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CHANGES IN THE HEPATOSOMATIC INDEX AND CONDITION FACTOR IN THE POPULATIONS OF *PELOPHYLAX RIDIBUNDUS* (AMPHIBIA: RANIDAE) FROM ANTHROPOGENICALLY POLLUTED BIOTOPES IN SOUTHERN BULGARIA. PART II

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Abstract

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The values of the hepatosomatic indexes (HIS) and the condition factor (CF) in populations of *Pelophylax ridibundus* living in conditions of anthropogenic pollution in Southern Bulgaria were studied and analyzed. The studied populations inhabit three biotopes: a relatively clean (control) one and two contaminated ones (domestic sewage pollution and heavy metal pollution). It was established that in individuals of both sexes in conditions of contamination the relative weight of the liver increases. In contaminated biotopes the CF values are lower in individuals of both sexes in comparison with those of the control one.

Key words: Anthropogenic pollution, *Pelophylax ridibundus*, hepatosomatic indexs, condition factor

Introduction

The condition of the liver and of the whole body, as measured with the hepatosomatic index (HSI) and "Factor for body nutritional status" of individuals = condition factor (CF), can provide information on potential pollution impacts. HIS values in Anura populations have long been a tool for bioindicational analysis and there are a significant number of publications on this problem already (Loumbourdis and Wray, 1998; Papadimitriu and Loumbordis, 2003; Zhelev and Mollov, 2004; Misyura et al., 2008; Stolyar et al., 2008; Peskova and Zhelev, 2009; Păunescu and Ponepal, 2011; Zaripova and Fayzulin, 2012). In ichthyology, the so-called Fulton condition factor – FCF (Eastwood and Couture, 2002) is used to assess the overall health of fish populations, on the one hand, and the quality of the habitats, on the other. In recent years, researchers from different parts of the world successfully demonstrate the practical application of the "Factor for body nutritional status" of individuals in populations of different Anura species for bioindication and biomonitoring (Spirina, 2009; Jelodar and Fazli, 2012; Thammachoti et al., 2012). The leading idea in bioindicational analyses is to examine the hypothesis that in amphibian populations living in anthropogenically transformed environments, the values of CF are lower than in populations inhabiting control areas. A similar bioindicational analysis using Anura individuals as a test object has not been made in Bulgaria. This provides justification for the present study.

The aim of the work is to present the research results of values of the hepatosomatic indices and the condition factor in *Pelophylax ridibundus* populations inhabiting biotopes in Southern Bulgaria. The biotopes are with varying degrees and different types of anthropogenic pollution. This work is an integral part of the research conducted in the period 2009-2012 in biotopes along the two most polluted river ecosystems in Bulgaria: the rivers of Sazliyka and Topolnitsa. The research results are shown in Part 1 (Zhelev et al., 2014).

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Materials and Methods

The area of investigation

The samples for our study were collected in the spring of 2012 in three biotopes (for convenience labelled as 1, 2 and 3) located along the courses of two rivers in Southern Bulgaria: Sazliyka River in its top part near the village of Rakitnitsa – biotope 1, in its middle part in the region of the town of Radnevo – biotope 2 and Topolnitsa River near the village of Poibrene, after the flowing of Medetska River into the Topolnitsa water reservoir – biotope 3.

A physical map with the geographic coordinates of each of the studied biotopes is presented in Part I (Zhelev et al., 2014).

Data from physicochemical analysis in the studied water ecosystems

In the present study, biotope 1 is relatively clean (control), biotopes 2 and 3 are polluted. In the biotope 2 the main pollution is of sewage-domestic type, while biotope 3 is polluted with heavy metals. The data from the physical-chemical analysis of the water in each of biotopes 1, 2 and 3 according to the reports of the Basin Directorate for water management – East Aegean Region – Plovdiv, Ministry of the Environment and Waters (http://www.bg-ibr.org) are presented in Part 1 (Zhelev et al., 2014).

Subject of study and methods for analyses

The subject of our study was the marsh frog *P. ridibundus.* Part 1 of the study (Zhelev et al., 2014) presents information on the terms of collection of the animals, their total count, gender distribution and the analysis methodology for each of biotopes 1, 2 and 3.

All animals (of both sexes) used for the analyses were adults the Snout-Vent Length (SVL) > 60.0 mm (control: ♂6.76±0.10, ♀7.76±0.14), domestic sewage pollution: ♂7.53±0.18, ♀9.47±0.28 and heavy metal pollution: ♂6.71±0.13, ♀8.16±0.23), sexually mature.

