

# DETECTION OF POP LEVELS IN SEVERAL MEDITERRANEAN SPECIES OF CETACEANS IN THE 2008-2010 TIME PERIOD.

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## ABSTRACT

Mediterranean cetaceans are known to accumulate very high levels of xenobiotic contaminants, such as organochlorines (OCs). In 1990-1992, many striped dolphins (*Stenella coeruleoalba*) were affected by a severe *Morbillivirus* infection epidemic along the Mediterranean coasts. In that period, the OC levels in blubber of stranded striped dolphin specimens resulted statistically higher than those found in specimens analysed before and after 1990-1992 years. Here we show that in blubber of stranded and in biopsies of free-ranging cetaceans sampled in the Mediterranean Sea between 2008 and 2010, the levels of some OCs (PCBs and DDTs) are very similar to the levels found in the 1991-1993 period, despite regulatory control on their production and use in Mediterranean area. In these specimens no evidence of *Morbillivirus*, but of other agents such as *Toxoplasma gondii* was found..

## KEYWORDS

MONITORING, MEDITERRANEAN SEA, CETACEANS, POLLUTANTS, PATHOLOGIES.

## INTRODUCTION

Environmental pressure on biodiversity in the Mediterranean Sea is intense. Over the past 100 years this basin has been affected by numerous types of human interventions that, combined with natural climatic factors, are having a negative impact on ecosystem quality, ecological status, and, consequently, on the health and the biodiversity of wildlife. Contaminants represent one of the many anthropogenic stressors. Contamination from (and potential effects of) the following compounds represent the principal causes of pollution in this area:

- Persistent organic pollutants (POPs) in particular DDT and polychlorinated biphenyls (PCBs);
- Polycyclic aromatic hydrocarbons (PAHs);
- Trace elements.

Lethal, sublethal or other detrimental effects of these contaminants on Mediterranean wildlife are reported, in particular regarding the potential of several environmental chemicals to cause endocrine disruption at environmentally realistic exposure levels (Fossi et al., 2006). Cetaceans, especially odontocetes, are particularly subject to chemical stress from xenobiotic compounds, since they accumulate large quantities of these contaminants. The impaired immune function, probably also connected to these immunosuppressive compounds, has likely contributed to the mass mortalities due to *Morbillivirus* infection in the striped dolphin (*Stenella coeruleoalba*) Mediterranean population (Di Guardo et al., 2005; Fossi and Marsili, 2003). In this paper we present the POP contamination status of some Mediterranean species of cetaceans sampled in the 2008-2010 period, and we explore the relationship with some highly immunosuppressive agents, such as morbilliviruses. Special reference to *Morbillivirus* is made since, by the end of 2006, a new morbilliviral epidemic occurred in the Mediterranean Sea, being caused by a *Dolphin Morbillivirus* (DMV) strain closely related to that responsible for the dramatic die-off among striped dolphins living in the same area (Raga et al., 2008; Fernandez et al., 2008).

## MATERIAL AND METHODS

Two sample typologies can be obtained from cetacean species in order to evaluate the levels of contaminants: those from stranded and those from free-ranging specimens.

- Stranded specimens

In theory, all stranded cetaceans in a relative good state of conservation can be used for ecotoxicological analysis. Brain, liver, lung, lymph nodes, spleen blood, skin, subcutaneous blubber, melon, heart, kidney and muscle were sampled in fin whale (*Balaenoptera physalus*), sperm whale (*Physeter macrocephalus*), common bottlenose dolphin (*Tursiops truncatus*) and striped dolphin. Various environmental pollutants such as OCs, heavy metals and PAHs were analysed in these samples, along with parasitological histopathological and immunohistochemical investigations

against *Morbillivirus* being also carried out from the above tissues (brain, lung, lymph nodes and spleen as far as *Morbillivirus* is concerned).

