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Trace metal blood concentrations in Scopoli's shearwaters (*Calonectris diomedea*) during 2007–2014: A systematic analysis of the largest species colony in Greece

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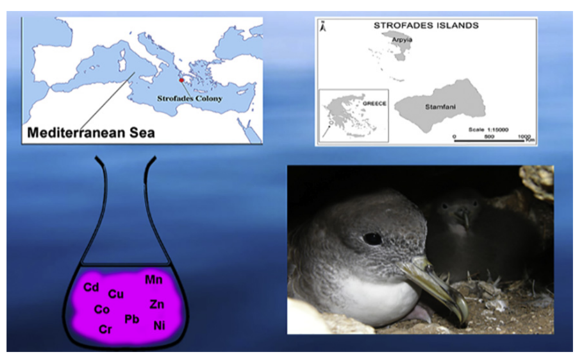
HIGHLIGHTS

- Sampled the largest colony of Scopoli's shearwaters for select trace metals
- Metal concentrations were measured in Scopoli's shearwater blood samples (N = 238).
- Covering seven breeding seasons in the Strofades Island complex, Greece
- Age- and gender- specific trace metal profiles were demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the concentrations of cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), cobalt (Co), nickel (Ni), manganese (Mn) and zinc (Zn) were investigated in the blood of Scopoli's shearwaters (*Calonectris diomedea*). Blood samples (N = 238) were collected from both juvenile and adult individuals during seven breeding seasons between 2007 and 2014, excluding 2013. Sampling was performed in the pristine environment of the Strofades island complex, Greece, where the largest colony of Scopoli's shearwaters is located in the Eastern Mediterranean basin. The median concentrations of the toxic metals, Cd and Pb, were 0.010 and 0.24 μg/g (dry weight; dw), respectively, which were in good agreement with previous studies. The median concentrations of Co, Cr, Cu, Mn, Ni, Zn were 0.18, 1.11, 3.41, 0.29, 0.61, and 22.9 μg/g dw, respectively. Inter-annual differences were observed among the concentrations of all assessed metals, except for Ni and Cd, which demonstrated similarities among female individuals. Age-group related differences were observed in both genders for Cd, Cu and Cr, but only among males for Zn. To the best of our knowledge, this is the longest multi-year biomonitoring study of select trace metals that has been conducted thus far on blood samples from Scopoli's shearwater species.

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1. Introduction

Seabirds reflect shifts in environmental conditions in a marine environment, including those occurring due to pollution pressures (Durant et al., 2009; Sponza et al., 2010; Frederiksen and Haug, 2016). Heavily industrialized/urbanized marine environments such as the Mediterranean Sea basin are impacted by chronic anthropogenic inputs of metals (Naimo, 1995; Ansari et al., 2004; Tsangaris et al., 2013). Nonetheless, metals are naturally occurring elements in aquatic systems, some of which (e.g., lead (Pb), cadmium (Cd)) are toxic to organisms (non-essential), while others (e.g. copper (Cu) and zinc (Zn)) are essential for life at trace concentrations, but again toxic above specific thresholds. Metal toxicity is influenced by several interacting factors in seawater environments such as pH, temperature and salinity (Burbidge et al., 1994). Nonetheless, scarce information is available concerning these synergistic or antagonistic effects of metals, but the synergistic toxicity can be accelerated significantly by the physiology of the marine organism involved (Stewart et al., 1996). The interactions between seabirds and their respective aquatic marine environment occur from the lowest to the upper trophic levels (Schreiber and Burger, 2002) of the ecosystem(s). Consequently, seabirds are reliable species that can be used as indicators for the ecological health of specific marine settings (Piatt et al., 2007).

Trace metals are known to accumulate in seabirds, although their biological function and effect mechanism remains largely obscure (Thompson et al., 1990). Certain seabird taxa, such as the procellariform species, including albatrosses, shearwaters and petrels, have been used up to now for the environmental monitoring of trace metals in the marine environment (Eisler, 1988, Von Schirnding and Fuggle, 1996; Pérez-López et al., 2006; Piatt et al., 2007; de Villiers et al., 2010; Cardoso et al., 2014). These seabird species can be exposed to chemicals through a multitude of pathways, including particle inhalation, digestion of specific pelagic fish species, and even by direct deposition on their feathers (which occurs mainly at their breeding grounds and/or wintering areas) (Elliott et al., 1992; Stewart et al., 1996, 1997; Monteiro et al., 1996; Thompson and Dowding, 1999; González-Solís et al., 2007; Bond and Lavers, 2010, Summers et al., 2014).

