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Bioaccumulation of mercury and other trace elements in bottom-dwelling omnivorous fishes: The case of *Diplodus sargus* (L.) (Osteichthyes: Sparidae)

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ABSTRACT

The presence of toxic elements in fish represents a hazard for human health, especially in Mediterranean countries and other regions with high per-capita fish consumption. The present research, carried out along the northern Catalan coast (NW Mediterranean), aimed to determine the levels of trace metals and arsenic in the muscle of white seabream *Diplodus sargus* (L.), a common demersal species of growing interest for fisheries and aquaculture. Average mercury concentrations widely exceeded the limits imposed by EU despite the low contamination levels previously reported for the study area, stressing the potential risk associated to the consumption of medium-sized, non-predatory fishes. The other analyzed elements fell within the recommended limits. Preliminary results about the feeding habits of *D. sargus* are reported, in order to determine feeding habitat and items of the analyzed specimens.

1. Introduction

Trace metals and arsenic are among the most spread and hazardous contaminants. Their concentrations in water, soil and atmosphere has increased sensitively during the industrial era, and they have entered the trophic webs, accumulating in organisms and representing a risk for humans feeding on contaminated animals, plants and fungi (Bradl, 2005; Castro-González and Méndez-Armenta, 2008). Fish are among the animal groups of greater concern, as they: i) are able to sequester relatively high amounts of pollutants in their tissues, as a defense against their toxic effects; ii) occupy high positions in aquatic trophic webs, being affected by lower levels; and iii) represent an important source of animal proteins for people (Dallinger et al., 1987).

Some trace elements (TE) have no recognized biological role (non-essential elements), whereas others are known as essential, since they are components of body fluids, cofactors in enzymatic reactions, structural units of non-enzymatic macromolecules, etc. (Watanabe et al., 1997). Non-essential elements can lead to oxidative stress in fish by increasing the cellular concentration of reactive oxygen species (ROS) and by reducing the cellular antioxidant capacity (Pinto et al., 2003). They may cause symptoms of chronic toxicity, affecting kidney functioning and reproductive capacity, and leading to hypertension, tumors and hepatic dysfunction (Luckey and Venugopal, 1978; Waalkes, 2000). Although fish regulate essential-metal loads in their organs by maintaining a delicate balance among uptake, storage and excretion, too high environmental concentrations of these elements may result toxic as well, damaging skin, gills, liver, kidney and nervous system, depressing embryonic development and growth, and leading to death in the most serious cases (Weis and Weis, 1989; Watanabe et al., 1997).

Trace-element uptake by aquatic organisms like fish may be either waterborne, i.e. directly from water to the organism, through the respiratory surfaces; or dietborne, i.e. with food, with consequent absorption in the digestive tract (Terra et al., 2008; Rozon-Ramilo et al., 2011). The bioaccumulation process is complex, depending on a large number of variables beyond environmental pollution: fish species, size and age, sex, feeding behavior, habitat, physiological conditions, spawning status or migration, and parasites (Andres et al., 2000; Canli and Atli, 2003; Merciai et al., 2014; Torres et al., 2015).

Fish is a traditional component of Mediterranean diet, and its consumption is generally recommended as an important source of high-quality proteins and omega-3 polyunsaturated fatty acids. However, the presence of highly toxic elements in fish muscle, sometimes at concentrations potentially harmful to humans, has led to questioning of the real benefits of fish consumption to human health. Relevant uptake of toxic non-essential elements such as Hg, Cd, Pb and As may lead to

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neurotoxicity of both the peripheral and central nervous system, especially in infants and developing embryos; damages to kidney, lung, skeletal, hematological and cardiovascular system, testes and placenta have been extensively documented, as well as carcinogenic effects (Castro-González and Méndez-Armenta, 2008).

In particular, Hg in its organic form (methylmercury: MeHg) is considered the metal of most concern, as it tends to accumulate in sediments and it is consequently uptaken by demersal fishes, especially those with benthivore and omnivore diets (Berge and Brevik, 1996; Storelli et al., 1998; Storelli and Marcotrigiano, 2000a; UNEP, 2013). Methylmercury binds to cysteine and accumulates in protein-rich tissues, like muscle, biomagnificating and achieving highest concentrations in large-sized top predators as tuna, swordfish and cod (Castro-González and Méndez-Armenta, 2008; EFSA, 2012; UNEP, 2013), but also in small-sized species searching for food on the deepness, close to the sediment (Berge and Brevik, 1996; Storelli et al., 1998; Storelli and Marcotrigiano, 2000a). The role of Se should also be considered when studying Hg bioaccumulation, as some studies suggest that a Se:Hg molar ratio > 1 could protect against potentially adverse Hg effects; therefore tissue MeHg loads alone would be no reliable predictor of risks of developing toxic symptoms due to MeHg exposure (Ralston et al., 2008; Burger and Gochfeld, 2011).

Diplodus sargus (L.) is a common demersal fish, inhabiting rocky infralittoral and circalittoral habitats in Mediterranean Sea and eastern Atlantic Ocean, including Macaronesian Archipelagos (Pérez et al., 2007). The total amount of captures of this species has almost doubled in the last decade, and its importance for aquaculture is increasing too (D'Anna et al., 2004; Sa et al., 2006, 2007; FAO, 2007; Ferreira et al., 2008). Despite being neither a predator nor large-sized, previous studies showed that *D. sargus* is capable of accumulating, in its edible part, hazardous elements like Hg beyond the limits established by European Union (EC, 2006, 2008), making its consumption potentially harmful (Tramati et al., 2012; Casadevall et al., 2017). *Diplodus sargus* is therefore a medium-sized species with a relatively long lifespan and a benthic omnivorous diet. The purpose of this study is to evaluate the importance of habitat, diet and size on the accumulation of TE in *D. sargus*, as an example of small-sized species searching for food close to the sediment, as well as the possible effects of toxic elements on its body condition (i.e. weight-length relationship).

