Science of the Total Environment 657 (2019) 200–212

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea

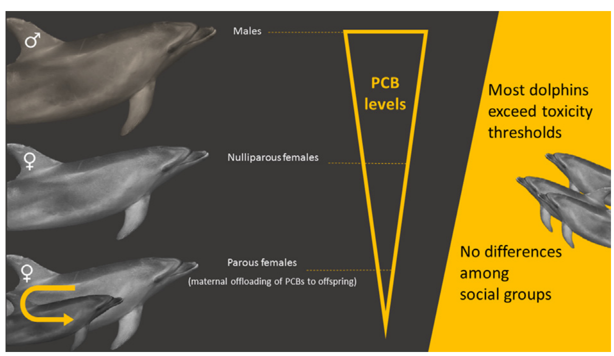
Tilen Genov [a,b,*], Paul D. Jepson [c], Jonathan L. Barber [d], Ana Hace [a], Stefania Gaspari [e], Tina Centrih [a], Jan Lesjak [a], Polona Kotnjek [a]

[a] Morigenos – Slovenian Marine Mammal Society, Kidričevo nabrežje 4, 6330 Piran, Slovenia
[b] Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, Fife KY16 8LB, UK
[c] Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
[d] Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Pakefield Road, Lowestoft NR33 0HT, UK
[e] Italian National Research Council, Institute of Marine Sciences (CNR-ISMAR), Arsenale - Tesa 104, Castello 2737/F, 30122 Venice, Italy

## 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.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 1 October 2018
Received in revised form 1 December 2018
Accepted 3 December 2018
Available online 04 December 2018

Editor: Adrian Covaci

*Keywords:*
Organochlorine contaminants
PCBs
Ecotoxicology
*Tursiops truncatus*
Adriatic Sea
Mediterranean Sea

## 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

* Corresponding author at: Morigenos – Slovenian Marine Mammal Society, Kidričevo nabrežje 4, 6330 Piran, Slovenia.
*E-mail address:* tilen.genov@gmail.com (T. Genov).

https://doi.org/10.1016/j.scitotenv.2018.12.025

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.



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

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

## 2. Material and methods

### 2.1. The study population

The Gulf of Trieste, together with its surrounding waters (Fig. 1), is probably one of the most heavily human-impacted areas within the Adriatic and Mediterranean Seas, due to shipping, fishing, industrialisation, tourism, aquaculture and agriculture (Horvat et al., 1999; Faganeli et al., 2003; David et al., 2007; Mozetič et al., 2008; Codarin et al., 2009; Grego et al., 2009). The dolphin population inhabiting these and surrounding waters (Fig. 1) has been the focus of a long-term study and monitoring by Morigenos – Slovenian Marine Mammal Society since 2002, primarily through boat-based surveys and photo-identification, and is now relatively well studied (Genov et al., 2008; Genov, 2011; Genov et al., 2016; Genov et al., 2017). The population is present within the area year-round (Genov et al., 2008; Genov, 2011) and appears to be demographically and genetically distinct (Genov et al., 2009; Gaspari et al., 2015). The annual abundance estimates range between about 70 and 150 animals (Genov, 2011;

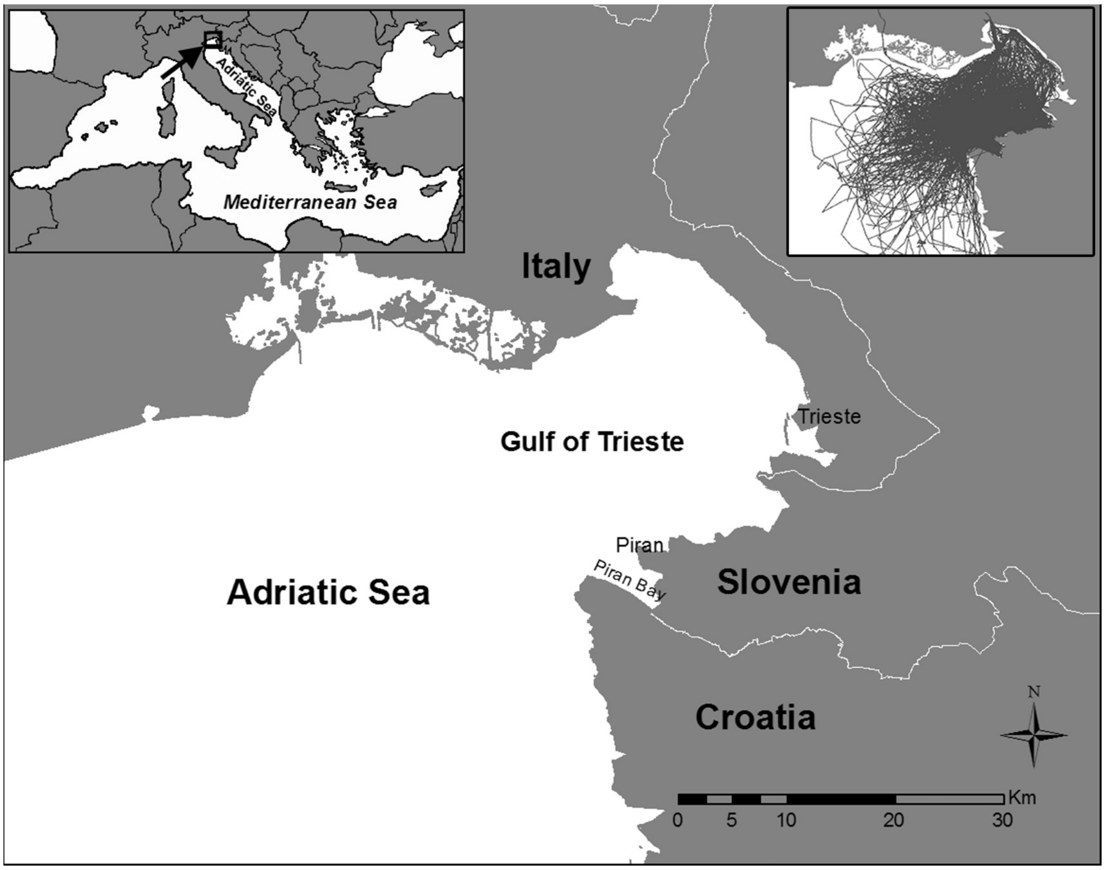



**Fig. 1.** Study area in the northern Adriatic Sea. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the survey effort (navigation tracks).

Morigenos, *unpublished data*). Most encountered individuals have extensive re-sighting histories over the study period, and several are of known sex and reproductive output.

### *2.2. 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

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

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.

### 2.3. 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).

### 2.4. 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 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 isooctane: 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 0.5–400 ng/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. LOQs are conservatively set at the lowest calibration standard concentration normalised to the sample multiplier (which varies depending on sample size and lipid content), which gives higher values than the alternative approach based on a S/N ratio of 10 would allow. 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.

