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Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*)

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ABSTRACT

The impact of microplastics (plastic fragments smaller than 5 mm) on large filter feeding marine organisms such as baleen whales and sharks are largely unknown. These species potentially are ingesting micro-litter by filter feeding activity. Here we present the case studies of the Mediterranean fin whale (*Balaenoptera physalus*) and basking shark (*Cetorhinus maximus*) exploring the toxicological effects of microplastics in these species measuring the levels of phthalates in both species. The results show higher concentration of MEHP in the muscle of basking shark in comparison to fin whale blubber. These species can be proposed as indicators of microplastics in the pelagic environment in the implementation of Descriptor 8 and 10 of the EU Marine Strategy Framework Directive (MSFD).

Keywords: microplastic, phthalates, organochlorines, basking shark, fin whale, Mediterranean Sea.

1. INTRODUCTION

Are the largest filter feeder marine organisms affected by any of the smallest human debris? Haw can 5 mm plastic debris affect 24 m long marine mammals and 7 m long sharks? In this paper we investigate the invisible war between the Mediterranean fin whale (*Balaenoptera physalus*) and basking shark (*Cetorhinus maximus*) against the smallest marine debris and their potential toxicological effects. Why microplastics may pose a threat to these species?

In 2009, 230 million tons of plastics were produced globally, Europe is the second larger producer of plastic (Plastics Europe, 2012). According to sea-based sources such as shipping, fishing and transport activities (Derraik, 2002) and land-based sources such as tourism, adjacent industries or river inputs (Browne et al., 2010), plastics are entering our seas and oceans, "posing a complex and multi-dimensional challenge with significant implications for the marine and coastal environment and human activities all over the world" (UNEP, 2009).

For the Mediterranean environment marine litter (including plastic) represents a serious concern (UNEP 2009, UNEP/MAP 2011, MSFD 2011). Three billions of litter items float or cover the sea bottom in the Mediterranean Sea, which 70-80% is plastic waste. The increasing of marine litter is mainly related to waste production in-land with an average amount of municipal solid waste in the EU of 520 kg per person/year and a projected increase to 680 kg per person/year by 2020.

The incidence of debris in the marine environment is cause for concern. It is known to be harmful to marine organisms and to human health (Derraik 2002; Gregory 2009; Wright et al., 2013), it represents a hazard to maritime transport, it is aesthetically detrimental, and may also have the potential to transport contaminants (Mato et al., 2001; Teuten et al., 2009). Marine debris, and in particular the accumulation of plastic debris, has been identified as a global problem alongside other key issues such as climate change, ocean acidification and loss of biodiversity. Impacts vary depending on the type and size of debris and the organisms affected.

The occurrence of microplastics (MPs - generally defined as fragments less than 5 mm in dimension - NOOA) in the ocean is an emerging world-wide concern. Due to high sorption capacity of plastics for hydrophobic organic chemicals, the adherent chemicals can be transported by MPs travelling long distances (Lee et al., 2013). MPs can serve as carrier of persistent organic pollutants (POPs) in marine ecosystems (Rochman et al., 2013; Koelmans et al., 2013). Small plastic particles in the environment are of particular concern as a wide range of organisms, from plankton to larger vertebrates such as turtles or whales, may ingest them (Wright et al, 2013). In particular, while evidence of macro and microplastic negative effects on marine organism is growing, little scientific investigation has focused on the problem in the Mediterranean. More information is required about plastic and microplastic inputs, spatial and temporal distributions, including transport dynamics, interactions with biota and potential accumulation areas.

Microplastics found in the marine environment are likely to be derived either directly or through the fragmentation of larger items. MPs can be subdivided by usage and origin as: i) Primary, pellets used in the plastics industry, and in certain applications such as abrasives; ii) Secondary, fragments resulting from the degradation and breakdown of larger items.

Microplastics floating over water are transported by ocean currents and are found in regions where water circulation is relatively stationary or on sea shores (Hidalgo-Ruz et al., 2012). A number of heavily produced low density plastics (e.g. polypropylene, polyethylene, and polystyrene) have been identified as the main components of MPs, and these have various shapes and sizes, ranging from a few micrometers to a few millimeters (Hidalgo-Ruz et al., 2012; Martins and Sobral, 2011).

Microplastics are accumulating at the sea surface, especially within the neustonic habitat (Ryan et al., 2009) that included a specifically adapted zooplankton fauna. Basking shark and particularly fin whale, being characterized by a long life span, could be chronically exposed to these persistent contaminants both leaching from microplastic ingestion and degradation and through the food chain.

Recent studies have identified potential effects of plastic particles mainly in invertebrates and fish, including: I) transport of persistent, bioaccumulating and toxic (PBT) substances from plastics; II) leaching of additives such as phthalates from the plastics; III) physical harm (Wright et al., 2013).

However, there is still little monitoring data on the occurrence of microplastics in large marine vertebrates. Until the paper of Fossi et al. (2012), no data were reported on the impacts of microplastics on large filter feeding marine organisms such as baleen whales or sharks. These species potentially undergo to the ingestion of micro-litter by filtrating feeding activity.

In this paper we focus on the case study of the two large Mediterranean filter feeders, the fin whale and basking shark.

