



Trace element concentrations in the Mediterranean monk seal (*Monachus monachus*) in the eastern Mediterranean Sea[☆]

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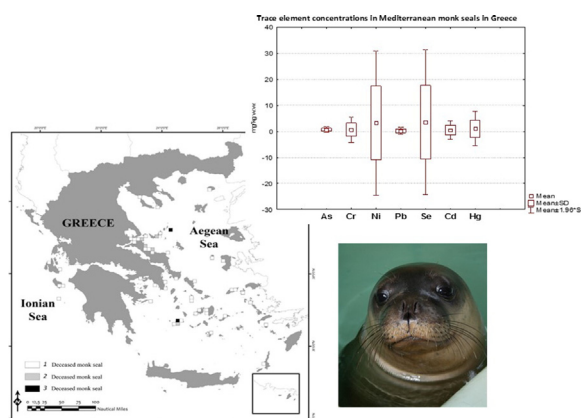
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HIGHLIGHTS

- First evaluation of trace element exposure in the endangered Mediterranean monk seal.
- Trace element exposure was low and within the non-acutely toxic levels for Pinnipeds.
- Adverse effects on the immune and endocrine system from some pollutants cannot be ruled out.
- Pollutant monitoring for monk seals in Greece is important.

GRAPHICAL ABSTRACT



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ABSTRACT

The Mediterranean monk seal (*Monachus monachus*) is one of the most endangered marine mammals in the world. The biggest sub-population of the species survives in Greece, where understanding the effects of pollution on the survival of the species has been identified as a national research and conservation priority. From 1990 to 2013 we collected tissue samples from 59 deceased monk seals in order to: (i) Define the concentration of trace elements (As, Pb, Cd, Hg, Se, Cr, Ni) in three different matrices (i.e., blubber, liver and kidney), (ii) Determine whether differences in trace element concentrations are age- or gender-related, (iii) Evaluate the potential effects of these pollutants. The study recorded differences in trace element concentrations among matrices, but in general, trace element exposure in Mediterranean monk seals in Greece was low and within the non-acutely toxic levels for Pinnipeds. Only arsenic concentrations were at the upper limit of the normal range observed in other marine mammals (0.69 ± 0.55 mg/kg w.w. in blubber, 0.79 ± 0.62 mg/kg w.w. in liver and 0.79 ± 0.59 mg/kg w.w. in kidney). We recorded also exceptionally high Hg concentrations in a single adult female (24.88 mg/kg w.w.). Age- and gender-related differences were also recorded and were due to various biological, ecological and chemical factors. Based on the results of the study, potentially adverse effects on the

[☆] Capsule: The exposure to trace elements and their potentially adverse effects in the endangered Mediterranean monk seal have been evaluated.

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immune and endocrine system of the Mediterranean monk seal from some pollutants (e.g., As, Cd, Se, Ni, Cr) cannot be ruled out, which may expose the Mediterranean seal population in Greece to epizootics and stochastic phenomena of mass mortality. It is therefore of utmost importance that pollutant monitoring becomes an integral component of the standard monitoring protocol of the endangered Mediterranean monk seal in the eastern Mediterranean.

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1. Introduction

Exposure to contaminants in the marine environment and their effects on wildlife is an issue of great concern in conservation and therefore a wide range of persistent chemical contaminants and their effects on marine organisms has been investigated (Chiuchiolo et al., 2004). These chemical contaminants affect mainly species inhabiting coastal areas, although wildlife from pelagic and other remote environments that are far from the source of emissions (e.g., Antarctica), appear to be affected as well (Braune et al., 2005; Chiuchiolo et al., 2004; Davis, 1993). Among these contaminants, trace elements are natural constituents of the earth's crust whose concentrations are increasing due to human activity (Maffucci et al., 2005; Singh et al., 2011) and are particularly important because of their persistence and potential toxicity (Barron et al., 2003; Das et al., 2003; Fossi et al., 1997; Storelli et al., 2005). Trace elements do not degrade through bacterial metabolic pathways like organic pollutants and undergo biomagnification processes throughout the food web. Above certain thresholds trace elements become toxic (Chronopoulos et al., 1997; O'Shea and Geraci, 1999) and cause adverse health effects [i.e., immune-, geno-, cyto-toxic effects, reproduction impairment and endocrine alterations (Cardellicchio et al., 2000; Das et al., 2003)] to long-lived, top marine consumers (Anan et al., 2002; Anan et al., 2001; Brunborg et al., 2006; Bustamante et al., 2003; Camacho et al., 2013; Drescher et al., 1977; Frank et al., 1992; Kakuschke et al., 2012; Medvedev et al., 1997; Poppi et al., 2012; Watanabe et al., 2002).

With an estimated population of <700 individuals, the Mediterranean monk seal (*Monachus monachus*) has been classified by the International Union for the Conservation of Nature (IUCN) as endangered (Karamanlidis and Dendrinis, 2015) and is considered one of the most threatened marine mammals in the world. Once abundant throughout the Black and Mediterranean Sea and the coasts and islands off northwest Africa, human persecution has led to a drastic reduction in the species' range, which is currently fragmented (Karamanlidis et al., 2016). The biggest subpopulation of the Mediterranean monk seal survives in the eastern Mediterranean Sea, a marine area heavily impacted by human activity (Halpern et al., 2008): the species survives mainly along the islands in the Ionian and Aegean Sea and the coasts of mainland Greece (Karamanlidis et al., 2016). Despite recent signs of population recovery of Mediterranean monk seals in Greece, the species' survival is still threatened by a number of factors and effective conservation measures are urgently required to safeguard its future. Understanding the effects of pollution has been identified as one of the national research and conservation priorities for the Mediterranean monk seal in Greece (Notarbartolo di Sciara et al., 2009).

Only two studies on trace element exposure have been conducted in the Mediterranean monk seal (Dosi, 2000; Yediler et al., 1993), using however a limited number of samples. The aims of this study were to use a large sample size to: (i) investigate the accumulation of trace elements (i.e., As, Pb, Cd, Hg, Se, Cr and Ni) in three different matrices (i.e., blubber, liver, kidney) of Mediterranean monk seals in Greece, (ii) determine if blubber, liver and kidney trace element concentrations differed with age and sex and (iii) evaluate the potential effects of these pollutants on this highly endangered species.

