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Ecological Status of the River Sazliyka and its Tributaries (Southern Bulgaria) as Indicated by Developmental Stability of *Pelophylax ridibundus* (Amphibia: Ranidae)

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Abstract: In the present work we did a bio-indicational assessment of the ecological status of the river Sazliyka (Southern Bulgaria) and two of its left tributaries (the rivers Blatnitsa and Sokolitsa), based on the integral indicator for the developmental stability – fluctuating asymmetry (FA) in the populations of the marsh frog *Pelophylax ridibundus*. Seven biotopes upstream to the mouth of the Sazliyka River, and one and four biotopes along the rivers Blatnisa and Skokolitsa, respectively, were examined. Based on the indicators for FA, grade rating was assigned, and it was also done as a characteristic of the living environment in these biotopes, parallel and independently of the data from the physicochemical analysis.

Key words: anthropogenic pollution, fluctuating asymmetry (FA), bio-indication, *Pelophylax ridibundus*, Sazliyka River, Southern Bulgaria

Introduction

Studying the responses of organisms in the populations that inhabit anthropogenically transformed ecosystems allows us not only to understand the causes for changes in biodiversity and the mechanisms of survival of certain components of a biotope under the influence of anthropogenic stressors, but also enables us to seek feedback and elicit information on the status of the environment. One of the most recent and promising methods for ecologic assessment of the environmental quality is bio-indication – a method that establishes the impact degree of pollutants, degradation of ecosystems in space and time and their manifestation in an integral form. The usage of bio-indicators for the integral assessment of ecosystems with different levels of complexity is also an advantage because they respond not only to individual pollutants but to the whole complex of the affecting substances as well.

One way of finding the changes that occur in the populations inhabiting anthropogenically transformed areas, before they can affect the viability of organisms, is to investigate the stability of the individual development of an organism – its genetically determined ability to form a phenotype in specific environmental conditions, without any ontogenetic disorders (Ustyuzhanina 2002). Pheno-deviations and ontogenetic noise are usually considered indicators of developmental stability. The expression of phenotypic differences reflects a certain level of developmental disorders (occurring in the first generation whose development has proceeded in suboptimal conditions and disappearing in the

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restoration of optimal conditions), that are reversible (Van Valen 1962, Parsons 1990, Palmer 1994, Zakharov 2001).

In the past decade there has been a growing interest in the group of amphibians, especially toward the tailless amphibians, with their use as test subjects for assessing the degree of anthropogenic pollution of the environment. It is particularly promising in this respect to investigate the manifestations of fluctuating asymmetry (minor, undirected deviations from the strict bilateral symmetry between the two sides of the body expressed by a sign) among the European green frogs *Pelophylax* kl. *esculentus* (Linnaeus, 1758): *P. lessonae* (Camerano, 1882), *P. ridibundus* (Pallas, 1771) and *P. esculentus* (Linnaeus, 1758). They are evolutionarily young and ecologically plastic species with a number of advantages over other amphibians: in a large area, of high density, tolerance, ability to inhabit cenoses with a high level of anthropogenic load. This allows bio-monitoring in large territories to be conducted, using the same features and obtaining comparable data (Chubinishvili 1997, 1998, Zakharov *et al.* 2000, Zamaletdinov 2001, Nikashin 2005, Spirina 2007, 2009, Peskova & Zhukova 2007, Lada *et al.* 2012, Zhelev *et al*. 2012, 2013).

In recent years in Bulgaria, the first experiments have been carried out by using the tailless amphibian for bio-indication assessment of the anthropogenic impact on many ecosystems in the country (Zhelev & Peskova 2010a, 2010b, Zhelev 2011a, 2011b, 2012). The marsh frog *P. ridibundus* is a widespread species throughout the country, *P. esculentus* occurs in the north – along the Danube, and *P. lessonae*, despite its assumed presence, has not been reliably documented (Biserkov *et al.* 2007, Stojanov *et al*. 2011).

The purpose of this work is to assess in ecological aspects one of the most heavily polluted rivers in Southern Bulgaria – the river Sazliyka and two of its large left tributaries (the rivers Blatnitsa and Sokolitsa), based on the analysis of the data on the integral indicator for developmental stability – fluctuating asymmetry (FA) in the populations of *P. ridibundus*.

