Nutritional benefits and heavy metal contents of freshwater fish species from Tonle Sap Lake with SAIN and LIM nutritional score

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ABSTRACT

Freshwater fishes from the Tonle Sap Lake are used in several programs to improve the nutritional status of children and pregnant women. Our aim was to characterize the overall nutritional profile and heavy metal contents of ten freshwater fish species. The lipid contents ranged from 1.4 to 10.0 g/100 g and fish can be considered rich in omega 3. The vitamin A content was ten times higher in small fish eaten whole than in fillets of large fish; the same applies to the Fe and Zn contents, but to a lesser extent. Mn, As and Pb contents were over the maximum permissible levels in several fishes. According to SAIN and LIM classification, the ten fish species belong to the food groups recommended for health. However, when heavy metals were integrated in LIM, seven species were ranked into the food to consume in small quantities. Globally, the most interesting fish species was *Henicorhynchus siamensis*.

Keywords: fatty acids, fat-soluble vitamins, minerals, proximate, maximum permissible limits, Cambodia.

1. Introduction

Several dietary guidelines in Europe and North America recommend the inclusion of "meat, fish, seafood and eggs" in a balanced and healthy diet (Hercberg et al., 2008). In many least developed countries of Africa and Asia, people with low incomes do not have access to dairy and animal products (Vilain et al., 2016). Among those developing countries, the Cambodian population is highly vulnerable to micronutrient deficiencies (vitamin A, iron, zinc and calcium), especially for children and women (Roos et al., 2007a, 2007b). Fish is a source of proteins and micronutrients such as calcium, iron, zinc, selenium, and vitamins A, B and D (Mohanty et al., 2019; Rehbein and Oehlenschläger, 2009; Roos et al., 2007c). Moreover, fish is also a source of lipids, which are important because they supply most of the calories necessary for growth and contribute to lipophilic vitamin transport. Lipids also provide essential polyunsaturated fatty acids (PUFA) such as linoleic acid C18:2 (n-6 family precursor) and linolenic acid C18:3 (n-3 family precursor) (Petenuci et al., 2016). n-3 PUFAs have several health benefits such as lowering the risk of heart disease, arthritis, mental illness, and improving brain function, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Zhang et al., 2020). PUFAs and particularly n-3 PUFAs are recommended during pregnancy, maternal breastfeeding for infant and child brain development (Ryan et al., 2010).

Tonle Sap Lake (TSL) is the largest wetland in Southeast Asia. TSL is a dynamic ecosystem that varies in size each year from 2500 km^2 to over 15000 km^2 by annual flooding from the Mekong River (Arias et al., 2014). The Mekong River and the TSL contribute to the subsistence of people living in floating villages for their domestic needs (water, fish), but also for agricultural and industrial activities (Nuorteva et al., 2010). Fish migration is based on the direction of water flow from the Mekong to the lake via the Tonle Sap River at the beginning of the rainy season (May-October), and in the opposite direction at the beginning of the dry season (November-February). Most fish species begin to breed at the beginning of the rainy season (May-June) in the floodplain and floodplain forest area of the lake (Lim, 1999). The fish diet in the TSL consists of zooplankton, prey fish, insects, plants, microfauna, crustaceans and molluscs (Heng et al., 2018). During the rainy season, flooding of the floodplain favours a more diverse diet of fish, especially crustaceans and insects (Heng et al., 2018; Pool et al., 2017). Some studies have shown that diet overlap tends to be lower during the dry season, due to competitive exclusion of resources between species, and that fish tend to concentrate in small, well-oxygenated areas (Heng et al., 2018). The diet and life history of the fish could determine fatty acid profiles (Iverson et al., 2002). Between 1936 and 1995, 50-66% of the previously known fish species in the TSL disappeared due to the construction of hydrological dams on the Mekong River, population pressure, overfishing with inappropriate catching methods and illegal fishing during the breeding season (Lim, 1999).

The lake pollution is still at an acceptable level, however, there are many concerns about waste management at the floating villages since currently they are not treated. Furthermore, agricultural residues are polluting the ecosystems and several heavy metals (arsenic, manganese and mercury) and pesticides (dichlorodiphenyltrichloroethane, hymexazol, pyridaben) were detected in water and fish (Kelly et al., 2018; Phat et al., 2018). Around 70% of fish from the TSL contained noticeable amounts of dichlorodiphenyltrichloroethane (Phat et al., 2018). Some amounts of pesticides or heavy metals exceeded the Maximum Permissible Limits (MPL). Mercury contamination was also identified along the Mekong River (Murphy et al., 2009).

Heavy metals are transferred to higher levels in the food chain and are ingested by humans through their diet (Schenone et al., 2014). Methylmercury and inorganic arsenic, the most toxic forms of mercury and arsenic, are a public health issue because of their respective neurotoxic and carcinogenic effects. Several recommendations were published to limit the consumption of heavy metals rich foods by the global population especially for pregnant women due to the risk of abnormal fetal development (Tchounwou et al., 2012).

In Cambodia, fishes are currently used in several programs for vulnerable people (infants, children, pregnant and lactating women) to improve their nutritional status and health. The aim of our research was to assess if the use of local fish is suitable for the nutrition and health of vulnerable people. According to the evolution of the water quality of the Mekong River and TSL, we wonder if it is still safe to use local fish for nutrition programs? To reach this objective, ten freshwater fish species were sampled and characterized in terms of nutritional value and contaminants. Special attention was paid to the profiles of fatty acids (FA), vitamin A, essential microelements and heavy metals taking into account the edible parts of fish (whole, with or without organs or fillet). This paper provides useful data for the fish supply chain stakeholders and the people in charge of nutrition programs in South East Asia.

2. Materials and methods

2.1 Chemicals

Solvents, reagents and pure standards (retinyl palmitate, retinol, fatty acid methyl esters, minerals, heavy metals) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). The polytetrafluoroethylene (PTFE) membranes were obtained from Sartorius (Palaiseau, France).

2.2 Selection of fish species

The ten fish species, which are most available, and consumed by people leaving around TSL in Cambodia were identified in a previous survey (unpublished data). The local, English and scientific names of these species are listed in Table 1. As known by Cambodian people, six belong to big-size species (*Cyclocheilichthys enoplos*, *Barbodes gonionotus*, *Puntioplites proctozysron*, *Channa micropeltes*, *Channa striata* and *Boesemania microlepis*) and four to small ones (*Mystus atrifasciantus*, *Trichogaster microlepis*, *Clupeoides borneensis* and *Henicorhynchus siamensis*). Some of these species (*Barbodes gonionotus*, *Channa micropeltes* and *Channa striata*) are also reared on farms, but only wild fish were sampled in this study because people living around TSL mainly eat wild fish.

