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Influence of Season and Sex on the Contents of Minerals and Trace Elements in Brown Crab (*Cancer pagurus*, Linnaeus, 1758)

SARA BARRENTO,[†] ANTÓNIO MARQUES,*[,†] BÁRBARA TEIXEIRA,[†] MARIA LUÍSA CARVALHO,[‡] PAULO VAZ-PIRES,[§,||] AND MARIA LEONOR NUNES[†]

Research Unit of Upgrading of Fishery and Aquaculture Products (U-VPPA), National Institute of Biological Resources (INRB-IPIMAR), Avenida de Brasília, 1449-006 Lisboa, Portugal, Centre of Atomic Physics, Faculty of Sciences, University of Lisboa, Avenida Professor Gama Pinto 2, 1649-003 Lisboa, Portugal, Institute of Biomedical Sciences Abel Salazar (ICBAS-UP), University of Porto, Largo Professor Abel Salazar 2, 4099-003 Porto, Portugal, and Centre of Marine and Environmental Research, University of Porto (CIIMAR-UP), Rua dos Bragas 289, 4050-123 Porto, Portugal

Cancer pagurus is much appreciated in Southern Europe, where the muscle, hepatopancreas, and gonads are consumed regularly with peaks in summer and December. The elemental contents of *C. pagurus* edible tissues were analyzed in this study during the four seasons. Results indicate that the content varied with tissue, season, and sex. The hepatopancreas had more S, Cl, Ca, Br, Sr, Fe, Cu, Cd, and Pb, the gonads had a higher concentration of Na, and the muscle was richer in Zn. Autumn and winter corresponded to a high Mg, S, Cl, K, Ca, Fe, and Zn content in both the muscle and hepatopancreas. Female gonads had more Fe, Zn, As, and Se than males but less Ca, Cl, Br, and Sr. Regarding toxic elements for human consumption, the levels of As, Hg, and Pb found in all edible tissues pose minimal risks to consumers. However, Cd concentration in the hepatopancreas was always above the action limit. Therefore, we recommend moderate hepatopancreas consumption.

KEYWORDS: *Cancer pagurus*; muscle; hepatopancreas; gonads; toxic and essential elements; FAAS; EDXRF; risk assessment

INTRODUCTION

In the last decades, much attention has been paid to the study of macro and trace element content in foodstuffs, as a result of a growing concern about health benefits and risk of food consumption. Health benefits are related to the essential elements content in food (e.g., Ca, Fe, and Se), which by definition are required for the maintenance of normal physiological functions. In opposition, risk assessment of inorganic elements has examined two ends of the toxicity spectrum: (a) those related with intakes that are too high and the resulting toxicity and (b) those associated with intakes that are too low and resulting in nutritional deficiencies (*1*). Food safety authorities around the world, namely, the U.S. Environmental Protection Agency (EPA), the European Food Safety Authority (EFSA), and the World Health Organization (WHO), have set limits for several chemicals, including essential and toxic elements. In particular, the evaluation of risks and benefits of seafood consumption has been controversial, and several studies have focused on the characterization of macro and trace element content of fish, molluscs, and crustaceans (*1*−*5*). Crustaceans are known to be an important supply of essential elements, including antioxidants such as Se and Zn, but also contaminants such as Cd (*5*). However, most wild marine animals have in general great variability in elemental content, mainly due to differences between populations and environments to which they are exposed (*6*). The elemental accumulation in aquatic animals is affected by endogenous (e.g., sex, age, condition, moulting, and tissue) and exogenous factors (e.g., elemental bioavailability in seawater and diet) (*7*). The exogenous factors are influenced by environmental changes (e.g., season, location, substrate, depth, salinity, temperature, and anthropogenic sources) (*7*). The edible crab *Cancer pagurus* is subjected to a large number of environmental variables following their annual and daily cycles (e.g., migration and habitats) that influence behavior, feeding, metabolism, and ultimately the elemental composition. This species is much appreciated in Southern European countries, being imported mostly from Britain, Ireland, Norway,

* To whom correspondence should be addressed. Tel: +351 21 3027025. Fax: +351 21 3015948. E-mail: amarques@ipimar.pt. URL: http://ipimar-iniap.ipimar.pt/departamentos/inovacao-tecnologica.html.

[†] National Institute of Biological Resources (INRB-IPIMAR).

[‡] University of Lisboa.

[§] nstitute of Biomedical Sciences Abel Salazar (ICBAS-UP), University of Porto.

[||] Centre of Marine and Environmental Research, University of Porto (CIIMAR-UP).

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Sweden, and France (*8*). In Spain, France, Portugal, and Italy, *C. pagurus* white meat (muscle) and brown meat (hepatopancreas and gonads) are consumed separately or as a mixture all year round, but with peak consumption usually occurring during summer holidays and Christmas festivities (*9*). In addition, larger males are usually more expensive than smaller males and females due to the larger size of claws and consequent meat yield (*10*). Considering consumers' habits and the potential variability in the elemental content of crabs, it is important to characterize and understand these variations in order to establish the benefits and risks of *C. pagurus* to human consumption. Therefore, the aims of this study were (a) to quantify the Na, Mg, S, Cl, K, Ca, Mn, Fe, Cu, Zn, As, Se, Br, Sr, Cd, Hg, and Pb content in the edible tissues of female and male *C. pagurus* during the four annual seasons and (b) compare the concentrations of essential elements with the recommended intake values as well as with the limits set by authorities for contaminants.

MATERIALS AND METHODS

Ethical Statement. All live animals utilized in the experiments have been treated with proper care, minimizing discomfort and distress, and were painlessly killed. Also, the number of animals was kept to the minimum necessary to obtain scientific results, considering that the gain in knowledge and long-term benefit to the subject species is high.

