BIOCHEMISTRY AND HEAVY METAL VALUES IN SOME GOBIIDAE SPECIES FROM THE ROMANIAN BLACK SEA COAST

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ABSTRACT

Gobiidae species are valuable for artisan fishing on the Romanian Black Sea coast. The aim of this study was the analysis of biochemical, energy and heavy metal values of three Gobiidae species: *Mesogobius batrachocephalus*, *Apollonia melanostomus* and *Neogobius cephalarges*. The results provided high values of proteins in the fish meat. Gobiidae females presented higher levels of lipids and energy values compared to the males. Among the analysed organs, the liver contained a high percentage of proteins, glucides and lipids. Heavy metals concentrations presented no significant differences between sampling stations, species and genders. Among the analysed metals, copper registered the highest values at the gobies. The heavy metal concentrations of the Gobiidae and their prey were significantly lower than those of the water and the sediments in the studied area.

KEY WORDS : Black Sea, Gobiidae, biochemistry, heavy metals

INTRODUCTION

Over the last decades, severe changes of the marine ecosystems structure resulted from anthropogenic pressure increase in the northwest area of the Black Sea. The Romanian coastal area is influenced by river inputs and coastal activities. The increase of heavy metals, resulted from domestic and industrial wastewater discharge, in the marine environment represents one of the most severe types of pollution. The majority of metals are natural components of the marine environment. However, anthropogenic activities determined an increase in heavy metals inputs on the Romanian coast (BAVARU *et al*., 2005). The Black Sea receives annually 60 tones of mercury, 240 tones of cadmium, 4000 tones of lead, 900 tones of chrome from the Danube River. The copper level in the Black Sea is 2-3 times higher than in the Mediterranean Sea (KONOVALOV, 1992). In the north area of the Romanian coast, the main source of pollution is represented by the Danube River, while the south area, situated between Capul Midia and Vama Veche, is affected by anthropogenic urban and harbour activities. The heavy metals tend to bioaccumulate along the trophic chains with negative consequences on the marine organisms.

The Black Sea studies on heavy metal levels started in 1975 and continued over the last two decades (MIHNEA *et al*., 1982; MIHNEA & PECHEANU, 1986, 1988; PECHEANU, 1998; PECHEANU & MIHNEA, 1986; MIHALCESCU, 2005). Most of these works treated heavy metals accumulation in molluscs and crustaceans and only a few studies concerned fishes (PECHEANU, 1998).

In the north-western Black Sea, Gobiidae represent the object of the artisan and commercial fishing performed in light watercrafts, equipped or not with engines, round turn and fishing lines, as well as seines. Within 2000-2007, the total gobies capture on the Romanian coast was 368 tones, with a maximum level in 2005 and a minimum one in 2007. This capture represented only 2.6% of the entire fish capture in the Romanian coastal area in that period.

This work presents a comparative evaluation of the biochemical value and the accumulation of heavy metals in three Gobiidae species: *Mesogobius batrachocephalus* (Pallas, 1814), *Apollonia melanostomus* (Pallas, 1814) and *Neogobius cephalarges* (Pallas, 1814) from the southern area of the Romanian Black Sea coast. The content in mineral residuum, proteins, lipids, glucides was analysed and reported to dry substance. Additionally, an evaluation of the level of organic substance and the energetic equivalent was performed. Based on the biochemical analyses results in muscle tissue, liver, milt or spawn, differences according to species, gender and area were exhibited.

MATERIALS AND METHODS

Gobiidae samples were collected in December 2006 and January 2007, by fishing with round turn and fishing lines in the southern area of the Romanian coast, in 23 August and Mangalia stations. Totally, 350 gobies, 263 males and 87 females were analysed.

The results of the biochemistry analyses concerned only *Mesogobius* sp. and *Apollonia* sp., whereas the results on heavy metal composition were acquired for each of the three species of gobies analysed:

- 86 specimens (54 males and 32 females) of *Mesogobius batrachocephalus* (knout goby) with the length and the weight of specimens ranging between 17.5-29.0 cm / 59.4-263.8 g for males and 16.1-29.6 cm / 43.4-245.2 g for females;
- 174 specimens (159 males and 15 females) of *Apollonia melanostomus* (round goby), with length and the weight of specimens ranging between 10.9-19.3 cm / 29.9-85.4 g for males and 11.0-15.0 cm / 20.4-52.9 g for females;
- 90 specimens (50 males and 40 females) of *Neogobius cephalarges* (mushroom goby), with length and the weight of specimens ranging between 10.6–19.0 cm / 14.9-114.4 g for males and 11.8-27.0 cm / 20.4-167.5 g for females.

