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Marine litter: One of the major threats for marine mammals. Outcomes from the European Cetacean Society workshop☆

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A B S T R A C T

Marine litter is a pollution problem affecting thousands of marine species in all the world's seas and oceans. Marine litter, in particular plastic, has negative impacts on marine wildlife primarily due to ingestion and entanglement. Since most marine mammal species negatively interact with marine litter, a first workshop under the framework of the European Cetacean Society Conference, was held in 2017 to bring together the main experts on the topic of marine mammals and marine litter from academic and research institutes, non-governmental organisations, foundations and International Agreements. The workshop was devoted to defining the impact of marine litter on marine mammals by reviewing current knowledge, methodological advances and new data available on this emerging issue. Some case studies were also presented from European waters, such as seals and cetaceans in the North, Baltic, and Mediterranean Seas. Here, we report the main findings of the workshop, including a discussion on the research needs, the main methodological gaps, an overview of new techniques for detecting the effects of marine litter (including microplastics) on marine mammals and, also, the use of citizen science to drive awareness. The final recommendations aim to establish priority research, to define harmonised methods to detect marine litter and microplastics, enforce networking among institutions and support data sharing. The information gathered will enhance awareness and communication between scientists, young people, citizens, other stakeholders and policy makers, and thereby facilitate better implementation of international directives (e.g., the Marine Strategy Framework Directive) in order to answer the question about the actual status of our oceans and finding solutions.

1. Introduction

Marine litter pervades and affects all the world's seas and a large number of marine species. Specifically, plastic debris affects marine mammals worldwide and microplastics have recently emerged as an additional threat within this topic. The development of protocols, which allow a harmonised approach to monitoring marine litter impact on marine mammals, including microplastics, has become essential for future research. The term microplastic used here refers to particles smaller than 5mm in size. Sources of microplastics have been discussed in several reviews including the fragmentation of larger items, as well as the introduction of micro-sized particles to the environment (GESAMP, 2016). It is widely documented that marine debris has negative impacts on marine mammals, primarily due to ingestion and entanglement (Baulch and Perry, 2014; Fossi et al., 2018a; Kühn et al., 2015). Macrolitter has been reported to be ingested by many species of marine mammals, such as baleen whales, beaked whales, dolphins and

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porpoises, and seals (Fossi et al., 2018b; Lusher et al., 2018; Unger et al., 2017, 2016), most of these are carried out through necropsies, using methods that target particles > 2.5 cm, therefore missing particles in the "micro" range. The absence of macrolitter in such studies does thus not imply the absence of microlitter (Lusher et al., 2018). Microplastics may present problems for biota if they are inhaled or ingested, including problems related to chemicals associated with the debris particles (Lusher, 2015). In order to achieve a more thorough understanding of the risk microplastic pose to marine mammals, a standardized protocol which is simple and cost-effective should be implemented to allow research teams to collect and analyse samples for the presence of microlitter in a comparable and transparent way, with a particular focus on microplastics.

In 2017, M.C. Fossi and colleagues from the University of Siena, Italy, brought together researchers investigating the impact of marine litter on marine mammals for a workshop at the European Cetacean Society (ECS), 31st Annual Conference in Middelfart (Denmark). The rationale of the workshop arises from the evidence that most marine mammal species are affected by plastic contamination, thus, the primary goal of the workshop was to explore the impact of marine litter on cetaceans and pinnipeds. The workshop was devoted to (1) defining the state of knowledge on the impact of marine litter to marine mammals; (2) presenting new and emerging data available ranging from entanglement in plastic debris to the ingestion of macro- and microplastics; (3) presenting the available methodological approach currently used to assess the impact of marine litter on diverse marine mammal species and (4) highlighting future perspectives and recommendations.

Forty attendees from eleven different countries participated in the workshop. They included representatives from universities, research institutes, non-governmental organisations, foundations and International Agreement representatives (e.g., Agreement on the Conservation of Cetaceans of Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS), Conservation on Migratory Species (CMS), International Whaling Commission (IWC)). The first half of the workshop consisted of invited presentations from participants which were subsequently followed by a panel-led discussion. Here we present the main outcomes from the workshop exploring the current state of knowledge and the methods available to study marine litter in marine mammals (both in dead stranded and live individuals) as well as future way forward for integrated and comparable monitoring of marine mammals and plastic debris on a global scale.

