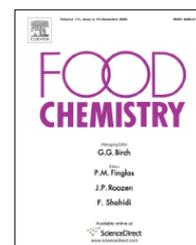


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## Essential elements and contaminants of clawed lobsters

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In several European countries, clawed lobsters such as the European (*Homarus gammarus*) and American (*H. americanus*) ones are widely consumed. Yet, information about essential elements and contaminants in both species is still scarce. Therefore, the aim of this work was to characterize the elemental content in the edible part (muscle, hepatopancreas, gonads and roe) of both homarids and to compare them with the daily intake recommendations and maximum allowed levels. Two techniques were employed: energy dispersive X-ray fluorescence (EDXRF) to quantify Cl, S, K, Ca, Fe, Cu, Zn, As, Se, Br and Sr; and flame atomic-absorption spectrometry (FAAS) to analyse Na, Mg, Mn, Cd, Hg and Pb. Significant differences were found in the elemental composition of edible tissues of both species, likely reflecting the distinct physiological role of those tissues: muscle (higher: Na, Mg, Ca and Sr; lower: Fe, Se, Cd); hepatopancreas (higher: Fe, Cu, Br and Cd); gonads (lower: Cl, Ca, Zn and Hg); and roe (higher: Na and Br; lower: K and As). Statistical differences in the elemental composition of each tissue were found between both homarids: muscle (Na, Se, As and Hg); hepatopancreas (Na, Mg, Fe, Cu, Se, Br and Hg); and gonads (S and Zn). Since the geographical distribution of both species is different, the differences likely reflect distinct elemental composition in the aquatic environment and, consequently in the feed chain. Both lobster species were rich sources of Na, Cl, Cu, Zn and Se for human consumption. Regarding contaminants, only Cd was detected at high concentrations in the hepatopancreas of both homarids. Despite Cd values were well above the maximum level set by the European Commission for crustaceans' muscle, so far any limit value was set for crustaceans' hepatopancreas. Nonetheless, the present study recommends avoiding or moderately consuming this tissue in homarids.

**Keywords:** *Homarus gammarus*, *H. americanus*, EDXRF, FAAS, macroelements, trace elements, contaminants.

## Introduction

In the last years, the health benefits related with seafood consumption have been extensively publicized. Seafood is rich in protein, contains low cholesterol and high percentage of (n-3) polyunsaturated fatty acids, liposoluble vitamins and essential minerals (Tahvonen *et al.*, 2000; Adeyeye, 2002; Chen *et al.*, 2006; Zalloua *et al.*, 2007). Epidemiological studies have indicated that populations with a rich seafood diet have low risk of coronary heart disease, hypertension and cancer (Simopoulos, 1997). In recent years, attention has been focused on determination of elements in seafood due to nutritional benefits of essential elements and toxicological concerns related to anthropogenic influx of contaminants. Lack of essential elements (e.g. Na, Mg, Cl, P, S, K, Ca, Mn, Fe, Cu, Zn, Se) leads to improper enzyme mediated metabolic functions and results in organ malfunctions, chronic diseases and ultimately death (Simopoulos, 1997; FAO/WHO, 2002). Other trace elements, such as Br and Sr, are still being categorised as essentials (Oehlenschläger, 1997). Therefore, regular intake of these elements via food ingestion is vital. On the other hand, environmental contaminants (e.g. As, Cd, Hg and Pb) are a major concern in aquatic environments, since they cause severe human health disorders (Carvalho *et al.*, 2005; Francesconi, 2007).

Clawed lobsters (*Homarus gammarus* and *H. americanus*) are worldwide highly prized delicacies (Holmyard and Franz, 2006). In Southern Europe, especially in coastal areas of Mediterranean countries (e.g. Italy, France, Spain, Greece and Portugal), these crustaceans are widely consumed. The European lobster *H. gammarus* production in 2005 was 2,326 t in all EU countries (EUROSTAT, 2007). This species typically inhabits the North Eastern Atlantic coast, Mediterranean Sea and Black Sea down to 100 m depth (Ingle, 1997; Holmyard and Franz, 2006). In order to satisfy the high consuming demands in Europe, American lobster *H. americanus* has been extensively imported from USA and Canada (16,000 t, corresponding to 140 million euros economic value; data from 2006; INFOFISH, 2007). *H. americanus* is mostly found along the North Western Atlantic coast down to 50 m depth (Holmyard and Franz, 2006).