Biological Material. *Cancer pagurus* harvested in the Scottish coast were sampled during spring (April), summer (August), and autumn (November) of 2007, and winter (February) of 2008. Every season, 20 intermolt crabs (10 females and 10 males) were purchased from a local importer and transported live to the laboratory. Animals were kept under refrigerated conditions (5 °C) during 1 h to decrease their metabolism and to stun them before being euthanized by piercing the two nerve centers by means of a stainless steel rod. The rod was inserted through one of the eyes and through the vent. Precautionary measures to prevent contamination during collection, dissection, and analyses were taken. Muscle from the claws, hepatopancreas, and gonads of every animal were individually separated by using sterilized stainless steel scalpels and forceps; disposable plastic containers were used for collection and plastic tubes for preservation and analytical purposes. Samples were pooled only when there was insufficient amount of tissue to perform all analyses (e.g., male gonads). All tissues were weighted and individually homogenized with a grinder (Retasch Grindomix GM200; 5000 rpm; material, PP cup and stainless steel knifes), vacuum packed, and stored at −20 °C. A portion of each frozen sample was 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; EXTRA II A, Atomika Instruments, Temple, Arizona, USA) was used to quantify the elements S, Cl, 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 molybdenum. The characteristic radiations emitted by the elements in the sample were detected by a Si(Li) detector, with a 30 mm^2 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 with the fundamental parameters method (*11*). The X-ray generator was operated at 50 kV, 20 mA, and an acquisition time of 1000 s. Each sample powder (1 g) was pressed into cylindrical 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. Flame atomic-absorption spectrometry (FAAS), through the spectrometer Varian (Australia) Spectr AA 20 with deuterium background correction (Varian), was employed to quantify Na, Mg, Mn, Cd, and Pb (*12*). Each sample (5 g wet weight to quantify Na, Mg, and Mn, and 10 g wet weight to quantify Cd and Pb) were dry-ashed at 450 °C under a gradual temperature increase (50 °C per hour). Ash was dissolved in concentrated nitric acid, and the solution obtained was evaporated to dryness. The final residue was dissolved with 12 or 5 mL of 15% nitric acid (v/v) and transferred to 25 or 10 mL volumetric flasks (10 mL for Pb and Cd and 25 mL for the other elements); final volumes were adjusted with Ultrapure water. A minimum of three replicate analyses were performed per sample. Concentrations were calculated from linear calibration plots obtained by measurement of standard solutions absorbance: $NaNO_3$ (Merck) dissolved in HNO_3 (0.5 M); Mg $(NO_3)_2$ (Merck) dissolved in HNO_3 (0.5 M); and Mn $(NO_3)_2$ (Merck) dissolved in HNO_3 (0.5 M), $Cd(NO3)_2$ (Merck) dissolved in HNO_3 (0.5 M), and $Pb(NO_3)_2$ (Merck) dissolved in HNO_3 (0.5 M). All glassware was cleaned with HNO_3 (10%) or HCl (20%) for 24−48 h and rinsed with Ultrapure water (18.2MX cm) to avoid contamination. Chemical reagents were pro analysis or superior. Total Hg was measured in triplicate with an AMA 254 Mercury Analyzer spectrometer that uses the mercury vapor generation technique. The procedure is based on dry sample decomposition (10 mg) by combustion, preconcentration of mercury by amalgamation with gold, and atomic absorption spectrometry. Concentrations were calculated from linear calibration plots obtained by measurement of the absorbance of an Hg standard solution (Hg diluted in HNO_3; 0.5 M) supplied by Merck.

Accuracy Tests. Accuracy was checked by analyses of certified biological reference material. The elemental concentrations obtained for canned matrix meat (SMRD-2000; Swedish Meats R & D and Scan Foods/National Food Administration, Sweden), nondefatted lobster hepatopancreas (LUTS-1; National Research Council of Canada), oyster tissue (SRM 1566; National Bureau of Standards), freeze-dried animal blood (IAEA-A-13; International Atomic Energy Agency), and lobster hepatopancreas (TORT-2; National Research Council of Canada) were compared with certified values. The detection limits (DL) of each element (**Table 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 establishes the minimum concentration at which the element is reliably detected and (b) FAAS with the residual standard deviation (RSD) of the response and the slope (S) of the calibration curve of each standard solution used ($DL = 3.3 \times RSD \div S$).

Nutritional Quality and Potential Hazards to Consumers. To evaluate the elemental nutritional quality and potential consumption hazards of *C. pagurus* during spring, summer, autumn, and winter, the concentration of elements per 100 g serving portion were calculated in the edible tissues and compared with the recommended intake and limits set by international authorities. The concentrations of Na, K, Ca, and Mn were compared with the daily adequate intakes (AI); Mg, Cl, Fe, Cu, Zn, and Se were balanced considering the recommended dietary allowances (RDA); Na, Ca, Cu, and Se were also compared with the daily tolerable upper intake levels (UL). AI, RDA, and UL were set by the U.S. Food and Nutrition Board of the Institute of Medicine for individual adults aged between 19 and 50 years old (*13*). The contaminants Cd, Hg, and Pb were compared with the maximum permissible concentrations (MPC) set by the European Commission, while As was compared with the action level (AL) set by the United States Food and Drug Administration. Since Cd and Hg MPC are only set for crustacean muscle, the levels of these contaminants were also compared with the AL set for the food commodity named Crustacea. Recommendations regarding As, S, Br, and Sr have not been set so far by food authorities, and therefore, these elements were not considered in the evaluation.