2. Part 1. Current state of knowledge and methods for monitoring the impact of marine litter on marine mammals

Impacts of litter on marine fauna occur throughout the food chain, with adverse impacts documented so far on over 800 species (Kühn et al., 2015). Impacts from entanglement can result in injury, drowning or strangulation, whereas those from ingestion range from no discernible impact through to blockage of the digestive tract, to suffocation and starvation (Laist, 1997). Both these interactions highlight the importance of implementing standardized protocols and programmes for monitoring this type of pollution.

Concerned by the huge potential for marine wildlife impacts, the International Whaling Commission (IWC) has held two marine debris workshops (Wright et al., 2016). The first, in 2013, focused on improving understanding of the threat posed by marine debris to cetaceans and discussed impacts from both macrodebris (e.g., fishing gear, plastic bags, and sheeting) and microplastics (e.g., plastic particles added to cosmetics and the pellet form of raw plastics) (IWC, 2013). The workshop made a number of recommendations and agreed that marine debris was both a welfare and a conservation issue for cetaceans on a global scale. The IWC's Scientific Committee subsequently endorsed the workshop's recommendation for more research and also agreed that:

- legacy and contemporary marine debris have the potential to be persistent, and have sub-lethal and lethal effects on cetaceans and thus represent a global management challenge; and
- entanglement in, and intake of, active fishing gear, ALDFG (abandoned, lost, or otherwise discarded fishing gear) and other marine debris have lethal and sub-lethal effects on cetaceans (IWC, 2014a).

The 2014 workshop gathered together several key international bodies already engaged in marine debris and agreed that the IWC's primary contribution should be to ensure that cetacean-related issues are adequately represented within existing initiatives and that the IWC Scientific Committee's expertise should be made available in collaborative efforts (IWC, 2014b). It also strongly recommended "as the highest priority" that the IWC and its Secretariat work together with the Secretariats of the other major Intergovernmental Organization (IGOs) and Regional Fisheries Management Organisations (RFMOs) relevant to this issue to ensure consistency of approach, synergy of effort and collection and exchange of information to develop appropriate mitigation strategies that recognise that: (a) prevention is the ultimate solution; but that (b) removal is important until that ideal is realised. Since these workshops, the Scientific Committee has continued its work on this topic and, at its 2018 meeting, recommended that a further workshop should be held (IWC, 2018).

Evidence of impacts on cetaceans comes from a variety of published and unpublished sources and Baulch and Perry (2014) collated over 500 records of marine litter interactions from the published literature and responses from stranding networks in eleven countries, showing an increase in the number of cases being reported over the last five decades. Among the 14 families of cetaceans (Committee on Taxonomy, 2017), 11 families have been reported to interact with marine litter (Fossi et al., 2018b). The number of records is unlikely to represent the extent of impact on marine mammals. Rather, what has been observed has strong bias based on the availability of the different species and other factors such as differential rates of stranding and necropsy.

Entanglement of marine mammals with marine litter, including ghost fishing nets, has been documented in 27 species and a total of 78 incidences were documented worldwide (Baulch and Perry, 2014; Kühn et al., 2015); 31.4% species have at least one documented occurrence of entanglement.

Ingestion of macrolitter has been documented frequently (in over 60% of all cetacean species), and in species employing a variety of feeding techniques at different levels of the feeding column (Baulch and Perry, 2014; Fossi et al., 2018a; Kühn et al., 2015; Puig-Lozano et al., 2018). Plastics were the most common item ingested and the size ranged from small fragments to large plastic sheets. In the 2014 review, relatively few stranding networks were found to collect data on rates of marine litter ingestion (Baulch and Perry, 2014). However, based on available data (considering more than ten organisms necropsied), ingestion rates varied from 0% to 31% of animals necropsied, with high geographic, intra- and inter-specific variations in rates.

The study of microplastic ingestion by marine mammals is a challenging task. Large cetaceans present difficulties in obtaining viable samples during necropsies due to large gut content volumes. Few studies have directly identified microplastics in the digestive tracts of stranded individuals. Applying standard protocols for the detection and identification of microplastics in the digestive tracts, microplastics were found throughout the stomach/intestine of eight odontocetes species: *Mesoplodon mirus, Ziphius cavirostris*, *Delphinus delphis*, *Stenella coeruleaolba*, *Phocoena phocoena*, *Orcinus orca* and *Tursiops truncatus* (Lusher et al., 2018, 2015; van Franeker et al., 2018). Only one study on mysticetes, a stranded humpback whale (*Megaptera novaeangliae*), has recorded microplastics in the intestines, including fragments and threads (Besseling et al., 2015).

