Contrasted accumulation patterns of persistent organic pollutants and mercury in sympatric tropical dolphins from the south-western Indian Ocean

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Abstract

Due to their high trophic position and long life span, small cetaceans are considered as suitable bioindicators to monitor the presence of contaminants in marine ecosystems. Here, we document the contamination with persistent organic pollutants (POPs) and total mercury (T-Hg) of spinner (*Stenella longirostris*, n=21) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*, n=32) sampled from the coastal waters of La Réunion (south-western Indian Ocean). In addition, seven co-occurring teleost fish species were sampled and analyzed as well. Blubber samples from living dolphins and muscle from teleosts were analyzed for polychlorinated biphenyls (PCBs), DDT and metabolites (DDTs), chlordanes (CHLs), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), and polybrominated diphenyl ethers (PBDEs). Methoxylated PBDEs (MeO-PBDEs), reported as having a natural origin, were also analyzed. T-Hg levels were measured in blubber and skin biopsies of the two dolphin species. Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ values were determined in skin of the dolphins and in the muscle of teleosts. For PCBs, HCHs and T-Hg, concentrations were significantly higher in *T. aduncus* than in *S. longirostris.* For other POP levels, intra-species variability was high. MeO-PBDEs were the dominant compounds (55% of the total POPs) in *S. longirostris*, while PCBs dominated (50% contribution) in *T. aduncus*. Other contaminants showed similar profiles between the two species. Given the different patterns of POPs and T-Hg contamination and the $\delta^{15}N$ values observed among analyzed teleosts, dietary and foraging habitat preferences most likely explain the contrasted contaminant profiles observed in the two dolphin species. Levels of each class of contaminants were significantly higher in males than females. Despite their spatial and temporal overlap in the waters of La Réunion, *S. longirostris* and *T. aduncus* are differently exposed to contaminant accumulation.

Keywords: POPs; Hg; stable isotopes; La Réunion; *Stenella longirostris*; *Tursiops aduncus*

Introduction

Due to their physicochemical properties and environmental behavior, Persistent Organic Pollutants (POPs) are one of the most intensively studied among the organohalogenated contaminants. Although POPs are regulated in many countries within the Stockholm Convention (www.pops.int), their resistance to degradation, persistence, and lipophilic properties facilitate their bioaccumulation and biomagnification in the environment. The marine environment is a global sink for legacy anthropogenic POPs, e.g. organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) [Dachs et al., 2002]. Although structurally similar to metabolites of anthropogenic PBDEs, methoxylated analogues (MeO-PBDEs) are of biogenic origin [Teuten et al., 2005]. Apart from POPs, mercury (Hg) pollution can also reach elevated concentrations worldwide including the open ocean [Hylander and Goodsite, 2006], although data on the oceanic distribution of Hg is limited [Sunderland et al., 2009; Savery et al., 2013]. Mercury biomonitoring might be used as an indicator of foraging habitats and trophic position of large marine predators, because body burden concentrations are highly correlated to size/age, environmental parameters and geographic location [Power et al. 2002; Cai et al. 2007]. Total Hg (T-Hg) levels in pelagic fishes increase with median depth of occurrence in the water column and mesopelagic habitats are probably major entry points of mercury into marine food webs as a result of increased methylation at these depths [Monteiro et al., 1996; Choy et al., 2009; Chouvelon et al., 2012]. Marine top predators feeding on mesopelagic prey, such as large predatory fishes, exhibit significantly higher T-Hg concentrations than epipelagic predators [Thompson et al., 1998; Kojadinovic et al., 2006; Choy et al.n 2009]. As a consequence, depending on their habitat influenced by their feeding ecology and diet, this might have a significant impact on the T-Hg, but also on the exposure to POPs of marine mammals [Balmer et al., 2011; Shaul et al., 2015].

The accumulation of organic and inorganic contaminants in marine food chains represents an important stress factor for marine mammals since they can have significant negative effects on health and reproductive ability [Wells et al., 2005; Schwacke et al., 2011; Murphy et al., 2015]. Cetaceans are particularly susceptible to POP accumulation in blubber [Ross et al., 2000; Pierce at al., 2008; Yordy et al., 2010; Ellisor et al., 2013;], since these species have long lifespans, large fat deposits and occupy high trophic positions. The adverse health effects of POPs on marine mammals are difficult to assess, although some studies have shown that for such contaminants toxicity thresholds are commonly exceeded [Kannan et al., 2000; Jepson et al., 2005; García-Alvarez et al., 2014; Murphy et al., 2015]. Consequently,

marine mammals such as cetaceans are considered as good indicators of POP contamination in aquatic ecosystems [Dachs et al., 2002; Bachman et al., 2014].

Apart from sampling of stranded or bycaught animals, non-lethal remote biopsy sampling of skin and blubber has become a routine method for sampling free-ranging cetaceans with relatively limited behavioral impact [Best et al., 2005; Jefferson and Hung 2008; Kiszka et al., 2010]. T-Hg measurements in skin reflected T-Hg in liver of small cetaceans and can provide valuable information on the status of Hg contamination, and even to inform on potential spatial variations [Aubail et al., 2013].

At least 10 species of cetaceans are regularly observed in the waters of La Réunion, in the south-western tropical Indian Ocean [Dulau-Drouot et al., 2008]. Spinner (*Stenella longirostris*) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) are the most common species found around the island year-round. Indo-Pacific bottlenose dolphins occur in shallow inshore waters (depth < 80 m) within 3 km of the coastline. Spinner dolphins have a wider depth range (<700 m) and use the coastal and insular slope waters of the island during daylight hours, mainly to rest and socialize [Dulau-Drouot et al., 2008]. At night, spinner dolphins feed upon mesopelagic organisms (primarily fish and squids) as deep as 400 m [Perrin et al., 1973; Dolar et al., 2003]. Indo-Pacific bottlenose dolphins can feed on a range of small- and medium-sized inshore prey [Amir et al., 2005a]. Some insular populations could be more specialized and feed on demersal and epipelagic predators [Kiszka et al., 2014].

The present study aimed to investigate anthropogenic POPs (OCPs, PCBs, and PBDEs), naturally-occurring MeO-PBDEs, and T-Hg concentrations in skin and blubber tissues of spinner and Indo-Pacific bottlenose dolphins around La Réunion. Given the differences reported in the foraging behavior of spinner (offshore mesopelagic prey) and Indo-Pacific bottlenose dolphins (coastal demersal prey), stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$ values) were used to investigate the effect of habitat preferences and diet on the bioaccumulation of these contaminants [Jardine et al., 2006].

