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Evaluation of lead, mercury, cadmium and arsenic accumulation, and fatty acids' profile in muscle and cephalothorax of *Parapenaeus longirostris* (Mediterranean shrimp) and of *Pandalus borealis* (northern shrimp)

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Abstract

The aim of the present work was to assess the nutritional value of shrimps in terms of the essential fatty acids versus the toxicological concern as regards elements' accumulation in their tissues. The concentrations of lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As), and the fatty acid (FA) profiles in the muscle and cephalothorax of *Parapenaeus longirostris* (Mediterranean shrimp) and *Pandalus borealis* (northern shrimp) were evaluated and comparatively studied. The results indicated a substantial association of the Cd, Pb and Hg concentrations with the shrimp fishing area and the tissue type. Moreover, Cd, Pb and Hg levels, found in the tissues of shrimps, were below EU maximum levels for human consumption. Total As concentration was highest ($p < 0.05$) in all tissues studied, mainly in the organic As form. Fatty acid patterns were found significantly different between shrimp species and tissues. Palmitic (C16:0), oleic (C18:1ω-9), eicosapentaenoic (C20:5ω-3, EPA) and docosahexaenoic (C22:6ω-3, DHA) acids were found to be the major FA in all tissues. The highest DHA/EPA and ω-3/ω-6 ratios were found in *Parapenaeus longirostris* and *Pandalus borealis* tissues, respectively. A positive feature, arising from the FA comparison, was the low values for both the atherogenic and thrombogenic indices, related to the high unsaturated/saturated FA ratio.

Introduction

Crustaceans, especially shrimps, are among the most popular shellfish for both commercial and economic reasons. They are an important source of biologically valued essential fatty acids, carotenoids, minerals and amino acids, which are all required for human health (Yerlikaya *et al*., 2013; Soultani *et al*., 2016). Shrimp muscle possesses low fat content (1.5-2.5% of total lipids) and high ω-3 polyunsaturated fatty acids (PUFA) content, constituting more than 29% of the total fatty acids (TFA) (Yerlikaya *et al*., 2013). Omega-3 PUFAs are known for their anti-atherogenic and anti-thrombotic roles in preventing cardiovascular diseases and maintaining the nervous system health (Simopoulos, 2001).

However, the sea pollution and the risk of exposure of contaminants to fish and shellfish have raised serious concerns among consumers. In this sense, shrimps might contain elements of toxicological concern such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) at potentially hazardous levels. Even though these elements exist naturally in the aquatic environment, the actual levels have increased due to industrial, agricultural and mining activities (Olmedo *et al*., 2013). Shrimps and other marine organisms accumulate pollutants through their diet, originating from waste disposal and human activities, and therefore can have cumulative effects and specific risk to humans when consumed. According

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to Dökmeci *et al*. (2014), shrimps could be used as bio-indicators in order to measure the concentrations of aquatic pollutants.

Parapenaeus longirostris (warm water species) and *Pandalus borealis* (cold water species) are the most common and commercially important shrimp species of the Mediterranean and North Atlantic Sea, respectively. *Parapenaeus longirostris* (Lucas 1846, commercial name: deep-sea rose shrimp; Penaeidae family) is usually found at depths ranging from 20 to 750 m, mainly between 150 and 400 m (Holthuis, 1980). *Pandalus borealis* (Krøyer 1838, commercial name: northern shrimp; Pandalidae family) is found at depths between 20 and 1,330 m (Holthuis, 1980). Both species show a characteristic colour, pink-orange carapace with reddish rostrum, and they are medium-sized, usually ranging from 12 to 18 cm in total length for adults (Holthuis, 1980).

The aim of the present work was to consider the health promoting effects in terms of fatty acid profile as opposed to the health risk of Pb, Hg, Cd and As accumulation, in muscle and cephalothorax of *Parapenaeus longirostris* and *Pandalus borealis*. Moreover, the present work was also intended to highlight the distribution of the essential fatty acid in muscle and cephalothorax, in regards to their nutritional value and their exploitation for the production of food supplements. An overall goal was to associate the element concentrations with the fishing area and the tissue type and to ascertain whether the levels of Pb, Hg and Cd exceed the EU standards which could be harmful to humans' health.

Materials and methods

Reagent and standards

All chemicals used were of analytical grade. Concentrated nitric acid (HNO_3), 30% hydrogen peroxide (H2O2) (Merck, Darmstadt, Germany), sulphuric acid (H_2SO_4) and bromine monochloride (BrCl) (Sigma–Aldrich Company, St. Louis, USA) were used for the digestion of samples prior to total element analysis. Standard solutions of Pb, Cd, As and Hg (Plasma CAL, SCP Science, Quebec, Canada) were used for establishing calibration curves. Methanol (MeOH) HPLC-grade (Rathburn Chemicals, Walkerburn, UK), 25% ammonia (suprapure grade, Merck) and ammonium carbonate (puratronic grade, Alfa Aesar, Karlsruhe, Germany) were used for production of the mobile-phase for inorganic As (iAs) determination. Fatty acid methyl esters used as gas chromatography (GC) standard mixtures were purchased from Sigma–Aldrich Company. Arsenobetaine (AB) (BCR CRM626, Institute for Reference Materials and Measurements; IRMM, Geel, Belgium) stock solution was used. Chloroform, methanol, t-butyl-hydroquinone and boron trifluoride used for lipid analysis were purchased from Merck and Sigma–Aldrich. All water used in this work was re-distilled.

