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COMPARATIVE ASPECTS OF THE DYNAMICS OF THE GENERAL BIOCHEMICAL COMPOSITION IN SOME BIVALVES OF THE ROMANIAN BLACK SEA LITTORAL

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A b s t r a c t

The authors present the dynamics of the content of water, mineral substances, total glucides, proteins and lipids throughout a biological year, in Mytilus galloprovincialis Lmk. (rock and deep-water breeds) and Mya arenaria L.. The significant variations in the quantity of some biochemical compounds are accounted for by the direct action of the main abiotic and biotic parameters of the environment on the vital activity cycle of the species.

Studies in the biochemical composition of some invertebrates from the northern and north-western areas of the Black Sea, including also some bivalves that may be turned to account, were conducted by Soviet researchers (ABLIAMITOVA-VINOGRADOVA, 1949; GOROMOSOVA, 1965, 1968 ; STEPANIUC, 1967 ; VINOGRADOVA, 1964).

These investigations underline the dynamic aspects of the biochemical composition, aspects closely linked with the influence of abiotic factors (temperature, salinity), biotic factors (quantity and quality of food) and with the annual and multi-annual ontogenetic cycle of the species.

The researches into the physiology of Black Sea animals have evidenced metabolic alterations in the marine invertebrates subjected to

the influence of variations in certain abiotic factors - under laboratory or natural conditions. It was thus possible to distinguish different physiological breeds among the populations of the continental shelf area of the Black Sea (PORA, 1946; 1961).

It is well known that the north-western area of the Black Sea is under the direct influence of the fresh water masses brought by the tributary rivers. These fresh water masses induce great variations in several abiotic or biotic parameters of the environment. Life on the Romanian shelf of the Black Sea is under the direct influence of these oscillations.

Our researches aim at supplying new data on the dynamics of the biochemical composition of some species of marine bivalves and we think it was absolutely necessary to carry out this study in the Romanian area of the Black Sea.

The biochemical analyses were conducted with the species : Mytilus galloprovincialis Lmk. (rock and deep-water breeds) and Mya arenaria L., a species which has come recently to the Black Sea. In view of a wider survey of certain ecological-physiological aspects we have carried out biochemical analyses of other bivalves : Cardium edule lamarcki Reeve, Paphia sp., Spisula subtruncata triangula (Renier) collected concomitantly with the two species mentioned above. The analyses material was collected along four north-south lines off the Romanian coast : off the Danube mouths, Constanţa, Agigea and Mangalia, at depths ranging between 0, 5 and 40 m. The samples have been collected and processed as follows : rock mussels and shallow water Mya, monthly ; deep-water mussels, more than 10 m. depth Mya and other bivalves, quarterly. In parallel one has collected water samples in wiew of current analyses, viz. : temperature, salinity, oxygen and organic substances.

The collected animals were kept 4-5 days in running water aquaria under best oxygenation conditions. This treatment aims at eliminating a wide range of organisms and foreign bodies from the paleal cavity of the bivalves.

For analysis purposes the animals were divided into two lots according to their size and age : young (valves length ranging between

20 and 40 mm) and adults (valves length ranging between 50 and 80 mm).

After removing the epibiosis from the valves, the animals were dissected. Macro-and microscopic examinations of their gonads were carried out. The adult lot animals were subdivided into two groups of 5-10 individuals each, according to the development stage of the gonads: the sexually undeveloped animals group and the sexually developed group.

The dissected animals were flushed under tap in order to achieve a total removal of foreign bodies. The soft part was removed from the valves, summarily dried on filter paper, weighted and reduced to a homogeneous paste in a mortar. The homogeneous paste was divided into small portions in view of the biochemical analyses. The analyses were carried out individually with the view of establishing the limits of quantitative variations within every lot.

The analyses had to determine : the content of water and mineral substances, total glucides, proteins and lipids. The total glucides were quantitatively determined using Selfter's anthrone method (DUMITRU, 1967). The proteins were quantitatively determinated employing the colorimetric method with Nessler reagent (ALTERAS, CAJAL, 1964). The total lipids were quantitatively determined utilizing the gravimetric method (DUMITRU, 1967).

The results are expressed in percentages for the water content and in g/100 g fresh homogeneous matter for the content of mineral substances and total glucides, proteins and lipids.

The data presented in this paper regrad a period of time ranging from July 1970 to December 1971.

The recordet salinity, organic substance and oxygen values presented - in general - normal variations, specific to the season and place where the water samples and animals subjected to analyses were collected. In spring 1971, temperatures below the average multi-annual temperatures were recorded in the Romanian litoral area (5-6^{o}C lower) The temperatures recordet in May 1971 correspond to the average multi-annual temperatures. These drops in temperature have brought about a delayed annual ontogenetic cycle in many invertevrates.

