Chemosphere 225 (2019) 139–149

Contents lists available at ScienceDirect

Chemosphere

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

Biochemical and molecular biomarkers in integument biopsies of free-ranging coastal bottlenose dolphins from southern Brazil

Barbara Pacheco Harrison Righetti [a], Jacó Joaquim Mattos [a], Marília Nardelli Siebert [a], Fábio Gonçalves Daura-Jorge [b], Carolina Bezamat [b], Pedro Friedrich Fruet [c, d, e], Rodrigo Cezar Genoves [c, d], Satie Taniguchi [f], Josilene da Silva [f], Rosalinda Carmela Montone [f], Paulo César de Azevedo Simões-Lopes [b], Afonso Celso Dias Bainy [a], Karim Hahn Lüchmann [g, *]

[a] Laboratório de Biomarcadores de Contaminação Aquática e Imunoquímica, Departamento de Bioquímica, Universidade Federal de Santa Catarina, Florianópolis, Brazil
[b] Laboratório de Mamíferos Aquáticos, Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Florianópolis, Brazil
[c] Museu Oceanográfico, Universidade Federal de Rio Grande, Rio Grande, Brazil
[d] Kaosa, Rio Grade, Brazil
[e] Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos – ICMBio/CMA, Santos, SP, Brazil
[f] Laboratório de Química Orgânica, Universidade de São Paulo, São Paulo, Brazil
[g] Departamento de Educação Científica e Tecnológica, Universidade do Estado de Santa Catarina, Florianópolis, Brazil

HIGHLIGHTS

- PCB levels were above the threshold established as risk limit for *Tursiops truncatus.*
- Transcript levels of *GR, GPx-4* and *IL-1α* were associated with PBDEs and pesticides.
- Higher GST activity was associated with higher pesticides levels.
- Data suggests a putative influence of seasonality on biomarker responses.

ARTICLE INFO

Article history:
Received 13 November 2018
Received in revised form
24 February 2019
Accepted 25 February 2019
Available online 2 March 2019

Handling Editor: Prof Willie Peijnenburg

Keywords:
Bottlenose dolphins
Biomarker response
Organic contaminants

ABSTRACT

Adverse effects of exposure to persistent organic pollutants (POPs) threaten the maintenance of odontocete populations. In southern Brazil, coastal bottlenose dolphins from the Laguna Estuarine System (LES) and Patos Lagoon Estuary (PLE) were sampled using remote biopsies during the winter and summer months. Levels of bioaccumulated POPs were measured in the blubber. The activities of glutathione *S*-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were also quantified, as were the mRNA transcript levels of *aryl hydrocarbon receptor* (*AhR*), *AhR nuclear translocator* (*ARNT*), *cytochrome P450 1A1-like* (*CYP1A1*), *metallothionein 2A* (*MT2A*), *GST-π, GPx-4, GR, interleukin 1 alpha* (*IL-1α*), and *major histocompatibility complex II* (*MHCII*) in the skin. In general, levels of POPs were similar among sites, sexes, ages and seasons. For most animals, total polychlorinated biphenyl (ΣPCBs) levels were above the threshold level have physiological effects and pose risks to cetaceans. The best-fitting generalized linear models (GLMs) found significant associations between *GR, IL-1α* and *GPx-4* transcript levels, SOD and GST activities, and total polybrominated diphenyl ether (ΣPBDEs) and pesticide levels. GLMs and Kruskal-Wallis analyses also indicated that there were higher transcript levels for most genes and lower GST activity in the winter. These results reinforce the need to consider the influence of environmental traits on biomarker values in wildlife assessments.

1. Introduction

Continuous human population growth and urbanization along

* Corresponding author.
E-mail addresses: karim.luchmann@udesc.br, khluchmann@gmail.com (K.H. Lüchmann).

https://doi.org/10.1016/j.chemosphere.2019.02.179
0045-6535/

the coastlines has led to the increasing release of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) like dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenyl ethers (PBDEs), into aquatic ecosystems. POPs are highly stable, toxic and lipophilic (Breivik et al., 2004), and thus accumulate in biota and biomagnify through the food chain to reach higher concentrations in top predators, such as odontocetes (Kelly, 2007; Tanabe, 2002). Previous studies demonstrated that continuous exposure to complex mixtures of POPs affected the health of a variety of odontocete species. For instance, the high incidence of carcinomas in belugas (*Delphinapterus leucas*) in the St. Lawrence Estuary, Canada, was associated with their exposure to OCPs, polycyclic aromatic hydrocarbons (PAHs), metals, and PCBs (De Guise et al., 1994). Furthermore, reduced activity of lymphocytes and natural killer (NK) cells, as well as increased susceptibility to viral infections, has been associated with exposure to POPs in bottlenose dolphins (*Tursiops truncatus*) (Lahvis et al., 1995). POPs were also associated with hormonal changes in delphinids (Schwacke et al., 2012; Trego et al., 2018), as well as to enhanced levels and activities of cytochromes P450, glutathione *S*-transferases (GSTs) (Bengtson Nash et al., 2014; Hooker et al., 2008; McKinney et al., 2004), and antioxidant enzymes (Fossi et al., 2013; Kanerva et al., 2012; Regoli et al., 2011), which are upregulated upon exposure to certain contaminants to facilitate their metabolism and eventual excretion or to cope with reactive molecules produced during their biotransformation (Regoli et al., 2011).

Changes at cellular, tissue, or systemic levels due to POP exposure may lead to higher mortality rates and reduced fecundity, resulting in adverse effects on population dynamics (Vasseur and Cossu-Leguille, 2006). This is of particular concern to small odontocete populations, which display a high degree of site fidelity to coastal areas close to POP sources and further harmful human activities. POP contamination is usually silent and cumulative, so exposure effects tend to become apparent only after longer exposure periods. Even then, the identification of cause-effect relationships may be hampered by confounding factors, such as the occurrence of diseases, fitness variations due to food availability, or normal aging effects (O'Shea, 1999). Therefore, the use of tools, such as biomarkers that indicate contaminant-induced biological responses, is necessary in the study of free-ranging cetaceans.

In recent years, many studies have searched for and measured levels of different biomarkers in wild populations of cetaceans, mainly in the skin tissue collected from live animals by remote biopsy. The main limitation facing these studies is that their sample sizes are usually small (Fossi et al., 1997, 2004; Jauniaux et al., 2011; Waugh, 2011), although the long-term collection of samples has provided some studies with greater sample sizes (Buckman et al., 2011; Fossi et al., 2003). Regardless of this challenge, this methodology is an invaluable tool for population assessment since it presents no bias in terms of the animals' health conditions or sample degradation, which are two of the main limitations in the use of stranded cetaceans for pollutant and biomarker analysis (Aubail et al., 2013; Kershaw et al., 2018).

The purpose of this study was thus to evaluate potential molecular and biochemical biomarkers in the skin and POP levels in the blubber of free-ranging bottlenose dolphins inhabiting coastal areas in southern Brazil, specifically the Laguna Estuarine System (LES) (28°30′S 48°46′W) and Patos Lagoon Estuary (PLE) (32°05′S 52°04′W), using samples obtained through remote biopsies. Both populations are small, with a high degree of site fidelity, and geographically and genetically isolated from other bottlenose dolphin populations (Borges Costa et al., 2015; Fruet et al., 2017). These characteristics, along with morphological differences among populations, have prompted researchers to discuss whether the bottlenose dolphins living along the Brazilian coast could be considered as belonging to a new subspecies or species; herein, we considered the studied dolphin populations to belong to *Tursiops truncatus gephyreus*, which was recently recognized as a valid subspecies by the Society for Marine Mammalogy Committee on Taxonomy (2017). Due to their habitats, coastal populations are at a particularly high risk due to their exposure to a variety of anthropogenic impacts, including POP release (O'Shea, 1999).

The LES dolphin population, estimated at 55 to 60 individuals (Daura-Jorge et al., 2013), has shown an increasing incidence in recent years of a lacaziosis-like disease or lobomycosis-like disease (LLD), which is an opportunistic fungal disease related to immune impairment (Van Bressem et al., 2015). Dolphins from the LES are also known for their specialized cooperative foraging with artisanal fishermen, a behavior displayed by a subset of this group (Simões-Lopes et al., 1998). This distinctive interaction has enormous cultural and economic value for the local community (Peterson et al., 2008), and reinforces the need for studies that monitor potential health risks to these animals. The LES is adjacent to the largest coal extraction and refining complex in southern Brazil and several rice farming properties (Rodriguez-Iruretagoiena et al., 2015; Vieira et al., 2016). Therefore, the runoff from such activities could potentially expose dolphins in the LES population to a suite of different POPs. No LLD occurrence has been recorded to date among the PLE dolphin population, which consists of about 90 individuals, but in spite of this it is also exposed to runoff from a highly developed petrochemical and industrial complex. PLE is one of the most productive fishing grounds in Brazil, with abundant fish assemblages inside the estuary and along the adjacent coasts (Rodrigues and Vieira, 2013). Intense rice farming is also present along the shores of nearby lagoons (Santos et al., 2008).

Thus, to understand whether POPs cause biological responses in the skin of free-ranging dolphins from the LES and PLE populations, remote biopsy sampling was conducted and the blubber obtained was analyzed to assess its concentrations of POPs, and the skin in the samples was also analyzed for the identification and quantification of various biochemical and molecular biomarkers. We further evaluated if sex, age, and season influenced the POP accumulation and biomarker responses in these bottlenose dolphins.

2. Materials and methods

2.1. Sample collection

Between June 2015 and March 2016, 17 remote biopsy samples were collected from resident bottlenose dolphins of the LES ($n = 7$) and PLE ($n = 10$), representing more than 10% of each population sampled (Fig. 1).

Sampling was conducted during boat surveys that were part of ongoing, long-term monitoring projects for both populations (Daura-Jorge et al., 2013; Fruet et al., 2015) following a well-established remote biopsy sampling protocol (Winn et al., 1973). Further details can be found in the Electronic Supplementary Material (SM1). Biopsy samples were fractionated into one blubber and two skin subsamples, with the former used for POP quantification and the two latter samples used for biomarker analyses.

