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Polychlorinated biphenyls (PCBs) in free-ranging common bottlenose dolphins (Tursiops truncatus) from the Gulf of Trieste, (northern Adriatic Sea), in relation to demographic parameters

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POLYCHLORINATED BIPHENYLS (PCBs) IN FREE-RANGING COMMON BOTTLENOSE DOLPHINS (*Tursiops truncatus*) FROM THE GULF OF TRIESTE, (NORTHERN ADRIATIC SEA), IN RELATION TO DEMOGRAPHIC PARAMETERS

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ABSTRACT

We evaluated PCB levels in free-ranging 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 ~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 significantly higher concentrations than parous ones, providing further support for maternal offloading of contaminants. Overall, 88% of dolphins had PCB concentrations above the toxicity threshold for physiological effects in experimental marine mammal studies (9 mg/kg lw), while 66% 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: Pollutants, organochlorines, biopsy sampling, photo-ID, Mediterranean Sea

INTRODUCTION

Cetaceans are known to bio-accumulate organochlorine contaminants, including polychlorinated biphenyls (PCBs), which may cause immunosuppression (Tanabe et al., 1994) and the subsequent increased vulnerability to infectious disease (Aguilar & Borrell, 1994; Jepson *et al.*, 2005), endocrine disruption (Tanabe *et al.*, 1994; Vos *et al.*, 2003), reproductive impairment (Schwacke *et al.*, 2002) and developmental abnormalities (Tanabe *et al.*, 1994; Vos *et al.*, 2003).

Although PCBs and other organochlorines declined in European seas since the 1970s-1980s ban, PCB pollution continues to be high in European and Mediterranean waters (Jepson *et al.*, 2016). However, linking PCB levels to potential individual-level effects and especially population-level effects in wild cetaceans is challenging.

Although stranded animals are a valuable source of samples for pollutant studies (Jepson *et al.*, 1999; Jepson *et al.*, 2005; Law *et al.*, 2012), they may carry biases in some contexts or in some areas. Blubber biopsy samples (Noren & Mocklin, 2012) collected from live, free-ranging cetaceans may offer a good alternative for evaluating the toxicological burden of populations (Fossi *et al.*, 2000), especially when combined with long-term re-sighting histories of known individuals.

Here, we evaluated PCB levels in free-ranging bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Trieste (northern Adriatic Sea), one of the most human-impacted areas within the Mediterranean Sea. The dolphin population inhabiting these waters has been the focus of a continuous long-term study since 2002 (Genov *et al.*, 2008; Genov *et al.*, 2016) and is relatively well studied.

METHODS

Sample collection

Biopsy samples were collected between 2011 and 2017 from free-ranging common bottlenose dolphins living in the Gulf of Trieste and adjacent waters (Fig. 1). Samples of blubber tissue were obtained using a crossbow and biopsy bolts with 25 mm sampling tips (Gorgone *et al.*, 2008; Kiszka *et al.*, 2010). Biopsy sampling was coupled with concurrent photo-identification, to make sure the identity of the sampled animal was known, to prevent re-sampling the same individuals, and to be able to link PCB levels to various individual-specific parameters.