In the Black Sea there is a single species of mussels - Mytilus-galloprovincialis Lmk., with a very wide-spread habitat (DRAGOLI, 1966 ; UYSAL, 1970). According to PORA (1946, 1961) within different species of mollusca, like in other invertebrate species, one could distinguish a range of physiological breeds.

Conducting parallel analyses with lots of rock and deep-water mussels collected along the same north-south line, one has found significant differences regarding the water and proteins content (Table 1). These differences are to be ascribed, in the first place, to the salinity factor, which, as is known, increases with the depth and which plays a dominant role in the osmotic adjustment of Black Sea fauna. FRAGA (1956) has shown the quantitative biochemical differences in lots of mussels collected at different depths. These observations corroborate the quantitative differences we have found in the two breeds of mussels.

The average values of the biochemical components analyses in young and adult specimens of mussels and Mya are shown in the same table. There are significant differences between the lot of young and the lot of adults. The young specimens of rock mussels show significant differences as regards the content of water, mineral substances, glucides and lipids, in comparison with the adult specimens. In the young deep-water mussel specimens we have found differences only as regards the glucides content in comparison with adult specimens. These significant differences between young and adult animals are accounted for by the much higher metabolism of the previous former. And if young specimens of rock mussels show more differences than young specimens of deep-water mussels this is accounted for by much wider variations in the environmental parameters of shallow water areas.

The young specimens of Mya arenaria show also significant differences of water and total proteins content, in comparison with the adult individuals. These differences will be explained in another section of this paper.

Temperature plays an important role in the metabolism of organisms. GOROMOSOVA (1965, 1968) and STEPANIUC (1967) have presented a series of aspects of the seasonal dynamics of the biochemical

composition in some invertebrate, as a function of this environmental parameter.

We are presenting in table 2 the seasonal dynamics of the biochemical composition in the mussels and Mya collected off the Romanian Black Sea coast. With the view of synthetizing the available data and attenuating the sharp individual variations, we have resorted to a quarterly presentation of the quantitative analysis data. These data represent a statistical processing of all monthly values. The average values presented in table 1 have been chosen as controls.

An analysis of table 2 leads to following conclusions :

- the water content of the species studied shows significant differences during the biological winter and spring periods ;

- the total mineral substances show significant drops during the summer period ;

- the total glucides assume minimum values throughout the winter period, values increasing gradually in the course of following months, to record a maximum in spring or summer - according to the species. In autumn the amount of glucides gradually diminishes reaching the minimal values, previously mentioned ;

- the protein content of the species is low during the winter, raise significantly in spring and score insignificant values in the other seasons ;

- the lipids show increased values throughout the biological winter period. In summer they score minimal values.

It therefore appears that most of the groups of biochemical compounds analysed show a seasonal dynamics which may be connected with the annual sexual development of the species (MASUMOTO, quoted by WILBUR and YONGE, 1966 ; STEPANIUC, 1967) The biochemical analyses conducted with adult animals, divided into different lots according to the development of their gonads, entirely corroborate this opinion.

One cannot overlook the influence of the abiotic factors on the seasonal dynamics of the biochemical composition of the animals. It is sufficiently to quote PORA's opinions (1961, b) on the ecological role of salinity and the same on the ionic ratios in the Black Sea, for

Table 1

Biochemical composition of studied species, by dimensions

Valve length	Statist. paramet.	Rock mussels					Deep-water mussels					Mya arenaria				
		Water	M. S.	T. G.	T. P.	T. L.	Water	M. S.	T. G.	T. P.	T. L.	Water	M. S.	T. G.	T. P.	T. L.
20-40 mm	N. A.	3	3	3	3	3	4	4	4	4	4	3	3	3	3	3
	$\bar{X}$	85, 03	0, 94	1, 27	9, 63	3, 14	87, 67	0, 71	1, 12	8, 72	1, 76	81, 85	0, 77	1, 94	12, 75	2, 69
	S. E.	±0, 60	±0, 09	±0, 08	±0, 62	±0, 30	±0, 76	±0, 08	±0, 10	±0, 61	±0, 50	±0, 65	±0, 03	±0, 16	±0, 75	±0, 78
	C. V.	0, 75	9, 57	6, 29	11, 83	9, 55	0, 86	11, 26	8, 92	6, 99	28, 40	0, 79	3, 89	8, 24	5, 88	12, 99
50-70 mm	N. A.	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	$\bar{X}$	86, 22	0, 71	1, 54	9, 52	1, 91	87, 20	0, 79	1, 52	8, 64	1, 85	83, 41	0, 84	1, 85	11, 34	2, 55
	S. E.	±0, 39	±0, 05	±0, 18	±0, 40	±0, 21	±0, 34	±0, 05	±0, 16	±0, 27	±0, 20	±0, 44	±0, 08	±0, 07	±0, 33	±0, 21
	C. V.	0, 45	6, 91	11, 68	4, 20	10, 99	0, 38	5, 81	10, 52	3, 12	10, 81	0, 52	8, 92	3, 80	2, 91	8, 23

