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Assessing levels of halogenated organic compounds in mass-stranded long-finned pilot whales (*Globicephala melas*) from Australia

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HIGHLIGHTS

- Victims of mass-stranding events provide fantastic opportunities for assessing pollution.
- Levels and trends of POPs and MeO-PBDEs were investigated in long-finned pilot whales.
- Levels of DDXs were highest, levels of PBDEs were lowest
- There is a selective and changing transfer of chemicals during pregnancy.
- Younger animals have the highest concentrations of all compounds.

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ABSTRACT

Pollution is a threat to the health of marine mammals worldwide. Mass-strandings are poorly understood, but often involve pilot whales. However, there is limited information regarding pollution in long-finned pilot whales from Australia. Consequently, the profiles and levels of several pollutant classes were investigated in blubber of Tasmanian long-finned pilot whales. DDX levels were highest in all groups, followed by PCBs or MeO-PBDEs and lowest for PBDEs. The concentrations of all pollutants decreased with age in males. This is at least partly due to the growth dilution effect although it might also be caused by decreasing levels of PCBs, PBDEs, DDXs, HCB and CHLs in the environment. Fetus/mother ratios of higher chlorinated PCBs increased with the duration of pregnancy suggesting a preference for offloading via gestation rather than through lactation. Overall, the highest pollutant levels were found in the youngest animals.

1. Introduction

Processes such as run-off, atmospheric deposition and oceanic currents ensure that pollutants, like polychlorinated biphenyls (PCBs) or polybrominated diphenyl ethers (PBDEs) become a part of the aquatic food chains. Once these persistent chemicals are in the environment, they move into the food chain via uptake from water and diet thereby increasing in concentration with every step. These biomagnification processes for persistent chemicals together with the relatively long life spans of most top predator species can lead to elevated levels of pollutants in top predators like marine mammals (Burreau et al., 2006; Kelly et al., 2008; Weijs et al., 2009a). Over the years, high levels of pollutants have been linked to various health effects potentially affecting the overall survival of marine animals (e.g. Ross et al., 1996; Hall and Thomas, 2007; Mos et al., 2007; Sonne et al., 2009; Beineke et al., 2010; Frouin et al., 2010).

PCBs, PBDEs and other halogenated compounds have been produced and used until their impact on the environment and wildlife became apparent. Since then, the production of these chemicals has been banned or limited and studies have been performed to measure the damage done to environment and biota. There are studies reporting levels of pollutants and their effects in a wide range of species other than marine mammals (Law et al., 2006; Shaw and Kannan, 2009). These marine mammal species, however, deserve some special attention as they have been suggested to be sentinels of the ocean's health (Ross, 2000; Bossart, 2011).

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In Tasmania (Australia), mass stranding events involving long-finned pilot whale (*Globicephala melas*) are quite common and perhaps even more frequent than in other places (Rudolph and Smeenk, 2009; DPIPWE, unpublished). Mass stranding events are defined as events in which groups of cetaceans come ashore alive (Geraci and Lounsbury, 2009). Social cetaceans are more likely to be involved in mass-stranding events as the tight group or family bonds force the animals to follow one or more individuals without questioning (Geraci and Lounsbury, 2009). These mass stranding events often result in large number of mortalities. Several plausible explanations for the mass stranding events have been explored so far: Animals appear to be disorientated or ill which can have both natural (e.g. escaping from predators, anomalous magnetic fields, naturally occurring biotoxins) or anthropogenic (e.g. military sonar exercises, pollution) causes (Hall and Harwood, 2009). Previous studies have suggested that pollution can lead to impaired immune systems in marine mammals (Ross et al., 1996; Beineke et al., 2010), thereby resulting in a higher susceptibility for infectious diseases or an increased sensitivity towards the harmful effects of pollutant and biotoxin (domoic acid) exposure. Mass stranding events are not common for all cetaceans, but seem to occur mostly for Odontocetes or toothed whales (Hall and Harwood, 2009) which often have the highest concentrations of pollutants among all cetaceans (Houde et al., 2005). However, although mass-stranding events are traumatic experiences for the marine mammals, they provide fantastic opportunities for scientists to investigate pollution in these animals.

Long-finned pilot whales can live in pods of up to one hundred individuals or occasionally even larger associations. They are distributed antitropically in contrast to the short-finned pilot whales (*Globicephala macrorhynchus*) which can be found in tropical and subtropical regions (Olson, 2009). Little information is available about levels of pollutants in southern hemisphere long-finned pilot whales (Gaus et al., 2005). They live too far away from their northern hemisphere counterparts to reasonably assume a similar diet and/or exposure. Moreover, it is unknown if and how much the long-finned pilot whales resemble the short-finned ones in terms of metabolic biotransformation capacities for pollutants. Studies reporting levels of pollutants in short-finned pilot whales from any region or in long-finned pilot whales from the northern hemisphere are, therefore, probably not applicable for long-finned pilot whales from the southern hemisphere.

Because of the limited information available about pollution in long-finned pilot whales from Australia, there is a clear interest to investigate and quantify contaminant levels in Australian long-finned pilot whales. The objective of the present study was, therefore, to investigate the bioaccumulation of persistent organic pollutants (POPs) in these animals.

