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# Comparison of the fatty acid profile of muscle neutral lipids and phospholipids of up-river anadromous sea lamprey (*Petromyzon marinus* L.) from three Portuguese river basins

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SUMMARY: Composition of fatty acid profile of muscle neutral lipids (NL) and phospholipids (PL) of sea lamprey that enter the Portuguese rivers Minho, Tagus and Guadiana during their non-trophic spawning migration was analysed. The fatty acid profile exhibited differences in the percentage among NL and PL and between river basins. Similarities were found in the fatty acid profile of NL. Monounsaturated fatty acids (MUFA) were the most representative, followed by saturated fatty acids (SFA) and finally by polyunsaturated fatty acids (PUFA). Monoenic 16:1 and 18:1ω9 formed a considerable percentage of total fatty acids, followed by SFA 14:0 and 16:0. EPA and DHA were the dominant PUFA fatty acids. In terms of NL, the fatty acid that contributed for the discrimination between the three river basins was 18:1ω7. Individuals from the Minho river basin exhibited a different fatty acid profile of PL characterised by a low PUFA percentage when compared with lampreys from the Tagus and Guadiana river basins. Muscle PL fraction showed that the two monoenes, 16:1 and 18:1ω9, occurred at high percentage, followed by 16:0 and 14:0 (SFA). Among PUFA, DHA was the most representative fatty acid. The fatty acids that contributed to the separation between the three river basins were 16:0, 18:4ω3 and 24:1ω9. Although the results point in the direction of a possible difference between the fatty acid composition of the NL and PL fractions in the muscle samples from the three river basins, further studies, especially in tissues where fatty acid composition will be less sensitive to diet and environmental factors, are necessary to confirm this hypothesis.

*Keywords*: *Petromyzon marinus*, fatty acids, spawning migration, Minho, Tagus, Guadiana, Portugal.

RESUMEN: COMPARACIÓN DEL PERFIL DE ÁCIDOS GRASOS EN LOS LÍPIDOS NEUTRALES Y EN LOS FOSFOLÍPIDOS DE LOS MÚSCULOS DE LA LAMPREA MARINA ANÁDROMA (*PETROMYZON MARINUS* L.) (AGNATHA) DE LA CUENCA HIDROGRÁFICA DE TRES RÍOS PORTUGUESES. – Se ha analizado la composición del perfil de ácidos grasos de los lípidos neutrales (NL) y fosfolípidos (PL) en el músculo de la lamprea marina que entra en los ríos Miño, Tajo y Guadiana durante su migración reproductora, no-trófica. El perfil de ácidos grasos presentaba diferencias en los porcentajes entre NL y PL y entre las distintas cuencas. Se encontraron semejanzas en el perfil de ácidos grasos de los NL. Los ácidos grasos monoinsaturados (MUFA) fueron los más representativos seguidos por los ácidos grasos saturados (SFA) y, finalmente, por los ácidos grasos poliinsaturados (PUFA). Los monoenoicos 16:1 y 18:1ω9 representaban un porcentaje considerable del total de ácidos grasos, seguidos por los SFA 14:0 y 16:0. EPA y DHA fueron los ácidos grasos PUFA dominantes. A nivel de NL, el ácido graso que permitió la discriminación entre las cuencas de los tres ríos fue el 18:1ω7. Los individuos de la cuenca del río Miño, en comparación con las lampreas de las cuencas de los ríos Tajo y Guadiana, presentaban un perfil de ácidos grasos distinto a nivel de los PL, que se caracterizaba por un bajo porcentaje de PUFA. La fracción PL del músculo mostró que los dos monoenoicos 16:1 y 18:1ω9 eran los que aparecían en mayor porcentaje, seguidos por los 16:0 y 14:0 (SFA). Entre los PUFA, los DHA fueron los más representativos. Los ácidos grasos que marcaban la separación entre las tres cuencas hidrográficas fueron los 16:0, 18:4ω3 y 24:1ω9. Aunque los resultados indican posibles diferencias en la composición de ácidos grasos de las fracciones NL y PL en el músculo de los individuos entre las tres cuencas hidrográficas, son necesarios posteriores estudios en tejidos en los que la composición de ácidos grasos sea menos sensible a factores ambientales y a la dieta, para confirmar esta hipótesis.

*Palabras clave*: *Petromyzon marinus,* ácidos grasos, migración reproductora, Miño, Tajo, Guadiana, Portugal.

## INTRODUCTION

The sea lamprey (*Petromyzon marinus* L.) is an anadromous species classified as "vulnerable" in the Portuguese red list of endangerous species (Rogado *et al.*, 2005). It has a high economical value, supporting commercial fisheries in most of the major Portuguese river systems (Almeida and Quintella, 2002; Quintella *et al.*, 2003). Mature sea lampreys are captured when entering the rivers during the upstream reproductive migration particularly in the peak of that movement, which occurs between February and April (Almeida *et al.*, 2000).