### 2.5. 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).

### 2.6. 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.

## 3. 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.

### 3.1. 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).

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

| Sample | Year | Sex | Parity | Source | % Lipid | Σ25PCB | ΣICES7 | *p,p'*-DDE | ΣDDT | HCB | LOQ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2011 | M | | Biopsy | 23.3 | 64.2 | 40.9 | 9.03 | | <0.098 | 0.098 |
| 2 | 2011 | M | | Biopsy | 9.7 | 80.2 | 50.9 | 11.3 | | <0.144 | 0.144 |
| 3 | 2011 | M | | Biopsy | 16.2 | 58.7 | 37.1 | 8.02 | | <0.166 | 0.166 |
| 4 | 2011 | M | | Biopsy | 11.7 | 139.8 | 94.8 | 13.7 | | 0.102 | 0.071 |
| 5 | 2011 | M | | Biopsy | 19.5 | 293 | 190 | 32.9 | | 0.128 | 0.066 |
| 6 | 2011 | F | + | Biopsy | 17.5 | 29.0 | 14.9 | 1.54 | | <0.091 | 0.091 |
| 7 | 2013 | M | | Biopsy | 15.2 | 34.2 | 21.2 | 4.49 | | <0.197 | 0.197 |
| 8 | 2013 | F | + | Biopsy | 12.9 | 7.96 | 3.96 | 0.44 | | <0.341 | 0.341 |
| 9 | 2013 | F | + | Biopsy | 10.9 | 17.9 | 9.89 | 0.95 | | <0.202 | 0.202 |
| 10 | 2013 | M | | Biopsy | 3.4 | 23.0 | 14.4 | 2.67 | | <0.414 | 0.414 |
| 11 | 2014 | F | – | Biopsy | 10.5 | 27.2 | 17.5 | 9.41 | | <0.208 | 0.208 |
| 12 | 2014 | F | + | Biopsy | 27.9 | 4.13 | 2.12 | 0.25 | | <0.093 | 0.093 |
| 13 | 2014 | M | | Biopsy | 6.6 | 32.2 | 20.2 | 16.7 | | <0.441 | 0.441 |
| 14 | 2014 | M | | Biopsy | 13.5 | 43.7 | 27.0 | 5.51 | | <0.228 | 0.228 |
| 15 | 2014 | M | | Biopsy | 6.9 | 56.7 | 35.6 | 7.72 | | <0.305 | 0.305 |
| 16 | 2014 | M | | Biopsy | 23.9 | 123 | 81.2 | 17.5 | | <0.092 | 0.092 |
| 17 | 2014 | F | – | Biopsy | 19.3 | 30.7 | 19.2 | 4.25 | | <0.124 | 0.124 |
| 18 | 2014 | F | – | Biopsy | 33.8 | 48.9 | 31.0 | 6.45 | | <0.141 | 0.141 |
| 19 | 2014 | M | | Biopsy | 10.1 | 131 | 84.8 | 21.9 | | <0.217 | 0.217 |
| 20 | 2014 | M | | Biopsy | 18.8 | 65.9 | 40.7 | 9.55 | | <0.333 | 0.333 |
| 21 | 2014 | M | | Biopsy | 9.3 | 93.8 | 60.9 | 13.5 | | <0.139 | 0.139 |
| 22 | 2014 | M | | Biopsy | 14.5 | 76.8 | 48.8 | 10.1 | | <0.200 | 0.200 |
| 23 | 2015 | M | | Bycatch | 6.6 | 152 | 96.5 | 25.9 | | <0.166 | 0.166 |
| 24 | 2015 | M | | Biopsy | 7.9 | 111 | 74.2 | 16.0 | 17.3 | <0.164 | 0.164 |
| 25 | 2015 | U | | Biopsy | 7.7 | 58.3 | 37.8 | 8.17 | | 0.195 | 0.128 |
| 26 | 2016 | U | | Biopsy | 13.7 | 145 | 96.6 | 20.3 | 22.04 | <0.080 | 0.080 |
| 27 | 2016 | F | + | Biopsy | 14.4 | 6.82 | 3.88 | 0.54 | 0.54 | <0.104 | 0.104 |
| 28 | 2016 | M | | Biopsy | 4.4 | 121 | 80.3 | 16.7 | 18.6 | <0.215 | 0.215 |
| 29 | 2016 | U | | Biopsy | 11.3 | 150 | 98.2 | 23.5 | | <0.194 | 0.194 |
| 30 | 2017 | U | | Biopsy | 18.9 | 157 | 102 | 23.5 | | <0.106 | 0.106 |
| 31 | 2017 | U | | Biopsy | 11.8 | 219 | 144 | 27.2 | | <0.126 | 0.126 |
| 32 | 2017 | F | + | Biopsy | 25.3 | 7.64 | 4.37 | 0.47 | | <0.059 | 0.059 |

Fig. 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 (Table 2, 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 (Table 2, Fig. 6). The lower confidence limit of ΣPCB in females was just below the lower toxicity threshold (Table 2, Fig. 6). The ΣICES7 concentrations follow a similar pattern and are presented in Tables 1 and 2.

**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 |

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.

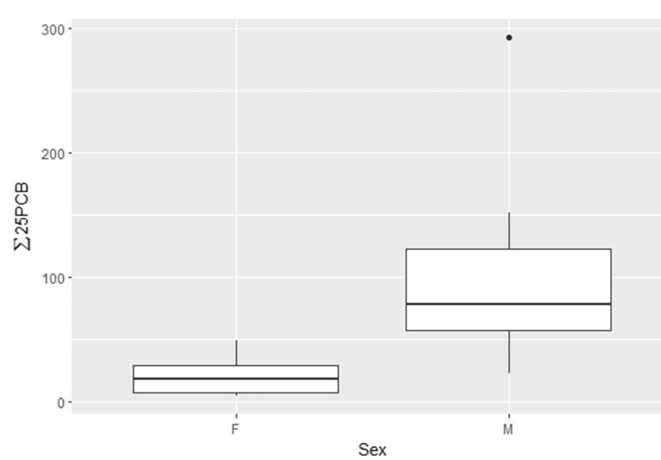



**Fig. 3.** Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between females (F, $n = 9$) and males (M, $n = 18$). The difference is statistically significant (Mann-Whitney U test, $U = 155$, $P < 0.001$).

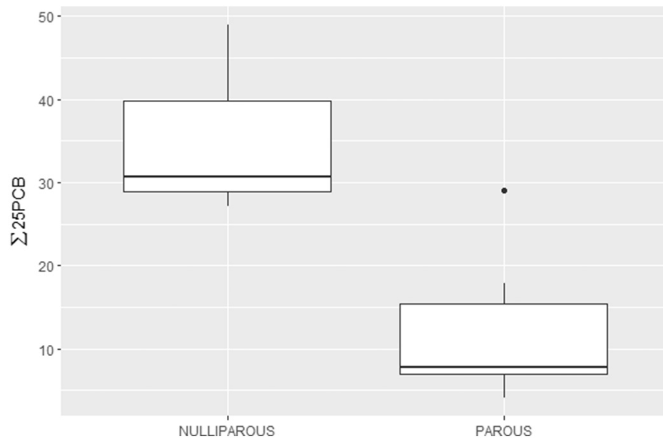



**Fig. 4.** Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between nulliparous ($n = 3$) and parous ($n = 6$) females. The difference is statistically significant (Mann-Whitney U test, $U = 17$, $P < 0.05$).

