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THE BIOCHEMICAL CHARACTERIZATION OF THE MARINE ENVIRONMENT AND THE MAIN COMMERCIAL MOLLUSKS FROM THE ROMANIAN BLACK SEA COAST AREA

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ABSTRACT

Within the requirements of implementing in Romania EU legislation is the EU Shellfish Waters Directive No. 79/923 EEC. The EU Directive No. 97/923 "Shellfish Waters" was implemented in Romania based on Government Decision No. 201/2002, amended and supplemented by Government Decision No. 467/2006 consisting of technical methods regarding the water quality for mollusks. The paper presents a biochemical characterization of the marine environment (water and sediments) and the main mollusks from the four areas designated by law for the development and exploitation of mollusks in the Romanian coastal area. The study brings supplementary data regarding ecological and physiological aspects of the Black Sea marine ecosystem, which are linked to the marine environment and organisms' health assessment and monitoring.

KEY-WORDS: seawater, marine sediment, mollusks, biochemistry, EU Shellfish Waters Directive

AIMS

The paper presents a biochemical characterization of the marine environment (seawater and marine sediments) and the main commercial mollusks (*Mytilus galloprovincialis* and *Rapana venosa*) from the Romanian Black Sea coast areas which are designated for the development and exploitation of mollusks according to the EU Shellfish Waters Directive No 79/923 EEC.

The EU Directive No. 97/923 "Shellfish Waters" was implemented in Romania based on Government Decision No. 201/2002, amended and supplemented by Government Decision No. 467/2006 consisting of technical methods regarding the water quality for mollusks.

For the implementation of the EU Directives, the following steps were accomplished: (i) the delimitation of four favorable areas for the development and exploitation of mollusks in the Romanian coastal area (*Order No. 1950/2007*); (ii) developing a monitoring system of the marine environment in the designated areas; (iii) establish the maximal allowed limits of organohalogenated substances and heavy metals in water, sediments and marine mollusks (*Order No. 1888/2007*)[1,2].

The biochemical data brings supplementary information regarding the organic load in the marine environment and organisms in correlation with ecological, physiological and health aspects.

BACKGROUND

Mollusks make up 9% of the total production of marine organisms in Europe[3].

M. galloprovincialis (Lamark, 1819) and *R. venosa* (Valenciennes, 1846) are marine mollusks with the highest ecological and economic importance in the Black Sea ecosystem.

Bivalve mollusks, particularly marine mussels such as *Mytilus* spp., have been used as indicator organisms in environmental monitoring programs due to their wide distribution, sedentary lifestyle, tolerance to a large range of environmental conditions[4].

M. galloprovincialis (mussel) is the most important mollusk in the Romanian marine area, known as mass species and marine bio-resource potentially exploitable for human consumption[5].

R. venosa is an invasive species originated from the Sea of Japan. It has formed important stocks by adapting to the brackish ecosystem of the Black Sea. This sea whelk is a carnivorous species, performing seasonal migration and preferring habitats occupied by bivalve and crustacean species in a prey-predator relationship[6]. Since the 1980s, *R. venosa* has become a valuable commercial resource: its meat is exported to Japan for food and it has recently also been included in the diet of those native to the Black Sea area.

R. venosa can be used as bioindicator, representing an important tool for the biomonitoring of environmental pollution in coastal areas.

As properties of living organisms, bioindicators could be affected by periodic variations in environmental factors (such as light, temperature, dissolved O2, and nutrient and contaminant input) and by changes in biological functions (e.g. rate of metabolic processes or the reproductive cycles usually connected to environmental changes). The ecological plasticity of the different organisms may also play a significant role[7].

In their environmental policy agenda, European maritime states have outlined the protection of species and habitats and the need to maintain quality standards in coastal and offshore waters as a priority[8].

The Water Framework Directive and Marine Strategy Directive call for the definition and application of biological quality elements and the development of early-warning approaches for environmental health assessment and monitoring.

EXPERIMENTAL

Sample collection

Biochemical parameters were measured twice, in summer and autumn, in 2009, from seawater, marine sediments and mussels (*M. galloprovincialis*, *R. venosa*), collected from the four designated areas for growth and commercial exploitation of mollusks (Table 1).

