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ARSENIC CONTENT IN THE PARASITE-HOST SYSTEMS: *POMPHORHYNCHUS LAEVIS-ABRAMIS BRAMA* AND *ACANTHOCEPHALUS LUCII-ABRAMIS BRAMA*

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Abstract

During the ecological study of 31 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) from Danube River, by applying standard techniques for parasites, an infestation was found with two acanthocephalan species – Pomphorhynchus laevis and Acanthocephalus lucii. The content of arsenic in water, sediments, parasites, tissues and organs of Abramis brama (infected and uninfected) were establishedin the present study. P. laevis showed 42-170 times higher content of arsenic than its host tissues and organs. The content of arsenic in A. lucii was 37-205 times higher than the content of arsenic in its host tissues and organs. The highest concentration of arsenic in uninfected specimens of A. brama was found in liver (1.35±1.29 mg.kg^{-1}). The highest concentration of arsenic for the infected with P. laevis specimens of freshwater bream was established for muscles (0.68±0.08mg.kg^{-1}), while for the infected with A. lucii specimens of fish, the highest concentration of arsenic was established for skin (1.75±1.32 mg.kg^{-1}). A highly significant correlation (p<0.01) was fixed for the relationship between $C_{As/P.\ laevis}$–$C_{As/Muscles}$.

Key words:*Abramis brama;Acanthocephalus lucii; arsenic;Danube River;Pomphorhynchus laevis.*

INTRODUCTION

The concentration of heavy metals in fish tissues and their parasites was studied by many authors (Turčekova et al., 2002; Kirin et al., 2014; Brázová et al., 2015; etc.). It was established that certain parasites had high accumulation possibilities and thus they could be used as bioindicators of heavy metals (Sures et al., 1997; Tenora et al., 2000; Thielen et al., 2004). This study aims to present the results of examinations of arsenic contents in water, sediments, *Pomphorhynchus laevis*, *Acanthocephalus lucii*, skin, muscles and liver of infected and uninfectedwith acanthocephalans specimens of freshwater bream from the Bulgarian part of the Danube River.

MATERIALS AND METHODS

In 2016, water, sediments, fish and fish parasites werecollected and examined from the Lower Danube River (village of Vetren, Bulgaria; 44^{0}133'N, 27^{0}033'E, Figure 1).

A total of 16 uninfected and 15 infected with acantocephalan specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) were collected and examined.

The fish samples were examined immediately after their capture for gastrointestinal parasites using standard techniques.

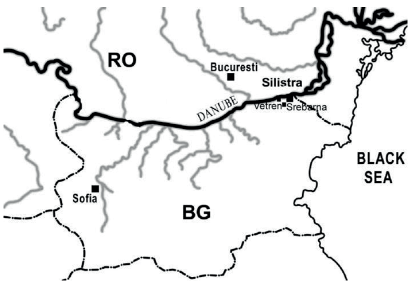

Figure 1. Danube River

The established acanthocephalan species were examined as temporary slides in ethanol-glycerin and identified (Petrochenko,1956; Bykhovskaya-Pavlovskaya, 1985). The ecological terms wereused and calculated (prevalence, mean intensity (MI) and mean abundance (MA), based on Bush et al. (1997). The dominant structure of the component helminth communities was determined based on the prevalence (P%) as: accidental (P% < 10), component (P% < 20) and core (P% >20) species according to the criteria proposed by Kennedy (1993).

The freshwater bream (*Abramis brama* (Linnaeus, 1758)) specimens chosen for the examination of arsenic (As) content in this study were weighed (total weight of the fish ranging from 24to 291 g) and measured (total length ranging from 13 to 26.5 cm). Samples of muscles, skin and liver were collected from all specimens of *A. brama*. The freshwater bream is freshwater, brackish, benthopelagic, potamodromous fish species that inhabit a wide variety of lakes and large to medium-sized rivers (Fröse and Pauly, 2020). It is estimated as least concern species (LC=Least Concern; IUCN Red List Status) and is not included in the Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011). The samples of water, sediment, fish tissues, organs and parasites were analyzed for content of arsenic (As) by the Inductively Coupled Plasma (ICP) Spectrometry.