Statistical Analysis. Main difference among the tissues, sexes, and seasons were tested with analysis of variance (ANOVA). Whenever necessary, data were transformed to satisfy normal distribution and homoscedasticity requirements, followed by nonparametric analysis of variance (Kruskall-Wallis), if transformed data could not meet these assumptions. Student's *t*-test and the equivalent nonparametric analysis' Mann−Whitney were also applied as appropriate. Principal component analysis (PCA) was also employed to reduce the multidimensional data sets of several elements to lower dimensions, thus simplifying the presentation and interpretation of data. All statistical analyses were tested at the 0.05 level of probability with the software STATISTICATM 6.1. (Statsoft, Inc., Tulsa, OK 74104, USA).

Table 1. Elemental Concentration ($\mu g \cdot g^{-1}$ DW; $n = 4$) and Detection Limits ($\mu g \cdot g^{-1}$ D.L.) of Certified Reference Material (± Standard Deviation) Analyzed by FAAS and EDXRF[a]

elements	technique	D.L.	certified reference material	certified value	present work
Na	FAAS	0.37	canned matrix meat (SMRD-2000)	8533 ± 281	8346 ± 280
Mg	FAAS	0.05	nondefatted lobster hepatopancreas (LUTS-1)	90 ± 4	91 ± 2
S	EDXRF	100	oyster tissue (SRM 1566)	7600*	8200 ± 500
Cl	EDXRF	100	oyster tissues (SRM 1566)	10000*	10200 ± 500
K	EDXRF	50	Oyster tissues (SRM 1566)	9690 ± 50	10000 ± 80
Ca	EDXRF	20	oyster tissues (SRM 1566)	1500 ± 200	1350 ± 50
Mn	FAAS	0.04	nondefatted lobster hepatopancreas (LUTS-1)	1.20 ± 0.13	1.28 ± 0.03
Fe	EDXRF	3.1	oyster tissues (SRM 1566)	195 ± 34	210 ± 15
Cu	EDXRF	0.7	oyster tissues (SRM 1566)	63 ± 4	64 ± 4
Zn	EDXRF	1.1	oyster tissue (SRM 1566)	852 ± 14	830 ± 40
As	EDXRF	0.7	oyster tissue (SRM 1566)	13 ± 2	13 ± 1
Se	EDXRF	0.5	oyster tissue (SRM 1566)	2.1 ± 0.5	2.3 ± 0.5
Br	EDXRF	0.8	freeze-dried animal blood (IAEA-A-13)	22 ± 3	22 ± 2
Sr	EDXRF	0.5	oyster tissue (SRM 1566)	10 ± 1	9.9 ± 0.8
Cd	FAAS	0.01	lobster hepatopancreas (TORT-2)	27 ± 1	27 ± 0
Hg	FAAS	0.02	lobster hepatopancreas (TORT-2)	0.27 ± 0.06	0.28 ± 0.00
Pb	FAAS	0.02	lobster hepatopancreas (TORT-2)	0.35 ± 0.13	0.35 ± 0.06

[a] Non-certified values were provided by the United States National Bureau of Standards.

Table 2. *Cancer pagurus* Biometric Data (Average ± Standard Deviation): Carapace Width (CW), Carapace Length (CL), Total Body Weight (BW), Muscle, Hepatopancreas, and Gonad Wet Weight of Female and Male Crabs Sampled during Spring, Summer, Autumn, and Winter

season	sex	CW (mm)	CL (mm)	BW (g)	muscle (g)	hepato (g)	gonads (g)
spring	F	163.9 ± 4.1	108.4 ± 0.4	770 ± 33	62.8 ± 5.7	85.3 ± 19.9	18.5 ± 7.7
	M	160.9 ± 4.7	100.2 ± 2.1	828 ± 44	99.1 ± 10.0	104.0 ± 19.3	9.0 ± 4.8
summer	F	163.3 ± 2.3	107.4 ± 1.8	748 ± 25	63.6 ± 7.8	77.5 ± 12.6	27.0 ± 14.8
	M	156.3 ± 3.0	98.4 ± 1.9	751 ± 27	89.2 ± 10.0	76.8 ± 16.3	10.4 ± 5.3
autumn	F	167.5 ± 18.2	107.7 ± 7.7	770 ± 153	60.2 ± 17.8	91.5 ± 21.5	54.0 ± 14.7
	M	167.7 ± 18.0	103.6 ± 8.5	869 ± 230	121.5 ± 42.1	88.3 ± 23.5	16.7 ± 8.5
winter	F	159.8 ± 7.0	99.4 ± 3.7	694 ± 59	55.2 ± 9.0	78.1 ± 14.8	12.6 ± 4.3
	M	153.7 ± 6.3	93.4 ± 3.4	650 ± 75	66.9 ± 14.5	85.9 ± 15.1	5.6 ± 1.7

RESULTS AND DISCUSSION

Influence of Tissue, Sex, and Season in Elemental Composition. This study evaluated the content of elements in the edible tissues of *C. pagurus* considering the effect of tissue and sex (endogenous factors), and season (exogenous factor). Biometric data of all specimens analyzed are shown in **Table 2**.

Statistical differences in the concentration of elements were found between the edible tissues: overall, the hepatopancreas had more S, Cl, Ca, Br, Sr, Fe, Cu, Cd, and Pb ($p < 0.01$), the gonads had higher concentration of Na ($p < 0.01$), while the muscle was richer in Zn ($p < 0.01$). No statistical differences were found between tissues in the concentration of K and Sr (**Table 3**). PCA analysis considering sex, season, and all elements was applied to differentiate tissues; factors one, two, and three yielded a total of 65% of explainable results (**Figure 1A**). Clear cluster separation is evidenced by the muscle, while the hepatopancreas and gonads overlap. Most elements loaded heavily on factor one, except for Na, K, As, and Se, which loaded on factor three, while Hg and Pb loaded on factor two, which is in agreement with the differences found with ANOVA (**Table 3**). The accumulation pattern of Fe, Cu, and Cd in the hepatopancreas is similar to that of other species of crabs, namely, *Portunus pelagicus*, *Pseudocarcinus gigas*, and *Eriocheir sinensis* (*14*−*17*). The hepatopancreas is a multifunctional organ that among several functions acts as a temporary reservoir for minerals, regulating physiologically important cations and detoxifying dietary contaminants such as Cd (*18*, *19*). Calcium and Cu are extremely important to crustaceans' homeostasis. Calcium takes part in the biomineralization process of the rigid exoskeleton by precipitation of calcium carbonate (*20*), while Cu is incorporated into the respiratory pigment hemocyanin that is responsible for gas transport in the hemolymph (*21*). The importance of other elements, such as K, Zn, Fe, Sr, Br, and Se, to the physiology of crabs is not well studied. Clearly, the pattern of elemental accumulation in tissues is complex, and it is difficult to arrive at generalizations that universally apply.