Trace metal concentrations are often reported for adult seabird individuals, but not often for chicks or fledglings (Walsh, 1990). Nonetheless, juveniles have been proposed as particularly useful indicators for pollution, as they demonstrate increased accumulation of metals during their time period spent in the breeding colonies (i.e. hatching to fledging) and the foraging areas (Walsh, 1990; Le Corre et al., 2012; Soanes et al., 2016; Karris et al., 2018a). Metal pollution has been previously studied in the Mediterranean basin region, mainly by monitoring seabird and waterfowl eggs (Fossi et al., 1984; Lambertini and Leonzio, 1986; Focardi et al., 1988; Leonzio et al., 1989; Ayaş et al., 2008; Pereira et al., 2019). In addition, a limited amount of references exists on trace metal concentrations (from eggs and feathers) of colonial seabirds that breed in the Aegean colonies of Greece (Ristow et al., 1992; Goutner et al., 2000, 2001, 2013; Escoruela et al., 2018). Moreover, relevant reports concerning trace metal concentrations in seabird blood samples originating from Hellenic colonies are lacking. In this context, this study focuses on investigating the occurrence and profiles of select trace metal concentrations, including those of lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn), over seven breeding seasons (spanning from 2007 to 2014), in blood samples from Scopoli's shearwater (*Calonectris diomedea*) in the pristine environment of the Strofades island complex (located in the Eastern Ionian Sea basin).

2. Materials and methods

2.1. Study area and species

The study area was the Strofades island complex (37°15′ N, 21°00′ E), located at the Eastern Ionian Sea; comprising of two small uninhabited flat islets (22 m above sea level), namely, Arpyia in the north and Stamfani in the south, and numerous rock formulations between those (Fig. 1). The remote insular area is located 32 nm south of Zakynthos Island and 26 nm west of the Peloponnese (mainland Greece), covering an area of 4 km^2 that constitutes part of the National Marine Park of Zakynthos. Updated estimates raise the Hellenic population of Scopoli's shearwater to 8400–10,000 pairs, while the study area hosts 50–60% of those, and currently constitutes the largest colony of the species in the Eastern Mediterranean basin (Karris et al., 2017). Fieldwork was carried out on the largest island (i.e. Stamfani 2.6 km^2) involving three study coastal breeding sectors, namely, the west, south and east coast of the islet, where different types of nesting shearwater habitats occur in terms of topography and vegetation, e.g., burrows under fallen boulders and bushes excavated by the birds, nests in crevices under rocks and natural deep cavities of coastal cliffs.

The Mediterranean Scopoli's shearwater is a pelagic, long-lived, monogamous, migratory seabird, with particularly high degree of site tenacity (Cramp and Simmons, 1983; Anselme and Durand, 2012). The species diet consists of pelagic and mesopelagic fishes, squids, crustaceans and occasionally zooplankton (Monteiro et al., 1996; Alonso et al., 2012; Neves et al., 2012; Afán et al., 2014), but also trawl fishery discards, which are mainly benthopelagic prey species and can be characterized as actually unavailable due to the foraging ecology of Scopoli's Shearwater (Karris et al., 2018b). Small prey is normally found in shallow waters and near reefs and is usually taken by shearwaters near the sea surface (Zino, 1971; Mougin et al., 1977; Granadeiro et al., 1998; Belda and Sánchez, 2001). The major threats of Scopoli's shearwater in its breeding grounds are: i) predation of eggs and chicks by invasive mammals (Pascal et al., 2008; Ruffino et al., 2009); ii) accidental entrapment (by-catch) in fishery gears (Belda and Sánchez, 2001; Ramos et al., 2003; Catry et al., 2006; García-Barcelona et al., 2010; Karris et al., 2013a; Báez et al., 2014); iii) human disturbance such as light pollution (Rodríguez et al., 2015); iv) marine pollution (Ristow et al., 1992; Roscales et al., 2010, 2011); and v) plastic debris (Codina-García et al., 2013).

2.2. Field sampling and sample handling

Fieldwork was carried out during the end of the fledging period (late September-mid of October) of the years 2007–2012 and 2014 as previously performed by Karris et al. (2018a). Overall, 238 individuals were extracted from their nesting burrows (i.e. 56 breeders and 182 fledglings, while 112 of them were males and 114 females according to gender determination) with either the aid of a projecting hook-like improvised tool or were captured on the ground by hand. From every captured individual, the weight was initially measured by using an electronic balance, and thereafter blood sampling followed. A handler extended the wing to expose the branchial vein and a sampler collected an amount of 0.2–0.5 ml of blood with a heparinized 28-gauge syringe. Blood was transferred and kept in Eppendorf vials (1.5 ml) with ethyl alcohol as preservative. After blood collection, the shearwaters were placed back to their nest burrows, while blood samples were kept in boxes with ice and transported directly at −8 °C to the laboratory for storage at −20 °C until further sample processing.