Materials and methods

Study area. La Réunion (21°07'S, 55°32'E) is a French oceanic volcanic island located in the south-west Indian Ocean, 700 km east of Madagascar and 60 km west of Mauritius (Figure 1). The island is relatively small, extending over 2,512 km^2, and is characterized by a steep insular slope and fringing reef off the west coast. The island faces increasing human pressures and coastal development. Very little is known on POP and trace element contamination of marine ecosystems and species, including marine fauna. Studies on seabirds and pelagic fishes from

oceanic islands, including La Réunion, suggest that mercury availability in the south-western Indian Ocean is relatively low compared to other regions [Kojadinovic et al., 2006; 2007a,b].

Sample collection. In order to document POP and Hg loadings from the coastal waters of the La Réunion, skin and subcutaneous blubber biopsy samples were collected between 2010 and 2011 from both spinner (n=21) and Indo-Pacific bottlenose dolphins (n=32) (Figure 1). A total of 62 boat-based surveys dedicated to biopsy sampling were conducted during the study period. Biopsies were collected by using a crossbow (BARNETT Veloci-Speed® Class, 150 lb draw weight) with Finn Larsen (Ceta-Dart, Copenhagen, Denmark) bolts and tips (dart 25-mm long, 7-mm-diameter). The dolphins are hit below the dorsal fin when sufficiently close (3-10 m) to the research boat. Samples were exclusively collected from adult or subadult individuals (based on body size). A maximum of 0.5×1cm of tissue was collected per individual biopsy. Samples were stored individually at –20°C and transported in dry ice. Biopsy permit was delivered by the French Ministry for Environment in November 2009 (reference number MC/2009/336). Supplementary information on the sampling (geographical coordinates, sampling date and time, species sampled and gender) is presented in Tables SI.1 and SI.2 from Supporting Information. Additionally, seven fish species were sampled in the same area and during the same campaign as the dolphin biopsies; additional information is given in Supplementary Information file. For each species, one composite muscle sample was prepared.

Target analytes. All samples were analyzed for T-Hg and organic contaminants, as follows: polychlorinated biphenyls (PCBs) – 37 tri- to deca-chlorinated congeners (IUPAC numbers: CB 18, 31, 28, 52, 49, 44, 74, 70, 66, 95, 101, 99, 87, 110, 105, 118, 151, 149, 146, 153, 138, 128, 167, 156, 187, 183, 174, 177, 171, 172, 180, 170, 199, 196/203, 194, 206, and 209), Dichlorodiphenyl trichloroethane (DDT) and metabolites (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD) discussed here as DDTs, chlordanes (*cis*-chlordane (CC), *trans*-chlordane (TC)) and metabolites (*oxy*-chlordane (OxC), *cis*-nonachlor (CN), *trans*-nonachlor (TN)) discussed in text as CHLs, hexachlorocyclohexanes (α-, β-, and γ-HCH) discussed as HCHs, hexachlorobenzene (HCB), and polybrominated diphenyl ethers (PBDEs) – 7 tri- to hepta-BDE congeners (BDE 28, 47, 100, 99, 154, 153, and 183). Two most abundant naturally-occurring MeO-PBDEs (2'-MeO-BDE68 and 6-MeO-BDE47) were also targeted.

153 ***Organohalogenated contaminants.*** Analyses of POPs in blubber were performed according
154 to the methods described in previous studies [Covaci et al., 2008; Weijs et al., 2009], with
155 minor modifications as presented below. Blubber samples (≈150 mg) were weighed, mixed
156 with anhydrous Na_2SO_4 and spiked with internal standards (CB 143, BDE 77 and BDE 128).
157 Further, the target analytes were extracted from samples using an automated Soxhlet extractor
158 (Büchi, Flawil, Switzerland) for 2 h (operated in hot-extraction mode) using approximately
159 100 mL hexane/acetone (3:1, *v/v*) as extraction solvents. The lipid content was determined
160 gravimetrically on an aliquot of the extract (105°C, 1h), while the rest of the extract was
161 transferred to 25 mL polypropylene columns (Alltech, Lokeren, Belgium) filled with ~8 g
162 acidified silica (44% H_2SO_4 by weight) and analytes were further eluted with 20 mL *n*-hexane
163 and 15 mL dichloromethane. The cleaned extract was evaporated to near dryness, re-dissolved
164 in 100 µL *iso*-octane and transferred to the injection vial. Identification and quantification of
165 the OCs was performed using a gas-chromatograph (GC; Agilent 6890) equipped with a
166 programmable-temperature vaporizer and a mass spectrometer detector (MS; Agilent 5973)
167 operated either in electron capture negative chemical ionization or electron impact mode.
168
169 ***Total mercury (T-Hg).*** Approximately 30 to 50 mg of freeze-dried skin or blubber were
170 weighed and loaded into quartz boats. Masses were recorded to the nearest 0.01 mg.
171 Concentrations of T-Hg were determined by atomic absorption spectroscopy (AAS; DMA-80,
172 Direct Mercury Analyzer; Milestone). The method has been validated for solid samples using
173 U.S. Environmental Protection Agency (U.S. EPA) method 7473.
174
175 ***Quality assurance/ Quality control (QA/QC).*** All analyzes were performed using validated
176 protocols routinely used in our laboratories. Each step applied over the entire procedure was
177 carefully evaluated (in terms of recoveries for each targeted analyte together with method
178 precision and accuracy). Each targeted analyte was identified and further quantified if the
179 retention time in GC matched that of the standard compound within ±0.1 min and the signal-
180 to-noise ratio (S/N) was higher than 3. The method limit of quantification (LOQ) was
181 calculated as three times the standard deviation of the mean of the blank measurements.
182 Procedural blanks were analyzed simultaneously with every batch of seven samples to check
183 for interferences or contamination from solvent and glassware. Since procedural blanks were
184 consistent (RSD<30%) the mean value was calculated for each compound and further
185 subtracted from the values measured in samples. The accuracy of the analytical procedure
186 described above was evaluated through the analysis of the certified material SRM 1945

(organic contaminants in whale blubber) for which deviations from certified values were less than 10%.

Rigorous QA/QC measures were also applied for the analysis of T-Hg including evaluation of procedural blanks, blind duplicate samples, and the analysis of several certified reference materials, such as NIST 1566b, BCR 60, BCR 61, BCR 62, and BCR 414.

