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Comparative study of the energy reserves of two populations of eels *Anguilla anguilla* (L. 1758) fished in the El Mellah Lagoon and Lake Tonga in northeastern Algeria

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ABSTRACT
This paper presents a comparative study of the energy reserves of two populations of eels *Anguilla anguilla* (L.1758) fished in the El Mellah Lagoon and Lake Tonga in northeastern Algeria. Results of the demographic structure of the population showed that the yellow eels predominated numerically in both plans of water. The monthly follow up of the (Kc) condition factor recorded no significant difference in the values of this index between the two stations. Compared to the extraction of metabolites, the protein content showed a significant increase at the end of autumn, which tended to decrease in winter. In addition, a significant increase was detected in the level of muscle lipids between the months, with higher levels observed in eels from El Mellah Lagoon. Hence, the eels fished at the site level proved to be particularly sensitive to spatio-temporal fluctuations in environmental factors.

INTRODUCTION

Once considered a harmful species, the European eel (*Anguilla anguilla*, L.1758) has been experiencing for more than 30 years a very significant decline throughout its range **(Bruslé, 1994).** It is now included in the Red List of Critically Endangered Species (UICN, France) and considered to be beyond its safe biological limits **(EIFAC, 2006)**. Thus, its presence provides good information on the physico-chemical quality of the environment and the accessibility of the habitat **(Feunteun, 2002).** Migration is one of the most visible and widespread phenomena in the animal world, particularly among insects, fish, whales and birds **(Alerstam *et al*., 2003).** Energy reserves are recognized as a key component necessary for migration. In eels, the energy storage characteristics are highly variable depending on the stage of the life cycle. The leptocephalus larva feeds mainly on sea snow **(Miller, 2003; Miller *et al*., 2013)** or planktonic organisms **(Riemann *et al*., 2010)**. Whereas the elver either feeds little on the aforementioned organisms or does not at all **(Bardonnet *et al*., 2005)**, and is therefore dependent on the reserves accumulated by the leptocephalus larva. During transoceanic migration, those reserves would also be catabolized to cover the energy expenditure necessary for swimming activities and salinity changes **(Baudrimont *et al*., 2006)**.

Studies have indicated that the energy reserves, accumulated by the yellow eel, cover 40% of the energy expenditure related to migration and 60% related to breeding **(Van Ginneken & Van Den Thillart, 2000)**. Its high percentage of lipids and its high place in the food web mean that it can dramatically accumulate lipophilic xenobiotic molecules during its long continental stay **(Bruslé, 1989)**. This would make it vulnerable to control the onset, duration, distance and physiological and behavioral adaptations of navigation **(Moore & Allen, 2002 ; Alerstam *et al*., 2003).**

This paper presents a comparative study of the overweight European eel *Anguilla anguilla* fished at two Ramsar sites, El Mellah Lagoon and Lake Tonga, at its yellow eel stage. Additionally, this study was conducted to quantify the lipid and protein energy reserves that are essential for breeding and migration to the Sargasso Sea, whose health is intimately linked to environmental factors.

MATERIALS AND METHODS

Study sites

Eels inhabiting the wetland complex of El Kala National Park, located in the northeastern tip of Algeria, including Lake Tonga (36°53'N; 8°31'E) and the El Mellah Lagoon (36°53'N; 8°13'E), were examined. Lake Tonga is a freshwater body of 2400 ha, which flows into the sea through the 1500 m long Wadi Messida. Whereas, the 850 ha long El Mellah Lagoon is a brackish water body fed by two main tributaries; Wadi Bouaroug and Wadi Mellah, and communicates with the sea through a 900 m long channel (Fig. 1).

Sampling

During September 2019 and February 2020, eighty fish, from each study site, were fished using fish traps and quickly transported to the marine science laboratory. The eels were still alive, kept in tanks containing water from their capture site. Upon arrival at the laboratory, the eels were cold-slaughtered and dried with filter paper (without anesthesia). Remarkably, the use of an anesthetic has a biochemical impact on the production of hormones of the endocrine system **(Rossi & Villani, 1980)**. The eels were then measured, weighed and classified by size class. Muscle fragments (100 mg) were collected and stored at -20°C until the time of dosing.