2.3. Sample extraction

Sample digestion was performed using a Teflon coated block digestion system (by Savillex, Minnesota, U.S.). The total amount of blood samples was transferred into polypropylene digestion tubes and the tubes were then placed on the block digestion system (set at 40 °C for 45 min) to evaporate ethyl alcohol (and the dry weight of the blood samples was noted). Thereafter, maintaining the samples at 60 °C for 60 min (on the digestion system), 1 ml of concentrated nitric acid (HNO_3) p.a. was added to each sample. After the sample digestion process was completed, the tubes were diluted up to a total volume of 5 ml

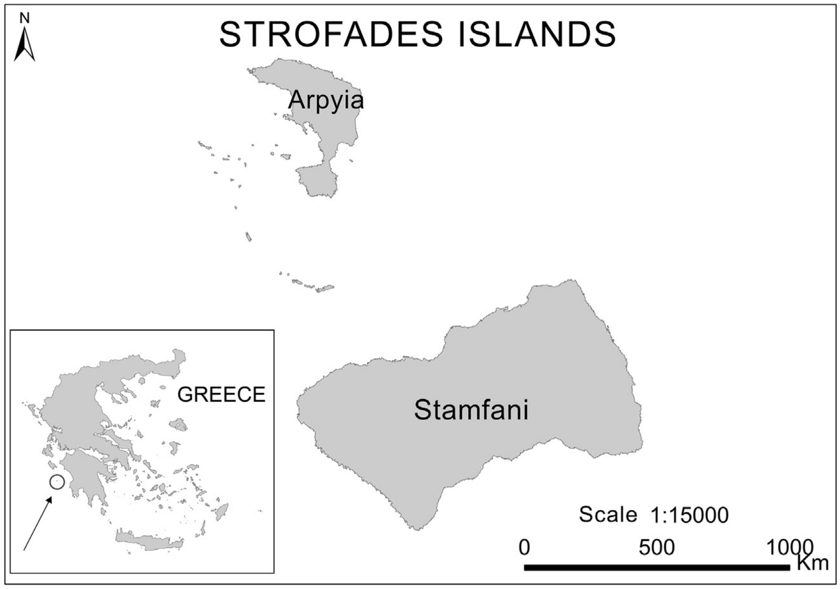

Fig. 1. Location of the study area in the South Ionian Sea, Greece.

by adding 0.2% nitric acid solution. Certified reference material (Seronorm™ Trace Elements Whole Blood, Sero, Billingstad, Norway) (duplicates; n = 2) and corresponding field blanks (n = 2) were analyzed for every sample batch (40 samples). Method blanks (n = 2) were also prepared for every batch and were treated in a similar manner as actual samples. The recoveries obtained for each metal based on fortified samples and the certified reference material were: Cd: 93.5–97.2%, Co: 90.1–94.7%, Cr: 98.4–102.5%, Cu: 95.2–101.0%, Mn: 94.3–98.0%, Ni: 91.2–95.4%, Pb: 90.8–93.1% and Zn: 102.4–105.1%.

2.4. Instrumental analysis

Analysis of Cd, Co, Cr, Cu, Mn, Ni and Pb was performed by graphite furnace atomic absorption spectroscopy, while the analysis of Zn was performed by flame atomic adsorption spectroscopy. 1 ml of each extract was transferred for graphite furnace atomic absorption spectroscopy (AS-800 THGA Furnace, Perkin Elmer. Massachusetts, U.S.) analysis. Calibration curves were prepared by using a commercial standard solution mixture of metals, specific for atomic absorption measurements (Merck CertiPUR Inorganic Mixes, Massachusetts, U.S.). The Analytik Jena nov AA 350 (Jena City, Germany) flame atomic adsorption spectroscopy instrument was used for the analysis of Zn (1 ml of each diluted sample), using the internal standards kit (Analytik Jena Company; Jena City, Germany) at the beginning and at the end of the analytical procedure. Solvent blanks were measured after every batch of 10 samples to monitor for carry-over effects. The limits of detection (LODs) were calculated for each target analyte as three times the signal from the baseline noise (S/N ratio; LOD = 3 S/N); Cd 0.38 10^{-3}, Co 8.28 10^{-3}, Cr 6.55 10^{-3}, Cu 8.28 10^{-3}, Mn 2.00 10^{-3}, Ni 6.90 10^{-3}, Pb 13.4 10^{-3}, and Zn 95.9 10^{-3} μg/g, while the limits of quantification (LOQs) were estimated as three times the LODs. The uncertainty (precision; RSD%) at the LOQ of each element was <20%.

