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Fatty acid composition of phospholipids and triacylglycerols in the flesh of the thick-lipped grey mullet *(Chelon labrosus)* living in Tunisian geothermal water and seawater: A comparative study

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SUMMARY: This study was conducted to elucidate the effects of rearing conditions on the composition of different phospholipid (PLs) classes and triacylglycerols (TAG) of the thick-lipped grey mullet (*Chelon labrosus*), a muscle originating from seawater and geothermal water. The major fatty acids in the examined lipid classes of the two fish groups were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6)**,** arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3). The analyses demonstrated that the fatty acid profiles of the PL classes in the seawater fish group were characterized by the predominance of n-3 polyunsaturated fatty acids (PUFA). By contrast, in geothermal fish, the distribution of PUFA series proportions differed between the phospholipid fractions. It was found PUFA n-3 was particularly abundant in PS and PI, while the n-6 series dominated the PC and PE PUFA group. Nonetheless, it was found that neutral lipid fatty acids were characterized by saturated fatty acids (SFA) followed by monounsaturated fatty acids (MUFA) in the seawater fish and by PUFA in the geothermal fish. The results presented here give useful information on the role of lipid classes in the physiological adaptation of *C. labrosus* which can serve for the optiminzation of these aquaculture systems.

KEYWORDS: *Fatty acid composition; Geothermal water, Phospholipids; Seawater thick-lipped grey mullet* (Chelon labrosus)*; Triacylglycerols.*

RESUMEN: ***Composición en ácidos grasos de fosfolípidos y triacilgliceroles de la carne del salmonete gris de labios gruesos*** **(Chelon labrosus)** ***que vive en agua geotérmica y agua de mar tunecina: un estudio comparativo.*** Este estudio se llevó a cabo para dilucidar los efectos de las condiciones de cría sobre la composición de diferentes clases de fosfolípidos (PL) y triacilgliceroles (TAG) del músculo de salmonetes de labios gruesos (*Chelon labrosus*) procedentes de agua de mar y de agua geotérmica. Los principales ácidos grasos en las clases de lípidos examinados de los dos grupos de peces fueron, palmítico (C16:0), esteárico (C18:0), oleico (C18:1n-9), linoleico (C18:2n-6), araquidónico (C20:4n-6), eicosapentaenoico (C20:5n-3) y ácido docosahexaenoico (C22:6n-3). Las determinaciones mostraron que los perfiles de ácidos grasos de los PL, en el grupo de peces de agua de mar, se caracterizaron por el predominio de ácidos grasos poliinsaturados n-3 (PUFA). Por el contrario, en los peces geotérmicos, la distribución de las proporciones de las series de PUFA difirió entre las fracciones de fosfolípidos. Se encontró que los PUFA n-3 eran particularmente abundantes en PS y PI, mientras que la serie n-6 dominaba el grupo de PUFA PC y PE. No obstante, se encontró que en lipidos neutros, los mayoritarios son los ácidos grasos saturados (SFA) seguidos de los ácidos grasos monoinsaturados (MUFA) en el pescado de agua de mar y los PUFA en el pescado geotérmico. Los resultados actuales brindan información útil sobre el papel de las clases de lípidos en la adaptación fisiológica de *C. labrosus* que puede servir para la optimización de estos sistemas de acuicultura.

PALABRAS CLAVE: *Agua geotérmica; Composición de ácidos grasos; Fosfolípidos; Salmonete gris de labios gruesos* (Chelon labrosus); *Triacilgliceroles.*

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1. INTRODUCTION

In order to meet the increased demand for seafood, aquaculture has experienced significant progress over recent decades. According to the latest FAO report, the world aquaculture production reached 114.5 million tonnes and was valued at 263.6 billion USD in 2018 (FAO, 2020). According to the same statistics, fish farming accounted for almost half (47%) of the global aquaculture production. Mullets are members of the Mugilidae family and are among the most ubiquitous fish resources worldwide. Their farming has been practiced for centuries (FAO,2015a). In the Mediterranean basin, the thick-lipped grey mullet *Chelon labrosus* is cultured in natural earthen ponds under extensive or semi-intensive regimes (De las Heras *et al*., 2015). It has been described as an easily cultivable species and constitutes a promising species for aquaculture diversification (Zouiten *et al.,* 2008; Ben Khemis *et al.,* 2013). Indeed, as other mullet species, *C. labrosus* is a low trophic level feeder, obtaining its energy directly from the first trophic level (Brusle, 1981). In addition, the eurytherm and euryhaline species, *C. labrosus* is able to tolerate wide ranges of temperature and salinity (Cardona, 2006; Rabeh *et al*., 2013). Furthermore, several authors have reported that *C. labrosus'* osmoregulation abilities appear early during in their development, allowing them to maintain elevated growth rates even under hyposaline conditions (Nordlie *et al.,* 1982; Cardona*,* 2006). In most North African regions, the production of *C. labrosus* may be carried out in a variety of ecosystems, such as coastal lagoons with brackish to hyper saline waters (Crosetti*,* 2016) or reservoirs and ponds with fresh waters (Losse *et al.,* 1991). For many years, Tunisia has opted to breed fish in reservoirs and artificial lakes as a strategy for suppling aquatic products to the interior regions and for providing work opportunities for local communities (Besbes *et al*., 2020). For instance, natural water flow from artesian sources is used directly as a culture medium in the south of Tunisia (Béchima locality) to facilitate in the raising of some freshwater and marine fish species. In this respect, recent investigation has shown the aptitude of *Oreochromis niloticus* and *C. labrosus* to live in geothermal waters (26 to 30°C) and to display good growth rates (Azaza *et al.,* 2008a; Azaza *et al.,* 2008b).