Evaluating the frequency and severity of impacts of marine litter on cetaceans is complicated by low sample sizes linked with to the low rate of detection (with as few as 0–6.2% of carcasses recovered from cetacean deaths at sea) and the compounding effects of a low necropsy and publication rate. New techniques have been developed to detect plastic tracers using non-lethal methods (e.g., skin biopsies, Fossi et al., 2016).

Sub-lethal impacts of plastic ingestion are more difficult to assess. Such impacts may include injury within the gastro-intestinal tracts (GITs), compromised feeding, malnutrition, disease and, reduced reproduction, growth and/or longevity; these issues may be reported with the evaluation of specific molecular markers (Allen et al., 2012; Fossi et al., 2018b; Katsanevakis, 2008; McCauley and Bjomdal, 1999; Moore et al., 2013; Puig-Lozano et al., 2018).

Field studies and monitoring indicates that interactions between marine litter and a mixture of chemical compounds are of significance. Laboratory studies could shed light over possible interactions (synergy or antagonism) learning from the field mixture toxicity (Syberg et al., 2017).

Given the multiple potential physical and ecotoxicological effects of marine litter interactions, the impact of litter on marine mammals should be assessed using a new threefold approach (Fossi et al., 2018c). The application of the threefold approach (discussed during the workshop) can add to the data on the rate of ingestion in cetaceans, data on the multiple sub-lethal stresses that marine litter ingestion can cause in the short and long term. Each of the three level of investigation tools that make up the threefold approach can be applied independently or simultaneously and whether the animals concerned are stranded or free ranging. The threefold approach comprises the following elements:

a) Analysis of gastro-intestinal content: Detection of the occurrence and rate of marine litter ingestion and any associated pathology through analysis of the gastro-intestinal content (with a particular focus on plastics and microplastics) in stranded cetaceans;
b) Analysis of the levels of plastic additives, as a proxy for ingestion: The plastic additives indirect quantification can be applied both to free-ranging as well as to stranded organisms. The levels of plastic additives (such as phthalates or PBDEs) and associated Persistent Bioaccumulative and Toxic (PBT) compounds allow to evaluate the exposure to marine plastic pollution.
c) Analysis of biomarker responses: Biological responses can be used to detect the potential toxicological effect related to PBT and plastic additives related to plastic ingestion in free-ranging individuals or in stranded organisms up to a few hours after death.

Further details on these three methodological phases will be described in the following sections, also focusing on specific case studies.

3. Part 2. Studying marine litter in stranded marine mammals

There are various ways to detect marine litter ingestion in marine mammals. Few standard protocols for the recording of plastic are currently available, and therefore the amount and size of plastic reported differs between research groups.

Nevertheless, collecting data from stranded marine mammals provides important information to researchers from different fields. For example, pathologists will open the GIT of stranded animals as part of an investigation into the reasons of stranding and/or death; in these cases, large marine litter items may be detected but smaller particles can be easily overlooked. Necropsies are typically conducted according to standard protocols (e.g. Kuiken and Hartmann García, 1993). In diet studies, usually only the stomachs of stranded animals are investigated in more detail than presented in pathological reports. Some diet studies are implementing an overflow technique which requires floatation for the removal of less dense particles; however, the method may see that floating particles are lost during the rinsing process (van Franeker et al., 2018). In these studies, the lack of a standardized protocol for the examination of microplastics might cause the loss of these smaller particles. With the ongoing interest in plastic ingestion, researchers have adapted dietary studies to understand the levels of plastics present in marine mammals. For plastic research, the complete GIT of the stranded animal will ideally be examined, as smaller plastic particles can easily pass through the stomach into the intestine. When the GIT is rinsed both the plastics and the prey remains can be examined by a standard protocol (Lusher et al., 2018).

