



## Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health

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### ABSTRACT

Among all available species, fish are a powerful model for risk-benefit assessments to study the effects of contaminants on human health. Gilthead seabream (*Sparus aurata*, Linnaeus 1758) and european seabass (*Dicentrarchus labrax*, Linnaeus 1758) are two species of great economic importance, representing very large production volumes in the Mediterranean. The objective of this study is (1) to analyze the concentrations of Trace Elements (TE) between wild and cultured seabream and seabass specimens, (2) to compare the determined concentrations with other studies, and (3) to increase the data about the potential risks to human health. Our results point to significant intra- and interspecies-specific differences between wild and cultured fish for several trace elements. Several strong and moderate inter-elemental correlations in fish muscle were observed through correlation analysis. In our study, the mean levels of trace elements were still below the standard safety values for fish intended for human consumption. The same results were reached for all the parameters analyzed (international legal limits, estimated weekly intake, provisional tolerable weekly intake, target hazard quotient, target cancer risk), with trace element levels in fish below those that could pose a risk to human health. Consequently, these fish can be considered safe for human consumption. A better understanding of the levels of trace elements in fish would also better inform consumers about the potential risks of exposure to contaminants.

### 1. Introduction

In recent years, there has been an increase in global fish consumption, mainly because human health benefits, such as the prevention of cardiovascular and other diseases (Lei et al., 2013). Likewise, fish are a good source of proteins and lipids of high biological value, with long-chain polyunsaturated fatty acids, liposoluble vitamins, and essential elements (Erkan and Özden, 2007; Mnari et al., 2012). At present, approximately 44% of the fish consumed by humans comes from aquaculture, and this percentage is predicted to reach 52% by 2025 (FAO, 2016). In spite of these advantages, the accumulation of trace elements in fish may pose a potential risk to human health (Medeiros et al., 2012). For a long time, however, pollution by trace elements (TE) of anthropogenic origin in the marine environment has been recognized as a serious threat to marine ecosystems (Dural et al., 2007).

Trace elements in the marine environment are either of natural origin (geochemical processes) or anthropogenic origin (e.g.

agriculture, transport, mining, metalworking and pharmaceutical products) (Castritsi-Catharios et al., 2015).

In aquaculture, there may be additional sources of TE, such as copper-based antifoulings used to retard the build-up of fouling organisms, fish excreta, and/or fish pellets enriched with TE to meet their nutritional requirements (Sapkota et al., 2008). These elements due to their persistence, bioaccumulation and possible biomagnification in the food web represent a significant threat to marine organisms (Demirak et al., 2006; Uysal et al., 2009). Several factors can influence the bioaccumulation of TE in fish tissues, such as environmental conditions (temperature, salinity, pH etc.), biological variations (species, sex, size and age), nourishment sources, and seasonal changes (Fallah et al., 2011).

The assimilation of TE by fish is carried out mainly by the ingestion of suspended particles in the water, ingestion of food, ion exchange of dissolved TE across lipophilic membranes (e.g., gills), and adsorption on tissue and membrane surfaces (Alam et al., 2002b). Some TE are

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essential for life (e.g. selenium, copper, cobalt, molybdenum, manganese, and zinc), with important nutritional functions that promote good health status in both fish and humans (Vandermeersch et al., 2015). However, other elements have no biological function (e.g. lead, cadmium, mercury, arsenic, tin, vanadium, and aluminum), and their consumption can cause adverse effects (e.g., renal dysfunction, lung disease, liver failure and dysfunctions in the kidneys, chronic damage to the central and peripheral nervous system) (Dadar et al., 2016; Squadrone et al., 2016). As they accumulate along the food chain, critical levels for human health can be reached (Yi et al., 2011). Thus, there is a growing interest in food safety with regard to the potential accumulation of these contaminants and its consequences (Lei et al., 2013). Fish represent an interesting model for risk–benefit assessments of the nature and probability of health effects in humans exposed to contaminants at present and in the future (Di Bella et al., 2015).

Gilthead seabream (*Sparus aurata*, Linnaeus 1758) and european seabass (*Dicentrarchus labrax*, Linnaeus 1758) are very important commercial species and are the two most widely farmed marine species in the Mediterranean Sea (Ferreira et al., 2010). Total fishery production (for both species) was 15,104 t in 2014, including a production of 6703 t for gilthead seabream and 8401 t for european seabass. Aquaculture production was 314,838 t, out of which 158,389 t were seabream and 156,449 t were seabass (FAO, 2017). Every year, between 400 and 600 million individual seabream and seabass are produced (Arechavala-Lopez et al., 2013). In the Mediterranean, their production has steadily increased in recent years, and these species are of great economic importance for the region (Squadrone et al., 2016). However, this intensive production in aquaculture generates many questions and concerns about the quality of farmed fish compared to wild fish (Alasalvar et al., 2002). For this reason, the assessment of differences in the quality of wild and farmed fish flesh is essential (Wang et al., 2014). It is therefore necessary to monitor and control the dangerous levels of fish contamination (Custódio et al., 2011).

