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UNIVERSIDADE Đ COIMBRA

Manuel José Pereira Rodrigues

FISH AS A SOURCE OF MINERALS IN A GROWING WORLD

Dissertação no âmbito do Mestrado em Ecologia, orientada pelo Professor Doutor Miguel Ângelo do Carmo Pardal (Universidade de Coimbra) e pelo Doutor João Pedro Martins Coelho (Universidade de Aveiro) e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Abstract

Fish and seafood are one of the most traded food commodities and their consumption is highly recommended because of its nutritional value. The Portuguese population is one of the largest fish consumers worldwide and the highest consumer in the European Union (55.9 kg per capita per year). Considering this, the present work focus on screening marine species, fished and consumed on the Portuguese coast, for the content of essential mineral elements, and evaluates how ecological traits influence their mineral content.

Data were collected along the Portuguese coast from traditional beach seine fisheries called "Arte-Xávega" and from fish markets, between 2016 and 2020. Mineral quantification (Ca, K, Mg, Na, P, Cu, Fe, Mn, Se, Zn) using ICP-MS was performed. Species were grouped according to taxonomy and ecological traits (feeding mode and vertical distribution) and a PERMANOVA analysis was used to evaluate differences among groups of species with different mineral content.

In general, K, P, and Na were the most abundant macro-minerals, while Zn, Fe, and Cu were the most abundant trace elements in the studied species. Results revealed significant differences in the mineral content between taxonomic groups, as a result of distinct characteristics and physiological needs, except for bivalves and gastropods (both molluscs) which share some morphological and ecological characteristics that may justify their similar mineral content. Also, differences in the mineral content of feeding mode groups were found, justified by the different food items, which will influence the mineral content of consumer species. Vertical distribution was also a significant factor for the mineral content, reflecting the variation in the mineral bioavailability and food items within the water column.

Overall, seafood was found to be an excellent source of essential elements. Moreover, were identified element-dense groups that may be suitable for the treatment of nutritional deficits and balanced diets.

Key-words: Fish consumption, seafood, mineral content, human health, nutrition

Resumo

Peixe e produtos alimentares marinhos são das mercadorias mais comercializadas e o seu consumo é altamente recomendado devido ao seu valor nutricional. A população portuguesa é um dos grandes consumidores de peixe a nível mundial e a maior consumidora da União Europeia (55.9kg per capita por ano). Tendo isto em conta, este estudo caracteriza o conteúdo em elementos minerais essenciais nas espécies marinhas, pescadas e/ou consumidas, na costa Portuguesa e avaliar como as características ecológicas, influenciam o conteúdo mineral nas espécies.

Os dados foram recolhidos ao longo da costa portuguesa recorrendo à pesca tradicional, Arte-Xávega e de mercados de peixe, entre 2016 e 2020. A quantificação mineral (Ca, K, Mg, Na, P, Cu, Fe, Mn, Se, Zn) foi realizada através de um ICP-MS. As espécies analisadas foram agrupadas de acordo com a sua taxonomia e com características ecológicas (alimentação e distribuição vertical) e realizou-se uma PERMANOVA para avaliar diferenças no conteúdo mineral.

De modo geral, K, P e Na foram os macro minerais mais abundantes, enquanto Zn, Fe e Cu foram os micro minerais mais abundantes nas espécies estudadas. Diferenças significativas no conteúdo mineral foram encontradas entre grupos taxonómicos, devido a características especificas e necessidades fisiológicas. No entanto, bivalves e gastrópodes (ambos moluscos) foram a exceção uma vez que partilham algumas caraterísticas morfológicas e ecológicas que podem justificar o semelhante conteúdo mineral. Foram também encontradas diferenças significativas entre espécies com diferentes modos de nutrição, justificadas pelo diferente conteúdo mineral dos itens alimentares que por sua vez, irão influenciar o conteúdo destes elementos nas espécies consumidoras. No que respeita à distribuição vertical na coluna de água das diferentes espécies verificaram-se igualmente diferenças no conteúdo mineral, que refletem a variação na biodisponibilidade dos minerais e dos itens alimentares ao longo da coluna de água.

Em resumo, os produtos alimentares marinhos mostraram ser uma excelente fonte de minerais essenciais, e foram identificados grupos ricos em determinados minerais que podem ser adequados para tratamentos de défices nutricionais e dietas equilibradas.

Palavras-chave: Consumo de peixe, produtos marinhos, conteúdo mineral, saúde humana, nutrição

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Abbreviations

Al	Aluminum
As	Arsenic
B	Boron
Ba	Barium
Br	Bromine
Ca	Calcium
Cd	Cadmium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
CRM	Certified Reference Material
Cs	Cesium
Cu	Copper
DGPA	Direção Geral das Pescas e Aquicultura (Directorate General for Fisheries and Aquaculture)
F	Fluorine
FAO	Food and Agriculture Organization
FCNAUP	Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto (The Faculty of Nutrition and Food Sciences from Oporto University)
Fe	Iron
Hg	Mercury
I	Iodine
ICP-MS	Inductively Coupled Plasma-Mass-Spectrometry
INE	Instituto Nacional de Estatística (National Institute of Statistics)

K	Potassium
Li	Lithium
LOD	Analytical Limits of Detention
MD	Mediterranean Diet
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
OECD	Organisation for Economic Co-operation and Development
P	Phosphorus
Pb	Lead
PCO	Principal coordinate analysis
RDI	Daily Recommended Intake
S	Sulfur
SD	Standard Deviation
Se	Selenium
Si	Silicon
Sn	Tin
Sr	Strontium
t	Tonnes
Ti	Titanium
USD	United States Dollars
W	Tungsten
WHO	World Health Organization
ww	Wet Weight
Zn	Zinc

1. Introduction

1.1 Importance of oceans to the human population

The world population has been growing more prominently since the 2000s, reaching nearly 7.6 billion people in 2017. In 2030, it is estimated to reach 8.6 billion (United Nations, Department of Economic and Social Affairs 2017). This will increase the pressure on the ecosystems since the demand for food resources will increase as well (Duarte et al. 2009; Béné et al. 2015).

Coastal and marine ecosystems yield different kinds of services and goods to the human population. Nutrient retention and cycling, flood control, energy sources, transportation, recreation, and food (fisheries and aquaculture) are some services these ecosystems provide (Barbier 2012). Although all of these services are important to humans, there is one that stands out. Fish production not only presents high economic importance but also provides people with almost 20% of their average intake of animal protein per capita (FAO 2018).

In total, the fish industry generated approximately 362 billion US dollars (USD) in 2016, equivalent to 171 million tonnes of fish. Of these 171 million, 151 were directly used for human consumption (FAO 2018), being 90.9 million tonnes from fisheries (131 billion USD) and 80 million tonnes from aquaculture (231,6 billion USD) (FAO 2018).

Over the last 20 years, aquaculture has continued to demonstrate sustained growth whereas capture fishery production kept stagnant, leading to increased consumption of aquaculture products (FAO 2018). Due to this, aquaculture already contributes with 46.4% of the total production of fish (FAO 2019). By 2030, it is estimated that it will contribute up to 63% of global fish consumption (Thilsted et al. 2016). Since global fish consumption is expected to increase from 20.2 kg in 2015 to 22kg per capita per year in 2024 (OECD and FAO 2015; FAO 2018), both aquaculture and capture fisheries will have a complementary role in increasing fish availability and access. Fish is one of the most traded food commodities in the world (Thilsted et al. 2016), reinforcing the idea that oceans have major importance as a vital component in people's lives.

1.2 Fish consumption in Portugal

Countries bordering the Mediterranean sea are known for a specific dietary pattern called the Mediterranean Diet (MD) (Boccardi et al. 2018; Guiné et al. 2019). MD is characterized by being rich in plants (vegetables, fruits, cereals, and seeds) and olive oil consumption. Also, moderate consumption of eggs, poultry, and dairy products (cheese and yogurt), low consumption of red meat, a moderate intake of alcohol (mainly wine at mealtimes), and moderate to high intakes of fish and seafood (Bach-faig et al., 2011; Boccardi et al., 2018; Ostan et al., 2015).

Countries like Italy, France, Greece, Morocco, Spain, and Portugal have this type of diet (Bach-Faig et al. 2011), which will influence their average annual fish consumption per capita. In the European Union, the average annual consumption of fish or seafood is around 25.1 kg. However, in countries like Portugal, Spain, and France the average consumption is much higher (55.9, 45.2, and 33.9 kg per capita per year respectively) (European Commission 2018).

In the case of Portugal, this value makes it the country in the European Union with the highest consumption of fish per capita per year, and one of the largest consumer in the world (European Commission 2018).

Portugal has a privileged position due to its geo-strategic front to the Atlantic Ocean. It has an exclusive economic zone currently around 1 700 000 km^2, a coastline of 2 830 km on the mainland, and two island areas (DGPA 2006). Fishing has always been an important source of livelihood, especially for coastal communities, many of which are almost dependent on fisheries and other related activities.

Based on data from The Portuguese National Institute of Statistics, INE (2019) in the year 2018, 177 685 tonnes (t) of fish were caught in Portugal. Although only 128 438 tonnes (t) were commercialized, it generated approximately 292 million euros (INE 2019). Meanwhile, aquaculture produced 12 549 tonnes (t) of fish that correspond to approximately 84 million euros (INE 2019).

1.3 Benefits of fish consumption

Nowadays, a healthy diet is a growing concern. The ingestion of nutrients in a good amount is important to healthy development and for that reason, fish is a product of high interest due to its nutritional quality (Carvalho et al. 2005). Fish is recognised as one of the most valuable sources of essential animal protein, lipids, vitamins, minerals, unsaturated fatty acids such as Omega-3, low fat, and cholesterol (Sidhu 2003; Torpy et al. 2006; Storelli 2008). Therefore, the benefits to nutrition and health in having a diet rich in fish are numerous (Weichselbaum et al. 2013; Thilsted et al. 2016), such as reducing the risk of developing coronary heart disease (He et al. 2004), one of the main health problems in the western world (Adeyemi et al. 2015). Additional benefits include reduced stroke risk and reduce the prevalence of some cancers, rheumatoid, and other inflammatory diseases (Lund 2013). A study conducted in the United States of America, associated fish consumption with long-term weight loss (Smith et al. 2015). Also, fish consumption slows cognitive decline with age (Morris et al. 2005). Raji et al. (2014) showed that consuming fish weekly is related to larger gray matter volumes in areas of the brain responsible for memory and cognition in cognitively normal elderly individuals. In pregnant women, a diet rich in seafood ensures optimal dietary intakes of key micronutrients (iron, zinc, selenium) important for fetal development (Bonham et al. 2009).

In 2010, diets low in seafood omega-3 fatty acids were responsible for 1.4 million deaths worldwide (Lim et al. 2012), showing how important seafood can be in improving the health of the human population. Besides, it prevents malnutrition due to the high content and wide range of essential elements (such as calcium, phosphorus, iron, magnesium, selenium, among others) (Martínez-Valverde et al. 2000; Béné et al. 2015).

Calcium is an important element in bone health and teeth. It plays an important role in bone mineralization since it is the main component of the skeleton and therefore prevents bone deformation and osteoporosis (Peterson 2010; Weichselbaum et al. 2013). Also, it is involved in the muscular system and controls essential processes like muscle contraction, blood clotting, brain cell activity, and cell growth (Belitz et al. 2009). Potassium is crucial to the normal function of the human body cells, including nerves, and has been associated with a decrease in blood pressure (Weichselbaum et al. 2013). It regulates the osmotic pressure within the cell, is involved in cell membrane transport, and in the activation of several glycolytic and respiratory enzymes (Belitz et al. 2009). Sodium is important in the transmission of nerve impulses and keeping electrolyte balance (Eti et al. 2019). Phosphorus has an important role in overall metabolism, it is also an integral part of the bone and tooth mineral as well as part of the structure of every cell (Belitz et al. 2009; Eti et al. 2019). Iron is a vital element to prevent anaemia, a disease that is estimated to affect 1.6-2 billion people, meanwhile iron deficiency affects approximately 40% of the total human population (McLean et al. 2009; Delforge et al. 2011). Magnesium is needed in RNA and DNA synthesis, maintenance of the electrical potential of nervous tissues and bone, and new cell formation (Joint FAO/WHO 2005). Copper is essential for the formation of several oxidoreductase enzymes and is fundamental for the oxidation of Fe^{2+} to Fe^{3+} since only Fe^{3+} can be transported from blood to the iron pool in the liver (Belitz et al.