The majority of lamprey species exhibit a similar life cycle that consists of two distinct trophic phases (Bird and Potter, 1983). The microphagic filter-feeding larvae (ammocoete) are relatively sedentary and live in areas of fine sediment in still water, where they burrow (Hardisty and Potter, 1971a; Almeida and Quintella, 2002). During the larval period, the ammocoete starts to accumulate lipids (Potter, 1980) in several depots, such as the liver, kidney, subcutaneous tissue and myosepta (Lowe *et al.*, 1973). After four to seven years, a metamorphosis period occurs which transforms the microphagic filter-feeding larvae into pelagic juveniles. This is a non-trophic period during which lamprey utilises the lipid reserves accumulated in the larval phase (Bird and Potter, 1983). Juvenile lampreys then migrate downstream to the sea, where they begin the parasitic stage of their life cycle, feeding primarily on blood and muscle tissue of marine and anadromous fish for at least 24 months (Beamish, 1980). This marine phase is accompanied by the deposition of large amounts of lipids, particularly in the musculature, the main store depot for these energy reserves (Sheridan, 1988), which act as the primary energy source during upstream spawning migration (Bird *et al.*, 1993). At the end of this phase the adults cease feeding and migrate upstream, where they spawn and die (Larsen, 1980). The spawning migration of sea lamprey, similar to that of other anadromous species such as the Atlantic salmon *Salmo salar* L. (Ballantyne *et al.*, 1996), involves sustained swimming for long distances (Quintella *et al.*, 2004), fasting and completion of the final stages of gonadal development. However, there is a considerable lack of knowledge with respect to the marine life history of anadromous sea lampreys. In fact, there is a scarcity of data in several aspects, such as the hosts of the sea lamprey and their feeding ecology (Farmer, 1980; Halliday, 1991). The migratory period entails costs in time and energy expenditure, and natural selection favours traits that increase the probability of successful migration and reproduction (Bernatchez and Dodson, 1987; Gross, 1987; Roff, 1988; Dodson, 1997). The use of lipids as biomarkers in marine ecosystems has provided reliable information not only on the dietary source but also on the nutritional status, lipid utilisation and fatty acid tissue composition of several species of fish (Varljen *et al.*, 2003). It is well known that there are considerable changes in the chemical composition of the fish tissues in each period of the life cycle as a result of feeding and sexual maturation, the most important being the marked changes in tissue content and fatty acid composition (Huynh *et al.*, 2007).

The objective of the present study was to characterise qualitatively the fatty acid profile of neutral lipids and phospholipids of muscle of sea lamprey *P. marinus* that enter the three major Portuguese river basins at the beginning of their spawning migration period. Hopefully, the results will foster a discussion on the use of fatty acid profiles as a means of identifying sea lamprey populations, and will give us some clues about anadromous sea lamprey hosts.

## MATERIALS AND METHODS

### Individuals

Adult sea lampreys were collected by local fishermen in three major Portuguese river basins located in the northern, central and southern regions of the country (i.e. the rivers Minho, Tagus and Guadiana, respectively) at the beginning of the reproductive upstream migration (March 2004). In each river basin, 15 individuals were caught and transported live to the laboratory in 0.4 $m^3$ capacity tanks equipped with a proper life support system.

### Sampling sites

The Minho river basin is located in the northwest area of Portugal (41° to 43°N; 06° to 08°W) and sea lampreys were collected near S. Pedro da Torre, approximately 25 km upstream from the river mouth. The Tagus river basin is located in the central region of Portugal (38° to 41°N; 01° to 10°W) and individuals were caught at Ortiga, immediately downstream

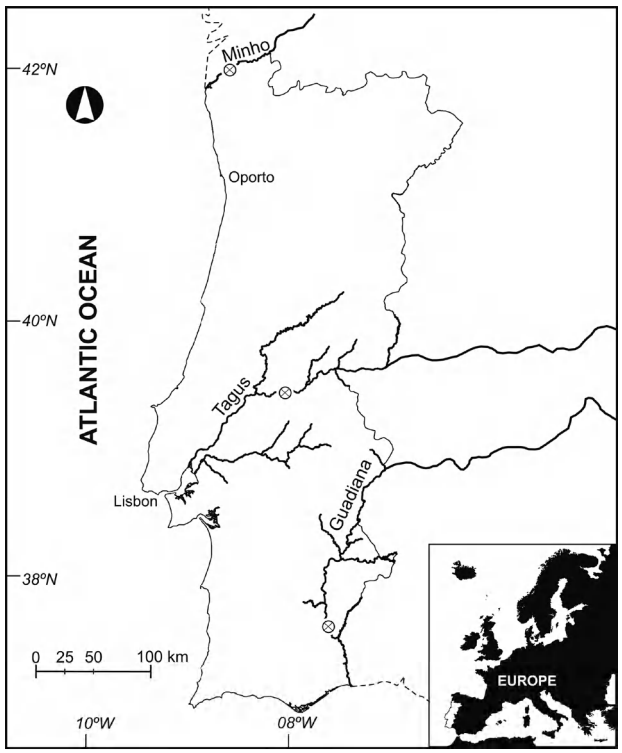


FIG. 1. – Location of the sampling sites (⊗) in the rivers Minho, Tagus and Guadiana.

of the Belver dam and 160 km upstream from the river mouth. The Guadiana river basin is situated in the southwest of Portugal (37° to 40°N and 02° to 08°W) and sea lampreys were collected in the fishing grounds of Mértola, 80 km upstream from the river mouth (Fig. 1).

## Sample selection

In the laboratory, total weight (TW, nearest g) and total length (TL, nearest mm—length between the beginning of the oral hood and the end of the caudal fin) were registered for each sea lamprey. Fulton's condition factor (K) was calculated using the following expression:

$$K = (TW/TL^3)\times 10^4.$$

Muscle samples were collected in the proximity of the mid-dorsal line, on the left flank of the animal, close to the dorsal fin. Three replicates were taken from each individual, with an average weight of 2.20 ± 0.13 g each (mean ± SD). Muscle samples were washed with physiologic saline and immediately stored in liquid nitrogen (-196°C) until laboratorial processing.