Table 1. The location, area and sampling sites along transects in each of the four designated areas

Areas	Geographic limits	Area (nautical mile)	Sampling transects (location and isobath)
1.	Sulina and Sfântu Gheorghe	142 Mm^2	Mila 9 45°01'N 29°39'E (5 m depth) 45°01'N 29°44'E (20 m depth) 45°01'N 29°51'E (30 m depth)
2.	Perişor and Chituc	215 Mm^2	Portiţa 44°40'N 29°00'E (5 m depth) 44°38'N 29°12'E (20 m depth) 44°37'N 29°20'E (30 m depth)
3.	Năvodari and Constanţa Harbor	109 Mm^2	Cazino-Mamaia 44°14'50''N 28°38'50''E (5 m depth) 44°14'50''N 28°42'50''E (20 m depth)

4	Agigea and Mangalia	101 Mm^2	44°14'50''N 28°51'E (30 m depth) Constanţa North 44°13'N 28°39'E (5 m depth) 44°13'N 28°42'E (20 m depth) Eforie South 44°02'N 28°39'E (5 m depth) 44°02'N 28°40'E (20 m depth) Costineşti 43°56'N 28°39'E (5 m depth) 43°56'N 28°41'E (20 m depth) 43°56'N 28°45'E (30 m depth) Mangalia 43°50'N 28°36'E (5 m depth) 43°50'N 28°41'E (20 m depth)

The mussels have been sampled from the sea bottom at different depths using the dredge of 60 cm and 100 cm opening on board the research vessel "Steaua de Mare 1" belonging to the National Institute for Marine Research and Development " Grigore Antipa" Constanța.

Biochemical Analyses

The marine water samples were filtered through filter paper with 0.45 μm pore size.

The marine sediments were dried on filter paper at room temperature before biochemical analyzing (dry weight, ash, lipid) and watery extract (1 g:10 ml distilled water) for protein and carbohydrate analysis.

Ten mussels within the size range of 40-70 mm and ten whelks were lyophilized (soft tissue) and used for biochemical analysis (protein, carbohydrate, lipid).

We measured the following variables: dry weight, organic substance content, ash content, total protein content, total carbohydrate content and total lipid content in seawater, marine sediment and mollusks (*M. galloprovincialis* and *R. venosa*).

The dry weight, organic substance and ash were determined according to the Manescu method[12]. The ash content was estimated by burning the material in a muffle furnace at 550°C for 24 hours. The difference between dry weight and ash weight was the organic matter. For protein analysis in seawater, the Kackar method was applied, cited by Iordachescu and Dumitru[9]. The method with L-Tryptophan was used for carbohydrates and is a universal method for carbohydrates content from seawater, suspensions and sediments, reading at 540 nm[10]. The lipids in seawater were analyzed with the Agatova method, which is based on the lipids extraction with a mixture of chloroform, methanol, ethanol (2:1:1), followed by spectro-photometrical determination with vaniline at 540 nm[11].

The protein[13] and carbohydrate in sediments were analyzed in watery extract.

The lipids in sediments were assessed by the gravimetrical method, which is based on the lipids extraction with a mixture of chloroform, methanol, (2:1), followed by drying and weighing[14].

The lipids[15], proteins[16], total carbohydrates[17] were assessed in lyophilized tissue of mollusks, according to "Les notes techniques de l'URAPC"- NT/URAPC/ 96-01-02-03, IFREMER[18].

RESULTS AND DISCUSSION

In seawater, dry weight and ash recorded higher values during the autumn compared to the summer period. The mean values for dry weight, ash and organic substance analyzed in seawater had the same tendency both seasons, in summer and autumn (Table 2, Table 3).

