SEA LAMPREY (*PETROMYZON MARINUS L.*), A DELICACY WITH HIGH GASTRONOMIC IMPACT IN SEVERAL COUNTRIES OF WESTERN EUROPE: NUTRITIONAL COMPOSITION AND HEALTHY LIPID INDEX OF FILLETS

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ABSTRACT - In some countries, sea lamprey (*Petromyzon marinus* L.) is considered a delicacy and its intensively captured during spawning migration, being its fishery an important source of income for inland commercial fishermen in Western Europe (Portugal, Spain, France), providing hundreds of thousands of sea lamprey to restaurants every year. In Portugal, it is sold at high prices in restaurants where tens of thousands of sea lamprey are consumed annually. This work characterizes sea lamprey proximate composition of fillets, providing the fatty acid profile, crude total protein, total cholesterol, macro and micro minerals, gross energy contents and lipid healthy indexes. 30# sea lamprey were captured during early spawning migration in Guadiana and Mondego River basins, Portugal. The results revealed that fillets present a high lipid content (50,7g/100g, dry weight), a high gross energy (684,5Kcal/100 g, dry weight), a low total cholesterol (0.056g/100 g, dry weight) and a very high ω3/ω6 ratio (11,2). Thus, sea lamprey could be a healthy lipid choice for consumers.

KEYWORDS: sea lamprey; proximate composition; healthy lipid indexes, muscle; fillets

LAMPREIA-MARINHA (*PETROMYZON MARINUS L.*), UMA IGUARIA COM ELEVADO IMPACTO GASTRONÓMICO EM VÁRIOS PAÍSES DA EUROPA OCIDENTAL: COMPOSIÇÃO NUTRICIONAL E ÍNDICE LIPÍDICO SAUDÁVEL DOS FILETES

RESUMO: Nalguns países, a lampreia-marinha (*Petromyzon marinus* L.) é considerada uma iguaria e é capturada intensivamente durante a migração reprodutiva, sendo a sua pesca uma importante fonte de rendimento para os pescadores comerciais de interior na Europa Ocidental (Portugal, Espanha, França), fornecendo centenas de milhares de lampreias-marinhas para restaurantes todos os anos. Em Portugal é vendida a preços elevados em restaurantes onde são consumidas anualmente dezenas de milhares de lampreias-marinhas. Este trabalho caracteriza a composição centesimal dos filetes de lampreia-marinha, fornecendo o perfil de ácidos gordos, proteína bruta total, colesterol total, macro e micro minerais, teor de energia bruta e índices lipídicos saudáveis. 30# lampreias-marinhas foram capturadas durante a migração inicial de desova nas bacias dos rios Guadiana e Mondego, Portugal. Os resultados revelaram que os filetes apresentam alto teor lipídico (50,7g/100g, peso seco), alta energia bruta (684,5Kcal/100 g, peso seco), baixo colesterol total (0,056g/100 g, peso seco) e uma relação ω3/ω6 muito elevada (11,2). Assim, a lampreia-marinha pode ser uma escolha lipídica saudável para os consumidores.
PALAVRAS-CHAVE: lampreia marinha; composição centesimal; índices lipídicos saudáveis, músculos; filetes

INTRODUCTION

Seafood is a major contributor to nutrition for many people worldwide (FAO, 2022). It is a particularly important source of high-quality protein, whilst being high in vitamins and minerals, low in saturated fat and cholesterol and the best global source of ω3 fatty acids (Kolakowska *et al.*, 2003). Marine halieutic resources play an extremely important role in the diet, particularly in countries with extensive coastal shores and in coastal communities where fish is the main source of animal protein (Afonso, 2009).

According to FAO reports, in 2016, the global *per capita* fish consumption increased above 20 kg *per* year for the first time. Japan has among the highest seafood consumption with an average of 79 kg *per capita per year* (FAO, 2018). This contrasts with the EU countries, where annual fish consumption averages 17 kg *per capita per year*, being Portugal, the EU country with the highest fish consumption per capita, *ca.* 57 kg/year (FAO, 2018). In 2030, 21,5 kg will the expected world fish consumption *per capita* according to FAO (2022).

To the best of our knowledge, information about the valorization of sea lamprey as an important nutritional resource is scarce (Araújo *et al.,* 2013; Araújo *et al.*, 2016; Ventura, 2014). Sea lamprey fisheries are a traditional activity in several western European countries (Portugal, Spain, and France) and artisanal sea lamprey fishery is an activity of high gastronomic and economic impact, despite its seasonality, particularly for coastal and

riverine communities (Ventura, 2014). In Portugal the value of a single specimen can range between €50 and €60, depending on the abundance of sea lampreys in each river basin each year or the time of capture throughout the fishing season (Pedro *et al*., 2013) being the trading price practiced outside of official fishing markets. In a restaurant, prices can vary between €90 and €150 (in the most expensive ones) during gastronomic festivals.