2.3 Fish sampling, fish morphometry and sample preparation

Fishes were bought directly from the fishermen during the fishing hours in Kompong Chhnang, Siem Reap and Battambang Province near the TSL (Fig.1). The fish collection was done three times during the dry season (from December 2018 to February 2019). For each sampling site, approximately six kilograms of each fish species were collected. The fishes were placed in a Ziploc bag to prevent any contamination and immediately stored under ice in a polystyrene box until arrival at the laboratory.

The morphometric characteristics (length, width and body weight) of the fish were determined using a ruler and a weight balance (Kern PCB 1000-1 Balance, Germany, precision ± 0.1 g). For each species, the individuals were divided into three groups according to their size. Then, three fishes from each group were randomly selected for measurement.

Samples were prepared from the edible parts of fishes as follows: only fillet for big-size species, the whole body for some small-size species and without organs for other small-size species (Table 1). The edible parts were cut into small pieces and chopped with a meat grinder (HR-12, China). Half of each sample was stored at -20 °C for determination of titratable acidity, pH, moisture, ash and vitamin A contents. The other part of the sample was lyophilized (Freeze dryer, Christ, Alpha 1-4 LD plus, Germany) for assessment of protein, lipid, fatty acid profile, minerals, and heavy metal contents. The analyses were done in triplicate. For the following analyses, an electronic balance (Intelligent-Lab Precisa XB 120A SCS, Switzerland) was used for sample weighing.

2.4 Determination of pH and titratable acidity

Five g of sample was homogenized with distilled water. pH was measured by using a pH meter (Hanna, pH 213, Italy) (Tsighe et al., 2018). Titratable acidity was determined by the titration method with 0.1N NaOH using phenolphthalein as the indicator and expressed in g lactic acid per 100 g of fish (Horwitz, 2002).

2.5 Proximate composition analysis

Moisture content of the sample was measured by drying 5 g of samples at 105 °C for 24 h in an oven (Memmert, UF B 500, Germany) (AOAC, 1990).

The ash content was determined according to the AOAC method (AOAC, 1990). Around 5 g of sample in a porcelain crucible was incinerated in a furnace at 550 °C for 4 h (Nabertherm muffle furnace, L 5/11/B410, Germany).

Total nitrogen content was determined by using the Dumas method with an element analyzer (FP528-LECO Trumac N, EVISA, Europe) (Edeling, 1968). The crude protein content was calculated using a conversion factor of 6.25.

The total lipid content was determined using the Folch method with slight modifications (Folch et al., 1957). Briefly, lipids were extracted from 1.5 g sample hydrated for 10 min in 10 ml distilled water before being dispersed in 30 ml chloroform/methanol (2:1, v/v) for 2 min at 10 000 rpm (Ultra-Turrax T8, IKA, Germany). The solution was sonicated for 5 min (Ultrasonic bath, Fisher Scientific, Germany) and then stirred (Heidolph, Reax 2, overhead shaker, Germany) for 1 h at room temperature. The mixture was centrifuged at 2500 × g for 30 min at 4 °C (Avanti J-E, Beckman Coulter, France) and the upper layer was discarded. The extract was washed with 0.9% NaCl and centrifuged again at 400 × g for 30 min at 4 °C to separate the phases. The upper layer was discarded and the lower layer was evaporated at 40 °C under 30 mbar vacuum (Genevac LTD, EZ-2 series, Sp Scientific, England).

2.6 Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared from the extracted lipids under alkaline and acid hydrolysis and separated and quantified using gas chromatography (Varian AC3800, England) equipped with a flame ionization detector as described by Servent et al., (2018). Briefly, 0.05 g of lipid was dissolved in 2 ml of 0.8% sodium methoxide in methanol. The mixture was placed under reflux at 80 °C for 15 min. After cooling, the mixture was neutralized with sulfuric acid (1 M) in the presence of phenolphthalein until colorless. The mixture was then placed again under reflux at 80 °C for 5 min, after which 4 ml of saturated chloride solution and 1 ml of hexane were successively added. The organic upper phases were analyzed using gas chromatography. For gas chromatography analysis, a DB-WAX non-polar column, 30 m×250 μm×0.25 μm (Agilent, Santa Clara, USA) was used with helium at 0.7 mL/min. The oven gradient was set as follows: initial temperature of 150 °C for 3 min, followed by an increase of 3 °C/min until 220 °C, with a step at 220 °C for 10 min. The injector temperature was set to 250 °C with a split of 1:80 with an injection of 1 μL. The detector temperature was set to 270 °C. Identification was based on retention time and elution order using a commercial standard of all FAMEs from octanoic acid (C8:0) to docosanoic acid (C22:0) (Sigma Aldrich, Saint Louis, USA). The profiles of fatty acids were presented as a percentage of total FAME according to their relative peak areas.

2.7 Determination of vitamin A content

Vitamin A was extracted by the saponification procedure by taking into account the previous recommendation (European Comittee for Standardization; EN12823-1, 2000). One g of sample was homogenized in 4 ml of 50% KOH (w/v) for 1 min (Ultra-Turrax T8, IKA

Labortechnick, Staufen, Germany) and heated for 43 min at 80 °C. The samples were cooled for 30 min and then lipophilic compounds were extracted with ethanol/hexane (4:3, v/v) and centrifuged at 20000 × g for 15 min at 4 °C (Heraeus Multifuge X1R, Thermo Fisher Scientific, Villebon sur Yvette, France). Afterwards, the upper layer was transferred into amber glass tubes and dried (Genevac LTD, EZ-2 series, Sp Scientific, England). The dried residues were dissolved in 400 µl of acetone and filtered with a 0.45 µm PTFE minisart SRP4 membrane (Sartorius). Sample (20 µl) was injected into the HPLC (Agilent System 1200 series, Massy, France). Chromatograms were recorded with a UV–visible photodiode array detector (Agilent Technologies 1200 series) at 325 nm, the wavelength of maximum absorption of the vitamin A in the mobile phase. External calibration was performed weekly with standard solutions of the pure chemical in acetone in the range of 0.5 to 25 mg/L. Vitamin A activity was expressed in retinol equivalents (RE).

