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Biological variables and health status affecting inorganic element concentrations in harbour porpoises (*Phocoena phocoena*) from Portugal (western Iberian Peninsula)

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ABSTRACT

The coastal preferences of harbour porpoise (*Phocoena phocoena*) intensify their exposure to human activities. The harbour porpoise Iberian population is presently very small and information about the threats it endures is vital for the conservation efforts that are being implemented to avoid local extinction. The present study explored the possible relation between the accumulation of trace elements by porpoises and their sex, body length, nutritional state, presence of parasites and gross pathologies. The concentrations of arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) and selenium (Se) were evaluated in 42 porpoises stranded in Portugal between 2005 and 2013. Considering European waters, porpoises stranded in Portugal present the highest Hg concentrations and the lowest Cd concentrations, which may reflect dietary preferences and the geographic availability of these pollutants. While no effect of sex on trace element concentrations was detected, there was a positive relationship between porpoise body length and the concentration of Cd, Hg and Pb. Animals in worse nutritional condition showed higher levels of Zn. Harbour porpoises with high parasite burdens showed lower levels of Zn and As in all analysed tissues and also lower levels of renal Ni, while those showing gross pathologies presented higher Zn and Hg levels. This is the first data on the relationship between trace elements and health-related variables in porpoises from southern European Atlantic waters, providing valuable baseline information about the contamination status of this vulnerable population.

1. Introduction

Marine mammals are long-lived top predators that may function as sentinel organisms of the marine ecosystem health (see Bossart, 2011). Several marine mammal species present a more coastal rather than oceanic behavior, inhabiting areas with elevated levels of human activities pressures (e.g. economic fisheries) (Halpern et al., 2008, 2015). Coastal habitats represent a depot for toxic agents from industrial, agricultural and urban sources, contributing to a contaminant-enriched environment (e.g. Delgado et al., 2011; Mil-Homens et al., 2014). Consequently, bio-accumulation and long-term exposure to pollutants could be expected to represent a threat to marine mammals, especially for those living in coastal areas (Bennett et al., 2001; Das et al., 2004a).

The variability of contaminant levels in cetaceans has been correlated with their ecological and biological characteristics such

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as diet, age, length, sex, location or tissue type (e.g. Aguilar et al., 1999; Das et al., 2003a; Wagemann and Muir, 1984). Additionally, several studies have found a relationship between concentration of contaminants and the health status of cetaceans (e.g. nutritional state, infectious disease, parasite burden; Beineke et al., 2005; Bennett et al., 2001; Das et al., 2004a; Lavery et al., 2009; Mahfouz et al., 2014; Pascual and Abollo, 2003; Siebert et al., 1999; Strand et al., 2005; Szefer et al., 1998). Along with the immunosuppressive effects and immunological dysfunctions of non-essential trace elements on marine mammals (e.g. Frouin et al., 2012; Mahfouz et al., 2014), other adverse effects include metal-induced mutagenic alterations (e.g. Mollenhauer et al., 2009), interference in neurological processes (e.g. Basu et al., 2006), renal and hepatic damage (e.g. Lavery et al., 2009; Rawson et al., 1993).

As such, monitoring the harbour porpoise (*Phocoena phocoena*) provides good information about the contamination status of the local population of the species, as well as public health problems. In fact, the coastal preferences of harbour porpoises (Hammond et al., 2013; López et al., 2004; Méndez-Fernandez et al., 2012; Pierce et al., 2010; Santos et al., 2012) intensify their exposure to negative effects of human activities. High levels of trace elements have been documented in various studies on harbour porpoises (e.g. Bennett et al., 2001; Das et al., 2004a; Lahaye et al., 2007; Mahfouz et al., 2014; Méndez-Fernández et al., 2014; Szefer et al., 2002).

Although the abundance of harbour porpoise has slightly increased over the last decade in higher north Atlantic latitudes (estimated abundance: 323,968 and 341,366 individuals in 1994 and 2005, respectively; Hammond et al., 2002, 2013), the Iberian population is presently very small with an estimated abundance of 2357 individuals in 2005 including the south of France (Hammond et al., 2013) and 2125 individuals in 2013 only in Portugal (Araújo et al., 2015), presenting the lowest densities ever recorded in European waters (0.017 and 0.085 ind./km2, in 2005 and 2013, respectively; Araújo et al., 2015; Hammond et al., 2013). The Iberian and north Atlantic populations represent separate ecotypes based on ecological, genetic and morphological differences (e.g. Fontaine et al., 2010, 2014), with restricted contact between the two Atlantic ecotypes in the Bay of Biscay indicating the onset of speciation (Fontaine et al., 2010, 2014; Nosil et al., 2009). Although further evaluation of their divergence is needed, the subspecies *Phocoena phocoena meriodionalis* spp, allocated to porpoises inhabiting upwelling waters (Iberia and Mauritania), was recently proposed (Fontaine et al., 2014).