2.5. Statistical treatment

All data were checked for normality by a set of two tests (Kolmogorov-Smirnov and Shapiro-Wilk) and pairwise correlation coefficients were applied to obtain relevant correlation matrix and identify possible associations between selected variables. One-way analysis of variance was used to determine significant interannual differences of metal concentrations and establish profile differences between age classes (juveniles and adults) and genders. Gender determination was achieved by following a polymerase chain reaction-based methodology combined with morphometric variables (Karris et al., 2013b; Appendix A).

Principal Component Analysis (PCA) was ultimately performed for the investigation of all variables (elements and body weight). Independent variables, including gender, age class and year of sampling were used to illustrate the results of the analysis on PCA bi-plots. Data handling was performed with the SPSS statistical package (IBM SPSS statistics 20 software) for descriptive and correlation statistics and the R (version 3.5.1) open source software package (R Development Core Team, 2015) for PCA.

3. Results and discussion

Metal concentrations in Scopoli's shearwater blood samples collected during 2007–2014 in the Strofades island group are presented in Table 1. Metal concentrations in circulating blood reflect recent exposure to the source of pollution through diet or accumulation (Furness, 1993; Kahle and Becker, 1999). The rank order of median concentrations of target metals in blood samples was: Zn (22.9) > Cu (3.41) > Cr (1.11) > Ni (0.61) > Mn (0.29) > Pb (0.24) > Co (0.18) > Cd (0.010 μg/g dw). The results are demonstrated by gender, age group and year of sampling in Table S1; Supplementary data. All relevant statistical tests demonstrated that the assumption of normality among elemental concentrations was not obtained ($P < 0.05$), and therefore relevant data were analyzed by using non-parametric tests. Spearman's correlation test revealed negative but no significant relationship between body mass and the concentrations of each metal, except for Cd ($r_s = -0.32$; $P < 0.01$) (Table S2; Supplementary data). Furthermore, significant inter-annual differences were detected in the concentration of all the metals examined (Kruskal-Wallis H test; $P < 0.001$; Fig. 2), except for Ni (Kruskal-Wallis H = 3.09; $P = 0.79$) and Cd among female individuals (multiple comparison post-hoc Kruskal-Wallis H = 10.3; $P = 0.11$). Regarding age and gender, statistically significant differences were detected for certain metals (Cd, Cr, Cu, Ni, Zn) among the two age groups (Table S3; Supplementary data). Significantly higher concentrations of Cd, Zn and Ni were found in adults compared to those found in juveniles (Kruskal-Wallis H tests $P < 0.05$), and these differences for Cd were consistent in both genders (Table S3; Supplementary data). These results were in accordance with relevant studies in other seabird species, e.g., Black-headed gull (*Larus ridibundus*) juveniles had statistically significant lower Cd accumulation in muscle, liver, lung and femur compared to adults (Orłowski et al., 2007). On the

Table 1
Concentrations of metals in 238 Scopoli's shearwater blood samples collected during 2007–2014 in the Strofades island complex, expressed as μg/g of dry weight. Non-detects are not included in the calculations of descriptive statistics.

	N (detected out of 238)	Detection frequency (%)	Mean	Median	Min.	Max.	Range (25%–75%)
Cd	125	52.5	0.040	0.010	<LOD	0.50	0.010–0.030
Co	104	43.7	0.40	0.18	<LOD	2.48	0.090–0.45
Cr	228	95.8	2.90	1.11	<LOD	78.0	0.31–2.87
Cu	235	98.7	9.11	3.41	0.040	211	1.70–9.53
Mn	217	91.2	0.73	0.29	0.010	30.4	0.11–0.57
Ni	165	69.3	5.47	0.61	0.030	203	0.21–0.62
Pb	91	38.2	0.81	0.24	<LOD	26.5	0.080–0.69
Zn	150	63.0	27.9	22.9	0.28	132	8.94–39.5

contrary, significantly higher concentrations of Cr and Cu were found in juveniles compared to adults (Kruskal-Wallis H tests $P < 0.05$), and these differences were consistent for both genders (Table S3; Supplementary data). In many relevant toxicological studies, blood samples were used to examine exposures to metals in seabirds (Wilson et al., 2004; Summers et al., 2014; Lerma et al., 2016; Fenstad et al., 2017). Blood concentrations of elements in birds are commonly reflected to current dietary preferences (Evers et al., 2008; Wayland and Scheuhammer, 2011). In avifauna, metals such as Pb and Cd are absorbed into the bloodstream and rapidly accumulate into the bones and growing feathers as well as to soft tissues, including liver and kidneys (Franson and Pain, 2011; Wayland and Scheuhammer, 2011). Overall, the results from all years collectively were lower compared to relevant blood concentrations of metals in other similar seabird species (Table 2). Among the seven breeding seasons, the metal concentrations in 2008 were the highest. Thresholds in contour feathers for metal adverse effects for seabirds have been previously defined, e.g., 4 μg/g for Pb and 2 μg/g for Cd (Burger and Gochfeld, 2000a, 2000b, 2002), but direct comparison with blood concentrations found in this study was avoided.