## Lipid extraction

Muscle lipids were extracted according to the procedure of Marmer and Maxwell (1981). This method permitted the total lipid separation into neutral lipids (NL) and polar lipids (PL) by a sequential elution procedure. Prior to analysis, each muscle sample (2.0 g) was ground for 30 s in an ice-chilled mortar with 8.0 g anhydrous $N_2SO_4$ (Merck, Darmstadt, Germany) and 0.1 ml BHT (20 mg/l dichloromethane, Merck, Darmstadt, Germany). Celite 545 (6.0 g) (Fisher Scientific, USA) was added and the mixture was ground again for 30 s in order to obtain a fine homogenised powder. A 16 mm × 30 cm glass column was packed with glass wool and 4.0 g of $CaHPO_4$/Celite 545 (1:9 w/w) at its tip. The NL fraction was eluted first with dichloromethane (Merck, Darmstadt, Germany) and then the PL fraction was eluted with a dichloromethane/methanol (9:1 v/v) mixture (Merck, Darmstadt, Germany). NL fraction and PL fraction were weighed after evaporation of the solvent in a water bath under nitrogen flushing. NL extracts were dissolved in 4 ml hexane (Merck, Darmstadt, Germany) and PL extracts in 4 ml methanol (Merck, Darmstadt, Germany). The extracts were then transferred to a vial, topped and stored at -70°C until analysis.

## Fatty acid composition

Aliquots 0.6 ml of NL extracts and PL extracts containing approximately 30 mg of lipids were saponified in methanolic NaOH 0.5 N at 70°C for 15 min. Fatty acids were then prepared with boron-trifluoride-methanol (14 g $BF_3$/l $CH_3OH$, Merk-Schuchardt, Germany) in order to give fatty methyl esters (FAMEs) according to the procedure of Morrison and Smith (1964).

The FAMEs were analysed by liquid-gas chromatography in a Hewlett Packard HP 6890 Series GC System equipped with a split-splitless injector, an auto-sampler, a flame-ionisation detector (FID), an Omegawax 320 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness, Supelco, Bellafonte, PA) and HPChem software (2002). Operating conditions were: He as carrier gas with a flow rate of 1.2 ml $min^{-1}$ and 200°C. The injector temperature and the detector temperature were 250°C and the column ran isothermally at 200°C. Each sample ran for 55 min and the split was 100:1. Fatty acid peaks were identified by the

comparison of their retention times with those of an external standard (PUFA-1, Supelco, Bellafonte, PA) chromatographed in identical gas chromatography conditions. Triplicate GC analysis was performed and the results were expressed in GC area percent as mean ± SD. The resulting peak areas were corrected by theoretical relative FID response factors (Ackman, 2002).

Fatty acids were designated according to the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) for carbon chain length: number of double bonds and position of the double bond closest to the omega carbon.

## Data analysis and interpretation

The statistical package SPSS for Windows (version 14.0) was used for data treatment and statistical analysis.

The integrated chromatogram values for each fatty acid were expressed as a percentage of the total sum of fatty acids identified in order to eliminate concentration effects. The relative values of fatty acids were logarithmically transformed prior to further statistical treatments.

For each river basin, differences in muscle fatty acid composition between genders were analysed by Wilcoxon rank-sum test.

Differences in the chemical composition of muscle from the lamprey from the three river basins were analysed by multivariate analysis of variance (MANOVA) and multiple discriminant analysis (MDA). MANOVA was used with river basins as the fixed factor and fatty acids as the dependent factor and was used to test our $H_0$: lampreys from different river basins showed similar muscle fatty acid composition. Significance of the MANOVA was evaluated with Wilk's lambda. MANOVA identifies the differences in muscle fatty acid composition between lampreys from different river basins but does not reveal which fatty acid contributed most to the differences observed. To identify which fatty acid contributed most to the differences between river basins, a MDA was employed.

The computational method used to derive the discriminant function was the stepwise method that involves entering the independent variables into the discriminant function one at the time on the basis of their discriminating strength. The selection rule in this procedure is to maximise Mahalanobis $D^2$ between groups (Hair *et al.*, 1998). A chi-square transformation ($\chi^2$) of Wilk's lambda ($\Lambda$) was used to test equality among the group centroids and whether all discriminant functions reflected population differences or only random variation (Hair *et al.*, 1998). Discriminant Z scores and group centroids were plotted for visual inspection of differences between groups on each function. The resultant discriminant functions were used to classify lamprey into groups. To assess group membership prediction accuracy, the expected actual error rates of the classification functions were estimated using cross-validation by the leaving-one-out procedure, in which the discriminant function is fitted to repeatedly drawn samples

TABLE 1. – Total length (expressed in mm), total weight (expressed in g), Fulton's condition factor (K) and sex of sea lampreys caught in the three Portuguese river basins

