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ORIGINAL RESEARCH PAPER

STUDIES ON THE NUTRIENT CONTENT OF SPECIES *Mytilus galloprovincialis* OF THE BLACK SEA♦

Rodica Sirbu[1]*, Anamaria Bechir[2], Ticuţa Negreanu-Pîrjol[1], Constanta Sava[1], Bogdan Negreanu-Pîrjol[1], Tania Zaharia[3], Cornel Ursache[3], Ramona M. Stoicescu[1]

[1] *"Ovidius" University of Constanţa, Faculty of Pharmacy, Department of Chemistry, University st. 1, Constanţa, Romania*
[2] *"Titu Maiorescu" University of Bucharest, Gh. Patrascu st. 67A, Sector 3, 031593, Bucharest, Romania*
[3] *"Grigore Antipa" National Institute for Marine Research and Development, Mamaia blvd. 300, Constanţa, Romania*

*Corresponding author: sirbu_27@yahoo.com

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Abstract: It is well known that, among the marine organisms, mollusks are highly appreciated in many European, Asian and North American countries. In the Romanian area of the Black Sea, mussel *Mytilus galloprovincialis* can be found in deep areas which form a belt around the sea on the continental platform. There are significant variations of the main environmental factors which directly influence the physiological behavior of the organisms and the accumulation of the biochemical components with nutritional value. This paper correlates the study of the dynamics of the main biochemical compounds with the environmental factors and the annual ontogenetic phases of the organisms. There are also presented comparative studies concerning the rock mollusks and mollusks of deep from the Black Sea. It was found variations in quantitative results for biochemical composition. Cases are due to seasonal variations of physical-chemical conditions of seawater.

Keywords: *Black Sea, marine, mussels, Mytilus galloprovincialis, nutrients*

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INTRODUCTION

Midia - *Mytilus galloprovincialis* - according to the classification order system introduced by Piveteanu and still using the nomenclature proposed by Theile for determinations at subordinate level and super families, is a bivalve lamelibranhiatea mollusc belonging to: - fillum. Mollusca - Ord. DYSODONTA - area MYTILACEA - fam. MYTILIDAE - gen. Mytilus [1, 2].

Bivalve species representative of the genus Mytilus, mussels are widespread in the seas and oceans. They can be found from the tropics to polar seas, and in terms of fixed horizon where mussels are present in the water until 10 to 15 m, but may fall more in depth, reaching 60 – 70 m, sometimes forming large areas or mussel banks 'true' facies with mussels. There are many species of *Mytilus* but only two are subject to exploitation in Europe: *Mytilus edulis Linne* and *Mytilus galloprovincialis Lamarck* [1, 2].

Mytilus edulis lives in northern regions and is found in the Baltic Sea, North Sea and Atlantic Ocean to Portugal; *Mytilus galloprovincialis* is common in the Mediterranean and Atlantic coasts to Western Channel area. *Mytilus galloprovincialis Lamarck* has been considered for a long time only as a variety of *Mytilus edulis Linne*; based on anatomical research it has been established that there are enough differences that clearly separate these species.

This problem is difficult to solve because there is convergence, due to same factors (especially salinity and temperature) that make the valves of these animals to have many similarities between them and both forms of *M. galloprovincialis* to be removed close to *M. edulis*. This has led many researchers, but not only for the Mediterranean and Black Sea, at the existence in this sea the *Mytilus edulis* species. What can still distinguish the two species are: biometric characters (in the case that two species coexist in the same conditions), the ratio height / length for the species *M. edulis*, mantle edge color (yellow – brown in *M. edulis* and black – purple to *M. galloprovincialis*) and *M. edulis* in the presence or absence of *M. galloprovincialis* on the shell of longitudinal purple bands to remove visible periostracum. A number of identical chromosomes in the two species *M. edulis* and *M. galloprovincialis,* but a different behavior regarding sexual cycle was observed [3, 4].

From over 20 species of economic value of the genre *Mytilus* (mussels) widespread in all temperate seas and of the world subboreale in the Black Sea species *Mytilus galloprovincialis* lives, represented by two environmental groups, rock mussels and mud mussels (broad, deep) [5, 6].

Rock Midia are found in shallow rocky areas - less than 20 m, and although they are in relatively large quantities is not yet a direct economic interest at least for two reasons: are strongly attached (cliffs, rock platforms, artificial concrete structures from dams, the so called "stabilopods") of hard substrate, can not be harvested mechanically (autonomous diver can only do the harvesting) and by being heavily covered by a rich epibiota and containing a high percentage of foreign bodies inside them (sand, small fragments of valves, pearls, etc.) have not a commercial aspect (or require a large amount of work for sale). Rocky mussel instead has an important role in maintaining healthy quality of water near tourist beaches by strong water biofiltration action.