Morpho-metric parameters

The different dimensional measurements were grouped into three size classes (10 cm class) of eels for each study site. This allowed to determine the most frequent sizes during the sampling phase.

Fig. 1: Geographical location of the wetlands of the Park National of El-Kala **(Benyacoub & Chabi, 2000)(Google modified)**

Condition coefficient (Kc) variation

The condition coefficient (Kc) is defined by the ratio between the weight and the size of the fish. It is given by the formula of **Postel (1973)**:

$$Kc = 100 * W/L^3 \quad (1)$$

where, ***W*** is the weight in grams, and ***L*** is the length in centimeters.

This condition or overweight coefficient (general state of health of the fish) allows to assess the morphological variations in relation to the physiological state of the individuals and the ecological conditions (temperature, salinity, etc.) to which the individuals are subjected.

Extraction of metabolites

Protein dosage

The protein content in the supernatant (S9) of the flesh of fish muscle was determined according to the method of **Bradford (1976)**. Bovine serum albumin was used as the standard and Coomassie Brilliant Blue as the reagent. The protein contents are expressed in mg/ml S9.

Lipid dosage

Total lipids were determined according to the method of **Goldsworthy *et al*. (1972)**, using vanillin as the reagent. Lipids form a pink complex with sulphuric acid in the presence of vanillin and ortho-phosphoric acid, detectable at 530nm. Lipid levels are expressed in gr/gr of fresh tissue.

Statistical analysis

One-factor analysis of variance (ANOVA) and Pearson correlation coefficient were performed on all the results obtained during this work to determine whether there were significant differences between the values of different samples. The comparison of means and the search for the significant effect were carried out using the Fisher and Tukey test. These studies were conducted using Minitab 13.31 for Windows (X, 2000).
Inter site and inter-season comparisons were made using the Kruskal-Wallis test. In addition, the PCA principal component analysis was also used to compare all variables in the study. Furthermore, statistical analyzes were performed with R Software (3.5.2. version).

RESULTS

Morpho-metric parameters

The different dimensional measurements were grouped into three size classes (10 cm class) for each study site to better assess the results and to compare the measurements. The analysis of the histograms indicated that the eels studied can be subdivided into 3 groups. The first group caught consisted of eels smaller than 39 cm; this group represented 47.50% of the total catch in Lake Tonga and 38.75% for El Mellah Lagoon (Fig. 2). The second group was comprised of eels between 39 and 59 cm; this group of medium size individuals represented 41.25% in El Mellah Lagoon and 35% in Lake Tonga. The third group was characterised by relatively large individuals; longer than 59 cm, with a percentage of 20% in El Mellah Lagoon and 17.50% in Lake Tonga.

Condition coefficient (Kc) variation

Fig.(3A) shows the variation in the condition coefficients of the different eels caught in the two bodies of water. For eels from Lake Tonga, the Kc coefficient varied between 0.13 and 0.18 with an average of 0.16 ± 0.02. While those from El Mellah Lagoon, Kc varied between 0.14 and 0.19 with a mean of 0.18 ± 0.01. The mean Kc was almost the same in both populations. However, the calculation of the Seasonal Condition Index indicate that the highest values are found in autumn for Lake Tonga eels and in autumn and winter for specimens collected from El Mellah Lagoon (Fig. 3B). The results of the index as a function of size classes indicate that the index varies significantly with size.

