



Biochemical Evaluation of Euro-Mediterranean Eel from Tonga Lake and El Mellah Lagoon (North-East of Algeria)

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Accepted on: 02-06-2016; Finalized on: 31-07-2016.

ABSTRACT

This study was carried out to investigate on seasonal variation some biochemical parameter in the muscle of Eel from Tonga lake (freshwater) and El Mellah lagoon (brackish water) in Algeria. Analysis of the various macronutrients showed that in both sites, total lipid levels are between 19.4% and 22.15% with a maximum in winter at Eels El Mellah. Findings indicated also that the two sites protein, ash, moisture and carbohydrate levels of Eel from Tonga present the maximum in spring and higher than in El Mellah. Qualitative analysis of fatty acids by gas chromatography revealed that the Eels captured in both lakes contain 23 fatty acids. The maximum rate of SFA observed for Eel El Mellah is 35.87% in winter and the maximum rate of UFA is 54.848% for Eel Tonga in spring. Among the saturated fatty acids, palmitic acid is majority and the maximum was observed in winter at Eels from El Mellah. Regarding the unsaturated fatty acids, the most dominant MUFA is oleic acid with a maximum rate of 36.968% for Eels of El Mellah in winter. At the level of PUFA, we only note the presence of linoleic acid and linolenic acid with a maximum observed 4,599 % (AL) and 2.872 % (ALA) for Eel from Tonga in winter and higher than in El Mellah. The n-3/n-6 ratio of Eel from Tonga is more important than El Mellah.

Keywords: *Anguilla Anguilla*, Seasonal variation, fatty acids, Tonga, El Mellah.

INTRODUCTION

The overall composition in most fish and shellfish is mainly water, proteins and lipids. These constituents, account for approximately 98% and other minor components include carbohydrates, vitamins, and minerals. However, the biochemical composition of fish usually varies depending on season, geographic areas, stages of maturity and size.¹ The specific composition of fish gives it a nutritional and sensory quality that looks for and value consumers. Fatty acids and lipids are generally present in the marine food chain represented by different organisms (bacteria, crustaceans, seaweed, fish, sponges). Fish have high proportions of polyunsaturated fatty acids to long chain because of their terminal position in the trophic chain. Furthermore, fish have enzymes (desaturase and elongases) for the biosynthesis and processing of certain fatty acids, compared to the living conditions marin, which affects the fatty acids content. These levels vary between 25 and 40% of polyunsaturated acids, eicosapentaenoic acid (EPA) and 5 to 8% docohexaénoïque acid (DHA).² In their natural environment, marine fish at the base of the trophic chain, are in the phytoplankton they feed, and which has a high concentration of polyunsaturated fatty acid.³ Thus same fish will be used as source poly unsaturated fatty acids (PUFA) of other higher trophic level fish, which reflects the composition of body fat,^{4,5} the natural diet of freshwater fish is rich in linoleic and linolenic acid.⁶ They must therefore be able to convert these C18 fatty acids EPA and DHA to maintain the fluidity of their membranes.

Consequently, and contrary to many marine species, most of the freshwater fish have good ability to lengthen and desaturate their C18 fatty acids long chain.⁷ In this context and from fatty fish, the species *Anguilla Anguilla* (Eel) is an amphihalin fish, which performs its life cycle in freshwater and saltwater. This fatty fish with a lipid levels in the muscle of up to 20% and its richness in polyunsaturated fatty acids of the omega-3 family (n-3) as the eicosapentaénoïque acid (EPA) and omega 6 (n-6) as the docohexaénoïque acid (DHA) makes a food's unique nutritional characteristics among animal products. The family of fatty acids omega 3 and omega 6 contribute to the development of the brain and sensory organs in infants; prevent some aspects of obstructive cardiovascular disease involved in various inflammatory diseases as well as diabetes and osteoporosis.^{8,9} In Algeria, the consumption of Eel is very low compared to other species: red mullet, whiting, bream, sardines, mullet and others. This is due firstly to the eating habits and also the lack of knowledge of this fish. Thus, the Eel is rather exported abroad especially to Italy of around 80 tons,¹⁰ where it is highly regarded by the population. Moreover, many scientific research work¹¹⁻¹⁴ conducted on this species, are more oriented towards biology and parasitological Eel.

