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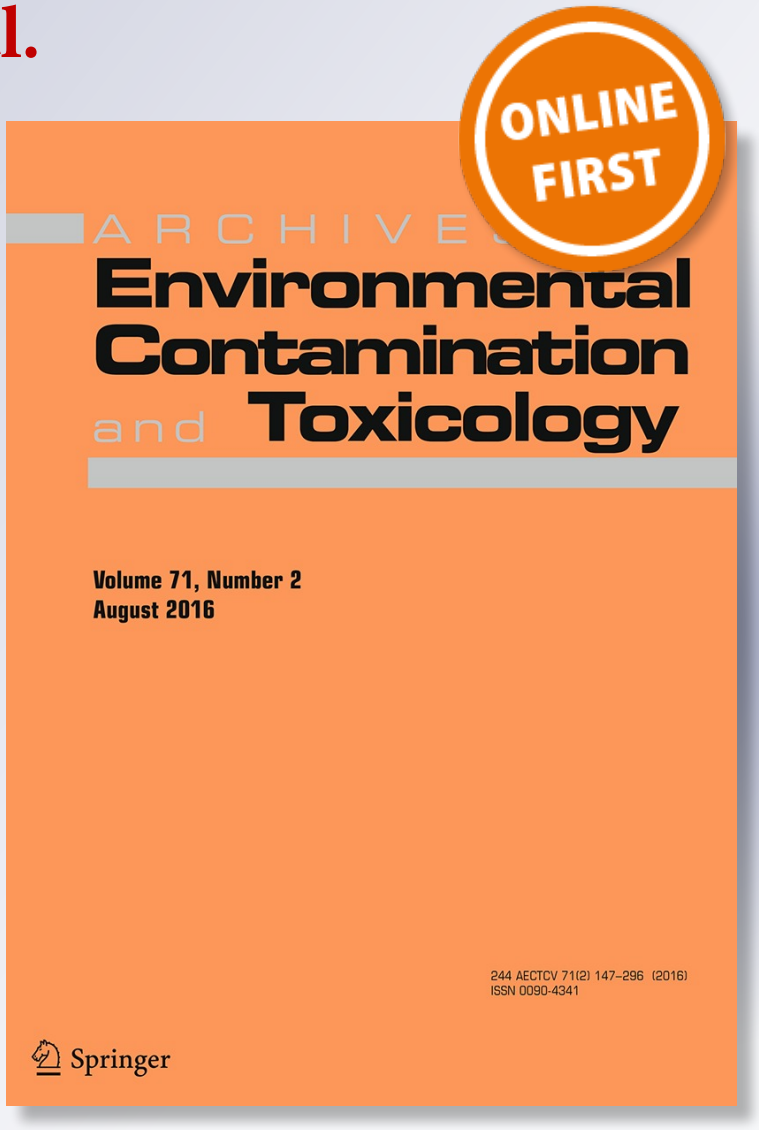

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Guiana Dolphins (*Sotalia guianensis*) and DR-CALUX for Screening Coastal Brazilian Environments for Dioxins and Related Compounds

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Abstract Guiana dolphin is the top predator of highest toxicological concern in Brazil and many studies on levels of persistent, bioaccumulative, and toxicant (PBT) pollutants have been performed on the species. However, due to high costs of the analyses, only one investigation comprised the determination of dioxins and related compounds (DRCs) in Guiana dolphin tissues. The dioxin responsive-chemically activated luciferase gene expression (DR-CALUX®) cell bioassay was used in the present study for the analyses of hepatic samples from 28 male Guiana dolphins in order to screen estuarine environments for DRCs, comprising three regions (Northeastern, Southeastern, and Southern) and four states [Paraná (PR), Rio de Janeiro (RJ), Espírito Santo (ES), and Ceará (CE)] of Brazil. High bioanalytical equivalent (BEQ) concentrations [dioxins (pg BEQ/g lipid)] were found, varying from 1.94 to 15.6 pg BEQ/g. A significant negative correlation between BEQ concentrations and total length was found in Guiana dolphins from Brazil (all analysed dolphins). This pattern also was verified for RJ state, pointing to (1) chemically induced developmental disruption or to (2) increasing efficiency of the detoxifying activity with the growth of the animal. Comparison was performed with literature data and significantly higher BEQ levels were found in Brazilian Guiana dolphins than in those reported for North Sea harbour porpoises. Higher levels were found in Southeastern (the most PBT-contaminated area of the country) than in Southern region. However, it is not possible to affirm that Guiana dolphins are more contaminated by DRCs in SE than in S region, because individuals were lengthier in S than in SE region. Our results seem to have mirrored dolphin exposure to PCBs in Brazil according to the literature. Further studies are required for investigating the hypotheses 1 and 2 mentioned above.

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The hotspots of chemical pollution along the Brazilian coast are related to the heterogeneity in the distribution of urban centres and industries, as well as to peculiarities of land use at the country (Alonso et al. 2012a; Bisi et al. 2012; Dorneles et al. 2007, 2008a, b, 2010, 2013; Lailson-Brito et al. 2010, 2011 Santos-Neto et al. 2014). Brazil is a newly industrialized nation, with few governmental restriction laws over production and use of endocrine disrupting chemicals (EDCs) (Almeida et al. 2007; Rodrigues et al. 2015). Marine environments worldwide have been receiving substantial inputs of metals and organohalogen compounds (OHCs), which turns coastal environments of

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urbanized and industrialized areas into contaminated bodies of water (Sun et al. 2012). Some Brazilian estuaries are among the most OHC-contaminated marine environments of the globe (Bícego et al. 2006; Souza et al. 2008). In this context, it is worth mentioning the case of the Guanabara Bay, an estuary regarded as the most dramatic example of manmade degradation along the Brazilian coast (Amador 2013; Azevedo et al. 2009; Bittencourt et al. 2014; Carreira et al. 2004).

