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Body burdens and distribution of mercury and selenium in bottlenose, striped and Risso's dolphins along the Adriatic coast: A 20-year retrospective

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ABSTRACT

Top marine predators present high mercury concentrations in their tissues due to biomagnification in the marine food chain. This study reports mercury (Hg) and selenium (Se) status, and the Hg:Se molar ratio assessment in bottlenose (*Tursiops truncatus*), striped (*Stenella coeruleoalba*) and Risso's dolphins (*Grampus griseus*). Total Hg and Se concentrations were determined in muscle, liver, kidney, lung, spleen, adipose tissue and skin collected from 186 specimens stranded in the Croatian part of Adriatic Sea from 1995 to 2014. Total Hg concentrations in tissue samples ranged from 0.001 in the spleen to 2238 mg/kg wet weight in liver. Se concentrations in dolphin samples ranged from 0.010 to 2916 mg/kg ww. Minimum Se concentration was found in muscle and maximum Se concentration were found in liver of bottlenose dolphin. Hg and Se levels in Risso's dolphins showed higher concentrations in all tissues in comparison to bottlenose and striped dolphins. Significant and positive correlations were observed between age and Hg concentrations ($P < 0.05$). In 66.6 % of Risso's, 15.3 % of bottlenose dolphins and one stranded striped dolphin in this study, the hepatic concentration of Hg exceeded the higher toxic thresholds (400 mg/kg w.w.) previously defined as evidence of liver damage in marine mammals. The Hg:Se molar ratio in the liver of Risso's dolphin was 0.670. The liver of adult bottlenose dolphins showed expected values (0.870), while the liver of young dolphins had a high ratio (0.750), non-specific for the age group. The Hg:Se molar ratio in the liver of striped dolphins was 0.390, which is lower than the literature values.

1. Introduction

Cetaceans are marine mammals most completely adapted to life in the water. They are listed as endangered and threatened species. For many years, a significant drop in the number of individuals in a population of almost all species of this order was determined. An example of this is the Adriatic Sea in which today appears to be present only one species of whales, bottlenose dolphin (Tursiops truncates), whose number is estimated at around 220 individuals. In the Adriatic Sea occasionally stay and some other whale species that regularly inhabit the Mediterranean Sea, which are usually striped dolphin (*Stenella coeruleoalba*) and Risso's dolphin (*Grampus griseus*).

Hg is a global environmental contaminant which belongs among 10 chemicals of public health concern (WHO, 2017). Human activities such as mining, and fuel combustion have released large quantities of Hg sequestered in the lithosphere to the biosphere (Streets et al., 2017). Various studies have shown that Hg concentrations in the tissues of cetaceans from the Mediterranean Sea are higher than those measured in organisms from the Atlantic and Pacific Oceans (Bellante et al., 2012; Savery et al., 2013). In aquatic ecosystems, some inorganic Hg is converted by microbes into MeHg, the only form of Hg that biomagnifies in food webs (Gilmour et al., 2013). Marine mammals are long-lived predators that ingest Hg through their diet and accumulating it in high concentrations in their organs. Inorganic Hg has been identified in tissues of dolphins, although they are primarily exposed to MeHg through the fish diet (Romero et al., 2016). Marine mammals may be

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partially protected from the negative effects of Hg through a number of mechanisms, including demethylation, excretion (eg, urine, feces, hair), interactions with proteins such as metallothioneins (MT), and metals such as Se. Hg has a strong affinity for MT. However, studies conducted on dolphins, California sea lions pilot whales, narwhales and sperm whales have shown that only a small fraction of Hg is bound to MT. The results on the mentioned organism were different compared to terrestrial mammals where almost all Hg is bound to MT. This difference is attributed to the different speciation of Hg in the diet. In marine mammals, almost all Hg is in the methylated form, small percentage of Hg is bound to MT in liver and kidney and the affinity of MetHg for MT is low (Romero et al., 2016). In mammals and fish, an important detoxification mechanism is the antagonistic influence of Se in relation to Hg, which is well documented in marine species, although there are various hypotheses related to the protective mechanism of Se (Kalisinska et al., 2017). The first antagonism study was carried out by Pařízek and Ostádalová (1967) who initiated research interests to evaluate the detoxification of Hg by Se. Since then, various studies have presented the Hg-Se antagonism phenomenon in aquatic organisms and mammals (Siscar et al., 2014). Se-containing biomolecules participate in the demethylation of MeHg, whereby HgSe is formed. In addition to binding and immobilizing Hg, Se plays an important role in the biosynthesis of essential amino acids and enzymes. Therefore, higher Se than Hg is required to support essential metabolic processes in biological tissues. Only specific selenium biomolecules such as Se-cysteine are directly involved in the demethylation of MeHg and the formation of HgSe. Extensive MeHg detoxification can deplete the biological pool of Se (Se-methionine) needed to maintain Se-cysteine levels in the brain and liver. Reduced Se availability can lead to a number of adverse health outcomes, including reduced coordination, autoimmune disorders, and increased susceptibility to infectious diseases (Li et al., 2020).

Se is trace element that has a very narrow range of safety between requirements and toxic levels of trace elements in animals and humans. Se contamination is an increasing environmental problem globally. Se pollution likely occurs as a result of anthropogenic activities such as copper refining coal combustion, agriculture, mining, oil and gas refineries (Okonji et al., 2021). It is recognized as an essential element, required for the metabolic activity of aquatic mammals, and may provide a protective role in Hg poisoning (Dauplais et al., 2013).

The study of toxicology draws mainly upon observations made in humans and laboratory animals such as rats and mice. While laboratory animals are extremely practical and useful experimental models, they are limited in their ability to reflect real world exposures, integrate multiple stressors, and predict population level effects. Mammalian wildlife possess many characteristics that can help to better understand the human health risks (Basu and Head, 2010).

Information on mercury levels in dolphins in the Croatian part of Adriatic Sea is scarce (Pompe-Gotal et al., 2009; Bilandžić et al., 2012), while the selenium and HgSe ratio has not been examined. In the available literature, there are only data on HgSe ratios along the Italian part of Adriatic coast (Palmisano et al., 1995; Storelli and Marcotrigiano, 2000).

The main goal of this study was to investigate the levels, relationship and toxicity of Hg and Se in bottlenose, striped and Risso's dolphins in the muscle, liver, kidney, lung, spleen, adipose tissue and skin of animals stranded in the Croatian part of Adriatic Sea, thus expanding the current knowledge on the contamination status of these cetacean species, which are frequently used as sentinel species for marine pollution. The specific goals of this study were: (a) to determine the Hg:Se molar ratio for each tissue to evaluate the potential protective effect of Se regarding Hg toxicity, and (b) to contribute to the literature regarding metal contents in the Adriatic Sea, especially in Croatian waters.