2009). Bioavailable selenium is an indispensable element in at least eleven seleno-proteins in the human body, some of them are antioxidant enzymes (Luten et al. 2008; Weichselbaum et al. 2013). Zinc is essential for the immune system, maintenance of organs and cells and is a component of numerous enzymes (Joint FAO/WHO 2005). Manganese acts like a cofactor activating a large number of enzymes such as arginase, aminopeptidase, alkaline phosphatase, lecithinase, or enolase (Belitz et al. 2009).

It is then important to highlight that the macronutrients like fats, proteins, and carbohydrates are not the only nutrients necessary to health development, growth, and maintenance of the human body (Martínez-Valverde et al. 2000). Micronutrients like essential elements are involved in the metabolic processes of the body and are essential for all living beings (Afonso et al. 2013; Eti et al. 2019). As a consequence, considerable research has been carried out to better understand the human micronutrient requirements and to develop dietary requirement guidelines.

The main goal of the dietary requirement for micronutrients is to minimize the risk of nutrient deficit or excess of it. The requirement is defined as an intake level that meets specific criteria for adequacy (Joint FAO/WHO 1998). Table 1 summarizes the daily recommended intake (RDI) of the most vital essential mineral elements in five major groups of the human population.

Table 1. Daily recommended intake of mineral elements (mg/day) (Mahan and Raymond 2017).

Mineral Elements	**Groups**				
	Children 0 - 9 years	**Adolescents 10 - 18 years**	**Adults**		**Pregnancy**
			19 - 70 years	**70+ years**	
Ca	200 – 1000	1300	1000 – 1200	1300	1000 – 1300
K	400 - 4500	4500 – 4700	4700	4700	4700
Mg	30 – 240	240 – 410	310 – 420	320 – 420	360 – 400
Na	120 – 1500	1500	1300 - 1500	1200	1500
P	100 – 1250	1250	700	700	700 – 1200
Cu	0.2 – 0.7	0.7 – 0.9	0.9	0.9	1
Fe	0.27 – 11	8 – 15	8 – 18	8	27
Mn	0.003 – 1.9	1.6 – 2.2	1.8 – 2.3	1.8 – 2.3	2.0
Se	0.015 – 0.04	0.04 – 0.055	0.055	0.055	0.06
Zn	2 – 8	8 – 11	8 – 11	8 – 11	11 – 12

1.4 Micronutrients – Minerals

Minerals are chemical elements that belong to the group of micronutrients because they are needed in smaller amounts relative to macronutrients (FCNAUP 2014).

According to Nurnadia et al. (2013), Belitz et al. (2009) and Zoroddu et al. (2019) minerals can be divided into 1) main elements or "macro-minerals", including Na, K, Ca, Mg, Cl, P, and S. These are essential for humans, with a well-known biological role; 2) Trace elements, also essential for humans, with a known biological role as well. However, these elements are needed in lower concentrations than "macro-minerals". In this group are included Mn, Fe, Cu, Zn, Se, Mo, Co, I, F, Cr, Ni; 3) Ultra-trace elements, with unknown function, if any, for humans. Here are included Al, Ba, B, Br, Cs, Li, Si, Sn, Sr, Ti, W, among others, and 4) Toxic elements, toxic to the human body like Pb, Cd, As, and Hg (Figure 1). From all these metallic elements some are well known for being the most important to the proper function of the human body, like Na, K, Mg, Ca, Mn, Fe, Cu, Zn, Se (Zoroddu et al. 2019).

Like humans, aquatic animals require minerals for their normal life processes (Craig and Helfrich 2009; Bhouri et al. 2010). Mineral elements are essential for growth, bone formation and integrity, reproduction, regulation of osmotic balance, metabolism, and in enzyme and hormone systems (Roy and Lall 2006; Yildiz 2008; Bhouri et al. 2010). Marine species can absorb and retain mineral from two main ways, natural diet and through the surrounding environment (water and sediments) and deposit them in their skeleton and organs (Alasalvar et al. 2002; Rainbow 2002; Roy and Lall 2006; Craig and Helfrich 2009).

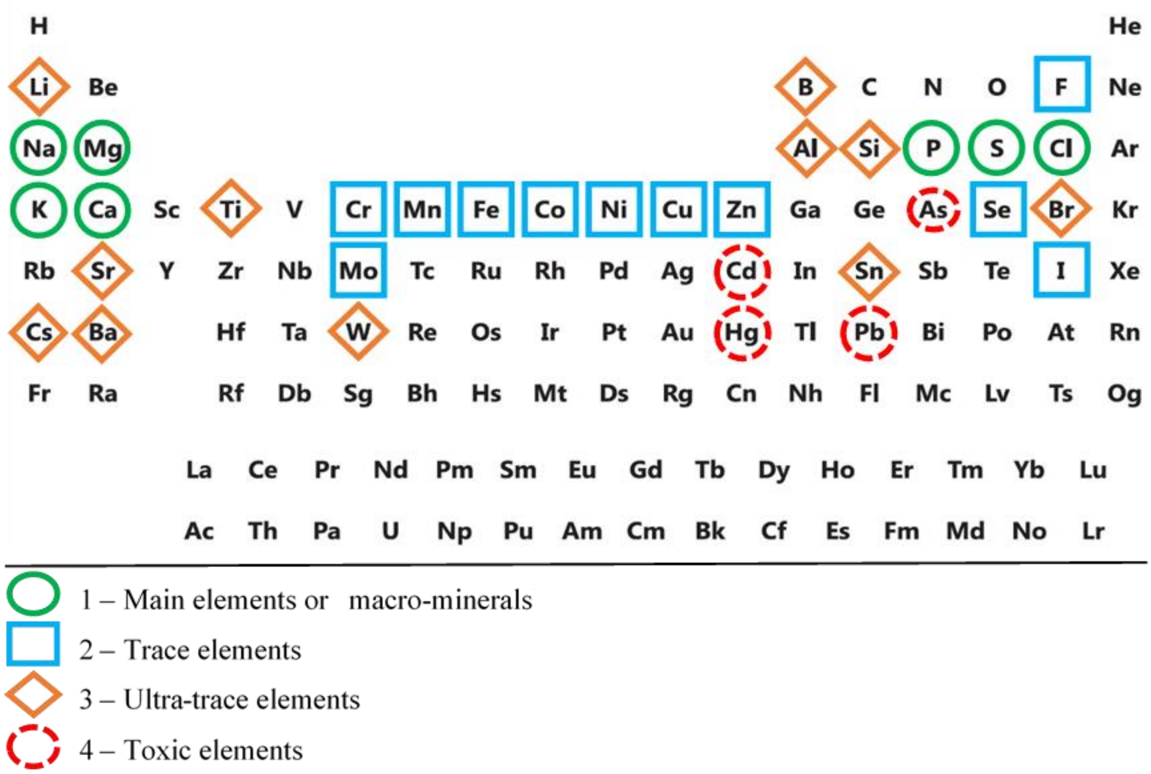

Figure 1. Periodic table of the chemical elements.

1.5 State of the art

The nutrient composition of seafood is highly variable among and within species due to several biological and environmental factors. Some of these are age, sex, size, sexual maturity, water temperature, and diet (Martínez-Valverde et al. 2000; Gökçe et al. 2004; Roy and Lall 2006; Rebolé et al. 2015). Nutrient composition data for common food are widely available, especially for seafood products (Carvalho et al. 2005), mainly because of the nutritional quality that seafood present. A wide range of studies on nutrient composition and metals in seafood species has been made (Sivaperumal et al. 2007; Storelli 2008; Nurnadia et al. 2013; Chahid et al. 2014; Bogard et al. 2015; Wheal et al. 2016; Stoyanova 2018; Afandi et al. 2018; Eti et al. 2019).

However, regarding mineral composition, the majority of studies are restricted to a few selected species (Carvalho et al. 2005). Therefore, the lack of understanding of the mineral composition of most seafood and how it differs among different traits has delayed the full potential of fisheries development for food nutrition and security.

In a country like Portugal, where seafood has an important role in peoples’ diet, scientific studies on fish and seafood mineral composition are scarce. Regarding fish species, Afonso et al. (2013) focussed on characterizing 15 elements in 6 deep-water fish species. Carvalho et al. (2005), determined the mineral content of 13 elements in 9 species of fish and one species of octopus. Lourenço et al. (2012) studied 11 elements of four farmed fish produced in Portugal. Concerning crustaceans, Barrento et al. (2009) analysed 10 minerals in the brown crab *Cancer pagurus*, while Marques et al. (2010) determined the content of 19 elements, including 4 toxic elements, in the species *Maja brachydactyla*. Also, Lourenço et al. (2009) focussed on 17 elements in the species *Nephrops norvegicus*. For cephalopods, Lourenço et al. (2009b) determined the concentration of 16 elements in three species of cephalopods (*Octopus vulgaris*, *Loligo vulgaris,* and *Sepia officinalis*) and, Napoleão et al. (2005) analysed 10 mineral in *Octopus vulgaris*. There were also other works in the mineral content of seafood, but focusing only on the analysis of toxic elements (Raimundo et al. 2004; Afonso et al. 2007, 2008; Anacleto et al. 2009)

Therefore, considering the amount of seafood consumed by the Portuguese population, it is extremely important to develop a wide and full characterization of the mineral content in seafood. This will allow the creation of seafood-based food policy guidelines where the types of seafood that should be consumed are specified. Ultimately, it will permit the consumers to know the principal nutritional characteristics of seafood, allowing for informed consumer choices and dietary adjustments to overcome nutritional deficits through fish and seafood consumption.

1.6 Objectives

Considering the purpose of the present study, two main objectives were established:

[1] To screen marine species, fished and/or consumed in the Portuguese coast, for the content of essential mineral elements;

[2] To evaluate how ecological traits influence mineral content in seafood species;

2. Materials and methods

2.1 Study area and fish sampling

Sampling took place in the Portuguese continental waters in the Northeast Atlantic Ocean, between 2016 and 2020. The majority of samples were collected from traditional beach seine fisheries called “Arte-Xávega”. Briefly, wooden boats went up to 2km from shore deploying the nets in the water and returning to shore. On the beach, with the help of mechanical tractors, the nets were dragged beachwards and the fish was collected (Cabral et al. 2003). Other samples were purchased from fish markets along the Portuguese coast, in the usual places where consumers most frequently obtain fish for domestic consumption. Overall, 96 species were collected (66 fish, 6 cephalopods, 10 crustaceans, 9 bivalves, and 5 gastropods). All specimens caught were within the commercial EU standards (DGPA 2001).

2.2 Laboratory procedures

2.2.1 Samples

A total of 530 individuals (n= 5 per species) were weighed (g) and measured (cm) before a sample of muscle tissue (edible portion) was collected. The samples were weighed to obtain the fresh weight and then frozen in plastic sample bags at -20°C, between 24h to 48h. Afterward, all samples were freeze-dried, between 48h to 72h, using a freeze-drier. Next, all samples were weighed again to know the dry weight and allow the calculation of the moisture content for each species. This was used after all samples were analysed. The results are multiplied by the specific moisture content, to convert the element concentrations from dry weight to fresh weight.

