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ASSESSMENT OF ECOLOGICAL CONDITIONS IN TWO WATER BASINS IN THE REGION OF GALABOVO (SOUTH BULGARIA) ON THE BASIS OF DEVELOPMENTAL STABILITY OF *CARASSIUS GIBELIO* (PISCES: CYPRINIDAE)

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Abstract

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The purpose of this work is to present the results of a study on the values of the integral indicator for developmental stability – Fluctuating Asymmetry (FA) in the populations of gibel carp *Carassius gibelio* that live in two anthropogenically polluted water basins in South Bulgaria: the Sazliyka River and "Rozov Kladenets" Dam Lake. The study was conducted over a period of three consecutive years: 2009–2011. The fluctuating asymmetry of 6 meristic traits was used as a method for evaluating of the developmental stability. In August each year, two indexes were analyzed for 50 individuals from a water basin: FAMI – Frequency of Asymmetric Manifestation of an Individual and FAMF – Frequency of Asymmetric Manifestation of a Feature. Throughout the period of the study, statistically significant higher levels of FA were found within the population of *C. gibelio* that lives in the Sazliyka River. Living conditions in the two basins are highly worsening (5th grade), and the population of *C. gibelio* in the Sazliyka River is in a critical condition, with a higher degree of genetic disorders of homeostasis than the population in "Rozov Kladenets" Dam Lake.

Key words: Gibel carp, fluctuating asymmetry, Sazliyka River, "RozovKladenets" Dam Lake, anthropogenic pollution

Introduction

The developmental stability is an ability of organisms to form genetically determined phenotype without ontogenetic disturbances (Leary et al., 1985; Zakharov and Graham, 1992; Klingenberg, 2003). Organisms maintain high level of developmental stability on the basis of genetic co-adaptation under optimal environmental conditions (Zakharov, 1987).

Ideal forms are rarely known a priori. However, bilateral structures in bilaterally symmetrical organisms' offer a precise ideal, perfect symmetry, against which departures may be compared (Palmer and Strobeck, 1986, 1992; Alford et al., 1999). Thus, they provide a very convenient method for assessing deviations from the norm, and studying the factors that might influence such deviations.Fluctuating asymmetry – FA is one of the most promising methods for evaluating the developmental stability of a big number of vertebrate animals (including hydrobionts: fish and amphibians) for organisms and populations as well (Van Valen, 1962, 1978; Tomkins and Kotiaho, 2001). FA (minor, untargeted deviations in the strict bilateral symmetry) is a result of body inability to develop in a specific plan and it can be determined according to the normal distribution (relating to zero) of the differences between the sides (Right-Left), expressed in a trait (Zakharov and Graham, 1992; Palmer, 1994; Alford et al., 1999). The differences between sides are not genetically determined and their nature is not adaptive. FA is a good marker for evaluating the developmental stability and it can characterize the morphogenetic state of homeostasis and deviations from it – pheno-deviations, ontogenetic noise (Palmer, 1994; Tomkins and Kotiaho, 2001; Zakharov, 2001). Asymmetry is a matter of great interest and it is worth studying it, especially fluctuating asymmetry which is believed to be the indicator that effectively defines the "environmental stress" (extreme temperatures, audiogenic stress, toxins) and reflects the instability of ontogenesis (Parsons, 1990; Markovski, 1993; Polak, 2003) and could be used for assessing the environmental influence on organisms (Zakharov, 2001).

Over the past 10 years there was a greater number of research works that have studied the manifestations of fluctuating asymmetry in populations of vertebrate animals (primarily mammals and amphibians) living in conditions of environmental stress: urbanization and anthropogenic pollution. Integrated assessment of the quality of environment has been conducted in some regions of Russia that are exposed to the impact of anthropogenic factors (chemical pollution, ionizing and non-ionizing radiation). The assessment showed that developmental stability can be used as a sensitive measure of a body's status. In these studies, indices of morphogenetic homeostasis changed synchronously with indices of cytogenetical, immunological, biochemical and physiological status. The morphogenetical approach is proposed as a basic method for biological monitoring the quality of environment (Dmitriev, 1997; Chubinishvili, 1997, 1998; Zakharov et al., 1997, 2000a, 2000b). A few bioindication studies have been conducted on saltwater and freshwater fish (Ruban, 1989, 1992; Lucentini et al., 1998; Lu and Bernatchez, 1999; Bergstrom and Reimchen, 2003; Romanov and Kovalev, 2003; Romanov, 2008; Kostyleva

