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Assessment of Ecological Status of Two Rivers with Different Types of Anthropogenic Pollution in Southern Bulgaria Based on the Level of Fluctuating Asymmetry in the Populations of Marsh Frog *Rana ridibunda* (Amphibia: Ranidae)

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Abstract: The degree of manifestation of the indicators of fluctuating asymmetry (FA) was studied in the populations of *Rana ridibunda* in two of the most polluted river ecosystems in Southern Bulgaria. The integral indicator of developmental stability, evaluation of the ecological condition parallel to the data of physicochemical analysis was performed. It was found that the variations in genetic homeostasis in the populations of *R. ridibunda* in a habitat under conditions of anthropogenic pollution occur independently on the type of toxicants, but the degree of violation expression in the form of FA depends on the nature of the pollution.

Key words: anthropogenic pollution, *Rana ridibunda*, toxicants, fluctuating asymmetry, South Bulgaria

Introduction

Fluctuating asymmetry (FA) is one of the modern and advanced methods of assessing the indicators of sustainable development in populations of amphibians. It provides an opportunity to identify the degree of disturbance in ecosystems exposed to anthropogenic influence, including pollution caused by human activities and evaluate them in an integral form (Van Valen 1962). Since FA reflects the changes in the state of the body during a long-term habitation in pollution conditions, it is a good illustration of the state of the biota and provides an objective assessment of the environmental state of the water (Zakharov *et al. 2000*). In this sense, the FA is an independent biomonitoring method, parallel to the physicochemical analysis of water, that reflects the state of the reservoir at the time of sampling and does not always catch those pollutants released by 'burst discharges' (even at high concentrations), especially from industrial enterprises.

It is known that the marsh frog *Rana ridibunda* Pallas, 1771 is a species highly resistant to anthropogenic influence (Vershinin 2007) and is able to live in water with high levels of pollution (Zhelev 2012). Symmetry breaking (in the form of FA), which is the result of environmental pollution in the lake, where there was the embryonic and larval development of amphibians, allows, using the manifestation of its parameters in the lake frog, the bioindication of the water body and the determination of the extent of its of anthropogenic pollution by catching animals (Peskova 2007).

The purpose of this paper is to identify both the degree of manifestation of FA indicators in the marsh frog populations living in two of the most polluted river ecosystems in Southern Bulgaria and, based on the integral index of rate stability, parallel to the physical and chemical analysis of the water, their ecological status.

Materials and Methods

Collection of material performed in the spring and summer of 2010, in habitats that were downstream (upper, middle and lower) of two rivers in southern Bulgaria with different types of anthropogenic pollution – the river Sazliyka (145.5 km) and river Topolnitsa (154.8 km) (Fig. 1). Sazliyka River (below the town of Stara Zagora) and Topolnitsa – one of the most polluted in Bulgaria (Irikov, Atanasova 2008). Sazliyka river is mainly contaminated by fecal household and industrial waters of Stara Zagora and Nova Zagora and respectively, through its left tributaries, the rivers Bedechka and Blatnitsa. Pollutants to the river Topolnitsa – copper mining plant 'Aurubis' (formerly 'Pirdop'), the mining and processing plant 'Asarel-Medet' and the tailing 'Chelopech Mining'.

