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Preliminary assessment of persistent organic pollutants (*POPs*) in tissues of Risso's dolphin (*Grampus griseus*) specimens stranded along the Italian coasts

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ABSTRACT

Ecotoxicological and pathological research on *Grampus griseus* (Cuvier, 1812) (Risso's dolphins) is scarce both globally and in the Mediterranean Sea. This species has been classified as "*Vulnerable*" by the *International Union for Conservation of Nature* (*IUCN*) in the Mediterranean Sea.

To evaluate the presence of "persistent organic pollutants" (*POPs*), especially organochlorine compounds (*OCs*), in the animals, chemical analyses were performed on tissues and organs of Risso's dolphin stranded along the Italian coasts between 1998 and 2021. Toxic contaminants such as hexachlorobenzene (*HCB*), polychlorinated biphenyls (*PCBs*), dichlorodiphenyltrichloroethane and its metabolites (*DDTs*) were examined in the blubber, liver, muscle, and brain of 20 animals, and data was correlated with sex, age, and stranding locations.

1. Introduction

On a global scale, studies on Risso's dolphin (*Grampus griseus* Cuvier, 1812) about distribution (Baumgartner, 1997; Bearzi et al., 2011; Hartman, 2018), feeding (Würtz et al., 1992; Blanco et al., 2006; Luna et al., 2022), biology (Azzellino et al., 2016) are more available than those on pollution. The *International Union for Conservation of Nature* (*IUCN*) classifies this species as "*Least Concern*" (Taylor et al., 2012), but the Mediterranean subpopulation is listed as "*Endangered*" (Lanfredi et al., 2022). Main threats for this subpopulation are bycatch (Zucca et al., 2005; Öztürk et al., 2007; Azzellino et al., 2016; Podestà and Pavan, 2018), climate change (Lanfredi et al., 2022), noise pollution (Jepson et al., 2005; Monaco et al., 2016) and, contamination from toxic substances (Marsili and Focardi, 1997; Storelli et al., 1999; Storelli and Marcotrigiano, 2000; Shoham-Frider et al., 2002, 2014a, 2014b; Fossi and Marsili, 2003; Zucca et al., 2005).

The latter threat can affect reproduction and health both the individual and population levels (Fossi and Marsili, 2003). Levels of organochlorine compounds in Mediterranean Risso's dolphin have already been described as "high" (Marsili and Focardi, 1996), and these cetaceans are known to be sensitive to environmental contaminants, especially organochlorine compounds (*OCs*) (Marsili et al., 2000). Some of these contaminants are known as endocrine disruptors, including dichlorodiphenyltrichloroethane (*DDT*) and its metabolites, some polychlorinated biphenyls (*PCBs*) and hexachlorobenzene (*HCB*) (Marsili et al., 2004), which induce adverse health effects, such as reproductive dysfunction (DeLong et al., 1973; Gilmartin et al., 1976; Reijnders, 1986; Fossi and Marsili, 2003; Murphy et al., 2015) and immunosuppression (Ross et al., 1994; Hammond et al., 2005; Jepson et al., 2005; Desforges et al., 2016; Centelleghe et al., 2019; Marsili et al., 2019).

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Apex predators, such as delphinids, which reside at the top of the food chain, accumulate the highest levels of contaminants through the biomagnification process depending upon age, gender, and species (Marsili and Focardi, 1997; Tuerk et al., 2005).

Cetaceans are excellent environmental indicators of chemical pollutants due to their position within the marine food web, their long lifespan, and their wide home ranges (García-Alvarez et al., 2014; Hutchinson et al., 2013). According to the Italian strandings database, between 1998 and 2021, 84 stranding events of *G. griseus* have been recorded along the Italian coasts (http://mammiferimarini.unipv.it/).

Since the scarcity of data on contaminant levels in Risso's dolphin tissues, the principal aim of this study was to determine the levels of some legacy contaminants (*HCB*, *PCBs* and *DDTs*) in tissues and organs of *Grampus griseus* stranded along the Italian coasts, increasing the interest of this still scarcely studied species as well as the knowledge of the impact of the analysed contaminants on it.

2. Material and methods

2.1. Samples collection

The ecotoxicological analysis was conducted on *Grampus griseus* individuals ($n = 20$) stranded along the Italian coast between 1998 and 2021 (Fig. 1). Four types of biological samples were collected from the 20 stranded animals: blubber ($n = 20$), liver ($n = 10$), muscle tissue ($n = 10$) and brain ($n = 6$).