2. Materials and methods

2.1. Samples

Tissue samples from blubber, liver and kidney were collected from 59 deceased monk seals (28 males, 29 females, 2 unknown sex) from the Aegean and Ionian Seas in Greece in 1990–2013 (Fig. 1). Based on external morphological criteria (Samaranch and González, 2000) Mediterranean monk seals were classified as pups, juveniles and adults. Due to the varying degrees of decomposition of the carcasses it was not possible to collect all the samples from all the individuals sampled (Table 1).

2.2. Sample preparation and analysis

Following a microwave digestion of 0.7 g of homogenized sample, a trace element analysis using the Inductively Coupled Plasma-Optic Emission Spectrometry (ICP-OES) technique on a Perkin Elmer Optima 2100 DV, coupled with a CETAC U5000AT + ultrasound nebulizer for mercury, was performed. Samples were microwave-digested in a Milestone ETHOS ONE oven using 6 ml nitric acid and 1 ml hydrogen peroxide. Digested samples were then diluted to 50 ml with suprapure water (18 mΩ) and injected in the ICP; a total of 5 ml were used for trace element analysis. All reagents were from Merck, Darmstadt (Germany); acids were of suprapure grade.

2.3. Quality assurance and quality control

To check for chemical purity two blanks were run during each set of analysis and the accuracy of the method was verified using reference materials (DORM-2 dogfish muscle, Measurement Standards Laboratory, Callaghan Innovation, Lower Hutt 5040, New Zealand). All the reference material values were within the certified limits. Instrumental Limits of Detection (LOD), expressed as wet weight (w.w.), were: 24 ng/ml for As; 11 ng/ml for Pb and Se; 1.80 ng/ml for Cd; 0.71 ng/ml for Cr; 1.80 ng/ml for Ni; and 0.06 ng/ml for Hg (see Supplementary material for details). Trace element concentrations in blubber, liver and kidney are expressed as mg/kg wet weight.

2.4. Statistics

Data were initially checked for normality by performing a Shapiro-Wilk test. As the data were not normally distributed, non-parametric statistics were used. We used Kruskal–Wallis Analysis of Variance to compare mean trace element values of blubber between age classes (i.e., pup vs. juvenile vs. adult) and gender. No age- and gender-specific comparisons were carried out for liver and kidney because of small sample sizes. In addition, a Spearman's rank correlation test was used in order to examine whether a relationship between the concentrations of different trace elements existed. The significance level was set at $p = 0.05$ and all analyses were conducted with STATISTICA 6.0 (StatSoft Italia S.r.l.).

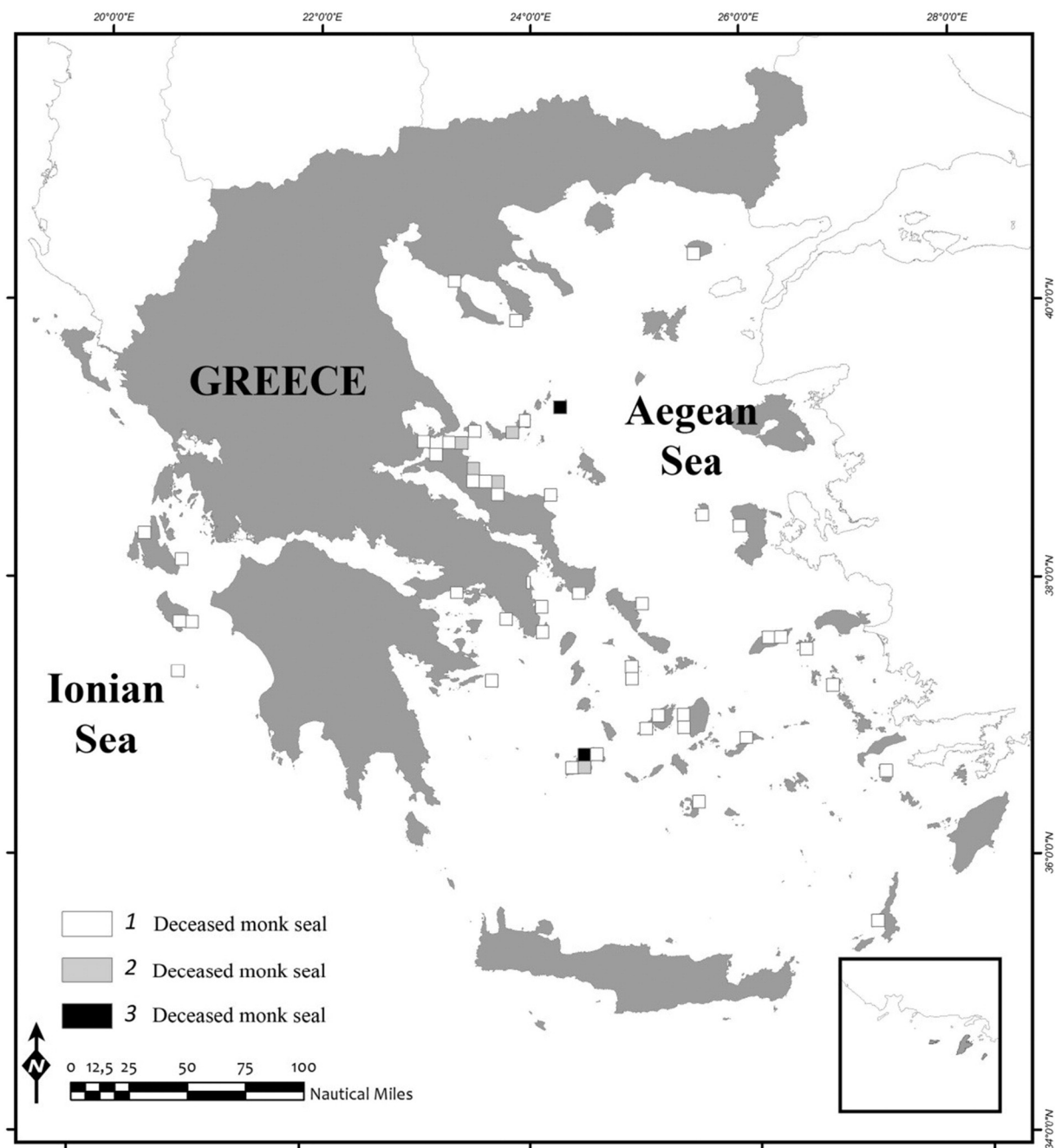


Fig. 1. Map indicating the locations and the number of deceased Mediterranean monk seals sampled in Greece (1990–2013).