Samples were sorted according to areas, species and genders. Fishes were dissected and liver, milt, spawn, as well as some muscle fillets were used for analysis.

The samples were analysed by current methods applied in the Measurement and Analysis Laboratory (LMA) of “Grigore Antipa” NIMRD, Constantza, as follows:

- total number of proteins, by multiplication of total N by factor 6.25 - Kjeldahl method,
- total number of lipids by means of their extraction - Keate method,
- total number of glucides - Dumas method.

The total content of organic substance was acquired by subtraction of the organic substance from the mineral residuum, whereas the energetic equivalent was calculated by multiplication of the protein, lipid and glucides concentrations by factors 4.5, 9.3 and 4.0.

The concentrations of five heavy metals with toxic potential on fishes: Cu, Cd, Ni, Pb and Cr were measured in Gobiidae muscle tissue, as well as in the surface sea water and the superficial sediments samples (5m and 20m depth). The values were expressed in % µg/g of fresh material. The analyses on the heavy metal composition were conducted in “Grigore Antipa” NIMRD Constantza laboratories by atomic absorption spectrophotometry (type ATI -

UNICAM 939 Z SOLAR with graphite cuvette and ZEEMAN background correction cells). Biological samples were processed by dry mineralization method in Teflon bombs on a hot plate (105^0C), in concentrated nitric acid. The mineralization process lasts until the resulted substance becomes transparent. In this phase, the residuum was used again in diluted solution of nitric acid 1N. It was filtered and finally put in the volumetric flask in order to be read at the atomic absorption spectrophotometer. This process is completely automated from the introduction of the samples up to the graphic interpretation of the results. The concentration was determined using a standard solution with known concentration.

The results acquired were statistically processed by Statistica 6.1. programme. The normality and the homogeneity characteristics of dataset variability were verified by Student and Levene tests. The effects of the analysed factors: sampling stations, species, genders and organs/tissues on protein, lipid, glucide and energy values, as well as studied compartments (water-sediment) and water depth factors were tested by 1 way ANOVA test (when Levene was negative) and by non-parametric Kruskal-Wallis test (when Levene was positive) and the significant different means were compared to the Newman-Keuls test.

RESULTS

Biochemistry

The biochemistry analyses were conducted on dry substance which ranged between 21.7% and 70.7%. The results showed a percentage of 95.4-97.8% of organic substance and 2.2-5.8% of inorganic residuum. Proteins represented the largest part of organic substance 75.9-94.0%, while lipids represented 2.5-10.8% and glucides only 0.4-1.7%. The energy value of Gobiidae ranged between 370 and 530 Kcal/g.

Statistical tests proved no significant differences concerning the biochemical parameters (proteins, lipids and glucides) and the energy values between the two sampling stations (23 August and Mangalia), nor between the two species studied for their biochemical composition (*Mesogobius* sp. and *Apollonia* sp.) ($p > 0.05$) (Table 1).

Table 1

Biochemical analyses results of *M. batrachocephalus* and *A. melanostomus* in Mangalia sampling area