The basking shark (*Cetorhinus maximus* Gunnerus, 1765) is a very large, filter-feeding cold-water and migratory pelagic species. It is widely distributed throughout temperate waters but only regularly seen in few favoured coastal locations. It may be considered frequently present in the Mediterranean, especially in the North-Western part, mainly in spring (Mancusi et al., 2005). Basking sharks are regular seasonal visitors in coastal waters of Sardinia, where between 2005-2012 a total of 111 individuals (including 14 captures) were recorded within "Operazione Squalo Elefante", the first dedicated basking shark research project in the Mediterranean basin (de Sabata and Clò, 2010). The basking shark is one of only three shark species that filter seawater for planktonic prey. It captures zooplankton by forward swimming with an open mouth, so that water passively flows across the gill-raker apparatus. The rates of gastro-intestinal evacuation in basking sharks are unknown; however, filtration rates have been estimated using measurements of swimming speed and mouth gape area. Seawater filtration rate for a 7m basking shark (mouth gape area ca. 0.4 m^2) swimming at a speed of 0.85 m s^{-1} was calculated to be 881 m^3 h^{-1}; if it fed constantly in food patches, a so 5–7 m long basking shark might consume 30.7kg of zooplankton in a day (Sims, 2008). During this massive filtering activities the basking shark could of the ingestion and degradation of microplastics.

Due to its slow growth rate, lengthy maturation time, long gestation period, probably low fecundity, probable small size of existing populations - some severely depleted by targeted fisheries - the basking shark is classified by the IUCN Red List of Threatened Species as "Endangered" in the North-East Atlantic Ocean and "Vulnerable" in the Mediterranean Sea (Fowler 2009; Cavanagh and Gibson, 2007). It is listed in all four major International conventions (Bern, CMS, CITES, Barcelona). Every year basking sharks are accidentally caught in small-scale fisheries throughout the Mediterranean region.

The fin whale (*Balaenoptera physalus,* Linnaeus 1758), one of the largest filter feeders in the world, feeds primarily on planktonic euphausiid species. This baleen whale, the only resident mysticete in the Mediterranean Sea, forms aggregations during the summer on the feeding grounds of the Pelagos Sanctuary Marine Protected Area (MPA). The fin whale is a wide ranging cetacean. It is found in largest water masses of the world, from the Equator to the polar regions, but, in spite of its cosmopolitan distribution, it is classified as "Endangered" by the IUCN Red List of Threatened Species. Fin whale feeding, in general, has been described as the largest biomechanical event that has ever existed on earth (Croll and Tershy, 2002). Fin whales capture food by initially swimming rapidly at a prey school and then decelerating while opening the mouth to gulp vast quantities of water and schooling prey. Fin and blue whales foraging on krill off the coast, concentrate their foraging effort on dense aggregations of krill (150–300 m) in the water column during the day, and near the surface at night (Croll et al., 2005).

With each mouthful, the fin whales can trap approximately 70,000 l of water. Since their feeding activities include surface feeding and, they undergo to the risk of the ingestion of MPs occurring in the sea surface and consequent degradation once ingested by the organism. Seawater filtration daily is 5893 m^3 rate with 913 kg of plankton consumed daily.

One major toxicological aspect of MPs in the marine environment and, consequentially, on filter-feeding organisms, is the influence that microplastics may have on enhancing the transport and bioavailability of PBT persistent, bioaccumulative, and toxic substances. These two large filter feeders species (fin whale and basking shark) could therefore face risks caused by the ingestion and degradation of microplastics.

PBT compounds, such as dichlorodiphenyltrichloroethane (DDT) or polychlorinated biphenyls (PCBs), are of particular concern for human health and the environment. Plastic debris can be a source of PBT chemicals. Some plastic debris can release toxic chemicals that have been added to enhance the performance of the plastic (such as phthalates, nonylphenol, bisphenol A, brominated flame retardants). Plastic debris may also be a sink for toxic chemicals: toxic chemicals from the environment can sorb to the debris and to be released once inside the organism (Engler 2012, Lithner et al., 2011). Since PBT chemicals, generally, have low solubility in marine water they tend to migrate into water microlayers where they tend to migrate to microdrebis or in the sediments also biomagnifing the concentration and effect in organisms that can ingest MP particles. Furthermore, plastic debris sorbs PCBs and DDE about one hundred times more than naturally suspended organic matter. 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 380–13 000-fold over seawater (Engler 2012).

Most of the chemicals absorbed or added to plastic that can potentially affect organisms (Teuten et al., 2007) have endocrine disruptors potency and affect population viability.

Phthalates are a class of chemicals commonly used to make rigid plastics softer to enhance the use of some plastic polymers. Phthalates generally do not persist in the environment, but may leach from plastic debris on a fairly steady basis. The dialkyl- or alkyl/aryl esters of 1,2-benzenedicarboxylic acid, commonly known as phthalates, are high-production volume synthetic chemicals. They are not covalently bound to plastic so can migrate from the products to the environment, thus becoming ubiquitous contaminants (Latini et al., 2004; Latini et al., 2009). Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment; DEHP, in organisms, 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.