2. Materials and methods

2.1. Samples

Blubber samples were collected from 55 long-finned pilot whales (*G. melas*) from Sandy Cape (SC), Tasmania and 53 long-finned pilot whales from Stanley (S), Tasmania (Fig. 1). Body sizes were recorded for all animals, except for a fetus and its mother from SC. The age of the animals could not be assessed through counting dentine layers, but was estimated via the recorded body size of each animal and growth equations for long-finned pilot whales (Bloch et al., 1993). The animals were divided in groups according to their estimated age, gender and lactation status (for females). All animals were victims of the mass-stranding events at Stanley and Sandy Cape, Tasmania in November and December 2008, respectively. In addition, two long-finned pilot whale males from Robbins Island (RI), found stranded in January 2011, and five male long-finned pilot whales from Butlers Beach (BB; Bruny Island), found stranded in March 2011, were analysed as well (Fig. 1). In all samples, 37 PCB congeners (PCB 18, 28, 49, 47, 44, 52, 74, 66, 95, 101, 99, 87, 110, 118, 105, 149, 146, 132, 153, 138, 128, 167, 156, 187, 183, 174, 177, 171, 172, 180, 170, 199, 196/203, 194, 206, 209), 7 PBDEs (PBDE 28, 47, 49, 100, 99, 154, 153), 6 DDXs (*o*, *p*-DDT, DDE and DDD, *p,p′*-DDT, DDE and DDD), HCB, chlordanes (CHLs: OxC (oxychlordane), CC (cis-chlordane), TC (trans-chlordane), TN (trans-nonachlor), CN (cis-nonachlor)) and 5 MeO-PBDEs (6-MeO-BDE 49, 2-MeO-BDE 68, 6-MeO-BDE 47, 5-MeO-BDE 47 + 4-MeO-BDE 49) were targeted.

2.2. Sample preparation and analysis

The method used for the sample extraction and clean-up has been previously described (Weijs et al., 2010a) and is briefly presented below. Approximately 0.2 g of blubber was spiked with internal standards BDE 77, BDE 128 and CB 143 and extracted by hot Soxhlet for 2 h with hexane/acetone (3/1; *v/v*). After lipid determination (performed on an aliquot of the extract), the extract was cleaned on ~8 g of acidified silica and analytes eluted with 20 mL hexane and 15 mL dichloromethane. The cleaned extract was evaporated to dryness and reconstituted in 150 μL iso-octane. PBDEs, MeO-PBDEs and CHLs were measured by GC-ECNI/MS on a 30 m × 0.25 mm × 0.25 μm DB-5 column by monitoring ions $m/z = 79$ and 81 (for PBDEs and

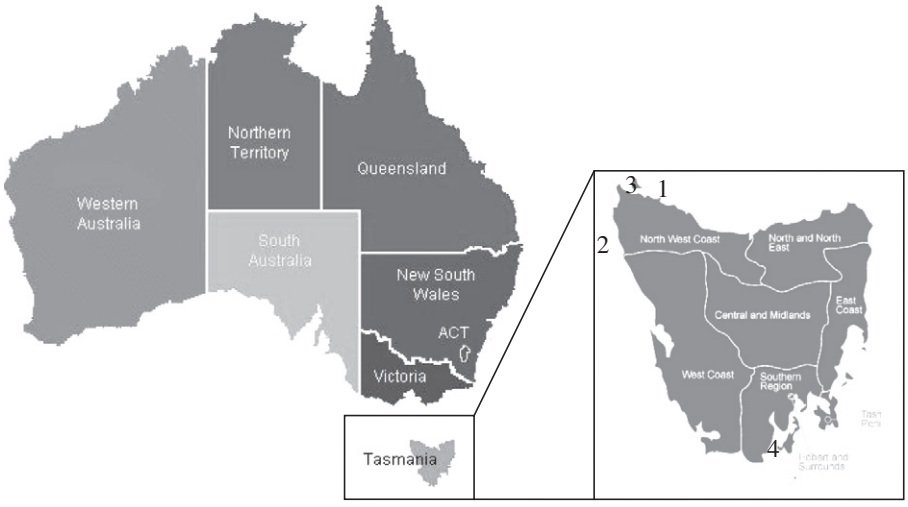

Fig. 1. Map of Australia and Tasmania showing the location of the long-finned pilot whale strandings. 1) Stanley, 2) Sandy Cape, 3) Robbins Island and 4) Butlers Beach.

MeO-PBDEs) and two most abundant and specific ions for each CHL. PCBs and DDXs were measured by GC-EI/MS on a 25 m × 0.22 mm × 0.25 μm HT-8 column by monitoring two most abundant and specific ions for each homologue group. This system was also used to confirm MeO-PBDEs.

2.3. Quality assurance/quality control (QA/QC)

Recoveries for individual PCB and PBDE congeners ranged between 75 and 104% (RSD < 12%). For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 4 ng/g lipid weight (lw). QC was performed by regular analyses of procedural blanks and by random injection of standards and solvent blanks. A standard reference material SRM 1945 (whale blubber) was used to test the method accuracy. Obtained values did not deviate more than 10% from the certified values.