Due to some features that include chemical stability and affinity for proteins or lipids, some PBTs are efficiently bioaccumulated and end up undergoing biomagnification, i.e., an amplification in chemical concentration at each trophic level in the food web (Connell 1989; Gobas et al. 2009; Gobas and Morrison 2000; Gray 2002). Therefore, high trophic level nektonic organisms are critical groups and may present high concentrations of PBT substances (Azevedo-Silva et al. 2009; Das et al. 2003; O'Shea and Brownell Jr 1994; Reijnders et al. 2009). In Brazil, the predator species of greatest ecotoxicological concern is the Guiana dolphin, *Sotalia guianensis* (Dorneles et al. 2013; Lailson-Brito et al. 2010), because this small marine mammal inhabits shallow waters and often is found year-round in bays and estuaries. The Guiana dolphin can be used as a sentinel of the health of the marine and estuarine environment because of the resident behaviour and habitat fidelity patterns described for this species along its distributional range in the region (Azevedo et al. 2009; Meirelles 2013).

Some studies have evaluated the exposure of Guiana dolphins to toxic trace elements (Dorneles et al. 2007, 2008b; Kunito et al. 2004; Lailson-Brito et al. 2012a; Seixas et al. 2009) and POPs (Dorneles et al. 2008a, 2013; Kajiwara et al. 2004; Lailson-Brito et al. 2010; Quinete et al. 2009, 2011 Santos-Neto et al. 2014; Yogui et al. 2003, 2010, 2011) in Brazilian environments. However, knowledge on dioxins and related compounds (DRCs), such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), as well as on non-ortho and mono-ortho polychlorinated biphenyls (dioxin-like PCBs, DL-PCBs) is still scarce in the country and only one investigation has comprised DRC measurements in Guiana dolphins (Dorneles et al. 2013). The reason for the paucity of data on DRCs is partially related to the high cost of the analyses. In this context, it is worth mentioning the existence of a less expensive approach for evaluating the exposure of organisms to DRCs. This cost-effective technique relies on the use of the chemically activated luciferase gene expression (CALUX) cell bioassay, more specifically, the dioxin responsive-CALUX (DR-CALUX®). The DR-CALUX® is an increasingly used bioanalytical tool for the screening and relative quantification of DRCs, such as PCDDs, PCDFs, and DL-PCBs (Scippo et al. 2004).

The purpose of this work was to evaluate the DRC contamination of coastal marine environments under the influence of highly urbanized and industrialized areas of Brazil through analyses of samples from a resident and top predator species. To achieve this goal, we have employed the DR-CALUX® to screen samples from Guiana dolphins for DRCs using a quantification approach relative to the tetrachlorodibenzodioxin (TCDD) response, allowing the estimation of bioanalytical equivalents (BEQ).

Materials and Methods

Sampling

Only samples from adult male dolphins were analysed in the present study. The rationale for this choice is given to the fact that transplacental and lactation transfer of organohalogen compounds (OHCs) constitute elimination and assimilation routes for these toxicants in female and young mammals, respectively (O'Shea and Tanabe 2003). Hence, female marine mammals frequently present oscillations in OHC concentrations in accordance to the reproduction history/status (Thron et al. 2004). Similarly, the pollutant concentrations of young individuals result from a higher or lower influence of the pollutant burden transferred from the mother depending on the age of the calf (Alonso et al. 2012b). In addition, metabolizing rates for xenobiotics may vary according to gender (Hall 2002). Therefore, samples from females and immature individuals were not analysed to avoid additional confounding factors.

Liver samples were obtained by different marine mammal research groups from four Brazilian states, including the Northeastern [Ceará (CE) state], the Southeastern [Espírito Santo (ES) and Rio de Janeiro (RJ) states], and the Southern [Paraná (PR) state] regions of the country (Fig. 1; Table 1). They were collected through the necropsy of 28 (i.e., 5 individuals from CE, 8 from ES, 7 from RJ, and 8 from PR) male Guiana dolphins that had been incidentally captured in fishing operations or found stranded dead on the beaches. The carcasses were classified as early decomposition stage (Geraci et al. 2009). After dissection, liver samples were stored in individual aluminium foil and kept frozen (−20 °C) until being dried at 50–55 °C (72 h) for the analyses.

Analytical Procedures

The DR-CALUX® was developed by Wageningen University (Aarts et al. 1993) and is distributed by BioDetection System (BDS, NL). This assay involves the