Samples	**Analytical results**						**Energy equivalent**
	Dry substance %	**Mineral residuum %**	**Organic substance %**	**Total proteins %**	**Total lipids %**	**Total glucides %**	**Kcal/g (100g/SU)**
	Mesogobius batrachocephalus - males						
Muscle	21.7±1.0	2.2±0.1	97.8±1.0	75.9±1.0	2.5±0.5	0.9±0.1	370±20
Liver	45.5±1.0	5.8±0.1	94.2±1.0	86.2±1.0	3.4±1.0	1.5±0.1	426±20
Milt	30.3±1.0	3.5±0.1	96.5±1.0	90.7±1.0	2.8±1.0	0.7±0.2	420±20
	Mesogobius batrachocephalus - females						
Muscle	25.0±1.0	2.4±0.1	97.6±1.0	85.5±1.0	5.5±1.0	0.7±0.1	440±20
Liver	48.2±1.0	3.8±0.1	96.3±1.0	92.5±1.0	10.5±0.5	1.6±0.1	520±20
Spawn	60.1±1.0	2.5±0.1	97.5±1.0	88.5±1.0	9.8±0.5	0.4±0.1	490±20
	Apollonia melanostomus - males						
Muscle	24.8±1.0	3.5±0.1	97.5±1.0	78.0±1.0	4.0±0.1	1.3±0.1	393±20
Liver	66.0±1.0	4.4±0.1	95.4±1.0	88.0±1.0	6.5±0.1	1.5±0.1	462±20
Milt	31.0±1.0	3.1±0.1	96.9±1.0	92.0±1.0	3.2±0.1	0.5±0.1	455±20
	Apollonia melanostomus - females						
Muscle	24.0±1.0	3.0±0.1	97.0±1.0	86.0±1.0	6.8±0.1	1.4±0.1	455±20
Liver	59.0±1.0	4.0±0.1	96.0±1.0	94.0±1.0	10.8±0.1	1.7±0.1	530±20
Spawn	70.7±1.0	3.5±0.1	96.5±1.0	90.0±1.0	6.7±0.1	0.4±0.1	470±20

Differences were observed between genders only for lipids ($F = 29.0$; $p < 0.001$) and energy values ($F = 14.9$; $p < 0.001$) which presented higher levels at females. The medium percentage of lipids for females was 7.6%, whereas for males was 3.6%. The energy value of the goby females was 470 Kcal/g, higher than that of the males which was 418 Kcal/g. The concentrations of proteins and glucides did not differ significantly according to gender.

The analysed organs and tissues presented significant differences in protein levels which were higher in milt, liver and spawn than in muscles ($F = 12.1$; $p < 0.001$). Lipids presented higher values in liver, spawn and muscles than in milt ($F = 4,2$; $p < 0.05$). The higher glucide concentrations were found in liver and the lowest ones in milt and spawn, whereas the muscle values were medium ($F = 23.3$; $p< 0.001$).

Heavy metals

Data statistical analyses proved that there were no significant differences in heavy metals at gobies according to the analysed species, gender and sampling stations (Table 2). Therefore, the global results are presented as minimum, maximum and average values for all analysed gobies.

Table 2

The results of the statistical tests on heavy metal variations according to sampling stations, species and genders in gobies from the Romanian Black Sea southern coast. F = ANOVA test; H = non-parametric Kruskal-Wallis test ; ns = non-significant ($p > 0.05$)

	Stations	**Species**	**Gender**
Cu	F = 0.3; ns	F = 0.2; ns	F = 0.1; ns
Cd	H = 1.1; ns	H = 4.1; ns	H = 0.2; ns
Ni	F = 0.1; ns	F = 0.6; ns	F = 0.6; ns
Pb	F = 1.0; ns	F = 0.4; ns	F = 1.0; ns
Cr	H = 0.1; ns	H = 4.6; ns	H = 1.0; ns

Table 3

Minimum, maximum, average and standard deviations of heavy metal concentrations for Romanian southern coast gobies (winter 2006 and 2007)

	Minimum/Maximum % µg/g	**Average % µg/g**
Cu	1.89 – 4.46	2.86 ± 0.98
Cd	0.00 – 0.11	0.05 ± 0.04
Ni	0.31 – 0.88	0.45 ± 0.19
Pb	0.01 – 0.04	0.01 ± 0.01
Cr	0.22 – 0.87	0.41 ± 0.23

The analysis of the heavy metal concentrations in the gobies from the Romanian southern coast area exhibited high values for copper, whereas the cadmium and lead levels were the lowest and the nickel and chrome values were medium (Table 3). In order to explain the values of the heavy metal concentrations in the analysed gobies, supplementary analyses were conducted on water, sediment and mussel (*Mytilus galloprovincialis*) - the principal prey of the gobies in the studied area. The results showed high values of heavy metal concentrations in water and sediments (Fig. 1). These values presented neither significant differences between the water and sediment, nor according to depth (5m and 20m) (Table 4). The heavy metal concentrations for *Mytilus* and Gobiidae were lower than those of the water and sediment (Fig. 1).