3. Results and discussion

Data on concentrations of trace elements in different tissues of Mediterranean monk seals, reported as total, age and gender-related mean \pm s.d., are summarized in Fig. 2 and Table 2, and presented in relation to other Pinnipeds in Table 3.

Table 1
Summary information of samples collected from 59 deceased Mediterranean monk seals in Greece (1990–2013).

Sample type	Total	Age class				Gender		
		Pup	Juvenile	Adult	Unknown	Male	Female	Unknown
Blubber	59	16	24	17	2	28	29	2
Liver	15	7	5	3	0	7	8	0
Kidney	13	7	4	2	0	6	7	0

3.1. Concentrations of trace elements in Mediterranean monk seal tissues

Arsenic concentrations in the blubber, liver and kidney of Mediterranean monk seals were similar (0.69 ± 0.55 mg/kg w.w., 0.79 ± 0.62 mg/kg w.w., 0.77 ± 2.56 mg/kg w.w.) (Fig. 2, Table 2). They were lower than those reported for Weddell (*Leptonychotes weddellii*) (Noda et al., 1993) and grey seals (*Halichoerus g. grypus*) (Frank et al., 1992), but higher than those reported for Caspian (*Pusa caspica*) (Anan et al., 2002), common (*Phoca v. vitulina*) (Skaare et al., 1990) and ringed seals (*Pusa hispida*) (Frank et al., 1992; Skaare et al., 1990), and by Dosi (2000) for the same Mediterranean monk seal population in Greece (Table 3). Arsenic concentrations in Mediterranean monk seals were considered to be relatively high on a global scale, and at the upper limit (i.e., 0.65–1.95 mg/kg w.w.) of the normal range observed in other marine mammals (Bellante et al., 2012; Carvalho et al., 2002; Frank et al., 1992).

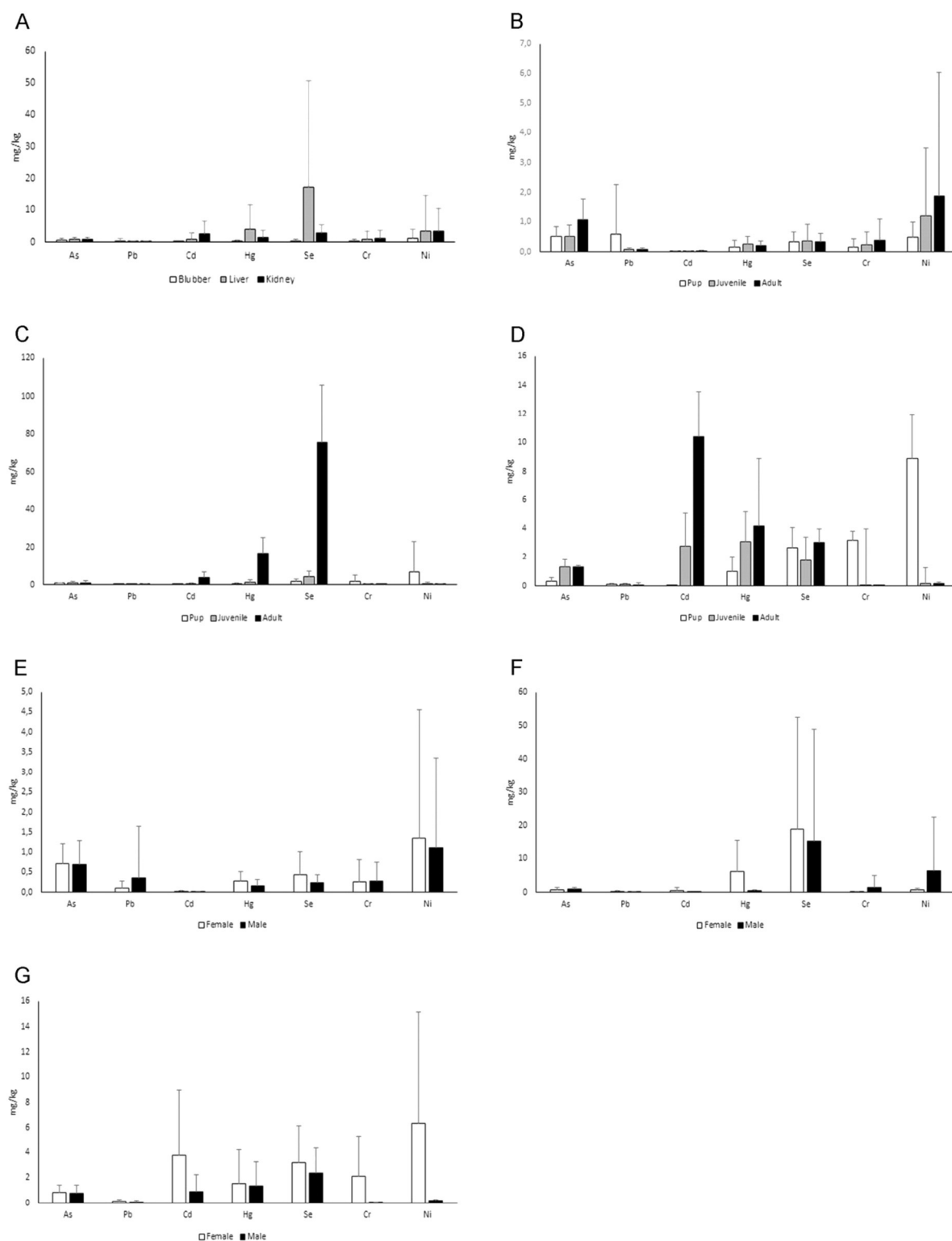


Fig. 2. A) Trace element concentration in tissues of Mediterranean monk seals; B) Trace element concentration in blubber according to age; C) Trace element concentration in livers according to age; D) Trace element concentration in kidneys according to age; E) Trace element concentration in blubber according to gender; F) Trace element concentration in livers according to gender; G) Trace element concentration in kidneys according to gender.

