LINKING ORGANOCHLORINE CONTAMINANTS WITH DEMOGRAPHIC PARAMETERS IN FREE-RANGING COMMON BOTTLENOSE DOLPHINS FROM THE NORTHERN ADRIATIC SEA

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HIGHLIGHTS

- Male bottlenose dolphins have significantly higher PCB concentrations than females
- Nulliparous females have significantly higher concentrations than parous ones
- There are no differences among social groups
- Majority of animals exceed the toxicity thresholds
- Pollutant concentrations can successfully be linked with demographic parameters

ABSTRACT

Marine top predators, including marine mammals, are known to bio-accumulate persistent pollutants such as polychlorinated biphenyls (PCBs), a serious conservation concern for these species. Although PCBs declined in European seas since the 1970s-1980s ban, considerable levels still persist in European and Mediterranean waters. In cetaceans, stranded animals are a valuable source of samples for pollutant studies, but may introduce both known and unknown biases. Biopsy samples from live, free-ranging cetaceans offer a better alternative for evaluating toxicological burdens of populations, especially when linked to known histories of identified individuals. We evaluated PCB and other organochlorine contaminants in free-ranging common bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Trieste (northern Adriatic Sea), one of the most human-impacted areas in the Mediterranean Sea. Biopsies were collected from 32 male and female dolphins during 2011–2017. All animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002. We tested for the effects of sex, parity and social group membership on contaminant concentrations. Males had significantly higher organochlorine concentrations than females, suggesting offloading from reproducing females to their offspring via gestation and/or lactation. Furthermore, nulliparous females had substantially higher concentrations than parous ones, providing further support for maternal offloading of contaminants. Overall, 87.5% of dolphins had PCB concentrations above the toxicity threshold for physiological effects in experimental marine mammal

studies (9 mg/kg lw), while 65.6% had concentrations above the highest threshold published for marine mammals based on reproductive impairment in ringed seals (41 mg/kg lw). The potential population-level effects of such high contaminant levels are of concern particularly in combination with other known or suspected threats to this population. We demonstrate the utility of combining contaminant data with demographic parameters such as sex, reproductive output, etc., resulting from long-term studies.

KEYWORDS:

Organochlorine contaminants, PCBs, ecotoxicology, *Tursiops truncatus*, Adriatic Sea, Mediterranean Sea

INTRODUCTION

Persistent organic pollutants (POPs) are chemical compounds that occur in the marine environment and have far-reaching consequences for human and ecosystem health. Marine top predators, including marine mammals, are known to bioaccumulate POPs, which represent a conservation and health concerns for these species and their environment (Tanabe et al. 1994, Aguilar et al. 2002, Vos et al. 2003, Jepson and Law 2016). Of these, organochlorines such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are of particular concern, as they are persistent in the environment, highly lipophilic, bioaccumulate in individuals over time, and biomagnify in marine top predators through trophic transfer (Green and Larson 2016). These toxic compounds may cause anaemia (Schwacke et al. 2012), immune system suppression (Tanabe et al., 1994) and the subsequent increased vulnerability to infectious disease (Aguilar and Borrell 1994a, Jepson et al. 2005, Randhawa et al. 2015), endocrine disruption (Tanabe et al. 1994, Vos et al. 2003, Schwacke et al. 2012), reproductive impairment (Schwacke et al. 2002) and developmental abnormalities (Tanabe et al. 1994, Vos et al. 2003) in marine mammals, thereby representing a serious health risk for these top predators. Such health risks are likely to have direct impacts on marine mammal abundance, through reduced reproduction or survival (Hall et al. 2006, Hall et al. 2017). Because of their trophic position, propensity for bio-accumulating organochlorines, and long life span, marine mammals are often considered ecosystem sentinels (Ross 2000, Wells et al. 2004, Moore 2008).

Due to concerns about toxicity and suspected carcinogenicity to humans, their effects on biota and environmental persistence, the use of PCBs and OCPs such as dichlorodiphenyltrichloroethane (DDT) was banned in most of Europe in the 1970s-1980s. Subsequent monitoring of POPs in tissues of several marine mammal species demonstrated their decline in several European seas (Law et al. 2012), including the Mediterranean Sea (Aguilar and Borrell 2005, Borrell and Aguilar 2007). However, a recent European-wide study showed that PCB levels continue to be high in European and Mediterranean cetaceans (Jepson et al. 2016). In particular, very high PCB concentrations were linked

120 to small populations, range contraction, or population declines in some striped dolphin (*Stenella*
121 *coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*) and killer whale (*Orcinus orca*)
122 populations (Jepson et al. 2016).

123 Linking organochlorine concentrations with individual-level effects in wild marine mammals
124 (and especially cetaceans) is challenging at best, while linking them with potential population-level
125 effects is extremely difficult. It is therefore unsurprising that few quantitative approaches for
126 estimating such effects have been developed (Hall et al. 2017). Stranded animals can be a valuable
127 source of samples for pollutant studies in wild populations (Geraci and Lounsbury 2005), and are often
128 the only source of samples used in toxicological analysis (Jepson et al. 1999, Jepson et al. 2005, Law et
129 al. 2012). However, the use of stranded animals, especially in some contexts or in some locations, may
130 introduce substantial biases. For example, stranded animals may not be representative of the
131 population or area of interest, but may originate from other areas, due to winds, currents, or abnormal
132 behaviour prior to stranding (Hansen et al. 2004). Moreover, putrefaction processes, resulting from
133 exposure to the sun, high temperatures, wind and bacterial activity, can lead to altered organochlorine
134 concentrations and potentially misleading results (Borrell and Aguilar 1990). Finally, it has also been
135 suggested that the presence of disease may lead to abnormal rates of pollutant metabolism or
136 excretion (Borrell and Aguilar 1990). On the other hand, blubber biopsy samples (Noren and Mocklin
137 2012) collected from live, free-ranging cetaceans offer a good alternative for evaluating the
138 toxicological burden of populations (Fossi et al. 2000), especially when linked to long-term re-sighting
139 histories of known individuals (Ross et al. 2000, Ylitalo et al. 2001, Wells et al. 2005). For example,
140 information on pollutant levels can be combined with mark-recapture techniques to estimate the
141 impact of contaminants on survival or reproduction (Hall et al. 2009). Moreover, an appropriate study
142 design can ensure that the sampling is representative of the population or area in question. It was
143 previously recognised that the proper evaluation of pollutants on marine mammals will require efforts
144 directed toward long-term studies of known individuals in wild populations (Hall et al. 2006).

The common bottlenose dolphin is a long-lived marine top predator (Wells and Scott 1999, 2009). In many parts of the world, including the Mediterranean Sea, it is essentially "coastal" and mainly found nearshore (Bearzi et al. 2009). This makes it particularly susceptible to a range of anthropogenic impacts, including the exposure to organochlorine contaminants. This species is regularly present in the Gulf of Trieste and adjacent waters, where it has been continuously studied since 2002 (Genov et al. 2008, Genov et al. 2016, Genov et al. 2017). As a coastal, mobile and long-lived top predator with strong site fidelity, it is a particularly good candidate for investigating the effects of organochlorine contaminants, and for regional monitoring of organochlorine pollution.

In this study, we evaluated organochlorine levels, particularly PCBs, in free-ranging common bottlenose dolphins in relation to demographic parameters, as part of a long-term investigation into their ecology, behaviour and conservation status in the Gulf of Trieste and adjacent waters in the northern Adriatic Sea. In particular, we tested for the effects of sex, parity and social group membership on organochlorine concentrations, in one of the most heavily human-impacted areas within the Mediterranean Sea.

171 MATERIAL AND METHODS

172 *The study population*

173 The Gulf of Trieste, together with its surrounding waters (Fig. 1), is probably one of the most heavily
174 human-impacted areas within the Adriatic and Mediterranean Seas, due to shipping, fishing,
175 industrialisation, tourism, aquaculture and agriculture (Horvat et al. 1999, Faganeli et al. 2003, David
176 et al. 2007, Mozetič et al. 2008, Codarin et al. 2009, Grego et al. 2009). The dolphin population
177 inhabiting these and surrounding waters (Fig. 1) has been the focus of a long-term study and
178 monitoring by Morigenos – Slovenian Marine Mammal Society since 2002, primarily through boat-
179 based surveys and photo-identification, and is now relatively well studied (Genov et al. 2008, Genov
180 2011, Genov et al. 2016, Genov et al. 2017). The population is present within the area year-round
181 (Genov et al. 2008, Genov 2011) and appears to be demographically and genetically distinct (Genov et
182 al. 2009, Gaspari et al. 2015). The annual abundance estimates range between about 70 and 150
183 animals (Genov 2011; Morigenos, *unpublished data)*. Most encountered individuals have extensive re-
184 sighting histories over the study period, and several are of known sex and reproductive output.