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).

### 3.2. 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.

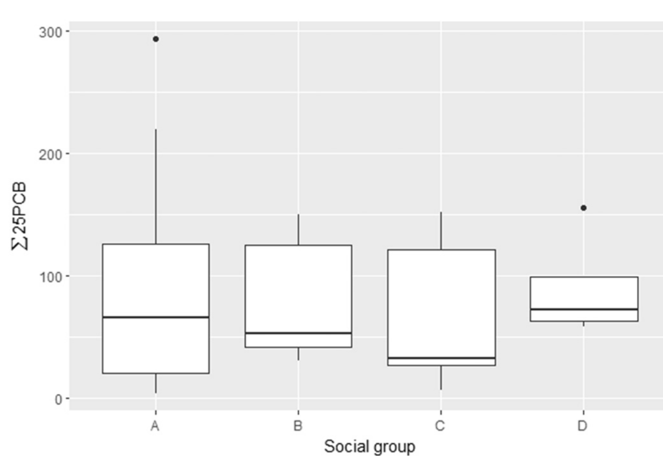



**Fig. 5.** Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) among social groups A ($n = 15$), B ($n = 8$), C ($n = 5$) and D ($n = 4$). Differences are not statistically significant (Kruskal-Wallis test, $H = 1.24$, $P = 0.743$).

### 3.3. 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.

## 4. Discussion

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

To date, a number of studies looked at contaminants in different cetacean species in the Adriatic Sea. Marsili and Focardi (1997) investigated organochlorines in cetaceans stranded around the Italian coasts, but only three samples were from bottlenose dolphins from the northern Adriatic. Storelli and Marcotrigiano (2000) assessed organochlorines from three Risso's dolphins (*Grampus griseus*) stranded in the southern Adriatic. Storelli and Marcotrigiano (2003) and Storelli et al. (2007) assessed organochlorines in bottlenose dolphins stranded on the southern Adriatic Sea coast, but the latter study did not include analysis of blubber tissue. In the same area, Storelli et al. (2012) measured organochlorines in stranded striped dolphins. In the northern Adriatic Sea, on its eastern side, Lazar et al. (2012) analysed different tissues in a single common dolphin (*Delphinus delphis*), a species considered extremely rare in the basin nowadays (Bearzi et al., 2004; Genov et al., 2012). Finally, Herceg Romanić et al. (2014) analysed organochlorine contaminants in various tissues in 13 bottlenose dolphins stranded along the Croatian coast in the northern Adriatic, providing the most comprehensive organochlorine assessment for dolphins in the northern part of the Adriatic Sea until now. All of these studies provided valuable insights, but due to limited sample sizes and the use of stranded animals, the inferences that can be made are somewhat limited.

In most cases, cetacean studies typically involve either a) collecting photo-identification data of free-ranging individuals, or b) analysing pollutant concentrations in stranded animals. However, studies combining these two important aspects, the analysis of pollutants in conjunction with long-term photo-identification of live animals (e.g. Ross et al., 2000; Ylitalo et al., 2001; Wells et al., 2005) are still relatively rare. In our study, all sampled animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002 (Genov et al., 2008; Genov et al., 2009; Genov et al., 2016; Genov et al., 2017), which adds additional value to this dataset. It allowed us to combine long-term records of identifiable individuals with individually-specific organochlorine concentrations, which in turn enabled us to link contaminant loads to certain demographic parameters in a known resident dolphin population. In the long term, the continued organochlorine monitoring in conjunction with photo-identification may provide further useful insights and we hope to be able to expand on this in the future by including additional parameters. Such integrated approach offers a lot of potential, as PCBs can be linked to sex, reproductive output and other parameters (Ross et al., 2000, Ylitalo et al., 2001, Wells et al., 2005). Such information is often lacking for wild populations and is of considerable importance for evaluating the impacts of pollutants on marine top predators.

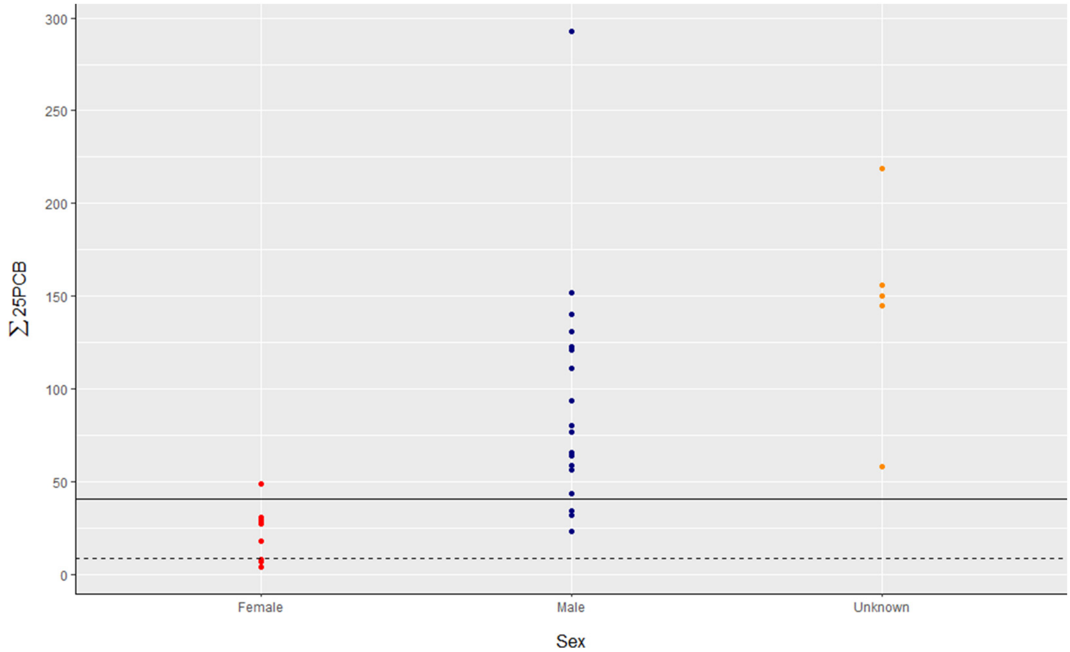



**Fig. 6.** Σ25PCB (mg/kg lipid weight) concentrations for females ($n = 9$), males ($n = 18$) and unknown sex ($n = 5$), in relation to published toxicity thresholds. The lower dashed line represents the lower toxicity threshold (9 mg/kg lw) for onset of physiological effects in experimental marine mammal studies (Kannan et al., 2000). The solid line represents the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals from the Baltic Sea (Helle et al., 1976).

When considering potential caveats, it should be noted that sampling live free-ranging animals meant there was some heterogeneity in the origin of samples with respect to the exact body location, despite the same general body area being targeted. This could potentially affect the resulting organochlorine concentrations, as these may vary across the body parts sampled (Aguilar, 1987). However, because we quantified the proportion of lipid and expressed the concentrations on a lipid weight basis, the resulting concentrations can be considered unbiased (Aguilar, 1987). Moreover, previous studies showed that biopsy samples yield representative details on chlorinated and brominated aromatic compounds in marine mammal blubber, regardless of the quantity and type of blubber sampled, provided that lipid normalization is performed on the resulting concentrations (Ikonomou et al., 2007).