Pb concentrations in Mediterranean monk seals were highest in blubber (0.23 ± 0.90 mg/kg w.w.), followed by liver and kidney (Fig. 2, Table 2). The same tissue distribution was observed in harp seals from Labrador (Yeats et al., 1999), although reported concentrations were almost two times lower than those found in Greek monk seals (0.15 mg/kg vs. 0.23 mg/kg). Lower Pb concentrations were found also in ringed and grey seals from Finland (0.05 mg/kg and

0.11 mg/kg respectively) (Pertilä et al., 1986). Pb concentrations in blubber were approximately three times lower than those reported by Dosi (2000) for the same seal population. The difference between the two studies is likely an effect of the small sample size of latter study, which included two individuals with exceptionally high Pb concentrations. Pb concentrations in liver and kidney in the present study were similar to those reported for Caspian (Anan et al., 2002; Watanabe

Table 2Concentrations of trace elements in Mediterranean monk seal tissues collected from 1990 to 2013 in Greece, expressed as mean \pm standard deviation (mg/kg w.w.).

Tissue	N ()	As	Pb	Cd	Hg	Se	Cr	Ni
Blubber	Total (59)	0.69 \pm 0.55	0.23 \pm 0.90	0.02 \pm 0.01	0.21 \pm 0.22	0.34 \pm 0.44	0.26 \pm 0.51	1.22 \pm 2.74
	Female (29)	0.71 \pm 0.50	0.10 \pm 0.17	0.03 \pm 0.02	0.27 \pm 0.24	0.44 \pm 0.57	0.25 \pm 0.55	1.35 \pm 3.20
	Male (28)	0.68 \pm 0.62	0.36 \pm 1.29	0.02 \pm 0.01	0.15 \pm 0.17	0.24 \pm 0.20	0.28 \pm 0.47	1.10 \pm 2.26
	Pup (16)	0.51 \pm 0.32	0.58 \pm 1.68	0.02 \pm 0.01	0.16 \pm 0.23	0.33 \pm 0.33	0.15 \pm 0.29	0.49 \pm 0.50
	Juvenile (24)	0.54 \pm 0.40	0.09 \pm 0.05	0.02 \pm 0.01	0.25 \pm 0.24	0.36 \pm 0.58	0.25 \pm 0.44	1.23 \pm 2.34
	Adult (17)	1.08 \pm 0.71	0.08 \pm 0.05	0.04 \pm 0.02	0.20 \pm 0.16	0.33 \pm 0.29	0.39 \pm 0.71	1.89 \pm 4.15
Liver	Total (15)	0.79 \pm 0.62	0.17 \pm 0.15	0.87 \pm 1.90	4.09 \pm 7.57	17.33 \pm 33.24	0.77 \pm 2.56	3.37 \pm 11.41
	Female (8)	0.72 \pm 0.61	0.18 \pm 0.20	0.60 \pm 0.87	6.11 \pm 9.56	18.91 \pm 33.51	0.17 \pm 0.15	0.70 \pm 0.50
	Male (7)	0.86 \pm 0.63	0.12 \pm 0.05	0.05 \pm 0.03	0.49 \pm 0.24	15.52 \pm 33.38	1.46 \pm 3.63	6.42 \pm 16.10
	Pup (7)	0.69 \pm 0.30	0.12 \pm 0.05	0.05 \pm 0.03	0.49 \pm 0.24	1.89 \pm 1.09	1.51 \pm 3.61	6.69 \pm 16.10
	Juvenile (5)	0.81 \pm 0.68	0.13 \pm 0.05	0.35 \pm 0.34	1.48 \pm 1.09	4.06 \pm 2.87	0.15 \pm 0.19	0.57 \pm 0.59
	Adult (3)	0.98 \pm 1.10	0.35 \pm 0.28	3.64 \pm 3.02	16.83 \pm 8.40	75.49 \pm 30.39	0.07 \pm 0.03	0.29 \pm 0.35
Kidney	Total (13)	0.79 \pm 0.59	0.12 \pm 0.10	2.46 \pm 4.03	1.45 \pm 2.28	2.84 \pm 2.46	1.15 \pm 2.47	3.49 \pm 7.03
	Female (7)	0.82 \pm 0.56	0.14 \pm 0.11	3.81 \pm 5.13	1.53 \pm 2.71	3.23 \pm 2.90	2.09 \pm 3.17	6.31 \pm 8.87
	Male (6)	0.75 \pm 0.68	0.09 \pm 0.08	0.89 \pm 1.35	1.36 \pm 1.93	2.39 \pm 1.99	0.06 \pm 0.02	0.20 \pm 0.08
	Pup (7)	0.33 \pm 0.23	0.12 \pm 0.06	4.89 \pm 0.01	1.01 \pm 1.04	2.68 \pm 1.38	3.17 \pm 0.65	8.88 \pm 3.07
	Juvenile (4)	1.31 \pm 0.51	0.10 \pm 0.08	0.36 \pm 2.30	3.11 \pm 2.09	1.77 \pm 1.62	0.01 \pm 3.97	0.17 \pm 11.10
	Adult (2)	1.32 \pm 0.08	0.04 \pm 0.18	4.74 \pm 3.13	4.18 \pm 4.71	3.06 \pm 0.93	0.01 \pm 0.01	0.15 \pm 0.14

et al., 2002), common (Drescher et al., 1977; Frank et al., 1992), ringed (Frank et al., 1992; Medvedev et al., 1997), grey (Frank et al., 1992; Nyman et al., 2002), hooded (*Cristophora cristata*) and harp seals (*Pagophilus groenlandicus*) (Brunborg et al., 2006) (Table 3), and were considered relatively low on a global scale.