- Free-ranging specimens

Subcutaneous tissues (skin and blubber) were obtained from i) striped dolphin and common dolphin (*Delphinus delphis*) by means of an aluminium pole armed with biopsy tips and ii) from fin whale, sperm whale, long-finned pilot whale (*Globicephala melas*) and common bottlenose dolphin with a biopsy dart launched with a crossbow (150-pound). Biopsy samples were taken in the dorsal area near the dorsal fin and on the upper part of the caudal peduncle. A little piece of biopsy was immediately stored in cell medium for cell cultures.

**Organochlorine contaminants:** Total PCBs were quantified as the sum of 30 congeners. The congeners constituted 80% of the total peak area of PCBs in all tissues. Total DDTs were calculated as the sum of op'DDT, pp'DDT, op'DDD, pp'DDD, op'DDE and pp'DDE. For DDT and PCB analysis, the samples were freeze-dried and extracted with n-hexane in a Soxhlet apparatus followed by sulphuric acid clean-up and Florisil chromatography (Marsili & Focardi, 1997).

## RESULTS AND DISCUSSION

The main results of this project were the following ones:

- high levels of DDTs and PCBs were recorded in the stranded specimens, in particular in common bottlenose dolphin (Fig. 1); these levels were very similar to those found in the 1990-1993 period, despite regulatory control on their production and use in Mediterranean area;
- the negativity to *Morbillivirus* was immunohistochemically obtained in all investigated tissues (brain, lung, lymph nodes and spleen) from all specimens under study. On the contrary, the serological and biomolecular investigations against *T. gondii* yielded positive results from different tissues of the stranded striped dolphins and bottlenose dolphins under study (Pretti et al., 2010; Di Guardo et al., 2010). *T. gondii* infection is commonly asymptomatic, although fever, lymphadenomegaly, encephalitis and severe ocular damage may occur mostly in immunocompromised individuals. In particular, concerns have been raised that *T. gondii* may be a major cause of mortality in sea otters (Miller et al., 2002);
- the high levels of DDTs and PCBs were also detected in the free ranging specimens, in particular in long-finned pilot whale (Fig. 2A-B), a finding that could be linked to the fact this species was the first one in which the 2006-2008 morbilliviral epidemic in the Mediterranean area occurred.

## CONCLUSION

The data obtained in this study still show the significant presence in the Mediterranean basin of xenobiotic compounds, such as DDTs and PCBs. These toxics are present with levels very similar to those found in the 1990-1993 period, when many striped dolphins were affected by a severe *Morbillivirus* infection epidemic along the Mediterranean coasts. Of the free-ranging specimens analyzed, pilot whale showed the highest levels of DDTs and PCBs. This finding could be linked to the fact this species was the first one in which the 2006-2008 morbilliviral epidemic in the Mediterranean area occurred. In the stranded cetaceans under study, no evidence of *Morbillivirus*, but of *T. gondii* infection was found, which could be related to a primary immunodeficiency condition caused by the high body loads of highly immunosuppressive xenobiotics - such as organochlorines – detected in these animals.

## ACKNOWLEDGEMENTS

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**CAPTIONS**

**Figure 1.** Levels of organochlorine contaminants (DDTs and PCBs) ( $\mu\text{g/g}$  lipid weight) in blubber of stranded specimens of Mediterranean *Stenella coeruleoalba* ( $n=7$ ), *Tursiops truncatus* ( $n=4$ ), *Physeter macrocephalus* ( $n=2$ ) and *Balaenoptera physalus* ( $n=1$ ) (2008-2010 period).

**Figure 2.** Levels of DDTs (A) and PCBs (B) ( $\mu\text{g/g}$  lipid weight) in subcutaneous blubber of free-ranging specimens of Mediterranean *Stenella coeruleoalba* ( $n=33$ ), *Tursiops truncatus* ( $n=1$ ), *Delphinus delphis* ( $n=8$ ), *Globicephala melas* ( $n=12$ ), *Physeter macrocephalus* ( $n=2$ ) and *Balaenoptera physalus* ( $n=6$ ) (2008-2010 period).