Concerning the problem of marine litter in EU waters, the amount of marine litter in the Mediterranean environment and the effects on sentinel organisms need to be reduced to achieve the GES (Good Environmental Status) as planned by the European Marine Strategy Framework Directive (MSFD) by 2020. As an amendment to the MSFD the "composition of micro-particles (in particular microplastics) has to be characterized in marine litter in the marine and coastal environment" (European Parliament and the Council, 2010). Currently, there is a severe gap in establishing the presence and effects of MPs on Mediterranean marine , which must be done with sentinel species to determine effects and implement future mitigation actions.

Here we present the case studies of the two Mediterranean larger filter feeders, the fin whale (*Balaenoptera physalus*) and basking shark (*Cetorhinus maximus*), exploring the toxicological effects of MPs in these species and suggesting the possible implication as indicators of MPs in the pelagic environment in the implementation of the European MSFD. We also suggest the possible implication of considering these species as indicators of MPs in the pelagic environment in the implementation of the European MSFD. We also suggest the use of phthalates and organochlorines in plankton, shark and whale, as a tracer of microplastics assumption by ingestion in these species.

2. MATERIAL AND METHODS

This work is implemented through three main steps: 1) detection of phthalates in *Euphausia krohni*; 2) detection of phthalates and organochlorine compounds (OCs) in accidentally caught basking shark in Italian waters; 3) detection of phthalate and OCs content in stranded fin whale specimens collected on the Italian coasts. Details on gender, size, date and location of the stranded animals are reported in Fig.2.

2.1 Specimens and sampling sites

Ten pools of 30-40 specimens of the Euphausiidae *Euphausia kronhi* were sampled during two expeditions in collaboration with National Council of Research (CNR) with the Oceanoghraphic ship "Urania" in the Channel of Sicily, South Mediterranean Sea.

Muscle samples were collected from accidentally caught specimen of basking shark in Italian waters within "Operazione Squalo Elefante", during the period 2007–2013: four in the Pelagos Sanctuary (3 in Sardinia, 1 in the Ligurian Sea) and one off the southern border (Latium), one in Mola di Bari (Puglia) (Fig.1). Blubber and muscle of five stranded fin whales were collected along the Italian coasts during the period 2006 –2013 in five different locations. Details on gender and location of the stranded whales are reported in Fig.1.

2.2 Detection of phthalate content in euphasiids, stranded fin whale and basking shark specimens

DEHP and MEHP were extracted from *E. krohni* samples (30-40 individuals), plus blubber samples (1 g) and muscle (1 g) of five stranded fin whales and in the muscle (0.5 g) of six accidentally caught basking sharks using the described method. DEHP (di-(2-ethylhexyl) phthalate) and MEHP (mono-(2-ethylhexyl) phthalate) were analyzed from the subsamples following a method described by Takatori and colleagues (2004) with few modifications. Each sample was thawed, weighted and transferred into a 15 ml tube. To this were added 4 ml of acetone. The sample thus obtained was sonicated for 2 minutes and stirred for 5 minutes and then centrifuged for 15 minutes at 3000 rpm to separate the organic part, containing DEHP and MEHP, from the remainder water. Then, 4 ml of supernatant were placed in a further 15 ml tube. Infranatant was again added to 1 ml of acetone, and was sonicated for 2 min, agitated for 5 minutes and centrifuged for 15 minutes at 3000 rpm for a further separation of the organic from aqueous medium. Then the supernatant phase was recovered and rebuilt with that resulting from the first extraction. The supernatants, mixed well, were then evaporated in a centrifugal evaporator. The extract was then resuspended with 0.5 mL of acetonitrile and passed through a nylon filter. Subsequently, the final volume was adjusted to 0.5 ml, which were placed in an autosampler vial and injected into a LC-ESI-MS system. The instrumental analysis was performed using a Finnigan LTQ Thermo LC/MSn 110 with ESI interface. 5 µl of extracted sample were injected via autosampler in the HPLC system. A reverse phase HPLC column (Wakosil3C18, 2.0×100 mm; Wako Pure Chemical Industries Ltd.) was used. The mobile phases consisted of 100% acetonitrile (A) and 0.05% aqueous acetic acid (B). Elution was performed using an isocratic mode (A/B: 15/85, v/v) at 0.25 ml/min. The chromatographic run for each sample had duration of 30 minutes. ESI-MS was operated in negative or positive ion mode depending on the analytes (MEHP was detected in negative mode, while DEHP in the positive mode). The heated capillary and voltage were maintained at 500 °C and ±4.0 kV, respectively. The daughter ions were formed in the collision cell using N2 gas as the collision gas. The ions used for identification were (parent ion/daughter ion) 277/134 120 and 391/149 for MEHP and DEHP respectively. For the quantitative analysis four-point calibration curve, prepared by progressive dilution of a solution of the two analytes of interest was used. Blanks were analyzed with each set of five samples as a check for possible laboratory contamination and interferences. Data quality assurance and quality control protocols included also matrix spikes, and continuing calibration verification. The limits of detection (LODs) and limits of quantification (LOQs) for the compounds analyzed are the value of the compound in the blanks +3 SD and +10 SD, respectively; LOD and LOQ were 1 and 2 ng/g respectively for MEHP and 5 and 10 ng/g respectively for DEHP. The analytes levels below the limits of detection (<LOD) were considered with a value equal to the value of the LOD, while, in the cases in which the analyte was present at levels between the LOD and the LOQ, the LOQ value was used.

