Advances in Bioresearch
Adv. Biores., Vol4 (3) September 2013: 14-21
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

ORIGINAL ARTICLE

Seasonal Variation of Fatty Acids Composition in European Eel Muscles from the North East of Tunisia: Bizerte Lagoon

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ABSTRACT
The aim of this study is to determine the seasonal variation of the total fat content and fatty acids (FA) Composition of European eel population caught from Bizerte Lagoon located in the north east of Tunisia. Analyses of environmental parameters were carried out throughout the sampling period ranging from October 2007 to June 2008. Mean weight and length of samples studied is about 198g±5g and 48.2cm±5cm. Total lipid extraction was carried out by Folch method, methyl esters preparation was performed by direct transesterification, gas chromatography analysis showed that the major fatty acids present in eel oil extract were: saturated, monounsaturated and polyunsaturated fatty acids which were significantly represented by eicosapentanoic (C20:5) and docosahexanoic acids (C22:6) of (n-3) family known as a major source of energy allowing eels growth and reproduction and with their benefic effect on human health; this component are influenced by environmental parameters as temperature and salinity.
Keywords: *Anguilla anguilla, fat content, fatty acids, seasonal variation, environmental parameters.*

Received 04/05/2013; Accepted 29/07/2013

INTRODUCTION

Generally fish use fat content as an energy source. They accumulate large amounts of lipid in their muscles and adipose tissue [1],[2] by this characteristic they can be classified into two groups, fatty fish and non-greasy. *Anguilla anguilla* is known for its higher fat content [3], [4] which is important for human diet [5]. Its fatty acids composition is rich source in palmitic (C16:0) and in oleic (C18:1) acids; it is also known to require both linolenic and linoleic acids [6] as well as in polyunsaturated fatty acids of the (n-3) family mainly represented by eicosapentaenoic (C20:5) EPA, and docosahexaenoic (C22: 6) DHA acids [7], [8] which have benefic effects on human health [9], [10], [11]. In addition lipid profile, proteins and fatty acids composition can be influenced by both of exogenous and endogenous parameters [12] including environmental parameters as temperature and salinity associated with the life cycle of fish and the fishing season. The purpose of this study is to follow the seasonal variation in fat content and fatty acids composition of European eel muscles caught from Bizerte lagoon.

MATERIALS AND METHODS

Site and sampling strategy

Anguilla anguilla population studied was captured from the Bizerte Lagoon, located in the extreme north of Tunisia between 37°08' and 37°15' north latitude and 9°45' and 9°57' east longitude (Figure 1). It communicates with the North Mediterranean Sea by a navigation channel (7km long) to the West Lake Ichkeul across the Tinja River. It is an elliptical basin area equal to (128 km^2) receives several rivers: to the south by the lower valley of the Mejerda, to the East by the watershed of the coastal area of Ras El Jebel and to the West by Ichkeul with which it communicates by the Tinja River. The later is a winding channel with a few meters wide and 5 km long and feed the Bizerte lagoon water during the rainy season [13]. The installation of dams upstream of Ichkeul strongly affected the natural balance by varying its water balance. These developments lead to profound changes on the hydrological characteristics of the lagoon especially salinity [14].

European eel samples were collected (N = 30) monthly during the period from October 2007 to June 2008, measured 48.2cm ± 5cm and weighed 198g ± 5g.