Concern for the species' status led to harbour porpoise (*P. phocoena*) being listed in many international conventions, directives and agreements (e.g. EU Habitats Directive, CITES, ASCOBANS). Specifically, the harbour porpoise is listed under the Annex II of the European Habitats Directive (Directive 92/43/CEE), which requires member states to designate special areas of conservation (SAC) to protect their populations. Therefore, data on harbour porpoise ecology and anthropogenic impacts are needed in order to build effective management plans including conservation and mitigation measures. This is particularly evident in the Iberian Atlantic coast, considering the ecological and genetic isolation of porpoises in this region, their "Vulnerable" status in Portugal (Cabral et al., 2006), their low abundance, their coastal preferences overlapping with human activities and the scarce information regarding the impact of human pressures on this species.

In this study, several trace element concentrations were evaluated in harbour porpoises stranded in Portugal in order to: 1) evaluate the potential influence of porpoise sex, body length, nutritional state, presence of parasites and gross pathologies in trace element burdens; 2) assess the population status in the Portuguese coast by comparing levels of metal contaminants in organs of porpoises stranded in other regions.

2. Methodology

2.1. Sample collection

Samples were collected from 42 harbour porpoises (average body length = 153.10 cm, SD = 23.06 cm) stranded in the Portuguese coast from 2005 to 2013 (Table 1). Strandings were attended by experienced personnel belonging to the Portuguese stranding network, coordinated by Institute for Nature Conservation and Forests (ICNF) and Sociedade Portuguesa de Vida Selvagem (SPVS). Detailed necropsies were performed, depending of the animal's decomposition state (Geraci and Lounsbury, 1993). The nutritional state (body condition) of the individuals was evaluated from visual examination of specific anatomical landmarks (see Joblon et al., 2014) and categorized into good, moderate and emaciated animals (Table 1). All organ systems were examined macroscopically, to detect the presence of parasites or evidence of gross pathologies (Young, 2007). The presence of parasites refers to severe parasite infestation found in any organ system or if a high diversity of parasites was collected in an individual. The occurrence of gross pathologies was recorded if any evidence of pathology (lesions or abnormal appearance) was found in any organ system (e.g. Pugliares et al., 2007; Vlasman and Campbell, 2004). Data and samples were collected according to standard protocols (Kuiken and Hartmann, 1991). In particular, kidney, liver and muscle samples were stored in glass vials and frozen (−20 °C) for posterior trace element analysis.

2.2. Analytical procedure

Approximately 100–150 mg (wet weight, ww) of kidney, liver and muscle tissues were digested in teflon vessels with 2 ml of HNO_3 and 1 ml of H_2O_2 (Merck, Suprapure). Acid digestion of the samples was performed in an drying oven at 90 °C, overnight (14 h). All materials used in the digestion process were thoroughly acid-rinsed. After digestion, samples were diluted with ultrapure water and analysed for nine trace elements [zinc (Zn), manganese (Mn), lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), mercury (Hg), nickel (Ni), selenium (Se)], by ICP-MS (Perkin Elmer Elan 6000). To determine analytical accuracy several blanks and standard reference material (*Squalus acanthias* – Dogfish liver (DOLT-3) and muscle (DORM-2)) (National Research Council, Canada) (Table S1, Supplementary material) were prepared and analysed along with samples. The accuracy of each trace element in respect to the standard reference material (DOLT-3 and DORM-2) is summarized in Table S1. ICP-MS analysis revealed accuracy rates ranging between 91.5% for Mn and 112.8% for Se.

Trace element concentrations are reported in $\mu g.g^{-1}$, based on wet weight values (ww).

2.3. Statistical procedure

All data series were explored for outliers, collinearity, heterogeneity of variance and for visualization of potential relationships between response and explanatory variables, following Zuur et al. (2010). Generalized Linear Models (GLMs) were used to determine the effect of explanatory variables on the concentration of trace elements in harbour porpoises. Since response variables (concentration of each trace element) were continuous, a Gaussian distribution was applied. The considered explanatory variables were sex (categorical), length (continuous), nutritional state (categorical), presence/absence of parasites (categorical) and presence/absence of gross pathologies (categorical). Forward selection was applied to identify the best models, with the optimum model being the one that presented the lowest Akaike Information Criterion