2.3 Detection of OC concentrations in stranded fin whale and basking shark specimens

Analysis for HCB, DDTs and PCBs were performed according to method of U.S. Environmental Protection Agency (EPA) 8081/8082 with modifications (Marsili and Focardi, 1997). The samples of 1g of blubbler (*B. physalus*) and 1g muscle (*C. maximus*) were lyophilized in an Edwards freeze drier for 2 days. The sample was extracted with n-hexane in a Whatman cellulose thimble (i.d.25 mm, e.d. 27 mm, length 100 mm) in the Soxhlet apparatus for 9 h. The sample was spiked with surrogate compound (2,4,6-trichlorobiphenyls - IUPAC number 30, Ballschmiter and Zell, 1980) prior to extraction. This compound was quantified and its recovery calculated. After the extraction, the sample was purified with sulphuric acid to obtain a first lipid sedimentation. The extract then underwent liquid chromatography on a column containing Florisil that had been dried for 1 h in an oven at 110°C. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. Decachlorobiphenyl (DCBP - IUPAC number 209) was used as an internal standard, added to each sample extract prior to analysis, and included in the calibration standard, a mixture of specific compounds (Aroclor 1260, HCB and pp'- and op'-DDT, DDD and DDE). The analytical method used was High Resolution Capillary Gas Chromatography with a Agilent 6890N and a 63Ni ECD and an SBP-5 bonded phase capillary column (30 m long, 0.2 mm i.d.). The carrier gas was N2 with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40 ml/min. Oven temperature was 100°C for the first 10 min, after which it was increased to 280°C at 5C°/min. Injector and detector temperatures were 200°C and 280°C respectively. The extracted organic material (EOM%) from freeze-dried samples was calculated in all samples. Capillary gas-chromatography revealed op'- and pp'- isomers of DDT and its derivatives DDD and DDE, and 30 PCB congeners. Total PCBs were quantified as the sum of all congeners (IUPAC no. 95, 101, 99,151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206). Total DDTs were calculated as the sum of op'DDT, pp'DDT, op'DDD, pp'DDD, op'DDE and pp'DDE. The results were expressed in ng/g lipid basis (l.b.). The detection limit was 0.1 ng/kg (ppt) for all the OCs analysed.

3. RESULTS AND DISCUSSION

In this study, six muscle samples of accidentally caught basking shark in Italian waters and, plus blubber and muscle samples from in five stranded fin whales (sub-adults and adults) between 2007–2012 in five different sites on the Italian coast were analyzed. All these samples were analyzed for phthalates and organochlorines (expressed in l.b.) used as potential tracers of assumption of microplastics during the filtering activities for feeding. Additionally, the crustacean *E. krohni* was analyzed as one of the major prey of the fin whale and as component of the zooplankton.

The DEHP primary metabolite, MEHP, was analyzed in the stranded specimens and *E. kronhi*. The analysis showed appreciable levels of MEHP in all of the samples, while DEHP was detected in only one sample (data not shown)(Tab.1).

Table 1.Organochlorine and MEHP concentrations (ng/g l.b.) in the blubber of Mediterranean *B. physalus* (BP) and muscle of *C. maximus* (CM).

Sample ID	Species	HCB (ng/g l.b.)	∑DDTs (ng/g l.b.)	∑PCBs (ng/g l.b.)	MEHP (ng/g l.b.)
BP 1	*B. physalus*	129.13	6580.67	9117.09	377.82*
BP 2	*B. physalus*	286.26	12284.32	8155.00	110.68*
BP 3	*B. physalus*	157.82	21404.45	42778.45	332.31*
BP 4	*B. physalus*	180.93	26833.64	24060.13	61.06*
BP 5	*B. physalus*	201.02	15357.34	16410.87	1.48*
CM 1	*C. maximus*	41.09	1890.42	1575.57	58.06
CM 2	*C. maximus*	9.52	2638.73	1710.69	113.94
CM 3	*C. maximus*	41.02	2177.60	1820.77	50.39
CM 4	*C. maximus*	10.74	1647.66	1970.62	156.67
CM 5	*C. maximus*	-	-	-	114.37
CM 6	*C. maximus*	21.11	1652.64	1820.73	11.17

(*) From Fossi et al. 2012.

Interestingly, concentrations of MEPH are twice as high in the cetacean species compared with the cartilaginous fish (Fig. 2a).

The same trend is shown for the organochlorine concentrations, where for the three classes of OCs investigated (HCB, DDTs and PCBs) were always markedly higher in fin whale specimens compared to basking sharks (Fig. 2b).

Moreover as previously published by Fossi et al. (2012), the presence of harmful chemicals in Mediterranean fin whales, that were hypothesized to be linked with intake of plastic derivatives by water filtering and plankton ingestion, are confirmed by the results of this study, which documents relevant concentrations of MEHP in the blubber of five out of six stranded fin whales. MEHP is a marker for exposure to DEHP, whereas DEHP was never detected in the fin whale samples.