Materials and Methods

The investigations were conducted in the spring and summer of the years 2010 and 2011. In 2010, 7 biotopes were investigated (Fig. 1) located on the left bank along the river stream of Sazliyka from

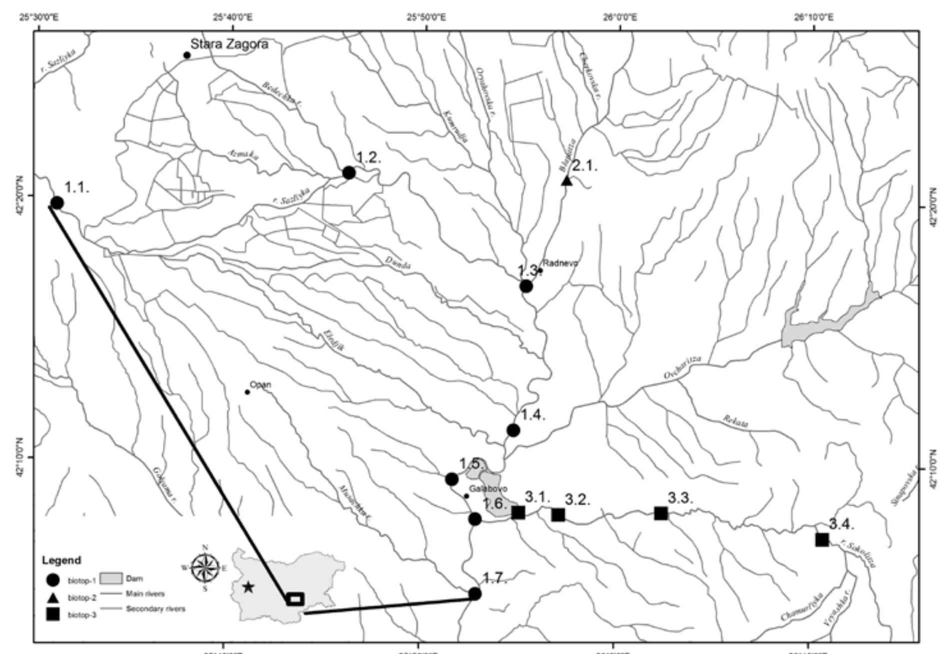

Fig. 1. Map of the investigated biotopes. Biotopes: 1.1 – Sazliyka River downstream of the village of Rakitnitsa; 1.2 – Sazliyka River downstream of the town of Stara Zagora (after the confluence with the Bedechka River); 1.3 – Sazliyka River in Radnevo (downstream of the confluence with the Blatnitsa River) 1.4 – Sazliyka River at the village Lyubenovo; 1.6 – Sazliyka River in Galabovo (downstream of the confluence with the Sokolitsa River) 1.7 – Sazliyska River to the mouth at the Maritsa River, 2.1 – Blatnitsa River in Lyubenova Mahala; 3.1 – Sokolitsa River in Obruchishte; 3.2 – dam “Rozov Kladenets”; 3.3 – Sokolitsa River at the village Iskritsa; 3.4 – Sokolitsa River near the village of Orlov Dol

Table 1. Recent data on the status of biotopes at the time of the study: Physicochemical analisis - surface water sample: mean annual parameters. The indicators that exceed permissible standards in Bulgaria from the total number of 21 indicators, monitored in each of the reservoirs, are shown