Even though known males were not preferentially targeted over known females, and several animals were of unknown sex at the time of sampling, the skewed sex ratio is likely driven by the fact that females with accompanying calves were not sampled.

**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 |

### 4.1. PCB concentrations

We detected relatively high PCB concentrations. This is in agreement with other studies that showed the continued persistence of PCBs in large marine predators in Europe (Law et al., 2012; Jepson et al., 2016). In a previous European-wide study (Jepson et al., 2016), PCB levels were shown to be high in six Gulf of Trieste bottlenose dolphins, but the sample size from this area was limited. Here, using a larger sample size, we corroborate that concentrations in this population are indeed high in relation to published reference values (Kannan et al., 2000; Jepson et al., 2016). It is probably safe to assume that organochlorine threats to this population are mainly restricted to PCBs, as is the case for other Mediterranean areas (Jepson et al., 2016). Other studies in Europe have shown that following the 1970s–1980s ban the declines of PCBs have been slower than those of DDTs (Aguilar and Borrell, 2005) and levels have subsequently reached a plateau in harbour porpoises (*Phocoena phocoena*) around the United Kingdom (Law et al., 2012) and in striped dolphins in the western Mediterranean Sea (Jepson et al., 2016).

The main part of the PCB profile was represented by congeners 153, 138 and 180 (Table 3, Fig. 7), which is in agreement with other studies from the region (Storelli and Marcotrigiano, 2003; Lazar et al., 2012; Herceg Romanić et al., 2014) and elsewhere (Fair et al., 2010; García-Álvarez et al., 2014).

Comparing organochlorine levels across various literature sources is not always straightforward and can in fact be challenging. The reasons for this include different methods of organochlorine quantification, differences in compounds analysed (e.g. the total number and selection of individual PCB congeners), the basis on which the concentrations are expressed (e.g. lipid, wet or dry weight basis - especially if the proportion of lipid or water is not reported), the summary statistics used

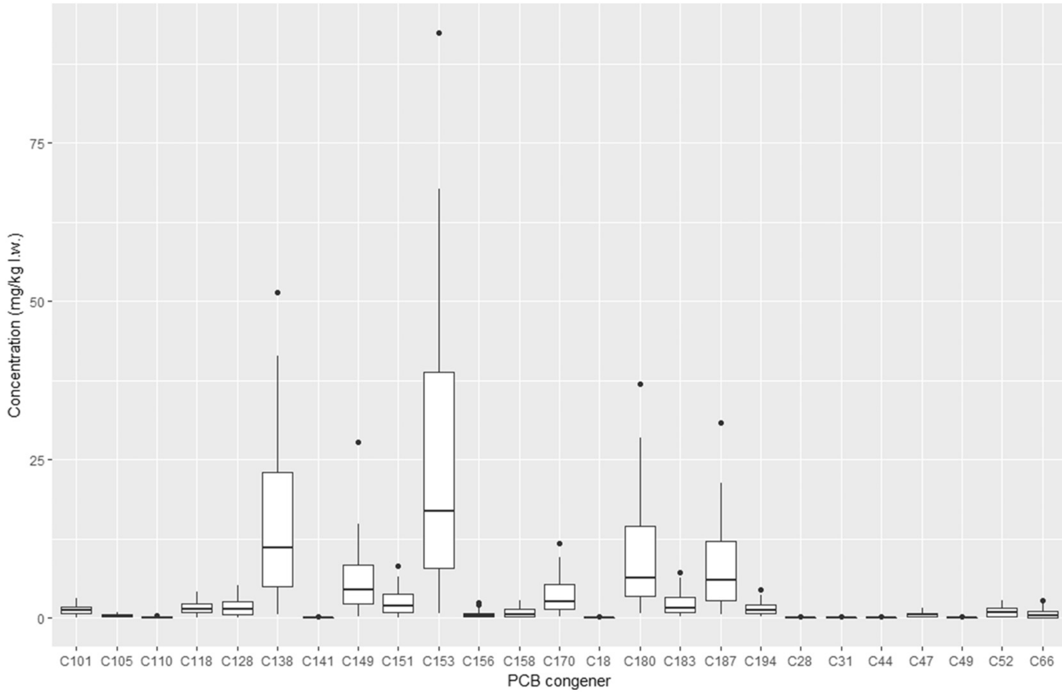



**Fig. 7.** Contribution of individual PCB congeners to the total PCB burden.

(e.g. arithmetic mean, geometric mean or median) together with measures of spread (e.g. standard deviation, confidence intervals or range); the sources of samples (controlled live captures, biopsies, bycaught animals or stranded animals), sample size, the sex and age classes included or excluded from the analysis, period of sampling, etc. For these reasons, not all studies are directly comparable.

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

On a global scale, our reported concentrations are higher than those found in bottlenose dolphins in Taiwan (Chou et al., 2004), around Canary Islands (García-Álvarez et al., 2014), off Rio de Janeiro, Brazil (Lailson-Brito et al., 2012), Bermuda (Kucklick et al., 2011), Beaufort, North Carolina, USA (Hansen et al., 2004), southern Biscayne Bay, Florida, USA (Kucklick et al., 2011), and along the coasts of Louisiana, Mississippi and northwestern Florida (Kucklick et al., 2011; Balmer et al., 2015), relatively similar to those from Indian River Lagoon, Florida, USA (Fair et al., 2010), Sarasota Bay, Florida, USA (Yordy et al., 2010) and Charleston, South Carolina, USA (Fair et al., 2010; Adams et al., 2014), and lower than in New Jersey (Kucklick et al., 2011), northern Biscayne Bay and Tampa Bay in Florida, USA (Kucklick et al., 2011), and waters of Georgia, USA (Balmer et al., 2011). With respect to other species, our bottlenose dolphins had higher PCB concentrations than striped dolphins from the southern Adriatic Sea (Storelli et al., 2012), harbour porpoises from the United Kingdom (Law et al., 2012), Guiana dolphins (*Sotalia guianensis*) from north-eastern Brazil (Santos-Neto et al., 2014), common dolphins (*Delphinus* sp.) from New Zealand (Stockin et al., 2007) or northern resident killer whales from British Columbia, Canada (Ross et al., 2000; Ylitalo et al., 2001), but substantially lower than striped dolphins from the western Mediterranean Sea (Jepson et al., 2016), killer whales from the United Kingdom, Canary Islands and the Strait of Gibraltar (Jepson et al., 2016), or southern resident and transient killer whales from the waters of British Columbia, Canada, and the states of Alaska and Washington, USA (Ross et al., 2000, Ylitalo et al., 2001). In addition, male dolphins in our study had higher concentrations than male pilot whales, male sperm whales and male fin whales from the western Mediterranean Sea (Pinzone et al., 2015), while female dolphins in our study had lower concentrations than female pilot whales, similar concentrations as female sperm whales and higher concentrations than female fin whales from the western Mediterranean Sea (Pinzone et al., 2015).

