# The nutritional quality of the red mangrove crab (*Ucides occidentalis),* harvested at two reserves in the Guayas estuary

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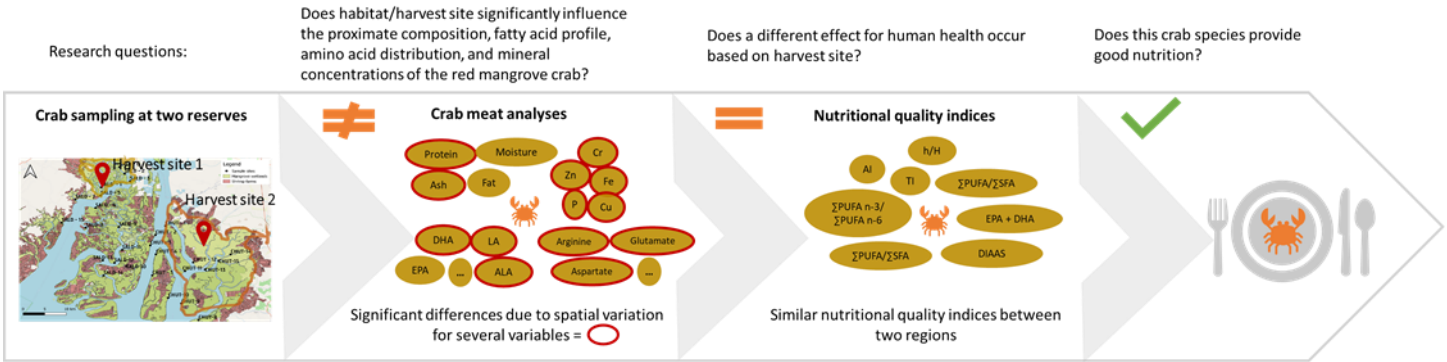
## HIGHLIGHTS

- Harvest site influenced the fatty and amino acid profiles in the crab meat.
- Harvest site influenced the essential mineral concentrations in the crabs.
- The final nutritional quality of the red crab did not differ based on harvest site.
- The red mangrove crab is a good source of nutrition for a balanced diet.

## ABSTRACT

Crabs are an outstanding source of many essential nutrients. Little research has been performed on the nutritional quality of the red mangrove crab (*Ucides occidentalis).* In this research we investigated the proximate composition, fatty acid profile, amino acid composition, and mineral concentrations of red mangrove crabs sampled at two reserves within the Guayas estuary in Ecuador. Furthermore, we evaluated the influence of spatial variation. We found that the red mangrove is a good source of nutrition for a balanced diet and can contribute to the recommended intake of essential nutrients. Also, the red mangrove crab obtained better lipid nutritional quality indices compared to other shellfish species, apart from the EPA+DHA index values. Current research indicated that the final nutritional quality indices of the red crab were not influenced by harvest site. More research into the influence of environmental and ecological factors on the nutritional composition of crabs is suggested.

## GRAPHICAL ABSTRACT



**KEYWORDS:** Red mangrove crab, spatial variation, nutritional value, Ecuador, El Salado reserve, Churute reserve.

## 1. Introduction

Over the years, research has shown that shellfish contains appreciable quantities of digestible proteins, essential amino acids, bioactive peptides, long-chain polyunsaturated fatty acids, vitamin B12, and other vitamins, minerals including micronutrients, and other nutrients, which offer a variety of health benefits to the consumer (Venugopal & Gopakumar, 2017). Belonging to the shellfish category, crab and crab meat are outstanding sources of many nutrients that are essential for human health. Additionally, because of their attractive flavor and taste, crab meat products are very popular worldwide (Nanda et al., 2021). This type of food contains a broad series of polyunsaturated fatty acids (PUFAs) in their tissues, more specifically n − 3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is shown that a balanced fatty acid composition in a human diet is essential for good health (WHO, 1994). The health benefits of highly unsaturated fatty acids, such as arachidonic acid (AA), EPA and DHA are widely recognized (Wu et al., 2010). Previous studies have indicated that the consumption of foods rich in long-chain n − 3 fatty acids is related to a decreased risk of coronary heart disease (Harper & Jacobson, 2005) and cancer (Çelik et al., 2004), while DHA and AA can promote the development of the central nervous system of fetuses and infants (Innis, 2000). On the contrary, excessive levels of AA have been linked with hypertension and Crohn's disease (Wu et al., 2010). Moreover, crustacea contain essential macro and micro minerals, such as calcium, sodium, magnesium, iron, zinc, copper, chromium, potassium, and phosphorus (Venugopal & Gopakumar, 2017).

The red mangrove crab (*Ucides occidentalis*) resides in the mangroves of the west coast of Latin America, from the Espiritu Santo Island of Mexico to the San Pedro estuary in Peru (Zambrano & Meiners, 2018). This species is a traditionally exploited crustacean with high economic importance. Despite being a very popular dish and part of the local culture, the nutritional value of the red mangrove crab has not been previously investigated. Several studies have reported the chemical indices (proximate composition and fatty acid profiles) and nutritive value of crab species in various parts of the world (Barrento et al., 2010; Çelik et al., 2004; Chen, Zhang, & Shrestha, 2007; Skonberg & Perkins, 2002; Wu et al., 2010). From those studies, it can be concluded that the fatty acid profiles and biochemical composition may vary from species to species. Little research has been performed on the differences of harvest habitat on the biochemical composition of a given crab species (Barrento et al., 2010). Also, previous research has shown the presence of metals and pesticides in the crab species under study (De Cock et al., 2021a; De Cock et al., 2021b).

Specifically, our goal was to identify the nutritional value of crab individuals caught at two mangrove forest reserves within the Guayas estuary in Ecuador. Samples were taken from the Salado reserve and the Churute reserve. The Churute reserve was created in 1979 as the first protected mangrove area on the continental coast of Ecuador. Due to the diversity and great extension of aquatic environments that protect the reserve, it was declared a wetland of international importance in 1990 under the Ramsar convention. The reserve contains a mix of diverse ecosystems: mangroves, which are thousands of hectares of trees growing in brackish waters; plains that flood with the tide; a large number of estuaries and arms of the sea; the freshwater lagoon El Canclón, and several hills belonging to the Churute mountain range (Ministerio de ambiente Ecuador, 2015b). The Salado reserve was created in 2002 to protect the mangrove remnants of the estuary from the growth of several urban and rural parishes that progressively have been occupying the mangrove ecosystem. The importance of the creation of the Salado reserve lies not only in being the vital space for the native flora and fauna

of the gulf, but also because of the great scenic, aesthetic and recreational value it has, even more so since it is situated within the largest and most populated city in the country, the city of Guayaquil (Ministerio de ambiente Ecuador, 2015a).

The results of this study provide essential information regarding the influence of harvest site on the nutritional composition of the red mangrove crab and its nutritional quality.

## 2. Materials and Methods

### 2.1. Study area and sampling method

The Guayas estuary is located in the coastal region in the central-western part of Ecuador, within the Gulf of Guayaquil (De Cock et al., 2021b). In November 2020, crab individuals coming from 28 sampling sites in the Guayas Estuary in Ecuador were collected along with water samples in the timespan of one week. Thirteen sampling sites were located in the "Churute mangroves ecological reserve" and surroundings while the other fifteen sampling sites were situated in the "El Salado Mangrove Fauna Production Reserve" and environs. Both reserves are mangrove areas in the estuarine system and are used by local fishermen communities for crab catch. At each site, 6 male crabs were bought from the crab-catchers. The capture of female crabs is prohibited by the Ecuadorian government (De Cock et al., 2021b). In that sense, the results of the present study indicate the nutritional characteristics of crabs that are available for consumers. Zambrano and Meiners (2018) report that the size at morphometric sexual maturity of male red mangrove crabs is 63.5 mm of cephalothorax width (AC) with a confidence interval (IC) between 62.9 and 64.5 mm AC. The minimum size of the crabs that were captured was 65.3mm, indicating that all crab samples were sexually mature. A knife was inserted through the rostrum of each crab. Afterward, the crabs were washed with distilled water, weighed, and the carapax width was measured. The crabs were labeled and stored in Ziplock bags at -4°C, transported frozen, and stored at -20°C upon arrival in Ghent, Belgium. Furthermore, physiochemical properties of water were measured at each site.

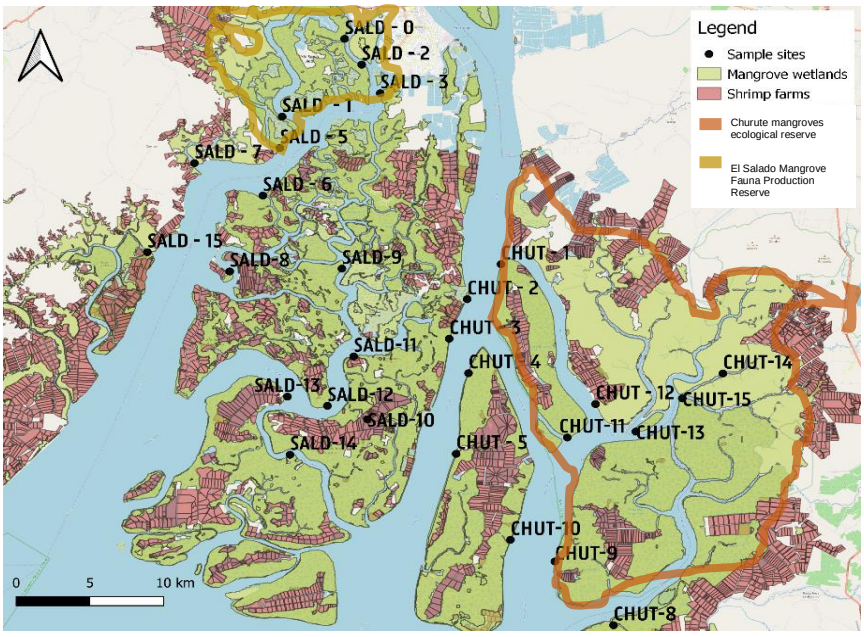



**Figure 1.** Sampling sites in Guayas estuary, Ecuador.

## 2.2. Sample processing

The crab samples were thawed overnight. The next day, the samples were washed and excess mud was removed carefully through water rinsing. Subsequently, all legs were pulled out and carefully crushed to extract the white meat with tweezers. The extracted crab meat was collected in a glass beaker and homogenized with an Ultra-Turrax (T 25 ultra Turrax - IKA) for 10 minutes until a smooth substance was obtained. After each sample, the mixer was carefully cleaned with distilled water and acetone, and after each 6 crab samples from the same site, the mixer was cleaned with 2 % HCl. Per sample, 2 g of crab meat was stored in a 5 mL Eppendorf for essential element analysis, and 6 g was stored as a backup sample. The remaining fractions of 6 crabs per sampling site were pooled. The pooled samples were divided into 5 g (in double) for fatty acid analysis, 1 g (in double) for protein analysis, 5 g ( in double) for ash content, and 7.5 g ( in double) for fat content and dry weight. All samples were stored at -20 °C until analysis.

## 2.3. Physiochemical properties of water

Some physiochemical properties of the water were measured *in situ* with the portable multiparameter HQ40d (HACH), at 50 cm under the water surface. For the dissolved oxygen (DO) and temperature the Intellical Optical / Luminescent Dissolved Oxygen Electrode LDO101 (HACH) (including a temperature sensor) was used, with an accuracy of ±0.1 mg/L and ±0.3 °C. For the pH, the Intellical PHC101 pH Electrode (HACH), with an accuracy of ± 0.02 pH was used and for the conductivity (EC) and salinity the Intellical CDC401, 4 Pole Graphite Conductivity Cell (HACH) was used with an accuracy of ± 0.5 % for EC and ± 0.1 for salinity. The probes were calibrated the day before sampling. All the measurements were performed simultaneously for crab sample collection at each site.

## 2.4. Proximate composition

Crude ash was determined by incineration at 550 °C for 3h in a combustion oven, after ISO 5984-2002. The moisture content was determined by the tissue weight difference after drying in a 105 °C oven until constant weight is reached, after ISO 1442-1973. Crude protein was determined using the Kjeldahl method, with a 6.25 nitrogen-to-protein conversion factor (Barrento et al., 2010). The analysis method of crude fat content was performed after ISO 1444-1973 using petroleum ether as an extraction solvent.

