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Trace elements in stomach oil of Scopoli's shearwater (*Calonectris diomedea*) from Linosa's colony

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ABSTRACT

Calonectris diomedea is a colonial Procellariiform breeding on Mediterranean islands. The stomach oil produced during chick rearing is a peculiar trait of this species. The composition of the stomach oil is likely to reflect the composition of the prey ingested and might reveal the contaminants uptake with prey becoming a possible tool for the marine pollution monitoring. We examined the concentration of 15 trace elements by ICP-MS and direct mercury analyser. The principal component analysis revealed a heterogeneous pattern of metal concentration, showing a significant separation between samples collected 20 and 70 days after hatching. The data obtained in this work give preliminary information on the feeding habits and breeding ecology of Linosa's colony of Scopoli's shearwater. The trace metals variability found suggest that the stomach oil may have a role as trophic markers to understand predator-prey relationships and to have evidence on the accumulation of pollutants in the latter.

1. Introduction

Seabirds' life-history traits are determined by the spatial and temporal variability of food resources in the marine environment (Ricklefs, 1990). Some species of seabirds nest in rock crevices and burrows under rocks or soil (Brooke, 2004; Ramos et al., 1997; Warham, 1990).

The Scopoli's shearwater (*Calonectris diomedea*) is a long-distance migrant and colonial Procellariiform breeding on Mediterranean islands (Sangster et al., 2012). The second-largest Scopoli's shearwater colony is in Linosa Island (Baccetti et al., 2009).

The two mates share the incubation (laying only one egg) and chick-rearing (Cecere et al., 2013). Apart from the incubation period, Scopoli's shearwaters visit the colony mainly at night to feed their chicks (Rubolini et al., 2014). In Atlantic waters, the diet of Cory's shearwater *Calonectris borealis*, a species close to the Scopoli's shearwaters (Sangster et al., 2012), comprises predominantly epipelagic and mesopelagic fish and squid (Thibault et al., 1997) which are capable of accumulating pollutants such as trace metals (Anan et al., 2005; Shalini et al., 2020; Carravieri et al., 2020). Contaminant uptake varies to some extent depending on the variability of the diet, both between individuals and across years (Furness and Camphuysen, 1997). Several methods are used to investigate the diet of seabirds; these include both the conventional sampling of food regurgitation, contents of pellets regurgitated by adults and samples offloaded from chicks by "stomach-pumping" (Furness and Camphuysen, 1997; Jarman et al., 1996).

The parents of Scopoli's shearwater feed their chicks with an oily paste obtained from the mechanical rupture of the prey in the proventriculus (Clarke and Prince, 1976; Roby and Place, 1997; Wang et al., 2007). Although quantitative work on trace metals has not been done, close resemblances have been noted between the chemical composition of Procellariiform stomach oil and their preys (Cheah and Hansen, 1970; Wang et al., 2007).

Given this, the feeding behavior of Scopoli's shearwater and the analysis of stomach oil could be useful tools for the monitoring of heavy metal pollution in marine environment (Cherel and Weimerskirch, 1995; Furness and Camphuysen, 1997). This work aimed at assessing the trace elements contents in the stomach oil collected from Scopoli's shearwater chicks of Linosa Island to deepen the possible risks of toxic metals accumulation in their chicks and have a possible description of the trace metals pollution levels of the study area.

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2. Materials and methods

2.1. Sampling plan

Fieldwork was carried out during the breeding season of 2017 in Linosa island (South Mediterranean Sea, Southern Italy, 35°52′30.2″N 12°52′13.5″E, Lat. 35.875056, Long. 12.870417) from July to October. Nests were monitored since egg laying period. Egg deposition occurred between 23 and 27 July. The stomach oil sampling occurred at 20th and 70th day after deposition for each nest/chick.

The chick rearing period last about 90 days (Becciu et al., 2011). Eight nests were monitored by sighting since the laying and hatching of the eggs. The stomach oil was collected from chicks by water off-loading technique using a disposable syringe with a silicon vesical catheter at 20 and 70 days after hatching according to the procedure reported by Connan et al. (2005). About 1 ± 0.5 g of stomach oil was collected from each chick and stored in 1.5 mL polypropylene microtubes. The stomach oil samples collected at day 20 (Fig. 1a) were characterized by a range of colours between deep orange and black due to the presence of calcareous fragments, probably attributable to mussel valves. The stomach oil samples collected at day 70 showed a range of colours between deep yellow and light orange (Fig. 1b). All the samples were transported at +4 °C to the laboratories and stored at −20 °C until the analysis. The analysis was conducted in November 2017.