### *4.2. Effects of demographic parameters on PCB concentrations*

Males had significantly higher PCB concentrations than females (Fig. 3). Animals of unknown sex also had high concentrations, with values more similar to males than to females (Table 2, Fig. 6). 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, particularly based on experimental laboratory or captive studies involving mammals (Kannan et al., 2000) and samples from whaling operations (Aguilar and Borrell, 1994b; Borrell et al., 1995), it

**Table 4**
PCB blubber concentrations in *Tursiops truncatus* across different studies for males, females and both sexes. Whenever possible, reported values pertain to adult animals. All concentrations are in mg/kg, and expressed on lipid weight basis, unless otherwise noted. Concentrations expressed in different units in source literature were converted to mg/kg. Concentrations are shown as either arithmetic mean (A) ± standard deviation, (or with range in parentheses), or geometric mean (G) with 95% confidence intervals in parentheses. Summary statistics were obtained from text or tables of cited sources, or calculated from raw data reported in tables. Note that both the number and choice of 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 and 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 and 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-west 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 |

is informative to be able to demonstrate that this is indeed happening in a wild, free-ranging cetacean population. In Sarasota Bay, Florida, research initiated in the 1970s, combining tagging, photo-identification monitoring and capture-release operations for health assessments, provided an unparalleled opportunity to investigate the relationships between organochlorine levels and life-history and reproductive parameters in the world's best-studied bottlenose dolphin population (Wells et al., 2005). In the eastern North Pacific, long-term identification records of one of the best-studied killer whale populations in the world enabled similar comparisons (Ross et al., 2000; Ylitalo et al., 2001). However, such studies remain relatively rare, especially in the Mediterranean Sea, the largest enclosed sea in the world, with substantial anthropogenic pressure.

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

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

### 4.3. Potential toxicological effects

The vast majority of animals in our study exceeded the lower toxicity threshold (Kannan et al., 2000), with >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).

### 4.4. 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 up as DDE (Aguilar and Borrell, 2005). Unless there is a recent source, DDE tends to be the highest concentration DDT metabolite present (Storelli et al., 2004; Pinzone et al., 2015), and can be used as an indicator of DDT contamination (but see Kljaković-Gašpić et al., 2010 on possible recent input). Our results are similar to several other studies and indicative of DDT ageing (Lailson-Brito et al., 2012; Adams et al., 2014; García-Álvarez et al., 2014; Gonzalvo et al., 2016). This suggests that DDE (and hence DDT) levels are relatively low, as is the case in the western Mediterranean Sea and around the United Kingdom (Aguilar and Borrell, 2005; Borrell and Aguilar, 2007; Law et al., 2012). In the Eastern Mediterranean Sea, however, levels of DDTs appear higher than those of PCBs (Shoham-Frider et al., 2009; Gonzalvo et al., 2016). For HCB, the extremely low levels in our study, consistent with studies on other biota from the Adriatic Sea (Storelli et al., 2004), suggest that recent environmental input of this compound is negligible (Borrell and Aguilar, 2007).

### 4.5. Future monitoring perspectives

Our results represent a useful baseline for future research and monitoring. With ongoing studies of this dolphin population and new insights into its ecology, future sampling may provide a better understanding of population-level impacts of pollutants. It should be noted that concentrations in top predators with high lipid stores inevitably lag behind any reductions in environmental concentrations (and those in prey), due to the slow depuration of POPs out of the population (through the legacy from female to calf, as well as the cycling of POPs in the marine environment). Nevertheless, this approach may represent a monitoring tool in relation to EU legislation such as the Habitats Directive and the Marine Strategy Framework Directive (MSFD). The presence of pollutants in tissues of marine biota is already included as a Descriptor 8 of MSFD, while marine mammals are one of the indicators of the "Good Environmental Status" under Descriptor 1 of MSFD. Jepson and Law (2016) proposed that at a European policy level, PCB levels in relation to established toxicity thresholds should also be used to assess "Favourable Conservation Status" of marine mammals under the EU Habitats Directive.

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

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

## 5. 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 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–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 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.

## Acknowledgements

We are grateful to all the Morigenos volunteers who helped with fieldwork over the years. Special thanks to Jeremy Kiszka for initial training on biopsy sampling procedures. We also thank the Scientific Committee of the International Whaling Commission, in particular the Standing Working Group on Environmental Concerns, for their helpful feedback on this study, and particularly to Ailsa Hall for further useful comments on the early manuscript draft. Three anonymous reviewers provided helpful and constructive feedback on the submitted manuscript. This study was partially supported through the development of the expert baselines for the implementation of the EU Marine Strategy Framework Directive in relation to marine mammals in Slovenia, for which we are grateful to Monika Peterlin, Elizabeta Gabrijelčič and the Institute for Water of the Republic of Slovenia. OceanCare and Earthwatch Institute provided additional valuable support.

## References

Adams, J., Speakman, T., Zolman, E., Mitchum, G., Wirth, E., Bossart, G.D., Fair, P.A., 2014. The relationship between land use and emerging and legacy contaminants in an Apex predator, the bottlenose dolphin (*Tursiops truncatus*), from two adjacent estuarine watersheds. Environ. Res. 135, 346–353.

Aguilar, A., 1987. Using organochlorine pollutants to discriminate marine mammal populations: a review and critique of the methods. Mar. Mamm. Sci. 3, 242–262.

Aguilar, A., Borrell, A., 1994a. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sci. Total Environ. 154, 237–247.

Aguilar, A., Borrell, A., 1994b. Reproductive transfer and variation of body load of organochlorine pollutants with age in fin whales (*Balaenoptera physalus*). Arch. Environ. Contam. Toxicol. 27, 546–554.

Aguilar, A., Borrell, A., 2005. DDT and PCB reduction in the western Mediterranean from 1987 to 2002, as shown by levels in striped dolphins (*Stenella coeruleoalba*). Mar. Environ. Res. 59, 391–404.

Aguilar, A., Borrell, A., Reijnders, P.J.H., 2002. Geographical and temporal variation in levels of organochlorine contaminants in marine mammals. Mar. Environ. Res. 53, 425–452.

Balmer, B.C., Schwacke, L.H., Wells, R.S., George, R.C., Hoguet, J., Kucklick, J.R., Lane, S.M., Martinez, A., McLellan, W.A., Rosel, P.E., Rowles, T.K., Sparks, K., Speakman, T., Zolman, E.S., Pabst, D.A., 2011. Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. Sci. Total Environ. 409, 2094–2101.

Balmer, B.C., Ylitalo, G.M., McGeorge, L.E., Baugh, K.A., Boyd, D., Mullin, K.D., Rosel, P.E., Sinclair, C., Wells, R.S., Zolman, E.S., Schwacke, L., 2015. Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (*Tursiops truncatus*) along the northern Gulf of Mexico coast, USA. Sci. Total Environ. 527-528, 306–312.

Bearzi, G., Holcer, D., Notarbartolo Di Sciara, G., 2004. The role of historical dolphin takes and habitat degradation in shaping the present status of northern Adriatic cetaceans. Aquat. Conserv. Mar. Freshwat. Ecosyst. 14 (4), 363–379.