Finally, all samples were homogenised to a fine powder and stored dry until the chemical digestion.

2.2.2 Chemical Digestion

The sample preparation for mineral analysis was made by wet digestion in a microwave accelerated reaction system (CEM Mars 5). From the homogenised samples, 200 mg were weighed and digested with two ml of ultrapure water, one ml of hydrogen peroxide (H_2O_2), and one ml of nitric acid (HNO_3), in Teflon vessels (XP-1500 High-pressure vessels). When the samples did not have 200 µg, the whole sample was used. The following microwave digestion program was used: ramp time: 3 min, temperature: 115°C; ramp time: 3 min, temperature: 150°C; ramp time: 3 min, temperature: 175°C; ramp time: 3 min, temperature: 190°C; hold time: 3 min, temperature: 190°C.

After cooling, the digest was collected to plastic flasks and diluted with ultrapure water until reaching approximately 25 g of the liquid sample and stored until further analysis.

2.2.3 Inductively Coupled Plasma Mass Spectrometry (IPC-MS)

The concentration of the mineral elements was measured through Inductively Coupled Plasma-Mass-Spectrometry (ICP-MS). This technique enables the determination of low concentrations (parts per billion or µg L^{-1}) of elements.

The sample solution is introduced in the IPC-MS by a peristaltic pump. There, it is nebulized, becoming an aerosol which is injected into the plasma base. As it travels along the different heating zones of the plasma torch the solution is atomized and ionized. The resulting ions are sorted on account of their atomic mass giving the concentration of the respective element in the sample.

2.2.4 Quality Control

Throughout the laboratory procedures, all efforts were made to prevent and minimize errors, to assure reliable results. Between each digestion cycle, a washing cycle was made. All Teflon vessels were washed using an acidic solution of 40% HNO_3 and 20% hydrofluoric (HF) and the following microwave program was used: ramp time: 10 min, temperature: 135°C; hold time: 10 min, temperature: 135°C.

The accuracy and precision of the method were assessed through the analysis of certified reference material (CRM) Dorm-4 (National Research Council of Canada, Ottawa, Canada), CRM used for fish muscle tissue samples (Table 2). Also, sample blanks were prepared similarly to the muscle samples and the analytical limits of detection (LOD), for each element, were calculated (Table 3).

Table 2. Laboratory performance of Certified Reference Material for the elements (mg kg^{-1} dry weight) measured in the present work (n= 58). Values are Mean (±SD).

Technique	Reference material	Element	Certified value (mg kg^{-1})	Present work (mg kg^{-1})	% recovery
		Ca	2360 ± 140	2310 ± 293	97.9
		K	15500 ± 1000	12390 ± 1146	79.9
		Mg	910 ± 80	892 ± 100	98.1
		Na	14000 ± 2400	13212 ± 1174	94.4
IPC-MS	Dorm-4 [a]	P	8000	7521 ± 1669	94.0
		Cu	15.7 ± 0.46	15.7 ± 1.7	100.3
		Fe	343 ± 20	338 ± 36.3	98.8
		Se	3.45 ± 0.40	4.00 ± 0.40	116.0
		Zn	51.6 ± 2.8	46.2 ± 5.9	89.6

[a] Fish muscle tissue, National Research Council of Canada, Ottawa, Canada.

Table 3. Analytical limits of detection (LOD) (µg L^{-1}) for the elements analysed, used in ICP-MS analysis.

Elements	LOD (µg L^{-1})
Calcium	100
Copper	2
Iron	10
Potassium	200
Magnesium	20
Manganese	0.5
Sodium	50
Phosphorus	50
Selenium	3
Zinc	2

2.3 Species traits

Species were grouped according taxonomy and two ecological traits: feeding mode, and vertical distribution in the water column, considering that life history, habitat characteristics, and its prevalent environmental conditions are known to affect mineral content (Martínez-Valverde et al. 2000; Gökçe et al. 2004; Rebolé et al. 2015) (Table 4).

Regarding taxonomy, five groups were considered: Pisces, Crustacea, Cephalopoda, Bivalvia, and Gastropoda. For the feeding mode, we considered zoobenthivorous - species feeding predominantly on invertebrates associated with the sediments; planktivorous - which feed predominantly on plankton; piscivorous - feed predominantly on fish; omnivorous - feed predominantly on algae, infauna, and epifauna; herbivorous - feed predominantly on plants; and filter feeders - feed predominantly on suspended particles in the water column (Froese et al. 2000; Elliott et al. 2007; Baptista et al. 2015). Considering vertical distribution, species were divided into six categories: demersal – species that feed and live near the bottom; pelagic – species that inhabit in the water column; benthopelagic - species that feed and live near the bottom as well as in the midwaters or near the surface; bathydemersal – species that feed and live on the bottom below 200 m; reef-associated – species that feed and live on or near coral reefs (rocky bottoms); and benthic – species that live in direct contact with the sediment (Elliott and Dewailly 1995; Froese et al. 2000; Baptista et al. 2015).

Table 4. Traits selected for the study, with the categories selection rationale.

Traits	Categories	Categories selection rationale
Taxonomy	Fish Crustaceans Cephalopods Bivalves Gastropods	Shared characteristics are based on the evolutionary relationships between organisms (Froese et al. 2000).
Feeding mode	Zoobenthivorous Planktivorous Piscivorous Omnivorous Herbivorous Filter feeders	Species predominant diet (Froese et al. 2000; Elliott et al. 2007; Baptista et al. 2015)
Vertical distribution	Demersal Pelagic Benthopelagic Bathydemersal Reef-associated Benthic	Main habitat used by each species, regarding its position in the water column and dependence to the bottom sediment (Elliott and Dewailly 1995; Froese et al. 2000; Baptista et al. 2015)

2.4 Data analysis

PERMANOVA (non-parametric permutational multivariate analysis of variance) was used to evaluate the different mineral content in the selected marine species, considering the following traits: taxonomy, feeding mode, and vertical distribution. Data was initially standardized so that all variables contributed equally to the results (element concentrations had varying scales and ranges). The analysis was performed based on the Euclidean distance between samples, and all the factors were considered as fixed, with 999 random permutations. To test for differences between groups, within each trait, we used multiple comparisons Pairwise test. Finally, a Principal Coordinates Analyses (PCO), based on Euclidean distance, was performed using PRIMERv6 and PERMANOVA+ routines (Anderson et al. 2008).

3. Results

3.1 Mineral Content – General

The concentration of 10 elements was analysed in the muscle of all studied species. From these, five were main elements or "macro-minerals", which are essential for humans and with a well-known biological role, such as Ca, K, Mg, Na, and P. The other five were trace elements, also essential for humans but needed in lower concentrations than "macro-minerals", including Cu, Fe, Mn, Se, and Zn.

In general, potassium, phosphorus, and sodium were the most abundant macro-minerals in the studied species. Meanwhile zinc, iron, and copper were the most abundant trace elements. Calcium concentration ranged from 18 mg kg^{-1} ww (*Pagrus Pagrus*) to 10283 mg kg^{-1} ww in *Crangon crangon*, which was the species with the highest mean concentration (9609 ± 649 mg kg^{-1} ww). Meanwhile, potassium ranged from 224 mg kg^{-1} ww (*Meretrix lyrata*) to 5860 mg kg^{-1} ww (*Sardina pilchardus*), with the highest mean concentration of 5411 ± 206 mg kg^{-1} ww in *Chelon ramada*. Concerning magnesium, the minimum, and maximum concentration were respectively 133 mg kg^{-1} ww (*Epinephelus aeneus*) and 2662 mg kg^{-1} ww in *Littorina littorea*, which recorded the highest mean concentration (2383 ± 307 mg kg^{-1} ww). For sodium, the minimum was 210 mg kg^{-1} ww (*Scomber colias*) while *Thunnus albacares* recorded the highest individual (8364 mg kg^{-1} ww) and mean concentration (7407 ± 1137mg kg^{-1} ww). The species with the highest mean phosphorus concentration was *Sardina pilchardus* (4501 ± 192 mg kg^{-1} ww), with individuals levels varying from 550 mg kg^{-1} ww (*Pollicipes pollicipes*) to 4725 mg kg^{-1} ww (*Sardina pilchardus*). Concentrations of copper, manganese, and zinc ranged from 0.061 mg kg^{-1} ww (*Scophthalmus maximus*) to 387 mg kg^{-1} ww (*Magallana angulata*), 0.029 mg kg^{-1} ww (*Epinephelus aeneus*) to 29 mg kg^{-1} ww (*Magallana angulata*) and 1.7 mg kg^{-1} ww (*Aphanopus carbo*) to 1991 mg kg^{-1} ww (*Magallana angulata*), respectively. *Magallana angulata* was the species with the highest mean value for the three minerals (318 ± 51 mg kg^{-1} ww, 18 ± 8.3 mg kg^{-1} ww, 1670 ± 281 mg kg^{-1} ww). For iron, concentrations varied from 0.55 mg kg^{-1} ww (*Aphanopus carbo*) to 552 mg kg^{-1} ww in *Patella vulgata*, which recorded the highest mean value (469 ± 57 mg kg^{-1} ww). Finally, the lowest selenium concentration was 0.084 mg kg^{-1} ww (*Microchirus variegatus*), while the maximum individual and mean concentration were recorded in *Phorcus lineatus* (11 mg kg^{-1} ww and 6.8 ± 4.2 mg kg^{-1} ww, respectively).

The mean and standard deviation from the concentration of the 10 elements in the edible portion of fish, cephalopods, crustaceans, bivalves, and gastropods are shown in Supplementary Tables 8–12, respectively.

3.2 Taxonomy

Fish was the taxonomic group with the highest mean concentration for potassium (3375 ± 986 mg kg^{-1} ww). For the majority of the remaining minerals, however, this group presented the lowest values. Meanwhile, crustaceans stand out by presenting the highest mean concentration for calcium (2343 ± 3242 mg kg^{-1} ww) and phosphorus (2181 ± 786 mg kg^{-1} ww). This group also presented high values in some of the remaining macro and micro minerals such as potassium, selenium, and zinc. Concerning bivalves and gastropods, these groups presented similar concentrations in their mineral content and were the groups that presented the highest mean concentration for all micro minerals. Bivalves showed the highest mean concentration for sodium (3830 ± 1820 mg kg^{-1} ww), copper (41 ± 107 mg kg^{-1} ww), manganese (3.7 ± 6.2 mg kg^{-1} ww), and zinc (226 ± 560 mg kg^{-1} ww). However, oysters had a great influence on the mean concentration of copper and zinc in bivalves. Gastropods presented the highest mean concentration for magnesium (1140 ± 668 mg kg^{-1} ww), iron (131 ± 175 mg kg^{-1} ww), and selenium (2.5 ± 3.0 mg kg^{-1} ww) (Table 5).

Mineral content in seafood varied among taxonomic groups (Pseudo-F = 104.64, $p(perm) < 0.01$), except for bivalves and gastropods (pairwise test; $p = 0.045$) (Supplementary Table 13). Axis 1 of the Principal Coordinate Analysis (PCO) explained 70.3% of the total variation, which was positively related to potassium and phosphorus, and negatively related to the remaining minerals. PCO axis 2 explained 19.6% of the total variation, which was mainly positively related to Ca content and negatively related to Na (Figure 2).

Along the PCO axis 1, we observed a clear taxonomical separation in the mineral content, especially between fish and the other taxonomic groups (Figure 2).