Table 4

Results of the statistical tests on heavy metal concentrations according to the compartment analysed (water and sediments) and depth (5 and 20 m) in Magalia sampling station (2006 and 2007 winter). F = ANOVA test ; H = non-parametric Kruskal-Wallis test; ns = insignificant $p > 0.05$

	Compartment	**Depth**
Cu	F = 0.5; ns	F = 1.0; ns
Cd	F = 2.3; ns	F = 0.0; ns
Ni	H = 3.0; ns	H = 1.3; ns
Pb	H = 1.3; ns	H = 2.1; ns
Cr	F = 3.0; ns	F = 0.7; ns

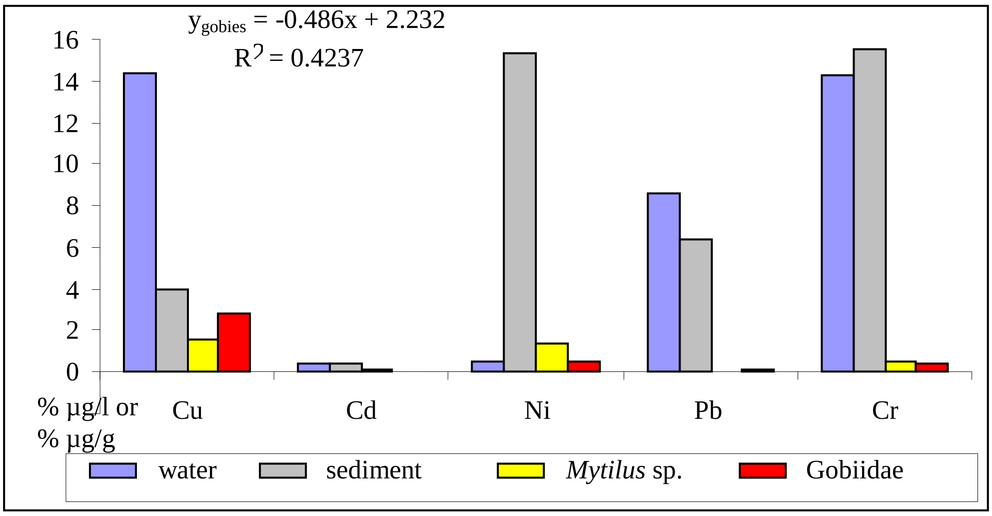

Fig. 1 - Graphic representation of heavy metal concentrations in water, sediments, mussels (*Mytilus galloprovincialis*) and Gobiidae in Mangalia sampling station (winter 2006-2007)

DISCUSSION

Gobiidae, even though fished only by artisan fishing are appreciated for their white and tasteful meat. This justifies the interest of this study on biochemical, energy and heavy metal values of the gobies from the Romanian Black Sea coast.

The comparative study of the biochemical and heavy metal composition of *Mesogobius batrachocephalus*, *Apollonia melanostomus* and *Neogobius cephalarges* proved that these species (the most frequent ones in the studied area) did not present specific differences; therefore, we may generally refer to Gobiidae. These species belong to similar ecological and trophic niches, which may explain the absence of specific differences. Gobiidae species are carnivorous fishes with a trophic level ranging between 2.6 and 3.3, feeding mainly on *Mytilus galloprovincialis*, but also on other invertebrates (*Mytilaster*, *Cerastoderma*, *Gammarus*, decapods) and fish (sprat) (BĂNARU *et al*., 2006 ; CREŢEANU & VASILESCU, 2006).

The two sampling stations, 23 August and Mangalia, are geographically close and present similar ecological conditions. This may explain the lack of significant differences concerning the biochemical and heavy metal values in gobies between the two sampling stations.

The muscle and the analysed organs of the Gobiidae contained high levels of proteins (seldom over 80%). Gobiidae presented a low level of lipids and glucides, and also a low caloric value and therefore it may be recommended as diet meat. Gobiidae females showed higher quantities of lipids and the energy values than males, as females fed more intense and prepared the spring reproduction period.

Analysed organs and tissues presented biochemical differences according to their role and metabolic activities. Protein levels in muscles were lower than in gametes (spawn and milt) and in liver, whereas the quantities of lipids and glucides in muscles were high in order to provide a supported energetic activity. Spawn and milt, which produce the future embryos, presented the highest protein deposits. The liver, with an important role in the digestive and metabolic activities, was rich in proteins, lipids and glucides.

