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

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Mercury and cadmium in striped dolphins (Stenella coeruleoalba) stranded along the Southern Tyrrenian 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; Pinitore 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 delphinid 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; Martinez-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.](http://epublishing.ekt.gr)
**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 | 16.110377 | 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| 39.716683 | 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 | 15.832413 | 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    | 40.753398 | 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   | Adult      | 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, the vessels were rinsed with water.

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 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=0.83).

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 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.

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.
The concentration of mercury was found to be extremely high in the organs, especially in the liver (mean 1.83 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

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

| Age-group | Cadmium | Mercury |
|-----------|---------|---------|
|           | Liver   | Kidney  | Muscle | Liver   | Kidney  | Muscle |
|           | mean ± sd | median | min-max | mean ± sd | median | min-max |
| Adult     | 2.96±1.91 | 2.94 | 0.237-6.07 | 2.16±2.32 | 1.78 | 0.005 | 0.095±0.200 | 0.005±0.040 | 0.005-0.619 | 0.005-8.95 |
| (n=11)    | 10.56±9.92 | 9.20 | 0.007-34.1 | 8.90±8.25 | 6.24 | 0.225-28.5 | 0.200±0.281 | 0.040 | 0.086±0.179 | 0.086±0.700 |
| Juvenile  | 1.037±0.261 | 0.047 | <loq-0.904 | 7.08±5.48 | 0.020 | <loq-8.95 | 0.012±0.015 | 0.002 | 1.60 | 1.27-22.3 |
| (n=15)    | 9.33±8.25 | 4.95 | 1.31-24.5 | 7.11±5.48 | 6.17 | 0.20-9.59 | 2.26±1.45 | 1.60 | 2.98±2.62 | 0.20-9.59 |
| Newborn   | 0.020 | 2.85 | 1.18-42.7 | 2.98±2.62 | 2.52 | 0.66-6.45 | 1.40±1.04 | 1.13±0.73 | 0.012±0.015 | 0.012-0.049 |
| (n=9)     | 4.79 | 4.19 | 0.12-16.4 | 2.87±1.55 | 2.89 | 1.00 | 1.17 | 1.00 | 2.80 | 0.93-5.40 |
| Total     | 1.78 | 2.89 | 2.87±1.55 | 2.89 | 4.19 | 0.93-5.40 | 1.40±1.04 | 1.13±0.73 | 2.80 | 0.33-3.08 |
| (n=35)    | 6.24 | 4.19 | 2.87±1.55 | 2.89 | 1.00 | 0.26-2.80 | 1.17 | 1.00 | 2.80 | 0.26-2.80 |

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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 (Suran 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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