2.8 Determination of mineral contents

Around 50 mg of sample was weighed into a Teflon microwave digestion vessel and digested with HNO_3:H_2O_2 (65:35, v/v) with a MARS Xpress microwave system (CEM Corporation, Mathews, NC, France) (Corns et al., 1993). All glassware was cleaned with 20% HNO_3 (v/v) for one day and rinsed with ultrapure water. The digestion conditions were as follows: up to 120 °C for 15 min and then constant for 10 min; up to 160 °C in 20 min and constant for 15 min; finally, samples were cooled to 22 °C for 30 min and diluted to 25 ml with deionized ultrapure water. The trace elements were analyzed by inductively coupled plasma mass spectrometry (Thermo Elemental, X-Series, Germany). The limits of detections were in µg per g: 22.1 for calcium (Ca); 8.6 for potassium (K); 0.5 for magnesium (Mg); 5.4 for sodium (Na); 9.8 for phosphorus (P); 2.6 for iron (Fe); 3.4 for zinc (Zn); 0.2 for aluminum (Al); 0.001 for cadmium (Cd); 0.001 for cobalt (Co); 0.02 for chromium (Cr); 0.2

for copper (Cu); 0.01 for manganese (Mn); 0.02 for nickel (Ni); 0.05 for lead (Pb) and 0.003 for total arsenic (tAs).

Total mercury (tHg) was quantified after combustion of samples (approximately 50 mg) at 750 °C. Mercury vapors were retained on a gold trap and tHg concentrations were determined with a mercury analyzer (Leco, France). The limits of detections were 0.005 µg per g for tHg.

2.9 Determination of methylmercury and inorganic arsenic contents

Methylmercury (MeHg) and inorganic arsenic (iAs) were estimated from the tHg and tAs contents. In fish, MeHg and iAs account for 92% of tHg and 10% of tAs, respectively (Kelly et al., 2018).

2.10 Calculation of energy value

The total energy value was calculated by adding up the calories provided by the proteins and lipids and expressed in kcal per 100 g.

2.11 Calculation of nutritional quality indexes of lipids

The quality of fatty acids was evaluated with five indicators: polyunsaturated fatty acids to saturated fatty acids ratio (PUFA/SFA); n-6/n-3, n-6 PUFA (sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:4n-6 and C22:2n-6) to n-3 PUFA (sum of C18:3n-3, C20:3n-3 C20:5n-3 and C22:6n-3) ratio; index of atherogenicity (IA); index of thrombogenicity (IT) and hypocholesterolemic/hypercholesterolemic ratio (HH) (Petenuci et al., 2016; Zhang et al., 2020). IA, IT and HH were calculated as follows:

$IA = [(C12{:}0 + (4 \times C14{:}0) + C16{:}0)]/(MUFA + n\text{-}6\ PUFA + n\text{-}3\ PUFA)$

$IT = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times MUFA) + (0.5 \times n\text{-}6\ PUFA) + (3 \times n\text{-}3\ PUFA) + (n\text{-}3\ PUFA/n\text{-}6\ PUFA)]$

HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)/(C14:0 + C16:0)

2.12 Calculation of SAIN and LIM scores

The SAIN and LIM scores were developed to describe the nutritional profile of food by taking into account the positive and negative nutrients (Darmon et al., 2009). The SAIN score corresponds to a nutrient density calculated by the arithmetic mean of the percentage adequacy for the 23 positive nutrients to assess a global evaluation of the nutritional quality.

$$\mathrm{SAIN} = \frac{\frac{\sum_{i=1}^{23} \frac{Nut_i}{RV_i}}{23}}{E} \times 100$$

where Nut_i is the quantity (g, mg or µg) of positive nutrient i in 100 g of food, RV_i is the daily recommended value for nutrient i and E is the energy (in kcal) in 100 g of food. The contents of vitamins C, E, B1, B2, B6 and B9 were obtained from the literature (Rehbein and Oehlenschläger, 2009).

The LIM score calculates the mean content of disqualifying nutrients in 100 g of foods.

$$\mathrm{LIM} = \frac{\sum_{j=1}^{3} \frac{Nut_j}{MRV_j}}{3} \times 100$$

where Nut_j is the quantity (g, mg or µg) of disqualifying nutrient j in 100 g of food and MRV_j is the daily maximal recommended value for nutrient j. The LIM 3 was calculated based on the SFA content, sodium and added sugars of the food. Added sugars were equal to zero as the fishes were not submitted to any formulation.

The number of nutrients involved in calculating the LIM score can be adapted according to the type of food and disqualifying nutrients. The LIM 11 was calculated by also taking into account eight heavy metals identified in the fish species of this study. Their MPL in µg per 100 g fish (wet basis) were as follows: Cr (5000), Cu (3000), Mn (100), Ni (50-100), tAs

(140), Cd (100), tHg (50) and Pb (200) (Agusa et al., 2005; Kelly et al., 2018; Moustafa et al., 2019).

This profiling allows classifying the food into four groups:

- SAIN > 5 and LIM < 7: food recommended for health
- SAIN < 5 and LIM < 7: neutral food
- SAIN > 5 and LIM > 7: food to consume in small quantities
- SAIN < 5 and LIM > 7: food to avoid or limit.

2.13 Data analysis

Fish morphometric characteristics were assessed on 81 individuals, while titratable acidity, pH, proximate composition, FA, vitamin A, minerals and heavy metals were determined on three pooled fish samples for each fish species. Data were analyzed using one-way analysis of variance (one-way ANOVA) using Statgraphics plus 5.1 (Virginia, USA). Significance was accepted at probability $P < 0.05$. Comparison of means was performed using the Tukey test. Hierarchical cluster analysis was used to group fish based on five nutritional quality indices (PUFA/SFA, n-6/n-3, IA, IT and HH).

3. Results

3.1 Fish morphometric

The average lengths, widths and weights of the ten fish species are presented in Table 1. The average length and weight of big-size species ranged respectively from 16.3 to 36.9 cm and 74.0 to 570.2 g, while those of the small-size species ranged from 7.3 to 12.7 cm and 2.9 to 23.5 g. Among big-size species, *Channa micropeltes* showed a significantly higher weight than the others. Weights of small-size species were not significantly different but *Henicorhynchus siamensis* showed the longest length.

3.2 pH, titratable acidity and proximate composition

The p-values of the effects of fish species and species size on titratable acidity, pH and proximate composition are shown in Table 2 as well as average values and ranges. Fish species significantly influence the moisture contents, the nutritional profile in macronutrients (protein, lipid and ash content) and energy values. The proximate composition of the ten fish species is described in Table S1 (supplementary material). Among the ten fish species, *Clupeoides borneensis* showed a low protein content, while *Boesemania microlepis* was low in lipid content. The effect of species size was significant on the protein and ash content. Overall, the protein content of fish samples varied from 11.8 to 20.9 g/100 g (Table 2) and average protein content of big-size species (18.6 g/100 g) was higher than average protein content of small-size species (15.8 g/100 g) (Table S1, supplementary material). The lipid content of fishes varied from 1.1 to 15.4 g/100 g (Table 2).