- Water in % ; M. S. = mineral substance in g/loog fresh substance ; T. G. = total glucides in g/loog fresh substance ; T. P. = total proteins in g/loog fresh substance ; T. L. = total lipids in g/loog fresh substance ; N. A. = number of analyses ; $\bar{X}$ = arithmetic mean ; S. E. = standard error ; C. V. = variability coefficient.

Table 2

Seasonal dynamics of biochemical composition in the mussels and Mya arenaria

Quarter	Statist. paramet.	Rock mussels					Deep-water mussels					Mya arenaria				
		Water	M. S.	T. G.	T. P.	T. L.	Water	M. S.	T. G.	T. P.	T. L.	Water	M. S.	T. G.	T. P.	T. L.
I	N. A.	6	6	6	6	6	10	10	10	10	10	6	6	6	6	6
	$\bar{X}$	87,32	0,55	0,98	9,33	1,82	86,07	0,88	1,37	9,06	2,62	84,43	0,76	1,65	10,73	2,43
	S. E.	±0,22	±0,08	±0,09	±0,29	±0,39	±0,40	±0,04	±0,06	±0,29	±0,22	±0,44	±0,06	±0,07	±0,48	±0,28
	C. V.	0,25	14,70	9,18	3,10	21,42	0,46	4,07	4,37	3,20	8,39	0,52	8,01	4,24	4,47	11,52
II	N. A.	6	6	6	6	6	15	15	15	15	15	6	6	6	6	6
	$\bar{X}$	84,80	0,80	1,43	10,81	2,16	88,42	0,80	1,05	8,26	1,47	83,67	0,87	1,84	11,47	2,41
	S. E.	±0,67	±0,11	±0,21	±0,59	±0,24	±0,21	±0,05	±0,05	±0,20	±0,07	±0,55	±0,08	±0,18	±0,72	±0,35
	C. V.	0,79	13,55	14,68	5,45	11,11	0,23	6,39	4,76	2,42	4,76	0,65	13,00	9,78	6,27	14,52
III	N. A.	6	6	6	6	6	10	10	10	10	10	10	10	10	10	10
	$\bar{X}$	86,16	0,81	1,97	9,60	1,46	87,34	0,63	1,43	9,14	1,46	81,72	0,61	2,07	12,49	2,85
	S. E.	±0,35	±0,09	±0,18	±0,33	±0,20	±0,36	±0,04	±0,11	±0,34	±0,12	±0,53	±0,06	±0,21	±0,78	±0,20
	C. V.	0,40	11,26	9,13	3,43	13,69	0,41	6,01	7,69	3,72	8,21	0,64	7,31	10,14	6,24	7,01
IV	N. A.	6	6	6	6	6	6	6	6	6	6	4	4	4	4	4
	$\bar{X}$	87,02	0,68	1,76	8,35	2,19	87,04	0,86	2,18	8,12	1,80	83,85	1,10	1,84	10,68	2,53
	S. E.	±0,40	±0,12	±0,25	±0,39	±0,27	±0,58	±0,08	±0,11	±0,42	±0,27	±0,76	±0,10	±0,21	±0,64	±0,60
	C. V.	0,45	17,62	14,20	4,67	12,32	0,66	9,83	5,04	5,18	15,00	0,90	8,90	11,41	5,99	23,71

– Water in % ; M. S. = mineral substance in g/100g fresh substance ; T. G. = total glucides in g/100g fresh substance ; T. P. = total proteins in g/100g fresh substance ; T. L. = total lipids in g/100g fresh substance ; N. A. = number of analyses ; $\bar{X}$ = arithmetic mean ; S. E. = standard error; C. V. = variability coefficient

explaining the significant variations in the content of water, mineral salts and proteins of the studied species.

Bivalves are filter-feeding animals, per excellentiam. VINOGRADOVA (1964) found a seasonal dynamics in the biochemical composition of many planktonic organisms. It appears therefore that the biochemical composition of the food of the filter-feeding animals has also a direct repercussion on their seasonal biochemical composition.

