SKIN AND BLUBBER BIOPSIES FROM WHALES AND DOLPHINS: IMPLICATIONS FOR THE STUDY OF POLLUTANT EXPOSURE

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Introduction

Skin and blubber biopsy sampling technique allows sampling from free-ranging whales and dolphins [1–7]. In many studies, this technique complements samples obtained from stranded and by-caught individuals, which provide access to internal tissues including muscle, liver or kidney [8–10]. These organs are essential to better assess biochemical pathways of toxicity and to build pharmacokinetic models [11–13]. Biopsies are convenient for the researchers as their sampling allow assessing exposure of free-ranging and healthy individuals. However, many biotic processes should be taken into account before interpreting data to assess exposure of the individuals. The present study gathers some of our data extracted from previously published, submitted or on-going studies on pollutants in free ranging whales and dolphins from various seas and oceans [14–18].

Materials and methods

Data on pollutants (POPs and Hg) and stable isotopes were extracted from previous studies, where the details of the entire procedures and quality assurance are given [4,15–18]. Briefly, biopsies of skin and blubber of various cetacean species including humpback whales *Megaptera novaeangliae* from the Indian Ocean, bottlenose dolphins *Tursiops truncatus* off Florida and off French coasts, Mediterranean sperm whales *Physeter macrocephalus*, long-finned pilot whales *Globicephala melas* and fin whales *Balaenoptera physalus*, were used to measure levels of persistent organic pollutants (POPs), total mercury (T-Hg) and stable isotopes. Skin biopsies were used to analyse $\delta^{13}C$ and $\delta^{15}N$ composition (delta (δ) notation in parts per thousand ‰) through Mass Spectrometer (IRMS) coupled in continuous flow to an elemental analyser (EA), and total-Hg content through atomic absorption spectrometry at 254 nm, in a Direct Mercury Analyser DMA 80. Blubber biopsies were used to measure POPs concentrations. After extraction of lipids through accelerated solvent extraction (ASE) or hot Soxhlet, samples were purified via multicolumn liquid-solid chromatography. POPs were then quantified using different techniques of Mass Spectrometry (GC-ECD, GC-ECNI/MS or GC-HRMS), resting on laboratories availabilities.

Results and discussion

Several biotic and non-biotic factors, such as gender, age, specific blubber metabolism, body size, nutritional status and species distribution, can influence marine mammals' final burden of contaminants, and explain interspecies differences.

- The **geographical location** can strongly determine contamination profile and levels, due to differences in historical usage and production of these contaminants between studied areas [4,15,19,20]. POP concentrations analysed in humpback whales from La Reunion (stock C4, IWC) are up 40 times lower than that described for humpback whales feeding near the Western Antarctic Peninsula waters (Stock G) [14], but similar to POP concentrations described in humpback whales breeding in Moreton Bay Marine Park, off Australia (stock E1) [20,21]. Migration, fasting and lactation obviously affect POP concentrations in tissues of marine mammals leading to an increase of concentrations because PCBs are less easily mobilised from blubber than lipids [22]. Humpback whales sampled off Australia, on their breeding zone, two dominant congeners, *p,p'*-DDE and HCB, exhibited a significant increase of concentration in late migrating cohorts (x1.6), while other compounds remained similar between cohorts [21]. Our results clearly reflect a lower POP exposure of humpback whales from stocks C4 and E1 compared to humpback whales sampled near the Antarctic Peninsula (stock G). The reason of such discrepancy is currently unknown, but it requires further investigation on humpback whales from Antarctica. A higher exposure of whales from Western Antarctic Peninsula cannot be excluded presumably linked to differential anthropogenic sources in Antarctic. However, many other factors should be taken into account when using data acquired from blubber biopsies

