effect by ingestion of contaminated microplastics.

This may be different for plastic additives (PAs) that are added in various quantities to polymers to modify their properties. This comprises pigments and dyestuffs as colorants, fillers and reinforcements to modify mechanical properties, antioxidants, UV stabilizers and flame retardants to provide resistance against heat, aging, light or flames, and anti-static/conductive additives, plasticisers, blowing agents, lubricants, mould release agents, surfactants or preservatives to improve the performance of the polymer (Gächter and Müller, 1993). It has been qualitatively shown that these additives can leach out of the matrix over time and exert toxic and endocrine disruptive effects on marine organisms when plastics are ingested (Oehlmann *et al.*, 2009). It is therefore essential to collect information on the nature and quantity of additives in microplastics and on their ability to leach out in the organisms' gut. As a first step, a method has been developed for the identification of additives in plastic particles extracted from natural sediment samples (Fries *et al.*, 2013). However, methods to determine the amount of additives potentially released from ingested plastics during the gut passage are not yet available.

The next steps would presumably be to identify potentially vulnerable species at different trophic levels and to rank POPs and additives according to their potential for enhanced bioaccumulation with plastic particles as transport vector. This information can then be overlaid to trigger targeted investigations such as analysis of high ranked chemicals in specific organisms. All in all, the question as to how far ingestion of microplastics by marine organisms constitutes a severe risk factor for individuals, populations or whole ecosystems cannot yet be fully answered at this time and requires further research.

One major toxicological aspect of plastic litter in the marine environment and, consequentially, on marine organisms, is enhancing the transport, accumulation, and bioavailability of Persistent, Bioaccumulative and Toxic (PBT) substances, in addition to toxic chemicals that have been added, during the production procedure, to enhance the performance of the plastic (such as phthalates, nonylphenol, bisphenol A, brominated flame retardants).

The direct and indirect ecotoxicological effects of micro- and macro-plastics exposure in marine organisms need to be investigated in depth, with a particular focus on:

a) Indirect toxicological effects. Plastic debris may be a sink for toxic chemicals from the environment as they can sorb to the debris and be released once inside the organism (Engler, 2012; Lithner *et al.*, 2011). Since PBT chemicals, generally, have low solubility in marine water, they tend to migrate into water microlayers where they may be biomagnified. PCBs and DDE sorb to debris with a partition coefficient, Kd, of approximately 100,000-1,000,000 over seawater. Similarly, phenanthrene, a PAH, partitions to plastic debris 13,000-fold over seawater (Engler, 2012). Most of these chemicals can potentially affect organisms (Teuten *et al.*, 2007) having endocrine disruptors potency and affect population viability.

b) Direct toxicological effects. These include mechanical/particulate problems and leaching of toxins. For instance, phthalates are a class of chemicals commonly used to make soften rigid plastics to enhance the use of some plastic polymers. Phthalates generally do not persist in the environment, but may leach from plastic debris on a fairly steady basis. Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment; DEHP, in both invertebrates and vertebrates, is rapidly metabolized in its primary metabolite, MEHP (mono-(2-ethylhexyl) phthalate) (Barron *et al.*, 1989), that can be used as marker of exposure to DEHP.

5.11 Harm: new habitats

In a manipulative field experiment on shallow soft substrata, Katsanevakis *et al.* (2007) found a marked gradual change in the community structure because of marine litter. They found that litter caused a clear successional pattern of change in the megafauna community composition, the establishment of new relationships in the modified communities with intraspecific and interspecific competition for hard substrates and shelter and new predator-prey interactions. Litter on the seafloor of soft bottoms may stimulate the invasion of many hard-substratum (native or alien) species. Indigenous soft bottom species might be displaced by invading species and the extent of such an impact is yet unpredictable but one may reasonably fear that many populations of soft-bottom species may be greatly affected and could even be driven to local extinction. Especially in the deep sea, litter may provide a unique substrate for colonisation. Further research is needed to evaluate the impact of native and alien invaders that colonize marine litter on soft bottoms, and assess the role of litter as stepping stones for invasions through unsuitable habitats.

5.12 Litter as vector for alien invasions?

The Mediterranean Sea is a receiver rather than a source of species – with marine aliens arriving by various vectors through Gibraltar and the Suez Canal (major pathways). Plastic litter provides more opportunities - in number, surface area, and diversity of surface characteristics-, a slower transition (less heat shock) and a greater variety of locations (both geographic and bathymetric) than other vectors (e.g., ships largely travel port to port). Thus, floating plastics can rapidly disperse a primary invasion to very many secondary spots. This can drastically increase the chances of a potential invader finding somewhere suitable substrates for initial establishment (in which case plastic becomes part of the primary pathway). Also spreading an alien to multiple locations drastically decreases (virtually to zero) any option for trying to contain or remove it and will rapidly decrease the time needed by the invader to significantly impact – e.g., fisheries, aquaculture, tourism, water treatment, etc. Mesoscale oceanographic models should provide reasonable probabilities, timelines and directionality of spread if validated.

The extent to which floating marine litter may contribute to the introduction of exotic species has been questioned. While ships create novel pathways, move across currents and often visit many locations over short periods of time, transport due to rafting on plastics occurs with speeds of the order of a few cm/s or tens of cm/s, i.e. of fractions of knots. This makes adaptation to different temperatures and salinities much more gradual, thus probably increasing the possibilities of survival in new environments, in comparison to ship-based transfer. Furthermore, the availability of floating litter, mostly plastics, has become huge, offering substantial rafting opportunities for encrusting fauna and flora, especially in areas where only a few natural sources of flotsam do occur. Plastics provide both new and expanding habitats depending on the species and environment. On the sea surface there is a wide variety of natural flotsam from algae (kelp rafts), plants (tree trunks, see pods) and animals (floating mollusc shells), but these have a smaller size range, are less abundant and ubiquitous, have a smaller surface area, sink more easily, and are typically harder to attach to. For such reasons some species have never been recorded in natural flotsam but do occur on plastics. Plastics can sink into soft sediment seabeds or beaches, providing the only hard surfaces there – so can act as stepping stones to allow species to jump over natural barriers; again allowing species to invade new regions.

Many questions remain open and need to be further studied: What is the increase in the probability of species translocation due to floating litter? Which species in the Mediterranean preferentially settle on marine litter rather than on natural flotsam? What are the constraints on the colonization of floating plastic? Which Mediterranean alien or native species colonize floating litter? Which Red

Sea species enter the Mediterranean via floating litter; what is the probability of their establishment and which are the relevant constraints?

Box: The *Rosalina* case

Among the rich fauna found on floating plastics sampled in the north western Mediterranean Sea, substantial specimens of a single species of benthic foraminifer, *Rosalina concinna,* were found (Jorissen, this volume). The occurrence of this monospecific foraminiferal assemblage is highly surprising in view of the large biodiversity of epiphytic benthic foraminifera found on Mediterranean algae and sea grass. The explanation is probably that *Rosalina* is one of the very rare foraminiferal taxa with a planktonic (*Tretomphalus*) stage. In fact, the species is characterized by an irregular alternation of sexual and asexual generations, with the sexual generation producing large floating chambers before the release of gametes in the surface waters. Laboratory experiments suggest that sexual reproduction (and construction of floating chambers) only occurs at elevated temperatures, above 18°C. This means that in the western Mediterranean, colonization of floating plastics by *R. concinna* is only possible part of the year.

In the Mediterranean, *Rosalina* is a common constituent of epithytic foraminiferal assemblages. In laboratory experiments, the closely related species *R. bradyi* stands out by its highly opportunistic behavior, surviving adverse conditions and attaining very high densities. Until recently, the planktonic *Tretomphalus* was never observed in the western Mediterranean, which contrasts with its common occurrence in the Adriatic Sea and eastern Mediterranean. Observations of this planktonic life stage in sediment samples from the western Mediterranean sampled in 2006 (Milker and Schmiedl, 2012), and on floating plastics (Katsanevakis and Crocetta, this volume), suggest that sexual reproduction is a recent phenomenon, maybe related to climate warming.

On the floating plastics we studied, *R. concinna* attained a density of about 20 individuals per 100 cm^2, comparable to its density on natural substrates. Its ability to colonize floating plastics leads to a significant extension of the available niches, which could substantially modify the dispersal efficiency of this highly opportunistic taxon.

6. Future directions for research and action

Both the implementation of the management schemes and improvement of knowledge on marine litter are long term processes. Research and monitoring have become critical for the Mediterranean Sea where not much information is available. To support this endeavour, our working group reviewed and discussed several options, and retained the following priorities that may be considered for short term projects:

- Repeatability, optimisation, robustness and reliability of methods require further research so as to develop large scale measurements and rapid interpretation of litter data. Further attention will be required to standardize and / harmonize methods.

- Increase coverage of survey sites, further development of data analysis in all regions. This may enable to map hot spots (including river plumes).

- Large assessment of species (also biofilms) settled on litter in the Med, including the development of standardised protocols. Development of a database on rafted species to better explain the risk of dispersion, the influence of climate change, the travel of lessepsian species through the Suez Canal and the possible colonisation of new deep sea areas.

- Microplastics in sediments: evaluate the distribution and changes of microplastics, from beaches to the seafloor/ deep seafloor. Quantify ingested microplastics in key species, from coastal epipelagic to demersal species.

- New indicator species, through laboratory and field evaluation, and definition of thresholds for harm.

- Education of public: tourists, fishermen, people from countries where the issue of litter has become critical.

- Understand interactions of nanoplastics and marine fauna and how these are affected by local conditions. This will need the development of original methods to identify micro/nano particles/fibers.

- Better understand the ecology of microorganisms living on/with litter, their role in the degradation of microplastics, identification of species involved and populations/assemblages in coastal waters, and finally develop strategies, methods and standards.

WORKSHOP COMMUNICATIONS

Distribution, composition and abundance of marine litter in the Mediterranean and Black Seas

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ABSTRACT

Anthropogenic litter on the ocean surface, beaches and seafloor has significantly increased in recent decades and both Mediterranean and Black Seas have been described as some of the most affected areas in the world.

Plastic, mainly bags and fishing equipment, is the largest part of debris at sea. Cleaning and regular surveys at sea are now providing information about temporal and spatial distribution that is related to the presence of large cities, large rivers and shore uses. Hydrodynamics, maritime activities and geomorphology of the sea floor are finally the main drivers affecting the distribution of litter at sea. On beaches, studies have demonstrated densities in the 1/m² range. Polymers can be then physically degraded into smaller fragments, the so called microplastics. All existing surveys on the surface, worldwide, have found average densities lower than in the NW Mediterranean Sea (115,000/km²). Microplastics are also found on beaches and sediments, including deep sea, reaching concentrations of 1,000 pellets/m² of beaches on the island of Malta.

Most of the studies on the deep sea floor were in the Mediterranean Sea where shelves are not extended. Recent studies concluded that coastal submarine canyons act as conduits for the transport of marine debris into the deep sea areas where densities were evaluated from 295 samples at an average density of 179 plastics/km².

The balance between the increase of waste and plastic productions, reduction measures and the quantities found at the surface and on shorelines is still not answered. Before a correct global estimation of debris is provided in the Mediterranean and Black Seas, basic information is still needed on sources, inputs, degradation processes and fluxes and they will need harmonized investigations and standardized approaches.

Keywords: Mediterranean Sea, marine litter, distribution, trends, beaches, sea floor, microplastics, floating litter

Anthropogenic litter on the ocean surface, beaches and sea floor has significantly increased in recent decades. Initially described in the marine environment in the 1960s, marine litter is now commonly observed across all oceans and the Mediterranean Sea recognized as one of the most affected areas in the world. Human activities generate considerable amounts of waste, in increasing quantities, although these vary between countries. Some of the largest amounts of Municipal Solid Waste (MSW) are generated annually per person in the Mediterranean Sea (208 – 760 kg/year, http://www.atlas.d-waste.com/). Plastic, which is the main litter component, has now become ubiquitous and accounts for up to 95% of the waste accumulated on shorelines, the ocean surface or sea floor.

In the Mediterranean Sea, plastic bags, fishing equipment and food & beverage packaging are the most commonly-found items, accounting for over 80% of litter stranded on beaches (Topcu *et al.*, 2012). A majority of these materials do not decompose, or decompose slowly. This phenomenon can also been observed on the sea floor, where 90% of litter caught in benthic trawls is plastic. (Galil *et al.*, 1995; Galgani *et al.*, 1995; 2000). This figure can reach up to 100% on the sea surface.

Strandline surveys, cleaning and regular surveys at sea, are in place in many Mediterranean countries with the aim of providing information on temporal and spatial distribution. The surveys conducted to date show considerable spatial variability. Accumulation rates vary widely and are influenced by many factors, such as the presence of large cities, shore use, hydrodynamics and maritime activities. They are higher in enclosed seas such as the Mediterranean or Black Sea. These seas are home to the highest densities of marine litter stranded on the sea floor, sometimes reaching over 100,000 items / km² (Galgani *et al.*, 2000). The issue of plastic fragments in surface waters has been exacerbated in recent decades and the quantities of microparticles in European seas have continued to increase (Thompson *et al.*, 2004) since the first reports published in 1972 (Wong *et al.*, 1974). However, recent data suggest that microparticle quantities have stabilized in the North Atlantic Ocean. Debris densities on the deep sea floor decreased between 1996 and 2003 in some areas, such as in the Bay of Tokyo; a decrease was also recorded between 1994 and 2009 in the Gulf of Lions (Kuriyama *et al.*, 2003; Galgani *et al.*, 2011). Conversely, in some areas around Greece, the abundance of debris in deep waters increased over an 8-year observation period (Koutsodendris *et al.*, 2008).

Individual litter items can, in most cases, be linked back to their sources and it is therefore most important to analyse marine litter composition.
Some items can be attributed, with a high level of confidence, to sources such as fishing activities, sewage-related debris and tourist litter. These so-called “use-categories” provide valuable information for setting reduction measures (Galgani *et al.*, 2011).

Marine litter is mainly transported to the sea or coasts by rivers, industrial discharges and runoffs, or can be blown by local winds. Land-based sources also include recreational use, public litter, industry, harbours and unprotected landfills, together with sewage overflows, accidental losses and extreme events. Ocean-based sources of marine litter include shipping, ferries, fishing vessels, pleasure boats and offshore installations, including aquaculture sites. Factors such as ocean current circulation, proximity to urban, industrial and recreational areas, shipping lanes and fishing grounds also influence the types and amounts of litter found in the open sea or collected along beaches.

Densities may be enhanced by up to 40 % in summer months due to high tourist influxes and over 75 % of overall annual waste production can be generated in the summer season (Galgani *et al.*, 2011). In the Mediterranean, reports from Greece (Koutsodendris *et al.*, 2008) classify land-based sources (69% of litter) and vessel-based sources (26%) as the two predominant litter sources. In addition, litter items have variable floatability and hence variable dispersal potential.

Marine litter stranded on beaches is found throughout the Mediterranean Sea and has become a permanent concern. Various strategies based on the measurement of quantities or fluxes have been adopted for data collection purposes. Most of these surveys are conducted by NGOs with a focus on cleaning. Standing stock evaluations of beach litter reflect the long-term balance between inputs, land-based sources or stranding, and outputs from export, burial, degradation and cleanups. Recording the rate at which litter accumulates on beaches through regular surveys is currently the most commonly-used approach for assessing long-term accumulation patterns and cycles.

A good correlation appears to exist between accumulated litter and arriving amounts, suggesting regular inputs and processes. Litter is often stranded on beaches devoid of strong currents or prevalent winds to flush them offshore (Costa *et al.*, 2011). For topographic reasons, patchiness is a common distribution scheme on beaches, especially for smaller and lighter items that are more easily dispersed or buried. This is also true for microparticles in sand (Turra *et al.*, 2014). Hard plastics also appear to accumulate more easily on rocky shores (Moore *et al.*, 2001).

The majority of studies performed to date have demonstrated densities in the order of one item/m², together with very high concentrations due to local conditions or flooding (5,058 items/m², Topçu *et al.*, 2013). Plastic accounts for a large proportion of the litter found on beaches in many areas, reaching up to 91 % in the southern Black Sea (Topçu *et al.*, 2013), although other specific types of plastic are widely-found in certain areas, according to type (Styrofoam, crafted wood) or use (fishing gear).

Small fragments measuring less than 2.5 cm (Galgani *et al.*, 2011), also referred to as meso particles or meso debris (versus macrodebris), are often buried and may not be targeted by cleanup campaigns. Stranding fluxes are therefore difficult to assess. Small items can comprise a large proportion of the debris found on beaches and very high densities have been found in some areas. These items, together with floating debris, can pose a direct threat to wildlife. As an example, up to 75% of the total debris recorded on beaches in the southern Black Sea measured less than 10 cm (Topçu *et al.*, 2013).

Floating debris comprises the mobile fraction of debris in the marine environment as it is less dense than seawater. However, the buoyancy and density of plastics may change during their stay in the sea due to weathering and biofouling (Barnes *et al.,* 2009; Barnes, this volume; Claessens *et al.,* 2011; Collignon *et al.,* 2012; Colton *et al.,* 1974; Law *et al.,* 2010).

Polymers comprise the majority of floating marine debris, with figures reaching up to 100%. Although polymers are resistant to biological or chemical degradation processes, they can be physically degraded into smaller fragments and hence turn into microlitter, defined as measuring less than 5 mm. They can also be transported by currents until they sink to the sea floor, are deposited on the shore or degrade over time. Although anthropogenic debris floating in the world ocean was reported decades ago, the existence of Floating Marine Debris accumulation zones in oceanic gyres has now gained worldwide attention. However, there are no permanent gyres in the Mediterranean Sea and local drivers may largely affect litter distribution (Zambianchi *et al.,* this volume).

Visual assessment approaches include the use of research vessels, marine mammal surveys, commercial shipping carriers and dedicated litter observations. Aerial surveys are now being employed for larger items. Although the basic principle of floating debris monitoring through visual observation is very simple, as for beaches, few datasets are available for the comparable assessment of debris abundance (see Table 1). The areas covered are extremely limited and monitoring is only performed occasionally.

Table 1. Comparison of mean litter densities from recent data (from 2000) in the Mediterranean Sea. Intervals of values are given in parentheses.

Location	Habitat	Date	Sampling	Depth	Density (min-max)	% plastics	References
Slovenia	Beaches	2007	3 beaches, 150 m-2 per transect	0	12158/km	64	Palatinus, 2009
Balearic	Beaches	2005	32 beaches	0	36000 /km (high season)	75 (46% cigarette butts)	Martinez *et al.*, 2009
France/Marseille	Beaches	2011-2012	10 beaches (30 in winter)	0	0,076 m-3/day/100m (stranding rates)	80-94	MerTerre 2013 - (www.mer-terre.org)
Turkey	Beaches	2008-2009	10 beaches	0	0,085 to 5,058 items m²	91	Topçu *et al.*, 2013
Croatia (Mjet island)	Beaches	2007	NA	0	NA	80	Cukrov and Kwokal, 2010
Mediterranean Sea (15 countries)	Beaches	2002-2006	Beaches	0	NA	>60	ICC, *in* UNEP, 2011
Kerch Strait/Black Sea	Floating	Before 2008	Visual	Aerial	66 /km²	nd	BSC, 2007
Ligurian coast	Floating	1997-2000	Visual	Surface	1.5-25 /km²	nd	Aliani and Molcart, 2011
North western	Floating	2013	Waveglider	0-4,5m	40,5 /km²	100	Galgani *et al.*, 2013a
Slovenia	Floating	2011	Visual	Surface	1.98 /km²	90	Vlachogianni and Kalampokis, 2014
Adriatic/Greek waters	Floating	Since 2008	Visual	Surface	5.66 /km²		Vlachogianni and Kalampokis, 2014
Aegean/Levantine	Floating	Since 2008	Visual (172.8 km²)	Surface	2.1 km²	83	UNEP, 2011
NW Mediterranean	Floating /Microplastics	2010	40 samples/Manta/330µm mesh	Surface	115000 /km²	> 90%	Collignon *et al.*, 2012
West Sardinia	Floating /Microplastics	2012	30 samples/Manta/500µm mesh	Surface	150 000 items /km²		Andrea /Lucia *et al.*, 2014
North western	Floating	2006-2008	Visual	Surface	3,13 /km²	85	Gerigny *et al.*, 2012 and unpublished data (www.Ecoocean.org)
Greece	Floating		Visual	Surface	2.1 items /km²	83	HELMEPA (Greece) *in* UNEP, 2011
Malta	Shelf	2005	Trawl (44 hauls, 20mm mesh)	50-700	102	47	Misfud *et al.*, 2013
Sicily/Tunisian channel	Shelf	1995	Trawl (fishermen)	0-200m	401 /km²	75	Cannizarro *et al.*, 1995
Adriatic Sea	Shelf	1997	12 hauls (trawling, 20mm mesh)	0-200m	378 +/- 251 /km²	69,5	Galgani *et al.*, 2000
Northern & central Adriatic	Shelf	2005-2010	Trawl trawling	0-200m	5-34 kg/ km²	NA	From Vlachogianni and Kalampokis, 2014
Montenegro	Shelf/slopes	2009	Trawling	48-746m	6-59% of total catches	NA	Petrovic and Marcovic, 2013
Slovenia	Shallow waters	2013	Diving	0-25m	Na	55	From Vlachogianni and Kalampokis, 2014
France - Mediterranean	Seabed, slopes	2009	17 canyons, 101 ROV dives	80-700m	3.01 /km survey (0-12)	12 (0-100)	Fabri *et al.*, 2013
Tyrrhenian Sea	Seabed, fishing grounds	2009	6 x 1.5 ha samples, trawl, 10mm mesh	40-80m	5960±3023 /km²	76	Sanchez *et al.*, 2013
Spain-Mediterranean	Seabed, fishing grounds	2009	Trawling (fishermen)	40-80m	4424±3743 /km²	NA	Sanchez *et al.*, 2013
Mediterranean Sea	Seabed, bathyal/abyssal	2007-2010	292 tows, Otter/agasiz trawl, 12mm mesh	900-3000m	0.02- 3264.6 kg/km² (including clinkers)	nd	Eva-Ramirez, 2013
Turkey/Levantine basin	Seabed, bottom/bathyal	2012	32 hauls (trawl, 24mm mesh)	200-800m	290 litter (3264.6 kg) /km²	81.1	Güven *et al.*, 2013
Turkey/North eastern basin	Shelf	2010-2012	132 hauls (2.5kts)	20-180	72(1-585 kg)/ hour	73	Eryasar *et al.*, 2014
Mediterranean, Southern France	Shelves & canyons	1994-2009 (16 years study)	90 sites (trawls, 0.045 km²/tow)	0-800m	76-146/ km² (0-2540)	29.5 -74	Galgani *et al.*, 2000 and unpublished data
Greece	Shelf	Before 2004	59 sites	30-200	4900 /km²	55.5	Katsanevakis and Katsarou, 2004
Greece	Shelf	2000-2003	54 hauls (trawl, 1,5mm mesh)	30-200	72-437 /km²	55,9	Koutsodendris *et al.*, 2008

The reported quantities of floating marine debris items measuring over 2 cm range from 0 to over 600 per square kilometre. Floating debris was quantified during marine mammal observation cruises (Figure 1) in the northern Mediterranean Sea, in a 100 x 200 km offshore area between Marseille and Nice and in the Corsican channel. A maximum density of 55 items/km² was found, with a clearly-discernible spatial variability relating to residual circulation and a Liguro-Provencal current vein routing debris to the West (Gerigny *et al.*, 2011).

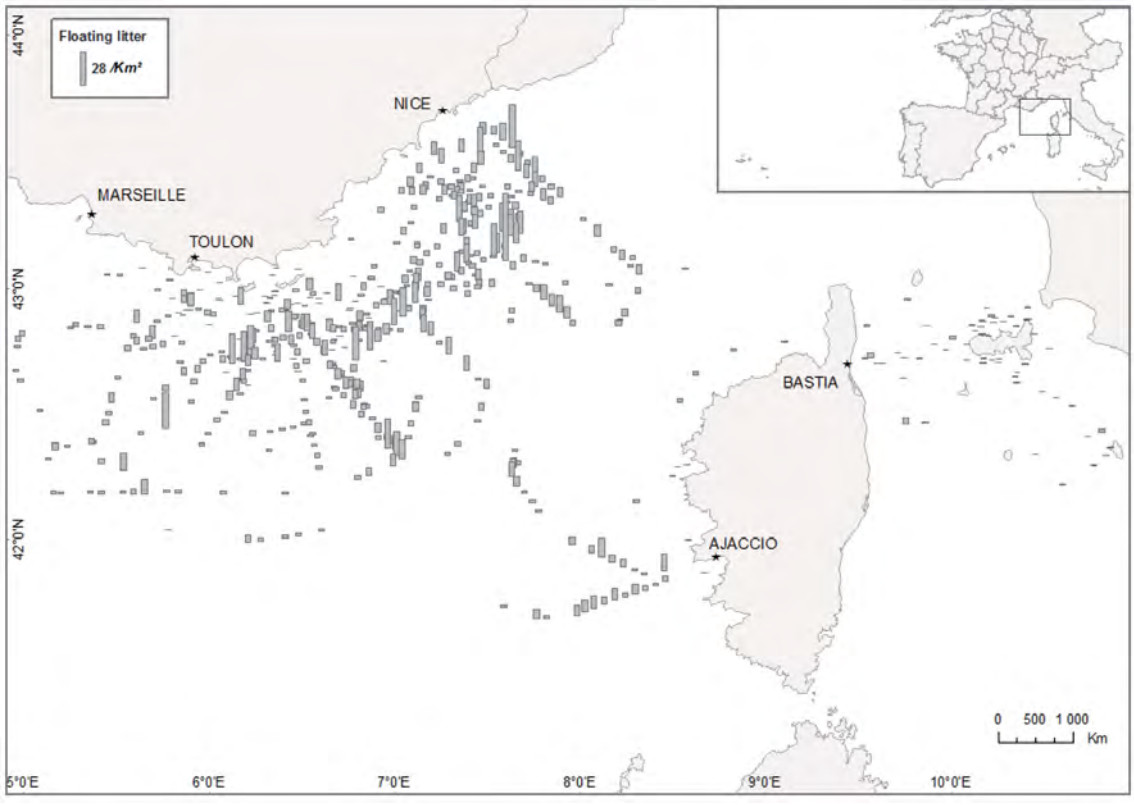

Figure 1. Distribution of floating litter in the northwestern Mediterranean Sea (2006-2008) (visual observations). IFREMER/SHOM map using data from the Ecocean/Participe Futur project for initial MSFD assessment (Gerigny *et al.*, 2011).

In the Ligurian Sea, data were collected through ship-based visual observations in 1997 and 2000. 15-25 items/km² were found in 1997, with a decrease to 1.5 – 3 items in 2000 (Aliani and Molcard, 2003). Voluntary observations in the Mediterranean Sea reported litter concentrations of 2.1 items/km², with plastic materials representing 83% and higher concentrations in coastal areas (Helmepa, *in* UNEP, 2011). Finally, high debris densities were found locally such as in the Adriatic Sea or in the Algerian basin, at up to 195 items/km² (for 25 in the Mediterranean Sea, Suaria and Aliani, 2014 *in* Zambianchi *et al.*, this volume).

Visual aerial surveys have been conducted in the Black Sea, with reported concentrations of 65.7 items/km² (BSC, 2007), far higher than in any other area worldwide found to range from 0.1 – 0.8 items/km² in Japan (Shiomoto and Kameda, 2005) to 1.48 items/km² in British Columbia (Williams *et al.*, 2011). Quantities have also been shown to increase in the presence of specific drivers, e.g. in the fjords of southern Chile, where 10-50 items/km² were measured due to fish farming activities (Hinojosa *et al.*, 2011).

Modelling oceanographic currents using input scenarios based on population densities and major shipping lines can help identify pathways and accumulation areas, thus enabling source attribution and the localization of areas harbouring high litter concentrations (Maximenko *et al.*, 2012). A 30-year circulation model using various input scenarios showed the accumulation of floating debris in ocean gyres and closed seas such as the Mediterranean Sea (Lebreton *et al.,* 2012). Modelling

is also used to predict the pathways and impacts of large debris quantities introduced through natural extreme events, runoffs (e.g. the discharge located in Saida, Lebanon) and transborder transportation (Zambianchi *et al.*, this volume). Deep sea surveys are of major importance, as most litter comprises high-density materials and hence sinks. Even low-density polymers, such as polyethylene and propylene, may sink under the weight of fouling. General strategies for the investigation of seabed debris are similar to those used to assess the abundance and type of benthic species. Although floating debris, such as that found in the highly publicized ‘‘gyres” and/or convergence zones, has attracted public attention, as debris accumulating on the sea floor can potentially impact benthic habitats and organisms. 47 studies were conducted between 2000-2013, but, until recently, very few covered extensive geographic areas or considerable depths. The Mediterranean Sea is a special case, as its shelves are not extensive and its deep sea environments can be influenced by the presence of coastal canyons. The geographical distribution of plastic debris is highly impacted by hydrodynamics, geomorphology and human factors. Continental shelves are proven accumulation zones, but they often gather smaller concentrations of debris than canyons: debris is washed offshore by currents associated with offshore winds and river plumes (see Figure 2).

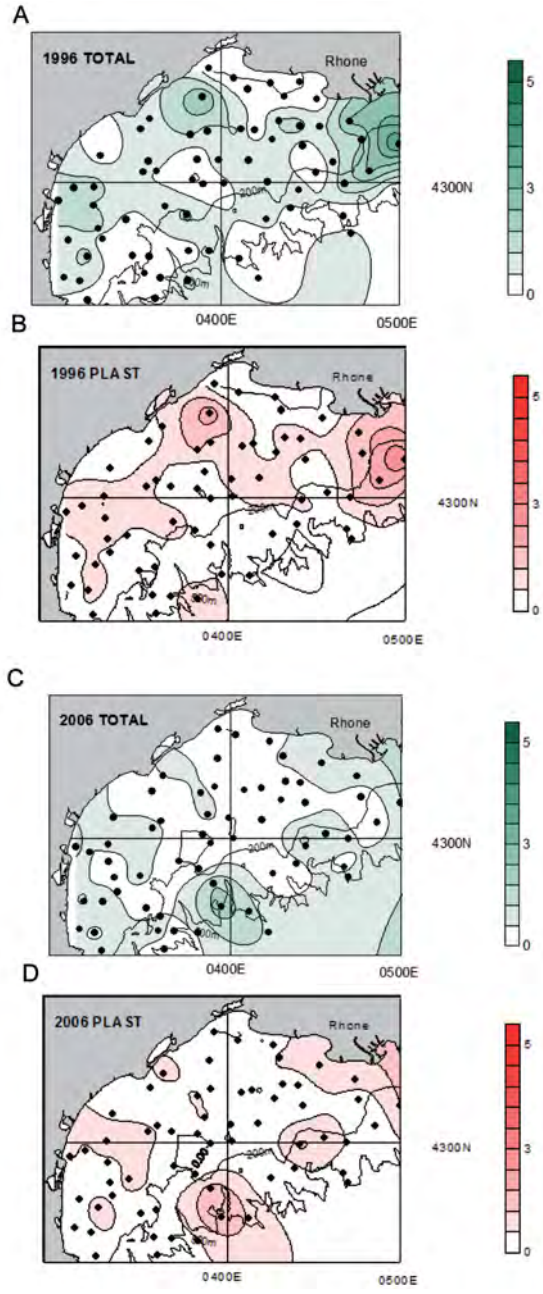

Figure 2. Total plastic debris (items/ha) on the sea bed in the Gulf of Lion, France.
A and B: 1996, total debris and plastic; C and D: 2006, total debris and plastic. Correlation between total debris and plastic items was 0.81 (n=63) in 1996 and 0.97 (n=60) in 2006. Extrapolation and mapping were performed using Surfer VII with a 50x50 grid and the Kriging algorythm.

Less than a dozen studies have focused on debris located at depths of over 500 m (Galil, 1995; Galgani *et al.*, 1996; 2000; 2004; Miyake *et al.*, 2011; Pham *et al.*, 2014; Ramirez-Llodra *et al.*, 2013; Schlining, 2013). Galgani *et al.* (2000 and Figure 2) observed decreasing trends in deep sea pollution over time off the European coast, with extremely variable distribution and debris aggregation in submarine canyons. Using a deep sea remote operated vehicle (ROV), video surveys in submarine canyons (Galgani *et al.*, 1996; Mordecai *et al.,* 2011; Watters *et al.*, 2010; Schlining *et al.*, 2013; Pham *et al.*, 2014) concluded that submarine canyons may act as a conduit for the transport of marine debris into the deep sea. Higher bottom densities are also found in particular areas, such as around rocks and wrecks, and in depressions and channels (Galgani *et al.*, 1996). In some areas, local water movements carry debris away from the coast to accumulate in high sedimentation zones. The distal deltas of rivers may also fan out into deeper waters, creating high accumulation areas.

A wide variety of human activities, such as fishing, urban development and tourism, contribute to these patterns of seabed debris distribution. Fishing debris prevails in commercial fishing zones (Watters *et al.*, 2010; Schlining *et al.*, 2013) and can constitute high percentages of total litter. More generally speaking, accumulation trends in the deep sea are of particular concern, as plastic longevity increases in deep waters and most polymers degrade slowly in areas devoid of light and with a lower oxygen content.

The abundance of plastic debris is very location-dependent, with mean values ranging from 0 to over 7,700 items per km² (Table 1). Mediterranean sites tend to show the highest densities, due to the combination of a populated coastline, coastal shipping, limited tidal flows and a closed basin, with exchanges limited to Gibraltar. In general, bottom debris tends to become trapped in areas with low circulation, where sediments accumulate.
Counts from seven surveys and 295 samples in the Mediterranean Sea and Black Sea (2,500,000 km² + 436,402 km², worldatlas.com) indicate an average density of 179 plastic items/km² for all compartments, including shelves, slopes, canyons and deep sea plains, in line with trawl data on three sites described by Pham *et al.* (2014). On the basis of these data, we can assume that over half a billion litter items are currently lying on the sea floor.

In addition to large debris, there is growing concern regarding microparticles measuring less than 5 mm and particles measuring as little as 1 m that have already been identified (Thompson *et al.*, 2004). Most, but not all microparticles consist of microplastics. The abundance and global distribution of microplastics in oceans has increased steadily in recent decades (Cole *et al.*, 2011). Microplastics comprise a very heterogeneous group, varying in size, shape, colour, chemical composition, density and other characteristics. They can be subdivided by use and source as (i) 'primary' microplastics, produced either for indirect use as precursors (nurdles or virgin resin pellets) for the production of polymer consumer products, or for direct use, such as in cosmetics, scrubs and abrasives and (ii) 'secondary' microplastics, resulting from the breakdown of larger plastic materials into increasingly small fragments. This is the result of a combination of mechanisms, including photo, biological, mechanical and chemical degradation.

To date, only a limited number of global surveys have been performed with the aim of quantifying microplastic distribution (Pedrotti *et al.*, this volume). The majority of existing surveys are localized and concentrate on specific areas around the world, such as regional seas, gyres or the poles. Most of these studies focus on sampling the sea surface and/or water column and intertidal sediments (Hidalgo-Ruz *et al.*, 2012; Klasmeier *et al.*, this volume). Mean sea surface plastic concentrations were found in concentrations up to 330,000 particles / km² in the California current system, with 334,000 particles / km² in some stations in the North Pacific and 115,000 particles / km² in the NW Mediterranean Sea (maximum 890,000 particles) (Collignon *et al.*, 2012; Moore *et al.*, 2001; Cole *et al.*, 2011). The highest microplastic concentrations in sediment (Claessens *et al.*, 2011) were found in beach and harbour sediments, with concentrations of up to 391 microplastics/kg of dry sediment in a harbour sediment sample from the southern North Sea (Belgium). Similarly, a beach survey on the Mediterranean island of Malta revealed an abundance of pellets on all of the studied beaches (Turner and Holmes, *in* Cole *et al.,* 2011), with the highest concentrations reaching 1,000 pellets/m² along the high-tide mark. Microplastic pollution has also

spread throughout the world's seas and oceans, into sediment and even deep seas (Van Cauwenberghe *et al.*, 2013).

Time trends relating to the composition and abundance of microplastics are scarce. However, available long-term trend data suggest various patterns in microplastic concentrations. A decade ago, Thompson (2004) revealed a significant increase in plastic particle abundance over time. More recent evidence indicates that microplastic concentrations in the North Pacific Subtropical Gyre have increased in the last four decades (Goldstein *et al.*, 2012), whereas no changes have been observed on the surface of the North Atlantic gyre over a 20-year period (Law *et al.*, 2010).

Conclusion

Accumulation rates vary widely in the Mediterranean Sea and are subject to factors such as adjacent urban activities, shore and coastal uses, wind and currents, leading to floating, beach and sea floor accumulation areas. Additional basic information is still required on sources, inputs, degradation processes and fluxes before a correct global debris assessment can be provided. For this purpose, and in view of the considerable variations in methodologies across regions and investigators, more valuable and comparable data could be obtained by standardizing our approaches. In terms of distribution and quantities, the overall balance between increased waste and plastic production, reduction measures and quantities found on the surface and shorelines has not been assessed to date, hence indicating the possible accumulation of large quantities, the locations of which have yet to be discovered. We clearly need to understand litter distribution better in order to accurately assess its impact.

Marine litter in the Mediterranean Sea: an oceanographic perspective

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Abstract

Floating debris is ubiquitous in the world ocean, and its presence is very widespread also in the Mediterranean Sea. The conservative nature of an important fraction of marine litter allows it to travel for distances up to the basin scale, causing local accumulation, posing a serious environmental threat, offering a rafting opportunity to marine organisms. In this paper we briefly review the main oceanographic processes responsible for marine debris transport at the surface and at depth. We also briefly review the main studies carried out on the oceanographic forcing on marine litter in the world ocean and we interpret the most recent findings on floating debris in the Mediterranean region in the light of the oceanographic knowledge of the basin.

1. Introduction

Finding objects washed ashore while walking on beaches may hold an aspect of great fascination (as noticed by Kako *et al.*, 2010, as witnessed by the captivating book by Ebbesmeyer and Scigliano, 2009, and by the popular website http://beachcombersalert.org/).

However, on a personal timescale, things have drastically changed in the last few decades: when we were children, the few glass and tile fragments smoothed by the never-ending effect of waves and currents were rare findings, much more sought after than the more common shells or wood pieces, to be then proudly displayed at our beach homes in transparent jars, sparkling with colours and with the mystery of their provenance.

At present, a stroll on the shore often represents a challenge in view of the huge quantity of stranded debris, which is typically produced far away (either on land or at sea) and that, after having travelled for hundreds, if not thousands of miles, eventually ends up on beaches and shores worldwide, posing environmental, economic, health and aesthetic problems (UNEP, 2009).

Elsewhere in this volume (Galgani, 2014; Pedrotti *et al.*, 2014; Sobral *et al.*, 2014) readers will find detailed information on origin, distribution, composition, abundance of marine litter in the Mediterranean and in (few) other regions of the world ocean. Its ubiquitous presence at sea and ashore is mainly due to the fact that after entering the marine environment, some very efficient transport mechanisms can convey floating litter considerably far from its sources, carrying it even to remote beaches and waters barely ever touched by any human being (Barnes, 2005; 2014 and this volume).

In the next pages we briefly review the main oceanographic processes responsible for marine debris transport at the surface and at depth. We then look at previous studies on the oceanographic forcing on marine litter in the world ocean and we interpret recent findings on floating debris in the Mediterranean region in the light of the oceanographic knowledge of the basin.

2. Transport processes for marine litter at sea

The study of displacement and accumulation of marine litter at the sea surface and at depth fits very well into what is generally known as environmental fluid mechanics – the investigation of processes that change concentrations of substances within a geophysical fluid environment (Socolofsky and Jirka, 2002; Cushman-Roisin *et al.*, 2008). They can be subdivided into two categories: transport and transformations.

Transport refers to those processes which move substances by physical means; as will be illustrated below, the two main physical transport modes are advection and diffusion.
Transformations change a substance into another one. Among them we mainly distinguish between physical and chemical transformations.

The classical definition of marine litter as "any persistent manufactured or processed solid material discarded, disposed of or abandoned in the marine environment" (UNEP, 2005) suggests that motion of such items can be described in terms of the motion of passive, conservative particles. Here passive means that their presence does not affect the dynamics of water masses carrying them, and conservative stands for not undergoing transformations. The latter is true in the sense that the typical time scales of marine litter transformation are much longer than those of transport: this is the case, for instance, for the breakdown of macroplastics into microplastics, see, e.g., Barnes (2009); Andrady (2011); Raddadi *et al.* (2014).

Generally speaking, the distribution of any substance (in fact, of any physical or chemical property, as well as of floating organisms and other particles) in a fluid environment can be described either in the Eulerian, i.e. at fixed point, or in the Lagrangian, i.e. following the substance parcels, context (e.g., Csanady, 1973; Kundu and Cohen, 2002). Both approaches are essentially equivalent, and the choice of the representation depends on the nature of the investigated substance and process, as well as on the type of output sought after.
The Eulerian description is based on the integration of the advection-diffusion equation, and its output is typically represented by maps of substance concentration. The Lagrangian description is based on the integration of stochastic models describing the motion of independent particles launched in the flow, and it provides trajectories. In the case of marine litter the latter approach is obviously more natural (as a semi-serious remark, we may notice that the very first Lagrangian instruments used at sea resemble one of the most ubiquitous litter items found in the ocean, i.e. bottles: results of very successful, early utilizations of drifting bottles may be found in Mazelle, 1914; a review of the subject in Penry, 2007; see also Davis, 1991; Joseph, 2013).

Lagrangian particle models describe the transport of tracer particles (or of their aggregates) carried by the flow. We can assume that the velocity field has two distinct components: a large-scale mean flow and an irregular (turbulent) smaller-scale field. The mean flow is at the basis of advection, which is the process responsible for the displacement of the bulk of substance, i.e. the center of mass of the particle cluster; while the turbulent field induces the dispersion, i.e. the spreading of particles from the center of mass (for recent discussions on critical aspects of this hypothesis see, e.g., Grooms and Majda, 2013; Majda and Grooms, 2014).
Our knowledge of the current field is generally limited to the advective flow, while turbulent effects are typically parameterized in terms of the so-called diffusivity, a coefficient representing the spreading rate of particle clusters.

The advective flow represents the main transport backbone for passive particles, and can be composed of parts that play very different roles, also in combination with the local diffusion properties: it may represent a conveyor belt, acting as the main pathway carrying particles over scales up to the basin and interbasin scales (the definition of ocean conveyor belt was first given in the seminal paper by Broecker, 1987; for applications to the Mediterranean, see Robinson *et al.*,

2001); on the other hand, advective flow may display structures acting as transport barriers or, on the opposite, flow features having the effect of blenders, convergence areas corresponding to accumulation zones, or divergence areas dominated by spreading. It is also worth adding that this portion of the flow may often show chaotic characteristics, i.e. a strong dependence on initial conditions, resulting in an irregular Lagrangian behaviour, which makes predicting advective transport a complicated job (Ottino, 1989).

At the same time, the role and the functioning of smaller scale, irregular motion, still has a number of obscure aspects, which make it difficult to conceive a general parameterization for it: as time and technology and computer power progress, the threshold between the resolved and the non-resolved velocity field is shifted towards smaller and smaller scales. However, what we call submesoscale may have very different characteristics in different areas of the ocean, and in particular may have a local or a non-local character, meaning that transport at scales smaller than a few kilometres in the ocean may be mainly determined by flow features occurring at those scales, or otherwise may be driven by the (slightly) larger scale velocity field, with obvious consequent different degrees of difficulty in finding a generally applicable parameterization (Schroeder *et al.*, 2011; 2012; Griffa *et al.*, 2013).

In addition to advection and diffusion, if we investigate the transport of litter particles floating at the very surface, there are two additional important factors to account for: the first is the direct effect of wind onto particles, called windage. It is here worth underlining that this effect does not correspond to the portion of surface flow field driven by the wind, which is already contained in the surface current, but to the very direct wind drag exerted to bodies laying at the sea surface. Windage depends quantitatively on the buoyancy ratio, i.e. on the ratio between the cross sections of floating objects normal to the wind direction above and below the sea surface; this aspect may be very relevant for marine litter. Aliani and Molcard (2003) pointed out the role of wind in sorting debris with different "free board", affecting their potential dispersal paths according to their buoyancy; Yoon *et al.* (2010) considered different possible buoyancy ratios of disposable plastic lighters in the ocean. Windage is a very important parameter in a number of further different applications, from oil spill studies to search and rescue situations; see for instance Liu *et al.* (2013); Ni *et al.* (2010) and references therein.
The second important additional effect acting at the sea surface is the Stokes' drift, which is the motion in the direction of propagation of surface waves. It is essentially due to the fact that particles subject to a surface wave field move forward at the top of their orbit faster than backward at the bottom; even if a second-order effect, its magnitude is frequently significant (Kundu and Cohen, 2002); Stokes' drift is also an important factor for oil spill prediction and search and rescue (see again Liu *et al.*, 2013, and Breivik *et al.*, 2013).

The study of advection and diffusion (plus, in case, wind and wave effects) allows to reconstruct individual debris trajectories, which may be replicated in order to mimic a number of different realizations of what is, at least in part, a stochastic process, and to infer some of its statistical properties. The deployment of large numbers of particles, advecting them with the (real or modelled) currents and diffusing them by a parameterization of processes occurring at scales smaller than the resolution of the advective field has been extensively carried out, apart from studies on marine litter (see below, section 3), in particular for studies on larvae or propagule dispersal and more generally on connectivity. Numerical circulation models coupled with Lagrangian particle-tracking algorithms following particles released at multiple locations and multiple times have proven instrumental in estimating relationships between particle distribution and physical processes and in the reconstruction of pathways of different kinds of passive floating particles in the marine environment (Cowen *et al.*, 2000; 2006).
This kind of approach is a particularly efficient tool to study transport processes over an entire basin, to examine the role played by various physical processes in the dispersion at basin scale (Cowen *et al.*, 2006; Guizien *et al.*, 2006; Serra *et al.*, 2010; Rossi *et al.*, 2014), and to simulate complex and interactive processes acting at different scales (Qiu *et al.*, 2010).

It is also possible to define integral properties of dispersion, which may allow us to characterize transport in a broader sense, without necessarily going into the details of specific drift and spreading patterns.
Transport timescales have a particular relevance in this context, and can be used to efficiently characterize the dispersion processes where the overall behaviour of the system, rather than its detailed description, is of interest. Andutta *et al.* (2014) revisited very recently the definitions of a few of them, including residence time, previously utilized in dispersion studies of idealized basins, as well as in sub-basins of the Mediterranean Sea (Buffoni *et al.*, 1997; Falco *et al.*, 2000; Bellucci *et al.*, 2001). In confined basins or areas (which can be confined by solid boundaries/coasts as well as by the presence of fluid boundaries i.e. by the flow field pattern), considerations based on residence time and input rate may provide information as to the possible local persistence of litter.
Looking, at time rather than, at space features (we use this term for contrast and in a very broad sense, refering in fact to spatial-temporal structures determining local transport characteristics of the flow), we need to mention another category of descriptors, closely related to the possible chaotic structure of even two dimensional (if time-dependent) fluid motion, thus to their Lagrangian irregular character: the Lagrangian Coherent Structures (LCSs: Haller, 2001; a simple illustration of the concept of LCSs can be found in Peacock and Haller, 2013), which are a powerful tool for understanding transport in complex fluid flows, and have been recently applied to studies on oceanic and Mediterranean connectivity (d'Ovidio *et al.*, 2004; Berta *et al.*, 2014). Their reconstruction requires a detailed synoptic knowledge of the current field, which may be achieved through ocean circulation models or current field data provided by remote sensing observations (land-based, especially: HF radars yield highly suitable, spatial and temporal high resolution synoptic data over coastal areas, e.g. Uttieri *et al.*, 2011).
The approach through LCSs allows to identify key features of transport in a fluid flow, that attract or repel neighboring fluid elements over a certain period of time, thus defining local retention or repulsion areas for passive particles. In this respect, LCSs have the potential to be an excellent tool for looking at accumulation or dispersion of marine debris.

Obviously, such integral descriptors are just two exemplary choices that we find particularly suitable for marine litter applications. Recent developments in dispersion studies in the ocean and in the Mediterranean Sea include coupling Lagrangian modeling and quantities borrowed from network theory to characterize marine connectivity at basin scale (Rossi *et al.*, 2014); tools derived from graph theory (Kininmonth *et al.*, 2010; Rozenfeld *et al.*, 2008) to describe and analyze connectivity patterns. We refer the readers to the review by LaCasce (2008) on statistics from Lagrangian observations that may contain further possible methods for such investigations.

3. Oceanographic studies on marine litter drift in the world ocean

Oceanographic studies focused on marine litter in the ocean (and in marginal seas) are typically limited to debris floating at the surface, and eventually washed ashore.
As mentioned above, given the nature of the material and the dynamics of its transport, the Lagrangian, i.e. particle-following, approach is best suited to study the fate of floating litter. The available literature provides examples of suitable approaches based on real and/or simulated Lagrangian data.