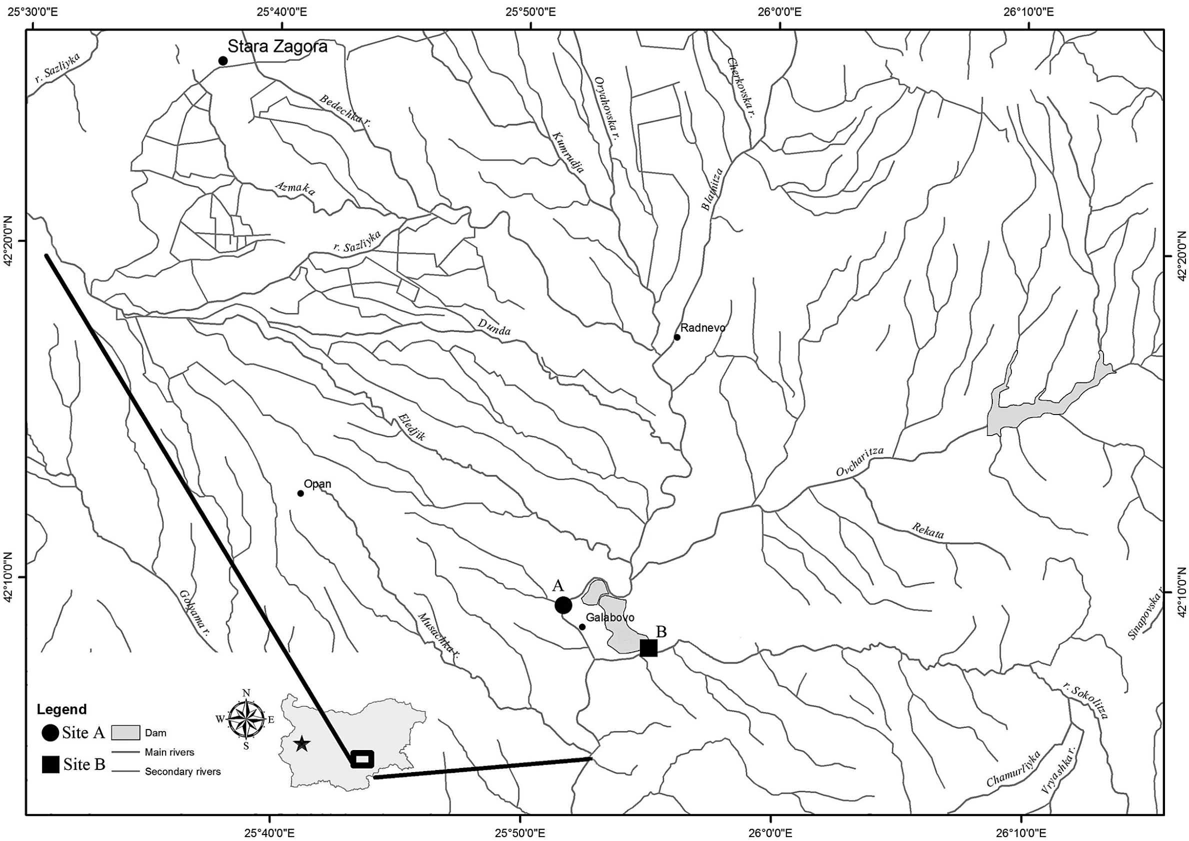

Fig. 1. Map of the investigated region: Site A – Sazliyka River; Site B – "Rozov Kladenets" Dam Lake

Table 1

Recent data on the status of water basins (sites) at the time of the study: Physicochemical analisis – surface water sample: mean annual parameters

