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Mercury and cadmium in striped dolphins (*Stenella coeruleoalba*) stranded along the Southern Tyrrhenian and Western Ionian coasts

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Abstract

Pollution by heavy metals is becoming a serious threat to food safety and the health of humans and marine animals in many areas, including the Mediterranean Sea. Cadmium and mercury are among the most toxic of these metals. Their presence in various environmental compartments, which is partly due to human activity, inevitably leads to their bioaccumulation in the food chain.

In this study, levels of cadmium and mercury were determined by means of Atomic Absorption Spectrophotometry in liver, kidney and muscle tissues of specimens the dolphinid *Stenella coeruleoalba* stranded at various locations in coastal areas of the Tyrrhenian and Ionian Sea in Southern Italy from 2015 to 2018.

The data were compared with those reported for other Mediterranean locations. Correlations between biometric data (body length, weight and gender) and cadmium and mercury concentrations in tissue samples from these specimens were statistically analyzed in order to investigate the risk that these contaminants may pose to the health of delphinids.

Examination of the pattern of contaminants revealed a significantly high concentration of mercury in all the matrices analyzed (liver, kidney and muscle tissue). By contrast, elevated concentrations of cadmium were found only in liver (range: 0.005 - 8.95 mg/kg w.w.) and kidney (range: 0.005 - 34.1 mg/kg w.w.), owing to the accumulator role of these organs during long-term exposure.

Keywords: Stenella coeruleoalba; mercury; cadmium; Tyrrhenian Sea; Ionian Sea.

Introduction

Metals are natural components of the earth's crust and may be present in sea water as a result of atmospheric fallout and erosion. However, industrialization and human activities can also release large quantities of these elements into marine environments (Bradl, 2005).

The Mediterranean Sea is an ecologically interesting environmental area. Being a closed sea surrounded by countries with a high degree of industrialization, high population density and many anthropogenic activities, such as fishing, tourism etc., it is at high risk of contamination by toxic compounds (Coll *et al.,* 2012; Karadirek *et al.,* 2019).

Metals are not biodegradable, are rapidly assimilated into the environment and can reach toxic levels in a short time. Some are considered non-essential, such as cadmium and mercury, and, if their concentrations exceed natural levels, they can cause serious damage to marine life.

Cadmium is an element with no known beneficial properties; it is highly toxic, even at low concentrations, and can cause cancer, birth defects and genetic mutations (Jarup *et al.,* 2009; Eisler *et al.,* 1985). Most cadmium in the environment is released by human activities, such as mining and smelting of sulfide ores, fuel combustion, and the use of phosphate fertilizers or sewage sludge in agriculture. Cadmium contamination in aquatic systems is of particular concern, as the metal is both persistent and toxic.

Mercury is also one of the most toxic heavy metals. Its sources are both natural (erosion of sediments, volcanic activity) and anthropogenic (urban discharges, agricultural materials, mining and combustion and industrial waste), from which it is released into the atmosphere and into the sea, where aquatic organisms live and feed (Pacyna *et al.,* 2010). Moreover, its concentration is biomagnified through food webs.

Marine organisms, such as swordfish (*Xiphias gladius*) and tuna (*Thunnus* spp.), which are at the apex of the aquatic food chain, can assimilate metals from the environments in which they live, bioaccumulating large amounts of pollutants (Esposito *et al.,* 2018). Similarly, cetaceans, which are also large predators and which feed on cephalopods and fish, can accumulate large amounts of mercury in their tissues as a result of their longevity and trophic level (Borrell *et al.,* 2014). For this reason, dolphins are considered to be sentinels in the monitoring of spatial and temporal trends in many aquatic pollutants (Augier *et al.,* 1993; Bellante *et al.,* 2011; Stavros *et al.,* 2011; Reif *et al.,* 2015).