PROVASOLI's (1966) and HAILOV's (1971) synthesis works have shown that many marine organisms have the capacity of releasing into the environment some of their metabolites of partially known chemical structures that have a strong biological impact on other organisms. GAVRILESCU (1964) has found important quantities of reducing glucides in sea water. In this author's opinion these substances are being released by phytoplanktonic organisms. DAKIN (1925) ; MITCHELL (1915) ; YONGE (1928) , quoted by WILBUR and YONGE (1966) have proved that certain species of mollusca directly absorb glucides from sea water through branchiae and mantle. All these findings bear out the idea that the metabolic activity of mollusca is directly or indirectly influenced by the organic fractions available in the sea water. Seeing that metabolic activity is based on the quantitative or qualitative alterations of various biochemical components it is normal that these components should undergo qualitative or quantitative variations.

Under normal conditions, in the marine environment, abiotic and biotic parameters interfere in the course of a season. The interpretation of biochemical dynamics should be based on the correlation of these factors.

The biochemical values presented in tables 1 and 2 show differences between the two species of mollusca : Mytilus galloprovincialis and Mya arenaria. These differences are particularly marked in the water, mineral salts and proteins content. Mya arenaria's water content is lower than that of most of the Black Sea bivalves (table 3).

Comparing the quantities of these three categories of substances in this Black Sea mollusc with the quantities found in the same species living in the North Sea (table 3) it appears that the Black Sea individuals

show a higher water content and a lower mineral substances and proteins content. It results that Mya arenaria which has recently entered the Black Sea tends to adjust its own biochemical composition, assuming that of the mollusca which have lived for a long time in this sea. The young Mya individuals show a lower water content and a higher protein content than the adult specimens (table 1).

Table 3

The content in the water, mineral substance and total proteins of some species of marine bivalves

Species	Marine area	Water %	Mineral substance g/loog	Total proteins g/loog
Mytilus galloprovincialis Lmk	Black Sea	86,76	0,75	9,08
Modiolus phaseolinus (Philippi)	Black Sea	86,54	1,15	6,79
Spisula subtruncata triangula (Renier)	Black Sea	88,45	0,77	8,43
Cardium edule lamarcki Reeve	Black Sea	86,91	1,21	8,42
Paphia sp.	Black Sea	86,88	1,56	8,70
Chione gallina L.	Black Sea	86,16	2,31	7,94
Pitar rudis (Poli)	Black Sea	88,65	1,98	7,19
Pecten ponticus (B.D.D.)	Black Sea	88,13	1,64	7,18
Mya arenaria L. (adult specimens)	Black Sea	83,44	0,84	11,34
Mya arenaria L. (young specimens)	Black Sea	81,85	0,77	12,78
Mya arenaria L. (adult specimens)	North Sea	78,50	1,70	15,6

The values of these two components in young animals are nearer to the values found in North Sea specimens.

The adult Black Sea Mya arenaria individuals show the lowest water content and the highest protein content in summer, the reproduction period in this species. These values approach those found in North Sea individuals. During the reproduction period it tends to assume the biochemical composition of the animals which have lived for a long time in an

other marine environment. Combining these observations one may assert that this species is underogoing now to a physiological and biochemical self-acclimatizing process. The inter- or intraspecific relations within the framework of a natural biotype correlated with the seasonal values recorded by the seasonal abiotic parameters of the environment, play an overwhelming role in the biological behaviour of the species, behaviour which is ultimately based on the dynamics of the biochemical components of the individuals.

The quantitative differences found in certains groups of biochemical components, analysed in the five species of bivalves collected along the four north-south lines, off the Romanian Black Sea coast, bear out the above mentioned point of view (fig. 1). The adult animals analysed were collected in June 1971. The trends of the quantitative biochemical alterations differ from a species to another, being directly linked to the annual ontogenetic stage of the animals and to the impact of the specific variations in the environmental factors of their habitat. The analyses conducted with these bivalves corroborate the opinion that different physiological and biochemical breeds may exist within these species.

From the presented data one may reach following conclusions:

- The general biochemical composition of the studied species has a strongly marked dynamic character .

- Within a species, one records high individual biochemical differences.

- The abiotic and biotic factors of the environment directly influence the physiological behaviour and biochemical composition of the studied species. The seasonal dynamics of the biochemical composition is determined, in the first place, by the temperature.

- The quantitative variations in the biochemical composition are accounted for by the age and the ontogenetic cycle of the species.

- The biochemical aspects presented emphasize the existence of physiological breeds among the mollusca populations.