Oceanic currents carry litter everywhere (Law *et al.*, 2010; Maximenko *et al.*, 2012), which permits plastic materials to reach remote areas, like islands or polar regions, noticeably distant from the sources of pollution (Derraik, 2002; Barnes, 2005; Barnes *et al.*, 2009; Ryan *et al.*, 2009). The best example for this is definitely the journey of some 29000 rubber duckies lost at sea from a shipping container in 1992 which kept travelling through the world ocean for the next two decades. They have become the symbol of the close relationship existing between floating litter distribution and the global circulation dynamics (Ebbesmeyer and Ingraham 1994; the same authors in 1992 studied the voyage of about 80000 sneakers around the Pacific Ocean, Ebbesmeyer and Ingraham, 1992; Ebbesmeyer *et al.*, 2007).
Large scale media interest in the accumulation of oceanic debris began in 2001 through the efforts of Moore *et al.* (2001) who presented data showing that in the North Pacific Gyre, plastic was

more abundant than plankton by as much as 6:1. Law *et al.* (2010) then provided the most comprehensive review of data on floating debris from the North Atlantic Ocean, summarising more than 6000 samples taken over 20 years, and very recently, Còzar *et al.* (2014) demonstrated how plastic debris is accumulating, with comparable densities, in the convergence zones of each of the five world's subtropical gyres.
From an oceanographic perspective, we can distinguish between two broad categories of marine litter oceanographic studies: global-scale investigations on the general characteristics of marine litter transport mechanisms, and studies on litter dispersal and distribution in individual areas of the world ocean.

i. Large scale studies

The general framework is set by the paper by Howell *et al.* (2012), who reviewed the main circulation patterns of the North Pacific Ocean, and outlined the possible mechanisms for marine litter transport and accumulation. It is worth mentioning their distinction, between two mechanisms enhancing local litter concentration: accumulation, characterized by convergent particle paths; and retention, promoted by tracer homogenization processes inside closed streamlines (see also Franks, 1996). There is a large body of studies describing the abundance, distribution and composition of floating debris contained within large scale accumulation zones (Carpenter and Smith, 1972; Law *et al.*, 2010; Maximenko *et al.*, 2012) studied the formation mechanisms of "garbage patches" using data on surface currents from a global set of historical trajectories of drifting buoys. The reason for such a choice lies in the above-mentioned peculiarity of Lagrangian measurements (earlier studies using an analogous approach had used ship drift data, as in Wakata and Sugimori, 1990; while large scale investigations of the tropical North Pacific were carried out numerically by Kubota, 1994). Maximenko *et al.* (2012) incorporated an idealized initial state with floating debris uniformly distributed over the global ocean's surface, before employing observed drifter data to advect marine debris forward in time. The authors took advantage of the fact that drifter observations account for the whole surface current field in both its geostrophic and in its locally wind-driven portion. They identified major garbage accumulation areas in the world ocean. These areas correspond to convergences of the portion of surface currents which are due to the effect of the local wind, which in their case includes windage, Stokes' drift and even Ekman currents, correctly identifying the different functioning of subtropical vs subpolar gyres in accumulating marine litter at the surface, which partly corresponded to well known accumulation areas at the time, and partly suggested where to look in order to find additional floating litter gathering zones in the world's ocean.

Other works described the global-scale transport of floating debris from its entry into the marine environment to its accumulation within the main oceanic gyres. Lebreton *et al.* (2012) proposed a methodology for tracking floating debris from source to sink based on realistic descriptions of global waste production and oceanic surface currents. The authors also extended in time their perspective, by looking at results of a 30-year long numerical simulation of surface circulation and passive transport pathways.
Van Sebille *et al.* (2012) also used observational drifters to study the fate of marine garbage patches in terms of aggregate of passive tracers away from the coastal margin and into the open ocean with a method that incorporates the seasonal cycle and uses a marine debris source function that scales with human population around the coast. This function allowed them to study the fate and mixing of marine debris in a more realistic scenario than previously achieved, and to estimate the pathway and ultimate location of litter retention zones. Their study revealed a sixth garbage patch in the Barents Sea not previously identified in addition to those in North and South Pacific, the North and South Atlantic and South Indian Oceans. They further examined, for the first time, how each of the garbage patches are connected, how inter-ocean exchange mixes debris from different regions, how 'leaky' patches result and how they will evolve over century timescales.

ii. Regional studies

At more local scale, two areas of the world ocean have been subject to extensive dedicated studies on litter transport driven by oceanographic factors: the East Asian marginal seas (Isobe *et al.*, 2009; Yoon *et al.*, 2010; Kako *et al.*, 2011; Kako *et al.*, 2014) and the ocean surrounding the Hawaiian Islands (Kubota, 1994; Carson *et al.*, 2013a). These studies typically stem from observations of beached litter at specific locations, and therefore focus on some particular types of plastic objects: for instance, Yoon *et al.* (2010) examined disposable lighters with three different buoyancy ratios, as well as plastic containers and bottles, while Kako *et al.* (2011) focused on plastic bottle caps and other debris to derive drifting objects trajectories.
Moreover, given the regional angle of these studies, and the fact that they apply to marginal seas enclosed by different countries, the identification of the main sources of debris play an important role in these investigations. Obviously, as noted in various studies (e.g. Yoon *et al.*, 2010) the key factor for solving issues of floating litter in semi-enclosed seas is a strong cooperation among source countries).
Kako *et al.* (2011) utilized a particle tracking model to reconstruct sources of marine litter that gets eventually stranded on Hassakubana beach (Goto Islands, Japan). They performed backward and forward simulations and verified beach litter input from different sources by using an inverse method introduced by the same authors (Kako *et al.*, 2010).
In a further, recent paper, Kako *et al.* (2014) extended their previous analyses by putting them in future perspective and investigated the potential risk created over a decade by the increasing level of floating plastic beached in the East Asian marginal seas, developing basin-wide budgets for the input of such material. Their results, considering the residence time of plastic litter in the area, suggest a likely future accumulation of potentially stranding material.

Among the studies on the Hawaiian coasts, we would like to mention the recent paper by Carson *et al.* (2013a; see references therein for earlier works in the region) is notable in evaluating the differences between observed and modelled drifter trajectories, which leads the authors to a number of critical points, such as the importance of nearshore and tidal dynamics, that even regional scale models typically do not account for, but which may be of great importance for coastal litter budgets.

4. Mediterranean Sea

The Mediterranean Sea is the largest and deepest enclosed sea on Earth. Its shores are home to 7% of the world's population and intense shipping, fishing and touristic activities take place in its waters and around its coastlines (UNEP/MAP, 2012). All these characteristics are key factors in the input of marine litter into the basin, and a large literature, dating back as early as the 1980s, concerns the presence of debris in Mediterranean waters.
Several studies have investigated the abundance and distribution of floating macro- and micro-debris in Mediterranean waters: Morris (1980b), Saydam *et al.* (1985); McCoy (1988), Kornilios *et al.* (1998); Tomàs *et al.* (2002); Aliani *et al.* (2003); Aliani and Molcard (2003); Topcu *et al.* (2010); Collignon *et al.* (2012); Hagmann *et al.* (2013) and Suaria and Aliani (2014). The litter stranded on Mediterranean beaches and coasts has been studied by Shiber (1982; 1987); Gabrielides *et al.* (1991); Golik and Gertner (1992); Tudor *et al.* (2002); Martinez-Ribes *et al.* (2007); Ariza *et al.* (2008); Turner and Holmes (2011); Kordella *et al.* (2013) de Lucia *et al.* (2014). Its distribution on the sea floor and in shelf habitats has been studied by Bingel *et al.* (1987); Galil *et al.* (1995); Galgani *et al.* (1995; 1996; 2000); Stefatos *et al.* (1999); Katsanevakis and Katsarou (2004); Koutsodendris *et al.* (2008); Guven *et al.* (2013); Mifsud *et al.* (2013) and Sánchez *et al.* (2013). Recently, deep sea habitats have also been considered (Ramirez-Llodra *et al.*, 2013) and attention starts to be focused on the drivers which lie behind the geographical patterns of debris abundance and distribution.

The retention of floating debris in the Mediterranean Sea may be facilitated by the structure of its conveyor belt: the basin imports surface waters from the Atlantic and has no significant surface outflow anywhere on its margins (apart from the limited exchange with the Black Sea); the return flow towards the Atlantic Ocean occurs at depth. As a consequence, once entered in the marine environment, floating material is almost inevitably destined to accumulate here, where it can be

retained within isolated eddies and convergence areas associated with fronts for long periods of time, before sinking, being washed ashore or breaking up into progressively smaller particles. In a recent global numerical model (Lebreton *et al.*, 2012) simulating 30 years of input, transport and accumulation of floating debris in the world ocean, the Mediterranean Sea was found to have one of the highest concentrations of marine litter in the world, potentially retaining between 6% and 8% of all particles introduced into the model.
Several of the above mentioned studies report a conspicuous spatial heterogeneity in litter distribution, and recently, clues about litter retention dynamics were proposed by Suaria and Aliani (2014) and Ramirez-Llodra *et al.* (2013). However, no evident pattern of surface debris accumulation has been identified as yet and evidence about the existence of one or more "Mediterranean garbage patches" is still lacking.

While the ubiquitous presence of marine litter both at the surface and at depth has been widely observed, the Mediterranean Sea has been so far only scantily investigated in terms of its transport mechanisms (an exception to this are the hints contained in Aliani *et al.*, 2003 and in Ramirez-Llodra *et al.*, 2013) and little is known on litter displacement and accumulation dynamics in the basin.
On the other hand, information of general interest for such studies, mainly derived from Lagrangian data and/or simulations is already available: as outlined in the previous section, the motion of plastic debris floating at the surface is best approximated by surface drifter trajectories, since those instruments undergo essentially the same transport mechanisms as marine litter. Over the last few decades, quite a few Lagrangian experiments on surface currents have been carried out in the Mediterranean, and their results have provided substantial information on transport patterns in its sub-basins (for a general view of the entire surface Lagrangian data set collected for the last 20 years in the Mediterranean Sea, see Poulain *et al.*, 2012).

In the absence of an encompassing description putting together oceanographic data and litter distribution in the basin, in the following we will briefly try and connect some Lagrangian observations gathered in the Mediterranean Sea with the results of a very recent survey of floating macro- and megadebris debris carried out in Mediterranean waters (Suaria and Aliani, 2014, refered as SA2014 heretofore), limiting our analysis to the regions sampled in that study.

SA2014 evidenced that natural and anthropogenic debris (>2 cm) are widespread all over the Central and Western sectors of the Mediterranean Sea. According to the authors, floating debris densities were ranging from 0 to 194.6 items/km^2, and, on average, 24.9 anthropogenic items/km^2 and 6.9 natural debris items/km^2 were found across the entire study area. The authors reported also that most of the floating objects (78%) were of anthropogenic origin, which 95.6% consisted of petrochemical derivatives (i.e. plastic and styrofoam). The highest densities of man-made objects (>52 items/km^2) were found in the Adriatic Sea and in the Algerian basin, while the lowest densities (<6.3 items/km^2) were observed in the Northern Tyrrhenian Sea and in the Strait of Sicily. All the other sectors surveyed by the authors had mean litter densities ranging from 10.9 to 30.7 items/km^2. The authors concluded that, if a comparable abundance of debris were floating in the Eastern part of the basin, at the time of their survey more than 62 million macro-litter items would have been afloat on the surface of the entire Mediterranean Sea. Their findings are summarized in Figure 1.

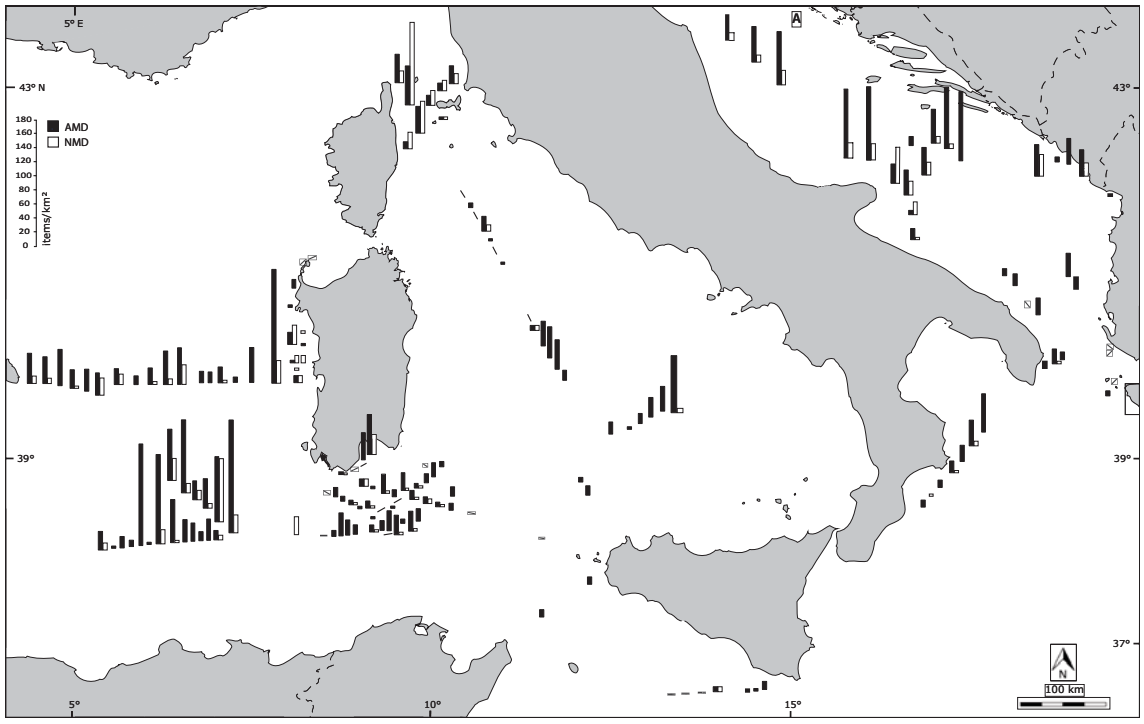

Figure 1. Map of the central-western Mediterranean Sea showing the observed distribution of Anthropogenic (black bars) and Natural Marine Debris (white bars) densities (expressed as number of items/km^2) estimated in SA2014's survey (adapted from Suaria and Aliani, 2014).

Our attempt to qualitatively put together results from SA2014's survey with Lagrangian observations in the Mediterranean Sea will proceed West to East, following the main path of the surface waters of Atlantic origin.

Atlantic surface waters enter the Mediterranean Sea from the strait of Gibraltar and circulate anticlockwise in the whole Algero-Provencal Basin. The southernmost branch of this circulation, Algerian Current, is clearly depicted drifter trajectories as a swift flow parallel to the north-western African coast, detaching (mainly anticyclonic) eddies which drift northwards and may trap floating debris. These eddies have been extensively studied through satellite observations, both of Sea Surface Temperature and of Sea Level Anomaly, hydrological measurements and surface drifter data (Millot, 1999 and references therein, Salas *et al.*, 2001; 2002; Salas, 2003; Isern-Fontanet *et al.*, 2004 and references therein). These features extend further northwards, in their meridional extension, approaching the Sardinia channel, and this might be reconnected to the very high density of both natural and anthropogenic marine debris found considerably far from shore by SA2014 south-west off the Sardinian coast and to the high density of benthic plastic litter found approximately in the same area by Ramirez-Llodra *et al.* (2013). Moreover, the further north-northwestward motion of these eddies is possibly at the origin of the relatively high density found by SA2014 between Sardinia and the Balearic Islands. Major sources of debris for this area are very likely the coastal urbanization, together with the very intense maritime traffic present along the Algerian slope, which represents the main shipping corridor used by ships entering and leaving the Mediterranean Sea.

Lagrangian measurements and marine debris observations in the Tyrrhenian Sea are also clearly compatible: Rinaldi *et al.* (2010) analysed surface drifters deployed in the Tyrrhenian Sea between 2001 and 2004, clearly showing the presence of particle retention areas in that basin, which might possibly result in litter accumulation zones: this is particularly true for the south-easternmost portion of the Tyrrhenian, which was characterized by a tendency to stagnation, as shown in their Fig. 4 which documents the fate of drifters deployed along the Naples-Palermo ferry track in subsequent deployments; but the whole southern portion of the basin is characterized by very low

surface velocities, i.e. by little efficient water renewal, measured by Lagrangian instruments. Although this area was poorly sampled during SA2014's survey, there is a correspondence between the surface current field in this part of the basin and the rather high debris density found by the authors along the southern, roughly zonal transect of their survey, as well as with the southernmost portion of the meridional one.
On the contrary, the low densities encountered in the northern part of their meridional transect might be associated with the crossing of the Bonifacio gyre, a cyclonic feature which, by causing upwelling and divergent surface flow, is not expected to accumulate material floating at the surface. Relatively high debris densities were again found by SA2014 in the Corsica Channel, quite understandably, as the channel represents the chokepoint for waters passing from the Tyrrhenian to the Ligurian Sea, thus possibly inducing an enhanced concentration of floating material transiting through the straits, which constitute a very narrow passage bordered by highly touristic areas (note that the SA2014 survey was carried out during the summer).

Proceeding eastwards, Lagrangian observations in the Strait of Sicily were collected in the 1990s by Poulain and Zambianchi (2007). In this case, no retention areas with a semi-permanent character were identified. These observations allowed to identify the main surface pathways linking the western and the eastern Mediterranean sub-basins, which most likely represent the preferred trajectories for floating marine debris across the two basins. They showed how the surface flow into the Ionian Sea occurs mainly in the northernmost part of the straits, and in particular in the narrow channel between Sicily and Malta, while south of Malta island drifter data show a very strong dependence upon the wind direction, with flow reversals (southeastwards/northwestwards) in the central part of the Straits. Very few data about floating debris are available in this area: results by SA2014 barely cover a small portion of it, and report low litter density, which might confirm the above mentioned absence of accumulation mechanisms. Old data from a survey performed by McCoy (1988) off the waters of Malta, east of the Strait of Sicily, reported an accumulation zone with extremely high concentrations of megalitter in the Central Ionian Sea (up to 2000 items/km^2), which is consistent with the presence of a gyre in the central Ionian Sea, and also possibly witnessed by the benthic findings of Ramirez-Llodra *et al.*, 2013 (even though the upper circulation in the Ionian Sea presents a strong and pervasive long-term variability, not allowing to draw conclusions in a generalized manner, see Gačić *et al.*, 2010).

SA2014 present extensive observations of floating debris in the Adriatic Sea. This basin has been subject to several investigations using Lagrangian instruments. Its surface circulation is overall cyclonic with waters entering the basin through the Otranto Strait along the Albanian coasts and exiting on the opposite side of the Strait in the form of an enhanced southeastward coastal current (the so-called WAC, Western Adriatic Current, see Poulain *et al.*, 2012 and references therein). This broad circulation is composed of three gyres in the northern, central and southern parts of the basin. The presence of a recirculation in correspondence to the outflow region of semi-enclosed basins has been studied in the past in terms of its influence on the residence time of tracer particles (Buffoni *et al.*, 1997; Bellucci, 2001); this methodology has been specifically applied to the Adriatic Sea (Falco *et al.*, 2000) showing that its surface circulation pattern strongly enhances the time spent by tracers inside the basin, thus accounting for the high abundance of floating litter found in this region by SA2014. In addition to this, it is worth noticing that the Adriatic Sea is characterized by the presence of multiple, interacting factors which are possibly responsible for the observed high density of debris. As underlined by SA2014, the Adriatic is a semi-enclosed sea subject to the inflow of one-third of the total mean annual river discharge into the whole Mediterranean basin (with the Po River being the primary actor); it is surrounded by a very populated coastline, (especially on the northern and western sides) and several important activities are extensively carried out at sea (i.e. fisheries, tourism and maritime transport), which likely represent a litter source.

The Levantine basin has not been sampled in the survey by SA2014 and more data about litter distribution in the Eastern part of the Mediterranean basin are urgently needed. However, Lagrangian measurements (Gerin *et al.*, 2011; Menna *et al.*, 2012; Poulain *et al.*, 2012), as well as earlier Eulerian studies (Robinson *et al.*, 2001) highlighted the presence of conveyor currents as well as several recirculations features, whose influence on floating debris aggregation is not

possible to evaluate at this point. We will limit ourselves to note the observations by Ramirez-Llodra *et al.* (2013) in correspondence to the anticyclonic Ierapetra gyre, which show an accumulation of plastic debris on the sea floor in the area, compatible with a gyre-induced process.

5. Discussion and concluding remarks

Available drifter trajectories over the Mediterranean show that no area is ideally free of transport tracks. Parallel data from visual sightings of floating natural and man-made debris demonstrate that debris is widespread all over the basin; despite its variability in abundance and distribution, marine litter is basically ubiquitous, and to date no study has reported a completely litter-free zone over the Mediterranean Sea (whether benthic, stranded or floating litter).

The general picture coming out of SA2014 is that of a very high local variability in floating debris abundance, distribution and composition across the surface of the entire central and western sectors of the Mediterranean Sea. However, their survey showed that, at the basin scale, natural and man-made objects tend to accumulate roughly in the same areas and concluded that generally, despite a very high variability within sectors, those areas with the highest densities of natural debris were showing also relatively high anthropogenic litter densities. This might suggest that the offshore distribution of floating debris in the Mediterranean is most likely controlled by the physical transport associated with the surface current field, which obviously does not distinguish between the different origins of litter.

On the other hand, extreme care must be used when comparing integrated results of a large-scale survey like SA2014 with information on the multi-scale surface current field, as we just did: the basin/sub-basin scale findings of SA2014 are the result of transport processes often developing also at smaller scales (such as the oceanic mesoscale). In order to truly understand the effect of physical transport, future surveys should be planned so as to resolve the scale of as many physical processes controlling the litter distribution as possible. A further important issue would be to plan investigations so as to account for the variability of the circulation on seasonal to interannual timescales.

In addition, little is known about debris input areas, small-scale geographical patterns and seasonal trends. Local processes such as riverine discharge, storm-related events, fishing grounds, shipping lanes, touristic zones and the proximity to urban centers may all be crucial for determining the regional abundance and composition of floating debris. Observational studies on river plumes, on the extension of filaments generated along the coast and protruding offshore, and more generally on in-/offshore exchanges have been generally carried out using the Eulerian approach and looking at individual regions and local phenomena (for a Mediterranean recent example see Iermano *et al.*, 2012, and numerous references therein; local in-/offshore exchanges can be studied by merging land-based Eulerian remote sensing data with Lagrangian measurements or simulations as was done by Uttieri *et al.*, 2011). More investigations of this kind would add a fundamental piece to the jigsaw puzzle of the marine litter budget and transport in the Mediterranean region.

An important addition to this would be represented by studies on the population dynamics of organisms carried by debris, which are necessary to properly understand the real ecological potential that an increase in rafting opportunities can have for Mediterranean ecosystems (as shown for other regions of the ocean, e.g. by Barnes, 2002 and 2014).

Even though marine litter represents a serious environmental concern, oceanographic studies on its motion, distribution and accumulation in the world ocean are still sparse, and typically restricted to the surface.

In the Mediterranean Sea this knowledge is even more limited, despite the fact that numerous measurements from surface drifters are available, which can be utilized as proxies for marine debris items or aggregates.

It is often said that the Mediterranean Sea may be utilized as a natural laboratory to investigate, in a more accessible environment, mechanisms of general interest which occur in the world ocean. This is true also for marine litter transport, since this Basin presents the whole suite of dynamics leading to the transport, dispersal or on the contrary accumulation of floating material. This lays the ground for future promising work on marine litter in the Mediterranean region, with outputs of much more general significance.

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Bacterial degradation of synthetic plastics

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Abstract

Synthetic plastics are emerging environmental contaminants that have been found to accumulate within marine waters worldwide. In marine environments, microorganisms function as pioneering surface colonizers and drive critical ecosystem processes including primary production, biogeochemical cycling and the biodegradation of anthropogenic pollutants. This paper reviews the current knowledge on the biodegradation of synthetic plastics by microorganisms. The microbial biodegradation of plastic materials is a complex phenomenon that includes several steps that are described here.

Introduction

Colonization of plastic marine debris by microorganisms was first reported in the 1970s, where authors mention diatoms and other microbes on the debris (Carpenter *et al.*, 1972; Colton *et al.*, 1974). In marine waters, plastic debris represents a novel ecological habitat for microorganisms since it entered the consumer arena less than 60 years ago, acting as new floating type of particles for microbial colonization and transportation. Plastic has become the most common form of marine debris and it presents a major and growing global pollution problem. In the north western Mediterranean Sea, plastics were found at concentrations of up to 3.6 10^5 pieces/km^2 (Collignon *et al.*, 2012), which is equivalent to what was found in the "great Pacific garbage patch" (5.0 10^5 items/km^2 were found in the North Atlantic Subtropical Gyre, Law *et al.*, 2010). Particles may serve as a niche for microorganisms, offering a support for growth especially when it concerns organic aggregates, but also a protected area with limited predation. The presence of particles in aquatic systems is known to stimulate microbial productivity and respiration (Simon, 2002; Ghiglione *et al.*, 2009). However, detailed analyses on sorted plastic particles are scarce. So far, the only study dealing with bacterial communities living in the so-called 'plastisphere' showed a high diversity composed of heterotrophs, autotrophs, predators, symbionts and also some opportunistic pathogens (Zettler *et al.*, 2013).

The research on degradability of plastics began in the early 1980s and we dispose of numerous papers providing information on the microbial biodegradation of a variety of plastics such as polyesters, polyhydroxybutyrate (PHB), polycaprolactone (PCL), polylactic acid (PLA), polyurethane PUR, polyvinyl alcohol (PVA), nylon, and polyethylene (PE) (for example Shimao *et al.*, 2001).

The descriptor 10 (D 10) of the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) concerns marine litter. Started in 2011, a technical subgroup on marine litter (TSML) aims to provide scientific and technical background for the implementation of MSFD requirements with regard to D 10. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Indeed, it is important to consider the microbial degradation of synthetic plastics in order to understand what is necessary for their biodegradation. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved.

This paper reviews the current research on the biodegradation of synthetic plastics by microorganisms. The microbial biodegradation of plastic materials includes several steps that are described here. Biodegradation is not disconnected from abiotic degradation, since several studies about biodegradation of some polymers show that the abiotic degradation (mechanical, light, thermal or chemical degradation) precedes microbial assimilation (Kister *et al.*, 2000; Proikakis *et al.*, 2006).

Different steps of plastic degradation by microorganisms

Several steps occur in the plastic biodegradation process (Figure 1) and could be identified by specific terminology (Lucas *et al.*, 2008):

-**Bio-deterioration** defines the action of microbial communities and other decomposer organisms responsible for the physical and chemical deterioration that result in a superficial degradation that modifies the mechanical, physical and chemical properties of the plastic.

-Bio-fragmentation refers to the catalytic actions that cleave polymeric plastics into oligomers, dimers or monomers by ecto-enzymes or free-radicals secreted by microorganisms.

-**Assimilation** characterizes the integration of molecules transported in the cytoplasm in the microbial metabolism.

-**Mineralisation** refers to the complete degradation of molecules, resulting in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, H_2O).

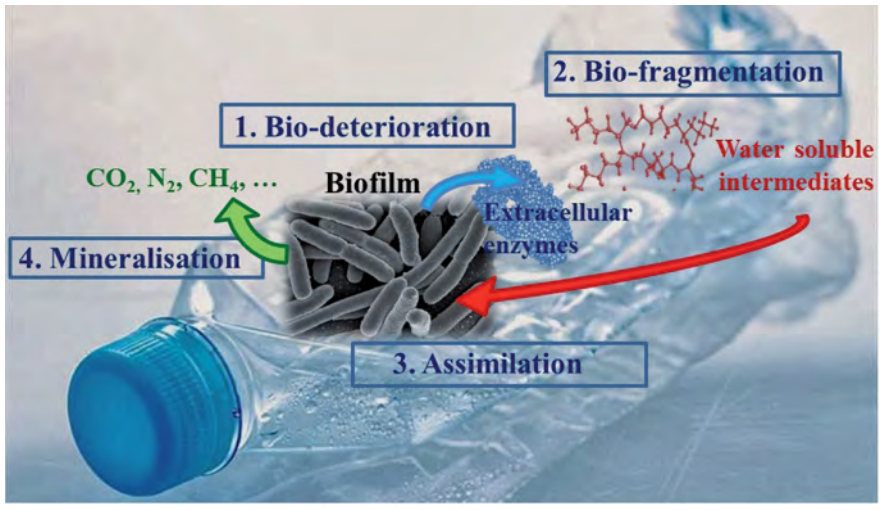

Figure 1. The different steps of plastic biodegradation by microorganisms.

We describe below the different degrees of the biodegradation process (bio-deterioration, bio-fragmentation, assimilation and mineralisation). The technical estimations related to each level of biodegradation are given in Table 1 and Table 2.

Table 1. Bio-degradability tests related to different steps of plastic degradation (left) and analytical techniques for bio-degradability estimation (right) (from Lucas *et al.*, 2008).

Tests	Norms	Characteristics		Estimating			
		Difficulty	Reality	AB[a]	BD[b]	BF[c]	A[d]
Out-door exposure		+	++++	X	X		
UV exposure	ISO 4582	+	++	X			
Suntest	ISO 4892 series	+	++	X			
Accelerated weathering chamber		++	+++	X			
Differential scanning calorimetry		++	+	X			
Thermogravimetric analysis		++	+	X			
Pyrolysis		++	+	X			
Microorganisms surface colonisation	ISO 846 ISO 11266 NF X41-513						
	NF X41-514 ASTM G22-76 ASTM G21-70 ASTM G21-90	+++	+++		X	X	
Weight loss	ISO 14852						
	ISO 14855 NF EN ISO 13432	+	+	X	X	X	
Significant enzymes in batch		++	++		X	X	
Clear zone test		+++	+++		X	X	
Respirometry	OECD series, ISO 14852, ISO 14855 ASTM D 5209	++	++				X

[a] Abiotic degradation.
[b] Biodeterioration.
[c] Biofragmentation.
[d] Assimilation.

Analytical techniques	Norms	Characteristics		Estimating			
		Cost	Difficulty	AB	BD	BF	A
Morphological							
Yellowness	ASTM D 1925	+	+	X			
Photonic microscopy		++	++	X	X		
Electronic microscopy		++++	++++	X	X		
Polarization microscopy		+++	++	X	X		
Rheological							
Tensile	ISO 527-3	++	+	X	X	X	
X-ray diffraction		++++	+++	X	X	X	
Differential scanning calorimetry		++++	++	X	X	X	
Thermogravimetric analysis		++++	++	X	X	X	
Gravimetric		+	+	X	X	X	
Spectroscopic							
Fluorescence		++	++	X	X	X	
UV–visible		+	+	X	X	X	
FTIR		++	++	X	X	X	
RMN		++++	++	X	X	X	
Mass spectrometry		++++	+++	X	X	X	
Chromatographic							
Gel permeation chromatography		+++	++	X	X	X	
Hight performance Liquid chromatography		+++	++	X	X	X	
Gas phase chromatography		+++	++	X	X	X	

Bio-deterioration

Deterioration is a superficial degradation that modifies mechanical, physical and chemical properties of the plastic. In most cases, abiotic parameters contribute to weaken the polymeric structure (Helbling *et al.*, 2006; Ipekoglu *et al.*, 2007). Sometimes, these abiotic parameters are useful either as a synergistic factor, or to initiate the biodegradation process (Jakubowicz *et al.*, 2006).

For more details on the role of pollutants attached to the plastic in the development of microbial biofilm, see Fotopoulou *et al*., this volume.

The bio-deterioration seems to be triggered by the formation of a microbial **biofilm** growing on the surface and inside the plastic. The development of the biofilm is dependent on the composition and the structure of the plastic, but also on the environmental conditions (Lugauskas *et al.*, 2003). Since plastic polymers such as PE and PS are hydrophobic, forming a stable biofilm requires that the bacterial surface will also be hydrophobic. For example, the biofilm of *Rhodococcus ruber* C208 formed on polyethylene showed high viability and even after 60 days of incubation adhered to the polyethylene without any supplementation of external carbon (Figure 2).

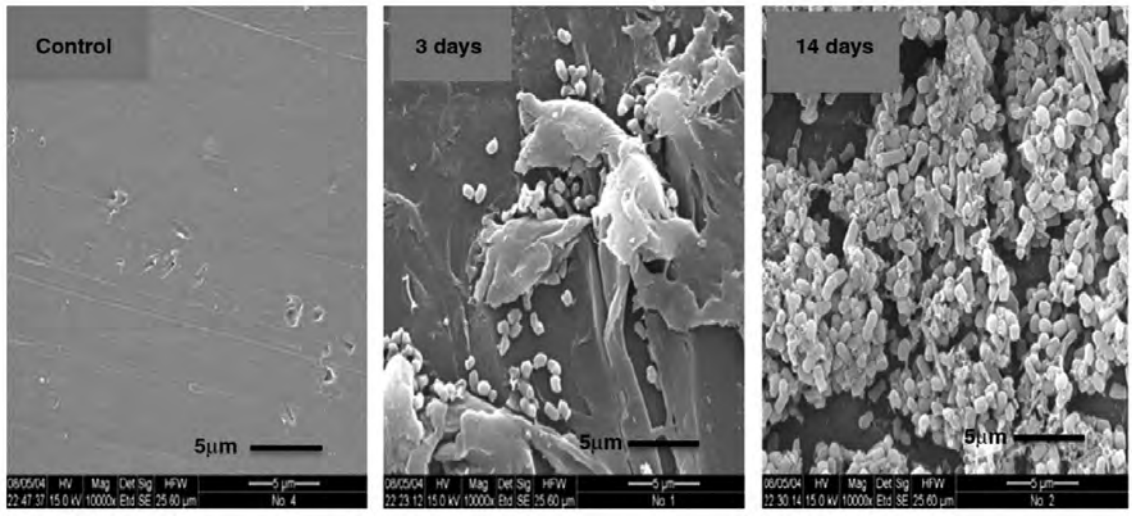

Figure 2. Scanning electron microscopy of biofilm formed by *Rhodococcus ruber* C208 on the surface of UV photo-oxidised polyethylene. Initiation of biodegradation was detected after 3 days. UV irradiated but not inoculated served as control (from Sivan, 2011).

The microbial biofilm provokes serious physical and chemical deterioration:

-physical deterioration: the formation of the microbial biofilm is associated to the secretion of extracellular polymeric substances (EPS) that reinforce the cohesion of the biofilm and the adhesion to the plastic surface. The EPS enters the pores, microorganisms can then grow inside, thus increasing the pore size and provoking cracks that weaken the physical properties of the plastic (Bonhomme *et al.*, 2003).

-chemical deterioration: the microbial communities that develop on plastic may be highly diverse (Zettler *et al.*, 2013) and the development of a biofilm may **release acid compounds** such as nitrous acid (e.g. *Nitrosomonas* spp.), nitric acid (e.g. *Nitrobacter* spp.) or sulphuric acid (e.g. *Thiobacillus* spp.) by chemolithotrophic bacteria. Organic acids such as oxalic, citric, fumaric, gluconic, glutaric, glyoxalic, oxalic and oxaloacetic acids may also be released by chemoorganotrophic communities. The pH inside the pores is then modified, resulting in a progressive degradation that changes the microstructure of the plastic matrix.

Bio-fragmentation

The fragmentation of plastic polymers into oligo- and monomers can be of various origins, i.e. mechanical, UV radiation, thermal, chemical and/or biological. Here we focus on the biological aspect; other reviews provide details about the abiotic fragmentation (see for example Singh and Sharma, 2008).

Plastic polymers are molecules with high molecular weight that cannot cross the cell wall. Microorganisms secrete extracellular enzymes (**exoenzymes)** that can catalyze reactions principally at the boundaries of the plastic polymer. They can perform many chemical reactions, but they generally need imbalance of electric charge to perform lysis. The main limit of bio-fragmentation is the stability of the plastic polymers, which are constituted by a long chain of carbons and hydrogens that contains very balanced charges. To destabilize the local electric charge, bacteria that can break down plastics usually contain enzymes called **oxygenases**, which can add oxygen to a long carbon chain. For instance, mono-oxygenases and di-oxygenases incorporate, respectively, one and two oxygen atoms, forming alcohol or peroxyl groups that are less recalcitrant for biodegradation. Other transformations are then catalysed by lipases and esterases after the formation of carboxylic groups, or by endopeptidases for amide groups (Lugauskas *et al.*, 2003). Some of the **well-known microbes** which have the capacity to degrade plastic polymers into their respective simple monomeric are shown in Table 2 (from Ghosh *et al.*, 2013). The 15 bacterial genera listed have the capacity to degrade various types of plastics. Among them, *Pseudomonas*

is dominant. It can degrade polythene, PVC, PHB, poly(3-hydroxybutyrate-co-3-mercaptopropionate), and poly(3-droxypropionate). *Bacillus brevis* can degrade only polycaprolactone while *Streptomyces* can degrade PHB, poly(3-hydoxybutyarate-co-3-hydroxyvalerate), and starch or polyester. *Ochrobactrum* TD is also able to degrade PVC. The majority of the strains that are able to degrade PHB belong to different taxa such as Gram-positive and Gram-negative bacteria, *Streptomyces*, and fungi (Mergaert and Swings, 1996). It has been reported that 39 bacterial strains of the classes Firmicutes and Proteobacteria can degrade PHB, PCL, and PBS, but not PLA (Suyama *et al.*, 1998). Other bacterial species identified as having the properties of degrading plastics are *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Diplococcus* sp., *Micrococcus* sp., *Pseudomonas* sp., and *Moraxella* sp. (Kathiresan, 2003).

For more details on the biodegradation of most prominent synthetic plastics (PEs, PP, PS and PVC), see Raddadi *et al.*, this volume.

Table 2. List of microbial strains and the types of plastic which they degrade (from Ghosh *et al.*, 2013).

Plastic	Microorganism	Reference
Polyethylene	*Brevibacillus borstelensis*	Hadad et al. (2005)
	Rhodococcus rubber	Sivan et al. (2006); Gilan et al. (2004)
	Pseudomonas chlororaphis	Zheng et al. (2005)
	Comamonas acidovorans TB-35	Akutsu et al. (1998)
Polyvinyl chloride	*Pseudomonas putida* AJ	Anthony et al. (2004)
	Ochrobactrum TD *Pseudomonas fluorescens* B – 22	Mogil'nitskii et al. (1987)
BTA copolyester	*Thermomonospora fusca*	Kleeberg et al. (1998)
Some biodegradable/natural plastics and their degrading microorganisms		
Poly(3-hydroxybutyrate-*co*-3-mercaptopropionate)	*Schlegelella thermodepolymerans*	Elbanna et al. (2004)
Poly(3-hydroxybutyrate)	*Pseudomonas lemoignei*	Jendrossek et al. (1995)
Poly(3-hydroxybutyrate-*co*-3-mercaptopropionate)	*Pseudomonas indica* K2	Elbanna et al. (2004)
Poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)	*Streptomyces* sp. SNG9	Mabrouk and Sabry (2001)
Poly(3-hydroxybutyrate-*co*-3-hydroxypropionate)	*Ralstonia pikettii* T1 *Acidovorax* sp. TP4	Wang et al. (2002)
Poly (3-hydroxybutyrate), poly(3-hydroxypropionate), poly(4-hydroxybutyrate), polyethylene succinate, polyethylene adipate	*Alcaligenes faecalis* *Pseudomonas stutzeri* *Comamonas acidovorans*	Kasuya et al. (1999)
Poly(3-hydroxybutyrate)	*Alcaligenes faecalis* *Schlegelella thermodepolymerans*	Kita et al. (1997)
	Caenibacterium thermophilum	Romen et al. (2004)
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)	*Clostridium botulinum* *Clostridium acetobutylicum*	Abou-Zeid et al. (2001)
Polycaprolactone	*Clostridium botulinum* *Clostridium acetobutylicum*	Abou-Zeid et al. (2001)
	Amycolatopsis sp.	
	Bacillus brevis	
Polymer blends and its degrading microorganisms		
Starch/polyester	*Streptomyces*	Lee et al. (1991)

Assimilation and mineralisation

The formation of monomer does not guaranty their assimilation by microorganisms. They have to use specific carriers to cross the cell wall and/or cytoplasmic membrane. Some monomers may stay in the surrounding of microbial cells without being assimilated. Inside cells, the plastic monomers are oxidized through catabolic pathways to produce energy, cell structure and new biomass. Depending on the microbial abilities to grow in aerobic or anaerobic conditions, there exist three essential catabolic pathways to produce the energy to maintain cellular activity, structure and

reproduction: aerobic respiration, anaerobic respiration and fermentation. The assimilation refers to the integration of atoms inside microbial cells, but the degradation of the monomers may not be complete. The assimilation results in numerous secondary metabolites that can be transported outside the microorganism that does not have the metabolic capability to transform it or that does not need to metabolize or store it. The secondary metabolites excreted may be used by another cell that can perform further degradation, or can stay further in the pool of non-assimilable compounds. The mineralization refers to the complete degradation of primary and secondary metabolites that result in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, H_2O).

Concluding remarks and perspectives

For the last 30 years, scientists have been trying to develop some alternative ways where they can use microbes to degrade these long chain synthetic polymers into their respective monomers. Until now, there is very little evidence that scientists were able to develop some alternative ways to enhance the mode of degradation and make it faster.

Biodegradability tests are necessary to estimate the environmental impact of plastic materials and to find solutions to avoid the disturbing accumulation of polymers. The augmentation of derived biodegradability tests has led to confused interpretations about biodegradation mechanisms. To compensate for this problem, it is necessary to explain the different steps involved in biodegradation (i.e. bio-deterioration, bio-fragmentation, assimilation and mineralization). In addition, each biodegradation stage should be associated with the adapted estimation technique.

Better knowledge on the different steps of plastic degradation by microorganisms may also help for improving biodegradation. Several factors may be explored for a better biodegradation of polymer plastics (i) by using surface active agents or inducing the microorganism to produce surfactant to allow better attachment of microorganisms on the polymer surface, (ii) by blending the polymers with biodegradable synthetic polymers such as polylactic acid or polycaprolactum (iii) by the pretreatment of the plastics which includes thermal, UV, high energy radiation and chemical treatment, (iv) by culturing those microorganisms that can efficiently degrade the plastics (bio-addition) and (v) by addition of nutrients that may be limiting in the environment (bio-stimulation).

The biodegradation of synthetic plastics is a complex phenomenon. Nature-like experiments are difficult to realize in laboratory due to the great number of parameters occurring during the biogeochemical recycling. Actually, all these parameters cannot be entirely reproduced and controlled *in vitro*. To date, most of the knowledge on the microbial ability to degrade synthetic plastics is based on few bacteria able to grow on culture media, that represent <0.1% of the total bacteria. Hence, the great natural source of high diversity of microorganisms is not fully exploited. By using -omics technologies (genomic, transcriptomic, proteomic, metabolomics), it is now possible to discover new non-culturable microorganisms involved in plastic colonization and degradation, and explore the new properties of microorganisms that arise from the interplay of genes, proteins, other macromolecules, small molecules, and the environment.

Low cost, efficient technology, eco-friendly treatments capable of reducing and even eliminating plastics, are of great environmental interest. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. There is a huge demand in exploring these microbes which can grow in different conditions and, under specific stress conditions, may be directed to grow and use the plastic carbon polymers as energy source.

Microbial biodegradation of synthetic plastic polymers: state of the art and perspectives from the BIOCLEAN project

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ABSTRACT

The dramatic increase in worldwide plastic production and the very low biodegradability in the environment of such plastics focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries. In EU, high amounts (10,3 mil ton) of post-consumer plastic waste is disposed of in landfills; where it partially undergoes photooxidation resulting in the production of small fragments. These can absorb toxins and toxic chemicals and together with plasticizers/plastics additives, can enter the marine environment and thus the food chain, where they can exert toxic effects. Moreover, marine litter has various impacts on the marine biota. Therefore, innovative eco-efficient solutions for degrading fossil plastics currently accumulated in landfills, intensifying the biodegradation of those entering composting/anaerobic digestion facilities and occurring in aquatic environments are sought in order to mitigate the environmental impact associated with plastic waste.

In this review, an overview of the biodegradation process of synthetic plastics based on literature search followed by a summary of the microorganisms reported to be involved in the biodegradation of four major plastics produced in EU i.e. PEs, PVC, PP and PS is provided. Moreover, the potential of environmental biotechnology approaches for plastics waste management and consequently for combatting marine litter is discussed.

1. INTRODUCTION

The worldwide plastic production was about 280 million tons in 2011. While the EU contributes 24% of this amount, it produces 75% of the most prominent petroleum-based plastics, i.e., polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS). Such plastics are extensively used not only for their excellent mechanical properties, low cost, light weight and high energy effectiveness, but also for their stability, durability and chemical and biological inertness. The dramatic increase in production and the very low biodegradability in the environment of such plastics is drawing increasing public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries.

In the EU, the post-consumer plastic waste is about 25,1 million ton/y among which ca.14,9 million ton is recovered (recycled or incinerated with energy recovery) and ca. 10,3 million ton is disposed of in landfills (Plastics-The fact 2012; www.plasticseurope.org). The plastics disposed of partially undergo photooxidation, producing small fragments which can absorb toxins and toxic chemicals and together with plasticizers/plastics additives enter the marine environment and thus the food

chain, where they can exert toxic effects (Tanaka *et al.*, 2013; Baztan *et al.*, 2014; Teuten *et al.*, 2007; Moore, 2008; Galgani, this volume; Fotopoulou *et al.*, this volume; Klasmeier *et al.*, this volume; Deudero and Alomar, this volume). Furthermore, colonization of plastic debris by sessile organisms may permit transport of alien species in the ocean environment and may threaten marine biodiversity (Gregory, 2009; Katsanevakis and Crocetta, this volume). This, in addition to the various impacts of marine litter on the marine biota (Fossi *et al.*, this volume; Sobral *et al.*, this volume).
Strategies addressed to increase the safe disposal of such materials in landfills as well as their recycling and incineration as benign smarter disposal procedures have been put in practice in several industrialized European countries. However, recycling into new products requires prior collection and sorting, which in turn increases the costs of the recycled products. In addition, the recycled products tend to have inferior long-term properties, and thus limited market applications. Consequently, quaternary recycling, i.e., incineration for recovering energy, is only practiced in a few European countries, while in most of them, a large fraction of plastic wastes ends up in landfills. Biobased and biodegradable plastics can represent interesting alternatives to synthetic plastics in specific applications but the sector is still in its infancy (<0.3% of total plastics produced in Europe).

Thus, in order to confine/mitigate the environmental impact associated with plastic waste, innovative eco-efficient solutions for degrading/detoxifying (hopefully also valorizing) fossil plastics currently accumulated in landfills, intensifying the biodegradation of those entering composting/anaerobic digestion facilities and occurring in aquatic environments are sought. Environmental biotechnology can offer promising tools/strategies in this field.

The scope of the present review is to outline of the biodegradation process of synthetic plastics based on published literature, followed by a comprehensive summary of the microorganisms reportedly involved with PEs, PVC, PP and PS biodegradation and to discuss the potential of environmental biotechnology approaches for plastics waste management and consequently for combatting marine litter.

2. Biodegradation of synthetic plastics: basics

Biodegradation of synthetic plastics can occur in nature, in sediments and marine environments as well as in landfills, compost and soil. The process is governed by the polymer characteristics, type of organisms available and environmental conditions (Artham and Doble, 2008). The primary mechanism for the biodegradation of a polymer is the oxidation or hydrolysis by enzymes that creates functional groups that improve polymer hydrophilicity; often the same enzyme breaks down the polymer structure by yielding shorter macromolecular chains that enter the cells, where they undergo further depolymerization by intracellular enzymes and mineralization (Albertsson and Karlsson, 1990; Huang *et al.*, 1990; Sivan, 2011; Santo *et al.*, 2012; Restrepo-Flóreza *et al.*, 2014). The biodegradation of plastics very often requires different organisms, some able to break down the polymer into its constituent monomers, others able to use the monomers or plastic additives as carbon source or to co-metabolize them by using simpler waste compounds as primary substrates. When oxygen is available, aerobic bacteria and fungi are mostly responsible for the degradation of polymers and plastics. In contrast, under anoxic conditions, anaerobic consortia of bacteria are responsible for polymer/plastic deterioration (Shah *et al.*, 2008). Plasticizers/additives can sometimes remarkably interfere with plastic biodegradation (Oehlmann *et al.*, 2009; Fontanella *et al.*, 2013). Generally, a decrease in molecular weight and/or in crystallinity results in a higher biodegradability of the polymer. An increase of surface area increases biodegradation too (Tokiwa *et al.*, 2009). Photo, thermal and chemical pretreatments can induce changes in the mechanical, electrical and chemical properties due to bond scission, cross linking, chemical transformation and formation of new functional groups, which in turn can remarkably increase the final biodegradability of polymers (Shah *et al.*, 2008). In general, physical and/or chemical treatments improve the hydrophilic character of the material (either by surface modification or by controlled oxidation), reduce the crystal/amorphous ratio and decrease the molecular weight, thus making the polymer more amenable to microbial attack. UV exposure, thermal treatment and gamma radiation rank among the major physical treatments. Upon exposure to UV radiation, polymers undergo photo oxidation, controlled by the intensity of light, leading to the formation of radicals

that propagate with the generation of shorter chains frequently terminated by carboxylic groups or esters, ketones, alcohols and double bonds, i.e., products with remarkable bioavailability and degradability. Thermal treatment normally oxidizes the polymer chain thereby introducing hydroxyl, carboxyl and hydroxyperoxyl groups which increase the hydrophilicity of the polymer and in turn its biodegradability. Gamma radiation can facilitate the biodegradation of polymer by inducing its oxidative fragmentation (Arkatkar *et al.*, 2009). Among the chemical treatments, ozonation can provide oxidation of different synthetic polymers, like PE, PP, PVC, with their partial scission and substitutions with ketonic, aldehydic and carboxylic groups (Ziani *et al.*, 2011).