Parameters SI		Reg. 7/ 8.8.1986 category		Biotopes -											
		II	III	year	1.1	1.2	1.3	1.4	1.6	1.7	2.1	3.1	3.2	3.3	3.4
Insoluble substances	mg/dm^3	50	100	2010	5.8	16.8	31.8	31.0	64.0*	39.0	56.75*	25.67	81.0*	62.21*	3.6
				2011	6.0	25.8	41.8	56.5*	52.0*	64.0*	50.75*	21.1	83.28	56.3*	3.3
Conductance	µS/cm	1300	1600	2010	751.5	612	643	924	955	861	1295	2082**	1706**	1437*	820
				2011	891	612	596	912	945	1021	1286	2740**	1731**	1372*	834
Biologic consumption of oxygen BCO_5	mgO_2/dm^3	15	25	2010	3.2	11	18.8*	8.06	10.5	12.0	7.54	6.3	6.9	2.45	3.1
				2011	1.8	9.7	9.3	10.35	7.0	8.2	4.69	6.21	6.4	2.34	3.0
Ammonium nitrogen $N-NH_4$	mg/dm^3	2	5	2010	0.07	2.18*	2.2*	1.04	0.6	0.301	2.04*	1.84	1.92	0.318	0.02
				2011	0.079	1.76	1.3	1.89	0.349	0.264	0.176	1,28	1.79	0.47	0.02
Nitrite nitrogen $N-NO_2$	mg/dm^3	0.04	0.06	2010	0.016	0.165**	0.2**	0.175**	0.148**	0.09**	0.235**	0.127**	0.079**	0.054*	0.011
				2011	0.012	0.138**	0.149**	0.158**	0.108**	0.087**	0.09**	0.1**	0.092**	0.042*	0.009
Orthophosphate P	mg/dm^3	1	2	2010	0.045	0.484	0.46	0.351	0.358	0.32	1.78*	-	-	1.09*	0.025
				2011	0.316	0.449	0.443	0.718	0.94	0.449	0.194	-	-	0.792	0.019
Total nitrogen	mg/dm^3	5	10	2010	2.4	6.0*	5.3*	6.3*	6.54*	5.4*	6.43*	-	-	-	-
				2011	1.8	5.43*	5.2*	6.3*	-	4.43	3.99	-	-	-	-
Sulphates	mg/dm^3	300	400	2010	-	-	55.7	181.0	281.0	178.0	306.0	578.0**	568.0**	484.0	-
				2011	-	-	57.8	247.0	46.3	-	450.0**	119.0**	573.0*	423.0*	-

Note* - above the maximum for category II; ** - above the maximum for category III; - no measurement were made.

the village of Rakitnitsa up to the mouth of the river which is in the south of the town of Simeonovgrad; and in 2011 – the biotopes located on the left banks of its two large tributaries: the rivers Blatnitsa and Sokolitsa (1 and 4 biotopes, respectively).

The Sazliyka River rises from the Sarnena Sredna Gora Mountain, north of the village Kazanka, under the name "Varbitsa", and reaches the mineral baths of Stara Zagora, where it is known as "Banyanska River". It passes through the village Rakitnitsa (as the Syutliyka River) and after the Bedechka River runs into it, south from the town of Stara Zagora, it flows south into a broad alluvial plain under the name Sazliyka. At the town of Radnevo the Blatnitsa River (length 54 km) empties into it along with the Sokolitsa River (60.5 km) in the south of Galabovo. The total length of the river up to its mouth is 145.4 km, with a catchment area of 3300 km^2.

On the basis of the data in the year-book for the state of the environment (waters) in the period 2001-2011 of EEA (http://eea.goverment.bg) and the data of the physicochemical analysis of water in the Sazliyka River (2009-2010) from the bulletins of the Basin Directorate for Water Management the Eastern Belomorski Region – Plovdiv (Bulletins about the condition of water in Sazliyka River in 2001-2010; Ministry of Environment and Water; Basin Directorate for Water Management; East Aegean Region, Plovdiv), the river is considered one of the most polluted in Bulgaria (Table 1). The main pollutants are nitrite nitrogen, phosphates, BOD_5 and irresolvable solids – by exceeding the permissible standards for the country I category and the project II and III categories under Regulation № 7 of 8.08.1986 about indicators and standards of running waters in the Republic of Bulgaria (State Gazette, issue 96 of 12.12.1986). Along the river, two "local hotspots" on a national scale are marked: the first is downstream of the town of Stara Zagora at the confluence with the Bedechka River, and the second – downstream of Radnevo at the confluence with the Blatnitsa River. The flow of the rivers Bedechka and Blatnitsa is formed almost entirely from the domestic sewage waste and industrial water of the towns Stara Zagora (12 km very severely affected area) and Nova Zagora (the course downstream of Nova Zagora) respectively.

The object of examination is *P. ridibundus*. The animals were collected in the evening, in the water and on the banks with electric torches. Sections of riverside strip with a length of 1 km and width of 4 m were passed a single time, along the river downstream of the respective town (according to Sutherland 2000). The biotopes of the Sazliyka River under examination are numbered conventionally as follows: biotope 1.1 – south of the village Rakitnitsa, 1.2 – downstream of the town of Stara Zagora and the confluence with Bedechka River; 1.3 – downstream of Radnevo and the confluence with Blatnitsa River; 1.4 – downstream of the village of Lyubenovo; 1.5 – north of the town of Galabovo (under the sluices which block the river to discharge part of it to "TPP Brikel") 1.6 – south of the town of Galabovo downstream of the confluence with Sokolitsa River; 1.7 – south of the village of Kalugerovo until it runs into Maritsa River. At the Blatnitsa River the examined population (2.1) occupies the south of the village Lyubenova Mahala, and at the Sokolitsa River the examined biotopes are located north of the settlements along the stream as follows: biotope 3.1 – the dam "Rozov Kladenets"; 3.2 – Obruchishte village; 3.3 – Iskritsa village; 3.4 – the village of Orlov Dol. The distance between the biotopes is minimum 12-15 km and maximum 40-42 km in a straight line, so an exchange of animals between them is most unlikely.