Biopsied animals were identified through photo-identification of natural marks on the dorsal fin (Würsig and Jefferson, 1990) to avoid re-sampling. The sex of each sampled dolphin was determined through the amplification of the *ZFX* and *SRY* genes (Rosel, 2003). The dolphin of undetermined sex was not included in analyses that included sex as a parameter. Age class was determined

Fig. 1. Study sites in southern Brazil, South America (A), and close-up view of southern Brazil (B). Red rectangles indicate sites where the remote biopsy sampling of resident bottlenose dolphins was undertaken. LES, Laguna Estuarine System, Santa Catarina state; PLE, Patos Lagoon Estuary, Rio Grande do Sul state. Sampling sites are shown in greater detail in C (PLE) and D (LES). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

based on the available data from long-term individual monitoring (see Electronic Supplementary Material for methodology details – SM2), and seasonality was based on the astronomical calendar and annual temperature fluctuations in the sampling areas. The information on each dolphin sampled is presented in Table 1.

2.2. Contaminant analysis

Levels of 7 PBDE congeners, 17 OCPs, and 51 PCB congeners were quantified in the blubber samples. PCB-198 and PCB-103 were added as surrogates to all samples analyzed, including the blanks and reference materials (SRM1245). ΣPCBs refers to the sum of the 33 PCB congeners (28, 52, 49, 44, 74, 95, 101, 99, 87, 110, 151, 149, 118, 153, 132, 105, 141, 138, 158, 187, 183, 128, 167, 174, 177, 156, 180, 170, 201, 195, 194, 206 and 209), ΣPBDEs represent the sum of all measured PBDEs, and ΣDDTs refers to the sum of o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT, and p,p'-DDT.

The lipid content of the blubber was measured gravimetrically, and its POP content was normalized by the lipid weight in the sample to avoid biases due to variations in lipid content among the biopsy samples (Méndez-Fernandez et al., 2016). POP concentrations were expressed as ng.g lipid weight^{-1}. Concentrations below the limit of detection were assigned random values between zero and the limit of detection for that compound. For details of sample preparation, chemical analyses, and a list of all the POPs quantified, see the Electronic Supplementary Material (SM3).

2.3. Biochemical biomarkers

The biochemical biomarkers evaluated were the activity of glutathione *S*-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). For all assays, approximately 100 mg of epidermal tissue was homogenized in a 1:7 (w:v) mixture of the proper buffer (50 mM Tris-HCl, pH 7.4, 150 mM KCl, 1 mM DTT and 0.5 mM PMSF). Disruption of tissues was carried out with a Tissue-Tearor (BioSpec Products) in

Table 1
Summary of sampling and individual data for sampled dolphins: Sample ID, established according to the location and sampling order; individual ID, obtained from the long-term monitoring catalog of both populations[a]; sex; age class (juvenile or adult); sampling date and levels of bioaccumulated POPs (ng.g lipid weight^{-1}).

Sample ID[a]	Ind. ID[b]	Sex	Age class	Sampling date	ΣDDT	ΣPCB	ΣPBDE	Mirex	HCB	Chlordanes
LES1	#3	Male	Adult	07-22-2015[w]	293.85	695.97	0.30	0.24	59.97	0.79
LES2	J42	Female	Juvenile	07-27-2015[w]	702.15	1267.7	52.94	34.33	35.88	16.28
LES3	#16	Male	Adult	07-27-2015[w]	8385.61	19336.72	312.94	167.92	29.32	31.51
LES4	#46	Male	Adult	07-29-2015[w]	827.62	2310.94	0.24	283.11	0.30	0.21
LES5	#48	Male	Adult	07-29-2015[w]	4146.20	10051.08	426.67	148.62	0.76	0.35
LES6	#32	Male	Adult	02-11-2015	17267.56	16369.67	238.75	212.62	181.99	257.94
LES7	#51	Female	Adult	03-01-2016	5505.28	14956.12	564.49	259.47	42.04	42.66
PLE1	#105	Male	Adult	06-01-2015[w]	2568.15	22962.99	413.33	567.55	1.01	0.56
PLE2	#207	Male	Juvenile	06-02-2015[w]	1694.03	12051.95	260	336.37	44.59	15.18
PLE3	#137	Male	Adult	06-02-2015[w]	5075.31	41093.43	472.67	295.05	42.07	90.13
PLE4	#208	Male	Juvenile	06-02-2015[w]	1053.24	7836.07	73.75	240.58	27.50	0.35
PLE5	SM1	Male	Juvenile	03-08-2016	2077.40	29827.26	269	301.52	55.91	24.58
PLE6	#23	Male	Adult	03-09-2016	3699.03	30988.08	534.19	561.55	34.45	33.20
PLE7	#123	Male	Adult	03-09-2016	5259.65	51491.59	590	492.46	41.34	30.28
PLE8	#012	Female	Adult	03-09-2016	99.49	5563.45	15	76.36	0.24	16.09
PLE9	#142	Female	Adult	03-09-2016	316.48	7345.97	66.47	135.44	0.89	14.87
PLE10	SM2	Unknownn	Juvenile	03-09-2016	435.47	5443.94	80	75.80	36.72	233.06

w: Winter.
[a] LES: Samples obtained from the LES population; PLE: Samples obtained from the PLE population.
[b] LES: Population catalog based on Daura-Jorge et al. (2013); PLE population catalog based on Fruet et al. (2015).

three cycles of 10 s duration each. Samples were maintained on ice throughout homogenization and between disruption cycles. The homogenate was centrifuged at 9000 g for 30 min at 4 °C, and the resulting supernatant was used in enzymatic assays.

The GST assay was performed according to Habig et al. (1974). The SOD assay was conducted as described by McCord and Fridovich (1969). GPx activity was measured according to Flohé and Gunzler (1984), and the measurement of GR activity followed the method established by Carlberg and Mannverick (1985). All enzymatic assays were performed in duplicate at 37 °C in a 96-well plate reader (SpectraMax 250, Molecular Devices, Sunnyvale, CA, USA). Total protein levels were quantified according to Bradford (1976). See the Electronic Supplementary Material (SM4) for the details of the methodology used in the above assays.

2.4. Molecular biomarkers

2.4.1. RNA extraction and cDNA synthesis

Total RNA was extracted from 30 mg of epidermis individually using a RNeasy Fibrous Tissue Mini Kit (Qiagen, US), according to the manufacture's instructions. Genomic DNA (gDNA) was eliminated by a DNase-on-column treatment supplied with the kit. The quantity of extracted RNA was determined with a NanoDrop ND-1000 UV–Vis spectrophotometer (ThermoScientific, UK) at 260 nm, and the absorbance ratios at 260/280 nm and 260/230 nm were used to assess the purity of the RNA samples.

To avoid any gDNA contamination before cDNA synthesis, the extracted RNA was treated with a gDNA wipeout buffer (QuantiTect Reverse Transcription Kit, Qiagen, US). For each of the 17 samples, 1 μg of treated RNA was retrotranscribed to cDNA with the QuantiTect Reverse Transcription Kit (Qiagen, US), following the manufacturer's instructions. The cDNA samples were then stored at −20 °C. The detailed methodology used is described in the Electronic Supplementary Material (SM5 and SM6).

2.4.2. Quantitative real-time polymerase chain reaction (qPCR)

Nine target genes were selected to provide biological information on the effects of POPs in the skin tissue of *T. truncatus*: *cytochrome P450 1A1-like* (*CYP1A1*), *aryl hydrocarbon receptor* (*AhR*), *AhR nuclear translocator* (*ARNT*), *metallothionein 2A* (*MT2A*), *major histocompatibility complex class II* (*MHCII*), *interleukin 1 alpha* (*IL-1α*), *glutathione S-transferase π* (*GST π*), *glutathione peroxidase 4* (*GPx-4*), and *glutathione reductase* (*GR*), along with the housekeeping gene, *ribosomal protein S18* (*RPS18*), for the correction of input variation. Primers specific to *T. truncatus* were designed from the gene sequences deposited in the NCBI database (https://www.ncbi.nlm.nih.gov/genbank/) using the PrimerQuest Tool® of Integrated DNA Technologies Inc. (IDT). Primers were designed for distinct exons, or exon-exon junctions, to minimize the risk of amplifying contaminating gDNA. Primers were evaluated for hairpin, self-dimer, and hetero-dimer formation, annealing temperatures, the similarity between forward and reverse primers, and primer quality using the FastPCR 6.5 software (PrimerDigital). Gene accession numbers, sequences from selected primers, and results for the primer parameters evaluated are reported in the Electronic Supplementary Material (Tables S1 and S2).

The qPCR assays were performed in duplicate in a Rotor-Gene 6000 system (Qiagen, US) using a QuantiNova SYBR Green PCR Kit (Qiagen, US). For each target gene, the reaction system consisted of 100 ng of cDNA (or 200 ng cDNA for low expression genes), QuantiNova SYBR Green Master Mix and 0.7 μM of forward and reverse primers. The amplification program for each transcript consisted of one cycle for enzyme activation at 95 °C (2 min), followed by 35 cycles of denaturation at 95 °C for 5 s, and a combined primer annealing/elongation step at 60 °C for 10 s. PCR products were submitted to a melting curve analysis from 55 to 98 °C to evaluate primer specificity. A standard curve was constructed in duplicate for all genes, with five known concentrations obtained from a pool of all samples. Reference blanks with molecular-grade water were also included in each curve and run. This curve was used to assess primer efficiency (E) and the linearity of the relationship between the Cq (quantification cycle) and cDNA concentration. This linear relationship was considered to be appropriate if the r^2 values obtained were above 0.97. An E-value of one represented a duplication of the transcripts in one PCR cycle.

The Cq values obtained for the target genes from each sample were normalized by the Cq of the housekeeping gene (*RPS18*), yielding ΔCq values. These ΔCq values were used in the estimation of relative transcription levels using the $2^{-\Delta Cq}$ method (Schmittgen and Livak, 2008).