Deep mussels form a ring around the Black Sea, located on the Continental Shelf between izobates 25 ~ 55 m deep, on muddy bottoms. The presence of mussels on

muddy bottoms of the Black Sea - an exception in this sense in the world, is one of the basic features of the basin [3 – 5]. Mussels living in the muddy areas in 'nests' consisting of a few specimens caught with bissus between them and the substrate, which forms the real core, "condensation" isolated specimens rarely meet. Epibiosis attached to deep mussels is generally poor, and foreign bodies of the valves are in a small percentage.

MATERIAL AND METHODS

The study material represented different samples of marine water samples, bivalves, crustaceans and fish, taken directly from the sea or obtained from areas where the sea it has landed being stored on an area within 5 m from shore sea. Sediment samples were collected by autonomous divers, area located on the characteristic profiles of the izobates of 5, 10 and 15 m and were represented by sediments, chemically, biochemically and biologically analyzed. Their processing was done in specialized laboratories of the *National Institute for Marine Research and Development Constanța*, and in the *"Ovidius" University* laboratories. The work has been done in two phases.
Working methods: for algae

- PHASE I :

- *drying*: 2-3 g of wet substance (mussel) was dried at 105 ± 20 ^{0}C in the oven for 3 - 4 hours

- PHASE II:

- *determination of moisture and ash*: moisture determination was made by drying the sample at 105ºC ± 2ºC (thermoregulation oven CALORIS EC 100, degree range of 50-240ºC) for 12 hours. The minerals content was determined by calcining the sample at 550ºC ± 10ºC for 12 hours. Organic substance (OS) was calculated as the difference between 100% and the sum of (%) moisture and ash.
- *determination of total nitrogen and protein*: total protein content of the powder substance was obtained by the method 981.10, AOAC "Official methods of analysis" (total nitrogen - Kjeldahl method) using the equipment Digestor DK6 and distillation unit UDK 127, VELP SCIENTIFICA. The method is based on determination of total nitrogen in the sample after it has been mineralized in the presence of sulfuric acid under the catalytic action of mercury and selenium. After alkalinization, ammonia is steam driven and captured in a boric acid solution which is dosed by titration with hydrochloric acid. Results were expressed as percentage relative to the amount of dry mass taken it.
- *lipid determination*: lipids from samples were extracted with dichloromethane in Soxhlet apparatus for 5 hours. After solvent evaporation fats were determined gravimetrically. Results were expressed as percentage relative to the amount of dry mass taken it.
- *determination of carbohydrates*: carbohydrate extraction was performed with acetic acid solution outlet 15%.Carbohydrate content was determined by the Dubois method (1956) with an maximum at absorption λ = 490 nm using UV-VIS spectrophotometer SPECORD 205 (measuring range 190-1000 nm). Results were calculated by a standard calibration curve with glucose.

An important role was played by the study of bibliography about the high recovery on marine products by identifying active compounds, and their interpretation of the results of investigation of marine resources from the Romanian coast [2 – 5].

RESULTS AND DISCUSSIONS

External morphology

Mytilus galloprovincialis is an equivalves species; the valves close to the front rectangular are sharp and elongated, dilated and flattened ventrally above the back side. The opening of the bissus is short and elongated. Peaks are less curved and remote terminals between them. The upper half of the body distinguishes two regions: the solid, compact and placed back flank, and abdominal thin, lamellar, empty region. Massive dorsal side include digestive with its annexes, heart, excretory and reproductive apparatus. From this massive body starts, in the ventral region, the foot. Of lateral, curved start, saccate, two passed on covering a bell-shaped cavity, called the Board blades - scaphium. As a limit between the two regions is considered an imaginary line passing through the underside of the adductor muscles and connecting the two ends. The top is solid body (body itself) and scaphium is the bottom, inside which are the foot, gills and labial palpii. The front of the panel formed by the mantle serves mainly to the leg and was called leg room and rear room is soda. Where the sheath is secured to the lower edge of valve using a muscle, valves remain imprinted on the form of a trench, more or less visible, that impression is called the mantle, [5, 6].