2.2. Organochlorine compound (OCs) analysis

Determination of *HCB*, *DDT*s and *PCB*s was performed at the Department of Physic Sciences, Earth and Environment at University of Siena, according to the modified U.S. Environmental Protection Agency (*EPA*) 8081/8082 method (Marsili and Focardi, 1996).

Specifically, samples (5–20 g) were lyophilized in an Edwards freeze drier for three days and extracted with n- hexane (PESTINORM, VWR Chemicals) in a Soxhlet apparatus. VWR cellulose thimbles (i.d. 25 mm, e.d. 27 mm, length 100 mm) used for extraction of the samples were preheated for about 30 min to 110 °C. Each sample was spiked prior to extraction with 2,4,6-trichlorobiphenyl (International Union of Pure and Applied Chemistry; *IUPAC* number 30) (Ballschmiter and Zell, 1980) as surrogate compound.

The concentration of *PCB30* was quantified and its recovery calculated for each sample. After 9-h extraction with *n*-hexane, samples were saponificated with sulphuric acid to obtain lipid sedimentation.

The extract then underwent liquid chromatography on a column containing Florisil that had been dried for 1 h at 110 °C. This further purified the apolar phase of lipids that could not be saponified, such as cholesterol.

Decachlorobiphenyl (*DecaPCB* – *IUPAC* number 209) was used as an internal standard, which was added to each sample prior to the extraction and included in the calibration standard (a mixture of Aroclor 1260, *HCB* and *pp*′- and *op*′*-DDT*, *DDD* and *DDE*). High resolution capillary gas chromatography was performed with an Agilent 6890 N and a 63Ni *ECD* and an SBP-5 bonded phase capillary column (30 m long, 0.25 mm internal diameter, film thickness 25 μm). The carrier gas was nitrogen with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40 ml/min.

Oven temperature was 100 °C for the first 10 min, after which it was increased to 280 °C at 5 °C/min. The injector and detector temperatures were 200 and 280 °C respectively. The extracted organic material (*EOM*