2.5. Statistical analyses

The distributions of the data for the different POPs and

biomarkers were analyzed using histograms and plots comparing the data to a theoretical normal distribution. Homoscedasticity was evaluated through the construction of boxplots, as recommended by Zuur et al. (2010). Statistical analyses were planned to answer two main questions: (1) Do dolphins present distinct levels of contaminants and biological responses based on individual characteristics, sites and/or sampling season, and (2) on top of the effects of such factors, can the measured biological responses be explained by the levels of POPs bioaccumulated in their tissues?

Both biomarkers and POPs presented non-Gaussian error distributions, so the first question was answered by performing Kruskal-Wallis tests comparing the data for all POP classes and biomarkers between groups defined based on sex, age class, site, and sampling season. Significance was established to occur at $p < 0.05$. For the second question, generalized linear models (GLMs) with gamma or Gaussian error distributions were fitted to the data for each biomarker. Season was the only categorical factor included as a predictor, given the results of the Kruskal-Wallis tests. Considering the strong influence of sex on POP levels reported in the literature (*i.e.* Aguilar et al., 1999; Jepson et al., 2016; Yordy et al., 2010), and the impossibility of including sex as a predictor within the models due to the small number of females sampled ($n = 4$), the GLMs were constructed using data from males only ($n = 12$). ΣPBDEs and ΣPCBs were not included in the same models due to their co-linearity ($r^2 = 0.85$).

Models were fit to the data with a stepwise procedure, using backward elimination from the full model in the MuMIn package version 1.40.0 (Bartón, 2017) in R ver.3.3.3 (R Core Team, 2017). Adjusted *p*-values were used to check the statistical significances of the variables included in each model. The best models were selected based on their values of Akaike's information criteria (AIC) (Zuur et al., 2009). Those with a ΔAIC from the AIC of the best model less than 2 were retained, and can be found in the Electronic Supplementary Material (Tables S4 and S5). Model goodness-of-fit was assessed based on pseudo-*r*-squared (r^2) values, which were calculated as (null deviance − residual deviance)/null deviance, and the models were validated by plotting their residuals vs. fitted values (Zuur et al., 2007).

A principal component analysis (PCA) was performed to visualize the relationships among the different contaminant classes and biomarkers, and also to investigate potential separations between groups of the sampled dolphins based on patterns in the variables used for the PCA. All data were normalized and scaled to unit variance. A preliminary PCA was conducted with all POPs and biomarkers (Electronic Supplementary Material, Fig. S1). To reduce the ratio between the number of variables and the number of samples, a second PCA was performed with variables selected from among those included in the preliminary PCA. Based on the preliminary PCA plots, we identified strongly correlated variables, and among these, selected those with the highest loading values. The selected variables for the second PCA were: ΣPBDEs, ΣDDTs, GST activity, and the transcript levels of *ARNT* and *GPx*.

All statistical analyses were conducted in R ver. 3.3.3 (R Core Team, 2017) and the R scripts used for the data analyses are supplied in the Electronic Supplementary Material (SM7).

3. Results and discussion

3.1. Contaminant concentrations in resident bottlenose dolphins

Levels of different POPs bioaccumulated in the sampled dolphins are presented in Table 1. In general, levels of POPs did not vary between dolphins from the LES and PLE populations, or between sampling seasons, with the exception of chlordane levels, which were significantly higher in dolphins sampled in summer (mean ± standard error = 81.58 ± 101.78) compared to those sampled in winter (17.26 ± 29.45) ($C-S = 4.9$, $df = 1$, $p = 0.02$) (Table S3 in the Electronic Supplementary Material shows a complete list of these results). POP levels were also similar between juveniles and adults, and despite of the vast body of literature on the influence of sex on the bioaccumulation of organic contaminants in cetaceans (*i.e.* Aguilar et al., 1999; Jepson et al., 2016; Yordy et al., 2010), POP levels did not vary between the males and females sampled in this study ($p > 0.05$; Table S3). This result was most likely due to the uneven sampling of males ($n = 12$) and females ($n = 4$) in this study, and may possibly also be attributed to the fact that, to our knowledge, only two out of the four females sampled were of reproductive age (samples ID: PLE8 and PLE9).

In dolphins from both sites, ΣPCBs were the prevalent class of POPs, with average levels of 9284 ± 7859 and 21,460 ± 16,441 in dolphins from the LES and PLE populations, respectively. The levels of ΣPCBs detected in *T. truncatus* from PLE were much lower than the levels previously found in dolphins from the Mediterranean Sea, Adriatic Sea, and waters surrounding Portugal and parts of the United Kingdom (UK), which ranged from 40,000 to over 180,000 ng g lipid weight^{-1}. The values obtained herein were also lower than those reported for dolphins from the coast of the United States of America (USA), which ranged from 50,000 to over 100,000 ng g lipid weight^{-1} (Borrell et al., 2006; Borrell and Aguilar, 2007; Fair et al., 2010; Genov et al., 2019; Gonzalvo et al., 2016; Jepson et al., 2016; Marsili et al., 2018). On the other hand, the levels of ΣPCBs in dolphins from the PLE herein were similar to those found in highly industrialized regions of Brazil, Ireland, and India (Berrow et al., 2002; Lailson-Brito et al., 2012; Tanabe et al., 1993; Yogui et al., 2010), and even higher than the values reported for bottlenose dolphins from Hawaii, Taiwan, and Australia (Bachman et al., 2014; Chou et al., 2004; Vetter et al., 2001). Such results indicate that there are likely relevant levels of PCB inputs in the PLE, which are most likely associated with the petrochemical complex and intense harbor activities in the region.

Hexachlorobenzenes (HCBs) were the most frequent PCB congeners found in all sampled dolphins, followed by pentachlorobiphenyls and heptachlorobiphenyls (see Electronic Supplementary Material, Table S6). Higher levels of highly chlorinated PCBs are recurrently seen in odontocete cetaceans (Lailson-Brito et al., 2012; Tornero et al., 2006; Yordy et al., 2010), which is primarily associated with the reduced capability of the cytochrome P450 enzyme superfamily in this group to detoxify specific PCB groups (Boon et al., 1997; Fossi et al., 1992; Tanabe et al., 1988). The lower dispersion rates of these PCB congeners may also contribute to their deposition in coastal environments (Combi et al., 2016) and enhanced bioaccumulation in coastal marine mammals (Brown et al., 2015).

Among pesticides, DDT metabolites were predominant in the dolphins sampled in this study, although the levels found herein were lower than those identified in other regions of the world (Fair et al., 2010; Berrow et al., 2002; Borrel, Aguilar, 2007; Borrell et al., 2006; Gozalvo et al., 2016; Marsili et al., 2018). Furthermore, p,p'DDE was the most abundant metabolite in all samples, reinforcing the fact that the ΣDDTs measured in dolphins likely originated from historical inputs. A similar pattern is frequently observed in cetacean studies (Borrell et al., 2007; Fair et al., 2010; Genov et al., 2019).

Concentrations of ΣPBDEs (LES: 228 ± 222; PLE: 277 ± 215) were similar to those previously reported in bottlenose dolphins from southern Europe, north of Florida, USA, and along the coast of São Paulo, Brazil (Barón et al., 2015; Wilson et al., 2012; Yogui et al., 2011). BDE47 was the most prevalent PBDE congener in all

samples, followed by BDE100. BDE153 and BDE99 were only detected in dolphins from LES. Furthermore, the levels of mirex measured in the blubber of PLE dolphins (PLE: 308 ± 105) were comparable to those previously found in bottlenose dolphins from estuaries in the southeastern USA (Fair et al., 2010).

Overall, our results indicate that, despite presenting lower POP levels than those previously reported from more highly impacted regions of the globe, the bottlenose dolphins from the PLE and LES still face health risks from ΣPBDEs, as they presented bioaccumulation values similar to those found in more industrialized regions, and also from ΣPCBs because they contained levels of these exceeding the threshold for the onset of physiological effects (e.g. hormonal imbalance and immune suppression), which is estimated to occur at 17,000 ng g lipid weight^{-1} of Aroclor (Kannan et al., 2000), equivalent to 9000 ng g lipid weight^{-1} of ΣPCBs (Jepson et al., 2016). For six out of the 17 dolphins sampled in the present study, PCB concentrations were above 14,800 ng g lipid weight^{-1}, which is considered a 'risk limit' for reproduction in *T. truncatus* based on the dose-response data for surrogate species (Schwacke et al., 2002). Interestingly, the PCB levels for two of the PLE dolphins (samples ID: PLE3 and PLE7) were above the 41,000 ng g lipid weight^{-1} threshold for reproductive impairment in ringed seals established by Helle et al. (1976).

3.2. Biomarker responses to organic contaminants

Considering the potential health risks associated with POP exposure, we chose a set of biomarkers, namely those involved in immune responses and antioxidant and biotransformation systems, to try to understand whether POPs contributed to biological changes in response to environmental stress in the skin of free-ranging dolphins from the PLE and LES populations. The biochemical and molecular data obtained for the sampled dolphins are presented in Table 2.

Among the GLMs tested in this study, most models presented high r^2-values (0.51–0.76), which indicated that the selected models explained the variance in the data well, with the exception of the data for transcription levels of *AhR* ($r^2 = 0.35$) and GPx activity ($r^2 = 0.16$) (Table 3). *ARNT*, *GST π*, and *MHCII* transcript levels were best explained by the null model, suggesting that the predictors assessed could not explain these responses. The best-fitting models obtained are presented in Table 3.

Overall, the best-fitting models indicated that POP levels were positively associated with GST and SOD activities and *GR* and *IL-1α* transcript levels, while a negative association was found between POP levels and *GPx-4* mRNA levels. The detailed results of these analyses are presented in the following sections, which are divided up based on the metabolic functions of the measured biomarkers.