Several studies have documented crustaceans' mineral value (Skonberg and Perkins, 2002; Gökoğlu and Yerlikaya, 2003; Küçükgülmez *et al.*, 2006) and contaminants content (Páez-Osuna and Ruiz-Fernández, 1995ab; Páez-Osuna and Bdjorquez-Leyva, 1999; Kádár *et al.*, 2006; Mishra *et al.*, 2007; Mohapatra *et al.*, 2007). Yet, as far as clawed lobsters are concerned there is still limited information on their essential elements and contaminants composition. This knowledge is fundamental, especially considering the high consumption of both homarids, but also because the edible tissues of *H. americanus* are available in a wide variety of forms for human consumption (Holmyard and Franz, 2006). These forms include processed hepatopancreas, presented as a green coloured meat (named tomalley), roe and muscle. Since each tissue has distinct functions, it is assumed that their mineral content also varies. In this context, the aim of the present work was to quantify the essential elements and contaminants contents in the muscle, hepatopancreas, gonads and roe of *H. americanus* and *H. gammarus*, and to compare such values with the daily intake recommendations and maximum limit levels.

## Material and methods

### Biological material

Twenty-one live American lobsters *H. americanus* (origin: USA and Canada;  $580.1 \pm 58$  g average weight) and 20 European lobsters *H. gammarus* (origin: Scotland;  $587 \pm 81$  g average weight) were purchased from a local company soon after importation and immediately transported to the laboratory. Animals were kept under refrigerated conditions during one hour to decrease their metabolism before being euthanized. The muscle tissue (from claws, legs and abdomen), hepatopancreas, gonads and roe (whenever present) were individually separated and weighed. Each tissue was subsequently homogenised with a grinder, vacuum packed and stored at  $-20$  °C, whereas the remaining was frozen at  $-80$  °C and subsequently freeze-dried for 48 h at  $-50$  °C and low pressure (approximately  $10^{-1}$  atm). Samples were powdered and stored at  $-20$  °C under controlled moisture conditions (vacuum packed) until further analyses.

### Element analyses

Energy dispersive X-ray fluorescence (EDXRF) spectrometer was used to quantify Cl, S, K, Ca, Fe, Cu, Zn, As, Se, Br and Sr. The EDXRF technique consists of an X-ray tube equipped with a changeable secondary target, normally of molybdenum (EXTRA II A, Atomika Instruments, Temple). The characteristic radiations emitted by the elements in the sample were detected by a Lithium drifted Silicon [Si (Li)] detector, with a  $30$  mm<sup>2</sup> active area and an  $8$  µm beryllium window. The energy resolution was  $135$  eV at  $5.9$  keV and the acquisition system was a Nucleus PCA card. Quantitative calculations were made by the fundamental parameters method (Mantler and Kawahara, 2004). Experimental parameters were obtained by calibration, using standard reference materials. The X-ray generator was operated at  $50$  kV and  $20$  mA and a typical acquisition time of  $1000$  s was used. Each sample powder ( $\sim 1$  g) was pressed into pellets of  $2$  cm diameter without any chemical treatment. A minimum of three pellets (replicates) per sample were glued onto Mylar films, on sample holders, and placed directly in the X-ray beam. There are several advantages of using EDXRF to determine elemental content: no sample preparation, less time consuming compared to conventional techniques, low amount of sample required, no need to use liquid reagents, thus eliminating reagent waste and reducing potential contamination. The elements Na, Mg, Mn, Cd and Pb were measured by flame atomic-absorption spectrometry (FAAS), with a Variant Spectr AA 20 spectrometer, according to official analytical methods (AOAC, 2005). The procedure is based on sample incineration ( $5$  g for Na, Mg and Mn;  $10$  g for Cd and Pb) and dissolution in  $\text{HNO}_3$ . A minimum of three replicate analyses was performed per sample. Concentrations were calculated from linear calibration plots obtained by measurement of the absorbance of standard solutions of  $\text{NaNO}_3$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{Mn}(\text{NO}_3)_2$ ,  $\text{Cd}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$  diluted in  $\text{HNO}_3$  ( $0.5$  M), all supplied by Merck. The element Hg was also measured in triplicate by flame atomic- absorption spectrometry (FAAS), but with AMA 254 Mercury Analyser spectrometer that uses the Hg vapour generation technique spectrometer, according to official methods of analysis (AOAC, 2005). The procedure is based on combustion of dry sample (hepatopancreas,  $3$  mg; muscle,  $6$  mg; and gonads,  $10$  mg), pre-concentration of Hg by amalgamation with gold and