The striped dolphin (*Stenella coeruleoalba,* Meyen 1833) is a small pelagic delphinid, and is common in the Mediterranean Sea. In these marine mammals, the marked accumulation of contaminants can act on the immune system, reducing the immune defenses and, therefore, increasing sensitivity to various types of infections (Aguilar & Borrell, 1994; Fossi *et al.,* 2004). Specifically, the accumulation of mercury in various tissues has been linked to renal and hepatic damage and to neurotoxic, genotoxic and immunotoxic effects (Frederick *et al.,* 2018; Kershaw & Hall, 2019), pathological conditions that can lead to the stranding and death of these mammals.

Episodes of unusual mortality, mainly involving striped dolphins along Italy's Tyrrhenian Sea coasts, have been attributed to infections, such as Morbillivirus infection (Casalone *et al.,* 2014, Pautasso *et al.,* 2019). However, neuropathological alterations have also been detected in stranded cetaceans (Profeta *et al.,* 2015; Pintore *et al.,* 2018). Moreover, marine mammals can easily become entangled in macroplastics, the main component of litter, and ingest microplastics (Napper *et al.,* 2019) that may carry or contain toxic pollutants (Brennecke *et al.,* 2016; Alimba *et al.,* 2019; Chen *et al.,* 2019).

In addition to concerns over the health of cetaceans, the issue of food safety also arises, as dolphinid species are, in some cases, used for human consumption (Endo *et al.,* 2004; Fielding *et al.,* 2014).

There are many studies on the determination of heavy metals in the organs and tissues of delphinids, especially *Stenella coeruleoalba*, beached alive or dead, in various oceans and seas (Law, 1992; Baptista *et al.,* 2016; Shoham-Frider *et al.,* 2016; Rojo-Nieto *et al.,* 2017; Martínez -Lopez *et al.,* 2019). Some papers report the concentrations of cadmium and mercury in delphinids from the northern Tyrrhenian Sea, stranded along the coasts of Liguria and Tuscany, from the Ionian Sea and Adriatic Sea, stranded along the coasts of the Puglia region, and in Sicily, particularly in the Strait of Sicily (Leonzio *et al.,* 1992; Monaci *et al.,* 1998; Bellante *et al.,* 2012; Cardellicchio *et al.,* 2000; Cardellicchio *et al.,* 2002). These studies have shown great variability in the concentrations of mercury and cadmium, according to both the marine areas in which these animals live and the type of diet, which is linked to the species.

To our knowledge, very little information on heavy metal concentrations in cetaceans beached along the coastal areas of the southern Tyrrhenian Sea and western Ionian Sea is available in the literature. The present study is the first to determine the content of mercury and cadmium in various organs and muscle tissues of specimens of *Stenella coeruleoalba* collected in the Calabria and Campania regions, in Southern Italy. The aims of the study were to ascertain the state of health of these animals and to use them as sentinels to monitor the presence of mercury and cadmium in the marine area selected, in order to provide baseline data for monitoring studies. In addition, we compared our results with data from other marine areas, in order to determine whether there were any differences in environmental contamination by cadmium and mercury.