Hepatosomatic index (HSI) was calculed as (LW/BW) x 100, where: LW – liver weight; BW – body weight.

For the animals from each biotope we calculated the "Factor for body nutritional status" of individuals = condition factor (CF) which is used in ichthyology, by the formula: (BW/SVL^3) x 10^2 proposed by Pauly (1983), also applicable for tailless amphibians and previously used in this frog species (Spirina, 2009; Jelodar and Fazli, 2012).

Statistical procedures

Mathematical data processing was performed using standard statistical procedures with STATISTIKA, Release 7.0 software (Statistika, 2004). The normalcy in the distribution and homogeneity of variance of the examined morphophysiological parameters was checked by using a Shapiro-Wilk test (Shapiro et al., 1968) that indicated normal distribution: $p > 0.05$. The statistical reliability of the differences in values of morphophysiological parameters and the value of the calculated condition factor received for individuals of both sexes in the biotopes compared was proved with a one-way ANOVA. LSD test was used as a post-hoc test. Results with $p < 0.05$ [$\alpha = 5\%$] were considered significant.

Results

Some of the basic statistical indicators of the two studied parameters (HIS and CF) in the populations of *P. ridibundus* are represented in Table 1.

For each of the two studied parameters we made a comparison to certify the statistically significant differences in *P. ridibundus* populations (post-hoc LSD test) from the three biotopes as follows: a) between individuals of both sexes in each of the three populations; b) between individuals of the respective sexes (♂/♂ and ♀/♀) from the populations in the polluted biotopes (2 and 3) and the control group (1); c) between individuals of the respective sexes from the populations in the biotopes with different type of toxins – 2 (domestic sewage polution) / 3 (heavy metal pollution). Our findings are presented below:

Liver

a) The hepatosomatic index did not show gender differences in population 1; in population 2, there were statistically reliable higher values in females (♀3.06±0.21/♂2.15±0.21,

Table 1
One-way ANOVA – Analysis of Variance (data: hepatosomatic index, condition factor) among populations *Pelophylax ridibundus* in the investigated biotopes in Southern Bulgaria

Parameters	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	P
HSI	296,6	5	59,321	329,25	196	1,6798	35,31347	0.00~1
CF	3154,1	5	630,821	4333,86	196	22,1115	28,52907	0.00~1

$p<0.01$), and in population 3 – in males (♂4.38±0.36 /♀3.27±0.31, $p<0.001$).

b) The values of HIS in male (1.00±0.38) and female (1.12±0.13) individuals from the control group (1) were statistically significantly lower than those of individuals in the respective gender in population 2 and 3 ($p<0.001$).

c) In the population from the biotope with heavy metal pollution (3), the value of HIS in males was statistically significantly higher than that in males from the biotope with domestic sewage pollution (2) ($p<0.001$); there was not reliable difference in the values of HIS in females from the two populations (Figure 1).

CF

a) In the population from the relatively clean biotope (♂19.38±0.89/♀12.71±0.68, $p<0.001$) and in that from the biotope with domestic sewage pollution (♂15.89±1.07/♀9.16±0.94), the value of CF is statistically reliably higher in male individuals. In the biotope with heavy metal pollution, the values of CF do not show statistically significant differences between individuals of both sexes.

b) In individuals of both sexes in the control group (1), the values of CF are statistically reliably higher than those of the individuals from the respective genders in the population from the anthropogenically polluted biotopes (2 and 3).

c) In the population from the biotope with heavy metal pollution, the value of CF in male individuals is statistically reliably lower (10.60±0.52) than that in males from biotope 2. The comparison between female individuals does not account statistically significant difference (Figure 2).

Discussion

The literature provides information on variations in the absolute and relative weight of the liver in amphibians inhabiting conditions of anthropogenic pollution. However, the diverse nature of the identified changes must be acknowledged.

The liver in Anura is the place where nutrients are stored, but also a place for accumulation and detoxification of xenobiotics (Crawshaw and Weinkle, 2000). Its mass changes according to the speed of processes of fat and hydrocarbon accumulation and decomposition (Schwartz et al., 1968), and in the Ranidae (Rafinesque-Schmaltz, 1814) family also depending on the respiratory conditions in the basin, at the expense of blood disposal (Frangioni and Borgioli, 1994). There are seasonal changes in liver weights of amphibians in relation to the changing nature of nutrition and energy costs in the process of reproduction in background territories (Brushko and Vashetko, 1974), as well as in living in biotopes with anthropogenic pollution (Zhelev and Mollov, 2004; Peskova and Zhelev, 2009; Jelodar and Fazli, 2012; Thammachoti et al., 2012). Generally, the literature data about the values of HSI in the family Ranidae form two groups: 1) The values of HIS in populations inhabiting relatively clean and anthropogenically polluted biotopes are the same or higher in amphibians than in clean biotopes and 2) HSI values are higher in amphibians from the contaminated biotopes. Among the main reasons that can explain the changes of HIS, which the first group of investigations assert, are the good nutritional base in the relatively clean waters and the depletion of glycogen stores with a subsequent mobilization of fat metabolism as a result of accelerating metabolism of long-term adverse