In order to determine the relative accumulation capability of the fish tissues and parasites in comparison with water and sediments, bioconcentrationfactors (BCF=[C host/parasites tissues]/[C water/sediments] were calculated (Sures et al., 1999). The bioconcentration factors were used for the estimation of trace metal pollution in the freshwater ecosystem through the examined fish and their parasites. The differences in concentration factors are discussed with respect to the bioavailability of arsenic from water and sediments. In order to determine the relative accumulation capability of the parasites and the host tissues, bioaccumulation factors (BAF=[C parasite]/[C host tissues]) were calculated. A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between parasites and their host tissues and organs.

RESULTS AND DISCUSSIONS

A total of 31 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) were collected and examined for parasites from the Danube River. Helminth parasites were recorded in 15 specimens of *A. brama* (48.39 %). Five specimens from the examined freshwater breams were infected with the acantocephalan *Pomphorhynchus laevis* (Table 1). *P. laevis* develops with the participation of an intermediate host – *Gamarus pulex* (Amphipoda), (Petrochenko, 1956).

Ten specimens from the examined *A. brama* were infected with the acantocephalan *Acanthocephalus lucii* (Table 1).

The intermediate host of *A. lucii* is *Asellus aquaticus,* and definitive hosts are fish species of different families as Cyprinidae, Salmonidae, Percidae, Anguillidae and others(Kakacheva-Avramova, 1983). Both acantocephalan species are autogenic, matured in fish.

The component community of freshwater bream from Danube River is presented only of acanthocephalan specimens. In the component community of *Abramis brama* from the Danube River, *A. lucii* (P%=32.26) is core species, and *P. laevis* (P%=16.13) is component parasite species for the helminth communities of *A. brama* (Table 1).

Table 1. Ecological indices of the helminth parasites of *A. brama* from the Danube River
(N – number of examined fish specimens, n – number of infected hosts, p – number of parasites, P% – prevalence, MA – mean abundance, MI – mean intensity)

Helminth species	N	n	p	P%	MA±SD	MI±SD	Range
Pomphorhynchus laevis (Zoega in Müller, 1776)	31	5	14	16.13	0.45±1.13	2.8±1.17	1-4
Acanhocephalus lucii (Müller, 1776)	31	10	19	32.26	0.61±1.04	1.9±0.94	1-4

Species richness in infracommunity of freshwater bream ranges from 0 to 1 species. Fifteen fishes (48.39%) were infected with one helminth species. The largest number of helminth specimens established in a single host specimen is 4. The average species richness (mean number of species for fish specimen) in the infracommunity of freshwater bream is 0.48±0.5 species.

The content of arsenic in samples of sediments was much higher than the content of arsenic in samples of water from the examined freshwater ecosystem – Biotope Vetren on the Danube River. From the tissues and organs of uninfected specimens of fish, the highest content of arsenic was determined in samples of liver, followed by those of skin and muscles. The highest bioconcentration factor (BCF) was in the liver, followed by the one for the skin and muscles (Table 2).

Table 2. Content of arsenic ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of arsenic in tissues and organs of uninfected *A. brama* and water and sediments

A.brama	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	1.35±1.29	C_{Liver}/C_{water}	103.85	$C_{Liver}/C_{Sediments}$	0.235
Muscles	0.41±0.17	$C_{Muscles}/C_{water}$	31.54	$C_{Muscles}/C_{Sediments}$	0.071
Skin	0.46±0.16	C_{Skin}/C_{water}	35.38	$C_{Skin}/C_{Sediments}$	0.080
River Danube		Water ($mg.l^{-1}$)	0.013	Sediments ($mg.kg^{-1}$)	5.74

Data are present as mean±standard deviation (SD)

From the tissues and organs of freshwater breams infected with *P. laevis* the highest content of arsenic was determined in samples of muscles, followed by those of liver and skin. The acanthocephalan *P. laevis* showed higher content of arsenic than its host *A. brama*. The highest bioconcentration factor (BCF) was in *P. laevis,* followed by the one in the muscles, liver and skin (Table 3).