Sampling and sample preparation

Parapenaeus longirostris were caught in the Ionian Sea close to the Kyllini area (Western Greece) (Fig. 1). Specimens were collected by using a net, at depths between 250-750 m during autumn (September-November) with two repetitions in 2013 and 2014. *Pandalus borealis* specimen were caught from the Skagerrak strait (Fig. 1) at similar depths and periods. *Parapenaeus longirostris* specimens (batches of 30-35 kg, per year) were stored on ice and immediately transferred to the laboratory, whereas *Pandalus borealis* samples (batches of 35-38 kg, per year) were boiled on-board, stored on ice and transferred to the laboratory. For a better estimation of shrimp nutritional value, the specimens were prepared as how they would normally be consumed in Greece and Denmark, according to the local preparation processes. In Denmark, shrimps are typically cooked immediately after capture, which helps the shrimp's meat to retain the best flavour and colour (Torry Research Station, 2001; Eigaard and Munch-Petersen, 2008). Shrimps were divided into six groups per species and per year, dissected, the muscles (by removal of the exoskeleton) and cephalothoraxes were weighed and separately homogenised. Next, the homogenised individual tissues from the three groups per species were used for lipid extraction and further analysis. The individual tissues from the other three groups per species were freeze-dried for Pb, Cd, Hg and As determinations. The analytical parameters were determined at least in triplicate for each group. For statistical analysis, $n = 6$ samples were used per species and tissue, for the two years' repetition.

Determination of Pb, Cd and total As (tAs)

The homogenised samples (muscle and cephalothorax) were weighed separately (~0.2 g) in triplicate, into quartz tubes. Solutions of HNO_3 (5.0 mL, 65% w/w) and H2O2 (1.0 mL, 30% w/w) were added prior to microwave digestion. TORT-2 (lobster hepatopancreas Reference Material for Trace Metals, National Research Council of Canada / NRC - CNRC) was used as certified reference material. The samples were digested in a Multiwave 3000 microwave system (Multiwave 3000, Anton Paar GmbH, Graz, Austria). Afterwards, Milli-Q

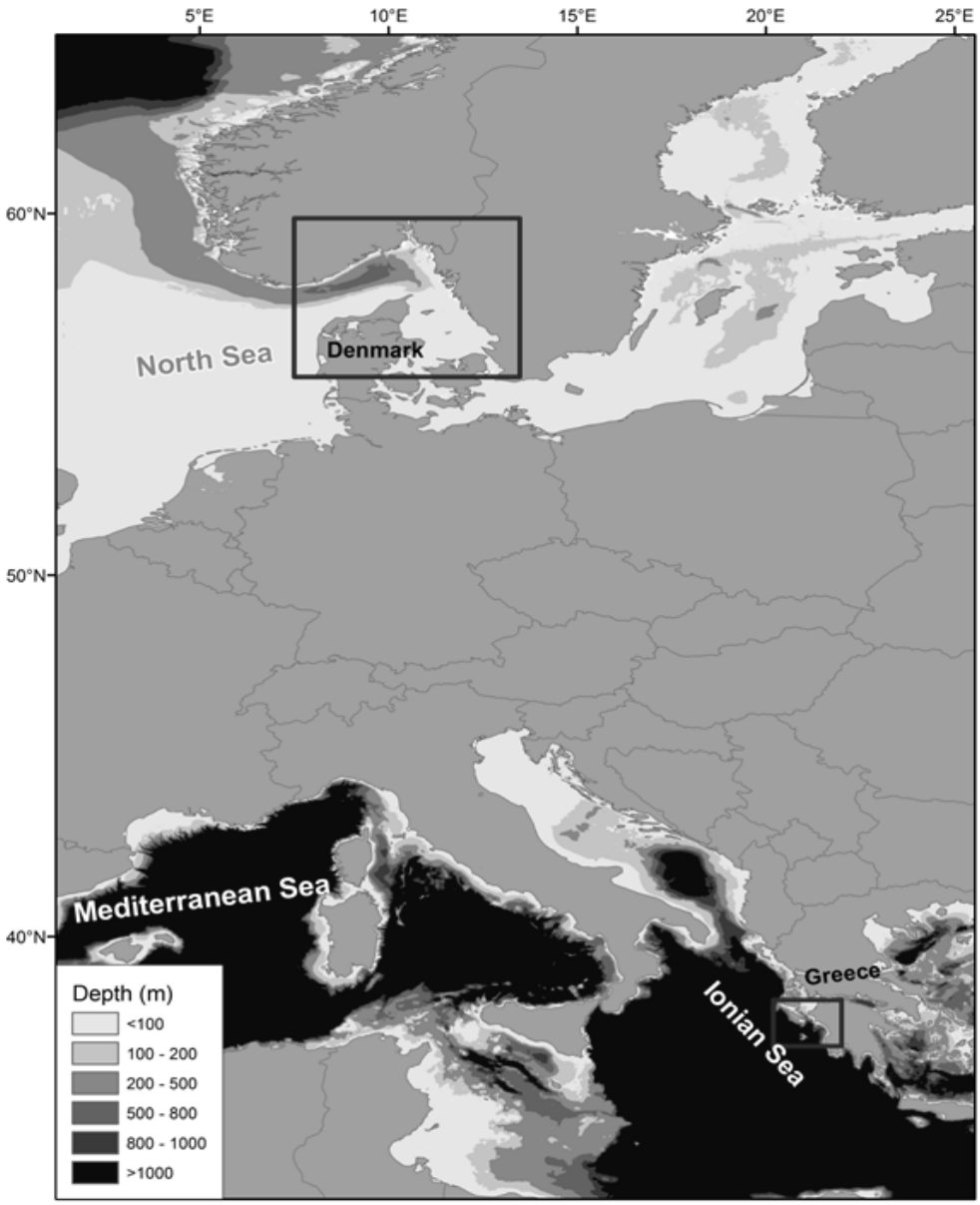

Figure 1: Sampling areas of Parapenaeus longirostris (Mediterranean shrimp) and Pandalus borealis (northern shrimp), respectively.