atomic-absorption spectrometry. A minimum of three replicate analyses was performed per sample. Concentrations were calculated from linear calibration plots obtained by measurement of the absorbance of an Hg standard solution (Hg diluted in HNO<sub>3</sub>, 0.5 M) supplied by Merck. Two groups of elements were distinguished in the analyses: elements considered essential for life maintenance (macroelements: Na, Mg, Cl, S, K and Ca; trace elements: Mn, Fe, Cu, Zn, Se, Br and Sr), and elements considered harmful for animals (contaminants: As, Cd, Hg and Pb), which are related with an exogenous influence (Belitz and Grosch, 1999; Francesconi, 2007).

### Accuracy levels

Accuracy was checked with certified biological material (Table 4.2.1). The elemental concentrations obtained for canned matrix meat (SMRD-2000; Swedish Meats R&D and Scan Foods/National Food Administration, Sweden; FAAS), non defatted lobster hepatopancreas (LUTS-1; National Research Council of Canada; FAAS), oyster tissue (SRM 1566; United States National Bureau of Standards; EDXRF), freeze-dried animal blood (IAEA-A-13; International Atomic Energy Agency; EDXRF) and lobster hepatopancreas (TORT-2; National Research Council of Canada; FAAS) were compared with certified values. The detection limits (DL) of each element (Table 4.2.1) were determined by two means: (a) EDXRF - with the signal-to-noise approach, where the equipment compares the signal of each element with blank samples and established the minimum concentration at which the element is reliably detected; (b) FAAS - with the residual standard deviation (RSD) of the response and the slope (S) of the calibration curve of each standard solution used ( $DL = 3.3 \times RSD/S$ ). The FAAS technique requires large amount of sample, which was not possible in gonads and roe.

### Nutritional quality and potential risks for consumers

To evaluate the elemental nutritional quality and potential risks of clawed lobsters' consumption, intake of elements per 100 g serving portion was estimated for the edible tissues (wet weight) (similar to the procedures used by the Food and Agriculture Organization of the United Nations, by the World Health Organization of the United Nations and by the United States Food and Drug Administration/USFDA for food risk-benefit analysis). For each homarid species, the average intake (AI) of each element per 100 g edible serving portion was calculated using the edible contributions of each tissue: *H. americanus* (muscle: 84.1 %; hepatopancreas: 14.2 %; gonads: 1.7 %) and *H. gammarus* (muscle: 85.7 %; hepatopancreas: 12.3 %; gonads: 2.0 %). For each homarid species the following equation was used to calculate the average intake (AI) of each element per 100 g portion:  $AI/portion = (AIM \times ECM) + (AIH \times ECH) + (AIG \times ECG)$ , where M = muscle, H = hepatopancreas, G = gonads, EC = edible contribution. The edible contribution of roe was not considered as this tissue was only available in few animals. The values of average intake of the essential element Na, Mg, Cl, K, Ca, Mn, Fe, Cu, Zn and Se were compared with the daily dietary reference intake (DRI) of adult females and males aged between 19 and 50 years set by the USA Food and Nutrition Board of the Institute of Medicine (IOM, 1997; 2001 and 2004). The contaminants Cd, Hg and Pb were compared with the maximum allowed level (ML) set by the

European Commission (EC), whilst As was evaluated in terms of the action level (AL; similar meaning to the ML) set by the United States Food and Drug Administration (USFDA). For each element, the ratio between the average intakes per portion and the DRI, ML or AL was calculated.