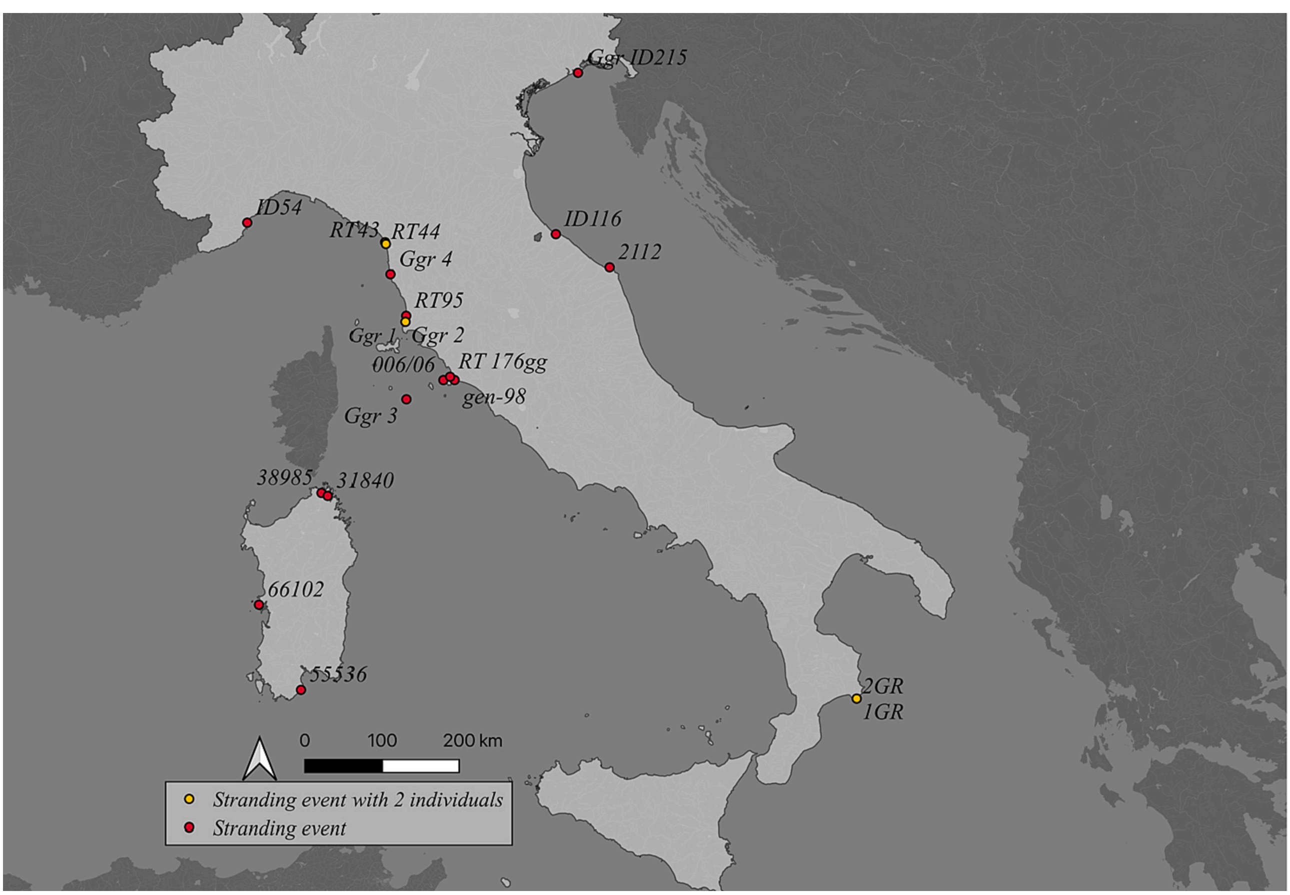

Fig. 1. Stranding events of *Grampus griseus* sampled in the study. Red dots indicate stranding events involving a single individual while yellow dots indicate stranding events involving two individuals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

% lipid content) was calculated gravimetrically after extraction with *n*-hexane.

Capillary gas-chromatography revealed 30 PCB congeners (IUPAC no. 95, 99, 101, 118 – pentachlorobiphenyls (Penta-CB); 128, 135, 138, 141, 144, 146, 149, 151, 153, 156 – hexachlorobiphenyls (Hexa-CB); 170, 171, 172, 174, 177, 178, 180, 183, 187 – heptachlorobiphenyls (Hepta-CB); 194, 195, 196, 199, 201, 202 – octachlorobiphenyls (Octa-CB); 206 – nonachlorobiphenyls (Nona-CB)). Total PCBs (PCBs) were quantified as the sum of all congeners. These congeners constituted 80 % of the total peak area of *PCB*s in the sample. Total *DDT*s were calculated as the sum of the isomers *op′DDT*, *pp′DDT*, *op′DDD*, *pp′DDD*, *op′DDE* and *pp′DDE*. The results were expressed in ng/g lipid weight (l.w.) unless otherwise specified. The limit of detection (LOD) was calculated by measuring replicates ($n = 20$) of blank samples, determining the mean value and SD, and calculating LOD as the mean + 2 SD. The limit of detection (LOD) for all compounds analysed was 0.1 ng/kg (ppt).

2.3. Statistical analysis

Statistical analysis was carried out with STATA 14 software (StataCorp, 2015). Data was analysed using descriptive statistics and non-parametric tests (Gibbons, 1997). In particular, a Kruskal-Wallis and Mann-Whitney *U* test were used to evaluate statistically significant differences between groups (Friedman, 1937). In addition, a Dunn's pairwise comparison test was used for multiple comparisons following the Kruskal-Wallis test in order to detect differences between pairs of groups. In order to validate the use of non-parametric approach preliminary analyses were also conducted for checking normality of data and presence of extreme values in each variable. Visual inspection of the empirical frequency distributions clearly showed lack of symmetry (positive skewness) and the Shapiro-Wilk test gave p-values lower than 0.05 for each variable (Peat and Barton, 2005). Moreover, box plots were used to identify extreme values of each variable by group of analysis. In these box plots, any data that lies outside the upper or lower fence lines was considered a possible outlier.

3. Results

OC levels were analysed in blubber, liver, muscle, and brain of *Grampus griseus* stranded along the Italian coasts between 1998 and 2021.

Table 1 shows the examined strandings events with relatives dates and place of stranding, the information on morphometric data (sex, length in cm, and age class), sampled tissues and status of the carcass at the time of stranding.

The age class was determined by length (Notarbartolo di Sciara and Demma, 1994) as follows: “Immature” individuals < 270 cm; “Mature” individuals > 270 cm.

3.1. OCs determination in tissues

Blubber was analysed in all the individuals ($n = 20$), while liver and muscle were analysed in 10, and brain tissue in 6 specimens. *HCB*, 30 *PCB* congeners and the op′ and pp′ forms of *DDT*, *DDE* and *DDD* were mostly detected in all the animals. In addition, the percentage of extracted organic material (*EOM*%) was calculated. This latter represents the fraction of lipid content in the tissues analysed and allows the calculation of values in lipid weight (l.w.) from values expressed in dry weight (d.w.).

