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The Pelagos Sanctuary for Mediterranean marine mammals: Marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (*Stenella coeruleoalba*)

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ABSTRACT

The concurrence of man-made pressures on cetaceans in the Mediterranean Sea is potentially affecting population stability and marine biodiversity. This needs to be proven for the only pelagic marine protected area in the Mediterranean Sea: the Pelagos Sanctuary for Mediterranean Marine Mammals. Here we applied a multidisciplinary tool, using diagnostic markers elaborated in a statistical model to rank toxicological stress in Mediterranean cetaceans. As a case study we analyzed persistent, bioaccumulative and toxic chemicals combined with a wide range of diagnostic markers of exposure to anthropogenic contaminants and genetic variation as marker of genetic erosion in striped dolphin (*Stenella coeruleoalba*) skin biopsies. Finally, a statistical model was applied to obtain a complete toxicological profile of the striped dolphin in the Pelagos Sanctuary and other Mediterranean areas (Ionian Sea and Strait of Gibraltar). Here we provide the first complete evidence of the toxicological stress in cetaceans living in Pelagos Sanctuary.

1. Introduction

Marine protected areas (MPAs) are not necessarily protected from man-made pollution. This is true for the only pelagic MPA in the Mediterranean Sea: the Pelagos Sanctuary (International Sanctuary for the Protection of Mediterranean Marine Mammals). The Pelagos Sanctuary hosts eight resident cetacean species (*Balaenoptera physalus*, *Physeter macrocephalus*, *Grampus griseus*, *Globicephala melas*, *Tursiops truncatus*, *Stenella coeruleoalba*, *Delphinus delphis*, *Ziphius cavirostris*) and was established in 1999. It is the first international high-seas MPA in the world and includes territorial waters of France, Italy and the Principality of Monaco. While the Pelagos Sanctuary represent a unique example of conservation in Mediterranean, without strong leadership and action, the risk of failure is ever-increasing (Notarbartolo di Sciara et al., 2008). Efforts to protect this valuable and complex ecosystem have not yet succeeded in blocking one of the major human impact: contamination by anthropogenic compounds.

Pressures on whales and dolphins in Mediterranean waters have dramatically increased in recent decades and have different origins (Notarbartolo di Sciara and Birkun, 2010; Van Bressem et al., 2009). The concurrence of different man-made pressures is potentially affecting cetacean population stability, community structure, food chain and marine biodiversity. Contaminants, infectious and immunosuppression diseases, bycatch, shipping, food depletion (by overfishing), noise pollution and climate change affect survival, recruitment, reproductive success, mutation rates and may play a significant role in the partitioning of genetic variation among populations exposed to high and less extreme stress (Whitehead et al., 2003).

The striped dolphin (*S. coeruleoalba)* is the most abundant cetacean species in the Pelagos Sanctuary and, indeed, the Mediterranean as a whole; although no overall population estimation for the entire region exists. The most recent survey estimates the abundance of striped dolphins in Pelagos Sanctuary population to be: in winter 19,462 (95% CI = 12,939–29,273) and in summer 38,488 (95% CI = 27,447–53,968) (Panigada et al., 2011). The Red

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List of the IUCN classifies Mediterranean striped dolphin subpopulation as *Vulnerable*. Striped dolphin, as other Mediterranean odontocetes, accumulates high concentrations of anthropogenic contaminants, many considered Persistent, Bioaccumulative and Toxic (PBT). There is still no evidence that PBT chemicals are causing direct mortality of marine mammals, however it is certain that lipophilic contaminants cause immune and reproductive dysfunction (Hammond et al., 2005; Ross et al., 1995). Polychlorinated biphenyl (PCB) loads in *S. coeruleoalba* that died in the 1990–1992 *Morbillivirus* epidemic were significantly higher than in surviving individuals. The well-known immunosuppressive effects of PCBs in mammals suggested that PCBs impaired immune responses and increased the severity of the outbreak (Aguilar and Borrell, 1994). Between 2007 and 2008 new cases of *Morbillivirus* infection (Raga et al., 2008), after the massive die-off of 1991–92, were detected in Mediterranean specimens. Contaminants such as Organochlorine Compounds (OCs) are also known to be endocrine disrupting chemicals (EDCs) (Fossi et al., 2003). Although OC contamination is decreasing, Polybrominated Diphenyl Ethers (PBDEs) and other emerging contaminants seem to be increasing in the environment, including the Mediterranean Sea (De Wit et al., 2010). PBDEs are lipophilic, persistent and toxic to wildlife and humans (Alaee et al., 2003; De Wit et al., 2010). The highest levels of PBDEs have been recorded in top marine predators, including Mediterranean odontocetes (Pettersson et al., 2004).

In this context international institutions, such as the International Whaling Commission (IWC) have encouraged research on panels of sensitive non-lethal biomarkers, combined with PBT detection in skin biopsies of free-ranging animals, to define the health status of cetacean species with respect to multiple threats, also supporting projects such as Pollution2000+(IWC). At the 2011 Scientific Committee of IWC held in Trømso, Hall et al. (2011) used two case study populations and species (*T. truncatus* and *Megaptera novaeangliae*) to describe and demonstrate an individual-based model that can be used to simulate a variety of pollutant impacts and their potential effects on population growth.

