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Cytochrome P450 1A1 expression in cetacean skin biopsies from the Indian Ocean

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ABSTRACT

The study describes cytochrome P450 1A1 (CYPA1) expression in the skin of different cetacean species (*Megaptera novaeangliae*, $n = 15$; *Stenella attenuata*, $n = 7$ and *Stenella longirostris*, $n = 24$) from the Mozambique Channel island of Mayotte. Immunohistochemical examination was performed with a monoclonal antibody against scup cytochrome CYPA1. The sex was determined using a molecular approach consisting in the genotyping sex-specific genes. CYPA1 was detected at the junction between epidermis and blubber on dolphins only, mostly in the endothelial cells. Similar observation was obtained in the dermis of one *M. novaeangliae*. Immunohistochemical slides were scored to evaluate the expression of the CYPA1 and a higher expression was observed in *S. longirostris*, suggesting a higher exposure to pollutants for this species. The difference of expression between sexes was not significant.

Cytochrome P450 1A proteins (CYP1A1) are induced by exposure to various environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and planar halogenated aromatic hydrocarbons (PHAHs) (e.g., non-ortho-polychlorinated biphenyls – PCBs), activating the aryl hydrocarbon receptor. These contaminants present worldwide and in all oceans, accumulate in the marine food web. High concentrations accumulate in the tissues of marine top predators, such as the blubber of marine mammals (Aguilar et al., 2002; Pierce et al., 2008). In addition, these stable contaminants are suspected to have adverse health effects mostly in marine top predators, especially on the immune and reproductive systems (Hall et al., 2006; Reijnders, 1986). In marine mammals such as cetaceans, stable contaminants are reported to induce sensitivity to infectious diseases, such as in the harbour porpoise (*Phocoena phocoena*) from the North-east Atlantic with high PCBs level. Indeed, high PCB levels have been recorded in harbour porpoises dying from an infectious disease compared with individuals dying from other causes on animals stranded on the coasts of the UK, on the continental southern North Sea coast (Jepson et al., 2005; Pierce et al., 2008) and for the German North and Baltic Seas coasts (Beineke et al., 2005). Morbillivirus epizootics in the harbour seal (*Phoca vitulina*) and striped dolphin (*Stenella coeruleoalba*) populations have been suspected to be associated with high level of PCBs (Aguilar and Borrell, 1994; Hall et al., 1992).

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CYP1A1 is expressed in all animal tissues including skin, liver, urine bladder and lung, and immunohistochemical investigation has be done to compare expression in tissues from beluga whales (Wilson et al., 2005). In the integument, analysis of CYP1A1 expression deserves attention as a potential non-lethal technique to study the impacts of contaminants on cetaceans and such expression was described by immunohistochemistry in the integument of 17 species (six mysticetes and 11 odontocetes), from various regions of the world (Angell et al., 2004). Wilson et al. (2007) correlated it with the PCB concentrations from bottlenose dolphins (*Tursiops truncatus*) resident in the Saratosa Bay. The detection of CYP1A1 has been also investigated on Mediterranean cetaceans (Fossi et al., 2008). Very little information is available on the exposure of cetaceans to contaminant and potential consequences the in Indian Ocean Sanctuary (Aguilar et al., 2002). The aim of the present study is to describe the skin histology and the cytochrome P450 1A1 expression in cutaneous biopsies of different cetacean species collected in the Mayotte waters. We hypothesised that a multi-species approach would help to understand interspecies variability of CYP1A1 expressions, with higher trophic level species having a higher expression than low trophic level species. Based on existing information in the literature and local information, we considered three species in the present study: the pantropical spotted dolphin (*Stenella attenuata* SA), the spinner dolphin (*Stenella longirostris* SL) and the humpback whale (*Megaptera novaeangliae* MN). The delphinids have the higher trophic level, feeding on epipelagic fishes and cephalopods (Norris and Dohl, 1980), while southern hemisphere humpback whales mostly feed on krill from high latitudes, being of a lower trophic level. Among the two delphinids considered in this study, the pantropical spotted dolphin has a higher trophic position than the spinner dolphin (Gross et al., 2009).