2.2. ICP-MS analysis

Water for trace metals analysis Suprapur® was obtained by Carlo Erba Reagents S.r.l. (Cornaredo, Italy). Standard stock solutions (1000 ppm) of 14 metals (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Pb) and ultrapure nitric acid were provided by Merck (Darmstadt, Germany). Tuning solutions for ICP-MS, capable of covering a wide range of masses (Ce, Co, Li, Mg, Tl, and Y 1 μg/l) were purchased from Agilent Technologies (Santa Monica, CA, USA). Ultrapure grade carrier gas Ar, He, H_2 were purchased from SOL S.p.a. (Monza, Mi, Italy).

The detection of metals was carried out by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The extraction of the samples was performed according to protocols reported before (Cammilleri et al., 2019b; Lo Dico et al., 2018). Briefly, approximately 1 g of the samples was transferred into decontaminated vessels with 3 mL of 60% (V/V) ultrapure nitric acid and 5 mL of deionized water. Subsequently, the samples were subjected to microwaves digestion by a Multiwave 3000 digester (Anton Paar, Graz, Austria) with a power ramp of 600 W in 10 min. Then, a power of 600 W was maintained for 10 min. Finally, the vessels were cooled for 15 min. All the analytes were determined using a 7700x series ICP-MS (Agilent Technologies, Santa Monica, CA, USA). The quality protocol included calibration with ≥98% pure standards, analysis of certified reference materials (DORM-4, fish protein) and duplicate samples. The method was validated by an in-house model according to the EC Regulation 657/2002.

2.3. Hg analysis

The Hg determination was carried out by a DMA-80 thermal direct mercury analyser (Milestone GmbH, Germany). The quality assurance protocols included a calibration with ≥98% pure standards and the analysis of certified reference materials from proficiency tests (Fapas, York, UK). About 0.1 ± 0.001 g of the samples was put onto nickel vessels, and introduced to the analyser (Cammilleri et al., 2019a). A calibration curve based on five concentration points (from 0.050 to 2 mg/kg) was carried out.

2.4. Data collection and statistical analysis

The results were expressed as mg/kg wet weight (w.w.). All the results under the limit of quantification (LOQ) of the method were considered for the statistical analysis as half of the LOQ values (Helsel, 2005).

All the variables were pre-treated before principal component analysis by Pareto-Scaling (van den Berg et al., 2006). A total of 2 principal components were selected after Kaiser–Harris criterion, Cattel Scree test and parallel analysis (n.iter = 100) (Kabacoff, 2015). Analytes with constant values (Cd and Co) were removed from the dataset. Statistical analysis was conducted with R software (3.6.2) using the R packages: Rcmdr, Leaflet for R and FactoMiner (Kassambara, 2017). The contribution of the variables was calculated as follow:

$$\text{Contrib} = [(C_1{*}E_1) + (C_2{*}E_2)]/(E_1 + E_2) \quad (1)$$

where C_1-C_2 are the contribution of variables and E_1-E_2 are the eigenvalues of the corresponding PC.

3. Results

3.1. Trace metals composition

The trace metals distributions of the samples examined are shown in Fig. 2. At day 20, the mean contents of trace elements followed the order Al > Fe > As>Sn > Zn > Ag > Sr > Cu > Se > Pb > Cr > Ni. No detectable Cd, Hg, and Mn levels were found. Aluminium was the most abundant element, showing a mean of 45.58 ± 14.23 mg/kg and a maximum of 67.19 mg/kg, followed by Fe (7.36 ± 8.04 mg/kg). Conversely, Pb, Cr, and Ni were the less abundant elements, with mean values of 0.052 ± 0.01 mg/kg, 0.04 ± 0.01 mg/kg, and 0.03 ± 0.02 mg/kg, respectively.