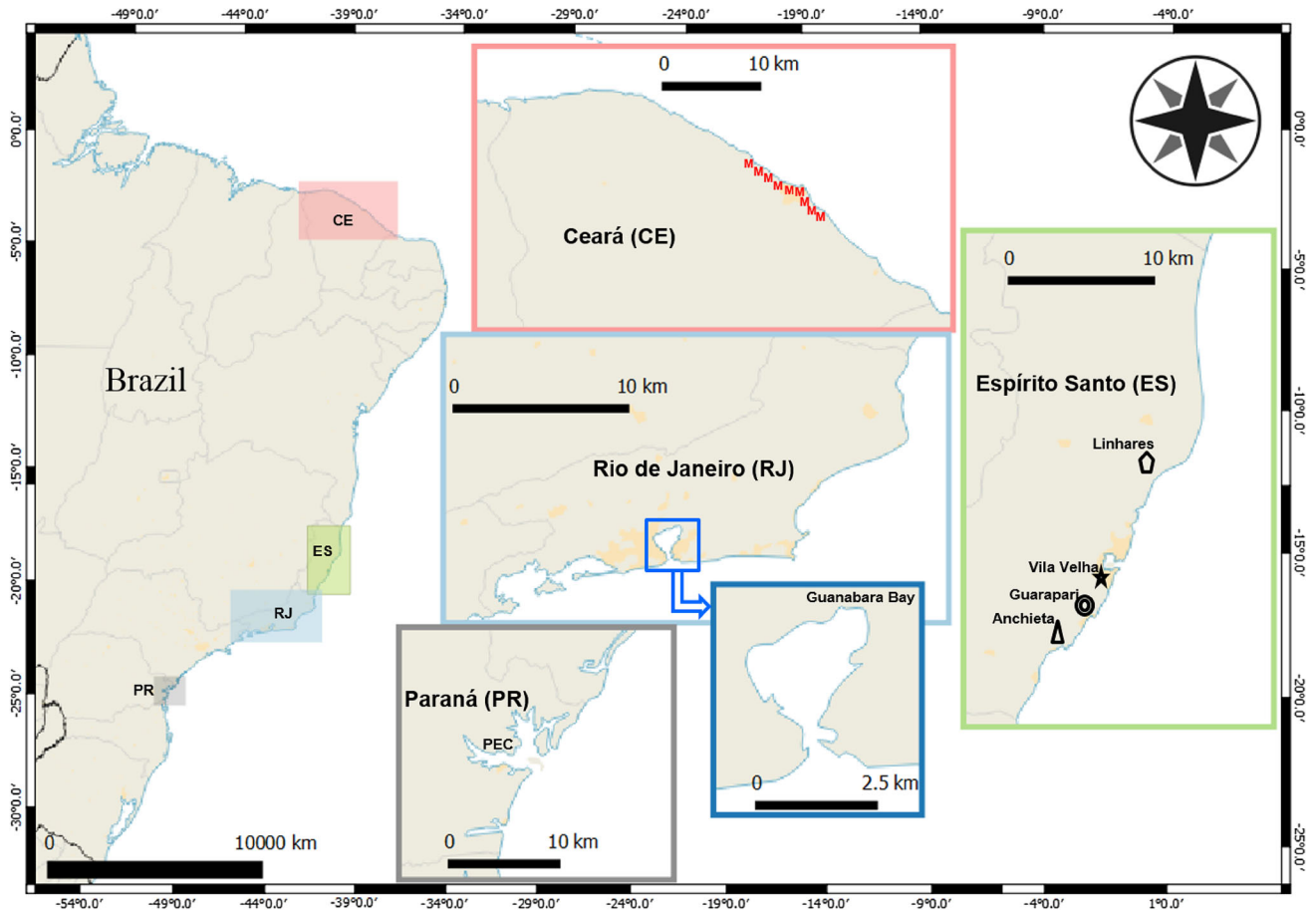

Fig. 1 Brazilian map amplifying the littoral of Paraná (PR), Rio de Janeiro (RJ), Espírito Santo (ES) and Ceará (CE) states. The metropolitan (M) area of the CE state and the ES state regions of Linhares (pentagon), Vila Velha (*star*), Guarapari (*circle*) and Anchieta (*triangle*) are indicated in the respective amplified maps, as well as the Paranaguá Estuarine Complex (PEC), in PR state. Guanabara Bay, in RJ state, is additionally amplified

rat hepatoma H4IIE cell line stably transformed with an AhR-controlled luciferase reporter gene construct. All of the steps of the procedure were performed as described by the manufacturer (details in: Scippo et al. 2004, 2006). Lipids were extracted by solid–liquid extraction with n-hexane/diethyl ether (97:3, v/v) (Merck, Darmstadt, Germany). A clean-up was performed on acidic silica columns; dioxins and similar compounds were eluted with n-hexane/diethyl ether (97:3, v/v). The cleaned extract containing dioxin-like compounds was evaporated to 50 µL, under a gentle stream of nitrogen. Before completing evaporation of the solvent, DMSO (Across organics) was added and the remaining solvent was evaporated. DR-CALUX® analysis was performed by exposing the cells (in triplicate, in 96-well plates) during 24 h to sample extracts or to standard TCDD solutions in DMSO diluted in culture medium (α-MEM, Invitrogen) containing 10 % (v/v) of foetal calf serum (FCS, Invitrogen). The final concentration of DMSO in culture medium was 0.4 % (v/v). Following cell lysis and substrate addition [buffer containing 1 % luciferin (Promega) and 0.5 mM ATP (Roche Diagnostics Belgium)], luminescence was measured using a luminometer Orion II (Berthold Detection System, Germany). DR-CALUX® concentrations were calculated from a standard calibration curve, ranging from 0 (blank DMS0) to 20 pg TCDD per well, and established in triplicate on each 96-well plates. Procedure blank (same procedure without any sample) and spiked beef fat (with 2 pg BEQ/g lipid of PCDD/Fs and DL-PCBs) were used as negative and positive controls respectively.

