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Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*)

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ABSTRACT

Baleen whales are potentially exposed to micro-litter ingestion as a result of their filter-feeding activity. However, the impacts of microplastics on baleen whales are largely unknown. In this case study of the Mediterranean fin whale (*Balaenoptera physalus*), we explore the toxicological effects of microplastics on mysticetes. The study included the following three steps: (1) the collection/count of microplastics in the Pelagos Sanctuary (Mediterranean Sea), (2) the detection of phthalates in surface neustonic/planktonic samples, and (3) the detection of phthalates in stranded fin whales. A total of 56% of the surface neustonic/planktonic samples contained microplastic particles. The highest abundance of microplastics (9.63 items/m^3) was found in the Portofino MPA (Ligurian Sea). High concentrations of phthalates (DEHP and MEHP) were detected in the neustonic/planktonic samples. The concentrations of MEHP found in the blubber of stranded fin whales suggested that phthalates could serve as a tracer of the intake of microplastics. The results of this study represent the first warning of this emerging threat to baleen whales.

1. Introduction

The emerging issue of microplastics (plastic fragments smaller than 5 mm) in the marine environment has recently received increasing attention (Hidalgo-Ruz et al., 2012). This ubiquitous, persistent form of micro-debris requires centuries to degrade completely. Microplastics are primarily the result of the degradation of plastics released into the environment since the beginning of the plastic age. Micro-debris floating in the Mediterranean Sea has reached maximum levels of 892,000 particles/km^2. Recently, Collignon et al. (2012) determined neustonic microplastic and zooplankton abundance in the northwestern Mediterranean Sea and showed that the estimated mean abundance of microplastics was of the same order of magnitude as that found for the North Pacific Gyre (0.334 particles/m^2, Moore et al., 2001), underscoring the high level of this emerging threat in the Mediterranean environment.

Microplastics accumulate at the sea surface, especially within the neustonic habitat (Ryan et al., 2009). This habit harbors a specifically adapted zooplankton fauna. There is increasing concern that a wide range of marine organisms are affected by plastic wastes in the sea. However, the mechanical, physical and toxicological impacts of these wastes are largely unknown. More than 180 species, including planktophagous species, have been shown to absorb plastic debris. Macrodebris ingestion and entanglement are well documented in sea birds, mammals and turtles and more recently in fishes (planktivorous and benthophagous) and invertebrates (Robards et al., 1995; Derraik, 2002; Thompson et al., 2004; Ryan et al., 2009; Boerger et al., 2010; Collignon et al., 2012; Possatto et al., 2011; Dantas et al., 2012; Murray and Cowie, 2011).

No information has previously been reported on the impacts of microplastics on baleen whales, such as fin whales (*Balaenoptera physalus*). The filter-feeding activities of these whales represent a potential source of exposure to micro-litter ingestion. The fin 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) (Notarbartolo di Sciara et al., 2003). These whales feed primarily on planktonic euphausiid species. With each mouthful, the whales can trap approximately 70,000 l of water, and their feeding activities include surface feeding. They could therefore face risks caused by the ingestion and degradation of microplastics. Micro-debris can be a significant source of lipophilic chemicals (primarily persistent organic pollutants – POPs) and a source of pollutants such as polyethylene, polypropylene and, particularly, phthalates. These chemical pollutants can potentially affect organisms (Teuten et al., 2007), are potential endocrine disruptors and can affect population viability. With their long lifespan, whales could be chronically

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exposed to these persistent contaminants derived from the ingestion and degradation of microplastics.

