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DETERMINATION OF THE MEAT BIOCHEMISTRY OF PONTIC SHAD *Alosa immaculata* (Bennett, 1835) DURING MIGRATION

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Abstract

The biochemical analysis of fish tissue is considered a dependable indicator of the fish`s quality, nutritional content, physiological condition, and environmental habitat. Alosa immaculata (Bennett, 1835) is a migratory fish species with great ecological and economic importance, which undergoes a remarkable journey during its migration. As A. immaculata embarks on its migration, the dynamic biochemical changes in its meat composition offer valuable information that contributes to a comprehensive understanding of the species` adaptation to the challenges posed by migration. In this context, this study aimed to investigate the meat biochemistry of Pontic Shad during the period of migration from three different points: Station 1 - Saint Gheorghe Branch, Station 2 - Sulina Branch, and Station 3 – Brăila, km 169 and km 197 of the Danube River. Significant differences (p<0.05) were recorded in the level of water, lipid, and ash, while no differences were recorded in the level of proteins (p>0.05). Notably, the lipid content in the meat samples from the Sulina exhibited the highest percentage, while the protein content in the samples from the St. Gheorghe arm surpassed that of the Sulina and Brăila, indicating differences in nutritional profiles that could be attributed to physiological adaptations during migration.

***Key words**: Danube, fish, lipids, proteins, reproduction migration.*

INTRODUCTION

The consumption of fish has attracted considerable attention owing to its wide range of health benefits and nutritional value. Well-known for its easily digestible protein of high biological value (De Smet, 2012; Mocanu et al., 2022), low saturated fat, and abundance of essential omega-3 fatty acids (Zuraini et al., 2006), fish presents an appealing dietary option to enhance overall well-being and mitigate the risk of numerous chronic illnesses (Raatz & Bibus, 2016; Li et al., 2020; Maulu et al., 2021).
Alosa immaculata belongs to the pelagic *Clupeidae* family. It is an anadromous fish, which it lives in winter scattered in the southern regions of the Black Sea, along the Bulgarian coast north of Burgas, at depths greater than 40 m (Niculescu-Duvăz, 1965). It is a commercially important species in Romania, with high-quality protein, omega-3 fatty acids, vitamins, and minerals (Năvodaru, 1997; Mocanu et al., 2022; Savin et al., 2020), essential for human overall health and well-being.
The proximate biochemical composition of a species serves as a crucial indicator for evaluating its nutritional and edible worth about energy units when compared with other species. Fluctuations in the biochemical composition of fish flesh can also be observed within the same species, influenced by factors such as the fishing location, season (Vollenweider et al., 2011), age (Breck, 2014), sex and reproductive condition of the individual (Yousaf et al., 2011). Also, the spawning cycle and food availability emerge as primary determinants contributing to such variations (Love et al., 1980).

The migration pattern of Pontic shad involves extensive journeys from the southern regions to the northern extents along the coastline of the Black Sea in Bulgaria and Romania, culminating at the mouths of the Danube River within Romania. This migratory behaviour reflects the species' adaptation to changing environmental conditions and resource availability throughout its life cycle. Knowing the meat biochemistry of Pontic shad during migration can offer valuable insights into the physiological changes occurring within this species as it undertakes its migratory journey.

In this context, this paper aimed to analyse the biochemical composition and the nutritional condition of Pontic shad during migration of reproduction

MATERIALS AND METHODS

Sample collection. *Alosa immaculata* specimens were collected by scientific fishing from Station 1 - Saint Gheorghe Branch, Station 2 - Sulina Branch, and Station 3 - Brăila (km 169 and km 197 of the Danube River) (Figure 1).

Figure 1. Sampling area of the study (source: https://www.google.com/maps)

All the samples were collected in April 2023. The fish samples (n = 20 fish/each station) were then transported to the laboratory in an icebox with a fish/ice ratio of 1:2, for further analysis. Before collecting the samples, all fish were weighed (g) and measured (cm). Following morphometric measurements, a clean stainless-steel knife dissected the fish. To determine the somatic indices, such as the viscerosomatic, hepatosomatic and gonadosomatic indices, fish viscera and liver were weighed:

Hepatosomatic index (HSI, %) = [liver weight (g)/body weight (g)] × 100;

Viscerosomatic index (VSI, %) = [viscera weight (g)/body weight (g)] × 100;

Gonadosomatic index (GSI, %) = [gonads weight (g)/body weight (g)]×100;

Proximate composition of fish. The biochemical composition of the meat was assessed through conventional analytical techniques (AOAC, 1990). Subsequently, solely the muscle tissue was homogenized using a blender and utilized for subsequent analyses.

To determine the protein content the Kjeldahl method was employed, involving the assessment of nitrogen content, which was then converted into protein equivalent using a multiplication factor of 6.25.

The lipid content (%) was measured using the Soxhlet method with an automated Soxtherm system. For ash content (%), the samples were incinerated in an electric oven at 550 ±1.0 °C for 4 hours, followed by weighing the sample crucibles at room temperature. Moisture content was determined by drying the samples at 105 ± 2°C until a constant mass was achieved.