Bearzi, G., Fortuna, C.M., Reeves, R.R., 2009. Ecology and conservation of common bottlenose dolphins *Tursiops truncatus* in the Mediterranean Sea. Mammal Rev. 39, 92–123.

Berrow, S., McHugh, B., Glynn, D., Mcgovern, E., Parsons, K., Baird, R., Hooker, S., 2002. Organochlorine concentrations in resident bottlenose dolphins (*Tursiops truncatus*) in the Shannon estuary, Ireland. Mar. Pollut. Bull. 44, 1296–1303.

Bérubé, M., Palsbøll, P., 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Mol. Ecol. 5, 283–287.

Borrell, A., Aguilar, A., 1990. Loss of organochlorine compounds in the tissues of a decomposing stranded dolphin. Bull. Environ. Contam. Toxicol. 45, 46–53.

Borrell, A., Aguilar, A., 2007. Organochlorine concentrations declined during 1987–2002 in western Mediterranean bottlenose dolphins, a coastal top predator. Chemosphere 66, 347–352.

Borrell, A., Bloch, D., Desportes, G., 1995. Age trends and reproductive transfer of organochlorine compounds in long-finned pilot whales from the Faroe Islands. Environ. Pollut. 88, 283–292.

Centrih, T., Kotnjek, P., Hace, A., Genov, T., 2013. A link between dolphin social structure and trawlers in the northern Adriatic Sea. Proceedings of the Biennial Conference on the Biology of Marine Mammals. Society for Marine Mammalogy, Dunedin, New Zealand.

Centrih, T., Kotnjek, P., Hace, A., Genov, T., 2014. Social structure of common bottlenose dolphins in the Gulf of Trieste, northern Adriatic Sea. Proceedings of the 28th Annual Conference of the European Cetacean Society. European Cetacean Society, Liege, Belgium.

Chou, C., Chen, Y., Li, C., 2004. Congener-specific polychlorinated biphenyls in cetaceans from Taiwan waters. Arch. Environ. Contam. Toxicol. 47, 551–560.

Cockcroft, V., De Kock, A., Lord, D., Ross, G., 1989. Organochlorines in bottlenose dolphins Tursiops truncatus from the east coast of South Africa. S. Afr. J. Mar. Sci. 8, 207–217.

Codarin, A., Wysocki, L.E., Ladich, F., Picciulin, M., 2009. Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880–1887.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W.W., Christensen, V., Karpouzi, V.S., Guilhaumon, F., Mouillot, D., Paleczny, M., Palomares, M.L., Steenbeek, J., Trujillo, P., Watson, R., Pauly, D., 2012. The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Glob. Ecol. Biogeogr. 21, 465–480.

Darnerud, P.O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Grawé, K.P., Becker, W., 2006. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. Food Chem. Toxicol. 44, 1597–1606.

David, M., Gollasch, S., Cabrini, M., Perkovič, M., Bošnjak, D., Virgilio, D., 2007. Results from the first ballast water sampling study in the Mediterranean Sea – the Port of Koper study. Mar. Pollut. Bull. 54, 53–65.

Ellis, S., Franks, D.W., Nattrass, S., Cant, M.A., Bradley, D.L., Giles, D., Balcomb, K.C., Croft, D.P., 2018. Postreproductive lifespans are rare in mammals. Ecol. Evol., 1–13 https://doi.org/10.1002/ece1003.3856.

Faganeli, J., Horvat, M., Covelli, S., Fajon, V., Logar, M., Lipej, L., Čermelj, B., 2003. Mercury and methylmercury in the Gulf of Trieste (northern Adriatic Sea). Sci. Total Environ. 304, 315–326.

Fair, P.A., Adams, J., Mitchum, G., Hulsey, T.C., Reif, J.S., Houde, M., Muir, D., Wirth, E., Wetzel, D., Zolman, E., McFee, W., Bossart, G.D., 2010. Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncatus*) from two southeastern US estuarine areas: concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. Sci. Total Environ. 408, 1577–1597.

Farrington, J.W., Tripp, B.W., Tanabe, S., Subramanian, A., Sericano, J.L., Wade, T.L., Knap, A.H., 2016. Edward D. Goldberg's proposal of "the Mussel Watch": reflections after 40 years. Mar. Pollut. Bull. 110, 501–510.

Fossi, M., Marsili, L., Neri, G., Casini, S., Bearzi, G., Politi, E., Zanardelli, M., Panigada, S., 2000. Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. Mar. Environ. Res. 50, 517–521.

García-Álvarez, N., Boada, L.D., Fernández, A., Zumbado, M., Arbelo, M., Sierra, E., Xuriach, A., Almunia, J., Camacho, M., Luzardo, O.P., 2014. Assessment of the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean. Mar. Environ. Res. 100, 48–56.

Gaspari, S., Scheinin, A., Holcer, D., Fortuna, C., Natali, C., Genov, T., Frantzis, A., Chelazzi, G., Moura, A.E., 2015. Drivers of population structure of the bottlenose dolphin (*Tursiops truncatus*) in the Eastern Mediterranean Sea. Evol. Biol. 42, 177–190.

Genov, T., 2011. Ecology of the bottlenose dolphin (*Tursiops truncatus*) in the northern Adriatic. (Graduation thesis). University of Ljubljana, Ljubljana.

Genov, T., Kotnjek, P., Lesjak, J., Hace, A., Fortuna, C.M., 2008. Bottlenose dolphins (*Tursiops truncatus*) in Slovenian and adjacent waters (northern Adriatic Sea). Annal. Ser. Hist. Nat. 18 (2), 227–244.

Genov, T., Wiemann, A., Fortuna, C.M., 2009. Towards identification of the bottlenose dolphin (*Tursiops truncatus*) population structure in the north-eastern Adriatic Sea: preliminary results. Varstvo narave 22, 73–80.

Genov, T., Bearzi, G., Bonizzoni, S., Tempesta, M., 2012. Long-distance movement of a lone short-beaked common dolphin *Delphinus delphis* in the central Mediterranean Sea. Mar. Biodivers. Rec. 5, e9.

Genov, T., Centrih, T., Kotnjek, P., Hace, A., 2014. Early dolphin catches the fishing boat: behaviour- and time-specific social structure in bottlenose dolphins. Proceedings of the 28th Annual Conference of the European Cetacean Society. European Cetacean Society, Liege, Belgium.

Genov, T., Centrih, T., Kotnjek, P., Hace, A., 2015. Conservation implications of complex social structure in Adriatic bottlenose dolphins. 27th International Congress for Conservation Biology. Society for Conservation Biology, Montpellier, France.

Genov, T., Angelini, V., Hace, A., Palmisano, G., Petelin, B., Malačič, V., Pari, S., Mazzariol, S., 2016. Mid-distance re-sighting of a common bottlenose dolphin in the northern Adriatic Sea: insight into regional movement patterns. J. Mar. Biol. Assoc. U. K. 96, 909–914.