Table 2. Mean values of the biochemical parameters in seawater in summer, year 2009

Mean ±SD SUMMER	Dry weight g/l	Organic substance g/l	Ash g/l	Protein mg/l	Carbohydrate mg/l	Lipid mg/l
Area 1	8.07±0.44	3.52±0.90	4.55±0.90	22.51±5.68	8.93±0.87	3.05±0.59
Area 2	9.59±0.92	4.00±0.62	5.59±0.30	15.46±3.41	5.00±1.58	2.83±0.43
Area 3	11.10±1.05	4.58±0.78	6.52±1.62	23.92±6.05	3.15±1.30	4.09±0.45
Area 4	12.21±0.63	4.36±0.26	7.85±0.78	20.52±10.82	4.69±2.54	4.48±1.88

The minimum values were achieved in Area 1 (dry weight 8.07±0.44 g/l, organic substance 3.52±0.90 g/l, ash 4.55±0.90 g/l-summer; dry weight 9.15±3.24 g/l, organic substance 2.77±0.20 g/l, ash 6.39±3.18 g/l-autumn) and the maximum values were registered for Area 4 (dry weight 12.21±0.63 g/l, organic substance 4.58±0.26 g/l, ash 7.85±0.78 g/l-summer; dry weight 19.79±0.77 g/l, organic substance 4.36±0.50 g/l, ash 14.53± 0.66 g/l-autumn) (Table 2, Table 3).

Table 3. Mean values of global biochemical parameters in seawater in autumn, year 2009

Mean ±SD AUTUMN	Dry weight g/l	Organic substance g/l	Ash g/l	Protein mg/l	Carbohydrate mg/l	Lipid mg/l
Area 1	9.15±3.24	2.77±0.20	6.39±3.18	35.83±4.78	4.42±2.34	3.74±0.13
Area 2	15.47±0.80	4.68±0.12	10.79±0.83	26.21±6.80	8.41±3.17	3.74±0.50
Area 3	18.85±0.27	5.28±0.34	13.57±0.30	15.48±7.14	6.28±3.06	1.52±0.29
Area 4	19.79±0.77	5.26±0.50	14.53±0.66	16.12±3.54	5.41±6.01	1.65±0.21

Regarding the biochemical composition in seawater, the general tendency showed that the higher values were registered for proteins, followed by carbohydrates and lipids (Table 2, Table 3).

During the summer season, protein content was minimum in Area 2 (15.46±3.41 mg/l) and maximum in Area 3 (23.92±6.05 mg/l) and, in the autumn, the minimum achieved was in Area 3 (15.48±7.14 mg/l) and the maximum in Area 1 (35.83±4.78 mg/l) (Table 2, Table 3).

For carbohydrates, in the summer period the minimum was registered in Area 3 (3.15±1.30 mg/l) and the maximum in Area 1 (8.93±0.87 mg/l); in autumn, the minimum recorded was in Area 1 (4.42±2.34 mg/l) and the maximum in Area 2 (8.41±3.17 mg/l) (Table 2, Table 3).

Lipids content indicated the minimum value in Area 2 (2.83±0.43 mg/l) and the maximum in Area 4 (4.48±1.88 mg/l) in summer. For autumn, lipids were minimum in Area 3 1.52±0.29 mg/l) and maximum in Area 1 (3.74±0.13 mg/l) and Area 2 (3.74±0.50 mg/l) (Table 2, Table 3).

Regarding the global biochemical parameters in marine sediments, the general tendency is the same for the both season (summer and autumn), exception for Area 1 and Area 2 are dry weight and ash with higher values during the summer, compared to autumn, and Area 4 indicated higher values for all global biochemical parameters in autumn (Table 4, Table 5).

Table 4. Mean values of the biochemical parameters in sea sediment in summer, year 2009

Mean ±SD SUMMER	Dry weight %	Organic substance %	Ash %	Protein %Dry weight	Carbohydrate %Dry weight	Lipid %Dry weight
Area 1	60.13±13.49	5.90±1.39	54.23±14.82	0.024±0.00	0.022±0.01	0.063±0.03
Area 2	61.97±10.03	9.31±4.85	52.67±5.59	0.020±0.00	0.020±0.00	0.050±0.04
Area 3	69.41±7.00	5.37±1.24	64.04±7.53	0.017±0.00	0.031±0.01	0.035±0.01
Area 4	74.17±8.15	9.52± 5.16	64.65±6.42	0.016±0.01	0.027±0.01	0.042±0.03

In this respect, the higher values were achieved in Area 4 for summer (dry weight 74.17±8.15 %, ash 64.65 ±6.42 %, organic substance 9.52±5.16 %) and autumn (dry weight 79.98±2.46%, ash 70.21 ±2.92%, organic substance 9.77±3.05%) (Table 4, Table 5). The minimum values were registered for dry substance in Area 1 (60.13±13.49% - summer, 52.77±19.55 % - autumn), Area 2 for ash (52.67±5.59% - summer, 46.92±16.47% - autumn).