## 2.5. Fatty acids

The lipids were extracted from the samples using chloroform/methanol (2/1; v/v), modified after Folch et al. (1957). Fatty acids were methylated according to Raes et al. (2001) and analyzed by gas chromatography (HP6890, Brussels, Belgium) equipped with a CP-Sil88 column (100 m x 0.25 mm x 0.25 mm; Chrompack, Agilent Technologies, Eindhoven, The Netherlands) and a flame ionization detector. The gas chromatography conditions were as follows: Injector (2µl), 250 °C (split 1/100); detector, 280 °C; hydrogen as carrier gas; temperature program: 150 °C for 2 min, followed by an increase of 1 °C/min to 200 °C and then 5 °C/min to 215 °C. Gas flows for the FID were nitrogen, air, and hydrogen. Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., Elysian, MN, USA; Sigma–Aldrich, Bornem, Belgium). C13:0 was used as an internal standard to quantify the individual and total fatty acids. The response factors can be found in **Table A.1**.

## 2.6. Essential minerals

Approximately 0.3 g sample was transferred into a digestion vessel; then, 7.5 ml of 37 % HCl and 2.5 ml of 67 % $HNO_3$ (pico pure quality) were added. Afterward, microwave destruction at 180 °C in closed

Teflon liners was performed, using a MARS-6 microwave digester (CEM Corporation, Matthews, NC, USA). After the destruction, the digestate was diluted to 50 mL. In the extracts, chromium (Cr) was determined using inductively coupled plasma mass spectrometry (ICP-MS Elan DRC-e, PerkinElmer, Waltham, MA, USA). Zn, P, Fe, Al, and Cu were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES Vista-PRO, Agilent Technologies, Santa Clara, CA, USA). The data are reported in µg/kg fresh weight (fw).

### 2.7. Amino acids

All amino acids were measured except for tryptophane, methionine, and cysteine. The method of the acid hydrolysis was based on "Commission directive 98/64/EC (1998) establishing Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC: Determination of amino acids" (European Commission, 1998). Firstly, we performed acid hydrolysis with 6M HCl. After neutralization with NaOH, the samples were brought to pH 2.2. Afterward, the samples were appropriately diluted, and then the samples were ready for online derivatization with OPA and FMOC (**Table A.2**). After chromatographic separation on an AMINO ADBIO AAA column (Agilent), the amino acids were detected with a fluorescence meter.

### 2.8. Nutritional quality

Regarding the fatty acid composition, several indices have been previously reported that indicate the nutritional quality of a certain diet. The Atherogenic index (AI) indicates the relationship between the sum of saturated fatty acids (SFAs) and the sum of unsaturated fatty acids (UFAs) as shown in **equation 1** (Ulbricht & Southgate, 1991).

$$IA = \frac{C12{:}0 + (4xC14{:}0) + C16{:}0}{\Sigma UFA} \quad (1)$$

The Thrombogenic index (TI) indicates the relationship between the pro-thrombogenic FAs (C12:0, C14:0, and C16:0) and the anti-thrombogenic FAs (MUFAs and the n-3 and n-6 families) as shown in **equation 2** (Ulbricht & Southgate, 1991).

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{(0.5x\Sigma MUFA) + (0.5x\Sigma n-6PUFA) + (3x\Sigma n-3PUFA) + (\Sigma n-3PUFA/\Sigma n-6PUFA)} \quad (2)$$

The ratio of hypo and hypercholesterolemia (h/H) characterizes the relationship between emic fatty acid (cis-C18:1 and PUFA) and hypercholesterolemic fatty acids as shown in **equation 3** (Chen & Liu, 2020).

$$hH = \frac{cis\ -\ C18{:}1\ +\ \Sigma PUFA}{C12{:}0\ +\ C14{:}0\ +\ C16{:}0} \quad (3)$$

Additional indices such as ∑PUFA n-3/ ∑PUFA n-6, ∑PUFA n-6/ ∑PUFA n-3, ∑PUFA/∑SFA, and EPA+DHA will be discussed in the results and discussion sections.

A daily recommended portion was determined for the red mangrove crab corresponding to the intake (expressed in g per day) needed to achieve the 250 mg EPA + DHA daily dietary requirement for an adult (Chen & Liu, 2020).

Based on the essential elements, the Recommended Intake Values (RIV) for men and women were calculated as shown by **equation 4** (Franco-Fuentes et al., 2021).

$$RIV = RWI\ x\ average\left(\frac{1000\ gr\ of\ fresh\ weight}{mg\ of\ metal}\right) \quad (4)$$

with RWI the recommended weekly intake as reported by Trumbo et al. (2001). In addition, the percentage of Weekly Contribution (WC) of each element was calculated with **equations 5 and 6** (Franco-Fuentes et al., 2021).

$$EWI = Res\ x\ CPW \quad (5)$$

$$WC = \frac{EWI}{RWI}\ x\ 100 \quad (6)$$

where Res is the concentration of the metal in the crab tissue (mg metal/kg fw). CPW is the crab Consumption Per Week. Based on pesticide and metal concentrations found in the crab tissue, previous research has indicated a maximum intake of 8 crabs per month for adults (De Cock et al., 2021a). Therefore, in this study, we assumed an intake of 2 crabs per week which coincides with 67.36 g/week based on the crab yield (**Table A.3**). Thus, the EWI indicates the weekly intake in grams per person for each metal.

Furthermore, to analyze the nutritional quality of proteins, various indices have been developed during the past years such as the amino acid score (AS), Protein-digestibility corrected amino acid score (PDCAAS), Digestible indispensable amino acid score (DIAAS), and the Essential Amino Acid Index (EAAI) . In this study, we calculated the most recently developed DIAAS % = 100 x lowest value [(mg of the digestible dietary indispensable amino acid score (DIAAS) in 1 g of the dietary protein)/(mg of the same dietary indispensable amino acid in 1g of the reference protein)] (FAO Expert Consultation, 2011). The digestible IAA content was calculated by multiplying the ileal digestible coefficients (Shaheen et al., 2016) with the concentration of each amino acid (mg/g protein).

### 2.9. Data analysis

All statistical analyses were conducted with R Version 4.1.2 (R Core Team, 2019). All data are presented as mean and standard deviation (SD). To verify the difference between the data collected in samples from Churute and Salado the Unpaired Two-Samples T-test was performed after confirming the data was normally distributed and no significant ($p < 0.05$) difference occurred between the variances of the two sets of data. The Welch t-test was used when the two samples had possibly unequal variances. In case the data was not normally distributed, the unpaired two-samples Wilcoxon test was computed. Moreover, Spearman correlation tests were performed to search for relations between the fatty acids in the crab meat and the physiochemical water parameters on the one hand and the crab size on the other hand. All statistical tests were evaluated at a significance level of 0.05. We applied an upper-limit scenario for the essential element data by setting all concentrations below the LOQ equal to the LOQ. PCA (Principal component analysis) in Rstudio was used to analyze data for possible clusters. All the obtained nutritional data (amino acids, fatty acids, proximate composition, and essential minerals) was subjected to this analysis. PCA was applied to reduce the dimensionality of the data and to explore patterns and relationships in the studied samples based on their nutritional characteristics. The sampling locations were graphically illustrated using QGIS version 3.16.14.

## 3. Results

### 3.1. Proximate composition

The proximate composition of the red mangrove crab was determined (**Table 1**). The average moisture, protein, fat, and ash content of the red mangrove crab were found to be 83.14 ± 1.18 %, 13.38 ± 0.85 % FW, 0.45 ± 0.49 % FW, and 2.49 ± 0.24 % FW respectively.

**Table 1:** The proximate composition of the red mangrove crab meat expressed as percentage and percentage of fresh weight (FW) for pooled samples from Churute reserve (n=13) and Salado reserve (n=15).

| | Churute | | Salado | | Average | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Moisture (%)** | 82.73 | 1.43 | 83.49 | 0.81 | 83.14 | 1.18 |
| **Protein (% FW)** | 13.87 | 0.92 | 12.96 | 0.52 | 13.38 | 0.85 |
| **Fat (% FW)** | 0.45 | 0.45 | 0.45 | 0.53 | 0.45 | 0.49 |
| **Ash (% FW)** | 2.30 | 0.15 | 2.66 | 0.19 | 2.49 | 0.24 |

3.2. Fatty acids

The main saturated fatty acids were palmitic (16:0) and stearic (18:0) acids, while oleic acid (18:1) was the dominant monounsaturated fatty acid (**Table 2**). The fatty acid profile of the red mangrove crab was dominated by polyunsaturated fatty acids (PUFA), which comprised 39.31 % of the total lipids. The dominant PUFAs were linoleic acid (LA, 18:2n – 6), α-linolenic acid (ALA, 18:3n – 3), arachidonic acid (AA, 20:4n – 6), eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acids (DHA, C22:6n-3) of which the contents averaged 11.26, 9.67, 4.97, 4.75, and 1.50 g/100 g fatty acid methyl esters, respectively. The n-3 fatty acids accounted for 19.33 % of the total FA and 49.16 % of all PUFAs. The n-6 PUFAs accounted for 19.33 % of the total fatty acids and 50.84 % of the total PUFA.

**Table 2**: The average fatty acid profile of the red mangrove crab meat expressed as percentage values (g/100 g fatty acid methyl esters) for pooled samples from Churute reserve (n=13) and Salado reserve (n=15), indicating mean and standard deviation (SD).

| | Churute | | Salado | | Average | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| *Saturated fatty acid (SFA) composition (%)* | | | | | | |
| **C10:0** | 1.16 | 0.39 | 0.95 | 0.36 | 1.05 | 0.38 |
| **C11:0** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **C12:0** | 0.04 | 0.11 | 0.03 | 0.08 | 0.04 | 0.09 |
| **C14:0** | 0.20 | 0.16 | 0.18 | 0.07 | 0.19 | 0.12 |
| **C15:0** | 0.21 | 0.19 | 0.20 | 0.18 | 0.20 | 0.18 |
| **C16:0** | 12.61 | 0.66 | 12.33 | 1.29 | 12.46 | 1.04 |
| **C17:0** | 0.44 | 0.11 | 0.41 | 0.11 | 0.42 | 0.11 |
| **C18:0** | 9.10 | 0.62 | 8.96 | 0.67 | 9.02 | 0.64 |
| **C19:0** | 0.90 | 0.56 | 0.92 | 0.60 | 0.91 | 0.57 |
| **C20:0** | 0.00 | 0.01 | 0.01 | 0.04 | 0.01 | 0.03 |
| **C22:0** | 0.03 | 0.02 | 0.05 | 0.04 | 0.04 | 0.03 |
| **C24:0** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **∑SFA** | 24.70 | 1.44 | 24.04 | 1.74 | 24.35 | 1.62 |
| *Monounsaturated fatty acid (MUFA) composition (%)* | | | | | | |
| **C14:1** | 1.17 | 0.19 | 1.13 | 0.19 | 1.15 | 0.19 |
| **C16:1** | 3.19 | 0.75 | 3.64 | 0.83 | 3.43 | 0.81 |
| **C17:1** | 0.41 | 0.16 | 0.45 | 0.13 | 0.43 | 0.14 |
| **c9C18:1** | 20.76 | 1.66 | 21.37 | 1.45 | 21.08 | 1.55 |
| **c11C18:1** | 0.49 | 0.13 | 0.53 | 0.16 | 0.51 | 0.15 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **C20:1** | 0.20 | 0.05 | 0.19 | 0.06 | 0.20 | 0.06 |
| **C22:1** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **C24:1** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **∑MUFA** | 26.22 | 1.61 | 27.31 | 2.25 | 26.80 | 2.02 |
| *Polyunsaturated fatty acid (PUFA) composition (%)* | | | | | | |
| **C18:2n-6** | 11.98 | 1.26 | 10.63 | 1.64 | 11.26 | 1.60 |
| **C18:3n-6** | 0.12 | 0.09 | 0.12 | 0.08 | 0.12 | 0.08 |
| **C20:2n-6** | 0.42 | 0.06 | 0.38 | 0.06 | 0.40 | 0.06 |
| **C20:3n-6** | 0.10 | 0.05 | 0.11 | 0.07 | 0.10 | 0.06 |
| **C20:4n-6** | 4.42 | 0.70 | 5.44 | 0.76 | 4.97 | 0.89 |
| **C22:4n-6** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **C22:5n-6** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **∑n-6 PUFA** | 17.04 | 1.33 | 16.68 | 1.45 | 16.85 | 1.38 |
| **C18:3n-3** | 10.88 | 2.11 | 8.62 | 1.23 | 9.67 | 2.02 |
| **C20:3n-3** | 0.46 | 0.16 | 0.26 | 0.15 | 0.35 | 0.18 |
| **C20:4n-3** | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 |
| **C20:5n-3** | 4.47 | 1.07 | 4.99 | 0.90 | 4.75 | 1.00 |
| **C22:5n-3** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **C22:6n-3** | 1.32 | 0.40 | 1.66 | 0.28 | 1.50 | 0.38 |
| **∑n-3 PUFA** | 17.15 | 1.72 | 15.54 | 1.71 | 16.29 | 1.87 |
| **∑PUFA** | 34.19 | 3.05 | 32.22 | 3.17 | 33.13 | 3.25 |
| **Sum** | 85.11 | 1.29 | 83.57 | 3.01 | 84.28 | 2.46 |

**Table 3**: Lipids nutritional quality indices of the red mangrove crab.

| | **Churute** | **Salado** | **Average** |
|---|---|---|---|
| **∑PUFA n-3/ ∑PUFA n-6** | 1.01 | 0.93 | 0.97 |
| **∑PUFA n-6/ ∑PUFA n-3** | 0.99 | 1.07 | 1.03 |
| **∑PUFA/∑SFA** | 1.38 | 1.34 | 1.36 |
| **Atherogenic index (AI)** | 0.22 | 0.22 | 0.22 |
| **Thrombogenic index (TI)** | 0.30 | 0.31 | 0.30 |
| **Ratio of hypo and hypercholesterolemia (h/H)** | 4.21 | 4.22 | 4.22 |
| **EPA+DHA** | 5.79 | 6.65 | 6.25 |

The values of the lipids nutritional quality indices of the red mangrove crab are reported in **Table 3**. The daily recommended intake value needed to achieve the 250 mg EPA + DHA/day dietary requirement resulted in 19.81 g crab meat/day since an average value of 12.62 EPA+DHA mg/100g was found (**Table A.4**). The daily recommended intake corresponds with around 58 crab per day.