**Table 4.2.1** Elemental concentration ( $\mu\text{g g}^{-1}$  DW;  $n = 4$ ) and detection limits of certified reference material ( $\pm$  sd) analysed by FAAS and EDXR.

Elements	Present work	Certified value	Detection limit
Na <sup>(a)</sup>	8346 $\pm$ 280	8533 $\pm$ 281	0.37
Mg <sup>(b)</sup>	91 $\pm$ 2	90 $\pm$ 4	0.05
Cl <sup>(c)</sup>	10200 $\pm$ 500	10000 <sup>(d)</sup>	100
S <sup>(c)</sup>	8200 $\pm$ 500	7600 <sup>(d)</sup>	100
K <sup>(c)</sup>	10000 $\pm$ 80	9690 $\pm$ 50	10
Ca <sup>(c)</sup>	1350 $\pm$ 50	1500 $\pm$ 200	20
Mn <sup>(b)</sup>	1.28 $\pm$ 0.03	1.20 $\pm$ 0.13	0.04
Fe <sup>(c)</sup>	210 $\pm$ 15	195 $\pm$ 34	3
Cu <sup>(c)</sup>	64 $\pm$ 4	63 $\pm$ 4	1
Zn <sup>(c)</sup>	830 $\pm$ 40	852 $\pm$ 14	1
As <sup>(c)</sup>	13 $\pm$ 1	13 $\pm$ 2	1
Se <sup>(c)</sup>	2.3 $\pm$ 0.5	2.1 $\pm$ 0.5	0.8
Br <sup>(e)</sup>	22 $\pm$ 2	22 $\pm$ 3	0.8
Sr <sup>(c)</sup>	9.9 $\pm$ 0.8	10 $\pm$ 1	0.8
Cd <sup>(f)</sup>	27 $\pm$ 0	26.7 $\pm$ 0.6	0.01
Hg <sup>(f)</sup>	0.28 $\pm$ 0.00	0.27 $\pm$ 0.06	0.02
Pb <sup>(f)</sup>	0.35 $\pm$ 0.06	0.35 $\pm$ 0.13	0.02

Superscripts: (a) canned matrix meat (SMRD-2000; FAAS); (b) non defatted lobster hepatopancreas (LUTS-1; FAAS); (c) oyster tissues (SRM 1566; EDXRF); (d) non-certified values provided by United States National Bureau of Standards; (e) freeze-dried animal blood (IAEA-A-13; EDXRF); (f) Lobster hepatopancreas (TORT-2; FAAS).

## Statistical analysis

All analyses were carried out in duplicate. Results were expressed as mean values  $\pm$  standard deviation (sd). Differences in the elemental concentration of edible tissues of *H. americanus* and *H. gammarus* were tested with analysis of variance (ANOVA) and Student's t-test. Whenever necessary, data were transformed to satisfy normal distribution and homoscedasticity requirements, followed by non-parametric analysis of variance (Kruskal-Wallis or Mann-Whitney tests), if transformed data could not meet these assumptions. All statistical analyses were tested at 0.05 level of probability with the software STATISTICA™ 6.1.

## Results and discussion

### Element composition in the edible tissues of each species

The mean elemental concentrations ( $\mu\text{g g}^{-1}$  wet weight) in the edible tissues of *H. americanus* and *H. gammarus* are listed in table 4.2.2.

**Table 4.2.2** Mean elemental composition ( $\pm$  sd) ( $\mu\text{g g}^{-1}$  wet weight) in the tissues of *H. americanus* and *H. gammarus*.