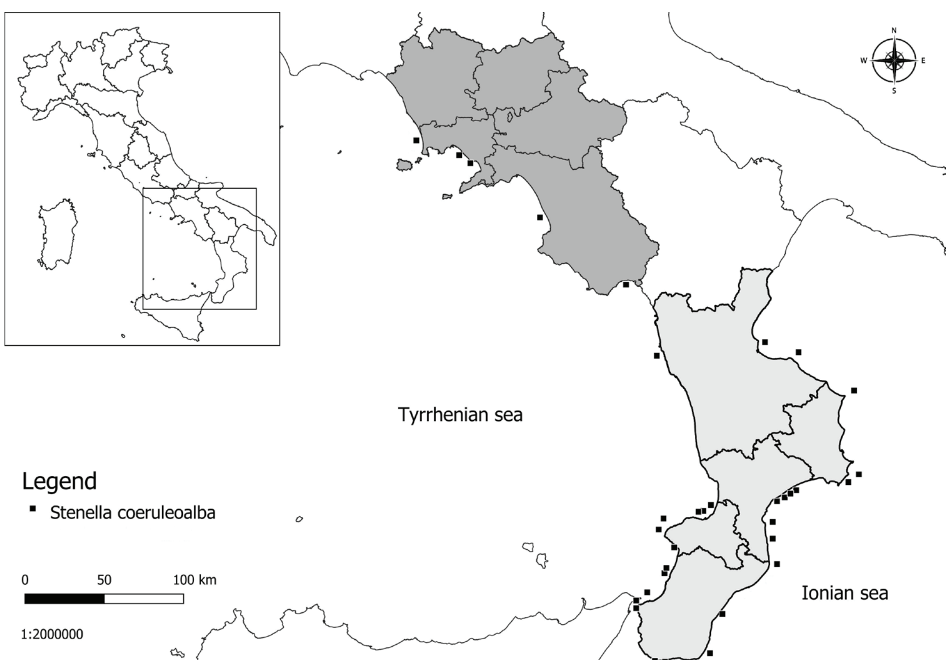

Fig. 1: Locations of stranding of 35 striped dolphins (*Stenella coeruleoalba*) between 2015 and 2018 in coastal areas of the Southern Tyrrhenian Sea and Western Ionian Sea.

Materials and Methods

Sampling

From 2015 to 2018, 35 stranded specimens of striped dolphins (*Stenella coeruleoalba*) were collected in different coastal areas of the Tyrrhenian Sea and Ionian Sea, in Southern Italy (Fig. 1). All the animals were found dead on beaches; details of the locations are reported in Table 1. All specimens were sent to the laboratory of the Istituto Zooprofilattico Sperimentale del Mezzogiorno, where they were sexed and their morphometric data were recorded.

For each specimen, the following key biometric parameters were recorded: body weight, body length, sex and life stage (Table 1). The life stage of each dolphin was determined from the total body length (BL): newborn (BL<100cm), juvenile (100≤BL≤190cm) and adult (BL>191 cm), in accordance with biometric measurement criteria (Carlini *et al.,* 2014). The age of each animal was confirmed by counting growth-layer groups in sections of its teeth.

Table 1. Dates and sites of collection and biometric parameters (sex, life stage, body weight, body length) of the 35 specimens of striped dolphin (*Stenella coeruleoalba*).

Location	Lat.	Long.	Sea	Sex	Life stage	Body weight (kg)	Body length (cm)
Ardore Marina	38.166128	16.199628	Ionian	F	Adult	105.0	194
Badolato Marina	38.573783	16.569739	Ionian	M	Adult	120.0	196
Bianco	38.099062	16.157191	Ionian	F	Newborn	6.7	84
Belvedere Marittimo	39.630401	15.8436715	Tyrrhenian	M	Adult	88.0	206
Brancaleone	37.975606	16.110737	Ionian	M	Juvenile	69.0	189
Brancaleone	37.947693	16.087468	Ionian	F	Juvenile	54.0	190
Capaccio	40.442720	14.971372	Tyrrhenian	M	Adult	89.5	208
Catanzaro Lido	38.837749	16.659988	Ionian	M	Newborn	10.0	90
Catanzaro lido	38.833853	16.651120	Ionian	F	Adult	55.0	201
Cirò Marina	39.392225	17.151162	Ionian	M	Juvenile	47.0	150
Corigliano Calabro	39.643940	16.561375	Ionian	M	Adult	85.0	208
Crosia	35.597731	16.804212	Ionian	M	Adult	110.0	200
Isola Capo Rizzuto	38.918747	17.120114	Ionian	M	Juvenile	60.0	180
Isola Capo Rizzuto	38.909890	17.089635	Ionian	M	Juvenile	94.0	189
Ispani	40.075926	15.561697	Tyrrhenian	F	Adult	82.0	203
Marina di S. Lorenzo	37.919284	15.835820	Ionian	M	Juvenile	49.4	162
Melito di Porto Salvo	37.916683	15.769675	Ionian	M	Newborn	6.8	88
Monasterace	38.443592	16.579153	Ionian	M	Juvenile	69.0	186
Nicotera	38.522649	15.926077	Tyrrhenian	F	Juvenile	69.7	182
Palmi	38.352102	15.835978	Tyrrhenian	M	Newborn	12.9	106
Palmi	38.366305	15.843347	Tyrrhenian	M	Juvenile	51.6	189
Pizzo Calabro	38.744423	16.177504	Tyrrhenian	M	Juvenile	21.2	104
Pozzuoli	40.820683	14.130655	Tyrrhenian	M	Adult	75.0	201
Ricadi	38.614720	15.838870	Tyrrhenian	M	Newborn	12.2	90
Ricadi	38.617380	15.834213	Tyrrhenian	F	Juvenile	44.0	147
Satriano	38.671347	16.557276	Ionian	F	Adult	65.0	201
Scilla	38.252715	15.710844	Tyrrhenian	M	Newborn	6.0	85
Sellia Marina	38.887202	16.756410	Ionian	M	Newborn	10.5	94
Simeri Crichi	38.859931	16.700979	Ionian	M	Newborn	10.0	95
Torre Annunziata	40753398	14.441511	Tyrrhenian	F	Juvenile	70.0	189
Torre del Greco	40.778314	14.373161	Tyrrhenian	F	Juvenile	12.7	108
Vibo Valentia	38.712551	16.105461	Tyrrhenian	F	Newborn	18.1	122
Vibo Valentia	38.714846	16.113375	Tyrrhenian	M	Adult	77.4	205
Villa S. Giovanni	38.213258	15.633398	Ionian	M	Juvenile	16.5	110
Villa S. Giovanni	38.204302	15.633489	Ionian	F	Juvenile	50.6	176