Fulton coefficient. To estimate changes in the nutritional condition of fish the Fulton coefficient (K) was calculated, according to Froese (2006): $K = W \times 100/Ls^3$, where Ls- standard length of fish (cm), W= fish weight (g).

Statistical Analysis. The results of the biochemical composition and the somatic indices are presented as mean ± standard deviation. To evaluate differences between the biochemical parameters of fish meat, the one-way ANOVA test was employed, with significance established at $p<0.05$. Statistical computations were conducted using SPSS 26.0 for Windows (SPSS, Chicago, IL, USA), and Microsoft Office 2019.

RESULTS AND DISCUSSIONS

Biometric measurements of fish samples taken in analysis are presented in Table 1.

Table 1. Biometric measurements of fish samples

Sample Stations	Mean W (g)	Mean Lt (cm)	Mean Ls (cm)	Mean Lf (cm)	Mean H (cm)	Fulton coefficient
Saint Gheorghe Branch	243.01± 4.60*	30.13±1.13*	27.38±1.07*	26.24±1.07*	6.21±0.34*	1.29±0.32*
Sulina Branch	261.54±46.34*	31.07±1.51*	27.02±1.35*	27.85±1.36*	6.52±0.57*	1.31±0.10*
Brăila (km 169-197 of the Danube River)	213.44±56.77**	29.93±2.53*	25.73±2.30**	28.24±1.21**	5.01±0.49**	1.20±0.11**

Note: N- number of specimens; Mean W- mean weight (g); Mean Lt- mean total weight (cm); Mean Ls- mean standard length (cm); Mean Lf – mean fork length (cm); Mean H- body depth (cm). *- no significant differences between the somatic measurements: ** - significant differences between the somatic measurements

Significant higher ($p<0.05$) weights were registered for fish provided from the station's Saint Gheorghe Branch and Sulina Branch, while at Brăila station, fish weight was significantly lower. Regarding the total fish length (Lt), Standard length (Ls), Fork length (Lf), and body depth (H), significantly lower values were recorded for the fish caught at Brăila (km 169-197 of the Danube River). Concerning the Fulton coefficient, there is a noticeable decrease observed as the migration progresses towards the spawning and egg-laying areas along the Brăila stretch (located between km 169-197 of the Danube River). Specifically, the Fulton coefficient shows values of 1.29 ± 0.32 and 1.31 ± 0.10 at the Saint Gheorghe Branch and Sulina Branch, respectively. At the Brăila station, there is a significant decrease in the Fulton coefficient, with a value of 1.20 ± 0.11. A similar study carried out by Savin et al., 2020 supports these findings, indicating higher Fulton coefficient values at the beginning of the migration, particularly at the St. Gheorghe Branch (1.28), and the lowest value (1.13) at kilometer 197 on the Danube River (Chiscani), while at km 103 (Isaccea village), the recorded value of Fulton was 1.15. Generally, when the Fulton coefficient values exceed or are equal to 1, it indicates a favourable condition within fish populations. In the case of Pontic shad, the Fulton coefficient demonstrates a decreasing trend in correlation with the migration distance to the Danube River and the reproductive process. This decline is attributed to the loss of biomass sustained as energy expenditure for both migration and spawning, as highlighted by Năvodaru (1997).

Figures 1-4 present the biochemical composition of the *Alosa immaculata* muscle. Significant differences ($p<0.05$) were recorded in the level of water, lipid, and ash, while no significant differences were recorded in the content of proteins ($p>0.05$).

The moisture content registered the highest value at Brăila station (66.16 ± 2.44 %). At the Sulina Branch (58.88 ± 0.65 %) and Saint Gheorge Branch (58.59 ± 0.72 %) the water content was significantly lower ($p<0.05$).

No significant changes ($p>0.05$) were observed in the protein content among the sampled fish from the three stations. Thus, the protein content recorded a value of 16.95 ± 0.26 % at Sulina Station, 17.42 ± 1.10 % at St. Gheorge Station, respectively of 16.89 ± 1.30 % at Brăila Station. Regarding the lipid content in Pontic shad meat, a significant decrease ($p<0.05$) was recorded at the Braila Station (15.61 ± 2.39 %), in comparation with the Sulina (22.17 ± 1.73 %) and St. Gheorghe (21.35 ± 2.10 %).

The ash content registered significant differences between the selected stations, with higher values at St. Gheorghe station (1.31 ±0.12 %).