It is well established that the capacity of an organism to efficiently adapt environmental changes is predominantly dependent on its metabolic flexibility (Smith *et al.,* 2018). This phenomenan includes a series of metabolic reorganizations and biochemical adjustments allowing the animal to meet increases in energy requirements and to maintain homeostasis under new environmental condition (Soengas *et al.,* 2007). As the densest form of energy in marine ecosystems and fundamental components of the cell membrane, lipids and their key components, fatty acids (FA), play key roles in the adaptation of aquatic organisms to new environmental conditions (Fokina *et al.,* 2017). In this regard, several studies have reported high variability in the FA composition of fish depending on different abiotic and biotic factors such as the type and amount of food available, water temperature, pH, salinity, and reproduction cycle (Shirai *et al.,* 2002; Kaushik *et al.,* 2006). Changes in FA composition are likely to induce conformational remodeling of membrane proteins, including receptors and channels, and ultimately affect cell responses to extracellular challenges (Brown, 1994). FA are distributed into two major sub-classes which represent an essential and integral part of these compounds. Lipid classes can be broadly divided into neutral, mainly triacylglycerol (TAGs), and polar lipids (such as phospholipids). Phospholipids (PLs), are the major structural constituents of biological membranes. They are involved in the maintenance of membrane integrity and permeability and provide the matrix for the function of a large variety of catalytic processes (Dowhan *et al*., 2008). The most biologically important phospholipids of organisms are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS). These lipid classes are particularly known for their role in the maintaining of membrane integrity and fluidity and in the ensuring of the fish acclimatization process to changes in environmental conditions (Murzina *et al.,* 2020). Neutral lipids which includeTAG, serve mainly as a depot for lipids and provide most of the energy consumed (Sargent *et al*., 1976). These molecules, which mainly come from food sources, tend to directly accumulate in fish muscle **(**Sushchik *et al*., 2020). The accumulation of these lipids can be differentiated by either external factors, such as fluctuations in environmental conditions, temperature, and food availability, or

by internal factors, such as metabolic and physiological activities.

Despite their commercial importance, there are few studies on the biochemical composition of grey mullets (Rabeh *et al*., 2015; Ben Khemis *et al*., 2019). According to our hypothesis, lipids could indicate the ability of *C. labrosus* to adapt to abiotic factors, including water temperature and salinity. Hence, the objective of the present study was to identify and compare the FA composition of individual PL (PC, PE, PI and PS) and NL (mainly TAG) classes in *C. labrosus* reared in geothermal and seawater conditions in order to gain further insight into the physiological fitness of mullets.

2. MATERIALS AND METHODS

2.1. Experimental material

Immature thick-lipped grey mullets *C. labrosus* (30 to 40 g body mass) were supplied by an experimental fish culturing center (National Institute of Marine Science and Technology (INSTM)- Tunisia). The specimens of *C. labrosus* provided by INSTM-Center de Monastir were reared in seawater conditions with a salinity of 35 ppt and an ambiant temperature ranging from18 to 20 °C. While the specimens supplied by INSTM-Center de Béchima were reared in geothermal water conditions with a salinity of 2 ppt and a temperature varying between a minimum of 23 °C and the maxima of 28 °C. During the period from June 2006 to May 2007, all fish were hand fed six times between 08.00 and 18.00 h every 2 h until satiation. To reduce the sampling differences or external influences, six individuals from each habit were randomly selected at the same age and all fed the same local diet. The diet was made of 45% fresh sardines, 40% soybean meal, 10% fish meal, 4% vegetable oil and 1% vitamin premix. The moisture, crude protein, crude lipid, crude ash and lipid composition of the diet are shown in Table 1. They were fasted for 24 h before sampling, and six specimens form each group were sacrificed and the dorsal muscles (without skin) were sampled and conserved at -30 °C until analysis.

Table 1. Ingredients of the experimental diet.

Ingredients	
Fatty acids composition %	
C14:0	4.59±0.19
C15:0	0.05±0.03
C16:0	28.80±0.90
C16:1	0.55±0.00
C16:2	1.25±0.01
C16:3	2.78±0.46
C16:4	0.39±0.01
C17:0	0.10±0.10
C18:1n-9	2.43±0.25
C18:2n-6	29.60±2.36
C18:3n-6	16.11±0.87
C18:3n-3	1.18±0.06
C18:4n-3	0.73±0.01
C20:0	0.83±0.01
C20:1n-9	25.32±2.07
C22:1n-11	0.71±0.07
C20:3n-6	0.18±0.02
C20:3n-3	0.44±0.03
C20:4n-3	0.07±0.05
C20:5n-3	0.37±0.02
C20:4n-6	0.55±0.07
C22:5n-3	2.74±0.55
C22:5n-6	1.06±0.18
C22:6n-3	1.25±0.01
C21:5	0.02±0.01
C24:1n-9	0.59±0.12
Proximate composition	
Humidity	35
Protein	37
Crude fat	13
Ash	2
Gross energy (kcal·g^{-1})	4.34

Note: Results are expressed as mole % of total FAME based on peak areas. Data are means ± standard deviations from triplicate estimations (n =3) using ANOVA (Tukey HSD test).

2.2. Lipid extraction

Lipids were extracted from fillet samples according to the method of Folch *et al.* (1957) with a mixture of chloroform:methanol (2:1, v/v) containing buthylated-hydroxy-toluene (BHT), which was added to the solvent mixture as an antioxidant. For 1 g of fresh sample (individually analyzed), 30 mL of the solvent mixture were used. After evaporation of the solvent mixture under nitrogen, the extracts were dried overnight in a vacuum desiccator and quantified gravimetrically. Once weighed, the lipids were re-dissolved

in the organic solvent and the obtained lipid extracts were conserved in -30 °C until analysis.