water was added to the digested samples to a final volume of 25.00 mL. External calibration curves were made from stock of standard solutions. Gallium (Ga) stock solution was added to all samples and served as an internal standard in order to correct for instrumental drift during the analytical procedure (Julshamn *et al*., 2007). Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7500 ce; Agilent Technologies, Waldbronn, Germany) equipped with an auto sampler (ASX-500, CETAC Technologies, Omaha, NE, USA) was used for the analysis. The instrumental settings are as follows: at RF power 1500 W, plasma gas flow 15 L/min, carrier gas flow 0.9 L/min, make up gas flow 0.16 L/min, torch 2.5 mm i.d., nebuliser Babington. The obtained results for these elements in TORT 2 were: Pb = 0.40 ± 0.06 mg/kg dry weight (dw) (nominally 0.35 ± 0.13 mg/kg), Cd = 27.8 ± 0.2 mg/kg dw (nominally 26.7 ± 0.6 mg/kg) and tAs = 21.2 ± 1.2 mg/kg dw (nominally 21.6 ± 1.8 mg/kg). The limits of detection (LOD) for Pb, Cd and tAs were defined as three times the standard deviation (S.D.) of the mean of three blanks. The limits of quantification (LOQ) were 0.001, 0.002 and 0.02 mg/kg wet weight (ww) for Pb, Cd and tAs respectively.

Determination of inorganic As (iAs)

For the determination of iAs, a method based on the principles of the fully validated European standard method EN16802 was used (CEN, 2016). Briefly, the freeze-dried and homogenised samples (muscle and cephalothorax separately) were weighed

(~0.2 g) in triplicate and placed in a 15 mL plastic tube, and 10.0 mL extraction solvent (0.1 mol/L HNO_3/3% H_2O_2) was added. A blank, calibration standards (As V) and TORT-2 were also used. The samples were heated in a water bath (Julabo, Buch and Holm, Denmark) at 90°C for 1 h. The extracts were further centrifuged (Sigma Laborzentrifugen, Germany) for 10 min at 4,000 rpm. Then, 300 µL sample solution was transferred into a HPLC filter vial. Final determination was performed by using high performance liquid chromatography coupled to ICP-MS (HPLC 1260; ICP-MS 7500ce, both Agilent Technologies, Waldbronn, Germany). The instrumental conditions were as follows- column: strong anion exchange (SAX) (ION120 from Transgenomic, 120 mm × 4.6 mm; 10 µm particles); elution method: isocratic; injection volume: 25 µL; flow rate: 1 mL/min; column temperature: 30°C; mobile phase: 50 mmol/L $(NH_4)_2CO_3$ in 3% MeOH; run time per injection: 10 min.

Determination of Hg

The samples (muscle and cephalothorax separately) were first lyophilised, homogenised and weighed (~0.2 g) in triplicate (each tissue and species) into the digestion vessels. Next, the digestion mixture (7.0 mL HNO_3 and 3.0 mL H_2SO_4) was carefully added. The vessels were left on the bench for 4 h for the excessive foaming to stop, and then the lids of the vessels were tightened. The vessels were then placed in a hot plate and after the digestion was completed, 40.0 mL of an aqueous solution of 0.02 mol/L BrCl was added. The technique of cold vapour atomic fluorescence spectrometry with a manual system of purge and trap dual amalgamation thermal extraction coupled with the model 2500 fluorescence mercury detector (Tekran 2500, Toronto, Canada) was used for the final determination of Hg concentrations, according to EPA method 1631 (USEPA, 2001). The LOQ for Hg was 0.01 mg/kg ww.

Risk-based consumption limits

The estimated weekly intake per meal size (EWIm) was calculated and compared with the tolerable exposure established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011a, 2011b) for Pb, Cd and iAs, and by the European Food Safety Authority (EFSA, 2012) for Hg. The element concentration reflects the value determined in the muscle tissue of shrimps and not in the cephalothorax tissue as it is accepted that only the muscle tissue is consumed. The EWIm was calculated for adults assuming a body weight (bw) of 70 kg and a meal size of 100 g of shrimps per day according to the following equation (Copat *et al*., 2012):

EWIm = meal size (g) × element's concentration (mg/kg) × 7 days/bw (kg)