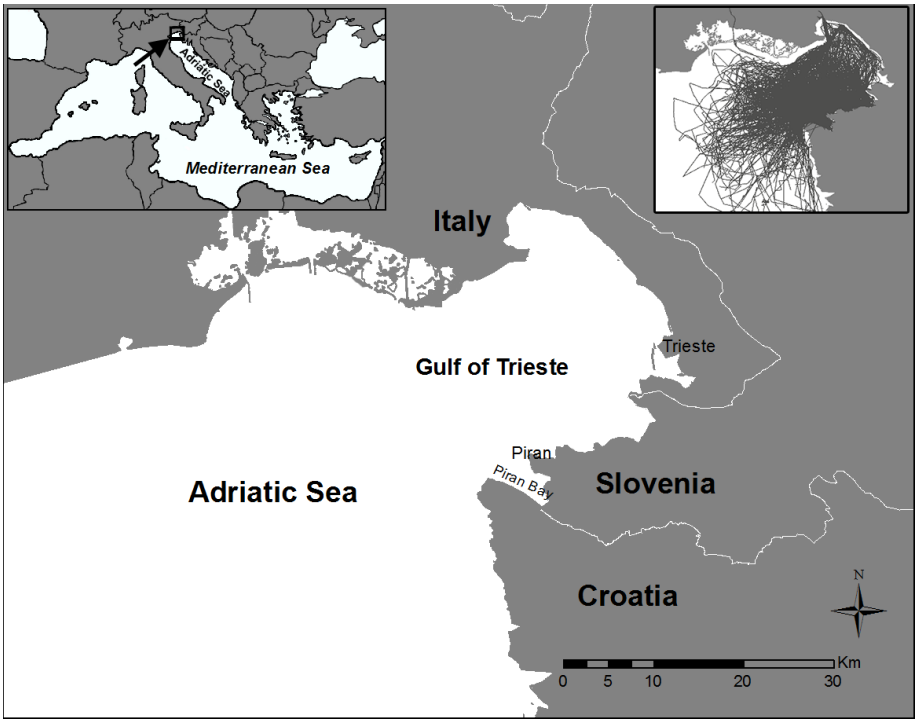

Fig. 1. Study area in the northern Adriatic Sea. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the survey effort (navigation tracks).

Sample collection

Biopsy samples were collected from free-ranging common bottlenose dolphins between 2011 and 2017. Sampling followed standard methodology (Gorgone et al. 2008, Kiszka et al. 2010) and was carried out exclusively in good weather conditions (Beaufort sea state ≤2, good visibility, no precipitation). Samples of skin and blubber tissue were obtained using custom made bolts and stainless steel sampling tips (tip length 25 mm, internal diameter 7 mm), made by Ceta Dart, Copenhagen, Denmark. Sampling tips were sterilised using 96% ethanol and burning prior to being used. Bolts with sterile sampling tips were fired into the dorso-lateral area below the dorsal fin (Fig. 2), at distances of

4–10 m, using a Barnett Panzer V crossbow with 68 kg draw weight. A high-pressure moulded stopper prevented the tip from penetrating more than about 20 mm and ensured the re-bouncing of the bolt. The floating bolt was retrieved from the water by hand. Blubber samples were removed and excised with sterilised forceps and surgical scissors, placed in aluminium foil and stored at −20°C until chemical analysis.

Sampling was only attempted on adults. No sampling was attempted on offspring or mothers with offspring. Care was taken not to attempt sampling of animals accompanied (followed) by another animal in their slipstream, to prevent potential shots in the head. All biopsy attempts were accompanied by concurrent photo-identification (Würsig and Jefferson 1990) of targeted individuals and other dolphins in their group. This ensured that the identity of the sampled animal was known, in order to prevent re-sampling the same individuals, and to be able to link organochlorine concentrations to various individual-specific parameters known from photo-identification. During each attempt, the behavioural reactions of the target animal and the focal group were recorded, together with information on distance of the target animal, the area hit and the sea state. Biopsy sampling was conducted under the permit 35601-102/2010-4 by the Slovenian Environmental Agency.

In addition to biopsies, one sample was collected from an adult male found entangled in fishing gear – due to the freshness of the carcass, it could be identified with confidence, determined to be one of the local dolphins, and therefore included in the analysis. Stranded animals too decomposed to be identified were not included in the analyses, as they were of unknown origin and may not be representative of the population in question.

Fig. 2. Biopsy sample collected from a free-ranging common bottlenose dolphin in the Gulf of Trieste, northern Adriatic Sea. Photo: Ana Hace, Morigenos

Demographic parameters

Sex of individuals was determined by a) observations of temporally stable adult-offspring associations (adults consistently accompanied by offspring were assumed to be mothers and therefore females); b) photographs of the genital area during bowriding or aerial behaviour and c) molecular methods from biopsy samples. For molecular sex determination, DNA was extracted with phenol/chloroform and ethanol precipitation from tissue samples preserved in 95 % ethanol. Sex was determined through

differential amplification of the zinc finger gene regions present in the X and Y chromosomes (ZFX and ZFY, respectively), as described by Bérubé and Palsbøll (1996).

Parity was assessed based on re-sighting histories and reproductive output of photo-identified females. Females known to have produced at least one offspring during the study period were considered parous. Females never observed with offspring were assumed to be nulliparous. One of these females appeared older based on external appearance, and could potentially be of post-reproductive age, although evidence for reproductive senescence in bottlenose dolphins is limited (Marsh and Kasuya 1986, Wells and Scott 1999, Ellis et al. 2018).

Previous work on social network analyses has shown that the local dolphin population is structured into distinct social groups, which exhibit temporal partitioning, differences in behaviour with respect to fisheries and may have different feeding preferences (Centrih et al. 2013; 2014; Genov et al. 2014; 2015; Genov et al., *in press*).

Chemical analysis

Blubber samples were stored frozen at –20.0 °C. Samples were analysed using the method reported in detail in Jepson et al. (2016). In brief, samples were subjected to Soxhlet extraction using of acetone: *n*-hexane 1:1 (*v:v*) and cleaned up and fractionated using alumina (5% deactivated) and silica (3% deactivated) columns, respectively. The total extractable lipid content was determined gravimetrically after evaporation of the solvent from an aliquot of the uncleaned extract. Lipid content varied from 3.4 to 33.8%. PCB concentrations in dolphin samples were determined with an Agilent 6890 GC with µECD. The PCB standard solutions contained the following 27 compounds in iso-octane: Hexachlorobenzene; *p,p'*-DDE; CB101; CB105; CB110; CB118; CB128; CB138; CB141; CB149; CB151; CB153; CB156; CB158; CB170; CB18; CB180; CB183; CB187; CB194; CB28; CB31; CB44; CB47; CB49; CB52; CB66, together with the internal standard CB53. Quantification was performed using internal standards and 11 calibration levels (range 0.5 – 400ng/ml). CEFAS follows a strict QA/QC regime for analysis of samples. The laboratory biannually participates in proficiency testing scheme Quasimeme

(Quality Assurance of Information for Marine Environmental Monitoring in Europe) as external quality assurance. All analyses were carried out under full analytical quality control procedures that included the analysis of a certified reference material (BCR349 cod liver oil; European Bureau of Community reference) and a blank sample with every batch samples analysed so that the day-to-day performance of the methods could be assessed. Wet weight analyte concentrations were converted to lipid-normalised concentrations using measured lipid contents. Values below the limit of quantification (LOQ) were reported as <LOQ. In addition to the compounds mentioned above, four samples (two males, one female and one animal of unknown sex) were also analysed for *p,p'*-TDE (also known as *p,p'*-DDD) and *p,p'*-DDT. The limited budget available for analysis prevented us from doing this for the entire sample set.