Table 2 shows that, on average, *HCB* shows the lowest and *PCB*s the highest levels in all the tissues. Data boxplots are shown in Fig. S1. Kruskal-Wallis (KW) test highlighted significant differences for *HCB* ($p = 0.0552$), *PCBs* ($p = 0.0572$) and *DDTs* ($p = 0.0798$). Moreover, the results of multiple pairwise comparison tests for HCB suggested that the brain to blubber comparison was significant at only 10 % level ($p = 0.0919$), considering that the comparison between muscle and blubber was significant at 5 % level ($p = 0.0598$). Pairwise multiple comparison for *DDT*s suggested that only the comparison between blubber and brain is significant ($p = 0.0390$). Finally, the multiple pairwise comparison test, for *PCB*s suggested that only the comparison between blubber and brain is significant ($p = 0.0220$).

The *DDTs/PCB*s ratio ranged between 0.52 and 0.77; the pp′DDE/

Table 1

Strandings of *G. griseus* individuals along the Italian coast between 1998 and 2021, arranged in chronological order. Each sample has its own identification code to which information on date and place of stranding, macro-sector area, sex, age, and length expressed in cm is related, sampled tissues and conservation status of the carcass. U=undetermined; M = male; F=female; Bl = blubber; Lv = liver; Mu = muscle; Br = brain.

Date of stranding	Macrosector area	Place of stranding	Sex	Length (cm)	Age class	Sample code	Sampled tissues	Status of the carcass
1998	Tyrrhenian Sea	Isola del Giglio (Grosseto)	U	U	U	Ggr 3	Bl	U
		Feniglia (Grosseto)	M	160	Immature	1/98	Bl, Lv	Advanced decomposition
2000	Ligurian Sea	Livorno (Livorno)	U	U	U	Ggr 4	Bl	U
2002	Ligurian Sea	Rimigliano (Livorno)	F	321	Mature	Ggr 1	Bl, Lv, Br	Decomposed
			M	115	Immature	Ggr 2	Bl	U
2004	Ligurian Sea	Ceriale (Savona)	M	325	Mature	ID54	Bl, Lv	Freshly dead
2005	Adriatic Sea	Ancona (Ancona)	F	290	Mature	2112	Bl, Lv, Mu	Rescued alive, died subsequently
2006	Tyrrhenian Sea	Isola dell'Argentarola (Grosseto)	M	202	Immature	006/06	Bl, Mu, Br	Decomposed
2007	Adriatic Sea	Cattolica (Rimini)	M	295	Mature	ID 116	Bl, Lv, Mu	Rescued alive, died subsequently
	Ionian Sea	Capo Rizzuto (Crotone)	F	U	U	1GR	Bl, Lv	U
			M	240	Mature	2GR	Bl, Lv, Mu, Br	U
2011	Tyrrhenian Sea	Barrabisa, Palau (Olbia-Tempio)	M	147	Immature	38,985	Bl, Lv, Mu	U
2012	Adriatic Sea	Bibione (Venezia)	F	299	Mature	Ggr ID215	Bl, Mu	U
	Ligurian Sea	Viareggio (Lucca)	F	305	Mature	RT43	Bl	Rescued alive, died subsequently
			F	320	Mature	RT44	Bl	Decomposed
2015	Ligurian Sea	San Vincenzo (Livorno)	F	293	Mature	RT95	Bl, Lv, Mu	Rescued alive, died subsequently
2016	Tyrrhenian Sea	Nora (Cagliari)	M	228	Immature	55,536	Bl	Freshly dead
		Cabras (Oristano)	F	168	Immature	66,102	Bl	Freshly dead
2018	Tyrrhenian Sea	Spiaggia Le Saline (Sassari)	F	290	Mature	31,840	Bl	Freshly dead
2021	Tyrrhenian Sea	Orbetello (Grosseto)	F	289	Mature	RT 176gg	Bl, Lv, Mu, Br	Rescued alive, died subsequently

Table 2
Summary statistics of organochlorine concentrations in tissues and organs of *G. griseus* expressed in μg/g l.w.