Dose response curves were fitted using a user-defined curve fit (Slide Write Plus v. 6.1, Advanced Graphics Software, USA), following:

$$y = a0\,[1 + (x/a1)a2]$$

where y is the measured response (the luminescence expressed in RLU for Relative Light Unit), x is the concentration of the test compound, $a0$ is the maximal

Table 1 Hepatic bioanalytical equivalent [dioxins (pg BEQ/g lipid)] concentrations, along with sample identity, area of origin within the state, death date and body length of male Guiana dolphins (*Sotalia guianensis*) from Northeastern - NE [Ceará (CE) state], Southeastern-SE [Espírito Santo (ES) and Rio de Janeiro (RJ) states], and Southern-S [Paraná (PR) state] regions

Sample identity	Brazilian region	Brazilian state	Area within the state	Death date (month/year)	Body length	BEQ conc. (pg BEQ/g lipid)
SgCE1	NE	CE	E from M	February 2007	177	3.05
SgCE2	NE	CE	W from M	July 2004	153	8.68
SgCE3	NE	CE	M	November 2007	173	14.2
SgES1	SE	ES	Linhares	January 2006	169	11.4
SgES2	SE	ES	Linhares	January 2006	169	11.2
SgES3	SE	ES	Guarapari	March 2006	178	14.8
SgES4	SE	ES	Vila Velha	January 2007	185	8.45
SgES5	SE	ES	Anchieta	August 2009	181	4.54
SgRJ1	SE	RJ	GB	May 2000	183	1.94
SgRJ2	SE	RJ	GB	September 2000	191	5.86
SgRJ3	SE	RJ	GB	July 2005	183	3.75
SgRJ4	SE	RJ	GB	July 2007	170	9.56
SgRJ5	SE	RJ	GB	July 2009	161	14.7
SgRJ6	SE	RJ	GB	October 2010	159	15.6
SgPR1	S	PR	PEC	October 2008	196	6.88
SgPR2	S	PR	PEC	October 2008	190	6.65
SgPR3	S	PR	PEC	November 2008	198	4.51
SgPR4	S	PR	PEC	January 2009	179	3.85
SgPR5	S	PR	PEC	February 2009	201	2.71
SgPR6	S	PR	PEC	August 2009	193	2.56
SgPR7	S	PR	PEC	August 2009	183	3.50
SgPR8	S	PR	PEC	November 2009	194	3.94

PEC, GB and M stand for Paranaguá Estuarine Complex, Guanabara Bay and Metropolitan, respectively

response, $a1$ is the EC50 (EC: Effective Concentration; EC50: concentration needed to reach 50 % of the maximal response), $a2$ is the slope of the curve.

All reported results met the quality criteria established by BDS: for the TCDD calibration curve, the maximum induction factor was at least sixfold, the EC50 value was in the range 0.4–2.4 pg TCDD per well, $R2$ of the fitted curve was >0.98, and the relative standard deviation of the mean of the RLU measured in triplicate was <15 %. For samples, the sample response was below the response corresponding to the TCDD EC50.

Statistical Treatment

First, the suitability of using parametric or non-parametric tests was evaluated. More specifically, Shapiro–Wilk's W test was used in order to test for normality of the data, whereas the Brown–Forsythe test was used for homogeneity of variance. The tests were applied to different data sets, i.e., bioanalytical equivalent (BEQ) concentrations or total length values of dolphins from different areas. Depending on the results of these tests, parametric [ANOVA, Student's t test, Pearson's (r) correlation test] or nonparametric [Kruskal–Wallis test, Mann–Whitney U test, Spearman's (Rs) correlation test] were applied. The variables generated for the investigation of possible year-to-year variations in ES, RJ, and PR states are exposed on Table 2. The level of significance was set at $p \leq 0.05$, and levels of significance at $0.05 < p \leq 0.10$ were considered a trend. The statistical software systems STATISTICA 7.0 and BioEstat 5.3 were used for statistical analyses.

Results and Discussion

Extracts of samples from six dolphins, two from CE, three from ES and one from RJ state, exhibited toxicity to the rat hepatoma H4IIE cell line. Therefore, it was not possible to obtain results from these six samples. Bioanalytical equivalent (BEQ) concentrations [dioxins (pg BEQ/g lipid)], in liver of Guiana dolphins from Northeastern (CE

Table 2 Death dates and the variables generated from this information, i.e., day, month and trimester of stranding of each Guiana dolphin (indicated by each individual code-Id Code) from the Brazilian states of Espírito Santo, Rio de Janeiro, and Paraná

Brazilian state	Id code	Death date	Day	Month	Trimester
Espírito Santo	SgES1	10-Jan-06	1	1	1
	SgES2	16-Jan-06	7	1	1
	SgES3	01-Mar-06	51	3	1
	SgES4	22-Jan-07	378	13	5
	SgES5	05-Aug-09	1304	44	15
Rio de Janeiro	SgRJ1	12-May-00	1	1	1
	SgRJ2	21-Sep-00	132	5	2
	SgRJ3	16-Jul-05	1892	63	21
	SgRJ4	06-Jul-07	2612	87	29
	SgRJ5	30-Jul-09	3367	111	37
	SgRJ6	17-Oct-09	3446	114	38
Paraná	SgPR1	29-Oct-08	1	1	1
	SgPR2	03-Nov-08	6	2	1
	SgPR3	11-Nov-08	14	2	1
	SgPR4	17-Jan-09	81	4	2
	SgPR5	18-Feb-09	113	5	2
	SgPR6	19-Aug-09	295	11	4
	SgPR7	19-Aug-09	295	11	4
	SgPR8	27-Nov-09	395	14	5

state), Southeastern (ES and RJ states) and Southern (PR state) Brazilian regions, are presented in Table 1.