3.3 Fatty acid profiles

The p-values of the effect of fish species and species size on FA profile are presented in Table 3 with their minimum, maximum and mean values. FA representing less than 1.5% on average are not shown. For FA for which the effect of fish species was significant, average levels for each species are presented in Table S2 (supplementary material). Overall, 34 FA were identified and constituted about 75.7% to 96.8% of total fatty acids. Overall SFA, MUFA and PUFA accounted for 36.9%, 31.7% and 17.9% on average, respectively. In all species, the main SFA, MUFA and PUFA were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2n-6), respectively. The proportions of n-3 PUFA and n-6 PUFA were on average 8.7% and 9.2%. The ratios of PUFA/SFA and n-6/n-3 were respectively equal to 0.5 and 1.2 on average. The effect of fish species was significant on all the above mentioned fatty acids except C18:2n-6 and C18:3n-3. The effect of fish species was significant on the ratio n-6/n-3 but not PUFA/SFA. Species size significant affected only C18:2n-6, SFA, n-6 PUFA and n-6/n-3.

Hierarchical clustering analysis of the ten freshwater fish species with the five lipid nutritional quality indexes was built (Fig. S1, supplementary material). The IA values ranged from 0.5 (*Channa striata*, *Henicorhynchus siamensis* and *Boesemania microlepis*) to 0.8 (*Clupeoides borneensis* and *Barbodes gonionotus*), IT values from 0.4 (*Boesemania microlepis*) to 0.8 (*Barbodes goninotus*), and HH ratios were 1.9 on average. According to the cluster analysis, fishes have been classified into three groups with increasing nutritional quality of lipids.

3.4 Micronutrient and heavy metal contents

Vitamin A varied according to species and species size and its average content in fish samples was 63.3 µg RE/100 g (Tables 2 and 4). Vitamin A contents were tenfold higher in whole body small-size species (*Henicorhynchus siamensis* and *Clupeoides borneensis*) (Tables 4 and S1). Species size determined also Ca and some essential microelements (Fe and Zn). Fish species significantly influenced the content of some minerals (Ca, Cu and Zn) and some heavy metals (Al, Cd, tAs and iAs, tHg and MeHg) (Table 2). In all fish species, Zn and Fe contents were high compared to other trace elements. Essential trace elements in fish muscle were on average by decreasing order: Zn > Fe > Mn > Cu > Ni > Cr > Co. Mn was detected in most of the samples and reached a high amount (252 µg/100 g). The majority of fish samples had Mn amounts higher than the limit set by the international recommendation (*i.e.* 100 µg/100 g wet weight). Heavy metal patterns of the ten fish species were variable and quantified elements were on average by decreasing order: Al > As > Pb > Hg > Cd.

3.4 SAIN and LIM nutritional profiling

SAIN and LIM scores were calculated according to our data (Fig. 2). The average value of SAIN, LIM 3 and LIM 11 for each fish species ranged respectively from 9.7 to 30.1, 1.4 to 5.1 and 8.2 to 60.5. According to SAIN and LIM 3, all the fish species belong to the food group recommended for health. When LIM 11 was calculated with eight negative heavy metals (harmful at high amounts), nine fish species were ranked into the food to consume in small quantities. Seven out of ten species had high LIM 11 values while three species had LIM 11 values close to 7.

4. Discussion

The pH and titratable acidity values confirm the freshness of fish in the study. The proximate composition is in agreement with previously reported values on other species (Mohanty et al., 2019). Fishes can be classified according to their lipid contents into lean (<5%), medium-fat (5-10%) and high-fat (>10%) (Durmuş, 2019). Four fish species (*Channa micropeltes, Channa striata, Boesemania microlepis* and *Mystus atrifasciantus*) belong to the lean class, while the other ones were medium-fat fish species (Table S1, supplementary material). Lipid contents may have been influenced by the preparation of the sample (skinned fillet or whole fish with viscera and fatty organs). Lipid content varied greatly among species because they are accumulated at distinct yields according to the species, seasons, life cycles and food availability in the environment (Özogul et al., 2007; Petenuci et al., 2016; Zhang et al., 2020). Moisture content were inversely proportional to lipid content and the sum of these two parameters reached approximately 80% of the total composition as previously observed (Mohanty et al., 2019).

The average ash content of big-size and small-size species were respectively 1.2 and 2.9 g/100 g (Table S1, supplement material). This is probably due to the presence of bones, head, skin and, for some fish, organs such as liver in their edible parts. Bogard et al. (2015) noted an increase in the ash content of the edible parts of certain species of fish depending on the presence of bones. Thus, although *Trichogaster microlepis* is a small fish eaten without organs, it is very rich in ash, which may reflect its richness in bones (Herawati et al., 2018). Indeed, in addition to the backbone, this species also contains many fins and small bones.

All fish species can be considered as a source of protein because their content is higher than 13 g/100 g (Table S1) (Bogard et al., 2015). Depending on their proximate composition, the energy content varies from 70.7 to 214.4 kcal/100 g of fish (Table 2). Bogard et al. (2015) also observed large variations in energy content ranging from 64 to 244 kcal/100 g depending on the fish species. The energy value of fish is associated with their lipid content and inversely proportional to their water content. For undernourished individuals, *Henicorhynchus siamensis* has an interesting nutritional profile with a high protein (17.9 g/100 g), lipid (10.0 g/100 g) and ash (2.8 g/100 g) levels.