2.4. Statistical analysis

Statistical analyses were conducted using the SPSS 18.0 statistical package (PASW Statistics 18). The level of statistical significance was defined at $p < 0.05$. For compounds detected in more than 50% of the samples, concentrations below LOQ were replaced by a value of f (frequency of detection) * LOQ. Only groups with samples sizes > 2 animals were included in the statistical analysis. Results were log-transformed in order to fit a normal distribution. The parametric ANOVA test was used to test the differences in lipid percentages, contaminant percentages and concentrations between the groups. Tests were also limited to the sums of PCBs, PBDEs, DDXs, CHLs and MeO-PBDEs, the most dominant compound of each contaminant class (PCB 153, PBDE 47, *p,p′*-DDE, TN and 6-MeO-PBDE 47, respectively) and the PCB groups (tetra, penta, hexa, hepta, octa, nona, and deca).

2.5. Groups

According to Olson (2009), long-finned pilot whale females reach sexual maturity at 8 years, males at about 12 years and lactation can go on for at least 3 years. The animals in the present study were therefore divided into groups following these life history guidelines. Females (F) or males (M) younger than 3 years were called juvenile (J) as they were probably drinking milk from their mothers, but also eating fish or squid occasionally. Sexually immature (I) females (age between 3 and 8 years) were those not drinking milk anymore, but reproducing. Sexually mature (A) females were females older than 8 years and this group can be divided into two subgroups, namely the animals that were lactating (L) at the time of death and those that were not (NL). Males with estimated ages between 3 and 12 years were the sexually immature (I) animals, whereas males older than 12 years were those considered to be sexually mature (A). Animals from RI and BB were pooled taking into account their respective age/gender group as the group sample sizes would otherwise have been too small to allow statistical comparisons with similar groups from other locations.

3. Results

3.1. Generalities

PCB congener 18 was not detected in any sample, whereas PCB 28, PCB 47, PCB 44 and TC (trans-chlordane) were detected in less than 50% of SC and S samples. Furthermore, PCB 209 and BDE 28 were detected in less than 50% of all SC samples, while PCB 49 and PBDE 49 were detected in less than 50% of all S samples. In addition to PCB 18, the congeners PCB 49, PCB 47 and TC were not detected in any animal from RI and BB and PCB 44 was only found in 1 out of 7 blubber samples of animals from RI and BB. PBDE 49 was detected in all 7 samples of animals from RI and BB in contrast to samples from SC and S, but was excluded from any further calculation or statistics to allow consistency between the number and type of compounds included in the mean and standard deviation calculations.

3.2. Overall contamination profiles

Fig. 2A (Sandy Cape) and B (Stanley and Robbins Island/Butlers Beach) show the overall contamination profile in all age–gender groups of the long-finned pilot whales from the present study. In all groups from all locations, DDXs represented the greatest proportion of the overall contamination profile (41–67%), whereas the proportions of PCBs and of MeO-PBDEs were often comparable. For tetra- and penta-PCBs, there were no differences in contaminant profile between the groups. For hexa-PCBs, statistically significant differences were found between juvenile females and lactating adult females from S ($p = 0.012$), between non-lactating adult females from S and SC ($p < 0.001$) and between juvenile and immature males from SC ($p = 0.009$). Contaminant profiles of hepta-PCBs in lactating adult females from S differed significantly from those in adult males ($p < 0.001$), juvenile females ($p < 0.001$), non-lactating adult females ($p < 0.001$) and pregnant adult females ($p = 0.001$) from S. There were also significant differences in proportions of hepta-PCBs between juvenile and non-lactating adult females from S ($p = 0.024$), between pregnant adult females and their fetuses from S ($p = 0.027$) and between juvenile and non-lactating adult females from SC ($p < 0.001$). The same differences were also found for octa-PCBs ($p = 0.001$, 0.008 and 0.001, respectively). For nona-PCBs, there were also differences between juvenile and non-lactating adult females from S ($p < 0.001$) and from SC ($p = 0.012$), but not between pregnant adult females and their foetuses from S ($p = 0.125$). In contrast to the hepta-PCBs, there was also a difference in octa-PCBs and nona-PCBs between lactating adult females from S and SC ($p = 0.001$). Percentages of HCB only differed significantly between juvenile and sexually immature males from SC ($p = 0.002$). For DDXs, differences were found between adult males and lactating adult females from S ($p < 0.001$) and between non-lactating adult females from S and SC ($p = 0.012$). CHL and PBDE contaminant profiles in adult males from SC differed from those in the same group of RI/BB and S ($p = 0.005$ and 0.001, respectively, for CHLs and $p = <0.001$ and <0.001, respectively, for PBDEs). Similar results were found for sexually immature males from RI/BB and SC ($p < 0.001$ for CHLs and PBDEs) and between non-lactating adult females from S and SC for CHLs only ($p = 0.001$). MeO-PBDE contaminant profiles only differed between adult males from RI/BB and S ($p = 0.012$). Contributions of PBDEs to the overall profile were <1%, except for the immature and mature males from RI/BB.