Blubber, liver and kidney Cd concentrations in Mediterranean monk seals were 0.02 ± 0.01 , 0.87 ± 1.90 and 2.46 ± 4.03 mg/kg w.w. respectively (Fig. 2, Table 2). These Cd concentrations were similar to those reported for Swedish common, ringed, Caspian and Baikal (*Pusa sibirica*) seals (Frank et al., 1992; Watanabe et al., 2002; Watanabe et al., 1998), lower than the concentrations reported for harp and hooded seals (Brunborg et al., 2006) (Table 3) and were considered to be overall low.

Hg concentrations in Mediterranean monk seals were 0.21 ± 0.22 mg/kg w.w., 4.09 ± 7.57 mg/kg w.w. and 1.45 ± 2.28 mg/kg w.w. in blubber, liver and kidney, respectively (Fig. 2, Table 2). In a

previous study on Mediterranean monk seals in Greece (Yediler et al., 1993), Hg concentration in hair was 5 to 10 times higher than the concentration recorded in the present study; because of the different tissues examined however data are not directly comparable. Liver Hg concentrations were similar to those reported for Caspian, ringed, Ladoga (*Pusa h. ladogensis*) and Baikal seals (Anan et al., 2002; Medvedev et al., 1997; Watanabe et al., 1998), and considerably lower than those reported for common, ringed, grey and hooded seals (Brunborg et al., 2006; Frank et al., 1992; Lake et al., 1995; Nyman et al., 2002) (Table 3). Overall, Hg concentrations in Mediterranean monk seals in Greece were considered to be relatively low.

Se concentration in Mediterranean monk seals in Greece was highest in liver (17.33 ± 33.24 mg/kg w.w.), followed by kidney (2.84 ± 2.46 mg/kg w.w.) and blubber (0.34 ± 0.44 mg/kg w.w.) (Fig. 2, Table 2). Selenium concentrations in livers and kidneys were similar to those reported for other phocids (Anan et al., 2002;

Table 3

Trace element concentrations in seal tissues, expressed as mean value/ranges (mg/kg w.w.). Values originally reported as dry weight were converted to wet weight applying a conversion factor of 4 (Yang and Miyazaki, 2003). N.d.: not detected.

Tissue	Species	Metal (mg/kg w.w.)							Reference
		As	Cd	Pb	Cr	Ni	Hg	Se	
Blubber	Mediterranean monk seal	0.66–2.48	0.013–0.05	0.004–6.79	0.001–20.26	0.003–125.82	0.003–0.93	0.02–2.89	Present study
	Mediterranean monk seal	0.16	0.06	1.51	1.68				1
	Common seal	1.6					0.11		2; 3
	Ringed seal	4.3							3; 15
	Grey seal	3.6							3; 15
Liver	Mediterranean monk seal	0.001–2.17	0.02–7.09	0.06–0.68	0.048–9.689	0.084–43.19	0.21–24.89	0.44–94.95	Present study
	Common seal	0.1–0.16	<0.1–0.2	0.05	0.16	0.03	0.49–69.9	2.13–11	2; 3; 4; 5; 6
	Ringed seal		0.18–0.65	0.044	0.16–0.45	0.019–0.3	0.45–44	2.53–19	3; 6; 7; 15
	Ladoga ringed seal		0.31		0.7	0.45	3.40		7
	Caspian seal	0.17	0.732–1.1	0.081–0.105	0.002–0.07	<0.07	5.8–27		8; 9
	Baikal seal		0.28				2.3		8
	Grey seal		0.018–1.8	0.106	n.d.–0.18	0.009	9.18–109	5.79–28	3; 6; 10; 15
	Harp seal	0.19–0.40	14–23	0.10	0.05		0.39–2	1.5–2.09	6; 11; 12
	Hooded seal	0.27–0.30	28–40	0.30	0.07		20–29	6–11	11; 12
	Weddell seal		2.25						13
	Mediterranean monk seal	0.03–1.94	0.012–12.64	0.03–0.35	0.04–8.33	0.10–23.51	0.09–7.64	0.46–8.02	Present study
Kidney	Common seal		0.46	0.15	0.05	<0.006	0.20–1.61	4.45–5.79	2; 3; 6
	Ringed seal		0.10–2.12	0.107	0.11–0.42	0.014–0.19	0.28–0.42	3.54	3; 6; 7; 15
	Ladoga ringed seal		0.5		0.56	0.31	6.15		7
	Caspian seal	0.16	6.99–12	0.07–0.08	0.01–0.08	0.07	1.6–1.9	2.8–3.6	8; 9
	Baikal seal		2				1.8		14
	Grey seal		1.26–10	0.11	n.d.–0.11	<0.006	1.62–4.7	2.3–3.40	3; 6; 10; 15
	Harp seal	0.23–0.40	43–50	0.20	0.09		0.22–0.70	1.4–2.66	6; 11; 12
	Hooded seal	0.36–20	93–140	0.20	0.04		2.1–3	2–2.2	11; 12
	Weddell seal		13.19						13

1: Dosi, 2000; 2: Brookens et al., 2008; 3: Frank et al., 1992; 4: Lake et al., 1995; 5: Skaare et al., 1990; 6: Skaare et al., 1994; 7: Medvedev et al., 1997; 8: Watanabe et al., 2002; 9: Anan et al., 2002; 10: Nyman et al., 2002; 11: Brunborg et al., 2006; 12: Julshamn and Grahl-Nielsen, 2000; 13: Noda et al., 1993; 14: Watanabe et al., 1998; 15: Perttälä et al., 1986.

Brunborg et al., 2006; Frank et al., 1992; Julshamn and Grahl-Nielsen, 2000; Nyman et al., 2002; Skaare et al., 1994; Skaare et al., 1990) (Table 3) and were considered to be relatively low.

Cr concentrations were highest in kidneys and livers (1.15 ± 2.47 mg/kg; 0.77 ± 2.56 mg/kg), followed by blubber (0.26 ± 0.51 mg/kg) (Fig. 2, Table 2). The liver and kidney Cr concentrations of Mediterranean monk seals in Greece were higher than those reported for other phocids (Anan et al., 2002; Frank et al., 1992), but lower than those reported by Dosi (2000) for the same monk seal population. Overall, Cr concentrations in Mediterranean monk seals in Greece were considered to be relatively low.