One toxicological feature of the marine environment that can affect filter-feeding organisms is the influence that microplastics may produce by enhancing the transport and bioavailability of persistent, bioaccumulative and toxic substances. In fact, chemicals for which the logarithm of the octanol/water partitioning coefficient (K(OW)) > 5 can potentially be partitioned >1% to polyethylene, a major component of microplastics. Moreover, contaminants such as phthalates and polycyclic aromatic hydrocarbons (PAHs) are among the principal constituents of plastics. The dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid, commonly known as phthalates, are high-production-volume synthetic chemicals; moreover, they are not covalently bound to plastic and migrate from the products to the environment, thus becoming ubiquitous contaminants (Latini et al., 2009). Public and scientific concern about the potential human and wildlife health risks associated with exposure to phthalates has increased in recent years. The primary focus has moved away from the hepatotoxic effects to the endocrine-disrupting potency of these chemicals (Latini, 2005), which have been shown to be reproductive toxicants in animals (Borch et al., 2006). Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment. In both invertebrates and vertebrates, DEHP is rapidly metabolized in the form of its primary metabolite, MEHP (mono-(2-ethylhexyl) phthalate) (Barron et al., 1989), which can be used as a marker of exposure to DEHP.

This case study examines the Mediterranean fin whale, one of the largest filter feeders in the world. This study is the first investigation of the potential impact of microplastics in a baleen whale and suggests the use of phthalates as a tracer of the intake of microplastics through the ingestion of micro-debris and plankton.

2. Methodology

The study included the following three steps: (1) the collection, counting and sorting of microplastics and planktonic organisms in surface neustonic/planktonic and water column samples from the Pelagos Sanctuary MPA (NW Mediterranean Sea); (2) the measurement of phthalate concentrations in surface neustonic/planktonic and water column samples; and (3) the measurement of phthalate concentrations in stranded fin whale specimens collected on the coasts of Italy.

2.1. Step I: collection and sorting of microplastics in surface neustonic/planktonic and water column samples in the Pelagos Sanctuary

Surface neustonic/planktonic and water column samples were collected in the Ligurian Sea and Sardinian Sea (Fig. 1a) in summer 2011 (June–July) during the day with a WP2 standard net (57 cm mouth diameter, 200 μm mesh size) equipped with a flowmeter for the measurement of the filtered volumes. For each surface sample (n = 23; MPM3–MPM26), the net was towed horizontally just below the water surface at a speed of approximately 1 knot for 15 min. For each water column sample (MPP3, MPP10 and MPP22, corresponding to the same geographical coordinates as MPM3, MPM10 and MPM22) (Fig. 1a), the same net was vertically towed from a depth of 50 m to the surface at a speed of 1 m/s. In both cases, the net was washed on board, and each 2-l sample was split into two separate aliquots of 1 l each with a Folsom splitter. One 1-l aliquot was filtered on a 200 μm mesh sieve and immediately frozen in liquid nitrogen for the subsequent analysis of phthalates. The second aliquot was preserved in 4% formaldehyde-seawater buffered solution for subsequent quali-quantitative analyses. A total of 26 frozen and preserved samples were used for this study. For the analysis of plankton and plastic particles, the samples were observed under a Leica Wild M10 stereomicroscope. The organisms were counted and taxonomically classified (Table 1, Supplementary data). The plastic particles were counted and measured, and those smaller than 5 mm were classified as microplastics. All the data were normalized to the total volume filtered and expressed as individuals and items/m^3. To compare the data with data expressed as items/m^2 in the literature, the present data can be converted by multiplying the values (items/m^3) by 0.5 m, the thickness of the water stratum sampled with the WP2 net as described above.