In each of the studied reservoirs was conducted a random trapping of adult (L> 60 mm), sexually mature individuals *R. ridibunda*. The animals were caught in the evening with the use of an electric torch in the water and along the shore in areas of shoreline 1 km in length and 4 m in width, downstream below the respective locality (Sutherland 2000). Because of the contradictory information in the literature regarding sex differences in FA values of the anurans of the genus Rana, and also because the majority of their populations inhabits anthropogenically transformed habitats, individuals of the *striata* morph, the sexual and phenetic identity of the animals is not included in the comparison. The conclusions are based only on the difference in the degree of pollution and habitat types of pollutants of the water body. As for the habitat in the upper reaches of the river Sazliyka, near the village of Rakitnitsa (marked as 1.1), there are no data about man-made pollution. In our work, it is considered as a background habitat. The remaining habitats of the river Sazliyka: 1.2 – the flow after the city of Stara Zagora; 1.3 – after the town of Radnevo; 1.4 – after the village of Lyubenovo and the flow of river Topolnitsa – 2.1 – after the village of Poibrene; 2.1 – after the bridge on the road from the town of Panagyurishte to the town of Pirdop; 2.3 – after the village of Chavdar the flow contains pollutant concentrations greater than the standards for categorizing water of the country – Category I (clean) and the estimated for the two rivers Categories II (slightly contaminated) and III (normally contaminated). In the river Sazliyka the main pollutants are nitrate nitrogen, phosphates, BOD_5 and suspended solids. In the river Topolnitsa they are some heavy metals (copper, iron, manganese, lead, arsenic). The degree of contamination of water bodies in the studied habitats and the nature of pollutants are obtained based on the data of the annual report of the state of the environment (water) in the Republic of Bulgaria for the period 2000–2010, the Executive Environment Agency, and the data from the physical and chemical analysis of the water in the rivers Sazliyka and Topolnitsa for 2010, from the ballot of the Basin Directorate of water management in the region of Eastern White Sea – Plovdiv, the Ministry of Environment and Water of Bulgaria (Table 1).

To determine the pattern of instability of the lake frog method FA 10 morphological indicators were used: 1 – the number of strips on the dorsal side of the thigh, 2 – the number of spots on the dorsal side of the thigh, 3 – the number of strips on the dorsal side of the tibia, and 4 – the number of spots on the dorsal side of the tibia, 5 – the number of strips on the metatarsus, 6 – the number of spots on the metatarsus, 7 – the number of spots on the back, 8– the number of white spots on the plantar side of the second finger of hind limbs, 9 – the number of white spots on the plantar side of the third finger of the hind limbs, 10 – the number of white spots on the plantar side of the fourth finger of the hind limbs (Nikashin 2007). We have counted the number of asymmetric features and the degree of their manifestation on the right and left sides of the body in each individual. According to the data, the frequency of the asymmetric manifestation in an individual (FAMI) and also the frequency of asymmetric manifestations of an indicator (FAMIndic) were found. A degree- based assessment of the indicators of FA was carried out using a dedicated scale for the marsh frog in the southern parts of its range of habitat (Peskova, Zhukova 2007) (Table 2).