	N	Mean	S.D.	Min	Q1	Median	Q3	Max
Blubber								
HCB	20	0.37	0.38	<LOD	0.06	0.32	0.48	1.51
DDTs	20	71.05	69.95	1.72	16.81	47.27	107.80	235.12
PCBs	20	137.5	193.07	2.21	16.23	70.58	183.589	853.82
EOM%	20	66.41	12.46	28.55	62.07	66.96	73.50	88.10
Liver								
HCB	10	0.22	0.22	<LOD	0.04	0.14	0.37	0.6
DDTs	10	118.23	118.23	2.54	5.68	24.69	77.10	381.99
PCBs	10	148.42	148.42	7.85	13.58	30.36	111.33	391.58
EOM%	10	10.31	10.31	5.80	12.04	17.87	21.18	41.18
Muscle								
HCB	10	0.11	0.11	<LOD	0.02	0.07	0.22	0.27
DDTs	10	49.2	68.43	1.31	2.84	7.15	93.17	200.81
PCBs	10	90.83	158.64	4.97	7.52	17.61	110.63	521.63
EOM%	10	6.79	3.48	2	5.40	6.10	8.48	13
Brain								
HCB	6	0.07	0.05	0.01	0.03	0.07	89.07	0.16
DDTs	6	18.39	31.46	0.72	1.63	2.58	17.75	80.32
PCBs	6	17.72	27.15	2.27	2.45	3.23	19.84	70.02
EOM%	6	43.52	3.67	40	41.13	43.53	43.52	50.22

DDTs ratio were all above 0.77; the pp′DDE/pp′DDT ratio ranged between 28.78 and 44.40 and, the Σop′DDT/*DDTs* ratio were all below 0.10.

Out of the 20 individuals examined, only in three specimens was possible to collect all the four tissues (Table 1). Results of OC levels are summarized in Tables S2 and S3.

Statistical analyses for the three classes of contaminants, using Friedman's test, showed no significant differences.

3.2. PCB and DDT profiles

The pattern of PCBs, divided by their chlorine content, was very similar in the blubber, liver, muscle and brain (Hexa-CB > Hepta-CB > Penta-CB > Octa-CB > Nona-CB) (Fig. 2A). In fact, the non-parametric Kruskal-Wallis test showed no significant evidence between the pattern of different tissues.

The fingerprints of the DDT isomers had different patterns in all the four tissues except for the pp′DDE isomer, which was always the most present (Fig. 2B). The Kruskal-Wallis test shows significant differences between the percentages of pp′DDD and op′DDT between tissues ($p < 0.05$). In particular, the Kolmogorov-Smirnov test shows differences between blubber-liver and blubber-brain ($p < 0.01$) for op′DDT, between brain-liver for op′DDT ($p < 0.005$) and pp′DDD ($p < 0.05$), and between brain-muscle for pp′DDD ($p < 0.05$).

3.3. OCs determination for sex and age class

The following results refer only to blubber tissue because was the only sampled from all the 20 individuals allowing the analysis by subgroups. Out of the 20 animals, due to the carcasses conditions, we were able to determine the sex only in 18 (females = 10; males = 8). Table S4 shows summary statistics of *HCB*, *PCBs*, and *DDTs* levels by sex. In general males showed mean values of all the three contaminant groups higher than females, although no significant differences were recorded (Fig. S2).

Individuals were divided by age class according to Notarbartolo di Sciara and Demma (1994). Correlation between mature females ($n = 10$) and others (the sum of immature males, immature females and mature males; $n = 8$) was calculated (Fig. 3).

3.4. OCs determination for stranding area

Results were also divided by the sea in which the stranding event occurred: Ligurian Sea ($n = 7$) and Tyrrhenian Sea ($n = 8$) (Table S5). We chose not to include the Adriatic Sea ($n = 3$) and Ionian Sea ($n = 2$) because there were few animals to make any conclusions about these areas.

Statistical analyses did not reveal any statistically significant differences (Fig. S3). However, the levels of *HCB*, *DDTs* and *PCBs* were higher in the Tyrrhenian Sea (*HCB* = 593.07 ng/g l.w.; *DDTs* = 90,442.85 ng/g l.w.; *PCBs* = 133,489.69 ng/g l.w) than in the Ligurian Sea (*HCB* = 276.40 ng/g l.w.; *DDTs* = 71,137.75 ng/g l.w.; *PCBs* = 105,521.32 ng/g l.w).

4. Discussion

The results showed the presence of all the three *OC* classes (*HCB* < *DDTs* < *PCBs*) which continue to be priority contaminants in the Mediterranean basin despite the restrictive regulation in their production and use in most areas of the world. Though the Stockholm Convention has regulated them at global level in 2001, Italy, until September 2022, was the only state in the European Community that has not yet ratified the convention (UNEP – United Nations Environment Programme – 2001) (Marsili et al., 2014; Law and Jepson, 2017; Jepson et al., 2016; Stuart-Smith and Jepson, 2017). The presence of these environmental pollutants poses a threat to Mediterranean cetaceans, determining the trends of the species and their populations (Stuart-Smith and Jepson, 2017).