The organic substance content was minimum in Area 3 (5.37±1.24 %) in summer and Area 1 (5.44±0.31%) in autumn (Table 4, Table 5).

Table 5. Mean values of the biochemical parameters in sea sediment in autumn, year 2009

Mean ±SD AUTUMN	Dry weight %	Organic substance %	Ash %	Protein %Dry weight	Carbohydrate %Dry weight	Lipid %Dry weight
Area 1	52.77±19.55	5.44±0.31	47.33±19.25	0.040±0.03	0.100±0.02	0.050±0.02
Area 2	53.35±20.44	6.43±4.02	46.92±16.47	0.050±0.01	0.110±0.04	0.060±0.02
Area 3	67.00±16.42	6.30±1.73	60.70±16.29	0.040±0.02	0.020±0.01	0.040±0.01
Area 4	79.98±2.46	9.77±3.05	70.21±2.92	0.008±0.00	0.005±0.00	0.071±0.01

The biochemical composition of sea sediment did not show the same evolution compared to seawater, the higher values are recorded by carbohydrates in autumn (exception: Area 3 and Area 4), followed by lipids and proteins in the both seasons (Table 4, Table 5).

The protein content was higher in Area 1 (0.024±0.00% dry weight) and minimum in Area 4 (0.016±0.01% dry weight) in summer. In autumn, the maximum value was in Area 2 (0.050±0.01% dry weight) and minimum in Area 4 (0.008±0.00% dry weight) (Table 4, Table 5).

The carbohydrate concentration recorded the maximum value in summer for Area 3 (0.031±0.01% dry weight) and minimum in Area 2 (0.020±0.00% dry weight). During the autumn season, carbohydrates indicated maximum in Area 2 (0.110±0.04% dry weight) and minimum in Area 4 (0.005±0.00% dry weight (Table 4, Table 5).

The lipid values were higher in summer for Area 1 (0.063±0.03% dry weight) and minimum in Area 3 (0.035±0.01% dry weight) and, in the autumn season, the maximum values were in Area 4 (0.071±0.01% dry weight) and minimum in Area 3 (0.040±0.01% dry weight) (Table 4, Table 5).

The presence of the organic molecules in seawater is an important ecological factor which influences the growing, development and death of the marine organisms[19]. In seawater, the organic dissolved and particulate substance is also important for chemical, biological, physiological and geological processes. In dissolved form, it influences the primary productivity, being a carbon source[20].

The biochemical composition of the organic substance characterizes the environment biochemical status. In the marine environment, proteins play an important role in the inside organisms reactions and in the interaction processes with the surrounding environment.

The glucides and their biochemical degradation constitute the main source of energy for the ecosystem and a real metabolic effector.

The natural lipids represent a heterogeneous mixture of compounds which contains the marine organisms complex lipids and derivatives and in the same time the combinations from the living organic substance transformation. A part of lipids, such as hydrocarbons, come in the seawater as a result of human activities[21].

Water and sediment quality along the Romanian coastline in 2009 was overall in conformity with the Shellfish Waters Directive. The only parameter below the minimal value was salinity in the northern part of the Romanian coastline, due to the freshwater transported by the Danube River[22].

During the summer period, the maximum values for all biochemical parameters in mussels were achieved in Area 4, followed by Area 3 and Area 2 (Table 6).

M. galloprovincialis was better represented in autumn than in summer, where no individuals were found in Area 1.

Table 6. The biochemical composition of *Mytilus galloprovincialis* in summer 2009

Mytilus galloprovincialis **SUMMER**	**Dry weight %**	**OS % Dry weight**	**Ash % Dry weight**	**Protein % Dry weight**	**Carbohydrate %Dry weight**	**Lipid %Dry weight**
Portița-18 m (Area 2)	15.37	13.91	1.46	37.78	21.48	0.82
Cazino Mamaia-17 m (Area 3)	16.77	15.36	1.39	39.44	29.20	3.27
Costinești-17 m (Area 4)	17.09	15.56	1.54	42.36	30.74	6.29
Mean	**16.41**	**14.94**	**1.46**	**39.86**	**27.14**	**3.46**

OS: organic substance

The low salinity (<12‰) in conformity with the Shellfish Waters Directive, recorded in the northern part of the Romanian coastline in summer 2009, influenced negatively the growth and reproduction of mussels[22].