Live organisms need for survival small quantities of essential heavy metals (cobalt, copper, iron, magnesium, strontium, zinc, molybdenum). High levels of essential metal concentrations in live organisms may produce strong disequilibrium and the presence of the non-essential metals (Cd, Cr, Pb, Hg, As etc) is harmful. Water, sediment and invertebrates represent sources of heavy metals for marine fishes. Fishes prey represent the main heavy metal sources. Gobies, situated at a superior level in the trophic chain, consumers of marine invertebrate are supposed to concentrate important heavy metal quantities by bio-accumulation (MIHALCESCU, 2005).

The results of this study showed the highest values for copper and the lowest one for cadmium and lead, whereas the nickel and chrome concentrations were medium. These results are similar to those presented by NIMRD in the report concerning the environment factors of the studied area (www.rmri). According to the same report, the highest heavy metal concentrations are registered in sediments (Cr, Ni, Pb), then in water (Cu, Cr) and the lowest ones are found in *Mytilus galloprovincialis* (Cu, Ni) (Fig. 2).

Traditionally, in oceans and seas, organisms accumulate heavy metals from the water and from the sediments and concentrate them by biomagnification and bioconcentration (RAND *et al.,* 1995). Biomagnification is the process where xenobiotic substances are transferred from food to an organism resulting in higher concentrations compared with the source (RAND *et al.,* 1995). Of studies on metals only organic mercury shows biomagnification and most metals are regulated and excreted and do not biomagnify (GRAY, 2002). Bioconcentration (uptake from the surrounding water) is the most usual way that organic compounds are accumulated in organisms from invertebrates to and including fish (RAND *et al.,* 1995). High trophic level predators have generally higher heavy metal values than the water and the sediments. According JOIRIS *et al.* (1995), primary production ('biomass effect') can influence pollutants' level, so that low environmental contamination, coupled with low primary production, can lead to high concentration in particulate matter and thus cause a high contamination of higher trophic levels.

In the northwest area of the Black Sea, a paradoxical phenomenon of heavy metal concentrations dilution between water-sediments and high trophic level organisms was evidenced by the present study. The heavy metal concentrations in water and sediments were much higher than in bivalves (*Mytilus* sp.) and Gobiidae. This characteristic may be explained by the frequent phytoplankton booms which assimilate the heavy metals, deposit and bury them in deeper sediment layers. As a result, they are no longer available to marine organisms for bio-concentration in the trophic chains. The special hydrological conditions in the Black Sea, with limited aerobic surface water mass and an important deeper anoxic zone, might be the reason for an important sink of organic particulate matter with its pollutants' load (JOIRIS *et al.,* 2001). Such phenomena could be the explanation for the generally low contamination levels in Romanian Black Sea Gobiidae and their prey.

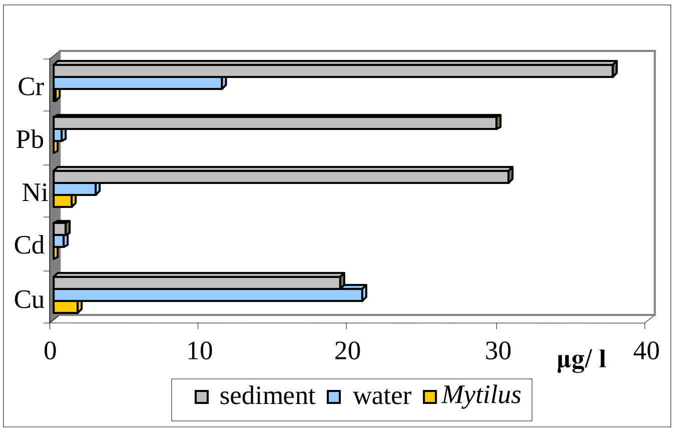

Fig. 2 - The medium concentration values of heavy metals in water, sediments and *Mytilus galloprovincialis* on the Romanian Black Sea coast in 2006 (NIMRD, 2006)

However, changes may occur in the functioning of this system as the reduction of phytoplankton blooms followed by the reduction of the heavy metal blocking in the sediments. This may increase heavy metal bio-availability and bio-accumulation in marine invertebrates and Gobiidae. Measures to reduce the heavy metal pollution of marine water and sediment are necessary, as well as a permanent monitoring of these metals concentrations evolution in water, sediments and marine organisms.

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