Parameters	Units	Order №7/8.8.1986			Site A: Sazliyka River (north of the town of Galabovo – under the sluices which block the river to discharge part of it to TPP "Brikel")			Site B: "Rozov Kladenets" Dam Lake		
	SI	categories								
		I	II	III	2009	2010	2011	2009	2010	2011
pH	pH units	6.5–8.5	6.0–8.5	6.0–9.0	7.83	7.97	7.81	8.19	8.04	8.19
Temperature	°C	to 3° middle of the season			13.57	13.6	14.33	17.2	15.9	16.5
Insoluble substances	mg/dm_3	30	50	100	40	29.4	56.5	80.2*	81.0*	62.4
Electroconductivity	μS/cm	700	1300	1600	654.3	924	912.2	1263	1706.0**	1128
Dissolved oxygen	mgO_2/dm^3	6	4	2	4.73	4.95	4.88	6.2	6.1	8.53
Oxygenation	%	75	40	20	48.7	48	48	73.6	76.5	86.2
$BOD_{5\text{ (biological oxygen demand)}}$	mgO_2/dm^3	5	15	25	15.7**	8.06	10.35	2.45	6.9	3.1
$COD_{\text{(chemical oxygen demand)}}$	mgO_2/dm^3	25	15	25	27.53	44	43	57.5	34.6	24.49
Nitrate ammonium $N – NH_4$	mg/dm^3	0,1	2	5	0.85	1.04	1.89	0.607	1.923	0.355
Nitrate nitrogen $N – NO_3$	mg/dm^3	5	10	20	2.7	2.68	1.78	0.318	2.4	1.93
Nitrite nitrogen $N – NO_2$	mg/dm^3	0,002	0,04	0,06	0.17**	0.19**	0.16**	0.077**	0.079**	0.083**
Orthophosphates	mg/dm^3	0,2	1	2	1.03**	0.35	0.72	–	–	–
Total nitrogen	mg/dm^3	1	5	10	4.44	6.3*	6.4*	–	–	–
Total phosphorus – as P	mg/dm^3	0,4	2	3	0.63	0.52	0.79	–	–	–
Sulphates (SO_4^{2-})	mg/dm^3	200	300	400	224	181	247.2	374.0**	568.0**	484.0**
Iron – total (Fe)	mg/dm^3	SKOS″ – 0.1								
Manganese (Mn)	mg/dm^3	SKOS – 0.05								
Copper (Cu)	mg/dm^3	SKOS – 0.02								
Arsenic (As)	mg/dm^3	SKOS – 0.02								
Lead and its compounds (Pb)	mg/dm^3	SKOS – 0.02								
Nickel and its compounds (Ni)	mg/dm^3	SKOS – 0.02								
Zinc (Zn)	mg/dm^3	SKOS – 1.0								
Chromium (Cr)	mg/dm^3	SKOS – 0.001								
Cadmium (Cd)	mg/dm^3	SKOS – 0.005				< 0.001			< 0.001	

Note: * – above the admissible concentration limit for category II; ** – above the admissible concentration limit for category III; – no measurements done; ″– above SKOS (assessment index): very poor condition

and Peskova, 2011; Khoroshenkov, 2013). Meristic traits are mostly used as diagnostic markers because they are more sensitive to environmental changes rather than the metric ones (Zakharov, 1987; Lajus, 2001). On the other hand, the method does not require killing of animals.

The purpose of the present work was to study the level of manifestation of fluctuating asymmetry in the populations *Carassiusgibelio* Bloch, 1782, that inhabit two water basins (the Sazliyka River and "Rozov Kladenets" Dam Lake) in South Bulgaria, as well as to make a bioindication that is based on the data for the ecological conditions of the water basins (in parallel and independently from the data of the physicochemical analysis). A similar study with a test subject *C. gibelio* has never been carried out in Bulgaria.

Materials and Methods

The area of investigation

The research work was conducted in two water basins near the town of Galabovo: the Sazliyka River and "Rozov Kladenets" Dam Lake. At the Sazliyka River the catch was done in the north of the city, near the sluices that deviate water to TPP "Brikel" and AES "Galabovo" – Site A. At the "Rozov Kladenets" Dam Lake the catch was done on the east bank of the dam lake, at the inflow of the Sokolitsa River – Site B (Figure 1).

The Sazliyka River (651 altitude) originates from the Sarnena Sredna Gora mountains, to the north of the village Kazanka, under the name Toplitsite (Varbitsa); it reaches the Stara Zagora mineral baths, where it is known as Banyanska

River; after that it flows through the village of Rakitnitsa (known as Syutliyka or Rakitnitsa) and after the inflow of the Bedechka River, south of the town Stara Zagora, it runs as Sazliyka to the south into a broad alluvial valley. The river Blatnitsa (length 54 km) inflows in Sazliyka near the town of Radnevo, and the river Sokolitsa (60.5 km) in the south of the town of Galabovo – they are its two largest left tributaries. The total length of the river to its estuary in the river Maritsa (78 m altitude), south from the town of Simeonovgrad, amounts to 145.4 km, with a catchment's basin of 3300 m^2 (the 16^{th} in length in Bulgaria). The average discharge of the river at the town of Galabovo is 18 m^3/s.