3.2.1. Biomarkers involved in xenobiotic biotransformation (AhR, ARNT, GST and GST-π)

Among the biomarkers assessed that were involved in xenobiotic metabolism, only GST activity varied with POP levels, and the best-fitting models indicated that the activity of GST was positively associated with the concentration of ΣDDTs and mirex (Table 3). GST is a classic biomarker of exposure to several classes of organic contaminants due to its role in phase II xenobiotic biotransformation. In this step, GST catalyzes the conjugation of endogenous glutathione (GSH) to electrophilic centers on a variety of compounds, both endogenous and exogenous, which are formed in phase I, contributing to their metabolism and excretion from the cell and organism (Regoli et al., 2011). Increased GST activity following exposure to POPs, namely PCBs, PAHs, and dioxin-like contaminants (and, to a lesser extent, OCPs) has been extensively reported in fish (for review, see Van der Oost et al., 2003). Increased GST activity has also been reported in amphibians (*Bufo regularis*) and earthworms (*Eisenia fetida*) following exposure to pesticides and DDTs, respectively (Ezemonye and Tongo, 2010; Shi et al., 2016).

It is well-known that GST expression is regulated by the aryl hydrocarbon receptor (AhR) protein (Nebert et al., 1990). AhR is a ligand-dependent transcription factor that dimerizes with ARNT to activate the transcription of the genes coding for xenobiotic phase I and phase II metabolizing enzymes (Dietrich and Kaina, 2010). Although some studies have reported higher *AhR* mRNA levels in killer whales (*Orcinus orca*) and ringed seals (*Phoca hispida*) exposed to PCBs (Brown et al., 2014; Buckman et al., 2011), in the present study, neither *AhR* nor *ARNT* transcript levels varied with the levels of pesticides or other POPs in the blubber. These findings are consistent with observations from a recent study that showed only a slight increase in the transcription of *AhRR* (AhR repressor, a feedback modulator of *AhR* transcriptional activity) in a bovine mammary epithelial cell line exposed to dioxin-like PCBs, which was coupled with there being no effects on the transcriptional activity of *AhR* and *ARNT* (Girolami et al., 2015). Similarly, the transcription of the *AhR* gene was not activated in mouse cells exposed to dioxins (Beedanagari et al., 2010). Our results indicate that the upregulation of AhR-targeted genes, particularly those involved in

Table 2
Values of biochemical and molecular biomarkers measured in all sampled bottlenose dolphins: SOD, GPx, GR and GST activity are presented in miliU per milligram of protein (mU.mgprt^{-1}) and Cq values are presented for all target genes and housekeeping gene *RPS18*.

Sample ID**	SOD (U SOD. mgprt^{-1})	GPx (mU. mgprt^{-1})	GR (mU. mgprt^{-1})	GST (mU. mgprt^{-1})	*AhR*	*ARNT*	*GPx-4*	*GR*	*GST π*	*IL-1α*	*MHCII*	*MT2A*	*RPS18*
LES1	184.83	11.79	71.74	48.00	22.37	27.87	24.6	27.42	26.11	18.75	23.26	13.55	14.97
LES2	179.46	25.04	87.70	67.92	21.98	26.85	23.53	26.15	25.27	18.98	23.29	13.51	15.44
LES3	143.31	20.14	75.44	72.60	22.72	27.87	24.86	27.61	26.3	20.53	24.85	13.5	15.49
LES4	96.84	22.43	81.20	49.07	22.72	27.63	24.34	27.25	26.6	19.93	23.64	13.69	15.81
LES5	158.29	16.98	95.95	66.97	21.14	24.68	23.5	24.99	24.91	17.38	22.65	13.71	15.64
LES6	164.26	16.38	62.70	74.86	23.02	27.45	24.94	28.88	26.88	22.49	22.99	14.76	15.39
LES7	205.87	19.65	85.94	95.46	23.72	27.2	27.35	29.21	29.06	23.49	25.77	14.62	15.76
PLE1	163.89	16.04	77.00	62.64	21.99	26.44	24.75	26.1	25.48	17.53	24.05	13.81	15.34
PLE2	195.14	29.87	72.90	61.02	21.74	26.04	24.53	25.99	25.77	18.09	23.8	13.5	15.05
PLE3	111.46	14.9	78.20	57.54	22.48	27.19	24.19	26.24	26.17	18.78	21.89	14.01	15.63
PLE4	208.63	15.66	77.38	63.80	22.35	26.89	24.68	26.85	25.42	18.72	20.41	14.37	15.41
PLE5	158.23	16.62	53.60	53.96	22.86	27.27	24.92	27.8	25.92	21.62	22.04	14.52	15.31
PLE6	195.47	23.47	82.43	75.52	23.94	28.24	26.03	29.19	27.75	23.22	27.57	14.32	14.83
PLE7	212.83	18.47	84.12	75.26	21.62	24.86	24.68	26.41	24.94	19.49	22.92	13.78	15.14
PLE8	156.62	18.61	56.24	81.23	24.35	28.32	25.85	30.1	28.87	22.33	28.08	14.31	14.94
PLE9	155.28	20.32	40.17	63.55	24.04	28.31	26.09	30.85	28.96	22.4	26.78	14.3	15.21
PLE10	161.78	23.47	67.01	90.89	22.86	27.06	25.65	28.24	26.13	20.24	25.09	13.89	14.97

Table 3
Generalized linear models (GLMs) fit to data for the biochemical and molecular biomarkers analyzed in bottlenose dolphins from the LES and PLE populations, including the best-fitting models of from those originally including ΣPBDES or ΣPCBs and a null model.

	Predictors	AIC	ΔAIC	r^2	w	df
AhR	PBDEs (+), Season W (+)*	−95.4	0	0.35	0.322	4
	Season W (+)	−94.6	0.79			
	Null model	−93.8				
ARNT	*Null model*	33.2	0		0.521	2
	PBDEs (+), HCB (−)	34.7	1.5			
GPx	Mirex (+)	72.9	0	0.16	0.348	3
	Mirex (+), PCBs (−)	73	0.15			
	Null model	73.1				
GPx-4	Mirex (−)**, HCB (−)**, Chlordanes (+)*	−139.7	0	0.66	0.609	5
	DDTs (−), Mirex (−)*, HCB (−)*, Chlordanes (+)	−137.9	1.82			
	Null model	−132.3				
GR	DDTs (+), HCB (−)*	209.1	0	0.51	0.443	4
	HCB (−)*	210.5	1.37			
	Null model	213.9				
GR	PBDEs (+)*, Season W (+)**	−166.7	0	0.58	0.452	4
	PBDEs (+), HCB (−), Season W (+)*	−165.7	0.97			
	Null model	−159.4				
GST	DDTs (+)**, Mirex (+)*, Chlordanes (−)*	81.2	0	0.76	0.407	5
	PBDEs (+), DDTs (+)*, Mirex (+), Chlordanes (−)	82	0.82			
	Null model	92.4				
GST-π	*Null model*	−80.7	0		0.352	2
	Chlordanes (−)	−80.3	0.35			
IL-1α	PBDEs (+)*, Season W (+)***	−40	0	0.68	0.478	4
	PBDEs (+)*, Chlordanes (−), Season W (+)***	−39.2	0.85			
	Null model	−29.5				
MHCII	*Null model*	−90.6	0		0.364	2
	Mirex (−)	−90.1	0.47			
MT2A	DDTs (+), HCB (−), Season W (+)*	26.3	0	0.71	0.361	5
	DDTs (+), HCB (−), Mirex (−), Season W (+)*	26.7	0.39			
	Null model	35.6				
SOD	PBDEs (+), Mirex (+), HCB (+)*, Chlordanes (−)*	118.6	0	0.65	0.361	6
	DDTs (+), Mirex (+), Chlordanes (−)*, HCB (+)*	119.1	0			
	Null model	123.2				

+ Indicates a positive contribution of the predictor to biomarker response.
- Indicates a negative contribution of the predictor to biomarker response.
Significance indicated through * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.
W: Winter.
w: Weight.
df: Degrees of freedom.

phase II xenobiotic biotransformation, does not implicate changes in the transcription rates of this receptor or its nuclear translocator genes.

3.2.2. Biomarkers involved in antioxidant defense (SOD, GR, GPx, GR and GPx-4)

The best-fitting models for antioxidant biomarkers pointed to there being a positive association between SOD activity in the skin and HCB levels in the blubber, which supports the onset of a prooxidant scenario in the animals presenting higher POP burdens. SOD is an enzyme that participates in converting highly toxic radicals, such as the superoxide anion, into the less toxic hydrogen peroxide, where it is further hydrolyzed into water and oxygen by the catalase (CAT) enzyme. These antioxidant enzymes are suggested to be the first line of defense against oxidative stress, and previous studies found increased SOD activity in predatory birds and broad-snouted caimans (*Caiman latirostris*) exposed to PBDEs and pesticides, respectively (Abbasi et al., 2017; Burella et al., 2018).

In our study, levels of ΣPBDEs were positively correlated with *GR* transcription in the skin of *T. truncatus* (Table 3). PBDEs have been associated with the onset of oxidative stress both *in vitro* and *in vivo*, which is related to the enhanced ROS production that is coupled with the biotransformation of PBDEs (Costa et al., 2014). PBDEs are structurally similar to PCBs (Hooper and McDonald, 2000), and some studies have indicated the existence of a similar CYP-mediated metabolism for the detoxification of PBDEs in odontocetes to that involved in PCB detoxification (Marsili et al., 2008; McKinney et al., 2006). CYP-mediated reactions contribute greatly to the production of ROS, and an increase in antioxidant responses is expected to occur to cope with this prooxidant scenario (Regoli et al., 2011). In fact, enhanced GR activity has been reported in rats and rainbow trout (*Oncorhynchus mykiss*) experimentally exposed to PCBs (Förlin et al., 1996; Twaroski et al., 2001). Moreover, Abbasi et al. (2017) reported a positive correlation between GR activity and BDE-100 levels in predatory birds. Increased GR expression associated with POP exposure, as was found in the present study, is likely due to there being a greater cellular requirement for endogenous reduced glutathione (GSH) to act as a non-enzymatic antioxidant and/or substrate for GST-mediated GSH conjugation (Regoli et al., 2011).