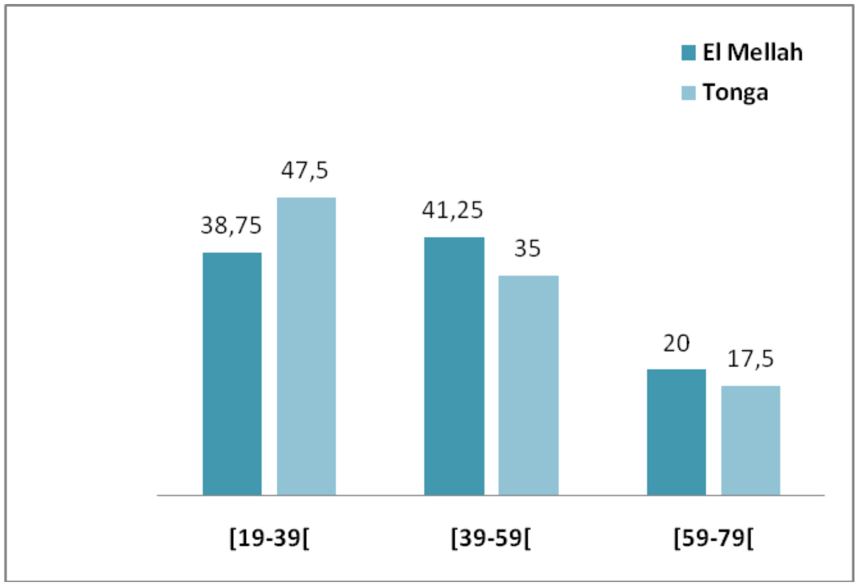

Fig. 2: Percentage of eel populations by size group in the waters of Tonga lake and El-Mellah lagoon.

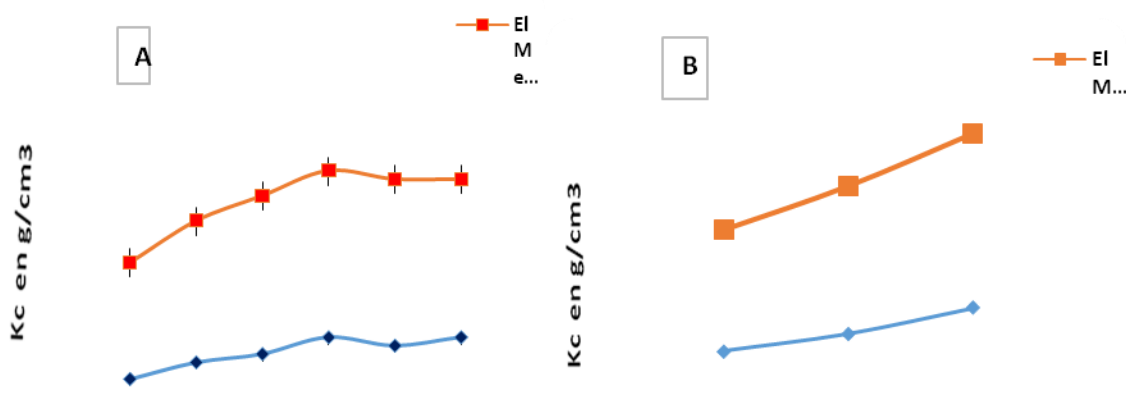

Fig. 3: Monthly evolution of the condition index (Kc) in *A. anguilla* according to the fishing period (A) and size classes (B) in the waters of Tonga lake and El-Mellah lagoon.

Total protein content

Fig.(4A) shows the protein assay evaluated at the eel muscle level, expressed in (gr/gr of fresh tissue). The results indicate monthly variations, marked by significant increases (p=0.019) in protein at the end of the fall season, which tends to decrease in winter, in eels from both water bodies.

