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**Ecological tracers and at-sea observations document the foraging ecology of
southern long-finned pilot whales (*Globicephala melas edwardii*) in
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Abstract :

The food and feeding ecology of the poorly known southern long-finned pilot whale (*Globicephala melas edwardii*) was investigated using ecological tracers (muscle and skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and total mercury, Hg) on individuals from two mass strandings together with at-sea observations of live animals in Kerguelen waters, southern Indian Ocean. Sightings of cetaceans from long-liners over 9 years (2003–2012) emphasized the regular occurrence of pilot whales in slope waters surrounding the archipelago. Tissue $\delta^{13}\text{C}$ values (a proxy of consumer foraging habitat) suggest that pilot whales fed in slope waters and in oceanic subantarctic waters over the last months preceding stranding. Tissue $\delta^{15}\text{N}$ values and Hg concentration (dietary proxies) indicate a high trophic position (~ 4.7) for the pilot whales, likely corresponding to a mixed diet of fish and squid (not crustaceans) of undetermined species. Both skin and muscle Hg concentrations were positively and linearly correlated to individual size with no concomitant $\delta^{15}\text{N}$ changes, which can be interpreted as a progressive Hg accumulation in tissues of individuals throughout life with no parallel dietary shift. Skin and muscle Hg concentrations were linearly and positively related; hence, Hg skin could be used as a proxy of Hg concentration in muscle (a main Hg reservoir of the body). Kerguelen southern long-finned pilot whales were less Hg contaminated than most pilot whale populations studied so far, thus suggesting that they are not at a high risk to Hg-induced damages in the remote islands of the Southern Ocean.

Keywords: stable isotopes; mercury; marine mammal

Introduction

Understanding the role of cetaceans in the structure and functioning of ecosystems is directly linked to a better knowledge of their foraging ecology that is often difficult to assess in the marine environment (Bowen 1997). Unlike breeding pinnipeds that can be handled ashore, cetaceans remain at sea all life long and, hence, investigating the food and feeding ecology of free-ranging odontocetes and mysticetes is especially challenging. Alternatively, mass stranding events offer a unique opportunity to collect large sets of biologically relevant data from dead specimens. Pilot whales (Genus *Globicephala*) are one of the most frequently reported cetaceans in mass strandings (Olson 2009). Two species of pilot whales are recognized: the short- and long-finned pilot whales (*G. macrorhynchus* and *G. melas*, respectively), with the latter species including two spatially segregated subspecies, namely *G. m. melas* in the North Atlantic and *G. m. edwardii* in the Southern Ocean (Olson 2009; Oremus et al. 2009). The overall biology of the northern subspecies is well known, e.g. mortality rate, life span (longevity up to 59 and 46 years in females and males, respectively) and adult length (up to 5.1 and 6.3 m in females and males) (Donovan et al. 1993). By contrast, the biology, including the food and feeding habits of the southern subspecies, is poorly documented. Dietary investigations on the southern long-finned pilot whale focused primarily on stomach content analysis of a few stranded individuals from Tasmania (Gales et al. 1992), New Zealand (Beatson et al. 2007a, b; Beatson and O'Shea 2009), Tierra del Fuego (Goodall and Galeazzi 1985; Clarke and Goodall 1994; Mansilla et al. 2012) and Brazil (dos Santos and Haimovici 2001), with no available information from the southern Indian Ocean. Cephalopods form the bulk of the food, but stomach content examination of stranded individuals suffers several drawbacks including overestimation of prey with hardly digestible elements that accumulate over time (e.g. cephalopod beaks), a bias towards the presence of near shore prey species, and the unknown health status of the animals (Santos et al. 2001).

Indirect trophic methods have been developed over the last 25 years to complement more conventional means. These methods use 'ecological tracers', such as stable isotopes, lipids and contaminants, and are all based on the common principle 'you are what you eat', i.e. the biochemical composition of molecules and tissues of consumers reflects that of their food in a predictable manner (Ramos and Gonzalez-Solis 2012). Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are increasingly popular to investigate the ecology of terrestrial and marine organisms. $\delta^{13}\text{C}$ values vary little along the food chain and are mainly used to determine primary sources in a trophic network (Kelly 2000; McCutchan et al. 2003). In the marine

environment, $\delta^{13}\text{C}$ values indicate consumer foraging areas (i.e. inshore versus offshore and pelagic versus benthic) or latitudinal variation in the contribution to food intake (Hobson et al. 1994, Cherel et al. 2000a; Pajuelo et al. 2012). In contrast, consumer tissues are enriched in ^{15}N relative to their food and $\delta^{15}\text{N}$ values are used as indicators of consumers' trophic positions (McCutchan et al. 2003; Vanderklift and Ponsard 2003). A main interest of the isotopic method is that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values provide dietary information over different temporal scales depending on tissue-specific isotopic turnover rates that range from a few days for plasma to several years for bone collagen (Dalerum and Angerbjörn 2005; Newsome et al. 2010).

Mercury (Hg) concentration can also be used as a biomarker of dietary preferences and trophic positions of consumers because the main source of Hg contamination is food and Hg biomagnifies along food webs, meaning that Hg level increases with increasing trophic position of consumers within a given ecosystem (Gray 2002; Ramos and Gonzalez-Solis 2012). Hg derives from both natural and anthropogenic sources. Owing to its high volatility and long atmospheric residence time, Hg reaches remote areas through long-range atmospheric transport, thus contaminating oceanic islands and Polar Regions (Fitzgerald et al. 1998). Hg levels vary with the marine environments, being enhanced in coastal waters relative to the surface ocean, higher at depths relative to oceanic surface waters and depending on oceanic water masses (Fitzgerald et al. 2007; Cossa et al. 2011). In addition, Hg is a persistent, toxic and highly mobile metal in the marine environment. In mammals, Hg toxicity is primarily manifested as central nervous system damage including sensory and motor deficits and behavioural impairments. Reliable toxicity data for marine mammals are scarce, but the slow rate of contaminant elimination and high contaminant levels of cetaceans make them good sentinel species for the monitoring of ecosystem health (Das et al. 2003).