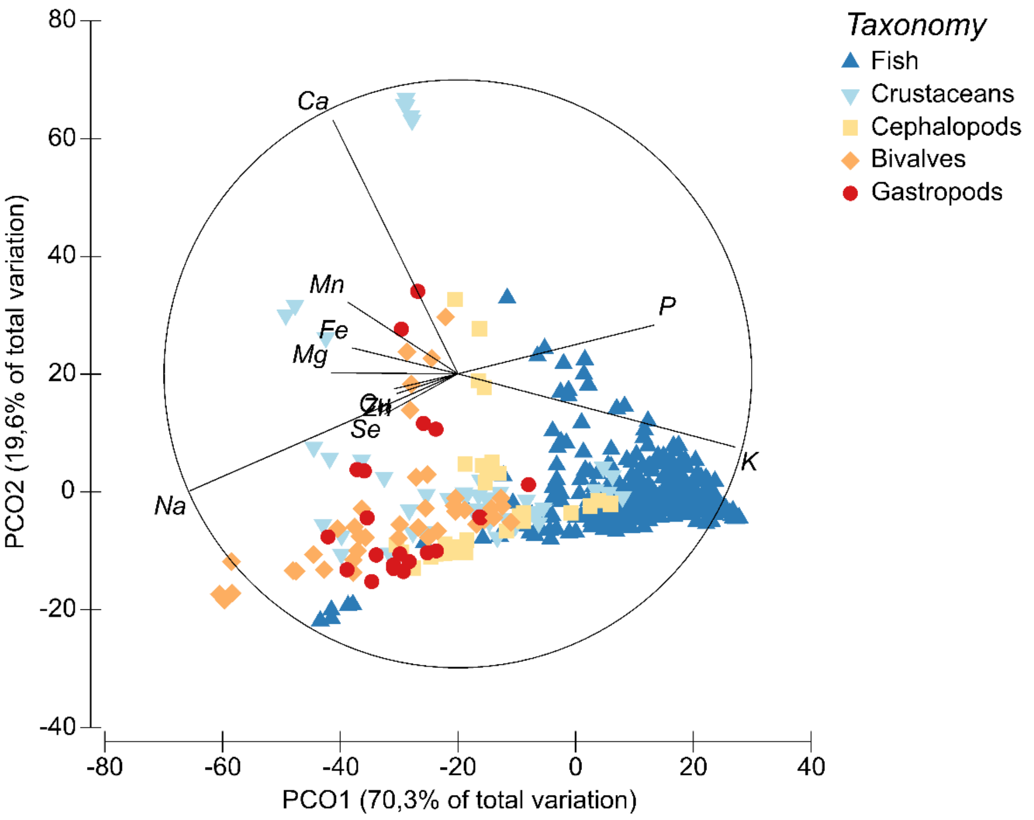

Figure 2. Principal coordinate analysis (PCO) performed with the selected trait taxonomy

Table 5. Mineral concentrations (mg kg^{-1} ww) in the muscle for each taxonomic group. Values are mean (±SD).

Taxonomic groups	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Fish	261 ± 334	3375 ± 986	282 ± 59	882 ± 922	2078 ± 707	0.29 ± 0.26	4.6 ± 4.8	0.22 ± 0.43	0.35 ± 0.17	4.4 ± 2.9
Crustaceans	2343 ± 3242	2679 ± 1054	504 ± 238	3207 ± 1727	2181 ± 786	5.5 ± 3.3	14 ± 22	0.89 ± 1.0	0.75 ± 0.35	28 ± 19
Cephalopods	308 ± 370	1745 ± 762	355 ± 48	1651 ± 554	1667 ± 572	5.0 ± 4.3	23 ± 44	0.48 ± 0.57	0.31 ± 0.056	10 ± 1.4
Bivalves	684 ± 331	1959 ± 1250	647 ± 254	3830 ± 1820	1615 ± 681	41 ± 107	47 ± 36	3.7 ± 6.2	0.74 ± 0.33	226 ± 560
Gastropods	951 ± 918	2234 ± 893	1140 ± 668	3727 ± 1905	1348 ± 295	11 ± 9.4	131 ± 175	3.3 ± 3.2	2.5 ± 3.0	58 ± 123

3.3 Feeding mode

Planktivorous species was the group that presented the highest mean concentration for phosphorus (3015 ± 932 mg kg^{-1} ww). Meanwhile omnivorous species presented the highest mean concentration for calcium (1083 ± 2024 mg kg^{-1} ww) and potassium (3776 ± 1373 mg kg^{-1} ww), and also presented high mean values for the remaining mineral elements. Herbivorous species showed the highest mean concentration for magnesium (856 ± 813 mg kg^{-1} ww) and selenium (2.1 ± 3.1 mg kg^{-1} ww). Also, high mean concentrations in minerals such as sodium, copper, iron, and manganese. Filter feeding was the feeding mode that presented the highest mean concentration for more mineral elements (four micro minerals, and one macro mineral). This group presented the highest mean concentration for sodium (3991 ± 1831), copper (37 ± 103 mg kg^{-1} ww), iron (85 ± 128 mg kg^{-1} ww), manganese (3.7 ± 5.9 mg kg^{-1} ww), and zinc (206 ± 537 mg kg^{-1} ww). Besides, it also presented high mean concentrations for minerals such as calcium, magnesium, and selenium (Table 6).

Mineral content in seafood showed statistically significant differences between feeding mode traits (Pseudo-F = 25.18, p(perm) < 0.01), except for zoobenthivorous and piscivorous (pairwise test; p = 0.056), and between omnivorous and herbivorous (pairwise test; p = 0.098) (Supplementary Table 14).

Axis 1 of the PCO explained 70.3% of the total variation, and was positively influenced by potassium and phosphorus, and negatively influenced by the remaining minerals. PCO axis 2 explained 19.6% of the total variation, which was mainly positively related to Ca and negatively related to Na content (Figure 3). Although not as clear as in the previous case, the PCO axis 1 also showed a separation in the mineral content by feeding mode (Figure 3).

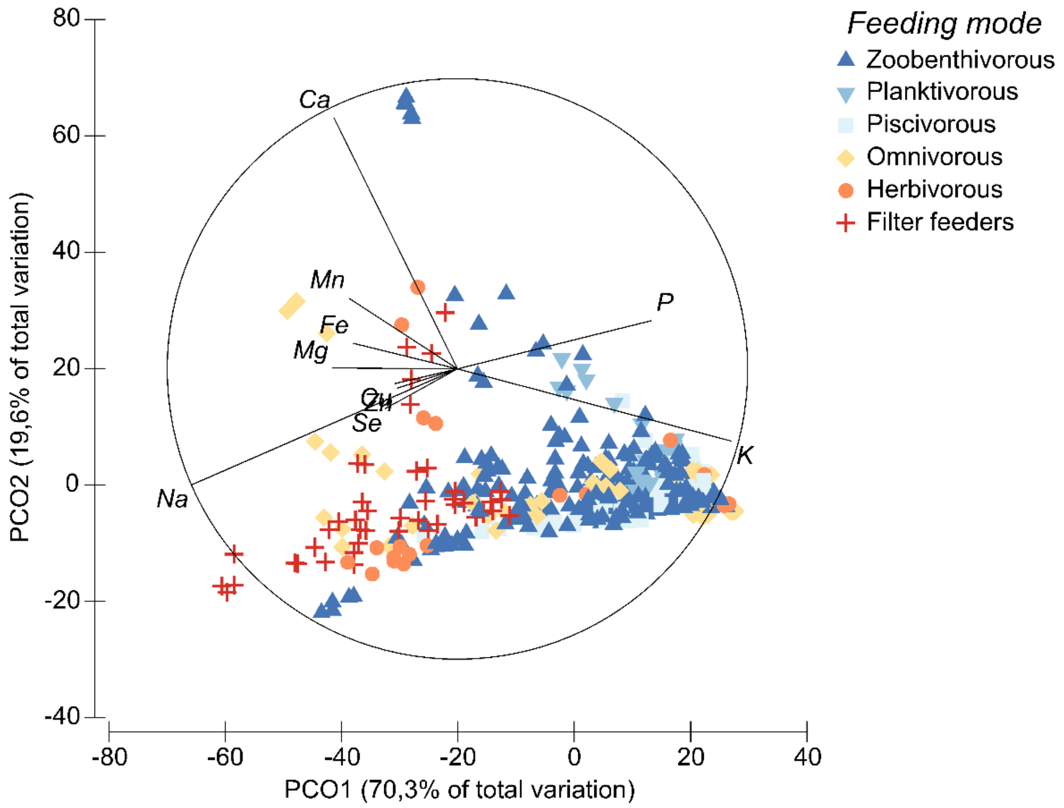

Figure 3. Principal coordinate analysis (PCO) performed with the selected trait feeding mode.

Table 6. Mineral concentrations (mg kg^{-1} ww) in the muscle for each feeding mode category. Values are mean (±SD).

Feeding mode categories	Mineral Elements									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Zoobenthivorous	474 ± 1332	3019 ± 957	307 ± 109	1172 ± 1146	1951 ± 515	1.5 ± 4.0	6.2 ± 16	0.35 ± 0.65	0.40 ± 0.25	10 ± 38
Planktivorous	501 ± 340	3512 ± 1306	336 ± 71	648 ± 150	3015 ± 932	0.60 ± 0.33	11 ± 10	0.31 ± 0.16	0.41 ± 0.26	8.4 ± 5.0
Piscivorous	186 ± 218	3192 ± 1092	265 ± 63	922 ± 436	2165 ± 1021	0.34 ± 0.46	4.3 ± 5.5	0.15 ± 0.11	0.37 ± 0.11	3.8 ± 1.4
Omnivorous	1083 ± 2024	3776 ± 1373	457 ± 240	2310 ± 2148	2122 ± 813	3.3 ± 3.6	15 ± 22	0.48 ± 0.78	0.54 ± 0.39	18 ± 19
Herbivorous	609 ± 810	2453 ± 1122	856 ± 813	2292 ± 2109	1364 ± 316	6.3 ± 6.9	37 ± 49	2.2 ± 3.5	2.1 ± 3.1	8.5 ± 4.4
Filter feeder	787 ± 480	1999 ± 1198	670 ± 256	3991 ± 1831	1591 ± 656	37 ± 103	85 ± 128	3.7 ± 5.9	0.71 ± 0.33	206 ± 537

3.5 Vertical distribution

Concerning the vertical distribution groups, the benthic group was the one that presented the highest mean concentrations for the majority of the mineral elements, comprising calcium (1326 ± 2216 mg kg^{-1} ww), magnesium (656 ± 425 mg kg^{-1} ww), sodium (3414 ± 1786 mg kg^{-1} ww), copper (19 ± 65 mg kg^{-1} ww) , iron (46 ± 89 mg kg^{-1} ww), manganese (2.2 ± 4.2 mg kg^{-1} ww), selenium (1.0 ± 1.5 mg kg^{-1} ww), and zinc (100 ± 343 mg kg^{-1} ww). Meanwhile, pelagic species presented the highest mean concentration for potassium (3497 ± 1265 mg kg^{-1} ww) and phosphorus (2509 ± 801 mg kg^{-1} ww) (Table 7).

Significant differences in the mineral content were also found considering species vertical distribution traits (Pseudo-F = 63.28, p(perm) < 0.01), except for demersal and benthopelagic species (pairwise test; p = 0.202), demersal and bathydemersal species (pairwise test; p = 0.078), pelagic and bathydemersal species (pairwise test; p = 0.207), benthopelagic and bathydemersal (pairwise test; p = 0.051), and bathydemersal and reef-associated species (pairwise test; p = 0.188) (Supplementary Table 15).

The PCO axis 1 explained 70.3% of the total variation, and was positively related to potassium and phosphorus content, and negatively related to the remaining minerals. PCO axis 2 explained 19.6% of the total variation, which was mainly positively related to Ca and negatively related to Na content (Figure 4). In this case, there was a clear separation between benthic species and the remaining ones (Figure 4).