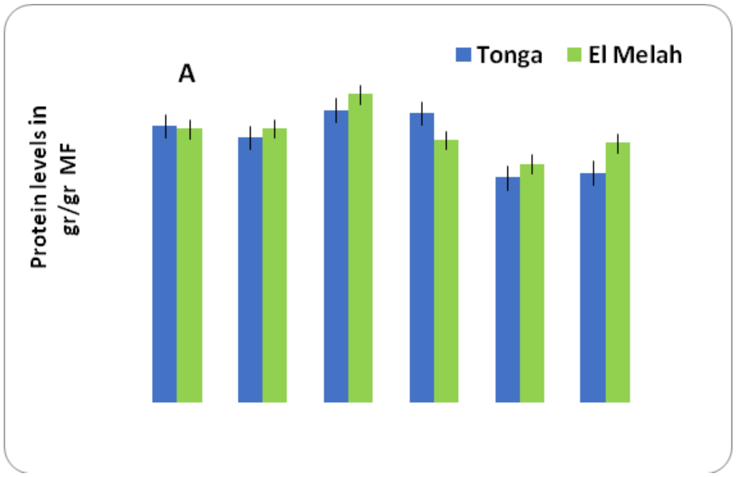

Fig. 4: Variations of protein (A) contents during the eel fishing period in Tonga lake and El Mellah lagoon (MF: fresh muscle).

Total lipid contents

Lipid concentrations (Fig. 5B), measured in muscle, increase significantly between months with higher levels observed in eels from El Mellah Lagoon. The results of the application of the Fisher test (single-factor ANOVA), reveal the existence of highly significant differences between months in lipids (p= 0.004).

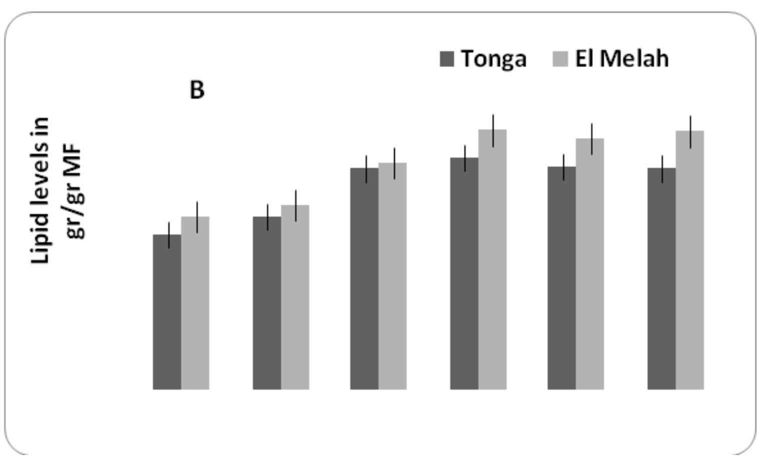

Fig. 5: Variations of lipid (B) contents during the eel fishing period in Tonga lake and El Mellah lagoon (MF: fresh muscle).

Multivariate statistical analysis: the analysis in main PCA component

Table (1) shows lists the results of the application of the one-factor ANOVA test. The results reveal the existence of highly significant differences between months for lipids (p=0.004) and significant differences for proteins (p=0.019). However, no significant difference was observed for the two variables (Kc). Moreover,the PCA analysis allowed to visualize the relationship between condition index, protein and lipids in fish by site and season. Indeed, this analysis represents 99.99% of the total variance with respectively 56.71% for axis 1 (F1) and 43.28% for axis 2 (F2) (Fig. 6).

Table 1: Inter-stations and inter-months comparison of lipids, proteins and Kc

Variables	**Factors**			
Anova one factor				
Months (df=5)			**Sites (df=1)**	
	P value	**Observation**	**P value**	**Observation**
Lipid	0.004	**	0.396	**ns**
Protein	0.019	*	0.683	**ns**
Kc	0.051	**ns**	0.156	**ns**
NB: * (P ≤0,05),	** (P ≤0,01),	*** (P ≤0,001),	ns (P >0,05)	

The 1st PCA axis (Fig. 6) alone explains 56.71% of the total variation. Thus, it is positively correlated with the following variables: Kc (r = 0.75) and lipids (r = 0.83). This first axis separates the two months, December and January (on the positive side of the axis), from the two other months September and October (on the negative side of the axis). Thus, it can be noted that the fish (of the present study) in winter (December and January) are characterized mainly by very high lipid levels and a very high Kc growth index. Furthermore, this axis reflects a clear structuring concerning the temporal variation, mainly with respect to the parameters: lipids and Kc.