The animals then underwent necropsy examination in authorized laboratories, to ascertain the probable causes of death. The organs and tissues were removed and sent to the laboratory for virological, microbiological and parasitological analyses; genetic analyses were also performed in order to confirm the species. Samples of liver, kidney and muscle were taken during necropsy and sent to the laboratory of the Department of Chemistry, where they were immediately frozen at -20°C until chemical analysis.

Reagents and chemicals

All reagents and solvents were of analytical grade. High-purity deionized water (resistivity 18.2 MΩ cm) was produced in-house by means of an Arium® pro purification system. Monobasic ammonium phosphate and magnesium nitrate, which were used as matrix modifiers, were purchased from Perkin Elmer. Standard solutions of cadmium and mercury were prepared by diluting elemental standard solutions to 1000 mg L^{-1}. Samples were diluted with water containing the same concentration of nitric acid as the samples. Before use, glassware was washed with a solution of nitric acid (10% w/v) and then rinsed with water.

Sample preparation

Samples were thawed and homogenized; 0.75 ± 0.01 g of homogenate was then weighed in a teflon reaction vessel, to which 5.0 mL of 70% nitric acid, 2.5 mL of 30% hydrogen peroxide and 2.5 mL of water were added. After being tightly closed, the vessels were placed in a microwave apparatus and the following thermal program was set: 5 min at 800 W, from T=25°C to T=120°C; 2 min at 1000 W, T=120°C; 7 min at 900 W, then from T=120°C to 190°C; and 10 min at 700 W, T=190°C. After acid digestion, the vessels were cooled to room temperature; the samples were quantitatively recovered by means of filtration in Class A volumetric flasks and then brought to 50 mL with water.

Solutions of ammonium dihydrogen phosphate and magnesium nitrate were used as matrix modifiers during the determination of cadmium.

Heavy metal analysis

In this study, heavy metal analysis was performed by means of atomic absorption spectrophotometry, as already reported by Esposito *et al.* (2020). The detection limits of the spectrophotometer were: 0.030 mg/kg and 0.005 mg/kg for Hg and Cd, respectively.

For quality assurance, blank chemical determinations were carried out regularly; these were run together with the determinations carried out on each batch of samples, in order to check the purity of reagents and to exclude possible laboratory contamination or interference in the whole analytical procedure. Accuracy and reproducibility were assessed through the analysis of certified material of mussel tissue ERM®-CE278k (Hg: 0.074 ± 0.011 mg kg^{-1} *vs* 0.071 mg kg^{-1}; Cd: 0.340 ± 0.035 mg kg^{-1} *vs* 0.336 mg kg^{-1}). The coefficient of variation of the replicates was always less than 20%. Data quality assurance also included participation in proficiency tests (Fapas ®), the results of which were satisfactory (z scores ≤ |2|).