Determination of fatty acids

Homogenised muscle and cephalothorax total lipids (TL) of shrimps were extracted (separately) with chloroform/methanol/water (1:2:0.8 v/v/v) according to the method described by Bligh and Dyer (1959). After phase equilibration, the lower chloroform layer containing TL was removed and dried in a rotary vacuum evaporator. The extracted lipids were weighed in order to determine the TL content, then re-dissolved in chloroform/methanol (9:1, v/v) and stored at 0°C until further use. To prevent oxidation, t-butyl-hydroquinone was added to all samples during preparation. Fatty acids were trans-esterified to methyl esters (FAME) using a base-catalysed trans-esterification followed by a borontrifluoride catalysed esterification according to the method described by Marinho *et al*. (2015). Tricosanoic acid (C23:0) methyl ester was used as internal standard to a final concentration of 0.5 mg/mL. The FAME solution (1.5 µL FAME) was injected into an Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto sampler and a flame ionisation detector. The separation was carried out on a DB-WAX fused silica capillary column (10 m x 0.1 mm, 0.1 µm; Agilent Technologies) using helium as carrier gas. The oven temperature program was: initial 160°C, 10.6°C/min to 200°C, hold 0.3 min and 10.6°C/min to 220°C, hold 1 min and 10.6°C/min to 240°C, hold 3.8 min (Marinho *et al*., 2015). A split ratio of 1:25 was used. Methyl esters were identified by comparison of the retention times with a mixture of standards, containing all the fatty acids identified in the present work. Fatty acids' proportions were expressed as fatty acid % weight of total fatty acids.

Lipid quality indices

Atherogenic (AI) and thrombogenic (TI) indices were calculated according to Ulbricht and Southgate (1991) (Equations 1 and 2, respectively) in order to calculate the propensity of shrimp's tissue of reducing the risk of coronary heart disease.

$$AI = \frac{[12:0+(4*14:0)+16:0]}{\omega3PUFA+\omega6PUFA+MUFA} \quad \text{(Equation 1)}$$

$$TI = \frac{[14:0+16:0+18:0]}{0.5*MUFA+0.5*\omega6PUFA+3*\omega3PUFA+\frac{\omega3PUFA}{\omega6PUFA}} \quad \text{(Equation 2)}$$

where MUFA: monounsaturated fatty acids

Statistical analysis

All measurements were carried out in triplicate. The values were averaged and reported along with their standard deviation (S.D.). All data concerning element composition and fatty acid proportion were analysed with one-way ANOVA test. The probabilities lower than 0.05 were considered as statistically significant ($p < 0.05$). All statistical calculations were performed with the SigmaStat statistical software package (Version 3.5 - 2007).

Results and discussion

Pb, Hg, Cd and As concentrations in shrimp muscle and cephalothorax

Mean concentrations and S.D. of Pb, Hg, Cd and tAs are presented in Table 1. Both shrimps' cephalothorax contained higher ($p < 0.05$) concentration (mg/kg ww) of Pb, Hg and Cd than the muscle tissues. These results were expected since cephalothorax included the hepatopancreas, where biochemical reactions such as the accumulation and detoxification of some metallic elements take place, while the muscle tissue does not play any active role in accumulation (Olgunoğlu *et al*., 2015). Other studies on different marine species also corroborate that the levels of elements of toxicological concern were higher in the liver than in the muscle tissue (Tapia *et al*., 2012; Olgunoğlu *et al*., 2015).

Furthermore, the concentrations of Pb and Hg were found significantly ($p < 0.05$) higher in the *Parapenaeus longirostris* tissues than in *Pandalus borealis*. According to Holthuis (1980), *Parapenaeus longirostris* is more abundant on sandy-muddy bottoms, which could be richer source of Pb and Hg than the seawater. Moreover, Pb and Hg levels' variation could be attributed to seawater contamination from anthropogenic sources or the occurrence of metalliferous sediments enriched in Hg and Pb, due to the geology and tectonic reconstructions of the fishing area (Kotnik and Horvat, 2013). However, both tissues of *Parapenaeus longirostris* and *Pandalus borealis* contained lower concentrations of Pb and Hg than the EU maximum levels (0.5 mg/kg ww for crustaceans) (ECR, 2006).

For Cd, the levels were about 10 and 4 times higher in the muscle and cephalothorax, respectively, in the *Pandalus borealis* as compared to *Parapenaeus longirostris* (Table 1). This finding might be associated with increased bioavailability of Cd in northern seas as compared to the Mediterranean Sea. Furthermore, Zauke and Schmalenbach (2006) reported that the levels of Cd in *Pandalus borealis* from Barents Sea (Arctic waters) varied from 0.7 to 4.7 mg/kg (dry weight) depending on the season and the origin (Cd levels increased moving from the south to the north of Barents Sea). According to the same authors, this phenomenon is referred to as a "Cd-anomaly" and is related to the effective but unselective absorption mechanisms of metals from the marine species. Therefore, the potential deficiency of copper (Cu) and zinc (Zn) in sea areas probably stimulates the Cd absorption from the shrimps. Either way, in the present work, the concentration of Cd in *Pandalus borealis* was quite high, but, it was still lower than the maximum levels (0.5 mg/kg ww) established by the European Commission (ECR, 2006). Furthermore, Pb, Hg and Cd concentrations found in *Parapenaeus longirostris* muscle (Table 1) were lower than those reported by Dökmeci *et al*. (2014) (2.1 ± 0.8, 0.18 ± 0.04, 0.11 ± 0.01 mg/kg wet weight, respectively) for the edible part of *Parapenaeus longirostris*.