"Rozov Kladenets" Dam Lake was built for the purposes of the neighboring TPP "Brikel" and AES "Galabovo", where lignite coal is burnt; the industrial effluents that are used for cooling the turbines flow in it. The dam lake is in direct link with the rivers Sazliyka and Sokolitsa.

With Order №РД-970/28.07.2000 the entire river ecosystem of Sazliyka (from its source to the estuary at the Maritsa River) was announced as a sensitive area under Directive 91/271/EEC and it was included in the Protected areas of the National ecological network "NATURA" 2000 (code BG0000425) under Directive 92/43/EEC for the conservation of natural habitats and wild fauna and flora (Management Plan for the river basins in the East Aegean region from 2010 to 2015). In 1997, "Rozov Kladenets" Dam Lake, located in connection with the Sazliyka River (near the town of Galabovo), and the adjacent terrains along its shores were declared as Place of ornithological importance (in the list of Bird Life International). In 1998, it was named "CORINE" biotope for its European importance for the preservation of rare and endangered bird species. It was declared as protected area by "NATURA" 2000 – code BG0002022.

Data from the physicochemical analysis of the water ecosystems

The monitoring and control of the status of surface waters in the Sazliyka River is done by the National System for Environmental Monitoring (NSEM). Based from the Executive Environmental Agency (http://eea.goverment.bg) and the data on the physicochemical analysis of Sazliyka River water for 2001–2012 in the newsletters of Basin Directorate of water management in the East Aegean Sea – Plovdiv (http://www.bg-ibr.org), the river is considered one of the most polluted in Bulgaria. The contamination exceeds the allowed values for Bulgaria – Water Category I (clean), and also the expected values – Water Category II (slightly contaminated) and Water Category III (moderately contaminated) under Regulation № 7 of 08.08.1986 on indicators and standards for running waters in Bulgaria (State Gazette, № 96.12.12.1986); the main pollutants are nitrite nitrogen, $BOD_{5,}$ sulphates and suspended solids. The main pollutants in "Rozov Kladenets" Dam Lake are nitrite nitrogen, sulphates and suspended solids (Table 1).

Subject of study and methods for analyses

Subjects of our study were the gibel (Prussian) carp *C. gibelio*. The ichthyologic material was collected over a period of three consecutive years: 2009–2011, in August of the respective year. The determination of the specimens was done according to Kottelat and Freyhof (2007) and Karapetkova and Zhivkov (2010). The catches of fish were performed with fishing nets, meshsize of 10.0-12.0 mm (Pravdin, 1966; Sutherland, 2000), in 1–2 km-long sections, against the stream of the water basins.

The samples fixed in 4% formalin were studied in the laboratory. The Ethics Board for Experimental Animals, Faculty of Biology at Plovdiv University, approved the animal handling and methodology. All experiments were conducted in accordance with the national and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU).

Samples with 50 individuals from each water basin were analyzed for every year of the study. The individuals were chosen selectively, and the selection aimed at working with larger, sexually mature individuals. Scales of the fish were used for age estimation (Kafanova, 1984; Howland et al., 2004). For this purpose, scales were taken from the left side of the fish, above the lateral line near the dorsal fin (De Vries and Frie, 1996) and they were put in 33% ethanol (Lorenzoni et al., 2010). The preparations were read under an Olympus SZ51 stereomicroscope. Specimens were assigned to age-classes on the basis of the number of winters that they had lived through before being caught (De Vries and Frie, 1996).

Sex of the fish was determined by macroscopic and microscopic investigations (Pravdin, 1966; Sarý et al., 2008).

The fluctuating asymmetry of 6 meristic traits was used as a method for assessing the developmental stability, according to Living beings' status as a tool for assessing the environmental quality: guidelines (2003) and Kostyleva and Peskova (2011): 1 – Number of rays in the pectoral fins, 2 – Number of rays in the pelvic fins, 3 – Number of rays in the septum between gills, 4 – Number of scales with sensory pores in the lateral line, 5 – Number of sensory pores on the gill cover, 6 – Number of sensory pores on the mandible (Figure 2).