3.3. Levels of PCBs, PBDEs and MeO-PBDEs

Levels of PCBs, PBDEs and MeO-PBDEs for long-finned pilot whales from RI/BB are given in Table 3. When comparing the sexually immature and adult males, there was a statistical significant difference only in the levels of tetra-PCBs ($p = 0.005$). For animals from S (Table 2), the most notable differences were between the non-lactating and lactating adult females which had differences in tetra-PCBs ($p = 0.039$), PBDE 47 ($p < 0.001$), sum of PBDEs ($p = 0.001$) and sum of MeO-PBDEs ($p = 0.004$). The penta PCB mixture only differed significantly between the juvenile females and the non-lactating adult females. Levels of nona- and deca-PCB were different between the adult males and non-lactating adult females, between juvenile females and

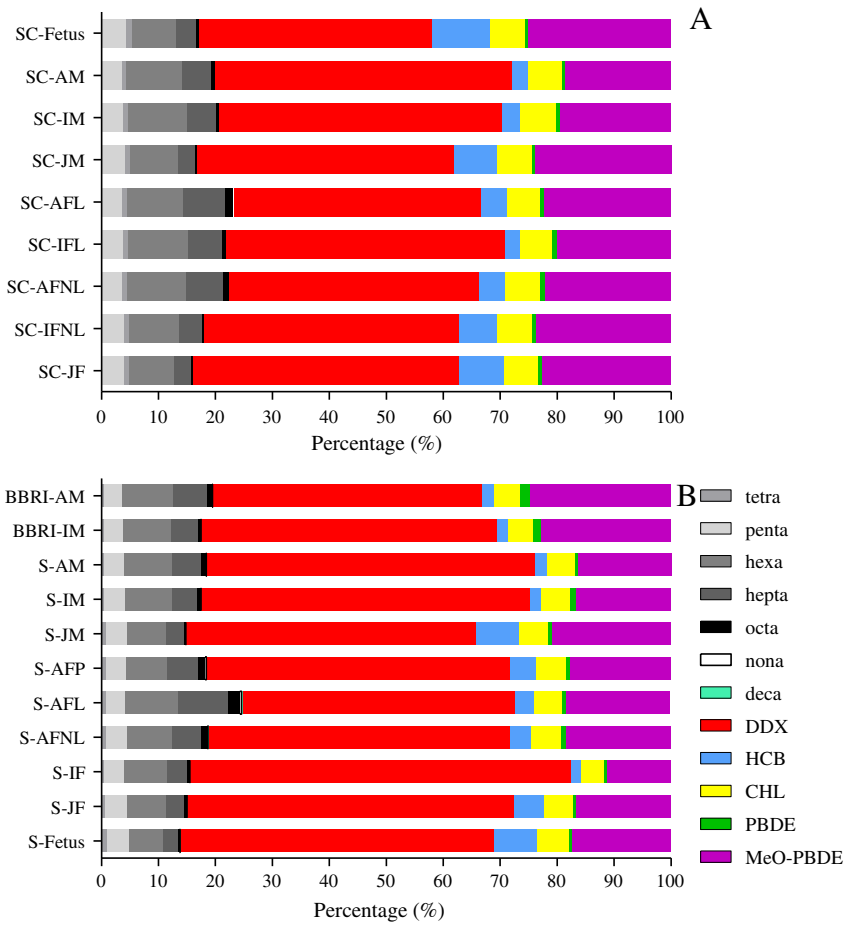

Fig. 2. Percentages of PCBs, PBDEs, MeO-PBDEs, DDXs, HCB and CHLs in blubber of A) 55 pilot whales from Sandy Cape (SC) and B) 53 pilot whales from Stanley (S), 2 pilot whales from Robbins Island (RI) and 5 pilot whales from Butlers Beach (BB). Animals from RI and BB were pooled according to age. I—sexually immature, A—sexually mature (adult), J—juvenile, F—female, M—male, NL—non-lactating, L—lactating, P—pregnant.

non-lactating adult females and between pregnant adult females and their fetuses. The octa-PCB concentrations in the fetuses were also significantly lower compared to their mothers ($p < 0.001$). MeO-PBDEs were found to differ significantly between the juvenile females and non-lactating adult females and between adult males and lactating adult females. For animals from SC (Table 1), there were statistically significant differences in PCB 153 levels between the juvenile females and the lactating adult females ($p = 0.027$) and between the immature females and the lactating adult females ($p = 0.028$). Levels of hepta- and octa-PCBs did not differ between the groups. However, there were differences between all other groups for all other compounds or sums tested. These differences could be attributed to the very low concentrations of contaminants in the lactating adult females.

To assess spatial trends, concentrations of PCBs, PBDEs and MeO-PBDEs were compared between adult males and the only statistically

Table 1
Concentrations, expressed in ng/g lw (lipid weight) in blubber of 55 long-finned pilot whales from Sandy Cape, Tasmania. Values are mean ± SD and minimum–maximum. I—sexually immature, J—juvenile, F—female, NL—non-lactating, L—lactating, A—sexually mature or adult, M—male.

	Fetus	JF	IFNL	AFNL	IFL[a]	AFL	JM	IM	AM
n	1	6	9	9	1	8	6	10	5
∑ PCBs[b]	80	676 ± 562 67–1382	418 ± 208 242–933	244 ± 150 77–472	306	167 ± 114 59–399	446 ± 223 97–709	404 ± 53 320–504	380 ± 88 287–512
∑ PBDEs[b]	3	25 ± 19 3–54	16 ± 7 10–31	8 ± 4 3–14	11	6 ± 4 2–15	17 ± 8 4–26	13 ± 3 10–20	10 ± 3 7–13
∑ MeO-PBDEs[b]	117	843 ± 561 105–1635	553 ± 262 292–1100	237 ± 142 82–431	280	157 ± 110 59–410	628 ± 297 157–1007	389 ± 72 267–492	353 ± 73 278–434
∑ DDXs[b]	191	2123 ± 1965 174–4748	1072 ± 623 543–2613	528 ± 408 133–1255	687	347 ± 311 92–1036	1229 ± 635 246–2071	983 ± 141 769–1177	997 ± 223 810–1366
∑ CHLs[b]	29	246 ± 205 27–530	144 ± 81 83–344	71 ± 51 20–152	78	43 ± 33 12–116	164 ± 88 38–287	125 ± 19 98–153	116 ± 19 92–139
HCB	47	244 ± 110 45–375	149 ± 69 78–254	47 ± 26 18–90	38	32 ± 24 15–86	171 ± 66 75–258	64 ± 24 23–113	52 ± 13 40–72