Using a combination of visual observations from fishing vessels together with stable isotopes and Hg from tissues of stranded animals as ecological tracers, the main aim of this exploratory study was to investigate the food and feeding ecology of southern long-finned pilot whales in a remote archipelago (Kerguelen), where almost nothing is known about the species. Only a few records of pilot whales have been documented in the southern Indian Ocean, including Kerguelen waters (Robineau 1989; Robineau and Duhamel 2006). The isotopic method was validated in the area, with $\delta^{13}\text{C}$ values of predators indicating their foraging habitats (Cherel and Hobson 2007; Jaeger et al. 2010) and their $\delta^{15}\text{N}$ values increasing with trophic level (Cherel et al. 2010). In brief, $\delta^{13}\text{C}$ values decrease from inshore to offshore consumers and they decrease with increasing oceanic latitudinal foraging habitats of the predators, while $\delta^{15}\text{N}$ values increase in the order crustacean-eaters < small fish consumers < large fish and squid

consumers. The pilot whale isotopic niche was determined using two tissues that record trophic information at different time scales, with skin and muscle integrating periods of weeks and months, respectively (Browning et al. 2014, Newsome et al. 2010). Previous and ongoing investigations on Hg exposure showed a large range of Hg concentrations within marine organisms living in Kerguelen waters that indicates Hg biomagnification in the corresponding oceanic ecosystem (Bustamante et al. 2003a; Bocher et al. 2003; Blévin et al. 2013; Carravieri et al. 2013, 2014). Ontogeny, age and sex modulates the foraging ecology, and thus the isotopic signature of cetaceans (Das et al. 2004; Nino-Torres et al. 2006; Newsome et al. 2009), and feeding habits, age, size and sex affect their Hg levels (Das et al. 2003; Lahaye et al. 2006). Hence, influences of individual traits (sex and size) on foraging habitat ($\delta^{13}\text{C}$) and trophic position ($\delta^{15}\text{N}$) as well as influences of both individual traits and foraging ecology on Hg concentrations were examined on a fairly large numbers of southern long-finned pilot whales that stranded in 2006 and 2010 at Kerguelen Islands.

Materials and methods

At-sea observations

Sightings of cetaceans were recorded by fishery observers working on-board seven French long-liners that fished within the Exclusive Economic Zone off Kerguelen Islands. The fishery targets the Patagonian toothfish (*Dissostichus eleginoides*) in slope waters (500–2,500 m depths) surrounding the archipelago. Observers recorded the occurrence, number and location (longitude and latitude) of any cetacean found in the area. Then, the geo-referenced data were included into the Pecheker data base (Martin and Pruvost 2007). All the pilot whale records during the period 2003–2012 were extracted from the data base and the software R.2.15.3 ('base', 'RcolorBrewer' and 'fields'; R Development Core Team 2013) was used to build a repartition map of the species in Kerguelen waters (Fig. 1). Bathymetric data around the Kerguelen Plateau were extracted from the General Bathymetric Chart of the Oceans (GEBCO) at the British Oceanographic Data Centre (BODC) website.

Study sites, stranding events and tissue sampling

Fieldwork was carried out at Kerguelen Islands (southern Indian Ocean), which is located in the southern part of the Polar Frontal Zone, in the immediate vicinity of the Polar Front (Park and Gambéroni 1997). Two mass strandings of 91 and 135 southern long-finned pilot whales occurred on remote beaches of the Courbet Peninsula (49°21'S, 70°18'E) in October 2006 and

January 2010, respectively. The precise dates of the two events are unknown. Noticeably, most internal organs from the carcasses were already scavenged by seabirds, thus precluding the collection of stomach contents, and hence, direct dietary analysis. All the specimens but four ($n = 222$) were measured (total length, TL). Small individuals with $TL \leq 3.0$ m were considered as suckling calves as the average (maximum) TL at the onset of weaning are 2.2 (3.2) and 2.2 (3.5) m for females and males, respectively (Desportes and Mouritsen 1993). Tissue sampling was performed on 193 specimens ($n = 91$ and 102 in 2006 and 2010, respectively). Both skin and muscle samples were collected from most pilot whales ($n = 137$), but some specimens were sampled for skin or muscle alone ($n = 29$ and 27, respectively). Tissue samples were either stored in 70 % ethanol (2006) or kept frozen at -20 °C (2010) until laboratory analyses in France.

Stable isotopes, mercury and molecular sexing

Any remain of subcutaneous white adipose tissue was removed from skin samples with a scalpel. The 2006 skin and muscle samples were first oven dried at 50 °C for 48 h for ethanol evaporation. Then, samples from both years were freeze-dried during 48 h and then grinded in a mortar. Since lipids are depleted in ^{13}C relative to other tissue components (DeNiro and Epstein 1977), they were extracted from skin and muscle samples using cyclohexane following Chouvelon et al. (2011). Powdered subsamples were weighed (0.3–0.4 mg) with a microbalance and packed into tin containers. Relative abundance of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were determined with a continuous flow mass spectrometer (Micromass Isoprime) coupled to an elemental analyser (Euro Vector EA 3024). Results are presented in the usual δ notation (in ‰) relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors <0.15 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Between 5 and 15 mg of freeze-dried and powdered samples were analysed in an advanced Hg analyser spectrophotometer (Altec AMA 254) following Bustamante et al. (2006). Hg determination involved evaporation of Hg by progressive heating until 800 °C under oxygen atmosphere for 2 min 30 s and subsequent amalgamation on a gold trap. The net was heated to liberate the collected Hg that was measured by UV atomic absorption spectrophotometry. Samples were analysed for total Hg. All analyses were repeated 2–3 times until having a relative standard deviation <10 %. Accuracy was checked using certified reference material [Tort-2 Lobster Hepatopancreas, NRC, Canada; mean 0.27 ± 0.06 $\mu\text{g g}^{-1}$ dry weight (dw)]. Our

measured values were $0.29 \pm 0.02 \mu\text{g g}^{-1} \text{ dw}$ ($n = 56$). Blanks were analysed at the beginning of each set of samples and the detection limit of the method was $0.005 \mu\text{g g}^{-1} \text{ dw}$.

Sampled individuals were sexed genetically using polymerase chain reaction (PCR) of introns within the Zfx and Zfy genes (Shaw et al. 2003). DNA was extracted and purified from a 25 mg aliquot of skin with the extraction kit 'NucleoSpin®Tissue'. PCR was performed on purified DNA ranging from 0.1 to 136 ng in a 20 μl reaction volume containing 12.5 μl of pure water, 2 μl of 1**Taq* polymerase reaction buffer, 1.5 μM MgCl_2 , 150 μM dNTPs, 1.5 U of *Taq* polymerase (Invitrogen) and either 0.3 μM of primers ZFX0582 forward, ZFX0928 reverse (for Zfx/Zfy amplification) and SRY reverse or 0.06 μM of primer SRY forward (for SRY amplification, Rosel 2003). PCRs were performed as follows: 1 cycle for 30 s at 92 °C, then 35 cycles of denaturation for 30 s at 94 °C, annealing for 45 s at 51 °C and amplification for 45 s at 72 °C. Then, each sample was loaded on a 3 % agarose gel and the fragments were separated by electrophoresis. Zfx-/Zfy-specific primers (Online Resource 1) allowed differentiating males (2 bands, 339 basepairs (bp) for the X-specific band and 382 bp for the Y-specific fragment) from females (1 band).

Methodological issue and statistical analyses

Since the precise dates of the two stranding events were unknown, the effect of the 'quality' of the skin samples was visually assessed. Samples were considered either 'bad' if skin was altered and not easily differentiable from the subcutaneous connective tissue or 'good' if skin was easily differentiable. The influence of sample quality on skin $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values within each stranding event was tested with Welch's *t* tests using R.2.15.3 ('stats'). In both 2006 and 2010, skin samples of bad quality had significantly higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and significantly lower Hg values than good quality samples (Online Resource 2).