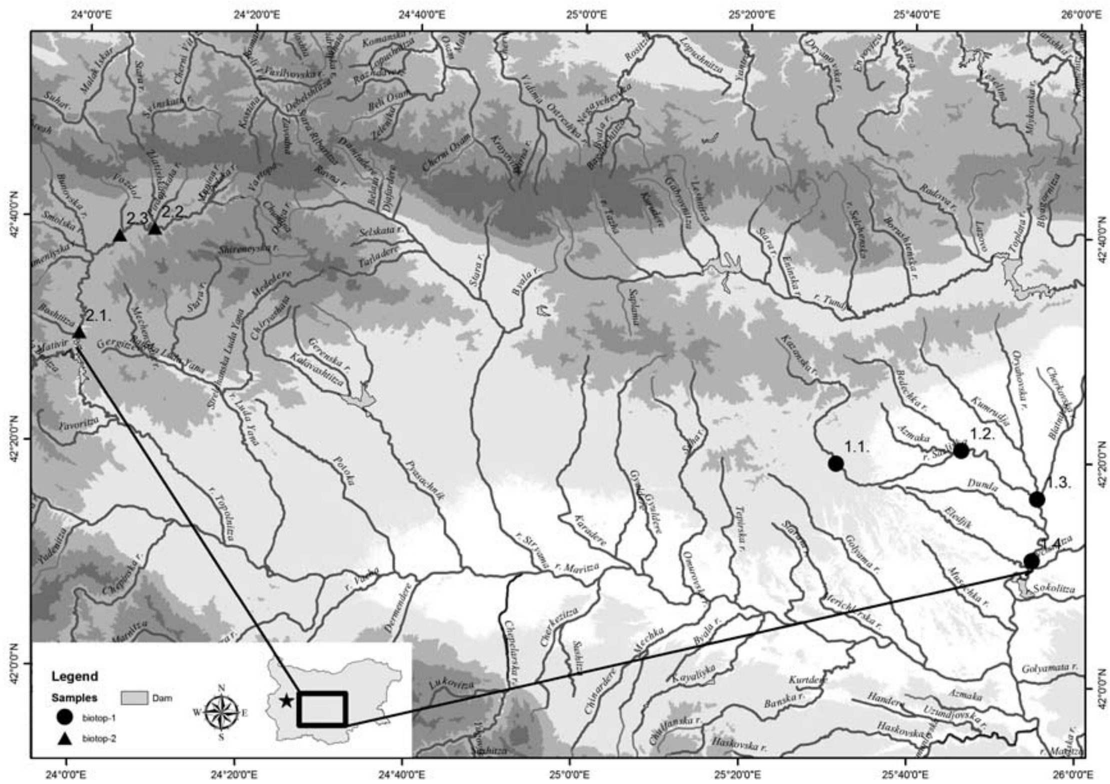

Fig. 1. Geographical location of the studied water ecosystems.

After the animals were captured, measured and the parameters of FA were taken, animals were returned to nature. The total number of marsh frogs examined for the morphological analysis was 205 individuals.

The numerical data was processed by standard statistical methods, using a statistical package STATISTICA for Windows 6.0. Data on the studied indicators were evenly distributed (Kolmogorov-Smirnov D – test; $p > 0.05$) which allowed the comparison of the average values of the parametric Student's t – test at a significance level $\alpha = 0.05$; ($p < 0.05$).

Results and Discussion

The results of the study of indicators of FA in the marsh frog from the studied habitats downstream of two rivers in southern Bulgaria are shown in Table 3.

In our study, the data on the integral indicator of the stability of development (discussed throughout the paper is only the FAMIndic) indicate that the only population of the habitat in the upper reaches of the river Sazliyka the values correspond to 2nd degree (based on FAMIndic) of development instability. All the other populations of lake frogs in the investigated habitats (in both rivers) deviate from the norm and correspond to 5th degree on the scale for the southern parts of the species habitat area.

For the biotope 1.1, the data from the physicochemical analysis (Table 1) does not indicate the presence of pollutants in excess of the maximum permissible limits for the river (categories II and III). Some concentrations of the studied substances are greater than the MPC for rivers in Bulgaria marked with category I, i.e. drinking water and some highland rivers in the country. Therefore, the water in this part of the river is clean enough and has no man-made toxins. But at the same time, the indicators of sustainable development of the population *R. ridibunda* are given grade 2 instead of 1 on the scale Peskova, Zhukova (2007). The population is growing in good conditions, but in the biotope there is a deviation from the optimal parameters for the environment, expressed in a minimal impact on the organism of the amphibians, affecting the degree-based assessment of FA.

The data from the physicochemical water analysis reveals the constant presence of toxins in the rest of the investigated habitats downstream the

Table 1. Results of physico-chemical analysis of surface water of river Sazliyka and river Topolnitsa.