Table 3. Statistical Differences between Tissues Considering a One Way ANOVA or Kruskall Wallis Test with the Respective *p* Values[a]

	tissue	*p*
Na	gonads	<0.01
Mg	hepatopancreas/gonads	<0.01
S	hepatopancreas	<0.01
Cl	hepatopancreas	<0.01
K	ne	0.814
Ca	hepatopancreas	<0.01
Zn	muscle	<0.01
Br	hepatopancreas	<0.01
Sr	hepatopancreas	<0.01
As	ne	0.13
Mn	hepatopancreas/gonads	<0.01
Fe	hepatopancreas	<0.01
Cu	hepatopancreas	<0.01
Se	hepatopancreas/gonads	<0.01
Cd	hepatopancreas	<0.01
Hg	muscle/hepatopancreas	<0.01
Pb	hepatopancreas	<0.01

[a] Each element was tested independently of the season and sex.

Generally, season and sex affected the concentration of most elements in the gonads (**Figure 1B**). In this PCA, a clear cluster separation is shown between sexes and seasons, with factors one, two, and three yielding 79% of explainable results, with Mg, Cl, K, Ca, Zn, Br, Sr, Fe, and Se loading heavily on factor 1, Na, As, Cd, Hg, and Pb loading on factor two, and S, Mn, and Cu on factor three. The concentration of Br and Sr (spring), Cl and K (summer), and Mg and Ca (winter) in male gonads

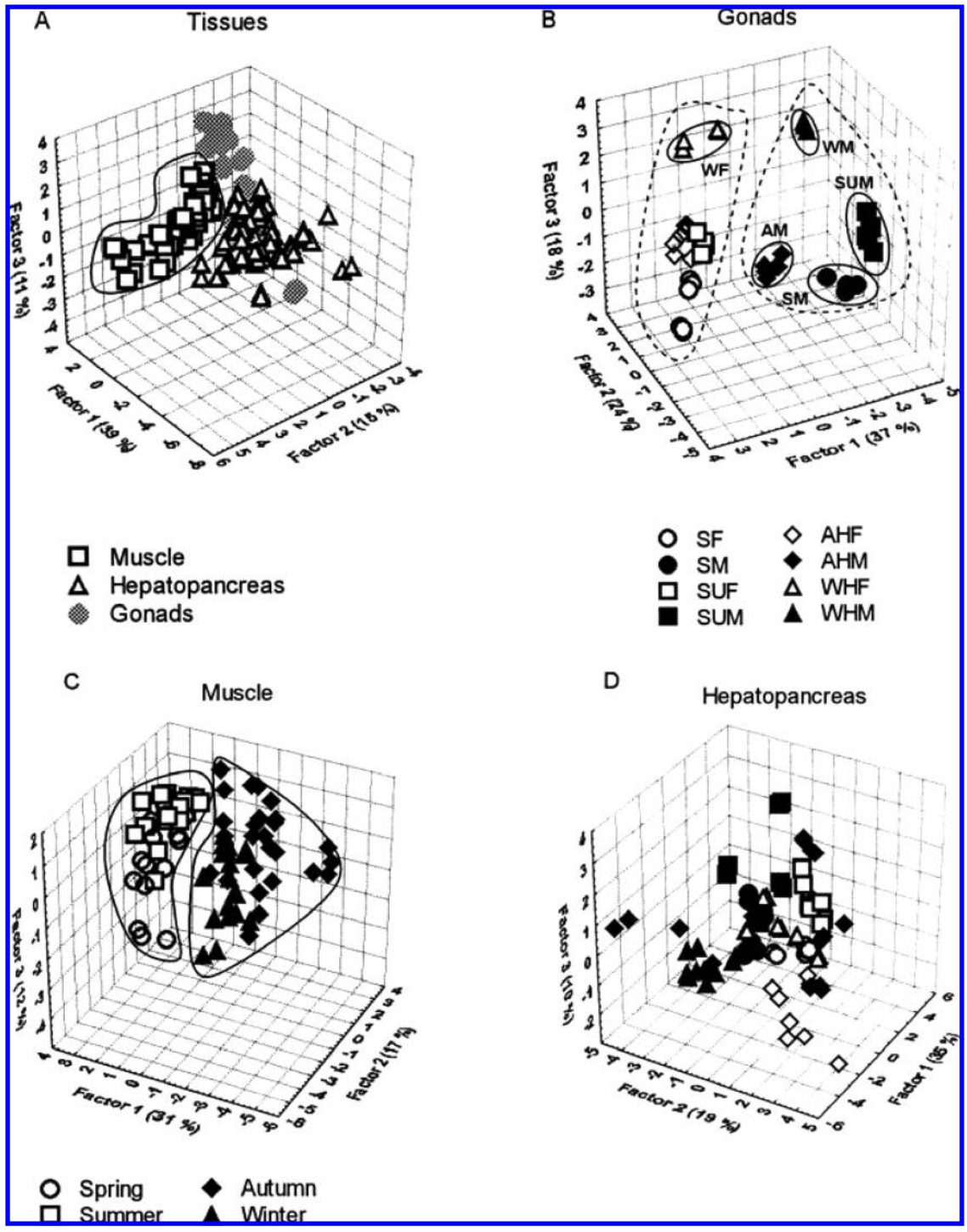

Figure 1. Result of four PCA analyses considering the concentration of all elements in (**A**) muscle, hepatopancreas, and gonads; (**B**) both sexes and four seasons in gonads; (**C**) four seasons in the muscle; and (**D**) both sexes and four seasons in the hepatopancreas. Abbreviations: SF, spring females; SM, spring males; SUF, summer females; SUM, summer males; AF, autumn females; AM, autumn males; WF, winter females; WM, winter males.