Stable isotopes. Stable carbon and nitrogen (hereafter noted $\delta^{13}C$ and $\delta^{15}N$, respectively) isotope analyses were performed in epidermis (hereafter skin) samples. Because lipids are highly depleted in $\delta^{13}C$ relative to other tissue components and are a large component of the tissues we collected [De Niro and Epstein, 1978; Tieszen et al., 1983], lipid-extractions were performed. For lipid-extractions, an aliquot of approximately 100 mg of fine powder was stirred with 4 mL of cyclohexane for 1h at room temperature, this operation being repeated three times. Next, the sample was centrifuged for 5 min at 4000g and the supernatant containing lipids was discarded. The sample was dried in an oven at 45°C for 48h, and 0.35±0.05 mg subsamples of lipid-free powder were then weighed in tin cups for stable isotope analyses. Stable isotope measurements were performed with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash EA1112 Thermo Scientific, Italy). Stable isotope ratios of carbon and nitrogen in fish samples were determined by analysing approximately 1.5 mg of powdered samples using an automate Vario MICRO Cube N-C-S elemental analyser (Elementar, Hanau, Germany) coupled to a continuous flow Isoprime 100 isotope ratio mass spectrometer (Isoprime, Cheadle, United Kingdom).

Results are expressed in δ notation relative to PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively, according to the equation:

$$\delta X = (R_{sample} / R_{standard} - 1) \times 10^3$$

where X is ^{13}C or ^{15}N and R is the isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ [Peterson and Fry, 1987]. Certified material from International Atomic Energy Agency (Vienna) (IAEA-C6 and IAEA-N2, for C and N respectively) were used to assess measurement reliability. Replicate measurements of internal laboratory standards (acetanilide and glycine) indicated that measurement repeatability was ±0.15 and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively in both laboratories. Percent carbon and nitrogen elemental composition of tissues were obtained using the elemental analyzer and used to calculate the sample C:N ratio, indicating a good lipid removal efficiency when C:N values were below 4 [Lesage et al., 2010].

Molecular sexing. Sex was determined genetically. DNA was extracted from skin samples using Qiagen DNeasy kits and following the manufacturer's instructions. The ZFX/ ZFY region of the sex chromosomes was amplified by polymerase chain reaction (PR), following the protocol described by Bérubé and Palsbøll (1996). PCR product were separated by electrophorese and visualized under UV-light to discriminate the males from the females (two and one PCR product respectively). Gender data was only available for *T. aduncus*.

Statistical analysis. All statistical analyses were performed using XLStat-Pro version 2013.5.01 (Addinsoft, 1995-2013). Levels below the method LOQ were assigned a value of DF×LOQ, with DF being the proportion (%) of measurements with levels above the LOQ or the detection frequency [Voorspoels et al., 2002]. Compounds with levels below the method LOQ in more than 50% of samples were excluded from further statistical analysis. For data which did not follow a normal distribution (Shapiro-Wilk test, $p>0.05$) they were log-transformed ($y=log(x+1)$) and further tested for normality. For data which did not show a normal distribution after *log*-transformation, non-parametric statistics was applied for comparison of concentrations (POPs and T-Hg) between dolphin species (the case for most of the PCB and PBDE congeners, HCHs and CHLs). For data which followed a normal distribution after *log*-transforming, parametric statistics was used on the new set of data (total lipids, DDTs and stable isotopes concentrations). Correlations were carried out using parametric Pearson correlations (for normally distributed *log*-transformed data) and non-parametric Spearman rank correlations (for data which were not normally distributed). Differences in levels among samples were tested on the *log*-transformed data (t-test comparison for means) or on original data for not normally distributed values (Mann-Whitney test for comparison of two independent samples). Linear regression models were applied to test for the influence of the $\delta^{13}C$ and $\delta^{15}N$ values on both POPs and T-Hg measured concentrations. Profiles of organic contaminants were investigated using principal component analysis (PCA) on normalized concentrations in order to remove concentration as a variable. Individual congeners were normalized by subtracting the mean and dividing by the standard deviation [Echols et al., 2000]. Factor loadings (plotted as lines) and factor scores (plotted as points) were determined and used in interpreting the PCA. The significance level was set at $\alpha=0.05$.

Results and Discussion

Levels and profiles of targeted contaminants

POPs. Median and range levels for the most abundant contaminants measured in samples (expressed in ng/g lipids weight (*lw*)) together with their detection frequencies (DF, as percentage measured at levels above LOQ) of chemicals analyzed in blubber samples collected from the two dolphin species are presented in Table 1.

The variation in the blubber concentrations of OHCs was large within each dolphin species and high DFs (%) were recorded for most of the targeted OHCs (Table 1). DDTs, PCBs together with MeO-PBDEs were the most abundant contaminants measured in dolphin samples (Figure 2). HCHs, CHLs, HCB and PBDEs were the minor contaminants in all blubber samples (Figure 2A). Our results shows that *p,p'*-DDE was measured at significantly higher levels than *p,p'*-DDT, which is consistent with literature data [Mwevura et al., 2010; Bachman et al., 2014; García-Alvarez et al., 2014]. No significant differences between the two dolphin species could be found for either *p,p'*-DDE or *p,p'*-DDT in blubber. The *p,p'*-DDT/*p,p'*-DDE ratios were significantly different ($p<0.05$) between the two species, with the median values 0.14 and 0.07 for *S. longirostris* and *T. aduncus*, respectively (Table 1). It suggests the existence of inter-species differences in the metabolism or intake of DDT. Our results show that both *p,p'*-DDT and *p,p'*-DDE were measured at lower levels in *S. longirostris*, but these differences were not statistically significant. A statistically significant positive relationship between concentrations of *p,p'*-DDT and *p,p'*-DDE was obtained for each dolphin species ($p<0.0001$ for *T. aduncus* and $p<0.001$ for *S. longirostris*). Moreover, significant positive relationships were found between the levels of PCBs, DDTs, PBDEs, and MeO-PBDEs measured in both species (Table SI.4-SI.7). The targeted compounds in our study belong to different classes of chemicals with their own physicochemical properties and they might reach the aquatic environment through different pathways.

In contrast to other compounds, ΣHCHs levels were significantly lower ($p<0.05$) in *T. aduncus* than in *S. longirostris* (Table 1). Additionally, HCH concentrations were not significantly correlated with any other POPs in *S. longirostris*, as opposed to *T. aduncus* samples, suggesting possible differences in the bioaccumulation of these compounds between the two species. However, the lack of correlation between HCHs and the other contaminants measured in *S. longirostris* might also indicate that the contamination source is different for these compounds when compared to the other OHCs targeted for analysis. The lower levels of ΣHCHs compared to other OHCs are possibly explained by the lower $\log K_{ow}$ values reported for ΣHCHs compared to PCBs, DDTs and PBDEs [IUCLID, 1996; Kelly and Gobas, 2000].