During the autumn season, the distribution of biochemical compounds in mussels was without correlation (Table 7). The mean values indicated that, in summer, all biochemical parameters (except ash) concentration achieved the highest values.

Table 7. The biochemical composition of *Mytilus galloprovincialis* in autumn 2009

Mytilus galloprovincialis **AUTUMN**	**Dry weight %**	**OS %Dry weight**	**Ash %Dry weight**	**Protein %Dry weight**	**Carbohydrate %Dry weight**	**Lipid %Dry weight**
Mila 9-20 m (Area 1)	14.95	12.72	2.23	28.58	13.3	6.15
Portița-20 m (Area 2)	14.32	12.86	1.47	37.94	17.36	3.25
Cazino Mamaia-28 m (Area 3)	17.245	11.75	5.49	39.28	6.60	2.30
Costinești-26 m (Area 4)	12.56	10.08	2.48	31.12	2.66	1.49
Mean	**14.77**	**11.85**	**2.91**	**34.23**	**9.98**	**3.31**

OS: organic substance

The whelk *R. venosa* was not well represented. It was found in catches in Area 4 during the summer and in Area 1 and Area 2 during the autumn (Table 8).

Table 8. The biochemical composition of *Rapana venosa* in summer and autumn 2009

Rapana venosa	**Dry weight %**	**OS %Dry weight**	**Ash %Dry weight**	**Protein %Dry weight**	**Carbohydrate %Dry weight**	**Lipid %Dry weight**
SUMMER						
Costinești-17m (Area 4)	27.19	25.76	1.43	45.89	39.63	0,32
AUTUMN						
Mila 9-20m (Area 1)	23.6	21.15	2.45	23.67	12.10	0,87
Portița-20m(Area 2)	23.74	21.73	2.01	40.28	17.72	1,43

OS: organic substance

By comparison, in summer, the biochemical parameters (except for ash and lipid) showed the highest concentrations. Also, it can be remarked that *R. venosa* is not rich in lipids, but it contains higher quantities of proteins and carbohydrates than *M. galloprovincialis* (Table 8).

Organochlorine substances, metals and faecal coliform content in mussels in 2009 were at concentrations below the threshold and in conformity with the EU Directives [22].

In benthic invertebrates, life cycles can be interpreted as cumulative responses to environmental fluctuations through the annual cycle[23, 24]. For example, tissue protein-carbohydrate-lipid concentrations are an excellent tool to understand the relationships between the feeding and reproductive cycles of benthic organisms and environmental factors in the water column [25, 26]. On the other hand, environmental changes are reflected in cellular signals[27].

Carbohydrates are used by mollusks as energy reserves that are metabolized into lipids during egg maturation. Protein and lipid reserves are also built mainly in the non-mantle tissue.

Seasonal changes in the biochemical composition may be of great importance in relation to (anaerobic) energy metabolism.

However, the fat content and the fatty acid composition of sea organisms are not constant. They are related to the life cycle and external factors, like temperature, salinity, and nutrition. The geographical origin of the organisms is an additional factor that affects the lipid content and the fatty acid composition of sea organisms[28].

CONCLUSIONS

1. Seawater registered high values for dry weight, organic substance, ash and protein content during the autumn season and are not correlated with biochemical accumulation in mollusks.

2. Marine sediments had a high accumulation of dry weight, organic substance and ash during the summer and are correlated with biochemical accumulation in marine mollusks.
3. The biochemical composition of *Mytillus galloprovincialis* and *Rapana venosa* showed high values for dry weight, organic substance protein and carbohydrate content for summer period in all four areas designated for growth and economic exploitation of mollusks.
4. Because of the low salinity (<12‰) in the northern part of the Romanian Black Sea coast, *M. galloprovincialis* has sometimes low densities.

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