Analyses were done during their lifetime, after that the animals were returned to nature. The age was determined on the basis of their body size. All examined animals were adults (L > 60.0 mm), sexually mature. As a method for assessing the stability of development we used fluctuating asymmetry in 10 morphological signs by Chubinishvili (1997) and Zakharov *et al*. (2000): 1 – number of stripes on the dorsal side of the thigh (femur), 2 – number of spots on the dorsal side of the thigh (femur), 3 – number of stripes on the dorsal side of the shank (crus), 4 – number of spots on the dorsal side of the shank (crus), 5 – number of stripes on the foot (pes), 6 – number of spots on the foot (pes), 7 – number of stripes and spots on the back (dorsum), 8 – number of white spots on the ventral side of the second finger of the hind leg, 9 – number of white spots on the ventral side of the third finger of the hind leg, 10 – number of white spots on the ventral side of the fourth finger of the hind leg, as for each individual we recognized the number of asymmetrical signs by the degree of their expression on the left and right side of their body. We defined the fluctuating asymmetry by the benchmarks FAMI = FAnm (according to Leary *et al*. 1985 and Palmer 1994) – frequency of asymmetric manifestation of an individual – the ra-

Table 2. Rating scale for the aberrations in the status of *Pelophylax ridibundus* from the conventional standard

Grade	The index value for stability of the development (FAMF or FAMI)		State of organism
	Northern and central parts of the area (Zakharov *et al.* 2000)	Southern part of the area (Peskova & Zhukova 2007)	
1	< 0.50	< 0.40	Conventional rate (clean water basin)
2	0.50 − 0.54	0.41 − 0.50	Minimal impact onorganisms (slightly polluted water basin)
3	0.55 − 0.59	0.51 − 0.60	Satisfactory condition of organisms (average polluted water basin)
4	0.60 − 0.64	0.61 − 0.70	Unfavourable condition of organisms (heavily polluted water basin)
5	≥ 0.65	≥ 0.71	Critical condition of organisms (very heavily polluted water basin)

tio of the number of individuals exhibiting the asymmetric sign towards the total number of tested individuals, and FAMF – frequency of the asymmetric manifestation of a feature – the ratio of the number of asymmetrical signs towards the total number of examined signs, generally for the individuals of both sexes (the literature rarely indicates gender differences in the manifestation of FA). The grade rating of the status of the population (respectively at the corresponding biotope), obtained on the basis of the features of FA, we did with an approved scale for *P. ridibundus* (Zakharov *et al*. 2000), and also with the specified scale for the population in the southern part of the habitat (Peskova & Zhukova 2007) – Table 2. For morphological analyses, 411 individuals of *P. ridibundus* were used.

The digital material was processed with standard mathematical methods using the software package "STATISTIKA" for Windows 7.0. (Statistica 2004). The data about the investigated features are evenly distributed Shapiro-Wilk's-test; $p > 0.05$ (Shapiro *et al.* 1968), and it allows comparing of the mean values with a parametric Student's t – test, at a level of significance $\alpha = 0.05$; [$p < 0.05$].

Results and Discussion

The results of the indicators for FA in *P. ridibundus* from the examined biotopes, along the Sazliyka River and two of its left tributaries (the rivers Blatnitsa and Sokolitsa), are presented in Table. 3.