2. Materials and methods

2.1. Sampling

Tissue samples were collected from dolphins stranded in the Croatian part of Adriatic Sea (Fig. 1) from 1995 to 2014. A total of 186 dolphins were included in the study: 155 bottlenose (*Tursiops truncatus*), 25 striped (*Stenella coeruleoalba*) and 6 Risso's dolphins (*Grampus griseus*). Stranded dolphins had a mean body length (cm, mean ± SD): bottlenose 245.4 ± 55.1, striped 191.5 ± 40.3, Risso's 300.2 ± 10.7. Mean weight values for the three dolphin species were (kg, mean ± SD): bottlenose 149.8 ± 81.1, striped 81.2 ± 15.5, Risso's 258 ± 45.5.

Prior to dissection, age, gender, weight and body sizes were recorded. During necropsy, teeth were collected for age determination. Tooth sections were prepared according to Slooten (1991) and age was estimated by counting growth layer groups (GLG) according to Hohn et al. (1998). Bottlenose dolphin samples were divided four groups based on gender and age, i.e. according to gender: males (N = 76) and females (N = 76) and according to age: adult males and females (≥7 years; N = 86), and young males and females (≤6 years; N = 69). The cutoff of seven years was selected as this is the approximate age of achieving sexual maturity in dolphins. Striped dolphin samples were divided into two groups based on gender: males (N = 15) and females (N = 10), while in Risso's dolphins there was only one group of adult individuals (N = 6). The Croatian part of Adriatic Sea is divided into the northern and southern part, with the location of demarcation at Maslenica. The time periods of collection of stranded dolphins were divided into three periods: 1995–2004, 2005–2010 and 2011–2014.

After sex and length determination, tissues were excised, placed in a plastic bag and preserved frozen at −20 °C until analysis.

2.2. Analytical procedure and quality assurance

Approximately 0.5 g (wet weight, w.w.) of tissue samples were digested in teflon vessels with 2 ml HNO_3 and 1 ml H_2O_2 (Merck, Suprapure). After digestion, samples were diluted with ultrapure water and analysed for Hg and Se.

Total Hg concentrations in muscle, liver, kidneys, lungs, spleen, adipose tissue and skin tissue were determined by cold vapor atomic

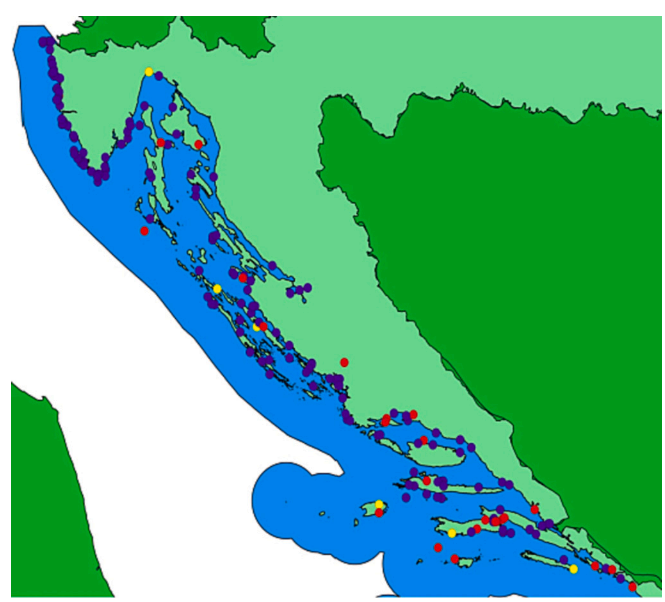

Fig. 1. Dolphin sites: BLUE- bottlenose dolphins (*Tursiops truncatus*), RED- striped dolphin (*Stenella coeruleoalba*), YELLOW- Risso dolphin (*Grampus griseus*). *(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

absorption spectrometry with a Flow Injection Mercury System (FIMS) e FIAS 400 (Perkin Elmer, USA) equipped with an auto sampler AS90 (Perkin Elmer, USA), using sodium borohydride as a reducing agent.

Se concentrations in liver, kidneys and lungs were determined by graphite furnace atomic absorption spectrometry, using a Perkin Elmer Model Aanalyst 600 spectrometer (Perkin Elmer, USA) with Zeeman Effect background correction equipped with AS-800 auto sampler. Palladium nitrate was used as a chemical modifier.

The entire analytical procedure was validated by analysing blanks, replicates and certified reference material. The data from this experiment are expressed as mg/kg on a wet weight (w.w.) basis. Replicates were found to differ <10 %, and the percent recovery was between 90 % and 110 %. The method detection limits (MDLs) were determined from 20 analyses of the blank solution taken through the preparation procedures described above. MDL, mg/kg: in muscle Hg 0.00005 and Se 0.005; in liver Hg 0.00003 and Se 0.005; in kidneys Hg 0.00004 and Se 0.005; in spleen Hg 0.00004 and Se 0.004; in lungs Hg 0.00002 and Se 0.004.

Precision and accuracy of the analytical methods were determined using two certified reference materials. The recovery result obtained for dogfish liver DOLT-4 (NRC Institute for National Measurement Standards, Ottawa, Canada) were (n = 5) 100.2 % for Hg and 103.9 % for Se. For fish protein DORM-4 (NRC Institute for National Measurement Standards, Ottawa, Canada), the following recovery values were obtained (n = 5): 99.6 % for Hg and 102.6 % for Se. The coefficients of variation (CV) in samples were <10 %. Therefore, results showed good accuracy of the methods used.

The molar ratio of Hg to Se was calculated as: Hg/Se = (Hg/200.59) / (Se/78.96), where 200.59 and 78.96 g/mol are the atomic masses of Hg and Se, respectively.

However, to allow comparison with other reports, the dry weight basis data from other studies was converted to wet weight basis data according to Becker et al. (1995) using a factor of 0.25.

2.3. Statistical analysis

All statistical analyses were performed using the software package Stata 13.1 (Stata Corp., USA). The results are presented as median, minimum and maximum value or as the arithmetic mean with corresponding standard deviation, depending on the distribution data. Since elements did not follow conditions of normality (Kolmogorov–Smirnov and Shapiro-Wilk tests) or homogeneity of variances in all variable

Table 1
Average concentration, standard deviation, median and range of Hg and Se (mg/kg, ww) in tissues of bottlenose, stripped and Risso's dolphins.