In this sense, this research has undertaken to study the seasonal variation of some biochemical parameters (total lipids, proteins, carbohydrates, moisture and ash) and the composition of the European eel muscle fatty acids captured in level the freshwater (Tonga) lake and brackish water (El Mellah) lagoon North-East (Algeria) for better



nutritional evaluation of the Eel which is an important economic source for this region.

MATERIALS AND METHODS

Sites of Study

Two sites (Figure 1) were chosen for the capture of the species *Anguilla Anguilla* (silver Eel) namely Tonga lake (freshwater) and El Mellah lagoon (brackish water) which are located in the national park of El -Kala. The wetland complex of El Kala National Park, located at the north-eastern end of Algeria, includes lakes Tonga (36 ° 51.511'N; 8 ° 30.100'E) Oubeira (36 ° 50.695'N; 8 ° 23.272'E) and El Mellah lagoon (36 ° 53.565'N; 8 ° 19,560 'E).¹⁵

Biological Material

The biological sample used in this study, is the fresh muscle of *Anguilla Anguilla* (silver Eels).

The different samples of Eel weight ranging from 250 to 500g were captured in a random manner at the two sites (Figure 1). Eels are 15 in number (N = 15) by site and season (winter and spring). The different samples collected are sacrificed and stored in the freezer at - 80°C and then used to assay some biochemical parameters including the fatty acid composition.

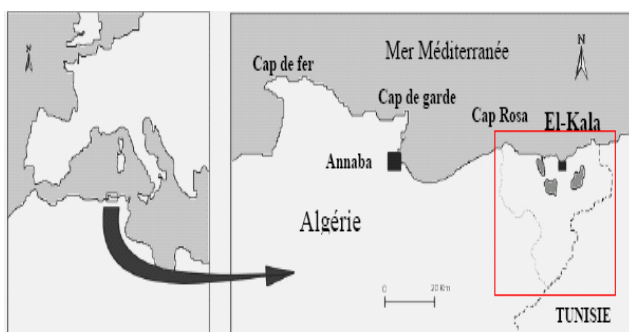


Figure 1: Red square show localization of sampling sites of European Eels. (North-East of Algeria).

Determination of Biochemical Parameters

Muscle from different samples (N = 15) of each site is collected, ground and homogenized. The total lipids are assayed by the method of Folch.¹⁶ The total soluble protein and total sugars were analyzed by the method of Bradford¹⁷ and Duchateau¹⁸ respectively. Moisture and

ash content are calculated according to AOAC¹⁹ and the results are expressed as a percentage.

Fatty Acid Composition

For fatty acid analysis, the fat content is extracted by the Soxhlet method (this method of total lipid extraction, using 5g of fresh muscle in an extraction solvent (hexane-acetone 4v/1v) of 300 ml for a time of 8h. Evaporation of oils is done by a rotary evaporator at 65°C. The resulting oil rate is also expressed as a percentage. After transesterification of fatty acids,^{20,21} ester methyl are injected into the gas chromatograph Agilent model HP 5890 equipped with a flame ionization detector and a column DB225 (20 m X 0.18 mm, 0.20 µm). The carrier gas used is hydrogen (1.0 ml/min); with an injection system in split mode (1: 60). The elution of the various fatty acids is done by programming temperatures of 50°C (2.7 min) to 180°C at 5°C/min to 230°C at 3°C/min. The temperature of the injection chamber and the detector is 250 to 250°C respectively. The qualitative analysis of different fatty acids on a chromatogram is given according to the retention time (Tr) from the standard (MIX37 Supelco). The quantitative analysis is expressed in relative percentage of fatty acids (% of fatty acids) by calculating the area of each peak.

Statistical Analysis

The "t" test of Student threshold at 5% was used for comparison of means for biochemical parameters and fatty acids in the flesh of the Eel between two season (winter-spring) and for the two sites (Tonga lake and lagoon EL Mellah). Analyses of variance of two factors were used to test the effect of the season on the increase or decrease of the contents of biochemical parameters and fatty acids. All statistical analysis were performed using the MINITAB 17 statistical software. The results are expressed as mean with a standard deviation standard error of the mean (mean ± D).