### 3.3. Essential elements

The concentrations of Zn, Cr, Cu, Fe, and P in the crab meat were quantified. Based on Fe concentrations, a recommended intake value of 748 g crab meat per week or 22 crabs per week is advised assuming crabs are the only source of iron in a diet. Of course, iron occurs in many nutrition sources and it appears that the crab intake does not contribute substantially to the recommended intake of iron as the weekly contribution percentage was 1.30 % for men and women. The EWI for phosphorus, being 73.6 mg/week, appeared higher than the EWI of the other elements. Copper presence in the mangrove crab appeared to contribute substantially with 16.7 % to the recommended

weekly dose of consumers. The Zn concentrations in the crab contributed between 4.8 and 6.6 % to the recommended Zn intake per capita.

**Table 4**: Average values of Recommended Weekly Intakes (RWI) (mg/week), Estimated Weekly Intake (EWI), Recommended Intake Value (RIV), and percentage of Weekly Contribution for essential elements present in the meat of the red mangrove crab for pooled samples from Churute reserve (n=13) and Salado reserve (n=15).

| | Mean (mg/kg fw) | SD | EWI (mg metal/week) | RWI (mg metal/week) | | RIV (g crabmeat/dag) | | RIV ( N°crabs/day) | | WC (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Men | Woman | Men | Woman | Men | Woman | Men | Woman |
| **Zn** | 55.2 | 6.0 | 3.7 | 77.0 | 56.0 | 199.2 | 144.9 | 5.9 | 4.3 | 4.8 | 6.6 |
| **Cr** | 0.1 | 0.2 | 0.0 | 0.2 | 0.2 | 308.5 | 220.4 | 9.2 | 6.5 | 3.1 | 4.4 |
| **Cu** | 15.6 | 3.1 | 1.1 | 6.3 | 6.3 | 57.6 | 57.6 | 1.7 | 1.7 | 16.7 | 16.7 |
| **Fe** | 10.7 | 10.4 | 0.7 | 56.0 | 56.0 | 748.4 | 748.4 | 22.2 | 22.2 | 1.3 | 1.3 |
| **P** | 1092.7 | 157.4 | 73.6 | 4900.0 | 4900.0 | 640.6 | 640.6 | 19.0 | 19.0 | 1.5 | 1.5 |

### 3.4. Amino acid composition

The amino acid compositions are shown in **Table A.5**. The major amino acids found were aspartate, glutamate, and arginine (with tryptophane, methionine, and cysteine not measured). The DIAAS value obtained for the samples from the Churute reserve was 95.8 % and for the Salado reserve was 81.9 % (**Table A.6**). Those indicate the protein quality of the red mangrove crab to be good. A DIAAS value greater than 100 refers to food with an "excellent" quality protein source. A food item can be considered a "good" quality protein source if the DIAAS value is between 75 and 99 (Shaheen et al., 2016).

### 3.5. Influence of harvest sites

#### 3.5.1. Nutritional data differences

The differences between the results of the proximate composition, a selection of fatty acids, the amino acids, the mineral content, and the carapax length obtained for the crab samples from the Churute reserve on the one hand, and the Salado reserve, on the other hand, were analyzed. Also, the physiochemical parameters measured at the sampling sites were analyzed to evaluate differences between the environmental conditions at the two reserves. **Figure 2** gives a graphical overview of all concentrations in the two reserves, the asterisk indicates those variables with a significant difference ($p < 0.05$) in results between the two reserves. The proximate composition, the ash, and protein content differed significantly. All minerals also differed significantly between the two harvest sites except for P. No consistent trend could be discovered in the differences in essential nutrients between the regions: Cr and Cu concentrations appeared higher in crabs from Salado, but Fe, Zn, and P concentrations in the red mangrove crabs were higher from the Churute reserve. Furthermore, a location difference was detected for several PUFAs, namely AA, ALA, LA, and DHA. AA and DHA appeared in higher concentrations in crabs from the Salado reserve while the opposite applied for ALA and LA. In general, a difference in the reserves between the n-3 PUFAs was found. We found that for 13 of the 16 amino acids a significant difference in the concentrations between the two reserves existed. Also, the conductivity and salinity values measured in the water samples were different between the two reserves with values significantly higher in the Salado reserve sampling sites (**Figure A.1**). The differences between the two regions regarding the lipids nutritional quality indices (**Table 3**) were small for all indices. Finally, the carapace width of the crabs harvested at the Churute reserve (average: 8.07 cm) appeared significantly higher than the carapace width of crabs from the Salado

reserve (average: 7.75 cm) (**Figure A.2**). The protein quality (DIAAS) for the samples obtained from the Churute reserve appeared to be better than those samples from the Salado reserve (**Table A.6**).

### 3.5.2. PCA plot

**Figure A.3** shows the score and loading plot of the PCA of the obtained nutritional data. PC1 explains 30.37 % and PC2 13.67 % of the variability in the data set, together they explain 44.04 % of the variability. This plot shows distinct clusters of the samples from Churute and Salado. This means the samples divided into groups based on harvest site appear to be similar in terms of their nutritional value. Since clustering occurs, it can be stated that significant differences exist in the nutritional data on which the harvest site groups can be separated. Furthermore, it can be seen that most variables have loadings between 0.1 and 0.4; this means the variables have a medium influence on the principal components and therefore on the samples.

## 3.6. Correlation tests

A negative correlation between the carapace width and the AA concentration in the crab meat was found (**Figure A.4**). Furthermore, both AA and DHA concentrations in crab meat were positively correlated with the conductivity and salinity measured in the water samples as well as the MUFAs. Also, a negative correlation was found between ALA and LA concentrations and the conductivity and salinity (**Figure A.5**). The same accounted for the n-3 PUFAs. The temperature of the water was not correlated with the fatty acid concentrations apart from the SFA group. A correlation was found between the turbidity and the EPA and LA fatty acids.

Interestingly, a positive correlation was found for all amino acids with the carapax width (**Figure A.6**). The temperature of the water was not correlated with the amino acid concentrations. All amino acids, except histidine, alanine, and proline, were negatively correlated with the conductivity measured in the water (**Figure A.7**). Furthermore, the same amino acids, excluding Aspartate, were also negatively correlated with the salinity.

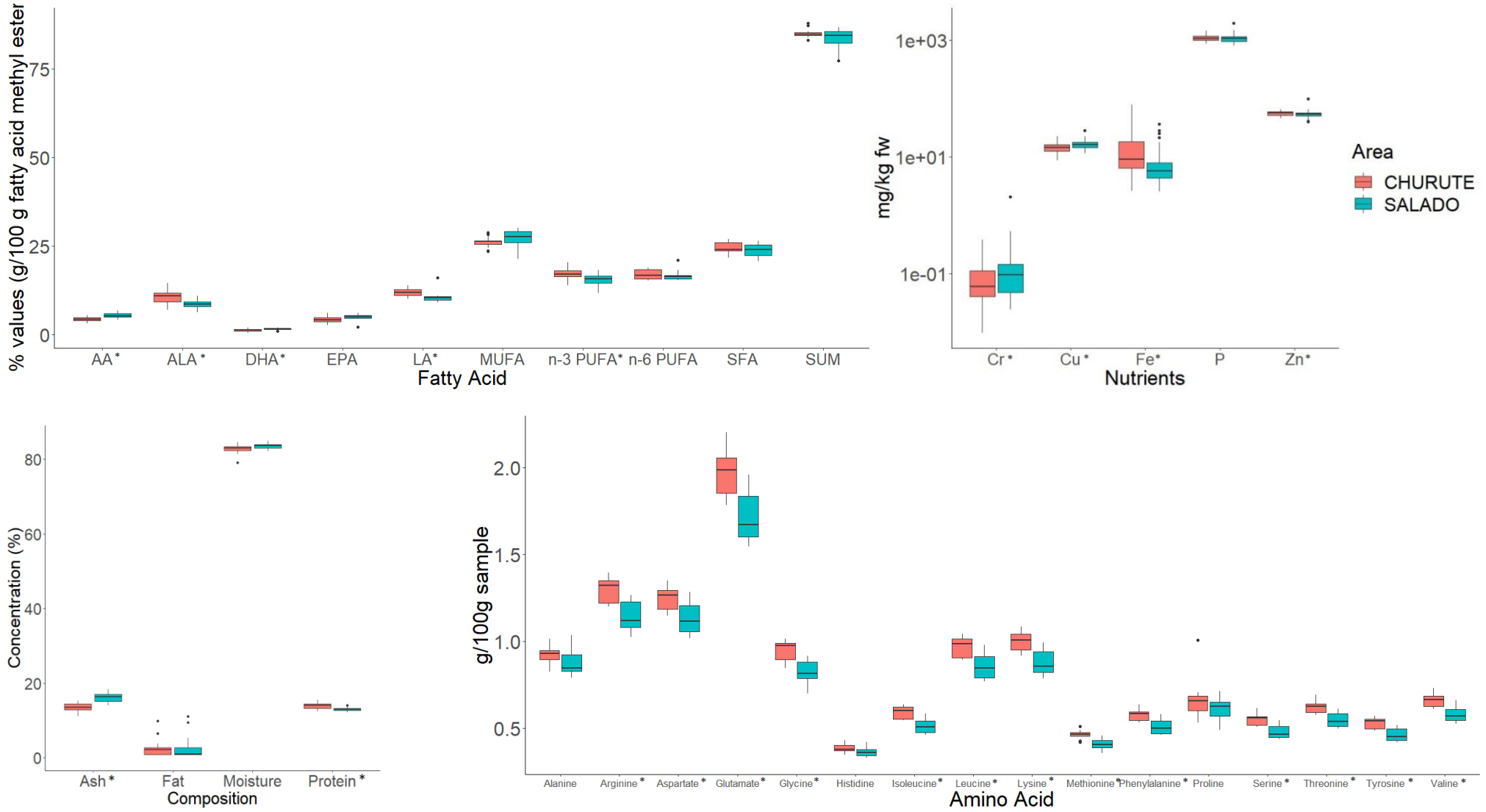



**Figure 2.** Fatty acid, proximate composition, and mineral value distributions in Churute and Salado reserve. The asterix (*) indicates a significant difference ($p < 0.05$) between the results for the Salado and Churute reserves.

## 4. Discussion

### 4.1. Nutritional quality comparison

Regarding the proximate composition, we found that the average moisture content of the red mangrove crab meat (83.14 %) is higher than the one of the Chinese mitten crab (78.8 %), the blue swimmer crab (79.5 %), the brown (74.6 %), and green crab (79.0 %)(Barrento et al., 2010; Chen, Zhang, & Shrestha, 2007; Skonberg & Perkins, 2002; Wu et al., 2010). Furthermore, the red mangrove crab protein content (13.38 %) was lower compared to the previously mentioned species (Chinese mitten crab: 18.9 %; brown crab: 20.5 %; blue swimmer crab: 16.9 %; green crab: 16.8 %). The nutritional differences between crab species could be caused by habitat characteristics, age, sex, season, water quality etc. (Barrento et al., 2010). The fat content of red mangrove crab (0.45%) was similar to that of green crab (0.50 %) investigated in the study by Nanda et al. (2021). On the other hand, it was lower than that found in the same study in the Chinese crab (0.90 %) and the blue crab (0.75 %), while the lowest content was observed in the brown crab (0.20 %). The ash content of the red mangrove crab was similar to the male brown crab (2.2 %) and higher than the Chinese mitten crab (1.90 %) (Barrento et al., 2010; Chen, Zhang, & Shrestha, 2007).