The samples of day 70 were characterized by the presence of Hg and Mn, showing mean concentrations of 0.47 ± 0.14 mg/kg and 0.23 ± 0.07 mg/kg, respectively, and a higher amount of Fe (24.22 ± 6.29 mg/kg), becoming the most abundant element. A relevant decrease of Al

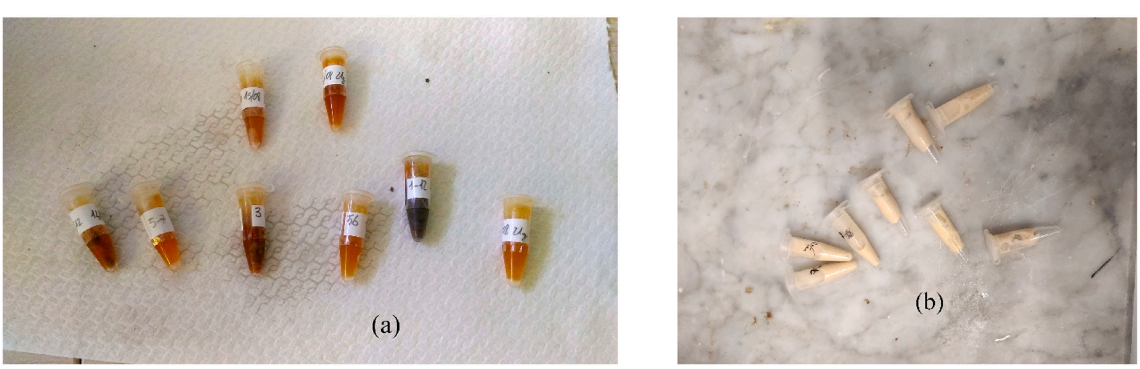

Fig. 1. Stomach oil samples of Scopoli's shearwater (*C. diomedea*) collected 20 days (a) and 70 days (b) after hatching. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