was statistically higher than that in females (**Tables 4** and **5**). In contrast, females had statistically higher Fe, Zn, As, and Se levels than males during all seasons (**Tables 4** and **5**). Earlier studies with the semaphore crab also reported higher Zn accumulation in female gonads compared to that in males (*22*).

The concentration of elements in the muscle did not vary significantly and was mostly affected by seasons: Mg, S, Cl, K, Ca, and Hg were more concentrated in autumn and winter (**Tables 4** and **5**). These results are also evidenced in the PCA analysis, where separation between summer and spring is clear from the autumn and winter cluster (**Figure 1C**). The three factors yielded 60% of explainable results, in which K, Zn, Mn, Fe, Hg, and Pb loaded on factor 1, Sr, As, and Se on factor 2, and Ca, Br, Cu, and Cd on factor 3. In contrast, the hepatopancreas was affected either by sex and season: higher concentrations of Cu (autumn, females), Zn (autumn and winter, both sexes), As (spring, females), Ca (winter, males), Mg, and Sr (winter, males) (**Tables 4** and **5**). The PCA analysis for the hepatopancreas considering all elements, sex, and season yielded 64% of explainable results and did not evidence any clear cluster separation between season and sex, which is in accordance with the results obtained (**Figure 1D**). Magne-

Table 4. Mean Macro Elemental Composition (mg per 100 g^{-1} Wet Weight; ± Standard Deviation) in Female and Male *C. pagurus* Edible Tissues during the Four Seasons[a]

tissue	season	sex	Na AI = 1500 UL = 2300	Mg RDA = 400	S	Cl RDA = 2300	K RDA = 4700	Ca RDA = 1000 UL = 2500
muscle	spring	F	351 ± 46 a	36 ± 4 ab	117 ± 20 b	243 ± 25 b	164 ± 26 b	48 ± 16 a
		M	241 ± 54 a	25 ± 7 b	162 ± 38 ab	244 ± 30 b	172 ± 24 b	47 ± 11 a
	summer	F	275 ± 54 a	30 ± 7 b	107 ± 31 b	283 ± 40 b	165 ± 19 b	20 ± 6 b
		M	278 ± 98 a	21 ± 10 b	135 ± 63 b	244 ± 33 b	179 ± 21 b	24 ± 5 b
	autumn	F	301 ± 54 a	39 ± 5 a	247 ± 51 a	430 ± 48 a	305 ± 25 a	38 ± 1 a
		M	354 ± 56 a	39 ± 4 a	251 ± 45 a	465 ± 96 a	286 ± 30 a	35 ± 4 a
	winter	F	306 ± 78 a	31 ± 6 b	240 ± 21 a	515 ± 56 a	294 ± 21 a	38 ± 8 a
		M	272 ± 11 a	29 ± 1 b	224 ± 61 a	508 ± 61 a	303 ± 12 a	35 ± 3 a
hepato	spring	F	223 ± 75 a	30 ± 2 c	164 ± 34 b	417 ± 64 c	162 ± 8 b	285 ± 58 c
		M	224 ± 67 a	50 ± 10 b	181 ± 45 b	326 ± 68 c	167 ± 12 b	631 ± 157 b
	summer	F	307 ± 79 a	33 ± 7 bc	206 ± 68 b	567 ± 64 b	185 ± 10 b	260 ± 79 c
		M	334 ± 118 a	42 ± 17 bc	234 ± 37 b	469 ± 64 b	201 ± 10 b	943 ± 79 b
	autumn	F	253 ± 27 a	53 ± 5 b	387 ± 81 a	600 ± 55 b	287 ± 36 a	642 ± 165 b
		M	279 ± 68 a	57 ± 23 ab	340 ± 81 a	668 ± 83 b	281 ± 48 a	919 ± 717 abc
	winter	F	281 ± 77 a	51 ± 7 b	345 ± 49 a	780 ± 34 a	288 ± 23 a	781 ± 214 b
		M	228 ± 53 a	79 ± 5 a	392 ± 92 a	664 ± 54 b	318 ± 25 a	1926 ± 368 a
gonads	spring	F	309 ± 155 c	7.2 ± 0.6 c	126 ± 51 b	154 ± 67 d	108 ± 49 d	8.5 ± 3.4 e
		M	110 ± 2 d	56 ± 1 b	152 ± 128 ab	673 ± 19 bcd	377 ± 34 b	59 ± 6 b
	summer	F	220 ± 22 c	17 ± 2 c	306 ± 61 a	380 ± 11 c	187 ± 7 d	20 ± 1 d
		M	281 ± 1 c	12 ± 1 d	291 ± 37 a	1114 ± 39 a	488 ± 18 a	57 ± 3 b
	autumn	F	167 ± 56 cd	23 ± 4 c	366 ± 69 a	333 ± 72 c	179 ± 16 d	21 ± 2 d
		M	502 ± 118 b	26 ± 4 c	161 ± 35 b	601 ± 60 b	293 ± 32 bc	33 ± 4 c
	winter	F	754 ± 246 b	65 ± 14 b	293 ± 61 ab	490 ± 53 b	274 ± 12 c	21 ± 1 d
		M	1879 ± 7 a	90 ± 1 a	210 ± 16 b	673 ± 19 b	348 ± 2 b	85 ± 2 a

[a] In each column, different letters indicate significant differences in the elemental concentration per tissue ($p < 0.05$). Abbreviations: F, female; M, male. Adequate intakes (AI), recommended dietary allowances (RDA), and tolerable upper intake levels (UL) set by authorities are also shown.