Furthermore, previous research focusing on OHC contamination levels in deep-sea ecosystems [Toyoshima et al., 2009] suggested that HCH concentrations decrease with an increase in the trophic level, implying dilution through food webs.

A larger variability was noticed for contaminant levels measured in *T. aduncus* blubber samples when compared to *S. longirostris* (Figure 3). Such variability on the measured contaminant levels might be driven by the higher levels of individual foraging specialization among *T. aduncus*. While factor scores obtained for *S. longirostris* are clearly grouped mostly on F1 axis, for *T. aduncus* the recorded factor scores are more heterogeneously distributed along both axes. The recorded variability in the distance between the groups may be related to the differences in their diet, as suggested in the literature [Ross et al., 2000; Hansen et al., 2004]. Information regarding the feeding ecology of delphinids around La Réunion remains unavailable, precluding further discussion. Nevertheless, published data on the diet of spinner dolphins suggests that they mainly feed offshore on mesopelagic fish and cephalopods [Dolar et al., 2003], which might have lower POP loads compared to coastal prey species. This might suggest a lower dietary intake of POPs in the spinner dolphins when compared to bottlenose dolphins, which feed on coastal prey [Amir et al., 2005a,b; Kiszka et al., 2014].

The most important PCB congeners in terms of percentage contribution relative to the total PCB levels are: CB 149, CB 153, 180, 138, 187, and 170 in blubber samples collected from each dolphin species. These PCB congeners contributed to 60% for *S. longirostris* and 64% for *T. aduncus* to the ΣPCBs (Figure 2B). For PBDEs, BDE 47 was the most important congener, while BDE 100, 154 and 99 contributed almost equally to the total PBDE levels, although they were measured at relatively low levels when compared to other OHCs (Figure 2C). Differences recorded between species did not affect the general profiles for both PCBs and PBDEs, which were almost similar for all dolphins (Figure 2) suggesting that both species accumulate similarly these contaminants independently of the contamination level or habitat preferences.

Regarding MeO-PBDEs, 6-MeO-BDE47 was measured in all samples at significantly higher levels ($p<0.05$) than 2′-MeO-BDE68. The presence of the naturally-occurring MeO-PBDEs in marine mammals was often reported, although there are few studies focusing on the analysis of MeO-PBDEs and anthropogenic POPs in *T. aduncus* and *S. longirostris* (Table 2). Since there are few studies on *T. aduncus*, studies on POPs on the common bottlenose dolphin (*Tursiops truncatus*) were also included in Table 2. Although highly variable, POPs concentrations reported for *S. longirostris* and *Tursiops* spp. from other locations were consistently higher than those found in La Réunion [Mwevura et al., 2010; Bachman et al.,

2014; García-Alvarez et al., 2014]. OHC profiles were consistent with other studies and confirmed that HCB, ∑HCHs and ∑PBDEs have the lowest contribution to the sum of POPs followed by PCBs, while DDTs appear as the most abundant anthropogenic contaminants (Table 2).

Total mercury (T-Hg). Consistent with other studies, a higher DF was recorded for T-Hg in skin when compared to blubber [Aubail et al., 2013; Borrell et al., 2015]. Due to low DF recorded for T-Hg in blubber samples of *S. longirostris* (T-Hg was measured in only 6 out of 21 samples), no further comparison was performed between paired blubber-skin samples for this species. However, in *T. aduncus*, a significant difference was found between T-Hg levels in skin and blubber ($p<0.0001$). Moreover, a strong correlation between skin and blubber T-Hg concentrations was found ($r=0.867$, $p<0.0001$). It suggests that T-Hg levels in skin can be a good predictor for the levels in blubber samples, which is in agreement with previous studies [Aubail et al., 2013; Borrell et al., 2015]. Significantly higher skin concentrations of T-Hg were found in *T. aduncus* compared to *S. longirostris* (Table 1), most probably due to their more coastal habitat and thus increased exposure to T-Hg from water runoff in coastal areas [Wang et al., 2012]. A significant relationship was observed between $\delta^{13}C$ and $\delta^{15}N$ values and T-Hg concentrations measured in fish (muscle) and in dolphins (skin) reflecting biomagnification of Hg through the food chain (Figure 4) [Atwell et al., 1998]. Although previous research suggests that Hg contamination is enhanced in mesopelagic organisms (potentially in *S. longirostris*) [Monteiro et al. 1996, Choy et al. 2009, Chouvelon et al. 2012; Aubail et al., 2013; Kiszka et al. 2015], our data highlight higher T-Hg concentration in the coastal *T. aduncus*. Given that La Réunion is a volcanic island, with the active volcano possibly representing a source of Hg emissions, and combined with increasing anthropogenic activities on this island, coastal habitats might be more exposed to Hg contamination [Wang et al., 2012].

Factors influencing bioaccumulation

Habitat preferences and relative trophic position. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values were measured in order to investigate the effect of habitat (inferred from $\delta^{13}C$) and relative trophic position (inferred from $\delta^{15}N$) on the observed POPs and T-Hg concentrations. Isotope values were significantly different between *S. longirostris* and *T. aduncus* (U-test, $\delta^{13}C$: $p=0.005$, $\delta^{15}N$: $p=0.003$). Values of $\delta^{15}N$ were significantly higher in *T. aduncus* than in *S. longirostris* (median and range values given in Table 1), while $\delta^{13}C$

values were more negative for *S. longirostris* than for *T. aduncus* (median and range values given in Table 1). It confirms that *S. longirostris* has a more pelagic foraging habitat than *T. aduncus*. Moreover, the slightly higher $\delta^{15}N$ values recorded in *T. aduncus* suggest that it has a higher relative trophic position, which is confirmed by existing studies [Dolar et al., 2003; Amir et al., 2005a,b; Kiszka et al., 2011,2014]. Similar differences were observed in *S. longirostris* and *T. aduncus* from the Mozambique Channel island of Mayotte [Kiszka et al., 2011]. Throughout the Indo-Pacific region, insular *S. longirostris* feed in deeper offshore waters on mesopelagic prey, including fish and cephalopods [Dolar et al., 2003], whereas coastal *T. aduncus* feed on both reef and coastal fishes of varying trophic levels [Amir et al., 2005a,b; Kiszka et al., 2014]. $\delta^{13}C$ and $\delta^{15}N$ values in fish varied widely, with the lowest values displayed by pelagic species such as the Skipjack tuna (*Katsuwonus pelamis*).