Mayotte (12°50′S, 45°10′E), the sampling area, is located in the northern Mozambique Channel between Madagascar and the African mainland, entirely surrounded by a 197 km long barrier reef. A high marine mammal diversity, 22 species including 12 delphinids (Kiszka et al., 2010) have been reported around Mayotte, the most common species being the spinner dolphin, the pantropical spotted dolphin, the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and the melon-headed whale (*Peponocephala electra*); these are resident year-round (Kiszka et al., 2010). The shallow and protected waters around the Comoros, including Mayotte, provide ideal habitat characteristics for wintering humpback whales during their reproductive cycle (Ersts et al., in press; Kiszka et al., 2010).

Skin biopsies (epidermis and underlying blubber) were collected from 46 free ranging cetaceans (MN, $n = 15$; SA, $n = 7$ and SL, $n = 24$) from Mayotte waters, during austral winter from 2004 to 2006. The biopsies were collected using a crossbow (*BARNETT Veloci-Speed® Class*, 68-kg draw weight) with Finn Larsen bolts and tips (dart 20 and 40-mm long, 5-mm-diameter). The largest tips were used for humpback whales, while the smallest were used for delphinids. Animals were hit below the dorsal fin and biopsies were preserved in 90° ethanol prior to analyses. For the delphinids, only adult individuals were sampled, whereas several calves were samples in humpback whales for adult/calves comparisons. For subsequent analyses, biopsies were divided in two: one for microscopical examination, one for sex determination.

For microscopy, skin samples were embedded in paraffin wax by routine procedures and sections (3 μm) were cut and stained with haematoxylin and eosin (HE). In addition, an immunohistochemical examination with a monoclonal antibody against the scup CYP1A1 was performed on paraffin embedded sections as described by Angell et al. (2004). The other immunohistochemical reagents used were those included in the Enhanced Polymer One-Step Staining Procedure (Dako Envision™, Dako, Glostrup, Denmark). Test sections, on which the first layer antibody was omitted, served as negative controls.

For every microscopic slide, a quality index (Q.I.) was given from 1 (well preserved and well differentiated tissue) to 3 (altered tissue). For the immunohistochemical examination, a staining score (S.S.) was given: 0 (absence of staining); 1 (slight or focal positive staining); 2 (moderate: mild and multifocal) and 3 (intense and multiple staining). The slides were scored in three different skin levels: epidermis, junction between epidermis and blubber and finally blubber. Statistical tests were used to evaluate the effects of species and gender. Due to the non-normality of the scores, permutation statistical tests were performed: the obtained difference between the score averages for males and females were compared to the differences distribution obtained by shuffling 1000 times the scores between males and females within each of the three species: the obtained p-value is the rank of the observed difference in this distribution. A similar procedure was used to test for species differences (Churgill and Doerge, 1994).

PCR-based sex determination was performed using the four primers described by Rosel (2003). The PCR products were separated by electrophoresis on 2% agarose gels and gender was determined from the resulting banding patterns.

In total, 46 biopsies were investigated and the Q.I. was 1 for 35 samples, 2 for 10 samples and 3 for one. Examination with haematoxylin and eosin staining revealed no abnormality or lesions but four MN samples were discarded since only the superficial epidermis had been biopsied. Histologically, the junction between epidermis and blubber was rich of vascular structure. There was no microscopic difference between individuals or species except that the epidermis of MN was thicker than in the delphinids.

The immunohistochemistry (Table 1) revealed a slight positive staining (S.S.1) only observed in the epidermis of two SL, most obvious in the cytoplasm of basal cells. At the junction between epidermis and blubber, a very slight staining was observed in the cytoplasm of endothelial cells of two MN and one SA while the staining was slight on 12 SL, moderate on three and intense on two. CYP1A1 expression was stronger and most frequent in endothelial cells and rarely in the fibrocytes. Finally, in the blubber, only one MN, one SA and one SL were slightly positive. The maximum global scores were 1 for the MN, 2 for the SA and 5 for the SL, the average by species was 0.27 for MN, 0.6 for SA and 1.29 for SL. The sex was determined on all cetaceans except on two SA. Among MN there were 12 females and three males (all calves); for SA, there were two females and three males and finally, there were nine females and 15 males among SL.