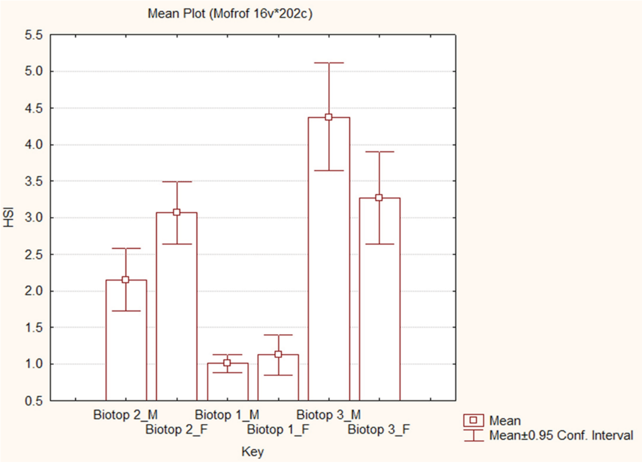

Fig. 1. Hepatosomatic index from the individuals (M – males, F – females) of *Pelophilax ridibundus* from populations in the investigated biotopes in southern Bulgaria

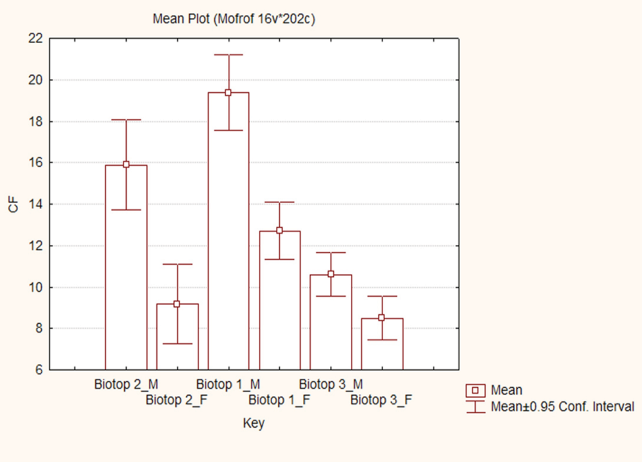

Fig. 2. Condition factor for the individuals (M – males, F – females) of *Pelophilax ridibundus* populations in the investigated biotopes in southern Bulgaria

effects of the environmental factors in the polluted biotopes (Zhukova and Kubancev, 1982; Kosareva and Vasiukov, 1976; Zhukova et al., 1986; Toktamysova, 2005; Spirina, 2009; Zaripova and Fayzulin, 2012). The second group of studies explain that the high values of HIS are caused by bioaccumulation of xenobiotics, mainly pesticides (Loumbourdis and Wray, 1998; Păunescu et al., 2008, 2009; Păunescu and Ponepal, 2011; Thammachoti et al., 2012), waste products of the chemical industry, phenols, oil, radioactive waste (Bolshakov et al., 1981; Misyura, 1985, 2006; Zhelev and Mollov, 2004; Misyura et al., 2007, 2008; Peskova and Zhelev, 2009), heavy metals (Loumbordis and Wray, 1998; Loumbordis and Vogiatzis, 2002; Papadimitriu and Loumbordis, 2003; Loumbordis et al., 2005; Wrobel et al., 2000; Sura et al., 2006; Stolyar et al., 2004, 2008; Marchenkovskaya, 2005; Zaripova et al., 2009; Karapetyan, et al., 2011; Jelodar and Fazli, 2012; Gürkan et al., 2014), as well as the related to this biochemical and histochemical changes in the organ. If living in conditions of pollution, in the family Ranidae there are increased average sizes of hepatocytes and nuclei in reduction of cytonuclear ratios (Pyastolova and Trubetskaya, 1989), tissue proliferation and reducing the amount of binucleated cells (Grigoryan and Karapetyan, 2006; Karapetyan et al., 2011), which is considered by the authors as a compensatory response to functioning of the organ in adverse conditions.