However, nutritional characterization of the sea lamprey fillets is scarce (Araújo *et al*., 2013) and in generally do not consider the sea lamprey`s capture location, which causes some bias in the nutritional profile analysis because during their upstream spawning migration, sea lamprey does not feed and greatly modify their lipid reserves (Lança *et al*., 2011).

Because sea lamprey fishery is an important source of income for inland commercial fishermen, it is therefore important to perform a characterization and an evaluation of the nutritional quality of sea lamprey´s fillets to assess if they could be considered a healthy practice in the diet of consumers and if will have value as a potential food and marketing item supported by nutritional quality analysis.

MATERIAL AND METHODS

All animal handling procedures were carried out in strict accordance with the recommendations present in the Guide for the Care and Use of Laboratory Animals of European Union 62/2010, in Portugal under DL nº 192/92, Portaria nº 1005/92 and DL 113/2013 and approved by the University of Évora ethics committee (ORBEA). Euthanasia was performed following the recommendations of the official regulations previously mentioned.

Sampling sites, animals, and selection of tissue samples

A total of #30 adult sea lampreys were captured by local fishermen in designated commercial fishing areas. In the Mondego River basin, sea lampreys were captured using fyke-nets, and in the Guadiana River basin, commercial fishermen used trammel nets to catch these fish. Of these, #15 were caught in the River Mondego at Figueira da Foz (40°7'0"N, 8°54'0"W) and #15 were caught at Mértola (37°38'0"N, 7°40'0"W), 80 km upstream from the mouth of the Guadiana River. No specific permissions were required for sampling in these locations because the adult sea lampreys were captured by local fishermen in designated commercial fishing areas. Moreover, the catch in these locations accurately reflects the nutritional characteristics of the individuals that will be used for gastronomic purposes.

After capture, all individuals were transported alive in tanks with adequate life support systems (i.e. aeration, filtration) to the laboratory. Upon arrival at the laboratory sea lampreys were first immersed in cold water to minimize handling stress and pain sensibility

and after that euthanized individually by contusion. For sea lamprey, data on total body length (*TL*, nearest millimeter) and total body mass (*TW*, nearest g) were recorded. Gonad was macroscopically examined to gender determination. The skin was removed, and the muscle tissue was exposed.

Muscle samples were collected in the proximity of the mid-dorsal line, on the left flank of the animal, close to the dorsal fin and were washed with physiologic saline and homogenized individually. Then, half of the homogenized sample was immediately stored at -80°C until laboratorial processing and the remaining portion was lyophilized for subsequent analysis.

Proximate Composition

Moisture content and Ash Content

Moisture content (water content) was determined according to IPQ (1991). Samples of 5-gram of homogenized fillet were defrosted and dried at 105°C ± 2°C until weight stabilization. Moisture content and dry matter were determined according to the following equations:

$$Moisture\ content\ (\%) = ((Wet\ sample - Dry\ sample)/\ Wet\ sample)) \times 100$$

and

$$Dry\ Matter\ (\%) = 100 - Moisture\ Content\ (\%)$$

Where: Wet sample – mass of wet sample (g); Dry sample – mass of dry sample (g)

The determination of the total ash content was carried out according to the method described in IPQ (1988).

Determination of macro and micro elements

Several elements [phosphorous (P), potassium (K), sulphur (S), calcium (Ca), iron (Fe) and zinc (Zn)] were determined by energy-dispersive x-ray fluorescence (EDXRF) according to Carvalho *et al*., (2005). Briefly, lyophilized muscle sample was pressed into circular tablets with a 2 cm diameter and 1 mm thickness under a pressure of 1333,224 Pa (Graseby, Speac, U.K.). The tablets were glued to a 50 x 50 mm Mylar film and exposed to radiation for 1000 s, using a PW 1140 x-ray tube (100 kV, 80 mA; Phillips, Eindhoven) equipped with a changeable molybdenum secondary target and Si(Li) detector (High Wycombe, Oxford, UK). The x-ray generator was operated at 20 mA and 50 kV and the spectra were analyzed using Quantum MCA software. The energy corresponding to each peak observed is determined by analysis of the energy versus intensity spectra obtained for each of the elements present in the sample. Each component is identified by comparing the amount of energy obtained with the amount of theoretical energy provided in reference tables. The concentration of each element was expressed in mg/kg wet weight. Further detail is provided in Carvalho et al., (2005).