Species	Tissue	Element	n	*mg/kg wet wt*				
				Mean	SD	Median	Min	Max
Bottlenose dolphins	Muscle	Hg	150	11.5	23.0	1.83	0.035	142
		Se	33	11.6	23.2	0.661	0.010	81.9
	Kidney	Hg	150	13.0	25.1	5.79	0.058	219
		Se	150	9.52	18.4	3.98	0.010	137
	Liver	Hg	144	199	322	44.5	0.069	2238
		Se	146	169	340	48.4	0.123	2916
	Spleen	Hg	140	31.9	84.2	2.19	0.001	519
		Se	33	20.2	36.6	1.72	0.089	143
	Lung	Hg	142	9.22	40.7	1.42	0.001	460
		Se	143	4.19	7.38	1.44	0.047	53.4
	Fat	Hg	90	3.34	12.7	0.468	0.010	83.6
		Se	4	1.07	0.96	0.921	0.216	2.24
	Skin	Hg	47	6.21	20.3	1.21	0.074	101
		Se		–	–	–	–	–
Risso's dolphins	Muscle	Hg	6	33.9	23.4	23.7	14.2	73.1
		Se	6	28.9	29.1	23.6	3.83	80.2
	Kidney	Hg	6	27.1	13.1	29.3	5.99	41.8
		Se	6	21.6	18.9	15.3	5.09	58.3
	Liver	Hg	6	807	806	870	64.9	1738
		Se	6	530	351	528	95.9	1069
	Spleen	Hg	5	534	502	378	18.56	1304
		Se	5	279	294	169	5.29	762
	Lung	Hg	5	60.4	32.1	68.9	14.7	102
		Se	5	35.5	18.5	38.1	7.65	58.0
	Fat	Hg	–	–	–	–	–	–
		Se	–	–	–	–	–	–
	Skin	Hg	–	–	–	–	–	–
		Se	–	–	–	–	–	–
Stripped dolphins	Muscle	Hg	24	11.0	12.3	7.11	0.319	48.4
		Se	10	5.23	3.59	5.84	0.010	9.55
	Kidney	Hg	24	8.51	9.38	4.55	0.455	38.5
		Se	24	5.39	2.86	5.02	1.38	11.6
	Liver	Hg	24	112	139	75.4	1.32	679
		Se	24	94.3	84.3	82.3	1.99	430
	Spleen	Hg	22	61.1	64.1	37.5	1.03	224
		Se	10	56.3	54.2	38.9	3.74	175
	Lung	Hg	22	26.1	23.9	20.6	0.072	78.6
		Se	23	15.4	12.9	11.5	2.19	66.7
	Fat	Hg	13	1.50	2.87	0.738	0.109	11.0
		Se	1	–	–	2.57	2.57	2.57
	Skin	Hg	4	1.42	0.97	1.32	0.386	2.66
		Se	–	–	–	–	–	–
			Muscle	Kidney	Liver	Spleen	Lung	Fat
P (Kruskal-Wallis) Hg			**0.0014***	**0.0214***	**0.0076***	**0.0001***	**0.0001***	0.484
P (Kruskal-Wallis) Se			**0.0133***	**0.0086***	**0.0049***	**0.0004***	**0.0001***	0.157

* $p<0.05$.

groups, non-parametric tests were used. Thus, the statistical significance between different categories was assessed using the Mann–Whitney *U* test and the Kruskal–Wallis test for differences between two or more independent groups, respectively. The level of statistical significance was set at $P \leq 0.05$.

3. Results and discussion

3.1. Hg concentrations in tissues of three dolphin species

The concentrations of total Hg (mean ± SD, median and range) in tissue samples of bottlenose, striped and Risso's dolphins in the Croatian part of Adriatic Sea are shown in Table 1 and Fig. 2.

The order of Hg concentrations in tissues of bottlenose dolphins from highest to lowest was liver > spleen > kidneys > muscle > lungs > skin > blubber, while for striped and Risso's dolphins, the order was liver > spleen > lungs > muscle > kidneys. According to Viale (1977), the skin essentially eliminates mercury through desquamation and, due to a short residence time, the accumulation of this metal is low for this particular organ.

Statistically significant differences in Hg concentrations were found in muscle ($P = 0.0014$), liver ($P = 0.0076$), kidneys ($P = 0.0214$), spleen and lungs ($P = 0.0001$, both) between the three dolphin species. There were no significant differences found for adipose tissue or skin.

Hg concentrations in bottlenose dolphins were measured in the range of 0.001–2238 mg/kg w.w. and showed the highest mean value in liver (199 mg/kg for total number dolphins), followed by spleen, muscle, kidneys and lungs. Mean Hg values of 356 mg/kg w.w. were found in the liver of mature females, which were higher than the mean Hg values in the liver of mature males (248 mg/kg). The lowest concentrations were found in adipose tissue and skin. Adult female dolphins compared to adult males have a higher concentration in muscle, liver, kidneys, spleen and lungs and lower in adipose tissue and skin. The maximum value of Hg (2238 mg/kg) was recorded in a female liver from a dolphin stranded on the Pelješac Peninsula. Hong et al. (2012) observed the highest Hg concentrations in pregnancy and lactating females and the authors attributed this finding to a feeding habit alteration due to a higher energetic demand. Life stage, such as pregnancy and nursing status, which requires more energy and higher fish consumption leading to higher Hg dose, should be considered.

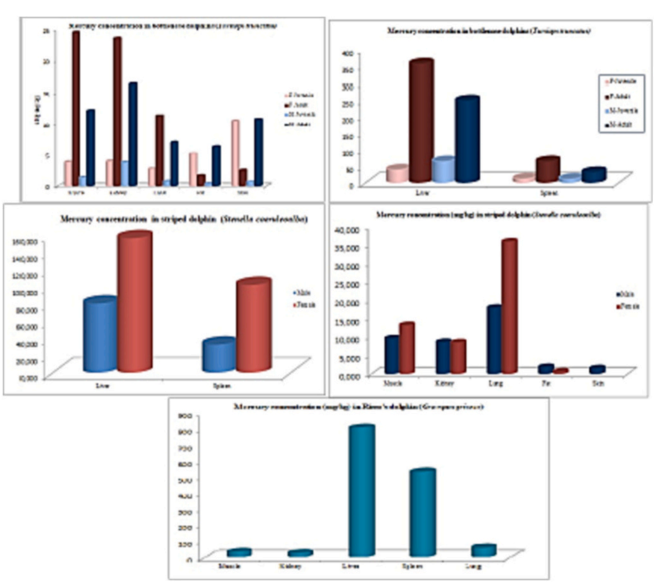

Fig. 2. The mean concentration of Hg in the tissues of bottlenose dolphins (*Tursiops truncates*), striped dolphin (*Stenella coeruleoalba*) and Risso dolphin (*Grampus griseus*).