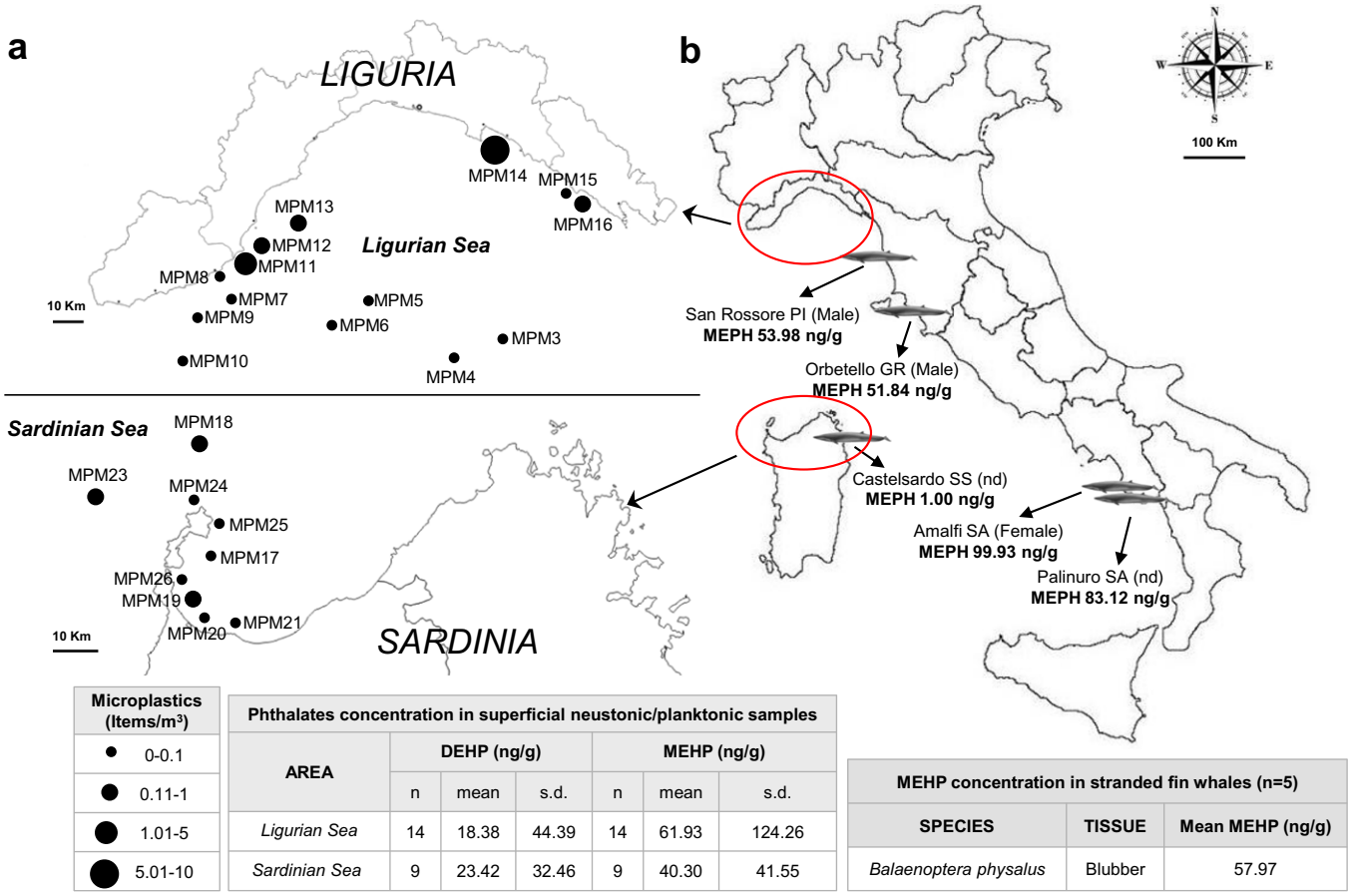
2.2. Step II: detection of phthalates in surface neustonic/planktonic and water column samples