- Mya arenaria, a species which has recently entered the Black Sea, is still undergoing an active biochemical and physiological self-acclimatizing process; we think that the effected biochemical analyses render this process evident.

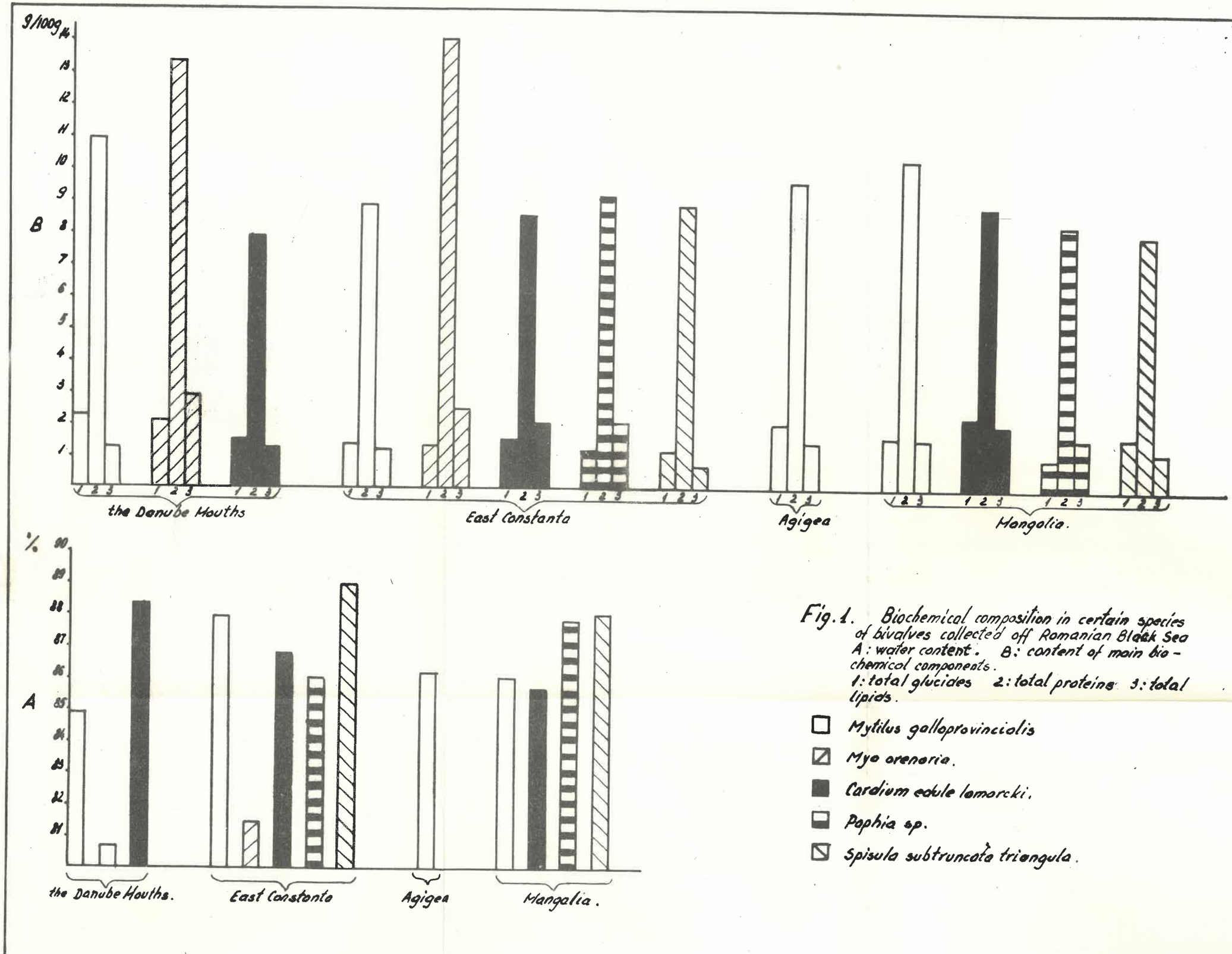

Fig. 1. Biochemical composition in certain species of bivalves collected off Romanian Black Sea
A: water content. B: content of main biochemical components.
1: total glucides 2: total proteins 3: total lipids.

- Mytilus galloprovinciolis
- Myo orenoria.
- Cardium edule lamarcki.
- Paphia sp.
- Spisula subtruncata triangula.

- The seasonal dynamics of the biochemical composition points out the best period when mussels and Mya can be turned to account. This period begins in April and end in November. In this period the animals show a valuable biochemical composition.

The elucidation of all the aspects of this biochemical dynamics calls for a complex survey to supply more knowledge on the biology of these species.

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