Contrastingly, transcript levels of *GPx-4* and GR activity were found to be negatively associated with pesticide levels (mirex, HCB, and chlordanes) in bottlenose dolphins sampled at the LES and PLE sites (Table 3). This outcome reveals the non-specificity of antioxidant defense biomarkers, as in the conflicting antioxidant results obtained in studies of different species upon exposure to POPs (Benedetti et al., 2009; Lushchak et al., 2009; Orbea and Cajaraville, 2006). Furthermore, it is important to consider the assumption of Regoli et al. (2011), who stated that antioxidant defenses frequently do not vary in their degree of synchronization, possibly due to complex interactions among antioxidant molecules or other confounding factors.

3.2.3. Biomarkers involved in immune response (IL-1α and MHCII)

The best-fitting models also pointed out that there was a positive association between levels of ΣPBDEs and *IL-1α* transcription (Table 3). In line with our observations, Brown et al. (2014) and Routti et al. (2010) found a positive correlation between hepatic *IL-1β* mRNA levels and ΣPCBs in ringed seals from the coasts of Labrador, Canada, as well as Svalbard and the Baltic Sea. Rajput et al. (2018) also found that expression of IL-1β was enhanced in fibroblast cell lines from the pantropical spotted dolphin (*Stenella attenuata*) exposed to BDE-47, BDE-100, and BDE-209. IL-1α and IL-1β are pro-inflammatory cytokines from the IL-1 family, which are involved in the stimulation of leukocyte activity and activation of lymphocytes, and thus act as major players in the immune system (Dinarello, 1988). Increased expression of inflammatory cytokines is usually observed in cases of cancer, bacterial infections, and inflammatory diseases (Dinarello, 1996; Yusa et al., 2017). The association between levels of ΣPBDEs and *IL-1α* expression found in the present study suggests that bottlenose dolphins with higher contaminant burdens are more prone to develop diseases, although such results do not necessarily support the occurrence of immunosuppression by exposure to these contaminants, as was previously found for free-ranging *T. truncatus* (Lahvis et al., 1995). It is important to mention that a high prevalence of a lacaziosis-like disease or lobomycosis-like disease (LLD) has been observed on the tegument of wild dolphins in the LES population (Van Bressem et al., 2015). However, whether this prevalence is related to environmental contamination needs to be investigated further.

Still with regard to the immune system, *MHCII* transcript levels exhibited no relationship with POPs in this study (Table 3). MHCII plays a key role in the onset of an immune response to bacteria and fungi, as it is responsible for the recognition of exogenous peptides and their presentation to T lymphocytes (Germain et al., 1996). Its expression level was previously found to be reduced in bottlenose dolphins affected by lacaziosis, suggesting that a reduced immune capacity favors the onset of such opportunistic disease (Reif et al., 2009). Once again, our results do not seem to support the hypothesis of immunosuppression due to contamination in coastal bottlenose dolphins.

In general, the best-fitting models indicated that biomarker responses to bioaccumulated ΣPBDEs and pesticides occurred in *T. truncatus* resident in estuarine systems in southern Brazil, suggesting that the contaminant levels in these dolphins are of biological relevance. For most dolphins from PLE, as well as some animals from the LES, the levels of ΣPCB exceeded thresholds for physiological effects and risks of reproductive impairment and mortality (Jepson et al., 2016; Schwacke et al., 2002). The measured values were also much higher than those reported by Brown et al. (2014) as thresholds for molecular responses in ringed seals, which were estimated to be 1680 ng lipid weight^{-1}.

While no 'risk' threshold has been established for concentrations of PBDEs in dolphins, previous studies have indicated that both contaminant classes (PCBs and PBDEs) display similar toxicological effects in murine models and fish, such as decreasing hormone production and potentially inducing carcinogenesis (Darnerud, 2003; Hallgren and Darnerud, 2002; Siddiqi et al., 2003). Furthermore, cetaceans seem to not be able to effectively metabolize specific PBDE congeners (McKinney et al., 2006), and *in vitro* assays with cetacean fibroblast cultures also suggested that PBDEs may elicit a stronger biomarker response when compared to a mixture of OCPs, even at much lower doses (Marsili et al., 2008). Such results reinforce the relevance of this contaminant class to cetacean toxicology and support our findings, suggesting that the PBDE levels measured in the sampled dolphins may be toxicologically relevant.

Hallgren and Darnerud (2002) also showed that the enzymatic responses and hormonal impacts in rats exposed to mixtures of PBDEs and PCBs were higher than those observed in animals exposed to different contaminant congeners individually. Such findings highlight the cumulative effects of contaminants, which can be attributed to their co-stimulation of similar cellular pathways, and shows that these are of particular relevance to environmental studies, where animals are exposed to myriad contaminants in the field at once.

3.3. Seasonal influence on biomarker responses

In addition to helping to improve our understanding of the effects of POPs on biomarker responses in the skin of *T. truncatus* from the LES and PLE populations, the Kruskal-Wallis test results revealed that sampling site, age class, and sex did not influence the biomarker responses examined (Electronic Supplementary Material, Table S1). In contrast, the results suggested that there was a putative influence of season on most molecular biomarkers and GST activity. For instance, the transcript levels of nearly all target genes (except *ARNT* and *MHCII*) were higher in dolphins sampled in winter in comparison to those in animals sampled in summer (the full set of results is shown in the Electronic Supplementary Material, Table S1). A similar pattern was visualized in the PCA plot (Fig. 2) and in the GLM results (Table 3), where the best-fitting models included season as a significant predictor for *AhR*, *GR*, *IL-1α*, and *MT2A* transcript levels. In all cases, winter samples exhibited higher transcript levels. However, GST activity presented the opposite pattern, with GST activity being higher in dolphins sampled in warmer months.

Interestingly, a recent study performed by Dopico et al. (2015) shed light on the role of season in the modulation of the expression of genes involved in human physiological processes, where the ambient temperature is a candidate environmental cue that can coordinate the transcription of different genes. In dolphins, shifts in water temperature are believed to significantly affect the

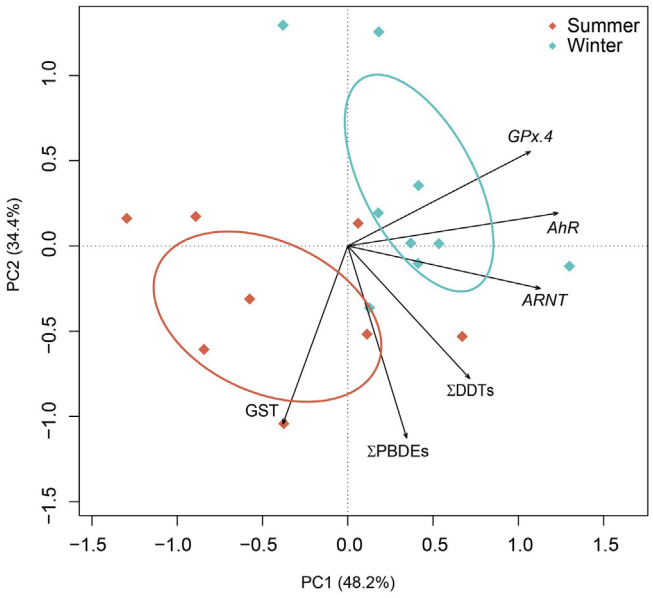

Fig. 2. Principal component analysis (PCA) of integument levels of representative molecular and biochemical biomarkers in coastal bottlenose dolphins (*Tursiops truncatus*) sampled in southern Brazil ($n = 17$). Each colored square indicates an individual dolphin. Solid lines indicate 95% confidence intervals of the biomarker values of dolphins sampled in the summer (orange) and winter (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

temperature of the epidermis, resulting in seasonal heat loss, as was previously observed at the surface of the skin in *T. truncatus* (Meagher et al., 2008). According to the authors of that study, such seasonal variation might indicate an enhancement of the metabolism during colder months. Van Dolah et al. (2015) also observed seasonal variation in biological responses in the skin of *T. truncatus*, with seasonal changes occurring in transcription rates of genes related to cell proliferation, motility, and differentiation. However, whether water temperature and/or changes in metabolism are involved in any mechanism maintaining the putative seasonal variation in biomarkers in *T. truncatus* from the LES (mean water temperature: ~23 °C in summer; ~18.5 °C in winter) and PLE (summer: ~25 °C; winter: ~15 °C) remains to be confirmed.

4. Conclusion

This study established the baseline for contaminant levels in the blubber of coastal bottlenose dolphins from the LES and PLE populations, and was the first to assess biomarker responses in this subspecies, which exhibits a restricted distribution in the western South Atlantic and is comprised of only a few discrete population units totaling no more than 300 individuals. Overall, our results point to an association between PBDEs and pesticides and biomarker responses in the skin of these free-ranging dolphins, suggesting that their levels of bioaccumulated POPs are biologically relevant. Given their high site fidelity to inshore areas, both populations are exposed to a variety of anthropogenic stressors, and long-term biomonitoring using biomarkers can be a powerful tool in determining to what extent such stressors challenge the maintenance of these populations. Considering the small sample size, we could not confirm the putative seasonal variation in biomarker responses. However, we suggest that seasonal effects should be taken into account when applying biochemical and molecular biomarkers to assess exposure to POP contaminants and its related effects in cetaceans.

Acknowledgments

The authors acknowledge the financial support of the National Council for Scientific and Technological Development (CNPq, grant number 428997/2016-3), and PAP-FAPESC (grant number 2017TR744) during the present study to K.H. Lüchmann and CAPES for Master's and PhD Scholarship to B.P.H. Righetti and C. Bezamat, respectively. R.C. Montone, P.C. Simões-Lopes and A.C.D. Bainy are recipients of CNPq productivity fellowship. This research was also partially supported by a Small Grant in Aid of Research from the Society for Marine Mammalogy, granted to B.P.H. Righetti, and Organization for the Conservation of South American Aquatic Mammals—YAQU PACHA e.V.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.02.179.