Table 3. Content of arsenic ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of arsenic in tissues, organs of *A. brama*and its parasite *P. laevis* and water and sediments

A. brama/P. laevis	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.39±0.10	C_{Liver}/C_{water}	30.00	$C_{Liver}/C_{Sediments}$	0.068
Muscles	0.68±0.08	$C_{Muscles}/C_{water}$	52.31	$C_{Muscles}/C_{Sediments}$	0.12
Skin	0.17±0.002	C_{Skin}/C_{water}	13.08	$C_{Skin}/C_{Sediments}$	0.03
P. laevis	29.01±0.97	$C_{P. laevis}/C_{water}$	2231.54	$C_{P. laevis}/C_{Sediments}$	5.06
River Danube		Water ($mg.l^{-1}$)	0.013	Sediments ($mg.kg^{-1}$)	5.74

Data are present as mean±standard deviation (SD)

From the tissues and organs of freshwater breams infected with *A. lucii* the highest content of arsenic was determined in the samples of skin, followed by those of liver and muscles. The acanthocephalan *A. lucii* showed significantly higher content of arsenic than its host *A. brama*. The highest bioconcentration factor (BCF) was for *A. lucii*, followed by the one in the skin, liver and muscles (Table 4). The highest content of arsenic in the liver was determined in samples of uninfected *A. brama* ($C_{As/Liver}$=1.35±1.29), followed by those of liver of *A. brama* infected with *A. lucii* ($C_{As/Liver}$=0.57±0.22) and infected with *P. laevis* ($C_{As/Liver}$=0.39±0.10). The highest content of arsenic in muscles was determined in samples of *A. brama* infected with *P. laevis* ($C_{As/Muscles}$=0.68±0.08), followed by those of muscles of uninfected *A. brama* ($C_{As/Muscles}$=0.41±0.17) and infected with *A. lucii* ($C_{As/Muscles}$=0.32±0.20). The highest content of arsenic in the skin was determined in samples of *A. brama* infected with *A. lucii* ($C_{As/Skin}$=1.75±1.32), followed by those of skin of uninfected *A. brama* ($C_{As/Skin}$=0.46±0.16) and infected with *P. laevis* ($C_{As/Skin}$=0.17±0.02).

Table 4. Content of arsenic ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of arsenic in tissues, organs of *A. brama* and its parasite, *A. lucii*, and water and sediments

A. brama/ A. lucii	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.57±0.22	C_{Liver}/C_{water}	43.85	$C_{Liver}/C_{Sediments}$	0.099
Muscles	0.32±0.20	$C_{Muscles}/C_{water}$	24.62	$C_{Muscles}/C_{Sediments}$	0.056
Skin	1.75±1.32	C_{Skin}/C_{water}	134.62	$C_{Skin}/C_{Sediments}$	0.305
A. lucii	65.78±0.97	$C_{A. lucii}/C_{water}$	5060.0	$C_{A. lucii}/C_{Sediments}$	11.46
River Danube		Water ($mg.l^{-1}$)	0.013	Sediments ($mg.kg^{-1}$)	5.74

Data are present as mean±standard deviation (SD)

The highest bioaccumulation factor (BAF) established for *P. laevis* was found inthe skin, followed by the one in the liver and muscles.

Regarding *A. lucii,* the highest bioaccumulation factor (BAF) was in the muscles, followed by the one in the liver and skin (Table 5).

Table 5. Bioaccumulation factors (BAF= [Cparasite]/[C host tissues]) of *P. laevis* and *A. lucii*

P. laevis/A. brama	BAF	*A. lucii /A. brama*	BAF
$C_{P.\ laevis}/C_{Liver}$	74.38	$C_{A.\ lucii}/C_{Liver}$	150.40
$C_{P.\ laevis}/C_{Muscles}$	42.61	$C_{A.\ lucii}/C_{Muscles}$	205.56
$C_{P.\ laevis}/C_{Skin}$	170.65	$C_{A.\ lucii}/C_{Skin}$	37.59

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between *P. laevis* and fish tissues and organs, and between *A. lucii* and fish tissues and organs. A highly significant correlation ($p<0.01$) was fixed for the relationship between $C_{As/P.\ laevis}$– $C_{As/Muscles}$.