Interestingly, the standard protocols for detecting plastics in other marine vertebrates (MSFD Technical Subgroup on Marine Litter, 2013; OSPAR, 2015), which have been adopted by European researchers, utilise a lower size limit of 1 mm; which has seen many research institutes develop closely aligned protocols investigating plastics > 1 mm. For example, in the Netherlands, the rinsing of the GIT of stranded whales and dolphins is carried out with a 1 mm sieve (Besseling et al., 2015; Bravo Rebolledo et al., 2016; Unger et al., 2017; van Franeker et al., 2018). Standardizing the method for recording the occurrence of plastic using dedicated protocols, will allow investigators to obtain results that can be compared between mammals, birds and turtles (Provencher et al., 2017). This methodology presents a problem because smaller microplastics can be lost during processing. Recently, research carried out in Ireland added an additional set of sieves to allow the collection of microplastics to 200 μm (Lusher et al., 2018, 2015). This procedure has been recommended for future investigations, not only in marine mammals but also seabirds and sea turtles to achieve a better understanding of the ingestion of microplastics.

Utilising stranding networks can provide further information of marine litter pollution and the exposure of plastics to these top predators. For example, Lusher and colleagues recently published the results of the incidence of microplastics in different cetacean species stranded on Irish coasts (Lusher et al., 2018, Fig. 1). A total of 410 digestive tracts were analysed for macroplastics, and 21 were investigated specifically for microplastics. All 21 digestive tracts contained microdebris, but only three of them contained macrodebris. More than three-quarters (84%) of the microplastics were classified as fibres. Blue was the most prominent colour (29%). Most of the fibres were less than 3mm in length. This information revealed the importance of using an adapted protocol for the detection of fibres, which are one of the most common microplastic items identified in the marine environment.

Two noteworthy studies of stranded animals impacted by marine litter were presented within the ECS2017 workshop. One study presents the marine debris findings in marine mammals from German waters of the North (NS) and Baltic Seas (BS), the other study evaluates marine debris occurrence in sperm whales stranded on the Italian coast between 2009 and 2016. In addition, a standardized protocol for dietary and marine litter studies, including microplastics was presented.