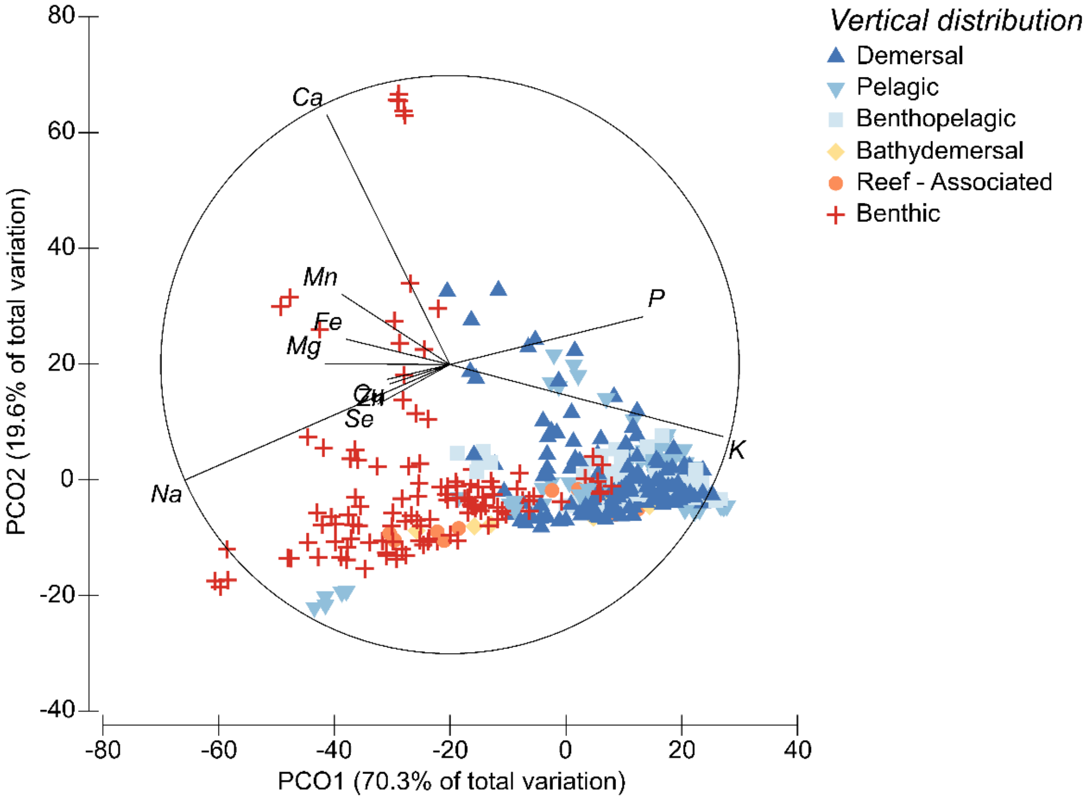

Figure 4. Principal coordinate analysis (PCO) performed with the selected trait vertical distribution.

Table 7. Mineral concentrations (mg kg^{-1} ww) in the muscle for each vertical distribution category. Values are mean (±SD).

Vertical distribution categories	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Demersal	315 ± 422	3243 ± 939	282 ± 55	839 ± 399	1962 ± 641	0.54 ± 2.0	5.0 ± 7.6	0.32 ± 0.63	0.35 ± 0.18	4.9 ± 3.4
Pelagic	241 ± 265	3497 ± 1265	305 ± 64	1154 ± 1709	2509 ± 801	0.68 ± 0.86	8.2 ± 7.7	0.19 ± 0.14	0.34 ± 0.19	5.1 ± 3.2
Benthopelagic	257 ± 236	3360 ± 992	288 ± 63	757 ± 350	1960 ± 519	0.77 ± 2.0	9.3 ± 30	0.16 ± 0.18	0.35 ± 0.13	3.7 ± 1.8
Bathydemersal	119 ± 61	2871 ± 1010	259 ± 18	981 ± 398	1704 ± 432	0.21 ± 0.21	2.3 ± 1.7	0.12 ± 0.050	0.50 ± 0.13	3.4 ± 1.4
Reef-associated	125 ± 74	2105 ± 847	251 ± 88	1183 ± 565	1285 ± 323	0.75 ± 1.0	2.1 ± 1.0	0.19 ± 0.11	0.31 ± 0.038	6.8 ± 4.9
Benthic	1326 ± 2216	2306 ± 1108	656 ± 425	3414 ± 1786	1794 ± 741	19 ± 65	46 ± 89	2.2 ± 4.2	1.0 ± 1.5	100 ± 343

4. Discussion

4.1 General

Concerning the macro-minerals, potassium, phosphorus, and sodium were the minerals with the highest concentration. Meanwhile, zinc, iron, and, copper were the trace elements with the highest concentrations. Similarly, Lourenço et al. (2009) reported comparable patterns in muscle tissue of three species of cephalopods with K, Na, and P among the main elements and Zn, Fe, and Cu among the trace elements with the highest concentrations. Also, Varol and Sünbül (2020) found that potassium and phosphorus were the mineral elements with the highest mean concentration in the muscle of two fish species from three different freshwater reservoirs in Turkey. Afonso et al. (2013) found that potassium and sodium were the main elements with the highest mean concentrations in the muscle of six fish species, while Zn and Fe were the trace elements with the highest mean concentration. Stoyanova (2018) also found that K, P, and Na were the macro-minerals that presented the highest mean concentration in four marine fish species. Carvalho et al. (2005) also found that the main element with the highest mean concentration present in the muscle of nine fish species and one species of octopus was potassium, while for trace elements were iron and zinc. Biandolino et al. (2019) showed that in seven species of bivalves, Na and P were the macro-minerals with the highest values, while for trace elements were Fe, Zn, and Cu the highest. All these studies agree with the present results, and the fact that this was observed in various species suggests these elements have similar distribution being an overall pattern to several taxonomic groups, since, these elements can have the same physiological importance to the organisms (Carvalho et al. 2005; Lourenço et al. 2009b).

4.2 How environmental and ecological traits influence mineral content

4.2.1. Taxonomy

Taxonomy focuses on grouping organisms related to each other, organisms that share similar characteristics. These can be phylogenetic, ecological, morphological, or physiological (Secretariat of the Convention on Biological Diversity 2007). Therefore, the differences found between taxonomic groups can be explained based on their specific characteristics and needs.

Fish presented the highest mean value for potassium. Similar contents in fish species were previously found by other authors (Vlieg et al. 1991; Martínez-Valverde et al. 2000; Afonso et al. 2013; Bogard et al. 2015). Potassium is a critical element for the physiological needs of fish, making it the most abundant intracellular ion in fish (Partridge and Lymbery 2008). This element has an important role in muscle functions, membrane potentials, and the transmission of impulses in the nerve system (McDonough et al. 2002; Eti et al. 2019). Besides, in fish, it is indispensable in osmo and ion-regulation and acid/base balance (Marshall and Bryson 1998; Evans et al. 2005). This high physiological dependence of potassium can explain its high levels.

Crustaceans were the taxonomic group that presented the highest mean value for calcium, even higher than previous reports on some of the species analysed on the present work (Karakoltsidis et al. 1995; Barrento et al. 2008, 2009; Lourenço et al. 2009a; Marques et al. 2010). Even so, higher levels of calcium in crustaceans can be explained because these organisms are covered by an exoskeleton composed of calcium (Anacleto et al. 2016). Crustaceans regularly shed its skeleton to increase in body size, making Ca metabolism much more active than in other groups (Greenaway 1985). This taxonomic group also presented the highest mean value for phosphorus. Levels of phosphorus are in the same range to those found by other authors in some species of crustaceans (Küçükgülmez et al. 2006; Lourenço et al. 2009a). This mineral is a strong component of hard tissues and therefore is important to the formation of the exoskeleton of crustaceans (Davis and Gatlin 1996).

Bivalves and gastropods were the only two taxonomic groups where differences in the mineral content were not found (both are molluscs), having high concentrations of sodium, copper, manganese, zinc, magnesium, iron, and selenium between the two groups. This can be explained by some similar characteristics shared by these two groups. First, both inhabit in close association with the bottom, where generally some elements are more available (Bruland and Franks 1983; Carvalho et al. 2005). Secondly, because both groups have filter feeder species and therefore can absorb minerals from inorganic particulate materials they ingest, making them especially predisposed to accumulate minerals (Nielsen and Nathan 1975; El-Sikaily et al. 2004).

In the present study, bivalves and gastropods presented the highest values of zinc and iron, respectively. Carvalho et al. (2005) found that benthic fish species presented higher levels of zinc and iron. Meanwhile, Vlieg et al. (1991) stated that levels of zinc and copper are often higher in shellfish organisms. Another study made by Bruland and Franks (1983) found that zinc and copper concentrations increased with depth. However, it is important to mention that oysters highly influenced the mean value of copper and zinc in bivalves because of its higher concentration of these minerals when compared with the other bivalves, since oysters are prone to copper and zinc accumulation (Nielsen and Nathan 1975; Vlieg et al. 1991) Regarding sodium and magnesium concentrations, Vlieg et al. (1991) found that they are higher in shellfish than in finfish, which agrees with the present study where the highest mean value for sodium and magnesium was found in bivalves and gastropods, respectively. Sodium is an important mineral in electrolyte balance (Eti et al. 2019) and in the case of bivalves is the principal responsible for osmotic regulation (Berger and Kharazova 1997) explaining the higher levels of this mineral in this group. Meanwhile, magnesium is one of the elements found in higher concentrations on the shells of molluscs (e.g. gastropods and, bivalves). Because they use this mineral as a component in the formation of their shells (Foster and Cravo 2003; Cobo et al. 2017) which can explain the higher concentration found in the muscle tissue of gastropods species. Bivalves also presented the highest mean value for manganese, while gastropods presented the highest mean value for selenium. For manganese results in the same range were found in different species of bivalves (Özden et al. 2009; Esposito et al. 2018). However, in other species higher and lower values were also found (Usero 1997; Özden et al. 2009). This can be linked to the fact that mineral concentrations in bivalves can change accordingly with environment concentrations (Usero 1997) For selenium, this mineral acts as an antioxidant, protecting cells against oxidative damage (Belitz et al. 2009) and has been associated with a protective effect against toxic elements in organisms (Barghigiani et al. 1991; Feroci et al. 2005). Once, gastropods presented higher levels of toxic compounds (unpublished data) it can explain the higher levels of selenium. Another explanation is environment pollution with selenium itself where these organisms inhabit (Carvalho et al. 2005; Barrento et al. 2008). Also, some gastropods species have an herbivorous diet, which can also influence their selenium concentrations, since plants have high concentrations of this mineral (Schiavon et al. 2017; Fox and Zimba 2018; Soares et al. 2020)

4.2.2. Feeding mode

Feeding mode was another trait that had an impact on the mineral content of seafood, showing significant differences between species with different feeding modes. Since the natural diet of marine species is one of the two main ways that organisms can absorb and retain mineral elements (Rainbow 2002; Roy and Lall 2006; Craig and Helfrich 2009), these differences were expected. Also, studies made in cultured fish found differences in the mineral content of cultured fish fed with different diets and also between wild and cultured fish (Alasalvar et al. 2002; Yildiz 2008; Bhouri et al. 2010; Siano et al. 2017), proving this way that diet can influence the mineral content of seafood. However, between zoobenthivorous and piscivorous species and between omnivorous and herbivorous species, differences in the mineral content were not found. Regarding zoobenthivorous and piscivorous species, the majority of zoobenthivorous species analysed were fish species. Once piscivorous species feed predominantly on fish (Elliott et al. 2007), they will inevitably feed on zoobenthivorous fish species and uptake minerals from these organisms. This can lead to a similar mineral content between these two groups. Concerning omnivorous and herbivorous species, one explanation is the overlap in the diet of these species, once omnivorous species also have a strong herbivorous component in their diet (Froese et al. 2000; Elliott et al. 2007). Another explanation is that omnivorous species have a diversified diet and consequently can absorb different minerals from different food items. Meanwhile, algae are a rich food item concerning minerals (Fox and Zimba 2018; Soares et al. 2020), and although they are two different diets, they may provide similar mineral contents.

