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Metals and elements in sardine and anchovy: Species specific differences and correlations with proximate composition and size

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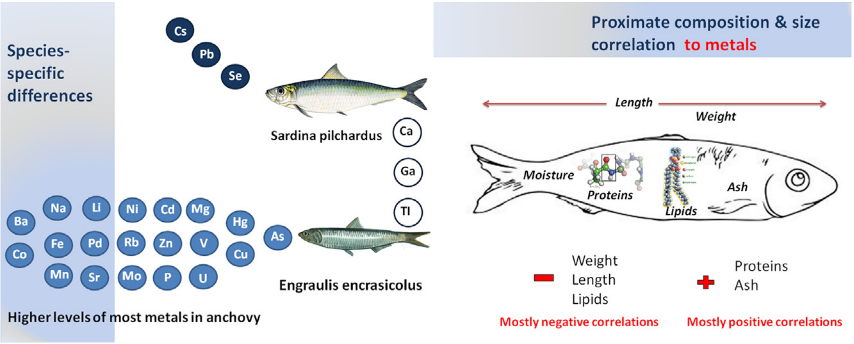
HIGHLIGHTS

- Metal and elemental content depends on fish species, proximate composition and size.
- Higher levels of most metals were observed in anchovy compared to sardine.
- Higher levels of metals and elements can be associated with lower lipid content.
- Higher levels of metals and elements can be associated with greater protein content.
- Higher levels of metals and elements can be associated with smaller fish size.

GRAPHICAL ABSTRACT



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ABSTRACT

Species – specific differences in the levels of 26 metals and elements in sardine and anchovy are investigated and the factors of proximate composition (proteins, lipids, ash) and body size (length, weight) that may affect the metal and elemental concentrations in fish are explored. Statistical analysis revealed that levels of metals and elements in fish seem to strongly depend on species. Significantly higher levels of most of the metals and elements studied have been observed in anchovy compared to sardine at each of six different sites. The observed species-specific differences in metal content could be attributed to different proximate composition and size among other factors. The significant (positive or negative) correlations found between lipids, proteins, ash, weight, length and metals or elements, suggest that higher levels of most of the metals and elements studied can be associated with lower lipid content, greater protein and ash content and smaller size.

1. Introduction

Marine metal pollution seems to have increased during the past decades on account of intense industrial development, agricultural and mining activities (Zhou et al., 2008; Olmedo et al., 2013; Renieri et al., 2014). Metals are included among the most dangerous pollutants of

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the marine environment since they are non-biodegradable, they may be toxic and may accumulate in sediment and marine life and may eventually be transferred, through the food chain, in humans (Zhou et al., 2008; Nadal et al., 2008; Vieira et al., 2011; Maanan, 2008). Thus, marine metal pollution may lead to ecosystem degradation, toxic effects on sealife and public health risks.

However, metal uptake by marine organisms does not necessarily lead to metal bioaccumulation in tissues, because the latter also depends on several processes such as metal storage or excretion or its transformation in the organism (Zhou et al., 2008). Various factors have been reported to affect fish metal and elemental bioaccumulation: (i) marine metal pollution at the sampling site (Zhou et al., 2008; Copat et al., 2012a; Renieri et al., 2014; Maanan, 2008) (ii) environmental factors such as water temperature, salinity, hardness, depth (Zhou et al., 2008; Copat et al., 2012a; Renieri et al., 2014) (iii) metal bioavailability and interactions (Metian et al., 2013) (iv) fish reproductive stage, age and sex (Zhou et al., 2008) (v) trophic level (Metian et al., 2013; Bat et al., 2014) (vi) fish diet (Renieri et al., 2014; Metian et al., 2013; Bat et al., 2014; Zhou et al., 2008) (vii) seasonal variations (Copat et al., 2012a; Sarkar et al., 2008; Maanan, 2008) (viii) fish species (Renieri et al., 2014; Galitsopoulou et al., 2012; Vieira et al., 2011; Onsanit et al., 2010) (ix) body size (length, weight) (Canli and Atli, 2003; Renieri et al., 2014; Sarkar et al., 2008; Vieira et al., 2011) (x) proximate composition of the fish tissues (Kalantzi et al., 2013; Marval-León et al., 2014). Among these factors there may be correlations (e.g. diet varies among species and can vary among sampling sites, trophic level is affected by diet). The current study focuses on investigating the effect of the three latter factors (fish species, proximate composition and body size) on the metal and elemental content of fish.

Two widely spread and consumed pelagic species (Galitsopoulou et al., 2012; Nunes et al., 2015; Bat et al., 2014) able to accumulate metals and elements have been chosen for the present study; anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*). Small pelagic species are considered of great importance in marine ecosystems due to their position in the food chain allowing them to influence the abundance of both their predators and of zooplankton on which they feed (Nunes et al., 2015; Karachle and Stergiou, 2013). Furthermore, these two pelagic species have been documented to have different proximate composition and size, which is expected to enrich the scope of the current investigation.