Fish samples were collected during the dry season when the lake temperature reaches its highest level around 31.6 °C (Campbell et al., 2006). Water temperature can affect the FA composition of fish and SFA proportion increases with temperature (Özogul et al., 2007; Petenuci et al., 2016). The main SFA and MUFA are the same as in other species (Emre et al., 2018). α-Linolenic acid (C18:3n-3), EPA (C20:5n-3) and DHA (C22:6n-3) were also abundant in PUFA. The fishes in this study can be considered rich in omega 3 fatty acids as the sum of EPA and DHA is above 80 mg/100 g or 100 kJ required by nutritional guidelines in Europe (Bucchini, 2019). Regarding PUFA, whose benefits have been mentioned in the introduction, the contents of n-6 fatty acids were always higher than n-3 fatty acids (Table 3). This richness in n-6 PUFA (especially linoleic acid, C18:2n-6) was already observed in freshwater fish. Compared to marine species, freshwater fish contain high levels of C18:2n-6 PUFA and low levels of EPA (C20:5n-3) and DHA (C22:3n-3) (Özogul et al., 2007). All fish species studied had a n-6/n-3 ratio of less than four, as recommended for the prevention of cardiovascular disease (Zhang et al., 2020). A minimum value of PUFA/SFA ratio recommended is 0.45 (Zhang et al., 2020). In our studies, the PUFA/SFA ratios ranged from 0.4 to 0.7 and were lower than the values reported for sardines (1.5), known to be high in PUFA (Petenuci et al., 2016). The evaluation of the nutritional value of lipids with the PUFA/SFA and n6/n3 ratio only lead to simplistic dietary advice as they both do not take into consideration MUFA. The IA, IT and HH indices were calculated to better characterize the lipid quality of the ten freshwater fish species. The calculation of the IA and IT indices takes into account the FA involved in the prevention of coronary heart disease and low values are desirable (Zhang et al., 2020). HH takes into account the FA involved in cholesterol metabolism and high HH values are considered more beneficial to human health (Petenuci et al., 2016; Zhang et al., 2020). The IA, IT and HH values were similar to previously published data on five freshwater fishes from the Amazon Basin, with IA ranging from 0.36 to 0.55, IT from 0.51 to 0.89 and HH from 1.69 to 2.46 (Petenuci et al., 2016). The ten freshwater fish species were clustered into three groups with a decreasing nutritional quality from group one to group three. The group one is more desirable for human consumption (Zhang et al., 2020), and includes *Channa striata*, *Henicorhynchus siamensis* and *Boesemania microlepis*. *Cyclocheilichthys enoplos* and *Barbodes gonionotus* have a similar trend of fatty acid composition (Table S2 and Fig. S1, supplementary material). This could be explained by the fact that the two species consume the same diet because their habitat and distribution ranges from the center to the extreme south of TSL (Chan et al., 2020).

Some essential micronutrients, particularly vitamin A were influenced by the size of species. The fish consumed with their liver and eyes (*i.e.* some small-size fish species) were analyzed as eaten and had a content of vitamin A ten-fold higher than the fish without these parts or organs. Many studies found that vitamin A is accumulated in the eyes and viscera of fish (Roos et al., 2007c). The vitamin A content of the two small fish eaten whole (*Clupeoides borneensis* and *Henicorhynchus siamensis*) is comparable to previously published data for the same fish from the Mekong River in Cambodia (100 to 500 RE µg/100 g) (Roos et al., 2007a). According to FAO and WHO, the recommended intake value of vitamin A ranges from 375 µg RE/day for infants to 600 µg RE/day for adults (Roos et al., 2002). *Henicorhynchus siamensis,* which showed a vitamin A content of 354.3 µg RE /100 g wet weight, is a good source of vitamin A compared to the other screened species. The consumption of fish species with high vitamin A content could improve the vitamin A status of Cambodian people, as fish is part of their daily dietary patterns.

The essential microelements with the highest content in the species studied (Zn, Fe, Mn and Cu) have a key role in the metabolism of organs and tissues and the maintenance of cellular functions (Alturiqi and Albedair, 2012). Zn and Fe occur in higher amounts in most aquatic organisms and are essential micronutrients required for their growth, physiological functions and metabolism (Kelly et al., 2018). As previously observed for vitamin A, the Zn and Fe amounts were higher in small fish species (doubled for Zn) compared to most of the big size species. Indeed, Zn and Fe are accumulated in the liver and used to produce blood cells and hemoglobin. Furthermore, Zn is associated with binding proteins such as hepatic metallothionein (Nargis et al., 2020). Small whole fish can be considered a good source of essential micronutrients, especially Zn, Fe, Ca and vitamin A.

The SAIN and LIM 11 scores highlight the fact that fishes have an interesting nutritional profile globally but can be contaminated by harmful heavy metals. *Henicorhynchus siamensis* used in several nutritional programs shows low LIM 3 and acceptable LIM 11 scores, while *Clupeoides borneensis* had the highest score of LIM 11. The high contamination of the latter fish could be due to its morphometry as it is the smallest fish species. The diet of *Clupeoides borneensis* is based on zooplankton, which is a good indicator of water quality as it can be highly contaminated by heavy metals (Gagneten, 2010; Lim, 1999). This could be one of the reasons why this fish species contains a high concentration of heavy metals.

According to their contents in food, some microelements (Cd, Cu, Cr and Mn) are beneficial or harmful for humans (Alturiqi and Albedair, 2012). Cd, Cu and Cr contents were below the limits set by international guidelines. Around 56% of the fish samples were exceeding the MPL for Mn. Excessive amounts of Mn were observed for all samples of *Clupeoides borneensis* (content up to six-fold-higher than MPL) and one sample of *Barbodes gonionotus* (content nine-fold-higher than MPL) and all samples of *Puntioplites proctozysron* (content up to five-fold-higher than MPL) (data not shown). *Henicorhynchus siamensis* was the only fish species with none of the samples contaminated with Mn. Mn is essential for humans but, at a high amount, it is responsible for psychological and neurological disorders, kidney failure and reduced immune function (Nargis et al., 2020).

Cadmium and mercury contents were always below the MPL and tAs was below the MPL (140 µg/100 g) except for *Channa striata* species (152 µg/100g wet weight). iAs amounts were all far below the limit. *Clupeoides borneensis* was the only fish species with a Pb content superior to FAO guideline (50 µg/ 100 g) but still below the WHO guideline (i.e. 200 µg/100 g) (Agusa et al., 2005; Kelly et al., 2018; Moustafa et al., 2019). As and Pb are among the three most toxic heavy metals with mercury with no biological role and only harmful risks for humans. Human long-term exposure to arsenic can form skin lesions, cancers, neurological problems, peripheral vascular disease, hypertension and diabetes mellitus (Schenone et al., 2014). Pb has long-term negative impacts on health causing anemia, encephalopathy, hepatitis and nephritic syndrome (Moustafa et al., 2019). Pb is highly harmful to the neonate because it crosses the blood-brain barrier and the placenta; it can cause reduce child cognitive development, increased blood pressure and cardiovascular diseases in adults (Alturiqi and Albedair, 2012). The variation in the amounts of heavy metals in fish depends on the species, metabolism, size, life cycle, habitat, environmental characteristics and feeding habits. In addition, the efficiency of heavy metal uptake by fish from polluted water and food depends on metabolism.