References

Abbasi, N.A., Arukwe, A., Jaspers, V.L.B., Eulaers, I., Mennilo, E., Ibor, O.R., Frantz, A., Covaci, A., Malik, R.N., 2017. Oxidative stress responses in relationship to persistent organic pollutant levels in feathers and blood of two predatory bird species from Pakistan. Sci. Total Environ. 580, 26–33. https://doi.org/10.1016/j.scitotenv.2016.11.197.

Aguilar, A., Borrell, A., Pastor, T., 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. J. Cetacean Res. Manag. 1, 83–116.

Aubail, A., Méndez-Fernandez, P., Bustamante, P., Churlaud, C., Ferreira, M., Vingada, J.V., Caurant, F., 2013. Use of skin and blubber tissues of small cetaceans to assess the trace element content of internal organs. Mar. Pollut. Bull. 76, 158–169. http://doi.org/10.1016/j.marpolbul.2013.09.008.

Bachman, M., Keller, J.M., West, K.L., Jensen, B.A., 2014. Persistent organic pollutant concentration in blubber of 16 species of cetaceans stranded in the Pacific Islands from 1997 through 2011. Sci. Total Environ. 488–489, 115–123. https://doi.org/10.1016/j.scitotenv.2014.04.073.

Barón, E., Giménez, J., Verborgh, P., Gauffier, P., De Stephanis, R., Eljarrat, E., Barceló, D., 2015. Bioaccumulation and biomagnification of classical flame retardants, related halogenated natural compounds and alternative flame retardants in three delphinids from Southern European waters. Environ. Pollut. 203, 107–115. https://doi.org/10.1016/j.envpol.2015.03.041.

Bartón, K., 2017. Multi-Model Inference.

Beedanagari, S.R., Taylor, R.T., Hankinson, O., 2010. Differential regulation of the dioxin-induced Cyp1a1 and Cyp1b1 genes in mouse hepatoma and fibroblast cell lines. Toxicol. Lett. 194, 26–33. https://doi.org/10.1016/j.toxlet.2010.01.019.

Benedetti, M., Fattorini, D., Martuccio, G., Nigro, M., Regoli, F., 2009. Interactions between trace metals (Cu, Hg, Ni, Pb) and 2,3,7,8- tetrachlorodibenzo-p-dioxin in the antarctic fish *Trematomus bernacchii*: oxidative effects on biotransformation pathway. Environ. Toxicol. Chem. 28, 818–825. https://doi.org/10.1897/08-066.1.

Bengtson Nash, S., Dawson, A., Burkhard, M., Waugh, C., Huston, W., 2014. Detoxification enzyme activities (CYP1A1 and GST) in the skin of humpback whales as a function of organochlorine burdens and migration status. Aquat. Toxicol. 155, 207–212. https://doi.org/10.1016/j.aquatox.2014.06.021.

Berrow, S.D., Mchugh, B., Glynn, D., Mcgovern, E., Parsons, K.M., Baird, R.W., Hooker, S.K., 2002. Organochlorine concentrations in resident bottlenose dolphins (*Tursiops truncatus*) in the Shannon estuary, Ireland. Mar. Pollut. Bull. 44, 1296–1303. https://doi.org/10.1016/S0025-326X(02)00215-1.

Boon, J.P., Van Der Meer, J., Allchin, C.R., Law, R.J., Klungsøyr, J., Leonards, P.E.G., Spliid, H., Storr-Hansen, E., Mckenzie, C., Wells, D.E., 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: Structural evidence for induction of cytochrome P450. Arch. Environ. Contam. Toxicol. 33, 298–311. https://doi.org/10.1007/s002449900257.

Borges Costa, A.P., Fruet, P., Daura-Jorge, F.G., Simões-Lopes, P.C., Ott, P.H., Valiati, V.H., De Oliveira, L.R., 2015. Bottlenose dolphin communities from the southern Brazilian coast: do they exchange genes or are they just neighbours? Mar. Freshw. Res. 66, 1201–1210. https://doi.org/10.1071/MF14007.

Borrell, A., Aguilar, A., Tornero, V., Sequeira, M., Fernandez, G., Al, S., 2006. Organochlorine compounds and stable isotopes indicate bottlenose dolphin subpopulation structure around the Iberian Peninsula. Environ. Int. 32, 516–523. https://doi.org/10.1016/j.envint.2005.12.001.

Borrell, A., Aguilar, A., 2007. Organochlorine concentrations declined during 1987-2002 in western Mediterranean bottlenose dolphins, a coastal top predator. Chemosphere 66, 347–352. https://doi.org/10.1016/j.chemosphere.2006.04.074.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Breivik, K., Alcock, R., Li, Y.F., Bailey, R.E., Fiedler, H., Pacyna, J.M., 2004. Primary sources of selected POPs: regional and global scale emission inventories. Environ. Pollut. 128, 3–16. https://doi.org/10.1016/j.envpol.2003.08.031.

Brown, T.M., Ross, P.S., Reimer, K.J., Veldhoen, N., Dangerfield, N.J., Fisk, A.T., Helbing, C.C., 2014. PCB related effects thresholds as derived through gene transcript profiles in locally contaminated ringed seals (*Pusa hispida*). Environ. Sci. Technol. 48, 12952–12961. https://doi.org/10.1021/es5032294.

Brown, T.M., Iverson, S.J., Fisk, A.T., Macdonald, R.W., Helbing, C.C., Reimer, K.J., 2015. Local contamination, and not feeding preferences, explains elevated PCB concentrations in Labrador ringed seals (*Pusa hispida*). Sci. Total Environ. 515–516, 188–197. https://doi.org/10.1016/j.scitotenv.2015.02.019.

Buckman, A.H., Veldhoen, N., Ellis, G., Ford, J.K.B., Helbing, C.C., Ross, P.S., 2011. PCB-associated changes in mRNA expression in killer whales (*Orcinus orca*) from the NE Pacific Ocean. Environ. Sci. Technol. 45, 10194–10202. https://doi.org/10.1021/es201541j.

Burella, P.M., Odetti, L.M., Simoniello, M.F., Poletta, G.L., 2018. Oxidative damage and antioxidant defense in *Caiman latirostris* (Broad-snouted caiman) exposed in ovo to pesticide formulations. Ecotoxicol. Environ. Saf. 161, 437–443. https://doi.org/10.1016/j.ecoenv.2018.06.006.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods Enzymol. 113, 484–490. https://doi.org/10.1002/0471684228.egp05186.

Chou, C.C., Chen, Y.N., Li, C.S., 2004. Congener-specific polychlorinated biphenyls in cetaceans from Taiwan waters. Arch. Environ. Contam. Toxicol. 47, 551–560. https://doi.org/10.1007/s00244-004-3214-y.

Combi, T., Miserochi, S., Langone, L., Guerra, R., 2016. Polychlorinated biphenyls (PCBs) in sediments from the western Adriatic Sea: sources, historical trends and inventories. Sci. Total Environ. 562, 580–587. https://doi.org/10.1016/j.scitotenv.2016.04.086.

Costa, L.G., de Laat, R., Tagliaferri, S., Pellacani, C., 2014. A mechanistic view of polybrominated diphenyl ether (PBDE) developmental neurotoxicity. Toxicol. Lett. 230, 282–294. https://doi.org/10.1016/j.toxlet.2013.11.011.

Darnerud, P.O., 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environ. Int. 29, 841–853. https://doi.org/10.1016/S0160-4120(03)00107-7.

Daura-Jorge, F.G., Ingram, S.N., Simões-Lopes, P.C., 2013. Seasonal abundance and adult survival of bottlenose dolphins (*Tursiops truncatus*) in a community that cooperatively forages with fishermen in southern Brazil. Mar. Mamm. Sci. 29, 293–311. https://doi.org/10.1111/j.1748-7692.2012.00571.x.