The strong negative association of body weight with Cd that was established, was found in accordance with previous studies on shearwaters (Ishii et al., 2017). It is worthwhile to mention that in our study fledglings represented the majority (80%) of the sampled individuals. Thus, this significant negative correlation between Cd and weight can be attributed to the fact that breeders mainly feed chicks with squids during the early rearing phase (Field Observation by Karris), which contain high Cd concentrations due to bioaccumulation capacity of Cd in the digestive gland of cephalopods (Bustamante et al., 1998). It is also noteworthy to report that as the fledging period progresses, and chicks increase their weight, breeders shift their meals to fish, and consequently, chicks demonstrate lower concentration of Cd in blood while it is gradually circulated to the growing feathers and tissues of the adult. Cd concentrations in Scopoli's shearwaters (0.010–0.020 μg/g) were comparable to other seabird populations, e.g., located in Marion Island, South Africa (range: 0.040–0.30 μg/g) (Table 2). Apart from the various well-established factors that may influence metal concentrations in marine feeding areas, such as the existence of wastewater effluent pressures (Topcuoğlu et al., 2002), another less-established parameter, is the season of foraging activity by the parent birds. It is notable that food provision to nestlings begins from mid-July, just after egg hatching, and lasts up to a few days before fledgling (late September-early October). This coincides with the time period where increased Cd concentrations are found in ichthyofauna in the Eastern Mediterranean, which spans from September to November (Galitsopoulou,

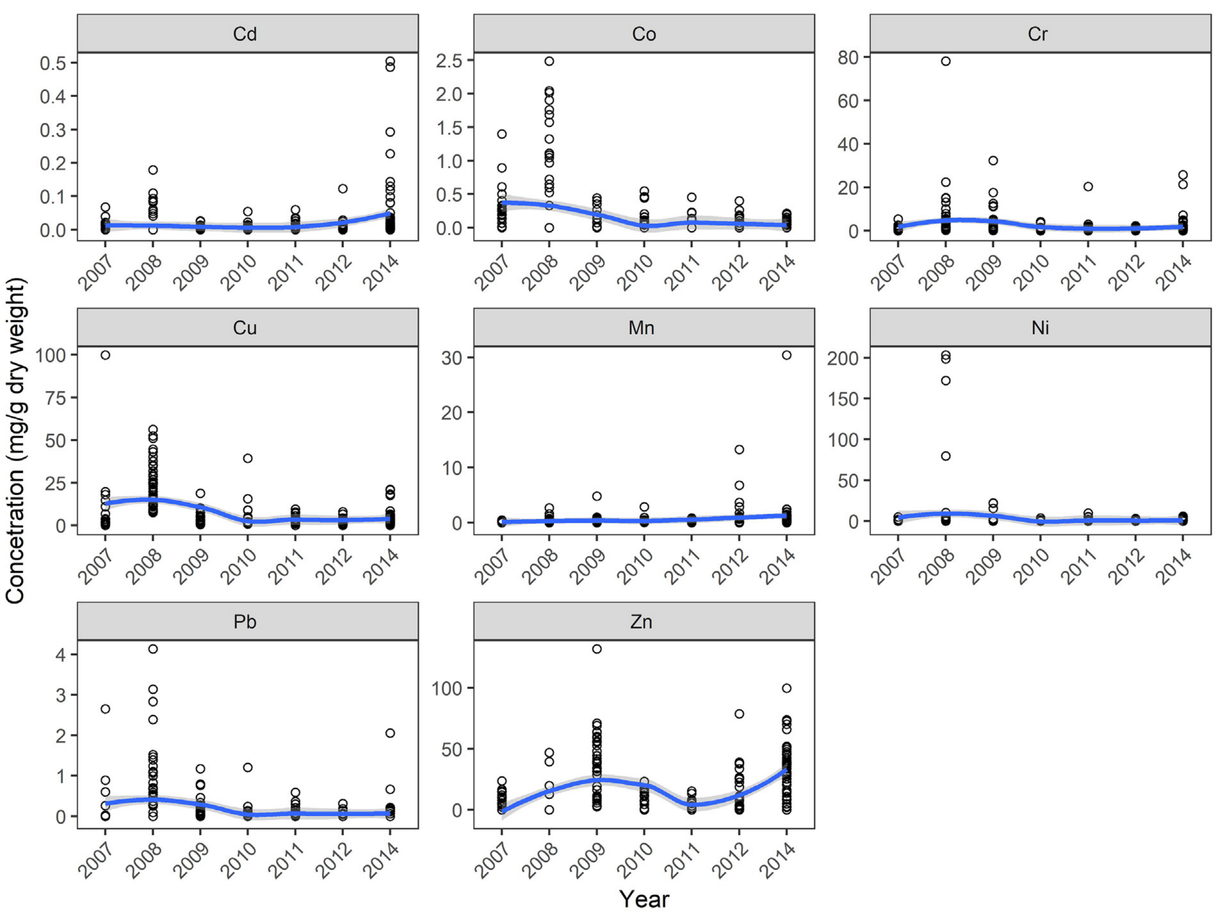

Fig. 2. Trace metal concentrations per sampling year in Scopoli's shearwater blood samples collected during 2007–2014. The blue lines go through the mean values of concentrations per year.