Significant differences between *T. aduncus* and *S. longirostris* were found for most of the individual PCB congeners, total PCB levels, total HCHs, 6MeO-BDE47 and T-Hg (Table 1, $p<0.05$). Except for ΣHCH levels, which were higher in *S. longirostris* than in *T. aduncus*, all other contaminants were present at higher concentrations in *T. aduncus*. Observed differences of measured OHCs between the two dolphin species are most likely attributable to the differences in their habitat and feeding preferences. Fish from coastal waters are more prone to anthropogenic contamination and therefore, this might explain higher OHC levels measured in *T. aduncus* blubber tissues.

Inter-species differences were also noticed between the profiles of anthropogenic POPs and naturally-occurring MeO-PBDEs (Figure 2A). MeO-PBDEs were dominant (55% of the total organic contaminants) in *S. longirostris*, while PCBs dominated (50% contribution) the profile in *T. aduncus*. As shown in Figure 3, *T. aduncus* was clearly separated from *S. longirostris* mainly due to the influence on the anthropogenic contaminants like PCB congeners *versus* PBDEs and MeO-PBDEs. Given the relatively low levels of PBDEs measured in both species, the differences in OHC profiles between the two dolphin species seem to be explained mainly by anthropogenic contaminants like PCBs (in *T. aduncus)* and naturally-occurring MeO-PBDEs (in *S. longirostris*) (Figure 3).

Therefore, the differences recorded in the levels of contaminants between the two dolphin species, but also in the profiles of the anthropogenic *vs.* naturally-occurring compounds, are consistent with an increased contamination from anthropogenic compounds, like PCBs, DDTs, PBDEs and T-Hg, in coastal ecosystems.

Gender differences. Previous studies on gender differences of contaminant loads in marine mammals have reported that OHC levels measured in blubber samples are higher in mature males compared to females [Dorneles et al., 2010; Mwevura et al. 2010]. In our study, gender of sampled individuals was determined only for *T. aduncus*. Data on the most common contaminant levels (ng/g *lw*) measured in *T. aduncus* samples in relation to gender is presented in Table SI.8. Except for HCHs for which no clear differences could be found, all other contaminants were measured at significantly (p<0.0001) higher levels in males than in females. Lower concentrations recorded in mature females are most likely due to the transfer of hydrophobic OHCs from the mother to the offspring during gestation and lactation [Kajiwara et al., 2008]. Regarding T-Hg levels in *T. aduncus* skin, higher median levels were found in females compared to males (Table SI.8), although these differences were not statistically significant (p=0.197).

Organic contaminants and mercury levels in pooled fish samples

For T-Hg measurements, the sample size of the composite fish allowed determination from a number of 11 samples (Table SI.10). Although each of the fish species included in our study may be part of the dolphins' diet, the percentage contribution of each fish relative to the total dolphin diet could not be estimated. The OHC concentrations and profiles in the composite fish samples are presented in Table SI.9. The OHC profiles in composite fish samples were heterogeneous, but given their reported habitat, fish reported to reach deep sea/ocean waters, e.g. *Thunnus albacares* (F-02), *Euthynnus affinis* (F-03) or *Katsuwonus pelamis* (F-04), the percentage contribution of sum MeO-PBDEs relatively to the total OHCs is considerably higher than for the other fish species included in our study. Inversely, for fish species with a more coastal habitat, e.g. *Decapturus macarellus* (F-01), *Variola louti* (F-05) and *Lethrinus miniatus* (F-07), the percentage contribution of PCBs is significantly higher than of the other measured OHCs. Although very difficult to expand the results obtained for the composite fish samples in order to fully explain the contamination status of dolphins' blubber samples analyzed for this study, the reported habitat for the fish seems to explain the differences in the profiles of PCBs and MeO-PBDEs in dolphins.

Spinner and bottlenose dolphins use very distinct foraging habitats leading to differences on their diet [Kiszka et al. 2011], most probably the most important contamination pathway of marine mammals to OHCs. Considering the habitat use of the prey fish species, the profiles in the dolphin's diet are consistent with the higher contribution of naturally-occurring MeO-PBDEs in spinner dolphins and with dominance of anthropogenic PCBs in the *T. aduncus*.

However, after plotting the measured $\delta^{15}N$ values for each of the fish and dolphin species included in this study against *log*-transformed OHC concentrations (Figure 5), significant positive relationships ($0.001<p<0.01$) were found for all OHCs, except for the total HCH levels ($p=0.097$). Similar findings were observed between the measured $\delta^{13}C$ values and the *log*-transformed OHC concentrations (Figure SI-1).

The T-Hg results in the composite fish samples presented a larger variability than the OHCs and did not evidenced a clear relationship with the fish habitat (Table SI.10). The variability in the T-Hg levels might be due to differences in fish length [Kojadinovic et al., 2006a] or to differences in feeding strategies among fish species [Kojadinovic et al., 2008]. The highest T-Hg concentration combined to their low $\delta^{13}C$ values were observed in *Katsuwonus pelamis* followed by *Thunnus albacares* and *Euthynnus affinis* reflecting their oceanic lifestyle.

While differences in the contamination with OHCs could be explained based on the dolphins' habitat, the differences in the T-Hg values between mesopelagic and coastal dolphins remained difficult to explain.

Conclusions

Significant differences among the levels and profiles of investigated contaminants in the two dolphin species are possibly attributed to their dietary and foraging habitat preferences. Despite their spatial and temporal overlap in the waters of La Réunion, *S. longirostris* and *T. aduncus* accumulate differently contaminants. The higher contribution of the anthropogenic PCBs in *T. aduncus* could be explained by their coastal habitat, while the important contribution of the naturally-occurring MeO-PBDEs in *S. longirostris* is related to their offshore habitat. Our data highlight higher T-Hg concentrations in the coastal dolphins, evidencing the importance of volcanic activity of La Réunion as an emission source of Hg.

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656

Table 1. Median and range levels (expressed in ng/g *lw*) together with their detection frequencies (DF, expressed as percentage measured at levels above the method limits of quantification) of the most important contaminants targeted for analysis in terms of levels measured in blubber samples collected from the two dolphin species. Significant differences (p<0.05) between the two species are marked (*) for each contaminant with data presented in bold.

