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 :
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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.