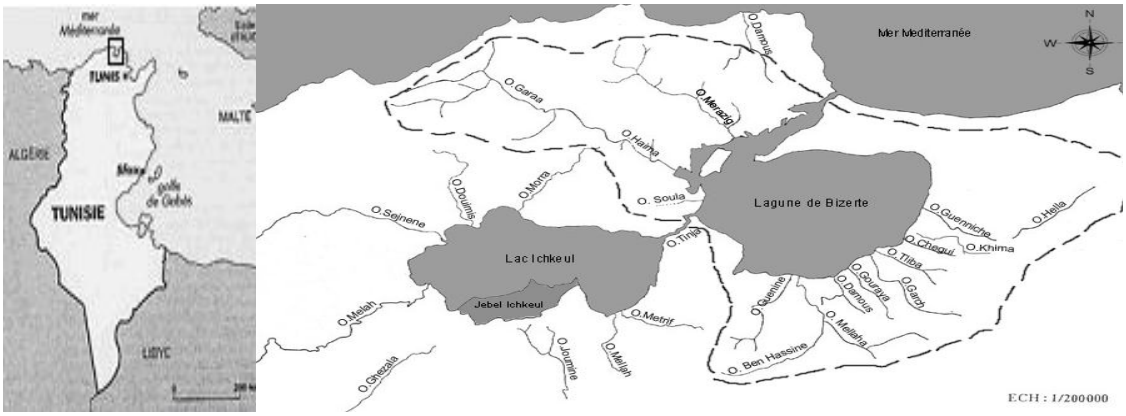

Figure 1: Geographical location of the lagoon of Bizerte

Physico-Chemical parameters

Temperature and salinity measurements were conducted every month using a precision electronic thermometer graduated to (1/10th°C) and a salino-meter type (WTW, LF191) previously calibrated by standard seawater.

European eel flesh Composition

Crude protein (N×6.25) was determined by the Kjeldahl method (ISO 5983-1997), moisture after drying for 4 h at 105°C by (ISO 6496-1999), total lipid extraction from eels tissues was carried out by Folch method [15] and results expressed as a percentage of wet tissue. Methyl esters preparation was performed by a direct transesterification [16], [17] and then analyzed by gas chromatography using a gas chromatograph type HP series 6890 with a split/splitless injector and a flame ionization detector was used for the analysis. The device includes a 30 m long HP Innowax capillary column with an internal diameter of 250 µm and a 0.25 µm film, the stationary polar phase of the column being polyethylene glycol. Comparison of the retention times of the fatty acids under study and those of standard fatty acid methyl esters Supelco (PUFA-3) allowed to identify the different fatty acids. An internal standard not existing in our sample which is nonadecanoate methyl (C19:0) served to quantify the fatty acids.

Statistical Analysis

To test the effect of season on fatty acids as well as environmental parameters on the mean levels of lipids statistical analyses were carried out with the SAS software program version 6.12. Different mean values were analyzed according to the Duncan's multiple range tests. The result is considered significant if ($p<0.05$).

RESULTS

During the study period, water temperature measured ranges from the minimum value of (12.2°C) recorded in January and the maximum of (21,7°C) in June (Figure 2). Salinity measurements ranged between (33.2‰) and (36.8‰) it peaked following the evaporation caused by higher summer heat, then decreases with the arrival of the rainy season.

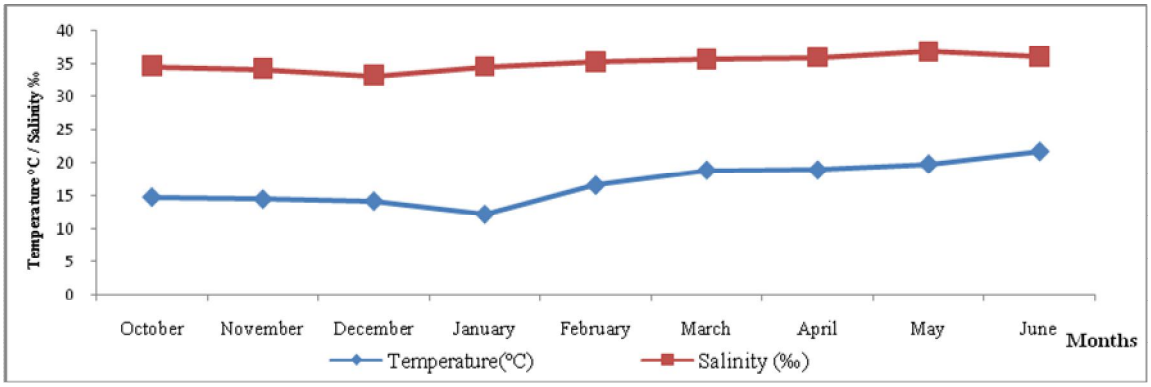

Figure 2: Monthly variation of temperature (°C) and salinity (‰) From October 2007 to June 2008