Target Compound	*Stenella longirostris* N=21			*Tursiops aduncus* N=32		
	DF, %	Median	Range	DF, %	Median	Range
Total lipids (%)		14.5	3.3 –46.5		12	3.0 – 51.7
PCB 99*	**89**	**15**	**<LOQ – 32**	**91**	**95**	**<LOQ – 1500**
PCB 101	95	21	<LOQ – 46	97	30	<LOQ – 710
PCB 153*	**100**	**175**	**3 – 390**	**100**	**1050**	**10 – 16000**
PCB 138*	**95**	**105**	**<LOQ – 245**	**94**	**580**	**<LOQ – 8900**
PCB 180*	**100**	**160**	**5 – 340**	**100**	**870**	**20 – 12500**
PCB 170*	**100**	**50**	**1 – 105**	**100**	**290**	**5 – 3900**
Σ PCBs*		**955**	**30 – 2170**		**5200**	**100 – 67500**
***pp'*-DDT**	100	50	7 – 145	97	85	<LOQ – 390
***pp'*-DDE**	100	350	40 – 1400	100	810	15 – 18500
pp'*-DDT/*pp'*-DDE		**0.14**	**0.06 – 0.30**		**0.07**	**0.01 – 0.74**
Σ HCHs*		**20**	**0.5 – 65**		**10**	**0.3 – 45**
BDE 47	95	20	<LOQ – 45	100	40	2 – 700
BDE 100	95	15	<LOQ – 30	84	45	<LOQ – 250
BDE 99	95	12	<LOQ – 30	88	7	<LOQ – 170
BDE 154	100	10	3 – 20	94	15	<LOQ – 100
BDE 153	84	5	<LOQ – 10	81	5	<LOQ – 50
Σ PBDEs		60	10 – 120		95	5 – 1200
2'-MeO-BDE68	100	935	140 – 4700	100	620	40 – 3800
6-MeO-BDE47*	**100**	**1040**	**135 – 3700**	**100**	**2280**	**70 – 25000**
Σ MeO-BDEs		2040	280 – 8450		3040	110 – 26130
T-Hg – skin (ng/g)*	**86**	**1410**	**284 – 3500**	**91**	**2850**	**723 – 6520**
T-Hg – blubber (ng/g)	29	939	534 – 2810	75	1350	342 – 4780
δ^{15}N (‰)		11.9	10.4 – 13.2		12.5	10.9 – 14.5
δ^{13}C (‰)		-16.3	-17.8 – -15.7		-16.0	-16.8 – -14.9

Table 2. Comparison between literature data and results of the present study on organohalogenated contaminants measured in blubber samples collected from bottlenose and spinner dolphins. Data is presented as median concentrations (ng/g lipids) and range levels (when units of measure are different, this is specified accordingly).

Dolphin specie	Sampling Information	ΣHCHs	HCB	ΣDDTs	ΣPCBs	ΣPBDEs	ΣMeO-PBDEs	Reference
Spinner dolphin, N=3 *Stenella longirostris*	Bay of Bengal, India 1990-1991	220 130–340	28 16–42	48000 32000–77000	1600 1100–2200	6.8 6.0–8.0	–	Kajiwara et al., 2006
Spinner dolphin, N=2 *Stenella longirostris*	Bay of Bengal, India 1990-1991	966	15	42100	1270	–	–	Tanabe et al., 1993
Spinner dolphin (stranded), N=3 *Stenella longirostris*	Philippines, 1996	110 66–190	220 110–430	16000 15000–17000	3600 2600–5400	36 20–64	–	Kajiwara et al., 2006
Bottlenose dolphin (biopsy blubber), N=64 *Tursiops truncatus*	Canary Archipelago, 2003-2011	147 <LOQ–855	48 <LOQ–3064	24236 189–1252210	30783 245–1016851	–	–	García-Alvarez et al., 2014
Bottlenose dolphin (stranded), N=3[a] *Tursiops truncatus*	Atlantic Ocean (South-West), 1994-2006	–	–	–	–	960 270–1350	19900 12500–32400	Dorneles et al., 2010
Spinner dolphin, (traped/killed), N=18 *Stenella longirostris*	Western Indian Ocean, 2000-2002	115 62–220	40 19–85	8900 1700–76000	–	–	58000 6800–210000	Mwevura et al., 2010
Indo-pacific bottlenose dolphin (traped/killed), N=18 *Tursiops aduncus*	Western Indian Ocean, 2000-2002	160 34–310	70 6–380	11500 500–93000	–	–	31000 600–190000	Mwevura et al., 2010
Spinner dolphin (stranded), N=10 *Stenella longirostris*	Pacific Island, 2007-2011	29.0 (<5.42–106)	134 (14.3–235)	2530 (267–15,700)	2090 (427–9730)	559 (46.4–10,100)	–	Bachman et al., 2014
Bottlenose dolphin (stranded), N=3 *Tursiops truncatus*	Pacific Island, 2009-2011	171 (27.9–210)	245 (144–745)	11,500 (9650–23,800)	7689 (7490–20,300)	1020 (927–1260)	–	Bachman et al., 2014
Spinner dolphin (biopsy blubber), N=21 *Stenella longirostris*	**Reunion Island, Indian Ocean, 2010-2011**	**20 0.5–65**	**8 1.5–29**	**432 49–1550**	**955 30–2175**	**60 10–120**	**2035 280–8450**	**Present study**
Indo-pacific **bottlenose dolphin (biopsy blubber), N=32 *Tursiops aduncus***	**Reunion Island, Indian Ocean, 2010-2011**	**10 0.3–45**	**8 0.2–51**	**837 26–19345**	**5200 100–67500**	**95 5–1200**	**2852 723–6516**	**Present study**

[a] – liver samples, results are given as mean values and range.

Figure 1. Map of Reunion island, showing the sampling locations of Spinner (*Stenella longitrostris)* and bottlenose (*Tursiops aduncus*) dolphin.