Finally, Ni concentrations in Mediterranean monk seals in Greece were highest in kidney (3.49 ± 7.03 mg/kg), followed by liver (3.37 ± 11.41 mg/kg) and blubber (1.22 ± 2.74 mg/kg) (Fig. 2, Table 2). Ni concentrations in all tissues of Mediterranean monk seals were higher than those reported from other seals (Frank et al., 1992; Medvedev et al., 1997; Watanabe et al., 2002) (Table 3) but still, were considered to be relatively low on a global scale.

3.2. Influence of age and gender on tissue distribution

In the present study age- and gender related variations in some trace element concentrations were detected. Age-related trends have already been reported in pinnipeds and other marine mammals (Agusa et al., 2008; Akmajian et al., 2014; García-Alvarez et al., 2015; Ikemoto et al., 2004b; Lavery et al., 2008; Pompe-Gotal et al., 2009; Stewardson et al., 1999). In contrast, marine mammals usually do not show sex-related differences in trace element concentrations, except in Hg (Aguilar et al., 1999; Agusa et al., 2008; García-Alvarez et al., 2015; Lavery et al., 2008; Méndez-Fernandez et al., 2014).

Arsenic concentrations in blubber showed an age-dependent trend, with adults showing significantly higher levels than juveniles and pups (adult vs. juvenile $p = 0.007$; adult vs. pup $p = 0.05$) (Fig. 2). In liver and kidney, pups had lower levels than juveniles and adults, a fact that is partially explained by an accumulation process and by a shift in diet composition. Pups are milk-fed, while after weaning juvenile Mediterranean monk seals in Greece switch to a fish- and cephalopod-rich diet (Karamanlidis et al., 2014; Pierce et al., 2011), which increases As intake (Klaassen, 2013). The fact that the highest As levels were observed in kidneys and livers (Table 2), but not in blubber, may be due to the different kinetics in the three tissues. Blubber is a storage tissue, where lipophilic nutrients and contaminants are deposited (Mullerova and Kopecky, 2007), while livers and kidneys are metabolically and excretive active organs with completely different functions, receiving large inputs of various substances including trace elements (Klaassen, 2013).

No significant gender-related differences were observed in the Pb concentrations in the blubber, livers and kidneys of Mediterranean monk seals in Greece (Fig. 2, Table 2), a fact that is consistent with observations in other seals (Agusa et al., 2008; Anan et al., 2001; Brookens et al., 2007; Nam et al., 2005). An age-dependent trend for Pb concentrations in livers was detected, with adults showing higher concentrations than juveniles and pups. An age-related accumulation has been reported in many other terrestrial and marine mammals as well, and is assumed to be the result of a long-term accumulation of contaminants from the diet and the environment in general (Agusa et al., 2008; Akmajian et al., 2014; Eisler, 1984; Monteiro et al., 2016).

Cd concentration in blubber was age-related, with adults having significantly higher concentrations than juveniles ($p = 0.0003$) and pups ($p = 0.001$). Similar trends in Cd concentrations were recorded also in the livers and kidneys analyzed (Fig. 2), which is consistent with age-related differences in Cd concentrations reported also in Baikal seals (Ciesielski et al., 2010; Ciesielski et al., 2006). High levels of Cd are often associated with diets rich in cephalopods (Bustamante et al., 1998), which are a key prey of the Mediterranean monk seal (Karamanlidis et al., 2014; Pierce et al., 2011). Another factor

contributing to the age-related differences in the Cd concentrations in Mediterranean monk seals in Greece is that Cd is not transferred through the placenta and therefore little or no toxic elements are accumulated during gestation in fetuses (Wagemann et al., 1988).

Blubber and liver Hg concentrations in Mediterranean monk seals showed gender-related differences, with females showing significantly higher concentrations than males ($p = 0.023$) (Fig. 2, Table 2). Female seals are known to accumulate higher mercury concentrations than males (Brookens et al., 2008; Ciesielski et al., 2010; Lyytikäinen et al., 2015); these gender-related differences are believed to be linked to the hyperactive metabolism of female seals, and to differences in reproduction and food intake (Ciesielski et al., 2010; Ciesielski et al., 2006; Watanabe et al., 1998). Liver and kidney Hg concentrations in Mediterranean monk seals showed also age-related differences: adults had a mean hepatic value of 16.83 ± 8.40 mg/kg, while juveniles and pups of 1.48 ± 1.09 mg/kg w.w. and 0.49 ± 0.24 mg/kg w.w., respectively. Similarly, renal concentrations were 4.18 ± 4.71 mg/kg, 3.11 ± 2.09 mg/kg and 1.01 ± 1.04 mg/kg in adults, juveniles and pups respectively (Fig. 2, Table 2). Age-related Hg concentrations in the livers and kidneys of Mediterranean monk seals were similar to those reported for Baikal and Saimaa seals (*Pusa h. saimensis*) (Ciesielski et al., 2010; Hyvärinen and Sipilä, 1984; Hyvärinen et al., 1998). Hg age-related increases have been reported for various marine mammals (Honda et al., 1983; Ikemoto et al., 2004b; Wagemann et al., 1996) as a result of the high affinity of Hg to the -SH groups of tissue proteins, leading to a continuous accumulation of this trace element (Haraguchi, 1999; Ikemoto et al., 2004b).

No statistical age- or gender-related difference was observed in blubber for Se (Fig. 2, Table 2), while an age-related trend was recorded in liver: Se concentrations were increased in adult livers (75.49 ± 30.39 mg/kg vs 4.06 ± 2.87 mg/kg and 1.89 ± 1.09 mg/kg in adults, juveniles and pups respectively, Fig. 2, Table 2). Hepatic accumulation of Se with age has been reported in harbor, Baikal and Caspian seals, and has been attributed to the interaction of Se with Hg: when Hg increases in liver, Se, which acts as a detoxifying agent for it, increases as well (Akmajian et al., 2014; Ikemoto et al., 2004b).