[a] In theory, this animal was not long enough to be an adult according to the growth equations from Bloch et al. (1993), but she was producing milk at the time of death.
[b] Information about the most dominant compound per class (per age/gender group) can be found in SI.

significant differences were found for tetra-PCBs ($p = 0.005$), BDE 47 ($p < 0.001$) and sum of PBDEs ($p = 0.001$). Levels of MeO-PBDEs were higher than sum of PCBs in the youngest animals compared to older animals, but MeO-PBDEs and PCBs reversed for the older animals from S and SC. Levels of PBDEs were lowest in all animals regardless of their location. At all locations, levels of PCBs, PBDEs and MeO-PBDEs were highest in the juvenile animals (male or female) and lowest in adult males and lactating adult females.

3.4. Levels of DDXs, CHLs and HCB

Regardless of the location, concentrations of DDXs were highest, followed by CHLs and HCB. Furthermore, concentrations of all compound classes were highest in the youngest animals and lowest in the oldest animals with emphasis on the lactating adult females. Despite this decrease in concentrations with age, no statistical significant differences were found between the groups for *p,p′*-DDE or sum of DDXs in the animals from S or RI/BB (all $p > 0.05$). The statistically significant differences between the DDXs in SC animals were all due to the very low concentrations in the lactating adult females. Furthermore, levels of DDXs did not differ between the three locations for the adult males. In contrast to DDXs, levels of HCB differed between the adult males and non-lactating adult females ($p = 0.005$), between juvenile females and non-lactating adult females ($p < 0.001$), between non-lactating adult females and lactating adult females ($p < 0.001$) and between lactating adult females and pregnant adult females ($p = 0.015$) from S. The low levels in lactating adult females from SC can be used as an explanation for the differences in HCB and CHL levels in animals from the SC. There were no differences in CHL levels between the groups from RI/BB whereas significant differences were found between juvenile females and non-lactating females ($p = 0.022$) and between non-lactating adult females and lactating adult females ($p = 0.029$) from S.

3.5. Gestational transfer of contaminants

There were 4 mother/fetus pairs of which the fetuses were, based on their body sizes, all in different stages of development (Table S1). In order to assess the transfer of pollutants from mothers to their offspring during gestation, fetus/mother ratios were calculated for all compounds studied. These ratios are shown in Fig. 3 for HCB and the sums of PCBs, PBDEs, DDXs, CHLs, MeO-PBDEs, but are given for each compounds separately in Table S1 (supporting information). Ratios for HCB were all >1 indicating a higher potential for bioaccumulation in the fetus compared to its mother (Fig. 3). In contrast, ratios of PBDEs were lowest (Fig. 3). If the body sizes of the fetuses are a good measure for their stage of development, it is worthwhile to note that ratios of PCB 101, 149, 132, 153, 138, 156, 183, 174, 177 and *o,p*-DDT follow the order of D (smallest fetus) < B < A < C (tallest fetus) (Table S1). In contrast, ratios of PCB 172, HCB, *o,p*-DDD, *p,p′*-DDD, OxC, CC, CN, PBDE 100, PBDE 99, 2-MeO-PBDE 68 and 6-MeO-PBDE 47 are lower in C than in D (Table S1).

4. Discussion and conclusions

To the best of our knowledge, this is the first study to report on several pollutant classes in victims of two mass-stranding events in Australia. Mass stranding events provide unique opportunities to study contaminant levels in a population in which all animals have been exposed to contaminants under similar environmental conditions.

4.1. General comparison with the literature

In the present study, there were almost no statistically significant differences for individual PCBs, PCB groups, the sum PCBs or other pollutant classes, between juvenile males and females and between

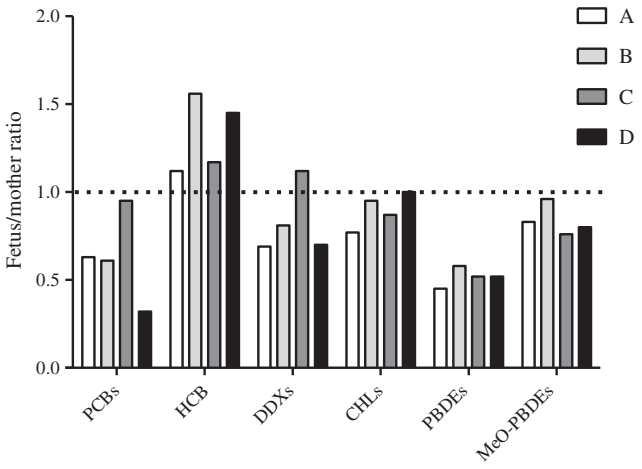

Fig. 3. Fetus/mother (F/M) ratios of PCBs, PBDEs, MeO-PBDEs, DDXs, HCB and CHLs in blubber of 4 fetus/mother long-finned pilot whales from Stanley, Tasmania. Gender of fetus, body size of mother and fetus as well as F/M ratios for all individual compounds are given in Table S1 (supporting information). The dotted line represents the threshold where fetuses have either higher contributions of a compound compared to their respective mothers (F/M ratio > 1) or where mothers have higher contributions of a specific compound compared to their fetus (F/M ratio < 1).