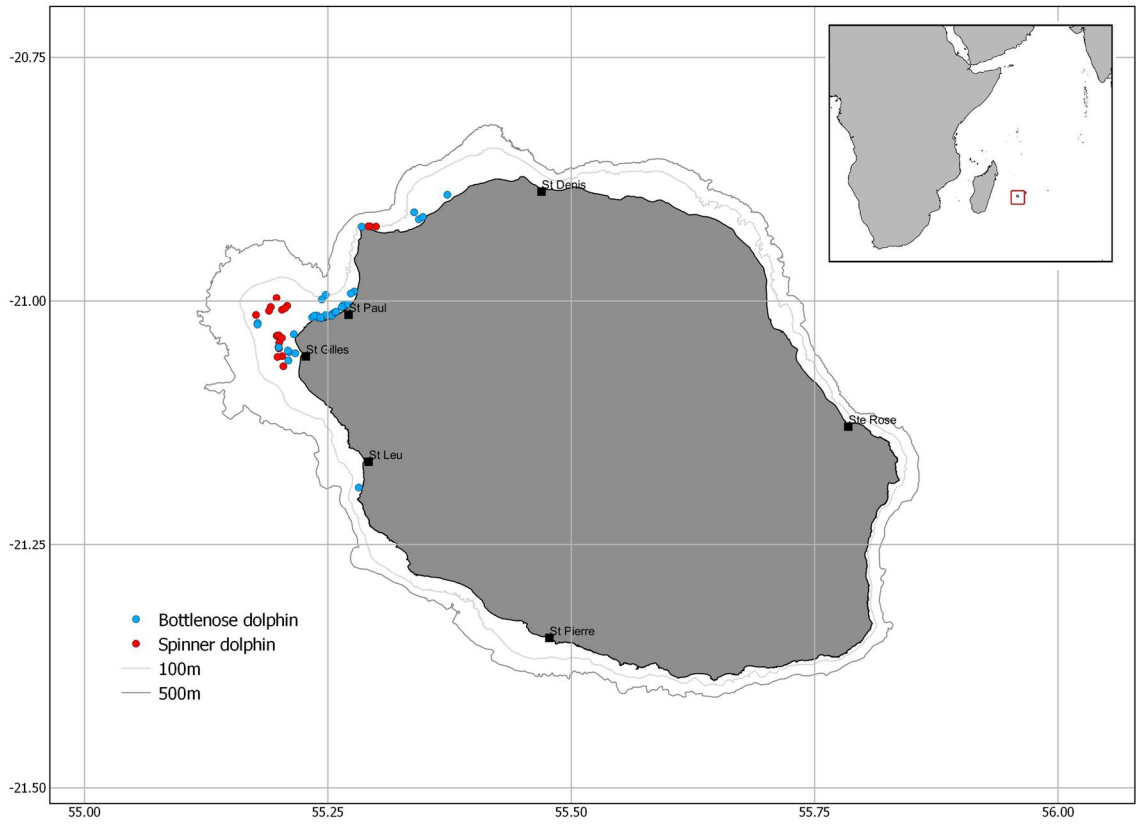

Figure 2. Profiles (percentage contribution to the sum concentration) of the organic contaminants function of the dolphin species included in this study (SL: *Stenella longirostris*; TA: *Tursiops aduncus*). The most important trends recorded for both species are highlighted in each graphical representation.

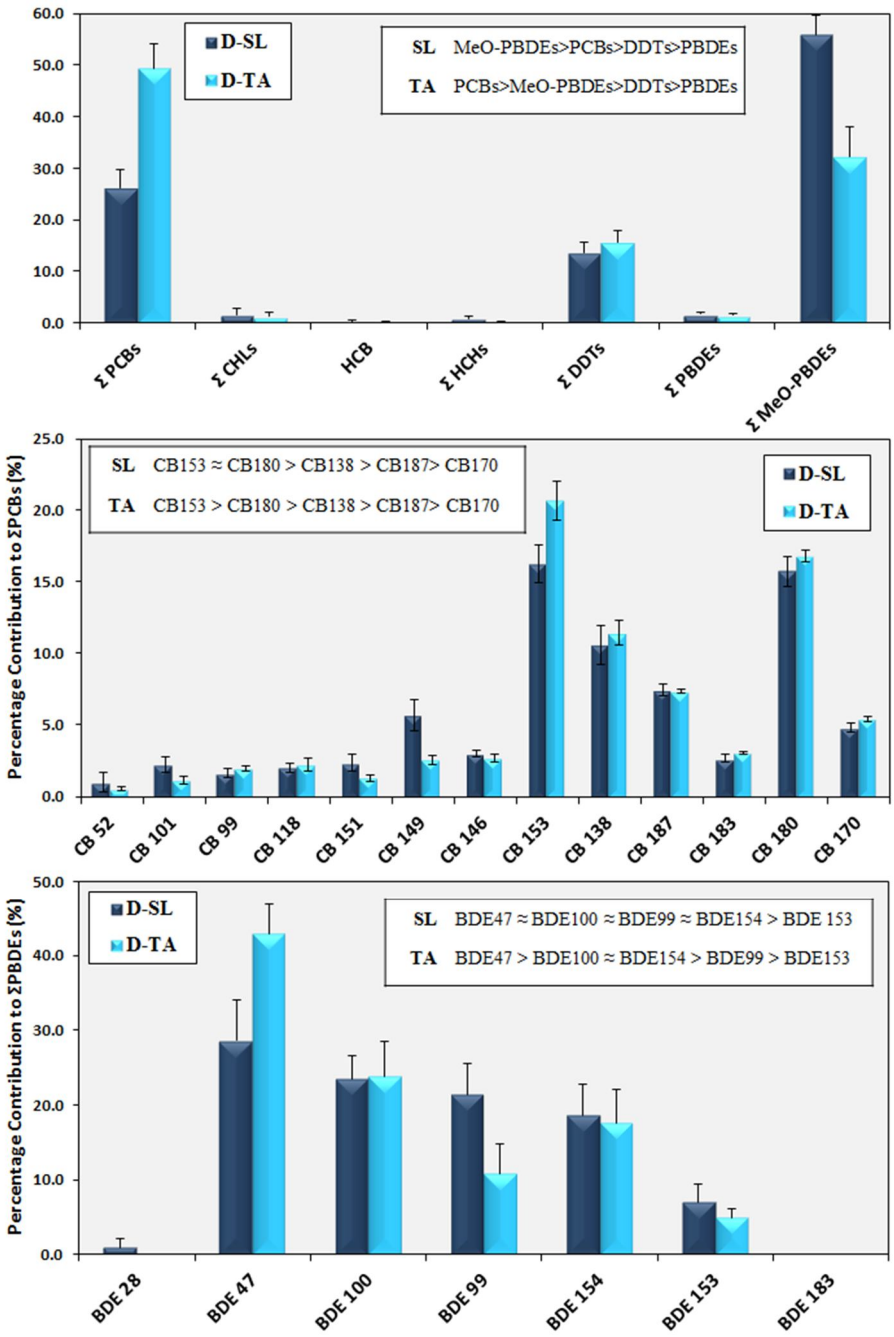

Figure 3. Distance biplot from the principal component analysis performed for organic contaminants measured in the blubber samples of two dolphin species (*Stenella longirostris* (SL) – 21 samples, *Tursiops aduncus* (TA) – 32 samples) from La Réunion Island (western Indian Ocean). Factor scores for each sample are presented as dots (● for SL dolphins and ● for TA dolphins), while factor loadings for the different compounds are presented as lines.