2.3. Lipid class separation

The lipid classes from total lipid samples were separated using thin-layer chromatography (TLC) with one dimensional double development following the method of Olsen and Henderson (1989). Concisely, 500 µL of lipid extracts from each sample were separated on silica gel plates (20 × 20 cm, Merck, Germany) into neutral and polar fractions. The plates were activated by heating at 105 °C for 1 h and developed with hexane/diethyl-ether/glacial-acetic-acid (80:20:2, v/v) for the neutral lipids (NL), and methyl acetate/isopropanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, v/v) for the polar lipids (PL). The individual lipid categories were detected under UV light after being sprayed with 0.1% 2'-7'dichloro-fluorescein in absolute methanol. Lipid fractions were identified by corresponding standards and scraped from the plate into separate tubes and their constitutive fatty acids were transmethylated.

2.4 Analysis of fatty acids

After evaporation to dryness, the lipid extracts and fractions were trans-esterified for fatty acid analysis according to the method of Cecchi *et al.* (1985). The resulting fatty acid methyl esters (FAME) were extracted using sodium methylate ($NaOCH_3$) in the presence of hexane and sulfuric acid (H_2SO_4). Methyl nonadecanoate 19:0 (Sigma-Aldrich, St. Louis, MO, USA), which was absent from our samples, was added as an internal standard product.

Fatty acid methyl esters (FAMEs) were separated by a HP6890 gas chromatograph (Agilent Technologies; Santa Clara, CA) with a split/splitless injector. Nitrogen was the gas carrier at a flow rate of 1.5 mL·min^{-1} in an Innowax 250 capillary column (0.25 inside diameter × 30m length, 0.25 µm film; Agilent Technologies). The gradient temperature program was set as follows: from 50 °C to 180 °C at a rate of 4 °C·min^{-1}, from 180 °C to 220 °C at a rate of 1.33 °C·min^{-1}, and finally to stabilize at 220 °C for 7 min. The detector and injector were maintained at 250 °C. The identification of fatty acid methyl esters was based on the comparison of their retention times with those of authentic standards (C4 C24 by SUPELCO) and a well-characterized fish oil (Menhaden oil by SUPELCO). FA peaks were integrated and analyzed using the Agilent G2070BA GC Hewlett-Packard Chemstation Software. All fatty acid data were reported as percentage of total fatty acids.

2.5 Calculation of indices and statistical analysis

The equations of unsaturation index (UI) and unsaturated-to-saturated FA ratio (U/S) were calculated according to Snyder and Hennessey (2003) and Wallaert and Babin (1994).

The unsaturation index (UI):

$$UI = \Sigma\ (\%monoenes + 2 \times \%dienes + 3 \times \%trienes\ldots)\ /100$$

where monoenes, diened, and trienes were fatty acids containing 1, 2, 3 double bonds, respectively.

The unsaturated-to-saturated FA ratio (U/S):

$$U/S = \Sigma(\%UFA)/\Sigma(\%SFA)$$

where %: weight percentage; UFA: unsaturated fatty acids; SFA: saturated fatty acid.

The statistical analyses were performed using R software version 3.3.3 (R Core Team 2017).

The data were checked for normality using the Shapiro-Wilk's test (Shapiro and Wilk, 1965) and Levene's tests, respectively. One-way analysis of variance (ANOVA) and Tukey HSD's test (at $p < 0.05$) were performed so as to detect significant statistical differences. The results are presented as means ± standard deviation (SD). Graphs were plotted using Prism. The covariance matrix was computed, and the Principal Component Analysis (PCA) was applied using the FactoMiner R Package (Lê *et al*., 2008) to evaluate the differences in the compositions of the samples under different conditions.

3. RESULTS

Four major classes of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) in the muscle of *C. labrosus* with a dominant neutral lipid (TAG) in different rearing conditions were identified. Indeed, *C. labrosus* from geothermal water was richer in PLs and TAG than those from seawater. A detailed distribution of the fat-

Table 2. Percentages of individual fatty acids in the TAG, PE, PC, PI and PS from the muscle tissue of *Chelon labrosus* reared in seawater conditions.