The concentrations of total OCs in the muscle of the three whale specimens are always markedly higher (DDTs mean value: 15956 ng/g l.b; PCBs mean value: 16692 ng/g l.b.) than those found in the muscle of the basking shark (DDTs mean value: 2001 ng/g l.b; PCBs mean value: 1779 ng/g l.b.). The difference between the two species in the bioaccumulation of fat-soluble contaminants can be linked to a different ability of excretion related to the potential excretory activity through the gills in fish (Barber, 2008) and bioaccumulation in adipose tissue especially in cetaceans.

The PCBs fingerprint of the two target species was compared with neustonic/planktonic and microplastic samples (NP-MPs) collected in the Pelagos Sanctuary (Fig. 3). Among the 30 PCB congeners analyzed, the highest percentage (43%) is represented by the PCB 195 in the NP-MPs samples that is the second most abundant congener in basking shark, while it was detected in very low percentage in fin whale. This preliminary evidence suggests the use of this PCB congener as tracer of the absorption of POPs through NP-MPs in surface feeding organisms. Moreover, the most abundant congeners in fin whale and basking shark is the PCB 153, a congener also abundant in NP-MPs samples (Fig. 3)

4. CONCLUSIONS

The initial insight into microplastic pollution on Mediterranean scale on the concentration levels and spatial distribution of microplastics in the area MPA of Pelagos Sanctuary underline that the mean abundance of microplastics estimated are of the same order of magnitude as that found for the North Pacific Gyre (Moore et al., 2001). This suggests the high occurrence of this emerging threat in the only pelagic MPA of the Mediterranean Sea (Collignon et al., 2012; Fossi et al., 2012). High presence of plastic particles have been detected in superficial neustonic/planktonic from the Pelagos Sanctuary areas investigated (mean value 0.62 items/m^3), with levels approximately seven time higher in the samples from the Ligurian Sea (mean value 0.94 items/m^3), than the samples compared to the Sardinian Sea (mean value 0.13 items/m^3). High concentration of phthalate MEHP and DEHP have been detected, in superficial NP-MPs samples collected in the Pelagos Sanctuary areas (MEHP 53.47 ng/g f.w., DEHP 20.36 ng/g f.w) (Fossi et al., 2012). Moreover, the levels of OCs and microplastic abundance in Mediterranean sea were recently detected in superficial neustonic/planktonic samples collected in Sardinian sea with PCBs ranging from 1889.6 ng/g d.w. to 3793.1 ng/g d.w. and DDTs from 185.0 ng/g d.w to 2130.1 ng/g d.w. (de Lucia et al., *this issue*).

Until now few studies have addressed the impact of microplastics on filter-feeding organisms or other planktivorous animals (Boerger et al., 2010; Cole et al., 2013; Lusher et al., 2013; Murray and Cowie, 2011; von Moos et al., 2012). A previous study by of Fossi and collaborators (2012) has reported on the potential impact on large filter-feeding organism such as baleen whales.

In the present paper, we explore the potential routes of exposure and or absorption of MPs in the Mediterranean fin whale and basking shark in relation to their different filter feeding activities (Tab. 2).

Tab.2. Comparison between total volume filter daily, total plankton consume daily and theoretical number of MP items assumed by *B. physalus* and *C. maximus.*

	Balaenoptera physalus	***Cetorhinus maximus***
Average adult body length	20 m	7 m
Average adult body mass	50,000 kg	4,000 kg
Engulfment volume	71 m^3	-
Filtration rate	-	881 m^3 h^{-1}
Number of lunges day^{-1}	83	-
Total volume filtered daily	5893 m^3	21,144 m^3
Total plankton consumed daily	913 kg	30.7 kg
Theoretical number of MPs items assume daily	3653	13,110

Basking sharks can sieve small organisms and microdebris from the water. Swimming with mouth open, masses of water fill the basking shark mouth with prey flow. After closing its mouth, the shark uses gill rakers that filter the nourishment from the water. Gill rakers have thousands of bristles in the shark's mouth that trap the small organisms and microdebris which the shark then swallows. The water is expelled through the shark's pairs of gill slits. The seawater filtration rate for a 7m basking shark (mouth gape area ca. 0.4 m^2) swimming at a speed of 0.85 m s^{-1} was calculated to be 881 m^3 h^{-1}; we can hypothesize that in the Pelagos Sanctuary areas (mean MPs value 0.62 items/m^3), this species could consume approximately 540 MPs items h^{-1}, for a total daily consumption of approximately 13,110 microdebris items, plus any related adherent or incorporated toxic chemicals such as OCs, PAHs and phthalates (Tab 2).