Recently, reviews have been published regarding the use of a broad range of extraction and quantification methods that vary in precision and accuracy, producing different nutrient estimations for a given sample, and across sample types and taxa (Zaguri, Kandel, Lavie, & Hawlena, 2021). In that sense, we should mention that the protein and lipids results reported in this study have been obtained using common methods based on standard practices, however, they are rather an estimation, and a comparison with other studies must be done carefully.

Concerning the fatty acid composition, similar results were reported by Barrento et al. (2010), the main SFA and MUFA in the brown crab (*Cancer pagurus*) meat were respectively palmitic acid and oleic acid. Çelik et al (2004) and Naczk et al. (2004) reported mostly lower values for the blue and green crab, namely 16:0 (13.5 % and 9.80 %), 18:0 (6.29 % and 6.31 %) and 18:1 (12.9 % and 10.3 %) respectively. Oleic acid appears higher in the red mangrove crab. It has been shown that the presence of this fatty acid in a certain diet is associated with a diminished risk of coronary heart disease (Harper & Jacobson, 2005). The sum of polyunsaturated fatty acids (PUFA) found in the red mangrove crab is higher compared to the one of the Chinese mitten crabs (23.87 %) as reported by Chen et al. (2007), but lower compared to the green crab (50.5 %) as reported by Nack et al. (2004). Barrento et al. (2010) reported that the main PUFA in the brown crab meat were eicosapentaenoic EPA and DHA, while based on our study, the main PUFA for the red mangrove crab were LA and ALA. The nutritional quality of fish and seafood is considerably linked to the content of essential fatty acids (EFAs), such as α-linolenic acid (ALA), linoleic acid (LA), and other omega-3 polyunsaturated fatty acids (n-3 PUFAs). ALA is a precursor of the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and LA is a precursor of arachidonic acid (AA) which, consecutively, is the main precursor of eicosanoids (Gil, 2002). AA and DHA are important elements of cell membrane phospholipids and are the main long-chain PUFAs of the central nervous system. Long-chain PUFAs assemble quickly in the brain during the period of maximal brain growth, which takes place from the last trimester of pregnancy until 2 years of age in humans (Innis, 2000). High levels of AA potentially benefit the pathogenesis of various diseases, such as Crohn's disease (Wu et al., 2010) and inflammatory diseases (Gil, 2002). All previously mentioned fatty acids were present in the red mangrove crab and therefore may promote the health of the consumers of this commercial crab species in Ecuador. Based on the lipids nutritional indices, the red mangrove crab showed to have similar and often better nutritional value compared to other shellfish and fish species worldwide. For example, the n-3/n-6 ratio is a valuable index to compare the relative nutritional value of the fatty acid composition of seafood oils. A higher ratio of n-3/n-6 PUFAs has been reported as an indication of high nutritional value (Chen, Zhang, & Shrestha, 2007). For the

red mangrove crab, the ratio of n-3/n-6 was 0.97 (**Table 3**). This ratio falls between the ratio of the Chinese crab (0.45) and the blue crab (3.18) (Çelik et al., 2004; Chen, Zhang, & Shrestha, 2007). Furthermore, an n-6/n-3 ratio below 5 is recommended for optimal health and growth (Chen, Zhang, & Shrestha, 2007). An increase of this ratio may cause adverse health effects, such as increased risks of cardiovascular diseases, obesity, cancer and autoimmune diseases. For the red mangrove crab, the ratio was far below (1.03). Next, the PUFA/SFA is an index regularly used to evaluate the impact of diet on cardiovascular health, the higher this ratio, the more positive the effect (Chen & Liu, 2020). The PUFA/SFA value for the red mangrove crab is 1.36, Barrento et al. (2010) reported a higher value of 2.8 for the male brown crab. Chen and Liu (2020) reported values for fish and shellfish similar to the red mangrove crab ratio. Also, the sum of EPA and DHA indicates the nutritional value, since these n-3 PUFAs play an essential role in the retina and nervous system functioning. EPA+DHA in the red crab yielded 6.25 %, a value that is lower compared to several crab species: the mud crab, swimming crab, blue crab, Southern king crab, and soldier crab as reported by Nanda et al. (2021). The recommended intake value based on the EPA+DHA diatary requirement indicated 58 crabs per day. This, however, exceeds the previously advised limit of 8 crabs/month, based on the metal presence, to prevent negative health effects in the long term (De Cock et al., 2021a). Also, it can be stated that no crab consumer would reach the requirement on a daily basis. Therefore, it is advised to red mangrove crab consumers to complement their diet with other food sources rich in EPA+DHA such as fish. The index of Atherogenicity (AI) defines the atherogenic capacity of the fatty acid composition in a certain diet. The index was developed by Ulbritcht and Southgate in 1991 (Ulbricht & Southgate, 1991). A low AI value suggests less adhesion of lipids to cells of the circulatory and immunological systems. The AI value for the red mangrove crab was 0.22, slightly higher than the AI for the male brown crab (0.18) but lower than the AI reported for various shellfish, and fish species by Chen et al. (2020). Also, the index of thrombogenicity (TI) was developed by Ulbritcht and Southgate in 1991 (Ulbricht & Southgate, 1991). The TI, in his turn, defines the thrombogenic potential of fatty acids, indicating the propensity to develop clots in blood vessels. So, the consumption of food products with a lower TI is beneficial for cardiovascular health. Anew, the thrombogenic index (TI) value for the red mangrove crab (0.30) appeared slightly higher than the TI for the male brown crab (0.12) (Barrento et al., 2010). The cholesterol metabolism is associated with the h/H ratio which characterizes the ratio of total hypocholesterolemic/hypercholesterolemic fatty acids (Özden, Erkan, Kaplan, & Karakulak, 2020). Unlike IA and TI, high h/H values are urged for improved health benefits. The h/H ratio for the red mangrove crab (4.22) is higher compared to various shellfish and fish species (range: 0.87-2.93) reported by Chen et al. (2020). Also, the h/H ratio for bluefin Tuna (3.02) appears to be lower (Özden, Erkan, Kaplan, & Karakulak, 2020). In general, the red mangrove crab scored generally better on all lipid nutritional quality indices compared to other crab species, apart from the EPA+DHA index. Based on all indices, it can be concluded that the red mangrove crab can be part of a healthy and balanced diet.

Zn and Cu concentrations found in the red crabs were higher compared to those found in 4 fish species (*C. princeps, S. violacea, T. albacares, and M. olfax*) from the Galapagos Archipelago as observed in the study of Franco-Fuentes et al. (2021). Fe and Cr concentrations in the red mangrove crab appeared lower compared to *C. princeps, S. violacea, T. albacares,* and *M. olfax.* Furthermore, all mineral concentrations found in the crabmeat in this study were higher compared to the mineral content of the Chinese mitten crab (Chen, Zhang, & Shrestha, 2007). Compared to three crab species described by Zotti et al. (2016b), the mineral content appeared similar in terms of Zn and Cr to the warty crab (*Eriphia verrucose*). The Fe and Cu concentrations in the red crab were higher compared to the three crab species (*Callinectes sapidus, Eriphia verrucosa; and Cancer pagurus*) (Zotti et al., 2016b). Shellfish minerals include both macro elements (Na, K, Ca, Mg) and micro elements (Cr, Co, Cu, F, Br, Fe, Se, Zn, and Mn) (Venugopal & Gopakumar, 2017). In this study, the concentrations of Zn, Cr, Cu,

Fe, and P in the crab meat were quantified. Of those, Zn, Cu, P, and Fe are considered essential elements. Zinc has a range of vital physiological functions and occurs within every cell in the body (EFSA, 2014). Zinc deficiency in developing countries can induce reduced resistance against infection, particularly in children, and on severe occasions, it may cause hypogonadism and dwarfism (Zoroddu et al., 2019). Iron forms part of at least hundred enzymatic reactions and is an essential element for almost all living organisms (Zoroddu et al., 2019). Copper is an essential micronutrient necessary for electron transfer processes. It is a central component of many enzymes, including those involved in neurotransmitter synthesis, energy metabolism and collagen and elastin cross-linking (EFSA, 2015a). Chromium, as the trivalent ion, was long considered an essential element, but the results of new studies indicate that chromium currently can only be considered pharmacologically active and not an essential element (Vincent, 2017). Phosphorus is part of many physiological processes, such as in the cell's energy cycle, regulation of the body's acid–base balance, as a component of the cell structure, cell regulation and signaling, and the mineralization of bones and teeth (EFSA, 2015b). The results indicated that the red mangrove crab meat is an excellent source of Cu and Zn, and a regular source of Fe, P, and Cr. In particular, due to the relatively high Cu concentrations, red mangrove crab meat could be recommended to people with this mineral deficiency. Based on the weekly contribution of the metals, it can be concluded that the mangrove crabs can attribute to the recommended intake of essential elements, but that other food sources should be considered to complete a balanced diet.

Importantly, apart from the fatty acids previously discussed, also protein is a fundamental nutrient for humans. Essential amino acid composition is one of the most important nutritional qualities of protein (Barrento et al., 2010). The amino acid composition found in this study was similar to previously reported values of the Chinese mitten crab (Chen, Zhang, & Shrestha, 2007). Similarly, the major amino acids found for the red mangrove crab as well as for the Chinese mitten crab were aspartate, glutamate, and arginine, (with tryptophane, methionine and cysteine not measured). Importantly, these crabs are a source of protein of high biological value and high digestibility, especially for fishing communities that survive on these foods for subsistence.

### 4.2. Harvest site effect

Differences in the protein,- and ash content, fatty acid,- amino acid,- and mineral composition of crabs from Churute on the one hand and Salado, on the other hand, were observed. Previous research has indicated that fatty acid profiles in fish depended on features such as fish size and lipid content (Usydus, Szlifder-Richert, & Adamczyk, 2012). In 2010, Barrento et al. (2010) described that the biochemical composition of edible tissues of marine invertebrates can be influenced by their nutritional habits, age, sex, season, seawater temperature, and salinity (Barrento et al., 2010). Also, Styrishave and Andersen (2000) reported the influence of intermoult duration on the fatty acid profiles in the hepatopancreas of the green crab (*C. maenas*) inhabiting the Ise-fjord, Denmark. Furthermore, Çelik et al. (2005) indicated that n-3 fatty acids in fish living in cold climatic conditions have a higher % composition. In the case of the red mangrove crab, the results indicated that the water temperature in the two reserves did not differ, additionally, the temperature of the water was not correlated with the fatty acid or the amino acid concentrations. Furthermore, researchers indicated that the difference in the EPA and DHA content could be due to the existing differences in the diet of crabs at the harvesting sites (Naczk et al., 2004). As the Salado reserve was more recently constructed and more affected by urbanization than the Churute reserve, the nutrition source of the crabs in the two reserves might be different and potentially influence the proximate, mineral, and fatty acid composition of the crab meat. Moreover, the results of our study indicated that other factors influencing the fatty acid and amino acid profiles of the crabs could potentially be the conductivity and the salinity of the water at the harvesting sites. For the mud crab, researchers have indicated that crabs rearing at high salinity

water possessed a superior nutritional quality to the ones in low salinity water (Wu et al., 2019). Furthermore, it has been reported that modulation of amino acidic deposits and nitrogen elements in crustaceans can be caused by environmental salinity variation generating osmo-adaptive processes (Zotti et al., 2016a). Regarding the influence of water conductivity on the biochemical composition of crabs, additional research is suggested.

Also, the PCA plot indicated that the two data sets based on the harvest site could be separated based on PC1. Also, from the results of our study (**Figure 2**), it can be concluded that the fatty acid distribution, amino acid profiles, several nutrients, and the ash,- and protein content can differ due to the spatial variation of crab habitats. However, the differences between crabs harvested at the two reserves regarding the nutritional indices (**Table 2**) were small for all lipid indices, indicating that even though a difference in fatty acid profile exists between crabs, the nutritional quality of the red crab did not differ between the locations studied, which were 40 km apart. Moreover, even though a small difference was found between the protein quality of the crabs harvested from the two reserves, it could be concluded that both reserves deliver crabs with good protein quality.