Determination of gross energy content

The gross energy content was measured using an automatic oxygen bomb calorimeter (Parr 6400, U.S.A.) according to ISO (1988). Briefly, 0.5 g portions of homogenized muscle were analyzed. The heat produced during combustion of the sample is absorbed by the metal pump and transmitted to water. The calorific value is automatically determined from the change in the water temperature. To directly compare the sample nutritional values with those provided in the literature, the results were converted from megajoules (MJ) per kg of dry matter to kilocalories (kcal) per 100 g of wet weight, according to the following equation:

$$Energy\left(\frac{Kcal}{100g}\right) = \frac{E_{MJ} \times 239}{10} \times DM$$

Where: E_{MJ} is the sample energy (MJ), 1 MJ = 239 kcal and *DM* is sample dry matter.

Determination of crude protein content

Crude protein content was determined by combustion according to AOAC (1990). Briefly, 100-mg of lyophilized homogenized fillet was combusted at high temperature (950°C) in the presence of oxygen to release carbon dioxide, water, and nitrogen. The thermal conductivity detector measures N_2, according to the equation:

$$\%\ N\ (DMbasis) = \frac{\%\ N\ Lab\ DM}{100}$$

Where: % *N* is percent nitrogen on a dry matter basis and *Lab DM* is the dry matter of a subsample determined in the laboratory.

The nitrogen was converted to protein content, using the following equation:

$$\%\ CP\ (DM\ basis) = \%\ N\ (DM\ basis) \times F$$

where: % *CP* is percent crude protein on a dry matter basis and *F* = 6.25.

Determination of total lipids and fatty acid fillet profile

Fillet´s total lipids were extracted using accelerated solvent extraction (ASE) as described in Jorge *et al*., (2021). Aliquots of individual homogenized and lyophilized samples with 500-mg (±0.005 g) were pulverized in an aluminum mortar with a stainless-steel pestle, both cooled in liquid nitrogen and the tissue powder was combined with a matrix drying agent (Diatomaceous Earth, hydro matrix Varian, P/N 049458). The total lipids were then extracted with a mixture of chloroform/methanol (60:40 vol: vol) (Merck; Darmstadt, Germany) at 100 °C and at 13.8 MPa. The crude extract was reconstituted in 0.5 N NaOH and the fatty acids derivatized with BF_3-CH_3OH (Merk-Schuchardt, Germany) to give fatty acid methyl esters (FAME), according to the procedure of Morrison and Smith (1964). The recovered organic phase was spiked with the methyl ester of C19:0 as the internal standard at a final concentration of 250 µg mL^{-1}. The FAME were analyzed using

a Hewlett Packard gas chromatograph (GC) instrument (HP 6890 series), equipped with a split-splitless injector, an auto-sampler, a flame-ionization detector (FID) and an Omega-wax 320 fused silica capillary column (Supelco, Bellefonte, PA). The chromatographic conditions were helium as carrier gas at a constant flow of 2.0 mL/min; injector operated in splitless mode for 1 minute, at 270 °C; MS interface: 240 °C and MS source: 220 °C. The oven temperature was held at 120 °C for 5-min and then increased from 120 to 250 °C with a ramping rate of 5 °C/min to 250 °C where it was maintained for 59 min. The peaks and their respective MS were analyzed by electronic impact at 70 eV, within the range of of m/z= 40 to 450 Da. The FAME standards used were 37-component FAME MIX (Ackman, 2002; Jorge *et al.*, 2021). FAME were identified by comparing their retention times with known standards (37-component FAME mix, Supelco plus C22:5ω3 fatty acid methyl ester) chromatographed in identical GC conditions. Each fatty acid concentration was converted to mg of fatty acid per 100 g of wet muscle weight (mg/100g) according to the equation:

$$\text{Fatty Acid (mg/100g)} = [\frac{(M_X \times M_{TL}) \times 100}{M_{LS}}] \times DM$$

Where: M_X=mass of the fatty acid *X* (mg/g of total lipid extract); M_{TL}=mass of the total lipids (g); M_{LS}=mass of the lyophilized sample (g) and DM=dry matter (%).

Determination of total cholesterol

Sea lamprey fillet´s total cholesterol content was determined using a cholesterol quantitation kit (Roche kit no. 10139050035, R-Biopharm, Germany) based on the principle described by Röschlau *et al.*, (1974). Briefly, 100 mg of the total lipids crude extract was reconstituted in a methanolic 2 M NaOH solution and saponified at 80° C for 30 min. The reaction was stopped by adding distilled water, cooled to 20 - 25° C and then, ether/petroleum ether solution (1/1; vol/vol) was added. The phases were stirred vigorously and then allowed to separate. The upper phase was evaporated under nitrogen flow. Then, $(CH3)_2CHOH$ at 20 - 25° C was added, the solution stirred and filtered. The total cholesterol was determined in the filtrate, according to the supplier's recommendations. The absorbance was determined at 450 nm (UV Beckman DU-530). The total cholesterol concentration in the muscle was calculated, according to the formula supplied with the kit. The total cholesterol content in muscle lipids (mg/100g) was calculated using the equation:

$$C\ mg/100g = c \times \frac{100 \times 50}{M}$$

Where: *c* = cholesterol concentration (g/L sample) and *M* = mass of the sample (g).