3. State of the art on microbial and enzymatic biodegradation of PEs, PP, PS and PVC

To date, most available information on biodegradation of petroleum-deriving polymers and plastics has been related to biodegradable synthetic polymers, such as poly(vinyl alcohol), aliphatic polyesters, polycaprolactone, polyamides, oligomeric ethylene, styrene, isoprene, butadiene, acrylonitrile, and acrylate (Shah *et al.*, 2008; Gąszczak *et al*., 2012; Ghosh *et al.*, 2013; Shah *et al.*, 2014). These polymers will not be considered in this paper which will focus on the biodegradation of four of the most prominent synthetic plastics, namely PE, PP, PS and PVC.

a) Polyethylenes (PEs):

The majority of studies on the biodegradation of synthetic polymers have been conducted on PE (Roy *et al.*, 2011; Ammala *et al.*, 2011; Restrepo-Flóreza *et al.*, 2014). Given its high hydrophobicity, molecular weight and crystallinity, PE is a very poorly biodegradable polymer. Considerable work addressed to develop biodegradable PE by incorporating starch or prooxidants during preparation has been conducted (Sudhakar *et al.*, 2008). However blends and prooxidant-based PEs constitute a minor portion of the current PE market and use (Ammala *et al.*, 2011). PE blends with natural polymers (starch, cellulose, chitin, etc.) are susceptible to biodegradation by amylase or carbohydrase enzymes. In case of pro-oxidant additive containing PE, biodegradation occurs following photodegradation and chemical degradation (Bonhomme *et al.*, 2003). Several studies documented the microbial degradability of PE, especially when tested in the form of film or pieces (Yamada-Onodera *et al.*, 2001; Gilan *et al.*, 2004; Balasubramanian *et al.*, 2010; Harshvardhan and Jha, 2013; Restrepo-Flóreza *et al.*, 2014). Although some branched hydrocarbons of PE have been reported to be biodegradable, it is the linear analogues (i.e., LLDPE), more susceptible to microbial attack, that normally proceed via fatty acids formation and complete mineralization (Ammala *et al.*, 2011). LDPE buried in soil mixed with sewage sludge were found extensively colonized by fungi of the genera *Fusarium, Aspergillus* and *Penicillum*, able to utilize plastic constituents as a source of nutrients by physically pitting and eroding the surface (Shah *et al.*, 2008). PE biodegradation was also studied under marine conditions (Sudhakar *et al.*, 2007; Artham *et al.*, 2009). Other pretreated PE degrading bacteria are the actinomycete *Rhodococcus ruber* and the thermophylic bacterium *Brevibacillus borstelensis* (Sivan, 2011). The ability of lignolytic fungi to degrade high molecular-weight PE via their Mn-dependent peroxidases was also demonstrated (Iioshi *et al.*, 1998). Direct biodegradation of PE by extracellular enzymes has been also reported in the literature (Sivan, 2011). Laccases are mainly responsible for this; they are known as copper lignin biodegrading enzymes able to catalyse the oxidation of aromatic compounds but also as nonaromatic substrates. Laccases from *Streptomyces lavendulae* and *Trametes versicolor* also displayed copper-induced activity towards PE films (Sivan, 2011). Further investigation on the exact role of reported enzymes is needed to better understand the mechanism of PE polymer degradation and thus the possible exploitation pathways of the resulting breakdown products (Ammala *et al.*, 2011).

b) Polypropylene (PP):

Until now, little is known about the biodegradation of PP polymers and plastics by bacteria and fungi. The first attempts at evaluating of microbial biodegradation of PP were performed by Cacciari *et al.* (1993) who reported that microbial communities were able to biodegrade isotactic PP. After 175 days incubation in the presence of bacterial consortia, the polymer had 40% methylene chloride extractable compounds and a mixture of hydrocarbons (between C10H22 and C31H64) were identified in the extract (Cacciari *et al.*, 1993). Recently, there have been few other

reports dealing with the biodegradation of pretreated and/or pro-oxidant additives -containing PPs. Thirty to sixty percent growth of *Aspergillus niger* was observed on gamma irradiated PP films (100 μm thickness) at the end of six weeks, which indicated that the fungus was able to utilize this polymer as its sole carbon source (Alariqi *et al.*, 2006). Only 0.5% gravimetric weight loss has been reported with unblended PP (1.5 mm thickness) deployed in marine waters for a period of six months although the formation of a high-density biofilm was noted when no blended or pretreated polymers were included in the experiment (Sudhakar *et al.*, 2007). Biodegradation of thermally pretreated 0.05 mm thick PP films by a soil microbial consortium after one year of incubation under aerobic conditions has been reported; in that case a 10.7% gravimetric weight loss was observed for the thermally pretreated PP compared to only 0.4% for the untreated polymer (Arkatkar *et al.*, 2009). The same group (Arkatkar *et al.*, 2010), evaluated the *in vitro* biodegradation of unpretreated (PPUT), chemically (Aquaregia pretreated or Fenton's pretreated) and physically pretreated (thermal and UV pretreated) polypropylene films with four bacterial species, namely one culture collection strain (*Pseudomonas azotoformans* MTCC 7616), and three isolates enriched from one soil sample recovered from a plastic dumping site after one year of incubation (*Pseudomonas stutzeri, Bacillus subtilis* and *Bacillus flexus*). After one year of monitoring, although three of the strains were able to form biofilm on the surface of all polymers, biodegradation of only UV-pretreated PP by *B. flexus* was observed as revealed by 2.5% weight loss of the polymer (Arkatkar *et al.*, 2010). About 18.8% and 9.42% gravimetric weight losses were observed with UV pretreated pro-oxidant blended-PP in 1 year with *Phanerochaete chrysosporium* NCIM 1170 and *Engyodontium album* MTP091 fungal strains, respectively (Jeyakumar *et al.*, 2013). Fontanella *et al.* (2013) compared the biodegradability of different polypropylene (PP) films (51 to 63 μm) containing various pro-oxidant additives (Mn + Fe, Mn or Co) and the additive-free films using *Rhodococcus rhodochrous* ATCC 29672, where the studied films were the only source of carbon and energy. After six months of incubation, they reported the biodegradability of PP films containing Mn + Fe or Mn additive, while no biodegradation was observed in the case of additives-free polymers. Longo *et al.* (2011) reported the biodegradability of 50 μm thick PP films and bioriented PP (BOPP) polymers after 11 months burial in landfill; they observed a reduction in the percentage of crystallinity of the polymers and an increase in the index of ketone groups.

c) Polystyrene (PS):

To date, literature reports on the biodegradation of PS are very scarce. Kaplan *et al.* (1979) reported on the biodegradation of 14C-labeled polystyrene in the presence of mixed microbial communities (which compositions were not determined) from activated sludge after 11 weeks of incubation. In the same study, pure fungal strains were reported to degrade 0 to 0.24% of polystyrene after 35 days of incubation. Mor and Sivan (2008) reported on biofilm formation on pure polystyrene flakes by the actinomycete *Rhodococcus ruber* isolate C208. Incubation of the biofilm for up to eight weeks resulted in partial biodegradation of the polymer as revealed by the 0.8% of gravimetric weight loss. Naz *et al.* (2013) reported the formation of biofilm on polystyrene cubes (cut from waste packaging polystyrene) by activated sludge under aerobic and anaerobic conditions. Moreover, monitoring for nine weeks, allowed the detection of biodegradative alterations exerted by biofilm growth on the surface of PS support as observed by SEM and revealed by FTIR analysis under both conditions. Recently, biodegradation of polystyrene blended with starch has been reported (Nikolic *et al.*, 2014).

With regard to the enzymatic degradation of PS, the depolymerisation of the polymer into small water-soluble molecules that could be detected by thin layer chromatography was also observed in the presence of a peroxidase from an *Azotobacter beijerinckii* HM121 strain applied in a two phase (aqueous and solvent) system (Gautam *et al.*, 2007).

d) Polyvinylchloride (PVC):

There are only few reports on the biodegradation of PVC polymers and plastics. Kirbas *et al.* (1999) reported on the biodegradation of PVC plastics by the white-rot fungi *Pleurotus* spp., *Polyporus versicolor* and *P. chrysosporium* and the bacterium *Pseudomonas putida*. Webb *et al.* (2000) reported that tested fungal strains of *Aureobasidium pullulans* were able to grow with the

intact plasticized PVC (pPVC) (0.5 mm thick films) as the sole source of carbon, degrade the plasticizer dioctyl adipate (DOA), produce extracellular esterase, and cause weight loss (max of about 7%) of the substratum during growth *in vitro*. Biodegradation of plasticized PVC by fungi (Kaczmarek and Krzysztof, 2007), by *Pseudomonas aeruginosa* and *Achromobacter* sp. bacterial strains isolated from soil samples (Das *et al.*, 2012) as well as after burial in soil for six months (Riaz *et al.*, 2010) also been reported. Finally, Ali *et al.* (2012) reported on the isolation of four fungal strains after burial of thin pure PVC films in sterilized soil soaked with sewage sludge for ten months. The isolates identified as *Phanerochaete chrysosporium, Lentinus tigrinus, Aspergillus niger,* and *Aspergillus sydowii* were able to grow in mineral salt medium and degrade PVC polymer after seven weeks of incubation. More specifically, *Phanerochaete chrysosporium* showed reduction of the polymer molecular weight (from 200,000 to 178,292 Da); changes on polymer surface and surface and NMR and FTIR profiles were also observed. This study is the only one reporting on effective PVC polymer degradation, while others reported on partial degradation of plasticized PVC where only plasticizers were degraded by the microorganisms.

4. Conclusions and perspectives in the light of the bioclean project

Research performed on four of the most prominent plastics, i.e. PS, PVC, PP and PEs biodegradation, both using pure strains as well as complex microbial communities has proved that biodegradation of this material is actually occurring in nature although with slow rates. The biodegradation rate is affected by abiotic factors such as UV irradiation or the presence of other prooxidant additives as well as by the physical and chemical properties of the polymer. However, biodegradation tests of synthetic plastics have been mainly performed with a limited number of pure cultures of common bacteria and fungi from collections or isolated mainly from the plastic waste soil buries. No systematic efforts were made so far to isolate microbial consortia and/or pure cultures from plastic wastes historically occurring in landfills and composting facilities or collected from marine habitats (plastic debris in this case) and biotopes where selective biogeochemical conditions might have forced native microorganisms to evolve aggressive enzymes and biodegradation pathways towards plastics.

Within the BIOCLEAN project (http://www.biocleanproject.eu/), new methodologies have been conceived and implemented for the enrichment, isolation and screening of PE, PP, PS and PVC polymer/plastic-degrading microorganisms (consortia and pure cultures) from plastic waste collected from different environments including two marine areas, anaerobic digesters, a variety of waste composting facilities and landfills. Microorganisms, i.e. bacteria (both anaerobic and aerobic) and fungi, but also enzymes produced by them, were thus selected as candidates for tailored polymer/plastic biodegradation screenings. Additional candidate organisms and enzymes (mainly peroxidases and laccases) were obtained from public/private collections, and subjected, along with the isolated strains, to screenings. Different microorganisms capable of pretreated and/or untreated target polymer degradation have been selected.

Based on the careful literature review reported here, to the best of our knowledge, no microbes able to completely degrade virgin un-pretreated PP or PEs have been reported so far; and only two reports, one on the effective biodegradation of pure PS (Mor and Sivan, 2008) and the other on PVC (Ali *et al.*, 2012) are available. On the light of the results obtained from the BIOCLEAN project, microbes apparently able to effectively degrade at least one of the four polymers were selected.

However, it should be noted that slow rates of biodegradation have been always observed, especially in the case of non-pre-treated polymers. Moreover, a careful and precise comparison of the efficiency of biodegradation between different research results is hampered by the absence of standardized procedures for the evaluation of biodegradation. Indeed, plastic films of different thickness as well as polymers of different molecular weight have been used in different experiments reported in literature. Moreover, due to the low degradation rates often observed, there is still a lack of appropriate analytical methods able to detect such low rates.
Regarding the applicability of biotechnological approaches for the mitigation of marine litter effects, microbial degradation of plastics could be considered as a long term measure rather than an immediate one since so little is known on the microbial degradation of synthetic plastics and

microbes with high biodegradation efficiency. The bioremediation of marine litter through the *in situ* application of plastic- degrading microorganisms, a strategy successfully applied in the case of oil spills, remains complicated to perform due also to the recalcitrance and poor bioavailability nature of the pollutant under study. Indeed, while hydrocarbon contamination can be contained/localized, floating plastics and microplastics are dispersed in the marine environment. However, application of microorganisms for the management/valorization of terrestrial plastic waste as an alternative/ or in parallel to landfilling would help in reducing the inputs of marine litter from land sources.

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Surface properties of marine microplastics that affect their interaction with pollutants and microbes

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Abstract

Interaction between pollutants and marine microplastics can occur under different modes. Partitioning of hydrophobic organic pollutants into the polymer is a common mechanism that can be found in the aquatic environment. However, the microplastic surface can change with time and exposure to marine environmental conditions or due to the formation of a biofilm. This could cause the microplastics to interact with metals, polar organic compounds, and microorganisms.

Introduction

Plastic pellets are a known solid waste widely found throughout the coastal zones worldwide. They are accidentally or intentionally released in the marine environment during transportation or plastic production (e.g. in order to avoid waste disposal fees, non-properly trained personnel, etc.). Those that have lower density than water, float on the sea surface and easily end up on the shore.

Plastic pellets have been collected and analyzed from several beaches on different continents in the world as presented in Figure 1 (Mato *et al.*, 2001; Endo *et al.*, 2005; Rios *et al.*, 2007; Ogata *et al.*, 2009; Rochman *et al.*, 2012; Antunes *et al.*, 2013) and on Greek Mediterranean beaches as well (Karapanagioti *et al.*, 2011; Barcelo *et al.*, 2012). These pellets were treated with solvents in order to extract organic pollutants. The pellets were tested for accumulation of hydrophobic organic compounds (HOCs) such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), hexachloro hexane (HCHs), polyaromatic hydrocarbons (PAHs), and perfluorinated alkylated substances (PFASs). A correlation was observed between the pollutant concentration on the plastic pellet and the sediment pollution as well as the land use in terms of possible pollution sources. This suggests that plastic pellets do in fact accumulate environmental pollutants.

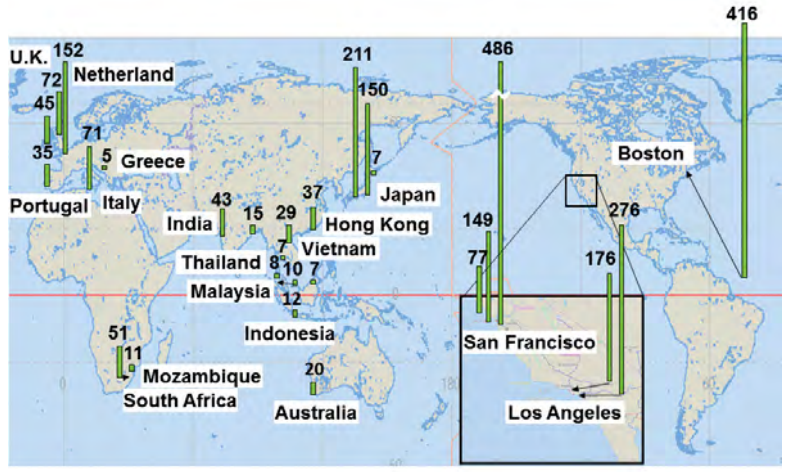


Figure 1. Levels of PCBs in (ng/g of pellet) found in polyethylene beached plastic pellets (Median concentrations of the sum of 13 PCB congeners) from Ogata *et al.*, 2009. Updated maps can be found in http://www.pelletwatch.org/

PARTITIONING AND DIFFUSION OF HOCS

Our previous studies have shown that plastic pellets can actually uptake HOCs from an aqueous solution (Karapanagioti and Klontza, 2008; Karapanagioti *et al.*, 2010). Once the pellets are in contact with water containing a HOC, the pollutant concentration starts to decrease with time (Figure 2). This process actually requires several months to reach equilibrium and the aqueous phase HOC concentration to stabilize. The proposed processes involved in such an experiment include aqueous diffusion, surface layer diffusion, surface adsorption and diffusion within the polymer (absorption). It seems that this last step is also the time limiting factor. Thus, the thicker the polymer the more time for sorption to reach equilibrium.

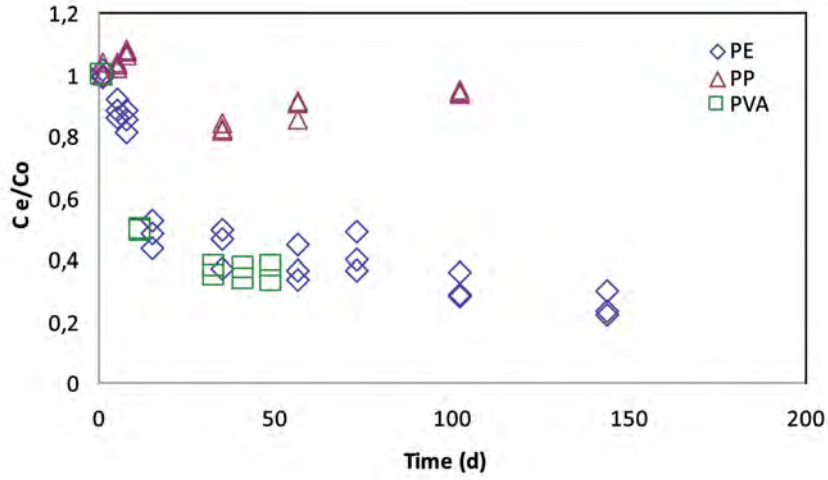


Figure 2. Relative phenanthrene concentration (Ce/Co) with a starting concentration equal to Co= 100 μg/L in saltwater solutions after the addition of polyethylene (PE) or polypropylene (PP) or polyvinyl alcohol (PVA) plastic pellets. Equal fractions of polymer mass per solution were used in each case.

However, sorption kinetics also varies based on the polymer type of the pellet (PE vs PP vs PVA in Figure 2). PVA and PP reach an equilibrium value faster than PE. However, PVA and PE sorb a similar amount of phenanthrene at equilibrium. Since PVA is a porous polymer as seen in Figure 3, phenanthrene will have to diffuse inside the macropores but after that the length that phenanthrene has to diffuse inside the polymer is short. Whereas PE and PP are both nonporous solids (Fotopoulou and Karapanagioti, 2012) but still PE sorbs more than PP. This suggests that

HOCs accumulate on the PP surface (adsorb) or its external layer and that they penetrate in the interior of the PE (absorb).

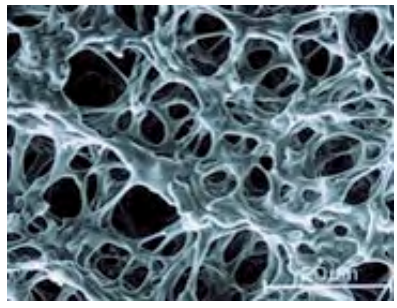


Figure 3. Photograph of PVA gel surface taken with an electron microscope as provided by the manufacturer (http://www.kuraray-aqua.co.jp/en/product/pvagel.html).

Previous studies have reported that the surface area of PE pellets is 0.13 m^2/g (Fotopoulou and Karapanagioti, 2012). Since phenanthrene molecular surface area is 198 $Å^2$ and its mol weights 178 g (Werner and Karapanagioti, 2005), the monolayer of flat phenanthrene molecules (assuming that there is no interaction among molecules) on surface area equal to the surface area of PE pellets weighs 0.6 μg/kg. The weight of the phenanthrene sorbed into PE pellets is equal to 1000000 μg/kg (Karapanagioti and Klontza, 2008) occupying 0,1% of the PE pellet volume (with phenanthrene density of 175 cm^3/mol; Werner and Karapanagioti, 2005). The difference of phenanthrene mass on the surface to the phenanthrene mass sorbed can explain why the surface area of the pellet is not important in terms of phenanthrene sorption.

The fact that phenanthrene can penetrate the polymer phase has been demonstrated in previous studies using polyoxymethylene pellets (POM) and PAHs (Ahn *et al.*, 2005). Using a highly specialized technique called microprobe two step laser-desorption laser-ionization mass spectrometry (μL^2MS) it was possible to measure PAHs on the solid surface at distances equal to 40 μm. The concentration of PAHs was measured on the interior surface of sliced POM pellets after various times of exposure to PAH aqueous solutions. It was observed that phenanthrene penetrated the POM pellet after more than 28 days and at equilibrium (238 days) it reached an equal value throughout the POM pellet cross section diameter (Figure 4). At the same time, during a parallel experiment, pyrene that is a larger molecule could not penetrate the POM pellet to reach its center. More studies are needed on this topic to better understand the penetration mechanism of the pollutant inside the polymer.

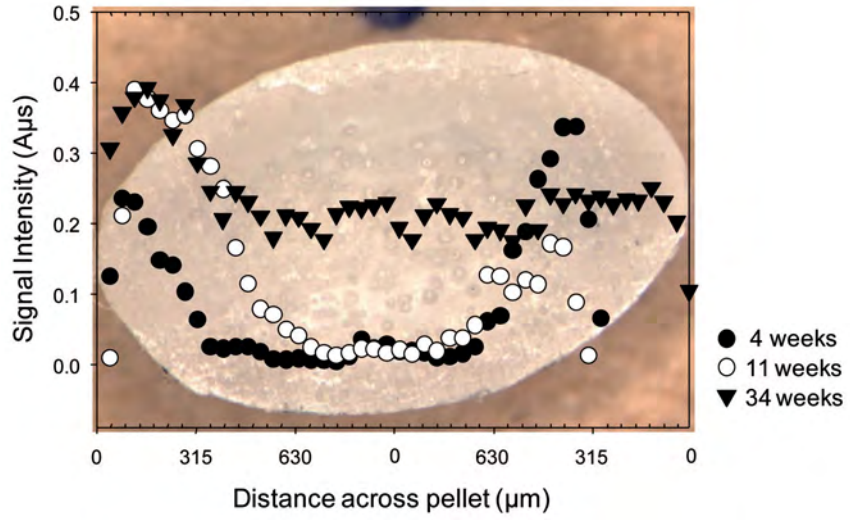


Figure 4. Phenanthrene concentration measured along the diameter of a sliced POM pellet with exposure time (original data presented in Ahn *et al.*, 2005).

PLASTIC SURFACE CHARACTERISTICS

Exposure of plastics to environmental conditions is expected to result in mechanical alteration of their surface. However, exposure to sunlight and saltwater can cause the surface to be oxidized and thus, result into the formation of functional groups containing oxygen on the surface. PE pellets collected on beaches were characterized with Scanning Electron Microscopy (SEM) as seen in Figure 5 and Fourier Transform Infrared (FTIR) spectroscopy as seen in Figure 6 and the chemical alteration was documented (Fotopoulou and Karapanagioti, 2012). It was observed that the deeper the yellowing color of the pellet, the bigger the ketone group signal in the FTIR analysis (Figure 7). This suggests that PE can now interact with species that can form bonds with ketone functional groups.

This observation was not evident for PP pellets in Figure 5 where only mechanical cracks were observed on the surface (Fotopoulou and Karapanagioti, 2012).

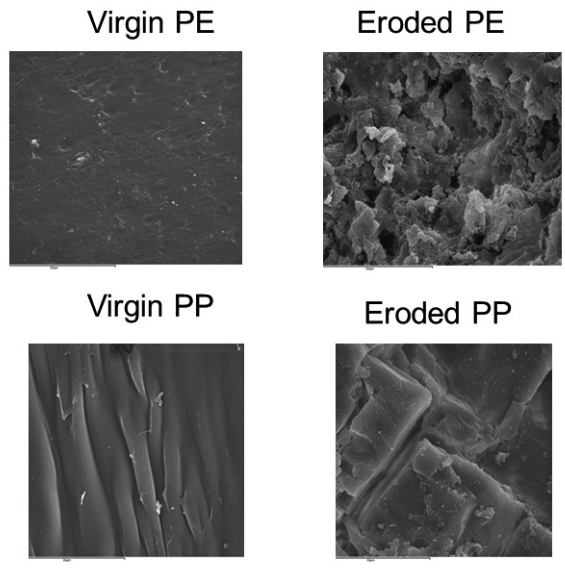
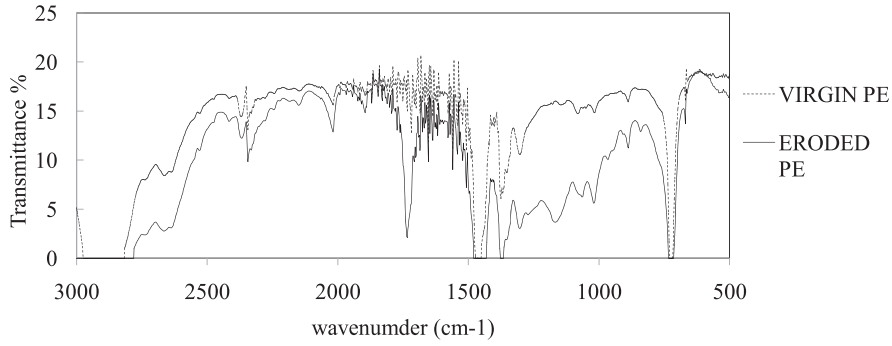


Figure 5. The surface topography of virgin plastic pellets from SEM for virgin and beached, PE and PP pellets enlarged 1000 times (Note the gray scale bar at the bottom of the image; scale bar 60 μm).



For the virgin PE pellets, three main peaks are identified at wavenumbers
722 :CH_2 rocking
1490 :CH_2 bending
3000 :CH_2 stretching
For eroded PE new peaks identified at wavenumders:
1734 cm^{-1}: ester carbonyl,
1715 cm^{-1}: ketone.

Figure 6. FTIR spectra of a virgin and an eroded beached PE pellet.

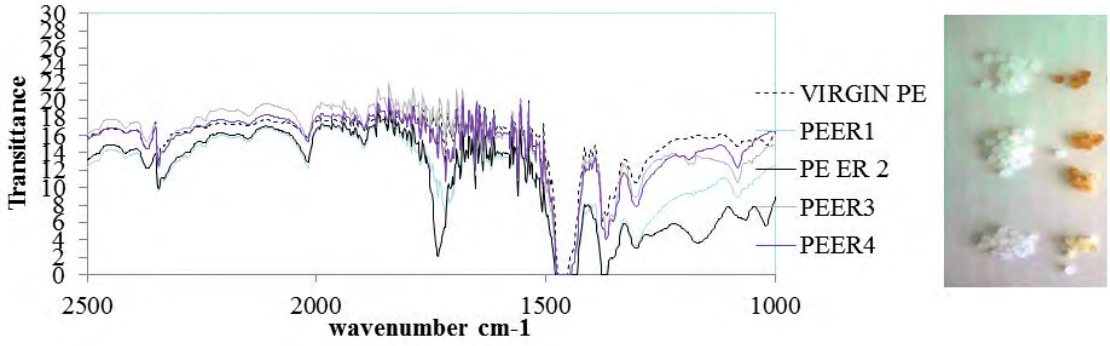


Figure 7. FTIR spectra of beached PE pellets demonstrating different yellowing.

Polymer acid base behavior

In all cases that virgin PE or PP was titrated in a non-reactive electrolyte solution (e.g. $NaNO_3$ or NaCl), no acid base behavior was observed. The titration curve of the electrolyte solution with the polymer is parallel to the titration curve of the electrolyte solution without the polymer (the blank in Figure 8). No intersection point between the two titration curves was observed either for virgin PE plastic pellets (Figure 8) or for PE biocarrier materials (Figure 9). This suggests that no functional groups can be found on the PE surface. If functional groups were present on the solid surface then, the two titration curves would intersect. The intersection point would be the pH at which the total charge of the surface is equal to zero (point of zero charge, pzc). Similar titrations with a virgin polar polymer biocarrier (PVA) resulted at a pzc value equal to 9.4. At pH lower than 9.4 the surface is positive and at pH higher than 9.4 the surface will be negative (Sfaelou *et al.*, 2011). Such a behavior was only observed for eroded PE pellets and the pzc determined was 6.1 as seen from the common intersection point in Figure 8.

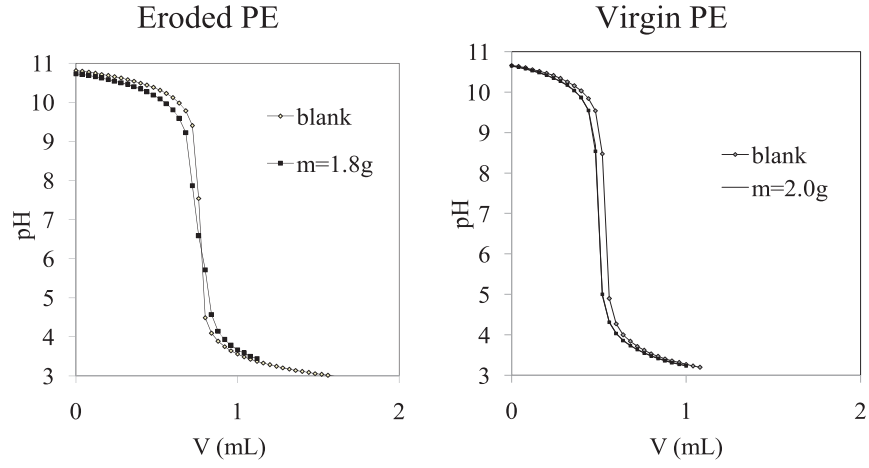


Figure 8. Potentiometric mass titrations of beached eroded PE and virgin PE pellets in $NaNO_3$ solution using HNO_3. m refers to the mass of pellets titrated.

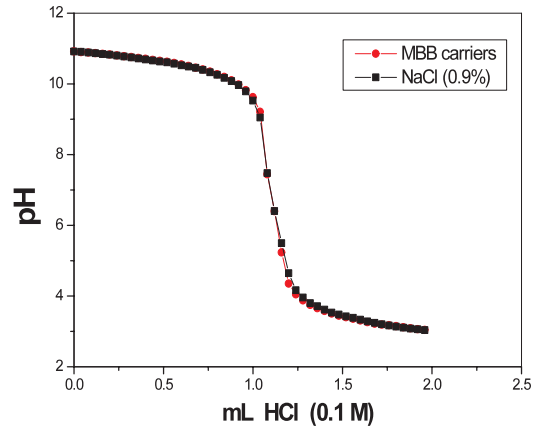


Figure 9. Potentiometric mass titration of PE biocarrier.

Biofilm formation on plastic surface

In another study, a mixture of microbes (activated sludge) with an apparent pzc value of 8.0 was kept in contact with PE biocarriers for two months. After this time, the PE biocarrier was titrated and the pzc obtained was 6.9 as can be seen in Figure 10 (Sfaelou *et al.*, 2013). This observation suggests that PE surface acid-base behavior was altered by the formation of biofilm. Either only the microbes that had a pzc value of 6.9 were preferentially attached to PE surface or the formation of biofilm resulted in the generation of an extracellular polymeric substance (EPS) matrix or a combination of both. In any case, the surface of PE was altered by the presence of the biofilm and thus, its ability to interact with pollutants has changed.

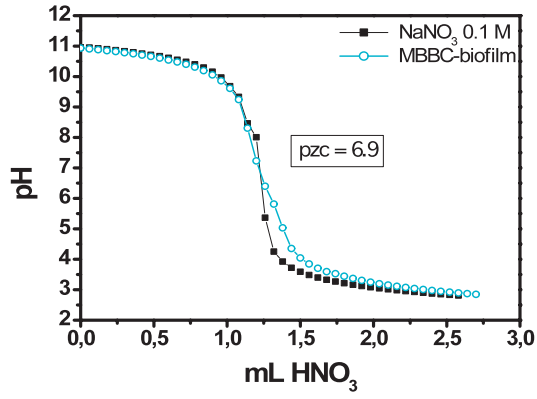


Figure 10. Potentiometric mass titration of PE biocarrier after 2 months in contact with a mixture of microbes.

The diffusion coefficient of organic pollutants is higher in the biofilm compared to the plastic. For example for phenanthrene, the diffusion coefficient in a biofilm was measured equal to $D = 4.5\ 10^{-10}$ cm^2/s (Wicke *et al.*, 2007) and in PE it was measured equal to $D = 3\ 10^{-11}$ cm^2/s (Karapanagioti and Klontza, 2008). This suggests that phenanthrene sorption kinetics are faster into the biofilm compared to PE. Thus, at the same time period, the plastic with the biofilm is expected to sorb a higher amount of a chemical than the plastic itself.

Conclusions

Although plastic is commonly considered an inert material, after its exposure to marine environmental conditions it can obtain different characteristics that potentially changes its interaction with pollutants and microbes.

Acknowledgements: The authors would like to thank S. Sfaelou from the University of Patras, D. Werner from Newcastle University, C. Rochman from University of California, Davis for providing part of the valuable data and figures.

Pathways of introduction of marine alien species in European waters and the Mediterranean – A possible undermined role of marine litter

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Abstract

In recent assessments of pathways of introduction of alien marine species in European seas, shipping, corridors (Suez canal and inland corridors), aquaculture, and aquarium trade have been identified as the most important (in decreasing order). In the Mediterranean Sea, the same pathways have been identified as the main ones, with the Suez Canal being the most important. The role of marine litter as a vector of introduction or secondary spread of alien species in the Mediterranean has not been considered and studied so far. Primary introductions of alien species in the Mediterranean Sea by rafting on floating litter through the Suez Canal or the Gibraltar strait could have occurred. Furthermore, the huge amounts of floating plastic in the Mediterranean offer increased opportunities for many alien species to further spread in the Basin. Thirteen established aliens in the Mediterranean are known to be able to colonize floating litter. Furthermore, as inferred from their life cycle and traits, more than 80% of the known alien species in the Mediterranean could potentially use litter for further expanding their range (after their initial introduction).

Introduction

Mediterranean marine ecoregions are amongst the most impacted ecoregions globally (Halpern *et al*., 2008) due to increasing levels of human pressures that affect all levels of biodiversity (Coll *et al*., 2012; Micheli *et al*., 2013), to severe impacts from climate change (Lejeusne *et al*., 2010), and to biological invasions (Zenetos *et al*., 2010; 2012; Katsanevakis *et al*., 2013). Nearly 1,000 marine alien species have been introduced in the Mediterranean up to now CIESM Atlas Series (Galil *et al.*, 2002; Golani *et al.*, 2002; Verlaque *et al.*, 2015 in press; Zenetos *et al.*, 2003), of which more than half are considered to be established and spreading. Some of these species have become invasive and substantially modify the recipient ecosystems acting as ecosystem engineers, change community structure, affect food-web properties and ecosystem processes, impede the provision of ecosystem services, impact human health, and cause substantial economic losses (Grosholz, 2002; Wallentinus and Nyberg, 2007; Molnar *et al*., 2008).

To address the problem of invasive species and to protect native biodiversity and ecosystem services, the Convention on Biological Diversity (CBD) has set the following target (Aichi Target 9): "By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment." In the European Union, this target has been adopted by the 'EU Biodiversity Strategy' (EU, 2011) and has led to the proposed new Regulation 'on the prevention and management of the introduction and spread of invasive alien species' (EU, 2013).

However, in the marine environment, eradication of invasive alien species is impossible in almost all cases, except in the very early stages of introduction (e.g. the eradication of *Caulerpa taxifolia* in California; Anderson, 2005). Prevention is by far more cost effective and environmentally desirable than post-introduction measures, which are both costly and of low probability of success. The only way to prevent new arrivals of alien marine species is by effectively managing the related pathways of introduction. Pathways and vectors of new arrivals (primary) and of further spread of established aliens (secondary) need to be first identified, and then proper management actions need to be decided.

Floating marine litter is a potentially important vector of primary introduction or of further (secondary) spread of alien species. The vast availability of anthropogenic rafting material (Galgani, this volume) can greatly assist the transport of species beyond their natural boundaries and their introduction to environments where they were previously absent (Winston, 1982; Barnes, 2002; Barnes and Milner, 2005; Barnes, this volume). Barnes (2002) estimated that human litter more than doubles the rafting opportunities for biota, assisting the dispersal of alien species. However, the role of marine litter for the introduction and spread of alien species has been understudied in the European Seas. Marine litter has not been included in any of the major recent assessments of pathways in Europe (Zenetos *et al*., 2012; Katsanevakis *et al*., 2013; Galil *et al*., 2014; Nunes *et al*., 2014).

The aim of this paper is to review our current knowledge on the pathways of introduction of alien marine species in Europe, focusing specifically in the Mediterranean, and to investigate the potential role of floating marine litter as a primary or secondary pathway.

Recent assessments of pathways of introduction in Europe

By critically reviewing related information in the scientific/grey literature and online resources, Katsanevakis *et al*. (2013) identified 1369 alien marine species in European seas, of which 92% were linked to the most probable pathway(s) of introduction (Fig. 1) the same percentage as found for the 986 alien species identified by Zenetos *et al*. (2012). Five categories of pathways of introduction were used in these assessments: 'shipping' (subdivided into 'ballasts' and 'fouling'), 'corridors' (subdivided into 'Suez', which is the only marine man-made corridor in Europe, and 'inland canals'), 'aquaculture' (subdivided into 'commodity' and 'contaminant'), 'aquarium trade' (also including escapes from public aquaria), and 'other' (including: live food/bait trade, floating objects, and import for military purposes).

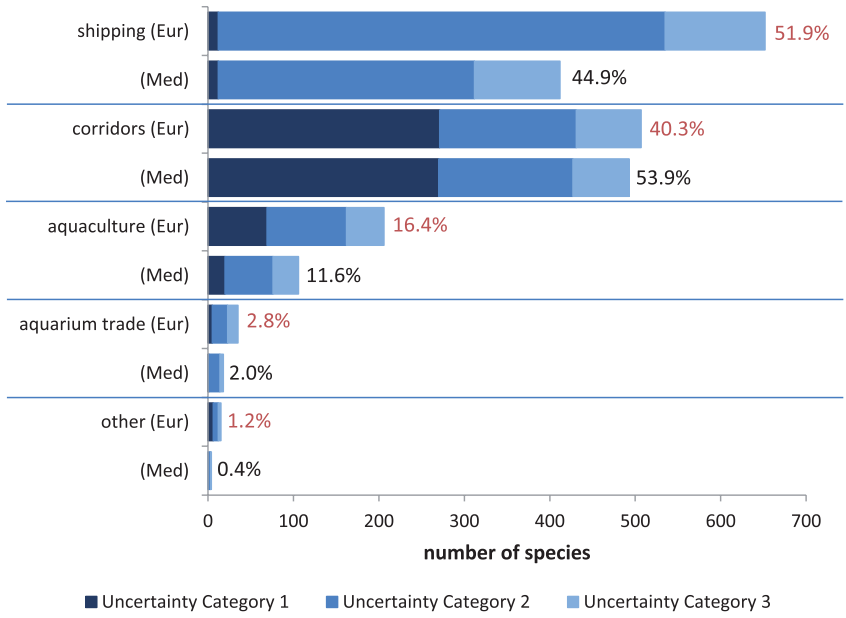

Figure 1. Number of marine alien species known or likely to be introduced by each of the main pathways, in Europe (Eur) and the Mediterranean (Med). Percentages add to more than 100% as some species are linked to more than one pathway (red percentages refer to the European total, black percentages to the Mediterranean total). Uncertainty categories: (1) there is direct evidence of a pathway/vector; (2) a most likely pathway/vector can be inferred; (3) one or more possible pathways/vectors can be inferred; (4) unknown (not shown in the graph). Modified from Katsanevakis *et al.* (2013) and Zenetos *et al.* (2012).

New introductions of alien species in Europe, and in the Mediterranean Sea in particular, have an increasing trend, reaching almost 200 new species introductions per decade (Fig. 2). Shipping and corridors are the two most important pathways in Europe and the Mediterranean Sea. Many more species are expected to invade the Mediterranean Sea through the Suez Canal, as it has been continuously enlarged and the barriers for the invasion of Red Sea species have substantially decreased (Katsanevakis *et al*., 2013). The increasing trend observed in new introductions by shipping is not expected to halt unless effective measures are taken, such as the ratification of IMO's (BWM Convention) (International Convention for the Control and Management of Ships' Ballast Water and Sediments). Nevertheless, introductions by hull-fouling, which was identified as the most common vector for marine alien species so far introduced in European seas (Katsanevakis *et al*., 2013), will remain or even increase due to the recent adoption of the IMO Anti-fouling Convention in 2004 and the banning of the most effective (i.e. most toxic) of the anti-fouling hull coatings.

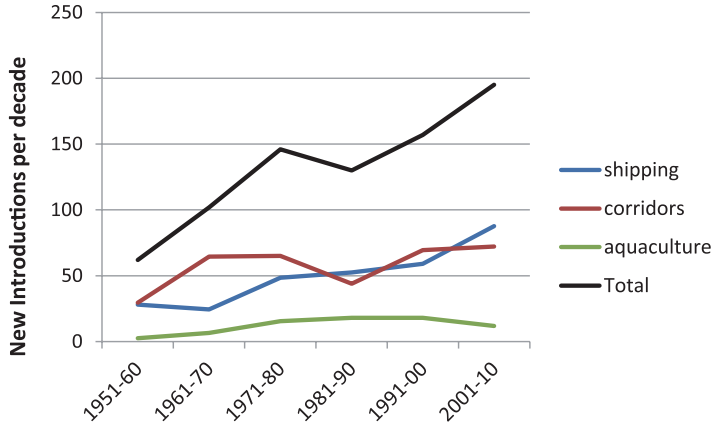

Figure 2. Trends in new introductions of alien marine species in the Mediterranean per decade (trends in total introductions and for the three most important pathways).

PLASTIC SURFERS: A GENERAL OVERVIEW

Many species may extend their distribution range through transport by floating rafts rather than through the active or passive dispersal of reproductive propagules (Highsmith, 1985; Aliani and Molcard, 2003; Barnes and Milner, 2005). Passive transport of juveniles or adult algae and invertebrates on natural floating debris (whether biotic or abiotic, such as trunks, seaweeds, seeds, volcanic pumice, cephalopods' sepion bones and buoyant corals) can last for years, with marine current-driven journeys covering vast distances across the ocean before landfall (De Vantier, 1992; Thiel and Gutow, 2005a, b).

With the onset of human travels at sea, especially commercial shipping, many marine organisms drastically increased their dispersal by travelling on vessel hulls or with ballast water. Nevertheless, over the past 4-5 decades anthropogenic floating litter offered a massive and historically unparalleled new opportunity for rafting species. During the last few decades, the availability of rafting material has increased dramatically, mostly because of the wide use of plastics, which currently constitute the larger percentage of floating litter (Katsanevakis, 2008).

Floating marine litter is commonly colonized by a wide variety of surfers, spanning almost all known taxa (e.g. bacteria, macro and micro-algae, barnacles, hydroids, bryozoa, sponges, polychaetes, sea-urchins, molluscs, and tunicates) (Carpenter *et al*., 1972; Carpenter and Smith, 1972; Winston, 1982; Minchin, 1996; Aliani and Molcard, 2003; Barnes and Fraser, 2003; Barnes and Milner, 2005; Katsanevakis, 2008). If favourable conditions are found in the new ground, the alien species may settle and become established. However, attributing a marine biological invasion to floating marine litter and not to other mechanisms is very difficult in most cases, and available data are generally insufficient. In the Mediterranean Sea, the role of marine litter as primary or secondary pathway of new introductions and translocations of species is largely neglected.

The impact of floating marine litter on biological invasions is difficult to measure and compare with other sources such as shipping or transportation on natural mobile hosts. Lewis *et al*. (2005) have questioned the extent to which floating marine litter may contribute to the introduction of exotic species in the Southern Ocean, and considered shipping as the most likely transport mechanism in that region. They stressed that floating litter follows already established passive dispersal routes, dependent on ocean currents and the wind, while ships create novel pathways, moving across currents and often visiting many locations over short periods of time. However, the availability of floating litter, mostly plastics, has become huge, offering substantial rafting opportunities for encrusting fauna and flora, especially in areas where there are only a few natural sources of flotsam. Marine litter should be considered as a serious potential vector for marine invasions, but further investigation is necessary.

TAXON-SPECIFIC OVERVIEW

The following review does not focus in the Mediterranean Sea and specifically on alien species. It intends to review the potential of species translocations via floating litter by reviewing taxa that have been reported to attach to drifting litter.

ALGAE and PLANTAE

Both unicellular and multicellular algae have been reported as living on plastic debris, including harmful algal bloom species. Masó *et al*. (2003) reported *Alexandrium taylori*, an *Ostreopsis* and a *Coolia* taxon living on plastic debris along the Catalan coast (north western Mediterranean), whilst diatoms have been often found on floating plastic spherules (*Cyclotella meneghiniana*, *Mastogloia angulata* and *Mastogloia hulburti*: see Carpenter and Smith, 1972). Pennate and centric diatoms have been also found by Carson *et al*. (2013b) between plastic-associated microorganisms of the North Pacific Gyre. Within Clorophyta, *Ulva rigida* has been reported from off Azores (Morton and Britton, 2000), and *Ulva* species have been also reported from off Chile, together with *Codium fragile* and *Bryopsis rhizophora* (Astudillo *et al*., 2009). The presence of several Rhodophyta living on floating plastics has been mostly stated, by genus only, by Winston *et al*. (1997): *Amphiroa*, *Fosliella*, *Jania*, *Lithophyllum* and *Mesophyllum*. To this list, Astudillo *et al*. (2009) added *Polysiphonia mollis*, *Antithamnion densum*, *Corallina officinalis* and a *Rhodymenia*

and *Gelidium* species. Aliani and Molcard (2003) reported *Hydrolithon farinosum* and unidentified algae from the Mediterranean Sea, and also included a *Cystoseira* sp. and *Posidonia oceanica* (including the bryozoan *Electra posidoniae* on his leaves, see below). Astudillo *et al*. (2009) also included *Zostera tasmanica* as found on detached plastic buoys, as well as three Ochrophyta species (*Scytosiphon lomentaria*, *Hincksia granulosa* and *Ectocarpus acutus*).

BRYOZOA

Bryozoan species are indeed among the most common plastic debris colonizers due to their ability to encrust nearly all hard and even soft surfaces. Stevens *et al*. (1996) reported 47 species from New Zealand, whilst Rocha Farrapeira (2011) reported 19 taxa and Goldstein *et al*. (2014) at least 16 taxa. All of them included widespread aliens and species with pelagic cyphonautes larvae such as *Electra tenella* and *Jellyella tuberculata*. Winston (1982) also often reported *E. tenella* as the only bryozoan species on plastic debris cast up on beaches along the Atlantic coast of Florida. Gregory (1978) suggested that the cosmopolitan *J. tuberculata* may have crossed the Tasman Sea from Australia to New Zealand via rafting on plastic pellets. Barnes and Fraser (2003) listed 5 different bryozoan species (*Aimulosia antarctica*, *Arachnopusia inchoata*, *Ellisina antarctica*, *Fenestrulina rugula* and *Micropora brevissima*) on a plastic packaging band in the Southern Ocean, and Thiel and Gutow (2005) reported that the erect bryozoan *Bugula neritina* can be found on a wide variety of different substrata, including plastic surfaces. It has also been reported by Astudillo *et al*. (2009), together with six other taxa (including *Cryptosula pallasiana*, *Membranipora isabelleana* and *Bugula flabellata*), from detached plastic buoys sampled in the Bay System of Coquimbo (Chile). Aliani and Molcard (2003) listed four bryozoan species on floating debris (*Bowerbankia gracilis*, *Callopora lineata*, *Electra posidoniae* - see above in algae and plantae - and *Membranipora membranacea*). *Thalamoporella evelinae* is well known to have been introduced to the Atlantic by drift plastics (Winston *et al*., 1997; Derraik, 2002). Another common Caribbean and South Floridian bryozoan, *Schizoporella pungens*, was first found in the Indian River area on plastic drift in 2002. The following year it appeared on fouling panels in the Fort Pierce inlet, and since the summer of 2003 has been found on natural and artificial substrata in the inlet and adjacent Indian River Lagoon. Winston (2012) suggested that its rapid northward spreading may have been expedited by rafting.

ARTHROPODA

Cirripedia are common surfers. Within Sessilia, both *Amphibalanus amphitrite* and *Amphibalanus eburneus* have been found off Florida (Winston *et al*., 1997), whilst Morton and Britton (2000) reported *Coronula diadema* and *Xenobalanus globicipitis* from the Azores, and Astudillo *et al*. (2009) *Balanus laevis*, *Balanus flosculoidus* and *Austromegabalanus psittacus* from the Chilean shores. Barnes and Milner (2005) found *Semibalanus balanoides* and the alien *Austrominius modestus* on plastic debris in the Shetland Islands (north Atlantic Ocean). *A. amphitrite*, *A. eburneus*, *Balanus trigonus*, *Chelonibia patula*, *Conchoderma auritum*, *Coronula diadema* and *Striatobalanus amaryllis* have been listed by Rocha Farrapeira (2011) in his review of species associated with floating plastic debris off Brasil. Finally, Goldstein *et al*. (2014) listed *Megabalanus rosa* and *Chthamalus* species, among other crustaceans. Within Lepadiformes, *Dosima fascicularis*, *Lepas anatifera*, *Lepas anserifera*, *Lepas australis*, *Lepas hilli*, *Lepas pacifica* and *Lepas pectinata* are well known to be transported throughout plastics, often together with their main predator (see below in Mollusca), and can form very dense assemblages on floating substrata (Thiel and Gutow, 2005b; Barnes and Milner, 2005; Astudillo *et al*., 2009; Rocha Farrapeira, 2011; Goldstein *et al*., 2014). Astudillo *et al*. (2009) also reported 17 decapoda from detached buoys, although within Brachyura the most commonly reported species are the pelagic *Planes marinus* and *Planes minutus*, to which Goldstein *et al*. (2014) added *Planes major*. Several Amphipoda (*Calliopius laeviusculus*, *Dexamine thea*, *Phtisica marina* and *Caprella andreae*, *Jassa marmorata*, *Jassa slatteryi*, *Paradexamine pacifica*, *Elasmopus rapax*, *Caprella equilibra*, *Caprella verrucosa*, *Caprella scaura*, *Deutella venenosa*, *Paracaprella pusilla* and *Zeuxo marmoratus*), Isopoda (*Idotea balthica*, *Idotea metallica* and *Synidotea marplatensis*) and Pycnogonida have been also reported from floating plastic debris, but mostly on early detached buoys (see Thiel and Gutow, 2005b; Astudillo *et al*., 2009; Rocha Farrapeira, 2011; Goldstein *et al*., 2014).

