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Assessing trace elements in striped dolphins from the Strait of Gibraltar: Clues to link the bioaccumulation in the westernmost Mediterranean Sea area and nearest Atlantic Ocean

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HIGHLIGHTS

- Stranded dolphins are good bioindicators showing great potential as monitoring tools.
- Trace elements were first measured in stranded dolphins in the Strait of Gibraltar.
- Trace elements are compared among target organs and correlated to biological parameters.
- First report of Se/Hg ratio in cetaceans from this relevant transitional region.
- This region can be considered a transitional area between Mediterranean and Atlantic basins.

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ABSTRACT

Dolphins are considered sentinel species in the marine environment. The Strait of Gibraltar is the only passage between the Mediterranean Sea and the Atlantic Ocean, being the transitional region which connects these two basins and one of the most important routes of cetacean migration worldwide. In this work, eight trace elements (TE) were studied in 45 samples of liver, kidney and muscle, from 15 specimens stranded in this study area. The preliminary results show, among others, the patterns of distribution of the TE in the target organs studied, the influence of sex, length and developmental stage in these TE concentrations and the Se/Hg ratio. Subsequently, the results of TE concentrations in liver have being compared to previous data on *S. coeruleoalba* from the westernmost Mediterranean Sea and the nearest Atlantic Ocean. For some elements (e.g. for As), concentrations are similar to those obtained from Atlantic samples, despite in other cases (e.g. for Cd) results are lined up with those observed in Mediterranean studies. In addition, in the case of some TE (e.g. Se and Zn) the results are in the middle of those reported for both basins, reinforcing the idea of the Strait of Gibraltar being a transitional zone. Present study is the first research regarding this issue in this outstanding region, aiming to give insights of how this matchless area can help to link TE concentrations observed in these Atlantic and Mediterranean threatened species.

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1. Introduction

Presently, several EU directives on protecting the marine environment are in force. The Water Framework Directive (WFD, Council Directive, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, Council Directive, 2008/56/EC) are regulatory frameworks, with the latter being aimed at achieving "Good Environmental Status" by 2020. The WFD has established a list of Priority Substances and Environmental Quality Standards (EQSs) for Priority Substances and certain other pollutants (reviewed and amended by Council Directive, 2013/39/EU). The MSFD considers this Priority Substances list for the assessment of chemical contaminants in the marine environment and emphasizes the need to

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monitor the concentration of contaminants in the marine environment and their effects, among others.

Some trace elements (e.g., zinc (Zn), copper (Cu), and selenium (Se)) are essential to organisms because such elements are involved in metabolic and biochemical processes (becoming toxic if present at too high concentrations). Those trace elements that are non-essential, i.e., mercury (Hg) and cadmium (Cd), are among the most toxic metals (Becker et al., 1997, Law, 1996; Wolfe et al., 1998; Borrell et al., 2015; Monteiro et al., 2016; inter alia). Hg is one of the compounds of greatest interest worldwide because it is one of the most toxic elements for marine mammals due to bioaccumulation and biomagnification through the marine food web (Das et al., 2003a; Stavros et al., 2007).

Odontocetes are considered sentinel species in the marine environment because of their high trophic position and long life-span as well as being prone to bioaccumulation and biomagnification of organic pollutants and trace elements (Bellante et al., 2011; Bossart, 2011; Borrell et al., 2014). The striped dolphin (*Stenella coeruleoalba*) is one of the most abundant species in the Strait of Gibraltar and surrounding areas and are also those most commonly found stranded in this region (Rojo-Nieto et al., 2011). This species is sentinel of the contamination status of marine ecosystems due to its exposure through diet to high levels of pollutants including trace elements (Monaci et al., 1998; Borrell et al., 2014, 2015, inter alia). This species is also considered to be a valuable biomarker of the health status of marine ecosystems because it inhabits the open water beyond the continental shelfs (Borrell et al., 2014). In addition, the striped dolphin is listed under Annex II (amended in 2013) of the Convention for the Protection of the Mediterranean Sea Against Pollution (Barcelona Convention, 1976; revised in 1995) which lists endangered or threatened species to be considered in protocols concerning specially protected areas and biological diversity in the Mediterranean. Therefore, data on the anthropogenic impacts on them are needed in order to build effective management plans that include conservation and mitigation measures.