~~Blubber samples were stored frozen at –20.0 °C. Samples were analysed using the method reported in Jepson et al., 2005 and Law et al., 2012. A total of 25 PCB congeners plus the organochlorines hexachlorobenzene and *p,p'* DDE were determined in the samples. Additionally, dichlorodiphenyltrichloroethane (DDT) was determined in four of the samples. In preparation for analysis, samples were defrosted and dissected in a strictly controlled, contaminant-free environment. Samples were chopped with solvent-rinsed scalpels and homogenised. Tissue samples were stored frozen at -20°C until required for analysis. After thawing, the homogenised subsamples were dried by mixing with anhydrous sodium sulphate and storing in a freezer for a minimum of 12 hours prior to further analysis. The samples were subjected to Soxhlet extraction using acetone: *n*-hexane 1:1 (*v:v*) for 5.5 hours. The total extractable lipid content was determined gravimetrically after evaporation of the solvent from an aliquot of the uncleaned extract. Lipid content varied from 3.4 to 33.8%. Depending on the lipid content of the samples, varying volumes of the blubber extracts were cleaned to have <50 mg of lipid in the samples for PCB analysis. Aliquots of the extracts were cleaned up and fractionated using alumina (5% deactivated) and silica (3% deactivated) columns, respectively. The~~

final gas chromatography (GC) ready fractions were spiked with PCB53 internal standard and made up to a final volume of 1 ml. PCB concentrations in dolphin samples were determined with an Agilent 6890 GC with μECD. The separation of analytes was performed on a 50.0 m × 200 μm, 0.33 μm film thickness DB-5 capillary column (J&W). The carrier and ECD make-up gas were hydrogen (32.2 psi constant pressure, initial velocity 50 cm/s) and argon/methane (95:5), respectively. The initial oven temperature was 90°C, held for 2.00min, then increased to 165°C at 15°C/min, to 285°C at 2°C/min, and finally held for 23 min. The injector temperature and detector temperature was 270°C and 300°C, respectively. A 1 μl extract was injected in splitless mode with a purge time of 2 min. The PCB standard solutions contained the following 27 compounds in iso-octane: Hexachlorobenzene; *p,p'* DDE; CB101; CB105; CB110; CB118; CB128; CB138; CB141; CB149; CB151; CB153; CB156; CB158; CB170; CB18; CB180; CB183; CB187; CB194; CB28; CB31; CB44; CB47; CB49; CB52; CB66, together with the internal standard CB53. Quantification was performed using internal standards and 11 calibration levels (range 0.5 – 400ng/ml). CEFAS follows a strict QA/QC regime for analysis of samples. The laboratory biannually participates in proficiency testing scheme Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) as external quality assurance. All analyses were carried out under full analytical quality control procedures that included the analysis of a certified reference material (BCR349 cod liver oil; European Bureau of Community reference) and a blank sample with every batch samples analysed so that the day-to-day performance of the methods could be assessed. If levels of target analytes in the samples were outside of the range of the instrument calibration, extracts were diluted to be within range and re-analysed. The results obtained for the reference materials were plotted as Shewhart quality control charts for each compound or determined. The charts had previously been created by the repeated analysis of the above certified reference materials in the CEFAS Lowestoft Laboratory using the North West Analytical Quality Analyst software™ (Northwest Analytical Inc., USA). Warning and control limits had been defined for the charts as 2σ and 3σ – 2x and 3x the standard deviation from the mean for each compound or trace element, respectively. The results obtained for all samples analysed were accepted as valid as the results for the certified

~~reference materials were within the limits set by the control charts. Concentrations were converted to on a lipid weight basis using measured lipid contents. Values below the limit of quantification (LOQ) were reported as <LOQ. In addition to the compounds mentioned above, four samples (two males, one female and one animal of unknown sex) were also analysed for *p,p'* TDE and *p,p'* DDT. The limited~~

Statistical analysis

For statistical analysis, congener concentrations below the limit of quantification (LOQ) were set to one-half of the LOQ (Darnerud et al. 2006, Lignell et al. 2009, Law et al. 2012). We compared this approach of treating <LOQ values with two alternative approaches: 1) replacing <LOQ values with zero and 2) keeping <LOQ values at the LOQ value. The choice of the approach had negligible effect on the results, and had no effect on conclusions. We therefore considered this approach the best compromise between underestimating and overestimating toxicological burden.

The values of individual 25 PCB congeners for each sample were summed to obtain the Σ25PCB for each individual. In addition, the sum of priority PCB congeners (28, 52, 101, 118, 138, 153 and 180) listed by the International Council for the Exploration of the Sea (ICES) was also calculated and displayed, for ease of comparison with some of the previous studies. The lipid content of each sample was used to obtain concentrations as mg/kg lipid weight (mg/kg lw).

Tests of normality revealed non-normal distribution of data. Both arithmetic and geometric means across individuals were calculated for Σ25PCB, ΣICES7 and *p,p'*-DDE. HCB values were too low (below the limit of quantification) to allow any useful analysis (Table 1). The contribution of each individual PCB congener to the Σ25PCB was also calculated across all individuals.

We tested for the effects of 1) sex, 2) parity (whether a female has previously had a calf or not) and 3) social group membership on contaminant concentrations. The Mann-Whitney U test was used to examine differences between males and females, and between nulliparous and parous females. The

Kruskal-Wallis test was used to examine differences among social groups. Statistical analysed were carried out in program R (R Core Team 2017).

Assessing toxicity

Two PCB toxicity thresholds or reference values were used, following Jepson et al. (2016). A lower PCB toxicity threshold was used for the onset of physiological endpoints in marine mammals of 17 mg/kg lipid weight (lw) (as Aroclor 1254, Kannan et al. 2000), that was calculated to be equivalent to 9.0 mg/kg lw (Σ25PCB) in Jepson et al. (2016) and in this study. A higher PCB toxicity threshold, the highest reported in marine mammal toxicology studies, of 77 mg/kg lw (as Clophen 50) for reproductive impairment in Baltic ringed seals (*Pusa hispida*, Helle et al. 1976) was calculated to be equivalent to 41 mg/kg lw (as Σ25PCB) in Jepson et al. (2016) and in this study.

RESULTS

Between 2011 and 2017, samples were obtained from 32 adult dolphins, including 18 males, 9 females and 5 animals of unknown sex (Table 1). Six of these samples were included in the study by Jepson et al. (2016). Six females were previously observed with offspring, while three were not.

PCBs

Σ25PCB ranged from 4.13 to 293 mg/kg lipid weight, with an arithmetic mean of 81.5 (95% CI = 57.2 – 105.8) and a geometric mean of 53.4 (95% CI = 36.9 – 77.3, Table 2). Males had significantly higher Σ25PCB concentrations than females (Mann-Whitney U test, $U = 155$, $P < 0.001$, Fig. 3). Furthermore, nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U test, $U = 17$, $P < 0.05$, Fig. 4). There were no statistically significant differences among social groups (Kruskal-Wallis test, $H = 1.21$, $P = 0.75$, Fig. 5).

Figure 6 shows female and male PCB concentrations in relation to two toxicity thresholds. Overall, 87.5% of dolphins had PCB blubber concentrations above the toxicity threshold of 9 mg/kg lw for physiological effects in experimental marine mammal studies (Kannan et al. 2000), while 65.6% had concentrations above the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals (Helle et al. 1976). In males, mean Σ25PCB were above the higher of the two thresholds, even when the lower confidence limit is considered (Fig. 6). One male had a Σ25PCB concentration of 293 mg/kg lw. In females, mean Σ25PCB were above the lower toxicity threshold of 9 mg/kg lw, but did not reach the higher one of 41 mg/kg lw, not even when the upper confidence limit is considered (Fig. 6). The lower confidence limit of ΣPCB in females was just below the lower toxicity threshold (Fig. 6). The ΣICES7 concentrations follow a similar pattern and are presented in Tables 1 and 2.

Among dioxin-like PCBs, these represented 2.3% (PCB 118, found in 90.6% of samples), 0.8% (PCB 156, found in 75% of samples) and 0.7% (PCB 105, found in 75% of samples) of the total PCB burden, respectively. Concentrations of the PCB congener 28 was below LOQ for all samples. PCB congeners 153, 138, 180, 187, 149 and 170 had the highest mean values across individual dolphins (Table 3, Fig. 7). Combined, they contributed 77.9% of the total PCB burden. Congeners 44, 31, 28, 18, 141, 49 and 110 had the lowest mean values, with a combined contribution of 2.2% to the total PCB burden (Table 3, Fig. 7).

~~samples), 0.8% (PCB 156, found in 75% of samples) and 0.7% (PCB 105, found in 75% of samples) of the total PCB burden, respectively. Concentrations of the PCB congener 28 was below LOQ for all~~

DDE and DDT

The concentrations of *p,p'*-DDE ranged from 0.3 to 32.9 mg/kg lw, with an arithmetic mean of 11.6 (95% CI = 8.3 – 14.8) and a geometric mean of 6.7 (95 % CI = 4.2 – 10.7, Table 2). As with PCBs, males

had significantly higher *p,p'*-DDE concentrations than females (Mann-Whitney U test, $U = 152$, $P < 0.001$, Table 2), and nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U test, $U = 18$, $P < 0.05$). Like for PCBs, there were no statistically significant differences among social groups (Kruskal-Wallis test, $H = 1.15$, P = 0.76). The values of total DDT (the sum of *p,p'*-DDE, *p,p'*-TDE and *p,p'*-DDT) for four individuals are shown in Table 1. For these four samples, the mean contribution of *p,p'*-DDE to total DDT was 89.7% (range = 83.9 – 92.6%), showing that *p,p'*-DDE is the predominant metabolite of total DDT.