Table 2
Comparative table of different seabird species, metal concentrations and type of sampled tissue.

Species	Tissue	Cd	Pb	Cu	Zn	Co	Ni	Mn	Cr	Area	Reference
Calonectris diomedea	Kidney (μg/g)	11.2 ± 8.74		21.1 ± 7.99	110 ± 32.3					Azores, Portugal	Stewart et al. (1997)
Calonectris diomedea	Kidney (μg/g)	9.3 ± 10.0		12.5 ± 3.54	114 ± 24.3					Pico Is., Portugal	Stewart et al. (1996)
Calonectris diomedea	Liver (μg/g)	3.03 ± 1.72		18.8 ± 5.29	198 ± 5.29					Azores, Portugal	Stewart et al. (1997)
Calonectris diomedea	Liver (μg/g)	2.03 ± 2.78		13.2 ± 7.38	176 ± 48.6					Pico Is., Portugal	Stewart et al. (1996)
Phoebetria fusca	Blood (μg/g)	0.19 ± 0.15	0.03 ± 0.02							Marion Is., S. Africa	Summers et al. (2014)
Phoebetria palpebrata	Blood (μg/g)	0.04 ± 0.02	0.05 ± 0.01							Marion Is., S. Africa	Summers et al. (2014)
Diomedea exulans	Blood (μg/g)	0.30 ± 0.12	0.14 ± 0.15							Marion Is., S. Africa	Summers et al. (2014)
Oceanodroma leucorhoa	Liver (μg/g)			20.7 ± 1.49	155 ± 12.6			15.8 ± 2.27		Gull Is., Can. Atl. Coast	Elliott et al. (1992)
Oceanodroma leucorhoa	Liver (μg/g)			20.7 ± 2.81	173 ± 40.3			16.0 ± 1.00		Kent Is., Can. Atl. Coast	Elliott et al. (1992)
Oceanodroma leucorhoa	Liver (μg/g)			17.8 ± 2.03	138 ± 14.6			17.3 ± 1.37		Ile Is., Can. Atl. Coast	Elliott et al. (1992)
Fratercula arctica	Liver (μg/g)			15.3 ± 0.69	99.5 ± 15.9			8.87 ± 1.55		Gull Is., Can. Atl. Coast	Elliott et al. (1992)
Fratercula arctica	Liver (μg/g)			23.3 ± 3.13	91.1 ± 8.94			11.3 ± 1.3		Ile Is., Can. Atl. Coast	Elliott et al., (1992)
Puffinus carneipes	Feathers (ng/g)	288 ± 816	515 ± 367	18,382 ± 3053	92,244 ± 33,945	257 ± 102	2649 ± 3593	2189 ± 1336		W. Australia	Bond and Lavers (2010)
Puffinus nativitalis	Feathers (ng/g)	950 ± 429	2380 ± 531					2050 ± 485	2350 ± 485	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Gygis alba	Feathers (ng/g)	216 ± 36.0	1380 ± 693					410 ± 53.2	1300 ± 102	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Sula sula	Feathers (ng/g)	51.3 ± 5,0.58	975 ± 97.9					1460 ± 314	2530 ± 576	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Phoebastria immutabilis	Feathers (ng/g)	364 ± 103	799 ± 5.8					1720 ± 255	6570 ± 2280	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Phoebastria nigripes	Feathers (ng/g)	152 ± 25.8	973 ± 125					1780 ± 195	1420 ± 160	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Fregata minor	Feathers (ng/g)	204 ± 127	1500 ± 143					590 ± 79.7	1120 ± 221	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Phaethon rubricauda	Feathers (ng/g)	55.2 ± 7.97	684 ± 115					678 ± 152	1670 ± 92.2	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Onychoprion fuscatus	Feathers (ng/g)	73.4 ± 6.62	519 ± 47.9					300 ± 53.0	502 ± 31.5	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Anous stolidus	Feathers (ng/g)	274 ± 207	289 ± 63.9					424 ± 148	5860 ± 1420	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Pterodroma hypoleuca	Feathers (ng/g)	129 ± 28.7	1350 ± 291					561 ± 196	1620 ± 69.5	Northern Pacific Ocean	Burger and Gochfeld (2000b)
Onychoprion lunatus	Feathers (ng/g)	95.0 ± 10.0	942 ± 312					1120 ± 340	820 ± 95.0	Northern Pacific Ocean	Burger and Gochfeld (2000b)