No age or gender-related differences were found in blubber for Cr and Ni, however these elements decreased in both liver and kidney with age (Cr: 0.07 ± 0.03 mg/kg vs 0.15 ± 0.19 mg/kg and 1.51 ± 3.61 mg/kg in adults, juveniles and pups respectively; Ni: 0.29 ± 0.35 mg/kg vs 0.57 ± 0.59 mg/kg and 6.69 ± 16.10 mg/kg in adults, juveniles and pups respectively; Table 2), a fact that is consistent with findings in other marine mammals (Eisler, 1984; Wise et al., 2009).

3.3. Potential effects of trace elements on Mediterranean monk seals

Despite the extensive literature regarding the analysis of trace elements in marine mammals, knowledge about their actual toxic effects remains limited, mostly due to the inherent difficulties of performing toxicological studies on cetaceans (Weijjs and Zaccaroni, 2016) and therefore values from humans or terrestrial mammals are used instead. These values however usually refer to overt toxicity and not to the sub-chronic effects due to long-term exposure to low levels of contaminants. Consequently, in many cases only inferences from laboratory animal studies can be made in order to evaluate the sub chronic toxicity in marine mammals (Monteiro et al., 2016).

Concentrations of 0.45 µg As/g are known to alter in vitro the production of steroid and adrenal hormones in cultured testicular cells from grey seals and consequently to impair their gonadal and adrenal function (Freeman and Sangalang, 1977). The As concentrations recorded in the present study (i.e., 0.79 ± 0.62 mg/kg in liver) are comparable to those reported by Freeman and Sangalang (1977) and therefore a potential effect of As on the reproductive biology of the Mediterranean monk seal in Greece cannot be ruled out. Although within the normal range for pinnipeds (Frouin et al., 2010), arsenic concentrations in all tissues of Greek Mediterranean monk seals were above the

0.375 mg/kg level at which a significant reduction in lymphocyte proliferation of common seal lymphocyte B 11B7501 cells has been recorded (Frouin et al., 2010).

It is difficult to evaluate the potential effect of increased Pb on the health of *Monachus monachus*, as only one case of Pb poisoning in marine mammals has been reported, a bottlenose dolphin (*Tursiops truncatus*) in captivity that died after the accidental ingestion of gun pellets (Shlosberg et al., 1997). However, when compared with the hepatic and renal Pb toxic thresholds (i.e., 9.6 mg/kg and 21.6 mg/kg w.w., respectively) established for terrestrial mammals (Ma, 1996), the observed concentrations are well below these levels, and can thus be considered as not capable of inducing acute toxicity. A comparison with studies performed in humans however gives reason for concern in regard to the reproductive health of seals, as the tissue concentrations recorded in the present study are within the range of exposure capable of negatively affecting the reproductive performance of an individual (i.e., median 49 µg/l, range 11–149 µg/l in blood) (Flora et al., 2012). A decrease in the reproductive performance, particularly of males, cannot be ruled out for Mediterranean monk seals in Greece.

Cd concentrations in this study were considerably lower than the concentrations (50 µg/g w.w.) recorded causing histopathological changes in the renal cortex of ringed seals and humans (Friberg et al., 1985; Sonne-Hansen et al., 2002; Sonne et al., 2000). They were however higher than the levels at which decreases in corticosterone (B) and aldosterone (ALDO) production and increases in dehydroandrosterone (DHA), testosterone (T) and cortisol (F) production have been recorded (Freeman and Sangalang, 1977) and therefore, an impairment of the adrenal and gonadal axes of Mediterranean monk seals cannot be ruled out, especially for older individuals with high Cd concentrations.

The Hg concentration recorded in this study is biased by the exceptionally high concentration of a single adult female (24.88 mg/kg w.w.). This concentration exceeds the threshold for subclinical toxicity for marine mammals suggested by the Arctic Monitoring and Assessment Program (i.e., 16.25 mg/kg w.w. in liver; AMAP, 2005). All other monk seals in the study had Hg concentrations well below this threshold and therefore Hg contamination should not be considered currently a risk for the Mediterranean monk seal in Greece. It should be acknowledged however that chronic exposures to even low concentrations of mercury have led to enzymatic and hormonal responses, changes in behavior and sensory functions, reduction in appetite and consequently weight loss in various mammals (Das et al., 2003; Freeman et al., 1975; Wolfe et al., 1998). It is therefore of utmost importance to constantly monitor mercury exposure in this endangered species.

The observed Se concentrations in liver and kidney were well above the 0.45 µg/g concentration used by Freeman and Sangalang (1977) in *in vitro* exposure of grey seal adrenal and testicular cells. This Se concentration proved to reduce δ 4-androstene-3, 17-dione (δ 4A), DHA, T, B and ALDO production, and to increase 11-ketotestosterone (11KT) and F and therefore a possible impairment in the adrenal and reproductive axes of Mediterranean monk seals in Greece cannot be ruled out. Furthermore, Se concentrations similar to the ones recorded in the present study have been found to decrease phagocytic activity and cell proliferation and to increase micronuclei in lymphoma B 11B7501 cell cultures of common seals (Frouin et al., 2010). The Se concentrations in the present study could have led Mediterranean monk seals to a severe immunodepression, thus making them more vulnerable to infectious diseases and it is therefore of utmost importance to constantly monitor selenium exposure of Mediterranean monk seals in Greece. Se has also an antagonistic action to the toxic effects of mercury. This detoxifying activity is attained only after total mercury concentrations have exceeded a specific threshold, which varies from species to species (Palmisano et al., 1995). The antagonistic action of Se to Hg in the liver of different marine mammals has been reported at a molar ratio Hg:Se of approximately 1 (Koeman et al., 1973). Considering the low Hg:Se ratios in the livers of Mediterranean monk seals in the present study (i.e., 0.04–0.5), no overt toxicological effects should be expected in the

species (Berry and Ralston, 2008; Boening, 2000; Ikemoto et al., 2004a). A positive correlation between Hg and Se was observed in blubber, liver and kidney ($p = 0.000$, $p = 0.003$ and $p = 0.001$ respectively), confirming the detoxifying role of Se versus Hg (Berry and Ralston, 2008; Boening, 2000; Ikemoto et al., 2004a). Positive correlations were observed also between Se and As ($p = 0.043$) and Se and Pb ($p = 0.016$) in kidney, and between Se and Pb ($p = 0.001$) in liver, but the biological meaning of such correlations is unknown. In the case of humans, such correlations are considered as indicative of a protective effect of essential elements (i.e., Se) against toxic elements (i.e., As, and Pb) (Yoo et al., 2000).