Elements	<i>H. americanus</i>			<i>H. gammarus</i>				<i>H. americanus</i> vs. <i>H. gammarus</i> (p-value)		
	Muscle	Hepat.	Gonads	Muscle	Hepat	Gonads	Roe	Muscle	Hepat	Gonads
<b>Macroelements</b>										
Na	3228 $\pm$ 329a	2842 $\pm$ 473b	1498 $\pm$ 7b	1962 $\pm$ 1090b	1608 $\pm$ 499b	1506 $\pm$ 32b	4006 $\pm$ 76a	0.004	0.008	0.326
Mg	252 $\pm$ 31a	158 $\pm$ 29b	112 $\pm$ 18b	222 $\pm$ 183ab	82 $\pm$ 48b	92 $\pm$ 26b	396 $\pm$ 209a	0.821	0.005	0.420
Cl	4419 $\pm$ 456a	4681 $\pm$ 305a	2316 $\pm$ 286b	4043 $\pm$ 695a	4756 $\pm$ 998a	2455 $\pm$ 478b	4803 $\pm$ 1496a	0.050	0.804	0.436
S	1716 $\pm$ 272a	1955 $\pm$ 300a	1320 $\pm$ 617a	1695 $\pm$ 251a	2029 $\pm$ 190a	2168 $\pm$ 301a	1696 $\pm$ 549a	0.808	0.476	0.003
K	2629 $\pm$ 185a	2694 $\pm$ 279a	2135 $\pm$ 589a	2613 $\pm$ 259a	2534 $\pm$ 494a	2314 $\pm$ 532a	644 $\pm$ 253b	0.821	0.339	0.474
Ca	473 $\pm$ 128a	293 $\pm$ 50b	173 $\pm$ 47c	456 $\pm$ 118a	335 $\pm$ 97b	173 $\pm$ 53c	446 $\pm$ 208ab	0.663	0.199	0.944
<b>Trace elements</b>										
Mn	0.61 $\pm$ 0.24b	2.7 $\pm$ 0.4a	2.1 $\pm$ 0.2a	0.40 $\pm$ 0.34b	1.9 $\pm$ 1.2a	2.2 $\pm$ 0.3a	0.80 $\pm$ 0.04ab	0.131	0.317	0.976
Fe	BDL	29 $\pm$ 4a	12 $\pm$ 8b	BDL	16 $\pm$ 3a	16 $\pm$ 5a	7.0 $\pm$ 3.4b	-	0.000	0.324
Cu	10 $\pm$ 2b	127 $\pm$ 87a	15 $\pm$ 10b	9.6 $\pm$ 5.2c	270 $\pm$ 141a	14 $\pm$ 7c	34 $\pm$ 4b	0.525	0.007	0.880
Zn	29 $\pm$ 4a	31 $\pm$ 6a	17 $\pm$ 5b	26 $\pm$ 3b	41 $\pm$ 11a	30 $\pm$ 8ab	35 $\pm$ 4a	0.004	0.009	0.000
Se	BDL	1.8 $\pm$ 0.6a	3.3 $\pm$ 2.0a	BDL	1.1 $\pm$ 0.3a	2.6 $\pm$ 1.8a	1.0 $\pm$ 0.2a	-	0.001	0.622
Br	23 $\pm$ 2b	48 $\pm$ 14a	25 $\pm$ 3b	22 $\pm$ 3c	74 $\pm$ 21b	26 $\pm$ 7c	114 $\pm$ 9a	0.411	0.002	0.888
Sr	34 $\pm$ 11a	14 $\pm$ 6b	12 $\pm$ 5b	35 $\pm$ 14a	20 $\pm$ 15ab	12 $\pm$ 8b	20 $\pm$ 4ab	0.784	0.151	0.833
<b>Contaminants</b>										
As	11 $\pm$ 3b	20 $\pm$ 5a	12 $\pm$ 4b	17 $\pm$ 3a	24 $\pm$ 5a	17 $\pm$ 3a	11 $\pm$ 1b	0.000	0.086	0.006
Cd	0.02 $\pm$ 0.00b	11 $\pm$ 5a	-	0.02 $\pm$ 0.01b	11 $\pm$ 9a	-	-	0.086	0.922	-
Hg	0.10 $\pm$ 0.02a	0.13 $\pm$ 0.03a	BDL	0.15 $\pm$ 0.04a	0.09 $\pm$ 0.02a	BDL	BDL	0.000	0.006	-
Pb	0.10 $\pm$ 0.00a	0.11 $\pm$ 0.03a	-	0.10 $\pm$ 0.00a	0.10 $\pm$ 0.06a	-	-	-	0.431	-