The concentration of total arsenic (tAs) was lower in *Parapenaeus longirostris* cephalothorax and muscle compared to those in *Pandalus borealis* tissues, ranging from 29.1 to 29.9 and from 33.4 to 62.4 mg/kg ww, respectively. Julshamn *et al*. (2004) reported considerable variations of tAs concentrations for *Pandalus borealis* edible part, ranging from 13 to 96 mg/kg ww. Moreover, it was observed that in the case of *Parapenaeus longirostris*, tAs was determined in similar levels in muscle and cephalothorax, while in *Pandalus borealis*, tAs concentration in the muscle tissue was twice as high as compared to the

Table 1: Concentration (mg/kg wet weight) of hazardous elements in muscle and cephalothorax tissue of *Parapenaeus longirostris* and *Pandalus borealis*.

Elements	Parapenaeus longirostris muscle	Pandalus borealis muscle	Parapenaeus longirostris cephalothorax	Pandalus borealis cephalothorax
Pb	0.012 ± 0.001^{a}	0.005 ± 0.002^{b}	0.090 ± 0.002^{c}	0.035 ± 0.016^{d}
Hg	0.097 ± 0.014^{a}	0.020 ± 0.009^{b}	0.114 ± 0.025^{c}	0.032 ± 0.018^{b}
Cd	0.012 ± 0.005^{a}	0.129 ± 0.038^{b}	0.120 ± 0.045^{c}	0.487 ± 0.045^{d}
iAs	n.d.	n.d.	0.0009 ± 0.0003^{a}	0.0007 ± 0.0002^{a}
tAs	29.9 ± 0.7^{a}	62.4 ± 0.3^{b}	29.1 ± 0.2^{a}	33.4 ± 0.4^{c}

Results represent means ± SD ($n = 6$)
Means in the same row bearing different letters differ significantly ($p < 0.05$)
n.d. not detected

cephalothorax tissue. The significant variation of tAs levels between species might be related to water salinity of the different fishing areas, which affects the mobility of arsenic complexes in sediment and to the methylation reactions by phytoplankton activity (probably to a lesser extent) (Chakraborty *et al*., 2012; Mason, 2013). The presence of As in seawater is mainly due to natural processes, such as volcanic activity and weathering of minerals. In addition, anthropogenic activities (pesticide use, burning of coal and ore smelting) might have increased As levels throughout the years (Smedley and Kinniburgh, 2002; Sele *et al*., 2012). Given the fact that the maximum levels for inorganic arsenic have been set only for rice and its products (EFSA, 2009), it was considered essential to determine separately both inorganic and organic As.

According to literature, more than 100 different naturally-occurring As compounds have been identified, in both inorganic and organic forms, such as AsIII, AsV, mono-, di- and tri-methyl arsenic, arsenobetaine, arsenosugars and As-containing carbohydrates (Mason, 2013). Inorganic forms of As (iAs) are considered to be more toxic than the organics; therefore, emphasis is given on the determination of iAs when assessing food safety (EFSA, 2009; JECFA, 2011a). Although iAs was found in low concentrations in most fish and shellfish (Julshamn *et al*., 2012), organoarsenic compounds, e.g. arsenobetaine (AB), were determined in high levels in marine organisms (Francesconi and Kuehnelt, 2002; Sele *et al*., 2012). In the present work, trace amounts of iAs were detected (Table 1). Specifically, in muscle tissues, the iAs concentrations were below the limit of detection (Table 1 and Fig. 2a), whereas in the cephalothorax somewhat higher levels were found (Table 1 and Fig. 2b), but lower than 1.0 μg/kg ww. According to the above results, it is clear that the concentration of As in the organic form was similar to the tAs levels (data calculated from Table 1). In this sense, ICP-MS analysis of shrimps' muscle and cephalothorax resulted an As compound having the same chromatographic characteristics with arsenobetaine (AB) standard (Fig. 2c).

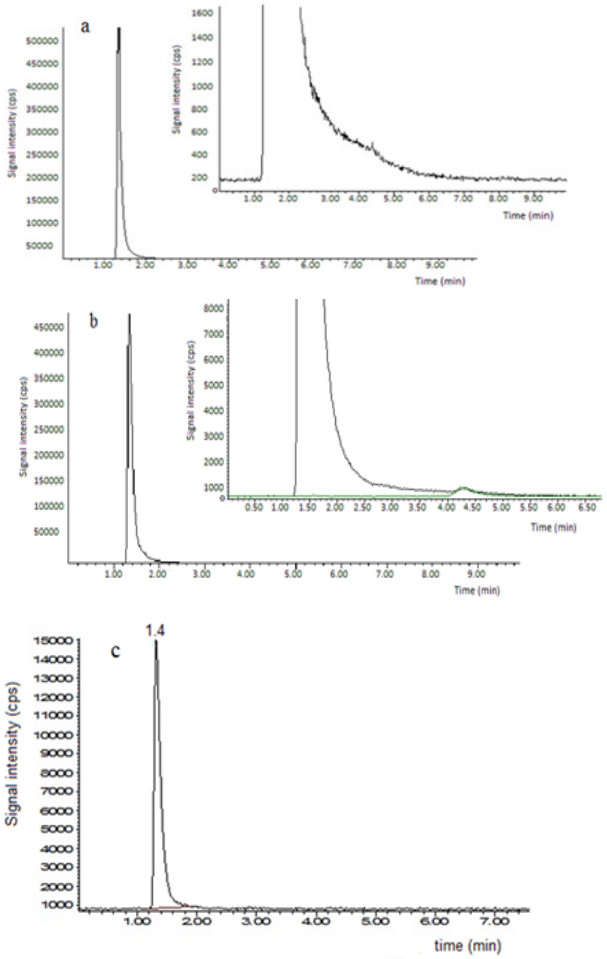

Figure 2(a-c): Chromatograms from the HPLC-ICPMS analysis of As of the northern shrimp (a) in the muscle, (b) in the cephalothorax, and (c) arsenobetaine (AB) standard eluting at 1.4 min.