In our study, the values of grade rating of the coefficient of asymmetry for all samples of the lake frog from the examined biotopes along the streams of the rivers Sazliyka, Blatnitsa and Sokolitsa match in both scales. In the biotope of the upper stream of the Sazliyka River at the village of Rakitnitsa, the indicators for FA (FAMI and FAMF) are minimal and correspond to grade 1 of violation in the stability of development (respectively the level of pollution of the water basin) by the scale of Zakharov *et al*. (2000), and grade 2 by the scale of Peskova & Zhukova (2007) for the southern part of the habitat. Regardless of the various assessments, the conclusion that can be made for this part of the population of *P. ridibundus* is that the population is in a stable condition i.e. here the anthropogenic pressure has not reached the level at which the processes that violate the stability of development start. In parallel, grade 1 was obtained for the population from the biotope in the upper stream of the Sokolitsa River – 3.4 (the village of Orlov Dol). The inter-population comparisons (Table 4) in the values of FAMI show that this is statistically significantly the lowest value of this feature, in comparison to all others, and this leads to the conclusion that the environmental conditions here are close to optimal and it can be regarded as a conventional "control" in our work. In an earlier study for South Bulgaria (Zhelev & Peskova 2010a), we assessed with a grade 1 the population of *P. ridibundus* inhabiting a natural lake, and the lake was filled by an underground artesian source in the region of the town of Galabovo (FAMI = 0.26 ± 0.01). In literature, values that respond to the conventional standard – grade 1 are marked for species populations inhabiting the water basins located in territories with a protected mode and those that are remote from the major cities in Voronezh region (Hitsova *et al*. 2004) and the West Predkavkazie of the Russian Federation (Peskova & Vasyutina 2005). The general ascertainment of the authors is that the low values of asymmetry, identified by them, is due to the

Table 3. Indicators for the fluctuating asymmetry in the *Pelophylax ridibundus* from the examined biotopes at the Sazliyka River and its left tributaries: the rivers Blatnitsa and Sokolitsa (range; mean ± standard errors of means, n ± number of parameters / individuals)

Indicators of the FA	Biotopes*											
	Sazliyka River (1)							Blatnitsa River (2)	Sokolitsa River (3)			
	1.1	1.2	1.3	1.4	1.5	1.6	1.7	2.1	3.1	3.2	3.3	3.4
FAMF-	0.33±0.67	0.66±0.89	0.65±0.96	0.59±0.81	0.53±0.85	0.57±0.93	0.45±0.79	0.73±0.93	0.34±0.78	0.44±0.81	0.28±0.69	0.24±0.45
	0.47±0.04	0.82±0.02	0.80±0.03	0.72±0.03	0.64±0.03	0.72±0.04	0.63±0.04	0.85±0.02	0.55±0.04	0.57±0.03	0.50±0.04	0.33±0.02
	10	10	10	10	10	10	10	10	10	10	10	10
FAMI-	0.3±0.6	0.7±1.0	0.7±1.0	0.5±0.9	0.5±0.9	0.6±1.0	0.5±0.9	0.7±1.0	0.4±0.7	0.4±0.7	0.4±0.6	0.2±0.4
	0.47±0.01	0.82±0.02	0.80±0.02	0.72±0.03	0.64±0.02	0.72±0.02	0.63±0.02	0.85±0.02	0.55±0.01	0.57±0.02	0.49±0.01	0.33±0.01
	39	35	26	37	34	30	38	30	32	36	36	38
Grade*	1	5	5	5	4	5	4	5	3	3	2	1
Grade**	2	5	5	5	4	5	4	5	3	3	2	1

Note: Grade - rating after * - Zakharov *et al.* 2000; ** - Peskova & Zhukova 2007

remoteness of the examined biotopes from anthropogenic sources of pollution.

In the biotope along the Sokolitsa River at the village of Iskritsa – 3.3, assessed by the value of FA, there is a violation in the stability of development corresponding to grade 2 – deviations characterizing the population as being in a tense ecological situation. Sewage from livestock farms influent in the river at several places could be a probable cause. The populations inhabiting the pond of the Pulp And Paper Factory in Stamboliyski (FAMI = 0.51 ± 0.01) and that on the left bank of the Chaya River at its confluence with the Maritza River (FAMI = 0.57 ± 0.02) were assessed with grade 2 in Bulgaria (Zhelev & Peskova 2010a), but it appears that in both cases the grades are higher than those reported for the biotope 3.3 (FAMI = 0.49 ± 0.01). In the literature, similar violations in the stability of development were noted for *P. ridibundus* inhabiting the right bank of the Oka River (Ustyuzhanina & Strelytsov 2001), the recreational areas of the city of Nizhny Novgorod (Loginov & Gelashvili 2001, 2005) and also the West Predkavkazie in ponds with pesticide contamination (Peskova & Zhukova 2007).