Seasonal variation in eels flesh composition (Table 1) captured from the Bizerte lagoon are leading to significant ($p<0.05$) fluctuation as well as for protein, water and fat. However, the maximum of protein was observed in spring (23.59% ± 0.08) and autumn (20.24% ± 0.02), while in winter it drops

significantly ($p<0.05$). The maximum rate of lipid is obtained in winter (10.75± 0.3) but decreased significantly ($p<0.05$) in spring (5.24 ± 0.04); whereas moisture variation was not statistically significant ($p<0.05$) during the studying period.

Table 1: Seasonal variation in the European eel flesh composition

	Autumn	Winter	Spring	Summer
Moisture (%)	63.14 ± 0.74	61.27 ± 5.97	64.27 ± 1.77	63.34 ± 0.55
Total Lipids (Folch)(g/100g)	8.94 ± 0.56	10.75 ± 0.30	5.24 ± 0.04	6.35 ± 0.77
Crude Proteins (N×6.25) (%)	20.24 ± 0.02	18.59 ± 0.3	23.59 ± 0.08	19.16 ± 1.18

Values given as mean ± SD.

We note that lipid level varies significantly ($p<0.05$) according to the seasons and essentially affected by environment parameters as temperature and salinity; in fact as shown in (Figure 3) for the minimum annual mean temperature about (14.3°C) in winter we have the highest lipid level value about (10.75g/100g) then an increase in temperature value in spring and in summer induce a significant ($p<0.05$) decrease in the rate of lipid about (5.24g/100g) and (6.35g/100g) respectively.

Salinity is directly related to rainy period, as shown in (Figure 4) lipid level and salinity are inversely proportional, in fact in winter for the minimal salinity value about (34.3‰) we have the maximum lipid level (10.75g/100g) a significant increase ($p<0.05$) in salinity medium (36,1‰) leads to a significant ($p<0.05$) drop in the lipid rate which is about (5.24g/100g).

Seasonal variation analysis of fatty acids composition in European eel flesh shows a predominance of monounsaturated fatty acids on saturated and polyunsaturated fatty acids.

During the study period seasonal variation of these component are shown in (Figure 5) in fact SFA variation was statistically significant ($p<0.05$) in autumn (37%) and in summer (27.6%), MUFA varies significantly ($p<0.05$) in winter (40.8%) and in spring (58.6%) whereas for PUFA statistical significant variation ($p<0.05$) is in winter (22.08%) and in spring (12.16%).

Gas chromatography analysis allows the identification of the following fatty acids as signaled in (Table 2) which are : myristic (14:0), pentadecanoic (15:0), palmitic (16:0), palmitoleic (16:1,n-7), stearic (18:0), oleic (18:1, n-9), linoleic (18:2, n-6), linolenic (18:3, n-3), eicosenoic (20:1, n-9), eicosadienoic (C20:2, n-6), eicosatrienoic (C20:3 n-6), arachidonic (20:4, n-6), docosapentaenoic (DPA) (22:5, n-3), and decosahexaenoic (DHA) (22:6, n-3). Percentage of identified fatty acids is illustrated in (Table 2) we note that saturated fatty acids (SFA) mainly represented by palmitic acid (C16:0), monounsaturated fatty acids (MUFA) represented in majority by C18:1(n-9), C16:1(n-7); whereas PUFA of (*n-6*) family are represented by (C18:2) and (C20:4) those of (*n-3*) family in majority represented by (C20 :5) and (C22 :6).