Indexes for lipid quality of sea lamprey fillets

Several indexes (PUFA/SFA, polyunsaturated saturated fatty acid ratio; h/H, hypocholesterolemic hypercholesterolemic ratio; TI, thrombotic index; AI, atherogenic index and w3/w6 ratio) were used to estimate the lipid quality of sea lamprey´s fillets (i.e., edible portion). The indexes were calculated according to the following equations:

$$\frac{\sum \text{PUFA}}{\sum \text{SFA}} = \frac{(\text{C18:2}\omega6 + \text{C18:3}\omega6 + \text{C18:3}\omega3 + \text{C20:2}\omega6 + \text{C20:3}\omega6 + \text{C20:3}\omega3 + \text{C20:4}\omega6 + \text{C20:5}\omega3 + \text{C22:5}\omega3)}{(\text{C6:0} + \text{C8:0} + \text{C10:0} + \text{C12:0} + \text{C13:0} + \text{C14:0} + \text{C15:0} + \text{C16:O} + \text{C17:0} + \text{C18:0} + \text{C20:0} + \text{C22:0})}$$

$$\text{h/H} = \frac{[18:1\omega9+18:2\omega6+20:4\omega6+18:3\omega3+20:5\omega3+22:5\omega3+22:6\omega3]}{[14:0+16:0]}$$

(Santos Silva *et al*., 2002)

$$\text{TI} = \frac{[14:0+16:0+18:0]}{[(0{,}5\times\sum \text{MUFA})+(0{,}5\times\sum \text{PUFA}\omega6)+(3\times\sum \text{PUFA}\omega3)+(\frac{\sum \text{PUFA}\omega3}{\sum \text{PUFA}\omega6})]}$$

(Ulbricht and Southgate, 1991)

$$(\text{IA} = \frac{[12:0+(4\times14:0)+16:0]}{[\sum \text{MUFA}+\sum \text{PUFA}\omega3+\sum \text{PUFA}\omega6]}$$

(Ulbricht and Southgate, 1991)

$$\frac{\omega3}{\omega6} = \frac{C18:3\omega3+C20:3\omega3+C20:5\omega3+C22:5\omega3+C22:6\omega3}{C18:2\omega6+C18:3\omega6+C20:2\omega6+C20:3\omega6+C20:4\omega6}$$

(Simopoulos, 2002)

Data Analysis

The SPSS for Windows (version 29.0) statistical package was used for data treatment and statistical analysis. Data of fatty acids was arcsine-transformed to meet assumptions of normality, independence, and homoscedasticity. The integrated chromatogram values for each fatty acid were expressed as a percentage of the total sum of fatty acids identified to eliminate concentration effects. A general linear model (GLM) was used. The data set comprised 30 observations × 19 variables [*TW*; *TL*; water content; gross energy content; crude protein content; total lipids; total cholesterol content; saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA); w3 fatty acids (w3-HUFA); w6 fatty acids (w6-HUFA) and P, K, S, Ca, Fe and Zn]. The design included two factors (i) river basin (two levels), and (ii) gender (two fixed levels). The Pearson product-moment correlation coefficient (*r*) was used to measure the linear dependence between some parameters. The results were considered significantly different for a significant level $P < 0.05$. However, for results in which significant level proved to be less than 1%, the $P<0.01$ notation was used. Eta^2 was used as the proportion of variance associated with or accounted for by each of the main effects, interactions, and error in GLM test used. Because interpretation of the values is easiest if the result can be interpreted as percentages of variance associated with each of the main effects, the interaction, and error, Eta^2 percentage was used.

RESULTS

The mean *TW* were 1336g and 1184g for animals captured at Mondego and Guadiana River basins, respectively, and the corresponding mean *TL* values were 89.6 and 83.8 cm, respectively. The results showed that river basin (sea lamprey capture location) had a significant effect on *TL* (P<0.05), with sea lampreys from Mondego River being slightly longer that those from Guadiana River, whilst *TW* were similar (NS). Gender factor had no significant effect (NS) on either of these parameters and, similarly, there was no significant interaction (NS) between river basin and gender. For individuals from both river basins, it was observed a positive and highly significant correlation (P<0.01; r= 0.934) between *TW* and *TL*. The proximate composition of fillets (muscle tissue) sampled from the sea lampreys captured at Guadiana River and Mondego River are shown in Table 1.