The prevalence of Hexa-CB and Hepta-CB is similar to the pattern of Arochlor 1260 and of other *G. griseus* specimens and cetacean species stranded in the Mediterranean Sea (Storelli and Marcotrigiano, 2000; Dron et al., 2022).

pp′DDE is the predominant isomer in all four tissues analysed, in fact it is the main metabolite of DDT (Ricking and Schwarzbauer, 2012). The brain has the largest differences in the metabolites of DDT from other tissues. This is due to the lipid composition of the tissues and the polarity of the components, which have different affinities for these compounds and their binding (Tanabe et al., 1981; Aguilar, 1985). The concentration of these compounds in different tissues also depends on their composition: 99 % of the total lipids in the blubber are triglycerides,

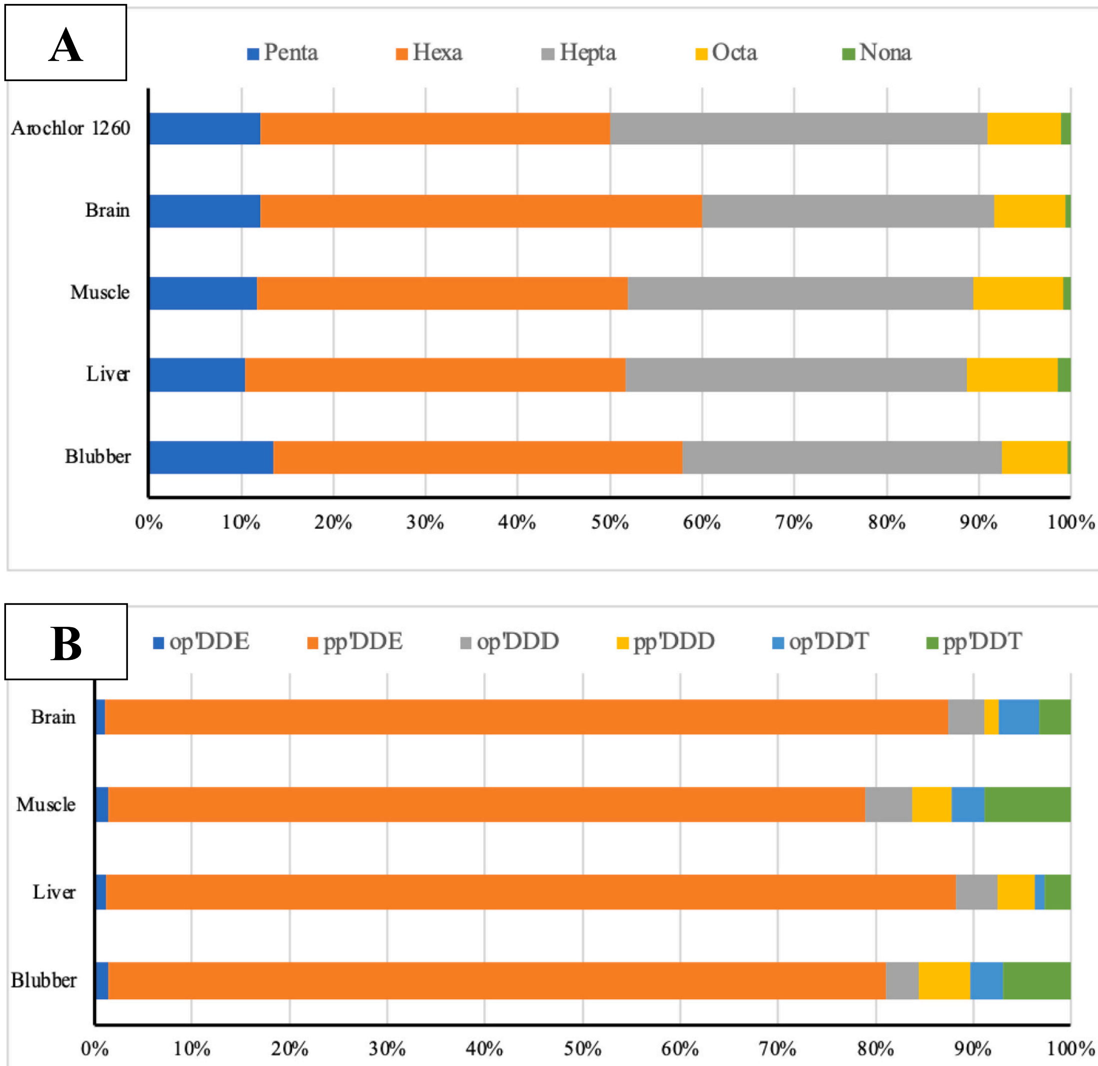

Fig. 2. Percentage composition of the polychlorinated biphenyls (*PCBs*) divided by chlorine content (Penta-CBs, Hexa-CBs, Hepta-CBs, Octa-CBs, Nona-CBs) on *PCBs* (A) and of the op′ and pp′ forms of DDT, DDE and DDD on *DDTs* (B), analysed in the brain, muscle, liver, and blubber of *G. griseus*.

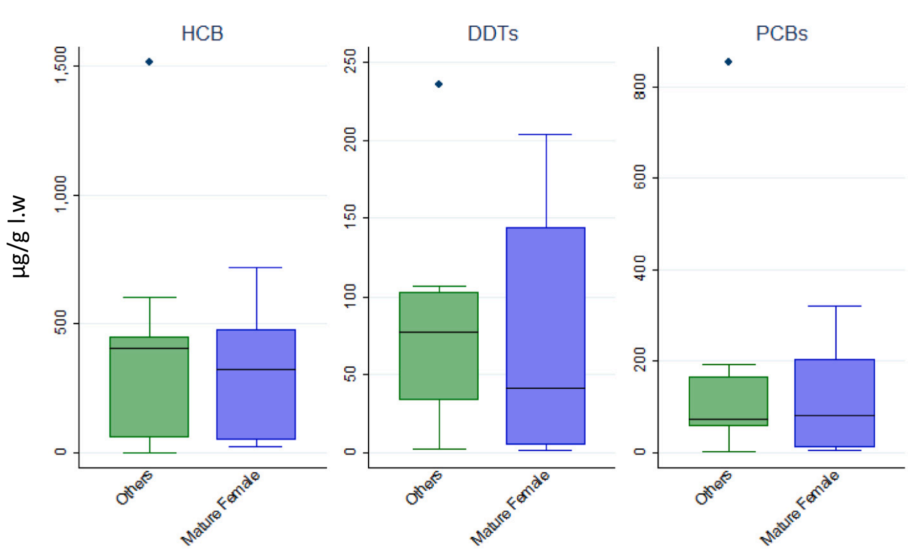

Fig. 3. Boxplots of the three organochlorine compounds (*HCB*, *DDTs*, *PCBs*) expressed in µg/g l.w. by Mature Female *vs* Others.

whereas the brain contains >50 % of phospholipids (Kawai et al., 1988).

The distribution of *OCs* in the four sampled tissues was similar to those recorded in previous studies (Marsili and Focardi, 1996). Higher levels in males can be explained by the fact that males continuously accumulate the contaminants in their tissues throughout their lives (Tanabe et al., 1982), while lower levels in females can be explained by the possibility to discharge (up to 90 %) contaminants to offspring during the period of gestation and lactation (Marsili and Focardi, 1996).