The level of asymmetric manifestation for each of the six traits was recorded for each individual; it may vary from 0 (no asymmetry) to 1 (all the traits are asymmetric). It is possible some of the traits not to express asymmetry, but hardly

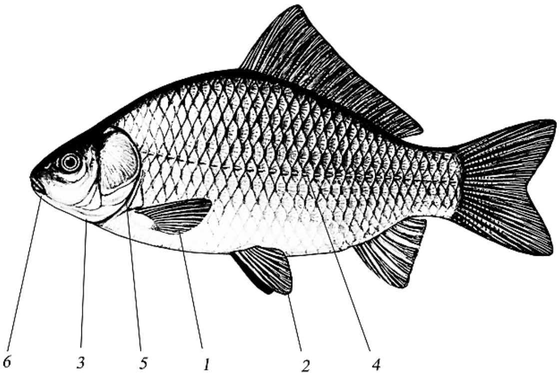

Fig. 2. Diagram of meristic traits for assessing the developmental stability in *Carassius gibelio* (according to: Living beings' status as a tool for assessing the environmental quality: guidelines, 2003; Kostyleva and Peskova, 2011)
1 **– Number of rays in the pectoral fins,** ***2*** **– Number of rays in the pelvic fins,** ***3*** **– Number of rays in the septum between gills,** ***4*** **– Number of scales with sensory pores in the lateral line,** ***5*** **– Number of sensory pores on the gill cover,** ***6*** **– Number of sensory pores on the mandible**

from the conventional standard of the fish status (Living beings' status as a tool for assessing the environmental quality: guidelines, 2003) (Table 2).

The digital material was processed with standard mathematical methods, according to Living beings' status as a tool for assessing the environmental quality: guidelines (2003), using the software package "STATISTIKA" for Windows, Release 7.0. (Statistica, 2004).

All the specimens that were caught in the Sazliyka River for the entire period of the study were female and at the age of 3^+. In "RozovKladenets" Dam Lake the specimens caught in 2009 were: 46♀ and 4 ♂, at the age of 3^+–5^+ (34 individuals were 3^+); in 2010: 41♀ and 9♂, at the age of 3^+–6^+ (36 individuals were 3^+); in 2011: 47♀ and 3♂, at the age of 3^+–6^+, (29 individuals were 3^+). Thus, the statistical processing and comparisons were done in total, without differentiating them in sex (♀+♂) and age (adults).

The descriptive statistics includes a minimal and maximal values (Range), mean value and error (Mean ± SEM). Mean value comparisons for the traits studied in the work were done with a parametric Student's t-test for independent samples, at a level of significance $\alpha = 0.05$; ($p < 0.05$), as the distribution normalcy of the parameters was checked with the Shapiro-Wilk test and a normal distribution was found: $p > 0.05$.

Table 2

Rating scale for the deviations in the status of *Carassius gibelio* from the conventional standard (according to Living beings' status as a tool for assessing the environmental quality: guidelines, 2003)

Grade	The index value for stability of the development (FAMF or FAMI)	State of organism
1	< 0.30	Conventional rate (clean water basin)
2	0.30 – 0.34	Minimal impact onorganisms (slightly polluted water basin)
3	0.35 – 0.39	Satis factory condition of organisms (average polluted water basin)
4	0.40 – 0.44	Unfavourable condition of organisms (heavily polluted water basin)
5	≥ 0.45	Critical condition of organisms (very heavily polluted water basin)

ever all the six traits are bilateral (Palmer and Strobeck, 1986, 1992; Palmer, 1994; Zakharov et al., 2000a, 2000b).

The fluctuating asymmetry was defined according to two indices: FAMI (Frequency of the Asymmetric Manifestation of an Individual) was calculated as the ratio of the sum of the number of individuals that express asymmetric traits towards the total number of individuals; and FAMF (Frequency of the Asymmetric Manifestation of a Feature) was calculated as the ratio of the sum of number of asymmetrical traits towards the total number of traits examined (Palmer, 1994; Zakharov et al., 2000a, 2000b). The grade for the state of individuals (populations), respectively the state of the corresponding water basin, was received on the basis of the indicators for FA, by the scale for the deviations

Results and Discussion

The values of the indicators examined for FA in the populations of *C. gibelio* from the two water basins near the town of Galabovo are presented in Tables 3 and 4.