As shown in (Table 2) maximum values of palmitic acid are recorded in autumn and in winter, it decrease significantly ($p<0.05$) in summer and in spring; for stearic acid (C18: 0) its maximum value is obtained in autumn (5.01%) and drops significantly ($p<0.05$) in all the other seasons. Palmitoleic acid (C16:1) and oleic acid (C18:1) maximum values are obtained in spring but decreases significantly ($p<0.05$) in autumn and in winter respectively; arachidonic acid (C20:4) increases significantly ($p<0.05$) in winter and drops in spring to minimal value of (2.01%); eicosapentanoic acid (C20:5) (*n-3*) is recorded in winter (4.86%) and decreases significantly ($p<0.05$) in the other seasons. The highest percentage of docosahexaenoic acid C22: 6 (*n-3*) was in spring (3.36%) decreasing significantly ($p<0.05$) in autumn.

The (*n-3*)/(*n-6*) report is represented by a maximum rate in spring (2.46%) and in summer, minimum value is obtained in winter (1.04%).

Variation of the different category of European eel lipid according to the salinity show that the later depend on water temperature in fact for a salinity (34.3‰) we have the mean minimal temperature value about (14.3°C) leading to an increase in PUFA percentage flowed by a decrease in MUFA (40.85%). An increase in water temperature in spring leads to a significant increase in MUFA/SFA report.

European eel fatty acids degree of saturation show that the evolution of the different ratio depending on the salinity varies with medium temperature, in fact low temperatures and salinity causes variations of PUFA/SFA ratio which increases in winter (0.57) this suggests that either the synthesis of polyunsaturated fatty acids is important, or there is a greater use of PUFA. The transition from high to low salinity in winter causes an increase in PUFA/SFA which indicates that there was a strong use of saturated fatty acids or an important MUFA and unsaturated fatty acids biosynthesis; in all cases salinity lowering seems to lead to greater unsaturation of fatty acids.