Planktivorous species presented the highest mean value for phosphorus concentration. This can be explained by the fact that phytoplankton needs phosphorus to grow (Broecker and Peng 1983; Garrison and Ellis 2016) and in the food chain phytoplankton is part of the zooplankton diet (Garrison and Ellis 2016). Consequently, it is expected that plankton will be a good source of phosphorus to species that feed on these organisms. Omnivorous species presented the highest mean concentration for calcium and potassium. These species have a diversified diet, eating from algae to epifauna and infauna (Elliott et al. 2007; Baptista et al. 2015) thus can accumulate minerals from diverse sources. This can explain the higher concentrations of calcium and potassium. For example, algae are a good source of both minerals (Csikkel-Szolnoki et al. 2000; Soares et al. 2020), while crustaceans are a good source of calcium and fish are a good source of potassium (Karakoltsidis et al. 1995; Barrento et al. 2008; Afonso et al. 2013; Stoyanova 2018). Herbivorous presented the highest mean concentration for selenium and magnesium. Selenium is an important mineral in the metabolism of several microalgae and also can accumulate and stimulate growth, in appropriated concentrations, in macroalgae (Araie and Shiraiwa 2009; Schiavon et al. 2017;

Wang et al. 2019). Consequently, the consumption of algae can work as a vector in the movement of selenium to animals that feed on them and other consumers of the food web (Schiavon et al. 2017) Regarding magnesium, this element is essential in high amounts for most algae, for chlorophyll and enzymatic processes (Fox and Zimba 2018). Due to this need, algae present higher concentrations of this mineral when compared with the concentrations found in other studies in different marine organisms (Csikkel-Szolnoki et al. 2000; Fox and Zimba 2018). This can explain the high mean concentration of magnesium in herbivorous species. Filter feeders were the group that presented the highest mean concentrations for five minerals, sodium, copper, iron, manganese, and zinc. This group filters the water feeding on suspended organic matter and inevitably ingests inorganic particulate materials (El-Sikaily et al. 2004). Also, they are known to easily accumulate trace minerals (Usero 1997; Bellante et al. 2016). This particular way of feeding explains why they presented the highest mean concentrations of the five minerals.

4.2.3. Vertical distribution

Vertical distribution of marine species is defined as the main habitat used by them, regarding its position in the water column and dependence to the bottom sediment (Elliott and Dewailly 1995; Froese et al. 2000; Baptista et al. 2015). Significant differences in the mineral content of the groups of species from different depths in the water column were also found. The mineral content along the water column can be affected by several sources as atmospheric transport, upwelling, and diagenetic exchanges at the water-sediment interface (Cotté-Krief et al. 2000). Thus species uptake minerals from their surrounding environment (water; sediments) and/or food (Rainbow 2002; Roy and Lall 2006; Craig and Helfrich 2009), and differences observed may result from variations in the bioavailability of minerals in the environment and food items along the water column. However, between demersal and benthopelagic species differences were not found. Demersal species are characterized for living and feeding near the bottom, while benthopelagic are characterized for living and feeding near the bottom as well as in the water column (Froese et al. 2000). Despite the wider distribution of the benthopelagic species, they have a strong overlap with demersal species, and this may explain why no differences were found. Besides these two groups, between bathydemersal species and all other groups of species (except benthic) no differences were found. Bathydemersal species are characterized for living and feeding on the bottom below 200 m (Froese et al. 2000). However, this habitat did not reveal an influence in the mineral content of species when compared with the remaining groups (except the benthic group) that use different habitats.

Benthic species presented a clear separation from the remaining groups, presenting the highest mean values for the majority of the elements. Bruland and Franks (1983) stated that some essential minerals have a nutrient-type distribution and are involved in a biogeochemical cycle, including their net removal from surface waters via sinking biological debris and subsequent regeneration at depth. Also, Carvalho et al. (2005) suggested that the mineral content is greater in the bottom and found that benthic fish species have the highest concentration of Fe and Zn. Vlieg et al. (1991) found that shellfish species, which are mainly benthic, have a higher concentration of sodium, magnesium, and copper. However, sodium and magnesium are minerals with similar concentrations along the water column (Broecker and Peng 1983), and therefore the higher concentrations found may be associated with the specific needs or diet of the species analysed. The concentrations of zinc, copper, and selenium are found to increase with depth (Bruland and Franks 1983, Broecker and Peng 1983). For manganese, it was suggested that this mineral is rapidly removed from the water to the sediments through its capture on particulate debris (Broecker and Peng 1983). Regarding calcium, it was found that in deeper waters this mineral has twice the concentration of surface waters (Broecker and Peng 1983). Also, the fact that a considerable number of benthic species analysed were crustaceans may contribute to the values of calcium.

Only two minerals did not present the highest mean concentrations in benthic species. Pelagic species had the highest mean concentrations of phosphorus and potassium. These two minerals have a distinct distribution in the water column. Phosphorus is considered to have higher concentrations in deeper waters because in the surface water it is used by photosynthetic organisms, decreasing its concentrations (Broecker and Peng 1983). Meanwhile, potassium has a similar distribution throughout the water column (Broecker and Peng 1983). However, the mineral distribution may not be the explanation for the higher concentrations of these two minerals in pelagic species. The species belonging to this group and their diet can be the reason for the higher levels of phosphorus and potassium. The highest levels of phosphorus can be explained because most of the pelagic species analysed were planktivorous. As previously discussed, plankton is a significant source of phosphorus, given that photosynthetic organisms extract phosphorus from the surface water to grow (Broecker and Peng 1983; Garrison and Ellis 2016). Meanwhile, the higher levels of potassium can be explained because the pelagic species analysed were mostly fish species, and fish, in general, are rich in potassium (Carvalho et al. 2005; Afonso et al. 2013; Stoyanova 2018).

4.3. Final considerations

The main purpose of this thesis was to characterize the essential mineral content of the marine species consumed in Portugal and to evaluate how ecological traits influence mineral content in seafood species.

This work allowed me to characterize the mineral content of 10 essential elements (Ca, K, Mg, Na, P, Cu, Fe, Mn, Se and, Zn) of most of the seafood caught and eaten in Portugal. Results showed that regardless of the species potassium, phosphorus and sodium were the most abundant macro-minerals while zinc, iron, and copper were the most abundant trace elements.

More importantly, permitted to clarify how ecological traits can influence the mineral content of seafood. Differences in the mineral content of taxonomic, feeding mode, and vertical distribution groups were found. These differences are attributed to specific characteristics and physiologic needs, different diets, and the bioavailability of minerals in and throughout the surrounding environment (water and sediments).

Element-dense groups were identified according to their mineral content. Fish can be a good source of K, while crustaceans presented high concentrations of Ca and P. Bivalves presented high concentrations of Na, Cu, Mn, and Zn, whereas gastropods have high concentrations of Mg, Fe, and Se. Meanwhile, planktivorous species can be a good source of P, while omnivorous can be a good source of Ca and K. Herbivorous presented high concentrations of Mg and Se, whereas filter feeders presented high concentrations of Na, Cu, Fe, Mn, and Zn. Finally, pelagic species can be a good source of K and P, while benthic species presented high concentrations of Ca, Mg, Na, Cu, Fe, Mn, Se, and Zn. Overall, seafood can be an excellent source of essential elements for the nutrition of the Portuguese population.

Finally, throughout this project new questions/objectives emerged to be developed in future works. First, using the database gathered in this work to calculate the contribution of each studied mineral, in each species, to the daily recommended intake (RDI) for the five major groups of the human population (children, adolescents, adults, elderly and, pregnancy). This way identifying the element-dense species who better contribute to the

RDI. Also, considering that mineral content in seafood can be influenced by other factors such as sex, age, and/or size, a detailed analysis to study and comprehend the effect these factors can have. This analysis can be a complement to the present work improving the knowledge of the factors that influence the mineral content of marine species. Additional future work could be the assessment of contaminant (toxic elements/non-essential elements such as As, Cd and Pb) bioaccessibility in seafood and evaluate if they pose a risk for human health. This because, marine species when exposed to contaminants can bioaccumulate and through the process of biomagnification transfer the contaminants along the food chain, until reaching humans.

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Supplementary material

Table 8. Mineral concentration (mg kg^{-1} ww) in the muscle of fish species. Values are mean (± SD).

Fish Species	Mineral Elements									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Conger conger	321 ± 176	2567 ± 468	238 ± 45.5	1050 ± 609	1907 ± 215	0.42 ± 0.075	3.5 ± 3.3	0.31 ± 0.21	0.52 ± 0.22	5.1 ± 0.37
Anguilla anguilla	419 ± 137	1714 ± 380	199 ± 36.2	689 ± 208	1666 ± 288	0.65 ± 0.22	7.1 ± 2.4	1.5 ± 1.1	0.45 ± 0.045	16.9 ± 3.4
Sardina pilchardus	612 ± 127	5172 ± 606	419 ± 31.1	653 ± 90	4500 ± 192	0.62 ± 0.35	21 ± 11	0.46 ± 0.15	0.23 ± 0.033	4.3 ± 1.0
Alosa alosa	706 ± 547	3522 ± 1603	263 ± 109.7	487 ± 134	2483 ± 822	0.33 ± 0.13	4.5 ± 1.9	0.25 ± 0.14	0.37 ± 0.076	3.3 ± 0.86
Alosa fallax	274 ± 46	4969 ± 536	366 ± 21.2	458 ± 165	3696 ± 191	0.47 ± 0.091	5.9 ± 3.3	0.35 ± 0.21	0.19 ± 0.021	3.6 ± 0.53
Salmo salar	56 ± 14	2258 ± 516	187 ± 37	534 ± 119	1660 ± 303	0.24 ± 0.064	1.6 ± 0.31	0.068 ± 0.028	0.19 ± 0.072	2.6 ± 0.29
Zeus faber	162 ± 34	2332 ± 484	196 ± 19	1155 ± 177	1506 ± 2251	0.15 ± 0.098	2.9 ± 1.6	0.11 ± 0.012	0.42 ± 0.024	4.4 ± 1.9
Merluccius merluccius	608 ± 513	4572 ± 396	329 ± 22	964 ± 246	3992 ± 497	0.21 ± 0.015	2.2 ± 0.23	0.14 ± 0.058	0.39 ± 0.066	3.3 ± 0.12
Trisopterus luscus	813 ± 186	4332 ± 393	340 ± 3.8	1212 ± 95	2152 ± 157	0.33 ± 0.11	2.7 ± 0.38	0.32 ± 0.029	0.25 ± 0.049	4.5 ± 0.52
Micromesistius poutassou	125 ± 9.6	1749 ± 446	261 ± 15	1090 ± 171	1753 ± 269	0.17 ± 0.036	1.5 ± 0.22	0.086 ± 0.017	0.36 ± 0.033	2.3 ± 0.21
Lepidopus caudatus	79 ± 7.1	2780 ± 613	238 ± 43	819 ± 90	1659 ± 266	0.22 ± 0.11	1.8 ± 0.78	0.97 ± 0.034	0.42 ± 0.099	2.2 ± 0.28
Aphanopus carbo	66 ± 13	2848 ± 191	256 ± 22	1300 ± 335	2523 ± 92	0.11 ± 0.014	1.1 ± 0.48	0.15 ± 0.031	0.52 ± 0.061	2.1 ± 0.44