Muscle samples were then grouped according to the corresponding quality of skin samples and an identical quality effect was found for $\delta^{15}\text{N}$ and Hg values but not for $\delta^{13}\text{C}$ values in 2010 samples. In contrast, no significant differences between muscle samples of good and bad quality were found in 2006. Consequently, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values from good 2006 and 2010 skin samples, all 2006 muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values, all 2010 muscle $\delta^{13}\text{C}$ values, but only $\delta^{15}\text{N}$ and Hg values from good 2010 muscle samples were used for statistical analyses (Table 1).

Welch's *t* tests (two-sample unpooled *t* tests for unequal variances) were used to check if the two tissues (skin and muscle) differed or not in various parameters ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg). The influence of size (TL), sex and year of stranding on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values were investigated using generalized linear models (GLMs) using R.2.15.3 ('stats'). In models where Hg

concentrations were the response variable, $\delta^{13}\text{C}$ (but not $\delta^{15}\text{N}$ values, see ‘Results’) was also incorporated as an explanatory variable. Models were constructed by incorporating the different variables and biologically significant interactions. Correlations between explanatory variables were tested using Pearson’s correlation tests. The most parsimonious models were selected through forward selection according to the bias-adjusted Akaike’s Information Criterion (AICc), which is a small sample size adjustment (Akaike 1973; Burnham and Anderson 2002). As a general guideline, if AICc values differ by more than 2, the model with the lowest AICc value is the most accurate, whereas models with AICc values differing by less than 2 are fairly similar in their ability to describe the data, and the model including the least number of parameters (the simplest) is the most accurate (Lebreton et al. 1992). Residuals of selected models were checked for normality using Shapiro–Wilk test and Q–Q plots. The explained variation is reported for each model (Table 2). The relationship between Hg concentrations in skin and muscle was best described by a linear model. Values are mean \pm SD.

Results

At-sea observations

A total of 131 at-sea observations of southern long-finned pilot whales were recorded within the Kerguelen Exclusive Economic Zone during the period from 31 October 2003 to 12 December 2012 (~9 years). The number of individuals per sighting averaged 53 individuals and ranged from 1 to ~300 individuals. The resulting map (Fig. 1) highlights the presence of the species in slope waters surrounding the Kerguelen Archipelago. Seasonal differences between winter and summer were found neither in pilot whale repartition nor in differences in the number of their at-sea observations (weighted by fishing effort that was quantified using the number of hooks).

Stranded individuals

TL of stranded southern long-finned pilot whales averaged 4.2 ± 1.1 m with no significant differences between the 2 years (Welch’s t test, $t = 0.22$, $p = 0.826$). TL ranged from 1.6 to 6.9 m with a mode at 4–5 m (Fig. 2), which corresponds to the size range of the species (Bloch et al. 1993a, b). Skin and muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values during the 2 years are presented in Table 1. In 2006, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly lower in muscle than in skin, while Hg concentration was higher in muscle ($t = -12.32$, -10.68 and 5.12 , respectively, all $p <$

0.0001). In 2010, $\delta^{13}\text{C}$ value was again lower in muscle ($t = -5.21$, $p < 0.0001$), but $\delta^{15}\text{N}$ values of the two tissues were not statistically different and Hg concentration was higher in skin than in muscle ($t = -2.93$, $p < 0.010$). A statistical comparison of the 2 years showed that skin $\delta^{13}\text{C}$ values, but not skin $\delta^{15}\text{N}$ and Hg values, were different ($t = 5.16$, $p < 0.0001$), and that muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values were all significantly different between 2006 and 2010 ($t = 5.96$, -3.59 and 10.19 , $p < 0.0001$, <0.01 and <0.0001 , respectively).

Statistical analyses of the influence of individual traits (sex and TL) and year of stranding on isotopic values (Table 2) showed that the most parsimonious GLM selected by AICc values included the effect of year in explaining tissue $\delta^{13}\text{C}$ values, with $\delta^{13}\text{C}$ values being significantly higher in 2006 than in 2010 (see above). Skin $\delta^{15}\text{N}$ values were best explained by an effect of sex, TL and year, and muscle $\delta^{15}\text{N}$ values by an effect of TL and year. Tissue $\delta^{15}\text{N}$ values decreased with increasing TL in small individuals (≤ 3 m), but did not change with increasing TL in mature individuals (> 3 m, Fig. 3). Indeed, skin and muscle $\delta^{15}\text{N}$ values were significantly higher in small pilot whales (≤ 3 m) than in larger individuals (Welch's t tests, $t = 3.28$ and 3.61 , $p < 0.001$ and <0.01 , respectively).

Hg concentrations in skin and muscle were positively and linearly related, with linear models explaining 68 and 50 % of Hg variability in 2006 and 2010, respectively (Fig. 4). Given the size-induced effect in tissue $\delta^{15}\text{N}$ values (see above), the effect of $\delta^{15}\text{N}$ values on tissue Hg concentrations was tested separately on mature individuals only. There was no correlation between Hg concentrations and $\delta^{15}\text{N}$ values in skin ($F_{1,60} = 0.146$, $R^2 \text{ adj} = 0$, $p = 0.704$), whereas in muscle a negative relationship was highlighted ($F_{1,75} = 19.33$, $p < 0.001$), but the explained variance was very low ($R^2 \text{ adj} = 0.19$). The influence of individual traits (sex and TL), year of stranding and $\delta^{13}\text{C}$ values on tissue Hg concentrations was tested through GLMs. For skin, the most parsimonious model selected by AICc values included the effect of TL and sex in explaining Hg values (Table 2). Females had significantly higher skin Hg concentrations than males (4.8 ± 2.1 vs. $3.6 \pm 2.7 \mu\text{g g}^{-1} \text{ dw}$), with concentrations increasing with TL (Fig. 5). For muscle, the best fit to the Hg data was obtained for the maximal model, which included the effect of sex, TL, year, $\delta^{13}\text{C}$ values and their interactions (Table 2). Muscle Hg concentration was significantly higher in females than in males (6.0 ± 2.8 vs. $5.1 \pm 3.1 \mu\text{g g}^{-1} \text{ dw}$) and higher in 2006 than in 2010 (Table 1).

Discussion

Foraging ecology and at-sea distribution

Sightings from long-liners emphasize the regular occurrence of southern long-finned pilot whales in slope waters around the Kerguelen Archipelago (Fig. 1). These at-sea observations were highly biased towards the Patagonian toothfish fishing grounds. However, the few previous sightings again points out the importance of slope waters for pilot whales in the area, with rare observations near the Kerguelen coastline (Robineau 1989; Robineau and Duhamel 2006). These observations are in agreement with long-finned pilot whales inhabiting deep oceanic waters and zones of higher productivity along the continental and island shelf breaks, apparently venturing sometimes into neritic waters (Abend and Smith 1999).

Muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of long-finned pilot whales from Kerguelen Islands are similar to the isotopic data from Australian specimens (Davenport and Bax 2002). However, spatial variations in isotopic baselines preclude direct comparison of raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of pilot whales living in different water masses (Table 3). In contrast, isotopic comparisons together with the known food and feeding ecology of air-breathing vertebrates from Kerguelen Islands helped delineating the whale foraging habitat ($\delta^{13}\text{C}$) and dietary habits ($\delta^{15}\text{N}$). In brief, many investigations were conducted over the last 20 years on various Kerguelen seabirds (e.g. penguins, albatrosses and petrels) and pinnipeds (e.g. elephant seal and fur seals) and their prey using a variety of complementary methods (dietary analyses, bio-logging and stable isotopes) thus allowing defining relevant control species from different habitats (benthic versus pelagic, coastal versus offshore) and with different diets (crustaceans, cephalopods and fish) (Online Resource 3).

The relatively high muscle $\delta^{13}\text{C}$ values of Kerguelen pilot whales preclude feeding in Antarctic waters (Cherel 2008). Instead, their muscle $\delta^{13}\text{C}$ values are similar to those of the Patagonian toothfish that feed over the Kerguelen outer shelf and slope waters (Cherel et al. 2008, unpublished data). Alternatively, since inshore-offshore and latitudinal $\delta^{13}\text{C}$ gradients overlap in the area (Cherel and Hobson 2007), the $\delta^{13}\text{C}$ values may also suggest that pilot whales migrated from the subtropical to the subantarctic zone. However, the lack of associated high muscle $\delta^{15}\text{N}$ values resulting from high $\delta^{15}\text{N}$ baseline level in the subtropics (Altabet and François 1994; Lourey et al. 2003) does not support the migration hypothesis. Hence, the most parsimonious explanation of their muscle $\delta^{13}\text{C}$ values is that southern long-finned pilot whales foraged primarily over the Kerguelen shelf break and nearby subantarctic oceanic waters in the months preceding their stranding, which is in agreement with at-sea observations of the species (see above).

Muscle $\delta^{15}\text{N}$ value of pilot whales was much higher than that of crustacean-eaters from Kerguelen Islands, namely the southern rockhopper penguin and Antarctic and thin-billed prions (Cherel et al. 2010, Online Resource 3). The whale $\delta^{15}\text{N}$ value was 1 ‰ above that of the myctophid fish consumers king penguin and Antarctic fur seal and 1 ‰ below the $\delta^{15}\text{N}$ value of black-browed albatross, which has a catholic diet including large fish and squid (Cherel et al. 2000b, 2008, 2010). Accordingly, pilot whale muscle Hg concentration was two and one order of magnitude higher than those of planktivorous petrels and of the crustacean- and myctophid-eater blue petrel, respectively (Bocher et al. 2003). Furthermore, the pilot whale Hg concentration was two times higher than that of the white-chinned petrel, which has a more diverse diet, including various fish and squid species (Delord et al. 2010; Cipro et al. 2014). Hence, both trophic markers ($\delta^{15}\text{N}$ and Hg) indicate that pilot whales did not prey primarily upon crustaceans, but favoured a mixed diet of fish and squid in Kerguelen waters. While prey cannot be determined at the species level using $\delta^{15}\text{N}$ values, feeding on a large spectrum of fish and squid is in general agreement with stomach content analysis of long-finned pilot whales elsewhere (Desportes and Mouritsen 1993; Pauly et al. 1998; Spitz et al. 2011). The trophic position of the long-finned pilot whale within the Kerguelen oceanic ecosystem can be estimated at 4.7, using the combination of the mean whale muscle $\delta^{15}\text{N}$ value, an assumed trophic level of 2.0 for the herbivorous salp *Salpa thompsoni* with a 3.4 ‰ $\delta^{15}\text{N}$ value (Cherel et al. 2008), and a 2.7 ‰ ^{15}N enrichment factor between prey and cetacean muscle (Borrell et al. 2012). The trophic position compares well with those previously estimated for the northern subspecies using muscle isotopic measurements (4.9), standardized diet composition (4.4) and ecosystem modelling (4.7) (Pauly et al. 1998; Lassalle et al. 2014). In Kerguelen waters, the southern long-finned pilot whale is a top consumer close to other marine mammals (e.g. southern elephant seal, 4.6) and large fish (e.g. Patagonian toothfish, 5.0), but the albatross trophic position is higher (5.3–5.7) and the oceanic ecosystem is dominated by the colossal squid (6.1; Cherel et al. 2008).

Tissue-related isotopic differences result from two nonexclusive explanations, namely different biochemical compositions leading to tissue-specific isotopic discrimination factors, and tissue-specific isotopic turn-over rates inducing different trophic temporal integrations (Wolf et al. 2009). Little or no differences were found in the isotopic ratios of lipid-extracted skin and muscle of cetaceans and hence in their discrimination factors (Borrell et al. 2012; Horstmann-Dehn et al. 2012). Consequently, the consistent ~ 0.7 ‰ $\delta^{13}\text{C}$ difference between the two tissues of southern long-finned pilot whales suggests a temporal shift associated with a spatial change in the main foraging habitat, since skin and muscle integrate periods of weeks and months,

respectively (Newsome et al. 2010; Browning et al. 2014). However, isotopic variability of Kerguelen southern long-finned pilot whales overall remained low ($<1\%$, Table 1) within and between tissues and when compared the two stranding events. Such a low degree of isotopic variability suggests no major changes in the whale feeding ecology in Kerguelen waters over the medium- and long-term (skin versus muscle, and 2006 versus 2010, respectively). Interestingly, most small individuals presented higher skin and muscle $\delta^{15}\text{N}$ values than larger pilot whales. An ontogenetic isotopic shift associated with nursing and weaning was previously described in pinnipeds and cetaceans, which can be explained by the consumption of milk by calves and the resulting higher trophic position ($\delta^{15}\text{N}$) of the young relative to its mother (Ducatez et al. 2008; Newsome et al. 2009). In pilot whales, juveniles are weaned during a protracted period during which they progressively increase their consumption of natural prey (Desportes and Mouritsen 1993), thus inducing a concomitant $\delta^{15}\text{N}$ decrease down to adult values. Neither tissue $\delta^{13}\text{C}$ nor $\delta^{15}\text{N}$ values changed with size thereafter, suggesting no major foraging variations associated with growth and age after weaning in Kerguelen pilot whales (Fig. 3).

Hg exposure

Exposure to Hg was previously investigated in many species of marine mammals, with liver exhibiting consistently the highest Hg concentrations, followed by kidney, muscle and skin (Thompson 1990; Das et al. 2003). Accordingly, long-finned pilot whales presented relatively low and almost similar Hg levels in muscle and skin samples (Frodello et al. 2000, this study). Cetacean skin is a fragile and highly vascularised surface tissue in which Hg is first sequestered into keratin and then eliminated by epidermal sloughing (Wagemann and Kozłowska 2005). Despite Hg excretion by desquamation, positive correlations had been reported between Hg levels in skin and internal organs like liver (e.g. Dall's porpoises and small delphinids in Yang et al. 2002 and Aubail et al. 2013, respectively). The equation between Hg levels in skin and liver of delphinids (Aubail et al. 2013) allows calculating an estimated mean value and range of 494 ± 490 ($0.3\text{--}2,210$) $\mu\text{g g}^{-1}$ dw in the liver of southern long-finned pilot whales from Kerguelen Islands. Within that context, the present study showed for the first time significant positive correlation between skin and muscle Hg values in cetaceans (Fig. 4). Hence, skin biopsy may be used as a non-destructive tool for assessing Hg concentrations of free-ranging pilot whales and for predicting their Hg concentrations in liver and muscle that are the two main Hg body reservoirs in marine mammals (Yamamoto et al. 1987).