The toxicity of Cr in marine mammals is not yet fully understood (Law, 1996). It has been reported that blood Cr concentrations of approximately 20 µg/l may induce hypersensitivity reactions in common seals (Kakuschke and Prange, 2007). These Cr concentrations are lower than those observed in the tissues of Mediterranean monk seals in Greece (Table 2). Considering that, due to tissue accumulation blood contaminant concentrations are generally lower than those of tissue, we cannot exclude the fact that some hypersensitivity reactions could have occurred also in the Mediterranean monk seals in our study (Klaassen, 2013). Hypersensitivity and autoimmune reactions can contribute to the etiology of serious systemic diseases not only in humans, but also in seals and marine mammals in general (Kakuschke et al., 2005).

Although higher than the levels reported from other seals (Frank et al., 1992; Medvedev et al., 1997; Watanabe et al., 2002), compared to all other species, Ni concentrations in all tissues of Mediterranean monk seals were considered to be relatively low. Vertical transmission of Ni has been reported for Saimaa ringed seals (Hyvärinen and Sipilä, 1984), where Ni levels in hairs of adult females were positively correlated with stillbirths. It is of utmost importance to constantly monitor Ni concentrations in Mediterranean monk seals in Greece, as Ni contamination may affect the reproductive activity of the species, reduce pupping rates ratio and impair population recovery. In addition, Kakuschke et al. (2005) pointed out that Ni can induce hypersensitivity reactions at blood concentrations lower than those reported in the present study. Hypersensitivity can cause severe reactions, comparable to immune depression effects. Positive correlations between Ni and Cr in blubber ($p = 0.00006$), liver ($p = 0.0034$) and kidney ($p = 0.003$) were recorded, leading to the assumption of a common source of exposure for these elements. Ni and Cr are used and are found together in fuels used in maritime traffic, which are considered an important source, not only for Ni and Cr but also for Pb and As (Legret and Pagotto, 1999; Winther and Slentø, 2010; Zaccaroni et al., 2014). The high maritime traffic in the eastern Mediterranean could be a potential source of both Ni and Cr, also explaining why these two elements are so strongly correlated. Co-exposure to Cr and Ni might lead to an additive or synergistic action of these two elements on the Mediterranean monk seals' immune system, amplifying the negative, hypersensitizing effect of both, Cr and Ni (Kakuschke et al., 2005).

4. Conclusion

This is the first assessment of trace element exposure in the endangered Mediterranean monk seal in the human-dominated environment of the eastern Mediterranean. Similar studies in the eastern Mediterranean have been carried out indicating that trace elements may have a negative effect on top marine consumers (Karapanagioti et al., 2011; Storelli et al., 2005; Vlahogianni et al., 2007). The assessment of trace element exposure alone however, i.e., without additional studies on their sub-chronic effects, can potentially provide incorrect information about their actual impact on a species' health and their role as a conservation threat. Despite this, it is important to determine the levels of trace element exposure, as they provide information on the thresholds that define the physiological and toxic values in animals (Wagemann and Muir, 1984). Unfortunately, currently only few studies

exist dealing with trace element exposure to Pinnipeds, making it difficult to accurately evaluate the significance of this threat to the survival of the endangered Mediterranean monk seal.

Trace element concentrations in the tissues of Mediterranean monk seals in our study were similar to those found in seals around the world and were considered to be generally low and below the overt toxicity thresholds reported from other pinnipeds or other species. Based on this finding we believe that exposure of Mediterranean monk seals to trace elements in Greece is currently not a major threat to the survival of the species in the country and that priority conservation efforts should focus on dealing with the major threats to the species, such as habitat destruction, deliberate killing and accidental entanglement in fishing gear (Karamanlidis et al., 2016). However, even the low trace element concentrations recorded in our study are within the ranges known to induce sub-chronic effects to the endocrine and immune system (Freeman and Sangalang, 1977; Frouin et al., 2010; Kakuschke et al., 2005) and therefore some potentially negative effects of some of the trace elements analyzed (e.g., As, Cd, Se, Ni, Cr) could not be ruled out. It is therefore important to continue monitoring trace element concentrations in Mediterranean monk seals in Greece. Interestingly, Hg, which is considered to be an important threat to several marine species, seems not to be a noxious agent to Greek monk seals (Dietz et al., 2013; Scheuhammer et al., 2015; Shoham-Frider et al., 2016; Shoham-Frider et al., 2014).

Immunodepression, which is one possible effect of trace element contamination, may expose Mediterranean monk seals to epizootics and stochastic events of mass mortality, such as the one that occurred in 1997 in the Atlantic monk seal colony at Cabo Blanco (Osterhaus et al., 1997; Osterhaus et al., 1998; Reyero et al., 2000). In addition, the simultaneous exposure to different trace elements can potentially lead to a final toxic effect which may either be additive, antagonistic or synergistic (Tchounwou et al., 2012), as shown for co-exposure to mixtures of arsenic, lead and cadmium (Wang and Fowler, 2008). Mediterranean monk seal populations have been showing recently encouraging signs of recovery, however they still remain endangered (Karamanlidis and Dendrinis, 2015; Karamanlidis et al., 2016). A detailed understanding of the species' biology and its threats is necessary to design and implement effective conservation measures. Considering the endangered status of the Mediterranean monk seal and the potentially adverse effects that trace element exposure can have on it, we believe that it is of utmost importance to integrate the analysis of trace element exposure in the standard monitoring protocol for the species. Considering furthermore the results of the study in regard to the age- and gender-related differences in the exposure to certain pollutants will help fine-tune research and management priorities, in view of establishing an effective conservation strategy that will safeguard the future of the endangered Mediterranean monk seal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.10.142>.

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