De Guise, S., Lagacé, A., Béland, P., 1994. Tumors in St. Lawrence beluga whales

(Delphinapterus leucas). Vet. Pathol. 31, 444–449. https://doi.org/10.1177/030098589403100406.
Dietrich, C., Kaina, B., 2010. The aryl hydrocarbon receptor (AhR) in the regulation of cell-cell contact and tumor growth. Carcinogenesis 31, 1319–1328. https://doi.org/10.1093/carcin/bgq028.
Dinarello, A., 1988. Biology of interleukin-1. FASEB J. 2, 108–115.
Dinarello, C.A., 1996. Biological basis for interleukin-1 in disease. J. Am. Soc. Hematol. 87, 2954–2956. https://doi.org/10.1136/bmj.s4-1.148.879-a.
Dopico, X.C., Evangelou, M., Ferreira, R.C., Guo, H., Pekalski, M.L., Smyth, D.J., Cooper, N., Burren, O.S., Fulford, A.J., Hennig, B.J., Prentice, A.M., Ziegler, A.G., Bonifacio, E., Wallace, C., Todd, J.A., 2015. Widespread seasonal gene expression reveals annual differences in human immunity and physiology. Nat. Commun. 6, 1–13. https://doi.org/10.1038/ncomms8000.
Ezemonye, L., Tongo, I., 2010. Sublethal effects of endosulfan and diazinon pesticides on glutathione-S-transferase (GST) in various tissues of adult amphibians (*Bufo regularis*). Chemosphere 81, 214–217. https://doi.org/10.1016/j.chemosphere.2010.06.039.
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. https://doi.org/10.1016/j.scitotenv.2009.12.021.
Flohé, L., Günzler, W.A., 1984. Assays of glutathione peroxidase. Methods Enzymol. 105, 114–120. https://doi.org/10.1016/S0076-6879(84)05015-1.
Förlin, L., Blom, S., Celander, M., Sturve, J., 1996. Effects on UDP glucuronosyl transferase, glutathione transferase, DT-diaphorase and glutathione reductase activities in rainbow trout liver after long-term exposure to PCB. Mar. Environ. Res. 42, 213–216. https://doi.org/10.1016/0141-1136(95)00061-5.
Fossi, M.C., Marsili, L., Lonzio, C., Sciara, G.N., Zanardelli, M., Silvano, F., 1992. The use of non-destructive biomarker in Mediterranean cetaceans: preliminary data on MFO activity in skin biopsy. Mar. Pollut. Bull. 24, 459–461. https://10.1016/0025-326X(92)90346-8.
Fossi, M.C., Marsili, L., 1997. The use of non destructive biomarkers in the study of marine mammals. Biomarkers 2, 205–216. https://doi.org/10.1080/135475097231571.
Fossi, M.C., Marsili, L., Neri, G., Natoli, A., Politi, E., Panigada, S., 2003. The use of a non-lethal tool for evaluating toxicological hazard of organochlorine contaminants in Mediterranean cetaceans: new data 10 years after the first paper published in MPB. Mar. Pollut. Bull. 46, 972–982. https://doi.org/10.1016/S0025-326X(03)00113-9.
Fossi, M.C., Marsili, L., Lauriano, G., Fortuna, C., Canese, S., Ancora, S., Leonzio, C., Romeo, T., Merino, R., Abad, E., Jimenez, B., 2004. Assessment of toxicological status of a SW Mediterranean segment population of striped dolphin (*Stenella coeruleoalba*) using skin biopsy. Mar. Environ. Res. 58, 269–274. https://doi.org/10.1016/j.marenvres.2004.03.070.
Fossi, M.C., Panti, C., Marsili, L., Maltese, S., Spinsanti, G., Casini, S., Caliani, I., Gaspari, S., Muñoz-Arnanz, J., Jimenez, B., Finoia, M.G., 2013. The Pelagos Sanctuary for Mediterranean marine mammals: marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (*Stenella coeruleoalba*). Mar. Pollut. Bull. 70, 64–72. https://doi.org/10.1016/j.marpolbul.2013.02.013.
Fruet, P.F., Genoves, R.C., Möller, L.M., Botta, S., Secchi, E.R., 2015. Using mark-recapture and stranding data to estimate reproductive traits in female bottlenose dolphins (*Tursiops truncatus*) of the Southwestern Atlantic Ocean. Mar. Biol. 162, 661–673. https://doi.org/10.1007/s00227-015-2613-0.
Fruet, P.F., Secchi, E.R., Di Tullio, J.C., Simões-Lopes, P.C., Daura-Jorge, F., Costa, A.P.B., Vermeulen, E., Flores, P.A.C., Genoves, R.C., Laporta, P., Beheregaray, L.B., Möller, L.M., 2017. Genetic divergence between two phenotypically distinct bottlenose dolphin ecotypes suggests separate evolutionary trajectories. Ecol. Evol. 1–13. https://doi.org/10.1002/ece3.3335.
Genov, T., Jepson, P.D., Barber, J.L., Hace, A., Gaspari, S., Centrih, T., Lesjak, J., Kotnjek, P., 2019. Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea. Sci. Total Environ. 657, 200–212. https://doi.org/10.1016/j.scitotenv.2018.12.025.
Germain, R.N., Castellino, F., Han, R., Sousa, C.R., Romagnoli, P., Sadegh-Nasseri, S., Zhunc, G.-M., 1996. Processing and presentation of endocytically acquired protein antigens by MHC class II and class I molecules. Immunol. Rev. 151, 5–30. https://doi.org/10.1111/j.1600-065X.1996.tb00701.x.
Girolami, F., Spalenza, V., Manzini, L., Carletti, M., Nebbia, C., 2015. Constitutive expression of the AHR signaling pathway in a bovine mammary epithelial cell line and modulation by dioxin-like PCB and other AHR ligands. Toxicol. Lett. 232, 98–105. https://doi.org/10.1016/j.toxlet.2014.09.013.
Gonzalvo, J., Lauriano, G., Hammond, P.S., Viaud-Martinez, K.A., Fossi, M.C., Natoli, A., Marsili, L., 2016. The gulf of Abracia's common bottlenose dolphins, *Tursiops truncatus*: a highly dense and yet threatened population. Adv. Mar. Biol. 75, 259–296. https://doi.org/10.1016/bs.amb.2016.07.002.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 7130–7140.
Hallgren, S., Darnerud, P.O., 2002. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats—testing interactions and mechanisms for thyroid hormone effects. Toxicology 177, 227–243. https://doi.org/10.1016/S0300-483X(02)00222-6.
Helle, E., Olsson, M., Jensen, S., 1976. PCB levels correlated with pathological changes in seal uteri. Ambio 5, 261–262.
Hooker, S.K., Metcalfe, T.L., Metcalfe, C.D., Angell, C.M., Wilson, J.Y., Moore, M.J., Whitehead, H., 2008. Changes in persistent contaminant concentration and CYP1A1 protein expression in biopsy samples from northern bottlenose whales, *Hyperoodon ampullatus*, following the onset of nearby oil and gas development. Environ. Pollut. 152, 205–216. https://doi.org/10.1016/j.envpol.2007.05.027.
Hooper, K., McDonald, T.A., 2000. The PBDEs: an emerging environmental challenge and another reason for breast-milk monitoring programs. Environ. Health Perspect. 108, 387–392. https://doi.org/10.1289/ehp.00108387.
Jauniaux, T., Farnir, F., Fontaine, M., Kiszka, J., Sarlet, M., 2011. Cytochrome P450 1A1 expression in cetacean skin biopsies from the Indian Ocean. Mar. Pollut. Bull. 62, 1317–1319. https://doi.org/10.1016/j.marpolbul.2011.04.005.
Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, A., Borrell, A., Murphy, S., Barry, J., Bronwlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Gimenez, J., Loveridge, J., Llavona, A., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., Stephanis, R., Tregenza, N., Verbogh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations or orcas and other dolphins in European waters. Sci. Rep. 6, 1–17. https://doi.org/10.1038/srep18573.
Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum Ecol. Risk Assess. 6, 181–201. https://doi.org/10.1080/10807030091124491.
Kanerva, M., Routti, H., Tamuz, Y., Nyman, M., Nikinmaa, M., 2012. Antioxidative defense and oxidative stress in ringed seals (*Pusa hispida*) from differently polluted areas. Aquat. Toxicol. 114–115, 67–72. https://doi.org/10.1016/j.aquatox.2012.02.013.
Kelly, B.C., 2007. Food web – specific biomagnification of persistent organic pollutants. Science 317 (80), 236–239. https://doi.org/10.1126/science.1138275.
Kershaw, J.L., Botting, C.H., Brownlow, A., Hall, A.J., 2018. Not just fat: investigating the proteome of cetacean blubber tissue. Conserv. Physiol. 6 (1) coy003. https://doi:10.1093/conphys/coy003.
Lahvis, G.P., Wells, R.S., Kuehl, D.W., Jennifer, L., Rhinehart, H.L., Via, C.S., 1995. Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environ. Health Perspect. 103, 67–72. https://doi:10.1289/ehp.95103s467.
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. https://doi.org/10.1016/j.scitotenv.2012.06.030.
Lushchak, O.V., Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I., 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76, 932–937. https://doi.org/10.1016/j.chemosphere.2009.04.045.
Marsili, L., Casini, S., Bucalossi, D., Porcelloni, S., Maltese, S., Fossi, M.C., 2008. Use of immunofluorescence technique in cultured fibroblasts from Mediterranean cetaceans as new "in vitro" tool to investigate effects of environmental contaminants. Mar. Environ. Res. 66, 151–153. https://doi.org/10.1016/j.marenvres.2008.02.068.
Marsili, L., Jiménez, B., Borrell, A., 2018. Persistent organic pollutants in cetaceans living in a hotspot area: the Mediterranean Sea. Mar. Mamm. Ecotoxicol. 185–212. https://doi.org/10.1016/B978-0-12-812144-3.00007-3.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.
McKinney, M.A., Arukwe, A., De Guise, S., Martineau, D., Béland, P., Dallaire, A., Lair, S., Lebeuf, M., Letcher, R.J., 2004. Characterization and profiling of hepatic cytochromes P450 and phase II xenobiotic-metabolizing enzymes in beluga whales (*Delphinapterus leucas*) from the St. Lawrence River Estuary and the Canadian Arctic. Aquat. Toxicol. 69, 35–49. https://doi.org/10.1016/j.aquatox.2004.04.010.
McKinney, M.A., De Guise, S., Martineau, D., Béland, P., Arukwe, A., Letcher, R.J., 2006. Biotransformation of polybrominated diphenyl ethers and polychlorinated biphenyls in beluga whale (*Delphinapterus leucas*) and rat mammalian model using an in vitro hepatic microsomal assay. Aquat. Toxicol. 77, 87–97. https://doi.org/10.1016/j.aquatox.2005.08.016.
Meagher, E.M., McLellan, W.A., Westgate, A.J., Wells, R.S., Blum, J.E., Pabst, D.A., 2008. Seasonal patterns of heat loss in wild bottlenose dolphins (*Tursiops truncatus*). J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 178, 529–543. https://doi.org/10.1007/s00360-007-0245-5.
Méndez-Fernandez, P., Galluzzi Polesi, P., Taniguchi, S., Marcos, M.C., Montone, R.C., 2016. Validating the use of biopsy sampling in contamination assessment studies of small cetaceans. Mar. Pollut. Bull. 107, 364–369. https://doi.org/10.1016/j.marpolbul.2016.04.021.
Nebert, D.W., Petersen, D.D., Fornace Jr., A.J., 1990. Cellular response to oxidative stress: the [Ah] gene battery as a paradigm. Environ. Health Perspect. 88, 13–25. https://doi.org/10.1289/ehp.908813.
O'Shea, T.J., 1999. Environmental contaminants and marine mammals. Biol. Mar. Mamm. 484–536.
Orbea, A., Cajaraville, M.P., 2006. Peroxisome proliferation and antioxidant enzymes in transplanted mussels of four Basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. Environ. Toxicol. Chem. 25, 1616–1626. https://doi.org/10.1897/04-520R2.1.
Peterson, D., Hanazaki, N., Simões-Lopes, P.C., 2008. Natural resource appropriation in cooperative artisanal fishing between fishermen and dolphins (*Tursiops truncatus*) in Laguna, Brazil. Ocean Coast. Manag. 51, 469–475. https://doi.org/

