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Bone as a surrogate tissue to monitor metals in baleen whales

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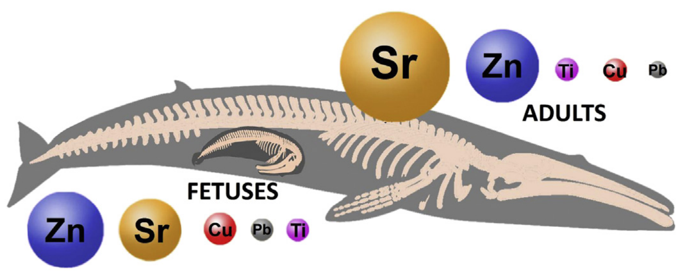
HIGHLIGHTS

- Zn, Cu, Pb, Ti, Sr concentrations were analyzed in the bone of two fin whale stocks.
- Sr and Pb were the only metals more concentrated in bone than in internal tissues.
- Whales from NW Spain showed higher Sr and Pb levels than those from W Iceland.
- Fetal bone showed higher Zn, Cu and Pb, and lower Sr levels, than the adults' bone.
- Zn and Pb concentrations in bone significantly increase with age.

GRAPHICAL ABSTRACT



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ABSTRACT

Metals are massively deposited in the marine environment through direct emissions or atmospheric dry and wet depositions, a process since long enhanced by human activities. Metal contamination in the marine organisms has been increasingly investigated, but most research focuses on few tissues, elements and species considered indicative. Baleen whales have been scarcely studied in this respect. Here we contribute to the fragmented knowledge on this field examining the concentrations of zinc, copper, lead, titanium and strontium in the bone of fin whales (*Balaenoptera physalus*) from NW Spain and W Iceland. Bone was selected because it is a tissue commonly available in archival historic collections, and it is therefore useful to examine long-term trends in metal pollution. We tested differences between populations and we investigated age- and sex-related accumulation trends, as well as the occurrence of placental transfer. Sr concentrations and Pb accumulation rates with age were significantly higher in individuals from NW Spain than in those from W Iceland. Placental transfer occurred, at different levels, for all metals: as a result fetuses showed significantly higher Cu, Pb and Zn concentrations than adults. After birth, only Zn and Pb concentrations significantly increased with age. Through this study we contributed to fill some gaps in the knowledge regarding metal contamination in marine mammals, and we concluded that bone can be a suitable surrogate tissue to monitor a number of trace elements, provided that dissimilarities in tissue-specific deposition are taken into account when comparing concentrations from different tissues.

1. Introduction

Metals reach the environment both through natural processes, such as volcanic activity or geological weathering, and

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anthropogenic processes, such as waste disposal, industrial emissions, mining operations or the use of fossil fuels (Bowles, 1999). The majority of metals are toxic for living organisms, although the level of such toxicity is variable. Some metals are essential at low concentrations due to their role in metabolism. These can present adverse effects to organisms both when their concentrations are too low and too high. Other metals do not have recorded functions in metabolism and can be toxic even at very low concentrations (Law, 1996).

The rapid and almost uncontrolled development of industrial activities in the last century has increased dramatically the emissions of heavy metals in the environment (Nriagu, 1990), raising concern about the potential threats of metal pollution to humans, other living organisms, and ecosystems as a whole.

The marine environment represents a sink where metals are deposited massively, through direct emissions or atmospheric dry and wet depositions (Thompson, 1990; Ansari et al., 2004). Despite metal contamination in the oceans has been increasingly studied in the last decades (*e.g.* Thompson, 1990; Eisler, 2010), most of the research has focused on a few elements, especially the most toxic ones, like mercury (Hg) or cadmium (Cd).

The importance of studying metal concentrations in marine mammals has been stressed since the late 1970s, when the FAO (Food and Agriculture Organization of the United Nations) working party on marine mammals stated that metals such as Hg, Cd, lead (Pb), arsenic (As), chrome (Cr), copper (Cu), nickel (Ni), iron (Fe) and titanium (Ti) were a potential threat to these key organisms and thus required special consideration (FAO, 1978). In 1999 the International Whaling Commission (IWC) further highlighted the lack of knowledge about metal contamination in marine mammals (except for Cd and Hg, studied in kidney and liver of some species) and stressed the need to expand the research to other metals and tissues (IWC, 1999).