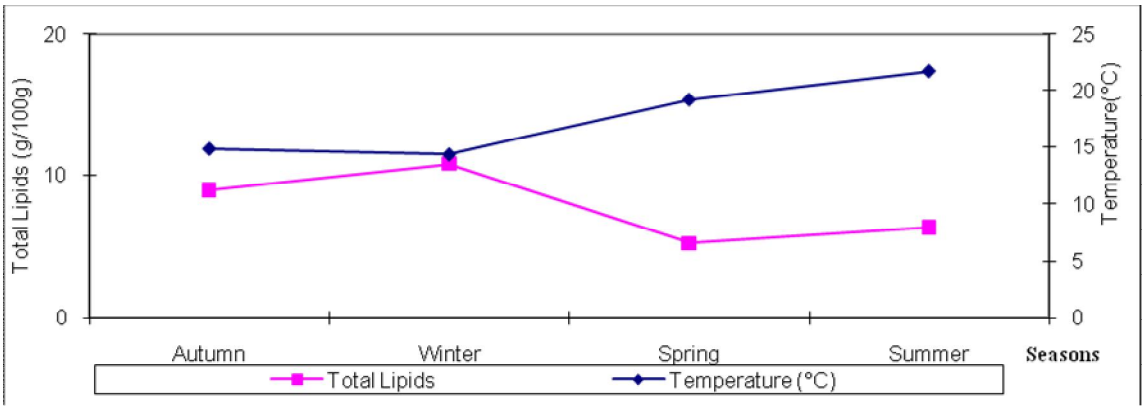

Figure 3: Variation of total lipids according to the temperature of the medium

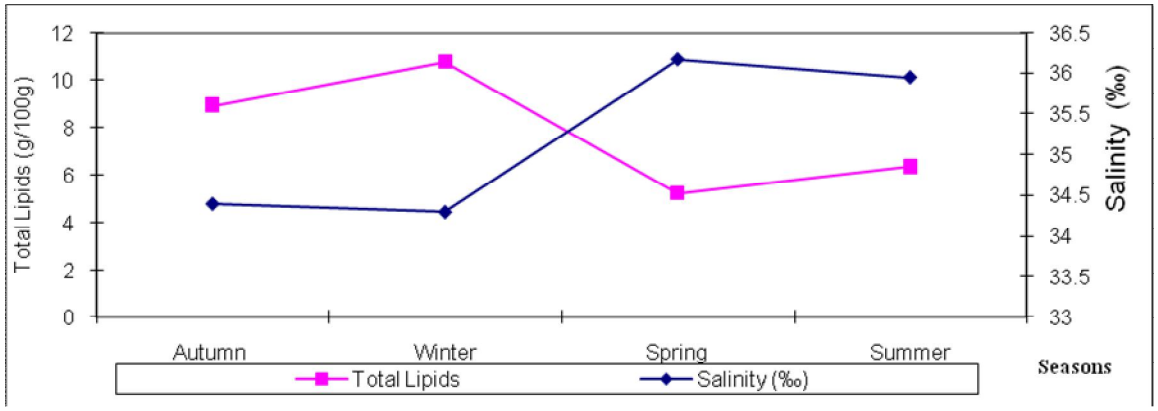

Figure 4: variation of total lipids according to the salinity of the medium

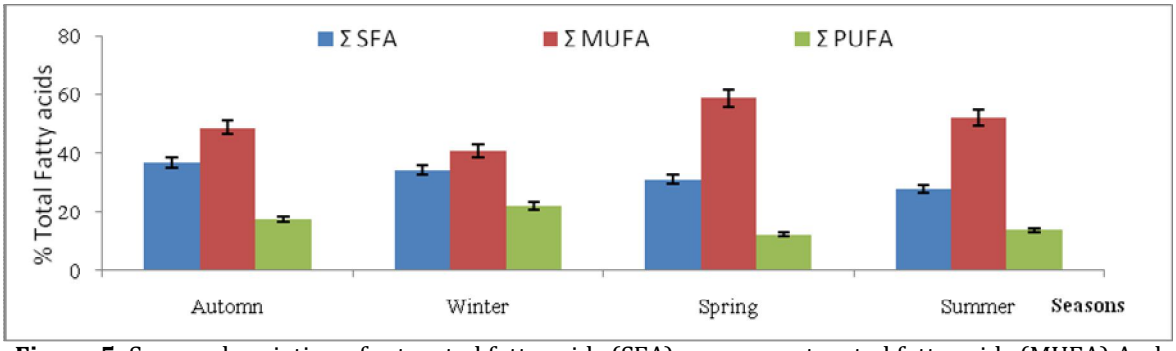

Figure 5: Seasonal variation of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) And polyunsaturated fatty acids (PUFA) in European eel flesh.

DISCUSSION

Generally biochemical composition of sea food products undergoes seasonal variations [18] [19]. From nutritional point of view the determination of total lipid content and proportions of the different fatty acids are important factors in flesh fish composition, however results obtained in (Table 1) show that European eel muscles possessed considerable higher fat content and lower moisture value than those of other fish species [3],[4],[20] it also has a significant proteins level (Table 1) there was no statistical significant ($p<0.05$) correlation either between the lipid and protein rate, or between protein and total moisture level [21], but there was reversed relationship between fat and moisture content however, an increase in fat content causes a decrease in the moisture content [22] in fact, contents values are considered in relation with the season of the year in which they are studied [23],[24] as signaled by [25],[26],[27]. Fish lipid profile depends on the season and in the characteristics of the sea area and on environment factors as salinity and temperature [28].