2. Material and methods

2.1. Data collection

Samples of *D. sargus* were obtained from four commercial ports located on the northern Catalan coast (NE Spain): Llançà, Port de la Selva, Roses and Palamós (Fig. 1), where the studied species is captured on a regular basis by the local fishing vessels. The above-mentioned area of study is found next to the Gulf of Lion, a highly productive zone in NW Mediterranean Sea, characterized by a wide continental shelf and high freshwater inputs (Radakovitch et al., 2008). The Cap de Creus zone, moreover, is affected by upwelling processes in correspondence of strong winds from NW (Millot, 1979).

Total length (TL), total weight (TW) and eviscerated weight (EW) of each specimen were recorded. Stomachs and guts were separated, opened and the content was observed under a stereo microscope in order to separate animal preys and macrophytes (algae and seagrasses). Animals were stored in 70% alcohol and macrophytes in 4% formaldehyde. All items were sorted and identified to the species level when possible. The contribution of each type of prey to the diet was expressed as the percentage of frequency of occurrence (OC%) i.e. the percentage of stomach with a specific prey in relation to number of stomachs with food.

In order to infer the habitat (infralittoral or circalittoral) of the captured specimens on the basis of consumed species, each macroalgae taxon was attributed to an ecological group (Boudouresque, 1984), whereas the seagrass *Posidonia oceanica*, whose leaves were frequently found in the digestive content, was treated as an infralittoral species. In the analyses, only the presence of *P. oceanica* and the macroalgae belonging to ecological groups restricted to a specific littoral level were counted, avoiding taxa with large environmental requirements that can be found both in the infralittoral and in the circalittoral level. Thus, for the infralittoral level, we included only photophilous species of large requirements (PhI), from wave-washed environments (PhIB), from quiet environments (PhIC), and thermophilous (PhIT), sciaphilous species of large requirements (SI), from wave washed waters (SIW), and from quiet environments (SIQ), species of hard bottoms (IHdB), epiphytes of the *P. oceanica* leaves (PL), species tionitrophilous (ETN) and species from small harbours (PhIH). For the circalittoral, only the macroalgae living in coralligenous concretions (CC) and sciaphilous, rheophilous (SRh) were taken into account.

2.2. Sample analysis

A portion of muscle tissue, between 150 and 250 mg wet weight (w.w.) was extracted and stored frozen, in order to analyze the muscular concentration of several elements (Cr, Mn, Cu, Zn, Se, Cd, Sn, Hg, Pb, As). These samples were digested in Teflon vessels with HNO_3 (2 ml) and H_2O_2 (1 ml) (Merck, Suprapure) at 90 °C in an oven and left overnight. All materials used in the digestion process were thoroughly acid-rinsed. After digestion, samples were diluted with 30 ml of Milli-Q water. Total concentrations of TE were quantified by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer Elan 6000). Mercury analyzed is the total Hg, or the amount of inorganic Hg (iHg), MeHg, and any other Hg forms present. The analytical procedure was checked using standard reference material Dogfish (*Squalus acanthias*) liver (DOLT-3) and muscle (DORM-2) (National Research Council, Canada). Several analytical blanks were prepared and analyzed, along with samples, in order to determine the detection limits. Limits of detection were lower than 0.1 ng ml^{-1} for all elements and their recovery rates always ranged from 90 to 110%. The analytical process was performed at the CCiTUB "Centres Científics i Tecnològics de la Universitat de Barcelona".

2.3. Data analysis

The total amount of TE in each sample was expressed as Metal Pollution Index (MPI) according to Usero et al. (2005): $MPI = (Cf_1 \times Cf_2 \ldots Cf_n)^{1/n}$, where Cf_n is the concentration of the element n in the sample. All element concentrations were expressed in micrograms of element per kilogram of fresh muscular tissue (parts per billion, ppb w.w.). Molar concentrations of Se and Hg were obtained by dividing their concentrations by each respective atomic mass (200.59 for Hg and 78.9 for Se) according to Burger and Gochfeld (2011). Fish body condition (relationship between weight and length) expresses the overall well-being of a fish (Bagenal and Tesh, 1978; Bolger and Connolly, 1989) and was computed as: i) Le Cren's relative condition factor: $Kn = W/aL^b$, where W is fish weight, L is fish length and a and b are constants; ii) length-adjusted fish weight, using the analysis of covariance (ANCOVA; García-Berthou and Moreno-Amich, 1993). Fish TL and EW were used for computations.

Trace-elements concentrations and Se:Hg molar ratio showed a right-skewed, non-normal distribution. So, they were Log_{10}-transformed in order to meet the assumptions of parametric statistical methods (normality, homoscedasticity and linearity). ANCOVA models were used to explore the variation of TE concentrations (dependent variable) among sampling sites (categorical factor) after controlling for the effect of fish length (covariate). These ANCOVA models included the interaction between covariate and categorical factors, which tests the equal slopes assumption of standard ANCOVA, and was only removed if it was non-significant ($P > 0.1$). If the interaction is significant, slopes are not homogeneous and the parallelism assumption of