Hg levels measured in all tissues of bottlenose dolphins were higher in adult dolphins (age ≥ 7) than in the tissues of younger animals (age ≤ 6). Hg concentrations in muscle, liver and kidneys of adult animals were 3.8 to 9.1 times higher than in young dolphins. Differences in Hg concentrations in all observed tissues were statistically significant between the age groups of bottlenose dolphins ($P < 0.05$, all; $P = 0.0008$ for skin). Fig. 3 shows the relationship between Hg concentrations and age in the liver of bottlenose dolphins. In marine mammals, the liver is the preferential organ for Hg accumulation, and therefore it appears to be the best indicator of long-term changes in Hg deposition. These results suggest the major impact of age on Hg accumulation. A similar observation was reported previously for bottlenose dolphins from the Mediterranean Sea (Shoham-Frider et al., 2002; Capelli et al., 2008). In general, total Hg content in all mammalian tissues increases with increasing animal age, due to bioaccumulation. Hg biomagnifies in marine food chains (Beldowska and Falkowska, 2016) and bioaccumulates in muscle tissue, and specially in the liver of marine mammals (Wagemann et al., 1998; Harms et al., 1978; Jones et al., 1976; Seixas et al., 2007).

The association of Hg content with the stranding location of the bottlenose dolphin was examined. No statistically significant differences ($P > 0.05$) were found in the concentration of metal in tissues of bottlenose dolphins from the north compared to the south.

Concentrations of Hg in striped dolphins were measured in the range from 0.010 to 679 mg/kg, with the highest mean level in liver (112 mg/kg), followed by spleen and lungs. Also in female dolphins, higher metal concentrations were measured in all examined tissues, except adipose tissue where the Hg values were higher in males. Mean Hg values of 156 mg/kg w.w. were found in the liver of females and 80.6 mg/kg w.w. in males. The highest value was measured in female striped dolphin (679 mg/kg) stranded in the Neretva River estuary. Mean Hg values found in liver, kidneys and muscle in both female and male striped dolphins were lower than those determined in tissues of bottlenose dolphins, while the opposite was true for Hg concentration in the lungs and spleen.

Mean Hg levels in Risso's dolphins were higher in muscle, kidneys, liver, spleen and lung tissues than in bottlenose and striped dolphins. The Hg concentrations of all Risso's dolphin tissues were found to be extremely high, especially in the liver (mean value 807 mg/kg w.w.), which was typically the main organ of accumulation. The lowest concentration of 5.99 mg/kg was measured in the kidneys of dolphin found on Korčula Island, and the highest value of 1738 mg/kg in liver of a dolphin found near Molat Island. The distribution of concentrations in tissues was the same as in striped dolphins. The results of this study confirmed that Risso's dolphins had the highest Hg concentrations, which is also due to the fact that this species has a greater body length and mass, while striped dolphins, due to the smaller body length and mass, had the lowest accumulated levels of Hg.

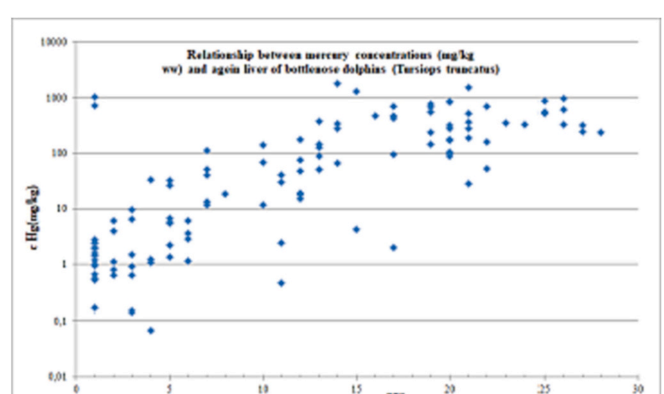

Fig. 3. Relationship between Hg concentrations (mg/kg ww) and ageing in liver of bottlenose dolphins (*Tursiops truncatus)*.

Reference values for toxicity in critical organs may be useful in evaluating the potential toxicological risk in dolphins. There is little information on the actual physiological tolerances to contaminants in free-living animals, though it has been noted that there is a limit to the concentration of Hg that an animal can tolerate. Elevated Hg concentrations in the tissues of marine mammals were also associated with poor health due to leptospirosis (Buhler et al., 1975), proximity to urbanized areas (Anas, 1974; Roberts et al., 1976), starvation (Jones et al., 1976), and with gender, as they were typically higher in females than males (Gaskin et al., 1972).

Fig. 4 *a, b* shows the Hg concentrations in the liver of bottlenose, striped and Risso's dolphins compared with the threshold levels of 100 mg/kg w.w. defined as the limit of liver damage, and 400 mg/kg w.w. as the upper limit of hepatic tissue damage in marine mammals (Wagemann and Muir, 1984). Comparing total hepatic Hg concentration to this threshold, liver concentrations exceeded 100 mg/kg Hg tolerance level in 83.4 % of Risso's, 37.5 % of striped and 32.7 % bottlenose dolphins. In 66.6 % of Risso's dolphins, 15.3 % bottlenose dolphins and one stranded striped dolphin that were studied, the hepatic concentration of Hg exceeded the higher toxic thresholds defined as evidence of liver damage in marine mammals.

However, in this range of concentrations, Rawson et al. (1993) found liver abnormalities in bottlenose dolphins associated with chronic Hg accumulation. It is interesting that a terrestrial mammal with a Hg concentration of 30.0 mg/kg of wet weight in the liver is likely to suffer from Hg intoxication (Thompson, 1996), although this is significantly lower than the estimated 100–400 mg/kg that causes damage in marine mammals (Wagemann and Muir, 1984). Lymphoma abnormalities consisting of lipofuscinosis, central necrosis and lymphocytic infiltration were associated with chronic Hg accumulation at concentrations above 60.0 mg/kg wet weight in bottlenose dolphins from the Atlantic Sea (Rawson et al., 1993) and *Mesoplodon densirostris* with a concentration of 248 mg/kg wet weight (Law et al., 1997). In addition, Hg levels found in dolphins are higher than those found in long-term marine predators, such as tuna or sharks (Storelli et al., 2002), likely due to their specific means of separation of Hg into inorganic form and the relatively lower ability for Hg excretion (Monaci et al., 1998).

Regarding the concentration of Hg in tissues of bottlenose dolphins stranded at different locations along Adriatic Sea, i.e. north and south, no significant differences were found in tissue Hg levels between locations. Contrary to this, a recent study in Portugal showed that stranding location had a significant effect on Hg concentrations in kidneys, liver and muscle of bottlenose dolphins (Monteiro et al., 2016), where dolphins stranded on the northwest coast had significantly higher Hg levels than those stranded along the southern coast of Portugal.