Indices	Units SI	Category according to №7/8.8.1986			Biotope № 1 (2010)				Biotope № 2 (2010)		
		I	III	II.	1.1	1.2	1.3	1.4	2.1	2.2	2.3
pH	pH units	6.5-8.5	6.0-9.0	6.0-8.5	8.34	7.8	7.8	7.97	7.82	5.84**	7.95
Temperature	°C	Deviation upto 3C° from the average of the season			13.9	15.7	14.1	13.6	12.5	12.3	11.4
Suspended Solids	mg/dm^3	30.0	100.0	50.0	5.8	16.8	28.8	29.4	1.51	26.5	10.7
Electrical conductivity	µS/cm	700.0	1600.0	1300.0	751.5	612.0	643.0	924.0	468.0	48.01	539.0
Dissolved oxygen	mgO_2/dm^3	6.0	2.0	4.0	8.1	7.1	5.2	4.95	5.92	7.61	5.5
Oxygen saturation	%	75.0	20.0	40.0	81.5	52.2	52.3	48.0	63.0	72.0	53.0
BOD_5	mgO_2/dm^3	5.0	25.0	15.0	3.2	11.0	18.8*	8.06	–	–	–
COD	mgO_2/dm^3	25.0	100.0	70.0	4.9	30.0	66.2	44.0	–	–	–
ammonia nitrogen $N–NH_4$	mg/dm^3	0.1	5.0	2.0	0.07	2.18*	2.2*	1.04	–	–	–
Nitrate nitrogen $N–NO_3$	mg/dm^3	5.0	20.0	10.0	2.1	1.67	2.2	2.68	–	–	–
Nitrate nitrogen $N – NO_2$	mg/dm^3	0.002	0.06	0.04	0.016	0.165**	0.2**	0.175**	0.005	–	0.0042
Orthophosphates	mg/dm^3	0.2	2.0	1.0	0.045	0.484	0.46	0.351	0.004	–	–
Total nitrogen	mg/dm^3	1.0	5.0	10.0	2.4	6.0*	5.3*	6.3*	–	–	–
Total phosphorus (as P)	mg/dm^3	0.4	3.0	2.0	0.07	0.585	0.734	0.516	–	–	–
Sulphates ($SO4^{2-}$)	mg/dm^3	200.0	400.0	300.0	–	–	55.7	181.0	111.0	221.0	201.0
Iron – total (Fe)	mg/dm^3	SKOS – 0.1			<0.001	<0.001	<0.001	<0.001	0.21″	0.324″	0.333″
Manganese (Mn)	mg/dm^3	SKOS – 0.05							0.22″	0.669″	0.254″
Copper (Cu)	mg/dm^3	SKOS – 0.022							0.06″	3.01″	0.117″
Arsenic (As)	mg/dm^3	SKOS – 0.25							0.003	0.0009	0.007
Lead (Pb)	mg/dm^3	SKOS – 0.0072							0.005	0.002	0.002
Nickel (Ni)	mg/dm^3	SKOS – 0.02							0.05″	0.023″	0.0107

Note:* – above the MPC for category II; ** – above the MPC for Category III; ″ – above SKOS: very poor condition; – not measured.

Table 2. Scale for assessing the state of the lake frog deviations from the conventional norms.

Degree	Value of the index of stability (FAMIndic or FAMI) (Peskova, Zhukova, 2007)	Meaning of the degree
1	< 0.40	conventional standard (clean pond)
2	0.41 − 0.50	minimal effect on the organisms (slightly dirty pond)
3	0.51 − 0.60	satisfactory condition of the organisms (medium dirty pond)
4	0.61 − 0.70	adverse condition of the organisms (very dirty pond)
5	≥ 0.71	critical condition of the organisms (very dirty pond)

Table 3. Indicators of fluctuating asymmetry in the investigated populations of the lake frog *R. ridibunda* from habitats downstream two rivers in Southern Bulgaria (Min – Max; $\tilde{O}$ ±*m; C_v* ± m_{cv} %; *n* – the number of indicators / number of individuals).