Fin whales exhibit one of the most extreme feeding methods among aquatic vertebrates. Fin whales, and other *Balaenopteridae*, lunge with their mouth fully agape, thereby generating dynamic pressure to stretch their mouth around a large volume of prey-laden water, which is then filtered by racks of baleen (Goldbogen et al., 2007). *Balaenopteridae* are intermittent filter feeders that ingest mouthfouls of water and separate food from the water before expelling it and, subsequently, swallowing the prey captured. The filtering apparatus of baleen whales can be compared to a net or a sieve, depending on the prey, microdebris and water condition through baleen fringes (Werth, 2001). Considering the seawater filtration rate approximately of 5893 m^3 daily we can hypothesize that fin whale surface feeding in the Pelagos Sanctuary areas (mean MPs value 0.62 items/m^3) could consume, a total daily amount of approximately 3653 items and the relative sink toxic chemicals (Tab 2). Experiment carried out on porosity of baleens in whales using polymer microsphere (mean particle size 710 µm) pointed out that suspended particles did not remain on baleen fringes and prey and items fall onto the tongue upon water expulsion (Werth, 2013). This mechanism suggest that microdebris can be ingested by the whale together with the prey. Considering this theoretical calculation, the basking shark can ingest daily approximately a total intake of 3,6 time more MP items than the fin whale. Although, this higher intake of MPs is however coupled to values of phthalates two time lower and of OCs three times lower than those found in fin whale. The marked difference between the two species in the bioaccumulation of phthalates and organochlorines can be linked both to a different ability of excretion of contaminants related to the presence of a high excretory activity through the gills in the basking shark but also to the massive ingestion of euphausiid species by fin whale (total plankton consume daily 913 kg) that show high concentrations of plastic additives (Fossi et al., 2012). It is well known that the fin whale in the Mediterranean Sea feeds preferentially on the planktonic euphausid *Meganyctiphanes norvegica,* even if it feeds on a wide spectrum of marine organisms, ranging from copepods to other euphausiid species, to small schooling fish (like *Thysanoessa inermis*, *Calanus finmarchicus, Euphausia krohni*) (Notarbartolo di Sciara et al., 2003; Relini et al., 1992). Preliminary data on MEHP concentration in samples of *Euphausia krohni* collected in Sicilian Channel show high concentration of this contaminant, ranging from 8.35 to 51.14 ng/g (mean values 36.92 ng/g) and suggesting the presence of plastic additives also in planktonic species living in the water column. Evidences of ingestion and impact of MPs by invertebrates, in particular zooplankton, have been reported (Cole et al., 2013; Murray and Cowie, 2011). Beside the physical harm and toxicological risk for invertebrates and zooplanktonic species themselves caused by MPs and through feeding activity, the trophic transfer across the food chain represent a serious concern, especially for planktivourous species such as baleen whales and basking sharks.

Considering both the high presence of MPs in the Mediterranean environment, and particularly in the MPA of Pelagos Sanctuary, and the detection of plastic additives and OCs in the tissues of basking sharks and fin whale, large filter feeding marine organisms appear to be chronically exposed to persistent and emerging contaminants related to prey and MPs ingestion. Rochman et al. (2014) underline that several classes of compounds can be carried and released by MPs since organisms living in high density MPs environment show higher plastic-derived chemical pollutants accumulation in their tissue. The dual sources of contamination could derive from direct leaching of contaminants (sorbed on or additive) from microplastics and assumption through already contaminated plankton prey.

In this context, the data in this paper suggest the use of phthalates as a tracer of microplastic ingestion by fin whale and basking sharks. The tracer can serve as a warning signal of exposure to endocrine disruptors such as MEHP in the endangered Mediterranean population of this baleen whale and cartilaginous fish.

Particular attention has also been given to this new field of research during the recent workshop organized by IWC and Woods Hole Oceanographic Institution in May 2013 in Woods Hole (MA, USA) on Assessing the Impacts of Marine Debris on Cetaceans. The workshop recommended that baleen whales and other large filter feeders should be considered as critical indicators of the presence and impact of microplastics in the marine environment, in national and international marine debris strategies. The workshop encouraged also further non-lethal research and the biomarker development on these endangered Mediterranean species (IWC, 2013).

The present study represents the first evidence of plastic additives (phthalates) in Mediterranean basking sharks and it underlines the importance of future research both on detecting the presence of and looking for toxicological impacts of microplastics in filter-feeders species such as cetaceans mysticete, basking shark and devil ray. Due to the wide home-range and high-mobility of these species, which move in the whole basin all year round, they could represent a wide scale integrator of the ecotoxicological status of the entire Mediterranean basin. Moreover, occupying these species the lowest position of the food web can be considered as an early warning of the presence of a mixture of contaminants in the marine food chain.

We highlight the value of these species in the implementation of the Descriptor 8 (contaminants) and Descriptor 10 (marine litter) in the European MSFD, as sentinels of the plastic-related contamination and presence and impact of micro-litter in the pelagic environment.