2014). The effect of seasonal foraging activity in the accumulation of Cd is attributed, mainly on physiologic differentiations of fish catches, throughout the year (Özden, 2013). Moreover, gender differentiation of Cd concentrations was demonstrated and was attributed to diet differences (Ishii et al., 2017). In some cases, male and female individuals in both sexually monomorphic and size dimorphic seabirds use different foraging areas, and this may be partially explained by clear differences in the reproductive roles during specific stages of the breeding cycle (Hedd et al., 2014; Matsumoto et al., 2017). Shearwater breeders employ a dual foraging strategy to serve the nutrition of their chicks and meet their own energy demands (Magalhães et al., 2008; Cecere et al., 2013). Possibly these different foraging areas can be characterized by different pollution loads. Therefore, foraging behavior of both genders must be verified with further tracking studies on Strofades breeders as well as elemental analysis on their diet items captured within their main foraging areas throughout the whole breeding period.

Regarding Pb, there is a substantial body of literature describing the effects of Pb contamination on seabird behavior and physiology (Burger and Gochfeld, 2000a, 2000b, 2002). In the present study, Pb blood concentrations ranged between 0.070 and 0.53 μg/g and were comparable to relevant results from other seabird species colonies, e.g., located in the Marion Island (0.034 μg/g–0.14 μg/g) (Table 2). Overall, Pb concentrations were higher than those of Cd; an observation consistent with previous results on related seabirds from different colonies (Table 2).

The absence of significant associations of the remaining trace metals with body mass can reflect either low metabolic rates (Colwell, 2010) or consumption of prey species of lower biomass as it is observed during trawler discarding (Karris et al., 2018b). However, it is suggested that when trace metal concentrations are not correlated with the body mass, it reflects of low pollution pressures in the corresponding marine habitats (Kim et al., 1996). Concentrations of Co, Cr, Cu, Mn, Zn and Ni were lower when compared to other biological media from related seabird species (Table 2). Zn concentrations were ranging from 0.28 to 132 μg/g dw. Zn is important during the fledging stage (Migula and Augustyniak, 2000) since it has been shown experimentally with poultry that higher demands of Zn are needed for feather growth, and potential deficiency in Zn can cause a frayed feather condition (Sunde, 1972). Consequently, juveniles herein showed significant lower blood concentrations of Zn compared to adults (16.9 vs 39.2 μg/g dw) potentially due to the higher demands of feather growth.

The PCA analysis (Fig. 3, Figs. S1 and S2; Supplementary data) was applied to identify the patterns and associations within the specific data set. It depicted a positive relationship of Cd and Zn, which is consistent with previous reports (Ishii et al., 2017), while it demonstrated the negative association between Cd and weight. Moreover, these metals are expected to interact with metallothionein (MT), a low molecular weight cysteine-rich protein involved in the homeostasis of essential metals. The synthesis of MT can be induced by several metals, such as Cd while Zn is essential for the synthesis of MT. When Cd is up taken, Zn is replaced by Cd in MT to achieve detoxification. It is reported that Cd and MT concentrations are positively associated in many seabirds (Elliott et al., 1992; Stewart et al., 1996) since high accumulation of Cd induces the synthesis of MT. Furthermore, from the PCA bi-plots it is evident that Pb and Cu demonstrated higher contributions to many samples that were collected during 2018. Higher Zn and Pb positive contributions were depicted for adult male and juvenile female individuals, respectively.

4. Conclusions

This work herein demonstrated the concentration profiles of Cd, Pb, Cr, Cu, Co, Ni, Mn and Zn between 2007 and 2014, excluding 2013 in the blood of Scopoli's shearwater species. This study highlighted the use of this species as a potential marker of current metal pollution. The Eastern Mediterranean basin and more specifically the pristine Ionian Sea area face new challenges due to increasing activities from the forthcoming developments of the energy industry in the area, including the construction of offshore wind farms (Bagiorgas et al., 2015; Soukissian et al., 2017) and hydrocarbons exploration (Karakitsios, 2013; Tsirambides, 2015); currently, Hellenic authorities are launching bidding processes for hydrocarbon exploratory and exploitation rights in the Ionian Sea marine area. Offshore windfarm developments are associated with numerous environmental concerns, including toxic metal pollution from increased vessel traffic and the shuffling of seabed sediments during developing and decommissioning procedures (Bailey et al., 2014; Topham and McMillan, 2017). In addition, oil-gas exploration and exploitation activities increase even more the pollution pressures towards the Ionian Sea marine environment (Cordes et al., 2016).