MOLLUSCA

Bivalves are common epibionts on floating plastic litter but gastropods may occur as well. Barnes and Fraser (2003) reported the gastropod *Laevilacunaria antarctica* on a plastic packaging band in the Southern Ocean, whilst Scarabino (2004) reported *Litiopa melanostoma* on a plastic tube. This species was also reported by Goldstein *et al*. (2014). Even vermetid gastropods may be associated with plastic debris, as reported in Breves and Skinner (2014) for *Petaloconchus varians*. Astudillo *et al*. (2009) reported 19 taxa on detached plastic buoys, including six different "opisthobranch" species. Aliani and Molcard (2003) also reported a *Doto* from the Mediterranean Sea. A usual species reported on plastic debris is the pelagic *Fiona pinnata*, often feeding on *Lepas* spp., that in turn are common cirripedia associated to both antropogenic and natural floating debris (see above in Arthropoda) (Aliani and Molcard, 2003; Scarabino, 2004; Thiel and Gutow, 2005b). Goldstein *et al*. (2014) also reported *Odostomia tenuisculpta*. It should have been transported in association with its bivalve prey. Bivalves attaching to plastic debris with byssal threads, such as Arcidae, Mitilidae, Anomiidae, Pectinidae or Pteridae have often been reported. *Semimytilus algosus*, *Brachidontes granulatus* and *Argopecten purpuratus* were found on detached plastic buoys (Astudillo *et al*., 2009), whilst Winston *et al*. (1997) reported *Anomia*, *Pinctada*, *Pteria* and *Isognomon* species. Also Gregory (2009) reported *Pinctada* from Bermuda, and *Pinctada imbricata* was found on floating plastic off Brasil (review in Rocha Farrapeira, 2011). Goldstein *et al*. (2014) listed an unidentified Arcidae, *Chlamys* and *Pinctada* species and *Mytilus galloprovincialis*. *Mytilus edulis* was also found on marine litter at several sites (review in Thiel and Gutow, 2005b). Even cemented species, such as Ostreidae and relatives, may be easily transported throughout floating plastic. Winston *et al*. (1997) reported the non-indigenous oyster *Lopha cristagalli* on plastics washed ashore in southern New Zealand, and a *Crassostrea* and a *Chama* species (Chamidae) in Florida. Goldstein *et al*. (2014) reported *Crassostrea gigas* on floating plastic.

CNIDARIA

Cnidarian polypoid phases or Anthozoa can be easily found on floating plastic debris. Several species of the genus Clytia (*Clytia gregaria*, *Clytia hemisphaerica* and *Clytia gracilis*) have been recorded as such (review in Thiel and Gutow, 2005b; Goldstein *et al*., 2014), while Aliani and Molcard (2003) also reported *Laomedea angulata*, *Obelia dichotoma*, *Eudendrium* sp. and *Gonothyraea loveni* from the Mediterranean Sea. In turn, a congeneric of the latter, *Gonothyraea hyalina*, has been found in the Sargasso Sea (Carpenter and Smith, 1972). Winston *et al*. (1997) also recorded a *Millepora* sp. and *Phyllangia americana* from off Florida, and the latter has been also recorded from off Brasil (see below). Jokiel (1984) reported a *Pocillopora* species. Hoeksema *et al*. (2012) reported trans-Atlantic rafting by the brooding reef coral *Favia fragum*. Astudillo *et al*. (2009) reported *Plumularia setacea*, an *Obelia* species and further hydrozoans, as well as the anthozoa *Anthothoe chilensis*, on detached buoys off Chile. Five cnidarians (*Aglaophenia latecarinata*, *Halecium nanum*, *Plumularia strictocarpa*, *Rhizogeton sterreri* and *Phyllangia americana*) have also been reported on floating plastic off Brasil (review in Rocha Farrapeira, 2011).

ANNELIDA

Several annellids have been reported on floating plastics. Winston *et al*. (1997) reported *Hydroides dianthus* and *Hydroides elegans* from off Florida and New Zealand, respectively. Aliani and Molcard (2003) listed *Nereis falsa* and *Spirobranchus polytrema*, whilst Rocha Farrapeira (2011) included *Hydroides dianthus* from off Brasil, a taxa well known as a worldwide invader. Three Spirorbinae species (*Spirorbis spirorbis*, *Spirorbis corrugatus* and *Circeis spirillum*) have also been recorded on plastic debris (see Thiel and Gutow, 2005b). Astudillo *et al*. (2009) listed 19 taxa from detached plastic buoys (including *Romanchella pustulata*, *Dodecaceria opulens*, *Autolytus simplex*, *Typosyllis magdalena*, *Platynereis australis*, *Pseudonereis gallapagensis*, *Halosydna patagonica* and *Steggoa magalhaensis*), whilst Goldstein *et al*. (2014) reported at least 14 taxa, including *Amphinome rostrata* and *Hipponoe gaudichaudi*.

OTHER

Carson *et al*. (2013b) reported *Bacillus* and coccoid bacteria, as well as the radiolarian *Circorrhegma dodecahedra*, between plastic-associated microorganisms of the North Pacific Gyre. Goldstein *et al*. (2014) found different folliculinid ciliates belonging to the genus *Halofolliculina*, pathogens that cause skeletal eroding band (SEB) disease in corals, and suggested that floating plastic debris facilitated the dispersal of *Halofolliculina* to new areas. Other taxa found on plastic floating debris include Echinodermata (*Arbacia lixula*: Aliani and Molcard, 2003; *Patiria chilensis* and *Tetrapygus niger*: Astudillo *et al*., 2009), Foraminifera (*Acervulina* sp. and *Homotrema rubra*: Winston *et al*., 1997; *Planulina ornata*: Goldstein *et al*., 2014; *Rosalina globularis*: Jorissen, this volume), Porifera (*Halichondria panacea* and *Sycon* spp.: Goldstein *et al*., 2014), Platyhelminthes (Astudillo *et al*., 2009; Goldstein *et al*., 2014), Nemertinea (Astudillo *et al*., 2009) and Chordata (*Pyura chilensis*, *Ciona intestinalis* and *Diplosoma* sp.: Astudillo *et al*., 2009).

Marine litter as a pathway in the Mediterranean

Based on the present review of the capacity of many species to use floating litter as a means to extend their distribution, we assessed which of the species reviewed by Zenetos *et al*. (2012) could have potentially used or can use litter as a primary or secondary means of translocation. In many cases, plastic can be colonized more easily than metals painted by anti-fouling paints (e.g. vessel hauls), and thus species that have been reported to foul the hauls of vessels can, quite probably, colonize floating plastic as well. We defined three categories of increasing probability of litter acting as a primary or secondary vector for the assessed species:

- category 1: the species has been reported to attach to floating marine litter;
- category 2: shipping/fouling has been reported as a probable vector of introduction;
- category 3: the species could potentially attach to floating marine litter, as judged based on its life cycle and traits, e.g. shallow water byssed bivalves, bryozoans, ascidians, hard-substrate polychaetes, barnacles, hydrozoans with a polypod stage.

We found that 81% of the species having been reported to be introduced in the Mediterranean Sea by a different pathway, might have been introduced by marine litter or could use litter for further expanding their range (Categories 1, 2, and 3; Fig. 3). Category 1 included thirteen species: the alga *Codium fragile fragile*, the bryozoans *Electra tenella* and *Rhynchozoon larreyi*, the crustaceans *Caprella scaura*, *Jassa marmorata*, *Amphibalanus eburneus*, *Austrominius modestus*, and *Balanus trigonus*, the molluscs *Crassostrea gigas* and *Pinctada imbricata radiata*, and the polychaetes *Platynereis australis*, *Hydroides dianthus*, and *Hydroides elegans*.

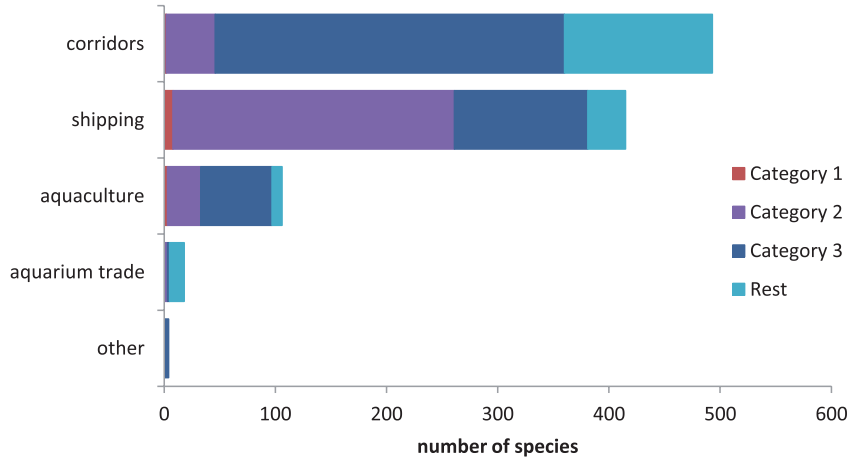

Figure 3. Number of alien marine species known or likely to be introduced by each of the main pathways (after Zenetos *et al.*, 2012). For each pathway, the number of aliens that could have been initially introduced by marine litter or may potentially use marine litter as a means of secondary range expansion are indicated (Categories 1–3). For the definition of the three categories of uncertainty see the text.

The role of marine litter as a vector of primary introductions or translocations of alien species in the Mediterranean Sea appears largely underestimated and needs further investigation. In the absence of any such targeted survey in the Mediterranean, we may not properly assess the potentially important role of marine litter.

Marine plastic debris and colonization by bryozoans in the South Atlantic

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Abstract

In the last half century the ubiquity, abundance and 'ecological problem' of synthetic debris has grown from negligible to global concern. Plastics are accumulating in considerable amounts even on the most remote island shores, mid-ocean, polar-regions and even on the seabed deeper than the continental shelf. Recent aircraft losses over oceans, and the search for any wreckage have led to public airing of satellite imagery of just how common and dense artefacts are in the sea. Patterns of accumulation have been examined, monitored and modeled by many researchers and patterns in the larger fragment sizes of plastic (mega, macro and meso-) are reported here for the South Atlantic Ocean. The implications of plastic debris inundating even non-urban environments are considerable, and it has led to massive entanglement, chocking, poisoning and change in the distribution possibilities of organisms. Many organisms (especially those with sessile adult phases) have had their dispersal possibilities greatly expanded by plastic debris. There is much more plastic afloat than natural flotsam, it is easy to colonise, may stay buoyant for decades and, at least in theory, can travel to anywhere from anywhere (rather than port to port as most ships do). Bryozoans, colonial and clonal benthic animals, are one particular taxon which have taken great advantage of this new habitat and as such have the potential to establish as aliens, possibly invasive pests, in many locations. Here the risk to mid Atlantic island and Southern Ocean coasts are highlighted – places with currently few if any marine aliens and rich, endemic faunas.

Introduction

In the 1970s people started becoming aware of anthropogenic debris accumulating on beaches, particularly plastics. Reports of plastic debris in the middle Atlantic started to appear and public concern grew. Since then the accumulation of plastics at sea and stranding on shorelines have been widely monitored, documented and analysed (see Thompson *et al.*, 2009). More than four decades have passed since the alarm bells started to ring and the extent of accumulation of mega- and macroplastics has lead to regular beach cleaning operations on every continent. The search for mid-ocean debris from the Air France and most recently, Malaysian missing airliners focused satellites, military surveillance, international co-operation at the highest level and public attention on marine debris at sea – such that it became apparent how common large anthropogenic items are in what has been thought of as 'ocean wilderness'.

Attempt at reduction of plastics debris in the environment varies highly within and between countries. Some nations have been placed taxes on some disposal plastics, such as plastic bags and international guidelines and laws such as MARPOL V have been introduced in attempts to

combat rising levels of waste plastics in the environment. Whilst the 'problem' of such debris seems most aesthetically acute near urban centres and semi-enclosed seas such as the Mediterranean (see Galgani; Pedrotti *et al.*, this volume), it is now clear that there are considerable accumulations of plastics far from sight on the seabed (deeper even than the continental shelf), remote island shores and even into the polar regions. Key evidence for the invasion of wilderness by plastic debris has been quantified in the South Atlantic (Ryan and Moloney, 1993) and in the Atlantic sector of the Southern Ocean (Barnes, 2002). In terms of both methodology for sampling and the nature of consequences, the current study distinguishes between microplastics (such as pellets) and larger fragments (>5mm in size) – and restricts consideration entirely to the latter. Here two decades of at-sea surveys of meso (5-25mm) and macro-plastics (25+mm) around the South-West Atlantic and Scotia Sea are reported as well as a recent mid-ocean transit from South Georgia to Ascension Island, via Gough and the Tristan da Cunha archipelago. This is contrasted with data on stranding of plastics on South Atlantic shores particularly on remote islands, based on recent surveys of Bird, East Falkland, Gough, Tristan da Cunha, Inaccessible, St Helena and Ascension island shores.

Beaches are both sources and sinks of plastic and other anthropogenic debris, but usually fishing and other shipping-related material and packaging are the main types of item. Apart from beaches, the sinks at sea vary from so called 'garbage patches' in the middle of gyres, sinking to the seabed, degrading or becoming associated with organisms – such as being swallowed or entangled. Such encounters have become serious issues with major implications for populations of seabirds (such as the Wandering Albatross in the Atlantic sector of the Southern Ocean – Fig. 1a), cetaceans and sea turtles. For a few organisms plastic debris could be argued as having some positives, such as provision of habitat for neustic (organisms living at the sea surface – Fig. 1b) or similarly increasing an otherwise limited resource on shore, such as for hermit crabs (Fig. 1c). The ubiquity, abundance, longevity and buoyancy of plastics means that organisms which colonise such debris may be transported considerable distances. In many cases these are probably native species travelling within their normal geographic range but this could then drastically alter connectivity and gene flow. Such an effect on native species is intuitively likely to be most severe in regions where there is little natural flotsam, such as the Southern Ocean, so the proportional change in opportunity is greatest (Fig. 1d). More serious is when species travel beyond their normal distributions thus becoming non-indigenous (see Katsanevakis and Crocetta, this volume). Famous recent examples of this are barges, containers, docks and other material washed out to sea during the Japanese tsunami of March 2011 – and some of the estimated 1.5 million tonnes of debris crossed the Pacific to arrive in US coastal waters with tens of non indigenous species attached. It is likely that only a small proportion become established and even fewer invasive but those proportions are very difficult to quantify as there are many vectors besides plastics (such as ship or flying boat hulls, ballast water, accidental transport with cargo and even deliberate introductions). 'Alien hitch-hikers' can be readily seen on floating debris (Fig. 1e) and their spread can only be enhanced by plastic carriers. It is clear that some taxa colonise plastics more readily than others; the secretariat of the Convention on Biological Diversity (2012) found that by far the most species reported on plastic debris were bryozoans. In the study region focused on here, the South Atlantic and the Southern Ocean, bryozoans often monopolise more space than all other taxa combined (Fig. 1f) – and are therefore a useful case study of macrofaunal colonization of macroplastics.

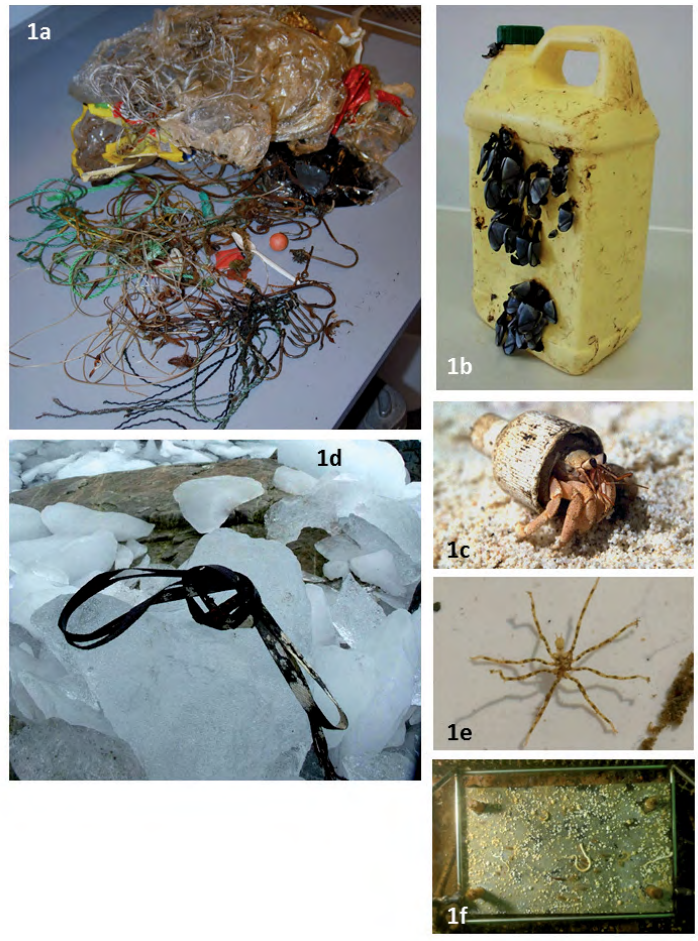

Figure 1. Plastic marine debris; from stomach contents (a), colonized by barnacles (b), a hermit crab (c), bryozoans (d), a sea spider (e), and after 6 years in the Southern Ocean (f).

Censi of mega- and macroplastics in the Atlantic Ocean

Investigations and censi of plastics floating in the North Atlantic have been patchily established in space and time, since the first interest in anthropogenic marine debris. These have been summarized at various times (see Morris, 1980) and show strong trends from urban to remote, coast to open ocean, gyre margins to centres (e.g. see Law *et al.*, 2010) as well as local effects such as local geography and wind effects (e.g. see Zambianchi *et al*., this volume). The South Atlantic has been much less well studied, apart from patchy areas of work mainly along Brazil and South Africa. As in the North Atlantic there is considerable variability in densities even across small spatial scales but overall Barnes (2005) found lower densities than in the North Atlantic and a steeper decline in density with latitude towards the Southern Ocean. Acha *et al.* (2003) have reported the highest densities of debris at sea (>150 km^{-2}) recorded in the South Atlantic (in the Rio Plata mouth, Argentina and Uruguay). They found a strong gradient from these densities rapidly decreasing to <10 km^{-2} offshore, about the levels found at a similar time by Barnes and Milner (2005). The Atlantic transect of marine debris reported by Barnes and Milner (2005) has been repeated twice – in 2006 and 2008 (Table 1a) and a new mid-ocean transect undertaken in 2013. The most recent published work, by Ryan (2014), found a similarly strong density gradient (to that out from Rio Plata) out to sea from Capetown. He reported ~100 items km^{-2} immediately around Capetown decreasing to <3 items km^{-2} until encountering a 'garbage patch' of higher accumulation with >6 items km^{-2}. Averages (in that case 67 items km^{-2} in South African coastal waters) are clearly inappropriate to describing marine debris patterns close to coastal urban areas where there are strong gradients. Ryan's (2014) study is the most detailed at sea observations recorded in the Southern Atlantic Ocean currently known.

Table 1.

a) Mega- and macroplastic density-at-sea surveys in the South Atlantic Ocean.

Latitude	**Plastic km^{-2}**			
	2002	**2006**	**2008**	**2013**
50–60 S	0-1	0-1	0-2	0-1
51 S Falkland Is	0–4	0-4	0-3	0-3
50–40 S	0–4	0-5	0-4	1-5
40–30 S	0–5	0-4	0-3	0-4
30–20 S	2–5	0-3	0-3	0-3
20–10 S	0–10	0-3	0-2	0-1
10–0 S	0	0-1	0	0-1
7 S Ascension Is	1–5	0-3	0-3	0-2

b) Macroplastic density on remote South Atlantic and Southern Ocean shores.

		Plastic m^{-1}	**% colonised**	
54 S	Bird Island	0.25-2.53	0-2	1990-present
51 S	Falkland Is	0.42-2.1	2-4	1993,2002-present
41 S	Gough Is	0.02	?	1984,2013
37 S	Tristan da Cunha	0.32-0.84	3-17	1984,2013
37 S	Inaccessible Is	0.56-2.3	24	1984,1987-1990,2013
16 S	St Helena Is	0.29-1.09	?	2003-2007
7 S	Ascension Is	1.8-3.4	19-36	2002-2004,2006,2009

Here two sets of new observations of plastic densities at sea are reported. 1) Repeat surveys of the Southwest Atlantic and Scotia Sea from RRS James Clark Ross revealed that the densities of larger fragments at sea have changed little between 1993 and 2013 (Fig. 2). Unfortunately the area surveyed is not always the same, though there is considerable overlap – but the observer, equipment, height above sea, distance from ship examined (60 m), duration of watch (3 x ~10 km distances) and size of item considered (10+ cm length) have remained constant. The most common items were packaging, shipping related and fragments. 2) RRS James Clark Ross undertook the first south-north transect of the mid-South Atlantic Ocean examining marine debris. This covered the area from South Georgia to Ascension Island via the Tristan da Cunha archipelago. From May to June 2013 daily observations of marine debris at sea were made by a single observer, during daylight, within 60 m of the vessel RRS James Clark Ross, using a pair of 12 x 50 binoculars for 3 x ~10 km distances. The minimum size of item detectable was considered to be 2-3cm in size. Only at 40-50°S were the densities different (higher) than along the South-North transect taken by the vessel in 2002, 2006 and 2008 (see Table 1a). This corresponds with both the East-West findings of Ryan (2014) of a South Atlantic 'garbage patch' concentrated by being in the approximate centre of a current gyre system.

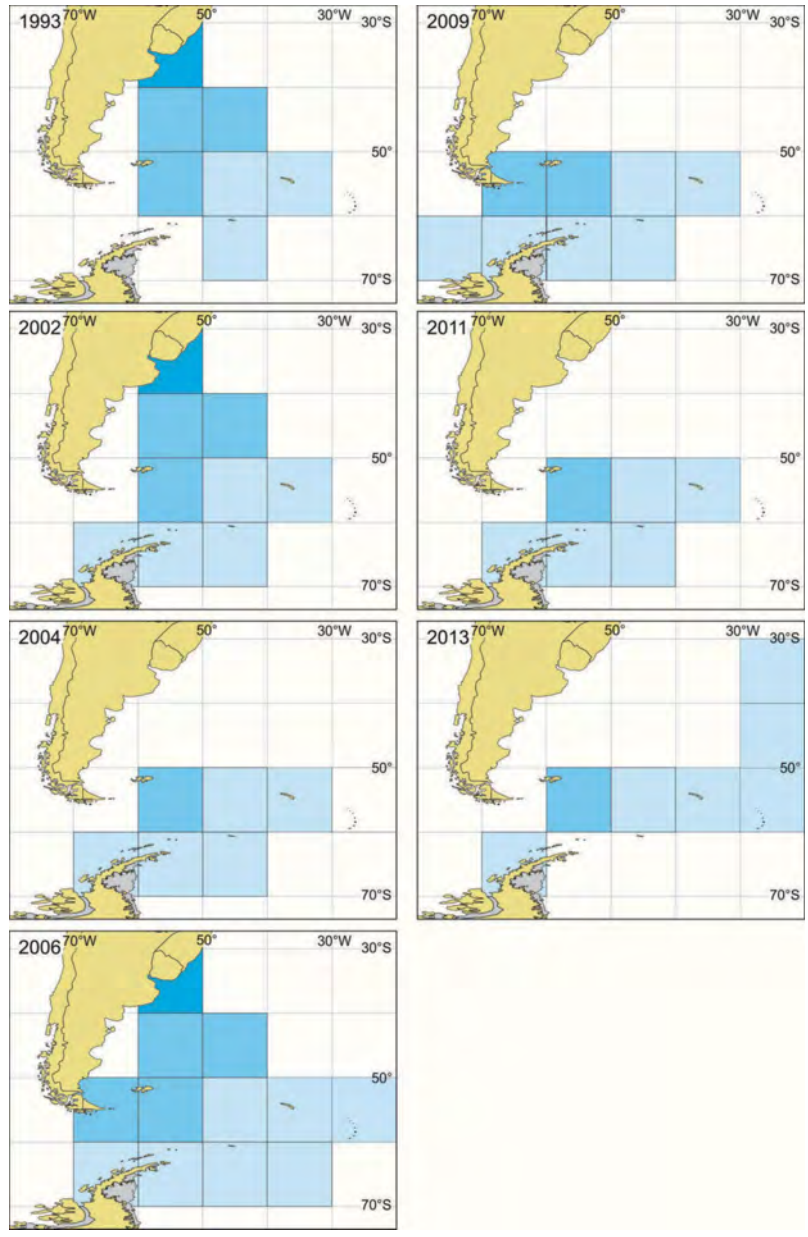

Figure 2. Marine debris densities by year and 10x10 degree area from surveys of the *RRS James Clark Ross*. Shades of light to dark blue are respectively 0-1, 2-10 and 11-100 items. km^2.

Censi of mega- and macroplastics stranding on South Atlantic Ocean shores

Far from most local sources of debris, remote island shores have been considered a valuable 'barometer' of floating debris densities (although of course stranding densities are influenced by the usual complex interaction of position relative to currents, winds, sources, etc.). Reports from surveys starting in the late 1980s showed that plastics were reaching even the most remote shores (Ryan, 1987; Benton, 1991; Ryan and Moloney, 1993). One of the longest running surveys anywhere is that at Bird Island, South Georgia (Fig. 3a). This is just one beach (albeit an important one because most of the world's population of Wandering Albatrosses occur there), but although no trend is obvious roughly 1 item has been stranding per m per year since the late 1980s. Impact, and decisive action because of impacts, can be seen in the drastic reduction of fishery - related plastics stranding since 2000 (Fig. 3b). In contrast on three Pacific shores Benton (1995) showed

that debris levels at remote Pitcairn were as high as those stranding on SW Irish shores in Europe. Wider surveys of remote Atlantic and Atlantic sector of the Southern Ocean islands showed levels one to two orders of magnitude lower than around Atlantic Europe (Barnes, 2005; see also Galgani, Sobral *et al.*, this volume). The latest data (Table 1b, Fig. 4a), from the current study, shows there is no longer a latitudinal trend for most of the South Atlantic - Atlantic Island shores as far south as South Georgia which now show similar densities of annual stranding of plastics. Furthermore, as Benton described a decade ago in the Pacific, remote shores now have similar levels of annual plastic stranding as continental shores (Fig. 4b). Only at and beyond 60°South do levels drop drastically.

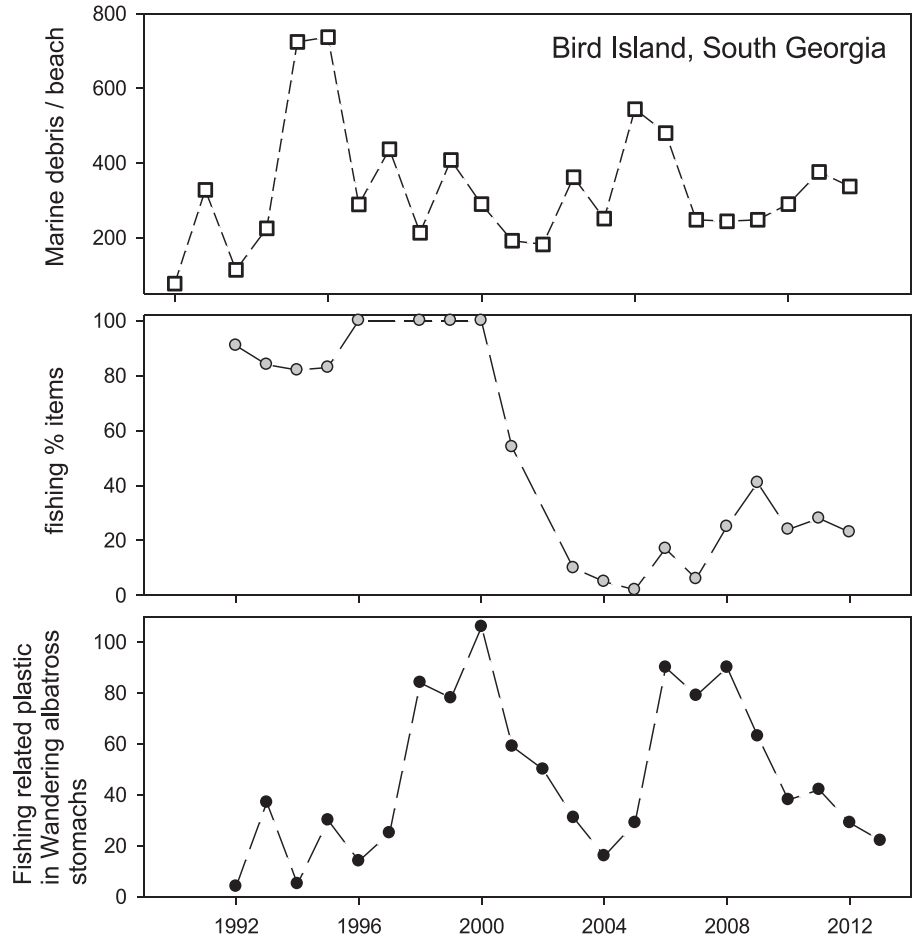

Figure 3. Marine debris on 'Big Beach' Bird Island, South Georgia. Number of items (per 291m survey) (top), proportion of fishing related items (middle), and number of items in Wandering Albatross stomachs per year (bottom).

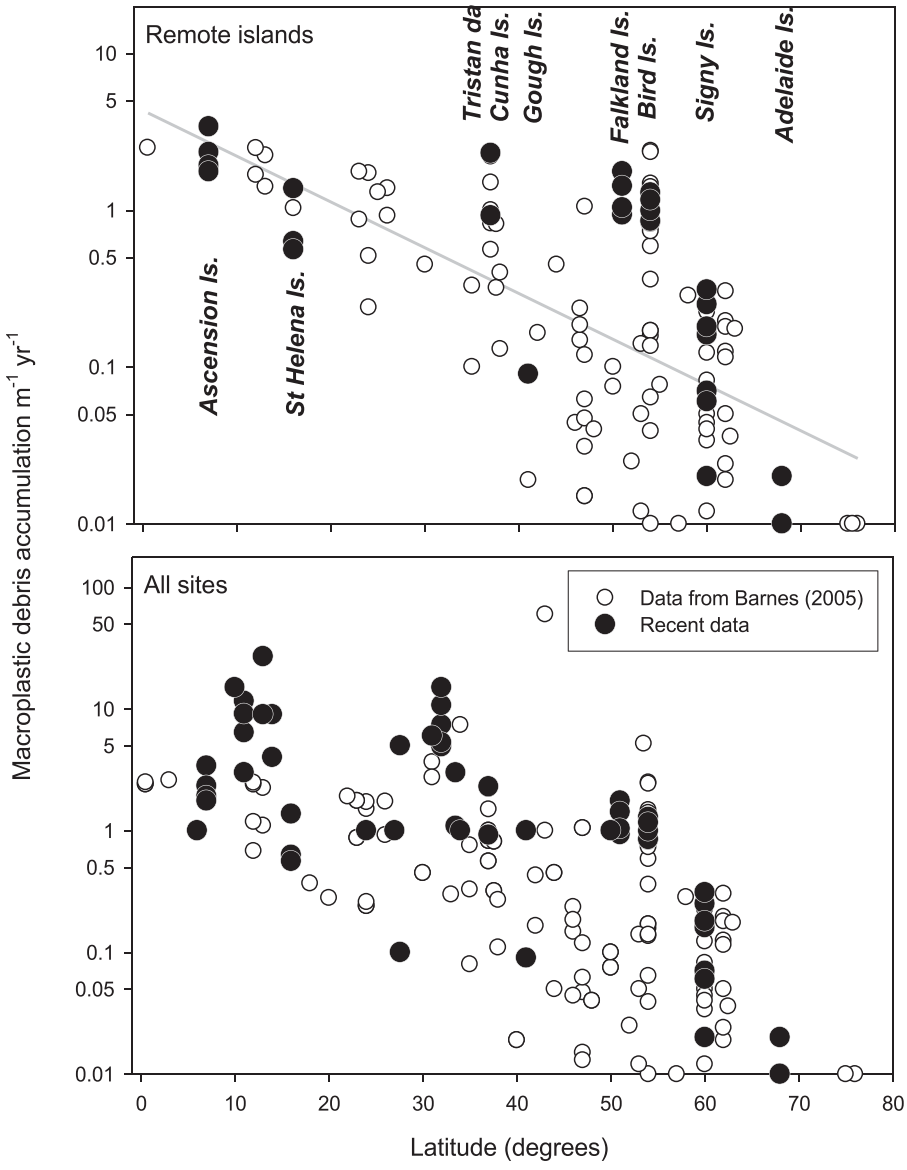

Figure 4. Debris accumulation on shores across the globe. Values are calculated from the literature[2-4] and present study from oceanic shores and the Mediterranean Sea. Hemispheres are identified by insert legend. Relationship for 1980-2000s data is significant (ANOVA, df = 1, *F ratio* = 37.4, $p < 0.001$, r^2 =40% (from Barnes, 2005). No trend with latitude is present in new data.

Continental shores do show some very high values of stranding debris close to urban centres but the variance in current data is reduced compared to values of Barnes (2005). Between 2001 and 2004 Santos *et al.* (2005) found macroplastics accumulating on Brazil beaches at 6.4-11.6 items km^{-1} yr^{-1}. Half to three quarters of stranding debris was plastic, with the most common items being bottles. On the other side of the northern part of the South Atlantic, in West Africa, plastic densities on beaches exceed 45 m^{-2} in urban areas and are still half of this on 'reference' beaches (Sheren *et al.*, 2002). Part of the explanation to the high values on continental shores is the shorter duration of survey. Very few remote locations have daily or weekly surveys, such as reported from Heard and Maquarie islands by Eriksson *et al.* (2013). They showed that estimates of stranding of debris may be an order of magnitude higher from daily rather than monthly or annual observations. The most regularly repeated observations in the South Atlantic and Southern Ocean remain those at Bird Island, where increasing frequency from monthly would prove difficult due to it being a secondary task of researchers present.

Composition and sources

Plastic dominates marine debris in the Atlantic. From the Southern Ocean to the equator about 86% of anthropogenic debris observed at sea by Barnes and Miler (2005) was plastic. Since then this survey has been conducted along approximately the same route twice in 2006 and 2008, record 92% and 89% of items as plastic respectively – with packaging, bottles and fishing/shipping related equipment (such as buoys and floating net) as the most abundant items. Ryan (2014) found that 97% and 98% of items in coastal and oceanic waters respectively were plastic and that packaging was by far the biggest source of these. On the 2013 South Georgia to Ascension Island mid-Atlantic transect 94% of items were plastic of which nearly half (44%) were some form of packaging material. The origin of material observed at sea is of course very hard to estimate without collection, which has rarely been attempted at sea in the South Atlantic.

Of material stranding on remote island shorelines, the proportion represented by plastic is unsurprisingly also high; 58-84% in the South Atlantic and 30-86% in the Southern Ocean (Barnes and Milner, 2005). The source of debris stranding at Bird Island, South Georgia was mainly fishing related, but since 2003 this has represented 40% or less of items. Packaging of some form has dominated items since and similarly represents most items recorded further south at Signy Island or Adelaide Island. On continental shore surveys plastic comprised 32-86% of items along Brazilian coasts (Santos *et al.*, 2005) but urban items, such as plastic bags, lids and food containers were found to be the biggest source – as found by Sheren *et al.* (2002) on West African shores.

Colonisation and rafting on plastic debris by bryozoans

There is a considerable, fast growing literature on the impacts of marine debris, particularly plastic. This covers ingestion (Fig. 1a) leading to choking, starvation or poisoning (see Deudero and Alomar, Fossi *et al.*, and von Moos, this volume); entanglement, absorption of pollutants, carriage of micro-organisms (see Jorissen, this volume) and disease and the transport of macrobiota (Fig. 1b-f). The proportion of debris colonized by macroscopic fauna can be low (1%) in Southern Ocean and Atlantic coastal waters (Barnes, 2002; Ryan, 2014). In Atlantic oceanic waters more than 30% of items can be colonized with thousands of individual organism recruits per square meter representing many phyla (Barnes and Milner, 2005). In 2013 the repeat megaplastics at sea survey in the Scotia Sea (reported for the first time here) recorded two items (a float and a fragment) with stalked (lepadomorph barnacles and bryozoans) in the Scotia Sea – the first observation [known to the author] of macrobiota colonizing flotsam at sea in the Southern Ocean. However colonized flotsam has washed ashore as far south as Adelaide Island on the Antarctic Peninsula. New data on the colonization of stranded plastics (Table 1b, Fig. 5) shows that, contrary to suggestions in Barnes (2005), there is a similar relationship of colonization with latitude. Of course there is a wide variety of both abiotic (e.g. pumice) and biotic (e.g. seeds) flotsam that is present naturally. However, the rapid and massive onset of floating plastics has changed the amount of this habitat by orders of magnitude and made the chance of reaching far locations and small islands much greater. This is of particular importance in the South Atlantic where there is little other anthropogenic aids to marine organism dispersal (e.g. sparse long distance shipping and tourism). Of the strandline debris examined in the literature and current study it was found that on remote Atlantic shores the primer space occupiers were cheilostome bryozoans with spirorbid and serpulid polychaetes being second most prevalent (Table 2). This is unsurprising as there is a considerable global literature showing them to be primary colonizers of newly available space in the shallows and particularly of plastic ‘settlement’ panels.

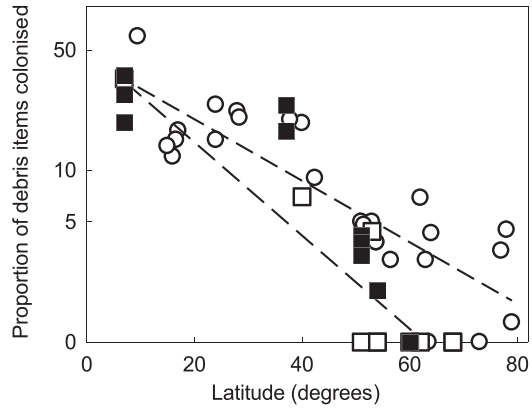

Figure 5. Percentage of marine debris colonized by fauna with latitude and island isolation in the Atlantic and Atlantic sectors of polar ocean. Symbols are squares from South and circles from North Atlantic. Filled symbols are data from current study and open symbols plus relationship lines (from Barnes, 2005).

Table 2.

Latitude	**Island**	**Colonising fauna dominance (most abundant left)**
54 S	Bird Is.	Bryozoan, polychaete
51 S	Falkland Is	Bryozoans, polychaetes, cnidarians, barnacles*, molluscs*
41 S	Gough Is	No data
37 S	Tristan da Cunha	Bryozoans, cnidarians, barnacles
37 S	Inaccessible Is	Bryozoans, barnacles, cnidarians, polychaetes
16 S	St Helena Is	No data
7 S	Ascension Is	Bryozoans, polychaetes, crustaceans (mainly barnacles), cnidarians, sponges

Placement of experimental plastics on shores, and submersed in subtidal environments in the South Atlantic and Southern Ocean, have similarly reported bryozoan and polychaete dominance in individuals, number of species and space occupation. Starting in 2001-2002 plastic panels were immersed at three sites each at 1) Rothera Research Station, Southern Ocean, for 10 years, 2) Stanley, East Falkland for 3 years, and 3) Ascension Island for 2 years. Bryozoans dominated space and numbers of species at all sites and by the end of the experimental periods had reached near total cover of some panels at each of the three regions. No known aliens were found to have recruited but it is clear that this surface is easily colonized quickly by a wide variety of species that can then potentially travel considerable distance. Non-indigenous species have been recorded on plastic debris in the North Atlantic (e.g. see Barnes and Milner, 2005), which includes many bryozoan species. For example all the non-indigenous bryozoans known to date around the UK occur on marine plastics and in at least one case these are known to have been the introduction vector (e.g. *Thalamoporella evelinae*) to the Atlantic (Winston *et al.*, 1997). The high colonization levels, concentration of debris by the South Atlantic gyre and otherwise little human transport means the native and endemic marine fauna at the Tristan da Cunha group and Gough Island are under threat from non-indigenous rafters on plastics. Barnes (2002) and Santos (2005) have argued, despite the low levels of debris densities and colonization levels by biota, that most at risk are the fauna of Southern Ocean coastlines, which to date have no known established non-indigenous marine fauna. There, where natural floating colonizable surfaces are very few, plastics have made the biggest change in potential opportunities for invasion – and the only confirmed report of an invader, the mussel *Mytilus edulis* at South Georgia in 1975, is a prolific fouler of plastics and common in South America. Plastics have washed ashore on remote coasts before humans even reached them and their increase in the South Atlantic, combined with climate-forced increase of sea surface temperatures, makes them a significant and growing threat in terms of organism transport.

Revising interactions of plastics with marine biota: evidence from the Mediterranean

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Abstract

Quantifying the interaction of marine biota with marine litter requires understanding the mechanisms of response of different taxa. Plastic ubiquity and size fraction preclude large impacts over the organisms exposed to marine litter. The Mediterranean, a semi-enclosed Sea, is one of the most affected by plastic concentrations. Different effects have been identified: ingestion, entanglement, toxicity, invasions, and physical harm, among others. This paper reviews studies on marine organisms and marine litter with emphasis on main taxa affected, habitats, feeding strategies, IUCN category. Further this review provides updated data, identifies knowledge gaps and provides suggestions for further research to be undertaken under the scenario of increasing plastic loads to the Mediterranean.

Keywords: litter, plastic, fish, marine mammals, invertebrates

Introduction

In the Mediterranean, a number of studies have been conducted to assess impacts of marine litter on marine biota (Akoumianaki *et al.*, 2008; Camedda *et al.*, 2014; Fossi *et al.*, 2014a). Most studies deal with mere quantification of debris (Sanchez *et al.*, 2013; Erya ar *et al.*, 2014) while others aim to investigate different sizes and classes of plastic material interacting with fauna (Tomás *et al.*, 2002; Campani *et al.*, 2013; de Stephanis *et al.*, 2013). Studies have tended to focus on marine mammals and turtles, with fewer on fishes or invertebrates, although the ingestion of microplastic by fish was discovered many years ago (Carpenter *et al.*, 1972; Hoss and Settle, 1990). Still in the Mediterranean, only one study did investigate plastic ingestion by fish (Anastasopoulou *et al.*, 2013) while several studies on diet and stomach content analysis of Mediterranean fish have detected marine litter (Deudero, 1998; Massutí *et al.*, 1998; Madurell, 2003). Research on litter impact on invertebrates is rather restricted to controlled laboratory experiments, mostly in UK (Browne *et al.*, 2008; Anastasopoulou *et al.*, 2013; Farrell and Nelson, 2013). In general, the bio-accumulation of plastic components along the food web are poorly understood.

Plastics may fragment but are not biodegradable, persisting in the environment for thousands of years (Derraik, 2002; Barnes *et al.*, 2009). Plastic debris enters in the marine environment in a wide range of sizes from micrometric to metric dimensions (Barnes *et al.*, 2009). In the

environment, microplastic litter (<5 mm-NOAA) proliferates, migrates and accumulates in natural habitats world-wide. Different potential trophic routes transfer microplastics across marine ecosystems and therefore, environmental microplastics are available to every level of the food web, from primary producers (Oliveira *et al.*, 2012) to higher trophic-level organisms (Wright *et al.*, 2013). Thus, marine organisms, either from pelagic to benthic compartments are under threat but our knowledge remains fragmented since most studies tackle groups of species (for example cetaceans, fish, reptiles) separately (Anastasopoulou *et al.*, 2013; Camedda *et al.*, 2014; de Stephanis *et al.*, 2013).

Various impacts have been described in the interaction of fauna and plastics: digestion, entanglement, toxicity, invasive species facilitators, carcinogenesis, endocrine disruption and physical harm, such as internal abrasion and blockage (Laist, 1997; Oehlmann *et al.*, 2009; Talsness *et al.*, 2009; Wright *et al.*, 2013). For marine fauna, the primary impacts of marine litter are ingestion and entanglement, especially in mammals and reptiles (Gregory, 2009). Additionally, hydroponic pollutants available in seawater may adsorb onto plastic litter in ordinary environmental conditions (Teuten *et al.*, 2007; Thompson *et al.*, 2009b; Cole *et al.*, 2011) and be transferred across organisms. As these contaminants are persistent, bioaccumulative and toxic, they are of particular concern for human and environmental health (Engler, 2012). Deciphering future scenarios of change is essential to predict ecological shifts and consequently address key issues with regard to minimising alteration of biota by marine litter interactions.

Material and methods

Experimental design and data analyses

A review of 79 documents concerning marine litter interactions with marine biota (scientific papers, grey literature, EU projects reports and personal observations) was conducted. Research criteria were based only on studies of the Mediterranean Sea without any date limitation.

Studies were included in the analysis if they contained quantitative data on marine litter (more specifically marine plastics) and their interaction with marine biota (macroalgae, seagrass, invertebrates, turtles, fish and cetacean). Only *in situ* studies or experimental work carried out at sea were incorporated, excluding all documents of litter in Mediterranean seashores and beaches. Litter affectation in seabirds was not evaluated in the search. The citation list was examined and in total 29 studies (37%) satisfied the above criteria for inclusion and analysis of our study.

Knowledge of the species was backed up by specified databases of species (fishbase (www.fishbase.org), the reptile base (www.reptile-database.org), world cetacean base (www.marinespecies.org/cetacea/) and the IUCN Red List of Threatened Species).

Only species registering over 5% of litter impact were considered. Presence or absence of general debris, plastic and non-plastic (wood, metal, glass, fishing gear, monofilaments and ropes and others) and ingestion, entanglement, colonization and rafting processes were defined as the dependent variables.

RESULTS

As shown in Table 1, and detailed further in Figure 1, litter impact in the Mediterranean has been more widely studied for cetacean (41.2%) followed by fish (24.1%), turtles (20.7%) and finally invertebrates (13.8%) and a majority of studies concerned some type of plastic litter (plastic bags, plastics sheets, plastic monofilaments and ropes).

Table 1. Range of studies covered in this review.