10.1016/j.ocecoaman.2008.04.003.
Rajput, I.R., Xiao, Z., Yajing, S., Yaqoob, S., Sanganyado, E., Ying, H., Fei, Y., Liu, W., 2018. Establishment of pantropic spotted dolphin (*Stenella attenuata*) fibroblast cell line and potential influence of polybrominated diphenyl ethers (PBDEs) on cytokines response. Aquat. Toxicol. 203, 1–9. https://doi.org/10.1016/j.aquatox.2018.07.017.
Regoli, F., Benedetti, M., Giuliani, M.E., 2011. Antioxidant defenses and acquisition of tolerance to chemical stress. In: Tolerance to Environmental Contaminants, pp. 153–173. https://doi.org/10.1201/b10519-9.
Reif, J.S., Peden-Adams, M.M., Romano, T.A., Rice, C.D., Fair, P.A., Bossart, G.D., 2009. Immune dysfunction in Atlantic bottlenose dolphins (*Tursiops truncatus*) with lobomycosis. Med. Mycol. 47, 125–135. https://doi.org/10.1080/13693780802178493.
Rodrigues, F.L., Vieira, J.P., 2013. Surf zone fish abundance and diversity at two sandy beaches separated by long rocky jetties. J. Mar. Biol. Assoc. U. K. 93, 867–875. https://doi.org/10.1017/S0025315412001531.
Rodriguez-Iruretagoiena, A., Fdez-Ortiz de Vallejuelo, S., Gredilla, A., Ramos, C.G., Oliveira, M.L.S., Arana, G., de Diego, A., Madariaga, J.M., Silva, L.F.O., 2015. Fate of hazardous elements in agricultural soils surrounding a coal power plant complex from Santa Catarina (Brazil). Sci. Total Environ. 508, 374–382. https://doi.org/10.1016/j.scitotenv.2014.12.015.
Rosel, P.E., 2003. PCR-based sex determination in Odontocete cetaceans. Conserv. Genet. 4, 647–649.
Routti, H., Arukwe, A., Jenssen, B.M., Letcher, R.J., Nyman, M., Bäckman, C., Gabrielsen, G.W., 2010. Comparative endocrine disruptive effects of contaminants in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 152, 306–312. https://doi.org/10.1016/j.cbpc.2010.05.006.
Santos, I.R., Niencheski, F., Burnett, W., Peterson, R., Chanton, J., Andrade, C.F.F., Milani, I.B., Schmidt, A., Knoeller, K., 2008. Tracing anthropogenically driven groundwater discharge into a coastal lagoon from southern Brazil. J. Hydrol. 353, 275–293. https://doi.org/10.1016/j.jhydrol.2008.02.010.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3, 1101–1108. https://doi.org/10.1038/nprot.2008.73.
Schwacke, L.H., Voit, E.O., Hansen, L.J., Wells, R.S., Mitchum, G.B., Hohn, A., Fair, P., 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. https://doi.org/10.1002/etc.5620211232.
Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn, 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. https://doi.org/10.1098/rspb.2011.0665.
Siddiqi, M.A., Laessig, R.H., Reed, K.D., 2003. Polybrominated diphenyl ethers (PBDEs): new pollutants – old diseases. Clin. Med. Res. 1, 281–290. https://doi.org/10.3121/cmr.1.4.281.
Simões-Lopes, P.C., Fabián, M.E., Menegheti, J.O., 1998. Dolphin interactions with the mullet artisanal fishing on Southern Brazil: a qualitative and quantitative approach. Rev. Bras. Zool. 15, 709–726. https://doi.org/10.1590/S0101-81751998000300016.
Shi, Y., Zhang, Q., Huang, D., Zheng, X., Shi, Y., 2016. Survival, growth, detoxifying and antioxidative responses of earthworms (*Eisenia fetida*) exposed to soils with industrial DDT contamination. Pestic. Biochem. Physiol. 128, 22–29. https://doi.org/10.1016/j.pestbp.2015.10.009.
Society for Marine Mammalogy Committee on Taxonomy, 2017. List of marine mammal species and subspecies. Society for Marine Mammalogy. Available at. www.marinemammalscience.org. Consulted on 10 October 2017.
Tanabe, S., Watanabe, S., Kan, H., Tatsukawa, R., 1988. Capacity and mode of PCB metabolism in small cetaceans. Mar. Mamm. Sci. 4, 103–124. https://doi.org/10.1111/j.1748-7692.1988.tb00191.x.
Tanabe, S., Subramanian, A., Ramesh, A., Kumaran, P.L., Miyazaki, N., Tatsukawa, R., 1993. Persistent organochlorine residues in dolphins from the bay of Bengal, South India. Mar. Pollut. Bull. 26, 311–316. https://doi.org/10.1016/0025-326x(93)90573-3.
Tanabe, S., 2002. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar. Pollut. Bull. 45, 69–77. https://doi.org/10.1016/S0025-326X(02)00175-3.
Tornero, V., Borrell, A., Aguilar, A., Forcada, J., Lockyer, C., 2006. Organochlorine contaminant and retinoid levels in blubber of common dolphins (*Delphinus delphis*) off northwestern Spain. Environ. Pollut. 140, 312–321. https://doi.org/10.1016/j.envpol.2005.07.006.
Trego, M.L., Hoh, E., Kellar, N.M., Meszaros, S., Robbins, M.N., Dodder, N.G., Whitehead, A., Lewison, R.L., 2018. Comprehensive Screening links halogenated organic compounds with testosterone levels in male *Delphinus delphis* from the southern California bight. Environ. Sci. Technol. 52, 3101–3109. https://doi.org/10.1021/acs.est.7b04652.
Twaroski, T.P., O'Brien, M.L., Robertson, L.W., 2001. Effects of selected polychlorinated biphenyl (PCB) congeners on hepatic glutathione, glutathione-related enzymes, and selenium status: implications for oxidative stress. Biochem. Pharmacol. 62, 273–281. https://doi.org/10.1016/S0006-2952(01)00668-2.
Van Bressem, M.F., Simões-Lopes, P.C., Félix, F., Kiszka, J.J., Daura-Jorge, F.G., Avila, I.C., Secchi, E.R., Flach, L., Fruet, P.F., Du Toit, K., Ott, P.H., Elwen, S., Di Giacomo, A.B., Wagner, J., Banks, A., Van Waerebeek, K., 2015. Epidemiology of lobomycosis-like disease in bottlenose dolphins *Tursiops* spp. from South America and southern Africa. Dis. Aquat. Org. 117, 59–75. https://doi.org/10.3354/dao02932.
Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.
Van Dolah, F.M., Neely, M.G., McGeorge, L.E., Balmer, B.C., Ylitalo, G.M., Zolman, E.S., Speakman, T., Sinclair, C., Kellar, N.M., Rosel, P.E., Mullin, K.D., Schwacke, L.H., 2015. Seasonal variation in the skin transcriptome of common bottlenose dolphins (*Tursiops truncatus*) from the northern Gulf of Mexico. PLoS One 10, 1–21. https://doi.org/10.1371/journal.pone.0130934.
Vasseur, P., Cossu-Leguille, C., 2006. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 62, 1033–1042. https://doi.org/10.1016/j.chemosphere.2005.05.043.
Vetter, W., Scholz, E., Gaus, C., Muller, J.F., Haynes, D., 2001. Anthropogenic and natural organohalogen compounds in blubber of dolphins and dugongs (*Gugong dugong*) from northeastern Australia. Arch. Environ. Contam. Toxicol. 41, 221–231. https://doi.org/10.1007/s002440010241.
Vieira, D.C., Noldin, J.A., Deschamps, F.C., Resgalla, C., 2016. Ecological risk analysis of pesticides used on irrigated rice crops in southern Brazil. Chemosphere 162, 48–54. https://doi.org/10.1016/j.chemosphere.2016.07.046.
Waugh, C.A., Huston, W.M., Noad, M.J., Bengston Nash, S., 2011. Cytochrome P450 isozyme protein verified in the skin of southern hemisphere humpback whales (*Megaptera novaeangliae*): implications for biochemical biomarker assessment. Mar. Pollut. Bull. 62, 758–761. https://doi.org/10.1016/j.marpolbul.2011.01.007.
Winn, H.E., Bischoff, W.L., Taruski, A.G., 1973. Cytological sexing of cetacea. Mar. Biol. 23, 343–346. https://doi.org/10.1007/BF00389342.
Wilson, R.M., Kucklick, J.R., Balmer, B.C., Wells, R.S., Chanton, J.P., Nowacek, D.P., 2012. Spatial distribution of bottlenose dolphins (*Tursiops truncatus*) inferred from stable isotopes and priority organic pollutants. Sci. Total Environ. 425, 223–230. https://doi.org/10.1016/j.scitotenv.2012.02.030.
Würsig, B., Jefferson, T., 1990. Methods of photo-identification for small cetaceans. Rep. Int. Whal. Comm. Special Issue 12, 43–52.
Yogui, G.T., Santos, M.C.O., Bertozzi, C.P., Montone, R.C., 2010. Levels of persistent organic pollutants and residual pattern of DDTs in small cetaceans from the coast of São Paulo, Brazil. Mar. Pollut. Bull. 60, 1862–1867. https://doi.org/10.1016/j.marpolbul.2010.07.022.
Yogui, G.T., Santos, M.C.O., Bertozzi, C.P., Sericano, J.L., Montone, R.C., 2011. PBDEs in the blubber of marine mammals from coastal areas of São Paulo, Brazil, southwestern Atlantic. Mar. Pollut. Bull. 62, 2666–2670. https://doi.org/10.1016/j.marpolbul.2011.09.024.
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. https://doi.org/10.1016/j.scitotenv.2010.01.032.
Yusa, T., Tateda, K., Ohara, A., Miyazaki, S., 2017. New possible biomarkers for diagnosis of infections and diagnostic distinction between bacterial and viral infections in children. J. Infect. Chemother. 23, 96–100. https://doi.org/10.1016/j.jiac.2016.11.002.
Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Analysing Ecological Data. Springer. https://doi.org/10.1016/S1573-4463(99)03094-1.
Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer-Verlag, New York.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.