Although there were no statistically significant differences in stranding areas, levels of *HCB*, *DDTs* and *PCBs* were higher in the Tyrrhenian Sea, which can also be considered a highly impacted area from an anthropic point of view thanks to the high rate of marine traffic, hence a hot spot for contaminants (Fossi et al., 2017).

The *DDTs/PCBs* ratio gives us the possibility to estimate the different sources of contamination. In all tissues, a value of <1 was found, thus a contamination of industrial origin. The pp′DDE/DDTs ratio, which gives us information on the historicity of the contamination (Aguilar, 1984) was higher in all tissues than the threshold value of 0.6, so no new releases of this insecticide into the environment are indicated.

Another indicator of the historicity of contamination is the pp′DDE/pp′DDT ratio; here too, all samples showed a high presence of pp′DDE compared to pp′DDT, confirming that *DDT* contamination is due to the historic use of this substance. The other ratio, Σop′DDT/DDTs was below the threshold of 0.20 in all tissues, so the source of *DDT* contamination is due to the insecticide mixture (Qiu et al., 2005).

Many studies have been previously conducted on organochlorine compounds in dolphins. A study carried out by Marsili and Focardi (1996) on the ecotoxicological analysis of an adult male Risso's dolphin stranded on Elba Island (Italy) showed high concentrations of organochlorine levels within the adipose tissue. Our results are in line with the conclusions of Marsili and Focardi (1996), who showed higher levels for *DDTs* and *PCBs*. High levels of HCBs were also found in this study.