Regarding risk assessment of shrimp consumption, the estimated weekly intake per meal size (EWIm) in adults was calculated and the results are presented in Table 2, in comparison with the food guideline values established by JECFA or EFSA (JECFA, 2011a, 2011b; EFSA, 2012). The EWIm depended on the element concentration in shrimp muscle and the muscle consuming amounts, whereas the provisional tolerable weekly intake (PTWI, μg/kg bw per week) and the tolerable weekly intake (TWI, μg/kg bw per week) were established by JECFA and EFSA based on epidemiological studies. For both Cd and Hg, the EWIm values were lower than PTWI and TWI, respectively (Table 2). Since the majority of Hg in seafood is methylmercury (MeHg), it was assumed that all Hg was present as MeHg, in accordance with EFSA scientific opinion (EFSA, 2012). Even though the EWIm for Pb had been calculated in the present work, it was not finally compared with the corresponding PTWI since the latter has been withdrawn by JECFA (2011b). Finally, in the case of iAs, EWIm was not calculated because in the muscle tissues the concentrations of iAs were below the limit of detection. According to JECFA (2011a), the PTWI for iAs has also been withdrawn and a benchmark dose lower confidence limit ($BMDL_{0.5}$) at 3 μg/kg bw per day was established (Table 2).

Total lipid content and fatty acid profile

The total lipid (TL) content of the cephalothorax of studied shrimps was found significantly ($p < 0.05$) higher than this of their muscle, whereas no significant difference was observed between species

Table 2: Estimated weekly intake per meal size (EWIm) in adults compared with the toxicological guideline values established by JECFA and EFSA (JECFA 2011a, 2011b; EFSA 2012).

Elements	Type	Value	EWIm (muscle of Parapenaeus longirostris)	EWIm (muscle of Pandalus borealis)
Pb	PTWI	withdrawn*	0.12	0.05
Cd	PTWI	6.25	0.12	1.29
Hg	TWI	1.3**	0.97	0.20
iAs	$BMDL_{0.5}$	21	n.c.	n.c.

BMDL0.5, benchmark dose level value (3 μg/kg bw per day); n.c. not calculated; PTWI, provisional tolerable weekly intake (μg/kg bw per week); TWI, tolerable weekly intake (μg/kg bw per week).
*Pb: withdrawn by JECFA (2011b)
** TWI value for methylmercury

for the same tissues. Cephalothorax tissue contained higher fat content as compared to the muscle, since cephalothorax also contains the hepatopancreas, where the fatty acid biosynthesis takes place (Jansen *et al*., 1968). In the muscle tissues, the mean values of TL were 1.05 ± 0.04% in *Parapenaeus longirostris* and 0.98 ± 0.06% in *Pandalus borealis*, while the corresponding levels for the cephalothorax were 5.00 ± 0.15% and 4.98 ± 0.05%, respectively. Results concerning muscle lipids were pointedly lower than those reported for the edible part of the deep-sea Mediterranean species: *Plesionica edwardsi*, *Plesionica martia* and *Aristaeomorpha foliacea* (1.86-2.46%) (Yerlikaya *et al*., 2013). Fishing season and area might play an important role to the TL composition. Therefore, the lipid content of the shrimp tissues varies before and after the spawning period (Rosa and Nunes, 2002) and it is also affected by the nutrients in specific sea areas (Iba *et al*., 2014).

The fatty acid profiles of muscle and cephalothorax TL in both shrimps are given in Table 3. Significant ($p < 0.05$) differences in the fatty acid proportions were found between species and tissues. Specifically, *Pandalus borealis* muscle TL had proportionally more PUFA, followed by MUFA and SFA whereas cephalothorax TL had significantly more MUFA, followed by PUFA and SFA. *Parapenaeus longirostris* both tissues TL showed a similar pattern dominated by PUFA, followed by MUFA and SFA. Furthermore, according to Table 3, *Pandalus borealis* both tissues TL had significantly higher unsaturated fatty acid (UFA) proportion than those of *Parapenaeus longirostris*. This finding could be attributed to the fact that *Pandalus borealis* is living in colder waters, where higher UFA levels are required to retain membrane fluidity (Hall *et al*., 2002). Moreover, it is reported that sea water temperature affects the action of lipogenic enzymes promoting the UFA synthesis in organisms living in cold waters (Hazel and Prosser, 1979).

Among the saturated fatty acids (SFA), palmitic acid (C16:0) was the most abundant in both species, ranging from 17.01 to 18.37% of TFA in the muscle and from 10.08 to 17.30% of TFA in the cephalothorax. The C16:0 percentages found in the studied muscle TL were lower than those determined in the edible part of various deep-sea species (varied from 19.03 to 21.16% of TFA) (Yerlikaya *et al*., 2013). A higher stearic (C18:0) acid proportion was noticeable in *Parapenaeus longirostris* both tissues TL as compared to that of *Pandalus borealis* tissues (Table 3). This result is in agreement with Huynh and Kitts (2009)'s findings in fish species caught from cold and warm waters. According to them, the metabolic differences due to water temperature might affect specific SFA content.