Because there is only one published study comprising PCDD/F measurements in dolphins from Brazil (Dorneles et al. 2013), the comparison between data generated in the present study and PCDD/F concentrations from the literature is not possible. The alternative would be to use PCBs for this type of comparison, as there is published information on PCB concentrations of dolphins from the four Brazilian states comprised in the present study (Dorneles et al. 2013; Lailson-Brito et al. 2010; Santos-Neto et al. 2014). Using the blubber (adipose tissue) PCB concentrations of mature male dolphins from the quoted studies, the median values of 4043 and 28,992 ng/g lipid were obtained for PR and RJ states (Lailson-Brito et al. 2010), as well as the median values of 7320 (M region), 3370 (W from M), and 850 (E from M) ng/g lipid were obtained for the three CE state regions (Santos-Neto et al. 2014) presented in Table 1. However, the PCB levels of dolphins from the ES state constitute a good example of the paucity of data on POP levels along the Brazilian coast. To the authors' knowledge, there is no published information on indicator PCBs (indPCBs) and the only data on PCBs in nektonic organisms from the ES state comprise hepatic DL-PCB concentrations in franciscana dolphins (Dorneles et al. 2013). Because PCB118 (IUPAC number) is not only a dioxin-like but also an indicator PCB, using the median value for this PCB congener (70.31 ng/g lipid), it was possible to estimate the blubber PCB concentrations for comparison purposes (6167 ng/g lipid). This estimation was based on the fact that PCB118 in average contributes to 6.69 % of ΣindPCB, which in turn constitutes 51.64 % of ΣPCB (sum of 36 congeners) in average, according to data on hepatic PCB concentrations of coastal dolphins from Brazil (Legat 2011). This estimation also was based on a liver/adipose tissue ratio of 0.33 for PCB118 in mammals (Kunisue et al. 2006). In possession of these values (Table 3), it was possible to perform statistical testing. No significant correlation was found between BEQ [dioxins (pg BEQ/g lipid)] and PCB concentrations (ng/g lipid) of dolphins from Brazilian coast ($p = 0.3$, $Rs = 0.54$). However, it is possible to verify through Table 3 that the extremely high PCB concentrations of dolphins from Guanabara Bay (Lailson-Brito et al. 2010) constitute atypical observations. The discrepancy between BEQ and PCB concentrations in Guanabara Bay (GB) may be explained by some features of this body of water that seem to influence the PCB profile reaching the top positions of its food chain. GB receives an extremely high load of particulate suspended matter (PSM) (SEMADS 2001) and the affinity for this material (particularly to organic and black carbon) is not the same among different PCB congeners. The more hydrophobic is the molecule the higher is its affinity to organic and black carbon. Hydrophobicity occurs in direct proportion to the degree of halogenation of the OHC molecule. Hence, the higher chlorinated PCB congeners would be removed faster from the water column than the lower chlorinated ones in bodies of water with high PSM concentrations (Dachs et al. 1996; Jonker and Koelmans 2002). It seems to be the case for GB, where the apparent lower bioavailability of PCBs of high degree of chlorination generated a PCB profile of lower percent contribution of penta-, hexa-, and heptachlorinated biphenyls in dolphins from GB than in those from PR state (Lailson-Brito et al. 2010). This also holds for the CE state regions, where octa-, hepta-, and hexachlorinated biphenyls constitutes more than 90 % of ΣPCB (Santos-Neto et al. 2014) in opposition to GB where PCBs with five or less chlorine atoms contributed to almost 50 % of ΣPCB (Lailson-Brito et al. 2010). Taking into account that among the 12 DL-PCBs there are only two tetrachlorinated biphenyls and no tri- or dichlorinated molecules (Van den Berg et al. 2006), it can be conclude that the bioavailability of DL-PCBs may be reduced in GB compared with other Brazilian coastal areas. It is important to keep in mind that BEQ values result from the concentrations of the different PCDD/Fs and DL-PCBs, as well as from the ability of each compound to induce the response mediated by the aryl

Table 3 PCB (data from the literature) and BEQ (data generated by the present study) concentrations of mature male dolphins from Brazilian coast

Brazilian state	Area/bay	ΣPCB conc. (ng/g lipid)	Sampling number	Published study	BEQ conc. (pg/Kg lipid)
PR	Paranaguá	4043[a]	9	Lailson-Brito et al. (2010)[c]	4325
RJ	Guanabara	28,992[a]	7	Lailson-Brito et al. (2010)[c]	8570
ES	E. Santo	6167[b]	2	Dorneles et al. (2013)	10,086
CE	W from M	3370[a]	1	Santos-Neto et al. (2014)[d]	8680
CE	M	7320[a]	3	Santos-Neto et al. (2014)[d]	14,200
CE	E from M	850[a]	3	Santos-Neto et al. (2014)[d]	3050

[a] Blubber PCB concentrations of Guiana dolphins

[b] Estimation based on: (1) hepatic PCB118 concentrations of Franciscana dolphins; (2) liver/adipose tissue ratio of 0.33 for PCB118 in mammals (Kunisue et al. 2006); (3) average contribution of 6.69 % from PCB118 to ΣindPCB and (4) 51.64 % from ΣindPCB to ΣPCB (sum of 36 congeners, Legat 2011)

[c] Sum of 27 PCB isomers and congeners

[d] Sum of 29 PCB isomers and congeners

hydrocarbon (Ah) receptor (Scippo et al. 2004). Although PCB concentrations are extremely high in GB, particular aspects of the estuary do not favour the arrival of compounds that present affinity to the Ah-receptor at the top of the food chain. Considering all this, statistical testing was performed with the information exhibited in Table 3 excluding the data from GB. In this scenario, a significant positive correlation was found between BEQ [dioxins (pg BEQ/g lipid)] and PCB concentrations (ng/g lipid) of dolphins from Brazilian coast when data from GB were excluded ($p = 0.037$, $Rs = 0.9$).