Valves

Mussels have valves placed symmetrically, joined together by a ligament in the hinge region called “cardinal plateau”. The two valves form two areas well limited, so the front, a smaller area called Arcola or lunula, then the rear is an area bounded higher, called area or corselet. In the back is almost always clearly visible, the ligament, and its back is so-called sinus, where the two valves are united, above umbon or peak of the shell is sinulus, small surfaces covered with a membrane. Valve ornamentation has great importance in describing a valve and can organize the following criteria: - Ornament by color. Mussels have uncolored valves, black violet. Valve surface is covered with a cuticle. - Interior decoration. Inside the valve there are some special lift used to support the muscles, being called miofore or apophyses, which serve to identify the species. - Ornamentation in relief. At mussels, valves are completely smooth and uncolored. Valves are completely closed, well stacked one above the other, (concha clausa), other species have an opening in the rear or front, or rarely, and front and rear.

Nutrients

Mussels ingest many particles from the environment: diatoms, dinoflagellate, organic debris, bacteria and flagellate protozoa, several spores, fragments of algae, inorganic debris, etc. Not yet been established how they are capable of sorting particles nutrients.

(a) (b)

Figure 1. *Mytilus edulis LINNE (a) and Mytilus galloprovincialis LAMARCK (b)*

Figure 2. *Artificially collector after seven months of immersion, detail*

Species found in stomach contents are generally simple forms without expansion or sharp edges with maximum size 20 ÷ 30 μm in length and diameter of 12 ÷ 15 μm. In addition to this selectivity mentioned factors, other factors work as were found in water and the liquid outer intervalvular many species of appropriate size and shape but were not found in stomach contents. There are many micro-organisms in water mass, with a size of up to several microns that can be ingested by the mussels. In plankton among species remain permanently planktonic stage (haloplancton) a large part of phytoplankton organisms have a role in diet nutrition and in particular diatoms representing the ruling class. Dinoflagellates and Piridineeles are also retained, but only copy the appropriate size that can be ingested. Some forms of haloplancton with size of 1-5 μm retained by mussels dominate problem, especially flagellate very small and numerous bacteria.

Chemical and biochemical studies of main compound determination on nutrition to Midia

Following its quality food that came into the concerns of many species of marine laboratories in the world where attention is given to analysis and environmental studies

- physiological and biochemical. Biochemical analysis shows that this species has higher food quality, with qualities comparable with preparations made from terrestrial animals, and some biochemical compounds are superior mussels - amino acids, vitamins. In the Romanian Black Sea sector, besides deep mussels' banks that can be exploited there are specific opportunities on intensive marine aquaculture installations. To determine the optimal period to harvest mussels for industrialization is necessary to determine the seasonal dynamics of physiological parameters and nutritive value of major compounds in mussels. Meat is 30 ÷ 31% by weight, valves 40 ÷ 41%, and 24.0 ÷ 26.3% interstitial juice, so a full recovery would contribute to efficient cultivation of mussels. Biochemical composition of biologically active points attributes support - protein, carbohydrates, lipids and their distribution in different extracts (aqueous and organic solvents) and enzymatic events adjuvant with anti-inflammatory qualities (Tables 1 – 5).

Table 1. *Physical and biological characteristics of aqueous extract of shellfish meat*

Characteristic	Value
*p*H	6.3 – 7.4
Electrical conductivity [μS]	8.3 – 8.5
Salinity [‰]	2.1 – 2.5
Proteins [mg·mL^{-1}]	7.5 – 7.8
Carbohydrates [mg·mL^{-1}]	2.8 – 3.0
Uronic acid [mg·mL^{-1}]	0.5
Enzyme activity and enzyme inhibition	
Superoxiddismutasa [U·mg^{-1} protein]	2.20 – 2.24
Alkaline phosphatase [mU·mg^{-1} protein]	3.9 – 5.7
Alkaline catalase [μmol H_2O_2·sec^{-1}]	0.65 – 0.70
Hyaluronidase [%] inhibition compared to incubation enzyme: protein 1:1; 2:1; 3:1	40 – 15

Processing this raw material will bring changes in the microbial load and the relationship between biochemical constituents detected in meaning, amplify the effects of using products to achieve desired bioproducts. As in the Romanian Black Sea coast there are wide variations of the main environmental factors that have direct impact on physiological behavior of organisms and the accumulation of biochemical components with nutritional value, key dynamics biochemical compounds was correlated with environmental factors and annual ontogenetic stages of an organism (Table 3). The ultimate aim of these biochemical tests was to specify the biological cycle of a year of major quantitative changes in biochemical components of food value and optimal timing of harvesting industry without jeopardizing the current stocks in the Romanian Black Sea coast. Biochemical composition of mussels and rock deep in the Black Sea has some seasonal dynamics (Table 5). These variations may be caused by: normal and abnormal seasonal physical factors - sea of chemicals, the existing density and high food quality, depth and where there are banks of mussels, age and annual physiological cycle of the animal. Between a southern and a northern population may be a lag of one month for the same stage of submission of laying. Concentrations in some essential amino acids in the mussel meat are much higher number like, terrestrial, currently used in the human diet.