The concentrations of Hg in the three dolphin species stranded in three observation periods were examined. Analysis of the obtained mean concentrations showed significant differences between the three observation periods for bottlenose dolphins in muscle (P = 0.0409) and fat tissues (P = 0.00280). For striped dolphins, significant differences in Hg levels between the observation periods were determined for all tissues (P values between 0.00390 and 0.0360), except skin. There was a clear reduction in Hg concentrations in the periods between 2005–2010 and 2011–2014 compared to 1995–2004 for both dolphin species. To the extent of our knowledge, there are several studies reporting temporal trends along the Southern European Mediterranean coast, although few examine the coastal waters of the region. Data on Hg concentrations for samples of *Mytilus galloprovincialis* collected at 21 stations in the period 1979–2006 along the French Mediterranean coast, showing a general, slow decline of Hg levels during this time span (UNEP, 2011). A similar decreasing trend was also observed along the northern Mediterranean Spanish coasts (Borrell et al., 2014). A similar decreasing trend in Hg levels as in this study has been reported for striped dolphins in Mediterranean open waters, showing that mean Hg concentrations in the liver and kidneys of dolphins stranded during the period 1990–93 were twofold and 1.22-fold higher, respectively, than those of the dolphins stranded during the 2007–09 period (Borrell et al., 2014). The author suggested that reduction-oriented activities of Western European countries resulted in effective Hg reduction in the Mediterranean basin. Industrial use of Hg has evidently decreased in recent decades due to increased awareness about Hg toxicity. In virtually all applications, Hg can be substituted by less harmful elements or alternative techniques (Hylander and Meili, 2003).

We compared Hg levels in dolphins from this study with those of different marine areas worldwide (Table 2). The mean Hg concentration measured in tissues of bottlenose dolphins were in accordance with the previous literature data for bottlenose dolphins from the southern Adriatic Sea and Croatian coast of the Adriatic Sea (Storelli and Marcotrigiano, 2000, 2002; Pompe-Gotal et al., 2009; Bilandžić et al., 2012), Israeli coasts of the Mediterranean (Roditi-Elasar et al., 2003; Shoham-Frider et al., 2009) and Australia (Lavery et al., 2008). In a previous study from the Adriatic Sea, stranded bottlenose dolphins had very high Hg concentrations: a 16-year old bottlenose dolphin found in the Split area had a Hg concentration of 1834 mg/kg in the liver (Pompe-Gotal et al., 2009) and a 14-year old male bottlenose dolphin found near the Island of Hvar had a liver concentration of 1790 mg/kg (Bilandžić et al., 2012). On the other hand, these levels were higher than tissues levels of bottlenose dolphins from different locations in the Atlantic Ocean, such as in USA (Meador et al., 1999; Durden et al., 2007; Stavros et al., 2011), Portugal (Carvalho et al., 2002), or the Hawaiian Islands (Hansen et al., 2016).

Higher Hg levels have been reported for dolphin tissues from the Mediterranean (Andre et al., 1991; Leonzio et al., 1992; Storelli and Marcotrigiano, 2002) than in the Atlantic (Holsbeek et al., 1998). Hg exposure in the Mediterranean basin is a consequence of its anthropogenic emissions or its input by atmosphere and rivers (Cossa et al., 1997; Pirrone et al., 2001). Large rivers that contribute to Hg exposure, such as the Rhone in France and Po in Italy, are the most highly polluted rivers with Hg (Rajar et al., 2007). The Adriatic Sea is exposed to this inflow from polluted rivers, especially in its northern and central parts. Studies have shown that the shore zone is saturated with Hg, and that the spatial distribution of Hg in water and sediment is influenced by sea water

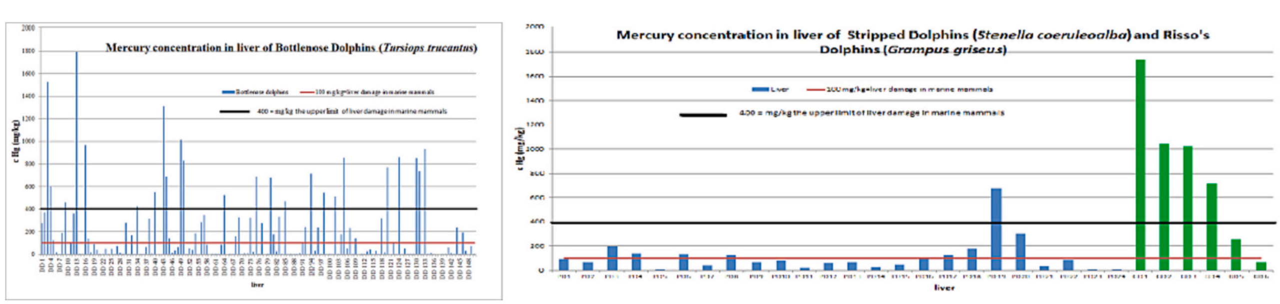

Fig. 4. ***a, b***: The individual concentrations of Hg in the liver of Stripped Dolphins (*Stenella coeruleoalba*)-PD, Risso's Dolphins (*Grampus griseus*)-GD and Bottlenose Dolphins (*Tursiops truncatus*) – DD in compared to critical concentrations.

Table 2
Concentrations of mercury and selenium (wet weight) in dolphins (conversion factor for dry to wet weight is 0.25).