| Guadiana | | | | Tagus | | | | Minho | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TL | TW | K | Sex | TL | TW | K | Sex | TL | TW | K | Sex |
| 770 | 888 | 0.19 | M | 780 | 867 | 0.18 | M | 740 | 820 | 0.20 | M |
| 788 | 854 | 0.17 | M | 780 | 940 | 0.20 | F | 750 | 829 | 0.20 | M |
| 790 | 879 | 0.18 | M | 793 | 1210 | 0.24 | M | 760 | 834 | 0.19 | F |
| 795 | 1022 | 0.20 | F | 805 | 929 | 0.18 | M | 780 | 917 | 0.19 | M |
| 800 | 944 | 0.18 | F | 810 | 989 | 0.19 | M | 815 | 930 | 0.17 | M |
| 805 | 940 | 0.18 | M | 810 | 1147 | 0.22 | F | 830 | 1115 | 0.20 | F |
| 810 | 1062 | 0.20 | M | 820 | 913 | 0.17 | F | 840 | 1179 | 0.20 | F |
| 820 | 880 | 0.16 | M | 845 | 1041 | 0.17 | M | 845 | 1195 | 0.20 | F |
| 820 | 968 | 0.18 | F | 855 | 1225 | 0.20 | F | 850 | 1096 | 0.18 | M |
| 825 | 916 | 0.16 | M | 885 | 1379 | 0.20 | M | 855 | 1234 | 0.20 | M |
| 855 | 1045 | 0.17 | F | 900 | 1301 | 0.18 | F | 860 | 1012 | 0.16 | M |
| 855 | 1119 | 0.18 | F | 920 | 1190 | 0.15 | M | 865 | 1239 | 0.19 | M |
| 870 | 1157 | 0.18 | F | 927 | 1606 | 0.20 | F | 885 | 1395 | 0.20 | M |
| 870 | 1169 | 0.18 | F | 930 | 1669 | 0.21 | M | 900 | 1534 | 0.21 | F |
| 875 | 1056 | 0.16 | M | 957 | 1495 | 0.17 | M | 970 | 1618 | 0.18 | M |
| Mean (± SD) | | | | Mean (± SD) | | | | Mean (± SD) | | | |
| 823.2 (±33.9) | 993.3 (±104.9) | 0.18 (±0.01) | | 854.5 (±60.6) | 1193.4 (±256.5) | 0.19 (±0.02) | | 836.6 (±61.3) | 1129.8 (±250.1) | 0.19 (±0.01) | |

TABLE 2. – Fatty acid composition of the neutral lipid (NL) and polar lipid (PL) fractions of sea lamprey muscle from the Guadiana, Tagus and Minho river basins. Values are expressed as percent of total fatty acids, mean ± SD, n=15

| Fatty Acid | NL | | | PL | | |
|---|---|---|---|---|---|---|
| | Guadiana | Tagus | Minho | Guadiana | Tagus | Minho |
| **SFA** | | | | | | |
| C14:0 | 19.821±0.492 | 20.599±0.339 | 19.642±0.455 | 6.280±0.515 | 6.058±0.478 | 8.348±0.640 |
| C16:0 | 15.541±0.626 | 16.259±0.287 | 14.554±1.200 | 23.832±0.670 | 24.689±0.816 | 29.267±1.414 |
| C18:0 | 0.102±0.022 | 0.094±0.012 | 0.100±0.008 | 1.061±0.986 | 0.177±0.033 | 0.221±0.028 |
| Σ | 35.464 | 36.952 | 34.295 | 31.173 | 30.925 | 37.836 |
| **MUFA** | | | | | | |
| C16:1 | 43.283±0.580 | 43.300±0.320 | 45.047±0.880 | 18.658±1.058 | 19.719±1.245 | 24.073±1.164 |
| C18:1ω9 | 18.155±0.346 | 16.502±0.295 | 17.983±0.590 | 12.484±1.213 | 14.458±0.587 | 17.385±0.550 |
| C18:1ω7 | 0.916±0.031 | 0.891±0.042 | 0.839±0.030 | 1.942±0.217 | 2.335±0.198 | 2.301±0.159 |
| C20:1ω9 | 0.412±0.045 | 0.331±0.025 | 0.335±0.039 | 0.434±0.042 | 0.439±0.029 | 0.505±0.048 |
| C22:1ω11 | 0.124±0.026 | 0.078±0.014 | 0.101±0.022 | 0.051±0.026 | 0.048±0.018 | 0.033±0.018 |
| C22:1ω9 | 0.000 | 0.003±0.002 | 0.000 | 0.008±0.006 | 0.009±0.006 | 0.000 |
| C24:1ω9 | 0.005±0.005 | 0.007±0.005 | 0.000 | 0.031±0.017 | 0.357±0.074 | 0.021±0.021 |
| Σ | 62.895 | 61.112 | 64.305 | 33.608 | 37.365 | 44.319 |
| **PUFA** | | | | | | |
| C18:2ω6 | 0.132±0.012 | 0.124±0.013 | 0.121±0.009 | 0.253±0.048 | 0.286±0.025 | 0.267±0.015 |
| C18:4ω3 | 0.075±0.003 | 0.061±0.005 | 0.079±0.005 | 0.037±0.013 | 0.163±0.044 | 0.444±0.089 |
| C20:5ω3 | 0.473±0.073 | 0.660±0.075 | 0.426±0.071 | 8.874±0.656 | 8.372±0.890 | 4.935±0.890 |
| C22:5ω3 | 0.446±0.064 | 0.477±0.046 | 0.364±0.045 | 6.379±0.436 | 5.637±0.708 | 3.435±0.724 |
| C22:6ω3 | 0.516±0.089 | 0.614±0.127 | 0.410±0.070 | 19.675±1.196 | 17.252±2.100 | 8.764±1.880 |
| Σ | 1.641 | 1.936 | 1.400 | 35.219 | 31.711 | 17.845 |
| Σ SAT/UNSAT | | | | 0.45 | 0.44 | 0.61 |
| EPA/DHA | 0.916 | 1.076 | 1.038 | 0.451 | 0.485 | 0.563 |

of the original sample. The predictive accuracy relative to chance, which would have a correct classification rate of 50 percent, was measured with Press's Q statistic. Discriminant loadings and potency index were used to assess the relative importance of each independent variable in discriminating between groups (Hair *et al.*, 1998).