References	Taxa	Study Area	Study depth (m)	Study year
Akoumianaki et al. (2008)	Invertebrates	Greece, Western Saronikos Gulf	16-20	2005-2006
Aliani and Molcard (2003)	Algae, Seagrass and Invertebrates	Ligurian Sea	0.10	1997
Anastasopoulou et al. (2013)	Fish	Greece, Cephalonia Island	300-850	2010
Aparicio, personal comm.	Cetacean	Spain		1990-2012
Camedda et al. (2013)	Turtles	Italy, Sardinia		2008-2012
Campani et al. (2013)	Turtles	Italy, Tuscany coast and Pelagos Sanctuary		2010-2011
Casale et al. (2008)	Turtles	Italy, Lampedusa		2001-2005
Colligon et al. (2012)	Fish	French-Italian coast	0.10	2010
de Stephanis et al. (2013)	Cetacean	Spain, Granada		2012
Deudero (2001)	Fish	Spain, Balearic Sea	0-30	1994-1998
Duras, personal comm.	Cetacean	Croatia		1990-2013
Estarellas personal comm. (2014)	Invertebrates	Spain, Balearic Sea	0-20	2014
Fossi et al. (2014)	Fish, cetacean	Italy, Pelagos Sanctuary		2007-2013
Gramentz (1988)	Turtles	Malta		1986
Katsanevakis (2008)	Cetacean	Greece, Mykonos Island		2006
Katsanevakis et al. (2007)	Invertebrates, Fish	Greece, Saronikos Gulf	16 -20	2005-2006
Lazar and Gracan (2011)	Turtles	Croatia and Slovenia		2001-2004
Levy et al. (2009)	Cetacean	Israel, Port Haifa		2007
Madurell (2003)	Fish	Greece	500	1999-2000
Massuti et al. (1998)	Fish	Spain, Balearic Sea	70-500	1990-1991
Mazzariol et al. (2011)	Cetacean	Italy, Adriatic coast		2009
MEDITS project	Fish	Spain, Balearic Sea	10-800	2007-2012
Nadal personal comm. (2014)	Fish	Spain, Balearic Sea	40-70	2014
Pace et al. (2008)	Cetacean	Italy, Capo Palinuro		2004
Roberts (2003)	Cetacean	Greece, south coast of Crete		2011
Shoham-Frider et al. (2002)	Cetacean	Israel		1993-1999
Tomás et al. (2002)	Turtles	Spain, catalonia		not available
Tonay et al. (2007)	Cetacean	Turkey, Black Sea		2002-2003
Viale et al. (1992)	Cetacean	France, Lavezzi Islands		1989

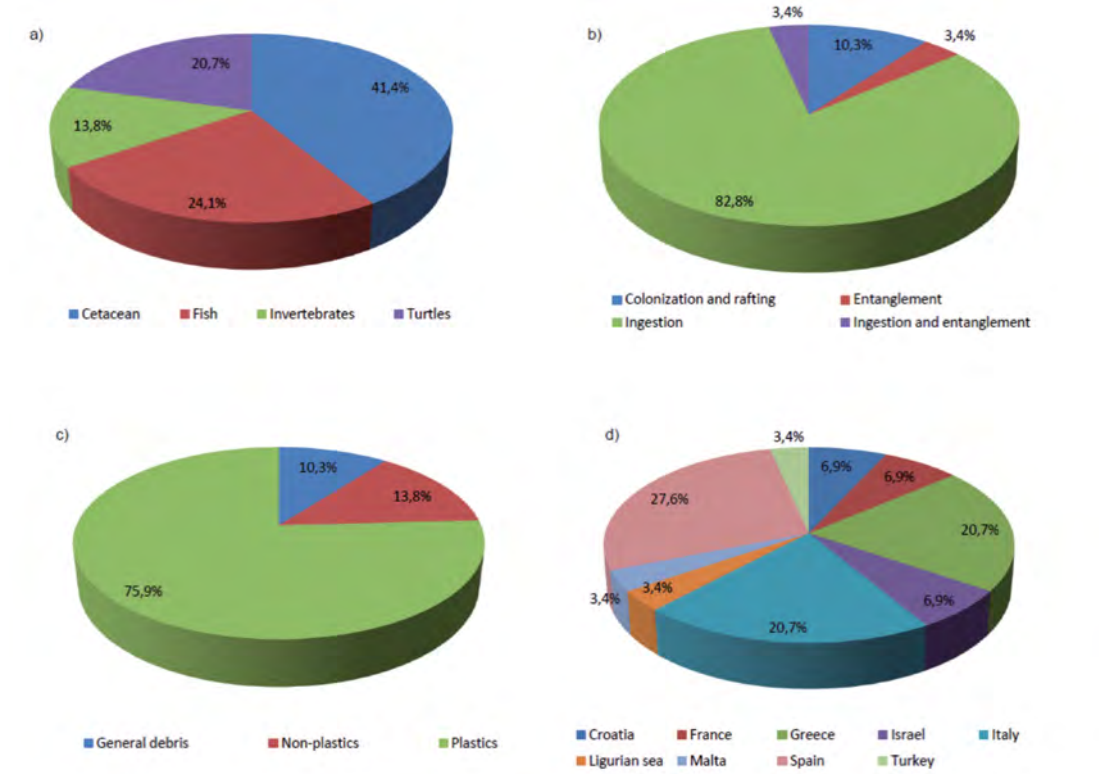

Figure 1 a,b,c,d. Bibliometric data classifying the documents reviewed according to taxa (a), type of impact (b) type of litter (c) and study area (d).

As seen in Figure 2, cetaceans are the most affected taxa with regard to ingestion and entanglement followed by turtles. Indeed half of the cetaceans studied presented plastic ingestion. Filter feeders were the most affected. No detritivorous organisms were affected by litter ingestion and

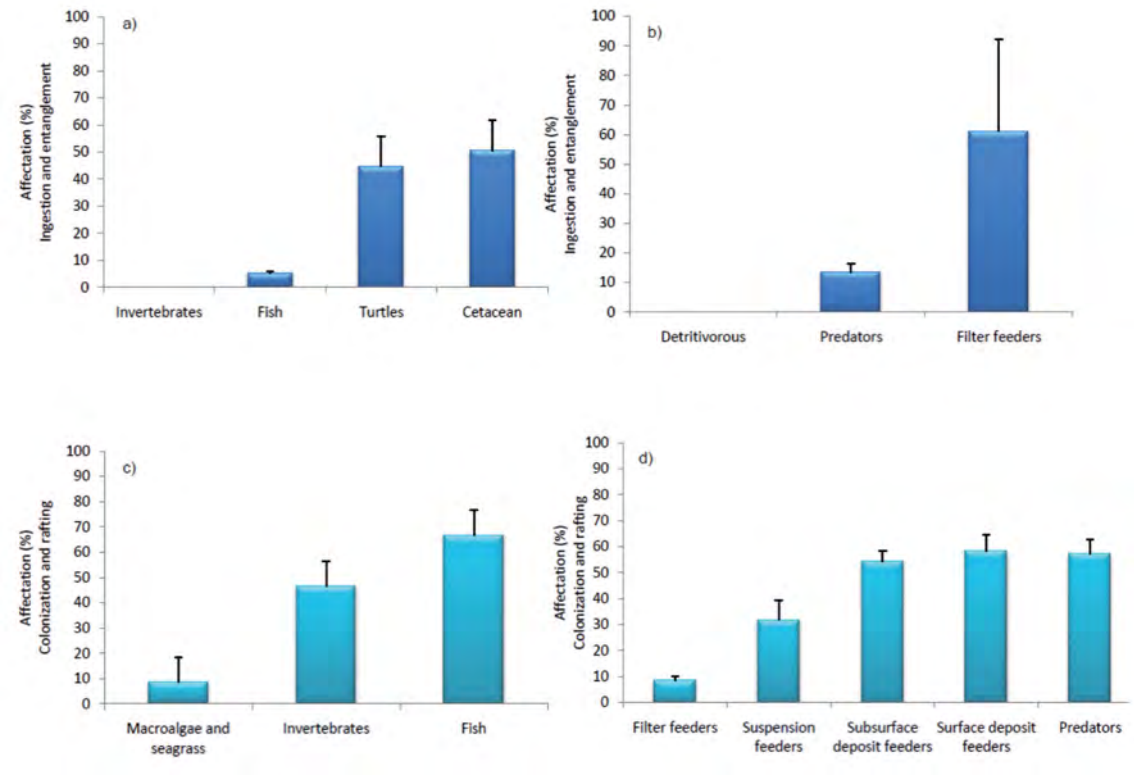

Figure 2 a,b. Impact of litter due to ingestion and entanglement for a) different taxa and b) according to different feeding strategies (Number of studies (n): invertebrates (n=1), fish (n=8), turtles (n=6), cetacean (n=12), detritivorous (n=1), predators (n=23) and filter feeders (n=2). Figure 2 c,d. Impact of litter due to colonization and rafting for c) different taxa and d) feeding strategies (Number of studies (n): macroalgae and seagrass (n=1), invertebrates (n=1), fish (n=1), filter feeders (n=1), suspension feeders (n=1), subsurface deposit feeder (n=1) study, surface deposit feeder (n=1) and predator (n=1)).

entanglement. Impact of litter due to colonization and rafting processes was large in fish (66.8%) followed by invertebrates (46.5%).

Non-multidimensional scaling showed dispersion of litter impact values according to pelagic species taxa (Figure 3a). Large filter feeders *Cetorhinus maximus* and *Balaenoptera physalus* grouped together while sea turtles were not bound to any other species, like *Coryphaena hippurus* which is the least affected pelagic species. Marine mammals such as *Grampus griseus* and *Phocoena phocoena*, which were sampled in the easternmost sector of the Mediterranean basin, were closely associated in the MDS. For benthic species, MDS results did not show a marked trend. However, bryozoans, macroalgae and seagrasses grouped together away from other taxa, while primary producers appear clumped with suspension feeders in the scaling graph (Figure 3b).

Figure 3 a,b. Non-multidimensional scaling (MDS) a) for pelagic species (cetaceans, fishes, turtles) after normalizing variables and applying the Euclidean distance according to % litter affectation (>5%) and b) for benthic species (macroalgae, seagrass, invertebrates (ascidiacea, bivalvia, bryozoa, cnidaria, crustacean, echinodermata, gasteropoda, polychaeta and porifera) and fish) after >5% litter impact data square root transformation with Euclidean distance according to feeding strategies.

Total litter impact (ingestion, entanglement, colonization and rafting) was as high as 73% for species classified by IUCN as Vulnerable (VU) and reached 41% in Endangered species (EN) (Table 2). In the endangered category, filter feeder species had 50% of litter affectation.

Table 2. Litter impact (%) in various taxa according to IUCN category, and feeding strategy.

	Macroalgae and Seagrass (%)	Detritivorous (%)	Subsurface deposit feedesr (%)	Surface deposit feeders (%)	Suspension feeders (%)	Filter feeders (%)	Predators (%)	Total Affectation (%)
Critically Endangered (CR)							**12**	**12**
Cetacean							12	12
Endangered (EN)						**50**	**38**	**41**
Cetacean						50	0	33
Turtles							45	45
Vulnerable (VU)						**83**	**72**	**73**
Cetacean							93	93
Fish						83	0	28
Near Threatened (NT)							**0**	**0**
Fish							0	0
Least Concern (LC)	**7**						**13**	**12**
Cetacean							21	21
Fish							9	9
Seagrass	7							7
Not Evaluated (NE)	**10**	**0**	**54**	**58**	**32**		**14**	**22**
Algae	10							10
Ascidiacea					98			98
Bivalvia				50				50
Bryozoa					7			7
Cnidaria					12		42	19
Cnidaria					7			7
Crustacea				68	56		62	61
Echinodermata		0			7			4
Fish							5	5
Gasteropoda							51	51
Polychaeta			54	57	56		56	56
Porifera					72			72
Data Deficient (DD)							**8**	**8**
Fish							8	8

DISCUSSION

Not surprisingly there is a vast array of taxa being affected by marine litter, and essentially by plastics, from invertebrates (polychaetes, ascidians, bryozoans, sponges, etc.), fishes, cetaceans up to the largest animals at sea (fin-whale *Balaenoptera physalus).* Consequences of marine debris vary from entanglement, ingestion, suffocation, and general debilitation, among others. In 1997 Laist reviewed over 250 marine species impacted by entanglement and ingestion. In this review, several affectations have been identified, and are split into ingestion and entanglement, colonization and rafting.

Ingestion and entanglement

Fishes

While plastic accumulation at the seafloor, continental shelf, submarine canyons (Ramirez-Llodra *et al.*, 2011) has been increasing in past decades, with important areas of accumulation of debris (Pham *et al.*, 2014), differential impacts have been detected among the different taxa surveyed. For instance most benthic fishes seem unaffected by plastic ingestion while pelagic fishes, such as *Boops boops*, present a high degree of plastic ingestion, with 70% of affected individuals. Possibly, prey selectivity on seafloor allows debris rejection. These results are relevant since bioaccumulation through the food web is known (Farrell and Nelson, 2012; Rochman *et al.*, 2013a; Wright *et al.*, 2013). Offshore epipelagic species are also exhibiting moderate levels of plastic ingestion, with several species ingesting marine litter such as *Coryphaena hippurus, Seriola*

dumerilii, Schedophilus ovalis, Naucrates ductor (Deudero, 2001). In the pelagic realm, optimised feeding is linked to a more voracious and visual behaviour for chasing the prey; in this sense, particle selection is linked more to mouth biometry than to nutritional quality.

Cetaceans

Cetaceans are highly affected by ingestion, at worldwide level (Baulch and Perry, 2014). Although most studies rely on stranded individuals, large filter feeders such as *Physeter macrocephalus,* the fin-whale *Balaenoptera physalus*, present large megaplastics, with dominance of plastic sheets. Entanglement is of concern in the sperm whale *Physeter macrocephalus* since many individuals are exposed to driftnets (Pace *et al.*, 2008). Most odontocetes are little affected by plastic ingestion, with the exception of *Grampsus griseus*, where plastics can be confused with squids (Shoham-Frieder *et al.*, 2002).

Turtles

All sea turtle species (*Caretta caretta*) surveyed are affected. Indeed, plastics ingestion in turtles has been chosen as a parameter for quantifying descriptor 10 in MSFD. Preferential ingestion towards white plastics blue and red plastics (Camedda *et al.*, 2014) has been shown, likely due to resemblance of white plastics with jellyfishes. From all turtle species present in the Mediterranean (*C. caretta*, *Chelonia mydas, Dermochelys coriacea, Eretmochelys imbricate, Lepidochelys kempii)* (Camiñas, 2004), only data has been reported from *C. caretta*. However, impact seems very probable in all turtle species.

Litter colonization and rafting

For benthic invertebrates colonization on rafting objects is a major issue, representing a vector of species introduction over large areas driven by current dynamics (Aliani *et al.*, 2003; Hoeksema *et al.*, 2012). Another impact is transport of pollutants (Rochman *et al.*, 2013a) along with these objects.

Experimental studies in aquarium under controlled conditions demonstrate ingestion of nylon plastics and PVC fragments (0.25 – 15 mm) over sediment grains at sedimentivorous benthic species such as holothurians under forced diets (Graham and Thompson, 2009). However, our own data on plastic ingestion analyses in holothurians *Holothuria forskalii* from coastal waters suggest that although microplastics (nylon filaments, plastic grains) are present in shallow-waters sediments (100% of the analysed sediment presented microplastics), mainly at grain fractions 0.5 mm to 1 mm, only one of the 50 *H. forskalii* individuals sampled presented plastics in the faecal pellets.

With regard to feeding strategies, filter feeders are highly affected by ingestion, while predators are more dispersed. Large filter feeders in the pelagic environment are highly exposed to either ingestion or entanglement. In this sense, some sharks such as basking shark *Cetorhinus maximus* are under threat (Fossi *et al.*, 2014a).

Many endangered species are impacted by plastic (41%), a factor to integrate when drafting conservation policies.

GAPS IN KNOWLEDGE AND RECOMMENDATIONS FOR PRIORITIES

Physical impacts of microplastics on marine organisms are well documented at global level (Wright *et al.*, 2013), but little is known of the impact of Nano and microplastics on the Mediterranean marine biota. Special attention should be given to micro plastic fibers ingested by pelagic species and to southern Mediterranean waters which remain data deficient.

Colonization by the benthic foraminifer *Rosalina (Tretomphalus) concinna* of Mediterranean drifting plastics

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Abstract

Floating plastic debris, sampled with a plankton tow around Corsica, in November 2011, January and March 2014, were investigated for the presence of benthic foraminifera. The sample of November 2011 yielded 144 live specimens of *Rosalina (Tretomphalus) concinna*, corresponding to 19.3 individuals per 100 cm^2, forming a monospecific assemblage. The appearance of monospecific assemblages of *R. concinna* can be explained by the fact that this taxon is one of the very few benthic foraminiferal species which produce a planktonic stage prior to sexual reproduction. Laboratory evidence has shown that in *Rosalina* sexual reproduction occurs only at temperatures above 18°C. This would mean that around Corsica, colonization of the plastic debris can only take place in late summer and autumn. It is possible that the critical temperature limit for sexual reproduction has only been reached recently, due to climate warming. Foraminiferal colonization would be a response to multiple factors: the presence of colonizable substrates in the surface waters and a recent temperature increase. At present, it is too early to conclude what is the impact of foraminiferal colonization on the degradation of the plastics, and on the dispersal capacities of *R. concinna*.

Introduction

Since the beginning of the 21st century, there is a growing concern for the harmful effects of floating plastic debris in the marine environment (e.g., Derraik, 2002). Floating plastics endanger marine life directly by entanglement, ingestion, or absorption of the organic components. They may also impact marine ecosystems indirectly, by offering available substrates for rafting species, some of which may be exotic, or even invasive. In fact, a wide range of epibiontic marine organisms colonize floating plastics, including bryozoans, hydroids, bivalves, barnacles, tube worms, coralline algae and foraminifera (e.g., Barbes and Fraser, 2003; Barnes and Milner, 2005; Gregory, 2009; Barnes, this volume). The ecology of these rafting taxa and the substrates they colonize have been extensively reviewed by Thiel and Gutow (2005a,b). Recently, Carson *et al.* (2013b) showed that this colonization is not limited to macrofauna, but that plastics are also colonized by micro-organisms, including coccoid bacteria, centric diatoms, dinoflagellates, coccolithophores and radiolarians. Collignon *et al.* (2013) studied floating micro- and meso-plastic particles in the Bay of Calvi, Corsica, between August 2011 and August 2012. They observed that 22% of the examined particles were colonized by fouling organisms, mainly small algae and foraminifera. Based on this intriguing report, we decided to study the presence of foraminifera on floating plastics in more detail. Here, we present the preliminary results of this study.

Material and methods

Three samples were collected in the surface waters at station BH2 off Bastia, Corsica, France, on 11 November, 2012, 8 January, 2014 and 12 March, 2014. In all three cases a Manta plankton net with a mesh size of 150 µm was applied for half an hour. In November 11, sea surface temperature (SST) was about 19°C vs. 15.2°C, in January 2014 and about 14°C in March 2014. Salinity was about 37.5 for all three sampling periods.

All samples where preserved in a mixture of 95% ethanol and 2g/l Rose Bengal (RB) stain. In the laboratory (always after more than one week of staining with RB) the samples were sieved over a 63 µm mesh, which retained all plastic fragments above this size and all adult and pre-adult foraminifera, not only those which were still attached to the plastic debris, but also those which were detached from the plastic fragments during sample transport and treatment. After this, all plastic fragments were very carefully examined on both sides under a binocular microscope. The surface of all fragments was measured (with a measurement objective in case of small pieces, with a ruler for the largest pieces), and foraminifera still attached to the plastic fragments were cut off in order to be stored (together with the plastic to which they were still attached) in Chapman slides. The remaining sieve residues, mainly containing biotic particles detached from the plastic debris during sample treatment was also studied carefully, and all RB-stained foraminifera were picked out (wet, in 50% ethanol) and stored in Chapman slides.

The Rose Bengal (RB) is a protein stain (Walton, 1952) that serves to verify whether the foraminifera were alive at the time of sampling. In the very well oxygenated surface waters of the Mediterranean, foraminiferal protoplasm (and the proteins is contains) will be degraded very quickly after the death of a foraminiferal individual, and the intense pink color of all stained foraminifera leaves no doubt that all stained individuals were alive at the time of sampling.

Results

The sample collected in November contained abundant plastic debris, of variable size (until 10 cm) and nature, whereas both samples taken in 2014 contained only some small plastic fragments (8 mm maximum length). For the sample taken in November 2012, the plastic fragments showed a large variety in faunal colonization; some fragments showed a dense cover of epibenthic (fixed) organisms, whereas other fragments were almost devoid of fauna.

Only some of the scarce fragments of both 2014 samples were colonized by sessile organisms. In view of the poverty of the 2014 samples we will base our discussion mainly on the November 2012 sample.

The November 2012 sample yielded a large amount of plastic remains, with a total estimated surface (only a single side quantified) of 749.6 cm^2. A wide variety of organisms was observed on the plastic fragments, with a strong dominance of benthic macroalgae, hydrozoans (order Leptothecata), gastropods (Nudibranchia) and bryozoans.

In the 2012 sample, a total of 144 Rose Bengal stained foraminifera were inventoried. Among these, 23 were still attached to the plastic fragments, whereas 121 were found detached from the plastic, apparently as a consequence of the sample treatment. No living foraminifera were found in the January 2014 sample, whereas 3 detached specimens were found in the March 2014 sample. All inventoried foraminifera belong to *Rosalina concinna* (Fig. 1), constituting a monospecific assemblage of this species. For the 2012 sample their density was 19 foraminifera per 100 cm^2.

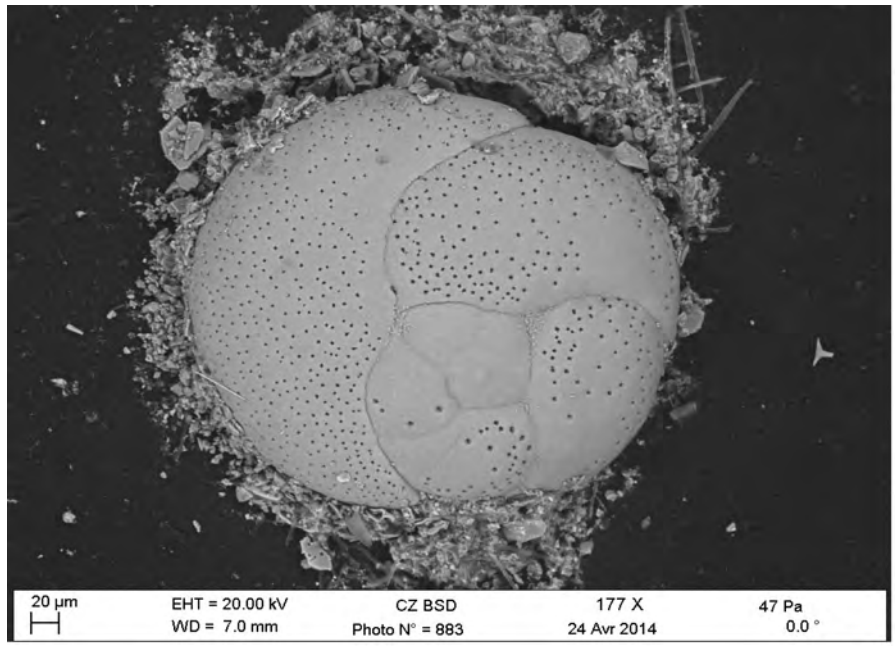

Figure 1. Adult specimen (350 µm) of *Rosalina concinna* fixed to a plastic debris. Note the organic remains concentrated around the test, which are the remnants of the feeding cyst, by which the foraminifer is fixed to its substrate.

Discussion

Life cycle, ecology, taxonomical aspects and molecular phylogeny of the genus *Rosalina*

The observation that benthic foraminiferal assemblages on floating plastics are monospecific is highly surprising in view of the wide range of epiphytic taxa that have been described on macroalgae in the Mediterranean (e.g. Langer, 1993). Apparently *Rosalina concinna* is the only benthic foraminiferal species capable to colonize available substrates at the sea surface. It appears that this unique capability is due to the highly particular reproductive cycle of this species (Fig. 2). In fact, some species of the genus *Rosalina* dispose of a planktonic stage, which is a very rare treat in benthic foraminifera.

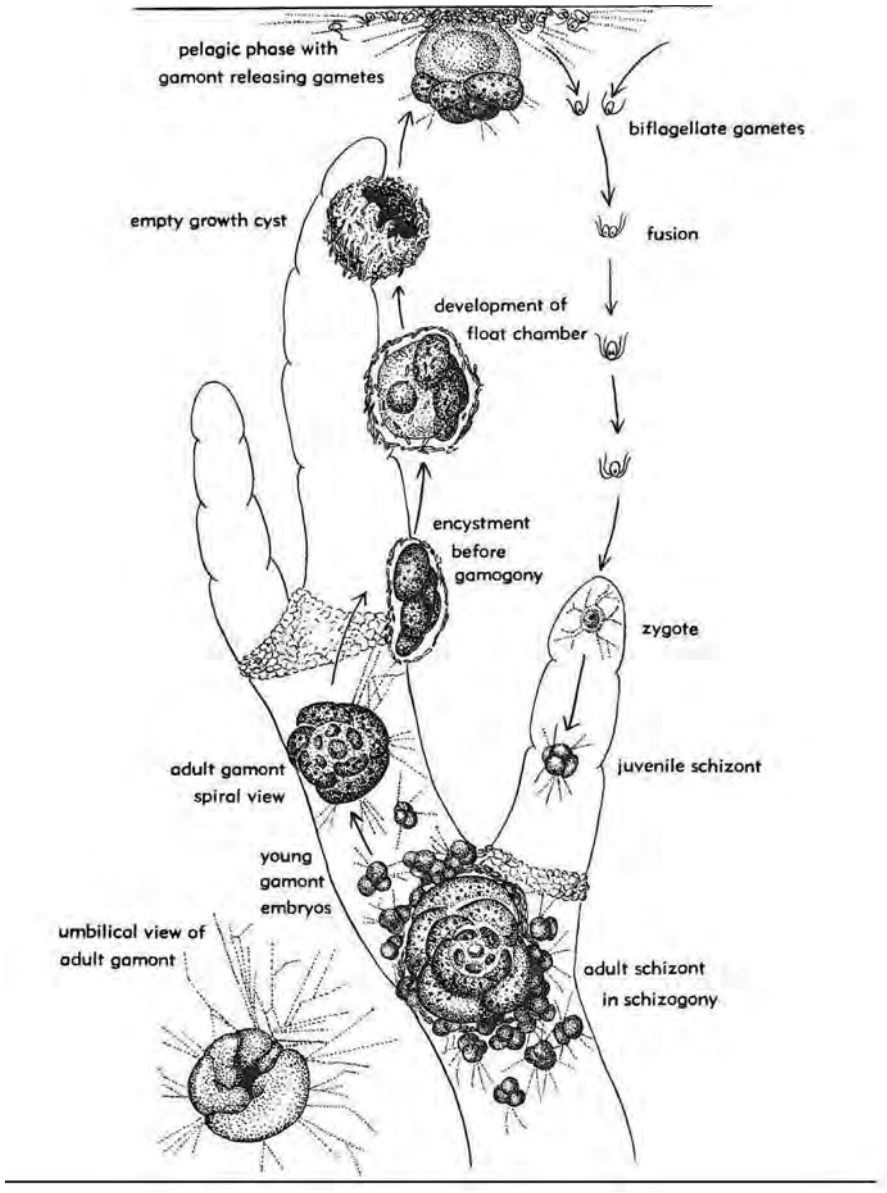

Figure 2. Life cycle of *Rosalina globularis* (Loeblich and Tappan, 1964, after Myers, 1943).

Most benthic foraminifera alternate sexual and asexual reproductive events. The schizont, characterised by a microspheric test, with a small initial chamber, reproduces asexually, whereas the gamont, with a megalospheric test (larger initial chamber), produces gametes, and reproduces sexually (Figs. 2,3). The particularity of some species of the genus *Rosalina* is that prior to producing gametes, the gamont produces a large floating chamber (the so-called *Tretomphalus*-stage), which allows it to liberate the gametes in the surface waters, optimizing their dispersal (Fig. 3).

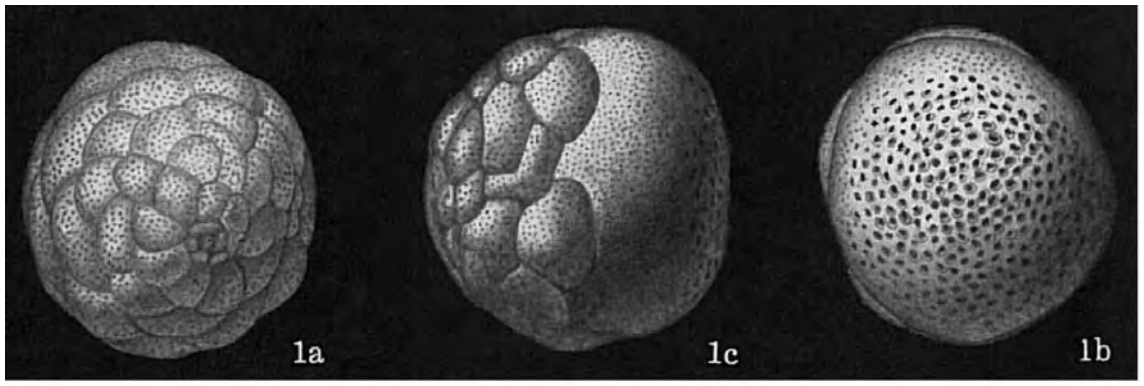

Figure 3. Floating chamber preceding sexual production (*Tretomphalus planus*, from Todd, 1965, The Foraminifera of the Tropical Pacific Collections of the "Albatross", 1899-1900. Part 4. Rotaliform families and planktonic families [End of Volume]. Bull. U.S. Nation. Mus., 161, 139 pp.)

As observed by Sliter (1965) in laboratory cultures, the strict alternation of sexual (gamogony) and asexual (schizogony) generations is not obligatory, and several successive asexual reproductions (apogamogeny) may take place if temperature is below optimal (Fig. 4). According to Myers (1943) and Sliter (1965, sexual reproduction and thus also the *Tretomphalus* floating chambers) only occurs at temperatures above 18°C. The presence of a high optimum temperature for *Rosalina globularis* was recently confirmed by Saraswat *et al.* (2011).

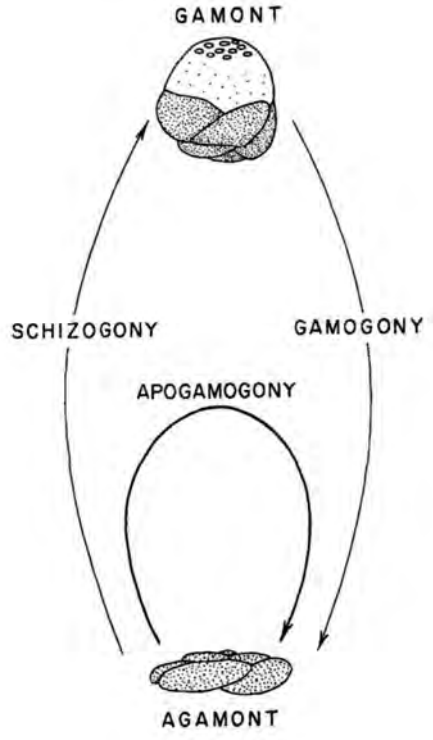

Figure 4. Life cycle of *Rosalina globularis* observed in laboratory cultures (Sliter, 1965).

In the traditional classification based on morphological criteria of the test, *Rosalina* d'Orbigny, 1826, belongs to the Discorbidae, in the order Rotaliida (Loeblich and Tappan 1964). Some authors place it in a separate family, the Rosalinidae. The planktonic stage is often considered as a separate

genus (*Tretomphalus*, Möbius, 1880) in the same family, although other authors prefer to use it as a subgenus (e.g. Banner *et al.*, 1985). Since it has not been shown conclusively that not all species of *Rosalina* are capable top form float chambers, we follow the paper of Banner *et al.* (1985), and use the name *Tretomphalus* at a subgenus level for the species of *Rosalina,* for which float chambers have been described. In their 1985 publication, Banner *et al.* proposed the subgenus *Tretomphaloides* for forms with a *Neoconorbina*-like benthic stage, which is caracterised by strongly lunate later chambers.
Since in *Rosalina concinna*, which was chosen as type species of this new subgenus, only the last chamber is slightly lunate, this species can in our opinion not be considered as a *Neoconorbina*. Therefore, *Tretomphaloides* is here considered as a junior synonym of *Tretomphalus*.
On the basis of a phylogenetic analysis focused on the complete gene of the small subunit of ribosomal DNA (SSU rDNA), rotaliids were divided into three clades (Schweizer *et al.*, 2008). *Rosalina* was placed in a subclade together with the genera *Discorbis, Planorbulina, Planorbulinella, Rupertina* and *Hyalinea* (Schweizer *et al.*, 2008; 2009). This subdivision fairly well corresponds to the traditional morphological classification. The first three genera are all continental shelf taxa, have a planoconvex morphology, and an epiphytic lifestyle. Conversely, *Rupertina* and *Hyalinea* live in bathyal environments, on elevated habitats and as surface dwellers, respectively. Rather surprisingly, within this group planktonic stages are only known for the genus *Rosalina* (Rückert-Helbig, 1983). The molecular data available for *Tretomphalus* concern the actin gene, which has a smaller sampling range than the SSU gene (Flakowski *et al.*, 2005). *Tretomphalus* DNA sequences grouped with a specimen originally identified as *Rosalina* sp. (Flakowski *et al.*, 2005, Fig. 3), but which turned out to be more closely related to *Rupertina* and *Planorbulina* in partial SSU phylogenies (Schweizer *et al.*, 2009, Fig. 6, "rotalid 3675").
Rosalina is a cosmopolitan genus, which is largely limited to the inner continental shelf. In dead faunas the genus appears also in marginal environments, but this is probably the consequence of an offshore transport of macroalgae, to which the foraminifera are attached. In fact, *Rosalina globularis* has been described (together with *Planorbulina acervalis)* attached to *Sargassum* seaweeds sampled off Bermuda by Spindler (1980). After the decomposition of the algae, the foraminiferal tests fall to the sea floor, and may be found in much deeper environments. *Rosalina* is very common in the Mediterranean, where it is mainly represented by the species *R. bradyi* and *R. globularis*. In an environmental survey of the living benthic foraminiferal faunas of the French Mediterranean coast performed in March-April 2009, *Rosalina* spp. was a prominent taxon (20 to 150 individuals per 100 cm^2) along the southeast French coast and around Corsica (Fig. 5), where it accounts for 5 to 20% of the total foraminiferal community. These areas are characterized by sandy substrates with abundant vegetation (macroalgae and *Posidonia*-meadows). In the western part of the French Mediterranean coast, characterized my muddy substrates with scarce vegetation, *Rosalina* is almost absent.

There are two observations of the appearance of the planktonic *Tretomphalus*-stages in massive amounts. Earland (1902) reports an observation of E.H. Mathew, who found a beach deposit almost entirely composed of *Tretomphalus*-stages on Corny Point, opposite Hardwicke Bay, on York peninsula, South Australia, in November 1880. A very similar observation was made by Todd (1971), who found a beach drift at Midway atoll (in the center of the Pacific!), composed of a nearly monospecific concentrate of *Tretomphalus*-stages.

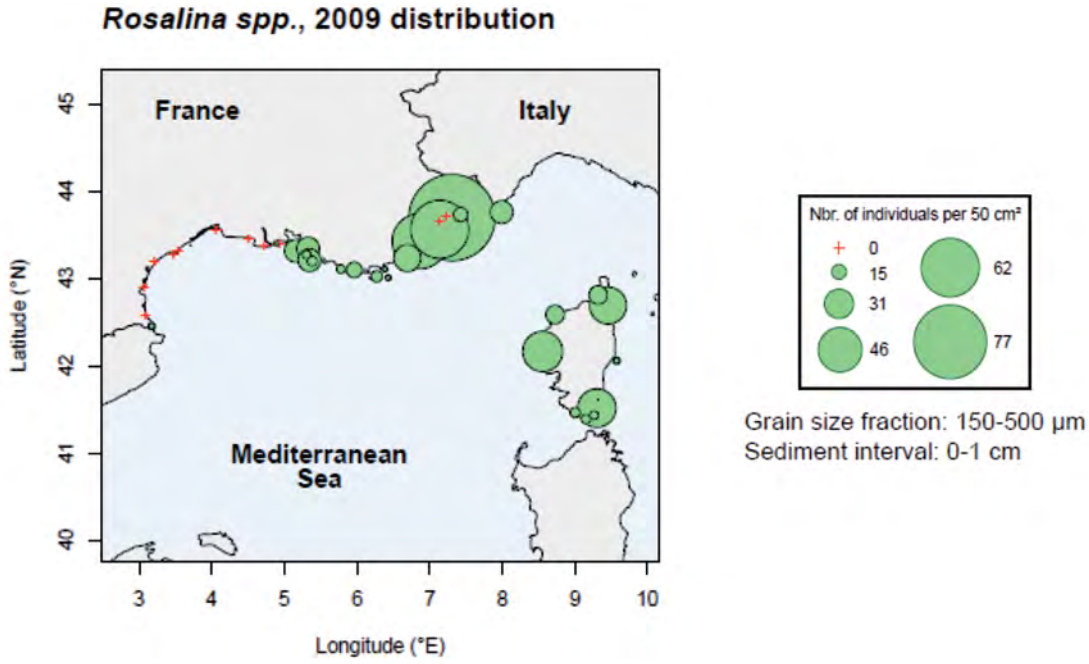

Figure 5. Distribution of *Rosalina* spp. along the French Mediterranean coast and around Corsica (Barras and Parent, pers. comm.). The circles indicate the foraminiferal density in the 150 – 500 µm size fraction of the sediment, standardized for a 50 cm² surface area.

In early publications on *R. globularis*, it was supposed that the European form of this species did not develop the planktonic *Tretomphalus*-stage. This idea was mainly based on the study of Vénec-Peyré (1981), who did not find any *Tretomphalus* stages in samples from Banyuls sur Mer (French Mediterranean coast), neither in the living nor in the dead assemblages. However, Tretomphalus stages have been described from the warmer eastern Mediterranean Sea (e.g. Frezza and Carboni, 2009, Northern Tyrrhenian Sea), and in a recent study, Milker and Schmiedl (2012) indicate the common occurrence of *Tretomphalus*-stages around Mallorca, offshore Oran as well as in the Alboran Sea.

Although *Rosalina* is one of the few benthic foraminiferal taxa which may develop a planktonic stage, this particularity cannot explain the very uncommon occurrence of this genus described by Fontanier *et al.* (2008). In a core sampled at 373 m depth in Saint-Tropez Canyon, which was incubated for 2.5 years with a salinity varying from 35 to 62, these authors found dense mono-specific assemblages of *R. bradyi*. In the incubated core, this species attained a density of about 500 individuals per 100 cm², about two orders of magnitude higher than the density found in a core sampled at the same site (3 ind/100 cm²). The authors suggest that contrary to all other taxa, *R. globularis* survived the strongly adverse laboratory conditions, with a very high salinity.

Heinz *et al.* (2001) made a very similar observation in a feeding experiment. Surface sediment sampled at 993 m depth in the Gulf of Taranto was incubated in the laboratory for 10 months. One half of the aquarium was fed monthly with deep-frozen algae (*Chlorella* sp.). At the end of the experiment, *R. bradyi,* which is normally not found alive at this water depth, strongly dominated the faunas, in the fed (40%), as well as in the unfed (25%) part of the aquarium. The faunas were only inventoried in the upper 2.5 cm of the 15 cm deep aquarium. The density of *R. bradyi* was 1450 individuals per 100 cm² in the fed part of the aquarium, compared to 400 individuals per 100 cm² in the unfed part.

These observations suggest a highly opportunistic behavior for the genus *Rosalina*. It appears that propagules of this taxon are available even in deep sea sediments, where this species usually does not occur. After incubation *R. bradyi* is much more resistant to conditions more or less adverse than for deep-sea taxa. It survives long incubation periods, and is apparently capable to reproduce asexually, which allows it to attain very high densities.

The densities of *R. bradyi* described in these two incubation experiments (400 to 1450 ind/100 cm^2) are at least 2 orders of magnitude higher than that observed in the field (3 ind/100 cm^2) at the 373m deep site studied by Fontanier *et al.*, 2008. The density of *R. concinna* on the floating plastics (19,3 ind/100 cm^2) is an order of magnitude lower than these spectacular values, and is similar to the minimal values found in sediment samples (Fig. 5).
However, these values cannot be compared directly. In the case of floating plastics, in most cases only a single side of the plastic is colonized, and the surface area represents the whole area available for colonization. In the case of the incubated cores or sediment samples, the standardized values represent the whole population living at and below the sediment surface, down to a depth of several cm. In view of this, the densities on the floating plastics appear to be very high.

R. concinna on plastic debris: a response to multiple stressors?

As explained in the previous chapter, it appears that *R. concinna* is the only benthic foraminiferal species capable to colonize floating plastics. Although the plastics could have been colonized by propagules, we think that the monospecific nature of the foraminiferal assemblages is a strong argument against this possibility. This monospecificity strongly contrasts with the observations made on macroalgae, on which normally fairly diverse assemblages (10 to 20 species) of epiphytic foraminifera are found (e.g., Langer, 1993). We think that the monospecific assemblages of *R. concinna* on floating plastics can only be explained by the fact that this species has a planktonic stage. Several experimental studies have indicated that in the closely related species *R. globularis*, sexual reproduction (and the formation of a floating chamber) only occurs above 18°C. Very probably, a similar lower temperature limit also exists for *R. concinna*. On the one hand, this would mean that at present, around Corsica, floating chambers can only occur in summer and autumn. On the other hand, this temperature limit could explain why the *Tretomphalus*-stage has until now not been observed on the French Mediterranean coast. It seems possible that the large scale colonization by *R. concinna* of floating plastics around Corsica is a fairly recent phenomenon, which has seen in late summer and autumn, the temperature of the surface waters rise above the critical limit for this species, allowing it to produce floating chambers and to reproduce sexually. As such, the appearance of *R. concinna* on floating plastics would be a response to the presence of floating plastic as well as a recent temperature rise.

Impact of colonization by _R. concinna_ on the degradation of plastic debris

At this moment, it is not clear whether the colonization of floating plastic debris by *R. concinna* has an impact on their degradation. Generally, *R. concinna* is fixed to the plastic by means of a feeding cyst (Fig. 1), consisting of organic particles collected by the foraminifer in its surrounding environment. According to Delaca and Lipps (1972), *R. globularis* is fixed to its substrate by means of an organic membrane composed of a sulfated acid mucopolysaccharide, similar to chondroitin sulfuric acid. It has been observed that on calcareous surfaces, pits are eroded under the area covered by the foraminifera (e.g. Todd, 1965). However, Sliter (1965) considered the presence of foraminifera in small pits as fortuitous. In view of the very scant knowledge of the composition of the substance allowing the foraminifer to be fixed on its substrate, and the lack of certainty concerning the capacity of the foraminifer to bio-erode the surface it colonizes, it is not yet possible to decide whether foraminiferal colonization contributes to the degradation of floating plastic debris.

Open questions and future research perspectives

The observation of dense monospecific assemblages of the foraminifer *R. concinna* on floating plastic debris lead to in a number of research questions, which have to be addressed in future studies:

• Is the colonization of plastic debris by *R. concinna* an indirect consequence of recent global warming, which caused an increase of summer and autumn SST above this species' lower limit for sexual reproduction? A detailed study of all available literature and a comparison with historical temperature records can help answer this question.

• Are the floating plastics only colonized following sexual reproduction (formation of *Tretomphalus* floating chambers), or are the individuals which colonized the plastics also reproducing asexually in situ, thereby maintaining viable populations of benthic organisms in a

surface water ecosystem ? A detailed morphological study of the early ontogenetic stages can answer this question.

• What is the impact of colonization by *R. concinna* on the degradation of the plastic debris? Do the chemical substances used by the foraminifer to attach itself, and the eventual mechanical usage, contribute to a more rapid degradation of these macro-debris? Further organic geochemical analysis of the membrane by which the foraminifera are fixed on the plastics can supply part of the answer.

• What is the impact of the colonization of plastic debris on the dispersal capacity of *R. concinna*? In this context, much depends on the dispersal patterns of the plastic debris and time involved in their degradation. However, the inferred presence of propagules of *Rosalina* even in deep-sea settings would suggest that this taxon already has an extraordinary dispersal capacity, which can hardly be increased.

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The toxicity of nanoplastics to marine organisms

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ABSTRACT

The abundance of nanoplastics in the marine environment is expected to increase due to the increasing use of engineered nanoplastics in various applications (primary nanoplastics), as well as due to the continuous degradation of macro- and microplastics at sea (giving rise to so called secondary nanoplastics).

Generally, the ability of (engineered) nanomaterials to interfere with cellular metabolic processes through the generation of reactive oxygen/nitrogen species (ROS, RNS), induction of oxidative stress and subsequent oxidative damage in exposed cells is widely considered to play a central role in their ecotoxicity and is thus an accepted means for testing and comparing the toxicity of various nanomaterials. What is more, the toxicity of a contaminant is directly affected by the chemical, physical and biological transformations it is subject in the environment, which in turn determine its "speciation" and fate, including its distribution, transport as well as partitioning into environmental compartments and lastly its bioavailability to living organisms.

The present document provides a brief synopsis of the (potential) mechanisms underlying the toxicity of synthetic, polymer (plastic) nanoparticles, i.e. nanoplastics, towards marine organisms. General hypotheses concerning material properties and their ROS generating capacities are roughly outlined, most of which remain to be verified for nanoplastics, and the concept of bioavailability is presented as a crucial concept for understanding and assessing toxicity, which allows to link chemical and physical speciation with possible biological effects.

Keywords: environmental nanotoxicology, bioavailability, mechanisms of toxicity, paradigm of oxidative stress

1. INTRODUCTION

The present document provides a brief synopsis of the mechanisms underlying the toxicity of synthetic polymer (plastic) nanoparticles, i.e. nanoplastics (NPs), towards marine organisms. Engineered nanomaterials[1] (ENMs) have unique, size-dependent physicochemical properties

[1] The European Commission has recently recommended the following definition of nanomaterials (European Commission 2011): "'*Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%.*"

(mechanical, optical, catalytic, electronic and magnetic material features) that arise at the nano-scale and significantly differ from their bulk equivalents. Advances in nanotechnology enable mankind to harness these very properties, making ENMs the next generation materials for various commercial, industrial and research applications (Bhatt and Tripathi, 2011; Seal and Karn, 2014).

These new material properties, in combination with expected elevated levels of ENMS and their likely persistence in the environment (especially metal and metal oxide ENMs) have raised concern with respect to their environmental impact (see also contributions by F. Galgani and others in this volume). Ecotoxicological ENM hazard assessment has been complicated by the fact that ENMs differ in two crucial ways from conventional pollutants such as trace metals or organic pollutants (Scown *et al.*, 2010):

• ENMs enter aquatic environments as colloids where they undergo physical and chemical transformation leading to a yet unknown "speciation" (by analogy to conventional pollutants such as metals) characterized by an inhomogeneous mix of homo- and hetero-agglomerates, free/complexed and partially dissolved/chemically transformed ENMs uncertain environmental fate and bioavailable fraction.

• ENMs are expected to have high surface reactivity due to their extremely high surface-to-weight/volume ratio (specific surface area) and potentially, electrically charged surfaces, which may also entail high redox activity enormous variety with uncertain reactivity.

A main ecotoxicological concern is that ENMs have the potential to physically interact with vital cellular and sub-cellular organelles, which in combination with their (potentially) very high reactivity could cause considerable harm to exposed organisms. It is assumed that the behavior and interactions of ENMs with living organisms, even those composed of low-toxic materials (in bulk) such as polystyrene or the metal oxide TiO_2, could distinctly differ from conventional (bulk) pollutants and cause harm due to 1) their small size, possibly facilitating access to subcellular compartments previously inaccessible to direct interactions with (conventional "bulk") contaminants, 2) their extraordinary high surface area and potentially enhanced reactivity and/or 3) other unique, physicochemical nano-scale properties (Scown *et al.*, 2010; Ward and Kach, 2009).

The abundance of NPs in the marine environment is expected to become higher due to the increasing use of engineered NPs in various applications (so called primary nanoplastics), such as polystyrene (Brijmohan *et al.*, 2005), and to the continuous degradation of macro- and microplastics at sea (giving rise to so called secondary nanoplastics) (Wright *et al.*, 2013). Synthetic polymers such as polyethylene (Karlsson and Albertsson, 1998; Zhao *et al.*, 2005) and polystyrene (Snell and Hicks, 2009) for example, are generally considered chemically and biologically inert (Andrady, 2011; Cole *et al.*, 2011; Karlsson and Albertsson, 1998; Welch, 1990), unless they possess reactive surface functional groups. Generally, adverse effects of ENMs to marine habitats can theoretically arise from either indirect or direct interactions. Possible indirect effects of NPs include the interference with and disruption of vital ecosystem services, such as for example biogeochemical cycles, abiotic processes (Bergmann and Klages, 2012) or changes in community structure and abundance (Thiel and Gutow, 2005b). Possible direct effects could result from direct physical interactions between NPs and biological entities (e.g. organisms, cells, organelles, proteins and molecules) via ad- and desorption, which may cause physical (e.g. clogging, obstruction, perforation, etc.) or chemical harm (e.g. generation of reactive oxygen/nitrogen species, denaturation of proteins, etc.) that can lead to toxic effects. There are two possible chemical effects distinctive of NPs. These include the leaching of constitutive toxic (monomer) additives (e.g. by polyvinylchloride, polystyrene, polycarbonate, polyethylene, polyester and polyurethane (Bhattacharya *et al.,* 2010)), of which some are known for their low-dose, endocrine disruptive mode of action in invertebrates, vertebrates, mammals and humans, e.g. Bisphenol A (Vom Saal and Hughes, 2005). The second important chemical effect involves their role as vectors of transport for persistent (often bioaccumulative and toxic) organic pollutants (e.g. polychlorinated biphenyls, dichlorodiphenyl, trichloroethane, nonylphenol) and other xenobiotics, endotoxins (e.g. lipopolysaccharides (Jovanović and Palić, 2012)) or even biofilms and microbial communities (Zettler *et al.,* 2013), which readily adsorb and concentrate on their surface due to their high surface

area and high sorption capacity for hydrophobic organic contaminants (Bergmann and Klages, 2012; Cole *et al.,* 2011; Koelmans *et al.,* 2014a; Teuten *et al.,* 2007).

The toxicity of a contaminant is directly linked to the chemical, physical and biological transformations it undergoes in the environment, which in turn determine its "speciation" and fate, including its distribution, transport, partitioning into environmental compartments and lastly bioavailability to living organisms. The concept of bioavailability provides an important means for linking chemical and physical speciation with possible biological effects and is a crucial concept for understanding toxicity.

2. BIOAVAILABILITY

Bioavailability is defined as "*the extent to which a contaminant in a source is free for uptake by an organism and to which it can cause an effect at the site of action.*" (Newman, 2010; Nordberg *et al.,* 2010). A fundamental premise of toxicology is that interactions, whether direct or indirect, between a contaminant and a target organism, are a prerequisite for the contaminant to elicit toxic effects and that these interactions are decisively influenced by three interfaces, viz. i) the physicochemical properties of the contaminant, ii) the abiotic and biotic properties of the ambient medium as well as iii) the biology of the target organism (Kashiwada, 2006; von Moos *et al.,* 2014; Ward and Kach, 2009). Table 1 provides examples of how these three interfaces may affect the bioavailability and toxicity of NPs specifically.

Table 1. Examples illustrating how the three interfaces affect the bioavailability and toxicity of nanoplastics.