FA	Biotopes						
	The Sazliyka river (1)				The Topolnitsa river (2)		
	1.1	1.2	1.3	1.4	2.1	2.2	2.3
FAM-Indic	0.33 – 0.67	0.66 – 0.89	0.65 – 0.96	0.59 – 0.81	0.57 – 0.83	0.65 – 0.87	0.59 – 0.91
	0.47 ± 0.04	0.82 ± 0.02	0.80 ± 0.03	0.72 ± 0.03	0.71 ± 0.01	0.73 ± 0.03	0.75 ± 0.01
	25.53 ± 5.70	8.54 ± 1.91	13.75 ± 3.07	11.11 ± 2.48	1.13 ± 0.25	10.96 ± 2.45	1.07 ± 0.24
	10	10	10	10	10	10	10
FAMI	0.30 – 0.60	0.70 – 1.0	0.70 –1.0	0.50 – 0.90	0.60 – 0.80	0.60 – 0.90	0.70 – 0.90
	0.47 ± 0.01	0.82 ± 0.02	0.80 ± 0.02	0.72 ± 0.03	0.71 ± 0.01	0.73 ± 0.02	0.75 ± 0.01
	17.02 ± 1.93	10.22 ± 0.16	10.0 ± 1.39	13.70 ± 1.59	9.86 ± 1.45	10.96 ± 1.62	9.33 ± 1.44
	39	35	26	37	23	23	22
De-grees*	2	5	5	5	5	5	5

Note* Degrees in Peskova, Zhukova, 2007

river Sazliyka (mainly N–NH_4; N–NO_2; BOD_5) and the river Topolnitsa (heavy metals). The maximum degree – 5, is given to the deviations in the stability of development of the populations of *R. ridibunda* from the habitats 1.2, 1.3, 1.4, 2.1, 2.2 and 2.3. This is to show that the environmental conditions in both river flows are greatly reduced and the populations of these species are in a critical situation with very severe environmental conditions. A confirmation of the serious violations of the development stability of populations from contaminated habitats in both rivers are not only the high values of the integral index of stability – in the river Sazliyka FAMIndic = 0.72–0.82 in the river Topolnitsa FAMIndic = 0.71–0.75. but also the constant appearance of an indicator in the individuals. Low values of the coefficients of the change can be seen: 8.54–11.11% and 1.13–10.96%, respectively, in the two rivers.

In the literature, there is a lot of information about the importance of the FA indicator for the lake frog living in the conditions of industrial pollution. For example, 5th degree (FAMIndic = 0,67 ± 0,01) was assigned to the population *R. ridibunda*, inhabiting the lake-sediment CHP 'Brikel' where in conditions of isolation in heavily polluted environments have been observed not only serious violations of stability, but also the course of an microevolutionary processes (Zhelev 2012). According to published data, similar to the rates we have reached for FA (FAMIndic = 0.65–0.87), and respectively, 5th degree are the populations *R. ridibunda* living on the right bank of Volga River, in the south of the city of Astrakhan (Erdneev, Zvolinsky 2002), in the river Sviyaga in the Ulyanovsk region (Spirina 2007) and in the Voronezh River in Novolipetsk Metallurgical Combine (Nikashin 2007) and in the river Hadazhka in the West Prikavkazie (Peskova 2007).

It is interesting to compare the values of the integral indicator of developmental stability (FAMIndic) in populations from contaminated habitats downstream of two rivers in Bulgaria against the toxicants in the environment. The analysis of the data in Table 4 indicates a statistically significant difference between the values of FAMIndic for all comparisons between populations of habitats 1.2 and 1.3 with those of habitats 2.1, 2.2 and 2.3. At the same time there is no significant difference between populations of biotope 1.4 and the populations from contaminated habitats downstream the river Topolnitsa. Although there is a known connection between the type of toxicants and the degree of manifestation of the FA indicators (they are higher in the river Sazliyka) such an opinion needs an additional confirmation and more accurate studies.

Conclusions

Except for the biotope in the upper reaches of the river Sazliyka (1.1), in which the conditions of the environment are relatively good and the *R. ridibunda* population is characterized by a high degree of stability, all other habitats downstream both rivers (Sazliyka and Topolnitsa) are highly contaminated, with lowered living conditions and the inhabiting marsh frog populations are characterized by serious violations of the stability of development, reflected in the high values of the indicators of FA.

Violation of the stability of development in habitats in conditions of anthropogenic pollution occurs regardless of the type of toxicants, but the extent of their occurrence (in the form of FA) depends on the nature of contamination.

The data of the physicochemical analysis of the samples taken from the water surface in differ-

Table 4. Comparison of the average values of FAMIndic in populations of *R. ridibunda* of the studied habitats.