Statistical analyses

All data regarding sex, age and concentrations of heavy metals in organs and tissues of the specimens were statistically evaluated by performing the non-parametric group-comparison test by means of Minitab Statistical Software (Minitab Inc.). Significant differences were calculated in order to test the significance of differences among the samples. A p-value of less than 0.05 was considered statistically significant ($p<0.05$); when the measured value was below the limit of quantification (LOQ) of the test method, we used the LOQ/2 value. A non-parametric study was also performed through box-and-whisker plots, which display variation in samples of a statistical population.

Results

From 2015 to 2018, 35 dolphins (*Stenella coeruleoalba*) were found dead on the beaches of Southern Italy: 20 on the coasts of the Ionian Sea and 15 on the coasts of the Tyrrhenian Sea.

On the basis of morphological data, these specimens were classified as 9 newborns, 15 juveniles and 11 adults; 23 animals were males and 12 were females. Table 1 reports the stranding locations, sex, life stage, body weight (BW) and body length (BL) of the dolphins.

The mean length of the females was 166.4 ± 41.0 cm and that of the males 153.5 ± 49.5 cm; the mean weight of the females was 52.7 ± 29.1 kg and that of the males 51.8 ± 37.0 kg. These results were similar to those reported in other studies on Mediterranean dolphins (Carlini *et al.,* 2014; Monaci *et al.,* 1998). A positive correlation between the length and weight of the 35 specimens was found ($R^2=0.83$).

Differences in the length and weight of specimens stranded in the two marine areas were not statistically significant; the dolphins collected from the coasts of the Ionian Sea were of slightly greater length (mean 159.2 cm) and weight (mean 54.7 kg) than those collected from the Tyrrhenian coasts (mean values 156.3 cm and 48.7 kg, respectively).

The concentrations of mercury and cadmium determined in organs and muscle tissue from the specimens varied markedly. Table 2 reports descriptive statistics (mean, median, standard deviation, minimum and maximum values) of mercury and cadmium concentrations both in the total sample and in the three age-groups: adult, juvenile and newborn.

Table 2. Cadmium and mercury concentrations (mg/kg wet weight) in tissues of the 35 stranded striped dolphins (*Stenella coeruleoalba*) according to age-group.

		Cadmium			Mercury		
		Liver	**Kidney**	**Muscle**	**Liver**	**Kidney**	**Muscle**
Adult	*mean ± sd*	2.96±1.91	10.56±9.92	0.137±0.261	9.33±8.25	7.81±11.9	5.05±4.61
(n=11)	*median*	2.94	9.20	0.047	4.95	2.85	4.19
	min-max	0.237-6.07	0.007-34.1	<loq-0.904	1.31-24.5	1.18-42.7	0.12-16.4
Juvenile	*mean ± sd*	2.16±2.32	8.90±8.25	0.086±0.179	7.11±5.48	2.98±2.62	2.87±1.55
(n=15)	*median*	1.78	6.24	0.020	6.17	2.52	2.89
	min-max	<loq-8.95	0.225-28.5	<loq-0.700	1.27-22.3	0.20-9.59	0.66-6.45
Newborn	*mean ± sd*	0.095±0.200	0.200±0.281	0.012±0.015	2.26±1.45	1.40±1.04	1.13±0.73
(n=9)	*median*	0.005	0.040	0.005	1.60	1.17	1.00
	min-max	<loq-0.619	<loq-0.687	<loq-0.049	0.93-5.40	0.33-3.08	0.26-2.80
Total	*mean ± sd*	1.88±2.13	7.03±8.60	0.083±0.188	6.56±6.35	4.09±7.21	3.11±3.11
(n=35)	*median*	1.06	5.23	0.020	4.79	2.30	2.80
	min-max	0.005-8.95	0.005-34.1	0.005-0.904	0.93-24.5	0.20-42.7	0.12-16.4

The concentration of mercury was found to be extremely high in the organs, especially in the liver (mean 6.56 mg/kg w.w.), which is typically the main organ of accumulation. High concentrations were also detected in kidney (mean 4.09 mg/kg w.w.) and muscle (mean 3.11 mg/kg w.w.), although to a lesser extent. The highest concentration of mercury (42.7 mg/kg w.w.) was found in the kidney of a male *Stenella* stranded at Ardore, on the Ionian coast, while the highest liver concentration (23.3 mg/kg w.w.) was detected in a female specimen from the beach of Catanzaro Lido, again on the Ionian coast.