HCB

Most HCB values were below the limit of quantification (Table 1). Using half the LOQ for calculations, the HCB concentrations ranged from 0.03 to 0.22 mg/kg lw, with an arithmetic mean of 0.09 (95% CI = 0.08 – 0.12) and a geometric mean of 0.09 (95% CI = 0.07 – 0.10, Table 2). Due to these low values, no further analysis was carried out on HCB concentrations.

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412 **Table 1.** Summary of common bottlenose dolphin samples from the Gulf of Trieste (northern Adriatic
413 Sea), analysed in this study. F = female, M = male. Parity is indicated by + (parous) and –
414 (nulliparous). Σ25PCB, ΣICES7, *p,p'*-DDE, DDT and HCB values expressed as mg/kg lipid weight. DDT
415 represents total DDT. The "<" indicates that the concentration was below the limit of quantification.

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Sample	Year	Sex	Parity	Source	% Lipid	Σ25PCB	ΣICES7	*p,p'*-DDE	ΣDDT	HCB
1	2011	M		Biopsy	23.3	64.2	40.9	9.03		<0.098
2	2011	M		Biopsy	9.7	80.2	50.9	11.3		<0.144
3	2011	M		Biopsy	16.2	58.7	37.1	8.02		<0.166
4	2011	M		Biopsy	11.7	139.8	94.8	13.7		0.102
5	2011	M		Biopsy	19.5	293	190	32.9		0.128
6	2011	F	+	Biopsy	17.5	29.0	14.9	1.54		<0.091
7	2013	M		Biopsy	15.2	34.2	21.2	4.49		<0.197
8	2013	F	+	Biopsy	12.9	7.96	3.96	0.44		<0.341
9	2013	F	+	Biopsy	10.9	17.9	9.89	0.95		<0.202
10	2013	M		Biopsy	3.4	23.0	14.4	2.67		<0.414
11	2014	F	–	Biopsy	10.5	27.2	17.5	9.41		<0.208
12	2014	F	+	Biopsy	27.9	4.13	2.12	0.25		<0.093
13	2014	M		Biopsy	6.6	32.2	20.2	16.7		<0.441
14	2014	M		Biopsy	13.5	43.7	27.0	5.51		<0.228
15	2014	M		Biopsy	6.9	56.7	35.6	7.72		<0.305
16	2014	M		Biopsy	23.9	123	81.2	17.5		<0.092
17	2014	F	–	Biopsy	19.3	30.7	19.2	4.25		<0.124
18	2014	F	–	Biopsy	33.8	48.9	31.0	6.45		<0.141
19	2014	M		Biopsy	10.1	131	84.8	21.9		<0.217
20	2014	M		Biopsy	18.8	65.9	40.7	9.55		<0.333
21	2014	M		Biopsy	9.3	93.8	60.9	13.5		<0.139
22	2014	M		Biopsy	14.5	76.8	48.8	10.1		<0.200
23	2015	M		Bycatch	6.6	152	96.5	25.9		<0.166
24	2015	M		Biopsy	7.9	111	74.2	16.0	17.3	<0.164
25	2015	U		Biopsy	7.7	58.3	37.8	8.17		0.195
26	2016	U		Biopsy	13.7	145	96.6	20.3	22.04	<0.080
27	2016	F	+	Biopsy	14.4	6.82	3.88	0.54	0.54	<0.104
28	2016	M		Biopsy	4.4	121	80.3	16.7	18.6	<0.215
29	2016	U		Biopsy	11.3	150	98.2	23.5		<0.194
30	2017	U		Biopsy	18.9	157	102	23.5		<0.106
31	2017	U		Biopsy	11.8	219	144	27.2		<0.126

32	2017	F	+	Biopsy	25.3	7.64	4.37	0.47	<0.059

Table 2. Σ25PCB, ΣICES7, *p,p*'-DDE and HCB concentrations by sex: mean, median, geometric mean with 95% confidence interval, and range. All values are in mg/kg lipid weight. "Mean" is arithmetic mean. "Geomean" is geometric mean.

	N	Mean	Median	Geomean	Geomean 95% CI	Range (min–max)
Σ25PCB						
Males	18	94.5	78.5	78.3	58.3 – 105.1	23.0 – 293.0
Females	9	20.0	17.9	14.9	8.5 – 26.1	4.1 – 48.9
Unknown	5	145.7	150	134.1	87.0 – 206.7	58.3 – 219.0
OVERALL	**32**	**81.5**	**61.5**	**53.4**	**36.9 – 77.3**	**4.1 – 293.0**
ΣICES7						
Males	18	61.1	49.9	50.1	37.0 – 67.9	14.4 – 190.0
Females	9	11.9	9.9	8.5	4.6 – 15.4	2.1 – 31.0
Unknown	5	95.7	98.2	88.0	56.8 – 136.3	37.8 – 144.0
OVERALL	**32**	**52.7**	**39.3**	**33.2**	**22.4 – 49.1**	**2.1 – 190.0**
***p,p'*-DDE**						
Males	18	13.5	12.4	11.4	8.5 – 15.3	2.7 – 32.9
Females	9	2.7	0.9	1.3	0.6 – 3.1	0.3 – 9.4
Unknown	5	20.5	23.5	19.0	12.5 – 29.1	8.2 –27.2
OVERALL	**32**	**11.6**	**9.5**	**6.7**	**4.2 – 10.7**	**0.3 – 32.9**
HCB						
Males	18	0.11	0.1	0.1	0.08 – 0.12	0.05 – 0.22
Females	9	0.07	0.06	0.07	0.05 – 0.09	0.03 – 0.17
Unknown	5	0.09	0.06	0.08	0.04 – 0.13	0.04 – 0.20
OVERALL	**32**	**0.09**	**0.09**	**0.09**	**0.07 – 0.10**	**0.03 – 0.22**

430

431

432 **Table 3.** Summary statistics for individual PCB congeners. All values are in mg/kg lipid weight.

PCB congener	Mean	Median	SD	Min	Max	Geomean	Geomean 95% CI
C101	1.35	1.33	0.89	0.05	3.16	0.93	0.64 - 1.35
C105	0.42	0.39	0.27	0.03	0.94	0.32	0.23 - 0.43
C110	0.14	0.10	0.10	0.03	0.35	0.11	0.09 - 0.14
C118	1.57	1.48	1.05	0.05	4.10	1.09	0.75 - 1.57
C128	1.67	1.40	1.31	0.05	5.13	1.01	0.66 - 1.56
C138	14.64	11.05	12.47	0.48	51.33	8.86	5.83 - 13.47
C141	0.10	0.09	0.05	0.03	0.22	0.09	0.07 - 0.1
C149	5.83	4.56	5.51	0.15	27.72	3.42	2.2 - 5.31
C151	2.40	1.92	1.97	0.05	8.21	1.45	0.94 - 2.24
C153	24.30	16.89	21.43	0.76	92.40	14.53	9.55 - 22.11
C156	0.61	0.43	0.56	0.03	2.41	0.39	0.27 - 0.56
C158	0.81	0.64	0.65	0.03	2.77	0.52	0.35 - 0.77
C170	3.52	2.61	2.89	0.25	11.81	2.39	1.69 - 3.37
C18	0.09	0.08	0.05	0.03	0.22	0.08	0.07 - 0.09
C180	9.71	6.34	8.72	0.68	36.96	6.31	4.42 - 8.99
C183	2.25	1.67	1.81	0.15	7.19	1.51	1.06 - 2.15
C187	8.07	6.09	6.76	0.58	30.80	5.45	3.86 - 7.7
C194	1.45	1.31	1.09	0.17	4.47	1.05	0.78 - 1.43
C28	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C31	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C44	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C47	0.57	0.55	0.42	0.03	1.51	0.38	0.26 - 0.56
C49	0.10	0.09	0.05	0.03	0.22	0.09	0.07 - 0.11
C52	0.99	0.91	0.76	0.03	2.71	0.6	0.39 - 0.92
C66	0.68	0.46	0.71	0.03	2.79	0.31	0.19 - 0.52