Table 5. Mean Trace Elemental Composition (mg per 100 g^{-1} Wet Weight; ± Standard Deviation) in Female and Male *C. pagurus* Edible Tissues during the Four Seasons[a]

tissue	season	sex	Fe RDA = 8	Cu RDA = 0.9 UL = 10	Zn RDA = 11	As AL = 7.6	Br	Sr
muscle	spring	F	0.37 ± 0.06 b	0.86 ± 0.13 a	5.5 ± 0.2 b	4.2 ± 1.1 ab	2.0 ± 0.3 b	1.6 ± 0.3 a
		M	0.48 ± 0.14 ab	0.93 ± 0.28 a	6.0 ± 1.0 ab	3.6 ± 1.1 b	2.3 ± 0.3 b	1.1 ± 0.3 ab
	summer	F	0.48 ± 0.30 ab	0.93 ± 0.21 a	5.5 ± 0.4 b	4.3 ± 0.9 a	2.2 ± 0.2 b	0.72 ± 0.29 b
		M	bdl	0.86 ± 0.25 a	5.9 ± 0.3 ab	4.0 ± 0.8 a	2.1 ± 0.3 b	1.0 ± 0.5 ab
	autumn	F	0.68 ± 0.10 a	1.0 ± 0.2 a	7.0 ± 0.9 a	2.4 ± 0.3 c	2.2 ± 0.1 b	0.82 ± 0.30 b
		M	0.38 ± 0.15 b	0.67 ± 0.25 a	7.4 ± 1.5 ab	3.5 ± 2.1 b	2.4 ± 0.2 b	0.73 ± 0.37 b
	winter	F	0.44 ± 0.09 b	0.90 ± 0.18 a	6.6 ± 0.4 a	2.7 ± 0.5 c	2.6 ± 0.2 ab	1.1 ± 0.4 ab
		M	0.32 ± 0.04 b	1.1 ± 0.2 a	6.1 ± 0.4 ab	1.8 ± 0.4 d	2.9 ± 0.3 a	0.59 ± 0.10 b
hepato	spring	F	1.6 ± 0.3 b	3.1 ± 0.2 b	2.2 ± 0.2 b	4.3 ± 0.4 a	5.5 ± 0.8 b	6.8 ± 1.1 c
		M	1.7 ± 0.1 b	1.8 ± 1.1 b	1.7 ± 0.2 b	2.4 ± 0.6 bc	6.4 ± 1.2 b	10 ± 2 b
	summer	F	1.8 ± 0.2 b	4.7 ± 1.9 ab	2.6 ± 0.5 a	4.9 ± 1.1 ab	8.1 ± 0.3 a	6.0 ± 0.9 c
		M	2.3 ± 1.0 ab	1.7 ± 0.6 b	2.0 ± 0.2 b	2.9 ± 0.4 b	8.3 ± 2.4 ab	12 ± 5 ab
	autumn	F	4.5 ± 1.1 a	10 ± 4 a	2.7 ± 0.5 a	2.6 ± 0.2 b	7.0 ± 0.5 b	9.1 ± 1.3 b
		M	3.2 ± 0.8 a	3.3 ± 3.9 ab	2.5 ± 0.8 a	2.0 ± 0.2 c	11 ± 4 a	11 ± 8 ab
	winter	F	3.0 ± 0.7 a	2.9 ± 2.0 b	2.6 ± 0.3 a	2.6 ± 0.5 bc	6.5 ± 1.0 b	8.9 ± 1.2 b
		M	4.3 ± 0.5 a	3.9 ± 1.7 b	2.7 ± 0.4 a	2.0 ± 0.3 c	7.7 ± 0.8 b	17 ± 2 a
gonads	spring	F	1.2 ± 0.4 a	0.56 ± 0.28 bc	4.7 ± 0.8 b	3.0 ± 1.8 ab	1.9 ± 0.9 c	0.25 ± 0.11 d
		M	bdl	0.75 ± 0.03 b	2.8 ± 0.1 c	2.1 ± 0.0 c	8.2 ± 0.2 a	1.6 ± 0.0 a
	summer	F	1.9 ± 0.1 a	1.2 ± 0.1 a	9.0 ± 0.4 a	4.7 ± 0.2 a	3.8 ± 0.2 b	0.41 ± 0.03 d
		M	0.45 ± 0.06 b	1.4 ± 0.0 a	3.3 ± 0.1 c	4.3 ± 0.1 b	8.8 ± 0.2 a	1.2 ± 0.0 b
	autumn	F	1.9 ± 0.2 a	0.96 ± 0.15 b	9.0 ± 1.3 a	1.8 ± 0.2 c	2.7 ± 0.3 c	0.31 ± 0.04 d
		M	bdl	0.44 ± 0.02 c	1.8 ± 0.1 d	1.5 ± 0.0 d	4.2 ± 0.1 b	0.61 ± 0.01 c
	winter	F	2.7 ± 0.5 a	0.83 ± 0.07 b	5.7 ± 1.3 b	2.5 ± 0.2 c	3.3 ± 0.3 c	0.39 ± 0.04 d
		M	0.32 ± 0.03 b	0.70 ± 0.02 b	2.0 ± 0.1 d	1.1 ± 0.0 d	4.1 ± 0.1 b	0.78 ± 0.01 c

[a] In each column, different letters indicate significant differences in the elemental concentration per tissue ($p < 0.05$). Abbreviations: F, female; M, male; bdl, below detection limit. Adequate intakes (AI), recommended dietary allowances (RDA), tolerable upper intake levels (UL), and action limits (AL) set per day by authorities are also shown.

sium, S, Cl, K, Ca, Zn, Sr, As, and Mn loaded on factor 1, Fe, Cu, Se, Hg, and Pb loaded on factor 2,while Na, Br, and Cd loaded on factor 3. The higher Ca content in males is likely due to the sexual dimorphism of crabs, in which males have bigger claws and harder exoskeletons (composed by calcium phosphate) (*23*).