In this study, we discovered that the carapace width of the red mangrove crab was correlated with the AA concentrations and all amino acids present in the crab meat. This could indicate that the age and growth of the red crab alternate its nutritional composition. Little research has been published regarding the influence of age on the amino acid composition of seafood. Hüssy et al. (2004) observed a change in amino acid composition over time for the Atlantic cod. Furthermore, it should be considered that crab physiology, and the reproductive cycle can alternate its biochemical composition. Previous research has reported that brachyuran species can modulate physiological metabolite concentration to face the variation of the abiotic factors in the habitats, the nature of available trophic resources, the genetic diversity, the life stages, and sex (Zotti et al., 2016a). Zotti et al. (2016b) compared the obtained proximate composition of the same crab species among researchers, and they noticed inter-species variability . Also, human errors should not be neglected.

## 5. Conclusion

Crustacea can be an important part of a balanced diet. The red mangrove crab (*Ucides occidentalis*) proved to be an adequate source of various nutrients. The indices indicated that the red mangrove crab contains many essential elements and could be a good source for a healthy diet. In general, the red mangrove crab obtained generally better lipid nutritional quality indices compared to other shellfish species, apart from the EPA+DHA index values. It is advised for red mangrove crab consumers to complement their diet with other food sources rich in EPA+DHA such as fish. Based on the weekly contribution of the metals, it can be concluded that the mangrove crabs can contribute to the recommended intake of essential elements. Current research confirmed that biochemical compositions can differ between crab species, and indicated that the nutritional composition of the red mangrove crab may vary between locations. Even though a difference in fatty acid and amino acid profile was found between the crab samples, the nutritional quality of the red crab did not differ due to spatial variation. More research into the influence of environmental and ecological factors on the nutritional composition of crabs is suggested.

**Acknowledgments**

The study was performed in the context of the VLIR-UOS Ecuador Biodiversity Network project. We acknowledge the ministry of environment and water of Ecuador as the research was implemented under the research permit 020-2015IC-FLO/FAU-DPG/MAE. All the crabs were bought from the freshly caught loot by the local crab-catchers. Finally, we would like to express gratitude to everyone that assisted during the sampling campaign and the analyses of the samples: Andrea Mishell Rosado, Nancy de Saeyer, and Els Vossen.

**Funding information/statement**

This research was funded by the Vlaamse Interuniversitaire Raad—Universitaire ontwikkelingssamenwerking (VLIR-UOS) (Belgium).

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Andrée De Cock: Conceptualization, Methodology, Investigation, Writing - original draft. Marie Anne Eurie Forio: Supervision, Writing - review & editing. Bruno De Meulenaer: Writing - review & editing. Filip Tack: Writing - review & editing. Luis Dominguez Granda: Project administration, Supervision, Writing - review & editing. Peter Goethals: Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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## Appendix

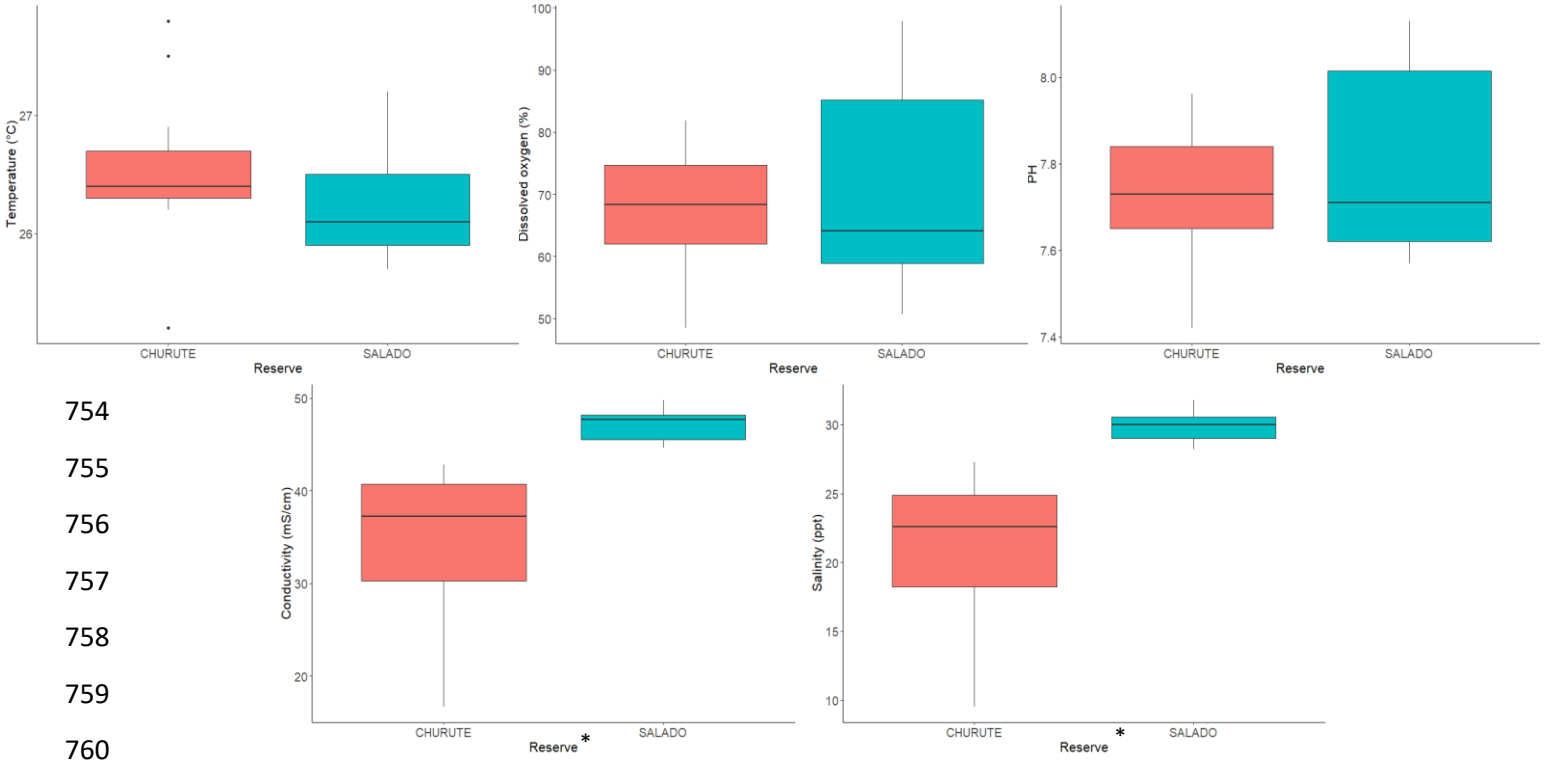



**Figure A.1** Physicochemical water parameters (Temperature, Disolved oxygen, PH, Conductivity, and Salinity) distributions in Churute and Salado reserve. The asterix (*) indicates a significant difference ($p < 0.05$) between the results for the Salado and Churute reserves.

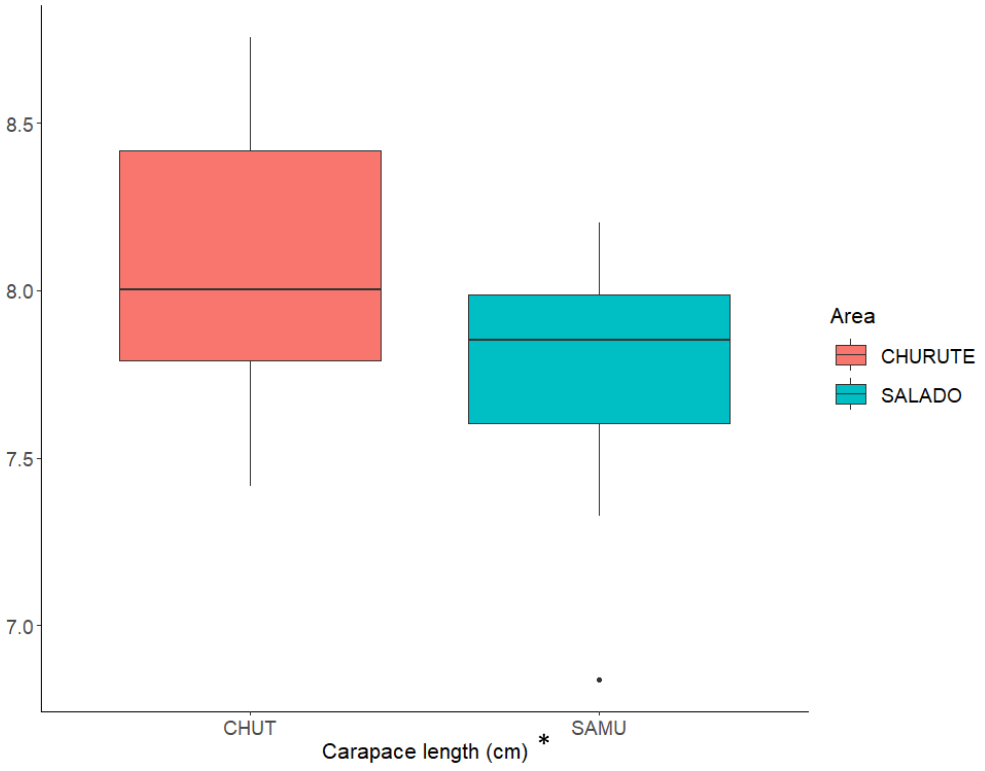
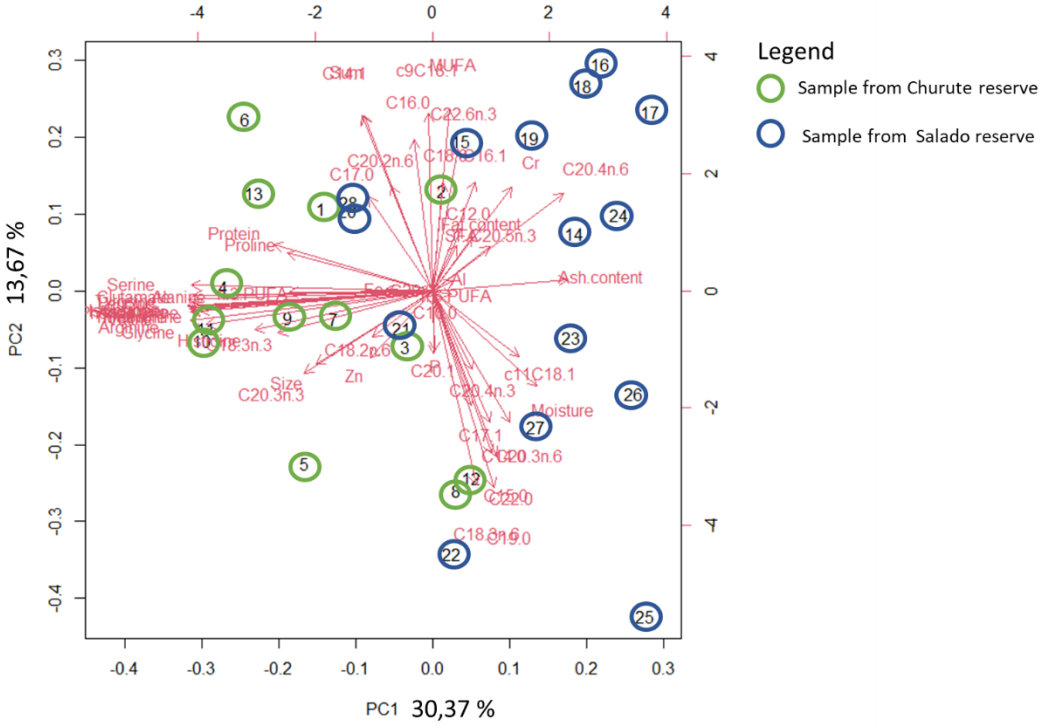



**Figure A.2** Mean carapace width of (pooled) crabs samples at Churute and Salado reserves. The asterix (*) indicates a significant difference ($p < 0.05$) between the results for the Salado and Churute reserves.




780

781 **Figure A.3** Score plot of the first two components of the principal components analysis (PCA) conducted
782 with the nutritional data (proximate composition, fatty acids, amino acids, and essential minerals) of
783 the red mangrove crab (*Ucides occidentalis*).