	Seawater				
% Fattyacids	**PC**	**PE**	**PS**	**PI**	**TAG**
C14:0	0.53±0.07^{a}	0.19±0.02^{b}	0.61±0.07^{c}	0.48±0.01^{a}	6.95± 0.54^{d}
C15:0	0.03±0.01^{a}	0.09±0.05^{b}	0.11±0.03^{b}	0.13±0.02^{b}	0.25±0.02^{c}
C16:0	37.28±2.06a	4.38±0.09^{b}	33.22±0.28^{c}	18.12±0.58^{d}	22.30±1.26^{d}
C17:0	0.41±0.11^{a}	0.73±0.07^{b}	0.46±0.24^{c}	0.39±0.10^{a}	0.45±0.03^{c}
C18:0	7.00±2.80^{a}	35.21±0.91^{b}	2.34±0.15^{c}	4.69±0.57^{d}	3.94±0.42^{d}
C20:0	0.09±0.02^{a}	0.27±0.08^{b}	0.06±0.01^{c}	0.07±0.02^{c}	0.13±0.03^{a}
C22:0	0.02±0.01^{a}	0.04±0.01^{b}	0.04±0.01^{b}	0.05±0.01^{b}	0.04±0.01^{b}
C24:0	0.11±0.04^{a}	ND	ND	0.01±0.00^{b}	0.04±0.01^{c}
C15:1	0.34±0.05^{a}	0.06±0.02^{b}	0.34±0.08^{a}	0.24±0.05^{c}	0.76±0.02^{d}
C16:1n-9	1.12±0.16^{a}	0.49±0.16^{b}	1.51±0.35^{c}	1.40±0.02^{c}	9.28±0.17^{d}
C18:1n-9	18.00±1.03^{a}	7.84±0.11^{b}	12.85±1.89^{c}	10.10±0.26^{c}	21.14±0.66^{a}
C20:1n-9	0.68±0.09^{a}	0.89±0.05^{b}	0.42±0.07^{c}	0.49±0.08^{c}	1.16±0.05^{b}
C22:1n-11	0.19±0.02^{a}	0.18±0.03^{a}	0.22±0.02^{a}	0.30±0.03^{b}	0.40±0.02^{c}
C24:1n-9	0.10±0.01^{a}	0.32±0.07^{b}	0.21±0.07^{c}	0.32±0.03^{b}	0.12±0.01^{a}
C18:2n-6	5.94±0.20^{a}	1.92±0.23^{b}	6.08±0.39^{a}	5.87±0.15^{a}	10.06±0.02^{c}
C18:3n-6	0.13±0.02^{a}	0.12±0.03^{a}	0.14±0.01^{a}	0.17±0.06^{a}	0.50±0.12^{b}
C20:2n-6	0.44±0.06^{a}	0.28±0.03^{b}	0.26±0.03^{b}	0.22±0.00^{b}	0.40±0.03^{a}
C20:3n-6	0.15±0.02^{a}	0.20±0.03^{b}	0.19±0.00^{b}	0.26±0.03^{c}	0.05±0.01^{d}
C20:4n-6	2.14±0.45^{a}	8.02±0.15^{b}	2.81±0.09^{a}	3.47±0.14^{c}	0.91±0.16^{d}
C22:5n-6	0.68±0.07^{a}	1.07±0.24^{b}	0.96±0.18^{b}	1.20±0.16^{b}	0.16±0.01^{c}
C18:3n-3	0.50±0.10^{a}	0.40±0.28^{a}	0.95±0.35^{b}	1.17±0.07^{b}	2.35±0.06^{c}
C18:4n-3	0.16±0.03^{a}	0.54±0.05^{b}	0.35±0.08^{c}	0.73±0.17^{d}	0.71±0.02^{d}
C20:3n-3	0.06±0.01^{a}	0.11±0.07^{b}	0.08±0.00^{b}	0.08±0.02^{b}	0.18±0.01^{c}
C20:4n-3	0.20±0.02^{a}	0.25±0.03^{a}	0.48±0.18^{b}	0.70±0.05^{c}	0.52±0.13^{b}
C20:5n-3	7.46±0.27^{a}	8.57±0.68^{a}	15.10±0.47^{b}	21.40±0.70^{c}	5.85±0.33^{d}
C22:5n-3	1.68±0.17^{a}	3.71±0.27^{b}	2.41±0.37^{c}	2.74±0.04^{c}	1.14±0.07^{d}
C22:6n-3	13.56±0.47^{a}	23.40±0.86^{b}	16.62±0.16^{c}	23.42±0.10^{b}	5.48±0.01^{d}
C16:2	0.71±0.28^{a}	0.30±0.04^{b}	0.80±0.17^{a}	1.09±0.05^{c}	1.40±0.07^{d}
C16:3	0.17±0.19^{a}	0.16±0.08^{a}	0.23±0.07^{b}	0.42±0.14^{c}	1.59±0.08^{d}
C16:4	0.03±0.01^{a}	0.05±0.01^{b}	0.04±0.02^{a}	0.09±0.01^{b}	0.88±0.06^{c}
C21:5	0.11±0.01^{a}	0.20±0.04^{b}	0.14±0.01^{c}	0.16±0.01^{c}	0.28±0.01^{d}
n-3/n-6	2.4±0.03^{a}	1.44±0.05^{b}	3.45±0.08^{c}	4.5±0.07^{d}	1.25±0.12^{b}
U/S	1.4±0.1^{a}	2.8±0.02^{b}	1.3±0.11^{a}	3.18±0.04^{c}	1.09±0.02^{d}
UI	1.06±0.01^{a}	1.7±0.02^{b}	2.3±0.1^{b}	2.9±0.02^{c}	0.9±0.1^{d}
Total PL (mg·g^{-1} Fw)			0.52±0.05		
TAG (mg·g^{-1} Fw)			0.12±0.01		

Note: Results are expressed as mole % of total FAME based on peak areas. Data are means ± standard deviations from triplicate estimations (n =3). Means followed by different letters in the same line are significantly different ($p < 0.05$) using ANOVA (Tukey HSD test). SFA, MUFA, and PUFA mean saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid, respectively. PC, PE, PI, PS and TAG mean phosphatidylcholine; phosphatidylethanolamine; phosphatidylinositol; phosphatidylserine and triacylglycerol, respectively. ND = not detected.

ty acids within the classes is given in Tables 2 and 3. The results show that the amount of neutral and polar lipids of *C. labrosus* from geothermal water was more important than those from seawater. The predominant FAs in the two groups (seawater and geothermal) were C16:0, C18:0, C18:1, C18:2n-6, C20:4n-6, C20:5n-3 and C22:6n-3 in PL and TAG. Among saturates, palmitic acid (C16:0) was the major fatty acid in all lipid classes and exhibited the highest levels, mainly in the PC, PS, and TAG of the two studied fish groups (Tables 2 and 3). In addition, stearic acid (C18:0) appeared to be particularly abundant in both polar and neutral lipid fractions. The main monounsaturated fatty acid (MUFA) was oleic acid (C18:1) and was found in high amounts in PC as well as in TAG (in both seawater and freshwater groups). Regarding polyunsaturated fatty acids (PUFA), we noted a significant increase in C18:2n-6

in the major polar lipid classes of the geothermal group. C22:6n-3 (DHA) and C20:5n-3 (EPA) were particularly abundant in PI. Indeed, 23 to 25% of PI were made of DHA for both fish groups.