In each line, different letters indicate significant differences ( $p < 0.05$ ) between the edible tissues of each homarid species. Additionally, the elements' composition in each tissue were compared between both homarids; results are presented as p-values. Abbreviations: (BDL) below detection limit.

Quantification of Pb and Cd was not possible in the gonads due to the small tissue sample obtained. Sodium, Cl, S and K were the most abundant elements in all tissues. Generally, significant differences were found in most elements' content amongst tissues of each homarid species, except S and Pb, suggesting distinct physiological role of each tissue. Sulfur is present in amino acids (e.g. cysteine, methionine), polypeptides (e.g. glutathione), proteins and enzymes, which might explain its ubiquity at similar concentrations in all tissues (Walker *et al.*, 2001). Similarly, the identical content of Pb in all homarids' tissues is likely explained by the fact that Pb is a toxic contaminant related with an environmental input and without any physiological function (Al-Mohanna and Subrahmanyam, 2001).

The muscle had significantly higher concentrations of Na, Mg, Ca and Sr. Since this tissue is mostly involved in the mechanical movements of appendages and claws, it is likely these elements are vital for neuromuscular functions. In contrast, Fe and Se were below the detection limit (BDL) in this tissue and Cd was close to BDL.

The hepatopancreas had statistically higher concentration of Fe, Cu, Br and Cd. The hepatopancreas is a multifunctional vital organ responsible for the regulation of essential mono and divalent ions and for detoxification of contaminants (Chavez-Crooker *et al.*, 2003). Earlier studies reported high contents of Cu in the hepatopancreas of other crustaceans, such as blue crab, swim crab, Chinese mitten crab and shrimps (Gökoölu and Yerlikaya, 2003; Chen *et al.*, 2006; Kádár *et al.*, 2006). Copper levels can reflect the active accumulation of this element by homarids for incorporation into the respiratory pigment haemocyanin found in crustacean's haemolymph (Lee and Shiao, 2002). Previous studies with *H. americanus* reported elevated levels of contaminants, namely Cd, in the hepatopancreas (Chou *et al.*, 2000; Engel *et al.*, 2001). The high concentration of Cd found in the hepatopancreas likely reflects the detoxifying role of this organ, particularly in the isolation of dietary contaminants and in complexing them with organic solutes, such as metallothionein, glutathione or inorganic anions (Chavez-Crooker *et al.*, 2003). In this way, the hepatopancreas reduces the load of contaminants that reach the haemolymph, tissues and organs. This role is also evidenced by the lower levels of Cd obtained in the remaining tissues.

Gonads are the reproductive organs where high nutritional investments are made to produce the reproductive cells. Selenium was detected at high concentrations in this organ, likely due to the important role of this element in the modulation of growth and development, and in oxidative stress defence (FAO/WHO, 2002). In contrast, low contents of Cl, Ca, Zn and Hg were found in gonads. The roe had significantly higher Na and Br contents and lower contents of K and Hg.

### **Differences in elements composition between species**

No statistical differences between *H. americanus* and *H. gammarus* were found in the content of Cl, K, Ca, Mn, and Sr in all edible tissues (Table 4.2.2). Such result likely reflects similar vital biochemical mechanisms in both homarids regulating these elements (Skinner *et al.*, 2004). *H. americanus* had significantly higher Na concentrations in the muscle and higher Na, Mg, Fe and Se

content in the hepatopancreas. In contrast, *H. gammarus* had statistically higher Cu and Br values in the hepatopancreas and higher S and Zn levels in gonads.