DEHP and MEHP were analyzed in the surface neustonic/planktonic and water column samples (0.5–0.7 g) from the two sampling sub-areas (Ligurian Sea and Sardinian Sea) following a method described by Takatori et al. (2004), with a few modifications described in Guerranti et al. (2012). Each sample was thawed and weighed, and acetone was added. The sample obtained in this way was sonicated. The organic part, containing DEHP and MEHP, was separated from the remaining water, and the supernatant was isolated. The supernatant phase was then recovered and combined with that resulting from the first extraction and was then evaporated in a centrifugal evaporator. The extract was then resuspended with 0.5 ml of acetonitrile and passed through a nylon filter with pores of 2 μm. Subsequently, the sample was placed in an autosampler vial and injected into an LC-ESI-MS system. The instrumental analysis was performed with a Finnigan LTQ Thermo LC/MSn 110 with an ESI interface. A total of 5 μl of the extracted sample was injected via the autosampler into the HPLC system. A reverse-phase HPLC column (Wakosil 3C18, 2.0 × 100 mm, 3 μm; 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. ESI-MS was operated in the negative or positive ion mode depending on the analytes (MEHP was detected in the negative mode, whereas DEHP was detected in the positive mode). The heated capillary and voltage were maintained at 500 °C and ±4.0 kV, respectively. The ions used for identification were (parent ion/daughter ion) 277/134 and 391/149 for MEHP and DEHP, respectively. For the quantitative analysis, a four-point calibration curve prepared by the 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 interference. The data quality assurance and quality control protocols also included matrix spikes and continuing calibration verification. The limits of detection (LODs) and limits of quantification (LOQs) for the compounds analyzed were the values of the compound in the +3 SD and +10 SD blanks, respectively. The LOD and LOQ were 1 and 2 ng/g, respectively, for MEHP and 5 and 10 ng/g, respectively, for DEHP.

The levels of analytes below the limits of detection (<LOD) were specified as values equal to the value of the LOD. If the analyte was present at levels between the LOD and the LOQ, the LOQ value was used. The values are expressed as fresh weight (f.w.).

2.3. Step III: measurement of phthalate concentrations in stranded fin whale specimens collected along the coasts of Italy

Blubber samples were collected close to the dorsal fin in five stranded fin whales (sub-adults and adults) during the period July 2007–June 2011 at five different sites on the Italian coast. The samples were stored at −20 °C prior to analysis. The details of the location and gender of the stranded whales are shown in Fig. 1b. DEHP



Microplastics (Items/m³)
0-0.1
0.11-1
1.01-5
5.01-10

Phthalates concentration in superficial neustonic/planktonic samples						
AREA	DEHP (ng/g)			MEHP (ng/g)		
	n	mean	s.d.	n	mean	s.d.
Ligurian Sea	14	18.38	44.39	14	61.93	124.26
Sardinian Sea	9	23.42	32.46	9	40.30	41.55

MEHP concentration in stranded fin whales (n=5)		
SPECIES	TISSUE	Mean MEHP (ng/g)
Balaenoptera physalus	Blubber	57.97

Fig. 1. (a) Microplastic particles in superficial neustonic/planktonic samples (items/m^3) collected in the Pelagos Sanctuary (Ligurian Sea and Sardinian Sea) and mean DEPH and MEPH concentrations (ng/g). Geographical coordinates of sampling sites are reported in Table 2 of Supplementary data. (b) DEHP concentrations (ng/g) in blubber samples of five stranded fin whales collected along the Italian coasts during the period July 2007–June 2011 in five different locations.

Table 1
Microplastic particles in superficial neustonic/planktonic samples (items/m^3) collected in the Pelagos Sanctuary, zooplankton abundance (ind/m^3), DEPH and MEPH concentrations (ng/g f.w.), mean values ± S.D. (see Fig. 1 for sampling sites).