Mn and As levels are high during the dry season when water levels are lower and pollutants are more concentrated (Kelly et al., 2018). Our fish were also sampled during this season. Mn and Fe could come from corrosion and abrasion from submerged hydraulic turbines along the Mekong River (Sim et al., 2016). Higher contents of Mn, Pb and tAs were found in Pearl River Delta in China (Leung et al., 2014). *Henicorhychus siamensis* is the only species, which showed contents below the legal values for all heavy metals.

Fish is important in the diets and livelihoods of many poor people suffering from vitamin and mineral deficiencies. It is very appreciated by local people living near the Mekong River and the TSL and available and affordable for them (Vilain et al., 2016). The most interesting fish species was *Henicorhynchus siamensis* as it provides high contents of protein, lipid, vitamin A, Zn and Fe with low heavy metal levels. In addition, it belongs to the most interesting group in terms of the quality of FA. This species is currently used in nutrition programs because the small-size species are preferred by Cambodia consumers in rural areas (Vilain et al., 2016).

TSL communities face social and economic challenges. With low incomes, people do not have access to a balanced diet that is conducive to nutrition and health. In rural areas, most Cambodians are affected by micronutrient deficiencies, especially women and children. About 40% of children are stunted and 28% are underweight, while 60% of women are anemic and 20% are underweight (Bagriansky et al., 2013). Iron deficiency has been recognized as a common disorder in ASEAN and Cambodia. Consumption of fish rich in Zn and Fe could improve these nutritional deficiencies. Nutritional strategies based on dietary diversity and fish consumption are sustainable as they enhance the value of local resources, generating income for small-scale fishers and promoting nutrition.

5. Conclusion

Fish species determine macronutrients and some microelements, especially heavy metals (Mn, As, Pb) while fish species size influences the essential microelements Fe, Zn and vitamin A. The ten fish species studied have good overall nutritional profile according to SAIN and LIM 3 classification. Small fish species contained several essential micronutrients (Fe, Zn and vitamin A) particularly when species are consumed as a whole with their head, eyes and organs such as *Henicorhynchus siamensis* and *Clupeoides borneensis*. All the fish species showed higher values of SFA and MUFA than PUFA and they can be considered rich n-3 fatty acids. In most samples, high amounts of Mn were quantified and this element should be monitored in the environment and food. In the same way, special attention has to be paid to total arsenic in *Channa striata* and Pb in *Clupeoides borneensis*.

In order to prevent human health problems related to these elements, it is important to enforce regulations to protect the Tonle Sap Lake ecosystem from pollution. *Henicorhynchus siamensis* was the most interesting species with a balanced composition (proteins, lipids, ash content), better FA quality, high levels of essential micronutrients Fe, Zn and vitamin A and low levels of harmful heavy metals.

CRediT authorship contribution statement

Sengly SROY: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing – review & editing. **Elodie ARNAUD:** Conceptualization, Methodology, Validation, Writing – review & editing. **Adrien SERVENT:** Methodology, Writing – review & editing. **Sokneang IN:** Conceptualization, Writing – review & editing. **Sylvie AVALLONE:** Conceptualization, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing.

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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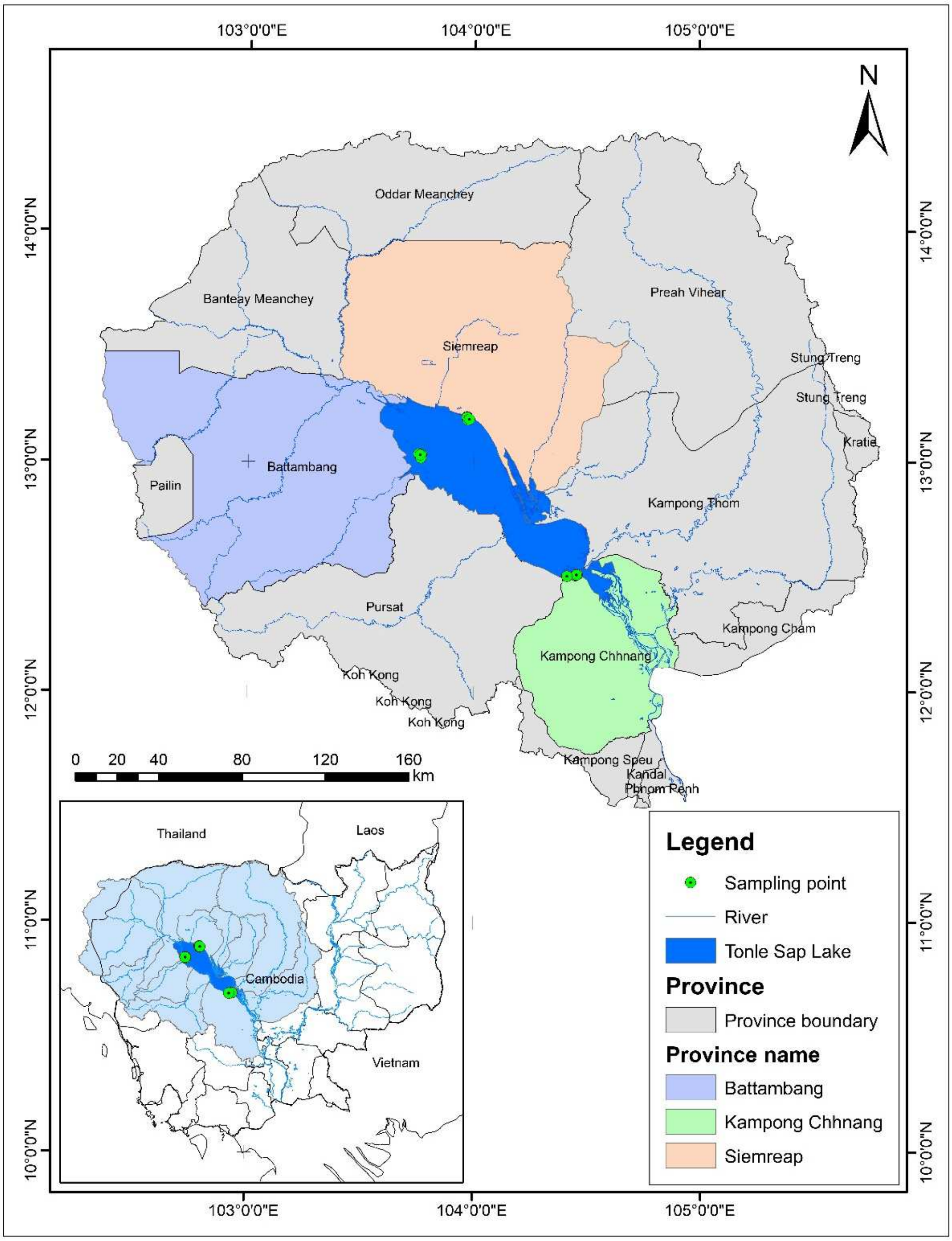
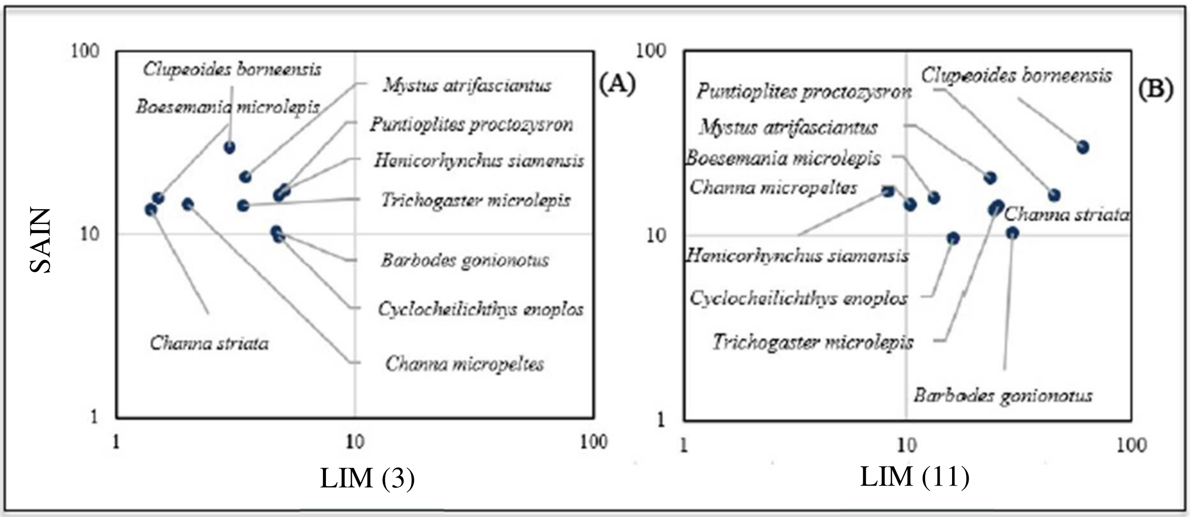
FIGURE CAPTION