River Basin	Water Content (%)	Gross Energy content (Kcal/100g)	Crude Protein (g/100g)	Total lipids (g/100 g)	Total cholesterol (g/ 100g)	Ash (g/100g)
Guadiana	68.51 ± 4.66*a	640.19± 0.07*a	17.45 ± 7.94*a	14.42± 0.02*a	0.056 ± 0.009	0.91±0.07
Mondego	61.34 ± 4.44	728.69± 0.07	17.21 ± 3.16	21.46± 0.13	0.059 ± 0.003	0.86±0.07

Note: Cases in which the chemical composition parameters are significantly different (P<0.05)

Table 1 – Proximal composition (n ± SD) of sea lamprey´s fillets for individuals captured in two river basins (n=30). All values are in dry weight. Between the two river basin individuals are marked with sign: *a: significantly different from Mondego.

The parameters that differed between river basins individuals (Tables 1 and 2), in descending order of their contribution to the differences were: muscle gross energy content (P<0.05; eta=44.3%) > Fe (P<0.05; eta=40.5%) > water content (P<0.05; eta=40.3%) > total lipids (P<0.05; eta=17.5%). However, the river basin revealed no significant effect (NS) on total cholesterol content. Once again, gender, and the interaction between gender and river basin did not have any significant effect (NS) for any of the parameters analyzed. The remaining concentrations of the macro (P, K, S, Ca) and micro essential minerals (Zn) were not significantly different (NS) between sea lampreys of the two capture locations (Table 2).

Macro e micro elements (mg/100 g)	Sea lamprey (*Petromyzon marinus*)		Salmon (*Atlantic salmon*)	Eel (*Anguilla anguilla*)
	Guadiana	**Mondego**		
P	1.073±92.87	1.069±77.71	209.0	246.0
K	763.59±144.69	706.66±177.71	301.0	179.0
S	360.49±146.54	315.05 ± 92.25	-	-
Ca	20.11 ± 4.25	16.80 ± 6.09	12.0	138.0
Fe	9.43 ± 1.99*a	6.93 ± 1.05	0.5 ±	0.5
Zn	4.50 ± 0.87	4.24 ± 0.83	0.5	2.5

Note: Cases in which the chemical composition parameters are significantly different (P<0.05) between the two river basin individuals are marked with sign: *a: significantly different from Mondego.

Table 2- Macro and micro elements (n ± SD, expressed in mg/100 g) of sea lamprey´s fillets for individuals of the two river basins analyzed (n=30) and for Atlantic salmon and eel (INSA, 2014).

Regarding to the macro and micro elements, only Fe recorded significant differences ($P<0.05$) between the sea lampreys of the two river basins (Mondego: 9.43 mg/100g; Guadiana: 6.93 mg/100g) (Table 2).

The sea lamprey fillet´s water content in Guadiana samples was significantly higher (68.5%) than that recorded for the fillets of the Mondego River individuals (61.4%) (Table 1). Total lipids and water content ($P <0.01$ and $r= -0.876$), as well as total lipids and total cholesterol ($P <0.01$ and $r= -0.730$), were highly inversely correlated in animals sampled from River Guadiana only. Concerning the crude protein levels, the values obtained for the fillets of the individuals from the two river basins did not appear to be significantly different (NS), with a mean value of 17.45 g / 100g dry weight in the Guadiana and 17.21 g / 100g dry weight for sea lampreys of Mondego. For the total lipids, the opposite situation was observed (Table 1). There was a highly negative correlation between the total lipid content and crude protein content of fillets of the individuals of the river basins analyzed ($P < 0.01$; $r = -0.846$).

Our results revealed a significant difference ($P<0.05$) for the energetic value of the fillets between animals of the two basins (an average of 640.19 kcal / 100g of muscle for the Guadiana and a mean of 728.69 kcal / 100g of muscle for Mondego). Moreover, for Guadiana animals, there was a positive and highly significant correlation ($P<0.01$; $r= 0.937$) between the fillet´s total lipid and gross energy content. For individuals sampled from Mondego River, there was a tendency for a positive but not significant correlation between fillet´s total lipids and gross energy content (Table 1).

Finaly, the fillet´s total lipid profile was characterized by a higher percentage of monounsaturated fatty acids (MUFA: 54.23%, and 57.52%) than saturated fatty acids (SFA:38.25% and 38.24%) and polyunsaturated fatty acids (PUFA: 7.73% and 4.42%) for Guadiana and Mondego respectively (Table 3). The fillet´s fatty acid profile revealed that differences between individuals from the two river basins were mainly related to the highly unsaturated fatty acids of omega-3 family (ω3-HUFA: $P<0.05$; eta=32.9%), PUFAs ($P<0.05$; eta=27.1%), ω6-HUFA ($P<0.05$; eta=24.6%) and MUFA ($P<0.05$; eta=14.7%) (Table 3). The ω3 fatty acids, DHA, and EPA, represented 22% and 40%, respectively, of the ω3-HUFA in Guadiana individuals, and 30% and 35% in Mondego animals (Table 3). Furthermore, for animals from both river basins, there was a highly negative correlation between DHA levels and total lipid content ($P<0.01$; $r= -0.785$ and $P<0.01$; $r= -0.575$, for Guadiana and Mondego, respectively). The average EPA+DHA value was 0.062g/100 g edible portion of sea lamprey muscle for individuals from both river basins.