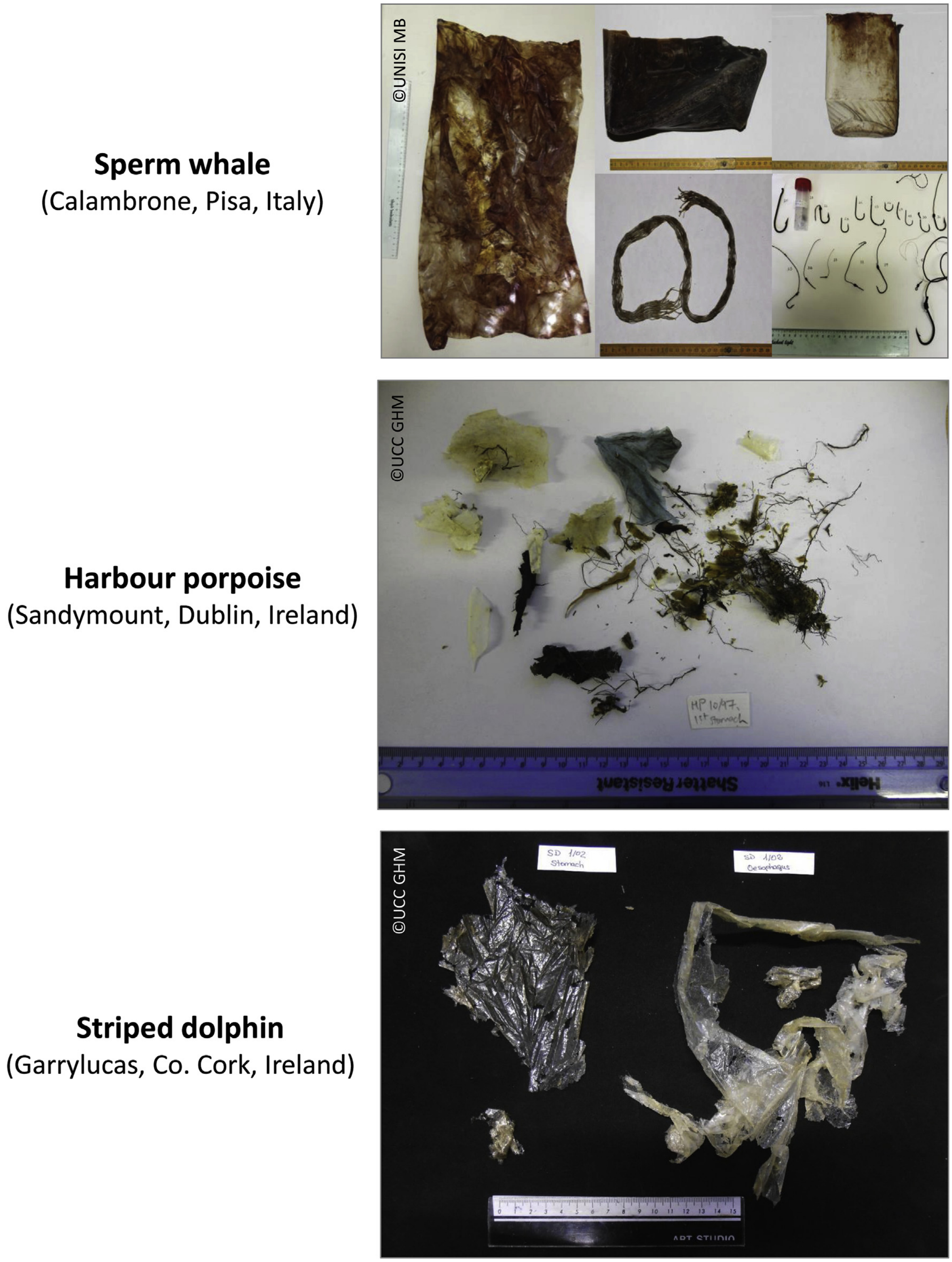

Fig. 1. Marine litter ingested by stranded cetaceans (sperm whale, harbour porpoise and striped dolphin) in European coasts.

Three marine mammal species inhabit the North and Baltic Seas: *Phocoena phocoena* (PP), *Phoca vitulina* (PV) and *Halichoerus grypus* (HG). Carcasses of harbour porpoises are collected since 1990, carcasses of seals since 1995. For this study data collected until 2014 were taken into account. Next to basic information such as sex, size and weight, additional information on marine litter items are noted during necropsies. From the 6587 collected individuals, a total of 1622 were necropsied on the GIT. Marine litter was found in 31 individuals either ingested (17 cases) or entangled around the body (14 cases) and a total of 37 items were recovered. External findings were then put into relation to the number of registered animals, internal ones to the number of individuals in which the GIT was necropsied. The prevalence in grey seals was higher for both, external (1.2%; PV: 0.3%; PP: 0.1%) and internal findings (2.4%; PV: 1.1%; HG: 0.7%). Comparing the North (NS) and Baltic (BS) Seas, the prevalence of ingestion and entanglement was higher in the Baltic Sea (Ingestion: BS: 1.8%, NS: 08%; Entanglement: BS: 0.3%, NS: 0.2%). The items mostly consist of synthetic materials, including plastic (73.0%) and 64.9% of all objects were fishing related. Impacts on marine mammals were identified, including perforation or rupture of the GIT, dermatitis, absecessation, peritonitis and septicaemia. Eight animals were either severely suffering or dying due to marine debris items. It must be noted that the result of this study is a minimum estimate of impacted animals, since not all carcasses are washed ashore and are available for further examination. This study provides valuable information on the occurrence and impact of marine debris on marine mammals in German waters. Although, the impact rates appear low, the possible consequences are of concern (Unger et al., 2017).

From 2009 to 2016, 13 sperm whales stranded along the Italian coast (Mediterranean Sea) were necropsied and their stomachs were collected for dietary and marine litter investigation. Initially, the contents were inspected for the presence of any tar, oil or particularly large material which were removed. Secondly, the stomach was washed, and the contents were rinsed and filtered through a 1 mm sieve. Marine litter items were identified and isolated for analysis following the “Litter in Biota” protocol (developed for seabirds and sea turtles and included in the “Monitoring Guidance for Marine Litter in European Seas”; Galgani et al., 2013). To better understand the composition and origin of the debris the protocol was implemented with the use of FT-IR spectroscopy technique. Marine debris was found in 10 out of 13 specimens (77%) and it was composed mainly of plastic (Fig. 1). Five user plastics categories were identified, and among these, the most abundant categories were the sheet/film, followed by thread, other plastic, fragments and foams. In the specimens analysed most items of isolated debris were black, transparent or white. The polymer analysis confirmed that isolated items, categorized by a visual analysis as plastic, were plastic polymers. The plastic items within the “sheets and fragments” category were mainly composed of polyethylene (PE) and, to a lesser extent, polypropylene (PP); these plastic types are widely used as packaging material worldwide both in sea and land-based activities.