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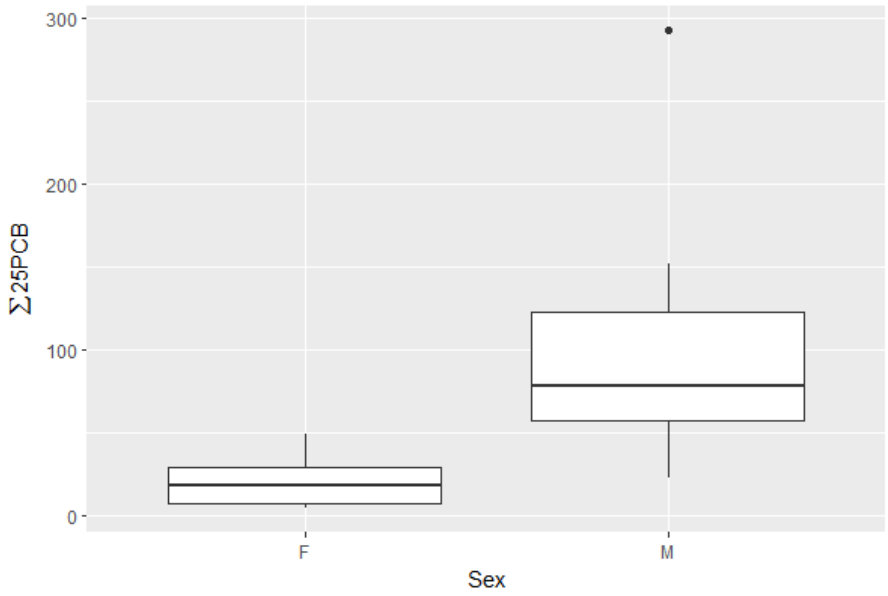


Fig. 3. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between females (F, n = 9) and males (M, n = 18). The difference is statistically significant (Mann-Whitney U test, U = 155, P < 0.001).

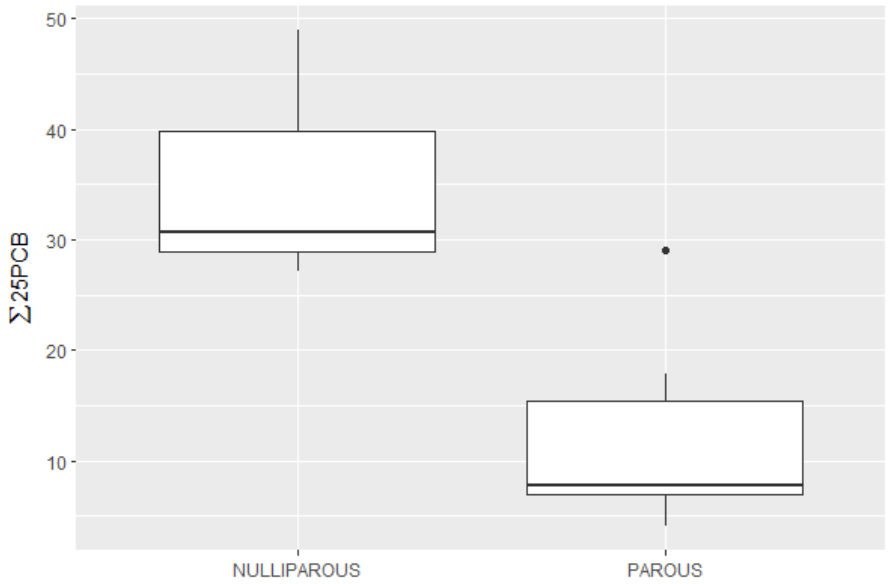
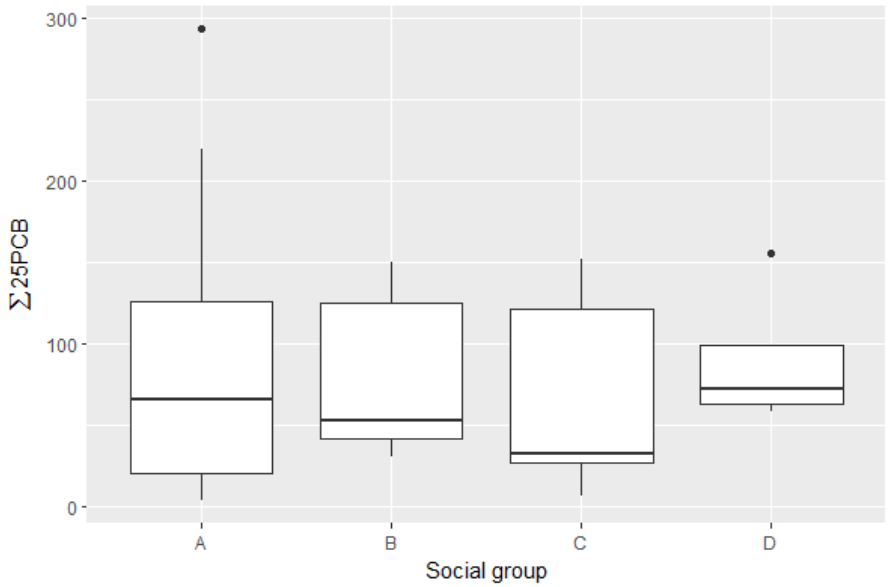


Fig. 4. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between nulliparous (n = 3) and parous (n = 6) females. The difference is statistically significant (Mann-Whitney U test, U = 17, P < 0.05).



448

449 **Fig. 5.** Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) among

450 social groups A (n =15), B (n = 8), C (n = 5) and D (n = 4). Differences are not statistically significant

451 (Kruskal-Wallis test, H = 1.24, P = 0.743).

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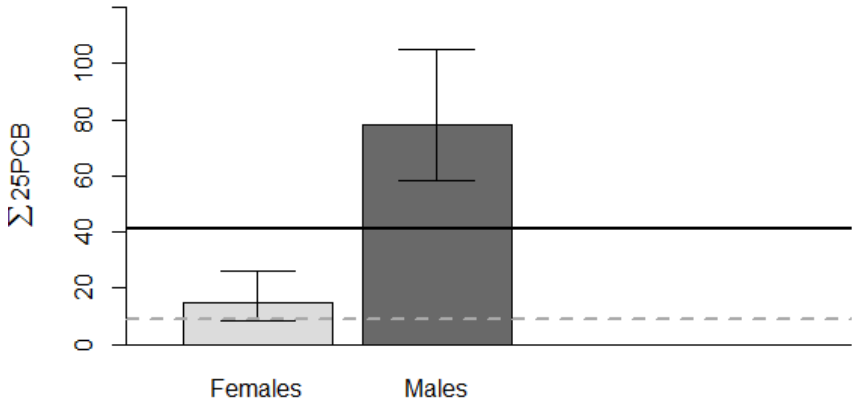

454

455 **Fig. 6.** Geometric mean Σ25PCB (mg/kg lipid weight) concentrations for females and males, in relation
456 to published toxicity thresholds. Error bars are the 95 % confidence intervals for geometric means. The
457 lower dashed grey line represents the lower toxicity threshold (9 mg/kg lw) for onset of physiological
458 effects in experimental marine mammal studies (Kannan et al. 2000). The solid black line represents
459 the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment
460 in ringed seals from the Baltic Sea (Helle et al. 1976).

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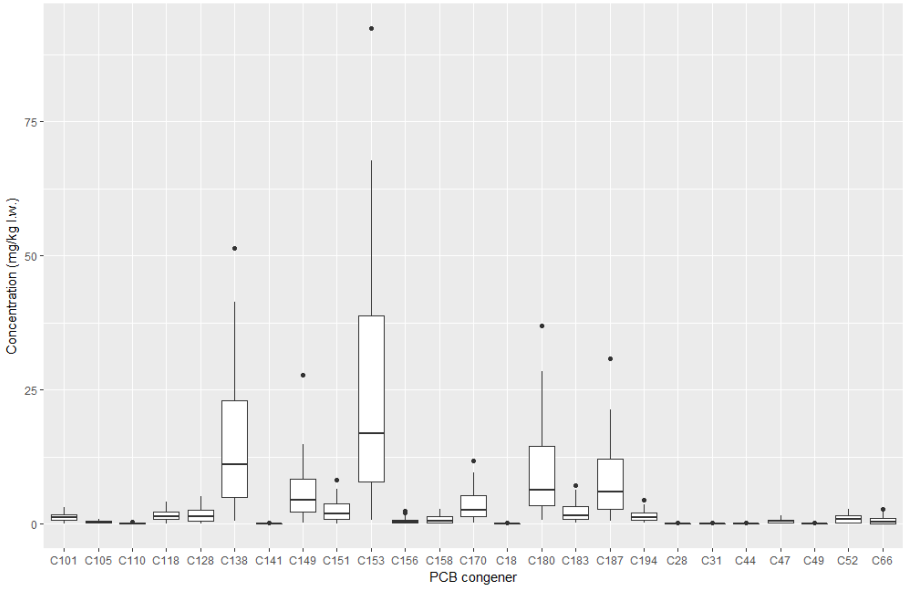

Fig. 7. Contribution of individual PCB congeners to the total PCB burden.