Sample	Items/m^3	Zooplankton abundance (ind/m^3)	DEHP (ng/g)	MEHP (ng/g)
Ligurian Sea				
MPM3	0.00	403.96	5.00	1.00
MPM4	0.10	167.78	5.00	55.20
MPM5	0.10	23.45	10.00	1.00
MPM6	0.00	43.67	172.41	3,12
MPM7	0.00	36.77	5.00	5.75
MPM8	0.05	204.71	5.00	454.07
MPM9	0.00	4275.51	5.00	1.00
MPM10	0.00	193.15	5.00	2.00
MPM11	1.35	377.49	5.00	37.64
MPM12	0.50	496.35	5.00	4.87
MPM13	0.33	6147.00	10.00	1.00
MPM14	9.67	4253.33	10.00	188.94
MPM15	0.04	179.51	10.00	25.68
MPM16	0.95	4645.71	5.00	85.78
Mean	**0.94 ± 2.55**	**1532.03**	**18.38 ± 44.39**	**61.93 ± 124.26**
Sardinian Sea				
MPM17	0.00	82.74	76,02	19.83
MPM18	0.83	27.07	10.00	1.00
MPM19	0.11	744.54	10.00	11.30
MPM20	0.00	668.66	5.00	107.11
MPM21	0.03	90.19	10.00	35.56
MPM23	0.24	102.73	5.00	1.00
MPM24	0.00	523.27	84.81	109.93
MPM25	0.00	15000.00	5.00	30.64
MPM26	0.00	3919.72	5.00	46.34
Mean	**0.13 ± 0.27**	**2350.99**	**23.42 ± 32.46**	**40.30 ± 41.55**
Total Mean	**0.62 ± 2.00**	**1852.49**	**20.36 ± 39.42**	**53.47 ± 99.34**

and MEHP were extracted from blubber (1 g), and phthalate concentrations were measured with the method described above.

3. Results

Of the 23 surface neustonic/planktonic samples, 13 contained plastic particles (Table 1, Fig. 1a). The highest microplastic abundance (9.67 items/m^3, equivalent to 4.83 items/m^2) was found in a sample collected near the Portofino MPA (Ligurian Sea). Large amounts of plastic particles were detected in the surface neustonic/planktonic samples collected in the Pelagos Sanctuary areas investigated (mean value 0.62 items/m^3). The amounts of plastic particles were approximately seven times higher in the samples from the Ligurian Sea (mean value 0.94 items/m^3) than in the samples from the Sardinian Sea (mean value 0.13 items/m^3) (Table 1). Plastic particles were not found in the three water column samples (Table 2). The planktonic species were taxonomically determined, and the results are shown in Table 1 of Supplementary data.

High concentrations of the phthalates MEHP and DEHP were detected for the first time in the surface neustonic/planktonic samples collected in the Pelagos Sanctuary areas. The values of MEHP were approximately 1.5 times higher in the samples from the Ligurian Sea than in the samples from the Sardinian Sea. Lower concentrations of MEHP were detected in the 3 water column samples than in the surface samples (Table 2).

The presence of harmful chemicals in Mediterranean fin whales, associated with the potential intake of plastic derivatives by water filtering and plankton ingestion, was demonstrated for the first time by the results of this study, which documented the presence of relevant concentrations of MEHP in the blubber of four out of five stranded fin whales (Fig. 1b). MEHP is a marker for exposure to DEHP, whereas DEHP was never detected in the samples. It is not surprising that DEHP was not detected in these samples, as it is well known that the DEHP is rapidly metabolized to MEHP, its primary metabolite (Latini et al., 2004). The preliminary data obtained by the current study suggest that phthalates can serve as a tracer of the intake of microplastics by fin whales resulting from the ingestion of micro-litter and plankton.

4. Discussion

The present study, following the recent publication by Collignon et al. (2012), provides an initial insight into microplastic pollution in the Mediterranean Sea by reporting the concentrations and spatial distribution of microplastics in the area of Pelagos Sanctuary. We emphasize that the mean abundance of microplastics estimated in this study is of the same order of magnitude as that found for the North Pacific Gyre (Collignon et al., 2012), suggesting the high level of this emerging threat in the only pelagic MPA of the Mediterranean Sea.