## RESULTS

From a total of 45 sea lampreys sampled, the total length (TL) ranged from 740 to 970 mm and the total weight (TW) ranged from 820 to 1669 g (Table 1). The smallest lampreys were from the Guadiana river basin, with a mean TL of 823 mm and a mean TW of 993 g. The largest lampreys were caught in the Tagus river basin, with a mean TL of 854 mm and a mean TW of 1193 g (Table 1). The sex ratios (males/females) of the sea lampreys captured during the peak of the spawning migration were 1.1:1, 1.5:1 and 2:1 in the Guadiana, Tagus and Minho river basins, respectively.

### Fatty acid composition of the NL fraction of muscle

Fifteen fatty acids were identified in the NL fraction of muscle samples of the sea lamprey *P. marinus* (Table 2). The analysis of the fatty acid composition of the NL fraction of muscle from individuals of each river basin sampled revealed that monounsaturated fatty acids (MUFA) were the most representative (*ca.* 60%), followed by saturated fatty acids (SFA) (*ca.* 35%) and finally by polyunsaturated fatty acids (PUFA) (*ca.* 1.5%). Among MUFA, monoenic 16:1 (ranging from 43% to 45%) and 18:1ω9 (ranging from 17% to 18%) formed a considerable percentage of the total fatty acids. The dominant species of SFA were 14:0 (ranging from 20% to 21%) and 16:0 (ranging from 15% to 16%). EPA (20:5ω3) and DHA (22:6ω3) were the dominant PUFA but percentages were lower than 1% (Table 2).

No significant differences were found for each fatty acid between genders. For Guadiana lampreys *P* values ranged between 0.068 and 0.34, for Minho lampreys they ranged between 0.093 and 0.71 and for Tagus lampreys they ranged between 0.15 and 0.87. MANOVA ($P \leq 0.001$) revealed differences in fatty acid composition of NL of lamprey muscle from the Minho, Tagus and Guadiana river basins. For most of the analysed fatty acids, lampreys from the Tagus river basin had significantly higher values than individuals from the other two river basins, as was shown by ANOVA ($P \leq 0.001$). No significant differences were observed for 22:1ω11, 22:1ω9 and 24:1ω9. The discriminant function based on the mean fatty acid composition of NL of muscle proved to be statistically

TABLE 3. – Results of Wilk's lambda test to verify: (1) the hypothesis that the means (centroids) of function are equal in the three groups when their neutral lipids (NL) fatty acids were separately compared by stepwise MDA; (2) the hypothesis that the means (centroids) of both functions are equal in the three groups when their polar lipid (PL) fatty acids were separately compared by stepwise MDA. The first function separates one group from the other two, and the second separates the remaining two groups

| Test of function(s) | Fatty acid analysis | | |
|---|---|---|---|
| | Λ | $\chi^2$ | df |
| NL | | | |
| 1 | 0.587 | 22.404*** | 2 |
| PL | | | |
| 1 through 2 | 0.259 | 55.456*** | 6 |
| 2 | 0.572 | 22.874*** | 2 |

*** - P ≤ 0.001.

TABLE 4. – Classification results obtained with the stepwise discriminant analysis cross-validation for neutral lipid (NL) and polar lipid (PL) fatty acids to determine the predictive accuracy level of the discriminant function.

| Groups | N | Percent correct | No. of individuals classified into group | | |
|---|---|---|---|---|---|
| | | | Guadiana | Tagus | Minho |
| **NL** | | | | | |
| Guadiana | 15 | 73.3 | 11 | 0 | 4 |
| Tagus | 15 | 66.7 | 2 | 10 | 3 |
| Minho | 15 | 73.3 | 3 | 1 | 11 |
| Total | 45 | — | — | — | — |
| **PL** | | | | | |
| Guadiana | 15 | 73.7 | 11 | 1 | 3 |
| Tagus | 15 | 60 | 2 | 9 | 4 |
| Minho | 15 | 100 | 0 | 0 | 15 |
| Total | 45 | — | — | — | — |

significant (Table 3) and accounted for 100% of total variation; the total classification rate estimated from the cross-validation procedure was 71%. About 73% of the lampreys from the Guadiana and Minho river basins were correctly classified despite some degree of overlap between individuals from these two river basins, and for lampreys from the Tagus river basin 68% of individuals were correctly classified despite some degree of overlap between individuals from the three river basins (Table 4). Press's Q test revealed that the classification accuracy was significantly better than chance (Press's Q = 32, 4, *d.f.* = 1, $P \leq 0.001$). The fatty acid that contributed to the separation of the three river basins was 18:1ω7.

## Fatty acid composition of the PL fraction of muscle

Fifteen fatty acids were identified in the PL fraction of muscle samples of the sea lamprey *P. marinus* (Table 2). The analysis of the fatty acid composition of the PL fraction of the muscle for each of the river basins revealed that MUFA and SFA were more representative than PUFA in individuals from the Minho river basin. In fact, in lampreys from the Minho river basin, MUFA (*ca.* 44%) and SFA (*ca.* 38%) formed a considerable percentage of total fatty acids of the PL fraction against 18% for PUFA. In individuals from the Tagus and Guadiana river basins, PUFA percentages were similar to MUFA and SFA percentages (*ca.* 30% each class). MUFA, monoenic acids were primarily represented by 16:1 (range: 19%-24%) and by 18:1ω9 (range: 12-17%). A high level of 16:0 (range: 24-29%) characterised the SFA, followed by 14:0 (range: 6-8%). Among PUFA, DHA (22:6ω3) dominated, with levels ranging between 17% and 20% in individuals from the Tagus and Guadiana river basins, respectively, and with lower levels (*ca.* 9%) in individuals from the Minho river basin (Table 2).