Globally, studies of pollutants and/or trace elements (TE) in target organs of stranded cetaceans have been developed especially in the last ten years (Bachman et al., 2014; Ko et al., 2014; Shoham-Frider et al., 2014; Nomiyama et al., 2011; Hoydal et al., 2015; Méndez-Fernandez et al., 2016). However, it is still a scope under development, and there are few studies taking into account a large number of samples, different compounds and/or different target organs, and their relationship to biological parameters. Even less are the works that link studies from different areas with possible common influences such as the South European Atlantic Ocean and the Westernmost Mediterranean Sea. In order to fill the gap, in this work, eight trace elements in three different target organs (liver, kidney, and muscle) of 15 striped dolphins stranded during 2012 and 2013 were studied. In addition, the Se/Hg molar ratio has been studied. According to García-Alvarez et al. (2015), to evaluate the health status of the ecosystems, the simultaneous study of Hg and Se and the relationships between them is of great interest, particularly in those species usually considered as sentinels of environmental pollution. To study this, the Se/Hg molar ratio has been widely used (García-Alvarez et al., 2015; McHuron et al., 2014; Méndez-Fernandez et al., 2014; Squadrone et al., 2015). Se molar excess could involve a protection action for methyl Hg toxicity by demethylating Hg to stable Se-Hg compounds (HgSe or tiemannite) (Decataldo et al., 2004). However, at the same time, this protective action can be harmful to the body because complex formation also results in the sequestration of Se which causes it to become biologically unavailable. The main goal of this study was to investigate the levels of trace elements in internal tissues of striped dolphins and the relationships with length, sex, and developmental stage by investigating samples from a unique area, the European coast of the Strait of Gibraltar, and connecting results with those from similar studies in the Southern European Atlantic Ocean and the Westernmost Mediterranean Sea. This strait connects the Mediterranean Sea and the Atlantic Ocean and is one of the most important routes of cetacean migration worldwide (de Stephanis et al., 2008). To the best of the author's knowledge, this study is the first research regarding this issue in this environment of enormous special interest.

2. Material and methods

2.1. Study area

The Strait of Gibraltar (Fig. 1), the only passage between the Mediterranean Sea and the Atlantic Ocean, is characterized by a surface inflow of Atlantic waters and a deep outflow of Mediterranean waters (Armi and Farmer, 1985). It is inhabited by a large number of cetacean species (de Stephanis et al., 2008). According to these authors, there is a group with a northward tendency that is composed of common and striped dolphins. Due to its at-sea location and feeding habits, this group is likely to feed on mesopelagic fish or squids associated with the surface Atlantic waters. Linking these results to those related to stranding frequencies (Rojo-Nieto et al., 2011; inter alia) points out the North coast of the Strait of Gibraltar and surrounding areas as an outstanding and representative zone to study samples of stranded specimens of striped dolphins. The most western set of samples included in this study was from Sanlúcar de Barrameda (Cádiz), and the most eastern samples were from Almuñécar (Granada) (see Fig. 1).

2.2. Sample collection

During 2012, 2013, 45 samples of liver, kidney, and muscle were collected from 15 striped dolphins (*Stenella coeruleoalba*) stranded on the Southwest coasts of Spain at the Strait of Gibraltar and surrounding areas. Necropsies were carried out on the stranded animals on 'very fresh, fresh, or moderate autolysis' by using a standardized protocol (Kuiken and García-Hartmann, 1991). The samples were collected by the veterinary staff of the Centre for Management of Marine Environment (CEGMA). All animals underwent necropsy as soon as possible after they were found/dead (time range from two to 48 h) and were kept cool in the interim period. Tissues samples from the liver, kidney, and muscle were excised, wrapped in a plastic bag, and preserved frozen at −25 °C according to Borrell et al. (2000, 2014). In the present study, samples from striped dolphins were transferred from CEGMA to CACYTMAR (Andalusian Centre for Marine Science and Technology) at the University of Cadiz where the analyses were carried out.

2.3. Sample preparation and analysis

The samples from target organs were stored in CACYTMAR facilities at −80 °C until the analyses. They were freeze-dried in a lyophilizer and milled using a ceramic mortar until they were reduced to a uniform powder. The samples were microwave-digested in an acid solution in Teflon vials and were analyzed both by ICP-MS and ICP-AES depending on the total concentration. Briefly, approximately 100 mg (dry weight, dw) of kidney, liver, and muscle tissues were oven-digested at 90° in closed teflon vessels overnight in an acid solution (H_2O_2/HNO_3, 1:2 ratio, Merck, Suprapure) as described by several authors (Borrell et al., 2015; Monteiro et al., 2016). All materials used in the digestion process were acid-rinsed according to a protocol developed specifically for trace elements analysis in the Central Services for Science and

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