To better visualize the differences in FA in the lipid classes, five-line charts referring to the major fatty acid groups, namely satured fatty acids (SFA), MUFAs and PUFAs, are presented in Figure 1. The results showed that in almost all lipid fractions of the seawater fishes, PUFA (representing up to 50% of the total FA of PS species) was by far the major FA group followed by SFA with corresponding increases in UI and U/S. By contrast, in geothermal fish the distribution proportions of the PUFA series substantially differed between polar lipid fractions. Indeed, PUFA n-3 was particularly abundant in PS and PI fractions while the n-6 series dominated the PC and PE subclass. Additionally, it was found that

Table 3. Percentages of individual fatty acids in the TAG, PE, PC, PI and PS from the muscle tissue of *Chelon labrosus* reared in geothermal water.

	Geothermal water				
% Fattyacids	**PC**	**PE**	**PS**	**PI**	**TAG**
C14:0	0.51±0.19^{a}	0.29±0.05^{b}	1.91±0.09^{c}	1.15±0.06^{d}	3.23±0.68^{e}
C15:0	0.65±0.03^{a}	0.68±0.28^{a}	0.44±0.07^{b}	0.40±0.06^{b}	7.92±0.65^{c}
C16:0	33.09±0.90^{a}	10.99±0.06^{b}	7.81±0.58^{b}	6.54±0.29^{b}	24.08±1.31^{a}
C17:0	0.74±0.10^{a}	0.19±0.07^{b}	0.46±0.07^{c}	1.19±0.04^{d}	2.60±0.27^{e}
C18:0	5.67±1.66^{a}	12.84±0.23^{b}	31.74±1.76^{c}	13.78±0.59^{b}	10.38±1.37^{b}
C20:0	0.10±0.01^{a}	0.47±0.01^{b}	0.07±0.01^{c}	0.22±0.04^{d}	0.10±0.01^{a}
C22:0	0.12±0.00^{a}	0.61±0.07^{b}	0.28±0.07^{c}	0.23±0.02^{c}	0,29±0.09^{c}
C15:1	0.55±0.00^{b}	0.29±0.02^{b}	0.31±0.05^{a}	0.64±0.14^{b}	0.75±0.03^{a}
C16:1n-9	2.43±0.25^{b}	1.94±0.06^{b}	0.93±0.06^{b}	1.41±0.08^{a}	6.03±0.33^{b}
C18:1n-9	25.32±2.07^{b}	15.54±0.32^{b}	9.00±0.52^{b}	11.06±0.14^{b}	15.41±0.32^{b}
C20:1n-9	0.71±0.07^{a}	0.83±0.13^{a}	0.81±0.12^{b}	0.45±0.13^{a}	1.20±0.34^{a}
C22:1n-11	0.59±0.12^{b}	0.53±0.07^{b}	0.17±0.01^{b}	0.06±0.01^{b}	0.37±0.02^{ab}
C24:1n-9	ND	ND	ND	1.31±0.24^{b}	ND
C18:2n-6	16.11±0.87^{b}	28.34±0.01^{b}	3.44±0.25^{b}	11.29±1.12^{b}	8.80±0.29^{b}
C18:3n-6	ND	ND	0.11±0.02^{a}	0.13±0.03^{a}	ND
C20:2n-6	0.18±0.02^{b}	1.09±0.11^{b}	0.14±0.02^{b}	0.26±0.11^{a}	1.49±0.29^{b}
C20:3n-6	0.55±0.07^{b}	2.16±0.01^{b}	0.94±0.06^{b}	0.76±0.15^{b}	0.36±0.05^{b}
C20:4n-6	1.06±0.18^{b}	1.53±0.35^{b}	9.34±0.91^{b}	5.22±0.44^{b}	1.98±0.28^{b}
C22:5n-6	1.18±0.06^{b}	1.23±0.27^{a}	1.46±0.21^{b}	3.17±0.30^{b}	0.69±0.15^{b}
C18:3n-3	0.73±0.01^{b}	1.27±0.05^{b}	0.26±0.04^{b}	0.56±0.05^{b}	1.17±0.25^{b}
C18:4n-3	0.44±0.03^{b}	1.37±0.03^{b}	0.08±0.01^{b}	0.49±0.05^{b}	1.46±0.22^{b}
C20:3n-3	0.07±0.05^{a}	1.21±0.07^{b}	0.36±0.03^{b}	0.37±0.05^{b}	0.23±0.08^{a}
C20:4n-3	0.37±0.02^{b}	1.20±0.19^{b}	0.62±0.09^{a}	0.69±0.08^{a}	0.63±0.20^{a}
C20:5n-3	2.74±0.55^{b}	5.16±0.51^{b}	5.54±0.55^{b}	6.44±0.08^{b}	2.47±0.41^{b}
C22:5n-3	1.25±0.01^{b}	1.67±0.05^{b}	5.10±0.45^{b}	4.12±0.07^{b}	0.94±0.13^{a}
C22:6n-3	2.78±0.46^{b}	6.59±0.16^{b}	17.36±1.45^{a}	25.63±0.66^{b}	4.96±0.12^{b}
C16:2	0.39±0.01^{b}	0.14±0.00^{b}	0.70±0.11^{a}	0.94±0.02^{b}	0.85±0.03^{b}
C16:3	0.84±0.07^{b}	1.14±0.03^{b}	0.23±0.03^{a}	0.87±0.07^{b}	1.20±0.19^{b}
C16:4	0.02±0.01^{a}	0.32±0.02^{b}	0.12±0.02^{b}	0.04±0.01^{b}	0.13±0.01^{b}
C21:5	0.83±0.11^{b}	0.36±0.04^{b}	0.24±0.03^{b}	0.42±0.05^{b}	0.27±0.02^{a}
n-3/n-6	0.44±0.02^{a}	2.83±0.02^{b}	1.89±0.03^{c}	1.7±0.09^{c}	0.8±0.01^{d}
U/S	1.2±0.03^{a}	1.44±0.1^{b}	1.7±0.02^{c}	3.17±0.1^{d}	1.8±0.05^{c}
UI	1.5±0.05^{a}	2.5±0.06^{b}	2.6±0.04^{b}	3.1±0.1^{c}	1.47±0.08^{c}
Total PL (mg·g^{-1} Fw)			0.64±0.02		
TAG (mg·g^{-1} Fw)			0.27±0.05		