Species (number of individuals)	Liver (mg/kg)	Kidney (mg/kg)	Lung (mg/kg)	Area/year	Reference
Stenella coeruleoalba (3)	Hg 79.1[a] Se 37.5[a]	Hg 9.3[a] Se 6.4[a]	Hg 25.6[a] Se 9.4[a]	Ligursko more/1991–2001	*Capelli i sur.. 2008*
Stenella coeruleoalba (10)	Hg 170.76 Se 63.18	Hg 8.99 Se 7.68	Hg 14.52 Se 5.47	South Italy/1991	*Cardellicchio i sur.. 2002*
Stenella coeruleoalba (18)	Hg 151.3[a] Se 57.8[a]	Hg 14.5[a] Se 7.25[a]	Hg 29.8[a] Se 12.3[a]	Ligurian sea/1986–1990	*Capelli i sur.. 2000*
Stenella coeruleoalba (6)	Hg 189.16	Hg 10.30	Hg 28.68	South Italy/1987	*Cardellicchio i sur.. 2000*
Stenella coeruleoalba (45)	Hg 148[a] Se 66.5[a]	Hg 11[a] Se 6.5[a]		Tyrrhenian and Ligurian sea/1984–1997	*Monaci i sur.. 1998*
Stenella coeruleoalba (20)	Hg 261[a] Se25.2[a]	Hg 15.7[a] Se 12.8[a]		Mediterranean coast-Spain/1984–1997	*Monaci i sur.. 1998*
Stenella coeruleoalba	Hg 17–568[a]	Hg 3.5–85[a]		France-Mediterranean coast	*Augier i sur.. 1993a*
Stenella coeruleoalba	Hg 3.15–1100[a]	Hg 1.45–51[a]		Tyrrhenian sea	*Leonzio i sur.. 1992*
Stenella coeruleoalba (4)	Hg 373			France-Mediterranean coast/1999–2004	*Lahaye i sur.. 2006*
Tursiops truncatus	Hg 35.6	Hg 6.7		Israeli coast	*Shoham-Frider i sur.. 2009*
Tursiops truncatus (2)	Hg 468.8[a] Se 214.6[a]	Hg 36.7[a] Se 13.6[a]	Hg 17.2[a] Se 6.4[a]	Ligurian sea/1999–2002	*Capelli i sur.. 2008*
Tursiops truncatus (14)	Hg 97	Hg 8.8		Israeli coast/1994–2001	*Roditi-Elasar i sur.. 2003.*
Tursiops truncatus	Hg 3–3289[a]	Hg 1.7–221[a]		Tyrrhenian sea	*Leonzio i sur.. 1992*
Grampus griseus (3)	Hg 408.1 Se 217.8[a]	Hg 12.1 Se 8.2[a]	Hg 33.2 Se 21.9[a]	Ligurian sea/1992–2004	*Capelli i sur.. 2008*
Grampus griseus (3)	Hg 14.3	Hg 7.63		Israeli coast/1993–1999	*Shoham-Frider i sur.. 2002*
Grampus griseus (2)	Hg 740.2 Se 189.9[a]	Hg 54.4 Se 16.5[a]	Hg 208.4 Se 45.1[a]	South Italy/1996	*Storeli i sur.. 1999*
Stenella coeruleoalba (33)	Hg 107.5[a] Se 35.0[a]			Japan/1977–1982	*Agusa i sur.. 2008*
Stenella coeruleoalba (13)	Hg 138			Atlantic ocean-France/1999–2004	*Lahaye i sur.. 2006*
Tursiops truncatus (8)	Hg 8.58[a] Se 3.63[a]			South Carolina/2000–2008	*Stavros i sur.. 2011*
Tursiops truncatus (15)	Hg 75[a] Se 27.25[a]			Florida/2000–2008	*Stavros i sur.. 2011*
Tursiops truncatus (10)	Hg 213.94 Se 70.19			Australia/1988–2004	*Lavery i sur.. 2008*
Tursiops truncatus (25)	Hg 53[a] Se 31[a]	Hg 8.25[a] Se 2.53[a]		Texas/1991–1992	*Meador i sur.. 1999*
Tursiops truncatus (13)	Hg 76[a] Se 16.25[a]	Hg 17[a] Se 5.5[a]		Florida/1991–1992	*Meador i sur.. 1999*
Grampus griseus (7)	Hg 232[a]	Hg 17.2[a]	Hg 41.2[a]	Japan/1999–2001	*Endo i sur.. 2004*

[a] Samples converted from dry weight to wet weight.

circulation and other geological factors (Kotnik et al., 2015). The Po River, together with the Soča River (Isonzo) affect exposure of the Adriatic Sea from Hg input, together with an evident rise of atmospheric Hg content due to numerous industrial plants located along the coast (Rajar et al., 2007; Sprovieri and Pirrone, 2007). In fact, one of the tributaries of the Soča River is the Idrijica, a river related to the well-known Idrija mine in Slovenia. In the past, the Idrija mine was one of the largest Hg mining areas in the world (Foucher et al., 2009). Therefore, the extremely polluted area around Idrija is the source of Hg in the Idrijica River, and is transported via the Isonzo (Soča) River into the Adriatic Sea near the town of Monfalcone, Italy in the Gulf of Trieste.

Other recorded zones with Hg pollution along the Croatian Adriatic coast are the urban zones or natural semi-closed zones that experience poor water flux. The Rijeka harbour is an urban polluted with Hg (Cukrov et al., 2011), with levels similar to those in the Venice Lagoon (Zonta et al., 2007), though much lower than the high concentrations measured in the Naples Port (Sprovieri et al., 2007) or Gulf of Trieste (Covelli et al., 2006). Kaštela Bay in the central eastern Adriatic coast near the city of Split was the site of a chlor-alkali plant that operated between 1949 and 1989 (Kotnik et al., 2015). Consequently, Hg is concentrated in the sediments near the plant and dispersed throughout the bay. However, analysis of Hg in different ecosystem compartments of Kaštela Bay suggests this improved in the years following plant closure in 1989 (Kljaković-Gašpić et al., 2006).

The above, particularly the last two facts, provide a possible explanation for the high Hg levels measured in dolphins in this study. It has been suggested that due to the geographical differences in Hg levels recorded in dolphins from different areas in the Mediterranean, this may in fact suggest the existence of a number of distinct dolphin populations, each characterized by a certain nutritional habitat and exposition to specific anthropogenic influences (Bellante et al., 2012). This can be confirmed by the existing evidence of genetic differences among populations of bottlenose dolphins (*T. truncatus*) from Gibraltar to the Black Sea (Borrell et al., 2006).

Range and mean Hg concentrations obtained in muscle, liver and kidneys of striped dolphins in the present study were similar to previously reported data for this species from Mediterranean sites, such as the Ligurian Sea (Capelli et al., 2000, 2008), Spanish coast (Borrell et al., 2015) and French Atlantic coast (Lahaye et al., 2006). In the present study, the maximal liver Hg level was 679 mg/kg, while the highest Hg concentrations of 1033 mg/kg were found in a 27-year-old striped dolphin from the Mediterranean (Lahaye et al., 2006) and 1500 mg/kg in dolphin from the French Mediterranean (Andre et al., 1991).

On the other hand, higher Hg levels in liver were found in striped dolphins from the southern Adriatic Sea and Croatian coast of the Adriatic Sea (Storelli et al., 1998; Pompe-Gotal et al., 2009; Bilandžić et al., 2012), southern Italian coast (Cardellicchio et al., 2002), French coast (Lahaye et al., 2006) and Israeli coasts (Roditi-Elasar et al., 2003). The maximal Hg levels measured in the liver of striped dolphins from the Hawaiian Islands are lower than in the present study (Lavery et al., 2008).

Hg content determined in muscle, liver and kidney tissues of Risso's dolphins were in line with levels found in the Mediterranean basin, i.e. from the Israeli coast (Shoham-Frider et al., 2002), Ligurian Sea (Capelli et al., 2008) and southern Adriatic Sea and Croatian coast (Storelli et al., 1998; Bilandžić et al., 2012). Muscle tissue of Risso's dolphins from Japan (Endo et al., 2004) showed lower concentrations than those found in the present study. However, the spleen Hg levels measured in this study (533 mg/kg) were significantly higher than those reported in dolphins from the Ligurian Sea (Capelli et al., 2008).

Very few studies have examined Hg concentrations in the lungs of cetaceans. Augier et al. (1993) hypothesized that Hg could penetrate from the atmosphere to the lungs, which could partly explain the relatively high values found in this organ. Authors determined Hg concentrations in the lungs of striped dolphins in the range 3.00–396 mg/g d.w. Frodello et al. (2000) found Hg concentration in the lungs of 264 mg/kg d.w. (66.0 mg/kg w.w.) in bottlenose dolphin. In this study, the concentrations in lungs were in the range of 1.79 to 60.0 mg/kg w.w. for male and from 0.0720 to 78.6 mg/kg w.w. for female striped dolphins. However, the high Hg content of 112 mg/kg was determined in the lungs of striped dolphins from the Ligurian Sea (Capelli et al., 2000).