For the statistics, four MN (only epidermis present) and two SA (without sexing) were not included in the analysis. Only the staining score of the junction between epidermis and blubber (endothelial cells) was considered and the permutation tests reveal that the difference was not significant among sexes ($P = 0.766$) but was very significant ($P = 0.002$) among species.

The CYP1A1 expression has been documented from North America humpback whales (Angell et al., 2004), but had never been reported in pantropical spotted dolphins and spinner dolphins. In addition, no data are available from cetaceans of the Indian Ocean. In the three species investigated, the expression of the CYP1A1 was strongest in endothelial cells at the junction between epidermis and dermis, followed by the dermis and very rarely in the epidermis. In addition, the expression was very mild in MN and in SA compared to SL. Such expression in the endothelial cells has been reported by Angell et al. (2004) and by Wilson et al. (2007) since CYP1A1 is highly inducible in the endothelium of vertebrates (Stegeman et al., 1989). Such induction in the integument endothelial cells of cetaceans may be related to the blood concentration of contaminants, agonist of the aryl hydrocarbon receptor (Angell et al., 2004). *In vitro* similar inductions were reported on cetacean

Table 1
Immunohistochemistry scoring. Means by species and by sex.

	Megaptera novaeangliae ($n = 15$)	*Stenella attenuata* ($n = 7$)	*Stenella longirostris* ($n = 24$)
Female ($n = 23$)	0.25	1.5	1.11
Male ($n = 21$)	0	0	1.4
Not-determined ($n = 2$)		0	
Total	0.2	0.42	1.29

endothelial cells from lung and kidney after exposure to aryl hydrocarbon receptor agonists (Garrick et al., 2006).

The difference of expression between the three species investigated was statistically significant while the difference between sexes was not significant.

For Wilson et al. (2007), the CYP1A1 expression 1n bottlenose dolphins from Sarasota was neither age nor sex related, and the reproductive status did not relate to the levels of CYP1A1 in females. But, the endothelial CYP1A1 expression was positively correlated with the blubber total PCBs, although the mono–ortho PCBs concentration did not show this relationship. The contaminant concentrations appear to be stronger determinants for the expression of CYP1A1 in the skin biopsies, more than are age, sex, or reproductive status (Wilson et al., 2007). In this study, contaminant concentrations were not investigated on the blubber. As pollutants induce the expression of CYP1A1 and as SA and SL are resident in the Mayotte waters all year-round, they could have been contaminated locally through the food by the process of bio-magnification (Pierce et al., 2008). Despite the fact that the southern hemisphere has a low level of pollution, some restricted areas such as river mouths or semi-closed water masses are contaminated as a result of point source pollution (Aguilar et al., 2002). The difference in the CYP1A1 expression between the two dolphin species could be linked to a different position in the trophic web (Kiszka & Martinez, unpublished data), revealing a higher trophic level of the spinner dolphin. However, existing information on the trophic position of these two delphinid species suggest that spotted dolphins have a higher trophic level than spinner dolphins (Gross et al., 2009). The lowest expression in humpback whales could be linked with the lowest trophic level of baleen whales in the marine food web compared to odontocetes (Pauly et al., 1998). In addition, MN feed abundantly in Antarctica where the level of contamination is very low (Aguilar et al., 2002) while they starve when they are on their wintering ground in subtropical waters such as Mayotte.

This study demonstrates the expression of CYP1A1 in endothelial cells of cetaceans of the Mayotte waters. The expression could be induced by environmental contaminants that are also suspected to result in adverse health effects in cetaceans (Hall et al., 2006; Jepson et al., 2005; Pierce et al., 2008). Further investigations will be helpful to identify the contaminants, their concentrations and to evaluate the potential consequences from an animal health standpoint.

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