Fatty Acids	River Guadiana (%)	River Mondego (%)
C6:0	0.15±0.01	0.011±0.005
C8:0	0.008±0.008	0.007±0.004
C10:0	0.03±0.01	0.025±0.002
C12:0	2.55±0.27	1.98±0.33
C13:0	0.18±0.02	0.18±0.03
C14:0	17.80±1.60	16.79±1.13
C15:0	0.08±0.04	0.07±0.08
C16:0	14.61±2.00	16.51±1.59
C17:0	0.03±0.02	0.03±0.02
C18:0	2.47±0.84	2.29±.020
C20:0	0.23±0.11	0.14±0.04
C22:0	0.11±0.17	0.10±0.07
∑SFA	38.25	38.24
C14:1	1.16±0.35	0.93±0.25
C16:1ω7	36.37±7.84	39.09±1.55
C17:1	0.15±0.04	0.14±0.09
C18:1ω9	15.92±1.88	17.01±1.19
C20:1ω9	0.44±0.21	0.29±0.07
C22:1ω9	0.19±0.15	0.06±0.03
∑MUFA	54.23*a	57.52
C18:2ω6	0.25±0.10	0.18±0.03
C18:3ω6	0.03±0.02	0.02±0.01
C18:3ω3	0.10±0.06	0.06±0.02
C20:2ω6	0.08±0.05	0.03±0.02
C20:3ω6	0.20±0.11	0.08±0.05
C20:3ω3	1.06±0.60	0.51±0.06
C20:4ω6	0.13±0.10	0.04±0.03
C20:5ω3	1.57±1.04	1.20±0.18
C22:5ω3	1.47±0.75	0.88±0.14
C22:6ω3	2.84±1.70	1.42±0.38
∑PUFA	7.73*a	4.42
∑ ω3	7.04*a	4.07
∑ ω6	0.69*a	0.35

Note: SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids;

ω3 - ω3 fatty acids sum and ω6 fatty acids sum. Cases in which the relative amounts of a fatty acid are significantly different (P< 0.05) between the groups are marked with sign: *a: significantly different from Mondego.

Table 3 – Fillets fatty acid profile, expressed in percentage relative to the total of identified fatty acids (n ± SD) of sea lampreys from Rivers Guadiana and Mondego (n=30)

The average yield contributions of sea lamprey´s fillets are shown in Table 4. Fillets presented a similar water content, crude protein and total lipid content to several other species typically consumed in Portugal (Afonso, 2009). However, the Fe values obtained for sea lamprey were 3.7 to 20-fold higher than referenced values for the species in the Table 4.

Species	Water (%)	Lipid (%)	Protein (%)	Ash (%)	Energetic Value (Kcal/100g)	Fe (mg/100g)	Cholesterol (g/100g)
Petromyzon marinus	64.9	18.2	17.1	0.9	243	8.18*	0.057
Sarda sarda	64.3	13.4	20.3	1.4	202	1.1***	0.045
Sparus aurata	68.9	9.8	19.7	1.4	167	0.4***	0.051
Salmo salar	60.5	21.9	16.2	1.3	262	0.5 **	0.040
Sardine pilchardus	63.4	16.4	18.4	1.7	221	1***	0.020
Anguilla anguilla	57.4	27.7	13.4	1.2	303	0.5 **	0.026
Thunnus thynnus	68.7	4.9	24.1	1.5	140	2.2***	0.030

Note: * Average value for sea lampreys from the two analyzed river basins

** According to Machado et al., (2010)

*** According to Beaulaton et al., (2008)

Table 4 - Proximate composition of the edible portion of sea lamprey and nutritional composition of some other species described in the "Table of Food Composition" of the National Health Institute Dr. Ricardo Jorge (INSA, 2014). Values of protein and lipids expressed in dry weight.

The PUFA/SFA (0.15) and hypocholesterolaemic/hypercholesterolaemic (h/H) indices (0.63) in sea lamprey muscle were markedly lower than those typical of marine fish species as those referenced in Table 5. The thrombotic and atherogenic indices (TI and AI, respectively) and ω3/ω6 index of 0.81, 1.46 and 11.15, respectively, in the sea lamprey were higher than those typical of marine fish species referenced in Table 5.