Figure 1. Fish sampling area.

Figure 2. Classification of the ten fish species according to SAIN–LIM scores: (A) SAIN LIM 3, (B) SAIN LIM 11. X axis represents LIM and Y axis represents SAIN.



103°0'0"E
104°0'0"E
105°0'0"E
N
14°0'0"N
13°0'0"N
12°0'0"N
11°0'0"N
10°0'0"N
Oddar Meanchey
Banteay Meanchey
Siemreap
Preah Vihear
Stung Treng
Stung Treng
Kratie
Battambang
Pailin
Kampong Thom
Pursat
Kampong Cham
Kampong Chhnang
Koh Kong
Koh Kong
Koh Kong
Kampong Speu
Kandal
Phnom Penh
0 20 40 80 120 160 km
Thailand
Laos
Cambodia
Vietnam
Legend
Sampling point
River
Tonle Sap Lake
Province
Province boundary
Province name
Battambang
Kampong Chhnang
Siemreap



(A)
SAIN
LIM (3)
100
10
1
1
10
100
Clupeoides borneensis
Boesemania microlepis
Mystus atrifasciantus
Puntioplites proctozysron
Henicorhynchus siamensis
Trichogaster microlepis
Barbodes gonionotus
Cyclocheilichthys enoplos
Channa striata
Channa micropeltes
(B)
LIM (11)
100
10
1
1
10
100
Puntioplites proctozysron
Clupeoides borneensis
Mystus atrifasciantus
Boesemania microlepis
Channa micropeltes
Channa striata
Henicorhynchus siamensis
Cyclocheilichthys enoplos
Trichogaster microlepis
Barbodes gonionotus

Table 1. Edible part, local, English and scientific names and morphometric characteristics of the ten fish species.

Fish size and edible part	Local name	English name	Scientific name	Length (cm)	Width (cm)	Weight (g)	N
Big-size species							
Fish fillet	Trey chhkok	Medium-sized cyprinids	*Cyclocheilichthys enoplos*	29.0±6.6[b]	7.4±2.0[abc]	282.1±275.7[b]	81
	Trey chhpin	Medium-sized cyprinids	*Barbodes gonionotus*	23.0±4.1[c]	8.3±1.7[a]	234.3±206.3[bcd]	81
	Trey chrakaing	Medium-sized cyprinids	*Puntioplites proctozysron*	16.3±3.6[d]	6.1±1.6[c]	74.0±55.3[cd]	81
	Trey diep	Indonesian snakehead	*Channa micropeltes*	36.9±9.9[a]	6.6±2.6[bc]	570.2±774.0[a]	81
	Trey phtouk	Striped snakehead	*Channa striata*	29.4±6.1[b]	4.3±1.4[d]	233.5±137.8[bcd]	81
	Trey promah	Boeseman croaker	*Boesemania microlepis*	32.3±8.7[ab]	7.7±2.7[ab]	266.9±280.8[bc]	81
Whole body small-size species							
Fish without organs	Trey kanchos	Mystus catfishes	*Mystus atrifasciantus*	11.3±2.5[ef]	2.1±0.7[ef]	12.2±8.8[d]	81
	Trey kawmpleanh	Gouramis	*Trichogaster microlepis*	9.5±2.0[ef]	3.4±0.8[de]	13.2±8.6[d]	81
Fish with organs	Trey bawndol ampeou	Thai river sprat	*Clupeoides borneensis*	7.3±1.4[f]	0.8±0.3[f]	2.9±1.3[d]	81
	Trey riel	*Siamese mud carps*	*Henicorhynchus siamensis*	12.7±2.2[d]	3.2±0.8[de]	23.5±15.4[cd]	81

N: number of specimens. Results are expressed as mean ± standard deviation. Different letters in the same column mean a significant difference (P<0.05) by Tukey's test throughout the different species.

Table 2. Statistical significance (one-way ANOVA) of fish species and species size effects on pH, titratable acidity, proximate composition, vitamin A, minerals and heavy metals of fish.