sexually immature males and females indicating that there is an equal bioaccumulation process in all animals as long as they are not reproducing (Weijs et al., 2009b). Compared to other marine mammal species such as harbour porpoises from the Black Sea and North Sea (Weijs et al., 2010a,b) and franciscana dolphins from Brazil (Leonel et al., 2010), the concentrations of the sum of PCBs in the pilot whales in the current study were more than 10 to 25 times lower. Apart from differences in diets and locations, harbour porpoises and franciscana dolphins are more coastal species than long-finned pilot whales which are living more in open waters. PCBs, as well as PBDEs, are known to penetrate the deeper waters as they were found in deep-diving species like sperm whales (de Boer et al., 1998). These compounds can, therefore, also be found in open waters. However, due to the differences in surface and volume of open waters versus coastal locations, levels of environmental pollutants are obviously more diluted in open waters than in coastal waters. Concentrations of PCBs and PBDEs are consequently higher in coastal species than in species inhabiting the open waters (Dorneles et al., 2010). The levels of PCBs in the long-finned pilot whales from the current study were higher than the concentrations of the sum PCBs in dugongs from the Northern Territory, sea lions from South Australia and sperm whales, beaked whales and pilot whales from Tasmania (Australia; Gaus et al., 2005) probably due to a combination of differences in diet among Australian marine mammal species and species-specific metabolic capacities for biotransformation of contaminants.

For PBDEs, BDE 47 was predominant in all animals from the present study. Similar to the sum of PCBs, the sum of PBDEs in the pilot whales was much lower than those in marine mammals worldwide (long-finned pilot whales from the Atlantic in Lindström et al. (1999) and Rotander et al. (2012a); harbour porpoises from the Black Sea and North Sea in Weijs et al. (2010a,b); Atlantic white-sided dolphin, hooded and ringed seal, harbour porpoise, minke and fin whale from the Atlantic in Rotander et al. (2012a) and delphinid species inhabiting the estuarine, coastal and open water areas from Brazil in Dorneles et al. (2010)). Low input levels of PBDEs in the Australian marine ecosystem were suggested earlier when Hermanussen et al. (2008) investigated PBDEs in marine turtles, dugongs and seafood from Australia, which is confirmed by the results of the concentration in the long-finned pilot whales from the present study. Despite the overall low PBDE levels, concentrations

in the long-finned pilot whales from the current study are still higher than levels reported by Hermanussen et al. (2008) in dugongs. However, this difference can possibly be attributed to the difference in diet between the dugongs and pilot whales. Considering the naturally produced MeO-PBDEs, levels of 6-MeO-BDE 47 were predominant, followed by 2-MeO-BDE 68 which is in contrast with previous reports for common dolphins, bottlenose dolphins, melonhead whales, pygmy sperm whales and humpback dolphins from Australia (Melcher et al., 2005) and for Atlantic spotted dolphins from Brazil (Dorneles et al., 2010). A predominance of 6-MeO-BDE 47 is in accordance with reports from the northern hemisphere (Rotander et al., 2012b). Because of the suggested different origins of both compounds (Vetter et al., 2002; Malmvärn et al., 2008) and a lack of information about migration of the pilot whales or movements about algae blooms and sponges, it is, however, difficult to point at a specific explanation. Most differences in levels of contaminants among the groups were caused by the lower concentrations found in the group of the lactating adult females in this study (Table 1). The levels of sum MeO-PBDEs in the pilot whales from Tasmania (Table 1) were (slightly) higher than those in harbour porpoises, harbour seals, long-finned pilot whales, Atlantic white-sided dolphins, hooded and ringed seals and Minke and fin whales from the Northern Hemisphere (Weijs et al., 2010a; Rotander et al., 2012b), similar to the levels in several marine mammal species from Australia such as common dolphins, bottlenose dolphins, melonhead whales, pygmy sperm whales and humpback dolphins (Melcher et al., 2005) and lower than the levels in marine mammal species (spotted dolphin, false killer whale, bottlenose dolphin, rough-toothed dolphin, common dolphin, spinner dolphin, striped dolphin, Fraser's dolphin) inhabiting the continental shelf and open ocean near Brazil (Dorneles et al., 2010).

p,p′-DDE had the highest concentration of the DDXs and most statistically significant differences involved the lactating adult female group. The lactating female group was significantly different for *p,p′*-DDE and the sum DDXs from all other female groups and from all male groups. With DDXs being detected in the fetus (Table 1) and in the milk of marine mammals (e.g. harbour porpoises; Weijs et al., 2010c), this finding was not a surprise. Compared to other marine mammal species and other areas around the globe, the pilot whales from the present study had lower concentrations of sum DDXs than harbour porpoises (Weijs et al., 2010a) and pilot whales from the Faroe Islands (Dam and Bloch, 2000), but similar concentrations to harbour porpoises from the North Sea (Weijs et al., 2010b) and other parts of Europe (Das et al., 2006), to franciscana dolphins from Brazil (Leonel et al., 2010) and several marine mammal species from Australia (Melcher et al., 2005).