Concerning possible correlations between BEQ concentrations [dioxins (pg BEQ/g lipid)] and total length of dolphins, a significant negative correlation was found when data from all analysed dolphins were considered ($p = 0.0017$, $Rs = -0.63$; Fig. 2a). Due to some features of Guiana dolphins, i.e., resident behaviour and habitat fidelity, the species presents different ecological populations along its distributional range (Azevedo et al. 2009; Meirelles 2013). An ecological population is comprehended in the present study as the main ecological impact unit regarding the use of environmental resources. As a regional concentration of individuals, the ecological population may not constitute a genetic unit (Lewontin 1974). This seems to be the case for the Guiana dolphin, for which a genetic unit may comprise a number of ecological populations (Cunha et al. 2005; Cunha and Watts 2007). Therefore, investigations on the possible correlations between BEQ concentrations and biological parameters within each evaluated region constitute a matter of interest. No significant correlations between BEQ concentrations [dioxins (pg BEQ/g lipid)] and total length of Guiana dolphins were found for ES ($p = 0.4$, $r = -0.46$) or PR ($p = 0.9$, $r = 0.04$) states; however, a significant negative correlation was found for RJ state ($p = 0.01$, $r = -0.90$; Fig. 2b). As mentioned, BEQ values result from the concentrations of the different DRCs, as well as from the ability of each compound to induce the Ah-receptor mediated response (Scippo et al. 2004). Therefore, these negative correlations between BEQ concentrations and total length of dolphins may be consequence of chemically induced developmental disruption. The latter hypothesis is based on the fact that inhibition of growth and development is among the effects attributed to exposure to DRCs (Sanderson and Van Den Berg 1999). Conversely, an increasing efficiency of the detoxifying activity with the growth of the animal may be a plausible explanation as well (Reijnders 2003). Another important aspect to consider while evaluating pollutant bioaccumulation through the life of dolphins is that the length is not the best proxy for age in mammals, because the aging process may continue for decades after the growing of the animal has ceased (Matthews 1978). Length, on the other hand, is a good proxy for the growth of the animal, as long as the length data constitute values below the asymptotic length (AL) verified for species and gender. The AL for male Guiana dolphins is 179 cm (Rosas et al. 2003). In this context, it should be highlighted for RJ and ES states that the highest concentration found among dolphins lengthier than 179 cm (5.86 and 8.45 pg/g lipid for RJ and ES, respectively) was still lower than the lowest level verified among those that were smaller than 179 cm (9.56 and 11.2 pg/g lipid for RJ and ES, respectively; Table 1). The fact that all dolphins from PR state were bigger than the AL helps to explain the absence of correlation between BEQ concentrations and total length (TL) in that state. The TL values of individuals from PR state are more likely to reflect typical variation in size of mammals whose growing had already ended than to demonstrate the growing process itself, as these dolphins were already lengthier than the AL of the species (Rosas et al. 2003).

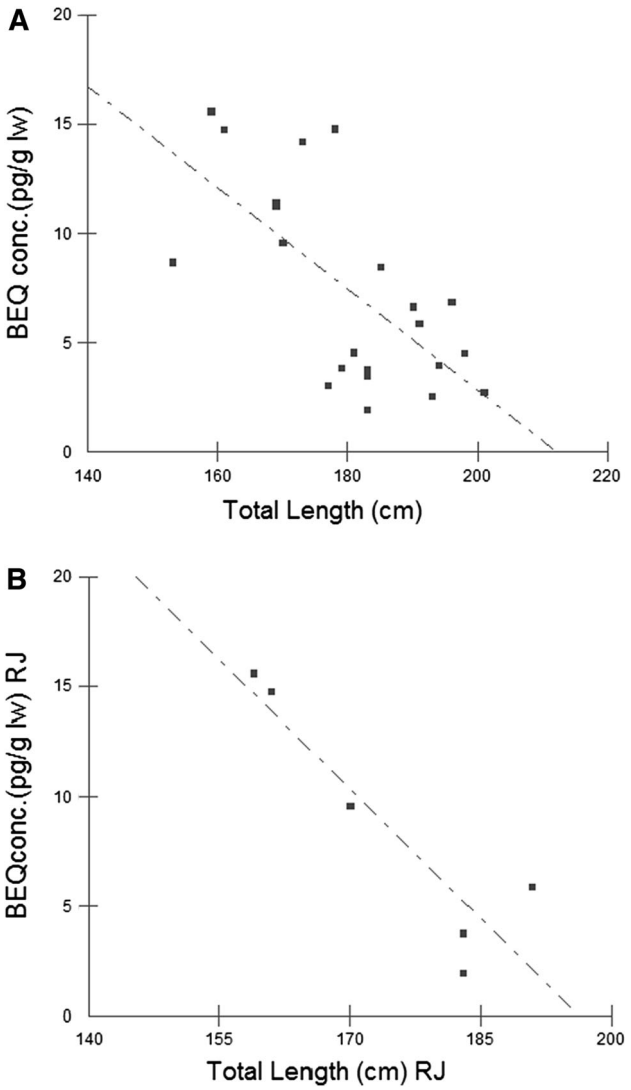

Fig. 2 Correlations between BEQ concentrations [dioxins (pg BEQ/g lipid)] and total length (cm) of Guiana dolphins, considering all individuals from Brazilian coast (**a**), as well as those from Guanabara Bay, Rio de Janeiro state, exclusively (**b**)