477 DISCUSSION

478 In this study, we assessed the organochlorine levels in free-ranging common bottlenose dolphins from
479 the Gulf of Trieste and adjacent waters in the northern Adriatic Sea. We show that organochlorine
480 concentrations vary with sex and reproductive status, but not with social group membership. With the
481 largest sample size analysed in the Adriatic Sea to date, and samples coming from live resident animals
482 with known resighting histories, this study provides an unprecedented insight into the organochlorine
483 burden in Adriatic dolphins. Judging from the literature, this study may also represent the largest
484 sample size of live free-ranging animals in the Mediterranean Sea or Europe published for this species
485 to date, and is comparable to some of the world's largest sample sizes analysed (Table 4).

486 To date, a number of studies looked at contaminant levels in different cetacean species in the
487 Adriatic Sea. Marsili and Focardi (1997) investigated organochlorines in cetaceans stranded around the
488 Italian coasts, but only three samples were from bottlenose dolphins from the northern Adriatic.
489 Storelli and Marcotrigiano (2000) assessed organochlorines from three Risso's dolphins (*Grampus*
490 *griseus*) stranded in the southern Adriatic. Storelli and Marcotrigiano (2003) and Storelli et al. (2007)
491 assessed organochlorines in bottlenose dolphins stranded on the southern Adriatic Sea coast, but the
492 latter study did not include analysis of blubber tissue. In the same area, Storelli et al. (2012) measured
493 organochlorines in stranded striped dolphins. In the northern Adriatic Sea, on its eastern side, Lazar et
494 al. (2012) analysed different tissues in a single common dolphin (*Delphinus delphis*), a species
495 considered extremely rare in the basin nowadays (Bearzi et al. 2004, Genov et al. 2012). Finally, Herceg
496 Romanić et al. (2014) analysed organochlorine contaminants in various tissues in thirteen bottlenose
497 dolphins stranded along the Croatian coast in the northern Adriatic, providing the most comprehensive
498 organochlorine assessment for dolphins in the northern part of the Adriatic Sea until now. All of these
499 studies provided valuable insights, but due to limited sample sizes and the use of stranded animals,
500 the inferences that can be made are somewhat limited.

501 In most cases, cetacean studies typically involve either a) collecting photo-identification data
502 of free-ranging individuals, or b) analysing pollutant concentrations in stranded animals. However,

503 studies combining these two important aspects, the analysis of pollutants in conjunction with long-
504 term photo-identification of live animals (e.g. Ross et al. 2000, Ylitalo et al. 2001, Wells et al. 2005) are
505 relatively rare. In our study, all biopsied animals were photo-identified and are part of a well-known
506 population of about 150 individuals monitored since 2002 (Genov et al. 2008, Genov et al. 2009, Genov
507 et al. 2016, Genov et al. 2017), which adds additional value to this dataset. This allowed us to
508 successfully combine long-term photographic records of identifiable individuals with individually-
509 specific organochlorine concentrations, which in turn enabled us to link contaminant loads to certain
510 demographic parameters in a known resident dolphin population. In the long term, the continued
511 organochlorine monitoring in conjunction with photo-identification may provide further useful insights
512 and we hope to be able to expand on this in the future by including additional parameters. Such
513 integrated information, linking pollutant levels to demographic and other parameters, holds a lot of
514 potential, as PCB concentrations can be linked to sex, reproductive output and other parameters (Ross
515 et al. 2000, Ylitalo et al. 2001, Wells et al. 2005). Such information is often lacking for wild populations
516 and is of considerable importance for evaluating the impacts of pollutants on marine top predators.
517 When considering potential caveats, it should be noted that the sampling of live free-ranging
518 animals meant that there was some heterogeneity in the origin of samples with respect to the exact
519 body location, despite the same general body area being targeted. This could potentially affect the
520 resulting organochlorine concentrations, as contaminant concentrations may vary across the body
521 parts sampled (Aguilar 1987). However, because we expressed the concentrations on a lipid weight
522 basis and quantified the proportion of lipid, the resulting concentrations can be considered unbiased
523 (Aguilar 1987). Moreover, previous studies have shown that biopsy samples yield representative
524 details on chlorinated and brominated aromatic compounds in marine mammal blubber, regardless of
525 the quantity and type of blubber sampled, provided that lipid normalization is performed on the
526 resulting concentrations (Ikonomou et al. 2007).

527 Even though known males were not preferentially targeted over known females, and several
528 animals were of unknown sex at the time of sampling, the skewed sex ratio is likely driven by the fact
529 that females with accompanying calves were not sampled.
530
531 *PCB concentrations*
532 We detected relatively high PCB concentrations. This is in agreement with other studies that showed
533 the continued persistence of PCBs in large marine predators in Europe (Law et al. 2012, Jepson et al.
534 2016). In a previous European-wide study (Jepson et al. 2016), PCB levels were shown to be high in six
535 Gulf of Trieste bottlenose dolphins, but the sample size from this area was limited. Here, using a larger
536 sample size, we corroborate that PCB levels in this population are indeed high in relation to published
537 reference values (Kannan et al. 2000, Jepson et al. 2016). It is probably safe to assume that
538 organochlorine threats to this population are mainly restricted to PCBs, as is the case for other
539 Mediterranean areas (Jepson et al. 2016). Other studies in Europe have shown that following the
540 1970s-1980s ban the declines of PCBs have been slower than those of DDTs (Aguilar and Borrell 2005)
541 and levels have subsequently reached a plateau in harbour porpoises (*Phocoena phocoena*) around
542 the United Kingdom (Law et al. 2012) and in striped dolphins (*Stenella coeruleoalba*) in the western
543 Mediterranean Sea (Jepson et al. 2016).
544 The main part of the PCB profile was represented by congeners 153, 138 and 180 (Table 3, Fig.
545 7), which is in agreement with other studies from the region (Storelli and Marcotrigiano 2003, Lazar et
546 al. 2012, Herceg Romanić et al. 2014) and elsewhere (Fair et al. 2010, García-Álvarez et al. 2014).
547 Comparing organochlorine levels across various literature sources is not always
548 straightforward and can in fact be challenging. The reasons for this include different methods of
549 organochlorine quantification, differences in compounds analysed (e.g. the total number and selection
550 of individual PCB congeners), the basis on which the concentrations are expressed (e.g. lipid, wet or
551 dry weight basis - especially if the proportion of lipid or water is not reported), the summary statistics
552 used (e.g. arithmetic mean, geometric mean or median) together with measures of spread (e.g.

553 standard deviation, confidence intervals or range); the sources of samples (controlled live captures,
554 biopsies, bycaught animals or stranded animals), sample size, the sex and age classes included or
555 excluded from the analysis, period of sampling, etc. For these reasons, not all studies are directly
556 comparable.

557 Still, considering these caveats, some general comparisons can be made (Table 4). Looking at
558 a regional perspective, it appears that PCB concentrations in our study are relatively similar to those
559 found in stranded bottlenose dolphins along the eastern Adriatic coast of Croatia (Herceg Romanić et
560 al. 2014), but substantially higher than in stranded bottlenose dolphins along the Adriatic coast of
561 south-eastern Italy (Storelli and Marcotrigiano 2003), stranded along the coast of Israel, eastern
562 Levantine Basin (but note the extremely small samples size, Shoham-Frider et al. 2009), or biopsied in
563 the Gulf of Ambracia, western Greece (Gonzalvo et al. 2016). Looking at the wider Mediterranean and
564 European picture, concentrations in our study are higher than those found in bottlenose dolphins from
565 Ireland (Berrow et al. 2002, Jepson et al. 2016), but lower than in bottlenose dolphins from western
566 Mediterranean (Borrell and Aguilar 2007, Jepson et al. 2016) and those from Portugal, north-western
567 Spain, Wales, England and Scotland (although note that the patterns are somewhat different between
568 males and females, Table 4, Jepson et al. 2016). Based on the above, it appears that within the
569 Mediterranean, generally speaking, PCB concentrations tend to decline from west to east, and from
570 north to south, which is consistent with the general geographical pattern of anthropogenic impacts
571 (particularly pollution and exploitation of marine resources) in the Mediterranean basin (Coll et al.
572 2012).