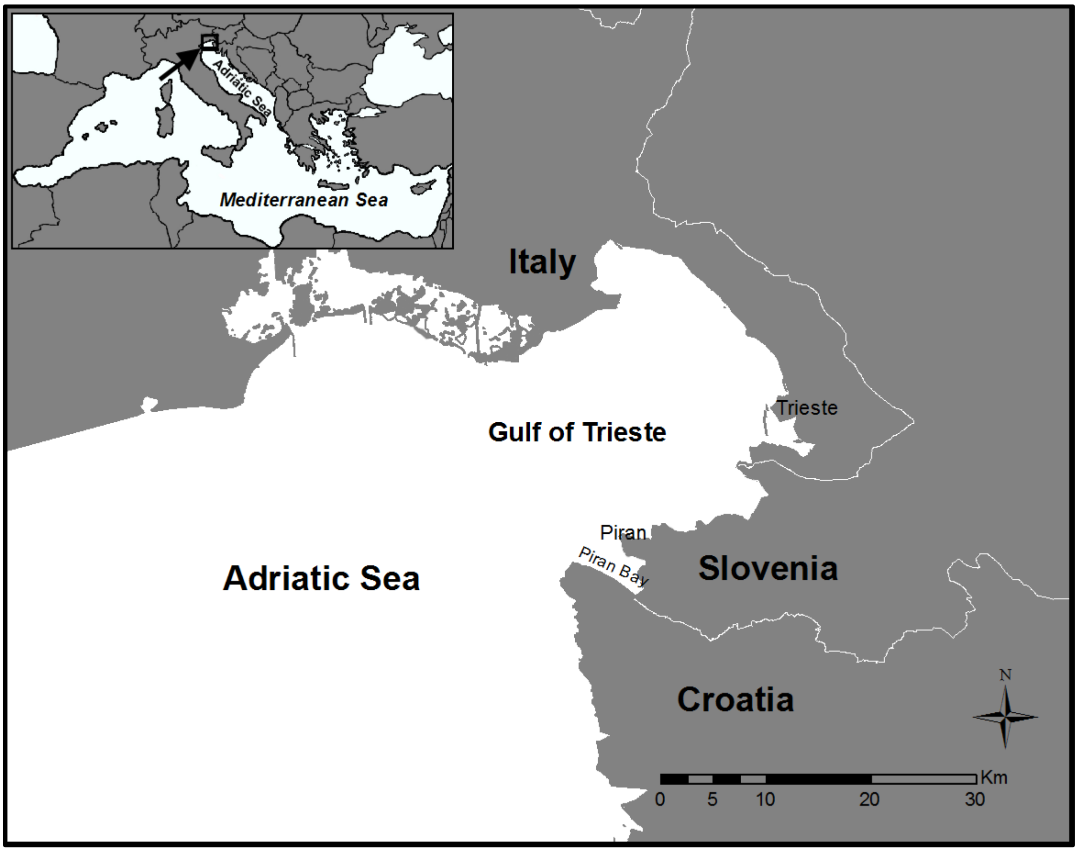

Figure 1. Study area in the northern Adriatic Sea.

Chemical analysis

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). 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 34%. PCB concentrations were determined with an Agilent 6890 GC with µECD. A total of 25 PCB congeners (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) were determined in the samples. Quantitation was performed using internal standards and 11 calibration levels. 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 10 samples analysed. Concentrations were converted to on a lipid weight basis using measured lipid contents.

Data analysis

The values of individual 25 PCB congeners for each sample were summed to obtain the Σ25PCB for each individual. The lipid content of each sample was used to obtain concentrations as mg/kg lipid weight (mg/kg lw). We tested for the effects of sex, parity and social group membership on contaminant concentrations.

Toxicity thresholds

Two PCB toxicity thresholds 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

Biopsy samples were obtained from 32 dolphins, including 18 males, 9 females and 5 animals of unknown sex (Table 1). Σ25PCB ranged from 3.34 to 293 mg/kg lipid weight, with an arithmetic mean of 80.7 (95 % CI = 56.3 – 105.1) and a geometric mean of 51.0 (95 % CI = 34.4 – 75.5, Table 1). Males had significantly higher Σ25PCB concentrations than females (Mann-Whitney U test, $U = 154$, $P < 0.001$, Fig. 2). Furthermore, nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U test, $U = 17$, $P < 0.05$, Fig. 3). There were no statistically significant differences among social groups (Kruskal-Wallis test, $H = 1.24$, $P = 0.743$, Fig. 4). Figure 5 shows female and male PCB concentrations in relation to two toxicity thresholds. Overall, 88% of dolphins had PCB concentrations above the toxicity threshold of 9 mg/kg lw for physiological effects in experimental marine mammal studies (Kannan *et al.*, 2000), while 66% 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).

Table 1. Σ25PCB 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.

Sex	N	Mean	Median	Geomean	Geomean 95% CI	Range (min–max)
Male	18	93.5	77.9	76.5	56.2 – 104.1	19.5 – 293.0
Female	9	19.1	16.4	13.2	7.0 – 24.9	3.3 – 48.4
Unknown	5	145.5	149.7	133.9	86.7 – 206.7	58.0 – 218.4
OVERALL	**32**	**80.7**	**60.9**	**51.0**	**34.4 – 75.5**	**3.3 – 293.0**