Regarding the geographical differences in Guiana dolphin exposure, a trend for differences in BEQ levels (pg BEQ/g lipid) among the Brazilian states (ANOVA, $F_{3,18} = 2.48$; $p = 0.094$) was observed, as well as significantly higher BEQ concentrations were verified in individuals from ES state (Southeastern region) than in those from PR state (Southern region) ($p = 0.003$, $T = -3.81$; Fig. 3). A similar trend (higher concentrations in SE than in S) was found when comparison was performed between RJ (SE) and PR (S) states ($p = 0.066$, $T = 2.02$; Fig. 3). Higher levels were expected to occur in SE Brazil, because it is the most industrialized region of the country, as well as the region that generally yields the highest PBT concentrations in the Brazilian coast (Alonso et al. 2012a; Dorneles et al. 2008a; b, 2010, 2013; Lailson-Brito et al.

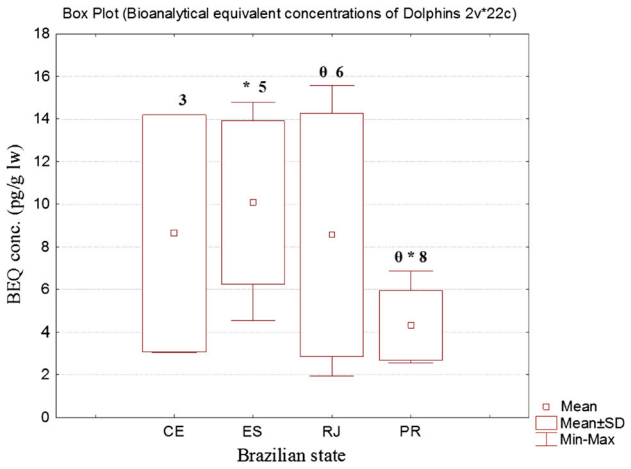

Fig. 3 *Box* and *whisker plots* of hepatic bioanalytical equivalent (BEQ) concentrations [dioxins (pg BEQ/g lipid)] of Guiana dolphins from the Brazilian states of Ceará (CE), Espírito Santo (ES), Rio de Janeiro (RJ), and Paraná (PR). The sampling number is shown over each superior whisker, as well as the symbols *greek small letter theta* or *asterisk*, which indicate a trend ($0.05 < p \leq 0.10$) or a significant difference ($p \leq 0.05$), respectively

2010, 2011, 2012b). However, significant differences in TL of dolphins were observed among the Brazilian states (ANOVA, $F_{3,18} = 6.06$; $p = 0.005$). Total length values were significantly higher in PR state than in both RJ ($p = 0.009$, $T = 3.13$) and ES ($p = 0.004$, $T = 3.65$) states (Fig. 4). Considering the general finding of higher BEQ concentrations in smaller individuals, it is not possible to affirm that Guiana dolphins are more contaminated by DRCs in SE than in S region due to the significant differences in TL between the two regions. In fact, there

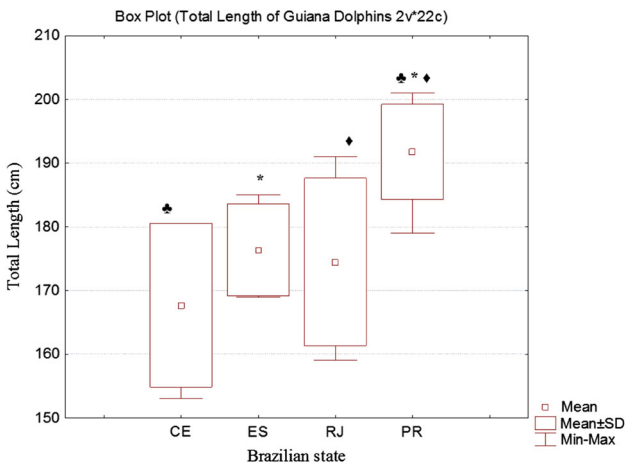

Fig. 4 *Box* and *whisker plots* of total length (cm) values of Guiana dolphins from the Brazilian states of Ceará (CE), Espírito Santo (ES), Rio de Janeiro (RJ), and Paraná (PR). The symbols *filled diamond*, *filled club*, and *asterisk* shown over each superior whisker indicate significant ($p \leq 0.05$) differences

was a significant difference in TL values between PR and CE states as well ($p = 0.003$, $T = 3.97$), with lengthier dolphins in the former state (Fig. 4). When data from the two areas of SE Brazil (ES and RJ states) were compared, no significant differences were found between the two states, considering either BEQ concentrations ($p = 0.6$, $T = -0.51$) or TL values ($p = 0.8$, $T = -0.29$). These later findings demonstrate the high exposure of Guiana dolphins from ES state, because the sample set of RJ state is entirely composed of Guiana dolphins from Guanabara Bay (GB). This region (i.e., GB) not only constitutes the most dramatic example of manmade degradation of the Brazilian coast (Amador 2013; Azevedo et al. 2009; Bittencourt et al. 2014; Carreira et al. 2004), but it also is an important hotspot of environmental contamination by PCBs (Dorneles et al. 2013; Lailson-Brito et al. 2010).