Oleic acid (C18:1ω-9) was the prevailing MUFA, mostly in *Parapenaeus longirostris* both tissues TL. The C18:1ω-9 was also found to be the major MUFA in other shrimp muscles such as in *A. foliacea* (19.95-28.34% of TFA) (Bono *et al*., 2012) and in *Penaeus monodon* and *Penaeus vannamei* (9.94 and 11.4% of TFA respectively) (Sriket *et al*., 2007). Gadoleic (C20:1ω-11) and cetoleic (C22:1ω-11) acids also contributed to the high proportion of MUFA in *Pandalus borealis* cephalothorax. Olsen *et al*. (2012) observed high levels of these specific fatty acids in *Pandalus borealis* which according to them was directly related to the shrimps' diet. Furthermore, the high C20:1ω-11 proportion in *Pandalus borealis* TL could be attributed to the increased consumption of zooplanktons (Sajjadi and Eghtesadi-Araghi, 2011).

The essential fatty acids, α-linolenic (C18:3ω-3) and linoleic (C18:2ω-6), were found in both *Parapenaeus longirostris* and *Pandalus borealis* tissues even in small percentages (≤1.3%). The comparison among the predominant PUFA of both tissue TL in shrimp species, showed that docosahexaenoic (C22:6ω-3, DHA) and arachidonic (C20:4ω-6) acids' proportions were significantly higher ($P<0.05$) in *Parapenaeus longirostris* than in *Pandalus borealis* tissues TL, while eicosapentaenoic (C20:5ω-3, EPA) acid was significantly higher ($P<0.05$) in *Pandalus borealis* lipids than in

Table 3: Fatty acid profile (%) and nutritional quality parameters of total lipid (TL) of muscle and cephalothorax of *Parapenaeus longirostris* and *Pandalus borealis*.

	Parapenaeus longirostris muscle	*Pandalus borealis* muscle	*Parapenaeus longirostris* cephalothorax	*Pandalus borealis* cephalothorax
C14:0	0.98 ± 0.00^{a}	2.32 ± 0.01^{b}	2.01 ± 0.02^{c}	3.19 ± 0.02^{d}
C14:1ω-5	0.14 ± 0.06^{a}	0.09 ± 0.01^{b}	0.32 ± 0.01^{c}	0.25 ± 0.03^{d}
C15:0	0.80 ± 0.06^{a}	0.42 ± 0.02^{b}	0.91 ± 0.02^{c}	0.31 ± 0.01^{d}
C16:0	18.37 ± 0.03^{a}	17.01 ± 0.01^{b}	17.30 ± 0.01^{c}	10.08 ± 0.04^{d}
C16:1ω-7	6.02 ± 0.00^{a}	6.26 ± 0.00^{b}	6.99 ± 0.06^{c}	11.00 ± 0.00^{d}
C16:2ω-4	0.17 ± 0.01^{a}	0.13 ± 0.02^{b}	0.61 ± 0.02^{c}	0.38 ± 0.01^{d}
C16:3ω-4	1.35 ± 0.02^{a}	0.37 ± 0.01^{b}	1.07 ± 0.02^{c}	0.17 ± 0.01^{d}
C16:4ω-1	0.49 ± 0.02^{a}	0.15 ± 0.01^{b}	0.61 ± 0.02^{c}	0.25 ± 0.00^{d}
C17:0	1.00 ± 0.01^{a}	0.25 ± 0.01^{b}	0.94 ± 0.02^{c}	0.27 ± 0.03^{d}
C18:0	5.62 ± 0.01^{a}	1.64 ± 0.00^{b}	4.97 ± 0.01^{c}	1.71 ± 0.01^{d}
C18:1ω-9	15.36 ± 0.01^{a}	11.85 ± 0.01^{b}	16.47 ± 0.04^{c}	12.55 ± 0.09^{d}
C18:1ω-7	3.75 ± 0.01^{a}	6.48 ± 0.00^{b}	5.11 ± 0.14^{c}	5.00 ± 0.08^{c}
C18:2ω-6	1.26 ± 0.01^{a}	1.19 ± 0.04^{b}	1.30 ± 0.01^{c}	1.07 ± 0.04^{d}
C18:3ω-6	0.06 ± 0.02^{a}	0.04 ± 0.03^{b}	0.14 ± 0.02^{c}	0.08 ± 0.02^{d}
C18:3ω-3	0.41 ± 0.01^{a}	0.50 ± 0.02^{b}	0.06 ± 0.02^{c}	0.55 ± 0.03^{d}
C18:3ω-4	0.29 ± 0.01^{a}	0.05 ± 0.01^{b}	0.42 ± 0.01^{c}	0.08 ± 0.01^{d}
C18:4ω-3	0.32 ± 0.03^{a}	0.58 ± 0.02^{b}	0.43 ± 0.01^{c}	1.29 ± 0.02^{d}
C20:0	0.23 ± 0.02^{a}	0.05 ± 0.02^{b}	0.54 ± 0.01^{c}	0.11 ± 0.01^{d}
C20:1ω-11	0.96 ± 0.00^{a}	3.56 ± 0.00^{b}	3.85 ± 0.07^{c}	10.66 ± 0.13^{d}
C20:1ω-7	0.49 ± 0.01^{a}	0.55 ± 0.00^{b}	1.66 ± 0.02^{c}	2.17 ± 0.00^{d}
C20:2ω-6	n.d.	n.d.	0.03 ± 0.02^{a}	0.02 ± 0.01^{a}
C20:3ω-6	n.d.	n.d.	0.29 ± 0.02	n.d.
C20:4ω-6	4.67 ± 0.01^{a}	1.35 ± 0.01^{b}	4.66 ± 0.04^{a}	0.97 ± 0.01^{c}
C20:3ω-3	0.21 ± 0.01^{a}	n.d.	0.35 ± 0.02^{b}	0.24 ± 0.01^{c}
C20:4ω-3	0.30 ± 0.01^{a}	0.34 ± 0.01^{b}	0.36 ± 0.01^{c}	0.41 ± 0.01^{d}
C20:5ω-3 (EPA)	13.46 ± 0.02^{a}	23.11 ± 0.00^{b}	9.84 ± 0.12^{c}	13.17 ± 0.03^{d}
C22:1ω-11	0.10 ± 0.04^{a}	1.61 ± 0.01^{b}	0.37 ± 0.04^{c}	11.65 ± 0.09^{d}
C21:5ω-3	0.16 ± 0.01^{a}	0.26 ± 0.01^{b}	0.19 ± 0.01^{c}	0.27 ± 0.01^{b}
C22:5ω-3	1.18 ± 0.02^{a}	0.64 ± 0.00^{b}	1.15 ± 0.01^{c}	0.63 ± 0.02^{b}
C22:6ω-3 (DHA)	21.51 ± 0.02^{a}	18.96 ± 0.01^{b}	16.59 ± 0.23^{c}	10.98 ± 0.01^{d}
C24:1ω-9	0.32 ± 0.01^{a}	0.23 ± 0.01^{b}	0.47 ± 0.03^{c}	0.52 ± 0.01^{d}
SFA	27.01	21.69	26.67	15.67
MUFA	27.14	30.63	35.23	53.79
PUFA	45.85	47.68	38.10	30.55
UFA	72.99	78.31	73.33	84.33
ω-3	37.55	44.39	28.97	27.54
ω-6	6.00	2.58	6.42	2.14
ω-3/ω-6	6.26	17.20	4.51	12.88
PUFA/SFA	1.70	2.20	1.43	1.95
DHA/EPA	1.60	0.82	1.69	0.83
AI	0.31	0.34	0.35	0.27
TI	0.18	0.13	0.22	0.12