With respect to the 2nd axis (Fig. 7), it alone explains 43.28% of the total variation. This axis clearly expresses the existence of spatial structuring between El Mellah Lagoon on the positive side of the axis and Lake Tonga on the negative side of the axis. This structuring could be explained by the positive correlation of Kc (r = 0.65). Thus, the fish in El Mellah Lagoon are characterized by a high Kc health index, compared to the fish in Lake Tonga. It should be noted that this axis also reveals a temporal structuring where the month of February is slightly separated (positive side of the axis).

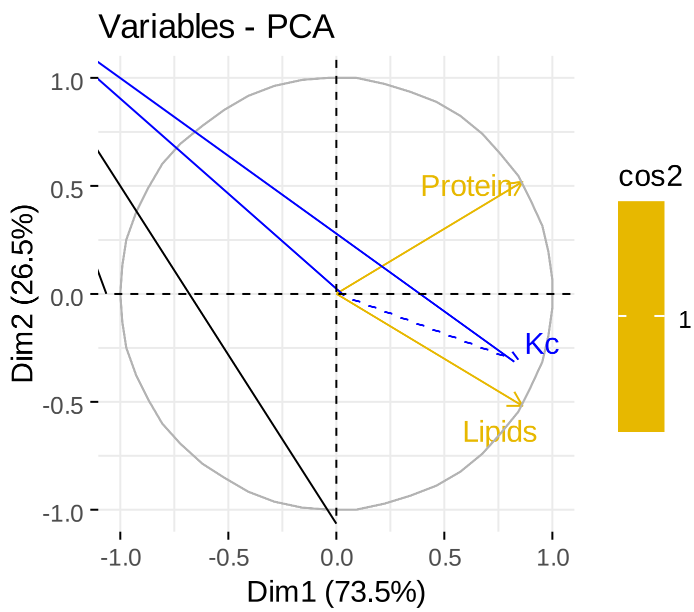

Fig. 6: PCA correlation circle for Axis 1 and Axis 2.

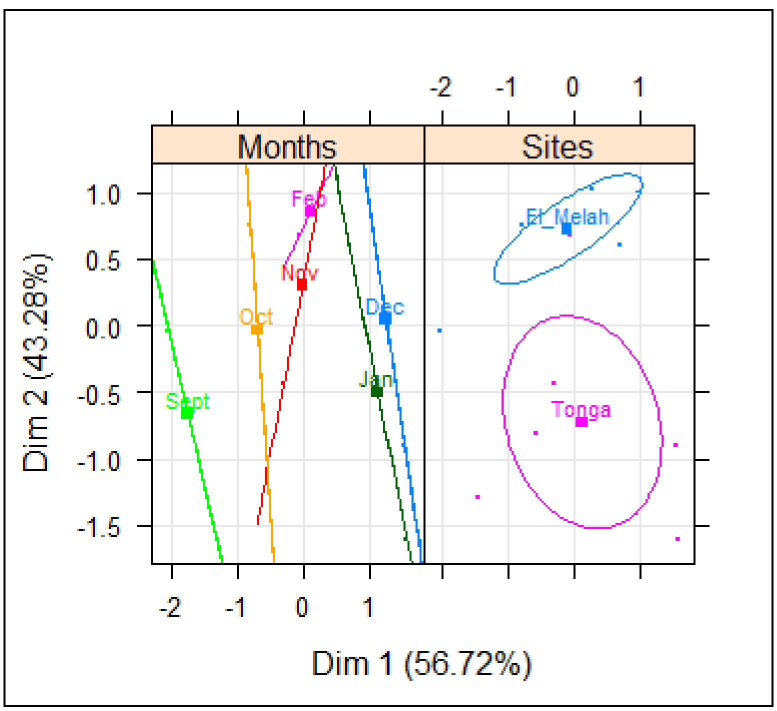

Fig. 7: PCA Ellipse for Axis 1 and Axis 2.