No significant differences were found for each fatty acid between genders. For Guadiana lampreys *P* values varied between 0.056 and 0.31; for Minho individuals they varied between 0.27 and 0.91 and for Tagus lampreys they varied between 0.095 and 0.64.

MANOVA ($P \leq 0.001$) revealed differences in the fatty acid composition of PL of lamprey muscle from the Minho, Tagus and Guadiana river basins. Sea lampreys from the Minho river basin had significantly lower values (ANOVA: $P \leq 0.001$) for nine of the fifteen analysed fatty acids than those from the other two watersheds. No significant differences were observed for 18:0; 22:1ω11 and 22:1ω9. Individuals from the Guadiana river basin had the lowest 18:4ω3 and lampreys from the Tagus river basin had the highest 24:1ω9.

The Wilks' lambda tests indicated differences between the three river basins when the fatty acid composition of PL was compared by means of discriminant analysis (Table 3). The first two discriminant functions accounted for 61.9% and 38% of total variation (Fig. 2). The overall corrected classification rate estimated from cross-validation procedure was 77.8%. All lampreys from the Minho River were correctly classified, and despite some degree of overlap *ca.* 74% and 60% of the lampreys from the Guadiana and Tagus river basins, respectively, were correctly classified (Table 4). Press's Q test revealed that the classification accuracy was significantly better than chance (Press's Q = 52.9; *d.f.* = 1; $P \leq 0.001$). The first discriminant function separated individuals from

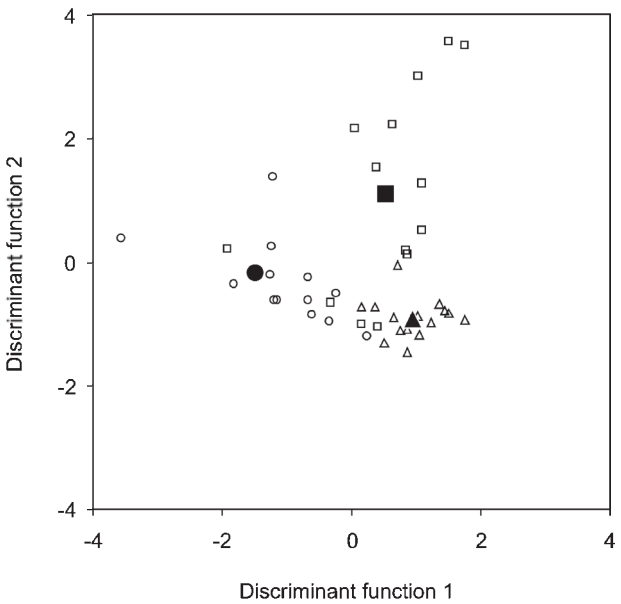


FIG. 2. – Bi-plot of the scores and group centroids for the discriminant functions 1 and 2 used to discriminate sea lampreys from different river basins (● - Guadiana; □ - Tagus; △ - Minho; group centroids are represented by the same symbols in black) based on the fatty acid composition of PL of muscle.

TABLE 5. – Summary of discriminant loadings and potency index for PL

| | Variables | Discriminant loadings Function 1 | Function 2 | Potency index |
|---|---|---|---|---|
| PL | c16:0 | -0.32 | 0.95* | 0.40 |
| PL | c24:1ω9 | 0.23 | 0.86 | 0.31 |
| PL | c18:4ω3 | 0.39* | 0.37 | 0.15 |

* - largest absolute correlation between each variable and any discriminant function.

the Guadiana river basin *versus* individuals from the Minho and Tagus river basins, while the second discriminant function separated individuals from the Minho and Tagus river basins. The fatty acids that contributed for the separation of the three river basins were 16:0, 18:4ω3 and 24:1ω9 (Table 5).

## DISCUSSION

In teleost fish, fatty acid composition has been suggested to reflect osmo-regulatory pre-adaptation (Sheridan *et al.*, 1985), dietary source, nutritional status, lipid utilisation, fatty acid metabolism and other environmental and physiological parameters (Henderson and Tocher, 1987; Hoch, 1988). It is well known that in fish tissues the fatty acid composition of triacylglycerols, and to a lesser extent of phospholipids, is determined by diet composition and lipid metabolism (Henderson and Tocher, 1987; Sargent *et al.*, 1989; Linko *et al.*, 1992; Peng *et al.*, 2003) and that the fatty acid composition of diet influences tissues differently, with great impact in triacylglycerol rich tissues (Joensen *et al.*, 2000).

The majority of lamprey species have a similar life cycle, which involves the migration of adults into rivers to reach the spawning areas and reproduce (Hardisty and Potter, 1971b) after a marine period characterised by the deposition of large amounts of lipids, used as the primary energy source during the upstream reproductive migration (Bird *et al.*, 1993). Several studies have investigated the lipid metabolism and the tissues lipid levels which occur in the life cycle of *P. marinus* but generally data are available only on the larval and metamorphosing phases (Kao *et al.*, 2002) or for the adult landlocked form (LeBlanc *et al.*, 1995). Beamish *et al.* (1979) described the large fluctuations in the total lipid content of various stages in the adult phase (small and large feeding adults, early immature and nearly mature migrants, spawning and spent reproducers) of the life cycle of anadromous sea lamprey and related them to feeding, migration and reproduction. However, this study did not characterise the fatty acid profile. Unlike mammals, whose main fat depots are located in the subcutaneous adipose tissue, in cyclostomes and fish there is a great diversity of depots. Lampreys store large amounts of triacylglycerols in liver and body wall muscles (Plisetskaya, 1980); their liver and somatic muscle are capable of lipid oxidation and fatty acids are used as oxidative substrates. Lampreys may also preferentially mobilise saturates (SFA), while specific PUFA fatty acids are needed for developing gonads (LeBlanc *et al.*, 1995).