Table 2. *Biochemical characterization of the lipid extract of mussels' meat [%]*

Characteristic	Value [%]
Neutral lipids	82.6 ± 0.3
Glycolipids	1.4 ± 0.1
Phospholipids of which:	17.0 ± 1.0
Phosphatidyl ethanolamine and lysophosphatidyl ethanolamine	31.5
Phosphatidyl choline and lysophosphatidyl choline	30.2
Phosphatidyl inozytol	2.4
Phosphatidylserine	14.2
Sphingomyelin	8.5
Unidentified lipids	15.0

Table 3. *Biochemical composition of* ***Mytilus galloprovincialis*** *from the Romanian Black Sea coast*

Species		Deep mussels			Rock mussels
Harvesting area		Sf. Gheorghe	East Constanța	Mangalia	Agigea
Depth [m]		30	30	30	2
Biochemical analysis [expressed in g/100 g of fresh substance]	Total carbohydrate	2.20	1.42	1.27	1.69
	Reducing carbohydrate	0.12	0.12	1.18	0.17
	Glycogen	2.08	1.30	1.09	1.52
	Total nitrogen	1.77	1.56	1.75	1.86
	Total protein	11.06	9.75	10.94	11.62
	Total fat	0.99	1.43	0.86	1.81
	Mineral	0.58	0.47	0.72	0.85
	Water [%]	85.17	86.93	86.21	84.05

Table 4. *Comparative quantitative biochemical data on* ***Mytilus galloprovincialis*** *from the Black Sea with the same species from other marine basins*

Species		*Mytilus galloprovincialis*		*Mytilus edulis*	
		(deep)	(rock)		
Marine basin		Black Sea	Black Sea	White Sea	North Sea
Biochemical analysis [expressed in g/100 g of fresh substance]	Total carbohydrate	1.87	1.57	-	2.60
	Total protein	8.98	9.81	8.60	5.63
	Total fat	1.75	2.18	-	1.04
	Mineral	0.76	0.73	-	-
	Water [%]	85.86	85.71	-	-
	Dry matter [%]	13.24	14.29	-	-

CONCLUSIONS

Biochemical compounds with high content in nutritional value (protein, carbohydrates, lipids) plus the presence of amino acids and vitamins, highlights the high nutritional

value of mussels from the Romanian Black Sea coastal area but also the possibility of their use for extracting active principles with uses in medicine.

Table 5. *Seasonal dynamics have major biochemical components in* ***Mytilus galloprovincialis*** *(averages)*

Season		**Jan - March**		**April - May**		**June - August**		**Sept. - Dec.**	
Examined species		Deep mussels	Rock mussels	Deep mussels	Rock mussels	Deep mussels	Rock mussels	Deep mussels	Rock mussels
Biochemical analysis [expressed in g/100 g of fresh substance]	Total carbohydrate	1.32	1.02	1.70	1.54	1.51	1.67	1.98	1.75
	Reducing carbohydrate	0.18	0.15	0.14	0.19	0.17	0.22	0.29	0.17
	Glycogen	1.14	0.88	1.56	1.35	1.34	1.45	1.69	0.58
	Total nitrogen	1.43	1.54	1.54	1.72	1.46	1.71	1.30	1.37
	Total protein	8.94	9.62	9.62	10.75	9.16	10.68	8.34	8.56
	Total fat	2.69	2.58	1.09	2.06	1.26	1.67	2.32	2.58
	Mineral	0.94	0.57	0.61	0.83	0.79	0.82	0.71	0.63
	Water [%]	86.11	86.20	86.98	84.82	87.28	85.16	86.63	86.48
	Dry matter [%]	13.89	13.80	13.02	13.18	12.72	14.84	13.35	13.52

Comparing the biochemical composition of mussels in the Romanian Black Sea coastal zone with mussels in the other marine basins we note that it is much richer in protein. Biochemical contents of mussels collected from the northern coast is higher in all parameters analyzed (protein, carbohydrates, lipids), to mussels harvest from the south. These quantitative biochemical differences demonstrate once again the existence of physiological races with superior nutritional qualities of existing populations on our coast.

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