Further work on Scopoli's Shearwater species should focus on their diet. Assessing the chick's diet, including the analysis of regurgitations, could provide insights on the dynamics and accumulation of metals in the study population. This, in turn, would allow a greater insight into the baseline metal concentrations in a relatively non-polluted environment such as the remote Strofades island complex, Greece.

Animal ethics permit

Research expeditions of the current study had specific permit every year (breeding season) from the Management Agency of the National Marin Park of Zakynthos (NMPZ), which as public service belongs to the Greek Ministry of Environment and Energy (e.g. ref. no. 579/NMPZ; 180468/657/Ministry of Environment and Energy). Additionally, the Natural History Museum of Crete (scientific institution code GR002), partner of the current research study, has a CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) sampling permit for wildlife (ref. no. 096860/2199/23-8-2005). All the relevant activities-samplings were carried out ensuring the maximum safety of Scopoli's Shearwater as well as the least possible disturbance to adults and fledglings of the target species and its breeding habitat.

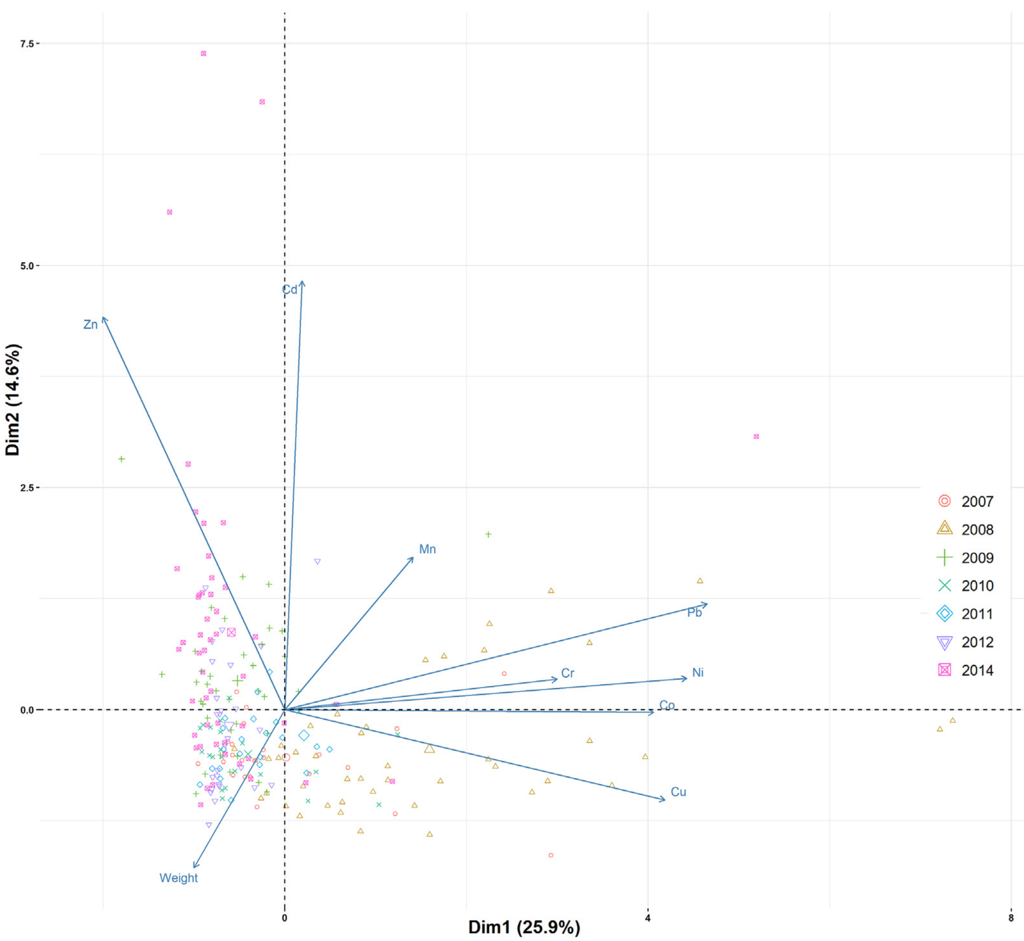

Fig. 3. Result of principal component analysis. xx′ axis shows component 1 and yy′ axis shows component 2. The blue arrows indicate the distribution of the tested metals. Samples per breeding season are also depicted with different colors.

Novelty statement

This is the first report for the biomonitoring of select trace metals in blood samples from the largest colony of Scopoli's shearwaters, covering seven breeding seasons. The study provides baseline data for the Ionian shearwaters that are located in the pristine environment of the Strofades Island complex, Greece, before the initiation of the forthcoming local developments from the wind and oil energy industry.

Declaration of Competing Interest

We have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.07.082.

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