Despite the body of knowledge on metal contamination in marine mammals has increased since then, the effort devoted to the various species, tissues and elements has been heterogeneous (for reviews, see Law, 1996; Aguilar et al., 1999; Das et al., 2003). Thus, most studies have focused on pinnipeds and odontocete cetaceans, while the groups of mysticete cetaceans and sirenians have been less investigated, due to their elusive nature and/or to the inherent difficulties in sampling. Metal analyses have been mostly conducted on protein-rich tissues such as liver, kidney and muscle, which accumulate and store the most toxic trace metals, while certain tissues, such as bone, teeth and skin, have received little attention. Finally, some metals raised a greater interest, particularly Hg, Cd, Cu or zinc (Zn), perceived as a potential hazard to marine mammals because of their higher toxicity and/or concentration in the tissues. Besides this, most studies have taken advantage of stranded, by-caught, or directly caught individuals, without any possibility to obtain sets of individuals of different ages from a given sampling area.

In the present study we contribute to complete this fragmented scenario investigating concentrations of Zn, Cu, Pb, Ti and strontium (Sr) in the bone of a mysticete cetacean, the fin whale (*Balaenoptera physalus*). Samples were available from a large tissue collection obtained from the whaling era that included individuals of both sexes and different ages taken from the waters of NW Spain and W Iceland. According to the IWC (IWC, 2009), these two areas represent the summer feeding grounds of two putative fin whale stocks, whose isolation is indicated by a number of evidences, ranging from genetics to chemical markers, morphologic data and satellite tracking studies (Lockyer, 1982; Sanpera et al., 1996; Bérubé et al., 1998; Víkingsson and Gunnlaugsson, 2005; Vighi et al., 2016). Previous investigation on metals in these whale stocks focused on determining the concentrations of Hg, Cd, Cu and Zn in liver, kidney and muscle (Sanpera et al., 1993, 1996), but data on metals such as Ti and Sr, as well as on any other metal in bone, were lacking. Indeed, information on Ti and Sr in cetaceans, as in other marine vertebrates, is overall very scarce (Viale, 1978; Holsbeek et al., 1999; Bryan et al., 2007; Griesel et al., 2008).

Bone has been scarcely used as a target tissue for cetacean research, due to the impossibility to take samples from alive specimens and the general difficulties related with its sampling even from dead animals. However, it is the main tissue preserved in museum and historical collections, which can typically include individuals from different regions, periods, and with a wide range of ages, allowing the investigation of long-term temporal trends. Moreover, due to its long physiological turnover rate, bone is less affected than other tissues to short-term variations in environmental baselines, diet composition or metabolic status. This characteristic makes bone liable to reflect exposure to metals over extended periods of time, suggesting that this tissue could be used to differentiate populations or detect differences between geographical regions.

The specific objectives of this study were: a) to determine metal concentrations in bone and compare them with those reported in other tissues in order to assess the potential of bone as a surrogate tissue to monitor trace elements in cetaceans; b) to test whether metal concentrations in bone may be used to differentiate fin whale populations possibly reflecting different environmental characteristic of their feeding areas; and c) to investigate the effect of sex, body length, age, and *trans*-placental transfer on the concentration of metals in the bone of fin whale.

2. Materials and methods

2.1. Sample collection and composition

Bone samples, made available by the biological tissue bank of the University of Barcelona (BMA Tissue Bank), were all composed of a central section of a thoracic rib. All samples had been collected from fin whales caught during the 1983–1984 summer whaling campaigns off NW Spain (Galicia Bank) and the 1986 summer whaling campaign off W Iceland (Denmark Strait) (Fig. 1).

In an attempt to mitigate any potential effect of sex and age in the comparison between areas, efforts were made to draw comparable subsets of samples from the overall collection. Thus, the final subsample sets used for this study included bone from 24 individuals from W Iceland (9 males and 15 females, with ages ranging from 7 to 27 years), and from 25 individuals from NW Spain (8 males and 14 females, with ages ranging from 6 to 26 years). Also, 3 bone samples from fin whale fetuses were analyzed, only from NW Spain.

All samples were preserved frozen until preparation for the analysis.

2.2. Age determination

Age determination was carried out according to the procedures described by Lockyer (1984). Growth layers were counted through a longitudinal section of the fin whales ear plug core. Each sample count was repeated by more than one reader, and for about 70% of samples the count was repeated twice by the same reader. Thus, if the difference between the age estimates for the same sample was higher than 10% of the lowest age estimate, the age determination of that sample was not considered valid; otherwise the mean of all readings was considered as a reliable estimate of the age of the sample.