RESULTS AND DISCUSSION

Biochemical Parameters

The results of various biochemical parameters obtained are shown in Table 1.

Table 1: Seasonal variation of biochemical parameters of European Eel muscles from lake Tonga and El Mellah lagoon

Biochemical paramètres (%)	Tonga Winter	Tonga Spring	P	Mellah Winter	Mellah Spring	P
Total lipids	21,37±0,13	19,4±0,20	0,000	22,15±0,11	21,80±0,10	0,000
Total protein	17,20±0,06	23±0,178	0,000	15,70±0,05	21,84±0,73	0,000
Total Carbohydrates	1,27±0,06	1,93±0,05	0,000	1,04±0,05	1,55±0,07	0,000
Moisture	74,52±0,12	71,08±0,12	0,000	66,71±0,12	73,05±1,27	0,000
Ash	1,34±0,06	1,48±0,07	0,000	1,19±0,10	1,32±0,08	0,000

p ≤ 0,001= highly significant Student test; Mean ± D; n = 15 / Site

Table 2: Seasonal variation of fatty acids of European Eel muscles from Tonga Lake and El Mellah lagoon

Fatty Acids	(% of Fatty Acids)			
	Tonga Winter	Mellah Winter	Tonga Spring	Mellah Spring
C12:0	0,208±0,005	0,075±0,007	0,214±0,009	0,077±0,007
C13:0	0,108±0,004	/	0,117±0,006	/
C14:0	3,294±0,006	3,431±0,019	3,231±0,011	4,446±0,007
C14:0	0,647±0,030	0,265±0,007	0,206±0,005	0,068±0,006
C15:0	0,786±0,277	0,835±0,011	0,592±0,007	0,984±0,006
C16:0	0,302±0,006	0,204±0,005	0,214±0,011	0,208±0,007
C16:0	19,301±0,003	21,529±0,010	18,511±0,011	18,987±0,008
C16:0	0,732±0,011	0,771±0,009	0,675±0,008	0,773±0,011
C16 :1n-7	7,091±0,041	7,258±0,072	7,655±0,008	9,840±0,029
C16 :0	0,303±0,005	0,081±0,012	0,285±0,009	0,195±0,007
C16 :0	1,005±0,030	0,743±0,005	0,631±0,018	0,777±0,027
C16 :0	1,092±0,054	0,656±0,006	0,993±0,008	0,823±0,010
C17 :0	0,854±0,013	0,556±0,013	0,478±0,010	0,367±0,007
C17 :0	1,020±0,257	0,812±0,008	0,914±0,009	0,736±0,012
C 18 :0	4,727±0,005	5,770±0,024	4,966±0,007	4,352±0,143
C18 :1n-9	27,697±0,006	36,968±0,04	33,351±0,009	26,947±0,077
C18 :1n-9 (trans)	5,069±0,039	3,334±0,008	5,653±0,020	3,762±0,087
C18 : 2n-6	4,599±0,007	0,678±0,011	3,738±0,008	0,770±0,006
C18 : 3n-3	2,872±0,061	0,432±0,013	2,613±0,009	0,435±0,008
C20 :0	0,213±0,007	0,142±0,007	0,234±0,010	0,135±0,006
C20 :1n-9	0,581±0,012	1,958±0,016	1,079±0,010	1,193±0,024
C20 :2n-6	0,787±0,007	0,430±0,010	0,756±0,008	0,564±0,011
C22 :0	0,233±0,006	/	0,055±0,011	0,071±0,005
ΣSFA	34,825±0,325	35,87±0,036	32,27±0,028	33,000±0,136
ΣUFA	48,699±0,08	51,106±0,103	54,848±0,035	43,520±0,082
ΣMUFA	40,334±0,061	49,518±0,101	47,738±0,027	41,742±0,08
Σ (n-6)	5,386±0,009	1,109±0,017	4,495±0,007	1,335±0,016
Σ (n-3)	2,872±0,061	0,432±0,013	2,613±0,009	0,435±0,008
UFA/SFA	1,398±0,013	1,425±0,008	1,697±0,002	1,318±0,006
n-3/n-6	0,533±0,011	0,389±0,009	0,581±0,002	0,325±0,015

Mean ± D; n = 15 / site.