With regard to cadmium, the kidney displayed the greatest accumulation (mean 6.99 mg/kg w.w.), followed by the liver (mean 1.83 mg/kg w.w.), while the concentrations determined in muscle were considerably lower (mean 0.082 mg/kg w.w.). The maximum value of cadmium (34.1 mg/kg) was recorded in the kidney of a female adult from the beach of Catanzaro Lido, on the Ionian Sea, while the highest hepatic concentration (8.95 mg/kg w.w.) was found in a juvenile male specimen collected at Marina di S. Lorenzo beach, on the Ionian Sea.

Discussion

The concentrations of mercury and cadmium in organs and muscle tissues were found to vary enormously from one specimen to another, which is in line with the findings of other studies on *Stenella* spp. (Pompe-Gotal *et al.,* 2009). Mercury levels ranged from 0.93 to 24.5 mg/kg w.w. in liver and from 0.20 to 42.7 mg/kg w.w. in kidney. Other studies carried out on striped dolphins stranded along the Mediterranean coasts have reported a similar pattern, with the highest concentrations of mercury being found in the liver and kidney, and the lowest in muscle (Borrell *et al.,* 2014; Capelli *et al.,* 2008). The wide variability of these data is shown in the boxplot chart (Fig. 2). Similarly, cadmium concentrations ranged from 0.005 to 34.1 mg/kg w.w. in kidney and from 0.005 to 8.95 mg/kg w.w. in liver. Very low levels of cadmium were measured in muscle, the maximum value observed being 0.90 mg/kg w.w. No statistically significant differences emerged between the cadmium and mercury concentrations in the different types of tissue.

Similarly, no statistically significant differences were observed between the two seas, in terms of mercury concentrations in the three types of tissue analyzed. Indeed, the median values in liver and kidney from delphinids collected in the Ionian Sea (4.43 and 1.95 mg/kg w.w., respectively) were similar to those found in the Tyrrhenian Sea (4.79 and 2.52 mg/kg w.w., respectively). By contrast, the median value of cadmium in the kidney was higher in specimens from the Ionian Sea (5.37 mg/kg w.w.) than in those from the Tyrrhenian Sea (3.16 mg/kg w.w.).

With regard to mercury, we compared the results obtained in this study with the data reported by other authors. However, this comparison could only be made with the results of those studies that expressed the concentration in relation to wet weight, as ours did. Our study found lower concentrations of mercury than other studies carried out on dolphins from the Mediterranean Sea. This confirms what has already been pointed out by some authors (Borrell *et al.,* 2014), i.e. that the level of mercury contamination in the Mediterranean has declined. Even so, a high degree of mercury pollution persists, as confirmed by the high concentrations found in the organs and muscle tissue of these animals. Therefore, despite reduction in the emission of mercury, cetaceans globally continue to bioaccumulate this element (Kershaw & Hall, 2019).

The relatively low mean concentrations of heavy metals detected in the present study can also be explained by the smaller size and earlier stage of development of