In Risso's dolphin, the maximum concentration in the lungs was 102 mg/kg w.w. Lungs of bottlenose dolphins also contained high concentrations of Hg, even in juveniles. The maximum value measured in a juvenile male was 460 mg/kg w.w. Since dolphins accumulate Hg to levels 10 to 100 times higher than fish from the same trophic level (tuna, swordfish) and since these organisms experience the same average life span, Leonzio et al. (1992) suggested that the difference between mammals and fish may depend on their different respiratory systems. Rawson et al. (1995) found Hg-Se in both the liver and respiratory system (lung and hilar lymph) of the bottlenose dolphin and short-finned pilot whale. This supports the earlier suggestion that in the liver Hg-Se may be a storage and product of Hg metabolism, adding the suggestion that Hg-Se may be inhaled in the respiratory system.

3.2. Se concentrations in the tissues of the three dolphin species

Se concentrations (mean ± SD, median and range) in tissues of bottlenose, striped and Risso's dolphin are presented in Table 1 and Fig. 5. Statistical differences in Se concentration between the three dolphin species were observed in the liver ($P = 0.0049$), kidneys ($P = 0.0086$) and lungs ($P = 0.0001$).

Concentrations of Se in bottlenose dolphins were measured in the range from 0.010 to 2916 mg/kg, with the highest mean level in liver (169 mg/kg), followed by spleen, lungs and muscle. The highest Se contents were found in adult female bottlenose dolphins for all tested tissues. These were followed by adult male dolphins, and finally by young males and females. The highest value of 2916 mg/kg was measured in the liver of an adult female dolphin from the Pelješac Peninsula.

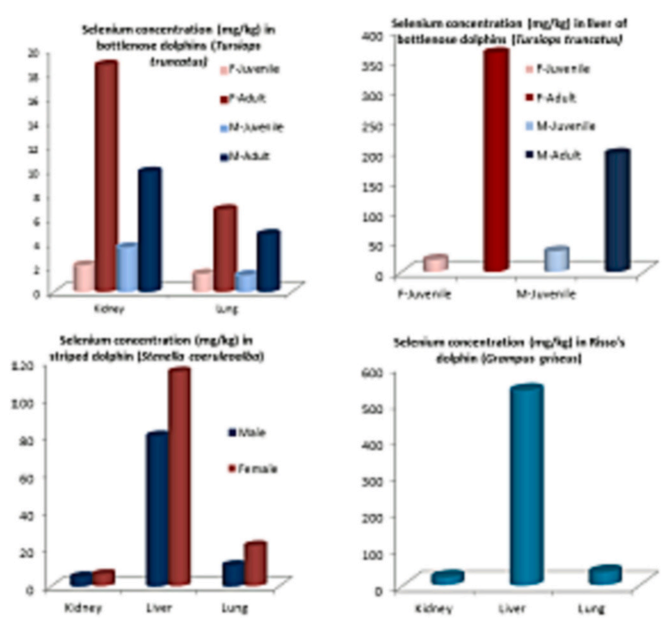

Fig. 5. The mean concentration of Se in the tissues of bottlenose dolphins (*Tursiops truncates*), striped (*Stenella coeruleoalba*) and Risso dolphin (*Grampus griseus*).

There were large differences in concentrations between adult bottlenose dolphins (age ≥ 7) compared to juvenile individuals (age ≤ 6). Se concentrations in the liver, kidneys and lungs of adult animals were 4.3- to 10-times higher than in young dolphins. Significant positive correlations were observed between age and Se concentrations in the liver, kidney and lung of bottlenose dolphins ($P < 0,05$).

Se levels in striped dolphins were measured in the range from 0.010 to 430 mg/kg. Female dolphins compared to males had 1.5–2 times higher mean Se concentrations in all analysed tissues. However, Se distribution in the examined tissues differed from bottlenose dolphins, with the highest concentrations in the liver (94.3 mg/kg) followed by spleen (56.3 mg/kg), lung (15.4 mg/kg) and kidney (5.39 mg/kg).

The tissues of Risso's dolphins showed the highest levels of Se in tissues compared to bottlenose and striped dolphins. Values were highest in liver, followed by spleen, equal in lung and muscle, and lowest in kidney. The highest mean Se values of 530 mg/kg w.w. were determined in the liver. The maximal measured concentration of 1069 mg/kg was found in the liver of a Risso's dolphin found at Molat Island.

Regarding the Se concentration in the tissues of bottlenose dolphins found in the northern part of the Adriatic Sea to those found in the south, statistically significant differences were observed only for Se content in the lungs ($P < 0.05$).

Se concentrations in kidney ($P < 0.001$), and lung tissue ($P < 0.05$) of bottlenose dolphins and kidney tissue ($P < 0.05$) of striped dolphins differed statistically between the periods of observation from 1998 to 2014. The following tissues in the first period show a maximum value while in the second period, the value were the lowest. The liver of bottlenose dolphins and liver and lungs of striped dolphins did not differ significantly between observation periods.

There are very few studies on Se levels in the liver of dolphins (Table 2). Concentrations of Se in the liver of striped and Risso's dolphins in this study were higher than values measured in the livers of these two species collected elsewhere in the Mediterranean, in the Ligurian Sea and southern Italy, though concentrations in kidneys and lungs were similar (Cardellicchio et al., 2002; Capelli et al., 2008). Se levels in liver and kidneys of bottlenose dolphins found in this study were comparable to those found in the Ligurian Sea (Capelli et al., 2008).

Se concentrations measured in the liver of striped and bottlenose dolphins in this study were markedly higher than reports from Japan, Australia and USA (Agusa et al., 2008; Lavery et al., 2008; Stavros et al., 2011). In some cases, concentrations of Se in dolphin liver from the Mediterranean Sea contained 240–600 mg/kg w.w. In general, liver Se concentrations were higher in older animals (Lavery et al., 2008; Seixas et al., 2007), and were strongly correlated with total Hg concentrations (Lavery et al., 2008; Storelli et al., 1998).

Depending on the concentration, Se can be an essential or toxic element, and it can act as an antagonist in relation to toxicity of heavy metals (Lemly, 1996). It is known that marine mammals contain high concentrations of Se in the liver and kidneys, because this element is involved in the detoxification mechanism of heavy metals, such as Hg, lead and cadmium (Arai et al., 2004). The mean hepatic concentration of Se was <100 mg/kg w.w. in most marine areas worldwide, but far exceeded this value in some locations, which also corresponded with those sites with the highest Hg burdens, such as the Ligurian Sea (Capelli et al., 2008) and Southern Italy (Storelli et al., 1999). These geographical differences are difficult to explain because Se is an essential element, and many factors, such as dietary intake or natural sources, but also differences in physiological requirements or retention of Se for detoxification processes, might influence its concentrations (McHuron et al., 2014).