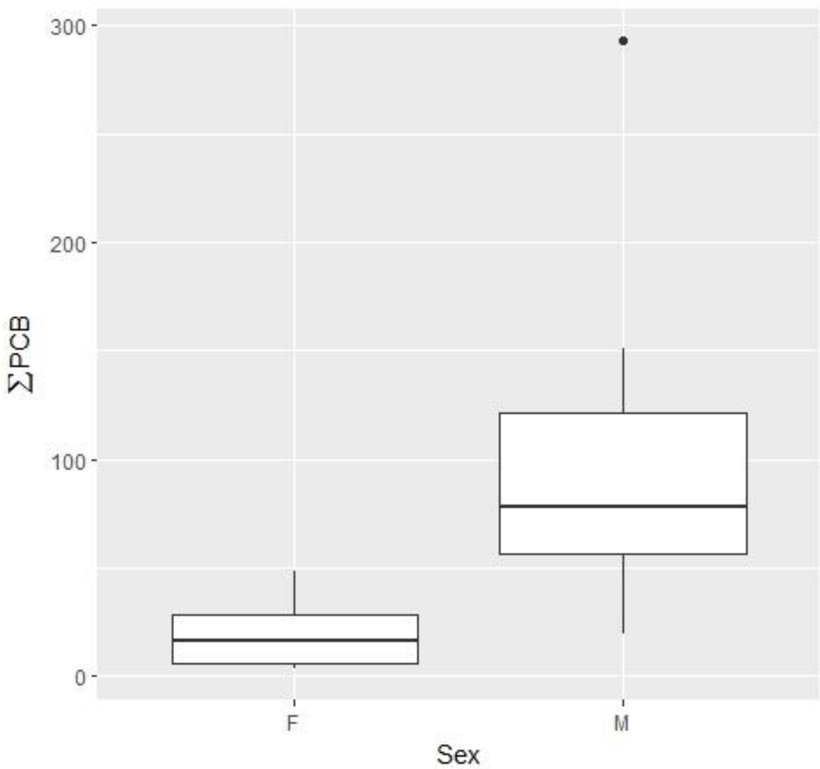

Figure 2. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between females and males. The difference is statistically significant (Mann-Whitney U test, $U = 154$, $P < 0.001$).

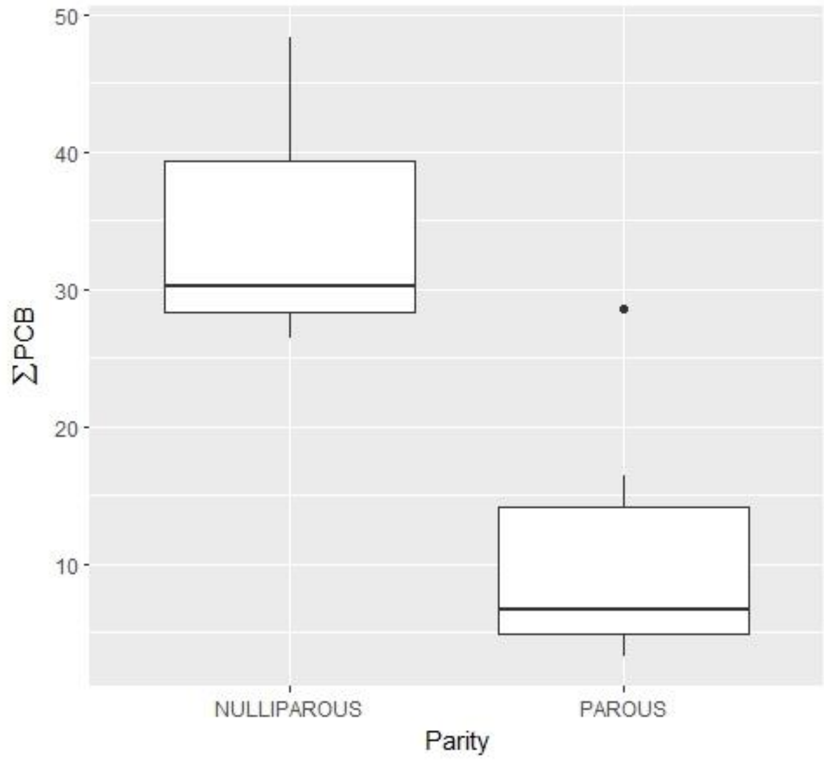

Figure 3. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between nulliparous and parous females. The difference is statistically significant (Mann-Whitney U test, $U = 17$, $P < 0.05$).