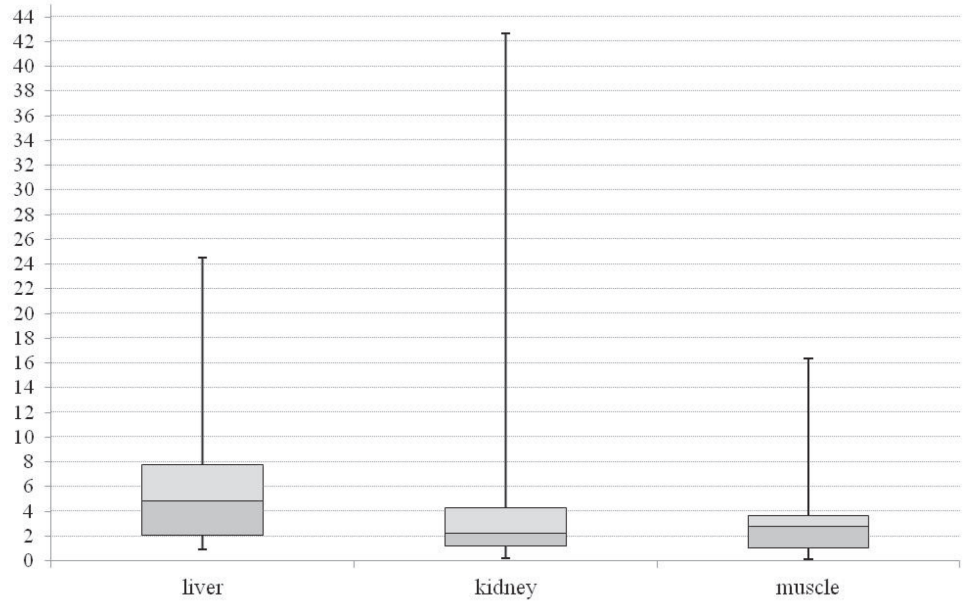

Fig. 2: Box-plot of median concentrations (mg/kg w.w.) of mercury in organs and muscle tissues of *Stenella coeruleoalba*.

the specimens analyzed. Indeed, Table 2 shows that the levels of mercury and cadmium were considerably higher in adult dolphins, confirming the age-related pattern of metal accumulation.

Concerning correlations between the length and weight of our specimens and the concentrations of the two heavy metals, the only positive correlation (r^2=0.27) that proved to be statistically significant was that between length and the concentration of mercury in muscle. This concentration increased with length probably as a function of age, and therefore of the bioaccumulation of mercury (Capelli *et al.,* 2000).

With regard to cadmium, the main organ of accumulation was the kidney. This finding is in agreement with what has already been reported by other authors in the same dolphin species. However, the values determined in the present study are well above those reported in the literature. Indeed, in dolphins stranded along the coasts of Puglia (Cardellicchio *et al.,* 2000), on the Croatian Adriatic coast (Šuran *et al.,* 2015) and along the Murcia coast (Martínez-Lopez *et al.,* 2019), the average concentrations of cadmium were always lower in all three organs than the values recorded in this study of dolphins stranded along the coasts of Campania and Calabria (Southern Italy).

This result is of particular importance since, although the metallothionein proteins capture cadmium from the body, thus mitigating its toxic effects (Das *et al.,* 2000), high levels of the metal can cause damage to the health of these marine mammals. The source of cadmium in these animals is their diet, which mainly consists of cephalopod molluscs (squid, cuttlefish, octopus), which are known to be cadmium accumulators (Aguiar dos Santos *et al.,* 2001). For this reason, such high levels could reflect an alarming contamination of sea water.

In our study, as in other studies on dolphins in the Mediterranean Sea, no statistically significant correlation was found between cadmium concentrations in either renal or hepatic tissues and biometric parameters such as weight and length, although the kidney is considered to be the main organ of bioaccumulation of this metal (Monaci *et al.,* 1998). However, as shown in Table 2, our data indicate that cadmium concentration in the storage organs (liver and kidney) increases with age.

Conclusions

This is the first paper to report the concentrations of mercury and cadmium in different organs and muscle tissues from striped dolphins stranded along the coasts of Southern Italy. The data obtained were elaborated with the aim of correlating the morphological parameters of the specimens collected with the concentrations of the two heavy metals.

The ranges of concentration of mercury and cadmium were very similar in the kidney and liver, and higher than in muscle tissues. Our results show good agreement with those of other studies conducted on the same species in different areas, and confirm that contamination by these toxic metals in the Mediterranean marine environment has decreased.

These results contribute to our knowledge of the exposure of dolphins to heavy metals in coastal and offshore areas. Moreover, they provide indications of the state of health of these animals, which are important bio-indicators of marine pollution and sentinel species for environmental health.

Conflict of interest

The authors declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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