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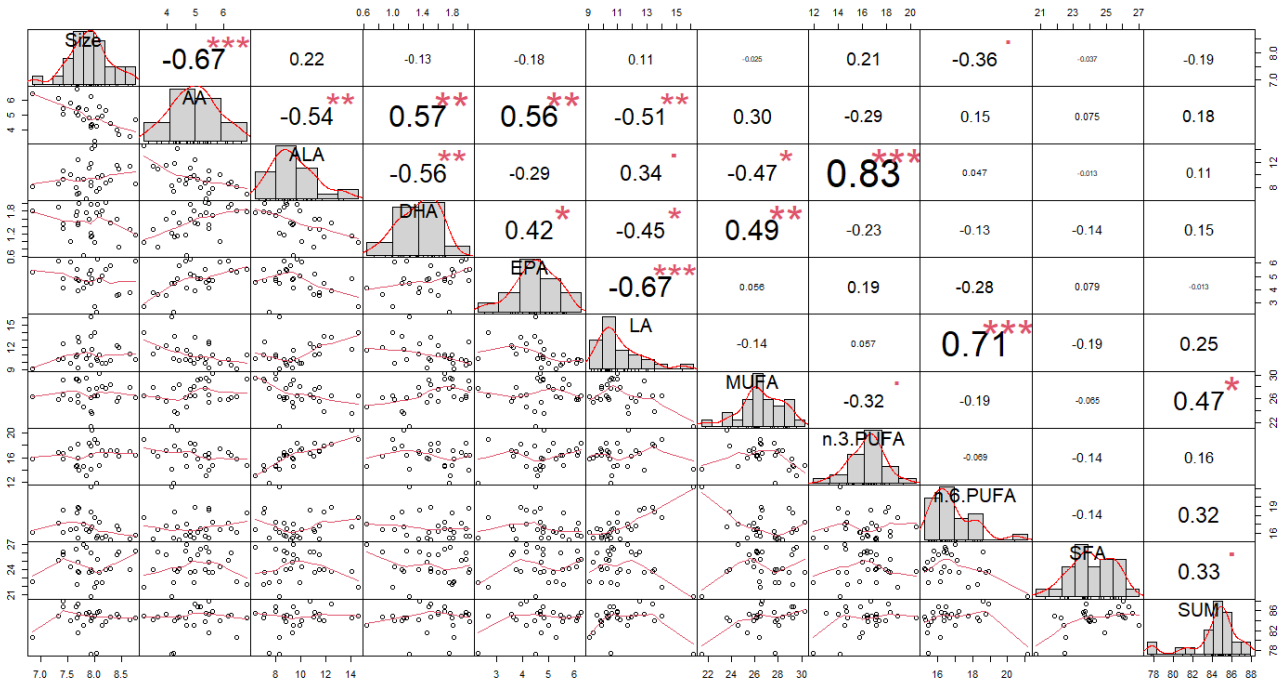



785 796

797 **Figure A.4** Correlation matrix of carapace ~~length~~ width of collected red mangrove crab and a selection of fatty
798 acid concentrations measured in crab meat. Each significance level is associated to a symbol p-values(0.001, 0.01,
799 0.05, 0.1) : symbols("***", "**", "*", ".").

800

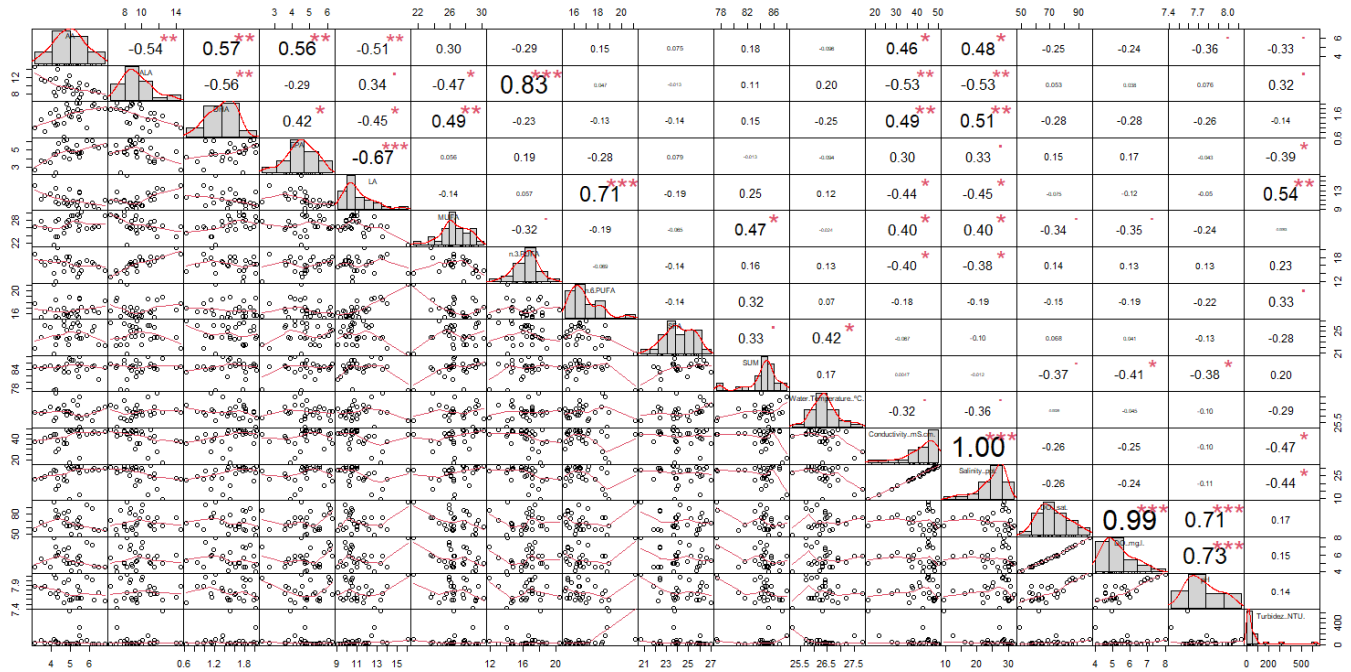


**Figure A.5** Correlation chart of a selection of fatty acid concentrations of the red mangrove crab and physiochemical water properties measured at the sampling sites. Each significance level is associated to a symbol p-values(0.001, 0.01, 0.05, 0.1) : symbols("***", "**", "*", ".").

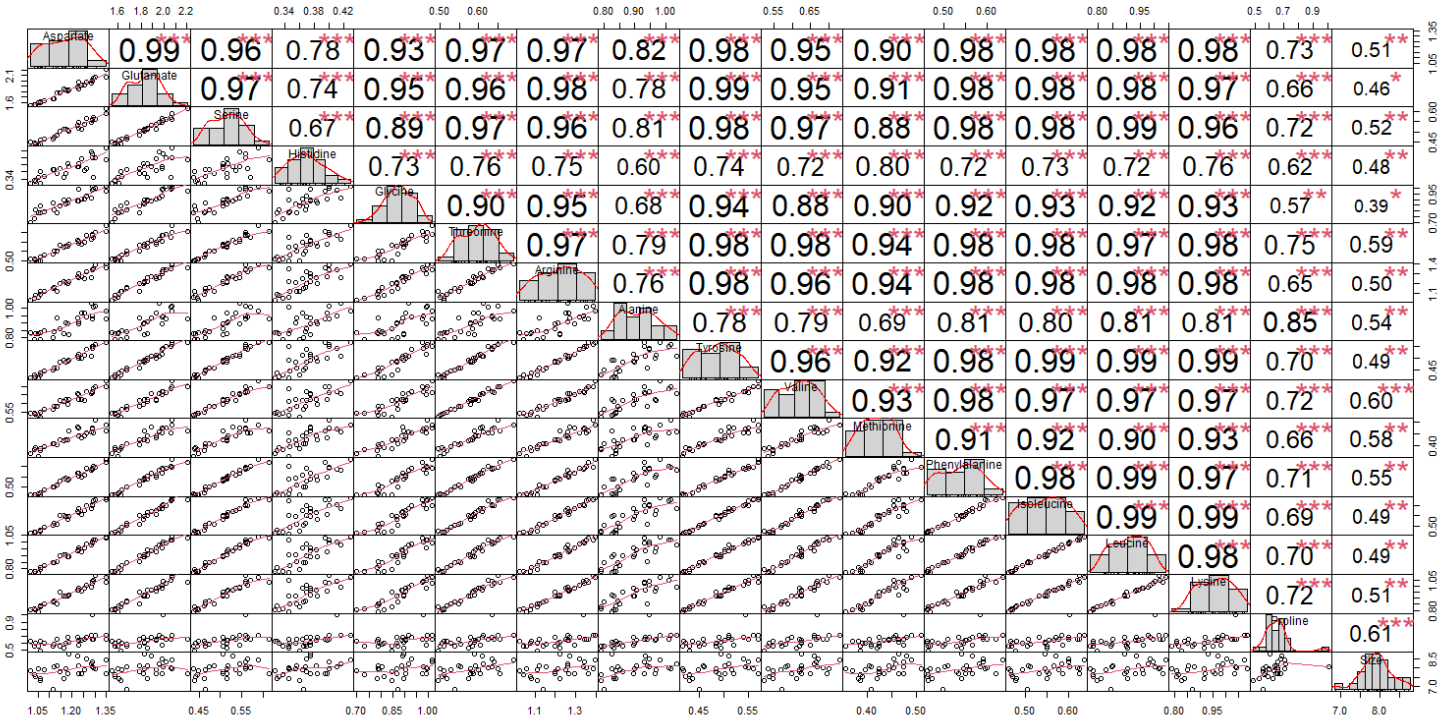
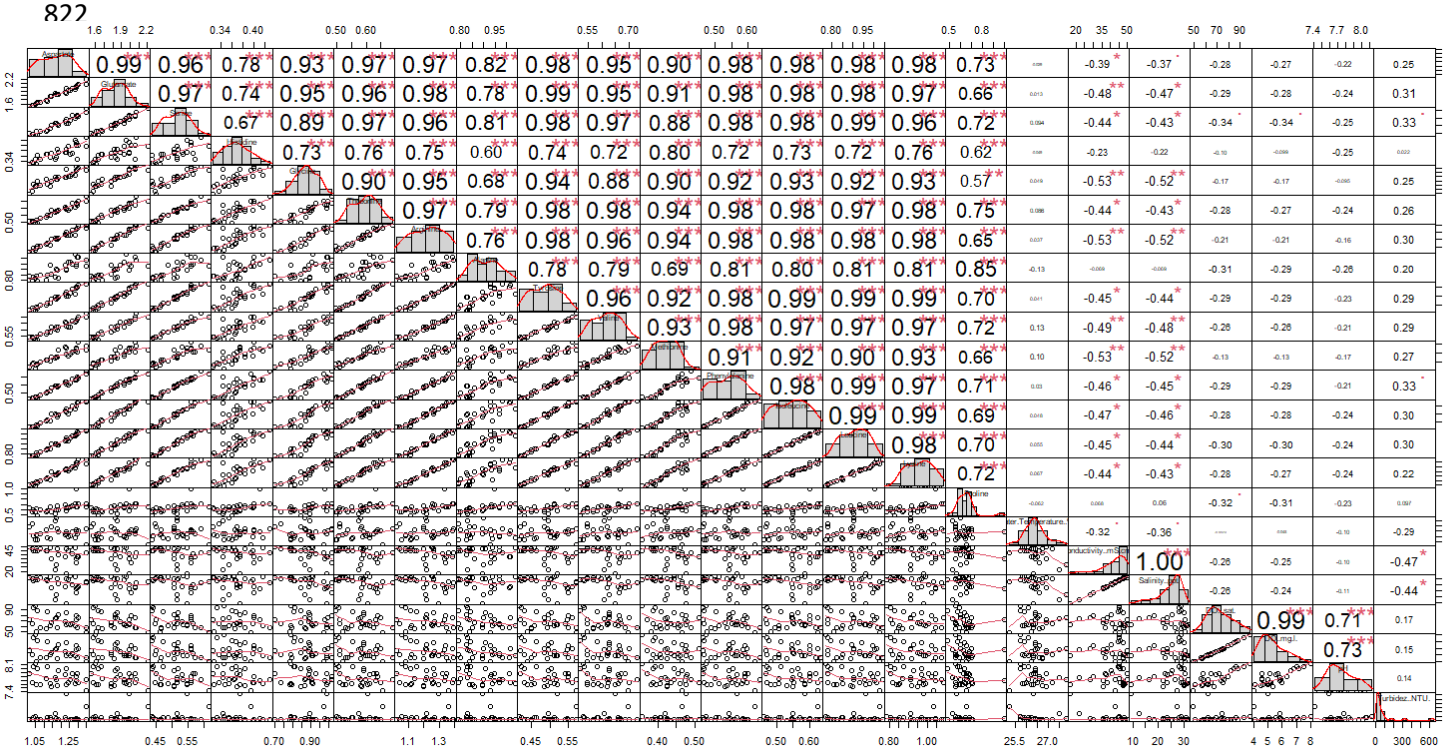


**Figure A.6** Correlation matrix of carapace width of collected red mangrove crab and the amino acid concentrations measured in crab meat. Each significance level is associated to a symbol p-values(0.001, 0.01, 0.05, 0.1) : symbols("***", "**", "*", ".").

822



836 **Figure A.7** Correlation chart of the amino acid concentrations of the red mangrove crab and physiochemical
837 water properties measured at the sampling sites. Each significance level is associated to a symbol p-values(0.001,
838 0.01, 0.05, 0.1) : symbols("***", "**", "*", ".").