Gamper and Savina (2000) observed that the main oxidised substrate in lamprey hepatocytes is fatty acids and they concluded that fatty acids are the major fuel for the oxidation process in lampreys during spawning migration period. For this reason, during spawning migration, the relative amount of saturated fatty acids and monoenic acids tended to decrease while the PUFA fatty acids rose. The constant swimming associated with the migration may speed up the general depletion of SFA because these acids are used as a high-energy fuel source for long-term sustained swimming (Bird and Potter, 1983; Ballantyne *et al.*, 1996).

## NL fraction of lamprey muscle

The fatty acid composition of the NL fraction of lamprey muscle revealed that MUFA were the most representative, followed by SFA and finally by PUFA in all the individuals from the river basins analysed. In fact, the distribution of the individual fatty acids in triacyglycerol fraction from muscle were characterised by high levels of 14:0, 16:0; 16:1 and 18:1ω9 and very low levels of PUFA, which are typical levels for neutral lipids of most marine fish (Cejas *et al.*, 2004). These results are also in concordance with the common opinion that fish species accumulate depot lipids composed mainly of SFA and MUFA (Kozlova and Khotimchenko, 2000).

In this study, percentages of SFA in NL muscle fractions from individuals of the three river basins exhibited notable similarities, which could be associated with the fact that SFA group comprises only three fatty acids (C14:0; C16:0 and C18:0) with a high C14:0 proportion in each of the studied river basins. No differences were observed between MUFA percentages of the three river basins and the fatty acid profile observed in the NL fraction (MUFA> SFA > PUFA) is a characteristic lipid profile for neutral lipids of most marine fish. This could mean that lampreys are at the beginning of their spawning migration, with no marked changes in NL fatty acid classes between the three river basins, and individuals still preserve the fatty acid profile of marine teleost fish .

Fellows and McLean (1982) also reported high percentages of SFA (ranging between 11% and 39%) and MUFA (ranging between 20% and 58%) in muscle of *Mordacia mordax* (Richardson) during upstream migration. A similar situation was found in the fatty acid composition of muscle NL in *Geotria australis* (Gray) (Bird and Potter, 1983). These authors also found an exceptionally high level of 16:1 (over 18%) in adult lamprey muscle at the end of the marine trophic phase compared with levels recorded for marine teleosts (Ackman *et al.*, 1967; Gruger *et al.*, 1964). They suggested that a high 16:1 value could be a characteristic of adult lamprey tissues. Our results reported a 16:1 ranging between 43% and 45%, which, according to Bird and Potter (1983), is still higher than the value found in the majority of marine teleosts. On the other hand, LeBlanc *et al.* (1995) observed very high values for 18:0 in plasma of landlocked sea lamprey during spawning migration, but they attributed it to the extreme energy demand during this phase rather than considering it to be a lamprey tissue characteristic. Bird *et al.* (1993) also studied the fatty acid compositions of the muscle and ovary of two lamprey species, *Lampetra planeri* (Bloch) and *Lampetra fluviatilis* (L.) and observed that the major fatty acid in the triacylglycerols of the muscle of adult *L. fluviatilis* was 18:1, with proportions higher than 28% followed by 20:5 and 22:6.

Ballantyne *et al.* (1996) found that the most important fatty acids in the plasma of sockeye salmon during the spawning migration were 16:0, 18:1, 20:5ω3 and 22:6ω3, which is in accordance with our findings regarding the sea lamprey. Previously, Ballantyne *et al.* (1993) concluded that the four fatty acids are the same as those reported for temperate-zone marine fish. For the authors, the availability of these fatty acids in the fatty acid plasma pool probably determines which fatty acids are important oxidative substrates for a variety of fish tissues.

The general pattern of fatty acid distribution in the NL of muscle was similar between the three river basin individuals. Nevertheless, for most of the analysed fatty acids the Tagus individuals had significantly higher amounts than the individuals from the other two river basins. This situation could be related to the lipid status and to a better condition of the individuals at the beginning of the upstream migration period. The fatty acids that contributed most to the differences found were 18:1ω7, 20:5ω3 and 22:6ω3, the last two with proportions three times higher in Tagus lampreys than in Minho and Guadiana lampreys.

Studies from Phleger *et al.* (1997, 1999a, 1999b) and Vlieg *et al.* (1993) reported the fatty acid profiles of a wide range of marine organisms, illustrating the great diversity of fatty acid profiles found in the marine food chain. Therefore, it is possible that the observed differences could also be due, at least in part, to lamprey's diet during the parasitic stage of their life cycle. Moreover, we also found that the fatty acid that had an influence in the separation of individuals from the three river basins was 18:1ω7, with lampreys of the Tagus river basin having an amount two times higher than those in the other river basins. Further studies will be necessary to confirm a possible 18:1ω7 rich sources in the diet of lampreys from the Tagus river basin since this fatty acid family may arise to some extent from biosynthesis but could also be highly indicative of differences in various lamprey´s hosts. A detailed assessment of the change of fatty acids during reproductive migra-

tion may also be necessary to understand metabolic demands as well as the importance of some specific fatty acids in the reproductive migration process of the sea lamprey.