Comparison	**t***	***P***
1.1/1.2	17.5*	< 0.001
1.1/1.3	16.5*	< 0.001
1.1/1.4	8.33*	< 0.001
1.2/1.3	0.67	> 0.05
1.2/1.4	2.5*	< 0.05
1.3/1.4	2.0*	< 0.05
1.1/2.1	24.0*	< 0.001
1.1/2.2	13.0*	< 0.001
1.1/2.3	28.0*	< 0.001
2.1/2.2	1.0	> 0.05
2.2/2.3	1.0	> 0.05
1.2/2.1	5.5*	< 0.001
1.2/2.2	3.0*	< 0.01
1.2/2.3	3.5*	< 0.001
1.3/2.1	4.5*	< 0.001
1.3/2.2	2.33*	< 0.05
1.3/2.3	2.5*	< 0.05
1.4/2.1	0.33	> 0.05
1.4/2.2	0.25	> 0.05
1.4/2.3	1.0	> 0.05

* Statistically significant difference
for the significance level $\alpha = 5$

ent habitats downstream of the two rivers correlate with the difference of the stability of the marsh frog populations found by us. This objectively confirms the informative and reliable assessment of the environmental quality of the bioindicative method of fluctuating asymmetry.

References

Annual Status of the Environment 2000-2011. Executive Environmental Agency. http://eea.goverment.bg. (In Bulgarian).

Bulletins on the state of water in the river Sazliika and Topolnitsa. 2010. Ministry of Environment and Water of Bulgaria. Basin Directorate for Water Management. Eastern Aegean region of Plovdiv, 23 p. (In Bulgarian).

Erdneev A. S., V. P. Zvolinsky 2002. Phenetic studies of natural populations of *Rana ridiunda*. Moscow, 44 p. (In Russian).

Irikov A., V. Atanasova 2008. Biomonitoring assessment of river ecosystems in East Bulgaria. – In: Velcheva A. Tzekov (Eds.): Jubilee Conference on Ecology. Plovdiv: 362-370.

Lakin G. F. 1990. Biometrics. Moscow. Vishaya shkola, 352 p. (In Russian).

Nikashin I. A. 2007. Ecological and morphological characteristics of populations of marsh frog (*Rana ridibunda* Pall) as a measurement device of the human impact on aquatic ecosystems (for example, the Lipetsk region). Summary of dissertation. Lipetsk, 17 p. (In Russian).

Peskova T. 2007. Evaluation of anthropogenic pollution of biotopes in West Asia Minor Caucasia frog (*Rana macrocnemis*). – *Mountain ecosystems and their components. Moscow.* 3:32-36. (In Russian).

Peskova T., T. Zhukova 2007. Using amphibians for bioindication of water pollution. – *Nuka Kubani*. **2**: 22-25. (In Russian).

Spirina E. V. 2007. Amphibians as bioindicative test system for the environmental assessment of aquatic habitats. Summary of dissertation. Ulyanovsk, 23 p. (In Russian).

statistica for Windows 6.0. 1993. StatSoftInc. Tulsa. USA.

Sutherland W. J. 2000. The Conservation Handbook: Research, Management and Policy. Blackwell Science. Oxford, 279 p.

Van Valen L. 1962. A study of fluctuating asymmetry. – *Evolution*. **16** (2): 125-145.

Vershinin V. L. 2007. Biota of urbanized areas. Ekatirinburg, 73 p. (In Russian).

Zakharov V. M., A. S. Baranov, V. I. Borisov (*et al.*) 2000. Environmental Health: a methodology for evaluation. Moscow. Nauka, 234 p. (In Russian).

Zhelev Zh. 2012. Ecology of tailless amphibians living under the influence of anthropogenic factors in Bulgaria. Usage of some population characteristics of three common amphibian species for the biomonitoring purposes. LAP LAMBERT Acad. Publsh. Saarbruken, 273 p. (In Russian).