839 **Table A.1** Response factors fatty acid analysis.

| compound | I.S. C13 |
|---|---|
| 04:0 | 1.34 |
| 04:1 | 1.31 |
| 04:2 | 1.28 |
| 04:3 | 1.25 |
| 04:4 | 1.21 |
| 04:5 | 1.18 |
| 04:6 | 1.15 |
| 05:0 | 1.24 |
| 05:1 | 1.21 |
| 05:2 | 1.19 |
| 05:3 | 1.17 |
| 05:4 | 1.14 |
| 05:5 | 1.12 |
| 05:6 | 1.09 |
| 06:0 | 1.17 |
| 06:1 | 1.15 |
| 06:2 | 1.13 |
| 06:3 | 1.11 |
| 06:4 | 1.09 |
| 06:5 | 1.07 |
| 06:6 | 1.05 |
| 07:0 | 1.13 |
| 07:1 | 1.11 |
| 07:2 | 1.09 |

| | |
|---|---|
| 07:3 | 1.08 |
| 07:4 | 1.06 |
| 07:5 | 1.04 |
| 07:6 | 1.02 |
| 08:0 | 1.09 |
| 08:1 | 1.08 |
| 08:2 | 1.06 |
| 08:3 | 1.05 |
| 08:4 | 1.03 |
| 08:5 | 1.02 |
| 08:6 | 1.00 |
| 09:0 | 1.07 |
| 09:1 | 1.05 |
| 09:2 | 1.04 |
| 09:3 | 1.03 |
| 09:4 | 1.01 |
| 09:5 | 1.00 |
| 09:6 | 0.99 |
| 10:0 | 1.04 |
| 10:1 | 1.03 |
| 10:2 | 1.02 |
| 10:3 | 1.01 |
| 10:4 | 1.00 |
| 10:5 | 0.98 |
| 10:6 | 0.97 |
| 11:0 | 1.03 |
| 11:1 | 1.02 |
| 11:2 | 1.01 |
| 11:3 | 0.99 |
| 11:4 | 0.98 |
| 11:5 | 0.97 |
| 11:6 | 0.96 |
| 12:0 | 1.01 |
| 12:1 | 1.00 |
| 12:2 | 0.99 |
| 12:3 | 0.98 |
| 12:4 | 0.97 |
| 12:5 | 0.96 |
| 12:6 | 0.95 |
| 13:0 | 1.00 |
| 13:1 | 0.99 |
| 13:2 | 0.98 |
| 13:3 | 0.97 |
| 13:4 | 0.96 |
| 13:5 | 0.95 |
| 13:6 | 0.94 |
| 14:0 | 0.99 |
| 14:1 | 0.98 |
| 14:2 | 0.97 |
| 14:3 | 0.96 |
| 14:4 | 0.95 |
| 14:5 | 0.95 |
| 14:6 | 0.94 |

| | |
|---|---|
| 15:0 | 0.98 |
| 15:1 | 0.97 |
| 15:2 | 0.96 |
| 15:3 | 0.96 |
| 15:4 | 0.95 |
| 15:5 | 0.94 |
| 15:6 | 0.93 |
| 16:0 | 0.97 |
| 16:1 | 0.96 |
| 16:1c11 | 0.96 |
| 16:1c7 | 0.96 |
| 16:2 | 0.96 |
| 16:3 | 0.95 |
| 16:4 | 0.94 |
| 16:5 | 0.93 |
| 16:6 | 0.93 |
| 17:0 | 0.96 |
| 17:1 | 0.96 |
| 17:1c9 | 0.96 |
| 17:2 | 0.95 |
| 17:3 | 0.94 |
| 17:4 | 0.94 |
| 17:5 | 0.93 |
| 17:6 | 0.92 |
| 18:0 | 0.96 |
| 18:1 | 0.95 |
| 18:1trans | 0.95 |
| 18:1c9 | 0.95 |
| 18:1c11 | 0.95 |
| 18:2 | 0.94 |
| 18:2n-6 | 0.94 |
| 18:3n-6 | 0.94 |
| 18:3n-3 | 0.94 |
| 18:4 | 0.93 |
| 18:5 | 0.92 |
| 18:6 | 0.92 |
| 19:0 | 0.95 |
| 19:1 | 0.95 |
| 19:2 | 0.94 |
| 19:3 | 0.93 |
| 19:4 | 0.93 |
| 19:5 | 0.92 |
| 19:6 | 0.91 |
| 20:0 | 0.95 |
| 20:1 | 0.94 |
| 20:2n-3 | 0.94 |
| 20:2n-6 | 0.94 |
| 20:3n-3 | 0.93 |
| 20:4n-3 | 0.92 |
| 20:5n-3 | 0.92 |
| 20:6n-6 | 0.91 |
| 20:3n-6 | 0.93 |
| 20:4n-6 | 0.92 |

| | |
|---|---|
| 20:5n-6 | 0.92 |
| 20:6n-6 | 0.91 |
| 21:0 | 0.94 |
| 21:1 | 0.94 |
| 21:2 | 0.93 |
| 21:3 | 0.93 |
| 21:4 | 0.92 |
| 21:5 | 0.91 |
| 21:6 | 0.91 |
| 22:0 | 0.94 |
| 22:1 | 0.93 |
| 22:2 | 0.93 |
| 22:3 | 0.92 |
| 22:4n-3 | 0.92 |
| 22:5n-3 | 0.91 |
| 22:6n-3 | 0.91 |
| 22:4n-6 | 0.92 |
| 22:5n-6 | 0.91 |
| 22:6n-6 | 0.91 |
| 23:0 | 0.93 |
| 23:1 | 0.93 |
| 23:2 | 0.92 |
| 23:3 | 0.92 |
| 23:4 | 0.91 |
| 23:5 | 0.91 |
| 23:6 | 0.90 |
| 24:0 | 0.93 |
| 24:1 | 0.93 |
| 24:2 | 0.92 |
| 24:3 | 0.92 |
| 24:4 | 0.91 |
| 24:5 | 0.91 |
| 24:6 | 0.90 |
| 25:0 | 0.93 |
| 25:1 | 0.92 |
| 25:2 | 0.92 |
| 25:3 | 0.91 |
| 25:4 | 0.91 |
| 25:5 | 0.90 |
| 25:6 | 0.90 |
| 26:0 | 0.93 |
| 26:1 | 0.92 |
| 26:2 | 0.92 |
| 26:3 | 0.91 |
| 26:4 | 0.91 |
| 26:5 | 0.90 |
| 26:6 | 0.90 |

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844 **Table A.2** Details on Ex and Em wavelength for the OPA and FMOC derivates for amino acid analysis.

| Time | Ex wavelength (nm) | Em wavelength (nm) |
|---|---|---|
| OPA derivates | 340 | 450 |
| FMOC derivates | 266 | 305 |

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846 **Table A.3.** Mean characteristics (weight, carapace width and yield) of the pooled crab samples.

| **Pooled sample code** | **Size (cm)** | **Weight (g)** | **Yield (g)** |
|---|---|---|---|
| SALD - 0 | 7.9 | 183.0 | 31.8 |
| SALD - 1 | 7.7 | 183.3 | 32.1 |
| SALD - 2 | 7.7 | 172.1 | 25.7 |
| SALD - 3 | 7.9 | 182.7 | 32.5 |
| SALD - 5 | 7.4 | 159.3 | 28.9 |
| SALD - 6 | 8.1 | 187.1 | 33.5 |
| SALD - 7 | 8.2 | 219.0 | 50.2 |
| SALD-8 | 8.1 | 179.1 | 32.5 |
| SALD-9 | 7.7 | 168.1 | 33.9 |
| SALD-10 | 6.8 | 142.5 | 29.9 |
| SALD-11 | 7.3 | 152.0 | 35.5 |
| SALD-12 | 7.9 | 195.3 | 44.9 |
| SALD-13 | 7.6 | 165.7 | 34.8 |
| SALD-14 | 8.0 | 194.7 | 33.1 |
| SALD - 15 | 7.9 | 188.3 | 34.7 |
| CHUT - 1 | 7.7 | 160.6 | 31.4 |
| CHUT - 2 | 7.4 | 151.3 | 27.7 |
| CHUT - 3 | 7.9 | 187.8 | 49.7 |
| CHUT - 4 | 8.0 | 178.3 | 34.1 |
| CHUT - 5 | 8.5 | 218.9 | 34.5 |
| CHUT-8 | 7.7 | 165.6 | 29.7 |
| CHUT-9 | 8.7 | 222.5 | 42.9 |
| CHUT-10 | 8.8 | 226.9 | 43.2 |
| CHUT-11 | 8.3 | 219.5 | 39.8 |
| CHUT - 12 | 8.4 | 202.7 | 35.2 |
| CHUT-13 | 8.0 | 170.6 | 27.2 |
| CHUT-14 | 7.8 | 177.9 | 35.0 |
| CHUT-15 | 8.1 | 178.9 | 31.9 |

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**Table A.4.** Fatty acid concentrations in all samples (mg/100g sample).