These differences might reflect distinct elemental requirements for the metabolism of each species or to different elemental availability in the aquatic environment, as the species are captured in different areas of the Atlantic Ocean. Worldwide, Se availability in sediments and live organisms show great variability due to distinct environmental conditions and agricultural practices (FAO/WHO, 2002). USA and Canada are seleniferous areas, whereas low Se concentrations are found in European countries (Popilac and Prpic-Majic, 2002). According to FAO/WHO, (2002), Se content in marine fish captured in USA is usually higher than the average international values. This fact may clarify the higher contents of Se found in the hepatopancreas of *H. americanus*.

Among contaminants, variations between both homarids were only found in the concentration of As and Hg: *H. gammarus* had statistically higher As and Hg content in muscle and lower Hg level in hepatopancreas than *H. americanus*. Such differences may indicate the degree of pollution in sediments and seawater of both regions of the Atlantic Ocean (Sadiq, 1992). Arsenic and Hg enter in coastal waters by sewage and other wastewater effluents, power plant cooling water discharges, vehicle emissions, petroleum and petrochemical industrial wastes, storm drain outfalls and solid waste landfills (Al-Mohanna and Subrahmanyam, 2001). Contaminants cannot be regulated by crustaceans and, therefore, bioaccumulation of these elements occurs in specimens' edible tissues (Al-Mohanna and Subrahmanyam, 2001).

### **Nutritional quality and potential risks for consumers**

Clawed lobsters are important diet sources in coastal areas of Mediterranean European countries. Therefore, intake of trace elements from *H. americanus* and *H. gammarus*, especially toxic elements, is of great concern for human health. The results obtained in table 4.2.3 indicate that both species are good sources of Na, Cl, Cu, Zn and Se, being *H. americanus* a better source of Na and Se than *H. gammarus*. Sodium plays an important role in many physiological processes: signal transduction in the human central nervous system, osmoregulation, arterial pressure maintenance and optimal salt and water balance in the body (Walker *et al.*, 2001). Zinc is an essential component of several enzymes, participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids (FAO/WHO, 2002). Additionally, this element has also an essential role in the process of gene expression, particularly in polynucleotide transcription (FAO/WHO, 2002). Fish, fruit and green leaf vegetables are considered poor sources of Zn (<10 mg kg<sup>-1</sup>), whilst lean meat, cereals and legumes are rich Zn sources (25-50 mg kg<sup>-1</sup>, compared to 30 mg kg<sup>-1</sup> in both clawed lobsters; FAO/WHO, 2002).

In both lobsters' species Cu and Se concentrations were above the DRI. Nevertheless, the tolerable upper limit (UL) set by the USDA for Cu (10 mg day<sup>-1</sup>) is well above the results found in the present study (2.7-4.0 mg 100 g<sup>-1</sup> portion). Copper is found in several enzymes, including the cytochrome c oxidase and the superoxide dismutase, and is used for biological electron transport (Walker *et al.*, 2001).

**Table 4.2.3** Average content of each element in 100 g edible serving portion (considering the contribution of all tissues) for each homarid species, and ratio between the average intake of each element per portion and the recommended daily dietary reference intakes.

Elements	DRI/ML/AL	<i>H. americanus</i>		<i>H. gamarus</i>	
		AI/portion	%	AI/portion	%
<b>Macroelements</b>					
Na	1500 mg	314	21	213	14
Mg*	(420-320) mg	24	(5.7-7.5)	22	(5.4-7.0)
Cl	2300 mg	442	19	417	18
S	NS	174	-	173	-
K	4700 mg	263	5.6	240	5.1
Ca	1000 mg	44	4.4	44	4.4
<b>Trace elements</b>					
Mn	(2.3-1.8) mg	0.09	(4.1-5.2)	0.06	(2.7-3.8)
Fe	(8-18) mg	0.63	(7.8-3.5)	0.42	(5.2-2.3)
Cu	900 µg	2706	301	4048	450
Zn	(11-8) mg	2.9	(27-37)	2.9	(26-36)
Se	55 µg	103	187	70	128
Br	NS	2.6	-	3.7	-
Sr	NS	3.1	-	3.2	-
<b>Contaminants</b>					
As	7.6 mg	1.2	16	1.7	23
Cd	50 µg	156	321	119	237
Hg	50 µg	11	22	2.8	5.5
Pb	50 µg	10	20	8.8	18