In our study total lipid content and fatty acids composition varies significantly ($p<0.05$) according to the seasons so it is affected by exogenous and endogenous factors [12] including water temperature and salinity as shown in (Figure 3, 4); water temperature has an influence in plankton production and

distribution [29], this fluctuations depend on compositions of food availability, and fishes adapt the composition of their lipids both to their own physiological and environment requirements [30].
The seasonal variations of fatty acids composition are illustrated in (Table 2) which show that The palmitic acid (C16:0) is the major fatty acid of the SFA family by its high level it is well distinguish between myristic (C14:0) and pentadecanoic acids (C15:0); results obtained are similar with those of [21], in fact the lowest SFA value is obtained in summer (27.25%) and the highest rate is obtained in autumn and winter (Table 2) and may be explained by tropic activity including feeding habit of the specimen studied which is in correlation by water temperature in fact eels stops feeding for temperature under 10°C and higher than 30°C [31].

Table 2: Seasonal variation of fatty acids of European eel oil extract

	(%) of Fatty acids			
Fatty acids	Autumn	Winter	Spring	Summer
C14 :0	5.54 ± 0.22	4.31 ± 1.32	5.25 ± 0.11	4.07 ± 0.34
C15 :0	0.65 ±0.20	0.6 ± 0.06	0.54 ± 0.09	0.48 ± 0.12
C16 :0	25.73 ±0.28	25.03 ± 6.51	21.44 ± 0.23	19.83 ± 0.94
C18 :0	5.01 ±0.05	4.26 ± 5.13	3.62 ± 0.38	3.29 ± 0.05
Σ SFA	36.93 ± 0.85	34.2 ± 0.65	30.85 ± 0.52	27.67 ± 0.35
C16 :1	8.78 ± 0.11	10.26 ± 2.50	14.65 ± 0.9	11.04 ± 0.11
C18 :1	37.55 ± 0.35	29.64 ± 1.23	42.89 ± 1.36	40.06 ± 0.53
C20 :1	1.8 ± 0.09	0.95 ± 0.36	1.1 ± 0.1	1.21 ± 0.53
Σ MUFA	48.13 ± 0.31	40.85 ± 0.51	58.64 ± 0.26	52.31 ± 0.12
C18 :2	3.36 ± 0.16	5.06 ± 1.23	0.92 ± 0.2	1.06 ± 0.16
C18 :3	0.21 ± 0.02	0.31 ± 0.13	0.12 ± 0.03	0.09 ± 0.02
C20 :2	0.75 ± 0.04	0.31 ± 0.14	0.17 ± 0.1	0.28 ± 0.04
C20 :3	0.43 ± 0.02	0.83 ± 0.43	0.29 ± 0.02	0.32 ± 0.02
C20 :4	2.91 ± 0.36	4.28 ± 1.18	2.01 ± 0.13	2.09 ± 0.36
ΣAGPI (n-6)	7.66 ± 0.21	10.79 ± 0.61	3.51 ± 0.12	3.84 ± 0.09
C18 :3	0.96 ± 0.04	0.69 ± 0.25	0.49 ± 0.16	1.09 ± 0.04
C20 :5	3.82 ± 0.23	4.86 ± 2.2	3.16 ± 0.17	3.2 ± 0.23
C22 :5	2.07 ± 0.11	2.46 ± 0.56	1.64 ± 0.23	2.22 ± 0.11
C22 :6	2.87 ± 0.32	3.28 ± 0.72	3.36 ± 0.16	3.23 ± 0.32
ΣAGPI (n-3)	9.72 ± 0.36	11.29 ±0.21	8.65 ±0.53	9.74 ±0.62
(n-3)/(n-6)	1.26	1.04	2.46	2.53
MUFA/SFA	1.3	1.05	1.9	1.44
PUFA/SFA	0.47	0.57	0.39	0.49

