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Opportunities for Further Qualification in Environmental Communication in Protected Areas

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Abstract. Environmental communication is a nature conservation tool that uses communication approaches, principles, strategies and techniques, involving different social and age groups. The interaction with people is essential for efficient environmental protection and prevention, thus practitioners more often need to communicate environmental problems and solutions with children, adults, citizens and the society as a whole. In this regard, the study aims to identify opportunities for further qualification of university students and to verify an environmental communication training program in protected areas. Based on a literature research, a training program was developed, implemented and evaluated in "Vrachansky Balkan" Nature Park with a group of 18-29 years old youngsters. The results show demand for environmental communication skills and confirm the potential of the training program. Young people share that the acquired competences open new perspectives for their professional development. The study underlines the importance of such qualification for environmentalists and recommends its integration in their curriculum, its provision from nature and national park administrations, regional inspectorates of environment and water, centers of continuing education.

Key words: environmental communication, protected areas, education for sustainable development.

Introduction

LUHMANN (1986) underlines that there would be no public impact on solving environmental issues, while they are not discussed.

Environmental communication is the exchange of information about environmental problems and solutions. This is a nature conservation tool, which uses communicainformational tional, and educational approaches, principles, strategies and techniques for environmental protection. It includes diverse forms of interpersonal, group, social. organizational media and

communication for prevention or solution of environmental threats. It covers knowledge in the fields of ecology, biology, geology, sociology, economics, politics, communications, etc. and is based on a professionally endorsed opinion, recommendation or a message, targeted to different age and social groups - children, youth, adults, local communities, local authorities, citizens, the public as a whole (FLOR, 2004).

According to MICHELSEN (2007), environmental problems require communication between different systems – political, legal, economic, educational, etc. and

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environmental communication is a "soft" instrument, which includes education for sustainable development, reports, initiatives, actions and campaigns for environmental protection, environmental consulting, etc. It is a very young scientific discipline and there is no certain theory for it, also as professional field, it is multifaceted. He defines four environmental communication methods, which social marketing, are civic empowerment, exhibitions and education.

predominant studies The in the education in environmental science in Bulgaria are focused on the high school stage and the fewest publications address the bachelor and master degrees. Non-formal education is insufficient explored. Studies about training of teachers, epistemology and philosophy of environmental science raise (HADJIALI & KOLAROVA, 2016). The research on information and digital technologies in environmental education also increase virtual courses, gamification, distance learning systems are implemented to develop students' social and professional skills (BREZIN et al., 2013a; b; ASENOVA & YOTOVSKA, 2014; TUPAROV & TUPAROVA, 2018; YOTOVSKA & GENOVA-KALOU, 2018).

Regarding environmental communication in particular, Bulgarian higher education institutions introduce it in the students' curriculum in different ways. As optional, in universities in Ecology some and Environmental Protection Studies, it is possible to choose a course of "public relations" or "communication skills", as part of their master's program (University of Forestry, South-West University "Neofit "Konstantin Preslavsky" Rilski"). In University of Shumen, students in bachelor could even choose among other disciplines "environmental education for high school students". In Sofia University "St. Kliment Ohridski" and Plovdiv University "Paisii Hilendarski" there are master programs "environmental/biology dedicated to education". Although there are possibilities for training in particular aspects, the efforts fragmented and complete remain а

specialized course, where pupils can explore and practice vary environmental communication approaches, is still not integrated.

At the same time, the professional realization of graduate environmentalists in various state, private and non-governmental organizations daily requires from them to communicate problems and solutions of environmental issues to diverse target groups, using different methods and approaches. For instance, experts from park administrations need to implement more and more communication activities, because in the management plans of the Bulgarian nature and national parks, by law (Bulgarian Protected Areas Act, 1998), in their long-term and operational objectives, it is set to develop information and interpretation infrastructure for better understanding of the protected areas' conservation significance among the public.

In this context, this case study aims to identify opportunities for further qualification of university students and to verify an environmental communication training program in protected areas.