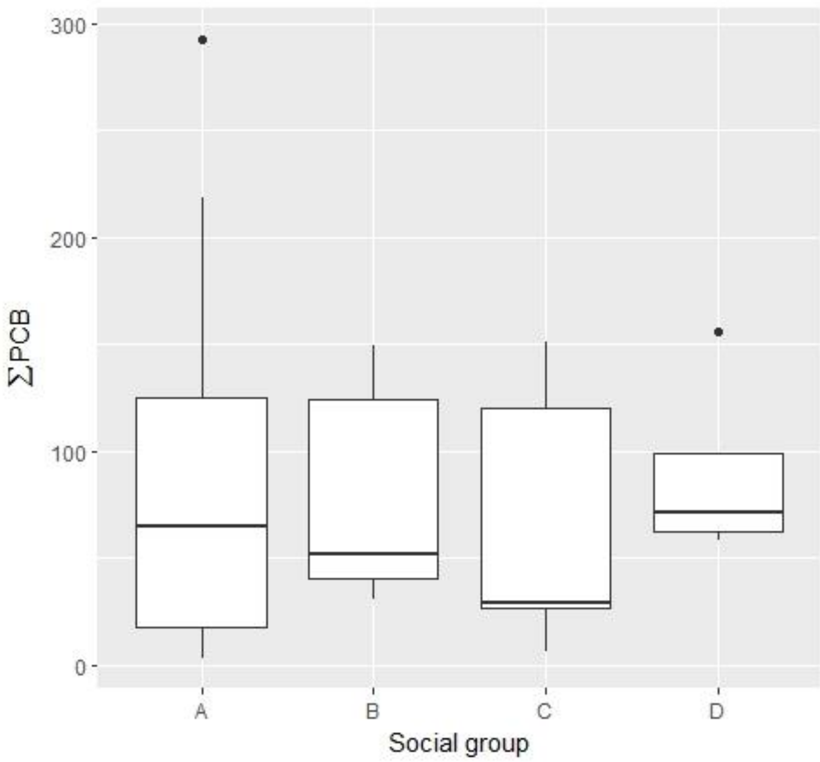

Figure 4. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) among different social groups. Differences are not statistically significant (Kruskal-Wallis test, $H = 1.24$, $P = 0.743$).

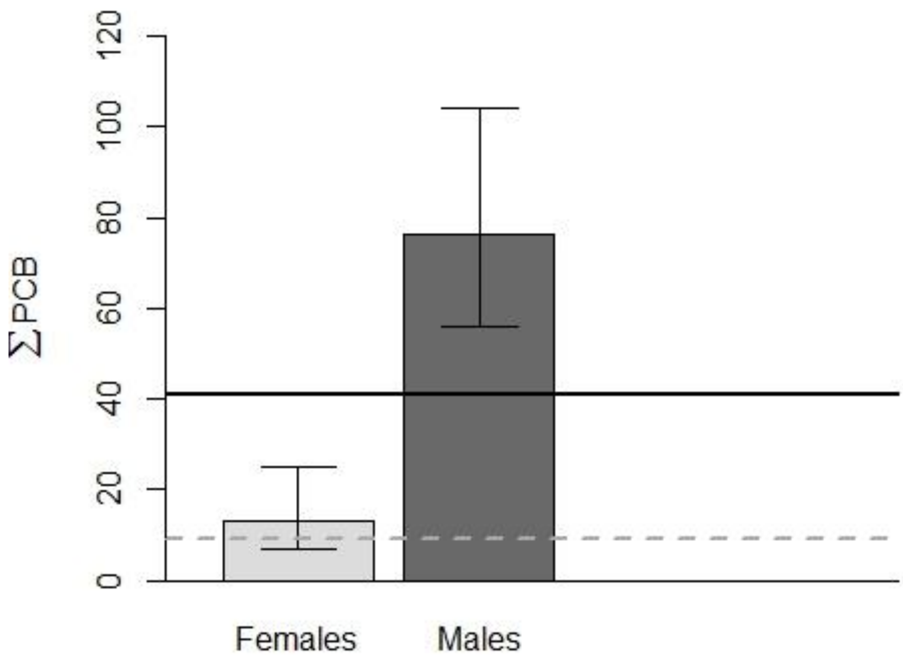

Figure 5. Geometric mean Σ25PCB (mg/kg lipid weight) concentrations for females and males, in relation to published toxicity thresholds. Error bars are the 95 % confidence intervals for geometric means. The lower dashed grey line represents the lower toxicity threshold (9 mg/kg lw) for onset of physiological effects in experimental marine mammal studies (Kannan et al. 2000). The solid black 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).

DISCUSSION

All biopsied animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002 (Genov *et al.*, 2008). 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.

In the long term, the continued PCB monitoring in conjunction with photo-identification may provide further useful insights. Such integrated information, linking pollutant levels to demographic and other parameters, holds a lot of potential, as PCB concentrations can be linked to sex, reproductive output and other parameters (Wells *et al.*, 2005). Such information is often lacking for wild populations and is of considerable importance for evaluating the impacts of pollutants on cetaceans.

Males had significantly higher PCB concentrations than females (Fig. 2), suggesting offloading of PCBs 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. 3) further support this, despite limited sample size. Given the long-term and ongoing monitoring of this population, future work incorporating individual re-sighting histories, information on reproductive rates and PCB monitoring may provide further insight into possible links between pollutant loads and recruitment.

Several social groups have previously been identified in this population, which display differences in behaviour as well as feeding strategies in relation to fisheries (Centrih *et al.*, 2013; Genov *et al.*, 2015). However, our results suggest that PCBs pose a threat to these animals regardless of social group membership and potential associated dietary differences (Fig. 4).

The vast majority of animals in our study exceeded the lower toxicity threshold, with more than half also exceeding the higher threshold (Fig. 5). This is 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, and occasional bycatch (Genov *et al*., 2008, Morigenos, unpublished).

In conclusion, PCBs are still a problem in Mediterranean Sea, and may be causing population-level effects in this population. PCB 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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