If the effects of toxins are chronical, the system for detoxication of the organ is activated: increased amounts of enzyme from the microsomal fraction – cytochromes P_{450} and B_5 (Misyura and Sporadets, 2005). According to Hamilton and Mehrle (1986), Loumbordis and Kyriakopoulou-Sklavounou (1991), Roesijadi (1992), Stoliar et al. (2008), fish and amphibian proteins have metal binding, such as liver metallothionein. These proteins bind metals causing liver to accumulate more Cu than other metal after Fe. Much of the above-mentioned authors opined that the increase in the values of HIS, as a unit of the overall mobilization of the body's defences aimed at disposing of toxins, can be regarded as a manifestation of adaptive response to the specific environmental conditions. This could hardly be accepted only because there are studies that show a completely different reaction of the organ to pollution of the environment with the same toxins, for example heavy metals – a decrease (Spirina, 2009; Zaripova and Fayzulin, 2012) or an increase in the values of HIS (Jelodar and Fazli, 2012). Herein, the course of pathological processes related to accumulation of heavy metals in the organ also has relation to the changes in the values of HIS. It is very likely the reaction of the liver in amphibians to pass through several stages, in accordance with the concentrations and length of exposure to toxins, and also the aforementioned studies that find a reduction in the values of HIS in permanent habitation in conditions of pollution, to indicate one of the final stages that are associated with a common energy exhaustion of the body. Our findings for population 3 from the biotope in southern Bulgaria come into line with that part of the literature data that refer to the role of heavy metals as a reason for higher values of HSI, while the high values of the index in population 2 cannot be uniquely related to the nature of pollution, although there are literature reports on the negative effects of nitrates and nitrites (part of pollutants in population 2) on the health of amphibians (Shinn et al., 2008; Mann et al., 2009). As confirmation of our proposition, namely that pollution is the reason for the increasing relative weight of the liver in the two populations from southern Bulgaria, are also the findings from our previous investigations in the same territories, in which we found serious violations in developmental stability of *P. rididundus* populations. There were statistically reliable increase, compared to the control from the relatively clean biotope, in the values of integral indicator for developmental stability – fluctuating asymmetry (Zhelev et al., 2012), as well as in the main haematological parameters: RBC, Hb, WBC, differential blood formula (Zhelev et al., 2013), which is particularly significant, as the liver in Anura is a major haemopoietic organ (Carmena, 1971). Such changes (increasing values of HSI and acute leukocytosis, but accompanied with anemia) as a result of acute chemical toxicosis have been reported for the treatment of *Clarias gariepinus* Burchell, 1822 (Gabriel et al., 2009) with aqueous extracts of leaves of *Lepidagathis alopecuroides* (Vahl) R. Br. ex Griseb and *P. ridibundus* with chronic doses of Roundup (Păunescu and Ponepal, 2011). We think that the increasing relative weight of the liver considered in our case is a manifestation of the body's reaction of adaptive nature, in a hostile environment with non-typical living conditions.

Our results for the values of CF reflect the deterioration of the general physical condition of individuals of both sexes in the populations of *P. ridibundus* which live in conditions of anthropogenic pollution. This could be due to the prevalence of individuals in these populations with accelerated level of metabolic processes, capable of bringing effectively eco-toxins, or due to deterioration of the nutritional base (insects and other invertebrates) in these biotopes. The first thesis explains betterthe changes in Population 2, where statistically significantly higher average BW values are measured – ♂61.54±1.62, ♀65.78±1.88. At the same time the second thesis is closer to the changes in Population 3 where average BW are the lowest – ♂31.43±1.53, ♀44.22±2.4, where the feature values in the control are – ♂56.91±1.89, ♀61.81±1.83 (F_5=42.646; p<0.001). Our data are consistent with the results established by other authors (Spirina, 2009; Jelodar and Fazli, 2012; Thammachoti et al., 2012). Basing on the results of our study, we support the idea that the negative human impact on the environment is one of the reasons for deterioration of environmental situation and the global reduction of the populations of Amphibians.

Conclusions

In *P. ridibundus* populations that inhabit anthropogenically polluted biotopes in Southern Bulgaria (whatever the nature of toxins is), in individuals of both sexes, the relative weight of liver increases. We believe that this is an adaptation to life in an environment with worsened life conditions.

In conditions of anthropogenic pollution, the overall physical condition of individuals of both sexes is deteriorated, and the degree of their "body nutritional status" is lower if compared to those inhabiting a relatively clean living environment.

Hepatosomatic indexs (HIS) and condition factor (CF) are informative parameters objectively reflecting the physiological state of amphibians in specific living environment conditions. They have their place as biomarkers in the biomonitoring system.

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