- **Age** is a crucial factor because PCBs, THg and other lipophilic persistent contaminants accumulate in blubber throughout the life [8,23,24]. However, when sampling is conducted through skin and blubber biopsies, no information is gathered on the age of the individuals. Age is usually calculated either using growth layers of teeth (toothed whales - odontocetes) or ear plugs (baleen whales - mysticetes). When the growth curve of the population is well known, body length could be used as a proxy for age for the smaller individuals (in mammals, the aging process may continue for decades after the growing of the animal has ceased). However, in the abovementioned pilot whale case, neither of these data was available, and during sampling it was only possible to approximately distinguish between adults and juveniles through visual observation of body size [16]. It is worth mentioning that it is possible to estimate the age of cetaceans using the fatty acid (FA) composition of blubber [25,26]. However, almost 20 years after the publication of the first study that draw attention to this possibility [27], the approach is still regarded as "a promising method" [28]. The main problem with the approach is related to the precision of the method, as it allows age assessment with an accuracy of ±7-10 years [28]. There is also a logistic constraint related to an incompatibility between the small amounts of blubber obtained with the biopsy and the mass of sample required for both types of analysis.
- **Sex** on the other hand, can be determined genetically in the lab through *karyotyping* done with blubber and skin biopsies [29]. PCBs, DDTs and PBDEs were significantly higher in males than females in Mediterranean whales[16]. Older males are generally more contaminated than females because significant burdens of lipophilic pollutants are transferred to calves from the mother through the highly fatty milk [23,30–32]. The high variability of pollutant concentrations in females long-finned pilot whales from the Mediterranean Sea could result from the fact that reproducing females impart up to 70% of their pollutant burden to calves either during gestation and lactation periods [16].
- Another main factor influencing the contaminant concentrations in marine mammals is the **trophic level** at which they feed [7,33,34]. Lipophilic POPs tend to biomagnify along the food webs resulting in greater concentrations with increasing trophic level. Subsequently, higher concentrations of POPs have been often related with higher $\delta^{15}N$ values [7,33,34]. However, this relationship was not found for pilot whales and sperm whales from the Mediterranean Sea [16]. Indeed, even if the resulting $\delta^{15}N$ values placed sperm whale at higher trophic level than long-finned pilot whales, the latter resulted as the most contaminated species for all classes of POPs [16].
- POPs in cetacean blubber are strongly influenced by its thickness, stratification and lipid profile [20,27]. In turn, blubber characteristics are very sensible to **sampling quality and technique** [19]. Several biases might occur when using biopsies[19]. Darted biopsies permit to collect only the outermost layer [19]. Even if this layer is metabolically inert, reflecting pollution exposure over a long time range, its particular composition in fatty acids that is dedicated more to diving and body structure support than energy storage, can prevent the accumulation of certain congeners with respect to others [35,36]. Secondly, the angle with which the dart arrives in the tissues of the animals, the particular body area and the time of the biopsy in water before recollection, can affect the blubber biopsy structure [36,37]. When blubber biopsies are in seawater, an important seepage of lipids occurs between the sampling and the collection moment. Moreover, the amount of tissue sampled with biopsies is very low (between 100 and 400 mg, personal observation). This leads to difficulties in performing good measurements of POP concentrations, especially for the detection of congeners that are present in very low levels, such as dioxins and furans [16].
- Finally, blubber structure and composition are influenced by multiple species-specific factors such as niches differentiation, migratory routes and diving behaviour, sex and age, but also nutritional status [38]. Capital breeders such as the fin whale use blubber mainly as energy storage during fasting period; therefore their lipid profile is mainly characterized by easily metabolized fatty acids, such as the triacylglycerides (TAG). On the contrary, odontocetes like sperm whales are income breeders and receive continual energy acquisition throughout the reproductive period. In these species, blubber acquires a wider role, mostly related with their deep foraging dives. For this reason the outermost layer of this species is characterized by more structurally complex fatty acids such as wax esters (WE), which are not easily metabolized [35]. Higher concentrations of DDT and PCB congeners are positively related with higher percentages of TAG and non-esterified fatty acids [35]. Based on these assumptions, sampling methods such as biopsies of free-ranging cetaceans are less efficient in representing pollutant burden of sperm whales as for other species. This could be thus one explanation for the significant difference showed in lipids content between the mysticete and the odontocete in our studies. In fact, humpback and fin whales present the highest lipids content (between 12 and 84%), followed by long-finned pilot whales (4 – 80%), bottlenose dolphins (2 – 46%) and in the end

by sperm whales (2 – 30%). Studies on stranded animals showed that blubber thickness of sperm whales presents high individual variability and can range between 43 to 168 mm [39]. The middle layer of sperm whale blubber contained double the amount of lipids found in the outer and inner layers [39]. Apparently thus, biopsies do not allow the acquisition of sufficient quantities of sperm whales blubber, enough to minimize the error related with the experimental analysis.

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