Daily recommended intake values (DRI) correspond to Na, Mg, Cl, K, Ca, Mn, Fe, Cu, Zn and Se; maximum level (ML) refers to Cd, Hg and Pb and, the action level refers to As. Values in brackets for Mg, Mn, Fe and Zn refer to differences in gender: the first and second values correspond to adult male and female aged between 19 and 50 years, respectively; Abbreviations: (AI) average intake; (NS) value not set; (\*) data refer to female and male adults aged between 31 and 50 years.

Despite the UL value for Se has not been set, it is important to stress that a normal adult can ingest up to 216 µg Se per day without any toxicological effect (FAO/WHO, 2002), which is higher than values found in the edible tissues of both clawed lobsters (55-70 µg 100 g<sup>-1</sup> portion). Several authors reported the Se protective action, especially selenite, (Se<sub>2</sub>O<sub>2</sub><sup>-3</sup>), on many toxicological effects of Cd and Hg (Gasiewicz and Smith, 1978; Early *et al.*, 1992; Skowerski *et al.*, 1997a, b). Selenium is also implicated in the protection of body tissues against oxidative stress, maintenance of defense against infection and modulation of growth and development (FAO/WHO, 2002).

Considering contaminants (As, Cd, Hg and Pb), only Cd content in both homarids was well above the ML/AL per 100 g portion (Table 4.2.3). The concentration obtained for As, Hg and Pb was in the range of the typical concentrations found in crustaceans muscle: 5-98 mg 100 g<sup>-1</sup> (As), 2-20 µg 100 g<sup>-1</sup> (Hg) and 2-10 µg 100 g<sup>-1</sup> (Pb; Francesconi, 2007).

In the present study, the main contribution to the high Cd concentration in both species was the hepatopancreas, whilst the Cd concentration in the muscle and gonads was below the ML (Table 4.2.2). Cadmium is toxic to many organisms since the ion form combines with sulphhydryl (thiol) groups of many enzymes preventing their normal function (Walker *et al.*, 2001). Yet, Cd absorption by humans is influenced by nutritional factors (*e.g.* Fe levels; Francesconi, 2007), and many toxicological effects of Cd are attenuated in the presence of Se (Gasiewicz and Smith, 1978; Early *et al.*, 1992; Skowerski *et al.*, 1997a, b). Since both homarids have high Fe and Se concentrations in the hepatopancreas (Table 4.2.2), the toxicological effects of Cd may be reduced. Moreover, despite *H. gammarus* and *H. americanus* are widely consumed in coastal areas of Mediterranean countries, the annual per capita consumption of these food items is relatively low (109 g in Spain; 64 g in France; 64 g in Italy; 23 g in Greece; 10 g in Portugal; data from 2005; EUROSTAT, 2007). Therefore, the risk of Cd contamination to homarids consumers is low.

## Conclusions

In general, Na, Cl, S and K were the most abundant elements in all tissues, evidencing their essential character to *H. americanus* and *H. gammarus*. Differences found in the elemental composition amongst tissues of the same species likely reflect distinct physiological roles of the tissues. Variations detected between both homarid species likely reflect distinct availability of elements in the aquatic environment, as the animals are distributed in different regions of the Atlantic Ocean. Both species can also require distinct elements to their metabolism.

Considering the daily intake recommendations, both homarids are good sources of Na, Cl, Cu, Zn, and Se. As to contaminants, only Cd was above the action level in the hepatopancreas of both homarids. Despite the low risk of Cd intake, consumers should avoid or moderately consume this tissue in both species. Future studies should evaluate the mineral composition of other crustacean species with relevance for human consumption.