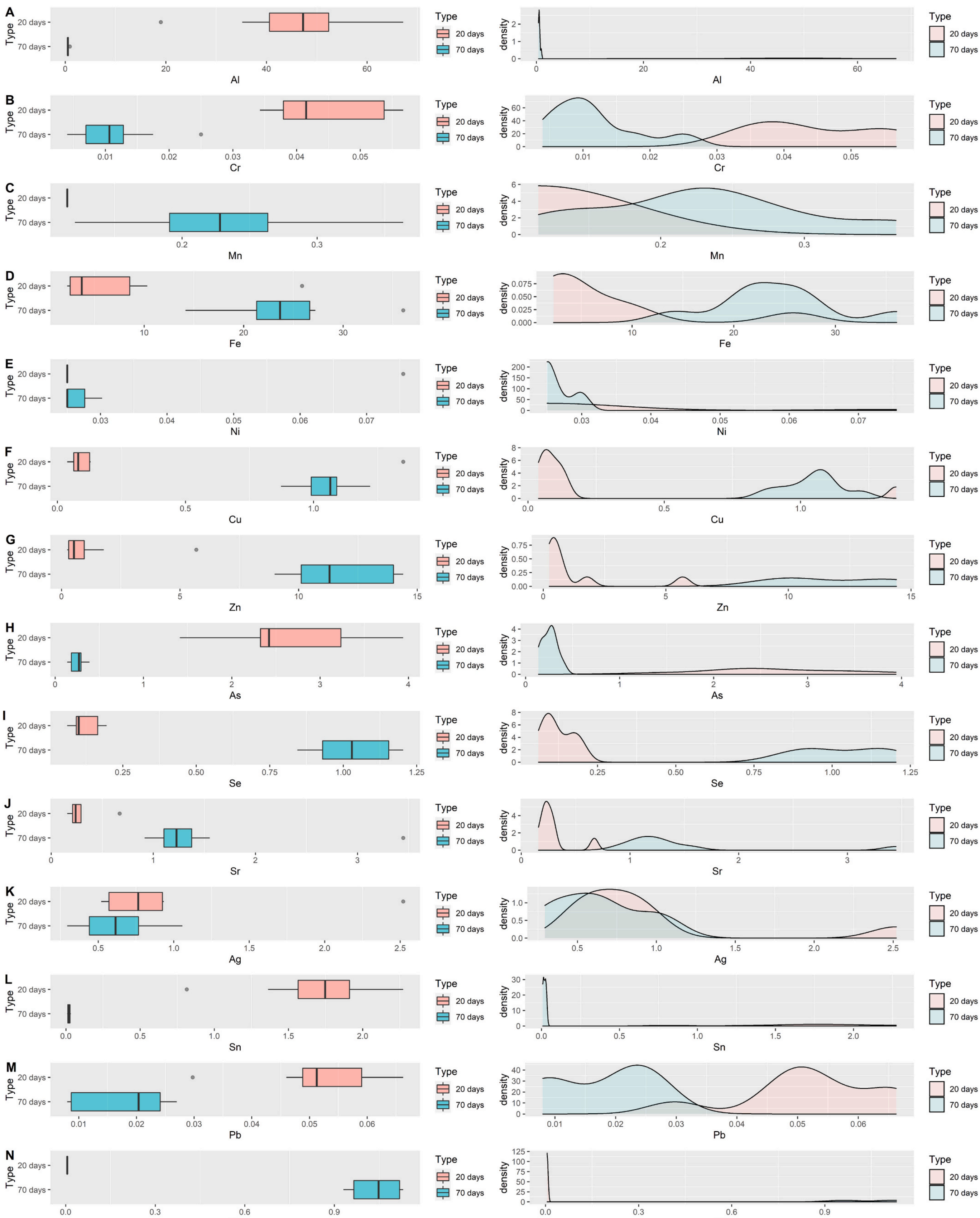

Fig. 2. Boxplot and distribution plot for each element divided for group (20 and 70 days).

contents was found (0.55 ± 0.16 mg/kg), showing mean values eighty times lower than samples of day 20. The mean contents of trace elements at day 70 followed the order Fe > Zn > Sr > Cu > Hg > Se > Ag > Al > As>Mn > Ni > Sn > Pb > Cr. Similarly to day 20, no Cd contents were found. Pb and Cr were the less abundant elements of day 70 samples, in accordance with the samples of day 20, showing mean values of 0.02 ± 0.008 mg/kg and 0.01 ± 0.006 mg/kg, respectively. The Zn contents showed an important increase, reaching mean values of 11.78 ± 2.17 mg/kg, nine times higher than samples of day 20.

The biplot of the trace elements content are shown in Fig. 3. PC1 accounts for 97.5% and PC2 for 2.2% (99.7% of total variability). Fe, Al, Zn, and Se were the trace elements with the higher influence on both PCs. The biplot enhances differences related to the period of collection, leading to the formation of two clusters. Trace elements on the right side of the biplot such as Al, As, and Sn were positively correlated with PC1, contributing to the variability between data groups. Zn, Cu, Se, Sr, and Fe were correlated negatively with PC1 and were responsible for the position on the left side of samples collected at day 70. Samples collected at 20 days were sharing high values of Sn, As, and Al and low values for Fe and Zn. Samples collected at 70 days had high values for the variables Zn, Fe, Cu, Se, Hg and Sr and low values for the variables Al, As and Sn.

4. Discussion

Stomach oil is the results of the mechanical rupture of the prey in the proventriculus of Procellariidae with the exception of diving petrels (Connan et al., 2005; Wang et al., 2007). The analysis of trace metals verified a clear division in terms of composition between stomach oil samples of day 20 and day 70.

The differences in trace metals contents between stomach oil samples collected at day 20 and day 70 suggest that Scopoli's shearwaters of Linosa island adopt a targeted strategy for provisioning their chicks, probably related to the different nutritional needs during the development. Parents can perceive the nutritional status of their chicks and adjust their provisioning rates accordingly (Ottosson et al., 1997).

Copper, zinc, and mercury were the elements that contributed most to the differentiation between day 20 and day 70. The higher levels of essential metals, such as Cu and Zn in the stomach oil samples of day 70 could be related to their important role in feather formation and growth (Stewart et al., 1996; Voulgaris et al., 2019). Experimental work showed that high levels of zinc are needed for feather growth, and zinc deficiency results in a frayed feather condition (Stewart et al., 1996; Voulgaris et al., 2019). Contrary to day 70, the stomach oil samples of day 20 are characterized by higher amounts of non-essential elements such as Al, As, Sn and Pb which, besides the need for essential metals such as Cu and Zn during the pre-flight development phase, could be explained by the chick provisioning behavior of Scopoli's shearwaters and the chemical composition of the prey ingested. It was proved that parents adopt a dual foraging strategy consisting of short-distance trips carried out mainly in the shoreface zone near the colonies and long-distance foraging trips offshore (Chaurand and Weimerskirch, 1994; Granadeiro et al., 1998). Scopoli's shearwater show its highest food delivery rates during the first 29 days after hatching (Ramos et al., 2003), suggesting a higher frequency of short trips for chicks provisioning, which appears to be more profitable (Schaffner, 1990). This condition leads the parents to catch prey predominantly in the intertidal zone.