Trans-nonachlor (TN) was the most dominant compound among the CHLs. For TN as well as for CHLs and HCB, the largest differences were found between the lactating adult females and the other groups. Information about CHLs and HCB in pilot whales is scarce. Levels of HCB in these pilot whales were lower than in harbour porpoises from the Black Sea (Weijs et al., 2010a). However, they were comparable to levels in harbour porpoises from the North Sea (Weijs et al., 2010b) and in pilot whales from the Faroe Islands (Dam and Bloch, 2000). The HCB levels in Tasmanian pilot whales (present study) were also comparable, and for some age classes even higher, than the levels found in franciscana dolphins from Brazil (Leonel et al., 2010). Levels of CHLs were lower in the Tasmanian pilot whales than in the pilot whales from the Faroe Islands (Dam and Bloch, 2000), whereas they were comparable to levels in harbour porpoises from the North Sea (Weijs et al., 2010b). For some age classes (e.g. the juvenile and sexually immature animals), levels of CHLs in the pilot whales from the present study were higher than the levels of CHLs in a dolphin species from Brazil (franciscana dolphin; Leonel et al., 2010).

Overall, levels of DDXs, CHLs and HCB in the Tasmanian long-finned pilot whales from the present study were in the same order of magnitude as reported in other marine mammal species from the southern hemisphere. Concentrations of PCBs, PBDEs and MeO-PBDEs were lower in the Tasmanian pilot whales compared to delphinids from Brazil. Long-finned pilot whales inhabit open oceanic waters, but besides that, there is hardly any information available about the migration of this species in Australian waters, which makes it hard to interpret the results in terms of ocean currents or local exposure.

4.2. Female pilot whales and reproduction

Adult females from Sandy Cape had similar contaminant profiles regardless of whether they were lactating or not, whereas adult females from Stanley differed only in the proportion of hepta-PCBs which was higher in the lactating adult females. The estimated ages for the adult females ranged from 7.5 to about 45 years, with the non-lactating adult females being the oldest. The similarity in contamination profiles independent of the age or lactation status is quite remarkable and may point towards a stable bioaccumulation profile coupled to a consistent and regular offloading of pollutants to their offspring throughout their lives. Long-finned pilot whales seem to reproduce more often than short-finned pilot whales (Kasuya and Marsh, 1984). Moreover, for several Odontocetes species, such as short-finned pilot whales and killer whales, it is not uncommon to find that non-reproductive older females are still producing milk for their offspring (Martin and Rothery, 1993). Throughout their pregnancy, long-finned pilot whale mothers seem to pass on increasing levels of higher chlorinated and persistent PCBs, such as PCB 153, 183 and 149, to their fetus during gestation. As a result, the fetus ends up with higher levels of those PCB congeners compared to its mother upon birth. According to Bloch et al. (1993), it is hard to draw a firm line between the body sizes of calves and fetuses. That study reported body sizes ranging between 163 and 191 cm for 49 fetuses and 39 calves of both genders and suggested a body size of 177.6 cm as the best estimate of the length at birth. The fetus of mother D (Table S1) is therefore definitely at the end of pregnancy. In contrast to the transfer of higher chlorinated and persistent PCBs during pregnancy, marine mammals seem to transfer more lower chlorinated PCBs via milk during lactation. In agreement with our findings, Debier et al. (2003) reported higher proportions of lower chlorinated PCBs than higher chlorinated PCBs in the milk of grey seals. A recent study also confirmed this and found stable percentages of hexa-PCBs in milk of grey seals (Vanden Berghe et al., 2012).

Milk samples of long-finned pilot whales were not available in the present study. Nevertheless, it looks like the conserved contamination profile in the long-finned pilot whale females, at least for PCBs, is a result of a balanced, selective and combined offloading of compounds during gestation and lactation. In both mass-stranding events, the lowest concentrations were found in lactating adult females. Multiple pregnancies with associated lactation periods are therefore capable of decreasing the levels of pollutants in the mothers, but are not capable of changing the contaminant profiles (Fig. 2). This opportunity for contaminant transfer to offspring probably stops as soon as the females are too old to reproduce. As mentioned previously, the non-lactating adult females were the oldest according to their body sizes and estimated age. In Tables 1 and 2, however, the levels of all contaminant groups are higher in the non-lactating adult females than in the lactating adult females although there were no changes in contaminant profile. Because of the higher levels, adult females might, therefore, face an increasing environmental stress later in life which is in contrast to the adult males of the same population.

There was a difference in contaminant profiles when comparing the adult females to the juvenile females and the sexually immature female groups. For the juvenile females, there was more similarity with the profile of the fetuses, whereas the profiles of the sexually immature females were intermediate between the profiles of the juveniles and

Table 2
Concentrations, expressed in ng/g lw (lipid weight) in blubber of 53 long-finned pilot whales from Stanley, Tasmania. Values are mean ± SD and minimum–maximum. I—sexually immature, J—juvenile, F—female, NL—non-lactating, L—lactating, A—sexually mature or adult, M—male, P—pregnant.