Previous studies with several crustacean species, such as *Dardanus arrosor*, *Carcinus mediterraneus*, *Homarus gammarus*, and *H. americanus*, also reported that most important changes in the concentration of elements are found in tissues involved in regulatory processes such as the hepatopancreas in opposition to structural tissues such as muscle (*5*, *24*, *25*).The seasonal and sex differences observed are most probably related with the spawning cycle and metabolism that influence feeding, reproductive state, and weight (*26*). *Cancer pagurus* mating season coincides with moulting periods and occurs during summer and early autumn; spawning occurs in late autumn and winter (*27*). After spawning, there is a period of incubation of the eggs that can last up to eight months before the broods hatch throughout spring, summer, and early autumn, depending on

Table 6. Mean Ultra-Trace Elemental Composition (mg per 100 g^{-1} Wet Weight; ± Standard Deviation) in Female and Male *C. pagurus* Edible Tissues during the Four Seasons[a]

tissue	season	sex	Mn AI = 2.3	Se RDA = 0.055UL = 0.4	Cd MPC = 0.05 AL = 0.3	Hg MPC = 0.05 AL = 0.1	Pb MPC = 0.05 AL = 0.15
muscle	spring	F	0.022 ± 0.003 b	0.11 ± 0.02 ab	0.003 ± 0.002 a	0.018 ± 0.001 b	bdl
		M	0.027 ± 0.005 b	0.09 ± 0.03 ab	0.002 ± 0.001 a	0.016 ± 0.004 b	0.002 ± 0.000 a
	summer	F	0.027 ± 0.003 b	0.09 ± 0.02 ab	0.002 ± 0.001 a	0.016 ± 0.001 b	0.002 ± 0.000 a
		M	0.024 ± 0.004 b	0.09 ± 0.02 ab	bdl	0.016 ± 0.001 b	0.002 ± 0.000 a
	autumn	F	0.062 ± 0.012 a	0.13 ± 0.01 a	bdl	0.061 ± 0.018 a	bdl
		M	0.026 ± 0.006 b	0.10 ± 0.06 ab	bdl	0.049 ± 0.015 a	bdl
	winter	F	0.024 ± 0.001 b	0.083 ± 0.17 b	0.002 ± 0.001 a	0.038 ± 0.008 a	0.003 ± 0.001 a
		M	0.029 ± 0.006 b	0.11 ± 0.02 ab	0.003 ± 0.003 a	0.021 ± 0.004 b	0.002 ± 0.000 a
hepato	spring	F	0.11 ± 0.01 c	0.19 ± 0.04 a	0.80 ± 0.70 b	0.021 ± 0.003 b	0.002 ± 0.001 b
		M	0.21 ± 0.02 b	0.15 ± 0.04 a	0.60 ± 0.30 b	0.015 ± 0.001 b	0.002 ± 0.001 b
	summer	F	0.20 ± 0.13 bc	0.16 ± 0.05 a	2.0 ± 1.2 ab	0.023 ± 0.006 b	0.003 ± 0.002 b
		M	0.17 ± 0.11 bc	0.11 ± 0.04 a	2.8 ± 1.1 a	0.018 ± 0.004 b	0.003 ± 0.002 b
	autumn	F	0.31 ± 0.05 b	0.20 ± 0.06 a	0.70 ± 0.30 b	0.051 ± 0.013 a	0.010 ± 0.004 a
		M	0.33 ± 0.32 abc	0.14 ± 0.07 a	1.6 ± 1.4 ab	0.039 ± 0.014 a	0.003 ± 0.002 b
	winter	F	0.22 ± 0.06 b	0.18 ± 0.04 a	2.7 ± 1.6 ab	0.033 ± 0.011 a	0.004 ± 0.002 b
		M	0.47 ± 0.06 a	0.17 ± 0.02 a	0.80 ± 0.30 b	0.030 ± 0.008 a	0.006 ± 0.003 b
gonads	spring	F	0.10 ± 0.00 d	0.20 ± 0.10 ab	0.013 ± 0.003 e	0.006 ± 0.004 b	bdl
		M	0.11 ± 0.00 d	0.13 ± 0.01 b	0.006 ± 0.000 f	0.006 ± 0.000 b	bdl
	summer	F	0.24 ± 0.01 b	0.26 ± 0.02 a	0.018 ± 0.001 d	0.006 ± 0.001 b	0.002 ± 0.000 a
		M	0.11 ± 0.03 d	0.14 ± 0.01 b	0.072 ± 0.020 c	0.007 ± 0.002 b	0.002 ± 0.000 a
	autumn	F	0.24 ± 0.06 b	0.29 ± 0.06 a	0.004 ± 0.002 e	0.007 ± 0.002 b	0.004 ± 0.002 a
		M	0.17 ± 0.02 c	0.070 ± 0.008 b	0.032 ± 0.001 d	0.014 ± 0.000 a	0.002 ± 0.000 a
	winter	F	0.41 ± 0.07 b	0.34 ± 0.06 a	0.20 ± 0.11 b	0.017 ± 0.004 a	bdl
		M	0.58 ± 0.00 a	0.12 ± 0.00 b	0.43 ± 0.01 a	0.009 ± 0.001 b	bdl