3.3. Hg:Se molar ratio in the tissues of the three dolphin species

The presence of HgSe was first demonstrated in livers, kidneys and brains, and more recently, in marine mammal muscles (Nakazawa et al., 2011), and it has already been shown that HgSe is the final product of the demethylation process as well as a defense mechanism against Hg toxicity in dolphins.

Detoxification processes would be achieved by the transformation of methylmercury in inorganic mercury via HgSe formation. These transformations would essentially occur when the level of organic mercury in the liver is high, and more commonly in the oldest organisms (Hansen et al., 2016). There is a significant correlation between Se and Hg concentrations in cetacean liver and kidney samples, with molar ratios close to 1 (Bustamante et al., 2003; Capelli et al., 2008; Hansen et al., 2016). It is assumed that an animal with a molar excess of Se in the liver (Hg:Se <1) is at a lower risk of direct Hg toxicity, while an animal with a molar excess of Hg (Hg:Se > 1) is at a higher risk (Hansen et al., 2016). It is more complicated to explain the toxicological significance of individuals in which Hg concentrations without Se concentrations are shown (Kershaw and Hall, 2019).

A 1:1 M Hg:Se ratio is often observed in the liver of marine mammals (Koeman et al., 1973). The ratio supports the hypothesis of MeHg detoxification by Se, although the ratio can vary depending on geographic location and marine mammal age. However, Hg:Se molar ratios in the liver may range from 0.20 (Hansen et al., 1990) to 2.49 (Caurant et al., 1996). Healthy beluga whales, for example, had liver Se:Hg ratios of about 10:1 (Dehn et al., 2006), while Se:Hg ratio in the livers of small cetaceans from the coast of Brazil was about 4:1 (Seixas et al., 2008).

Palmisano et al. (1995) suggest that the Hg:Se molar ratio approaches 1.0 in striped dolphins only at high hepatic concentrations of Hg (close to 100 mg/kg). Therefore, it is possible that the Hg:Se molar ratio approaches 1.0 even below the concentration suggested by the authors. Some studies also indicate a molar ratio of 1:1 between Hg and Se for different marine mammal species (Cardellicchio et al., 2002; Law et al., 2003; Kehrig et al., 2004), especially when liver Hg measurements exceed 200 mg/kg d.w. (Ikemoto et al., 2004). Therefore, in order to compare these data, the molar ratio between Hg and Se was also calculated.

Table 3 shows the molar ratio of Hg:Se in the liver, kidneys and lungs of bottlenose, striped and Risso's dolphins. The ratio for adult bottlenose dolphins showed ranges of: liver 0.47–1.0, kidneys 0.53–1.2 and lungs 0.47–1.0, while in young animals the ranges are: liver 0.28–1.26, kidney 0.10–0.71 and lung 0.10–0.62. In liver of the adult bottlenose dolphins, the Hg:Se molar ratio showed the expected values (0.87), while the liver of young dolphins obtained a high ratio (0.75), non-specific for the age group. In the lungs and kidneys of bottlenose dolphins, the molar ratio also differed between young and adult individuals, where the ratio was much higher in adult specimens. In general, a Hg:Se ratio between 0.20 and 0.40 was found in younger animals (Stavros et al., 2011).

In striped dolphins, the ratio ranged from: liver 0.180–1.0, kidneys 0.130–1.68 and lungs 0.260–0.940, while for Risso's dolphins, the ranges were: liver 0.270–0.870, kidney 0.280–1.16 and lungs 0.60–0.750. In the present study, the Hg:Se molar ratio in the liver of Risso's dolphin was 0.670 and the Hg:Se molar ratio in the liver of striped dolphins was 0.390, which is lower than the values obtained in the literature. The decreasing order of the Hg:Se molar ratio in striped dolphins was kidney (0.850) > lung (0.50) > liver (0.390). Aquatic mammals accumulate high Hg concentrations in their tissues, particularly in liver (Leonzio et al., 1992). Despite this common finding, investigations that describe the deleterious effects of Hg on the health of marine mammals are scarce. Several studies have shown that high Hg levels in liver were associated with liver abnormalities in stranded Atlantic bottlenose dolphins (Rawson et al., 1993) and were correlated with infectious diseases in stranded harbour porpoises (Siebert et al., 1999). Additionally, a lower Hg:Se molar ratio was observed in stranded harbour porpoises that perished due to physical trauma, such as entrapment in fishing gear (0.450), compared to those that perished from infectious disease (0.70) (Bennett et al., 2001).

Table 3
Molar ratio of Hg:Se in the liver, kidney and lungs of dolphins.

Dolphin species	Hg:Se molar ratio Hg:Se molarni odnos		
	Kidney	Liver	Lung
Bottlenose dolphin (*Tursiops truncatus)* ≥ 7	0.710	0.870	0.720
Bottlenose dolphin (*Tursiops truncatus)* ≤ 6	0.430	0.750	0.220
Stripped dolphin *(Stenella coeruleoalba)*	0.850	0.390	0.50
Risso's dolphin *(Grampus griseus)*	0.660	0.670	0.480

Dolphin species	Se:Hg molar ratio Se:Hg molarni odnos		
	Kidney	Liver	Lung
Bottlenose dolphin (*Tursiops truncatus)* ≥ 7	2.44	4.49	2.43
Bottlenose dolphin (*Tursiops truncatus)* ≤ 6	4.87	4.21	16.7
Stripped dolphin *(Stenella coeruleoalba)*	3.47	2.96	2.90
Risso's dolphin *(Grampus griseus)*	2.05	2.11	1.49

4. Conclusions

The reported data set clearly documents that the populations of bottlenose, striped and Risso's dolphin from the Croatian part of Adriatic Sea were affected by very high Hg concentrations in the examined tissues, reflecting the highly contaminated status of these areas. These concentrations exceed the threshold limits previously reported for mammalian Hg toxicity and pose a serious health risk for this species.

In this study, the amounts of Hg and Se in dolphins living on the eastern Adriatic coast are most comprehensively presented. Based on these data, it is possible to form a database that can help assess the health status of a protected species in the Adriatic Sea. Therefore, marine mammals can serve as a toxicological model to assess the risk of harmful substances because they share the coastal environment with humans and consume the same type of seafood (Bossart, 2011).

CRediT authorship contribution statement

Marija Sedak wrote the manuscript with support from **Nina Bilandžić. Marija Sedak** and **Maja Đokić** carried out the experiment. **Martina Đuras** ·**and Tomislav Gomerčić** contributed to sample collection and preparation. **Miroslav Benić** carried out the statistical analysis of the results. All authors discussed the results and contributed to the final manuscript.

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.

Data availability

The authors do not have permission to share data.

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