573 On a global scale, our reported concentrations are higher than those found in bottlenose
574 dolphins in Taiwan (Chou et al. 2004), around Canary Islands (García-Álvarez et al. 2014), off Rio de
575 Janeiro, Brazil (Lailson-Brito et al. 2012), Bermuda (Kucklick et al. 2011), Beaufort, North Carolina,
576 USA (Hansen et al. 2004), southern Biscayne Bay, Florida, USA (Kucklick et al. 2011), and along the
577 coasts of Louisiana, Mississippi and northwestern Florida (Kucklick et al. 2011, Balmer et al. 2015),
578 relatively similar to those from Indian River Lagoon, Florida, USA (Fair et al. 2010), Sarasota Bay,

579 Florida, USA (Yordy et al. 2010) and Charleston, South Carolina, USA (Fair et al. 2010, Adams et al.
580 2014), and lower than in New Jersey (Kucklick et al. 2011), northern Biscayne Bay and Tampa Bay in
581 Florida, USA (Kucklick et al. 2011), and waters of Georgia, USA (Balmer et al. 2011). With respect to
582 other species, our bottlenose dolphins had higher PCB concentrations than striped dolphins from the
583 southern Adriatic Sea (Storelli et al. 2012), harbour porpoises from the United Kingdom (Law et al.
584 2012), Guiana dolphins (*Sotalia guianensis*) from north-eastern Brazil (Santos-Neto et al. 2014),
585 common dolphins (*Delphinus* sp.) from New Zealand (Stockin et al. 2007) or northern resident killer
586 whales from British Columbia, Canada (Ross et al. 2000, Ylitalo et al. 2001), but substantially lower
587 than striped dolphins from the western Mediterranean Sea (Jepson et al. 2016), killer whales from the
588 United Kingdom, Canary Islands and the Strait of Gibraltar (Jepson et al. 2016), or southern resident
589 and transient killer whales from the waters of British Columbia, Canada, and the states of Alaska and
590 Washington, USA (Ross et al. 2000, Ylitalo et al. 2001). In addition, male dolphins in our study had
591 higher concentrations than male pilot whales, male sperm whales and male fin whales from the
592 western Mediterranean Sea (Pinzone et al. 2015), while female dolphins in our study had lower
593 concentrations than female pilot whales, similar concentrations as female sperm whales and higher
594 concentrations than female fin whales from the western Mediterranean Sea (Pinzone et al. 2015).
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608 **Table 4.** PCB blubber concentrations in *Tursiops truncatus* across different studies for males, females
609 and both sexes. Whenever possible, reported values pertain to adult animals. All concentrations are in
610 mg/kg, and expressed on lipid weight basis, unless otherwise noted. Concentrations expressed in
611 different units in source literature were converted to mg/kg. Concentrations are shown as either
612 arithmetic mean (A) ± standard deviation, (or with range in parentheses), or geometric mean (G) with
613 95% confidence intervals in parentheses. Summary statistics were obtained from text or tables of cited
614 sources, or calculated from raw data reported in tables. Note that both the number and choice of
615 individual PCB congeners tested varied across studies. See cited sources for details.

Location	N	Mean	M	F	M-F	Source
Croatia, north-eastern Adriatic Sea	13	A	-	-	97 ± 133	Herceg-Romanić et al. 2014
Italy, southern Adriatic Sea	9	A	30.3	28.8	32.7 (7.3–53)	Storelli & Marcotrigiano 2003
Gulf of Ambracia, western Greece	14	A	23.4 ± 18.0	32.9 ± 43.3	26.9 ± 28.3	Gonzalvo et al. 2016
Israel, eastern Levantine Basin	2	A, wet weight	6.3 ± 2.3	-	-	Shoham-Frider et al. 2009
South-east Spain, western Mediterranean	36	A	336.0 ± 241.1	246.4 ± 183.5	286.6 ± 274.6	Borrell & Aguilar 2007
Spain, western Mediterranean	27	A	182.7 (27.4–399)	193.2 (45.3–601.4)	-	Jepson et al. 2016
Strait of Gibraltar	8	A	324.0 (28.3–879.3)	123.1 (20.8–179.7)	-	Jepson et al. 2016
Gulf of Cadiz, south-west Spain	21	A	247.3 (98.5–445.3)	150 (3.7–426.4)	-	Jepson et al. 2016
Portugal	12	A	85.7 (19.4–164.7)	88.5 (35.0–226.8)	-	Jepson et al. 2016
North-western Spain	11	A	118.9 (5.1–382.2)	34.7 (5.4–82.0)	-	Jepson et al. 2016
Wales, UK	7	A	91.8 (8.2–175.4)	111.9 (9.1–307.5)	-	Jepson et al. 2016
England, UK	10	A	176.9 (22.1–446.6)	91.2 (4.1–358.5)	-	Jepson et al. 2016
Scotland, UK	21	A	96.6 (1.8–698.0)	46.1 (8.5–125.1)	-	Jepson et al. 2016

Shannon Estuary, Ireland	8	A	29.5 ± 21.0	7.1 ± 8.7	23.9 ± 20.8	Berrow et al. 2002
Shannon Estuary, Ireland	8	A	46.9 (13.0–95.1)	11.4 (1.5 21.2)	-	Jepson et al. 2016
Canary Islands	25	A	-	-	47.2 ± 53.9	García-Álvarez et al. 2014
Cape May, New Jersey, USA	3	G	139 (95% CI 62.8–130)	-	-	Kucklick et al. 2011
Beaufort, North Carolina, USA	5	G	53.3 (15.9–52.2)	11.6 (3.3–40.6)	-	Hansen et al. 2004
Charleston, South Carolina, USA	9	G	50.4 (23.6–84.6)	7.9 (2.7–31.2)	-	Hansen et al. 2004
Charleston, South Carolina, USA	47	G	94 (28.6–255)	14.3 (4.5–131)	-	Fair et al. 2010
Charleston, South Carolina, USA	40	G	76.6 (25.9–246)	-	-	Adams et al. 2014
Sapelo area, Georgia, USA	46	G	115.7 (95% CI 91.7–146.1)	48.3 (95% CI 27.3–85.5)	-	Balmer et al. 2011
Mixed area, Georgia, USA	22	G	253.6 (95% CI 177.9–361.5)	45.9 (95% CI 20.8–101.7)	-	Balmer et al. 2011
Brunswick area, Georgia, USA	34	G	509.6 (95% CI 369.0–703.6)	116.5 (95% CI 78.1–173.6)	-	Balmer et al. 2011
Indian River Lagoon, Florida, USA	11	G	20 (14.7–27.9)	9.3 (5.0–17.0)	-	Hansen et al. 2004
Indian River Lagoon, Florida, USA	48	G	79.8 (35–227)	25.5 (1.5–105)	-	Fair et al. 2010
Biscayne Bay – North, Florida, USA	15	G	157 (95% CI 110–224)	-	-	Kucklick et al. 2011
Biscayne Bay – South, Florida, USA	15	G	33.7 (95% CI 23.6–48.2)	-	-	Kucklick et al. 2011
Sarasota Bay, Florida, USA	47	G	98.6 ± 159	4.7 ± 5.4	-	Yordy et al. 2010
Tampa Bay, Florida, USA	5	G	109 (95% CI 58.9–203)	-	-	Kucklick et al. 2011
East of Apalachicola Bay, Florida, USA	20	G	33.1 (95% CI 24.3–45.1)	-	-	Kucklick et al. 2011

St. Joseph Bay to St. Andrews Bay, Florida, USA	38	G	63 (95% CI 50.4–78.9)	-	-	Kucklick et al. 2011
Mississippi Sound, Mississipi, USA	55	G	68 (95% CI 56.4–81.9)	-	-	Kucklick et al. 2011
Barataria Bay, Louisiana, USA	19	G	51.4 (95% CI 38.5–68.6)	-	-	Balmer et al. 2015
Bermuda	3	G	38.8 (95% CI 17.4–86.1)	-	-	Kucklick et al. 2011
Rio de Janeiro State, Brazil	2	A	11.8 ± 2.4	-	-	Lailson Brito et al. 2012
Taiwan	6	A	6.78	2.3	5.4 ± 3.6	Chou et al. 2004

Effects of demographic parameters on PCB concentrations

Males had significantly higher PCB concentrations than females (Fig. 3). Animals of unknown sex also had high PCB levels, with values more similar to known males than to females (Table 2). This suggests most of these animals were likely also males. The significant differences between males and females are suggestive of PCB offloading from reproducing females to their offspring via gestation and/or lactation (Borrell et al. 1995, Schwacke et al. 2002, Wells et al. 2005, Weijs et al. 2013). The significant differences in PCB concentrations between nulliparous and parous females (Fig. 4) further support this, despite limited sample size. Even though the premise of maternal offloading is well established,

634 particularly based on experimental laboratory or captive studies involving mammals (Kannan et al.
635 2000) and samples from whaling operations (Aguilar and Borrell 1994b, Borrell et al. 1995), it is
636 informative to be able to demonstrate that this is indeed happening in a wild, free-ranging cetacean
637 population. In Sarasota Bay, Florida, research initiated in the 1970s, combining tagging, photo-
638 identification monitoring and capture-release operations for health assessments, provided an
639 unparalleled opportunity to investigate the relationships between organochlorine levels and life-
640 history and reproductive parameters in the world's best-studied bottlenose dolphin population (Wells
641 et al. 2005). In the eastern North Pacific, long-term identification records of one of the best-studied
642 killer whale populations in the world enabled similar comparisons (Ross et al. 2000, Ylitalo et al. 2001).
643 However, such studies remain relatively rare, especially in the Mediterranean Sea, the largest enclosed
644 sea in the world, with substantial anthropogenic pressure.