Cetacean skin biopsies are suitable for hazard assessment of free-ranging cetaceans (Fossi et al., 1992, 2010a) obtainable with minimum disturbance for animals. In this paper the markers measured in skin biopsies were general and specific diagnostic signals at different hierarchical levels. A brief description of the mechanisms of action and the link with the toxic compounds is described in Table 1. The biomarkers are subdivided in: (1) markers of exposure to anthropogenic contaminants, (2) markers of general stress; and (3) markers of genetic erosion.

The main aim of this project, supported by the Italian Ministry of the Environment within the ACCOBAMS (Agreement on the Conservation of Cetaceans in the Black Sea Mediterranean Sea and Contiguous Atlantic Area), was to apply a multidisciplinary diagnostic tool, using specific and general biomarkers in striped dolphin in order to verify the toxicological stress in cetaceans living in the Pelagos Sanctuary MPA. In skin biopsy we analyzed persistent, bioaccumulative and toxic chemicals (PBT) combined with a wide range of diagnostic markers of exposure to anthropogenic contaminants and genetic variation as marker of possible genetic erosion. Finally, a statistical model was applied to obtain a complete profile of toxicological status of the striped dolphin in the Pelagos Sanctuary and in two other Mediterranean areas (Ionian Sea and Strait of Gibraltar).

2. Materials and methods

2.1. Sampling areas

The study area was the Mediterranean Sea (Fig. 1C), where we selected three areas with different types of human pressure/stressors for cetacean populations: Pelagos Sanctuary (P) (Italy–France), Western-Ionian Sea (Italy) (I) and Strait of Gibraltar (G) (Spain). *Pelagos Sanctuary* (Ligurian Sea) – The Pelagos Sanctuary is a MPA of about 90,000 km^2 in the north-western Mediterranean Sea between Italy, France and the Island of Sardinia, encompassing Corsica and the Tuscan Archipelago. Compared to the rest of the Mediterranean, this marine area is characterized by high offshore primary productivity with a large biomass of highly diversified zooplankton, which attracts various levels of predators, marine mammals included, to the area. All eight regular cetaceans present in the Mediterranean sea can be found in this area. The remarkable cetacean faunal diversity in the Pelagos Sanctuary has coexisted with very high levels of human pressure such as: intensive maritime traffic, industry and agriculture run-off, military exercises, seismic prospecting, over-fishing, oil–gas exploration and whale watching. *Western-Ionian Sea* – This pelagic area lies between eastern Sicily and south-western Calabria and hosts the eight cetacean species commonly present in the Mediterranean Sea. The area has a medium anthropogenic impact. Threats to cetaceans include drift-nets, maritime traffic and pollution of terrestrial and marine origin. *Strait of Gibraltar* – Since the Strait is the only connection between the Mediterranean Sea and the Atlantic Ocean, maritime traffic is intense. The area is a critical habitat and migration corridor for Mediterranean and Atlantic cetacean species, and is the most diverse cetacean habitat in the Mediterranean Sea. Threats for cetaceans include drift nets, noisy shipping traffic and pollution of terrestrial and marine origin (Cañadas et al., 2005).

2.2. Biopsy collection and analyses

Integument biopsies were obtained from free-ranging striped dolphins in the Pelagos Sanctuary (P) (Italy–France) ($n = 20$, Males = 12, Females = 8) in Western Ionian Sea (I) (Italy) ($n = 12$, Males = 7, Females = 5) and the Strait of Gibraltar (G) (Spain) ($n = 15$, Males = 7, Females = 8) (Fig. 1) during spring–summer 2007, using biopsy tips mounted on a pole (CITES Nat. IT025IS, Int. CITES IT 007 issued to Accademia dei Fisiocritici and University of Siena). Sex was determined by PCR according to Bérubé and Palsbøll (1996). A set of major PBT chemicals (OCs and PBDEs) and a set of biomarkers (CYP1A1, CYP2B, CAT) were analyzed in skin biopsies of three striped dolphin populations and integrated into the statistical model together with the values of gene expression biomarkers (CYP1A1, Aryl hydrocarbon Receptor-AHR, Estrogen Receptor α-ERα, E2F-1 transcription factor, Heat shock Protein 70-HSP70) used as variables (Panti et al., 2011). A screening of the genetic variability was also performed.

2.3. Detection of anthropogenic contaminants

A set of PBTs were analyzed in skin biopsies of three striped dolphin populations. Organochlorine Compounds (OCs): the analytical method used for quantitative and qualitative analysis of hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane and its metabolites (DDTs), and polychlorinated biphenyls (PCBs) is high resolution capillary gas chromatography with electron capture detector (^{63}Ni ECD)(AGILENT 6890/N), according to US Environmental Protection Agency (EPA) 8081/8082 modified (Marsili and Focardi, 1996). The GC has a SPB-5 bonded phase in a fused silica capillary column, 30 m long. Polybrominated Diphenil Ethers (PBDEs): fifteen brominated tri- to deca-substituted BDE congeners (# 17, 28, 47, 66, 85, 99, 100, 153,154, 183, 184, 191, 196, 197, 209) were analyzed by high resolution gas chromatography and low resolution mass spectrometry (HRGC–LRMS). A 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in selected ion monitoring mode (SIM) with negative chemical ionization (NCI) was used.

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