Means±standard deviation

SFA considered as a key part in fish lipids metabolism [32], it depends on food availability [33]. They are not considered as a whole because they differ in their: structure, metabolism, cell functions and their effects in case of excess [34].
Monounsaturated fatty acids highest proportion is correlated by oleic acid, it is considered as the main MUFA in Sparidae mussels [35], in our study it decreases in winter and in autumn and this may be explained by its intervention as a source of metabolic energy.
Polyunsaturated fatty acids (PUFA) represent the most important fatty acids in fish muscles their abundance is in accordance with (n-3) PUFA witch level varies according to the seasons; peaks are observed in winter (11.29%), in autumn (9.72%) and in summer (9.74%), alpha linolenic (ALA) acid considered as (n-3) precursor is converted into docosahexaenoic acid (DHA) which daily recommended dietary is about 1.8g [34], whereas aracidonic and linoleic acids are considered as the most important (n-6) PUFA which level varies significantly ($p<0.05$) according to the seasons, both of them peaks are obtained in: winter, autumn and in summer.

The (n-3) / (n-6) ratio is very important to evaluate oil fish nutritional value [36], it is benefic for human health to consume sea food product because of their high (n-3) PUFA and low (n-6) PUFA contents [37]. Modification in lipid composition of some fish including European eel under varying environmental conditions has been reported by some authors [38], [39], [40], [41].

Water salinity impact on different lipid classes shows that its action depends on water temperature. The increase in polyunsaturated fatty acids content of fish oil in cold water has been the subject of several studies [42], [43], [44], [45], [46].

In his investigation on carp [38], the level of polyunsaturated fatty acids, total lipids and phospholipids is higher in fish adapted to cold water than those adapted to warmer waters; radioactive labeling shows that at low temperatures, fish directs its metabolism towards a greater biosynthesis of polyunsaturated fatty acids, since they found a higher percentage of radioactivity in these fatty acids as at 5°C and at 20°C.

The study on *Seriola dumerili* shows that the variation of water temperature from December to July leads to a modification of the lipid composition of the liver. Temperature decrease leads to an increase in lipids degree of unsaturation [47].

Fatty acids analysis in muscle and liver of *Dicenlrarchus labrax*, shows that from winter to spring, saturated fatty acids increase while polyunsaturated fatty acids decrease [48].

In our study, low water temperature (14.3°C) in winter causes an increase in polyunsaturated fatty acids followed by a decrease in saturated fatty acids. We can deduce that an increase in polyunsaturated fatty acids may be a response to the decrease in water temperature. This increase indicates that low temperatures stimulate the function of desaturases and elongase involved in this fatty acids biosynthesis. These changes in fatty acid composition are mainly in relation with fluidity membrane maintenance. Low temperature causes an increase in polyunsaturated fatty acids and a decrease in saturated fatty acids [40]. These changes to the fatty acid composition of polar lipids designed to maintain membrane fluidity and cell function at low temperature [49].

Desaturation process seems to be more effective in freshwater, because monounsaturated fatty acids as oleic acid (C18: 1) and palmitoleic acid (C16: 1), can be derived from the power supply which decrease at low salinity medium and can be explained by their energetic consumption for the fight against the environment osmotic conditions.

CONCLUSION

This study describes the great degree of variation in fat, protein contents and fatty acids composition according to the seasons and the environment parameters as (temperature and salinity); gas chromatography analysis show that European eel oil extract is composed by saturated, monounsaturated and polyunsaturated fatty acids higher proportion are represented by palmitic, oleic, arachidonic, docosahexanoic and eicosapentanoic acids which seasonal variation is statistically significant equally for precursors of synthesis chains of (*n-3*) and (*n-6*) PUFA family. From nutritional point of view and according to our finding European eels muscles are considered as high source of proteins, fat content and PUFA making up the biggest part in fish muscles due to their abundance it is recommended and profitable for human health consummation.

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Citation of This Article
EL Oudiani Salma and Hechmi Missaoui. Seasonal Variation of Fatty Acids Composition in European Eel Muscles from the North East of Tunisia: Bizerte Lagoon. Adv. Biores., Vol4 (3) September 2013: 14-21.