The Pelagos Sanctuary for Mediterranean Marine Mammals is a marine protected area of approximately 90,000 km^2 in the northwestern Mediterranean Sea. A remarkable cetacean fauna consisting of 8 species, including the baleen whale *B. physalus*, coexists in the Sanctuary with very high levels of human pressure. Plastic from coastal tourism, recreational and commercial fishing, marine vessels and marine industries can directly enter the marine environment and pose a risk to biota both as macroplastics and, following long-term degradation, as microplastics. Within the Pelagos Sanctuary, the Portofino MPA showed the highest values of microplastic items/m^3. This area was also confirmed as a "hot spot" for microplastics by Collignon et al. (2012). These results serve to focus particular attention on the conservation status of an area with a high level of exploitation by tourists and on the balance between conservation measures and management.

Previously, very few studies have addressed the impact of microplastics on filter-feeding organisms or other planktivorous animals. No previous studies have assessed the potential impact of microplastics on large filter-feeding organisms, such as baleen whales.

At the lowest level of the food web, the great abundance of microplastics in the photic zone could both interfere with and be a severe threat to plankton viability. Microplastic debris has been found in the gastrointestinal tracts of several planktivorous fishes (*Myctophidae*, *Stomiidae* and *Scomberesocidae*) in the North Pacific Gyre (Boerger et al., 2010). In the Mediterranean Sea, during the survey recently carried out by Collignon et al. (2012), plastic micro-debris was found in the stomachs of myctophids (*Myctophum punctatum*). Moreover, several studies report the ingestion of plastic debris of different sizes, colors and shapes by both epibenthophagous and hyperbenthophagous fish species (*Ariidae*, *Scianidae*) inhabiting a demersal estuarine environment in the tropical Western South Atlantic (Costa et al., 2011; Possatto et al., 2011; Dantas et al., 2012). The occurrence of interactions between several species of marine mammals and marine debris (Williams et al., 2011) and of plastic ingestion in Franciscana dolphins were also recently reported (Denuncio et al., 2011). However, the physiological and toxicological effects of plastic ingestion by filter-feeding organisms are poorly investigated and understood, as are the implications of plastic ingestion occurring through the food chain.

Marine plastics have been found to adsorb and transport chemicals, including high concentrations of organochlorines such as polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT) and PAHs (Teuten et al., 2007). After the ingestion of plastics by an organism, the presence of digestive surfactants is known to increase the bioavailability of these compounds sorbed to plastics (Voparil and Mayer, 2000) by markedly increasing the desorption rate of plastics compared with that found in sea water (Teuten et al., 2007). Due to the large surface-area-to-volume ratio of microplastics, marine organisms may be particularly at risk of exposure to leached additives after microplastics are ingested. Such additives may interfere with biologically important processes, potentially resulting in endocrine disruption (Barnes et al., 2009; Lithner et al., 2009, 2011). In this context, it is known that commonly used additives, such as brominated flame retardants, phthalates and the constituent monomer bisphenol A, can act as endocrine-disrupting chemicals because they can mimic, compete with or disrupt the synthesis of endogenous hormones (Talsness et al., 2009). In particular, phthalates have been associated with a range of molecular, cellular and organ effects in aquatic invertebrates and fish (Oehlmann et al., 2009). Bisphenol A is both

Table 2
Microplastic particles in water column samples (items/m^3) collected in the Pelagos Sanctuary, zooplankton abundance (ind/m^3), DEPH and MEPH concentrations (ng/g f.w.), mean values ± S.D (see Fig. 1 for sampling sites).

Sample	Items/m^3	Zooplankton abundance (ind/m^3)	DEHP (ng/g)	MEHP (ng/g)
MPP3	0.00	49.71	5.00	1.00
MPP10	0.00	1266.05	5.00	4.32
MPP22	0.00	864.88	5.00	1.00
Mean	**0.00**	**726.88**	**5.00 ± 0.00**	**2.11 ± 1.92**

an estrogen agonist and an androgen antagonist, and it can differentially affect reproduction and development, depending on its concentration and the species affected. Nevertheless, Oehlmann et al. (2009) note that there has been relatively little research into the chronic effects of long-term exposure to these additives in aquatic organisms.