There were no statistically significant differences found between the values of FAMI and FAMF, for the entire period of the study ($p > 0.05$), either in the sample from the population inhabiting the Sazliyka River – Site A and in that from "Rozov Kladenets" Dam Lake – Site B. For this reason, the grade for the disorders in the developmental stability of *C. gibelio* populations (the state of the water basins, respectively) was assumed on the basis of the values of FAMI (Table 3).

Table 3

Value of the indicator of FA – FAMI (Frequency of Asymmetric Manifestation of an Individual) in *Carassius gibelio* from the researched water basins. Descriptive statistics: Range; Mean ± standard errors of means

Year	Site A: Sazliyka River (north of the town of Galabovo – under the sluices which block the river to discharge part of it to TPP "Brikel")		Site B: "Rozov Kladenets" Dam Lake		Comparisons
	n = 50 samples from each years		n = 50 samples from each years		FAMI: Sates A/B
	FAMI	Grade	FAMI	Grade	
	0.50–1.0		0.50–0.83		t (2009/2009) = 3.57***
2009	0.73 ± 0.02	5	0.63 ± 0.02	5	t (2010/2010) = 3.93***
	0.67–1.0		0.50–0.83		t (2011/2011) = 4.65***
2010	0.80 ± 0.02	5	0.69 ± 0.02	5	
	0.50–1.0		0.50–0.83		
2011	0.74 ± 0.02	5	0.61 ± 0.02	5	
Comparisons	t (2009/2010) = 2.51**		t (2009/2010) = 2.14*		
FAMI	t (2010/2011) = 2.14*		t (2010/2011) = 2.86**		
	t (2009/2011) = 0.36ns		t (2009/2011) = 0.71ns		

Note: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; ns – $p > 0.05$; n – number of individuals

Table 4

Value of the indicator of FA – FAMF (Frequency of the Asymmetric Manifestation of a Feature) in *Carassius gibelio* from the research water basins

Years	Sites*	The traits **						FAMF
		1	2	3	4	5	6	Mean ± SEM
2009	A	0.96	0.2	0.48	0.98	0.8	0.92	0.72 ± 0.28
	B	0.96	0.1	0.42	0.8	0.8	0.68	0.63 ± 0.26
2010	A	0.94	0.34	0.68	1	0.92	0.94	0.80 ± 0.22
	B	0.98	0.18	0.46	0.86	0.84	0.82	0.69 ± 0.27
2011	A	0.96	0.16	0.5	0.98	0.86	0.98	0.74 ± 0.30
	B	0.92	0.14	0.44	0.78	0.76	0.6	0.61 ± 0.23

Note: * – Sites: A (Sazliyka River), B ("Rozov Kladenets" Dam Lake); ** – The traits: 1 (Number of rays in the pectoral fins), 2 (Number of rays in the pelvic fins), 3 (Number of rays in the septum between gills), 4 (Number of scales with sensory pores in the lateral line), 5 (Number of sensory pores on the gill cover), 6 (Number of sensory pores on the mandible).

It is obvious from the data in Table 3 that the grades during the period 2009–2011 were the same – 5, nevertheless, the mean values of the integral parameter for FA in the population from Site A were statistically significantly higher than those in the population from Site B. The maximum grade (on a five-point scale) for the disorders in the developmental stability of *C. gibelio* populations could be interpreted with the fact that there are serious disturbances in their genetic homeostasis and the living conditions in the two basins near the town of Galabovo are getting worse. On the other hand, the results obtained provide reason to claim that the population of *C. gibelio* in habiting the Sazliyka River is in a much more severe (critical) condition than that in "Rozov Kladenets" Dam Lake. What also deserves consideration is the fact that, for the entire period of the study, all the individuals caught in the Sazliyka River were female and of age group 3^+. It is known that in populations of vertebrate animals, living in "ecological stress", males are selectively eliminated and these of "old age groups" decrease sharply (Kalmus and Smith, 1960). On the other hand, it has been found that *C. gibelio* is a species that can successfully reproduce through gónogenesis (Buthet al., 1991) – another reason for the successful survival of this species in terms of worsening living conditions. The presence of males and individuals of old age (3^+–6^+) in the populations *C. gibelio* in "Rozov Kladenets" Dam Lake confirms the fact that in this water basin, regardless of the deteriorating parameters of the environment, living conditions are better than those in the Sazliyka River.