Hg burden was not previously investigated in the southern long-finned pilot whale but was determined in the northern subspecies and in the congeneric short-finned pilot whale (*Globicephala macrorhynchus*) that lives in warmer waters. Muscle Hg concentration of pilot whales from Kerguelen Islands was close to the levels measured in populations inhabiting the Faroe Islands and northern Japan. It was 2.5 times lower than Hg level in specimens from the Lesser Antilles and 4.4–7.8 times lower than Hg concentrations in animals living in the Mediterranean Sea (Corsica), southern Japan and New Caledonia (Table 3). Local biogeochemistry, geology and anthropogenic activities indirectly affect Hg content of cetaceans through Hg concentrations of their prey. For example, pelagic organisms are naturally Hg enriched in the Mediterranean Sea due to high organic Hg availability, and consequently Mediterranean predators, including cetaceans, contain high Hg levels (Cossa and Coquery 2005; Savery et al. 2013). Conversely, the comparatively low Hg burden of Kerguelen pilot whales can be explained by the combination of low organic Hg availability in subantarctic waters (Cossa et al. 2011) together with the absence of human activities releasing Hg in the area. Within odontocetes, the Kerguelen long-finned pilot whale was less contaminated or their muscle Hg concentrations were within the same range of values than most species so far investigated, but their muscle Hg level was consistently higher than that of mysticetes (Thompson 1990; Endo et al. 2003, 2004, 2012). These taxa-related differences can be explained by different feeding habits, with baleen whales generally preying upon lower trophic level prey than toothed whales, i.e. crustaceans and small fish *versus* squid and larger fish, respectively.

Significant variations in Hg burdens occurred between the two mass-stranding events. Muscle Hg concentration was three times lower in 2010 than in 2006, with no corresponding change in skin values. These inter-annual differences are difficult to interpret, but they might result from differential Hg turnover rates between skin and muscle that remain to be determined. Tissue Hg concentrations were positively correlated to individual TL in southern long-finned pilot whales, which is consistent with previous findings on cetaceans (Honda et al. 1983; Thompson 1990; Leonzio et al. 1992). Since tissue $\delta^{15}\text{N}$ values did not increase with TL, the size-related Hg change was not a consequence of a progressive dietary shift and there was therefore a decoupling between Hg concentrations and trophic position. Compared to males, female pilot whales grow more slowly, are smaller (mean adult lengths 4.5 versus 5.7 m) and live longer (up to 59 and 46 years, respectively) (Bloch et al. 1993b). Hence, for a given body size, females are older than males and, accordingly, more Hg contaminated (Fig. 5). Overall, skin and muscle

Hg concentrations were also higher in females, which is in agreement with higher liver Hg levels in females than in males of the northern subspecies (Caurant et al. 1993).

In conclusion, both ecological tracers ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, Hg) and at-sea observations indicate that Kerguelen southern long-finned pilot whales foraged primarily in slope waters and oceanic subantarctic waters where they occupy a high trophic position. Their precise feeding habits remain to be determined, but it is likely that they prey on a mixed diet of fish and squid in the deep sea, as other populations of pilot whales do (Aguilar de Soto et al. 2008; Spitz et al. 2011). Muscle Hg concentration of southern long-finned pilot whales is close to or lower than that of many odontocete species. Noticeably, they are less Hg contaminated than most pilot whale populations studied so far, thus suggesting that the southern subspecies is not at a high risk to Hg-induced damages in the remote islands of the Southern Ocean.

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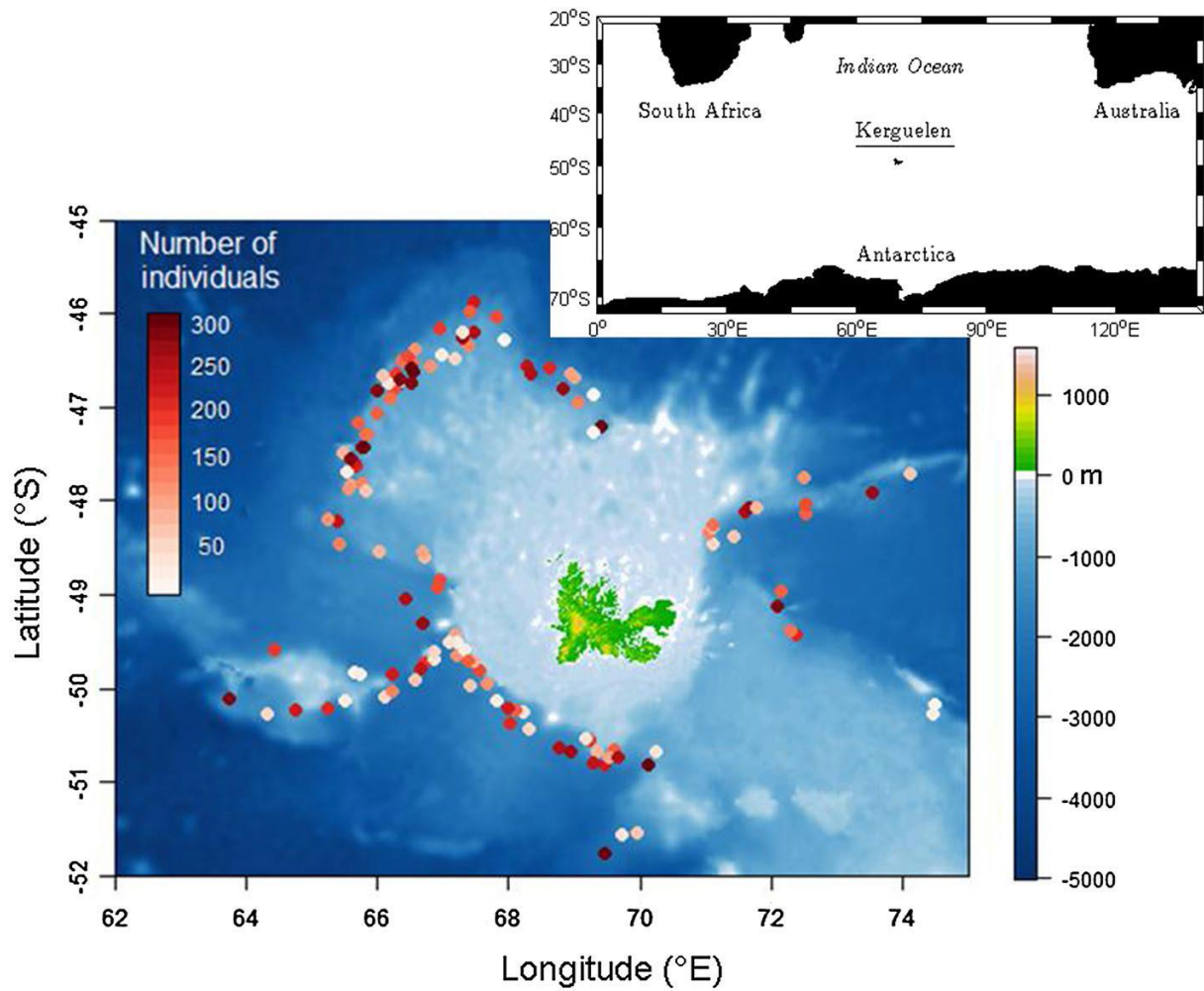