Note: Results are expressed as mole % of total FAME based on peak areas. Data are means ± standard deviations from triplicate estimations (n =3). Means followed by different letters in the same line are significantly different ($p < 0.05$) using ANOVA (Tukey HSD test). SFA, MUFA, and PUFA mean saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid, respectively. PC, PE, PI, PS and TAG mean phosphatidylcholine; phosphatidylethanolamine; phosphatidylinositol; phosphatidylserine and triacylglycerol, respectively. ND = not detected.

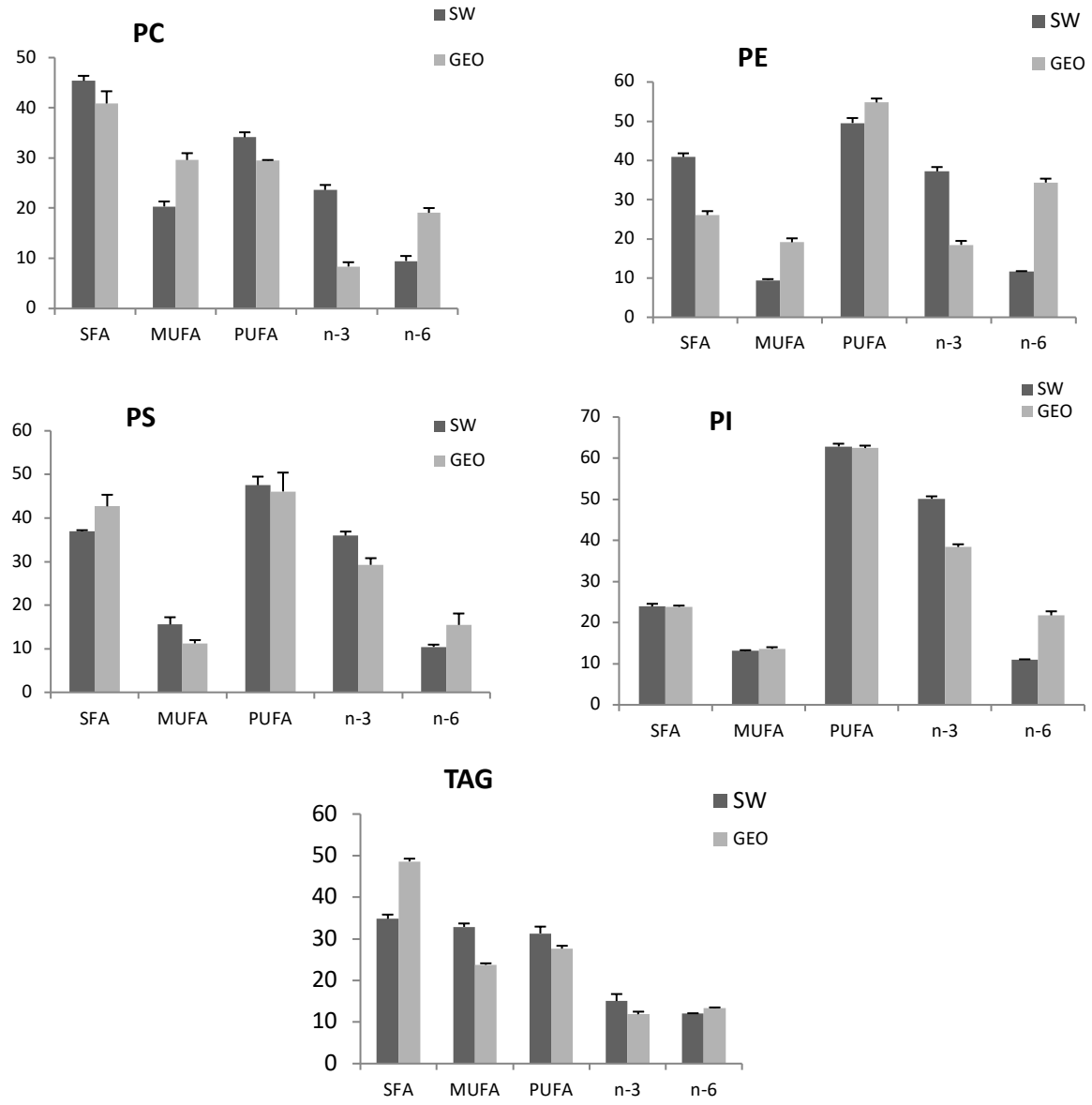

Figure 1. Changes in SFA, MUFA, PUFA, n-3 and n-6 of PC, PE, PS, PI, and TAG in the muscle of *Chelon labrosus* reared in seawater and geothermal water. Values reported are mean ± SD from triplicate estimations (n =3) using ANOVA (Tukey HSD test).

the level of n-3/n-6 ratio was markedly increased in the PE fraction of the geothermal group. It is also important to note that, SFA stood out as the most abundant fatty acid group in TAG mainly in the geothermal group.