A study conducted by Hayteas and Duffield (1997) showed the presence of *PCBs* and pp′DDE in the blubber of a newborn male stranded along the Oregon (USA) coast between 1991 and 1995. Compared to the newborn stranded in the waters of Rimigliano (Italy) in 2002 analysed in our study, the Oregon newborn showed higher levels of contaminants: the *PCB* value was 18,600 ng/g l.w., while the *DDT* isomer, pp′DDE, it was 44,500 ng/g l.w.

A study conducted by Jarman et al. (1996), regarding the levels of organochlorines compounds in the blubber of a cetacean from the West Coast of North America, examined in one mature male of Risso's dolphin, collected in March 1988, a *HCB* concentration level of 33 μg/kg l.w., a *DDTs*, and its respective isomers level, of 5000 μg/kg l.w. and finally the *PCBs*, and its congeners, of 1700 μg/kg l.w. The mean concentrations of the OCs, in the mature male individuals ($n = 3$) analysed (*HCB* 220.58 ng/g l.w., *PCBs* 327,516.78 ng/g l.w. *DDTs* 72,805.29 ng/g l.w.), are all higher than those reported by Jarman et al. (1996).

The Mediterranean individuals analysed in this study have much higher concentrations of contaminants than those recorded by Jarman et al. (1996). This finding is consistent considering that the Mediterranean Sea is particularly distressed by environmental contamination because of its half-closed basin geomorphology and its high coastal anthropization (Marsili et al., 2018).

In the study by Corsolini et al. (1995), in the blubber of two Risso's dolphin stranded along the Italian coast in 1992, the mean concentration for *PCBs* was 520,000 ng/g l.w, while for *DDTs* it is 340,000 ng/g l.w, higher which are than those recorded in our study.

However, lower values were reported by Storelli and Marcotrigiano (2000), who analysed the blubber of three individuals: *HCB* 20.9 μg/kg l.w., DDT and its respective isomers 7493 μg/kg l.w., and *PCB* 1028 μg/kg l.w.

Moreover, the *PCB* levels reported in the same study, can be compared to the levels reported by Jarman et al. (1996) for the animals of the west coast of North America (1700 μg/kg l.w.).

Analyses of contaminants in the adipose tissues of different species with the same feeding characteristics were compared such as the sperm whale (*Physeter macrocephalus*). The study conducted by Pinzone et al. (2015), in sperm whale's blubber ($n = 7$) detected a mean value for *PCBs* of 190,635 ng/g l.w. and a mean of 205,176 ng/g l.w. for *DDTs*.

The *OC* concentrations analysed in our study did not differ from those found in sperm whales. Both *P. macrocephalus* and *G. griseus* have a mainly teutophagous diet. This absence of differences could be due to the ingestion of the similar prey including the genus Histioteuthidae, Ommastrephidae and Onychoteuthidae (Bearzi et al., 2011; Blanco et al., 2006; Foskolos et al., 2020).

5. Conclusion

The results of the analysis carried out on individuals of *Grampus griseus* stranded along the Italian coasts from 1998 to 2021 showed the presence, in all the animals, of all the three classes of contaminants examined. Not significant differences in contaminant concentrations were related to age, sex and location of stranding.

In the literature until now there is little information, not only on the ecotoxicology and biology of the species, in general but also on the distribution and use of the habitat which, have not allowed us to fully understand the ecological and biological aspects of the species.

To date, no specific conservation measures have been adopted for Risso's dolphins in the Mediterranean Sea.

We insist that research and study on this species should be extended, and additional areas where protection measures would benefit the species should be identified, especially considering that the species has been ranked on the *IUCN* Red List as "*Least Concern*" globally and "*Vulnerable*" in the Mediterranean Sea.

The results of this study may help to plan legislation for conservation purposes. This study is the first of its kind highlighting the potential toxicological and biological information on Risso's dolphin can offer, constituting a great improvement for the knowledge of this species.

CRediT authorship contribution statement

L.M., G.C. and L.M. conceived the study, L.M., G.C. and L.M. designed the experimental approach. L.M., G.C, S.M., C.M., G.T., I.C., F. C., A. N. and L.M. conducted sampling activity. L.M., G.C. I.C., F.C., and L.M. contributed to the experimental work and laboratory analyses. L. M., G.C., I.C., F.C., A. D'A. and L.M. contributed to data elaboration and data interpretation. All the authors contributed to write and to finalize the 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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114470.

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