Figure 1. Location of Kerguelen Islands within the Indian Ocean (*inset*) and spatial at-sea observations of long-finned pilot whales from long-liners targeting Patagonian toothfish in Kerguelen waters during the period 2003–2012. *Colour scale on the right* indicates bathymetry (m)

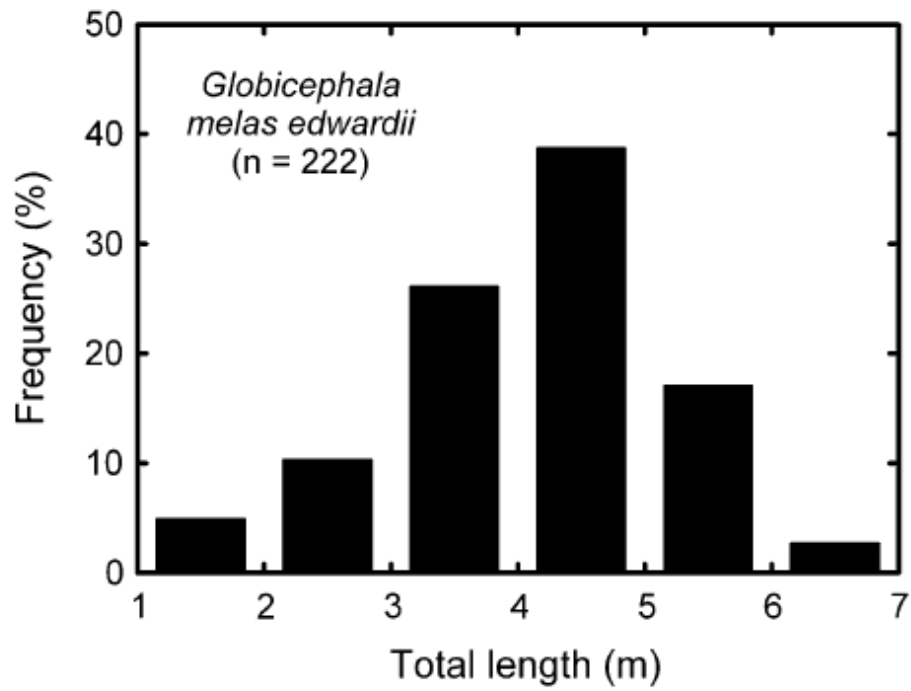


Figure 2. Frequency distribution of total length of stranded long-finned pilot whales from Kerguelen Islands (pooled data from the two mass stranding events)

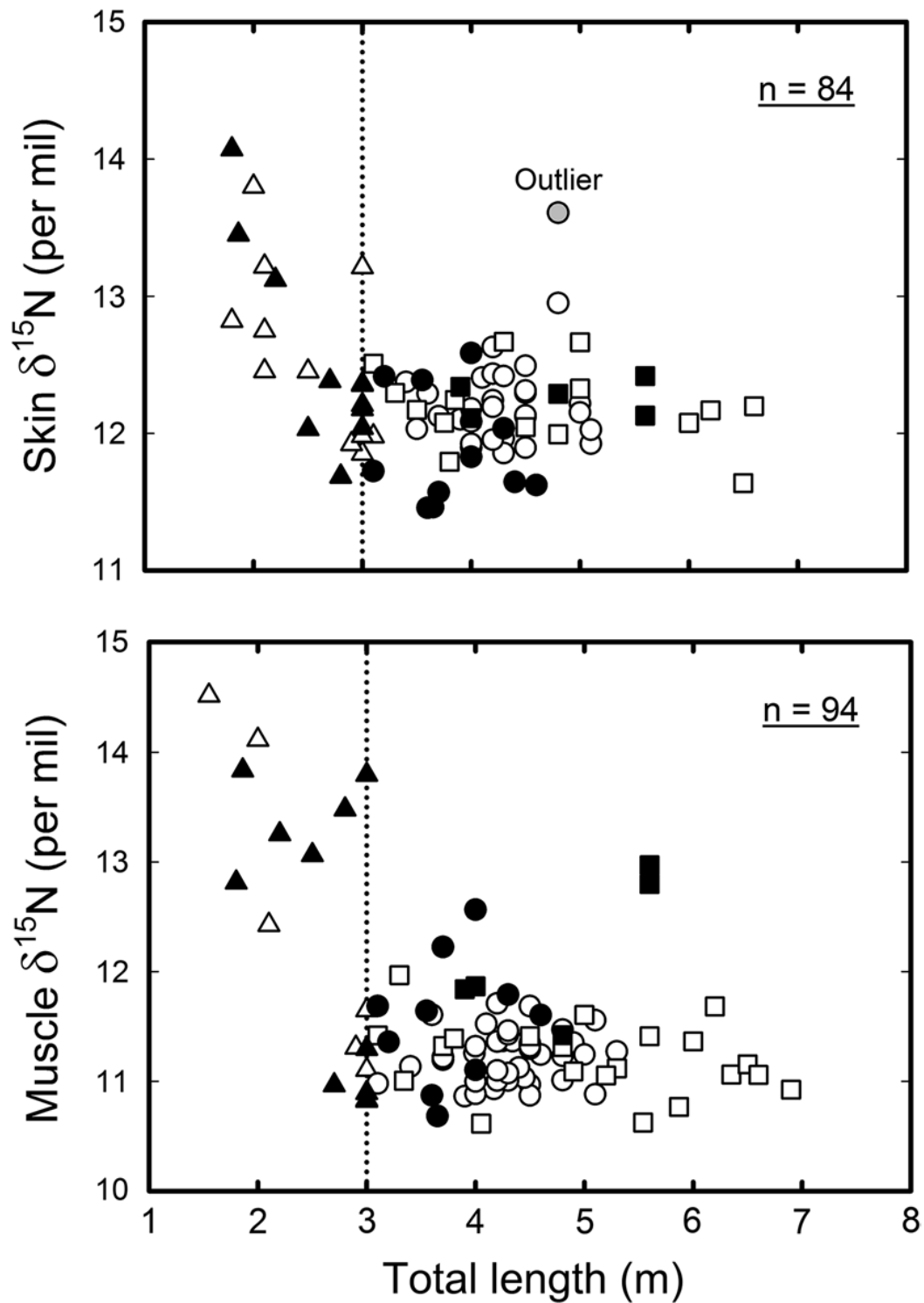


Figure 3. Tissue $\delta^{15}\text{N}$ values versus total length of calves (*triangles*), females (*circles*) and males (*squares*) long-finned pilot whales that stranded in 2006 (*white*) and 2010 (*black*). *Dotted lines* refer to the length at weaning (3 m) and outlier depicts an individual with both high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggesting that it migrated from warmer northern waters to the Kerguelen Islands