[a] In each column, different letters indicate significant differences in the elemental concentration per tissue ($p < 0.05$). Abbreviations: F, female; M, male; bdl, below detection limit. Adequate intakes (AI), recommended dietary allowances (RDA), tolerable upper intake levels (UL), maximum permissible concentrations (MPC), and action limits (AL) set per day by authorities are also shown.

latitude and temperature (*27*). During the premoult period of *C. pagurus* (winter to spring), Ca is accumulated in the hepatopancreas probably to be used in exoskeleton calcification (*20*). Throughout spawning, large females tend to migrate long distances, thus diversifying their feeding grounds, while males are more stationary, staying within the same habitats (*23*). During the incubation period (winter/spring), females hardly eat (*23*). Feeding deprivation probably explains the acute low levels of K, Cl, and Br in female gonads and of Mg, Ca, and Sr in both female gonads and the hepatopancreas during spring compared to the high levels in males. In contrast, autumn is a high feeding period for both males and females, resulting in high concentrations of Mg, S, Cl, K, and Zn content in the muscle and hepatopancreas. The high concentration of all macro elements and Fe during winter might be a consequence of the intense feeding during autumn.

No seasonal changes in the content of some elements were observed in the muscle (Na, Fe, Cu, and Se), hepatopancreas (Cu, Se, and Hg) and gonads (K, Ca, Zn, Br, Fe, and Se) (**Table 6**). It is likely that these elements are vital for edible tissues all year long, while others such as contaminants are bioaccumulated in edible tissues.

As far as contaminants are concerned, As concentration was higher in summer (all tissues), while Cd (gonads) and Hg (all tissues) were more concentrated in winter. Lead concentration did not vary in muscle and gonads but had a peak in female hepatopancreas during autumn. The bioavailability and uptake of nonessential elements by crustaceans depends on environmental factors such as temperature, salinity, and chelating agents (*15*, *28*). In this study, the higher concentrations of Cd and Hg coincided with winter, a period of lower seawater temperatures and salinities, factors that have been reported to be inversely related with Cd uptake rate by crabs (*26*, *28*). Additionally, adult crabs have annual migrations, moving to deeper waters during winter and shallower waters during summer, thus diversifying their feeding grounds; the females bearing eggs that do not eat during the incubation period (*23*)are the exception. This migration pattern might also explain the differences obtained in the present study.

Comparison with Intake Recommendations and Limits. Worldwide, authorities have set recommendations and limits for elements in food to guarantee its safety and quality. Results presented in **Tables 3**−**5** for *C. pagurus* tissues, indicate that the average elemental intake is rather variable depending on the tissue, season, and sex.

Regarding the nutritional quality of *C. pagurus*, the muscle is an excellent source of Cu and Se (as values can reach the RDA/AI and are below the UL) and a good source of Na and Zn in both sexes all year long (as values are higher than 20% of the recommended values). The hepatopancreas is an excellent source of Ca (males all year long and females in winter), Cu, Zn, and Se (all year long), Mg and Mn (winter males), Cl (winter), and Fe (autumn and winter). Gonads are excellent sources of Na (winter), Cu (summer), and Se (all year long), and good sources of Mg (winter), Cl (summer males), Fe (summer/autumn/winter females), Zn (females all year long and spring/summer males), and Mn (winter males).

Concerning the potential hazards of *C. pagurus* consumption, only Cu, Cd, and Hg levels were above the limits set by authorities. Copper was above the UL in the hepatopancreas of autumn females. Cadmium was above the AL and MPC in the hepatopancreas and in male gonads in winter; it was also above the MPC of female gonads in winter. Mercury was above the MPC in the muscle and hepatopancreas of most autumn crabs but always below the AL limit. It is important to remember that MPC is only set for muscle and not for other tissues. Several authors have reported high Cd values in the hepatopancreas of crabs such as *Cancer pagurus* (4.9 mg per 100 g wet weight (*29*), *Pseudocarcinus gigas* (2.24 mg per 100 g dry weight (*17*)), *Carcinus maenas* (0.3 mg per 100 g dry weight (*30*)), and *Calinectes sapidus* (0.4 mg per 100 g wet wight (*31*)), while there are no reports of high Cd values in crabs' gonads so far.

Exceeding the UL, MPC, and AL does not necessarily mean that the item is unfit for consumption, as the levels assume the worst case scenarios of food items constituting a major part of the total diet (*17*), which is unlikely the case in *C. pagurus*. A more meaningful hazard assessment may be obtained by calculating the amount of toxic elements expected in a typical diet and comparing this to the provisional maximum tolerable daily intake (PMTDI) set by the Joint Expert Committee on Food Additives of the Food and Agriculture Organization of the United Nations and the World Health Organization (*32*). Assuming the consumption of three crabs per year (equivalent to 1.5 g portion per day) by a 60 kg adult, the daily Cd intake due to *C. pagurus* hepatopancreas (0.03−0.42 mg) still exceeds this element PMTDI (0.06 mg), particularly in summer (both sexes), autumn (males), and winter (females). Considering this scenario, Cd can be a concern for regular consumers of *C. pagurus*. Yet, several authors reported that Se has a protective action, especially selenite ($Se_2O_3^{2-}$), on many toxicological effects of Cd and Hg (*33*, *34*). Since the hepatopancreas of *C. pagurus* is rich in Se, this element might counteract Cd and Hg effects. Nevertheless, it is recommended to avoid the regular consumption of *C. pagurus* hepatopancreas since Cd is known to exert a variety of toxic effects, including nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity, genotoxicity, teratogenicity, endocrine disruptions, and reproductive disfunctions (*35*). As far as S, Br, and Sr are concerned, there is still a lack of information regarding their essentiality or toxicity. Considering that these elements are commonly found in marine organisms, there is an urgent need to evaluate their impact on human consumption.

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