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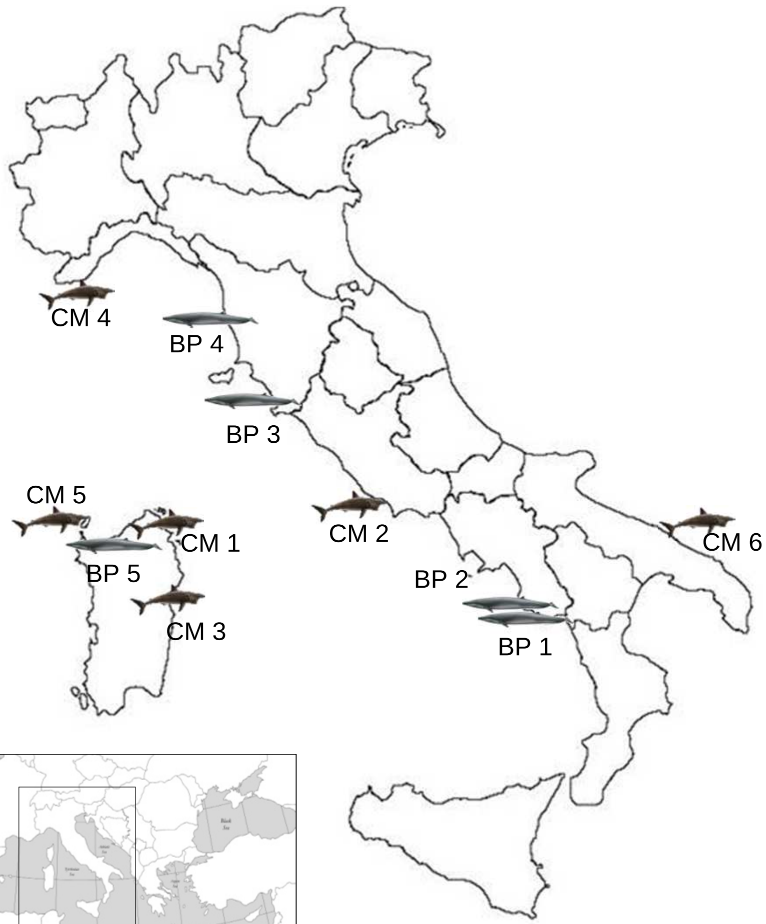
Captions

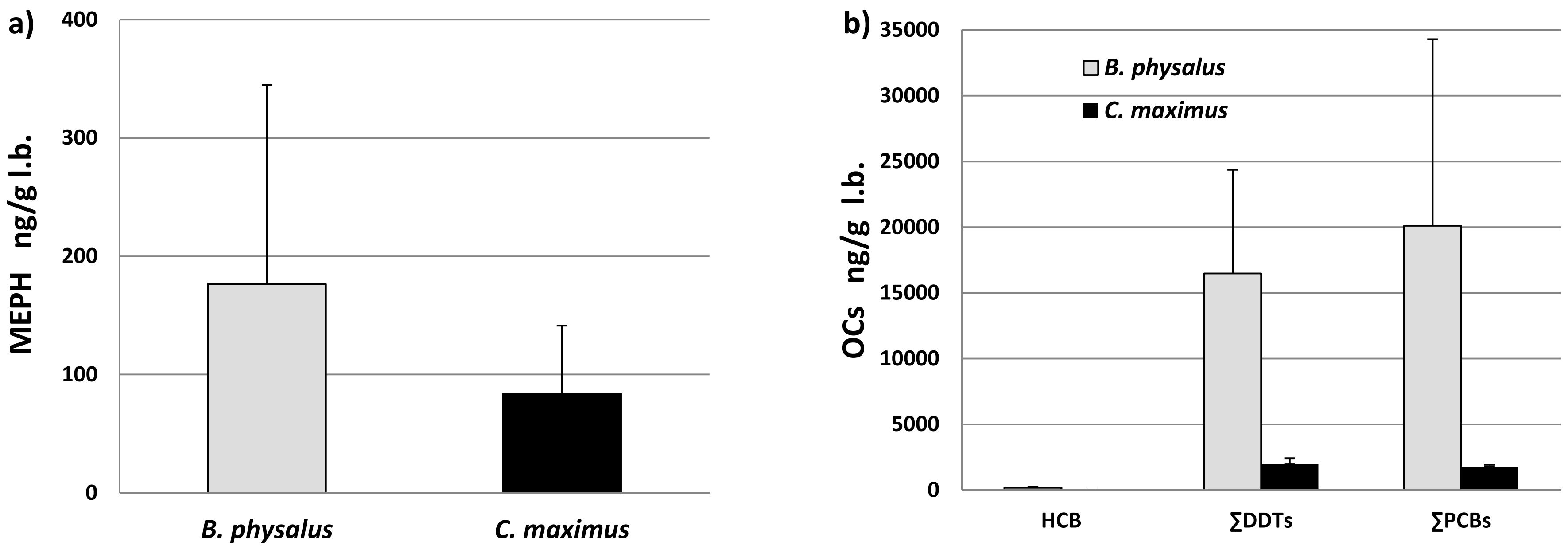
Figure 1. Details on gender, size, date and location of the stranded specimens of *Balaenoptera physalus* and *Cetorhinus maximus.*