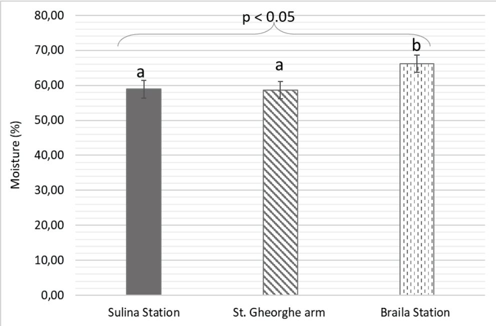

Figure 1. Moisture content of *Alosa immaculata*

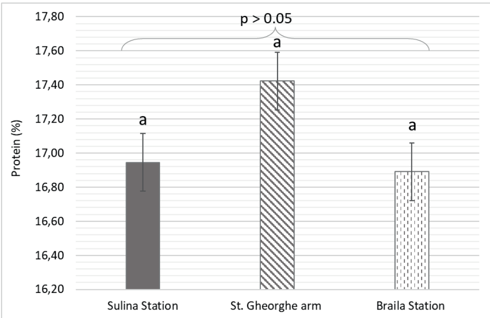

Figure 2. Protein content of *Alosa immaculata*

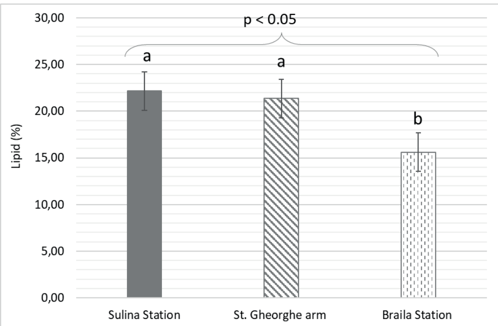

Figure 3. Lipid content of *Alosa immaculata*

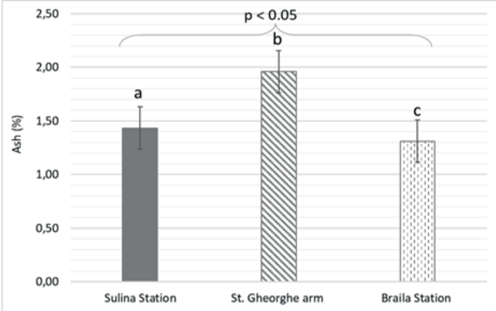

Figure 4. Ash content of *Alosa immaculata*

In general, fish meat exhibits high moisture content, typically ranging between 60-80%, with protein content falling within the range of 15-26 % and fat content ranging from 2-13% (Pearson & Cox, 1976). Notably, *Alosa immaculata* is distinguished among fish species for its remarkable lipid content, which varies between 15.12% and 25.8% (Savin et al., 2020; Ionescu et al., 2006). According to Ionescu et al., 2006, the Pontic shad has a proximate composition of 58.8% water, 25.8% lipids, and 14.2% protein. However, the lipid composition of fish meat is subject to various influencing factors, including species, age, diet, size, and seasonal fluctuations (Všetičková et al., 2020; Popa et al., 2022).

In Romania, for spawning migration, *Alosa immaculata* covers long distances, migrating from the Black Sea to the Danube River. First, it enters from the Black Sea to the Saint Gheorghe Branch, Chilia Branch, and Sulina Branch. After covering a considerable distance, it arrives at Brăila (km 169 - 197 of the Danube River), but the migration continues to the Iron Gate II. Throughout this migration process, numerous physiological and metabolic changes occur. Thus, the lower lipid content from the Brăila station can be explained by consumption of the lipid reserves during the reproductive migration. Generally, the lipid content serves as the primary energy reserve in anadromous fish and plays a critical role in their survival, migration, and reproductive success (Bayse et al., 2018).

Moving upstream in the Danube River requires a significant amount of energy to overcome the water current and for the maturation of the gonads, especially considering that the Pontic shad does not feed. This aspect entails the consumption of nutrients accumulated during feeding, leading to the loss of individuals' body biomass and lipid reserves.

This aspect is further highlighted by the values of hepatosomatic indices. The observed decrease in hepatosomatic indices at Brăila Station underscores the significant energy expenditure required for upstream migration and gonad maturation of *Alosa immaculata* during its spawning migration in the Danube River. This depletion of hepatic reserves emphasizes the critical role of lipid metabolism in facilitating the physiological adaptations necessary for successful reproduction and migration in anadromous fish species. (Table 2).

On the other hand, the increase in viscerosomatic and gonadosomatic indices at Brăila is mainly influenced by the intensification of gonad maturation processes during the reproductive migration of *Alosa immaculata* in the Danube River.

Table 2. Viscerosomatic and hepatosomatic indices of *Alosa immaculata*

Sampling stations	VSI (%)	HSI (%)	GSI (%)	N
Saint George Branch	5.10± 0.66*	2.02 ± 0.47*	2.12±1.14*	20
Sulina Branch	4.76±0.37*	2.14 ± 0.64*	2.37 ±1.14*	20
Braila	9.53±0.16**	1.73± 0.72**	4.51±1.14**	20

Note: VSI - Viscerosomatic index; HIS – Hepatosomatic index; N = number of fish taken in analysis; * - no significant changes were recorded between the stations ($p<0.05$); ** - significant changes were recorded between the stations ($p>0.05$).

CONCLUSIONS

In conclusion, the composition of fish meat, particularly the lipid content, varies significantly between the selected stations. *Alosa immaculata*, known for its high lipid content, undergoes physiological and metabolic changes during its spawning migration in the Danube River, leading to the depletion of lipid reserves. The observed decrease in lipid content at the Brăila station indicates the energy expenditure required for upstream migration and gonad maturation, further supported by the decline in hepatosomatic indices.

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