From the parallel data in Table 1 of the physicochemical analysis done in the two basins for every year it becomes clear that there were no significant differences in quantitative characteristics of their main pollutants: nitrates, nitrites, total nitrogen (the content of sulphates was higher in Site B), but regardless of this, the disorders in developmental stability were constantly higher in the population that inhabits the Sazliyka River, throughout the period of the study. It is hard to explain the reason for these differences; probably it is due to the larger volume of water mass in "Rozov Kladenets" Dam Lake and also due to the water entering it from another independent source – the Sokolitsa River. During 2010, when the highest average annual values of major toxicants were reported for both basins (Table 1), there were statistically significant higher values of FAMI in the two *C. gibelio* populations than in the previous year – 2009 (Table 3). This confirms the high sensitivity of the method of FA and its objectivity for bioindication analyses. Levels of FAMI, which are very similar to ours (respectively, grade 5), have been reported for *C. gibelio* populations that inhabit rivers in the southern part of the Russian Federation: the Don River – 0.69 ± 1.88 (Kostyleva and Peskova, 2011), the Kuban River – 0.66 ± 0.51, the Psekups River – 0.73 ± 0.34 and the Chituk River – 0.73 ± 0.26 (Khoroshenkov, 2013).

A matter of big interest is the comparison in the results from the parallel investigation on the parameters of developmental stability in the populations of another hydrobiont – *Pelophylax ridibundux* (Amphibia: Anura: Ranidae); a study conducted by the authors in the same water basins at the same time period (Zhelev, 2012; Zhelev et al., 2012, 2013). The values of FAMI for *P. ridibundus* populations that live in the Sazliyka River (in the region of Site A) received a grade 4, by a five-point scale, for assessing the developmental stability of the populations in the southern part of the species habitat (Peskova and Zhukova, 2007), while these of the population that live in "Rozov Kladenets" Dam Lake (at Site B) – a grade 3. The results support the view that the environmental conditions are better for the biota in "Rozov Kladenets" Dam Lake than in the Sazliyka River. On the other hand, the results show that the damage in *C. gibelio* populations (higher levels of FA, respectively severe disorders of the genetic homeostasis) is bigger than that the populations of *P. ridibundus* experience (they live in water and on land) in habitat conditions with a permanent anthropogenic pollution.

The analysis of FAMF indicator values allowed us to find which of the traits expressed differences in the manifestations of fluctuating asymmetry in the populations of *C. gibelio* from the two anthropogenically polluted water basins in South Bulgaria. It is notable that in both populations the maximum manifestation of asymmetry was found in 4 traits: the number of rays in the pectoral fins (trait 1), the number of scales with sensory pores in the lateral line (trait 4), the number of sensory pores on the gill cover (trait 5) and the number of sensory pores on the mandible (trait 6). For the entire period of the study 2009–2011, practically there were no differences in both populations in FA manifestation in regard to the trait – the changes in the trait in Site A were within 0.94–0.96, and in Site B – 0.92–0.98, respectively. For every year of the study the three other traits (4, 5 and 6) showed higher levels of asymmetry in the population that inhabits Site A (table 4). Whilst the lowest manifestation of asymmetry in the two *C. gibelio* populations was found for the traits 2 (number of rays in the pelvic fins) and 3 (number of rays in the partition between gills) (Table 4). For the entire period of the study the fluctuation limits of the trait 2 (0.10–0.18) and the trait 3 (0.42–0.46) in the population from Site B were lower than in the population from Site A – the traits 2 (0.16–0.34) and 3 (0.48–0.68), respectively.

Similar to ours high levels of asymmetry for the traits 4, 5 and 6 (fluctuations within 0.75–1.0) was also reported for *C. gibelro* populations from the lower course of the Don River (Kostyleva and Peskova, 2011), as well as in the populations living in the Kuban River (fluctuations within 0.80–1.0) in the Republic of Adygeya in the Russian Federation (Khoroshenkov, 2013). The authors found high levels of anthropogenic pollution in both locations (moreover, in the Kuban River the toxicants were identical to these from the two water basins explored by us near the town of Galabovo – nitrates and nitrites). Another study, conducted in the Far East of the Russian Federation, found maximal manifestation of FA in *C. gibelio* populations in the trait 5, and minimal one – in the trait 6 (Romanov and Kovalev, 2003).