Species	PUFA/SFA	h/H	TI	AI	ω3/ω6	EPA+DHA
Petromyzon marinus	0.15	0.63	0.81	1.46	11.15	0.062
Aphanopus carbo	1.13	2.82	0.23	0.26	3.6	0.161
Lepidopus caudatus	1.44	2.6	0.21	0.37	6.8	0.0819
Lophius piscatorius	1.42	2.43	0.23	0.37	5.1	0.0681
Merluccius merluccius	0.95	1.92	0.3	0.51	7.6	0.1938

Table 5 - Polyunsaturated/saturated (PUFA/SFA); hipo/hipercholesterolaemic (h/H); Thrombogenic (TI); Atherogenic (AI); ω3/ω6 indexes and EPA+DHA (expressed in g/100 g) for sea lamprey and other species according referenced in Afonso (2003).

DISCUSSION

The aim of the present work was the characterization of the nutritional value of sea lamprey fillets to confirm its contribution to a balanced and healthy consumer diet since artisanal fishery for sea lamprey is an activity with high gastronomic and economic impact in several western European countries (Duarte *et al*., 2003).

The *TW* and *TL* results for the sea lamprey captured during reproductive migration agreed with values reported by Duarte *et al*., (2003) and Machado *et al*., (2008), which refer a mean weight between 1077-1334 g for adult sea lamprey at this phase of their life cycle (spawning migration). Similarly, the results were consistent with other several studies that indicate an association among individuals *TL* and the latitude of the rivers where they undergo reproductive migration. Indeed, there is a tendency for larger animals to be found at higher latitudes Bandarra *et al*., (2004), although between these two basins the insignificant difference in latitude is not a crucial factor.

It is known that fish muscle tissue is characterized by low amounts of carbohydrate (less than 2%) instead relying on lipids and to a lesser extent, proteins (15 - 20%) (Tocher, 2003). In general, sea lampreys caught in Guadiana had statistically lower values of total lipids than Mondego individuals which could indicate that animals were already mobilizing their lipid reserves either to support the period of reproductive migration or to the gonads Lança *et al*., (2011). Thus, the fillets of these animals to be used in gastronomy will be significantly poorer in total lipids than that of animals from the Mondego River, which were captured immediately when they entered the mouth of the river at the beginning of the reproductive migration. Moreover, the fillet´s total lipid was inversely correlated with water content, as has been observed for several other species and reported by Huss (1995) and Osman *et al*., (2001).

The fillet´s total lipid revealed a negative linear correlation with total cholesterol, which is typical of fatty fish species as cholesterol plays an important role in the structure of biological membranes, explaining, in part, its absence in muscle tissue and on perivisceral fat (Oehlenschlager, 2000).

Differences in the contents of the elements found in the fillets of the fish are attributed either to intrinsic factors or to extrinsic factors (Lall, 1995; Martinez-Valverde *et al*., 2000; Belitz *et al*., 2004; Capelli *et al*., 2008). Furthermore, there are several factors that may be the cause of mineral content variation in wild fish populations such as specie and its biological cycle; size of animal; gender; age; and the phase of its life cycle (status of sexual maturity). Concerning to ecological factors the most crucial are geographical area; time of the year; nutrient availability and water temperature and salinity (Lall, 1995; Martinez-Valverde *et al*., 2000; Belitz *et al*., 2004). So, it was not surprising that sea lamprey fillet´s mineral results revealed great SD values.

Concerning to macro and micro elements, the Fe contents were considerably higher than those reported in the literature (INSA, 2014; Bandarra *et al*., 2004). The sea lamprey is a hematophagous species during the adult phase of its life cycle and consumes the blood of its host species (Macey and Potter, 1986; Andersen *et al*., 1998; Lança *et al*., 2013; Quintella *et al*., 2021), contributing to the high Fe content present in its erythrocytes and may explain the high Fe levels found. Concerning to Fe results, the possible explanations for the observed significantly different Fe values could be either essentially related to the large number of hosts that may be parasitized by sea lampreys during the oceanic phase (Quintella *et al*., 2021) or also be associated to the fact that sea lampreys of Guadiana and Mondego River basins belong to different stocks as described in Lança *et al*., (2014).

The concentration of Ca in the muscle was like values referenced for the fish in general, and it was verified that the Ca concentration in the edible part is low and very variable (Lall, 1995). Because most of the Ca is deposited in the bony skeleton of the fish and in the scales, these low values are not uncommon for the sea lamprey (Lall, 1995). In relation to the other minerals there is a great lack of literature related to sea lamprey. In general, fish muscle is considered a recommended source of Zn when compared to the muscle of animals of production (Lall, 1995). The values recorded in individuals of the two capture locations were similar, which is according to results reported for different species of fish, in which it is verified that the concentration of Zn varies very little (Lall, 1995). In relation to the elements P, K and S there is a great lack of literature related to sea lamprey.