In Brazilian states for which the area of origin of dolphin carcasses comprises a long coastal extension (i.e., CE and ES states) the sample set may not be composed of individuals from the same ecological population. The latter observation is based on the abovementioned site fidelity and small home ranges exhibited by Guiana dolphins in different areas along the distribution of the species (Azevedo et al. 2009; Bazzalo et al. 2008; Meirelles 2013). This allows for further analyses within each state. Concerning the ES state, the dolphin that provided the lowest level (4.54 pg BEQ/g lipid) was the only individual originating from an area regarded as a nonurbanized region, the Anchieta City (Carvalho 2013). Regarding CE state (Fig. 1), a recent investigation on OHCs in dolphins has drawn attention to the dissimilarities between the Metropolitan (M) and the other regions of that state (Santos-Neto et al. 2014). As the name suggests, the M region corresponds to a highly industrialized and urbanized area, while the adjacent regions present only 10 % of human population density found in the M region. In addition, the most important economic activities for these adjacent regions are agriculture, fishing, and tourism (Campos and Polette 2003). In this context, it is important to mention that the highest concentration among the CE dolphins (14.2 pg BEQ/g lipid) was found in the individual originated from the M region, an area that harbours a reduced Guiana dolphin population, assessed to be around 40 individuals (Meirelles 2013). No significant difference in BEQ concentrations was found between CE and the three other Brazilian states ($p = 0.2$, $U = 6$ for PR; $p = 0.8$, $U = 8$ for RJ; and $p = 0.6$, $U = 6$ for ES). The absence of difference between Northeastern (CE state) and Southeastern (ES and RJ states) regions may be a result of a combination of two factors: (1) the small sampling number ($n = 3$) from CE state and (2) the presence of an individual from a highly impacted area of this state (M region). In fact, it should be highlighted that any conclusions on Ceará should be seen with caution considering the small sampling number from this Brazilian state.

Because year-to-year variations and temporal trends in POP levels have been found in studies on marine mammals from different regions of the globe (Dorneles et al. 2015; Isobe et al. 2009; Law et al. 2010a, 2010b), this subject was evaluated in the present study as well. This evaluation was performed using three different variables for time, i.e., day, month, and trimester since the first stranding. A decreasing trend in BEQ concentrations was found between October 2008 and November 2009 for PR state, considering both month ($p = 0.092$, $r = -0.63$) and day of stranding ($p = 0.096$, $r = -0.63$). Regarding RJ state, a significant positive correlation was found between BEQ levels and the time variables ($p = 0.025$, $r = 0.87$; in the three scenarios, i.e., day, month, and trimester); however, significant negative correlations between total length of dolphins and the same time variables ($p < 0.01$, $r = -0.92$; in the three scenarios) also were observed. Concerning ES state, a significant negative correlation was found between BEQ concentrations and trimester of stranding ($p = 0.04$, $Rs = -0.89$); however, there was a significant positive correlation between TL and month of stranding ($p = 0.04$, $Rs = 0.89$) in this Brazilian state. Taking into account the general finding of higher BEQ concentrations in smaller dolphins discussed in a previous paragraph, the significant correlations found between total length and time variables suggest that our sample sets are not appropriated for investigations related to time trends.

Comparison between levels found in our investigation and concentrations in other cetaceans from different areas constitutes a difficult task due to the scarcity of data. However, crude data on BEQ concentrations [dioxins (pg BEQ/g lipid)] in liver samples from North Sea harbour porpoises (*Phocoena phocoena*) were available (Das et al. 2005), comprising BEQ levels of ten individuals (mean ± S.D., median, minimum, and maximum concentrations of 2.13 ± 0.71, 2.2, 0.9, and 3.3 pgBEQ/g lipid, respectively). Therefore, those ten values were statistically compared with the data generated by the present study. Significantly higher concentrations were found in Guiana dolphins from Brazil than in N. Sea porpoises in all four scenarios, i.e., when levels from the four Brazilian states where considered ($p = 0.0015$, $T = 3.84$ for PR; $p = 0.003$, $T = 3.61$ for RJ; $p = 0.00{,}002$, $T = 6.6$ for ES; and $p = 0.02$, $U = 1$ for CE), demonstrating the high exposure of coastal top marine predators to DRCs in Brazil.

Conclusions

It is important to highlight that BEQ concentrations [dioxins (pg BEQ/g lipid)] in liver of dolphins from Northeastern, Southeastern, and Southern Brazilian regions seem to

indicate the expected environmental contamination by some DRCs. However, additional investigations are required for detailed information on the exposure of top marine predators to DRCs in the different areas from CE and ES states before strong conclusions can be reached. The same holds for investigating the hypotheses raised as a consequence of our findings: (1) inhibition of growth of Guiana dolphins as a result of chemically induced developmental disruption; and (2) an increasing efficiency of the capacity of detoxifying DRCs with the growth of the Guiana dolphin. A wider sampling would be necessary to supporting such strong conclusions. This research need is reinforced when the site fidelity exhibited by the Guiana dolphin is taken into account (Azevedo et al. 2009). The large BEQ concentration range observed in CE state constitutes an important example of this requirement. Considering the difficulties associated with sampling marine mammals, including legal and ethical issues, a similar approach using a high trophic level fish species is strongly recommended. In this context, it is important to mention that the whitemouth croaker (*Micropogonias furnieri*) would be an interesting sentinel species, as this scianid fish not only is a crucial prey species for Guiana dolphins but also occupies (the adult whitemouth croakers) high trophic positions in Brazilian coastal bays (Bisi et al. 2012). Considering the importance of monitoring studies for the assessment of environmental distributions of POPs, as well as the scarcity of data on these contaminants in Southwest Atlantic coastal waters, the present study could constitute a first step for future risk assessment investigations. This study also could be used by the authorities from the assessed regions as an initial stimulus for the strengthening of legal obstacles to the generation of unwanted toxic compounds.

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