The findings from the analysis of the frequency of occurrence of FA in various traits, among the individuals of *C. gibelio* populations in the two water basins in South Bulgaria for the time period 2009–2011 (Figure 3) are the following:

- there were no individuals that express asymmetry in one and two traits in the two populations.
- in the population from Site A, the percentage of individuals (44.0%) which express FA in four traits is the highest, followed by these in five (38.66%), six (10.67) and three (6.67%) asymmetric traits.
- in the population from Site B, most individuals express FA in four asymmetric traits (56.67%), followed by the individuals that are asymmetric in three (30.0%) and five traits (13.33%). There were no individuals expressing asymmetry in six traits.

The data obtained support the view that living conditions in "Rozov Kladenets" Dam Lake are better than these in the Sazliyka River.

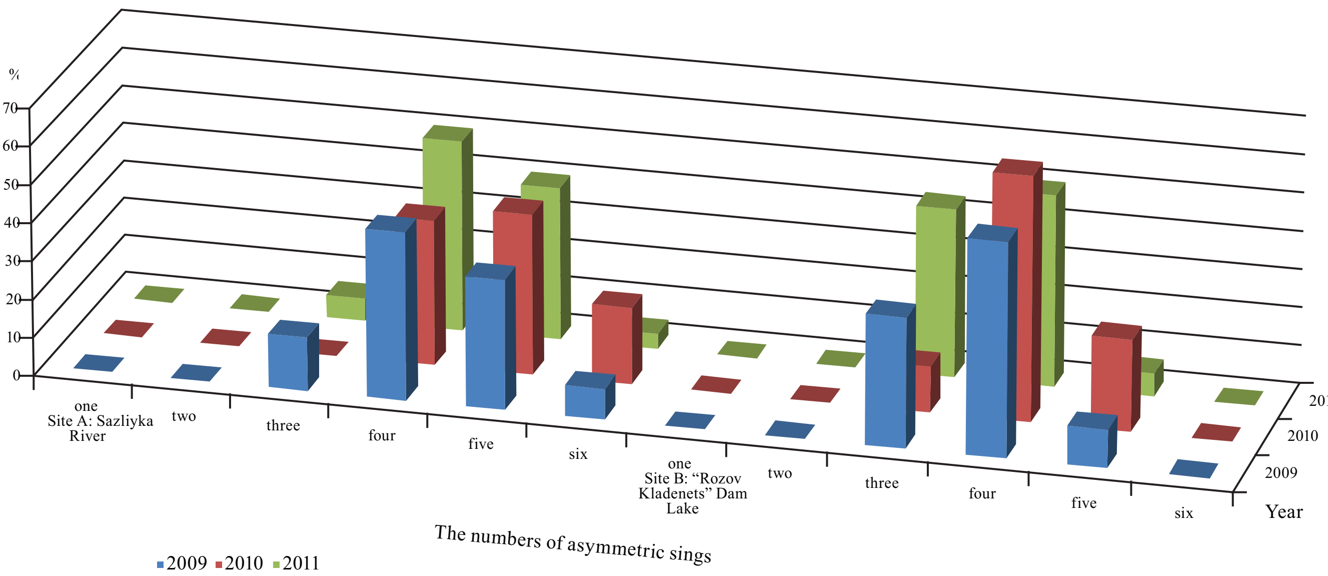

Fig. 3. Ratio (%) of the number of asymmetric individuals to different traits

Conclusions

For the entire period of the study (2009-2011) statistically significant higher values of FA were found in the population of *C. gibelio* that inhabits the Sazliyka River near the town of Galabovo than in the population that lives in "Rozov Kladenets" Dam Lake.

The living conditions in both water basins are highly deteriorated (grade 5), however, the population of *C. gibelio* in the Sazliyka River is in a critical state, with a higher level of disorders of the genetic homeostasis than in the population in "Rozov Kladenets" Dam Lake.

We can confirm the objectivity of the method of FA for assessing the developmental stability in *C. gibelio* populations as well as its informativeness for defining the ecological status of water basins.

We think that the method is a good opportunity for using FA in practice in the system of bioindication (*C. gibelio* as a biomonitor) for initial (ahead of physicochemical analyses) general diagnosis of the environmental condition.

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