Materials and Methods

"Vrachansky Balkan" Nature Park and its biodiversity conservation is selected as an environmental communication object for target group of children and youth. The choice is related to the need for support in the multiplication of nature conservation activities among school pupils in the Vratsa region, expressed by the Nature Park Administration. Important reasons are also the continuous effective collaboration with the Administration and the possibility for advancement of previous results in this protected area.

This study is based on the existing and verified Methodologies: for conducting educational activities in the field of ecology and nature protection through non-formal education for sustainable development in "Vrachansky Balkan" Nature Park; for training of trainers and for educational tourism in the Park (BANCHEVA-PRESLAVSKA, 2019).

An additional literature review is carried out and various theoretical and practical sources of information about methods and techniques in communicating environmental problems and solutions to children and youth are analyzed. Respectively, an educational program consisting of theoretical practical training is elaborated, and implemented and evaluated in Vrachansky Balkan with 15 young people between 18 and 29 years old.

The participants in the educational program are randomly gathered during its popularization in the Vrachansky Balkan region. The information about the training is spread by local media channels (radio, TV, newspapers), via e-mail straight to 713 interested contacts and during personal meetings in 3 high schools in Vratsa and the pedagogical university department there. Out of 21 candidates, 15 young people from 18 to 29 years old are selected by motivational letters. Unintentionally, about 30% of the participants study Ecology and Environmental Protection (EEP). The rest are freshmen in other sciences (biology, tourism, etc.), high school students and employed from Vratsa, Mezdra and Sofia.

The theoretical training includes 8 hours to broaden and deepen the knowledge of ecology and biodiversity conservation for the specific nature park and 24 hours of methods and approaches to work with children and youth in environmental protection. Both theoretical parts are integrated and presented in a complex four-day educational program. The program is built by three interlaced interpenetrating thematic elements: ecology and environmental protection, environmental education and non-formal learning methods (Table 1).

	Day 1	Day 2	Day 3	Day 4
Aim	Introduction in the theoretical topics. Teambuilding	Deepening the knowledge in ecology and non-formal learning methods	Deepening the knowledge and developing skills for environmental education	Consolidation of the new learned
Morning activities	 Arrival Outdoor non-formal learning methods for knowing each other. 	 Excursion with environmental education games - experiencing and reflecting. Fauna of the "Vrachansky Balkan" Nature Park - typical species, terms. 	 Threats for biodiversity in "Vrachansky Balkan" Nature Park. Challenges and possibili- ties for environmental protection and nature conservation. Best practices in environ- mental education for youth – projects, programs, etc. 	 Practice of educational environmental communication among each other. Reflection and analysis. Feedback on the training.
Afternoon activities	 Trainers' introduction. Rules of the group. Knowing each other & teambuilding games. Protected areas and "Vrachansky Balkan" Nature Park. Basics of environmental communication - theory and practice. Environmental teambuilding games. Teamwork. Feedback and reflection on the day. 	 Flora of the "Vrachansky Balkan" Nature Park - typical species, terms. Educational communication - dimensions, functions, practical areas. Tools for non-formal environmental education. Teamwork on environmental scenarios. Feedback and reflection on the day. 	 Recommendations and rules for organizing environmental education initiatives. Practical tasks - independent work outdoor. Preparation of a training program for a specific age group. Feedback and reflection on the day. 	• Departure
At night	• Environmental teambuilding games.	• Sharing skills' night. Self-organization	Free time	

Table 1. Schedule of the theoretical training.

The theoretical training is conducted according to the "flow learning" principles of CORNELL (2015): inspiration – concentration – nature experience – sharing/reflection and according to the KOLB's (1984) four-stage experiential learning cycle: concrete experience – reflective observation of the new experience – abstract conceptualization – active experimentation. Non-formal interactive teaching methods are used.

The practical training consist of 16 hours of developing and implementing their own communication strategies and direct work with school pupils. In this part, participants are mentored in 3 groups per 5 person to apply independently environmental education programs with 81 high school students and to establish their own campaigns and initiatives for environmental protection, targeted to children and youth.