In order to collect viable data across different species and different geographical areas of plastic ingestion by large marine mammals, Lusher et al. (2018) proposed an approach utilising strandings networks. They use the full GIT dissecting each stomach chamber individually and rinsed with pre-filtered water through a set of nested sieves of different sizes (e.g. 1000, 500 and 200 μm). Samples in the smaller mesh size sieve will be analysed for microplastics. Intestines are recommended to be divided in 20 equal pieces following Lusher et al. (2018). Scats can be processed in the same way. Any material retained on the sieves is transferred to a sterilised glass container for biological digestion. A solution of 10% KOH was recommended, being a simple and cost-effective method (Kühn et al., 2017; Lusher et al., 2017). Following digestion, the remaining solution is rinsed and filtered under vacuum onto a filter paper where is it subsequently analysed under a microscope. Particles are quantified and sorted into shape, colour and size categories. Where possible a subsample of particles will undergo further analysis to confirm polymer identity or plastic presence.

4. Part 3. Assessing marine litter interactions using live individuals

Plastic marine litter is well known to be associated with chemical contaminants. Therefore, the ingestion of plastic litter could cause severe toxicological effects due to the exposure to both chemicals absorbed by plastics and plastic components. Plastic additives are chemical compounds which are used to give specific properties to a plastic polymer and are incorporated during the manufacturing process (OECD, 2014). The most common compounds used are brominated flame retardants (BFR), stabilizers, phthalate esters (PAEs), bisphenol A (BPA), and nonylphenols (NPs) (Hermabessiere et al., 2017). Once in the environment, these compounds may leach out from plastic litter (both macro and microplastics) or be accumulated on the surface of plastic items. Tracers of plastic additives present in animal tissues can be used as an indirect method for detecting plastic ingestion, in particular phthalate esters (PAEs). For example, eight different phthalates (MBZP, MBP, MEHP, DNHP, BBzP, DEHP, DIOIP, DNDP) were detected both in neustonic/planktonic samples and four cetacean species (blubber from skin biopsies) sampled in the Pelagos Sanctuary (North-Western Mediterranean Sea) (Baini et al., 2017; Fossi et al., 2016). The results showed different fingerprints and levels across the neustonic/planktonic samples, indicating a heterogeneous pattern of phthalates in the environment, which may be associated with microplastics (Baini et al., 2017). In addition, seven out of eight PAEs were also detected in the blubber of *Balaenoptera physalus*, *Tursiops truncatus*, *Grampus griseus* and *Stenella coeruleoalba* sampled in the same area, which might therefore indicate plastic ingestion. MBzP, MBP, MEHP and BBzP were significantly correlated to the size and abundance of microplastics in the neustonic/planktonic samples (Baini et al., 2017).

Uptake and accumulation of plastic-associated chemical contaminants may produce undesirable biological effects. For example, when fin whale and sperm whale organotypic skin cell cultures were treated with increasing doses of PAEs, it showed an upregulation of the mRNA levels of the Peroxisome proliferator-activated receptor gamma (PPAR-γ) gene (Fossi et al., 2018a); these results suggests that PAEs play an important role in the alteration of the PPAR-γ, which regulates physiological processes of lipids homeostasis, inflammation, adipogenesis, reproduction, etc. (Schupp and Lazar, 2010).

Another approach has been applied to the *ex vivo* assay using organotypic skin cell cultures from the bottlenose dolphin, cultured and treated with different perfluorooctanoic acid (PFOA) and BPA concentrations. The microarray assay could represent an additional application to analyse global gene expression for assessing the exposure to a certain class (or a mixture) of compounds. RNA labelled and hybridized to a species-specific oligomicroarray showed that the skin transcriptome could hold information on the contaminant exposure. Using such assays may allow researchers to predict about long-term effects on health, being the genes affected involved in immunity modulation, response to stress, lipid homeostasis, and development (Lunardi et al., 2016). The transcriptomic signature of dolphin skin could be therefore relevant as classifier for a specific contaminant such as plastic-associated contaminants.

Further research on biomarkers targeting the exposure of plastic ingestion and their additives is required.

5. Part 4. Utilising citizen science projects to address marine litter

Plastic pollution, as part of marine debris, is widely known to impact many different ecosystems from land to sea. This implies that the solution to the problem must be addressed in a broad societal context. Involvement of people in citizen science (CSci) projects, such as beach clean-up projects has proven valuable, not just as a mitigation effort but also to generate awareness (Wyles et al., 2017).