The principal component analysis (PCA) was performed to gain better insight into the effects of rearing conditions on the fatty acid composition of lipid classes in the flesh of the thick-lipped grey mullet, *C. labrosus*. Figure 2 depicts the twoprincipal-components that described 57.72% of the total data variability (PC1 34.22% and PC2 23.5%). The PCA biplot of the overall data described a clear separation between samples from the seawater and the geothermal water. The projection of individuals (each fatty acid from each lipid class of the different fish groups) on the same factorial plan (1:2) showed that samples could be clustered into two groups (I and II). Group I, which consisted of individuals sampled from geothermal water, showed a positive contribution to the first component (PC1), which was characterized by the substantial PUFA n-6 in the PE class. Group II was, however, characterized by high PUFA, particularly n-3 PUFA, DHA and EPA of the phospholipid

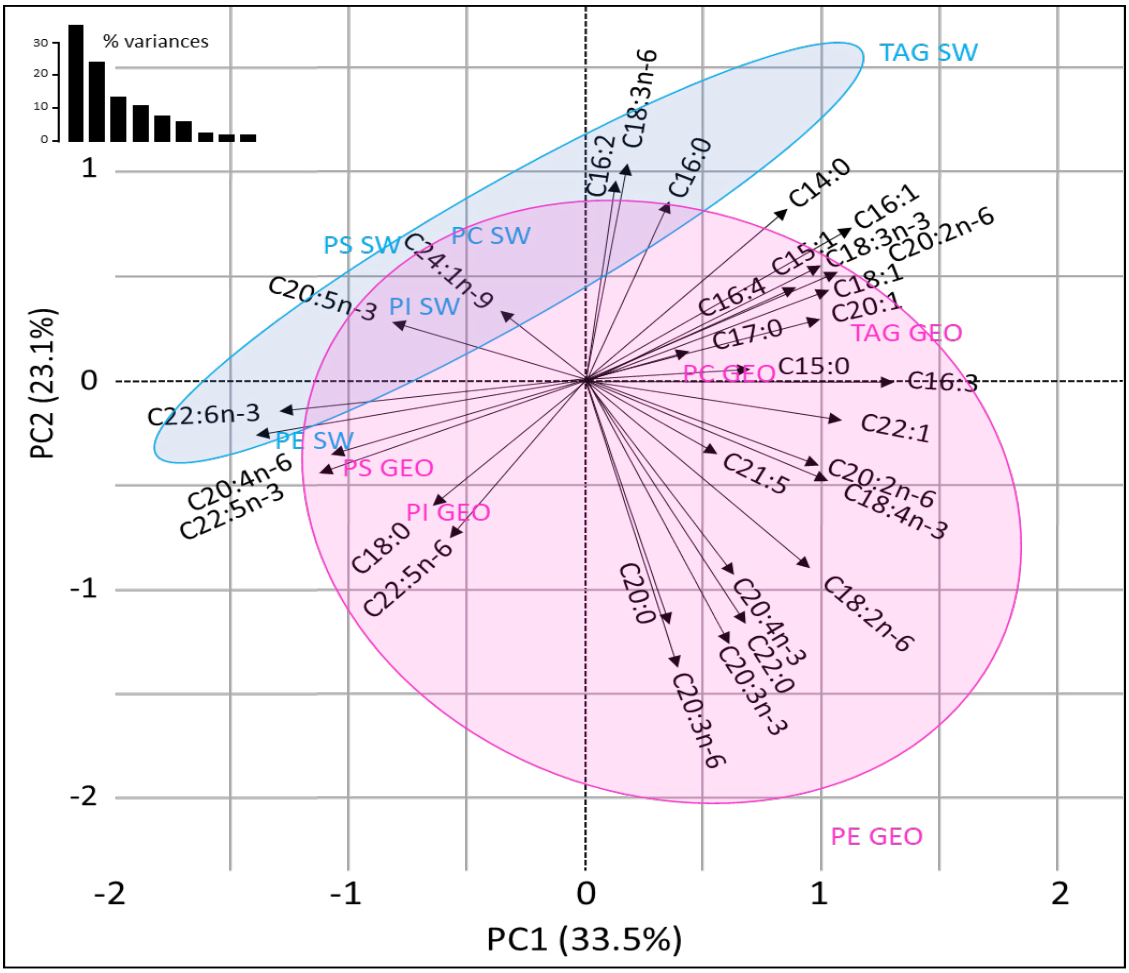

Figure 2. Principal analysis component (PCA) represented by two factors F1 and F2 and produced by lipid class and fatty acid composition in *Chelon labrosus* muscle reared in seawater and geothermal water.

class representing individuals sampled from seawater (Figure 2).

4. DISCUSSION

Teleosteen fish are exposed to varying and occasionally extreme environmental conditions that can produce potent effects on their physiology (Somero, 2004). The Thick-lipped grey mullet *C. labrosus*, is an euryhaline and eurytherm teleost that presents the ability to live under different environmental conditions of salinity and temperature (Azaza *et al*., 2008; Cardona, 2006; Rabeh *et al*., 2013). It is known that temperature and salinity are the key factors in teleost fish that cause fluctuations in the fluidity of cell membranes (Soengas *et al*., 2007) which largely depends on their lipid components. In this context, changes in membrane lipid composition is a key molecular mechanism of adaptation that is commonly called homeoviscous adaptation (Hazel,1995).