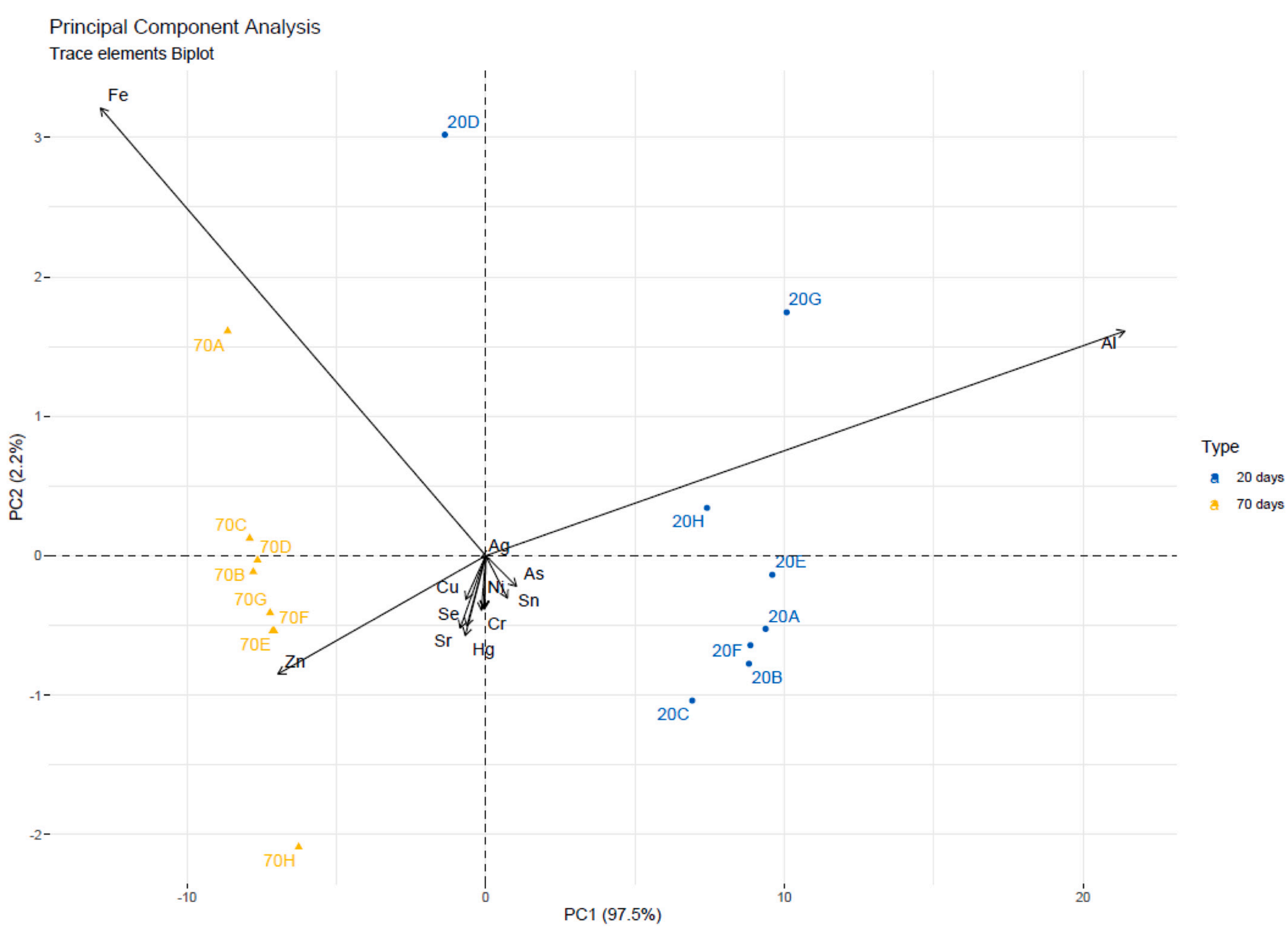

Fig. 3. *C. diomedea*. PC1 vs. PC2 biplot of trace elements content of the stomach oil samples analysed, according to the sampling period (day 20 vs. day 70).