Parameters	Fish species	Species size	Mean	Minimum	Maximum
pH	NS	NS	6.5±0.3	5.9	7.0
Titratable acidity (%)	NS	NS	0.6±0.1	0.4	0.7
Moisture (g/100 g)	***	NS	75.5±3.0	70.5	80.8
Protein (g/100 g)	***	***	17.5±2.2	11.8	20.9
Lipid (g/100 g)	**	NS	5.3±2.2	1.1	15.4
Ash (g/100 g)	***	***	1.9±0.9	0.7	3.7
Energy (kcal/100 g)	**	NS	117.5±29.3	70.7	214.4
Vitamin A (RE µg/100 g)	***	**	63.3±109.6	6.1	422.8
Major elements (mg/100 g)					
Ca	*	*	453.3±383.8	0.0	1123.1
K	***	***	254.5±79.9	69.0	381.9
Mg	NS	*	33.5±6.2	19.1	47.3
Na	**	NS	48.9±13.1	12.4	71.7
P	***	***	399.1±176.9	139.7	690.4
Trace elements (µg/ 100 g)					
Fe (MPL 10000 µg/100 g)	NS	*	645.7±354.7	0.0	1448.1
Zn (MPL 10000 µg/100 g)	***	***	1014.7±640.6	1.2	2601.2
Al	**	***	257.4±236.8	0.0	862.8
Cd (MPL 100 µg/100 g)	***	NS	0.2±0.3	0.0	0.9
Co	NS	NS	1.7±2.5	0.0	11.9
Cr (MPL 5000 µg/100 g)	NS	NS	2.7±1.9	0.0	7.1
Cu (MPL 3000 µg/100 g)	**	NS	22.1±22.4	0.0	100.8
Mn (MPL 100 µg/100 g)	NS	NS	213.2±227.9	0.0	897.5
Ni (MPL 50 to 100 µg/100 g)	NS	NS	18.7±63.7	0.0	343.8
Pb (MPL 50-200 µg/100 g)	NS	NS	14.1±48.5	0.0	259.4
Arsenic and Mercury (µg/100 g)					
tAs (MPL 140 µg/100 g)	***	NS	24.9±56.6	0.0	244.7
iAs	***	NS	2.5±5.7	0.0	24.5
tHg (MPL 50 µg/100 g)	***	NS	3.0±2.0	0.5	7.5
MeHg	***	NS	2.7±1.9	0.4	6.9

NS, $P \geq 0.050$; $*P<0.050$; $**P<0.010$; $***P<0.001$. Mean values were calculated irrespective of significant effects of fish species (FS) or size (S). Data are presented as mean ± standard deviation. Contents are expressed per 100 g of fresh weight. tAs: total arsenic. iAs: inorganic arsenic. tHg: total mercury. MeHg: methyl mercury. MPL (Maximum Permissible Limit) are indicated in bracket after each element. RE: Retinol equivalent.

Table 3. Statistical significance (one-way ANOVA) of fish species and species size effects on fatty acid profile of fish.

Fatty Acid (%)	Fish species	Species size	Mean	Minimum	Maximum
C14:0 (myristic acid)	NS	*	2.7±0.7	1.5	4.7
C15:0 (pentadecylic acid)	*	NS	1.9±0.8	0.5	3.4
C16:0 (palmitic acid)	**	NS	18.3±3.0	12.5	26.3
C17:0 (heptadecanoic acid)	NS	NS	2.4±0.7	0.9	3.9
C18:0 (stearic acid)	***	NS	7.2±2.4	4.7	12.2
C16:1n-7 (palmitoleic acid)	***	***	6.4±2.8	1.7	13.4
C18:1n-9 cis (oleic acid)	***	NS	18.5±5.7	7.72	35.77
C18:1n-9 trans (elaidic acid)	***	NS	3.6±1.3	0.7	6.4
C18:2n-6 (linoleic acid)	NS	*	6.4±2.0	3.7	11.7
C18:3n-3 (alpha linolenic acid)	NS	NS	3.3±1.4	1.8	8.3
C20:5n-3 (eicosapentaenoic acid)	***	NS	1.6±1.1	0.4	4.3
C22:6n-3 (docosahexaenoic acid)	***	NS	3.5±2.1	1.0	9.0
SFA	NS	**	36.9±2.9	29.4	42.0
MUFA	NS	NS	31.7±4.2	22.7	41.9
PUFA	NS	*	17.9±2.8	12.9	26.8
PUFA/SFA	NS	NS	0.5±0.08	0.4	0.7
n-6 PUFA	**	***	9.2±2.4	4.8	13.8
n-3 PUFA	**	NS	8.7±2.7	4.4	15.9
n-6/n-3	**	**	1.2±0.6	0.3	2.6
Unidentified FA	**	***	12.8±4.1	3.7	23.5
IA	*	NS	0.6±0.1	0.4	0.9
IT	***	NS	0.6±0.2	0.4	0.9
HH	NS	NS	1.9±0.3	1.2	2.6

NS, P≥0.050; *P<0.050; **P<0.010; ***p<0.001. Mean values were calculated irrespective of significant effects of fish species or species size. Data are presented as mean ± standard deviation, expressed as percentage (%). FA, fatty acids; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids. Only mean of FA representing more than 1.5% is shown. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. n-6/n-3, omega-6 (sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:4n-6 and C22:2n-6) to omega-3 (sum of C18:3n-3, C20:3n-6, C20:5n-3 and C22:6n-3) ratio. IA: index of atherogenicity. IT: index of thrombogenicity. HH: hypocholesterolemic/hypercholesterolemic ratio.

Table 4. Vitamin A content of fish species classified according to their edible parts and iron and zinc contents of big and small-size species.

Type of fish	Mean	Minimum	Maximum
Vitamin A (µg RE/100 g)			
Fish fillet	13.8±8.4[b]	5.2	45.0
Whole body fish without organs	25.0±6.3[b]	16.8	35.3
Whole body fish	250.1±122.5[a]	59.5	424.4
Iron (µg/100 g)			
Big-size-species	535.9±325.0[b]	0.0	1073.8
Small-size-species	810.3±345.3[a]	166.2	1448.2
Zinc (µg/ 100 g)			
Big-size-species	729.8±484.6[b]	1.2	1501.2
Small-size-species	1442.1±0.6[a]	328.3	2601.2

Data are presented as mean ± standard deviation. Contents are expressed per 100 g of fresh weight. Different letters in the same column of each component mean significant difference ($P<0.05$) by Tukey's test throughout the different species.