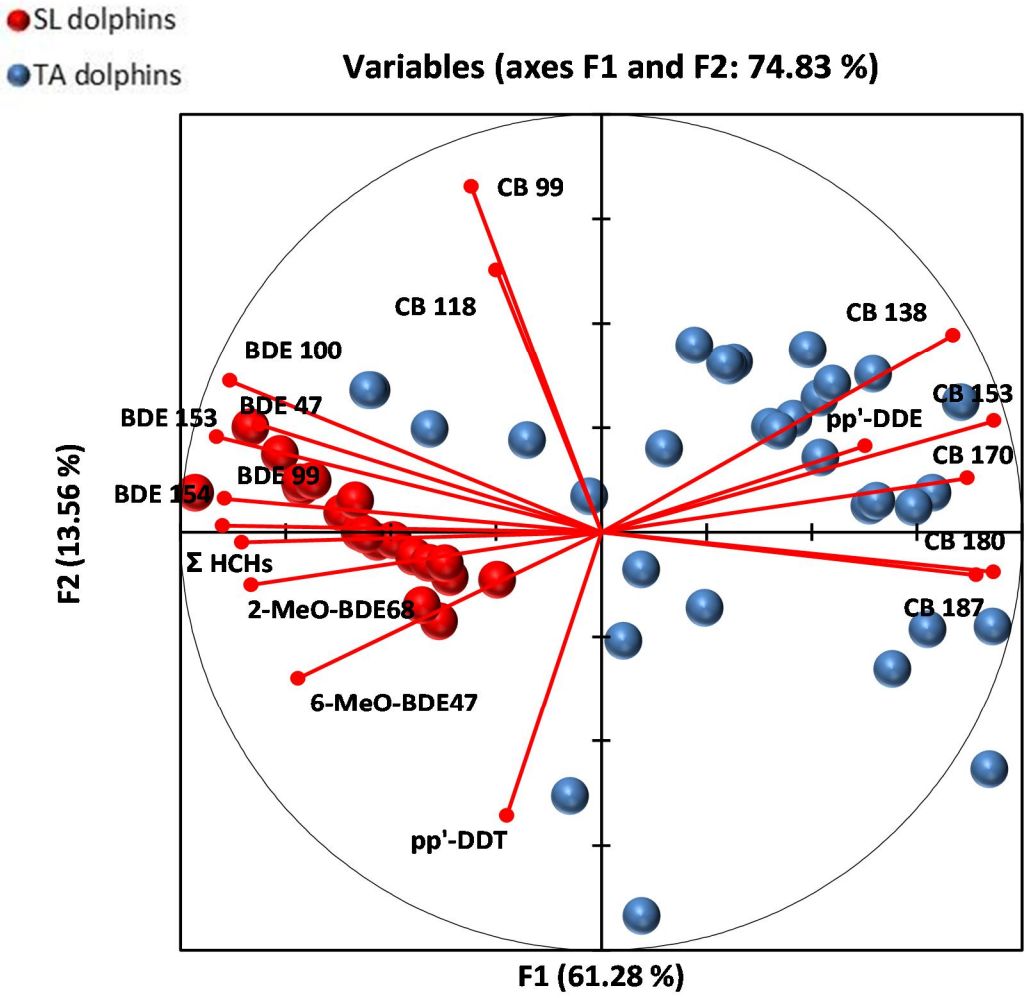

Figure 4. Relationship between $\delta^{15}N$ values and T-Hg concentration (logarithmic scale) in fish (muscle) and dolphins (skin) from la Réunion.

●*Tursiops aduncus;* ●*Stenella longirostris;* ●*Variola louti;* □*Decapterus macarellus;* ■*Gymnosarda unicolor;* ◆*Thunnus albacares;* ▯*Lethrinus miniatus;* ***o*** *Balistes capriscus;*△*Katsuwonus pelamis ;*▽Selar crumenophtalmus; ▲*Pseudanthias evansi;* ▼*Euthynnus affinis ;* ◇*Coryphaena hippurus*

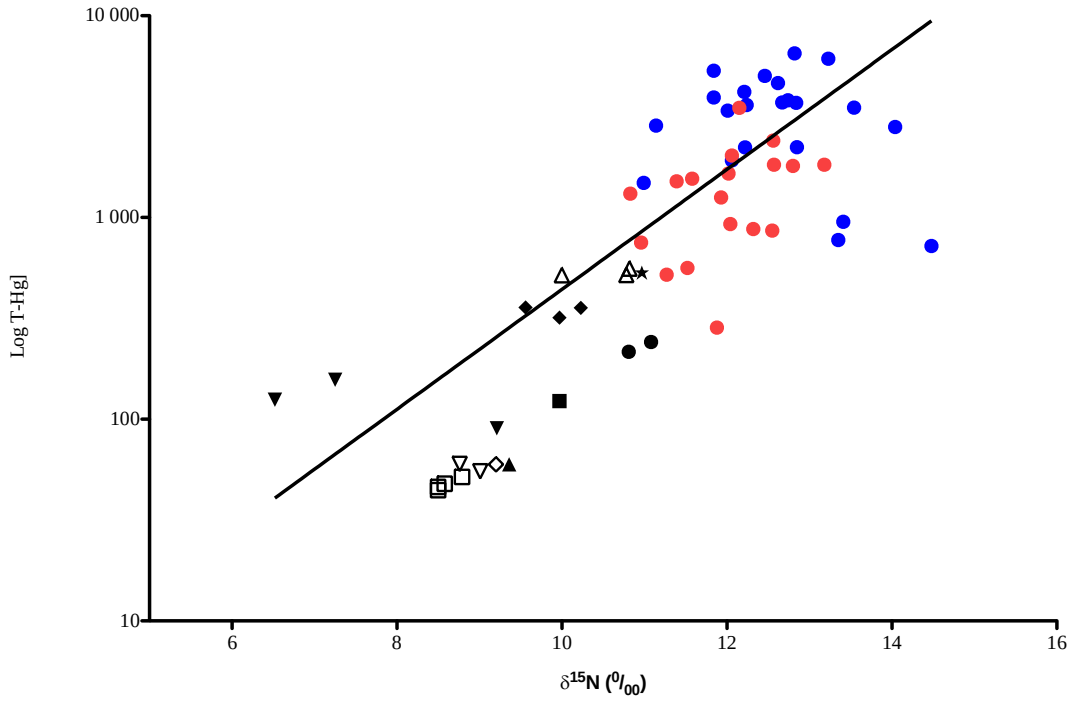

Figure 5. Relationship between $\delta^{15}N$ values and *log*-transformed concentrations for the sum of the most important contaminants measured in blubber samples collected from two dolphin species (*Stenella longirostris* (SL) – 21 samples, *Tursiops aduncus* (TA) – 32 samples) from La Réunion Island (western Indian Ocean).