Results represent means ± SD (n = 6)
Means in the same row bearing different letters differ significantly ($p < 0.05$)
AI, atherogenic index; MUFA, monounsaturated fatty acids; n.d. not detected; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenic index; UFA, unsaturated fatty acids

Parapenaeus longirostris. DHA and EPA have also been reported as the main PUFA in several shrimp species (Sriket *et al*., 2007; Tsape *et al*., 2010; Bono *et al*., 2012; Yerlikaya *et al*., 2013; Soultani *et al*., 2016), but no correlation was observed between the DHA/EPA ratio and the family of shrimp species. In this sense, Parrish *et al*. (2009) reported that the DHA/EPA ratio could be used as an indicator of the consumption of dinoflagellates and diatoms by shrimps. Therefore, the results could be attributed to the dominance of dinoflagellates in the nutrition of *Parapenaeus longirostris*.

The PUFA/SFA and the ω-3/ω-6 ratios were found significantly higher in both shrimp muscle TL than the respective in cephalothorax TL, presenting the highest value in *Pandalus borealis* muscle (Table 3). Both PUFA/SFA and ω-3/ω-6 ratios were found much higher to the recommended values of 0.45 and 4:1, respectively and therefore can be deemed beneficial for a balanced diet (Simopoulos, 2002; Soultani *et al*., 2016).

The atherogenic (AI) and thrombogenic (TI) indices reflect the different effects that specific fatty acid might have on the probability of increasing the incidence of atherosclerosis or thrombosis (Ulbrich and Southgate, 1991). Muscle TL of both shrimp species showed exceptionally low values of AI and TI, which is appropriate for a healthy diet (Table 3). The above results are similar to those reported by Rosa and Nunes (2003) and Bono *et al*. (2012) for the deep-sea decapod *Parapenaeus longirostris* and the central Mediterranean giant red shrimp (Aristaeomorpha foliacea), respectively.

Conclusion

In the present work, the concentrations of Pb, Hg, Cd and As as well as the fatty acid profile of muscle and cephalothorax of *Parapenaeus longirostris* (Mediterranean shrimp) and *Pandalus borealis* (northern shrimp) were comparatively studied. The identified differences in the element contents and fatty acid proportions seem to be attributed mainly to the fishing area, dietary sources and water temperature. The results indicated low contamination of shrimp samples with Pb, Hg and Cd since their concentrations were found lower than the maximum allowable levels reported by the European Commission. Furthermore, the major fraction of tAs in the shrimp tissues was present as arsenobetaine, which is considered inert and non-toxic. The inorganic form of As was detected at very low amounts only in the cephalothorax tissue. Moreover, results confirmed that the muscle and cephalothorax TL of the studied shrimps were characterised by a low proportion of SFA and a relatively high level of MUFA and PUFA. The fatty acid profile indicated that shrimp muscle constitutes a valuable source of essential fatty acids appropriate for human consumption, whereas cephalothorax might be used by the pharmaceutical industry for the production of medicines and food supplements.

Conflict of interest

The authors declare that there are no conflicts of interest.

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