Downstream of the Sokolitsa River in two biotopes (3.1 – the river at the village of Obruchishte and 3.2 – the dam “Rozov Kladenets”) the violations in the stability of development are even more expressed, (grade 3), as evidence of their more intensive pollution. Analogous grade rating, earlier in Bulgaria (Zhelev & Peskova 2010a), was given for the population of *P. ridibundus* inhabiting the area around the waste manifolds of the chemical plant near the town of Dimitrovgrad (FAMI = 0.54 ± 0.02). In the literature, violations similar to those found by us in the stability of development in *P. ridibundus* (FAMI = 0.56-60.0) were recorded for the populations inhabiting the left bank of the Oka River, which the authors associated with pesticide contamination in the area (Ustyuzhanina & Streltsov 2001), the region of the outgoing-cleaning facilities of TPP-1 and TPP-2 of the Voronezh reservoir (Hitsova *et al*. 2004) and the Sorokin lake in the Western Predkavkazie surrounded by a pasture for cattle (Peskova 2007).

Two biotopes, located downstream of the Sazliyka River – 1.5 (over the barrier sluices that throw away water to cool the turbines of thermal power plants “Brikel”) and 1.7 (the region of the river south of the village Kalugerovo to its influx into the Maritsa River), were assessed with a grade 4,

Table 4. Comparisons of mean FMFI values in populations of *Pelophylax ridibundus* from the investigated biotopes

Sazliyka River (1) Biotopes 1.1; 1.2; 1.3; 1.4; 1.5; 1.6; 1.7									
Comparisons	t	Comparisons	t	Comparisons	t	Comparisons	t	Comparisons	t
1.1/1.2	17.5^{***}	1.1/1.6	12.5^{***}	1.2/1.5	6.0^{***}	1.3/1.5	5.33^{***}	1.4/1.6	ns
1.1/1.3	16.5^{***}	1.1/1.7	8.0^{***}	1.2/1.6	3.33^{**}	1.3/1.6	2.67^{**}	1.4/1.7	2.25^{*}
1.1/1.4	8.33^{***}	1.2/1.3	0.67 ns	1.2/1.7	6.33^{***}	1.3/1.7	5.67^{***}	1.5/1.6	2.67^{**}
1.1/1.5	8.5^{***}	1.2/1.4	2.5^{**}	1.3/1.4	2.0^{*}	1.4/1.5	2.0^{*}	1.5/1.7	0.33 ns
								1.6/1.7	3.0^{**}
Blatnitsa River (2) Biotopes 2.1									
2.1/1.1	19.0***	2.1/1.3	1.67 ns	2.1/1.5	7.0^{***}	2.1/1.7	7.33^{***}	-	
2.1/1.2	1.0 ns	2.1/1.4	3.25^{**}	2.1/1.6	4.33^{***}	-			
Sokolitsa River (3) Biotopes 3.1; 3.2; 3.3; 3.4									
3.1/1.1	8.0^{***}	3.2/1.1	5.0^{***}	3.2/3.1	1.0 ns	3.3/2.1	18.0^{***}	3.4/1.6	19.5^{***}
3.1/1.2	13.5^{***}	3.2/1.2	8.33^{***}	3.3/1.1	2.0^{*}	3.3/3.1	6.0^{***}	3.4/1.7	15.0^{***}
3.1/1.3	12.5^{***}	3.2/1.3	7.67^{***}	3.3/1.2	16.5^{***}	3.3/3.2	4.0^{***}	3.4/2.1	26.0^{***}
3.1/1.4	5.66^{***}	3.2/1.4	3.75^{***}	3.3/1.3	15.5^{***}	3.4/1.1	14.0^{***}	3.4/3.1	22.0^{***}
3.1/1.5	4.5^{***}	3.2/1.5	2.33^{*}	3.3/1.4	7.67^{***}	3.4/1.2	24.5^{***}	3.4/3.2	12.0^{***}
3.1/1.6	8.5^{***}	3.2/1.6	5.0^{***}	3.3/1.5	7.5^{***}	3.4/1.3	23.5^{***}	3.4/3.3	16.0^{***}
3.1/1.7	4.0^{***}	3.2/1.7	2.0^{*}	3.3/1.6	11.5^{***}	3.4/1.4	13.0^{***}	-	
3.1/2.1	15.0^{***}	3.2/2.1	9.33^{***}	3.3/1.7	7.0^{***}	3.4/1.5	15.5^{***}		

Note: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; ns - $p > 0.05$.

testifying to the deteriorated condition of the population and the strong water pollution. A value close to ours obtained for the indicators of stability of development (FAMI = 0.61 ± 0.04) was found in the West Predkavkazie (Severskiy region) for a water basin polluted with sewage waters from a pig farm (Peskova & Zhukova 2007).