The evaluation of the educational program is based on specially prepared tests and self-assessment surveys. The tests check the level of knowledge after the theoretical training about ecology and biodiversity in "Vrachansky Balkan" Nature Park and about methods and approaches for environmental communication with children and youth. Self-assessment surveys measure certain knowledge and skills before and after the entire educational program. The analysis is made according to the grading scale in the Bulgarian education system from 2 (failed) to 6 (excellent) and the results are processed statistically in MS Excel.

Results and Discussion

The tests of the theoretical training show that the group's results in both training topics do not differ significantly. In the theoretical training about ecology and biodiversity conservation in "Vrachansky Balkan" Nature Park, not surprisingly, the students in EEP have full excellent mark (6), but in the tests about methods and approaches in communication environmental with

children and youth – 5,5 – less than the group result (Fig. 1).

Based on the survey, that measures certain parameters from 2 to 6 before and after the training, it was found that the self-assessment of the participants increased by 0.27 to 1.33 for essential knowledge and skills. These include knowledge in ecology and environmental protection, knowledge and skills for working with children and young people, communication skills, confidence, teamwork (Fig. 2).

Students in EEP have individual differences regarding some questions of the survey. For example, one student assesses his/her knowledge of ecology and environmental protection after the training lower, another – higher, and by the rest the assessment remains the same. The situation is similar to other questions of the survey, but benefits of the training are clearly highlighted, namely, with 1.50 and 1.25 respectively increase their overall assessment about their knowledge of methods and approaches and their skills to work with children and youth for environmental protection (Fig. 3). They categorically point out that the participation in this training broadens their professional horizons.

Still in their cover letters, students in EEP state the belief that this participation will extend their professional horizons. In their motivation to participate they also share, that they want to gain new knowledge and experience in their specialty, increase their professional confidence, and improve their communication skills. While their motivation is professional, the other participants show a need for information in environmental protection.

environmental The own communication strategies, which the participants developed during the practical training, include 3 campaigns initiatives environmental and for protection.

Hristina Bancheva-Preslavska



Fig. 1. Marks from the tests of the theoretical training.



Fig. 2. Self-assessment of knowledge and skills acquired during the educational program.



Fig. 3. Environmental communication with children and youth.

An award competition "The greenest class in Vratsa region" involves all local schools and requires from the school classes to proof their sustainability lifestyle and environmental protection activities through presentations, pictures, essays, videos, etc. 92 children (under 15 years old) and 76 youth (above 15 years old) take part.

Reality game in the nature "Hunting for adventure" for youth teams presents the biodiversity of "Vrachansky Balkan" Nature Park and the threats to it through experiential learning, with the purpose to motivate youths for its protection. It involves indirectly 12 742 followers from Bulgaria in Facebook and directly 64 young people from Vratsa who compete live in games.

"Otter in class" is an initiative of practical lessons for high school students from Vratsa, which introduces the anthropogenic impact on the biodiversity in "Vrachansky Balkan" Nature Park through non-formal education methods. It involves 124 pupils from three high schools.

Besides enhancing their knowledge environmental about protection and exercising independence in accomplishing their own project ideas, the participants in the environmental communication training program improve their social skills, their ability to work in teams, with and for other young people, develop their interests, their confidence and practical skills. This in turn helps young people to establish themselves individuals competitive as and professionals.

Environmental communication is becoming an increasingly topical issue faced by graduate environmentalists and the need to further their competencies in this area for better competitiveness in the labor market is growing.

Conclusions

The case study presents environmental communication, as a nature conservation tool. It proves the efficiency of a training program with university students for communication of environmental issues with children and youth in protected areas, after the example of "Vrachansky Balkan" Nature Park. It indicates a demand for such qualification from students in Ecology and Environmental Protection and underlines the importance of such a training for them.

training in environmental А communication could be offered as a specialized course for further qualification at national the nature and park administrations, regional inspectorates of water, continuing environment and education centers, etc. It could be complemented with more communication approaches and techniques not only for children and youth, but also for other target groups - adults, citizens, local authorities and communities, etc. to provide wider support to graduate environmentalists. It could be included in existing disciplines or be a separate one in the curriculum of students in Ecology and Environmental Protection.