645 There is some evidence of first-born offspring mortality in our dolphin population, as a few of
646 the observed newborns (presumed to be the first offspring of respective females) did not survive to
647 the following year (T. Genov, *pers. obs.*). This would support the notion that first-borns may receive a
648 very high (or even lethal) dose of PCBs from their mothers, as females may transfer up to 80% of their
649 burden to the offspring (Cockcroft et al. 1989). This may lead to poor survival of first-borns, with an
650 improved survival of subsequent offspring (Schwacke et al. 2002, Wells et al. 2005). However, related
651 evidence from our study area is limited and circumstantial, so further inferences are not possible.
652 Given the long-term and ongoing monitoring of this population, future work incorporating PCB
653 monitoring, individual re-sighting histories and information on reproductive rates may provide further
654 insight into the temporal accumulation of PCBs by females and the possible links between pollutant
655 loads and recruitment, as recommended by Hall et al. (2006).

656 Even though this dolphin population is structured into several social groups that display
657 differences in behaviour as well as feeding strategies in relation to fisheries (Centrih et al. 2013, Genov
658 et al. 2015), it appears that PCBs pose a threat to these animals regardless of social group membership
659 and potential associated dietary differences (Fig. 5).

Potential toxicological effects

The vast majority of animals in our study exceeded the lower toxicity threshold (Kannan et al. 2000), with more than 50% also exceeding the higher threshold (Helle et al. 1976, Fig. 6). As discussed by Jepson et al. (2016), the lower toxicity threshold may in fact overestimate the true PCB risk to cetaceans, but PCB levels reported here nevertheless provide a compelling case for the inherent PCB toxicity risk to these animals. In previous studies, high PCB levels were linked to pathological findings consistent with immunosuppression and increased susceptibility to disease, including macro-parasitic and bacterial pneumonias, high lung and gastric macro-parasite burdens, and generalised bacterial infections in harbour porpoises (Jepson et al. 2016). In Mediterranean striped dolphins, high levels of PCBs were associated to increased mortality during a morbillivirus epizootic outbreak, possibly due to immunosuppression (Aguilar and Borrell 1994a).

Our results are of concern, particularly in combination with other known or suspected threats to this population, including marine litter, disturbance from boat traffic, frequent interactions with fisheries, overfishing and occasional bycatch (Genov et al. 2008, Hace et al. 2015, Genov et al. 2016, Kotnjek et al. 2017). Hopefully, the quantification of organochlorine concentrations and establishing links with various demographic parameters as presented here, will enable placing the effects of contaminants in context with other anthropogenic stressors (Hall et al. 2017).

DDE and DDT

We were only able to determine PCB concentrations, but not DDT in our samples, except for four samples referred to above. DDE concentrations could be determined as they were obtained as a "side product" of PCB analyses. In these four samples, DDE was the majority component of the total DDT, representing 89.7% (Table 1). Biotransformation processes of DDT in vertebrates largely end

686 up as DDE (Aguilar and Borrell 2005). Unless there is a recent source, DDE tends to be the highest
687 concentration DDT metabolite present (Storelli et al. 2004, Pinzone et al. 2015), and can be used as an
688 indicator of DDT contamination (but see Kljaković-Gašpić *et al*. 2010 on possible recent input).
689 ~~supported by the four samples in which total DDT could be determined, and which showed that DDE~~
690 Our results are
691 similar to several other studies and is indicative of DDT ageing (Lailson-Brito et al. 2012, Adams et al.
692 2014, García-Álvarez et al. 2014, Gonzalvo et al. 2016). This suggests that DDE
693 (and hence DDT) levels are fairly low, as is the case in the western Mediterranean Sea and around the
694 United Kingdom (Aguilar and Borrell 2005, Borrell and Aguilar 2007, Law et al. 2012). In the Eastern
695 Mediterranean Sea, however, levels of DDTs appear higher than those of PCBs (Shoham-Frider et al.
696 2009, Gonzalvo et al. 2016). For HCB, the extremely low levels in our study, consistent with studies on
697 other biota from the Adriatic Sea (Storelli et al. 2004), suggest that recent environmental input of this
698 compound is negligible (Borrell and Aguilar 2007).
699
700 *Future monitoring perspectives*
701 Our results represent a useful baseline for future research and monitoring. With the ongoing
702 monitoring of this local dolphin population and new insights into its ecology, future sampling may
703 provide a better understanding of the population-level impacts of organochlorine pollution. It should
704 be noted that concentrations in top predators with high lipid stores will inevitably lag behind any
705 reductions in environmental concentrations (and those in the prey), due to the slow depuration of
706 POPs out of the population (through the legacy from female to calf, as well as the cycling of POPs in
707 the marine environment). Nevertheless, this approach may represent a tool for monitoring of
708 pollutants in relation to EU legislation such as the Habitats Directive and the Marine Strategy
709 Framework Directive. The presence of pollutants in tissues of marine biota is already included as a
710 Descriptor 8 of the EU European Marine Strategy Framework Directive (MSFD), while marine mammals
711 are one of the indicators of the "Good Environmental Status" under Descriptor 1 of the MSFD. Jepson

712 and Law (2016) proposed that at a European policy level, PCB levels in relation to established toxicity
713 thresholds should also be used to assess "Favourable Conservation Status" of marine mammals under
714 the EU Habitats Directive.

715 Even though biopsy sampling took place within Slovenian waters, the extensive spatial survey
716 coverage (Fig. 1) and the fact that sampled dolphins have been re-sighted throughout the study area
717 shown in Figure 1 (Genov et al. 2008), the reported organochlorine levels can likely be considered
718 representative of this part of the northern Adriatic Sea. At the same time, individual dolphin re-sighting
719 frequencies have shown that the sampled individuals are part of a resident population inhabiting this
720 area over the long term (Genov et al. 2008, Genov 2011), while both photo-identification (Genov et al.
721 2009) and genetic data (Gaspari et al. 2015) suggest that this population is distinct. This adds
722 confidence to the notion that the reported PCB levels are representative of the area in question, rather
723 than being a result of acute PCB exposure elsewhere (Phillips and Segar 1986).

724 Molluscs have typically been used as model species to monitor contaminants in the Gulf of
725 Trieste, elsewhere in the Adriatic Sea (Kljaković-Gašpić et al. 2010), and other parts of the world
726 (Phillips and Segar 1986, Farrington et al. 2016). This is primarily due to their widespread distribution,
727 abundance, sessile nature, tolerance to various types of stress, and the ability to accumulate a wide
728 range of contaminants (Phillips and Segar 1986, Kljaković-Gašpić et al. 2010), but probably also due to
729 ease of access to the animals. However, while molluscs may be better indicators for local point sources
730 of contamination, cetaceans may be more representative over larger spatial and temporal scales. For
731 example, dolphins are long-lived predators that integrate contaminant concentrations over time. They
732 have been shown to be incapable of metabolizing certain PCB congeners, making them accumulate
733 these compounds more readily than other mammals or taxa of comparable life history (Aguilar and
734 Borrell 2005). Moreover, being highly mobile, they are likely better regional rather than local
735 indicators, due to their propensity to move around more. Finally, as top predators, they are likely
736 representative of the ecosystem as a whole (Borrell and Aguilar 2007).
737

CONCLUSIONS

It is important to review current methods of PCB mitigation in the marine environment, at a European and international level. In Europe, much greater compliance with the Stockholm Convention is urgently needed and by many EU member states, in order to significantly reduce PCB contamination of the marine and terrestrial environment by 2028 (Jepson et al. 2016, Jepson and Law 2016, Stuart-Smith and Jepson 2017). Measures may include the safe disposal or destruction of large stocks of PCBs and PCB-containing equipment, limiting the dredging of PCB-laden rivers and estuaries, reducing PCB leakage from old landfills, limiting PCB mobilization in marine sediments, and regulating demolition of PCB-containing precast buildings such as tower blocks built in the 1950s to 1980s (Jepson et al. 2016, Jepson and Law 2016, Stuart-Smith and Jepson 2017).

Our results show that PCB levels are relatively high in northern Adriatic dolphins, and may be high enough to potentially cause population-level effects in this dolphin population. We provide important baseline data of a considerable sample size, against which future trends can be assessed. We demonstrate that POP monitoring combined with long-term photo-identification and population ecology studies can be highly informative for assessing the impacts of organochlorine pollution.

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