The present data represent the first evidence of the potential impact of the most abundant plastic derivatives (phthalates) in a baleen whale, the second-largest filter feeder in the world: the Mediterranean fin whale. The fin whale is a cosmopolitan cetacean. It is found in the largest water masses of the world, from the equator to the polar regions. Despite its cosmopolitan distribution, it is classified as Endangered on the IUCN Red List. In general, rorqual feeding 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 toward a school of prey 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 feed near the surface at night (Croll et al., 2005).

It is well known that the fin whale in the Mediterranean Sea feeds preferentially on the planktonic euphausiid *Meganyctiphanes norvegica*. Nevertheless, depending on the area and the season, the whale feeds on a wide spectrum of marine organisms, including copepods, other euphausiid species (e.g., *Thysanoessa inermis*, *Calanus finmarchicus*, *Euphausia krohni*) and small schooling fish (Orsi Relini and Giordano, 1992; Relini et al., 1992; Notarbartolo di Sciara et al., 2003). With each mouthful, a fin whale can trap approximately 70,000 l of water. For this reason, a whale could risk ingesting a great amount of microplastic debris, both directly from the water and indirectly from the plankton (during both surface feeding and deeper feeding activity). After microplastics are ingested, a fin whale may be exposed directly to leached additives, such as polybrominated diphenyl ethers, phthalates and bisphenolA and their potential toxicological effects.

Preliminary data on MEHP in 5 samples of *Euphausia krohni* collected in the Sicilian Channel reported high concentrations of this contaminant ranging from 8.35 to 51.14 ng/g. These results suggested that plastic derivatives also occur in planktonic species inhabiting the water column (unpublished data, Guerranti personal communication).

In view of the presence of microplastics in the Mediterranean environment, the detection of plastic additives in the blubber of fin whales and the long lifespan of the species, fin whales appear to be chronically exposed to persistent and emerging contaminants as a result of microplastic ingestion. In this context, the preliminary observations presented in this paper suggest that phthalates can serve as a tracer for the intake of microplastics in micro-litter and in plankton by fin whales. These observations represent a warning that the endangered Mediterranean population of this baleen whale is exposed to endocrine disruptors such as MEHP. The results of this study are consistent with the evidence previously reported by Fossi et al. (2010) of an early warning signal of endocrine interference furnished by the up-regulation of the estrogen receptor alpha gene detected in skin biopsies of male Mediterranean fin whales compared with fin whales from the Sea of Cortez (Mexico). This "undesirable biological effect" (in agreement with the description of the concept of biomarkers in Descriptor 8 of the Marine Strategy Framework Directive) can suggest that the Mediterranean population is exposed to a mixture of persistent and emerging contaminants, such as endocrine disruptors, that may impair the population viability of this already endangered species.

In this context, surveys covering much of the western Mediterranean basin have estimated the fin whale population to be 3.583 individuals (Forcada et al., 1996), 901 of which inhabit the Corsican-Ligurian-Provencal basin (Forcada et al., 1995). However, according to more recent data on the Pelagos Sanctuary, the estimated population has decreased markedly (approximately by a factor of six) in the past 20 years (Panigada et al., 2011) raising particular concerns about the status of this species.

In conclusion, the present data represent the first evidence of the potential impact of plastic additives (phthalates) in baleen whales. These results underscore the importance of future research on the detection of the toxicological impact of micro-plastics in filter-feeding species such as mysticete cetaceans, the basking shark and the devil ray. The results also underscore the potential use of these species in the implementation of Descriptor 10 (marine litter) in the EU Marine Strategy Framework Directive as indicators of the presence and impact of micro-litter in the pelagic environment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2012.08.013.

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