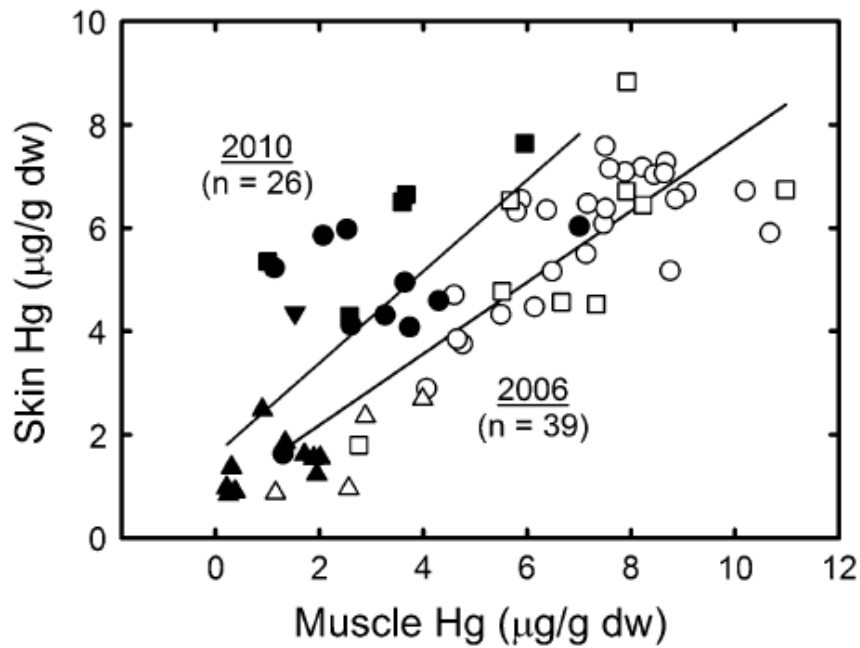


Figure 4. Relationship between skin and muscle Hg concentrations of calves (*triangles up*), females (*circles*), males (*squares*) and unsexed (*triangle down*) long-finned pilot whales that stranded in 2006 (*white*) and 2010 (*black*). Linear regressions: $y = 0.69x + 0.80$, $r^2 = 0.68$, $F_{1,37} = 78.36$, $p < 0.0001$ and $y = 0.89x + 1.62$, $r^2 = 0.50$, $F_{1,24} = 23.90$, $p < 0.0001$ in 2006 and 2010, respectively.

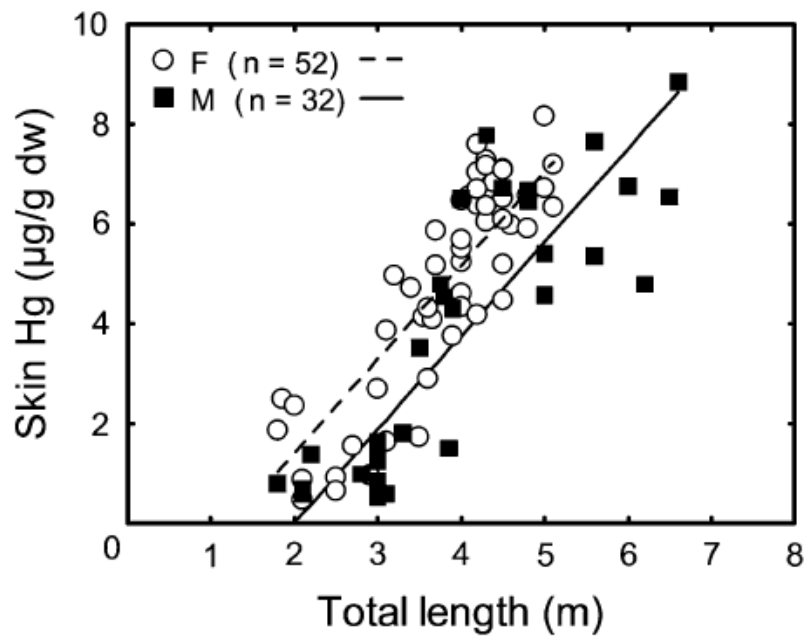


Figure 5. Relationship between skin Hg concentrations and total length of long-finned pilot whales (individuals from the two stranding events were pooled according to sexes and including calves). Best GLM model (Gaussian distribution, identity link function, 73 % of explained variation): y (females, *white circles*) = $1.88x - 2.34$, y (males, *black squares*) = $1.88x - 3.75$

Table 1. Tissue $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values in southern long-finned pilot whales from Kerguelen Islands. Values are mean \pm SD with ranges in parentheses. It was not possible to sex some individuals from the 2010 stranding event.

Tissues	Years	Sexes	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		Hg	
			<i>n</i>	(‰)	<i>n</i>	(‰)	<i>n</i>	($\mu\text{g g}^{-1}$ dw)
Skin	2006	Both	58	-18.3 ± 0.3 (−19.2 to −17.3)	58	12.3 ± 0.4 (11.6–13.8)	58	4.6 ± 2.5 (0.5–8.8)
		Females	37	-18.3 ± 0.3 (−19.2 to −17.3)	37	12.3 ± 0.5 (11.9–13.8)	37	5.1 ± 2.2 (0.5–8.1)
		Males	21	-18.2 ± 0.3 (−18.8 to −17.8)	21	12.3 ± 0.4 (11.6–13.2)	21	3.7 ± 2.8 (0.5–8.8)
	2010	Both	28	-18.9 ± 0.5 (−19.7 to −18.1)	28	12.2 ± 0.6 (11.5–14.1)	28	3.9 ± 2.2 (0.8–7.6)
		Females	15	-18.9 ± 0.5 (−19.7 to −18.1)	15	12.2 ± 0.8 (11.5–14.1)	15	4.0 ± 1.9 (0.9–6.8)
		Males	11	-18.7 ± 0.5 (−19.6 to −18.1)	11	12.3 ± 0.3 (11.7–13.1)	11	3.5 ± 2.7 (0.8–7.6)
	Average	Both	86	-18.5 ± 0.5 (−19.7 to −17.3)	86	12.3 ± 0.5 (11.5–14.1)	86	4.4 ± 2.4 (0.5–8.8)
Muscle	2006	Both	69	-19.0 ± 0.4 (−20.2 to −18.3)	69	11.3 ± 0.6 (10.6–14.5)	69	6.8 ± 2.4 (1.2–11.0)
		Females	45	-19.0 ± 0.4 (−20.2 to −18.3)	45	11.3 ± 0.5 (10.9–14.1)	45	7.0 ± 2.2 (1.2–11.0)
		Males	24	-19.0 ± 0.4 (−20.0 to −18.5)	24	11.3 ± 0.8 (10.6–14.5)	24	6.4 ± 2.7 (1.5–11.0)
	2010	Both	95	-19.4 ± 0.4 (−20.2 to −17.3)	26	12.1 ± 1.0 (10.7–13.8)	26	2.3 ± 1.7 (0.2–7.0)
		Females	43	-19.3 ± 0.3 (−20.1 to −18.7)	14	11.9 ± 0.9 (10.7–13.8)	14	2.6 ± 1.7 (0.4–7.0)
		Males	34	-19.4 ± 0.5 (−20.2 to −17.3)	11	12.2 ± 1.1 (10.8–13.8)	11	2.1 ± 1.8 (0.2–6.0)
	Average	Both	164	-19.2 ± 0.4 (−20.2 to −17.3)	95	11.5 ± 0.8 (10.6–14.5)	95	5.6 ± 3.0 (0.2–11.0)