Material properties	Exposure conditions	Biology of species
- NP size-dependent uptake Size dependent adsorption and accumulation of latex NPs by Medaka eggs (Kashiwada, 2006). **- NP size-dependent effects** Rotifers ingested polystyrene NPs ca. 30 – 3000 nm in size. Larger particles remained confined in the gut and caused no reduction in population growth rate. The smallest particles penetrated the gut wall, entered tissues and reduced population growth rate significantly (Snell and Hicks, 2009). **- NP surface charge dependent adsorption onto microalgal cells** Positively charged polystyrene (amidine-latex) beads more readily adsorbed onto microalgal cells due to the electrostatic attraction to the negatively charged cellulosic cell wall (Bhattacharya *et al.,* 2010).	**- Salinity dependent effects** Lethal effects of latex NPs increased dramatically with increasing salinity and simultaneously, aggregation of NPs occurred and their adsorption as well as accumulation by fertilized Medaka eggs decreased (Kashiwada, 2006). **- Marine aggregates facilitate ingestion** Mussels and oysters more efficiently capture and ingest NPs that are incorporated into aggregates compared to those freely suspended (Ward and Kach, 2009). **- Microalgae facilitate ingestion.** The uptake of PS NPs and the production of pseudofeces by the blue mussels were higher in the presence of algae, which probably formed large aggregates with the NPs that were more bioavailable (Wegner *et al.,* 2012).	**- different species uptake rates** Capture efficiencies for particle mass, shape and size vary between species of the same taxon (Ward and Kach, 2009). Medaka adsorbed 494 nm PS beads much more readily than larger (42 000, 18 600, 932 nm) or smaller beads (39 nm) (Kashiwada, 2006). Rotifers absorbed 37-nm particles much more readily than particles larger than 83 nm (Snell and Hicks, 2009). **- differential species sensitivities**

For an interaction to take place, a contaminant must first translate from the bulk solution to the immediate vicinity of a cell/organism via diffusion or mass transport, during which physical, chemical and biological transformations can occur as a function of material properties and the abiotic factors of the ambient medium (Fig. 1). Possible chemical transformations include dissolution, sulfidation, redox surface reactions, (de-)protonation, ligand exchange, photodegradation or complexation, for example. Physical transformations may involve homo- and heteroaggregation with inorganic (e.g. metal) and organic (natural organic matter, polysaccharides, proteins, etc.) colloids. Direct interactions with biological surfaces occur via adsorption and desorption, either as released ions, transformed single nanoparticles, agglomerates or complexes. Cells can modify these interactions by their specific cell surface properties (e.g. by the presence of extracellular polymeric substances, etc.) or by the release of small molecules/proteins. Following adsorption, engineered nanomaterials may, depending on their material properties and the biological target organism, actively or passively be internalized by cells and induce biological responses, which however can also be independent of direct interactions and internalization (von Moos *et al.,* 2014). Hence, interactions occur between an heterogeneous assembly of differently

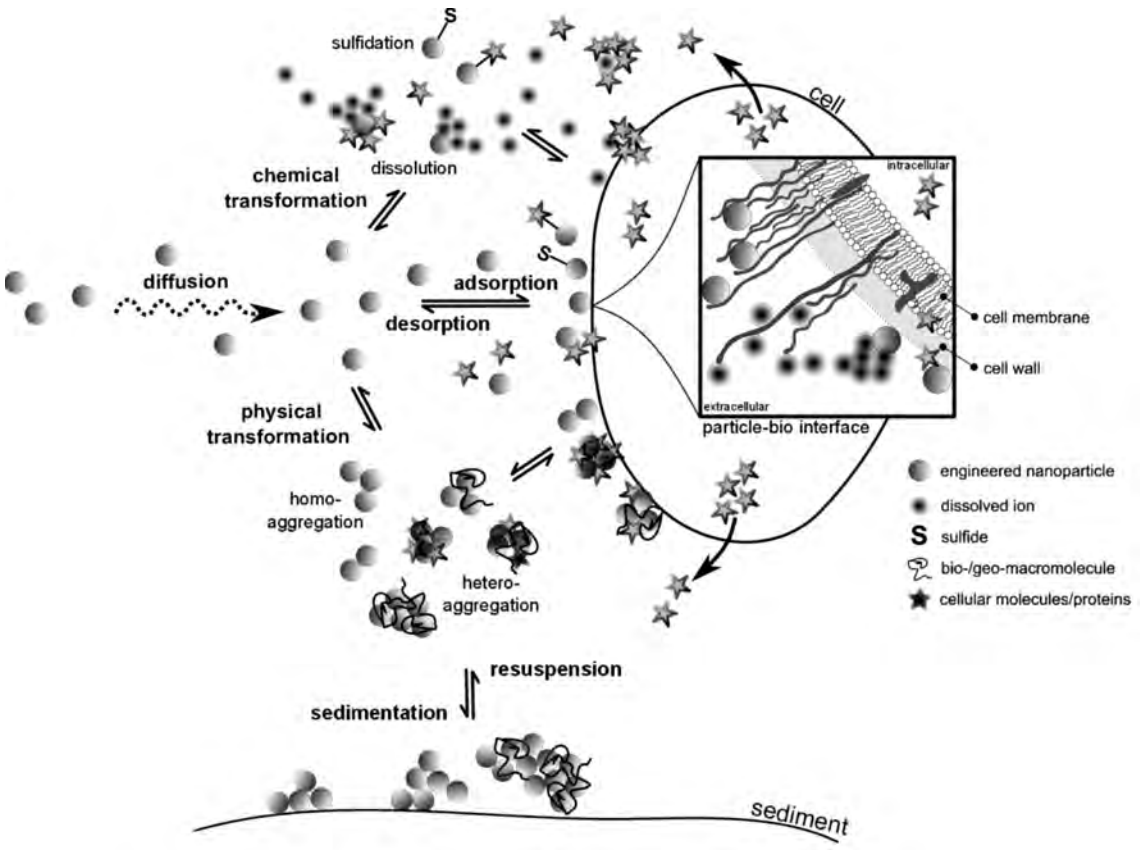

Figure 1. Processes at the medium–bio-interface underlying the bioavailability of engineered nanomaterials to aquatic microorganisms. All processes are highly dynamic. Adapted from von Moos *et al.* (2014), not to scale.

transformed nanomaterials and living organisms. The bioavailability and toxicity of NPs is ultimately shaped by the sum of the above processes at the particle, medium and biological interfaces (Fig. 1) occurring successively or simultaneously and subject to the spatial and temporal dynamics of natural water bodies (von Moos *et al.,* 2014).

The potential environmental transformations of NPs have major implications in terms of exposure of aquatic organisms and communities (Scown *et al.,* 2010). On the basis of laboratory studies, mainly performed on metal ENMs, and on some field studies on microplastics, we can infer the following behavior of NPs, which however, remains to be confirmed by conclusive experimental evidence and field work.

Low-density NPs such as polypropylene and polyethylene can predominantly be expected in the sea-surface microlayer where they are more likely to interact with pelagic organisms. However, their position in the water column can vary due to the accumulation of microbial biofilms, which propagate surface fouling and colonization, and lead to increases in density. Further, wind stress can significantly enhance the vertical mixing of buoyant micro-plastic in the water column (Bergmann and Klages, 2012). High-density NPs (e.g. polyvinylchloride, polyester and polyamide) can predominantly be expected in the benthos in which case sediment-dwelling and benthic organisms become the primary targets of exposure (Scown *et al.,* 2010), with (temporary) remobilization events possibly triggered by turbulence (e.g. storms) or physical perturbation by organisms (Montes *et al.,* 2012). Nonetheless, they could also remain suspended in estuaries due to tidal fronts, high-flow rates or sufficient momentum (Cole *et al.,* 2011). Generally however, high ionic strength favors the agglomeration of ENMs (at least in absence of counteracting surface coatings) and thus cation-rich marine and estuarine environments probably favor sedimentation

(Canesi *et al.,* 2012; Scown *et al.,* 2010). However, aggregation modifying interactions are very complex and hence are still difficult to generalize. It has for example been documented that water stirring and bubbling of exposure tanks during *in vivo* laboratory exposure actually lead to the disaggregation of previously aggregated metal oxide NPs and at the same time also to an increase of organic compounds released by the test species (e.g. mucus, fecal pellets, gametes), which in turn favor the formation of larger agglomerates (Canesi *et al.,* 2012). The relative bioavailability of single *vs* agglomerated NPs will strongly depend on the target organism. Free or embedded aggregated NPs have higher fluxes to the benthos and are generally more available to suspension-feeding bivalves whose particle capture efficiencies decrease with size (Ward and Kach, 2009). Agglomerates are captured and then broken down by the action of cilica on the gills and labial palps and the constituent particles ingested. It has been shown that NPs had longer gut retention times than microplastics (Canesi *et al.,* 2012; Ward and Kach, 2009). By capturing NPs from the water column and depositing pseudofeces on the benthos, mussels also represent an important pathway rendering suspended NPs bioavailable to the benthos (Montes *et al.,* 2012).

For a detailed description of colloidal stability and aggregation, I refer readers to Handy *et al.* (2008, pp. 298). With respect to NPs in oceans, the following two points seem to be key knowledge gaps:

• Development of standardized sampling and detection methods to measure environmental concentrations of NPs.

• Investigate their chemical and physical surface transformations, fate and behavior at sea.

• Adapt laboratory toxicity studies to insights gained from field monitoring.

For a more detailed discussion on technical limitations of ENM detection in organisms and environmental matrices, I direct readers to Powers *et al.* (2006), Bhatt and Triphathi (2011), section "9. Ecotoxicity test strategies and biological hazard assessment" as well as to Scown *et al.* (2010), last section "The future". Table 2 hereafter provides a more detailed listing of knowledge gaps concerning the environmental fate and behavior of NPs specifically.

Table 2. Knowledge gaps concerning environmental transformations, adsorption and uptake of nanoplastics.

Transformations and partitioning of NPs	
Known	**Unknown**
• Increasing input and continuous fragmentation of already present microplastics.	• Effective concentrations of nanoplastics in oceans (Wegner *et al.,* 2012) → exposure concentrations? • Predominant shapes and size distributions, chemical and physical transformations affecting bioavailability and toxicity.
• Trend: low-density plastics are buoyant, or neutrally buoyant. High-density plastics settle (Wegner *et al.,* 2012).	• Persistence, fate and partitioning/distribution (sinking rates) of nanoplastics in marine habitats and compartments. → main target organisms/communities according to plastic types?
• Algae can facilitate aggregation and possibly settling of nano-PS (Wegner *et al.,* 2012).	• General effects of biotic (biofilms, microbial communities, exopolymeric substances) and abiotic (salinity, ionic strength, pH, natural organic matter) factors on agglomeration, sedimentation and lastly on bioavailability and toxicity.
• Aggregation increases bioavailability to benthic organisms and suspension-feeding bivalves (Ward and Kach, 2009).	• Effect of aggregation on bioavailability. • How particle properties affect partitioning and bioavailability.
• Sediments predominantly contain fibrous NPs (Browne *et al.,* 2011; Claessens *et al.,* 2011).	• Bioavailability and toxicity of fibrous NPs?
• Concentration of POPs on the surface of nanoplastics. • Transfer of pollutants and additives from NPs to tissue is possible (Browne *et al.,* 2013; Koelmans *et al.,* 2013).	• Very little data on transfer of POPs from NPs to biota and across trophic levels. • Effects of aggregation on release kinetics. • Relative contributions of particle and POP toxicities?

Adsorption onto organisms/cells	
Known	**Unknown**
• Positively charged NPs adsorb onto negatively charged microorganisms and cause negative effects (Bhattacharya *et al.,* 2010).	• Uptake of secondary nanoplastics? Uptake routes? Retention, egestion and toxicity? • Effects on organism/cellular (behavior, feeding) and subcellular level (stress and damage?).
Ingestion and internalization	
Known	**Unknown**
• Ingestion of microplastics and metal(oxide) NPs occurs across different feeding guilds. Ingestion of nanoplastics has been shown for several organisms (Canesi *et al.,* 2012; Cole *et al.,* 2011; Matranga and Corsi, 2012; Ward and Kach, 2009; Wegner *et al.,* 2012; Wright *et al.,* 2013). • Trophic transfer probable (Matranga and Corsi, 2012).	• Uptake routes • Retention, egestion & bioaccumulation • Trophic transfer
• Mussels and oysters more easily ingest nanoplastic aggregates (Ward and Kach, 2009).	• Effect of agglomeration on bioaccumulation and toxicity?
• Nanoplastics translocate to the circulatory system/blood and penetrate tissue (Kashiwada, 2006; Snell and Hicks, 2009). • Nanoplastics are internalized by cells and single-cell organisms (e.g. ciliates) (Jovanović and Palić, 2012; Pace, 1987).	• Mechanisms of internalization? • Intracellular localization? • Effects of protein corona on toxicity? • Accumulation in tissue and organs? • Toxic effects of accumulation?
• Size ranges of 30 – 50 nm are the optimum for endocytic pathways. Hence, they are theoretically accessible to nanoplastics (von Moos *et al.,* 2014).	• Effective uptake routes.

Adsorption is a prerequisite for particle uptake and often also toxicity. Thus, particle charge crucially affects direct interactions with biological surfaces (e.g. membranes and organelles). Generally, the adsorption of ENMs onto surfaces is governed by van der Waals and hydrophobic forces, electrostatic attraction, hydrogen bonding and receptor-ligand interactions. Most inorganic ENPs are negatively charged in physiological media and natural pH values (Gregory, 2006), and most microorganisms, particularly bacteria and microalgae (Neal, 2008), and biological surfaces also exhibit a net negative surface charge (Hu *et al.,* 2009) due to the dissociation of carboxylic, phosphate and other acidic functional groups of the membrane. The plasma membrane (i.e. phospholipids), intracellular milieu and DNA are negatively charged, which favours interactions with cationic ENMs but does not preclude the uptake of anionic ENMs (Singh *et al.,* 2009). Natural waters are oxidizing environments, while carbon-rich sediments, groundwater (Lowry *et al.,* 2012), and physiological, intracellular conditions are reducing environments (Boxall *et al.,* 2007). Redox reactions highly depend on a compound's redox potential, the presence of redox agents, pH and on the ENM surface properties including the presence of adsorbed substances (Nowack *et al.*, 2012). Sunlight and saltwater are conducive to oxidation and may introduce oxygen containing functional groups on a particle's surface, hence introducing surface charge and enabling electrostatic interactions with biological surfaces. However, while electrostatic attraction is often considered a main driving force for adsorption/adhesion and for direct physical interaction, it does not account for all observed direct interactions between metal ENMs and aquatic microorganisms (von Moos *et al.,* 2014). Currently, literature is still very limited, especially with respect to secondary NPs. For more details on the chemistry and biology of surface modifying processes, readers are directed to contributions by Dussud and Ghighlione, Fotopoulou *et al.* and Raddadi *et al.* in this volume.

The internalization of metal and metal oxide ENMs by bacteria and microalgae has been shown by numerous studies, indicating that cell walls can be penetrated without prior damage. In most cases however, the exact uptake pathways remain unknown (von Moos *et al.,* 2014). Once the first barrier – the cell wall – is overcome, uptake must occur through the semi-permeable phospholipid bilayer, either by non-invasive passive diffusion (i.e. for very small, non-polar particles), membrane fusion or transport proteins. However, endocytic pathways (i.e. clathrin- and cavelolae-mediated) with optimal uptake sizes of 30 – 50 nm are generally considered the most likely internalization routes for ENMs into the cytoplasm (von Moos *et al.,* 2014). This, however, remains to be shown.

3. Mechanisms of toxicity of plastic nanoparticles

The field of nanotoxicology began with research on the toxicity of air-borne NPs (originally ultrafines) towards mammals and mammalian cell lines and more recently developed into what is now known as the field of nanoecotoxicology, dedicated to the ecotoxicity of ENMs towards aquatic freshwater and marine organisms/communities, primarily focusing on metal and metal oxide nanomaterials, which currently also represent the major manufactured class of ENMs. Though the proportion of manufactured NPs is certainly small, they are likely to gain in importance as a secondary byproduct emerging from the ongoing degradation of increasing amounts of macro- and microplastics in marine habitats. Very few studies, in fact I have only found a handful (summarized in Table 4), have specifically investigated the toxicity of NPs towards aquatic marine organisms (Ward and Kach, 2009). Early research on the toxicity of ultrafines lead to the insight that the ability of particles to generate reactive oxygen and nitrogen radical species (ROS and RNS, respectively) at or near their surfaces is a central mechanism by which ENMs engender toxicity (Donaldson *et al.,* 1996; Oberdorster *et al.,* 2005; Oberdorster *et al.,* 2007; Unfried *et al.*, 2007). This "oxidative stress hypothesis" then became one of the best-accepted paradigms for the assessment and comparison of ENM toxicity and was also successfully applied to nanoecotoxicological hazard assessment, and has been demonstrated for various (micro-) pollutants such as synthetic organic substances and trace metals.

The hierarchical oxidative stress hypothesis defines three levels of oxidative stress according to the predominating active biological processes and cellular responses with distinct enzymes and signaling pathways. The lowest level is associated with the induction of the protective antioxidant system to restore the cellular redox equilibrium. In the intermediate level, the activated cellular antioxidant system is overwhelmed and pro-inflammatory responses induce inflammation and cytotoxicity. During inflammation responses the signaling pathways mitogen activated protein kinase (MAPK) and the nuclear factor (NF)-κB are activated and trigger defense reactions that involve the release of pro-inflammatory cytokines (interleukins (IL), tumor necrosis factor (TNFs)) and additional ROS, which creates a negative ROS feedback loop. In the third and highest tier toxic oxidative stress, characterized by increased cytotoxicity and apoptosis, sets in and culminates in oxidative damage (Nel *et al.,* 2006; Singh *et al.,* 2009; Xia *et al.,* 2009; Zhang *et al.,* 2012). A non-exhaustive list of toxic effects of nanomaterials on different levels of biological organization is provided in Table 3.

Table 3. Toxic effects of engineered nanomaterials on different levels of biological organization (non-exhaustive). (Adapted from Jovanović and Palić, 2012; Matranga and Corsi, 2012; Scown *et al.*, 2010)

Subcellular level	Cellular/tissue level	Organism level
• Increase/Decrease in Na+,K+-ATPase activity • Generation of ROS • Oxidative stress • Lipid peroxidation - Thiobarbituric acid reactive substances ↑ - Malondialdehyde ↑ • Activation of antioxidant enzymes (e.g. superoxide dismutase, catalase) • Depletion of glutathione (GSH) • Genotoxicity - hydroxylation of guanine - DNA strand breaks - Changes in gene expression patterns • Immunotoxicity - Immnomodulation - Immune deficiency - Inflammatory responses - Frustrated phagocytosis - Impaired neutrophil function - Altered Immune gene transcription - Lysosomal destabilization • Endocrine disruption	• physical obstruction and damage of organs (pores, gills, digestive tract) • Deformations (e.g. notochord in fish) • Changed haematological parameters • Bradycardia (fish) • Cardiac arrhythmia • Slowed blood flow • Proliferation/hypertrophy (e.g. epithelial cells in fish) • Apoptosis • Oedema (fish) • Increased mucus secretion (fish) • Altered mucocytes (fish) • Hyperplasia in gills • thickening of the gill lamellae	• Alterations in behavior • Immobilization • Changes in morphology • Decreased reproduction • Growth inhibition • Increased mortality/ decreased survival • Developmental abnormalities • Delays in embryonic & larval development • Delay in hatching of eggs • Changes in (embryo) morphology

To date, the ability of ENMs to interfere with cellular metabolic processes through the generation of ROS/RNS, induction of oxidative stress and subsequent oxidative damage in exposed cells is widely considered to play a central role in their ecotoxicity. The present body of evidence seems to support this notion for the toxicity of carbon, metal and metal (oxide) ENMs to aquatic, marine and freshwater model invertebrates (e.g. microalgae, bacteria, crustaceans, etc.) and vertebrates (e.g. fish) (Bhatt and Tripathi, 2011; Matranga and Corsi, 2012; Scown *et al.,* 2010; von Moos and Slaveykova, 2013). Out of the four studies I have found explicitly examining the toxicity of NPs towards marine organisms (Table 4), two reported ROS generation and oxidative stress as observed biological effects of exposure, indicating that the paradigm of oxidative stress may also apply to the effects of NPs in marine organisms.

Table 4. Toxicity of nanoplastics to marine organisms.

Model organism	nanoparticle	particle size & zeta potential	Exposure concentration, time & medium	Biological effects	Mechanism of toxicity	Reference
blue mussel *Mytilus edulis*	Polystyrene beads + *Pavlova lutheri*	1° size: 30 nm 2° size: 1000 nm	0, 0.1, 0.2, and 0.3 g L^{-1}, 8h in seawater	Reduced filtering activity, production of pseudofeces.	NA	Wegner *et al.*, 2012
freshwater, single-celled microalga *Chlorella* freshwater/marine, multicelled microalga *Scenedesmus*	Charged polystyrene nanospheres/beads: - amidine latex (positive) - carboxyl latex (negative)	1° size: 20 nm Zeta: 106 & -40 mV	1.8 - 6.5 mg L^{-1}, 3 - 60 h in algal growth medium	Adsorption to cell walls, reduction of photosynthesis, increased **ROS** production	reduced photosynthesis lead to increased respiration and increased O_2 activity.	Bhattacharya *et al.*, 2010
marine rotifer *Brachionus manjavacas*	polystyrene	37, 83, 217, 546 and 2980 nm	5.74, 2.87, 1.43, 0.96, 0.72, and 0 µg mL^{-1}, 2h and 48h in artificial sea water	Ingestion.Smallest NP reduced population growth rate.	Possibly suppressed ingestion or direct interference with metabolism. Oxidative stress.	Snell and Hicks, 2009
Medaka/Japanese rice fish, eggs, larvae & adult fish *Oryzias latipes*	Fluorescent solid latex (polystyrene) water solutions	39 – 42000 nm	1mg L^{-1}, 24 h - 7 d in embryo rearing medium	Size-dependent adsorption of NPs onto chorion and accumulation of NPs in oil droplets of eggs. Uptake through gills and intestine by adult fish and entry into various organs via gill-blood route. Elimination via feces. Penetration of blood-brain barrier.	NA	Kashiwada, 2006

There are two main theoretical mechanisms, by which ENMs in general can generate ROS, namely i) via chemical reactivity or ii) via direct physical interactions with subcellular compartments, especially electron transport chains (von Moos and Slaveykova, 2013). In the case of NPs, the second mechanism seems more likely, since plastics, and probably NPs too, are generally considered bio-inert, at least in their bulk form. Hence, it seems plausible that NPs are capable of appropriating previously inaccessible (to their equivalent bulk formulations) access ways to cells (e.g. endocytosis, pores, channels, etc.), which may enable direct, contact-mediated interactions that lead to the generation of ROS. This is supported by findings from the exposure of rotifera to different sized polystyrene beads, where the authors Snell and Hicks (2009) observed the following: "*The 2-day reproductive bioassays for 100-, 200-, 500-,and 3000-nm diameter particles, for exposures up to 1.2 µg mL^{-1}, had no statistically significant effect on population growth rate. Since all of these particles are composed of the same material, which lack chemical toxicity at the exposure concentrations, the adverse effects of the 37-nm nanoparticles must be due to the size of the particle; not its chemical composition.*" and concluded *"Larger particles remain confined in the intestinal tract (Fig. 1) and were eventually defecated away. Rotifers therefore can eliminate larger particles from their guts through defecation without absorption, but nanoparticles of about 37 nm are strongly absorbed. Because all of the nanoparticles tested were of identical chemical composition, this result indicates that below a threshold diameter (37–83 nm), nanoscale particles are able to pass through the gut wall and enter the tissues, perhaps via membrane pores or uptake by epithelial phagocytosis."*. It remains to be shown if this observation can be considered a more general mode of NP action concerning the generation of ROS or if in other cases, there may in addition actually also be nano-scale effects affecting the reactivity of NPs in general (e.g. due to increased surface area), especially with respect to the leaching of constituents and the sorption and desorption of xenobiotics.

Current efforts are directed to linking (measurable) material properties to ROS/RNS inducing abilities to enable more rapid and efficient hazard assessment and prediction of the overwhelming variability of emerging materials and exposure scenarios. These principally include properties such as chemical composition, purity and solubility, size/aggregation, surface area and shape, primary and secondary surface coatings and surface functionalization, surface chemistry and charge,

photochemistry and band gap energy (Scown *et al.,* 2010; von Moos and Slaveykova, 2013). Detailed considerations on how these factors affect toxicity can, for example be found in Scown *et al.* (2010) and von Moos and Slaveykova (2013). General hypotheses concerning material properties and their ROS generating capacities are roughly outlined in Table 5, some of which remain to be verified for NPs specifically and in general. In the case of metal (oxide) ENMs, contradicting findings have been reported for practically every one of these hypotheses. This is thought to be mainly due to the extremely high material diversity as well as the high sensitivity of the exposure conditions to abiotic factors and treatment (exposure media, ENM preparation and handling, etc.).

Table 5. General hypotheses concerning ROS generating capacities.

Property	Hypothesis
Size & surface area	• Smaller particles are more toxic than larger particles. • Size-dependent generation of ROS (larger surface area).
Surface chemistry	• Bulk = non-toxic • Nano = toxic
Surface functionalization	• Increases or decreases ROS generation
Acquired surface coatings	• Increases or decreases ROS generation
Charge & Aggregation	• Opposite charge can favor electrostatic attraction and increase ROS generation. • Same charge decreases ROS generation due to increased repulsion. • Aggregation decreases ROS generation (decreased surface area).
Shape	• Rod shaped NPs are more toxic than spherical (frustrated phagocytosis).

4. CONCLUSION

The vast majority of studies have investigated the effects of microplastics or metal and metal oxide nanoparticles towards marine organisms. Some general concepts may also apply to nanoplastics, but there is a need to fill this research deficit to better understand their toxic potential. Most research has also focused on effects on single cells and organisms and their overall impact remains elusive. Studies investigating effects of chronic exposure to low, more realistic environmental exposure concentrations of nanoplastics are completely lacking.

Microplastics in the marine environment – How can we identify potential risks?

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Abstract

A novel analytical method for extraction of microplastics (< 5 mm) from sediment samples using two-step density separation has been developed. The method allows for simultaneous identification of plastic additives and specific identification of the polymer material. This is achieved by sequential thermodesorption (to identify PAs and sorbed POPs) and pyrolysis (for revealing the polymer material) of the plastic particles both coupled with GC/MS analysis. The method was exemplary applied to environmental samples. Extracted particles could be identified as plastics and a number of additives potentially leaching out of the particles under environmental conditions was detected. A model approach was applied to investigate the bioaccumulation potential of additives and co-transported organic pollutants from plastics in fish during their lifetime. Model simulations for two organic pollutants (1,2,3-Trichlorobenzene and PCB-153) revealed that bioaccumulation of organic pollutants through a carrier mechanism is probably of minor concern. Body burden of persistent substances in fish is likely to be reduced by dilution effects while non-persistent pollutants were predicted to accumulate slightly stronger in case of plastics ingestion due to the larger substance intake.

1. Introduction

Marine plastic debris of all size classes contaminates marine waters and coastlines worldwide (Barnes *et al.*, 2009; Andrady, 2001). Among the larger items, waste products of consumption and remains of fishing activities are dominating. In the smaller size fraction, plastic pellets (raw material) and micro-beads as additives in personal care products have been frequently detected (Cole *et al.*, 2011). Although plastic material is chemically persistent in the environment, larger items may be fragmented under environmental influences such as UV light or mechanical pressure exerted by water currents, rocks or sediments working together or in sequence. As a consequence, weathering, abrasion or disruption result in an increasing number of smaller polymer fragments (secondary plastic debris). Monitoring programmes are important to determine the actual contamination of the environment and to evaluate the efficiency of reduction measures. Hence, the development of analytical methods to determine the microplastics contamination in the environment is one of the major challenges to date. An extensive review on currently used methods was provided by Hidalgo-Ruz *et al.* (2012).

A major problem in this respect is the lack of standardization of protocols for sampling, extraction and detection of microplastic debris in water and sediment. As a consequence, existing data from various studies generally suffer from restricted comparability due to inconsistent methods and data reporting (e.g. different size class definitions and quantification units). The smaller the particles the more difficult the reliable quantification of plastic particles in sediment samples. While mesoplastics (5 – 25 mm) can be extracted relatively simply by sieving, separation of the microplastics fraction from the sediment matrix is more difficult. Currently used extraction methods for microplastic particles from sediments are based on the principles of fluidisation or flotation (Imhof *et al.*, 2012; Claessens *et al.*, 2013; Nuelle *et al.*, 2013). After extraction, quantification requires weighing or counting of the extracted particles which is a difficult task for microparticles barely visible by the naked eye. Microscopic inspection of the sample extracts has been shown to bear a high risk of overestimation due to large amounts of natural substances (animal parts, minerals) still present in the extracts (Dekiff *et al.*, 2014). Hence, chemical identification of particles suspected to be plastics is indispensable after visual pre-sorting. Recommended techniques for proper identification of plastics are Raman or FT-IR spectroscopy (Imhof *et al.*, 2012; Claessens *et al.*, 2013).

Another important issue is the danger for marine organisms emanating from the presence of microplastic debris in the environment. It has been shown that microplastics can generally be ingested by marine organisms such as mussels or fish. In lab experiments, blue mussels reacted with inflammative transformation of cells when exposed to small microplastics (von Moos *et al.*, 2012). However, experiments had been conducted under extremely high and unrealistic plastic particle concentrations of 500 mg/L in water so that transfer of the results to environmental conditions is difficult. Investigations for the presence of microplastics in fish showed relatively low contamination of less than three particles in the gut (Foekema *et al.*, 2013) which is most likely due to excretion of ingested particles with feces.

In conjunction with plastic ingestion by organisms it is also discussed whether transfer or enhanced bioaccumulation of persistent organic pollutants (POPs) may occur as a consequence of the high sorption capacity of many plastics for lipophilic compounds (Rochman *et al.*, 2013a). Koelmans *et al.* (2013) performed a model analysis to simulate the effect of plastics on bioaccumulation of selected POPs in the lugworm *A. marina.* They showed that for this species ingestion of plastics will often lead to decreasing bioaccumulation due to 'dilution' of the sediment contamination and 'cleaning' mechanisms that outweigh the carrier effect of contaminated microparticles. On the other hand, detected concentrations of organic chemicals such as DDT, HCB or PCBs in large filter feeding organisms in the Med Sea hint at an increasing effect of microplastic particles on bioaccumulation of organic chemicals and plastic additives (Fossi *et al.*, this volume).

Plastic additives are often added to polymers to modify their properties. The substance class comprises many different chemicals such as pigments and dyestuffs as colorants, fillers and reinforcements to modify mechanical properties, antioxidants, UV stabilizers and flame retardants to provide resistance against heat, aging, light or flames, and anti-static/conductive additives, plasticisers, blowing agents, lubricants, mould release agents, surfactants or preservatives to improve the performance of the polymer (Gächter and Müller, 1993). It has been shown that these additives can leach out of the matrix over time and exert toxic and endocrine disruptive effects on marine organisms when plastics are ingested (Oehlmann *et al.*, 2009). A model investigation of the potential exposure of organisms to plastic additives leaching out of the material in the gut was performed by Koelmans *et al.* (2014b). Using nonylphenol and bisohenol A as examples, they concluded that this pathway is probably of minor relevance for the North Sea cod. However, due to the wide variety of chemicals used as plastic additives, it is essential to collect more information on the nature and quantity of additives and their ability to leach out in the organisms' gut.

In this presentation, a novel analytical method is presented that combines density separation of microplastics (< 5 mm) with subsequent simultaneous identification of plastic additives and specific identification of the polymer material. One objective was to decrease analytical costs from huge amounts of high density salts by decreasing the initial sediment mass using a two-step density separation. The novelty of the method is the possibility of identifying incorporated plastic additives and the polymer material within the same analysis. In the second part of the presentation, the bioaccumulation issue is tackled by a modelling study. To do so, we adapted an existing bioaccumulation model for fish to allow for investigation of the effect of microplastics ingestion

on the bioaccumulation of organic pollutants in herring. The model was exemplarily applied for two organic pollutants (1,2,3-Trichlorobenzene and PCB-153) which are diffferent with respect to lipophilicity and persistence in the environment and the investigated organism.

2. Analytical method for microplastics in sediment

The developed method allows for simultaneous identification of polymer types of extracted plastic particles and associated plastic additives (PAs) using sequential thermodesorption and pyrolysis-gas chromatography coupled with mass spectrometry (Pyr-GC/MS). Extraction from the sediment matrix is performed by density separation and particles suspected to be plastics are pre-sorted by visual inspection supported by microscopy prior to specific identification.

2.1. Extraction procedure

Microplastics were extracted from sediment samples by applying a two-step extraction procedure (Nuelle *et al.*, 2013). In order to avoid background contamination during the analysis, all materials and vessels were thoroughly cleaned prior to use and covered with aluminium foil after each single step.

Air-induced overflow (AIO) method. In the first step, a fluidisation method was used to separate the major fraction of the sediment matrix from particles with lower density. The principle of the AIO method is shown in Figure 1. Approximately 1.5 L of saturated sodium chloride (NaCl) solution was pumped from a glass storage tank (H) into a 2 L glass beaker (J) that had been placed inside a larger glass vessel (I). Moderate bubbling was generated by a constant air flow (air flow rate: 0.1 L/s) and 1 kg sediment sample was added quickly to the NaCl solution. The air-generated tiny gas bubbles adhere to suspended particles, thereby lowering their density and support floating of the lighter plastic particles to the surface of the water. The high density sediment matrix settled on the bottom of the beaker. The air flow rate was increased to 0.2 L/s to ensure maximum recovery and another 2.5 L of NaCl solution was pumped into the beaker (near the bottom) with a constant flow rate of 0.040 L/s inducing an overflow of solution at the top into the outer glass vessel. This overflow contains the majority of low density particles. After the separation process, a series of rinsing steps was performed to enable efficient collection of low density particles that may have had adhered onto the glass wall of the beaker.
The final NaCl solution in the outer vessel was sieved through a 25 µm stainless-steel sieve. The residue containing sediments and microplastics was rinsed back into the outer vessel with 250 ml distilled water. The residue was then decanted from the outer glass vessel by thoroughly rinsing with about 800 ml NaCl solution onto a folded filter paper (pore size: 5 µm) placed in a glass funnel.The filter cake was rinsed with 700 ml distilled water to clean the sample material from NaCl. The filter was oven-dried at 60°C overnight and stored in a glass beaker for further extraction.

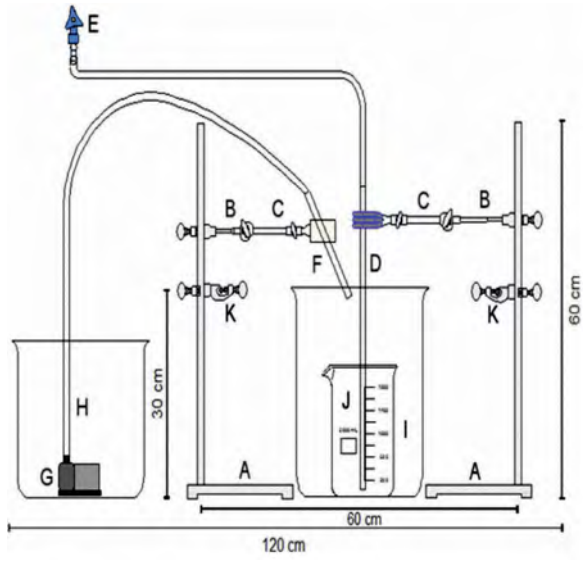

Figure 1. Experimental setup of the AIO method (from Nuelle *et al.*, 2013).
A: laboratory stands, B, C: clamps, D, F: glass tube, E: laboratory fume, G: fountain pump, H: storage tank for NaCl, I: outer glass vessel, J: inner glass beaker (2 L), K: double sockets with parallel metal rods.

Flotation method. The second extraction step was performed using the principle of flotation. To enhance the separation efficiency saturated sodium iodide (NaI) solution with a density of 1.8 g cm^{-3} was used in this step. The remainder of the first extraction step was filled into a volumetric flask and saturated NaI solution was added to roughly fill the flask to three-quarter. The volume of the flask was selected according to the remaining mass of the sediment sample. The flask was shaken by hand for about 20 seconds and then filled up to its nominal volume with NaI solution. After shaking for another 20 seconds to reduce the amount of minerals on the solution surface due to adhesion and surface tension, the flask was completely filled with NaI solution. The suspension was allowed to settle for 10 minutes and the supernatant (approximately 20% of total volume) was then decanted into another flask which was filled up with NaI solution. The flask was shaken for 20 seconds, let rest for 10 minutes and the supernatant was decanted. This procedure was repeated five times. The final supernatant was filtered through nitrocellulose filter (0.45 mm pore width). The beaker was rinsed thoroughly with 250 ml distilled water which was added to the filter. The filter was washed with 750 ml distilled water to remove any salt residues and finally air-dried. Optical analysis of the filters was performed using a stereomicroscope (Wild M3Z, Leica Microsystems, Wetzlar, Germany) providing 6.5-fold up to 40-fold magnification. Optical images of particles were taken using a digital camera, which was connected to the microscope via a phototube. Particles that were visually identified as potential plastics were separated using tweezers and stored for further analysis.

Recovery. The recovery of the method was tested by adding known quantities of plastic particles (approximately 1 mm in size) of ten different polymer materials to pre-cleaned sand. Applying the extraction and detection method described above, recovery rates were consistently between 91% and 99% (Nuelle *et al.*, 2013).

2.2 Detection of plastic additives and polymer material

The particles suspected to be plastics were analysed by sequential thermodesorption (to identify PAs and sorbed POPs) and pyrolysis (for revealing the polymer material) both coupled with GC/MS analysis. A detailed description of the method can be found in Fries *et al.* (2013).

Thermodesorption. A particle was placed in a thermal desorption tube, which was pre-conditioned in a first step at 40 °C for 60 min in order to remove any sorbed contaminants. Then thermodesorption at a temperature of 350°C was applied to remove PAs from the plastic particle. The thermally desorbed chemicals were trapped on a cold injection system (CIS) and analysed by GC/MS. The MS was run in the full scan mode for identification of thermally desorbed compounds by comparison with data from the NIST05 mass spectra libraries. For verification, authentical standards of the suspected chemicals were analysed under the same conditions. Verification requires agreement of gaschromatographic retention times and the mass spectra.

Pyrolysis. Subsequently, the same plastic particle was pyrolysed at 700°C for 60 seconds. Thermal decomposition products were again trapped using the CIS and analysed by GC/MS. Structure identification relies on the occurrence of polymer-specific patterns of pyrolysis products that were identified by consulting the mass spectra library and spectra published in the literature (Tsuge *et al.*, 2011; Fries *et al.*, 2013). After pyrolysis, typical GC/MS chromatograms (pyrograms) are observed for many plastic materials (fingerprint). Ten different types of the most common standard polymers (PVC, PC, PUR, PA, ABS, PET, LDPE/EVA, PP, PS and EVA) were analysed and the outcome was used as reference pyrograms for comparison with real samples.

2.3 Application of the method

The method was applied to selected sediment samples collected from two German islands, namely Norderney in the North Sea (Dekiff *et al.*, 2014) and Fehmarn in the Baltic Sea. The two step extraction method was applied to 1 kg of dry sediment each. After filtration of the final supernatant, particles potentially being microplastics were sorted out by visual inspection. Figure 2 shows a typical microscopic picture of the filtrated final supernatant from a sediment beach sample from Norderney.

Identification of polymer. Pyrolysis-GC/MS of the particles successfully identified items consisting of polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polyvinylchloride

(PVC), polystyrene (PS) and polyamide (PA). However, approximately 50% of the particles could not be assigned to a specific polymer type indicating that they may have been of natural origin. On average, Norderney beach samples contained 0 – 4 (average: 1.6) unequivocally identified microplastic particles per kg dry sediment, while the Fehmarn samples showed slightly higher numbers from 0 – 8 particles (average: 3.2). These concentrations were at the lower end of published data for microplastics in beach samples which may be due to overestimations in other studies as a consequence of the lack of specific identification of extracted particles. This application clearly shows the necessity for specific identification of extracted particles as microplastics to avoid overestimation of environmental contamination.

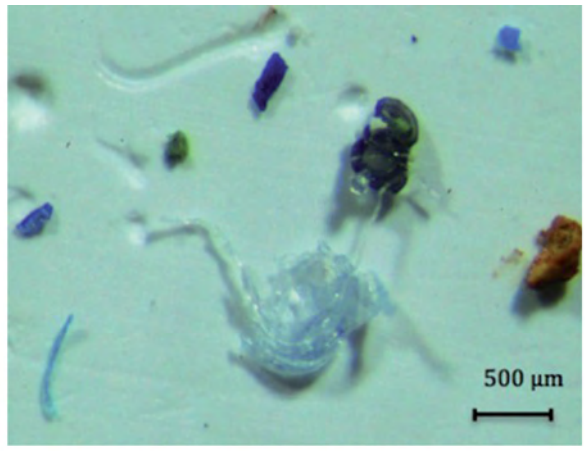

Figure 2. Suspected microplastics from a Norderney sample.

Fibres. The extraction of the first four samples from Norderney beaches revealed numerous fibres of different colors visually detected on the filters. However, inspection of filters from blank samples that had been openly exposed to the laboratory air for 24 hours, revealed a high risk of sample contamination. As an example, the left panel of Figure 3 shows the microscopic image of a filter from a Norderney beach sample, while the right panel shows the respective laboratory blank. We suspect that sample contamination via air can be an important pathway leading to erroneously high numbers of fibres in samples. Therefore, we urgently recommend taking precautious action like working in clean benches and sealing all samples and extracts during the whole analytical procedure to minimize laboratory-specific background contamination of samples. Additionally, analysis of procedural blanks is strongly recommended.

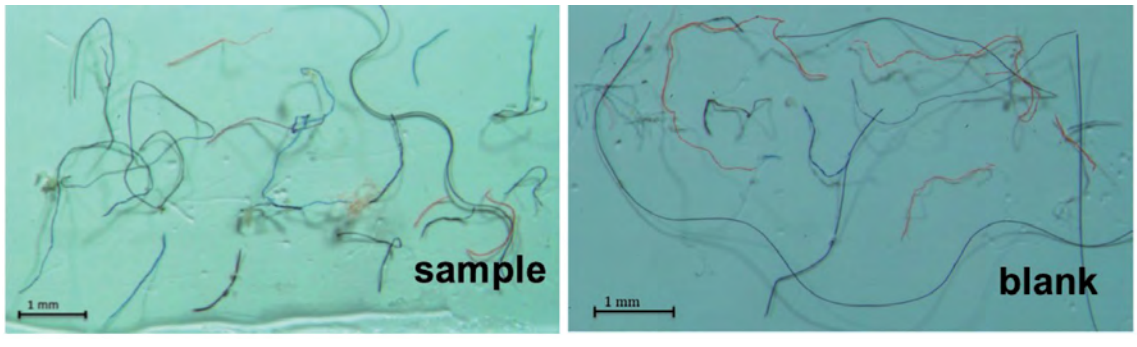

Figure 3. Microscopic images of fibres extracted from a sediment sample (left) and of a laboratory blank (right).

Plastic additives. For a total of ten investigated particles, thermodesorption-GC/MS allowed for identification of at least one additive. Among the detected compounds were benzophenone, 1,2-benzenedicarboxylic acid, dimethyl phthalate, diethylhexyl phthalate, dibutyl phthalate, diethyl phthalate, diisobutyl phthalate, dimethyl phthalate, benzaldehyde and 2,4-di-tert-butylphenol – all well-known plastic additives. The combination of thermodesorption and subsequent pyrolysis allows for additional identification of the thermally desorbable fraction of plastic additives even in the full scan mode of the mass spectrometer. The method needs to be further improved to allow for at least semi-quantitative analysis of the additives. Studies that focus on the potential effect of plastic additives on marine organisms could then make use of this method.

3. Effect of microplastics' ingestion on fish bioaccumulation

So far, only little information is available on the potential effect of ingestion of microplastics on bioaccumulation of organic chemicals in marine organisms. Since it is known that polymer surfaces have large capacities for sorption of organic pollutants (Fotopoulou *et al.*, this volume), this could be an important exposure route for active and passive feeders. Physico-chemical substance properties are important factors determining the environmental fate of chemicals probably also playing a role for the microplastics' effect on bioaccumulation. As a first step towards prioritization of chemicals in this respect, we performed a model analysis investigating the effect of microplastics ingestion on bioaccumaulation of chemicals with different lipophilicity and persistence.

3.1 Bioaccumulation model

The environmental fate of chemicals is commonly described by fugacity models of the Mackay type (Mackay, 2001). In these models, fugacity is a relative descriptor for the concentration of a chemical in an environmental compartment defined as the escape tendency of the chemical from the compartment expressed in units of pressure. In case of equilibrium between compartments, the fugacities of the compound in the compartments equal each other. As part of the well-acknowledged bioaccumulation model ACC-Human (Czub *et al.*, 2004) the dynamic Gobas fugacity model (Gobas *et al.*, 1988) describes the bioaccumulation of pollutants by baltic herring growing from birth to the age of five years. To model the effect of plastic ingestion on bioaccumulation of organic substances, we introduced a number of modifications and made the following additional assumptions on intake and partitioning behaviour of organic substances to plastics:

• In addition to plankton, herring ingests microplastics (10 % of the plankton volume). Ingested plastic material is completely excreted and so there is no plastic accumulation.

• The presence of microplastics in the water column does not alter the steady-state distribution of organic chemicals in the marine environment.

• Plankton, microplastics and the fish at birth are in equilibrium with the surrounding water. The fugacity (concentration) of water is fixed at 1 Pa.

• Within the gastrointestinal tract, plankton and microplastics are homogeneously mixed, i.e. pollutant exchange between microplastics and plankton is fast in comparison to dietary absorption.

• Partitiong coefficients for plankton/water and fish/water are assumed to be correlated with the octanol/water partitioning coefficient (K_{OW}).

• It is assumed that ingested microplastics consist of polyethylene (PE). The fugacity capacity of PE is determined by the partitioning coefficient between PE and water ($K_{PE,w}$) which is also assumed to be correlated with K_{OW} (Adams *et al.*, 2007) unless explicit measured values exist.

• Whole body transformation rate constants for fish (normalized to fish body weight of 10 g) were taken from the database of Arnot *et al.* (2008).

The modified model considers age-dependent growth of the organism (fish), intake of chemical via ingestion of plankton (food) and plastics, accumulation during gill breathing, loss by metabolic transformation and excretion. The model originally delivers body burden (mass) of the investigated compounds over the first five years of the herring's life. To illustrate the accumulation of the compounds in herring relative to its natural environment (water), we calculated the fugacity in

fish at each time point. Since the fugacity of water is fixed at 1 Pa, values in fish above unity indicate accumulation beyond equilibrium, while at values below 1.0 the herring's body burden is lower than it would be if in equilibrium with water.

3.2 Model application

We selected 1,2,3-Trichlorobenzene and 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB-153) as exemplary compounds. 1,2,3-Trichlorobenzene is moderately lipophilic (log K_{OW} = 4.05) and non-persistent in fish with a metabolic transformation half-life of 18 days. In contrast, PCB-153 is metabolized much more slowly with an estimated half-life of 212 days. With log K_{OW} being 6.87 (Li *et al.*, 2003) it is also more lipophilic, resulting in higher sorption to microplastics. The equilibrium partitioning coefficient between polyethylene and seawater of log $K_{PE,w}$ = 6.7 has been measured by Choi *et al.* (2013).

To demonstrate the effect of microplastics ingestion on bioaccumulation the model was run twice for each compound, namely with and without the assumption of additional PE ingestion. In Figure 4, the different accumulation behavior of the two compounds for the two scenarios is displayed by the fugacity ratios between herring and water.

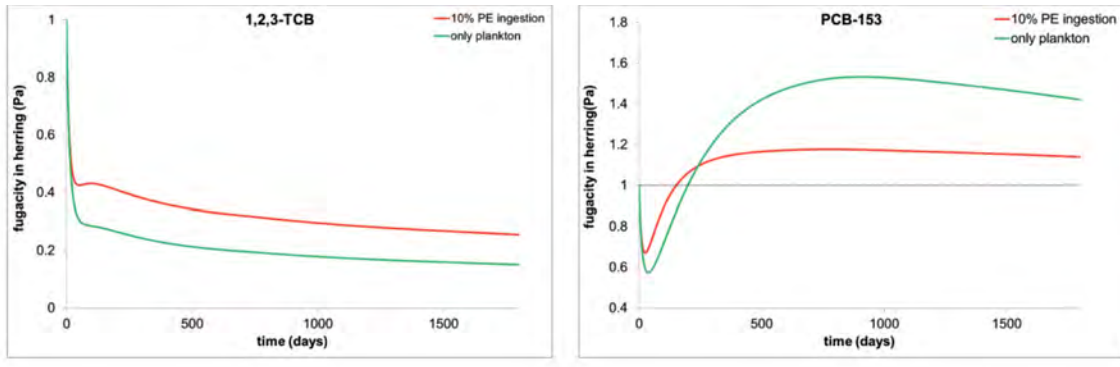

Figure 4. Simulated fugacity ratio herring / water with and without 10% PE ingestion for 1,2,3-Trichlorobenzene (left panel) and PCB-153 (right panel).

In general, 1,2,3-TCB is strongly diluted in the first months of the herring's lifetime and approaches steady-state values clearly below equilibrium in the long run. Metabolization of the compound is fast enough to compensate for further intake of the chemical via gill breathing and ingestion. The additional uptake of plastics (PE) leads to an increased body burden, but the herring is still below equilibrium during its whole lifetime. Since metabolic transformation is the major loss process, excretion has almost no effect on total removal from the body.