In general, the content of arsenic in tissues and organs of infected and uninfected specimens of *A. brama* decreased in a different order. For the uninfected specimens of *A. brama,* the order is: $C_{As/Liver}>C_{As/Skin}>C_{As/Muscles}$, for the specimens infected with *P. laevis* the order is $C_{As/Muscles}>C_{As/Liver}>C_{As/Skin}$, and for the ones infected with *A. lucii* it is: $C_{As/Skin}>C_{As/Liver}>C_{As/Muscles}$. The values of the bioaccumulation factor for *P. laevis* were the highest for skin and ranged as follows: $BAF_{Skin}>BAF_{Liver}>BAF_{Muscles}$. The values of the bioaccumulation factor for *A. lucii* were the highest for muscles and ranged as follows: $BAF_{Muscles}>BAF_{Liver}>BAF_{Skin}$.

In the scientific papers, there are a relatively small number of researches regarding the arsenic content in *A. brama* from the Danube River.

For example, Jovičić et al. (2015) studied the concentrations of 11 elements in tissues and organs of four commercial fish species from the Danube River (Serbia). They reported that the arsenic concentrations were in muscles– 0.325±0.145 μg g^{-1} dry weight and in the liver – 7.522±4.008μg g^{-1} dry weight of *A. brama*. Zrnčić et al. (2013) studied the heavy metal contamination of fish in the Croatian part of the Danube River. In their study, the concentration of arsenic in muscles of *A. brama* was 0.035±0.024 microgram per dry weight.

Kirin et al. (2014) studied the concentration of Pb, Zn and Cu in muscles, liver, intestine and bones of *A. brama* and its parasite *Pomphorhynchus tereticollis* from the Danube River, Bulgaria. In their study, highly significant correlations were determined for *P. tereticollis* as a sensitive indicator of Pb, Cu and Zn.

The ability of *Acanthocephalus lucii* to accumulate heavy metals was studied mostly as a parasite of *Perca fluviatilis* (Sures et al., 1994a; Sures et al., 1997a; Turčeková et al., 2002; Jankovská et al., 2011; Jankovská et al., 2012).

In the scientific papers, there are studies on how two helminth species from the same fish host accumulate heavy metals and impact their concentrations in host tissues, or they compare to the concentrations in host tissues. Some of them refer to acanthocephalans and cestodes (Turčekova et al., 2002; Brázová et al., 2015) and some to acanthocephalans and nematodes (Sures et al., 1994b; Nachev et al. 2013). Our research did not reveal studies for two acanthocephalan species from the same fish host. The studies mentioned above were based on parasite species that were either in different localization in the host body or were in different development stages. In this study, the investigated species (*A. lucii* and *P. laevis*) were in the same localization and development stage, which is why it is not possible to make a comparison with them. The obtained results for the differences in accumulation of arsenic for *P. laevis* and *A. lucii* from the same fish host (*A. brama*) might be explained with the differences in biology and selective capabilities to accumulate heavy metals between those two acanthocephalan species.

CONCLUSIONS

The highly significant correlation ($p<0.01$) fixed for the relationship between $C_{As/P.\ laevis}$– $C_{As/Muscles}$, determines *P. laevis* as a sensitive indicator for arsenic. The obtained results allow the two acanthocephalan species (*A. lucii* and *P. laevis*) to be proposed as useful for the evaluation of arsenic exposure in freshwater ecosystems. However, this should be confirmed with additional studies.

The results in the present study revealed that attention should be drawn not only to differences in bioaccumulation potential between the different parasite classes but also between the different species of the same parasite class (class *Acanthocephala*).

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