In the current study, lipid class composition and the fatty acid profiles of the two fish groups analyzed were shown to respond selectively and significantly to environmental conditions. Our data revealed that the lipids of the examined fractions from the seawater fishes were characterized by higher values for PUFA n-3, particularly in the PI fraction. However, a high content of PUFA n-6 was recorded in the PI fraction of the geothermal group. In addition, a substantial amount of SFA was observed in the TAG for the two groups. Our findings corroborate with those recorded by some authors who have reported that long-chain fatty acids were probably important for PI to successfully conduct particular cellular functions, including the role in cell growth, signal transduction processes and the membrane anchoring

of proteins in plants (Riekhof and Benning, 2009). Consequently, we hypothesize that an increase in PUFA n-6 in the PE of geothermal water fish might compensate for the effects of warm and lower salinity water, which tends to increase lipid rigidity.

The detailed investigation of *C. labrosus* groups considered in this study indicated that among the major fatty acids of the different lipid classes are C16:0, C18:1, C22:5n3, C18:2n6, C20:4n6, EPA, and DHA. First, with respect to saturates, the highest levels are usally contained in PE and TAG in the two *C. labrosus* groups. Among SFA, the major components were C16:0, followed by C18:0. Previous studies have reported that SFA, mainly palmitic acid, which is the main fatty acid synthesized *de novo* in fish, is classically associated with HUFAs in the diacyl phospholipids of fish (Steffens, 1997; Li *et al*., 2011). In fact, the important levels of SFA may result from a lipogenic activity (Dias *et al.,* 1998) that can be biosynthesized by fish through a conventional pathway catalyzed by cytosolic fatty acid synthetase (Sargent *et al.,* 2002). In addition, the TAG fraction in fish can also contain high levels of monoenoic C16-C18 fatty acids that are intensively synthesized in so-called "fatty" fish species to provide energy reserves. Likewise, the findings of Arts *et al.* (2009) revealed that triacylglycerol synthesis notably begins with a polar lipid that mostly has one of five common FA (e.g., myristic, C14:0; palmitic, C16:0; palmitoleic, C16:1n-7; stearic, C18:0; or oleic, C18:1n-9) in the *sn-1* position.

The highest MUFA detected in the lipid class of both fish groups was C18:1 with markedly impotant levels in the PC of the two studied groups. This is presumably due to its dominance in the commercial feed used in this survey. Another aspect to be taken into account is that C18:1 is a typical MUFA in fish which is most often considered from the standpoint of its energy importance (Sargent *et al.,*1989). It is also interesting to point out that PC in fish tissues is commonly rich in C18:1 and appears to be more easily influenced by dietary fatty acids than other phosphoglycerides (Tocher, 2003) and it should be the precise reason for the preponderance of these fatty acids.

It is remarkable that the phospholipids of the geothermal groups were found to contain significantly higher contents of C18:2n-6 compared to the seawater groups. The same conclusion was reached in a experiment in which *M. cephalus* fry were acclimatized to freshwater (El Cafsi *et al.,* 2003). The drop in salinity in the geothermal habit may have led to an increase in the percentages of PUFAs and particularly the n-6 series in the PE fraction. On the other hand, it is also possible that the mechanism involved in the catabolism of C18:2n-6 was more active in the fresh water fish than those from seawater (Sargent *et al.,* 1989; Kheriji *et al.,* 2003). It is also noteworthy that C18:2n-6, which is a well-known key dietary compoment constitutes a good energy source from fish (Castell *et al.,* 1972; Glencross, 2014). Herein, the dominant FA in the food is C18:2n-6 (29%), which allow us to explain the high levels of PUFA n-6 in the fillet of *C. labrosus*. Indeed, dietary lipids are a source of fatty acidswhich are required for the synthesis of new cellular lipids and for the turnover of existing lipids.

In the present study, an increased proportion of physiologically significant FAs (C22:6n-3 and C20:5n-3) in the phospholipid class was observed for both groups. It has been well established that fish phospholipids are usually considered as a physiologically crucial lipid classes since they are rich in PUFA, predominately DHA and EPA (Sargent *et al.,* 1993). Typically, EPA occurs in lower proportions than DHA due to the availability of these FA as dietary sources. Interestingly, C22:6n-3 is abundant in PI and PS particularly in geothermal specimens. The importance of DHA in PS was previously attributed to the ability of *C. labrosus* to assimilate this FA. The abundance of DHA observed in the polar lipids started with recognition that this FA has a unique conformation dictacted by helical or an angle iron shape with an overall length similar to that of C16:0 (Applegate *et al.,* 1986). This structure favors the formation of the hexagonal phase in phosphoglycerides, above all in C22:6n-3 phosphoglycerides containing small head groups such as phosphatidylserine and this will facilate very fast conformational changes in membranes (Brown,1994). In a general way, such essential FA are, for example, involved in the modulation of the properties of the lipid phase of the cell membrane, membrane bound enzymes as well as the precursor of functionally important lipooxygenase products (Koven *et al.,* 2011). Previous investigations have shown that variations in polar lipid contents and their individual fractions particularly in muscle are key compensatory mechanisms in organisms that guarantee optimum performance of several membrane-bound enzymes under different environmental conditions (Los, 2001; Cengiz *et al*., 2012).

5. CONCLUSIONS

This study provides initial insight into the lipid class composition of *C. labrosus* reared under different conditions. From the obtained results we can conclude that variations in the salinity and temperature chiefly affected the PUFA group. Indeed, we noticed that the juvenile *C. labrosus* reared in seawater conditions were characterized by the predominance of the n-3 series in phospholipid fractions. As for specimens from the geothermal system, high levels of n-3 PUFA were recorded in the PS and PI fraction, while PC and PE were dominated by the n-6 series. This suggests the aibility of *C. labrosus* to remodel its lipid composition to adapt to extreme environmental conditions. The obtained results can provide useful basic information that can help in the management of inland aquaculture practices. However, futher investigations are needed to better understand the impact of abiotic parameters on the lipid metabolism of of this promising species.

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