Experience from other environmental fields has shown that combining top down CSci with a more direct bottom up CSci can allow people to start an array of impacting initiatives. Beach clean-ups can typically be characterized as top-down CSci, where scientists (or other organisations such as NGOs) ask people to participate (Syberg et al., 2018). These projects can thus have a double impact since, on the one hand they can remediate plastic pollution before it enters the ocean, where it is much harder to clean it up than on the beaches, and on the other hand raise awareness, which can facilitate other societal activities such as regulatory measures. As an example, a Swedish study showed that local historical knowledge could be used to conceptualize reference conditions of a lake's environmental state and provide a more detailed description of the lake (Valinia et al., 2014). This enabled an assessment of the water quality leading to a better foundation for regulation under the Water Framework Directive (2000/60/EC).

Marine mammals are not only key species for marine ecosystems. In fact, most people have a strong emotional attachment to marine mammals which results in high involvement and commitment for their protection. Therefore, generating political awareness which can lead to measures to prevent plastic pollution, can help to protect marine mammals both directly (e.g. cleaning waste before it enters the oceans) and indirectly. Many marine mammal species investigated related to marine litter are charismatic and iconic indicators that can serve as flagship species for marine conservation. While umbrella species are useful for directing intervention strategies, flagship species can provide a mechanism for communicating awareness and stimulating action to tackle marine plastic pollution in all the marine ecosystems (Germanov et al., 2018). Furthermore, since plastic pollution is already of great public concern this provides an opportunity to engage a broad array of the public. Such raised awareness does not only lead to societal action but potentially also help raise awareness on other environmental problems of equal concern but with less public attention such as chemical pollution or ocean acidification.

6. Discussion and concluding remarks

It is clear that marine mammals are impacted by marine litter through many different ways. To understand the level of these impacts a consistent monitoring approach is required, especially as marine litter pollution is estimated to increase in the future. There are a number of approaches, as discussed here that can support researchers and environmental organisations to assess the impact of marine litter, in particular plastics, on marine mammals. Current methods use direct and indirect approaches (strandings and biopsies respectively; Table 1).

Direct approaches allow researchers to investigate the consequences of ingestion and entanglement in marine litter on individual organisms and researchers can gather information not only on litter but trophic ecology, habitat used, pathological condition, etc., which can benefit a wider researcher community. Estimation of microplastic intake is another gap requiring further investigation. For example, using a simple mathematical estimation rule, Lusher et al. (2016) estimated that a single Striped dolphin (*Stenella coeruleoalba*) could be exposed annually to ~463 million microplastics based on its diet on mesopelagic fish. Methodologies related to this issue should be improve and applied to all species in order to understand the exposure of top predators to plastic litter and the trophic transfer.

In addition, assessing the impact of this type of pollution on living organisms needs an indirect approach, based on the detection of biological responses related to the physical and chemical exposure and the accumulation of plastic associated contaminants. Since 2012, biomarkers have been investigated as an appropriate method to monitor plastic ingestion (Fossi et al., 2016, 2012). These authors used biopsies of whales and sharks to detect plastic additives in different areas. In a similar way, Baini et al. (2017) found these plastic additives in four cetacean species. The importance of these findings encourages researchers to develop more sophisticated approaches accordingly.

On the other hand, CSci has become a valuable resource to protect marine mammals and raise awareness within society. Including CSci in studies of marine pollution can help to reduce the impacts of this type of pollution in our environments using marine mammals as flagship species and help generate environmental awareness.

To date, in many cases the origin of plastics is still unknown. Identification of polymers and chemicals may allow researchers to identify the type of plastic; however, most of the time it is not possible to identify their source (including country of origin and product use). The majority of plastics are predicted to come from non-coastal areas (Jambeck et al., 2015), but once they reach the sea waters they can be transported by currents to different parts of the world (van Sebille et al., 2012). Further research on plastic release, transport and distribution mechanisms in aquatic ecosystems is needed to help better assess the impacts of marine mammals.

It is incredibly hard to understand uptake levels of plastics in marine mammals and monitoring their feeding in the environment is difficult. Therefore, uptake can be monitored through investigations of GITs of stranded individuals (e.g., Lusher et al., 2018; Unger et al., 2017) or indirectly utilising biomarkers or plastic additives (e.g., Baini et al., 2017; Fossi et al., 2018a, 2016). An alternative approach is to investigate estimated update through diets, as presented in Lusher et al. (2016). Understanding plastic levels in prey species may give some indication of plastic transfer to predatory marine mammals. However, this approach must be used with caution as uptake, retention and egestion rates may vary between individuals, their level of exposure in the environment and their ability to remove undesired items following feeding.