Table 8. Continued

Fish Species	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Scomber scombrus	183 ± 63	3711 ± 403	331 ± 14	766 ± 103	3179 ± 217	0.78 ± 0.36	7.8 ± 3.6	0.15 ± 0.024	0.33 ± 0.084	6.3 ± 2.1
Scomber colias	70 ± 43	2600 ± 369	214 ± 31	230 ± 15	2257 ± 193	0.54 ± 0.085	10 ± 7.6	0.064 ± 0.0059	0.35 ± 0.097	3.9 ± 1.2
Thunnus albacares	54 ± 7.4	2790 ± 356	255 ± 25	7407 ± 1136	2015 ± 229	0.26 ± 0.012	6.4 ± 1.7	0.093 ± 0.031	0.85 ± 0.34	2.7 ± 0.22
Belone belone	170 ± 14	3042 ± 288	330 ± 25	682 ± 73	1655 ± 107	1.6 ± 0.78	18 ± 9.3	0.21 ± 0.033	0.29 ± 0.033	6.7 ± 0.93
Chelon auratus	75 ± 7.6	5402 ± 120	307 ± 5.6	328 ± 23	2020 ± 49	0.31 ± 0.14	11 ± 4.0	0.13 ± 0.093	0.19 ± 0.019	3.2 ± 0.49
Chelon ramada	126 ± 27	5410 ± 206	335 ± 26	669 ± 78	2015 ± 109	0.40 ± 0.27	9.7 ± 2.7	0.11 ± 0.036	0.17 ± 0.016	5.3 ± 1.8
Lophius piscatorius	112 ± 28	1592 ± 508	260 ± 30	1414 ± 362	1169 ± 188	0.14 ± 0.091	3.1 ± 3.0	0.11 ± 0.038	0.39 ± 0.12	4.1 ± 2.4
Balistes capriscus	59 ± 14	3007 ± 530	203 ± 20	763 ± 233	1627 ± 279	0.19 ± 0.034	2.4 ± 1.1	0.15 ± 0.15	0.32 ± 0.029	6.0 ± 6.7
Helicolenus dactylopterus	55 ± 14	2967 ± 256	255 ± 11	986 ± 177	1801 ± 311	0.15 ± 0.051	2.6 ± 0.36	0.082 ± 0.039	0.63 ± 0.11	3.2 ± 0.41
Chelidonichthys obscurus	231 ± 96	4304 ± 247	352 ± 3.6	435 ± 96	2183 ± 36	0.063 ± 0.00*	2.7 ± 1.0	0.18 ± 0.077	0.28 ± 0.034	2.8 ± 0.18
Chelidonichthys lucerna	250 ± 122	2660 ± 407	285 ± 28	826 ± 117	1603 ± 193	0.33 ± 0.19	4.2 ± 0.75	0.19 ± 0.066	0.19 ± 0.049	3.5 ± 0.32

Table 8. Continued

Fish Species	Mineral Elements									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Trigla lyra	177 ± 46	3817 ± 234	260 ± 13	630 ± 117	2054 ± 109	0.31 ± 0.31	1.5 ± 0.56	0.17 ± 0.023	0.48 ± 0.059	2.8 ±0.41
Chelidonichthys lastoviza	98 ± 14	3809 ± 474	277 ± 25	661 ± 184	2167 ± 230	0.18 ± 0.027	2.2 ± 1.0	0.11 ± 0.016	0.34 ± 0.044	3.1 ± 0.36
Dicentrarchus labrax	137 ± 39	4580 ± 135	315 ± 11	399 ± 100	1804 ± 86	0.32 ± 0.091	6.6 ± 4.8	0.13 ± 0.065	0.27 ± 0.029	3.2 ± 0.67
Dicentrarchus punctatus	285 ± 128	3890 ± 341	332 ± 7.8	516 ± 27	1727 ± 86	0.34 ± 0.054	3.8 ± 0.18	0.23 ± 0.036	0.32 ± 0.032	4.8 ± 0.72
Epinephelus aeneus	122 ± 53	2222 ± 308	178 ± 32	618 ± 145	1224 ± 170	0.14 ± 0.031	1.6 ± 1.0	0.046 ± 0.013	0.41 ± 0.081	3.2 ± 0.21
Engraulis encrasicolus	799 ± 211	1804 ± 425	298 ± 40	523 ± 88	2631 ± 462	0.92 ± 0.16	17 ± 10	0.41 ± 0.17	0.25 ± 0.039	13 ± 3.5
Trachurus trachurus	236 ± 50	3400 ± 221	295 ± 24	670 ± 144	3037 ± 255	0.70 ± 0.18	6.8 ± 1.5	0.12 ± 0.031	0.21 ± 0.014	3.8 ± 0.43
Brama brama	59 ± 5.6	2960 ± 98	224 ± 6.4	806 ± 93	2100 ± 51	0.25 ± 0.027	3.4 ± 0.42	0.071 ± 0.015	0.43 ± 0.031	5.1 ± 0.19
Sparus aurata	100 ± 25	3809 ± 298	281 ± 3.2	795 ± 336	2470 ± 134	0.19 ± 0.025	4.6 ± 1.7	0.13 ± 0.036	0.44 ± 0.058	7.4 ± 0.65
Pagrus pagrus	28 ± 7.6	4765 ± 72	319 ± 12	430 ± 70	3012 ± 59	0.16 ± 0.022	1.2 ± 0.19	0.092 ± 0.028	0.36 ± 0.062	2.8 ± 0.46
Pagrus caeruleostictus	132 ± 19	4422 ± 390	300 ± 14	956 ± 147	2806 ± 200	0.18 ± 0.013	3.1 ± 1.1	0.059 ± 0.0078	0.41 ± 0.059	2.9 ± 0.12

Table 8. Continued

Fish Species	Mineral Elements									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Plectorhinchus mediterraneus	96 ± 11	3276 ± 162	262 ± 9.9	994 ± 172	1734 ± 98	0.29 ± 0.029	3.3 ± 0.51	0.057 ± 0.013	0.41 ± 0.051	3.0 ± 0.46
Dentex canariensis	93 ± 48	3337 ± 190	289 ± 23	931 ± 224	2129 ± 93	0.14 ± 0.021	1.8 ± 0.27	0.13 ± 0.012	0.56 ± 0.0095	2.5 ± 0.12
Dentex macrophthalmus	128 ± 16	2922 ± 254	259 ± 19	833 ± 151	1553 ± 135	0.15 ± 0.023	2.3 ± 0.76	0.056 ± 0.0088	0.47 ± 0.0091	2.1 ± 0.14
Pagellus bogaraveo	476 ± 152	3220 ± 251	295 ± 36	820 ± 49	1826 ± 305	0.42 ± 0.16	4.7 ± 0.93	0.17 ± 0.028	0.43 ± 0.076	3.8 ± 0.38
Pagellus erythrinus	395 ± 167	3418 ± 236	279 ± 15	539 ± 50	1823 ± 336	0.16 ± 0.029	1.9 ± 0.41	0.15 ± 0.061	0.25 ± 0.027	2.8 ± 0.19
Boops boops	98 ± 49	4709 ± 349	350 ± 18	339 ± 61	3397 ± 197	0.35 ± 0.058	5.8 ± 3.1	0.12 ± 0.079	0.27 ± 0.061	3.1 ± 0.59
Spondyliosoma cantharus	216 ± 109	4018 ± 333	296 ± 15	464 ± 82	1863 ± 449	0.11 ± 0.047	1.7 ± 0.25	0.12 ± 0.029	0.35 ± 0.064	2.6 ± 0.24
Sarpa salpa	308 ± 297	3827 ± 112	254 ± 18	295 ± 95	1672 ± 92	0.29 ± 0.15	5.4 ± 3.3	0.094 ± 0.049	0.11 ± 0.031	4.2 ± 1.1
Diplodus sargus	250 ± 49	4151 ± 310	329 ± 30	578 ± 89	1650 ± 169	0.27 ± 0.13	3.9 ± 1.0	0.16 ± 0.051	0.32 ± 0.14	4.7 ± 0.65
Diplodus vulgaris	320 ± 62	3272 ± 583	346 ± 23	754 ± 133	1703 ± 174	0.13 ± 0.066	4.0 ± 0.56	0.18 ± 0.027	0.48 ± 0.18	5.1 ± 1.0
Argyrosomus regius	84 ± 1.0	4175 ± 247	291 ± 16	420 ± 102	2515 ± 79	0.48 ± 0.19	3.5 ± 1.4	0.087 ± 0.031	0.41 ± 0.18	3.9 ± 0.87

Table 8. Continued

Fish Species	Mineral Elements									
	Ca	K	Mg	Na	P	Cu	Fe	Mn	Se	Zn
Cynoscion regalis	189 ± 33	3939 ± 373	317 ± 23	702 ± 161	2152 ± 189	0.17 ± 0.049	4.2 ± 1.4	0.089 ± 0.017	0.32 ± 0.028	4.4 ± 0.32
Mullus surmuletus	217 ± 9.5	3932 ± 501	429 ± 31	756 ± 79	2243 ± 230	0.29 ± 0.12	5.0 ± 0.91	0.21 ± 0.015	0.29 ± 0.038	4.1 ± 0.72
Sparisoma cretense	211 ± 81	2018 ± 346	174 ± 23	838 ± 133	1154 ± 174	0.19 ± 0.048	2.9 ± 1.1	0.22 ± 0.13	0.29 ± 0.046	3.1 ± 0.57
Lates niloticus	128 ± 56	2538 ± 303	217 ± 15	599 ± 159	1312 ± 153	0.19 ± 0.054	1.7 ± 0.16	0.072 ± 0.015	0.35 ± 0.064	2.6 ± 0.13
Scyliorhinus canicula	92 ± 31	2477 ± 156	202 ± 19	1605 ± 357	1965 ± 150	0.43 ± 0.11	8.1 ± 3.1	0.23 ± 0.11	0.41 ± 0.072	12 ± 1.3
Raja microocellata	128 ± 74	3260 ± 654	194 ± 11	1061 ± 461	2443 ± 387	0.30 ± 0.12	6.5 ± 3.6	0.16 ± 0.069	0.36 ± 0.12	3.5 ± 0.89
Uranoscopus scaber	64 ± 8.2	3097 ± 190	234 ± 8.8	1575 ± 314	2373 ± 194	0.16 ± 0.027	1.3 ± 0.28	0.095 ± 0.032	0.43 ± 0.045	2.5 ± 0.19
Ammodytes tobianus	246 ± 44	3351 ± 65	352 ± 30	777 ± 79	2176 ± 225	0.32 ± 0.066	3.4 ± 0.56	0.26 ± 0.076	0.89 ± 0.089	14 ± 2.2
Trachinus draco	122 ± 25	3488 ± 133	308 ± 12	469 ± 46	1889 ± 135	0.066 ± 0.016*	3.1 ± 2.2	0.11 ± 0.039	0.34 ± 0.071	3.2 ± 0.44
Echiichthys vipera	283 ± 100	4193 ± 299	316 ± 14	622 ± 80	3110 ± 86	0.21 ± 0.026	3.1 ± 0.88	0.23 ± 0.18	0.21 ± 0.026	4.3 ± 0.52
Scophthalmus rhombus	478 ± 120	2148 ± 401	313 ± 8.9	1038 ± 64	1381 ± 182	0.23 ± 0.076	3.3 ± 0.91	0.33 ± 0.091	0.28 ± 0.017	6.8 ± 0.71