The examination of Table 1 shows that the total lipid levels are higher in the Eel El Mellah lagoon; El Mellah as compared to Tonga lake and that for two seasons (winter and spring) with a significant difference ($p \leq 0.001$). The peak values recorded are for the winter season. These lipids decrease from winter to spring indicates a highly significant seasonal variation ($p \leq 0.001$). This can be explained by the increase in temperature and salinity in the spring which degrades fat.²² These results in agreement with those of EL Oudiani.²³ The analysis of the seasonal variation of proteins, sugars and ash at the two sites showed also that the Eel from Lake Tonga is more

rich in nutrients than the El Mellah lagoon and rates significantly increase ($p \leq 0.001$) in spring. According to Lecomte–Finiger,²⁴ Eel feed mainly at night in warm period (spring and summer). Also the maximum food activities observed in the European Eel occur at temperatures between 25 and 26°C, which explain the high levels of these biochemical parameters in the spring. it was also noted that during the spring, the total sugar content of the Eel from Tonga lake and El Mellah lagoon are high while in fatty fish, carbohydrates are in the form of glycogen are generally low 1%.²⁵ This biochemical parameter gives an excellent organoleptic characteristic

and specific nutritional quality particularly in Eels captured at Tonga lake which records carbohydrate rate (1.93%) significantly ($p \leq 0.001$) higher than those of Eel Mellah lagoon with a rate is 1.55%.

Composition of Fatty Acids

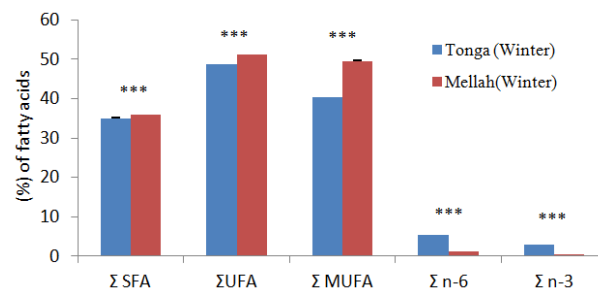
The different results of the study of the seasonal variation of the fatty acid composition of the lake European eel captured Tonga lake and El Mellah lagoon are presented in Table 2.

Qualitative analysis of the fatty acids profile showed that for two seasons (winter and spring), muscle Eel from Tonga lake and El Mellah lagoon contains 22 fatty acids ranging from C12 to C22, including 7 unsaturated fatty acids (Table 2). At the level of saturated fatty acids, we note that palmitic acid is the most dominant at the two sites and two seasons (winter and spring) up to a maximum of 21.52% for Eel El mellah observed in winter. For each site, palmitic acid decreases significantly ($p \leq 0.001$) from winter to spring. Also the rate of palmitic acid was significantly ($p \leq 0.001$) high in the lagoon El Mellah. The palmitic acid is indeed known as a key fatty acid metabolism in fish and it is generally not influenced by diet.²⁶ These findings are similar to other work on the European eel and carp.^{22,27} Among the identified unsaturated compounds. It was also observed the presence of monounsaturated fatty acids, C16 palmitoleate (n-7), oleic acid C18 (n-9), Methyl octadecenoate C18 (n-9) and C20 (n-9) cis-11-Eicosenoate. Oleic acid is the majority and the maximum (36.968%) was achieved in Eel El Mellah during the winter season with significant effect ($p \leq 0.001$). Monounsaturated fatty acids especially oleic acid amounts changes greatly depending on the species and feeding copepods.³ The analysis of these results also indicated the presence of polyunsaturated fatty acids such as linolenic acid C 18 (3n-3) omega 3, linoleic acid C18 (2n-6) and eicosadienoic acid C20 methyl (2n-6) omega 6. Essential fatty acids (linoleic and linolenic acid) play an important role in the synthesis of other polyunsaturated fatty acids. Moreover, the research work of Oudiani^{22,23} carried out on the muscle Eel Tunisia mentioned the presence of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are very important for the development and functioning of the brain and retina²⁸ which is not in line our report.