Maximum values of the parameters for FA were recorded along the Sazliyka River in the biotopes 1.2; 1.3; 1.4; 1.6 and 2.1 at the Blatnitsa River. The violations in the stability of development of *P. ridibundus* were assessed with grade 5: populations are in critical condition. From the data presented in Table 4 it is noteworthy that among the biotopes receiving a grade 5 for the parameters of FA, first, there are no statistically significant differences between the biotopes 1.2, 1.3 and 2.1, and second, each of them is reliably distinguished from biotopes 1.4 and 1.6, but despite the differences in values of the integral indicator of the stability of development, the effect of anthropogenic stressors on populations inhabiting them are comparable. The population of *P. ridibundus* inhabiting the pond-settler of the TPP "Brickel" was assessed with a grade 5 (FAMI = 0.67 ± 0.01), where in isolation in the habitat of a highly polluted environment, not only were there serious violations in the stability of development, but also in the course of micro-evolutionary processes (Zhelev & Peskova 2010a). According to the literature data that are close to the reported by us features of the FA (FAMF = 0.65 – 0.87), respectively a grade 5 was obtained for the populations of *P. ridibundus* inhabiting the right bank of the Volga River in the south of the city of Astrakhan (Erdeneev & Zvolinski 2002); Sviyaga River in Ulyanovskiy region (Spirina 2007), Voronezh River near the Novolipetsk Metallurgical Plant (Nikashin 2007) and Hadazhka River in the Western Predkavkazie (Peskova 2007).

Conclusions

Basing on these results, we can draw the following conclusions:

1) Sazliyka River in its upper stream (near the town of Stara Zagora) is in a good ecological condition. This level of anthropogenic impact is not strong

enough to cause deviations in the stability of the development of *P. ridibundus*. The good condition of the living environment supports healthy populations of the species without any serious phenotypic manifestations of asymmetry among the individuals. After the confluence of Bedechka River a sharp change occurs in the condition of the water-basin as a result of the strong anthropogenic pollution. An expression of the aggravated living conditions is the profound disruption in the stability of development of *P. ridibundus*, with very high values of the integral indicator of FA. The condition of the river is not getting better and it is overloaded with new doses of toxicant along 40 km downstream with the inclusion of highly contaminated waters of the Blatnitsa River at the town of Radnevo, and it continues to be critical along 12-15 km in the south (at the village Lyubenovo, despite the reduction in the values of parameters for FA – 0.72 ± 0.03, the grade rate remains maximum – 5). In the lower river, in the area of sluices of TPP "Brickel", due to the unnatural water level rising and sedimentation processes, there are acts of self-purification, an expression of which are the reduced values of the integral indicator for developmental stability: 0.64 ± 0.03 and respectively a grade 4. The next stretch of the river, 10-12 km in the south, including the exhaust domestic sewage of Galabovo, again show living conditions that get much worse, resulting in increased FA indicators corresponding to the grade 5. In the section after Kalugerovo village, to the mouth of the river at the Maritsa River, in the south of the town Simeonovgrad, processes of self-purification are undergoing again, leading to an improved living environment, but nevertheless, there the population of *P. ridibundus* is in a condition of high anthropopression and serious violations of stability in development. The data presented in Table 1 from the physicochemical analysis of the surface water samples, from the various points along the river, correlate with the disorders in the stability of development in populations of *P. ridibundus* found by us, which objectively confirms the good information and evaluation reliability for the quality of environment of the bio-indication method for fluctuating asymmetry.

2) Blatnitsa River is highly polluted and it is in an extremely severe environmental situation (the population at the village of Lyubenova Mahala has the highest values of the indicators of stability in development of all examined – 0.85 ± 0.02).

3) Sokolitsa River and the dam "Rozov Kladenets" on it are in a relatively good condition, but the living conditions in its lower stream and in the artificial closed water-basin itself get worse, compared with the middle and upper stream, where the living conditions are close to the optimal ones.

4) Parameters of FA in the adult, sexually mature representatives of the marsh frog are a very good bio-indication method, which add to the data of physicochemical analysis, and simultaneously, give an independent, parallel assessment of the state of the environment.

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