Genov, T., Centrih, T., Wright, A.J., Wu, G.-M., 2017. Novel method for identifying individual cetaceans using facial features and symmetry: a test case using dolphins. Mar. Mamm. Sci. https://doi.org/10.1111/mms.12451.

Genov, T., Centrih, T., Kotnjek, P., Hace, A., 2018. Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea. Mar. Biol. https://doi.org/10.1007/s00227-018-3450-8.

Geraci, J.R., Lounsbury, V.J., 2005. Marine Mammals Ashore: A Field Guide for Strandings. Second edition. National Aquarium in Baltimore, Baltimore, MD.

Gonzalvo, J., Lauriano, G., Hammond, P.S., Viaud-Martinez, K.A., Fossi, M.C., Natoli, A., Marsili, L., 2016. The Gulf of Ambracia's common bottlenose dolphins, *Tursiops truncatus*: a highly dense and yet threatened population. Adv. Mar. Biol. 75, 259–296.

Gorgone, A.M., Haase, P.A., Griffith, E.S., Hohn, A.A., 2008. Modeling response of target and nontarget dolphins to biopsy darting. J. Wildl. Manag. 72, 926–932.

Green, A., Larson, S., 2016. A review of organochlorine contaminants in nearshore marine mammal predators. J. Environ. Anal. Toxicol. 6, 370.

Grego, M., De Troch, M., Forte, J., Malej, A., 2009. Main meiofauna taxa as an indicator for assessing the spatial and seasonal impact of fish farming. Mar. Pollut. Bull. 58, 1178–1186.

Hace, A., Kotnjek, P., Centrih, T., Genov, T., 2015. Caught in the net: prolonged partial entanglement of a bottlenose dolphin calf in fishing gear. Proceedings of the 29th Annual Conference of the European Cetacean Society. European Cetacean Society, St Julians, Malta.

Hall, A.J., McConnell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., Reijnders, P.J.H., Wells, R.S., 2006. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environ. Health Perspect. 114, 60–64.

Hall, A.J., Thomas, G.O., McConnell, B.J., 2009. Exposure to persistent organic pollutants and first-year survival probability in gray seal pups. Environ. Sci. Technol. 43, 6364–6369.

Hall, A.J., McConnell, B.J., Schwacke, L.H., Ylitalo, G.M., Williams, R., Rowles, T.K., 2017. Predicting the effects of polychlorinated biphenyls on cetacean populations through impacts on immunity and calf survival. Environ. Pollut. 233, 407–418.

Hansen, L.J., Schwacke, L.H., Mitchum, G.B., Hohn, A.A., Wells, R.S., Zolman, E.S., Fair, P.A., 2004. Geographic variation in polychorinated biphenyl and organochlorine pesticide concentrations in the blubber of bottlenose dolphins from the US Atlantic coast. Sci. Total Environ. 319, 147–172.

Helle, E., Olsson, M., Jensen, S., 1976. PCB levels correlated with pathological changes in seal uteri. Ambio 5, 261–262.

Herceg Romanić, S., Holcer, D., Lazar, B., Klinčić, D., Mackelworth, P., Fortuna, C.M., 2014. Organochlorine contaminants in tissues of common bottlenose dolphins *Tursiops truncatus* from the northeastern part of the Adriatic Sea. Environ. Toxicol. Pharmacol. 38, 469–479.

Horvat, M., Covelli, S., Faganeli, J., Logar, M., Mandić, V., Rajar, R., Širca, A., Žagar, D., 1999. Mercury in contaminated coastal environments; a case study: the Gulf of Trieste. Sci. Total Environ. 237/238, 43–56.

Ikonomou, M.G., Rayne, S., Crewe, N.F., 2007. Concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), and polybrominated diphenyl ethers (PBDEs) as functions of sample depth in killer whale (*Orcinus orca*) blubber. J. Appl. Sci. Environ. Manag. 11, 147–151.

Jepson, P.D., Law, R.J., 2016. Persistent pollutants, persistent threats. Science 352, 1388–1389.

Jepson, P.D., Bennet, P.M., Allchin, C.R., Law, R.J., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 1999. Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. Sci. Total Environ. 243/244, 339–348.

Jepson, P.D., Bennett, P.M., Deaville, R., Allchin, C.R., Baker, J.R., Law, R.J., 2005. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ. Toxicol. Chem. 24, 238–248.

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, À., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Giménez, J., Loveridge, J., Llavona, Á., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci. Rep. 6, 18573.

Kannan, K., Blankenship, A., Jones, P., Giesy, J., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk. Assess. 6, 181–201.

Kiszka, J., Simon-Bouhet, B., Charlier, F., Pusineri, C., Ridoux, V., 2010. Individual and group behavioural reactions of small delphinids to remote biopsy sampling. Anim. Welf. 19, 411–417.

Kljaković-Gašpić, Z., Herceg-Romanić, S., Kožul, D., Veža, J., 2010. Biomonitoring of organochlorine compounds and trace metals along the Eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 1879–1889.

Kotnjek, P., Hace, A., Centrih Genov, T., Genov, T., 2017. Interactions between bottlenose dolphins (*Tursiops truncatus*) and trawlers in the Gulf of Trieste. Proceedings of the 31st Annual Conference of the European Cetacean Society. European Cetacean Society, Middelfart, Denmark.

Kucklick, J., Schwacke, L., Wells, R., Hohn, A., Guichard, A., Yordy, J., Hansen, L., Zolman, E., Wilson, R., Litz, J., Nowacek, D., Rowles, T., Pugh, R., Balmer, B., Sinclair, C., Rosel, P., 2011. Bottlenose dolphins as indicators of persistent organic pollutants in the western North Atlantic Ocean and northern Gulf of Mexico. Environ. Sci. Technol. 45, 4270–4277.

Lailson-Brito, J., Dorneles, P.R., Azevedo-Silva, C.E., Bisi, T.L., Vidal, L.G., Legat, L.N., Azevedo, A.F., Torres, J.P.M., Malm, O., 2012. Organochlorine compound accumulation in delphinids from Rio de Janeiro State, southeastern Brazilian coast. Sci. Total Environ. 433, 123–131.

Law, R.J., Barry, J., Barber, J.L., Bersuder, P., Deaville, R., Reid, R.J., Brownlow, A., Penrose, R., Barnett, J., Loveridge, J., Smith, B., Jepson, P.D., 2012. Contaminants in cetaceans from UK waters: status as assessed within the Cetacean Strandings Investigation Programme from 1990 to 2008. Mar. Pollut. Bull. 64, 1485–1494.

Lazar, B., Holcer, D., Mackelworth, P., Klinčić, D., Herceg Romanić, S., 2012. Organochlorine contaminant levels in tissues of a short-beaked common dolphin, *Delphinus delphis*, from the northern Adriatic Sea. Nat. Croat. 21, 391–401.

Lignell, S., Aune, M., Darnerud, P.O., Cnattingius, S., Glynn, A., 2009. Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996–2006: compound-specific temporal trends. Environ. Res. 109, 760–767.

Marsh, H., Kasuya, T., 1986. Evidence for reproductive senescence in female cetaceans. Report of the International Whaling Commission (Special Issue 8), pp. 57–74.