Overall, the objectives of the current study are to: 1) investigate the difference in the levels of metals and elements in sardine and anchovy and 2) to investigate the factors of proximate composition (proteins, lipids, ash) and body size (length, weight) that may affect the levels of metals and elements in fish. Several metals and elements (Li, Na, Mg, P, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Pd, Cd, Cs, Ba, Hg, Tl, Pb, U) have been measured in sardine and anchovy selected from 6 areas along the Greek coastline (having maximum stock density and different geophysical characteristics and degree of human environmental impact).

2. Materials and methods

2.1. Study areas and samples collection and pretreatment

Sardine (*S. pilchardus*) and anchovy (*E. encrasicolus*) samples ($n = 180$; gonad stage 1) were collected from six Greek sites (Thermaikos Gulf, Amvrakikos Gulf, Elefsina Gulf, Strymonian Gulf, Thracian Sea and Artemisium Straits) (Fig. S1).

Time and date of harvest was the same for both species in each site and didn't vary much among sites. Sampling data (dates and geographic coordinates) are provided in Table 1. Total lengths and body weights were recorded for each specimen (Table 1). Analysis was conducted in the edible tissues (skin, muscle and spine) processed altogether. Fish were beheaded and gutted and edible parts of each individual were stored in labeled, zip-lock bags at −20 °C until laboratory analysis. Samples were freeze-dried to constant weight and stored under a dry atmosphere until chemical analysis. For each species and site ($n = 15$ individuals), three composites were prepared by mixing and homogenising five individuals (edible tissues), chosen for each composite according to their weight (smaller, medium-sized, larger). Chemical determinations were carried out in triplicate for each composite, thus providing nine replicates for each species and site.

2.2. Proximate analysis

Total moisture content was determined by weighing mass differences before and after oven drying at 90 °C until constant weight was attained (around 4 h needed) (AOAC, 1990). Residual moisture content in the freeze-dried samples was determined accordingly and was used to convert on weight basis all chemical determinations that were carried out on freeze-dried samples. Ash content was estimated by weighing mass differences before and after incinerating the samples at 700 °C for 7 h in a muffle furnace (Heraeus D-6450 Hanau M110, Heraeus Instruments) (AOAC, 1990). Lipid content of the freeze-dried samples was estimated gravimetrically according to Folch et al. (1957). Protein content of the freeze-dried samples was estimated as N × 6.25, where N was the total nitrogen content determined according to AOAC methods using the Dumas combustion procedure (Nitrogen Analyzer: Leco Model FP-528). All proximate composition values were expressed in wet weight.

2.3. Metals and minerals analysis

Metal and other elements concentrations were determined according to a modified version of the USEPA method 3052 (1996) for

Table 1
Sampling data and size of the fish samples analyzed (mean ± SD and range; n = number of individuals).

Sites	Latitude	Longitude	Sampling date	Species	n	Total weight (wet) (g)		Total length (mm)	
						Mean ± SD	Range	Mean ± SD	Range
Elefsina Gulf	37°59′71.00″N	23°27′52.00″E	7/9/2013	Sardine	15	9.5 ± 2.1	5.6–12.7	108.2 ± 7.7	91.0–119.0
				Anchovy	15	4.6 ± 2.0	3.2–10.3	91.3 ± 10.9	82.0–120.0
Thermaikos Gulf	40°20′24.00″N	22°53′32.00″E	25/9/2013	Sardine	15	10.3 ± 3.3	7.4–19.1	111.7 ± 10.4	102.0–139.0
				Anchovy	15	6.8 ± 0.8	5.0–8.2	107.5 ± 3.4	101.0–114.0
Strymonian Gulf	40°41′66.00″N	23°53′25.00″E	22/9/2013	Sardine	15	11.7 ± 3.6	7.3–19.0	117.1 ± 10.2	105.0–138.0
				Anchovy	15	7.3 ± 2.1	4.4–11.7	107.0 ± 9.8	93.0–125.0
Thracian Sea	40°37′39.00″N	25°44′24.00″E	16/9/2013	Sardine	15	13.9 ± 2.5	9.8–18.5	120.1 ± 5.8	113.0–130.0
				Anchovy	15	8.2 ± 1.3	6.0–10.3	113.3 ± 5.2	104.0–121.0
Artemisium Straits	39°03′55.00″N	23°08′86.00″E	1/10/2013	Sardine	15	12.1 ± 3.1	8.0–18.5	121.5 ± 10.0	107.0–141.0
				Anchovy	15	5.5 ± 1.0	4.3–8.1	102.4 ± 4.9	95.0–114.0
Amvrakikos Gulf	38°58′44.00″N	20°54′81.00″E	11/10/2013	Sardine	15	12.1 ± 2.1	9.1–17.9	115.7 ± 7.3	108.0–138.0
				Anchovy	15	3.9 ± 0.6	2.9–5.1	87.1 ± 4.6	78.0–96.0