Table 2. Generalized linear models (Gaussian distribution, identity link function) selected for each tissue (skin: $\delta^{13}\text{C}_s$, $\delta^{15}\text{N}_s$, Hg_s; muscle: $\delta^{13}\text{C}_m$, $\delta^{15}\text{N}_m$, Hg_m) with their corresponding AIC. *AICc* Akaike's Information Criteria adjusted for small sample sizes, *Exp. Var.* explained variation adjusted by sample size and number of parameters, *TL* total length

Response variable	Selected GLM	AICc	Exp. Var. (%)
Isotopes			
Explanatory variables: size, year, sex			
$\delta^{13}\text{C}_s$	$\delta^{13}\text{C}_s \sim \text{year}$	48.80	30
$\delta^{13}\text{C}_m$	$\delta^{13}\text{C}_m \sim \text{year}$	119.00	19
$\delta^{15}\text{N}_s$	$\delta^{15}\text{N}_s \sim \text{year} + \text{TL} + \text{sex}$	53.58	19
$\delta^{15}\text{N}_m$	$\delta^{15}\text{N}_m \sim \text{year} + \text{TL}$	155.91	28
Hg			
Explanatory variables: size, year, sex, $\delta^{13}\text{C}$			
Hg _s	Hg _s ~ TL + sex	282.56	73
Hg _m	Hg _m ~ TL + sex + year + $\delta^{13}\text{C}_m$ + TL: $\delta^{13}\text{C}_m$ + sex: $\delta^{13}\text{C}_m$ + year : $\delta^{13}\text{C}_m$ + sex: size	327.36	81

Table 3. A review of skin and muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values in pilot whales

Table 3. A review of skin and muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg values in pilot whales. Values are mean \pm SD; *nm* no measurements ; a Values are mean \pm SE; b Results were initially reported in wet weight and were converted here to dry weight using a factor of 0.285 (Dam and Bloch 2000)

Tissue	Pilot whales	Location	<i>n</i>	Lipid extraction or normalization	$\delta^{13}\text{C}$ (‰)	<i>n</i>	$\delta^{15}\text{N}$ (‰)	<i>n</i>	Total Hg ($\mu\text{g g}^{-1}$ ww)	Total Hg ($\mu\text{g g}^{-1}$ dw)	References
Skin	Southern long-finned	Kerguelen	86	Extraction	-18.5 ± 0.5	86	12.3 ± 0.5	86		4.4 ± 2.4	This study
		Northern long-finned	51	Extraction	-16.4 ± 0.4	51	11.3 ± 0.4			nm	de Stephanis et al. (2008)
	Eastern USA				nm	3	13.8 ± 0.4			nm	Abend and Smith (1995)
					nm	3	13.9 ± 0.4			nm	Abend and Smith (1995)
			6	No	-18.8 ± 0.1^a	6	13.9 ± 0.1^a			nm	Abend and Smith (1997)
					nm	3	11.7 ± 0.5			nm	Abend and Smith (1995)
		Faroe Islands	5	Normalization	nm					nm	Praca et al. (2011)
		Southern France			nm	5	9.8 ± 0.3			nm	Frodello et al. (2000)
		Corsica			nm			1		27.0	
	Short-finned	Polynesia	12	Extraction	-14.7 ± 0.5	12	15.2 ± 0.7			nm	Kiszka et al. (2010)
Muscle	Southern long-finned	Southern Australia	94	No	-19.6 ± 0.4	94	10.7 ± 0.9			nm	Davenport and Bax (2002)
		Kerguelen	164	Extraction	-19.2 ± 0.4	95	11.5 ± 0.8	95		5.6 ± 3.0	This study
	Northern long-finned	Eastern USA			nm	3	13.2 ± 0.1			nm	Abend and Smith (1995)
					nm	3	13.4 ± 0.2			nm	Abend and Smith (1995)
	Faroe Islands		6	No	-18.0 ± 0.1^a	6	13.3 ± 0.1^a			nm	Abend and Smith (1997)
					nm			31	1.4 ± 1.8	4.7^b	Caurant et al. (1993)
					nm			50	0.4 ± 0.4	1.4^b	Caurant et al. (1993)
					nm	3	11.5 ± 0.5			nm	Abend and Smith (1995)
					nm			15	1.8 ± 0.5	6.3^b	Julshamn et al. (1987)
					nm			10	3.3 ± 1.7	11.6^b	Julshamn et al. (1987)
					nm			33	2.7 ± 0.2	9.4^b	Caurant et al. (1996)
					nm			417	1.9	6.6^b	Dam and Bloch (2000)
					nm						Lassalle et al. (2014)
					nm						Mendez-Fernandez et al. (2012, 2013)
		Corsica			nm		nm	1		25.0	Frodello et al. (2000)
	Short-finned	Japan			nm		nm	12	4.2 ± 0.7	14.6^b	Arima and Nagakura (1979)
					nm		nm	23	7.6 ± 6.1	26.6^b	Endo et al. (2003)
					nm		nm	4	$5.4-13.8$	$18.9-48.4^b$	Endo et al. (2004)
					nm		nm	31	2.8 ± 0.8	9.8^b	Honda (1990) in Endo et al. (2005)
		Northern Japan			nm		nm	8	1.5 ± 0.5	5.3^b	Endo et al. (2005)
					nm	5	13.3 ± 0.8	5	1.3 ± 0.4	4.6^b	Endo et al. (2010)
		Southern Japan			nm		nm	4	7.1 ± 1.9	24.9^b	Anonymous (2003) in Endo et al. (2005)
					nm		nm	34	11.6 ± 8.2	40.7^b	Endo et al. (2005)
		New Caledonia	18	Extraction	-16.5 ± 0.5	18	12.2 ± 0.6	18	12.4 ± 8.6	43.5^b	Endo et al. (2010)
		Lesser Antilles			nm		nm	2		$27.3-32.8$	Bustamante et al. (2003b)
					nm		nm	5	4.0 ± 0.9	14.0^b	Gaskin et al. (1974)