The Al and Cr values obtained in the stomach oil samples of day 20, together with the absence of mercury contents, showed a high probability of resemblance with signatures recorded in black mussels; the presence of valve fragments in the stomach oil samples seems to confirm this assumption (Cammilleri et al., 2019b; Mol and Alakavuk, 2011; Ranau et al., 2001).

Even the arsenic contents found in stomach oil showed a high resemblance with signatures recorded in Mediterranean mussels (1.7 to 3.7 mg/kg; Klarić et al., 2004; Ünlü and Fowler, 1979).

The Hg, Cu and Zn levels of the stomach oil samples of day 70 showed a high probability of resemblance with pelagic fish such as horse mackerel (*Trachurus trachurus*) and anchovies (*Engraulis encrasicolus*) (Cammilleri et al., 2019b; Capelli et al., 2004; Türkmen et al., 2008; Yaman et al., 2013), in accordance with the experimental study conducted by Sarà (1983), indicating a progressive shift from short trips near the colony to long trips involving prey of the pelagic zone. A higher concentration of iron was found in samples of day 70, comparable with those found in pelagic and mesopelagic fish species of the Mediterranean (Canli and Atli, 2003).

Seabirds are often used for marine environment monitoring (Furness and Camphuysen, 1997; Montevecchi, 1993; Camphuysen and Van Franeker, 1992). Cadmium and mercury can bioaccumulate the most in the long-lived predatory species that exhibit high concentrations of these toxic metals. This process is most evident at high latitudes (Bustamante et al., 1998; Dietz, 1998), where the baseline concentrations could be higher than in the temperate latitudes. It has been presumed that dietary was one of the more discriminant factors for the differences in mercury and cadmium concentrations. Cephalopods have been determined to be an important vector for transferring cadmium to top marine predators (Bustamante et al., 1998; Muirhead and Furness, 1988). Studies reported before have shown that seabird species which include an appreciable amount of crustacea in their diet, had lower cadmium and mercury concentrations than those predated predominantly on squids (Stewart and Furness, 1998). The absence of cadmium seems to exclude the provisioning of squid or other cephalopods for the Scopoli's shearwater colony of Linosa island, in contrast to what was found in the Cory's shearwater colony of the Azores (Alonso et al., 2014; Granadeiro et al., 1998). Furthermore, the very low levels of cadmium and other toxic metals such as Hg and Pb could be traced back to the absence of industrial processes in the study areas.

This hypothesis can be improved in the future by analysing the levels of Cd in marine organisms of Linosa's coasts. Therefore, low levels of Hg, Pb, As and the absence of Cd indicate an unremarkable pollution in this area.

5. Conclusions

To the best of our knowledge, the present work reports for the first time the trace metals composition of the stomach oil of Scopoli's shearwater colony in Linosa island. Our results showed a marked temporal difference in the chick provisioning activity of this species, suggesting a dual provisioning strategy based on the nutritional needs of the chicks and food availability. It could be hypothesized that the stomach oils of Procellariiformes may have an important role as trophic markers to understand predator-prey relationships and to have evidence on the accumulation of pollutants in the latter. The study also stresses the need to have references on trace metals patterns of potential prey in Mediterranean, in order to deepen the ecological status of this area. The results of this work suggest that Linosa island and its marine ecosystem has low level of pollution.

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CRediT authorship contribution statement

Valentina Cumbo: Conceptualization and original draft preparation. Gaetano Cammilleri and Francesco Giuseppe Galluzzo: Methodology, statistical analysis and data curation. Antonietta Mascetti: Investigation. Giovanni Lo Cascio: Validation. Innocenzo Ezio Giangrosso: Formal analysis. Andrea Pulvirenti: Writing, review and editing. Salvatore Seminara: Supervision. Vincenzo Ferrantelli: Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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