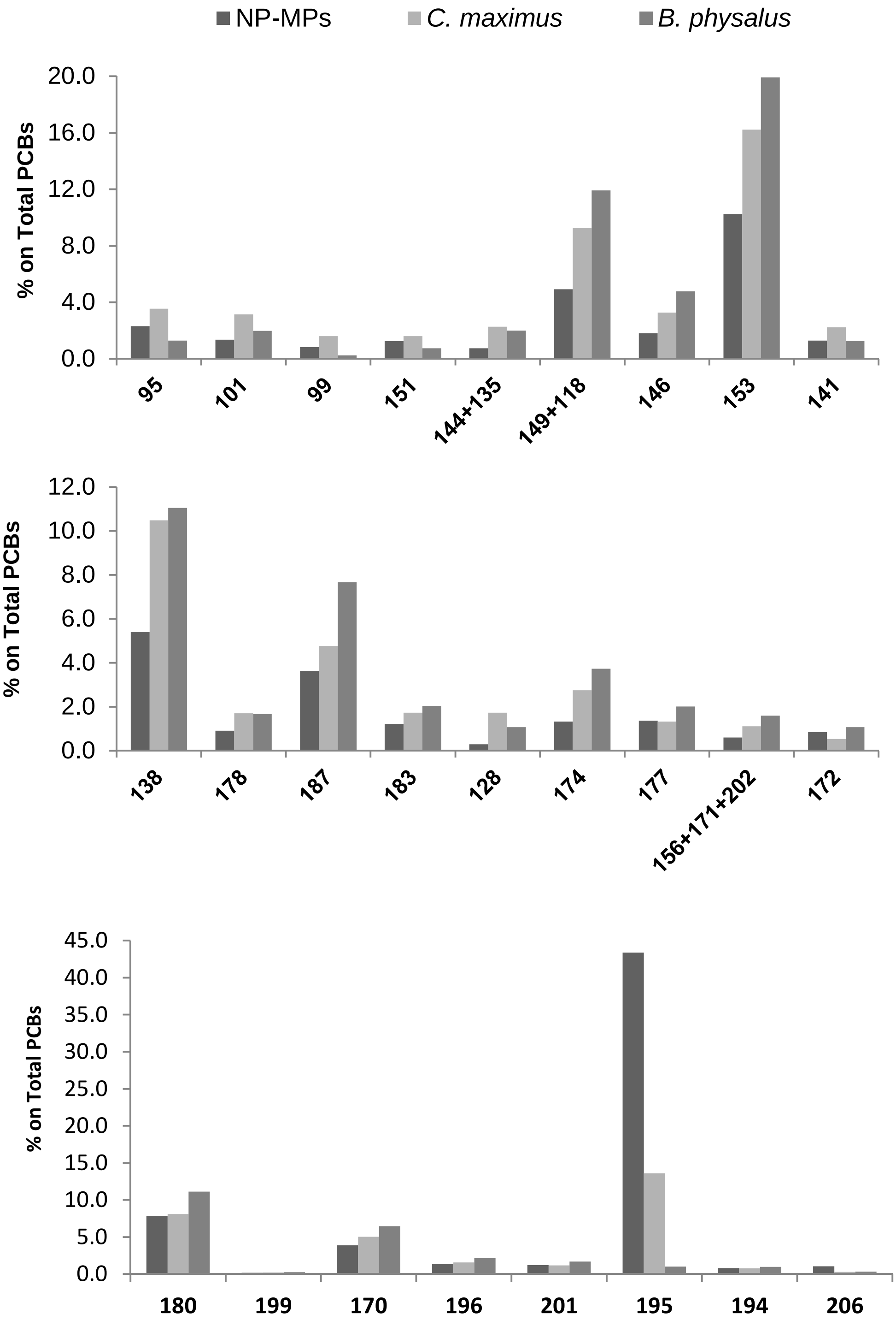
Figure 2. a) Phthalate (MEHP) and b) Organochlorines concentration (ng/g l.b.) in blubber of Mediterranean *B. physalus* and muscle of *C. maximus*. Bars show mean value ± standard deviation.

Figure 3. PCBs fingerprint (30 congeners): bars show the percentage of each congeners calculated on the total concentration of all the congeners analyzed in neustonic/planktonic and microplastic samples (NP-MPs), *C. maximus* and *B. physalus*. Each graph (a-c) show congeners in the same order as they are revealed by the instrument.

Sample ID	Area	Date	Lenght (m)	Sex
		Balaenoptera physalus		
BP 1	Palinuro (Campania)	2007	13.00	F
BP 2	Amalfi (Campania)	2007	13.50	F
BP 3	Giannella (Tuscany)	2008	10.40	M
BP 4	S. Rossore (Tuscany)	2011	16,70	M
BP 5	Castelsardo (Sardinia)	2011	8.00	-
		Cetorhinus maximus		
CM 1	Capo Figari (Sardinia)	2006	4.50	F
CM 2	Anzio (Latium)	2007	3.50	M
CM 3	Cala Gonone (Sardinia)	2010	8.00	F
CM 4	Ospedaletti (Liguria)	2010	3.90	M
CM 5	Porto Torres (Sardinia)	2006	-	-
CM 6	Mola di Bari (Puglia)	2013	7.00	M




a)
400
300
200
100
0
MEPH ng/g l.b.
B. physalus
C. maximus
b)
35000
30000
25000
20000
15000
10000
5000
0
OCs ng/g l.b.
B. physalus
C. maximus
HCB
∑DDTs
∑PCBs


NP-MPs
C. maximus
B. physalus
% on Total PCBs
20.0
16.0
12.0
8.0
4.0
0.0
95
101
99
151
144+135
149+118
146
153
141
% on Total PCBs
12.0
10.0
8.0
6.0
4.0
2.0
0.0
138
178
187
183
128
174
177
156+171+202
172
% on Total PCBs
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
180
199
170
196
201
195
194
206

Highlights

- Large filter feeding marine vertebrates can assume microplastics during feeding
- Micro-debris can be carrier of plastic additives and persistent organic pollutants
- Planktivorous species can accumulate high levels of contaminants released by microdebris
- Phthalates can be used as tracer of plastic ingestion in whales and basking sharks
- Basking shark and fin whale as sentinel species for descriptors 8 and 10 EU MSFD