Table 8. Continued

Fish Species	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Scophthalmus maximus	115 ± 44	2679 ± 512	282 ± 18	1096 ± 230	1266 ± 172	0.061 ± 0.022*	1.8 ± 0.28	0.17 ± 0.022	0.43 ± 0.14	4.0 ± 0.58
Arnoglossus laterna	772 ± 131	2419 ± 663	282 ± 9.1	816 ± 101	1258 ± 200	0.18 ± 0.036	3.9 ± 2.5	0.41 ± 0.099	0.29 ± 0.036	3.8 ± 0.27
Platichthys flesus	78 ± 22	4049 ± 124	247 ± 12	397 ± 75	1967 ± 39	0.24 ± 0.046	4.2 ± 1.9	0.12 ± 0.0051	0.19 ± 0.018	4.4 ± 0.45
Solea solea	2058 ± 766	2235 ± 169	264 ± 21	782 ± 86	1783 ± 320	0.11 ± 0.023	8.1 ± 5.4	3.1 ± 1.6	0.25 ± 0.032	6.2 ± 1.4
Solea senegalensis	106 ± 18	3718 ± 137	245 ± 6.2	586 ± 69	1745 ± 96	0.27 ± 0.15	1.8 ± 0.58	0.079 ± 0.018	0.26 ± 0.042	2.9 ± 0.46
Pegusa lascaris	136 ± 23	2924 ± 249	242 ± 18	1145 ± 73	1453 ± 97	0.22 ± 0.087	2.1 ± 0.41	0.071 ± 0.0079	0.17 ± 0.012	3.1 ± 0.22
Microchirus azevia	1196 ± 640	3410 ± 283	263 ± 6.7	426 ± 123	1751 ± 235	0.13 ± 0.052	4.1 ± 2.8	0.45 ± 0.21	0.12 ± 0.046	2.8 ± 0.39
Microchirus variegatus	231 ± 53	2789 ± 152	230 ± 15	846 ± 78	1399 ± 70	0.13 ± 0.018	1.7 ± 0.27	0.086 ± 0.026	0.27 ± 0.051	4.8 ± 0.53
Dicologlossa cuneate	198 ± 90	3574 ± 142	300 ± 19	1624 ± 447	1883 ± 53	0.24 ± 0.045	2.7 ± 0.86	0.098 ± 0.036	0.28 ± 0.028	2.5 ± 0.089
Synaptura cadenati	360 ± 241	2426 ± 308	272 ± 38	1603 ± 271	1400 ± 17	0.21 ± 0.069	3.1 ± 0.62	0.15 ± 0.079	0.82 ± 0.39	5.7 ± 0.86

*Values under the limit of detection

Table 9. Mineral concentration (mg kg^{-1} ww) in the muscle of cephalopods species. Values are mean (±SD).

Cephalopods Species	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Loligo vulgaris	469 ± 185	1427 ± 258	381 ± 65	1471 ± 190	1697 ± 287	7.3 ± 2.5	89 ± 78	0.68 ± 0.32	0.37 ± 0.049	8.7 ± 0.82
Alloteuthis subulata	964 ± 425	863 ± 221	290 ± 15	866 ± 267	1479 ± 88	10 ± 6.1	44 ± 8.7	1.5 ± 0.65	0.29 ± 0.057	9.9 ± 0.84
Illex coindetii	93 ± 24	2583 ± 568	386 ± 48	1653 ± 223	2369 ± 399	3.3 ± 1.4	1.6 ± 0.44	0.16 ± 0.032	0.32 ± 0.051	10 ± 2.3
Sepia officinalis	75 ± 24	2709 ± 496	337 ± 31	1842 ± 840	2293 ± 319	1.1 ± 0.25	0.92 ± 0.098	0.12 ± 0.035	0.24 ± 0.049	11 ± 1.1
Octopus vulgaris	123 ± 9.5	1273 ± 273	362 ± 20	1879 ± 161	1048 ± 73	1.7 ± 1.1	1.3 ± 0.16	0.21 ± 0.049	0.31 ± 0.039	10 ± 1.4
Eledone cirrhosa	126 ± 12	1617 ± 115	374 ± 23	2195 ± 210	1116 ± 96	6.5 ± 3.0	1.3 ± 0.086	0.22 ± 0.031	0.31 ± 0.017	10 ± 0.51

Table 10. Mineral concentration (mg kg^{-1} ww) in the muscle of crustaceans species. Values are mean (±SD).

Crustaceans	**Mineral Elements**									
Species	**Ca**	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Crangon crangon	9609 ± 649	1078 ± 81	309 ± 22	714 ± 53	2441 ± 124	2.3 ± 0.26	11 ± 0.88	2.9 ± 0.25	0.86 ± 0.14	8.2 ± 0.17
Penaeus vannamei	578 ± 227	4536 ± 291	440 ± 24	1523 ± 80	3006 ± 252	7.5 ± 1.7	60 ± 17	1.0 ± 0.56	0.26 ± 0.0074	13 ± 0.39
Pleoticus muelleri	705 ± 128	2864 ± 88	607 ± 62	3130 ± 382	2870 ± 157	4.4 ± 0.55	5.1 ± 1.6	0.57 ± 0.32	0.48 ± 0.056	11 ± 0.57
Penaeus monodon	820 ± 297	2858 ± 351	503 ± 88	3257 ± 744	2922 ± 270	10 ± 3.5	2.0 ± 0.49	0.49 ± 0.28	0.59 ± 0.056	22 ± 3.6
Maja squinado	1726 ± 767	2022 ± 567	697 ± 241	4551 ± 1642	1430 ± 307	3.7 ± 1.8	3.3 ± 0.44	0.33 ± 0.11	0.95 ± 0.29	45 ± 7.6
Cancer pagurus	651 ± 327	2688 ± 222	317 ± 71	2938 ± 1096	1744 ± 142	4.2 ± 1.7	5.5 ± 1.4	0.25 ± 0.046	0.97 ± 0.43	59 ± 7.4
Necora puber	1237 ± 525	2555 ± 166	486 ± 120	4359 ± 1709	1575 ± 169	4.6 ± 1.9	2.8 ± 0.86	0.19 ± 0.066	0.83 ± 0.21	21 ± 3.3
Homarus gammarus	360 ± 216	2164 ± 114	230 ± 16	2761 ± 1111	1613 ± 60	8.5 ± 0.98	1.9 ± 1.9	0.42 ± 0.29	0.67 ± 0.26	31 ± 9.9
Nephrops norvegicus	549 ± 48	4196 ± 139	510 ± 40	3372 ± 549	3163 ± 161	8.5 ± 2.0	2.5 ± 1.2	0.29 ± 0.029	0.71 ± 0.061	15 ± 0.74
Pollicipes pollicipes	6900 ± 2657	1750 ± 506	1056 ± 185	6278 ± 628	850 ± 230	0.52 ± 0.17	52 ± 14	2.3 ± 1.6	1.3 ± 0.38	60 ± 16

Table 11. Mineral concentration (mg kg^{-1} ww) in the muscle of bivalves species. Values are mean (±SD).

Bivalves Species	Mineral Elements									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Magallana angulata	442 ± 164	2338 ± 330	667 ± 163	4543 ± 1368	1645 ± 250	318 ± 51	39 ± 13	18 ± 8.3	0.26 ± 0.17	1670 ± 280
Donax trunculus	807 ± 202	2090 ± 158	467 ± 12	2382 ± 100	1224 ± 130	1.2 ± 0.37	53 ± 29	1.6 ± 0.51	0.93 ± 0.25	10 ± 1.4
Spisula solida	433 ± 55	2373 ± 280	491 ± 43	2354 ± 290	1402 ± 178	1.4 ± 0.44	21 ± 12	0.59 ± 0.29	0.66 ± 0.18	9.8 ± 1.2
Meretrix lyrata	805 ± 107	296 ± 73	214 ± 40	961 ± 249	1543 ± 42	1.6 ± 0.51	42 ± 4.2	3.3 ± 1.9	0.35 ± 0.092	13 ± 1.4
Ruditapes decussatus	1094 ± 559	2428 ± 129	662 ± 78	4582 ± 1068	1439 ± 133	1.1 ± 0.31	30 ± 8.0	1.0 ± 0.24	1.0 ± 0.12	10 ± 0.54
Ruditapes philippinarum	497 ± 37	1293 ± 71	952 ± 121	5558.1 ± 528	2155 ± 684	0.86 ± 0.14	26 ± 8.8	1.5 ± 0.88	1.1 ± 0.22	21 ± 4.7
Scrobicularia plana	716 ± 294	888 ± 71	992 ± 75	6722 ± 631	645 ± 58	1.6 ± 0.52	25 ± 4.4	1.6 ± 0.12	0.85 ± 0.068	53 ± 11
Mytilus edulis	423 ± 242	1554 ± 299	607 ± 143	3347 ± 699	1314 ± 160	0.71 ± 0.18	53 ± 19	1.5 ± 0.29	0.76 ± 0.23	16 ± 8.4
Solen marginatus	1077 ± 159	4955 ± 91	812 ± 16	4250 ± 181	3117 ± 91	1.4 ± 0.48	140 ± 35	2.1 ± 0.52	0.82 ± 0.16	20 ± 3.7

Table 12. Mineral concentration (mg kg^{-1} ww) in the muscle of gastropods species. Values are mean (±SD).

Gastropods Species	**Mineral Elements**									
	Ca	**K**	**Mg**	**Na**	**P**	**Cu**	**Fe**	**Mn**	**Se**	**Zn**
Littorina littorea	1905 ± 1284	1052 ± 138	2383 ± 306	1485 ± 127	1320 ± 101	17 ± 5.1	45 ± 6.9	9.2 ± 1.1	0.44 ± 0.061	10 ± 0.45
Phorcus lineatus	243 ± 47	1468 ± 209	536 ± 35	2710 ± 432	891 ± 40	10 ± 2.8	20 ± 2.1	0.52 ± 0.11	6.8 ± 4.2	9.6 ± 0.74
Patella vulgata	1824 ± 554	2407 ± 113	902 ± 157	5592 ± 1123	1353 ± 233	0.79 ± 0.13	468 ± 56	3.8 ± 1.0	0.36 ± 0.073	11 ± 2.0
Buccinum undatum	412 ± 51	2632 ± 561	865 ± 336	2575 ± 1038	1464 ± 272	24 ± 8.3	22 ± 14	1.6 ± 0.89	1.4 ± 0.59	258 ± 191
Steromphala umbilicalis	486 ± 116	3336 ± 55	1038 ± 44	5763 ± 442	1638 ± 56	5.4 ± 0.66	103 ± 67	1.7 ± 0.69	3.1 ± 1.4	14 ± 0.39

Table 13. Pairwise test for the selected trait taxonomy.

Groups	P(perm)
Fish, Crustaceans	0.001
Fish, Cephalopods	0.001
Fish, Bivalves	0.001
Fish, Gastropods	0.001
Crustaceans, Cephalopods	0.005
Crustaceans, Bivalves	0.001
Crustaceans, Gastropods	0.004
Cephalopods, Bivalves	0.001
Cephalopods, Gastropods	0.001
Bivalves, Gastropods	0.045

Table 14. Pairwise test for the selected trait feeding mode

Groups	P(perm)
Zoobenthivorous, Planktivorous	0.003
Zoobenthivorous, Piscivorous	0.056
Zoobenthivorous, Omnivorous	0.006
Zoobenthivorous, Herbivorous	0.001
Zoobenthivorous, Filter feeders	0.001
Planktivorous, Piscivorous	0.001
Planktivorous, Omnivorous	0.001
Planktivorous, Herbivorous	0.001
Planktivorous, Filter feeders	0.001
Piscivorous, Omnivorous	0.004
Piscivorous, Herbivorous	0.001
Piscivorous, Filter feeder	0.001
Omnivorous, Herbivorous	0.098
Omnivorous, Filter feeders	0.001
Herbivorous, Filter feeders	0.001

Table 15. Pairwise test for the selected trait vertical distribution.

Groups	**P(perm)**
Demersal, Pelagic	0.006
Demersal, Benthopelagic	0.202
Demersal, Bathydemersal	0.078
Demersal, Reef-associated	0.001
Demersal, Benthic	0.001
Pelagic, Benthopelagic	0.017
Pelagic, Bathydemersal	0.207
Pelagic, Reef-associated	0.007
Pelagic, Benthic	0.001
Benthopelagic, Bathydemersal	0.051
Benthopelagic- Reef-associated	0.001
Benthopelagic, Benthic	0.001
Bathydemersal, Reef-associated	0.188
Bathydemersal, Benthic	0.001
Reef-associated, Benthic	0.001