### PL fraction of lamprey muscle

Phospholipids serve as structural components in membranes and have several important biological functions, such as being sources of second messengers in cell signalling (Stoknes *et al.*, 2004). They play also an important role in the response of most poikilotherms to thermal changes by promoting the adaptation of the physical properties of membranes to the new situation in order to preserve functional and structural membrane integrity (Buda *et al.*, 1994).

It is also evident that the fatty acid composition of muscle PL differs markedly from that of muscle NL, especially because the PL fraction was characterised by higher PUFA percentages than the NL fraction, in particular high proportions of EPA and DHA. As described in the results section, our data clearly showed a great similarity in the fatty acid profile of the PL fraction of muscle between lampreys from the Tagus and Guadiana river basins with respect to percentages of MUFA, SFA and PUFA (*ca.* 30%). However, considerable differences appeared between the individuals of these two river basins and lampreys from the Minho. In fact, the Minho individuals exhibited low percentages of PUFA (*ca.* 9%), with DHA having the low levels and high percentages of SFA and MUFA, especially because of the high proportions of C16:0; C16:1 and C18:1ϖ9.

Takama *et al.* (1999) determined the percentages of each fatty acid class on phosphatidylcholine in several tissues of 27 species of teleosts, and SFA percentages were almost constant, ranging between 35% and 38% in all migratory fish tissues analysed, whereas PUFA represented 40-55% of phosphatidylcholine fatty acids. Bird and Potter (1983) also found large amounts of long-chain PUFA in the fatty acid composition of muscle phospholipids in adult *Geotria australis* and related that situation to the importance of PUFA for individuals that live in cold environments, as it enables membranes to remain fluid and function efficiently. In fact, EPA and DHA are usually conserved in comparison with MUFA during gonad development and migration, because these acids are involved in normal growth and development including reprodution and in characterization of fish cell membranes in order to maintaining cell membrane structure and function (Cejas *et al.*, 2004; Sargent *et al.*, 1995).

Davidson and Cliff (2002) reported that membrane phosphoglyceride structure requires a balance between SFA, MUFA and PUFA to maintain an appropriate degree of membrane fluidity in the presence of several environmental conditions.

In ectothermic individuals, changes in water salinity and temperature affect length and degree of unsaturation of the fatty acid tails present in membrane phospholipids (Cordier *et al.*, 2002). Hazel (1984) and Hazel and Williams (1990) showed that membrane activities could be modulated by temperature-induced alteration in membrane composition.

Our results seem to reflect the appropriate balance between the PL fatty acids classes in order to maintain the membrane integrity in the presence of distinct environmental conditions. The presence of a higher percentage of PUFA in the composition of muscle PL from Tagus and Guadiana lampreys than in that of Minho lampreys seems to reflect the importance of PUFA, particularly DHA, in the lipid structure of the membranes for maintaining membrane fluidity at lower temperatures, typical in the upper reaches of the rivers during this time of the year in Portugal (mean water temperature: Tagus upper reaches, 13.8ºC; Guadiana upper reaches, 12.0ºC; Minho estuary, 14.5ºC). It is possible that higher PUFA percentages observed in PL of lampreys from Tagus and Guadiana corresponded to an adaptation of these populations to specific temperature conditions since these individuals were at different migration distances from the river mouth (i.e. at 160 and 80 km, respectively, against 25 km for the Minho lampreys). Moreover, our data showed the lowest saturated to unsaturated fatty acid ratio in individuals from the Tagus and Guadiana river basins (0.44 and 0.45, respectively), against 0.61 obtained in lampreys from the Minho river basin. This is in agreement with studies of Dey *et al.* (1993) and Buda *et al.* (1994), which revealed that the ratio of saturated to unsaturated fatty acids varied with temperature, being lower in cold-adapted fish.

The high PUFA percentages in the PL of Tagus and Guadiana lampreys and the low SFA and MUFA percentages in comparison with Minho lampreys seems to reflect an appropriate balance between PL fatty acid classes in order to maintain the membrane integrity in the presence of different environmental conditions during the upstream migration of the sea lamprey rather than reproductive alterations, be-

cause depleted changes in SFA and MUFA were not yet observed in neutral lipid classes. This situation is typical when reproductive tissues develop at the expense of the stored triacylglycerides.

The distributions of the individual fatty acids in the PL fraction from muscle of individuals from the three river basins were similar to the results of Bird *et al.* (1993) in muscle of *L. fluviatilis*. Takama *et al.* (1999) found that in all samples of muscle tested, 16:0, 18:1, 20:5ω3 and 22:6ω3 were the principal fatty acids present. Several studies reported C16:0 as the predominant source of potential metabolic energy in fish during roe formation stage in female fish, mentioning the role of C18: 1ϖ9 during the course of gonad development and the fact that PUFA are also an important source for metabolic energy for reproduction (Huynh *et al.*, 2007).

Our data showed that DHA and EPA are the dominant PUFA fatty acids and DHA values exceed the level of EPA by twofold. This is in agreement with Ackman (1980, 1982) and Henderson and Tocher (1987), who stated that DHA rarely exceeds the level of EPA by more than two- or threefold. Nevertheless, for ten of the fatty acids analysed, Minho lampreys had significantly lower values than Tagus and Guadiana lampreys.

Our results showed that 16:0, 18:4ω3 and 24:1ω9 were the fatty acids that contributed to the separation of individuals from the three river basins based on the mean fatty acid composition of PL of their muscle. The importance of 24:1ω9 should be stressed, since 73% of Tagus lampreys had this fatty acid against 20% and 6.7% for Guadiana and Minho lampreys, respectively. Further studies, especially in tissues in which fatty acid composition is less sensitive to diet and environmental factors, are necessary to confirm the origin of this fatty acid.

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