DISCUSSION

The demographic structure of the population showed that yellow eels predominated numerically (47.50% in Lake Tonga and 38.75% in El Mellah Lagoon) in the size class below 39 cm. Similar results were observed by **Ladjama (2016)**, who reported that 75% of the eels, caught in the two water bodies, were between 37 and 57 cm in size. These results are also in good agreement with those of **Zemouchi (2012)**, confirming the predominance of yellow eels (79.42%) in the size limit classes between 30 and 60 cm. In addition, **Tahri (2016)** reported that the sizes of eels caught from Lake Oubeira were between 30 and 89.5 cm during the 1st and 2nd seasons of the year. Moreover, the previous author noticed the absence of eels smaller than 30 cm, and that the dominant class was represented by individuals between 70-80 cm in size.

In the same body of water, **Boudjadi (2010)** reported eel sizes between 31 cm and 89.4 cm. On the other hand, in eels from the Mafragh estuary, **Mardja (2009)** recorded sizes ranging from 24 cm to 74 cm. In Morocco, **El Hilali (2007)** and **Wariaghli (2013),** noted that the population of the Sebou and Loukous estuary is made up of small individuals with average sizes between 20 and 40 cm. This indicates that the eel population dynamics change a lot depending on environmental factors. Notably, this distinction of eel size classes is based on a relevant distribution associated with different phases of the species' life cycle characterized by different ecology and behavior, where the yellow eel represents the main stage of the species' growth that occurs after the migration phase **(Canal *et al*., 2013).**

In Tunisia, eels caught in Ghar El Melh Lagoon are mainly represented by medium-sized individuals (30-50 cm) **(Dhaouadi *et al*., 2014).** According to **Moriarty (1974)**, catches of yellow eels in this size category are larger in lagoon and coastal marine ecosystems (lagoons, estuaries) than in freshwater environments where catches are relatively limited. A heterogeneous distribution of eels of different size classes, according to bathymetry, has also been observed, with an absence of small individuals in deep habitats in *A. anguilla*, *A. australis* and *A. dieffenbachia* **(Baisez *et al*., 2000; Baisez, 2001).** According to **Oliveira (2008),** gender segregation is closely related to size, which explains the presence of young eels along the banks.

In the case of Lake Tonga, the hypothesis of a segregation of eel size classes according to the bathymetry of the habitats is probable, hence the importance of catches in shallow depths all along the periphery of this ecosystem. However, it would appear that medium and large size class eels have a higher percentage in El Mellah Lagoon than in Lake Tonga. In fact, fishing and poaching at all stages are frequent in Lake Tonga and more or less respected in the El Mellah Lagoon. In addition, the draining of marshes, especially estuarine marshes, would contribute to modify the spatio-temporal distribution of eels (**Gosset *et al.*, 2002).**

Considerably, the condition factor is an indicator of the quality of fish population growth. It describes the quality of growth according to environmental conditions. For eels, this index is 0.16 by good standards **(Bauchot & Bauchot, 1978).** In eels, overweight varies according to developmental stages. Thus, several authors have shown that the value of the coefficient (Kc) increases with growth, and thus as a function of size and weight has been elaborated (**El-Hilali, 1998**). **Affandi (1986)** highlighted that (Kc) may show small discrepancies in relation to the trophic status of the individual. Indeed, overweight depends on several ecological (environment, season, quantity of food) (**Mallawa, 1987; El-Hilali, 1992**) and physiological **(Rossi & Villani, 1980)** factors that influence the nutritional status and hence the health of the fish. Furthemore, **El Hilali (2007)** reported that the value of (Kc) in European eel followed growth in size and weight more than the increase in age. In the present study, the condition factor (Kc) was almost the same in both sites. Its averages were 0.16 ± 0.02 in Lake Tonga and 0.18 ± 0.01 in El Mellah Lagoon.