The fatty acid profile of fillets was like that of several fish species because most fish primarily accumulate lipid reserves as SFA and MUFA (Tocher, 2003; Pinela *et al*., 2009). The lower proportion of MUFA in the muscle profile of individuals from Guadiana River compared to individuals from Mondego River is probably due to the location of capture, as sea lampreys of Guadiana were captured 80 km from the river mouth at Mértola. This explains their low quantity of several MUFA, such as C18:1w9 (oleic acid), C20:1w9 (eicosenoic acid) and C20:1w11 (gadoleic acid), which are the predominant sources of metabolic energy during spawning migrations (Tocher, 2003; Lança *et al*., 2011).

The predominance of DHA (C22:6w3) relative to EPA (C20:5w3) in the sea lamprey´s fillets has been reported previously (Pinela *et al*., 2009; Lança *et al*., 2013) and verified in several species of fish (Ozogul *et al*., 2007; 2011; Prato and Biandolino, 2012). Furthermore, for both river basin individuals, there was a negative correlation between DHA levels and total lipid content, which is common in some fish species (Tocher, 2003; Afonso, 2009). In Portugal, commercial sea lamprey catches occur exclusively during the adult's upstream spawning migration in which sea lampreys do not feed and consume muscle´s lipid reserves but retain DHA as it is crucial for the development of gonads and future offspring (Tocher, 2003).

Compared with other species (Table 4), sea lamprey can be included in the very fatty fish category, which is characterized by lipid content higher than 8% of total weight. Total cholesterol values were lower than most fish species commonly consumed in Portugal but like *Sardina pilchardus* (Walbaum, 1792) and comply with the consumption standards recommended by the Portuguese Cardiology Foundation.

The PUFA/SFA index is widely used to assess the nutritional quality of the fillet´s lipid profile. A minimum PUFA/SFA of 0.45 is recommended for a balanced diet, according to the United Kingdom Department of Health. The sea lamprey muscle PUFA/SFA indices were substantially lower than the average range of 0.64 - 1.92 typical for marine fish species (Ozogul *et al*., 2011). However, this index only considers the chemical structures of the fatty acids. All saturated fatty acids are considered possible contributors to total cholesterol and conversely, the protective effects of MUFA, particularly oleic acid (C18:1w9), is underestimated or not considered at all (Santos-Silva *et al*., 2002). Hence, several authors prefer the use of indices based on the functional effects of fatty acids, such as the h/H index, which associates a higher h/H value with a lower risk of developing cholesterolaemia (Santos-Silva *et al*., 2002). The sea lamprey had a lower h/H value than found in some fish species as those referenced in Table 5.

The TI value for sea lamprey was like other fish species commonly consumed in Portugal, which ranges between 0.21 for the silver scabbardfish (*Lepidopus caudatus* (Euphrasen, 1788)) to 0.3 for the European hake (*Merluccius merluccius* (L.). The AI value for sea lamprey was higher than that commonly found in several marine fish species (Afonso, 2009). Sea lamprey and most spawning species, use C18, C16:0 and C14:0 fatty acids for their metabolic energy production during reproductive migration and this should be reflected in their muscle fatty acid profile (Tocher, 2003). Therefore, the TI and AI values reflect the time of sea lamprey capture, which coincided with the beginning of their spawning migration period when the muscle fatty acid profile is rich in these fatty acids.

The ω3/ω6 index is also widely used to assess the nutritional value of the lipids in food (Simopoulos, 2006). The importance of this ratio is associated with the low ω3 fatty acid intake and excessive consumption of ω6 fatty acids in modern western societies (Simopoulos, 1999b; 1999c). Our results for this index show the predominance of ω3 fatty acids relative to ω6 fatty acids, confirming that the ω3 fatty acid content in sea lamprey´s fillets result from their ω3-rich diet during the oceanic phase of their life cycle (Lança *et al*., 2013; Quintella, *et al*., 2021).

The EPA+DHA value for sea lamprey individuals of the two river basins was much lower than the 650 mg/day recommended for preventing cardiovascular diseases (Simopoulos, 1999a). However, the values obtained are consistent with those typical of other fish species, which may vary between 84 - 431 mg/100 g edible portion (Afonso, 2009).

CONCLUSION

The chemical composition of fillets of sea lamprey captured at the beginning of their spawning migration revealed that individuals from the Guadiana River basin were different than those from the Mondego River basin because animals caught were from different stocks. The lipid nutritional quality of sea lamprey´s fillets was typical for fatty fish species, particularly its high lipid and gross energy contents. In contrast, the total cholesterol values were low, and negatively correlated with the total lipids but were like that found in *S. pilchardus*, a species highly recommended to consumers by the Portuguese Cardiology Foundation. The high ω3/ω6 value found is a consequence of the oceanic diet of sea lamprey.

These results suggest that the flesh of sea lamprey provides important nutritional benefits based on its lipid profile. This information is crucial to consumers in that sea lamprey is traded outside the official markets during traditional gastronomic events in several western European countries.

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CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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