For PCB-153, diluton only occurs in the first month of the herring's lifetime. Thereafter, body burden increases due to uptake with plankton (biomagnification) and in the long run PCB-153 is accumulated even beyond the equilibrium state. Since PCB-153 is rather persistent in herring, the only depuration process is egestion with feces, which is not effective enough to prevent the fish from the simulated accumulation. Interestingly, for such a persistent compound PE ingestion has a depurating effect indicated by the lower accumulation of PCB-153 in the PE ingestion scenario. This is due to the fact that ingested PE is assumed to be 100% excreted again, thereby enhancing clearance of the compound from the fish body.

Figure 5 further illustrates the different effect of PE ingestion for the accumulation of the two chemicals in herring. The simulated body burden ratio with and without PE ingestion shows the above described opposite behavior. While PE ingestion is predicted to increase bioaccumulation for 1,2,3-TCB in herring by a factor of 1.5 to 1.7 over almost the whole lifetime, the model predicts lower PCB-153 accumulation with ingested plastics in the long run. Only for the first nine months

of the herring's lifetime a maximum increase of 25% is predicted. Due to the slow metabolic metabolization of PCB-153 the major route for removal is excretion. PE has a larger sorption capacity compared to feces which allows for more effective clearance from the body and eventually reduces observed bioaccumulation with increasing age. After two years the body burden is simulated to be approximately 25% less when PE ingestion is assumed.

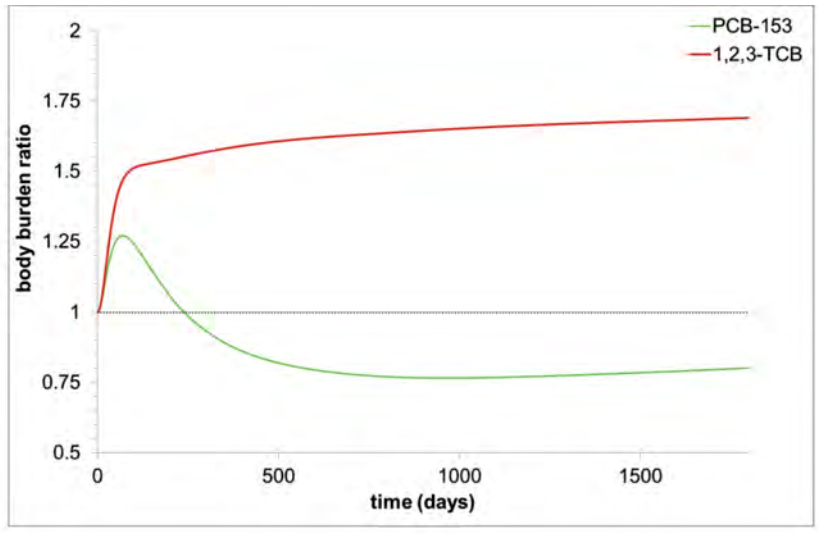

Figure 5. Simulated body burden ratio of herring with and without plastics ingestion for 1,2,3-Trichlorobenzene (left panel) and PCB-153 (right panel).

However, it has to be pointed out that PCB-153 shows higher absolute bioaccumulation compared to 1,2,3-Trichlorobenzene due to its higher K_{OW} and its resistance against metabolic transformation. Although PE ingestion is predicted to decrease PCB-153 bioaccumulation while increasing that of 1,2,3-Trichlorobenzene, the rank order for absolute bioaccumulation is still conserved.

4. Discussion

Information on microplastics abundance in the environment is not yet sufficient due to a lack of simple extraction and identification methods for routine monitoring. Existing methods are still extremely time-consuming. Indispensable specific identification of the particles as plastics is requiring advanced instrumentation such as Raman or FT-IR spectroscopy or Pyrolysis-GC/MS. Although published methods offer the chance of producing robust and comparable data on microplastics contamination in the marine environment, the required effort (time and money) is still extremely high. Particles extracted from beach sediments were analysed with thermodesorption coupled with GC/MS. It could be shown that additives are thermally desorbable from microplastics and therefore potentially bioavailable. To exclude the potential risk of additive leaching during the gut passage of organisms more research is necessary.

The issue of enhanced POP bioaccumulation through plastics ingestion was investigated by model analyses. Results for two exemplary substances indicate that bioaccumulation of organic pollutants through a carrier mechanism is probably of minor concern. For persistent substances the dilution effect dominates over the carrier mechanism, leading to reduced bioaccumulation. More effective accumulation of pollutants in the organisms in case of plastics ingestion is predicted for non-persistent pollutants where the increased intake can outweigh removal by metabolic transformation.

Plastic fragments on the surface of Mediterranean waters

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Abstract

The Mediterranean Sea is one of the most affected by floating plastic debris however few studies were performed on the impact of microplastics fragments. Data obtained up now suggests that neustonic microplastics are widespread in the North and Central Western Mediterranean Sea. The highest density (300,000 particles/ km^2) detected during a survey in Ligurian Sea is of the same order of magnitude as that found in the North Pacific Gyre. Microplastics debris composed mainly of polyamides (53%), polystyrenes, polyolefins and polyesters, were present in all Manta tows. The size distribution frequency showed that fragments are of small size (1-2.5mm). The average ratio between microplastics and mesozooplankton surface area was 0.2 for the whole survey. Copepods were the most abundant organisms in the surface layer but neustonic mollusks and cladocerans were also abundant. Due to of the fragmentation, small microplastics may be ingested by organisms commonly unaffected by the larger marine debris. The magnitude, distribution and especially the potential impact of microplastics on the environment and their interactions with the biota need to be better assessed.

Keywords: microplastic particles, zooplankton, Ligurian Sea, Mediterranean Sea

Introduction

Microplastics are now ubiquitous in the marine environment, found in seawater at the surface and at depth, in high seas and coastal waters from the equator to the poles. Several studies have reported high concentrations of surface microplastics in many of the world's oceans (see review of Barnes *et al.,* 2009) with a large impact on Gyres as North Pacific Ocean (Doyle *et al.,* 2011, Goldstein *et al.,* 2013), the central Northern Pacific Ocean (Moore *et al.,* 2001), the Sargasso Sea (Law *et al.,* 2010) and along coastlines and estuaries (Lima *et al.,* 2014). In the marine environment a wide range of organisms, from plankton to larger vertebrates such as fish, turtles or whales are "confronted to this abiotic plankton" and may ingest them (Wright *et al.,* 2013). The potential confusion with plankton by filter feeders in the neuston, their association with chemical contaminants and their possible role of vector of microorganisms make these particles potentially "harmful" for the ecosystem (MSFD, 2013). In addition, disintegrated plastic can be absorbed by

marine life and therefore likely incorporated into the pelagic marine food webs with multiple unknown consequences (Teuten *et al.,* 2009; Fotopoulou *et al.,* this volume). Thus understanding the mechanisms by which microplastics are distributed, transported and enter our food chain is essential to assess its effects on habitat degradation and to develop policies for their management.

Scientific investigation on the impact of neustonic microplastics (0.3–5 mm) in Mediterranean Sea is recent, existing data originate mainly from summer cruises performed from 2010 to 2013 in the Ligurian and Sardinian Seas (Collignon *et al.,* 2012; Fossi *et al.*, 2012; de Lucia *et al.*, 2013). There is only one annual survey describing the variations in microplastics and the neuston zooplankton from August 2011-2012 in the bay of Calvi (Collignon *et al.,* 2013). Additionally Fossi *et al.* (2012, 2014a and this volume) reported on the impacts of microplastics on large filter feeding marine organisms such as Mediterranean fin whale and basking shark. They showed that the presence of harmful chemicals in Mediterranean fin whales was linked with intake of plastic derivatives by water filtering and plankton ingestion. Here we report on the distribution and concentration of floating microplastics and zooplankton using a Manta collector in the Ligurian Sea in the summer of 2013. Surface floating microplastics abundance and area per square kilometer were calculated and compared to the abundance of the neustonic zooplankton.

Material and methods

Sampling

Surface floating microplastics were collected in the Ligurian Sea (NW Mediterranean Sea) in the framework of the participative science activities of ExpéditionMED association (www.expeditionmed.eu) from July 6th to August 6th 2013. Sampling was performed at different locations across the Northern (Liguro-Provençal) current and front, near urban centers, harbors, and in the open sea. Eco-volunteers were involved in assisting scientists in sampling. Throughout the cruise, weather conditions were calm (Beaufort Sea State from 0 to 2) and nets were towed in calm sea conditions. The samples were collected with a 330 µm Manta trawl with the size of the rectangular net opening of 60 x 20 cm (Fig. 1A). The net was towed at an average speed of 2.5 knots during 30 or 60 min at the top 10cm of the sea surface. The particles abundance was calculated per square kilometer. The content of the collector was sieved through a 150 µm mesh and fixed in 2 % buffered formalin.

Sample processing

In the lab, samples were gently mixed and transferred into a 2 L glass jar in order to separate by gravity the floating particles from the sedimented material containing zooplankton and organic tissues. This process was repeated from 3 to 6 times until no microplastic was observed in the supernatant. Two fractions were then obtained: the sediment and the supernatant with plastic particles. Both fractions were rinsed with filtered seawater before manual sorting of the plastic particles. Using a dissecting microscope, plastic particles were removed from preserved organic material in both fractions obtained by density separation. Microplastics and zooplankton were enumerated, sorted and measured by imaging analysis using the Zooscan system (Gorsky *et al.,* 2010; Goldstein *et al.,* 2013, Fig. 1B).

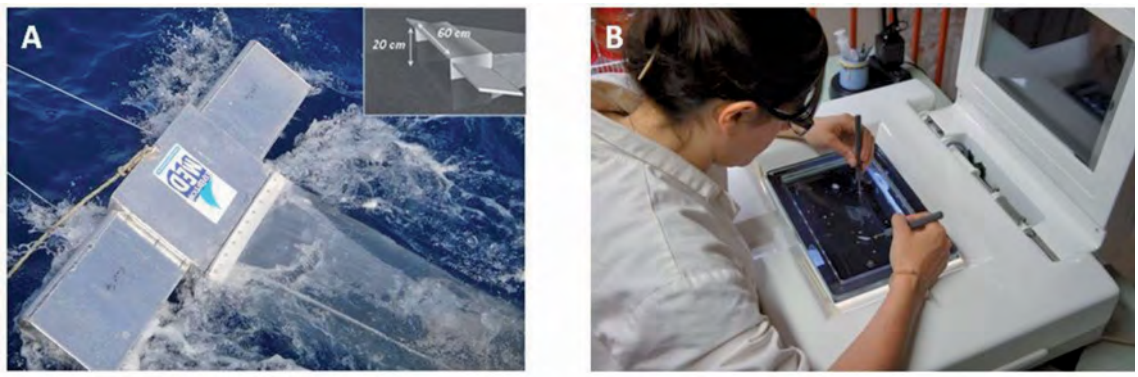

Figure 1. Methods for collection and analysis of microplastics. Samples were collected using (A) a Manta trawl net with 333µm mesh size and 60 x 20 cm opening. Trawl shots were performed at 2.5 knots from 30 to 60 min covering a known area at the top 10cm of the sea surface. (B).Back to the lab, plastic particles and zooplankton were enumerated, sorted and measured by digital imaging analysis using a data acquisition system and the Zooscan.

Zooscan analysis

Zooplankton and plastic particles were rinsed with 0.2 µm filtered seawater and then digitally imaged with a Zooscan digital scanner with a resolution of 2400 dpi; each pixel is about 10 microns wide. Image post-processing was performed with the Zooprocess & Plankton Identifier software that enumerates and gives a large set of morphological parameters for each object such as the ferret length (approximately equivalent to particle length), circularity and two- dimensional surface area (mm), etc. For zooplankton the biovolumes, concentration and surface areas were calculated and taxonomic identification was also determined (Fig. 2). After analysis, zooplankton samples were reconditioned in 4 % formalin solution whereas microplastic particles were split and conserved for further chemical analysis.

Figure 2. Plastic fragments imaged by the Zooscan (A & B) and by stereo microscope (C).

Results and discussion

We were able to enumerate plastic fragments and determine their size spectra. In our survey, microplastic were present in all Manta tows varying from 1.3 x 10^4 to more than 3.6 x 10^5 plastic debris per km^2 observed in a coastal station. This result suggests that plastic fragments are widespread in the Ligurian Sea as all studied 35 stations between Toulon and Genoa had detectable plastic micro-debris.

The average concentration was 1.03 x 10^5 plastic micro-debris per km^2 (Table 1A, Fig.3 A). The area covered by plastic represented in average 4.2 x 10^5 mm² / km² with a maximum of 1.72 x 10^6 mm² / km² and a minimum of 8.58 x10^4 mm² / km² (Fig.3 B). The average abundance value in our study is comparable to averages obtained in the same areas by Collignon *et al.* (2012) although maximal concentration of this study is lower (Table 1). Concerning the other studies, they are made with different sampling devices or mesh aperture, and results are expressed in cubic meters (Table1B). Overall, the average abundance of microplastics found in Ligurian Sea is higher than in Sardinian Sea with levels approximately seven times higher in the samples from the same survey in both sites (Fossi *et al.,* 2014a).

The spatial distribution of microplastics varies greatly across the Ligurian Sea spanning over one order of magnitude among the studied stations; high concentration and surface area of debris were detected in the inshore coastal stations, and were lower in the bays with open sea connections (Figs. 3, 4). Our results corroborate those of Collignon *et al.* (2012), which showed higher concentration of microplastics along shorelines adjacent to densely populated areas. In fact, higher densities of debris in coastal waters were correlated with proximity of human population centers (Browne *et al.*, 2011). This could explain the differences in the seasonal distribution observed by Collignon *et al.* (2013) in the Bay of Calvi, Corsica. In this touristic location, Collignon *et al.* (2013) found the highest abundance of microplastic during the summer with a decreasing concentration in autumn and levels close to zero in winter and spring. In the bay of Oristano (Sadinian Coast) while de Lucia *et al.* (2013) showed that spatial location of the different sampling sites can influence the abundance of microparticles, no significant difference in particle concentration was found between the coastal and offshore stations.

Table 1. (A) Microplastics and zooplankton abundance (items / km²) and (B) Average microplastic concentration (items /m³) in the Mediterranean Sea. Survey were performed between 2010 and 2013.

A	Microplastics (items / km²)			Zooplankton (items / km²)			Ref
	Mean	Max	Min	Mean	Max	Min	
	1.03E+05	3.66E+05	1.35E+04	2.96E+08	2.33E+09	7.75E+05	Our study
	1.16E+05	8.92E+05	0.00E+00	NA	NA	NA	Collignon, 2012
	6.20E+04	6.88E+05	0.00E+00	1.12E+08	9.86E+08	3.42E+06	Collignon, 2013

B	Microplastics (items /m3)		
	Location	Mean	Ref
	Ligurian Sea	0.373	Our study
	Ligurian Sea	0.116	Collignon, 2012
	Ligurian Sea	0.940	Fossi et al, 2012
	Sardinia Sea	0.130	Fossi et al, 2012
	Sardinia Sea	0.150	de Lucia et al 2014

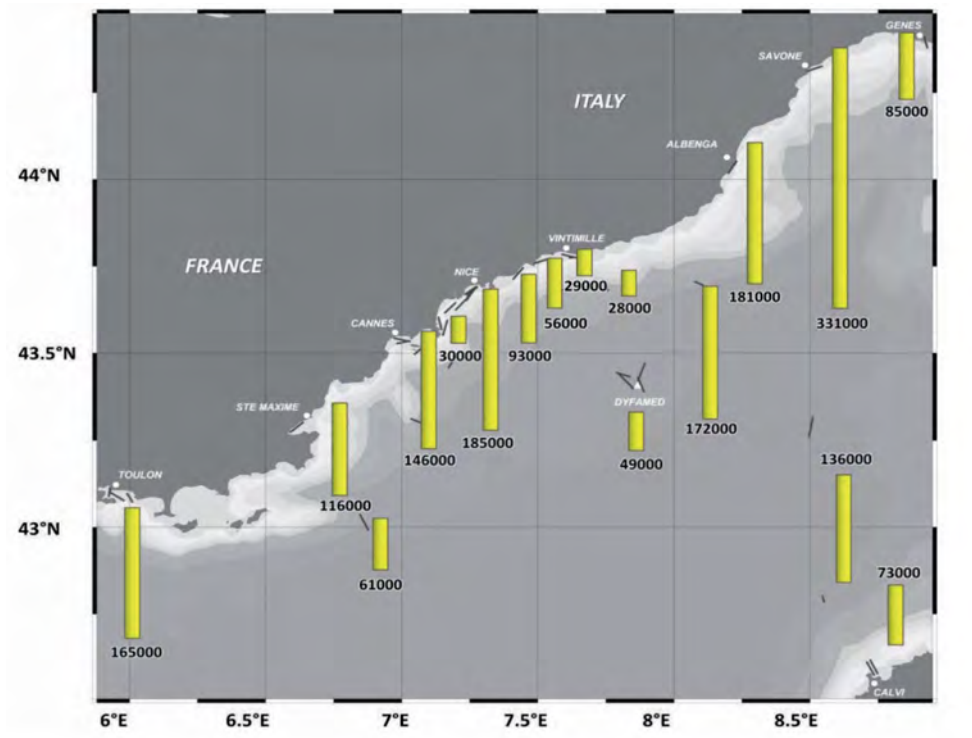

Figure 3. Distribution of the microplastic particles (size <0.5 cm) present in the top 10 cm of sea surface water collected in the Ligurian sea in July and August 2013 by ExpeditionMED.

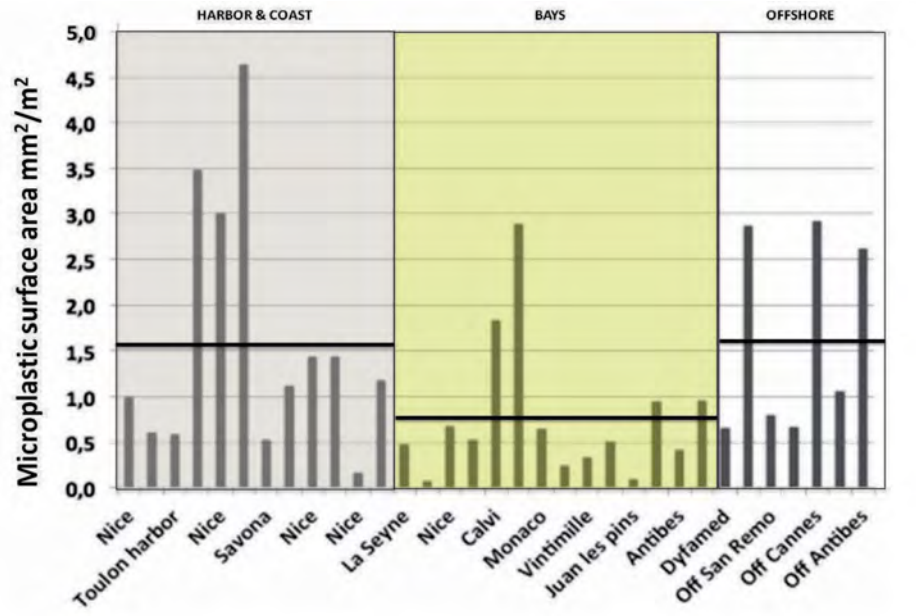

Figure 4. Microplastic surface area (mm²/m²) from ZooScan imaging collected in different station of the Ligurian sea in July and August 2013, in harbors and near coast (grey), bays (yellow zone) and offshore (white). Horizontal lines = mean values.

Data collected during our survey completed the results obtained from the other sampling surveys in the north and central Western Mediterranean (Collignon *et al.,* 2012; 13; Fossi *et al.,* 2012; de Lucia *et al.,* 2014b) and corroborate the initial insight of a significant distribution of fragments floating in a basin wide surface layer in the Western part of Mediterranean Sea, even if we cannot extrapolate to the other areas, as it is known from several studies that microplastic patterns in the ocean are patchy (Gordstein *et al.*, 2013). Maximum concentrations detected in several studies in this area are of the same order of magnitude as those found in the Pacific and Atlantic North subtropical gyre by Law *et al.* (2010; 2014). These authors analyzed data from more than 20 years in the North Atlantic gyre (6135 surface plankton tows) and 11 years in the eastern Pacific Ocean (2,529 surface plankton tows) and estimated a maximum concentration of 100,000 and 500,000 pieces of plastic km^2 respectively in the two gyres that correspond to centers of accumulation resulting from the convergence of ocean surface currents predicted by several oceanographic numerical models. Compared to others areas, the concentration detected in the Med surveys are lower than in the South California Current and Bering Sea (Gilfiland *et al.,* 2009; Doyle *et al.,* 2011) and in the California Coast (Moore *et al.,* 2002); but higher than in the Caribbean Sea, the Gulf of Maine and in the North Atlantic Gyre (Law *et al.,* 2010). The spatial heterogeneity in microplastic distribution is show to be linked to mesoscale and regional oceanographic conditions, such as gyres, eddy formation, upwelling and convergences areas (Law *et al.,* 2010; Collignon *et al.*, 2012; Ribic *et al.,* 2012; Goldstein *et al.,* 2013). On a smaller spatial scale, wind patterns affect the distribution of debris by differentially moving or mixing particles of different densities (Browne *et al.,* 2011; Kukulka *et al.,* 2012). Collignon *et al.* (2012) observed that concentrations of neustonic plastic particles are five times higher before than after a strong wind event. In areas as the North Pacific Subtropical Gyre (NPSG) Goldstein *et al.,* 2013 found an inverse relationship between wind and plastic concentration suggesting that microplastic is mixed down in high wind conditions, and is thus undersampled in the neuston tow.

Size and area distribution of microplastic particles

The ongoing analysis of individual particles (n=2,578), showed that the median size values of microplastics during the whole survey was 2.5 mm, however, we found a broad range of sizes (0.10 to 200 mm) with an asymmetrical frequency distribution skewed toward smaller diameters (size class 1-2.5 mm), (Fig.5 A). The median microplastic surface area was 2.3 mm^2, with microplastic areas ranging from 0.1 to 200 mm^2 (Fig. 5B). In our survey microplastic particles detected in the coastal station, in the bays, and offshore regions showed the same size pattern, 52 % of the total particles analyzed are microplastics smaller than 2.5mm in diameter (Fig. 6A). The majority of the microplastics (53 %) are smaller particles with a surface smaller than 2.5 mm^2 (Fig. 6B). There was also an increase in circularity (roundness) of microplastic with smaller particle size. The dominance of smaller particles in the neustonic samples suggests that the dominant pathway of microplastic formation is fragmentation from large plastics.

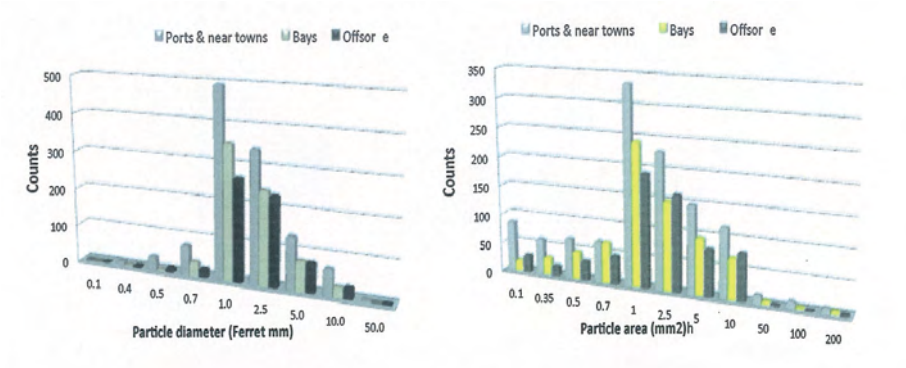

Figure 5. Microplastic size spectra. A-Histogram of frequency distribution of particle diameter (ferret mm). B-Histogram of particle surface area (mm^2).

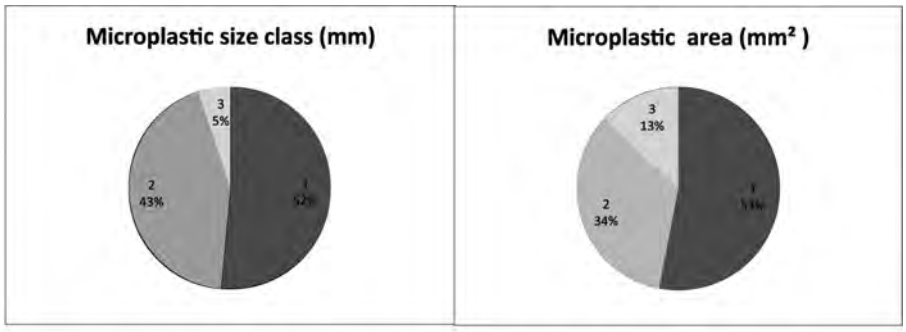

Figure 6. Proportion of the total abundance of microplastic for each size class and area during the whole study: A- Microplastic size class (1) <2.5mm; (2)2.5-5,0mm; (3)>5.0mm. B- Microplastic area (1)<2.5 (2) 2.5-5.0mm; (3) >5.0mm.

In the Southern California Current, using the same Zooscan method, Gilfilland *et al.,* (2009) found the same median size (2.62 mm) and area (2.24 mm^2) values of particles. In the North Pacific Gyre plastic particles less than 3 mm accounted for 82 % by number of the plastic observed (Moore *et al.,* 2001) with an area approximately of 1 mm^2 (Goldstein *et al.,* 2012). However, the size of microplastics observed in our survey is smaller than size values obtained by Collignon *et al.* (2013), which found that 54 % of particles were large within a size class of 2-5 mm. Data from the Malaspina 2010 circumnavigation, showed that the size distribution of surface microplastics, when analyzed separately by ocean, is around 2 mm with a pronounced gap below 1 mm sizes observed (Cozar *et al.,* 2014). The progressive fragmentation of plastic objects from millimeter to micrometer scale should lead to a gradual increase of fragments toward small sizes, rendering the very small pieces undetectable using convectional sampling nets, and/or may be transferred to the ocean interior. These findings (concerning the size distribution of floating plastic) provide strong support to the hypothesis launched by Cozar *et al.* (2014) of substantial size-selective losses of plastic on a large scale of the surface ocean. The tendency observed in our study of increasing particle concentration with decreasing size suggests that a continual fragmentation of plastic items may occur in Mediterranean waters. This agrees with the general trend observed in global environments postulated by Barnes *et al.* (2009) that the average size of plastic particles seems to be decreasing, while abundance of such particles is increasing due to continuous breaking down. This could have important consequences regarding ingestion by small planktonic organisms serving as prey for larger animals.

Chemical characterization of microplastic

The chemical characterization of plastic items was performed simultaneously. Fourier Transform Infrared Spectroscopy (FT-IR) spectra of various samples were recorded with a FT-IR spectrometer (Shimadzu 8400 M), using 4 cm^{-1} resolution and 40 scans. Thermogravimetric analysis (TGA) was carried out on each microplastic sample. Finally, the thermal characteristics of microplastics, i.e. glass transition temperatures (Tg), cristallinity and melting temperatures (Tm), were collected using differential scanning calorimetry (DSC). Preliminary results from our ongoing analyses using the combination of the characterization techniques allow us to initiate and to develop an unambiguous methodology for classifying microplastic samples collected in the NW Mediterranean Sea. A data bank containing the spectra of main marine microplastics has been established (Fig. 7A). These spectroscopic results associated with the thermal data obtained using TGA and DSC revealed that the most frequent plastic types were polyamides (comprising 52 % of the plastic present), polystyrenics (different kinds of polystyrene and also copolymers of polystyrene), polyolefins (polyethylene and polypropylene) and polyesters (Fig. 7B). As shown by numerous recent studies, polyethylene, polypropylene and polystyrene were usually the most abundant types of debris (Frias *et al.*, 2014; Cozar *et al.*, 2014) but in that special case, the plastic predominantly recovered is polyamide. Note that polymers such as polyamides and polyesters are denser than seawater and their presence in our samples indicates that the transport of debris is influenced by factors other than density alone, as previously noted by Sadry *et al.* (2014). One

likely explanation could be the introduction of some of these fibres via the sewage outlets onto shorelines and/or their re-suspension in water column as a result of turbulent mixing induced by wind and tidal currents (Browne *et al*., 2011). Further work on this question is in progress.

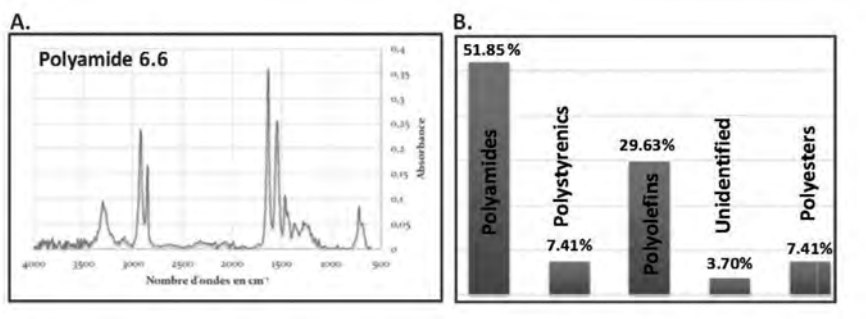

Figure 7. Chemical characterization of microplastic particles. As an example, IR spectrum of polyamide 6,6 (Nylon®) was shown (A). Distribution of plastic polymers in the microplastic samples is shown.

Microplastic and zooplankton interactions

Macroscopic observation of different microplastic showed that marine organisms were associated with and transported by floating plastic fragments (Fig. 2C). In order to evaluate the potential interaction between microplastics on zooplankton we calculated a microplastic: zooplankton ratio. The average ratio between microplastics and mesozooplankton surface area was 0.2 for the whole survey. This ratio was based on the average surface in mm^2 of microplastic and the zooplankton surface area. The mean value indicates that the area occupied by plastic is neustonic biota is five times lower that zooplankton. Copepods were the most abundant organisms in the surface layer but neustonic mollusks and cladocerans were also abundant. To illustrate, the particles surface area of one sample collected in the Toulon harbor are represented by nearly 5 % of plastic particles, 22.74 % of copepods and 71.53 % of other organisms (Fig. 8).

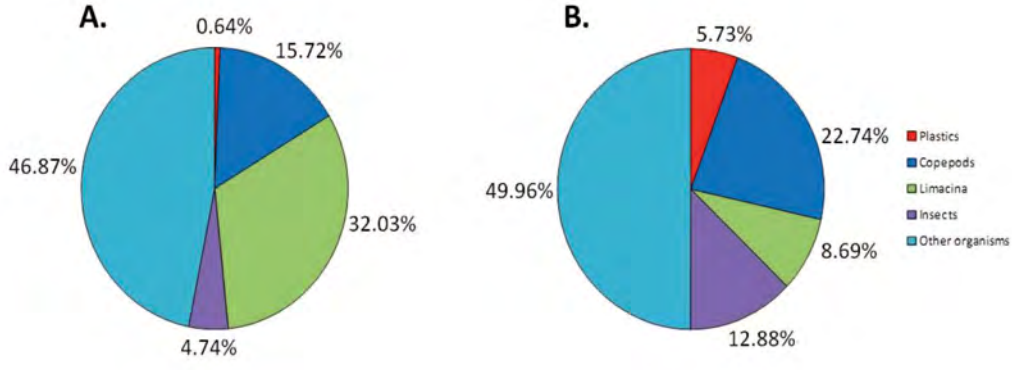

Figure 8. Proportion of the abundance (A) and the surface area (B) of plastic particles and of zooplankton for a sample collected in Toulon harbor the 7th July 2013.

This ratio is intermediary between the total dry weight ratio of 0.5 calculated by Collignon *et al.* (2012) and those calculated for three different size classes of zooplankton based on biovolumes (Collignon *et al.,* 2013). In the present case, the average number ratio between the abundance of small microplastics (0.2–2 mm) and zooplankton (e.g., copepods, cladocerans) are very low (0.002). This could imply that neustonic zooplankton rarely encounter or interact with small microplastic debris. On the other hand, the ratio calculated by Collignon *et al.* (2013) reached 2.63

for the large microplastics (2-5 mm) and zooplankton (decapod larvae, fish larvae). This would suggest a potential confusion for predators regarding planktonic prey of this size class.

The ratios published are often based on calculations between abundances or dry weight of both plankton and plastic particles (Moore *et al.,* 2001; 2002; Lattin *et al.,* 2004; Collignon *et al.,* 2012; Goldstein *et al.,* 2013). In the North Pacific Gyre, Golstein *et al.* (2013) obtained ratios varying from 0.01 to 10. Along the California Coast Moore *et al.* (2002) obtained a ratio of 0.6 and Lattin *et al.* (2004) in South California shore obtained a ratio of 0.3 for the size class of plastics smaller than 4.75 mm. These ratios are difficult to compare as different sizes of plastic were integrated in calculation and the areas studies have contrasted trophic state; in the California Current subjected to nutrient upwelling has a much higher biological productivity than the North Pacific central gyre. According to Doyle *et al.* (2011), this method is inappropriate due to high variance of both plastic and zooplankton in space and time, selective sampling by nets, and selective feeding by zooplankton.

Due to fragmentation, small microplastics may be ingested by organisms commonly unaffected by larger marine debris. The potential for ingestion of microplastic by the biota needs to be better assessed and efforts need to be made to establish an index for zooplankton encounter rates with various size ranges of microplastic in the marine pelagic environment. The first estimations obtained in our survey based on surface areas of both plastic and zooplankton give us an indication of the instantaneous encounter rate between plastic and zooplankton. This can be improved and proposed as an indicator to resolve at the small-scale microplastic and zooplankton interactions.

Microplastics – A never-ending story

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Abstract

Microplastics constituted about ~70% of plastic items found in a survey of the Portuguese coast. Pellets, within the size range of 3-5 mm, were the more abundant size class, and represented 57% of all plastic collected per m^2. Beaches near industrial sites or port facilities had significantly higher amounts of resin pellets.

Beaches located downstream from industries and/or port facilities presented higher quantity of plastic debris and microplastics as well as higher concentrations of POP (PAH, PCB and DDT). PAH contamination was mostly from petrogenic origin. Sines, an industrial site with port facilities, presented the highest PAH concentration. The highest PCB and DDT contamination were found at Matosinhos, an important port facility. White translucent PE pellets showed lower contaminant values when compared to yellow-brown (aged) ones, reflecting their residence time in the waters. Microplastic: zooplankton ratios were higher in areas under the influence of river input and the proximity of densely populated areas draining to the Tejo and Sado river estuaries. Most common polymers in our samples were polyethylene (PP) and polypropylene (PE) and to a lesser extent polyacrylates (PA) and polyurethane (PU). These results reflect the present and past decades of worldwide industrial production and intensive use.

Though research on microplastics has grown consistently over the last decade there are still important gaps in knowledge, namely those concerning the extent of the harm to marine organisms, the toxic effects of these particles towards the biota, and how this may affect humans, e.g., through the consumption of fish.

1. Introduction

Dispersal and accumulation of plastic debris is a growing problem on a global scale, affecting all marine habitats (Gregory, 2009; Moore, 2008). Plastic litter has been found stranded on beaches all over the world, floating on the surface, at mid-water and on the bottom of the oceans, not only on coastal areas but also at great depths (Barnes *et al.*, 2009; Thompson *et al.*, 2009b; Martins and Sobral, 2011; Lee *et al.*, 2013a; Galgani *et al.*, 2014; Pham *et al.*, 2014).

The impact of plastic litter on marine organisms has been widely reported specially when related to sizable items, such as lost or discarded fishing gear, plastic bags or waste from packaging (Butterworth *et al.*, 2012).

Ocean pollution by microplastics i.e. all plastic items with diameter below 5 mm (Arthur *et al.*, 2009), is of great concern due to the ubiquity of these particles, their persistence and also because they represent a potential vector of exposure and transfer of persistent bioacumulative and toxic pollutants to marine organisms (Thompson *et al.*, 2004). Regarding the impacts of microplastics

in the marine environment, the information has increased substantially in the last years and both field and laboratory work have provided new evidence on the fate of plastic particles and show that most groups of marine organisms are at risk of interacting with microplastics.

A study by Ivar do Sul and Costa (2014) analyzed more than 100 peer-reviewed papers investigating microplastic pollution in relation to plankton samples, sandy and muddy sediments, vertebrate and invertebrate ingestion, and chemical pollutant interactions, as well as other work dealing with polymer decay at sea, new sampling and laboratory methods, emerging sources.
Although our knowledge of microplastics distribution in the oceans is fast increasing, uncertainty on the harm they may cause to the organisms still remains.

1.1 A few numbers on plastic production and demand

According to data from Plastics Europe (2012) the world production has increased from 5 million tons in 1950 to 280 million tons in 2011, representing a steady 9 % increase per year with China being the new lead producer (23 %).

Europe is responsible for 21 % of the world production (58 million tons), a little less than in previous years with packaging remaining the largest segment and representing more than 39% of the overall demand (47 million tons: 40% short-service, 60 % long-service life). In fact roughly 40% of the plastic is discarded within one year of service and mostly from the packaging segment, and though there are no precise data on the amounts of plastic that reach the oceans, Derraik (2002) estimated 60 to 80% of all marine litter to be made of plastic.

In 2011, post-consumer plastic waste levels in Europe rose by 2.4% from the year before, and 25.1 millions tons entered the waste stream, of which 59.1 % were recovered, through recycling (25.1 %) and energy recovery (34 %).

If it is true that in the last years many countries in the world invested in efficient waste management systems and in plastic recovery through recycling and energy recovery, it is also true that a large amount of plastic litter escapes this cycle, not to mention the quantities disposed throughout decades when waste management was incipient or inexistent.

Even if plastic degradation rates are low, it will continue to degrade in the oceans resulting in fragments of smaller and smaller dimensions and increase the risk of ingestion by marine organisms at low trophic levels, with unknown impacts on marine food webs.

1.2 Microplastics, contaminants and ingestion by organisms

According to their origin microplastics are divided into (a) primary, i.e. those manufactured as virgin or recycled resin pellets, raw material for the industry of plastic converters, small particles to produce scrubbers for cosmetics, or abrasives for blasting, and (b) secondary microplastics which result from fragmentation of larger plastic products (Gregory, 1996; Andrady, 2011; Fendall and Sewell, 2009; Corcoran *et al.*, 2009; Cole *et al.*, 2011).

Pellets of polyethylene (PE) and polypropylene (PP) are the most common as these materials are widely employed by the packaging industry. PE is one of the main components of plastic scrubbers, though these particles are difficult to assess in the field due to their minute size (<1 mm).

Plastic resin pellets are small granules, generally in the shape of a cylinder or disk with a diameter of a few millimeters. A small portion of them are spilled or lost into the environment during land handling and transportation, and during operation at the converters facilities. Because PE and PP pellets are lighter than water, such spilled pellets are carried easily by surface runoff, streams and rivers, and finally reach the ocean. Because of their persistent nature, such pellets are distributed worldwide.
Due to the nature of the plastic surface, persistent, bioaccumulative, and toxic substances (PBTs), such as polychlorinated biphenyls (PCBs), DDTs, and dioxins, are sorbed from the water or sediment and concentrated in the pellets (Mato *et al.*, 2001; Endo, 2005; Teuten *et al.*, 2007; Ogata, 2009; Thompson *et al.*, 2009b; Cole *et al.*, 2011) by factors of up to 10^6 (Wurl and Obbard, 2005). Therefore plastics not only transport persistent pollutants, but they can also make these available at higher concentrations to marine organisms and seabirds through ingestion (Teuten *et al.*, 2009;

Tanaka *et al.*, 2013; Fossi *et al.*, this volume), increasing risk throughout the marine food web, and ultimately to humans (Engler, 2012).
Some pellets act as a source of toxic chemicals added in the manufacture to enhance performance (i.e. phthalates and bisphenol A). These may be released from plastics and impact organisms and humans, though to an extent that remains unknown (Andrady and Neal, 2009; Teuten *et al.*, 2009; Thompson *et al.*, 2009b; Lithner *et al.*, 2009; 2011; Cole *et al.*, 2011).
Studies on marine organisms have shown birds, turtles and mammals to be affected by entanglement and ingestion of marine litter, decreasing their ability to move and/or feeding resulting in reduced reproductive output, and injuries that sometimes lead to death (Laist, 1997; Derraik, 2002; Mallory, 2008; Moore, 2008; Gregory, 2009; Fossi *et al.*, 2012; Butterworth *et al.*, 2012; Deudero and Alomar, this volume). Laboratory studies have shown that various groups of invertebrates ingest microplastic fragments (Thompson *et al.*, 2004; Browne *et al.*, 2008; Graham and Thompson, 2009; Cole *et al.*, 2013; Fossi *et al.*, this volume). A recent study by Rochman *et al.* (2014b), reported signs of endocrine disruption in the Japanese medaka after ingestion of polyethylene particles. However there have been few studies documenting the ingestion of microplastics in the natural environment. Boerger *et al.* (2010) and Lusher *et al.* (2013), reported plastic ingestion by several fish species, raising a potential concern regarding the human diet.

1.3 Microplastics on the Portuguese coast

Here we present selected results from surveys on the Portuguese coast and the research project POIZON - "Microplastics and persistent pollutants - a double threat to marine life", funded by FCT-MEC, Portugal, under the reference PTDC/MAR/102677/2008. Two of the objectives of this project were to characterize beach-stranded microplastics on the coast of Portugal, size and quantities, and determine adsorbed pollutants (polycyclic aromatic hydrocarbons - PAH; polychlorinated biphenyls– PCB and pesticides – DDT and metabolites).

In order to contribute to the implementation of the Marine Strategy Framework Directive (2008/56/EC) zooplankton samples collected off the Portuguese coast during surveys for fish stock assessment were investigated for plastics detection. The main goals of this exploratory work were to detect and quantify microplastic particles and identify the plastic polymers present, using a spectroscopy technique, the Fourier Transform Infrared Spectroscopy (FTIR).

Further studies are being put in place by our team, to assess harm from ingestion of microplastics by invertebrates and fish and obtain a broader view of the possible effects at the sub-cellular level using biomarkers. Results are not available at this time.

2. Material and methods

2.1. Microplastics on the beach

Sampling sites

We started sampling marine litter from selected sites along the coast of Portugal in 2008, first from sites near Lisbon and then extending our campaigns to 11 beaches, 10 on the high energy west coast and 1 on the more sheltered south. Microplastics and contamination by organic persistent pollutants were our main focus, because plastic pellets and fragments are a common finding on many beaches in Portugal as seen in previous observations.

Beaches were selected according to factors that maximize accumulation, i. e. exposure to dominant northerly winds, proximity of important river mouths, proximity to industries and commercial ports (Fig.1).

Code	Sampled sites	GPS coordinates	Activities/influences
MT	Matosinhos	N41° 10' 29'', W08° 41' 28''	
ES	Espinho	N41°00'24'' W08°38'39''	
MI	Mira	N40° 27' 21'' W08° 48' 06''	
VL	Vieira de Leiria	N39° 52' 26'' W08° 58' 10''	
PV	Paredes de Vitória	N39° 42' 10'' W09° 02' 59''	
PEN	Peniche	N39° 21' 39'' W09° 22' 04''	
CR	Cresmina	N38° 43' 28'' W09° 28' 36''	
FT	Fonte da Telha	N38° 34' 04'' W09° 11' 38''	
SI	Sines	N37° 55' 23'' W08° 48' 20''	
BOR	Bordeira	N37° 11' 49'' W08° 54' 12''	
ANC	Ancão	N37° 02' 11'' W08° 02' 32''	

Figure 1. Sampling sites on the Portuguese coast: Matosinhos (Mt), Espinho (Es), Mira (Mi), Vieira de Leiria (VL), Paredes de Vitória (PV), Peniche (Pen), Cresmina (Cr), Fonte da Telha(FT), Sines (Si) and Bordeira (Bor). Code, names, GPS coordinates and activities and sites influences (wind, industry, rivers and ports) are presented in the table. RH are hydrographic regions divisions.

Marine litter was collected from 2 - 2 m squares, with 3 to 5 replicates from each site along the high tidal mark. The top 2 cm of sand was sieved through a commercial metal mesh of 2.5 mm. Samples were stored in paper bags to avoid potential contamination. In the laboratory plastic litter was separated (Fig. 2), according to density differences using the methods described in Martins and Sobral (2011).

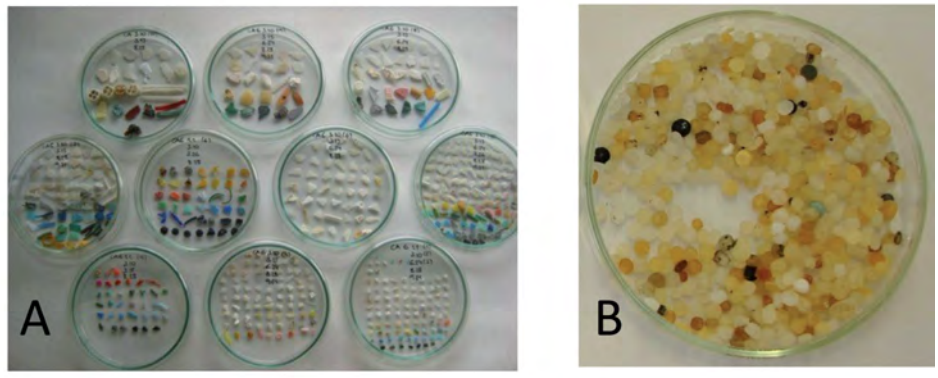

Figure 2. Plastic fragments organized according to size (A); industrial plastic pellets (B).

All microplastics were measured individually and classified into 1 mm size classes, from 1 - 10 mm, according to the methods described in Ogi and Fukumoto (2000) and adapted from Endo *et al.* (2005).

Resin pellets were separated from the remaining plastic litter and divided in four classes according to a classification adapted from Endo *et al.* (2005), white translucent pellets being those recently discarded in the environment, aged pellets were those having remained in the oceans for some

time and thus having acquired a yellow-brown tonality, colored pellets included pellets with various pigments (not shown). The black pellets class was created due to its different composition, later identified as being mostly polyurethane (PU).

2.2 Microplastics off the coast of Portugal

Zooplankton samples were collected between 2002 and 2008, in four areas of Portuguese coastal waters: Aveiro (Av), Lisboa (Lx), Costa Vicentina (Cv) and Algarve (Al) (Fig. 3) using three different sampling methods, W (WP2 net – mesh size 180 µm), N (Neuston net – mesh size 280 µm) and L (Longhurst Hardy Plankton Recorder, Pro-LHPRm -mesh size 335 µm). W and N samples were towed horizontally for 3 min at ship speed of approximately 1.5 knots, in the upper 20 cm of the water column. L samples were collected for approximately 30 min at a ship speed of approximately 4 knots, at 25 m deep.

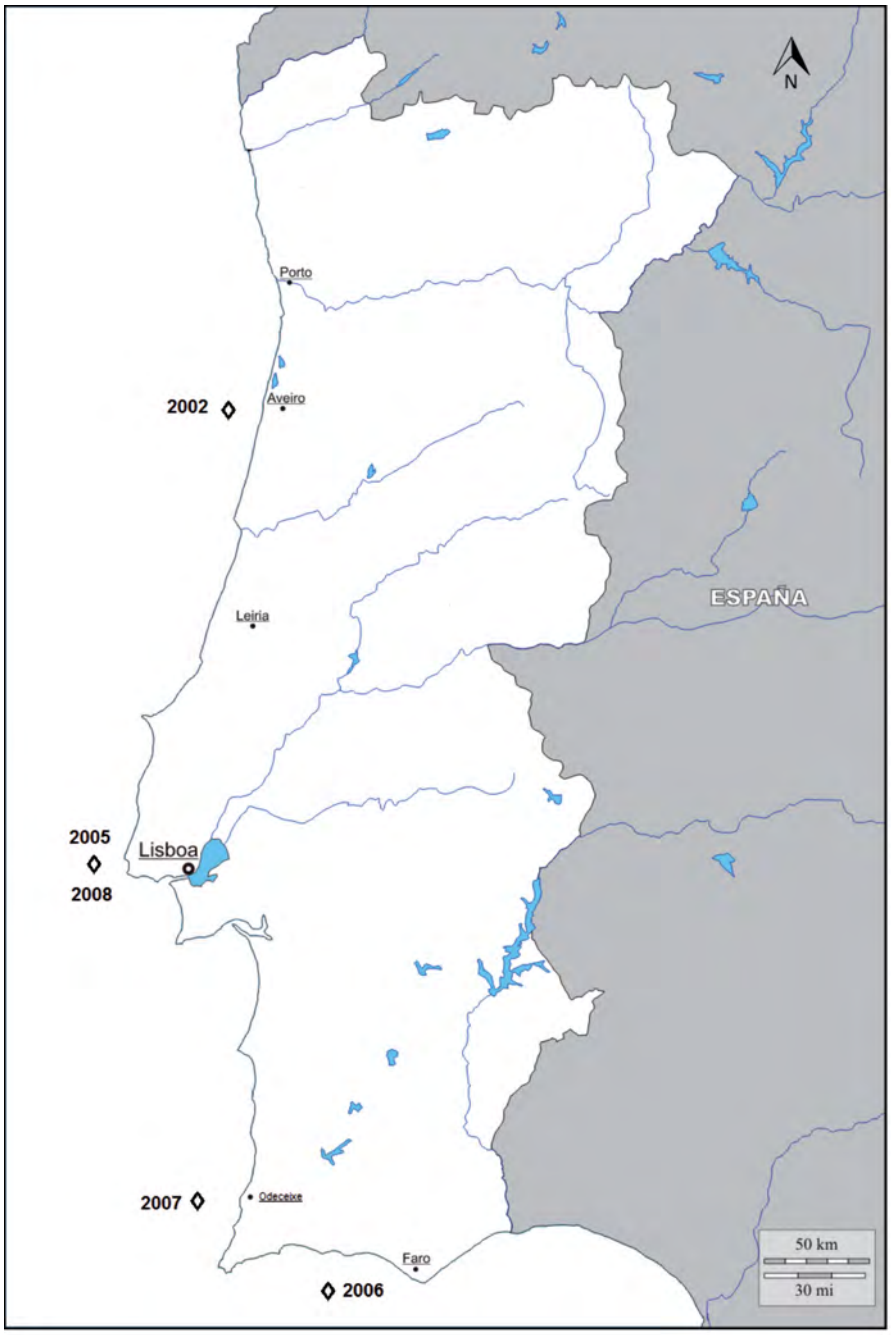

Figure 3. Location of sampling sites and survey years.