| | CHNU1 | CHNU2 | CHNU3 | CHNU4 | CHNU5 | CHNU8 | CHNU9 | CHNU10 | CHNU11 | CHNU12 | CHNU13 | CHNU14 | CHNU15 | SANU0 | SANU1 | SANU2 | SANU3 | SANU5 | SANU6 | SANU7 | SANU8 | SANU9 | SANU10 | SANU11 | SANU12 | SANU13 | SANU14 | SANU15 | AVERAGE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Saturated fatty acid (SFA) composition (%)* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **C10:0** | 1.63 | 2.42 | 2.61 | 1.80 | 2.15 | 1.79 | 2.32 | 1.73 | 1.35 | 2.42 | 1.93 | 3.18 | 2.65 | 2.17 | 1.24 | 2.33 | 3.42 | 2.27 | 2.24 | 2.94 | 1.58 | 1.07 | 1.68 | 2.02 | 0.98 | 0.88 | 2.47 | 1.61 | 2.03 |
| **C11:0** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **C12:0** | - | 0.88 | - | - | - | - | - | - | - | 0.26 | - | - | - | - | - | 0.45 | - | - | - | - | - | - | - | 0.43 | - | - | - | - | 0.07 |
| **C14:0** | 0.30 | 0.62 | 0.34 | 0.26 | 0.56 | 0.21 | 0.19 | 0.93 | 0.21 | 0.32 | 0.20 | 0.53 | 0.14 | 0.42 | 0.32 | 0.21 | 0.47 | 0.43 | 0.13 | 0.41 | 0.47 | 0.34 | 0.25 | 0.26 | 0.36 | 0.52 | 0.39 | 0.41 | 0.36 |
| **C15:0** | 0.33 | 0.32 | 0.14 | 0.09 | 0.53 | 0.16 | 0.34 | 0.84 | 0.79 | 0.26 | 0.09 | 0.91 | 0.09 | 0.23 | 0.05 | 0.43 | 0.30 | 0.42 | 0.21 | 0.39 | 0.05 | 0.76 | 1.03 | 0.10 | 0.67 | 0.43 | 0.13 | 0.23 | 0.37 |
| **C16:0** | 26.19 | 31.77 | 22.51 | 25.62 | 20.59 | 29.11 | 22.72 | 19.14 | 27.88 | 21.39 | 24.09 | 18.69 | 29.45 | 21.86 | 27.91 | 34.30 | 25.24 | 33.18 | 31.44 | 31.34 | 25.30 | 14.46 | 20.09 | 23.07 | 17.00 | 20.11 | 23.91 | 32.87 | 25.04 |
| **C17:0** | 1.10 | 1.17 | 1.16 | 0.95 | 0.60 | 0.96 | 0.82 | 0.27 | 0.65 | 0.79 | 0.89 | 0.80 | 0.98 | 0.53 | 1.12 | 1.31 | 0.55 | 1.24 | 0.87 | 1.37 | 0.63 | 0.44 | 0.65 | 0.75 | 0.53 | 0.72 | 0.63 | 1.58 | 0.86 |
| **C18:0** | 19.75 | 22.29 | 15.96 | 16.29 | 12.90 | 21.87 | 15.85 | 14.40 | 23.53 | 15.30 | 16.89 | 14.30 | 21.89 | 15.44 | 20.88 | 22.20 | 18.48 | 22.21 | 22.13 | 21.71 | 18.80 | 11.54 | 15.74 | 17.59 | 12.58 | 15.53 | 18.22 | 22.68 | 18.11 |
| **C19:0** | 1.13 | 1.24 | 2.89 | 0.24 | 1.09 | 1.19 | 2.92 | 2.42 | 2.51 | 1.24 | 0.88 | 2.92 | 0.82 | 1.44 | 0.85 | 1.38 | - | 1.93 | 1.07 | 1.13 | 2.78 | 2.62 | 2.89 | 0.94 | 3.02 | 2.21 | 3.06 | 1.15 | 1.71 |
| **C20:0** | - | - | - | - | - | 0.06 | - | - | - | - | - | - | - | - | - | - | - | 0.02 | - | - | 0.32 | - | - | - | - | - | - | - | 0.01 |
| **C22:0** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **C24:0** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **∑SFA** | 50.43 | 60.70 | 45.61 | 45.26 | 38.42 | 55.35 | 45.16 | 39.73 | 56.91 | 41.97 | 44.97 | 41.32 | 56.03 | 42.08 | 52.38 | 62.62 | 48.46 | 61.70 | 58.07 | 59.29 | 49.93 | 31.22 | 42.33 | 45.16 | 35.13 | 40.40 | 48.81 | 60.53 | 48.57 |
| *Monounsaturated fatty acid (MUFA) composition (%)* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **C14:1** | 2.62 | 3.03 | 2.26 | 2.83 | 1.65 | 3.74 | 2.24 | 1.39 | 2.68 | 1.71 | 2.36 | 1.25 | 2.45 | 1.78 | 3.22 | 3.48 | 2.24 | 2.66 | 3.21 | 2.73 | 2.87 | 1.07 | 2.27 | 1.73 | 1.44 | 1.50 | 2.50 | 2.91 | 2.35 |
| **C16:1** | 3.22 | 7.26 | 4.72 | 7.06 | 5.34 | 9.16 | 7.85 | 4.60 | 7.34 | 5.01 | 5.07 | 6.59 | 7.04 | 5.79 | 10.47 | 9.86 | 8.66 | 10.26 | 12.16 | 9.71 | 8.04 | 3.44 | 6.07 | 4.31 | 3.53 | 5.10 | 8.36 | 9.40 | 6.98 |
| **C17:1** | 0.55 | 0.48 | 1.14 | 0.45 | 0.87 | 0.59 | 0.88 | 0.45 | 0.77 | 0.77 | 0.99 | 1.04 | 0.94 | 0.77 | 0.72 | 0.96 | 0.63 | 1.09 | 1.24 | 1.00 | 1.03 | 0.80 | 0.46 | 0.56 | 0.81 | 1.31 | 0.99 | 1.14 | 0.84 |
| **c9C18:1** | 48.97 | 50.08 | 36.29 | 43.07 | 29.96 | 52.95 | 40.03 | 31.92 | 55.14 | 32.79 | 35.02 | 27.28 | 47.27 | 32.77 | 53.45 | 53.93 | 40.40 | 54.80 | 55.61 | 53.73 | 49.14 | 25.63 | 38.89 | 38.92 | 29.46 | 33.92 | 47.19 | 56.09 | 42.67 |
| **c11C18:1** | 0.73 | 0.80 | 0.76 | 0.84 | 0.91 | 1.08 | 1.13 | 0.96 | 1.22 | 0.98 | 0.91 | 1.05 | 0.66 | 1.17 | 0.96 | 1.20 | 1.63 | 0.74 | 1.65 | 0.81 | 0.95 | 0.76 | 1.23 | 0.70 | 0.86 | 1.04 | 1.16 | 1.09 | 1.00 |
| **C20:1** | 0.34 | 0.46 | 0.33 | 0.57 | 0.17 | 0.45 | 0.52 | 0.31 | 0.41 | 0.42 | 0.34 | 0.40 | 0.39 | 0.38 | 0.27 | 0.29 | 0.23 | 0.46 | 0.38 | 0.38 | 0.51 | 0.29 | 0.57 | 0.55 | 0.30 | 0.37 | 0.31 | 0.42 | 0.39 |
| **C22:1** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **C24:1** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **∑MUFA** | 56.42 | 62.10 | 45.49 | 54.82 | 38.91 | 67.95 | 52.64 | 39.64 | 67.55 | 41.68 | 44.70 | 37.61 | 58.75 | 42.67 | 69.09 | 69.72 | 53.80 | 70.02 | 74.25 | 68.37 | 62.54 | 31.98 | 49.49 | 46.77 | 36.40 | 43.25 | 60.52 | 71.05 | 54.22 |
| *Polyunsaturated fatty acid (PUFA) composition (%)* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **C18:2 n-6** | 28.42 | 29.17 | 25.85 | 29.12 | 17.79 | 30.05 | 21.02 | 15.75 | 24.38 | 19.98 | 22.19 | 17.84 | 22.72 | 15.65 | 25.92 | 25.53 | 19.24 | 25.10 | 26.91 | 24.49 | 22.10 | 11.72 | 17.14 | 18.80 | 27.60 | 16.37 | 22.96 | 28.17 | 22.57 |
| **C18:3 n-6** | 0.11 | 0.14 | 0.07 | 0.07 | 0.34 | 0.03 | 0.21 | 0.46 | 0.54 | 0.18 | 0.22 | 0.31 | 0.23 | 0.31 | 0.12 | 0.27 | 0.07 | 0.01 | 0.22 | 0.09 | 0.38 | 0.25 | 0.25 | 0.13 | 0.46 | 0.17 | 0.44 | 0.12 | 0.22 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C20:2 n-6** | 0.96 | 1.01 | 0.87 | 0.98 | 0.50 | 1.06 | 0.96 | 0.53 | 0.73 | 0.65 | 0.83 | 0.63 | 0.99 | 0.71 | 0.96 | 1.03 | 0.62 | 1.16 | 1.13 | 0.81 | 0.83 | 0.30 | 0.76 | 0.65 | 0.70 | 0.62 | 0.73 | 0.92 | 0.81 |
| **C20:3 n-6** | 0.15 | 0.31 | 0.16 | 0.13 | 0.33 | 0.05 | 0.14 | 0.22 | 0.12 | 0.15 | 0.11 | 0.11 | 0.32 | 0.12 | 0.15 | 0.12 | 0.25 | 0.11 | 0.27 | 0.14 | 0.23 | 0.28 | 0.13 | 0.27 | 0.23 | 0.45 | 0.38 | 0.13 | 0.20 |
| **C20:4 n-6** | 10.56 | 12.78 | 8.16 | 6.56 | 5.50 | 12.88 | 6.63 | 7.09 | 10.51 | 6.49 | 8.27 | 7.18 | 10.71 | 10.15 | 16.06 | 12.31 | 9.79 | 12.18 | 13.70 | 13.08 | 10.99 | 6.88 | 12.14 | 11.12 | 7.06 | 9.76 | 9.24 | 12.34 | 10.01 |
| **C22:4 n-6** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **C22:5 n-6** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **∑n-6 PUFA** | 40.19 | 43.40 | 35.11 | 36.86 | 24.46 | 44.08 | 28.96 | 24.05 | 36.28 | 27.46 | 31.61 | 26.06 | 34.97 | 26.94 | 43.20 | 39.26 | 29.97 | 38.57 | 42.23 | 38.60 | 34.54 | 19.42 | 30.43 | 30.95 | 36.04 | 27.38 | 33.75 | 41.69 | 33.80 |
| **C18:3 n-3** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **C20:3 n-3** | 26.43 | 24.94 | 22.54 | 30.25 | 16.33 | 16.72 | 21.56 | 13.08 | 21.74 | 18.72 | 26.63 | 14.39 | 21.57 | 14.40 | 16.55 | 18.99 | 11.55 | 22.48 | 19.51 | 21.72 | 22.14 | 11.73 | 15.46 | 15.60 | 14.02 | 16.79 | 16.27 | 28.45 | 19.31 |
| **C20:4 n-3** | 0.62 | 1.19 | 0.98 | 1.32 | 0.98 | 0.29 | 0.96 | 0.71 | 1.62 | 0.82 | 0.80 | 0.56 | 0.67 | 0.13 | 0.89 | 0.36 | 0.54 | 0.38 | 0.83 | 0.11 | 0.89 | 0.60 | 0.72 | 0.09 | 0.17 | 0.40 | 0.80 | 1.03 | 0.70 |
| **C20:5 n-3** | 0.01 | 0.05 | 0.02 | 0.04 | 0.07 | 0.02 | 0.05 | 0.01 | 0.05 | 0.01 | 0.03 | 0.02 | 0.08 | 0.02 | 0.04 | 0.04 | 0.05 | 0.04 | 0.03 | 0.05 | 0.05 | 0.07 | 0.04 | 0.05 | 0.05 | 0.06 | 0.03 | 0.04 | 0.04 |
| **C22:5 n-3** | 8.33 | 9.69 | 8.53 | 5.58 | 5.41 | 11.05 | 7.11 | 9.33 | 13.73 | 5.80 | 9.13 | 6.36 | 13.97 | 8.90 | 13.14 | 11.44 | 8.33 | 11.56 | 11.55 | 13.71 | 11.19 | 6.00 | 9.97 | 11.14 | 7.69 | 10.15 | 5.00 | 13.38 | 9.54 |
| **C22:6 n-3** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **∑n-3 PUFA** | 3.18 | 3.15 | 2.13 | 2.05 | 1.27 | 4.72 | 2.52 | 1.79 | 3.53 | 2.62 | 2.16 | 0.97 | 4.40 | 3.20 | 4.21 | 4.01 | 3.22 | 3.53 | 4.14 | 4.99 | 4.03 | 1.82 | 3.40 | 3.44 | 2.07 | 1.67 | 3.82 | 4.11 | 3.08 |
| **∑PUFA** | 38.56 | 39.01 | 34.20 | 39.24 | 24.06 | 32.81 | 32.19 | 24.92 | 40.67 | 27.99 | 38.75 | 22.29 | 40.68 | 26.65 | 34.83 | 34.85 | 23.69 | 37.99 | 36.06 | 40.58 | 38.30 | 20.21 | 29.60 | 30.32 | 24.01 | 29.07 | 25.93 | 47.01 | 32.66 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sum** | 185.60 | 205.22 | 160.40 | 176.17 | 125.85 | 200.19 | 158.95 | 128.34 | 201.41 | 139.10 | 160.03 | 127.29 | 190.43 | 138.34 | 199.49 | 206.44 | 155.92 | 208.28 | 210.60 | 206.84 | 185.31 | 102.84 | 151.84 | 153.21 | 131.58 | 140.10 | 169.01 | 220.28 | 169.25 |

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852 **Table A.5.** Amino acid concentrations and standard deviation (SD) in crabmeat (mg/kg fw).

| | Mean (g/100g sample) | SD |
|---|---|---|
| **Aspartate** | 1.18 | 0.10 |
| **Glutamate** | 1.83 | 0.18 |
| **Serine** | 0.51 | 0.05 |
| **Histidine** | 0.37 | 0.03 |
| **Glycine** | 0.88 | 0.09 |
| **Threonine** | 0.58 | 0.05 |
| **Arginine** | 1.22 | 0.11 |
| **Alanine** | 0.90 | 0.07 |
| **Tyrosine** | 0.49 | 0.05 |
| **Valine** | 0.62 | 0.06 |
| **Methionine** | 0.43 | 0.04 |
| **Phenylalanine** | 0.54 | 0.05 |
| **Isoleucine** | 0.55 | 0.05 |
| **Leucine** | 0.91 | 0.09 |
| **Lysine** | 0.93 | 0.09 |
| **Proline** | 0.64 | 0.09 |

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854 **Table A.6.** Adult daily recommended allowances of Indispensable amino acids (IAA), true ileal digestible
855 coefficients (%), and Dietary IAA (DIAAS) reference ratios for minimal IAA and DIAAS.

| | | | | Salado | Churute |
|---|---|---|---|---|---|
| **Indispensable amino acid** | **Minimum (mg/ g protein)** | **Adult IAA requirement (mg/g protein)[a]** | **True ileal digestible coefficients (%)[b]** | **DIAA ratio** | **DIAA Ratio** |
| **Histidine** | 23.91 | 16 | 0.84 | 1.26 | 1.29 |
| **Isoleucine** | 33.31 | 30 | 0.92 | 1.02 | 1.19 |
| **Leucine** | 55.49 | 61 | 0.9 | 0.82 | 0.96 |
| **Lysine** | 57.93 | 48 | 0.92 | 1.11 | 1.29 |
| **Phenylalanine + Tyrosine (AAA)** | 63.57 | 41 | 0.87 | 1.34 | 1.54 |
| **Threonine** | 36.19 | 25 | 0.94 | 1.36 | 1.54 |
| **Valine** | 38.72 | 40 | 0.89 | 0.86 | 0.96 |
| | | | DIAAS (%) | 81.9 | 95.8 |

856 [a](FAO Expert Consultation, 2011), [b](Shaheen et al., 2016)

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