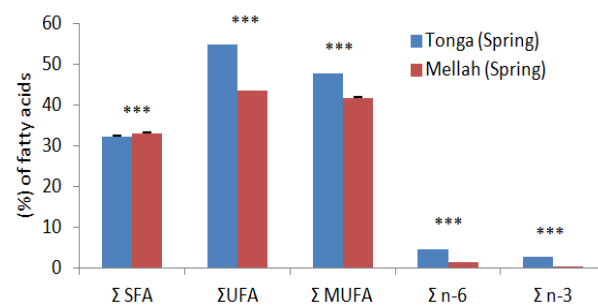
The Saturated Fatty Acids (SFA)

Table 2; Figures 2 (a, b); Figures 3 (a, b) summarize seasonal variation and spring percentage of saturated fatty acids (SFA) at Tonga lake and the lagoon El Mellah. The recorded rates is 34.82% in winter (Tonga) and a rate of 35.87% in winter (El Mellah). Statistical analysis showed that there is a statistically significant difference both in each site for two seasons between the two sites ($P \leq 0.001$). This result indicated that the season (winter) seems to influence the synthesis of these saturated fatty acids. Indeed, the relative abundance of the SFA could be

explained by their endogenous and food available at the lakes. However, according to Sargent,²⁹ saturated fatty acids are little influenced by the nature of fatty acids found in fish feed.

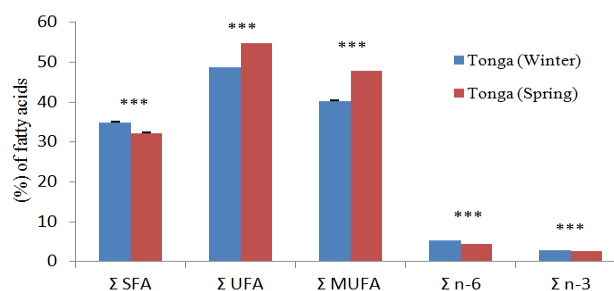


(a)

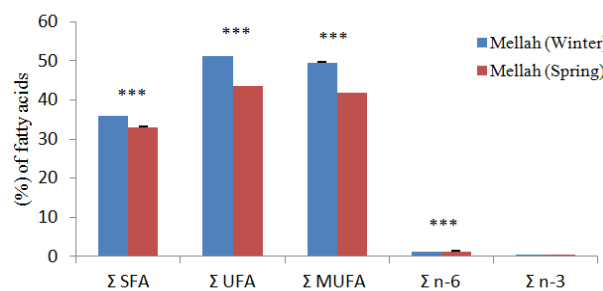


(b)

Figures 2: Fatty acids composition (%) Saturated (SFA), Unsaturated (UFA), Monounsaturated (MUFA), Σ n-6, Σ n-3 of Eel muscles from lake Tonga and the lagoon El Mellah: (a) Winter / (b) Spring. (Vertical Bars = \pm Standard Deviation. n=15, ***: $p \leq 0.001$).



(a)



(b)

Figures 3: Fatty acids composition (%) : Saturated (SFA), Unsaturated (UFA), Monounsaturated (MUFA), Σ n-6, Σ n-3 of Eel muscles from lake Tonga and the lagoon El Mellah: (a) Tonga Winter / Spring, (b) El Mellah Winter /

Spring. (Vertical Bars = \pm Standard Deviation. n=15, ***: $p \leq 0,001$).

The Unsaturated Fatty Acids (UFA)

Examining the results from Table 2, Figures 2 (a, b) and Figures 3 (a, b) obtained showed that the polyunsaturated fatty acid levels was height (54, 848%) in Eels from Tonga lake and that during the spring with a significant effect ($p \leq 0.001$). As for the winter season, the analysis shows that the highest rate (51.058%) was obtained in Eels El Mellah with a significant difference ($p \leq 0.001$). According to Lecomte–Finiger,²⁴ freshwater Eels prey consist mainly insect larvae, crustaceans, fish, but also rodents, by cons at sea Eel feeds on small fish and crustaceans. In the spring, Eels prepare their migration and adults do not feed and live on reserves accumulated during the yellow Eel stage. Furthermore, research Takeuchi³⁰ showed that increasing salinity induces enrichment in polyunsaturated fatty acids in the phospholipids of salmon gills. All these variations due to environmental factors are important in wild fish.