Marsili, L., Focardi, S., 1997. Chlorinated hydrocarbon (HCB, DDTs and PCBs) levels in cetaceans stranded along the Italian coasts: an overview. Environ. Monit. Assess. 45, 129–180.

Moore, S.E., 2008. Marine mammals as ecosystem sentinels. J. Mammal. 89, 534–540.

Mozetič, P., Malačič, V., Turk, V., 2008. A case study of sewage discharge in the shallow coastal area of the Northern Adriatic Sea (Gulf of Trieste). Mar. Ecol. 29, 483–494.

Noren, D.P., Mocklin, J.A., 2012. Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. Mar. Mamm. Sci. 28, 154–199.

Phillips, D.J.H., Segar, D.A., 1986. Use of bio-indicators in monitoring conservative contaminants: Programme design imperatives. Mar. Pollut. Bull. 17, 10–17.

Pinzone, M., Budzinski, H., Tasciotti, A., Ody, D., Lepoint, G., Schnitzler, J., Scholl, G., Thomé, J.-P., Tapie, N., Eppe, G., Das, K., 2015. POPs in free-ranging pilot whales, sperm whales and fin whales from the Mediterranean Sea: influence of biological and ecological factors. Environ. Res. 142, 185–196.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. https://www.R-project.org/.

Randhawa, N., Gulland, F., Ylitalo, G.M., DeLong, R., Mazet, J.A., 2015. Sentinel California sea lions provide insight into legacy organochlorine exposure trends and their association with cancer and infectious disease. One Health 1, 37–43.

Ross, P.S., 2000. Marine mammals as sentinels in ecological risk assessment. Hum. Ecol. Risk. Assess. 6, 29–46.

Ross, P.S., Ellis, G., Ikonomou, M., Barrett-Lennard, L., Addison, R., 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Mar. Pollut. Bull. 40, 504–515.

Santos-Neto, E.B., Azevedo-Silva, C.E., Bisi, T.L., Santos, J., Meirelles, A.C.O., Carvalho, V.L., Azevedo, A.F., Guimarães, J.E., Lailson-Brito, J., 2014. Organochlorine concentrations (PCBs, DDTs, HCHs, HCB and MIREX) in delphinids stranded at the northeastern Brazil. Sci. Total Environ. 472, 194–203.

Schwacke, L.H., Voit, E.O., Hansen, L.J., Wells, R.S., Mitchum, G.B., Hohn, A.A., Fair, P.A., 2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the Southeast United States coast. Environ. Toxicol. Chem. 21, 2752–2764.

Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn, A.A., Kucklick, J.R., Lamb, S., Levin, M., Litz, J.A., McFee, W.E., Place, N.J., Townsend, F.I., Wells, R.S., Rowles, T.K., 2012. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). Proc. R. Soc. B Biol. Sci. 279, 48–57.

Shoham-Frider, E., Kress, N., Wynne, D., Scheinin, A., Roditi-Elsar, M., Kerem, D., 2009. Persistent organochlorine pollutants and heavy metals in tissues of common bottlenose dolphin (*Tursiops truncatus*) from the Levantine Basin of the eastern Mediterranean. Chemosphere 77, 621–627.

Stockin, K., Law, R., Duignan, P., Jones, G., Porter, L., Mirimin, L., Meynier, L., Orams, M., 2007. Trace elements, PCBs and organochlorine pesticides in New Zealand common dolphins (*Delphinus* sp.). Sci. Total Environ. 387, 333–345.

Storelli, M.M., Marcotrigiano, G.O., 2000. Persistent organochlorine residues in Risso's dolphins (*Grampus griseus*) from the Mediterranean Sea (Italy). Mar. Pollut. Bull. 40, 555–558.

Storelli, M., Marcotrigiano, G., 2003. Levels and congener pattern of polychlorinated biphenyls in the blubber of the Mediterranean bottlenose dolphins *Tursiops truncatus*. Environ. Int. 28, 559–565.

Storelli, M., D'Addabbo, R., Marcotrigiano, G., 2004. Temporal trend of persistent organic pollutants in codfish-liver from the Adriatic Sea, Mediterranean Sea, 1993–2003. Bull. Environ. Contam. Toxicol. 73, 331–338.

Storelli, M., Barone, G., Piscitelli, G., Storelli, A., Marcotrigiano, G., 2007. Tissue-related polychlorinated biphenyls accumulation in Mediterranean cetaceans: assessment of toxicological status. Bull. Environ. Contam. Toxicol. 78, 206–210.

Storelli, M.M., Barone, G., Giacominelli-Stuffler, R., Marcotrigiano, G.O., 2012. Contamination by polychlorinated biphenyls (PCBs) in striped dolphins (*Stenella coeruleoalba*) from the Southeastern Mediterranean Sea. Environ. Monit. Assess. 184, 5797–5805.

Stuart-Smith, S.J., Jepson, P.D., 2017. Persistent threats need persistent counteraction: responding to PCB pollution in marine mammals. Mar. Policy 84, 69–75.

Tanabe, S., Iwata, H., Tatsukawa, R., 1994. Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. Sci. Total Environ. 154, 163–177.

Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J., 2003. Toxicology of Marine Mammals. Taylor & Francis, London and New York.

Weijs, L., Tibax, D., Roach, A.C., Manning, T.M., Chapman, J.C., Edge, K., Blust, R., Covaci, A., 2013. Assessing levels of halogenated organic compounds in mass-stranded long-finned pilot whales (*Globicephala melas*) from Australia. Sci. Total Environ. 461-462, 117–125.

Wells, R.S., Scott, M.D., 1999. Bottlenose dolphin *Tursiops truncatus* (Montagu, 1821). In: Ridgway, S.H., Harrison, R. (Eds.), Handbook of Marine Mammals. The Second Book of Dolphins and Porpoises vol. VI. Academic Press, San Diego, pp. 137–182.

Wells, R.S., Scott, M.D., 2009. Common bottlenose dolphin *Tursiops truncatus*. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals, 2nd ed. Academic Press, San Diego, pp. 249–255.

Wells, R.S., Rhinehart, H.L., Hansen, L.J., Sweeney, J.C., Townsend, F.I., Stone, R., Casper, D.R., Scott, M.D., Hohn, A.A., Rowles, T.K., 2004. Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth 1, 246–254.

Wells, R.S., Tornero, V., Borrell, A., Aguilar, A., Rowles, T.K., Rhinehart, H.L., Hofmann, S., Jarman, W.M., Hohn, A.A., Sweeney, J.C., 2005. Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Sci. Total Environ. 349, 106–119.

Würsig, B., Jefferson, T.A., 1990. Methods of photo-identification for small cetaceans. In: Hammond, P.S., Mizroch, S.A., Donovan, G.P. (Eds.), Individual Recognition of Cetaceans: Use of Photo-Identification and Other Techniques to Estimate Population Parameters. Report of the International Whaling Commission, Special Issue 12, Cambridge, UK, pp. 43–52.

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E., 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Sci. Total Environ. 281, 183–203.

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncatus*) resident to Sarasota Bay, FL. Sci. Total Environ. 408, 2163–2172.