The aim of this study is to examine the concentration of 19 trace elements (Ag, Al, As, Ba, Bi, Cd, Cr, Cu, Fe, Li, Mn, Mo, Pb, Sb, Se, Sn, U, V, and Zn) found in wild and farmed *S. aurata* and *D. labrax* collected from the Mediterranean Sea around Corsica. The study consisted of: (1) an analysis of the concentrations of trace elements in wild and cultured seabream and seabass specimens, (2) a comparison of the concentrations determined with those reported in other areas and with respect to international food safety regulations, and (3) enrichment of the data available on the potential risks to human health associated with the consumption of marine fish.

## 2. Material and methods

### 2.1. Sample collection

One-hundred and twenty fish [seabass (n = 60) and seabream (n = 60)] have been studied from different origins (wild and farmed) ranging from 26 to 60 cm total length (TL). Fish collection was carried out from populations located off the Corsican coast (Northwestern Mediterranean). The sampling was conducted from October to December 2016. Various fish farms located along the Corsican coast provided the cultured specimens. The anonymity of the farms sampled is preserved, according to the wishes of the fish farmers. Catches of wild specimens were made by fishermen using nets mainly in the Biguglia and Urbino lagoons and were transported directly to the laboratory. The method of slaughter was immersion in ice-cold water (hypothermia) for both wild and farmed fish. The analyzes were performed on the muscles of the fish.

### 2.2. Trace element analysis

According to the method described by (Gobert et al., 2017; Richir and Gobert, 2014), samples (approximately 200 mg) were mineralized

in Teflon digestion vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc. Sorisole, Italy), using 2 ml of nitric acid (HNO<sub>3</sub>, 60%) and 1 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) as reagents (suprapur grade, Merck, Darmstadt, Germany). Analyses of 19 trace elements (Ag, Al, As, Ba, Bi, Cd, Cr, Cu, Fe, Li, Mn, Mo, Pb, Sb, Se, Sn, U, V, Zn) was conducted using inductively coupled plasma mass spectrometry using dynamic reaction cell technology (ICP-MS ELAN DRC II, PerkinElmer®, Wellesley, United States). Analytical quality control was ensured using Certified Reference Materials (CRM), DORM-4: Fish protein and NIST 2976: Muscle tissue. Analysis of the CRM revealed a high level of agreement between the certified values for all TE (global mean recovery was 94 ± 12%). No certified values were reported for Ba, Bi, and Sb. For each TE, detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution (Currie, 1999). The detection limits in mg.kg<sup>-1</sup> dry weight were: Ag = 0.0011; Al = 0.0690; As = 0.0036; Ba = 0.0008; Bi = 0.0013; Cd = 0.0018; Cr = 0.0041; Cu = 0.0047; Fe = 0.1162; Li = 0.0018; Mn = 0.0010; Mo = 0.0014; Pb = 0.0037; Sb = 0.0024; Se = 0.0822; Sn = 0.0029; U = 0.0001; V = 0.0009; Zn = 0.0208. We converted our dry weight to wet weight data (using a conversion factor of 4) in order to compare with previously published studies (e.g. Anan et al., 2005). Our results are expressed in milligrams of element per kilogram of body wet weight (mg.kg<sup>-1</sup> ww).

### 2.3. Data analysis

During data statistical treatment, for the samples with values below their analytical limit of detection (LD), half of the respective LD was substituted. Normality and homogeneity of variances were tested using Kolmogorov–Smirnov and Levene tests respectively. To better meet the assumptions of standard parametric statistical tests, to reduce the effect of outliers skewing the data distribution, and to bring elemental concentrations within the same range, the data were natural-log transformed (Gobert et al., 2017). To explore the differences in concentrations obtained from TE analysis, a multivariate analysis of variance (MANOVA) method was applied: species (2 levels: seabass/seabream) and fish origin (4 levels: farmed and wild for each species). To identify the influence of the factor on the response variables, when the MANOVA tests showed a significant effect, posteriori univariate ANOVA and post-hoc Tukey's honestly significant difference (HSD) tests were performed. To highlight the major TE differences between fish species and origins, the principal component analysis (PCA) was performed with TE concentrations as dependent variables. In addition, to investigate the relationship between the trace element levels (inter-element correlations) and the biological data (length), Pearson rank correlation tests were performed.

For example, high correlation between two TE implies that these two elements may share similar pollution sources or analogous transformation and migration processes in certain circumstances (Ragi et al., 2017). To determine the significance and strength of each relationship, the correlation coefficient (r) was calculated together with p-values. A significant difference was considered a p-value less than 0.05. Analyses were performed using XLSTAT software (Addinsoft, Paris, France).

### 2.4. Human risk assessment analysis

#### 2.4.1. Metal pollution index (MPI)

The Metal Pollution Index (MPI) method was used to compare the overall TE contents in the different fish studied. This index was obtained by calculating the geometrical mean of concentrations of all the TE (Usero et al., 1997).

$$MPI = (C_1 \times C_2 \times C_3 \times \dots \times C_n)^{1/n}$$

where C<sub>n</sub> are the mean concentrations of trace element (n) in the examined tissue (mg kg<sup>-1</sup> dry weight).

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