The Monounsaturated Fatty Acids (MUFA)

The results are shown in Table 2, Figures 2 (a, b); Figures 3 (a, b). MUFA exhibits sufficiently a high levels, the maximum (49.51%) was observed in the Eel El Mellah lagoon during the winter. whereas, in Eels from Tonga lake, the maximum (47.738%) is obtained during the spring with a high significant ($p \leq 0.001$). These results are similar to those obtained by other scientists.^{23,31} in the European Eel. Indeed, the monounsaturated fatty acids vary greatly in amounts depending on the species and feeding pattern. The most abundant are to 18; 20 and 22 carbons and especially oleic acid (18: 1 n-9) and cetoleic acid (22: 1 n-11) from power crustaceans and cold water fish.³

Omega Σ n-6 and Σ n-3

The study of the seasonal variation of the family of omega 6 and omega 3 (Table 2, Figures 2 (a, b); Figures 3 (a, b) shows that the level of lake Tonga, the maximum amount recorded in winter is $5,386 \pm 0,009$ % (omega 6) and $1,335 \pm 0,016$ % (omega 6) for the lagoon El Mellah) in spring with high significant differences ($p \leq 0.001$).

Omega 3 Tonga site record the maximum with 2,872% observed in winter, these rates decrease significantly ($p \leq 0,001$) from winter to spring, but in lake El Mellah, both seasons do not affect the rate of omega 3 with a non significant ($p > 0.05$).

This results seem somewhat similar to other research that claim that freshwater fish are relatively high in omega 6, while marine fish are relatively rich in omega3.²⁶ These differences in the nature of lipids to various salinities suitable fabrics have often been attributed to oils ingested in the diet^{32,33} and the ability to convert linoleic and linolenic acids in long chain.³⁴ Other research works showed that in the yellow Eel, there is a particular effect

of salinity that primarily affects the nature of the fatty acids of the fabric.³⁵

The UFA/SFA Ratio

The ratio obtained (Table 2) at the two sites vary between 1.318 and 1.697% and the maximum ratio (1.697%) was observed in the spring at Lake Tonga with significant effect ($p \leq 0,001$). These ratios exceed the minimum threshold of 0.45 recommended by the HMSO.³⁶ These results showed that muscle Eel from Tonga Lake and El Mellah lagoon are rich in unsaturated fatty acids with predominance in Eel Tonga. Thus these findings are relatively similar to other studies on European eel.^{23,31}

The n-3 /n-6 ratio is very important to evaluate oil fish nutritional value it is benefic for human health to consume sea food product because of their high (n-3) PUFA and low (n-6) PUFA contents. These reports (Table 2) vary between 0,325 (El Mellah spring) and 0.581% (Tonga spring) with highly significant effect ($p \leq 0.001$). Belgian recommendations³⁷ give a $\omega 6 / \omega 3$ ratio between 2 and 6. Thus, the review of the results show that the values obtained are 3.06 for the lagoon El Mellah and 2.56 for Tonga lake are in this range which shows the good quality of oil Eel captured in the spring. So, a dietary fish high ratio n-3 / n-6 would be beneficial for human health.³⁸

CONCLUSION

The study of the seasonal variation of some biochemical parameters and the fatty acids composition in the European Eel (silver Eel) Tonga lake (freshwater) and El Mellah lagoon (brackish water) in the wilaya El Tarf (Algeria) has elucidated these nutritional parameters. Thus, various investigations showed that qualitatively, the muscle of silver Eels captured at two sites contain the essential unsaturated fatty acids: linolenic acid (Omega 3) and linoleic acid (Omega 6). These fatty acids are not synthesized by humans, but they are the precursors of eicosapentaenoic acid (EPA) and docohexaénoïque (DHA), which play an important role in vascular diseases. Registered rates are high and vary according to season and seem to be the maximum in winter.

Moreover, the comparative study of two sites showed that the Eel from lake Tonga has a better organoleptic and nutritional quality than Eels from lake El Mellah.

These differences appear to be due to environmental factors, primarily the availability and diversity of food.

Acknowledgement: This work is dedicated to the memory of my father Professor DAIF LADJAMA who passed away on January 30th, 1999.

Thank you for everything and we think about you every day.

Many thanks to Professor ALI TAHAR for helping us in the choice of different statistical analysis adapted to our experimentation.



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Source of Support: Nil, Conflict of Interest: None.