Samples were manually filtered through Whatman® glass microfiber filters with a diameter of 47 mm and were then examined under a stereoscopic microscope to sort and measure the microscopic plastic particles. Particles were photographed and recovered onto concave slides covered and stored until polymer analysis (see section 3). The microplastic:zooplankton ratio, based on standardized volumes ($cm^3\ m^{-3}$), was calculated.

2.3 Polymer identification

To identify the composition of polymers, a spectroscopy technique - μ-FTIR - was used. This is a fingerprinting technique that provides characterization at the molecular level, allowing the identification and distinction of the different materials, through the interaction between infrared radiation and matter. This interaction is different for each material resulting in a fingerprint spectrum with specific and characteristic bands for each one (Hummel, 2002). When using a microscope coupled to the m-FTIR spectrophotometer it is possible to go to the micro-scale and work with pieces with a size range of micrometers (Afremow *et al.*, 1969; Hummel, 2002), which is very convenient when you have few small pieces of plastic. To guarantee representativeness, micro samples were carefully cut under the Leica KL 1500 LCD microscope, and for each plastic depending on its heterogeneity (including degradation status) 2-3 micro samples were analyzed. These were compressed in a diamond anvil compression cell, and infrared spectra were acquired in a Nicolet® Nexus spectrophotometer coupled to a Continumm microscope (32 x objective) with an MCT detector. Spectra were collected in transmission mode in 128 scans, with a resolution of 4 cm^{-1} and are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO2 absorption at ca. 2300-2400 cm^{-1}. The identification of polymers was first made by searching the extensive polymer spectral database OMNIC® and by comparison analysis of the polymer characteristic band with spectral assignments.

2.4 Contaminant analysis

Polycyclic aromatic hydrocarbons (PAH) and legacy pollutants such as polychlorinated biphenyls (PCB) and pesticides (DDT and metabolites) were chosen to assess plastic pellets contamination. Analysis was performed on 1 g sample for each pellets category (white, aged, colored and black) according to each site. Detailed procedures for PAH, PCB and DDT determination is described in Antunes *et al.* (2013).

2.4.1 Polycyclic aromatic hydrocarbons – PAH

The determination of PAHs was performed on a Thermo_ DSQ Trace GC Ultra gas chromatography - mass spectrometry (GCeMS) system with a 30 m _ 0.25 mm _ 0.25 mm film thickness with capilar column J&W, DB5mn (Argilent, USA) in selected ion monitoring mode (SIM), according to Martins *et al.* (2012).
Seventeen individual PAH were analyzed: acenaphthylene (ANY), acenapthene (ANA), fluorene (F), phenantrene (P), anthracene (A) (three-ring compounds), fluoranthene (FL), pyrene (PY), benzo(a)anthracene (BA), chrysene (C) (four rings), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzon(a)pyrene (BAP), benzo(e)pyrene (BEP), dibenzo(ah) anthracene (DBA), perylene (Per) (five rings), indeno(1,2,3-cd) pyrene (IN) and benzo(g,h,i)perylene (BPE) (six rings). Total PAH (tPAH) is the sum of all the analysed compounds and all results are expressed on a dry weight basis.

2.4.2 Polychlorinated biphenyls (PCB) and pesticides (DDT and metabolites)

For PCB determination, pellet organochlorines were Soxhlet extracted with hexane for 17 h. The extraction was fractioned with a Florisil glass column, and then eluted with n-hexane, followed by a clean-up with sulphuric acid (H2SO4). The extracts were then injected in a Hewlett Packard_ chromatographer (ECD), model 6890 with a capilar column J&W, DB5 (60 m) and automatic sampler. Eighteen PCB congeners were analysed: CB18 (2,2',5-trichlorobiphenyl), CB26 (2,3',5-trichlorobiphenyl), CB31 (2,4',5-trichlorobiphenyl), CB44 (2,2',3,5'-tetrachlorobiphenyl), CB49 (2,2',4,5'-tetrachlorobiphenyl), CB52 (2,2',5,5'-tetrachlorobiphenyl), CB101 (2,2',4,5,5'-pentachloro biphenyl), CB105 (2,3,3',4,4'-pentachlorobiphenyl), CB118 (2,3',4,4', 5-pentachlorobiphenyl), CB128 (2,2',3,3',4,4'-hexachlorobiphenyl), CB138 (2,2',3,4,4',5'-hexachlorobiphenyl), CB149 (2,2',3,4',5',6-hexachlorobiphenyl), CB151

(2,2',3,5,5',6-hexachlorobiphenyl), CB153 (2,2',4,4',5,5'-hexachlorobiphenyl), CB170 (2,2',3,3',4,4',5-heptachlorobiphenyl), CB180 (2,2',3,4,4',5,5'-heptachlorobiphenyl), CB187 (2,2',3,4',5,5',6-heptaclorobiphenyl) and CB194 (2,2',3,3',4,4',5,5'- octachlorobiphenyl). Following the procedure for PCB analysis, a second extraction was made to determine pp'-DDE, pp'-DDD and pp'-DDT. The detection limit for these compounds is 0.01 ng g^{-1} (dry weight basis) (Ferreira and Vale, 2001).

3. Results

3.1. Microplastics on the beach

Plastic is the most important material accumulated on Portuguese beaches (85-96 %) and items < 2.5 cm are dominant. Microplastics make up ~70% of plastic items found. Pellets, within the size range of 3-5 mm, were the more abundant size class (Fig. 4), and represented 57% of all plastic collected per m^2. Number of pellets per beach (items m^{-2}) is shown in Fig. 5.

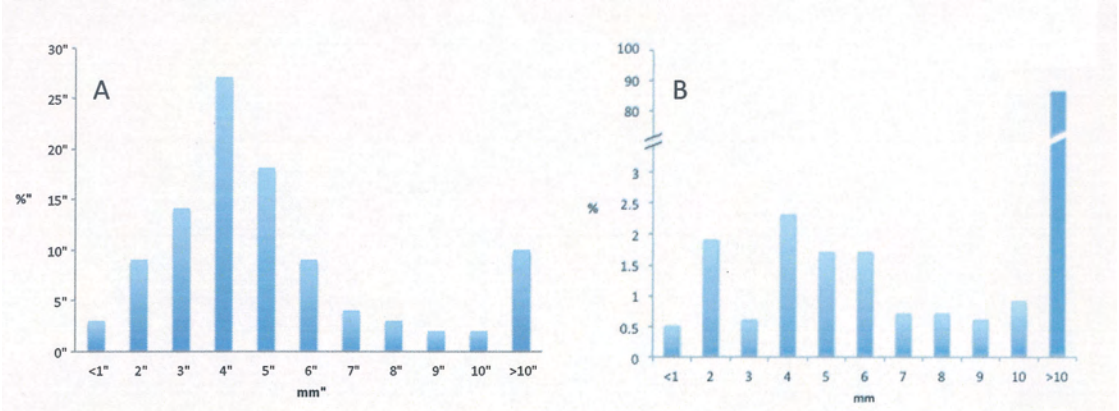

Figure 4. Size distribution of plastic items (A) % by number and (B) % by weight, in 5 beaches studied in 2009 (redrawn from Martins and Sobral, 2011). In terms of weight, large items are clearly more important, but regrading the number of items the highest percentage is made up by pellets lost from industry operations and carried to the water courses and thus to the oceans.

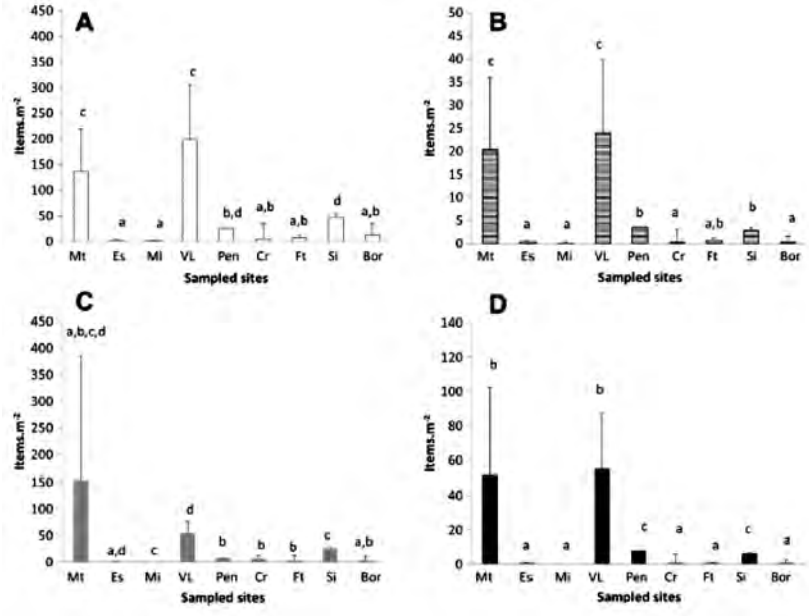

Figure 5. Resin pellets (items m^{-2} mean± SD), collected in the Portuguese beaches of Matosinhos (Mt) (n = 3), Espinho (Es) (n = 4), Mira (Mi) (n = 4), Vieira de Leiria (VL) (n = 3), Peniche (Pen) (n = 4), Cresmina (Cr) (n = 5), Fonte da Telha (FT) (n = 3), Sines (Si) (n = 4) and Bordeira (Bor) (n = 5): white pellets (A); aged pellets (B); colored pellets (C) and black pellets (D). Sites with different letters are significantly different (Mann-Whitney U test, $p < 0.05$) (from Antunes *et al.*, 2013).

Beaches near industrial sites or port facilities (Fig. 1) had significantly higher amounts of resin pellets: Matosinhos (Mt) (362 ± 41 items m^{-2}), Vieira de Leiria (VL) (332 ± 174 items m^{-2}). Quantities varied widely among beaches, as there is a wide range of factors controlling litter deposition on sand, either arriving from the sea or from land.
Comparison with other studies on microplastics is difficult due to the different methodologies used (Hidalgo-Ruz *et al.*, 2012) and so we need to agree on common protocols for sampling and collecting data on microplastics, either at the beach or at sea (Klasmeier *et al.*, this volume).

3.2 Microplastics off the coast of portugal

A total of 152 samples were processed and a total number of 684 microplastics (volume 113.01 cm^3) were identified in 93 of them, corresponding to 61% of the total. Costa Vicentina, followed by Lisboa, were the regions with higher microplastics concentrations (0.036 and 0.033 no. m^{-3}) and abundances (0.07 and 0.06 cm^3 m^{-3}), respectively (Figs. 6 and 7).
Microplastic: zooplankton ratios varied from 0.04 to 0.14 (in volume) and were also higher in these two regions, which is probably related river input and the proximity of densely populated areas draining to the Tejo and Sado river estuaries. The same pattern was found in Mediterranean waters (Collignon *et al.*, 2012; Pedrotti *et al.*, this volume), Microplastics polymers were identified using Micro Fourier Transformed Infrared Spectroscopy (μ-FTIR), (see section 3).

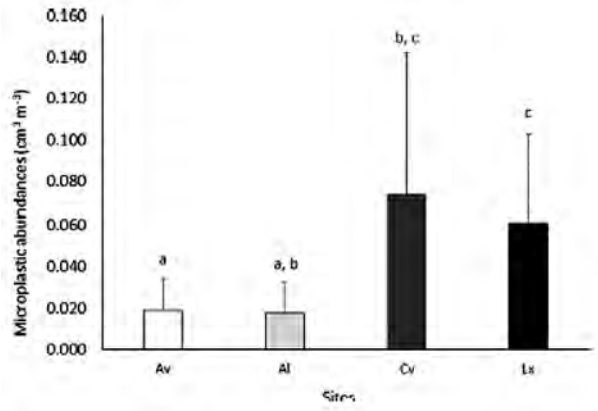

Figure 6. Microplastics abundance (cm^3m^{-3}, mean±sd) in neuston nets and LHPR. Significant differences are represented by different letters (Mann-Whitney U test $p<0.05$) (from Frias *et al.*, 2014).

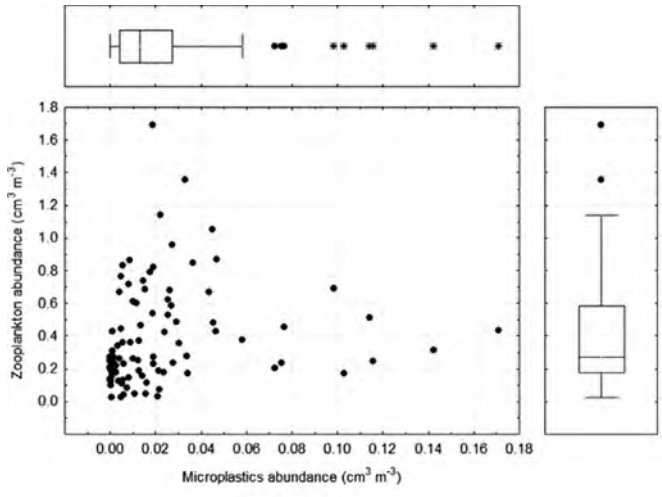

Figure 7. Zooplankton and microplastics abundances (cm^3 m^{-3}) and boxplot distributions. No significant relation was found between the two variables (from Frias *et al.*, 2014).

3.3 Polymer identification

Most common polymers in our samples were polyethylene (PP) and polypropylene (PE) (Figs. 8 and 9), though others were present. This was expected, since PP and PE are two of most widely used polymers, with multiple applications including in the single use packaging.

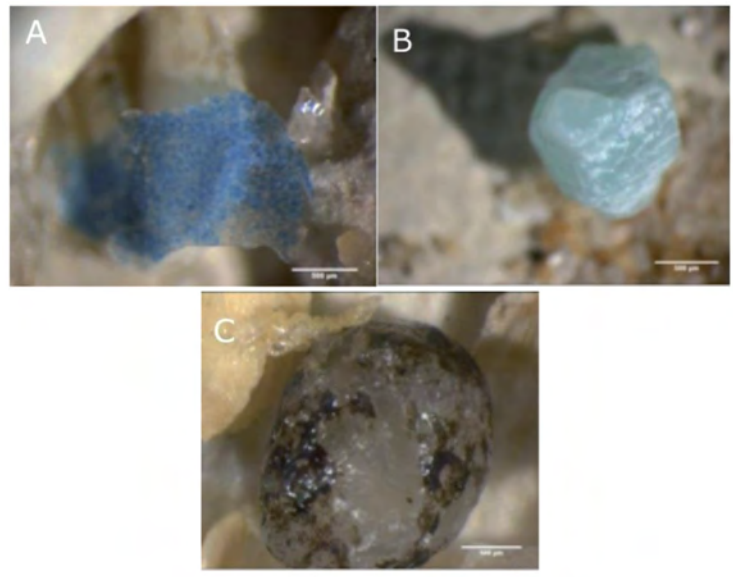

Figure 8. Microplastics found on the beach A) poypropylene PP; B) polyethylene PE and C) PE with visible contamination identified using the μ-FTIR technique. (segment 500 μm) (from Frias *et al.*, 2010).

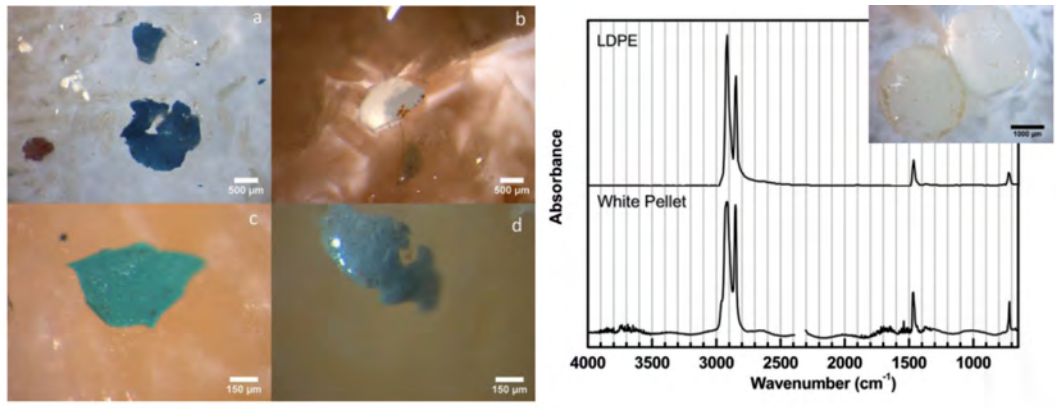

Figure 9. Left: Microplastics found in zooplankton samples: a – poypropylene PP, b – Polyacrylate; c – polyethylene PE; d – alkyd resin, identified using the μ-FTIR technique. (segment a, b – 500μm; c, d – 150μm). Right: infrared reference spectrum for low density polyethylene (LDPE) and spectrum from white pellet micro sample (from Frias *et al.*, 2014).

3.4 Contaminant analysis

Contaminant concentrations found on translucent white and aged pellets and in black pellets are presented in Fig. 10.

PAH concentrations ranged between 53 ng g^{-1} and 44 800 ng g^{-1}. Sines (Si) was the most PAH contaminated beach (686 ng g^{-1} in white pellets, 11860 ng g^{-1} in aged pellets and 44800 ng g^{-1} in black pellets) contrasting with the remaining beaches. Bordeira (Bor) (554 ng g^{-1} black pellets) and Matosinhos (Mt) (377 ng g^{-1} black pellets) also had high PAH concentrations. The majority of PAH present are from petrogenic sources. PCB contamination (Fig. 6) was higher in aged pellets

than in any of the other classes. Matosinhos (Mt) (223 ng g^{-1}), Peniche (Pen) (77 ng g^{-1}), Cresmina (Cr) (69 ng g^{-1}) and Fonte da Telha (FT) (52 ng g^{-1}) beaches recorded the highest concentrations. However, high concentrations of PCB were also found in black pellets from Sines (Si) (89 ng g_1) and colored pellets from Matosinhos (Mt) (108 ng g_1). White pellets showed significantly lower PCB concentrations than black and aged pellets (ManneWhitney U test, $p < 0.05$). PCB congeners 138, 153 and 180 presented the highest concentrations in Matosinhos (Mt) (71 ng g_1, 69 ng g_1 and 71 ng g_1). Concentrations of the less chlorinated congeners were higher near rural and less populated areas (Mira (Mi) and Sines (Si)) and the more chlorinated congeners recorded higher concentrations in the proximity of urban areas (Matosinhos (Mt), Espinho (Es), Paredes de Vitória (Pv) and Cresmina (Cr)). The highest total DDT (tDDT) concentrations (Fig. 8) were observed in aged pellets. Matosinhos (Mt) (41 ng g_1) recorded highest levels of DDTcontamination. Fonte da Telha (FT) (25 ng g_1) and Peniche (Pen) (21 ng g_1) also recorded significantly higher values. White pellets recorded significantly lower concentrations than the other classes (Mann-Whitney U, $p < 0.05$). DDT composition is shown in Fig. 9. Percentages of pp'-DDE, pp'-DDD and pp'-DDT represented higher concentration in those three sites.

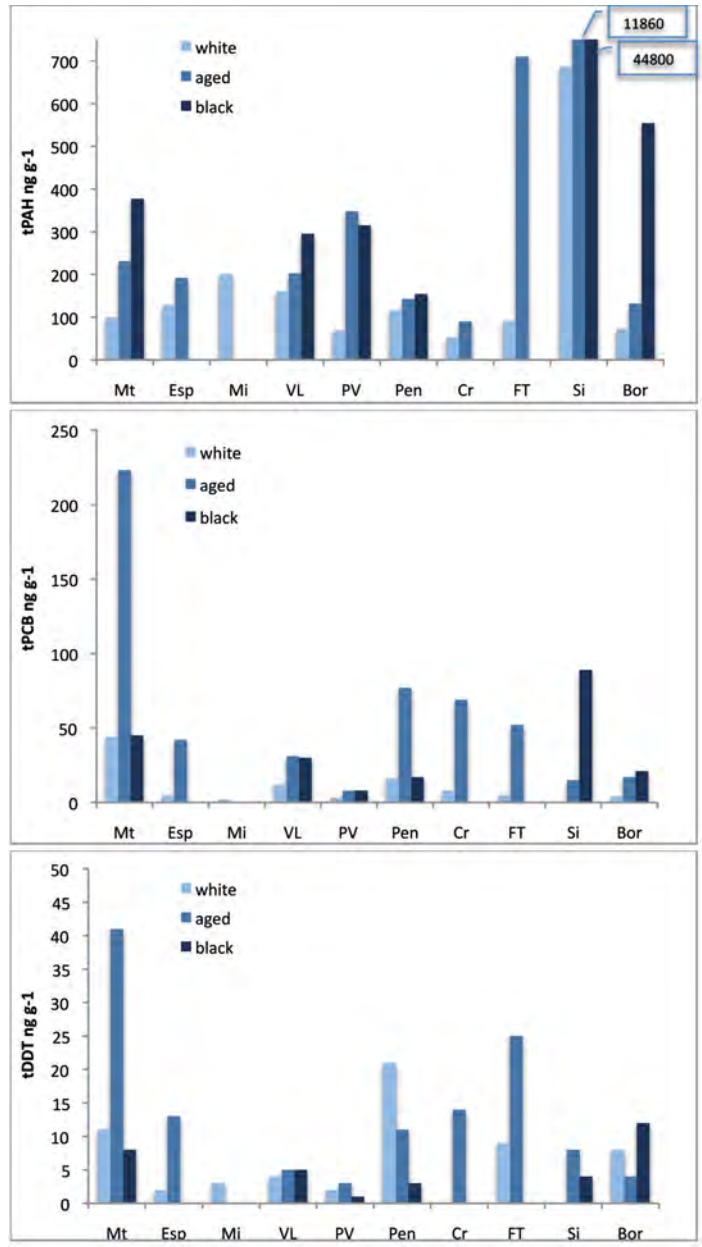

Figure 10. Total PAH, total PCB and total DDT (ng g^{-1}) concentrations recorded in white, aged and black resin pellets from Portuguese beaches, legend as in Fig. 5 (redrawn from Antunes *et al.*, 2013).

In general aged pellets showed higher contamination than white ones due to their longer exposure time while in the oceans. Higher POP concentrations were found near beaches downstream of plastic industries and port facilities. Sines, an industrial site with port facilities, presented the highest PAH concentrations in all pellets classes (white translucent, aged and black). The black pellets class contained a high percentage of polyurethane (PU) and due to the higher surface for adsorption registered high PAH contamination (the highest at Sines, 44800 ng g^{-1}). The highest PCB and DDT contamination were found at Matosinhos, an important port facility, but no clear pattern was found regarding black pellets. High concentration of PAH probably result from the engine oils spills from ships and industrial activities such as petrochemical refining and chemical manufacturing (Li et *al.*, 2007).

The majority of PAH present in our samples are from petrogenic sources. PCB contamination was higher in aged pellets than in any of the other classes. Concentrations of the less chlorinated congeners were higher near less populated areas and the more chlorinated congeners recorded higher concentrations in the proximity of urban areas. The highest total DDT was found near industrial sites and port facilities. These results are in the same range as those found by Karapanagioti *et al.* (2011) in pellets from Greek beaches, however results for PCB and PAH in aged pellets are higher than those found by Rochman *et al.* (2013c).
Depending on polymer type and hydrophobicity, lighter PAH and PCB compounds may reach the adsorption equilibrium faster than the heavier ones, and moreover antagonistic/synergistic effects are known to occur and affect sorption/desorption capacities as shown by Bakir *et al.* (2012) and Endo *et al.* (2013). Microbial degradation of polymers increases surface for adsorption and can improve their capacity to carry pollutants (Raddadi *et al.*, this volume). Microorganisms, attached to the plastic surface, form a biofilm, which modifies the interaction of microplastics with metals and organic compounds (Fotopoulou *et al.*, this volume) and further complicates the relation between pollutants in the water and those adsorbed or leached from plastics.
However there seems to be no doubt that these pollutants may pose a risk of toxicity towards marine organisms, and thresholds for no-effect levels of toxicity for persistent organic pollutants adsorbed to plastic articles are needed.

4. Conclusions and further considerations

On beaches from the coast of Portugal 85-96% of all the accumulated marine litter is made of plastic (items m^{-2}) and those plastics measuring less than 2.5 cm are dominant (90 %). Microplastics make up an average 70% of the plastic items and pellets, within the size range of 3-5 mm, were the most abundant category, (57% of all plastic collected per m^2).

Beaches located downstream from industries and/or port facilities presented higher quantity of plastic debris and microplastics as well as higher concentrations of POP (PAH, PCB and DDT). White translucent PE pellets showed lower contaminant values when compared to yellow-brown (aged) ones, reflecting their residence time in the waters. Black pellets mainly composed by PU also showed high values probably due to the increased area of their surface. Clearly, plastics in the oceans are vectors for these highly toxic pollutants, and bioaccumulation is likely to pose a long-term risk to the environment with unpredictable consequences, therefore research should address the need to establish no-effects levels of toxicity to biota for pollutants adsorbed to plastics.

Floating pellets have been found in plankton samples in Portuguese surface waters which contributes to enforce the role of routine plankton surveys performed by the national authorities as a useful tool in the assessment of marine litter, and particularly microplastics, without further costs of days at sea. This is particularly important for the implementation of the Marine Strategy Framework Directive (2008/56/EC) (MSFD).

In the ocean, changes in concentrations of pollutant mixtures, environmental conditions and distance to sources co-vary in time, and their role on the contamination of plastics and microplastics remains unknown.

Plastic polymers, identified using μ- FTIR analysis, proved to be PE, PP and polyacrylates. Polyurethane (PU) was also found frequently on beaches. These results reflect the present and past decades of worldwide industrial production and intensive use.

Though research on microplastics has grown consistently over the last decade there are still important gaps in knowledge, namely those concerning the extent of the harm to marine organisms, the toxic effects of these particles towards the biota, and how this may affect humans through the consumption of fish. As plastic degrades slowly in the oceans the amount of microplastics is bound to increase and effects on the food-web, still uncertain, must be investigated.

A final note to stress the absolute need to harmonize methodologies to address the issue of marine litter, including microplastics, at the beach and in the water, at the surface and at the bottom, so that results among different studies can be compared and a robust picture of marine litter in the world's oceans emerges. The implementation of the Regional Plan on Marine Litter Management in the Mediterranean as well as in the other European Regional Seas within the scope of the Marine Strategy Framework Directive will be a crucial step to increase knowledge and promote a concerted strategy among stakeholders in order to reduce marine litter. This strategy must be supported worldwide by outreach and awareness activities, to promote literacy and consistent public engagement regarding this global problem.

The impact of macro and micro-plastics on Mediterranean large vertebrates: persistent Bioaccumulative Toxic (PBT) substances, plastic additives and related toxicological effects

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Abstract

This paper focuses on three main aspects related to the impact of macro and micro-plastics on Mediterranean large vertebrates: a) the direct and indirect effects of micro- and macro-plastics exposure and related contaminants, on large marine vertebrates; b) the case studies of three large marine vertebrates in the Mediterranean, the fin whale (*Balaenoptera physalus)*, the basking shark (*Cetorhinus maximus*) and a reptile, the loggerhead sea turtle (*Caretta caretta*); c) future developments on how to detect the toxicological effects in these species.

1. Introduction

From sea-based sources such as shipping, fishing and transport activities (Derraik, 2002) and land-based sources such as tourism, adjacent industries or river inputs (Browne *et al.*, 2010), plastics are entering our seas and oceans, "posing a complex and multi-dimensional challenge with significant implications for the marine and coastal environment and human activities all over the world" (UNEP, 2009) and in particular in the Mediterranean (UNEP/MAP, 2011; MSFD, 2013). The occurrence of microplastics (MPs, generally defined as fragments less than 5mm in dimension) in sea and oceans is an emerging world-wide concern.

Due to high sorption capacity of plastics for hydrophobic organic chemicals, the adherent chemicals can be transported by MPs travelling long distances (Lee *et al.*, 2013b). MPs can serve as carrier of persistent organic pollutants (POPs) in marine ecosystems (Rochman *et al.*, 2013b; Koelmans *et al.*, 2013). Small plastic particles in the environment are of particular concern as a wide range of organisms, from plankton to larger vertebrates such as turtles or whales, may ingest them (Wright *et al.*, 2013; Deudero and Alomar, this volume). Microplastics found in the marine environment are likely to be derived either directly or through the fragmentation of larger items. MPs can be subdivided by usage and origin as: i) primary, pellets used in the plastics industry, and in certain applications such as abrasives; ii) secondary, fragments resulting from the degradation and breakdown of larger items.

A number of heavily produced low density plastics (e.g. polypropylene, polyethylene, and polystyrene) have been identified as the main components of plastic debris and MPs, and these have various shapes and sizes, ranging from a few micrometers to a few millimeters (Hidalgo-Ruz *et*

al., 2012; Martins and Sobral, 2011). Microplastics are accumulating at the sea surface, especially within the neustonic habitat (Ryan *et al.*, 2009) which hosts a specifically adapted zooplankton fauna. While recent studies have identified potential effects of plastic particles in invertebrates and fish, there is still little monitoring data on the occurrence of macro and microplastics in large marine vertebrates in the Mediterranean Sea.

2. Persistent Bioaccumulative Toxic (PBT) substances and plastic additives related to plastics

One major toxicological aspect of plastic litter in the marine environment and, consequentially, on marine organisms, is enhancing the transport, accumulation, and bioavailability of Persistent, Bioaccumulative and Toxic (PBT) substances in addition to toxic chemicals that have been added, during the production procedure, to enhance the performance of the plastic (such as phthalates, nonylphenol, bisphenol A, brominated flame retardants).

In large marine organisms one must distinguish between the direct and indirect ecotoxicological effects of micro- and macro-plastics exposure:

a. Indirect effects: include the problems associated with adsorbed PBT contaminants.

b. Direct effects: include mechanical/particulate problems and leaching of additives (e.g. phthalates) and PBT compounds.

Indirect toxicological effects - plastic debris may be a sink for toxic chemicals. Toxic chemicals from the environment can sorb to the debris and to be released once inside the organism (Engler, 2012; Lithner *et al.*, 2011). Since PBT chemicals, generally, have low solubility in marine water they tend to migrate into water microlayers where they tend to be biomagnified. PCBs and DDTs sorb to debris with a partition coefficient, Kd, of approximately 100,000-1,000,000 over seawater. Similarly, phenanthrene, a PAH, partitions to plastic debris 13,000-fold over seawater (Engler, 2012). Most of these chemicals can potentially affect organisms (Teuten *et al.*, 2007) having endocrine disruptors potency and affect population viability.
Direct toxicological effects - include mechanical/particulate problems and leaching of toxins. For instance, phthalates are a class of chemicals commonly used to make rigid plastics softer to enhance the use of some plastic polymers. Phthalates generally do not persist in the environment, but may leach from plastic debris on a fairly steady basis. Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment. In organisms, both invertebrates and vertebrates, DEHP is rapidly metabolized in its primary metabolite, MEHP (mono-(2-ethylhexyl) phthalate) (Barron *et al.*, 1989), that can be used as marker of exposure to DEHP.

3. Marine vertebrates as indicators in MSFD Descriptor 10

Concerning the problem of marine litter in EU waters, the amount of marine litter in the Mediterranean environment and the effects on sentinel organisms need to be reduced to achieve the GES (Good Environmental Status) as planned by the European Marine Strategy Framework Directive (MSFD) by 2020. As an amendment to the MSFD the "composition of micro-particles (in particular microplastics) has to be characterized in marine litter in the marine and coastal environment" and, for the litter in biota the implementation of descriptor 10 MSFD "Trends in the amount and composition of litter ingested by marine animals", requires "the need for further development based on the experience in some sub-regions" (including Mediterranean Sea). Currently, there is a severe gap in establishing the presence and effects of marine litter in Mediterranean marine organisms. This gap must be covered using sentinel species to determine effects and implement future mitigation actions (Galgani *et al.*, 2014). Here we present the case studies for three large vertebrate species and the potential risk posed to these organisms.

3.1 Large filter feeding marine organisms

Marine organisms with different feeding habits and ecology can be affected by the ingestion of plastic debris and plastic associated contaminants. The impact of microplastics on large filter feeding marine organisms such as baleen whales and sharks is largely unknown (Fossi *et al.*, 2012). These species potentially ingest micro-litter by filter feeding activity. Here we present the case studies of the Mediterranean fin whale (*Balaenoptera physalus*) and basking shark (*Cetorhinus*

maximus). We explore the toxicological effects of microplastics in these species by measuring their levels of phthalates in both species (Fossi *et al.*, 2014). Basking shark and particularly fin whale, characterized by a long life span, could be chronically exposed to these persistent contaminants both leaching from microplastic ingestion and degradation and through the food chain.

The basking shark (*C. maximus* Gunnerus, 1765) is a very large, filter-feeding cold-water and migratory pelagic species. It is widely distributed throughout temperate waters but only regularly seen in a few favoured coastal locations. It may be considered as frequent in the Mediterranean, especially in the north-western sector, mainly in spring (Mancusi *et al.*, 2005). The basking shark is one of only three shark species that filter seawater for planktonic prey. It captures zooplankton by forward swimming with an open mouth, so that water passively flows across the gill-raker apparatus. Filtration rates have been estimated using measurements of swimming speed and mouth gape area (see Table 1). This massive filtering activities imply ingestion and degradation of microplastics. Due to its slow growth rate, lengthy maturation time, long gestation period, low fecundity, probable small size of existing populations, the basking shark is classified by the IUCN Red List of Threatened Species as "Endangered" in the North-East Atlantic Ocean and "Vulnerable" in the Mediterranean Sea (Fowler, 2009; Cavanagh and Gibson, 2007).

Table 1. Comparison between total volume filter daily, total plankton consumed daily and theoretical number of MP items ingested by *B. physalus* and *C. maximus* (Fossi *et al.*, 2014).

	Balaenoptera physalus	***Cetorhinus maximus***
Average adult body length	20 m	7 m
Average adult body mass	50,000 kg	4,000 kg
Engulfment volume	71 m^3	-
Filtration rate	-	881 m^3 h^{-1}
Total volume filtered daily	5,893 m^3	21,144 m^3
Total plankton consumed daily	913 kg	30,7 kg
Theoretical number of MPs items ingested daily	3,653	13,110

The fin whale (*B. physalus*, Linnaeus 1758), the second largest filter feeders in the world, feeds primarily on planktonic euphausiid species. This baleen whale, the only resident mysticete in the Mediterranean Sea, forms aggregations during the summer on the feeding grounds of the Pelagos Sanctuary. The fin whale is a wide ranging cetacean, found from the Equator to the polar regions, but, in spite of its cosmopolitan distribution, it is classified as "Endangered" worldwide, the Mediterranean sub-population is considered "Vulnerable" by IUCN (Panigada and Notarbartolo di Sciara, 2012). Fin whale feeding, in general, has been described as the largest biomechanical event that has ever existed on earth (Croll and Tershy, 2002). Fin and blue whales foraging on krill off the coast, concentrate their foraging effort on dense aggregations of krill in the water column during the day, and near the surface at night (Croll *et al.*, 2005). With each mouthful, the fin whales can trap approximately 70,000 l of water (see Table 1) with a high risk of ingesting MPs through their surface feeding.

In a recent paper (Fossi *et al.*, 2014), the use of phthalates and organochlorines in plankton, shark and whale was proposed as a tracer of microplastics ingestion in these species. The work was implemented through two main steps: 1) detection of phthalates and organochlorine compounds (OCs) in accidentally caught basking sharks in Italian waters; 2) detection of phthalate and OCs content in stranded fin whale specimens collected on the Italian coasts.

The analysis showed appreciable levels of MEHP in all of the specimens analyzed, with concentrations twice higher in cetacean species than in cartilaginous fish (Fig. 1A). The same trend is shown for the organochlorine concentrations, where for the three classes of OCs investigated (HCB, DDTs and PCBs) were always markedly higher in fin whale specimens compared to basking sharks (Fig. 1B). In conclusion, the results show higher concentration of MEHP and OCs in the fin whale blubber in comparison to the muscle of basking shark. Considering both the high presence of MPs and plastic additives in the Mediterranean environment, particularly in the

cetacean Pelagos Sanctuary (Fossi *et al.*, 2012), and the detection of plastic additives and OCs in the tissues of basking sharks and fin whale, large filter feeding marine organisms appear to be chronically exposed to persistent and emerging contaminants related to prey and MPs ingestion. Rochman *et al.* (2014a) underline that several classes of compounds can be carried and released by MPs since organisms living in high density MPs environment show higher plastic-derived chemical pollutants accumulation in their tissue. The dual sources of contamination could derive from direct leaching of contaminants (sorbed on or additive) from microplastics and through already contaminated plankton prey. These species can be also proposed as indicators of microplastics in the pelagic environment in the implementation of Descriptor 8 and 10 of the EU Marine Strategy Framework Directive (MSFD).

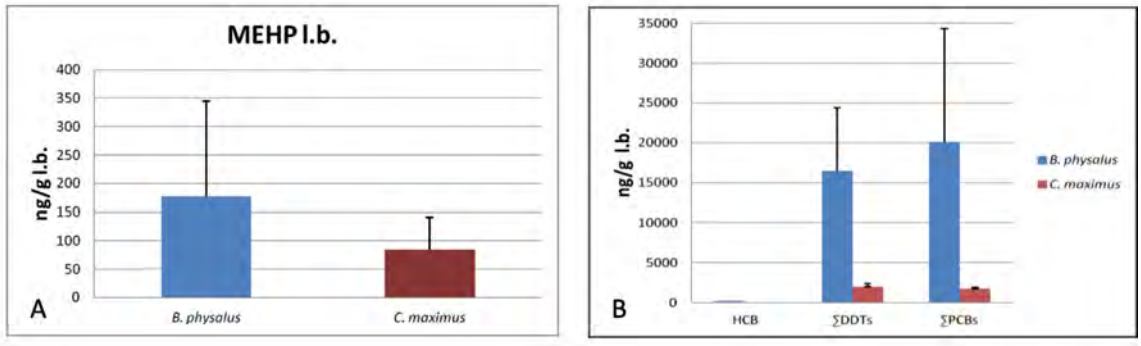

Figure 1. MEHP (a) and Organochlorines (b) concentration (ng/g l.b.) in blubber of *B. physalus* and muscle of *C. maximus.* Bars show mean value ± SD stranded along the Mediterranean coasts.

3.2 Loggerhead sea turtle *(Caretta caretta)*

Currently the only mature methodology for indicator 10.2.1 is the OSPAR Ecological Quality Objective (EcoQO) for litter particles in stomachs of northern fulmars, but this single species cannot provide full coverage over all European marine sectors, and so a range of species is needed to monitor ingested litter. The loggerhead sea turtle (*Caretta caretta*) is the most common and widespread marine turtle species in the entire Mediterranean basin, listed as "Endangered" in the IUCN Red List (IUCN, 2014). Being a generalist, it ingests a high amount of debris that is mistaken for food, causing in the worst case the death of the animal due to entanglement in marine debris or occlusion of gastro-intestinal tract. For this reason the loggerhead sea turtle was recently proposed by MSFD Technical Subgroup of Marine Litter (2013) as indicator to detect the presence of Litter (ingested) in biota in the Mediterranean and nearby Atlantic areas. The sea turtle tool is classified as under development, indicating that enough information exists to be able to suggest a monitoring approach for this species.

As a case study in the Pelagos Sanctuary, Campani *et al.* (2013) evaluated the presence and the frequency of occurrence of marine litter in the gastrointestinal tract of 31 *Caretta caretta* found stranded or accidentally bycaught in the North Tyrrhenian Sea. Marine debris were present in 71% of specimens and were subdivided in different categories according to Fulmar Protocol (Van Franeker *et al.*, 2011). The main type of marine debris found was user plastic, with the main occurrence of sheetlike user plastic. The small juveniles showed a mean +-SD of marine debris items of 19.00 +- 23.84, while the adult specimens showed higher values of marine litter if compared with the juveniles (26.87 +- 35.85) (see Figs. 2, 3, 4). The occurrence of marine debris observed in this study confirms the high impact of marine debris in the Mediterranean Sea in respect to other seas and oceans, and highlights the importance of *Caretta caretta* as good indicator for Descriptor 10 (marine litter) in the Marine Strategy Framework Directive (MSFD).

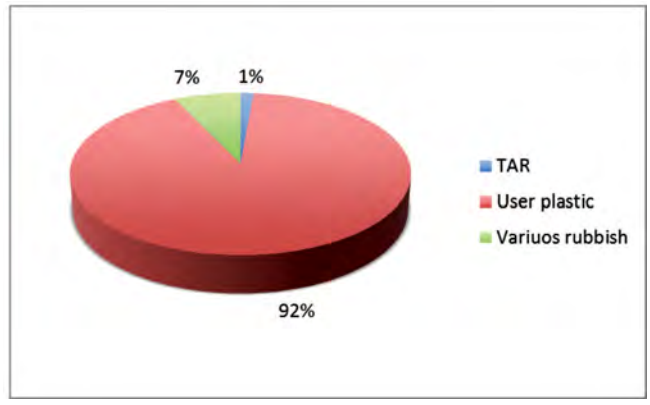

Figure 2. Frequency of different types of marine litter ingested by *C. caretta* (n=31) in the north Tyrrhenian Sea.

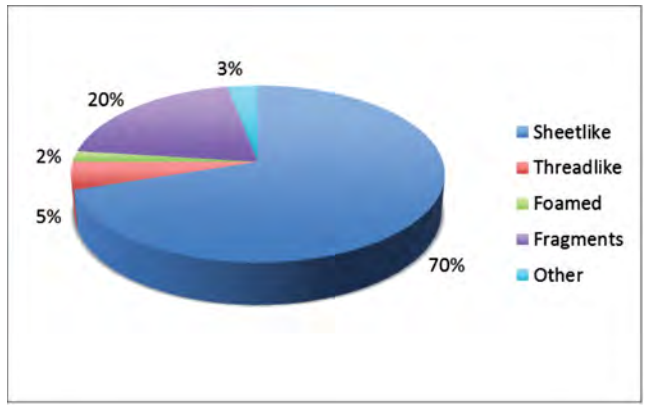

Figure 3. Frequency of user plastic categories ingested by *C. caretta* (n=31) in the north Tyrrhenian Sea.

Figure 4. Types of user plastic found in the specimens of *Caretta caretta* in the north Tyrrhenian Sea.

4. A WAY FORWARD TO EXPLORE THE TOXICOLOGICAL EFFECTS

4.1. PBT substances and plastic additives related to plastics: toxicological effects

The main toxicological threats related to plastics are mainly linked to microplastics and to related contaminants (PBT substances and additives). The direct and indirect effects of microplastic exposure in large filter feeding marine organisms and sea turtles need to be better defined and understood. Specifically:

- Direct effects include mechanical/particulate problems and leaching of toxic compounds (e.g. phthalates).
- Indirect effects include the problems associated with adsorbed contaminants (PBT substances). The effects of these contaminants associated to plastics need to be addressed to distinguish from the mixture of contaminants already present in marine environment.
- Need to develop (also using *in vitro* approach in laboratory studies) the specificity of biomarkers to microplastic exposure (plastic additives and PBT substances). This would help track a cause and effect relationship.
- Using a fresh carcass to investigate the potential biomarkers in the 'target / ideal' organ and then the same biomarker on tissues from free-ranging organisms (e.g. Cetaceans).

4.2. Large filter feeding marine organisms

Large filter feeding marine organisms, such as baleen whales and sharks, can be selected as wide-scale indicators of the presence and impact of microplastic in the whole Mediterranean pelagic environment. The monitoring activities on these species could be implemented through two steps: 1) detection of macro- and micro-plastic in stomach contents and detection of phthalates and PBT substances in stranded fin whales and basking sharks along the Mediterranean coast; and 2) detection of phthalates, PBT substances and biomarkers responses linked to the metabolism of specific toxic compounds (CYP1A1, CYP2B, PPAR G, ER, AHR, E2F1, lipid peroxidation) (Fossi *et al.*, 2010; Panti *et al.*, 2011; Fossi *et al.*, 2012) in skin biopsies of fin whales (Fig. 5) and basking sharks collected from free ranging organisms.

Figure 5. Skin biopsy of a fin whale.

4.3. Loggerhead sea turtle

The loggerhead sea turtle (*Caretta caretta*), could be used as wide-scale indicator of the presence and effects of plastics, particularly macro-plastics in the whole Mediterranean pelagic environment. The monitoring activities on this species might be implemented through three steps:

1) Detection of macro- and micro-plastic in stomach contents, phthalates and PBT substances, in loggerhead turtles stranded along the Mediterranean coast.
2) Development/implementation of non destructive ecotoxicological biomarkers of direct/indirect effects in blood, skin biopsy and excreta samples from loggerhead turtles hospitalized in rescue centers in the Mediterranean areas. A set of biomarkers of indirect effects, indicating exposure to lipophilic contaminants - PBT (induction of CYP1A, accumulation and excretion of porphyrins), endocrine disruption (Vitellogenin, Zona Radiata Proteins, Estradiol, Estrogen and Progesteron Receptors, Aromatase), oxidative stress (lipid peroxidation), genotoxicity (Comet, diffusion and ENA assays) and liver damage (y-GT) can be investigated in conjunction to the analysis of contaminant levels (phthalates and PBT compounds) (Fig. 6). There is a strong need for the development of biomarkers of direct effects. Biopsy slices (organothipic cell lines) can be exposed to specific plastic tracers and new biomarkers can be developed.
3) Non destructive approaches, including biomarker and contaminant analysis, should be applied to free-ranging loggerhead turtles in order to investigate the toxicological impact of plastics on wild populations.

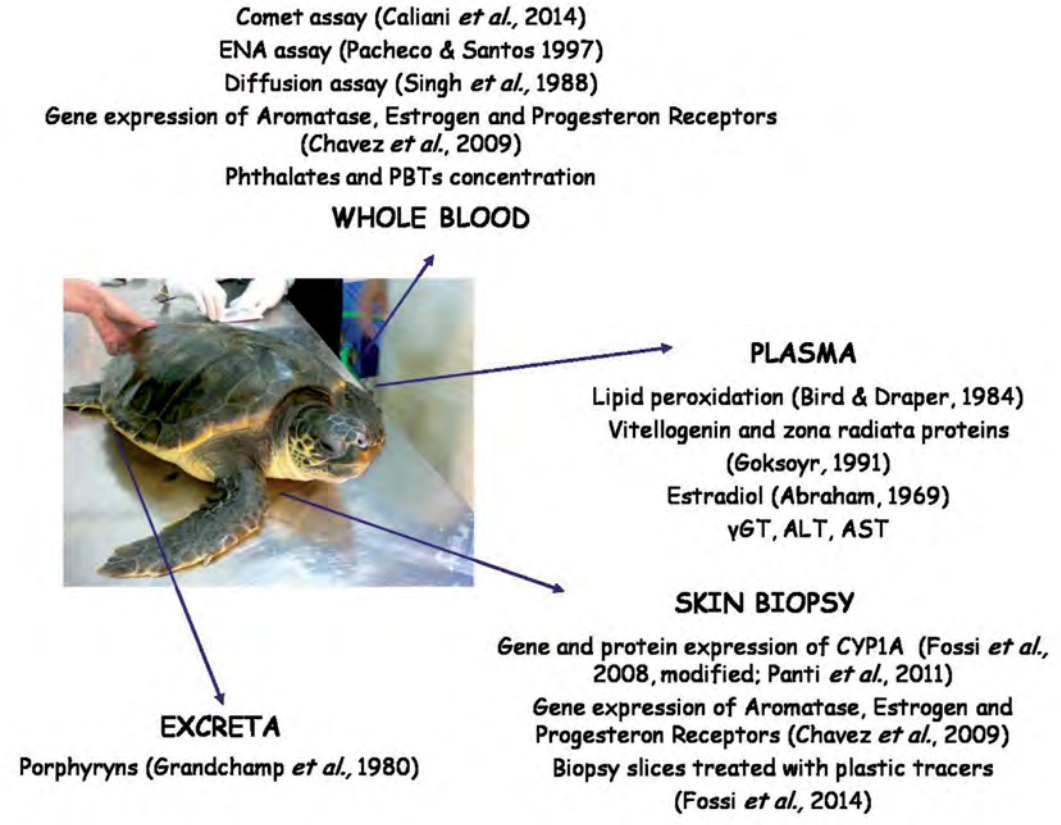

Figure 6. Array of tests available on *Caretta caretta*.

In conclusion one of the main outcomes of this paper is to propose the further development of scientific research on large marine vertebrates as indicators of medium and long term marine environmental change for incorporation under the MSFD. This might then be employed in the assessment of the quality of pelagic marine ecosystems elsewhere.

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