	Fetus	JF	IFNL	AFNL	AFL	AFP
n	4	6	1	16	6	5
∑ PCBs[a]	196 ± 154 45–368	830 ± 604 412–2005	942	393 ± 237 140–996	193 ± 69 119–316	292 ± 215 95–580
∑ PBDEs[a]	5 ± 3 2–9	31 ± 13 18–53	25	17 ± 7 5–27	6 ± 4 4–13	10 ± 6 5–16
∑ MeO-PBDEs[a]	218 ± 138 98–357	856 ± 462 392–1676	680	359 ± 161 134–653	144 ± 62 106–269	242 ± 127 123–381
∑ DDXs[a]	813 ± 655 201–1414	3678 ± 3612 1207–10,681	4061	1187 ± 901 353–3661	397 ± 225 245–808	949 ± 838 261–2038
∑ CHLs[a]	78 ± 59 26–135	276 ± 181 132–608	258	109 ± 63 37–214	41 ± 22 28–84	84 ± 66 26–176
HCB	77 ± 35 46–121	257 ± 126 83–416	102	67 ± 26 27–120	23 ± 5 17–31	56 ± 20 37–78

	JM	IM	AM
n	1	3	11
∑ PCBs[a]	249	388 ± 239 199–657	320 ± 93 210–509
∑ PBDEs[a]	12	19 ± 7 13–27	10 ± 2 7–13
∑ MeO-PBDEs[a]	356	332 ± 122 242–471	272 ± 48 207–372
∑ DDXs[a]	862	1338 ± 984 591–2452	1020 ± 402 640–1931
∑ CHLs[a]	87	111 ± 60 61–177	83 ± 18 58–107
HCB	129	36 ± 7 27–42	35 ± 8 27–58

[a] Information about the most dominant compound per class (per age/gender group) can be found in SI.

the adults. The changes in contaminant profiles are generally characterized by an increase in PCB proportions and a decrease in HCB proportions, whereas percentages of DDXs and CHLs remain similar. With respect to the levels of all contaminant classes, all levels decreased from juvenile females to adult females in both populations because of the gestational/lactational transfer at older age as well as the growth dilution effect at a younger age. Also, there were no differences between the same groups of the Stanley and Sandy Cape populations. The highest levels in the youngest animals of both populations are results of concern, since these animals are still in a critical stage of development. Nevertheless, it is unknown what impact this might have on their development or survival on the longer term.

4.3. Bioaccumulation in males

In terms of bioaccumulation in males, concentrations of PCBs, PBDEs, MeO-PBDEs, CHLs, DDXs and HCB decreased with age. Elimination pathways, such as gestation and lactation do not exist for males. For these animals, there are a number of explanations as to why the concentrations of these chemicals decreased with age. For sexually immature animals, the growth dilution effect plays an important role as the animals experience a rather steep growth curve until the age of 10–15 years (Bloch et al., 1993). After that age, a possible explanation is the change in ability to metabolise and eliminate these chemicals that occurs across a lifespan. This would mean that juveniles have a limited capacity to eliminate the chemicals while adults may have much more capacity which is an explanation that deserves some more investigation. Another explanation would be the worldwide bans and control actions on several of these chemicals that have been introduced over the last decennia which may have lowered the input in the environment and decreased the exposure of this species.

4.4. Influence of location and time

Overall, there were little differences between the locations for the same groups. Furthermore, levels of pollutants between 2008 (Stanley and Sandy Cape) and 2011 (Robbins Island/Butlers Beach) were comparable leading to the conclusion that contaminant exposure does not seem to decline over a period of 3 years. But, regarding the long life

Table 3
Concentrations, expressed in ng/g lw (lipid weight), in blubber of long-finned pilot whales from Butlers Beach and Robbins Island, Tasmania. Values are mean ± SD and minimum–maximum. I—sexually immature, A—sexually mature or adult, M—male.

	IM	AM
n	3	4
∑ PCBs[a]	408 ± 136 252–499	286 ± 67 222–366
∑ PBDEs[a]	32 ± 12 18–41	26 ± 11 11–37
∑ MeO-PBDEs[a]	471 ± 119 366–601	355 ± 67 261–413
∑ DDXs[a]	1249 ± 628 633–1889	683 ± 137 486–788
∑ CHLs[a]	99 ± 35 66–135	68 ± 10 54–75
HCB	40 ± 21 19–62	29 ± 1 28–31

[a] Information about the most dominant compound per class (per age/gender group) can be found in SI.

spans of the animals and the decreasing levels with age in males, it would take longer than 3 years to see any decrease in pollutant levels.

4.5. Implications for health and survival

The levels of DDE found in these animals are similar to those found in harbour porpoises from various European waters which were shown to have adverse effects on the thyroids of the porpoises (Das et al., 2006). In laboratory animals, PCBs and other POPs also have an endocrine-disrupting potential even at low concentrations (Arsenescu et al., 2008; Lee et al., 2011). Although the toxicological understanding of dose response is limited in marine mammals and average concentrations of chemicals in the present study seem lower than concentrations reported worldwide, it is of concern that the highest levels of POPs were found in the youngest animals. It is unknown whether these levels are toxic for this particular group or whether these levels induce changes that are compromising the well-being of pilot whales in the longer term.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.04.090.

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