According to **Ladjama (2016)**, eels from Lake Tonga and El Mellah Lagoon present their best condition of overweight in autumn and winter. In comparison, the current findings are similar to those of **Wariaghli (2013)** in Morocco, who noted that the coefficient (Kc) was almost the same in the two populations of Loukkos and Sebou with an average of (0.17±0.01 and 0.18±0.02) respectively. In Lake Oubeira, **Mardja (2016)** noted, according to the values of the condition coefficient obtained, that the eels caught in this body of water had an important energetic potential, with fairly high values (1st season: Kc between 1.69 and 1.89; 2nd season: Kcbetween 1.78 and 2.26). The author reported that the condition coefficient was positively correlated with the size and weight of the eels caught. However, **Boudjadi (2010)** reported the existence of a weak relationship of the condition coefficient (Kc) with the size, weight and even age of eels in the Mafrag (freshwater). Additionally, the same author highlighted that the condition coefficient was relatively high in small and large eels. Nevertheless, it was the oldest eels that recorded the highest values of the condition coefficient. This is due to the fact that the yellow eel spends its entire growth phase in the freshwater of lake rivers or in coastal environments for decades.Then it leaves the continental or estuarine environment when it accumulates sufficient energy reserves, and becomes sexually mature to reproduce in the sea **(Ranson, 2003).**

The eel represents not only a product of high socio-economic value but also a food of high nutritional value due to its richness in essential nutrients (proteins, lipids, vitamins, minerals, etc.). Those energy products are present in marine organisms and are controlled by various biological factors (growth, breeding, etc.) as well as numerous environmental factors (temperature, pollution, etc.), which determine the quality and availability of the food present in the environment (**Borresen, 1992**). As eels are long migrating fish, before reaching specific spawning sites, they use proteins in addition to lipids for energy, thus consuming their reserves. This would lead to a general reduction in the physical condition of the fish during migration. It is worthy to consider that the lipid fraction is the compound that undergoes the most variation, often seasonal, since it is related to the availability of food. Therefore, the present findings showed significant variations between months during the study.

The PCA and the Tukey test clearly indicated highly significant differences between months. Indeed, the period of the present study corresponds to the period of intense feeding, which would be marked by increases in lipid and protein levels. During the growth phase, yellow eels accumulate lipids in the muscle and liver, mainly in the form of triglycerides **(Lewande *et al.*, 1974; Dave *et al.*, 1975)**, and proteins for swim-related physical activities. In migratory fish in general, this accumulation of reserves is essential for transoceanic migration and gonad maturation **(Van Ginneken & Van Den Thillart, 2000)**.

A significant increase in muscle and protein lipid levels was recorded between months, with higher levels observed in eels from El Mellah Lagoon. Moreover, the results are consistent with those reported by **Ladjama (2016**), where the highest total lipid levels were recorded in El Malleh Lagoon eels with a maximum level obtained in winter. Markedly, the energy cost allocated to swimming Algerian eels would logically be lower, allowing the surplus to be used for gonad maturation and breeding during this reserve accumulation period. **Larsson *et al*., (1990)** attributed the observed differences in lipid levels between eel populations to environmental factors such as temperature, food availability (interspecific competition) and pollution. **Pierron *et al*., (2008)** stated that Cd causes an increase in lipolysis at the genetic and enzymatic level, which alters lipid storage in yellow eel muscle. Several authors have described the energy requirements of European eel broodstock for migration and breeding.

CONCLUSION

This study compared the overweight European eel *Anguilla anguilla*, fished at two Ramsar sites in northeastern Algeria, the El Mellah Lagoon and Lake Tonga, at its yellow eel stage. In addition, this study aimed to quantify the lipid and protein energy reserves that are essential for breeding and migration to the Sargasso Sea, whose health is intimately linked to environmental factors. The results showed that eels fished at the site level were particularly sensitive to spatio-temporal fluctuations of environmental factors, and consequently adjust their metabolism according to energy demands on the one hand and variations in the physicochemical parameters of the environment on the other. Indeed, these particularities mean that this species can accumulate significant quantities of xenobiotics during its long continental stay. Thus meeting the criteria of a good biomonitor and constituting a good indicator of the quality of the environment in which it lives.

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