Although this workshop was focused on marine litter, the outputs highlighted that researchers should take into account other information (e.g. diet, habitat, pathological condition) to understand the sources, the transfer and the effects of marine litter, and therefore their impacts on marine mammals. In addition, it was highlighted that further research and standardization of protocols are essential to understand these impacts.

It is therefore recommended that moving forwarded seven steps are required:

(1) To harmonize/standardized protocols for the analysis of marine litter in stranded organisms and share knowledge, facilities and samples. In particular, it is important to standardize methodologies for microplastic analysis on marine mammals simplifying and reducing the cost of these analysis; some research groups may have economic constraints and the microplastic methodology proposed in this workshop has been adapted to these requirements to allow future comparisons between research groups;
(2) Enforcing national stranding networks to collect/share samples for different marine litter analysis and establishing an international network of all marine mammals and marine litter people (MML group/community);
(3) To share information, scientific results, images in a database (to be hosted in a web platform);
(4) To define the actual threat to organisms (amount of debris ingested? Weight? Volume? Chemical transfer?) and to identify the most threatened species and hot spot areas according to season and species habitat use in EU waters;

Table 1
Summary of the studies presented and related methodological approach used to assess the impact (entanglement and ingestion) of marine litter on marine mammals.

Impact	Species	Location	Sampling method	Organs investigated	Litter size class	Technique	Reference
Entanglement	11 species of cetaceans (2 baleen whales, 9 delphinids)	Atlantic ocean (Irish coast)	Necropsy	Whole body	Macro	External visual inspection for by-catch, mutilations or entanglements.	Lusher et al., 2018
Ingestion/ Entanglement	Harbour porpoise, Harbour seal, Grey seal	North Sea, Baltic Sea (German coast)	Necropsy	Whole body and gastro intestinal tract	Micro; Macro	External and internal visual inspection	Unger et al. (2017)
Ingestion	Harbour porpoise	North Sea (Dutch coast)	Necropsy	Stomach	Micro; Macro	Visual inspection adopting the OSPAR protocol and near-infrared. (NIR)	van Franeker et al. (2018)
Ingestion	19 species of cetaceans (4 species of baleen whales, 6 species of deep diving whales, and 9 species of delphinids)	Atlantic ocean (Irish coast)	Necropsy	Oesophagous, Stomach or intestine or entire gastro intestinal tract	Micro; Macro	Visual inspection and/or filtration of the content through nested sieves of different sizes (up to 200 μm) followed by 10% KOH digestion.	Lusher et al. (2018)
Ingestion	Sperm whale	Mediterranean Sea (Italian coast)	Necropsy	Stomachs	Micro; Macro	Visual inspection adopting the MSFD protocol and FT-IR spectroscopy	Present study
Ingestion (Indirect)	Fin whale, bottlenose dolphin, Risso's dolphin, striped dolphin	Mediterranean Sea (Ligurian Sea coastal and pelagic waters)	Skin biopsy	Blubber	N/A	Evaluation of Plastic tracers (PAEs) using GC-MS	Baini et al. (2017)
Ingestion (Indirect)	Fin whale, sperm whale	Mediterranean Sea (Ligurian Sea coastal and pelagic waters)	Skin biopsy	Dermal tissue (organotypic skin cell cultures)	N/A	Biomarkers of Exposure to PAEs using qRT-PCR	Fossi et al. (2018a)
Ingestion (Indirect)	Bottlenose dolphin	Mediterranean Sea (Italian coast)	Skin biopsy from stranded organisms	Dermal tissue (organotypic skin cell cultures)	N/A	Biomarkers of Exposure to PFOA and BPA using oligomicroarray	Lunardi et al. (2016)

(5) To define new methods to evaluate the exposure to plastics and plastic additives in free-ranging organisms;
(6) To evaluate the presence and effects of micro and nanoscale plastics, including sub-lethal effects; and
(7) To enhance awareness raising communicating to other scientists, young people and, other citizens, stakeholders and policy makers

All the information gathered through **the studies used as examples at the ECS 2017 workshop are valuable** in the implementation the European Marine Strategy Framework Directive (MSFD).

These studies can also contribute to answering the key question about the actual status of our oceans and to finding solutions for achieving the demanded "*Good Environmental Status*".

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Appendix A. Supplementary data

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