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Current Ecological Status of Lotic Ecosystems in Vitosha Mountain Reserves (Torfeno Branishte and Bistrishko Branishte)

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Abstract. The national legislation on surface waters characterization (Regulation N4/2012) was applied for the lotic ecosystems in Torfeno Branishte (Vladayska and Boyanska Rivers) and Bistrishko Branishte Reserves (Bistritsa and Yanchovska Rivers) in view of ecological status assessment. Water samples were collected and analysed both for physicochemical elements (pH, dissolved oxygen, conductivity, biological oxygen demand, nitrate and ammonium nitrogen, chlorides, sulphates) and macrozoobenthos (by calculating the regulated indices Total number of taxa and Biotic index). The assessment based on Total Number of Taxa, Biotic index and the supporting physicochemical parameters showed a "good" and "high" ecological status for four rivers. The results obtained for the representative studied water bodies, can be utilized in decisionmaking process to insure the relevant measures for prevention the anthropogenic pressure and further maintaining the high water quality of the aquatic ecosystems in the two reserves.

Key words: reserves, macrozoobenthos, biological quality element, physicochemical elements, rivers, ecological status.

Introduction

Branishte Reserves are unique reserves located reserves have not been a subject of prior on the territory of Vitosha Nature Park. Torfeno Branishte preserves the significant complex of mountain peatlands in running outside protected areas have been Bulgaria, which supply the city of Sofia with carried out. RUSEV (1961) conducted the first drinking water. The Bistrishko Branishte benthic study of four Vitosha rivers, including Reserve is included in the UNESCO Biosphere Vladayska and Boyanska, in parallel with

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Reserve Network and protects valuable old-The Bistrishko Branishte and Torfeno growth spruce ecosystems. The rivers in both systematized hydrobiological studies. More most targeted studies on the lotic ecosystems

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parameters of the aquatic environment. Data on physicochemical water parameters were found in the later study of BLASKOVA & IORDANOVA (2007), which were conducted seasonal monitoring of five main rivers in the central part of the mountain. Subsequent physicochemical studies on the waters of the Vladayska and Boyanska rivers also were made by KIROVA (2012).

The Vitosha Park Management Plan (2005previous information 2014) summarized sources that have been used to analyze the characteristics of the rivers flowing through the mountain. In the period May-June 1996-1991, the data showed values of parameters comparable to the first category for water quality, the status of the surveyed sites was defined as oligosabrobic. Later hydrobiological (benthological) studies that concerned the ecological status of the Bistritsa River were found in the publication of MITKOVA (2013). GEORGIEVA et. al. (2013) made an analysis of the trophic structure of the macrozoobenthos in the Bistritza River at 5 points, the highest of which was situated immediately below the boundary of the Bistirshko Branishte Reserve. The assessment at that point was defined as "high" according to the trophic index RETI/PETI (CHESHMEDJIEV & VARADINOVA, 2013). KANEV (2014) performed research specifically on the Austropotamobius torrentium population from some Vitosha rivers, including the Bistrihka and Yanchovska rivers in stretches running through the territory and below the border of Vitosha Park.

The national regulations for characterization of the running waters in Torfeno Branishte Reserve (TBR) and Bistirshko Branishte (BBR) Reserve by physicochemical and biological quality elements (macrozoobenthos) was applied in view of their ecological status assessment.

Material and Methods

The benthic sampling and in situ of the physicochemical measurements parameters were carried out in June 2015 at 4 selected representative sites: two for TBR-on determined as "good". According to the

measurements of the basic physicochemical the Vladayska and Boyanska Rivers, both defined as a mountain rivers type R2, according national typology and two for BBR mountain streams forming the Vitoshka Bistritsa and Yanchovska Rivers found to be river type R15 Karst spring (Fig. 1; Table 1).

physicochemical The basic water parameters (pH, electrical conductivity (µS.cm⁻ ¹), dissolved oxygen concentration (mg.dm⁻³) and oxygen saturation were measured in situ portable with calibrated equipment. Concentrations of some major inorganic ions, as nitrate (NO_3^{2-}) , ammonium (NH_4^+) , sulfates (SO_4^{2-}) and chlorides (Cl^-) were measured photometrically. The permanganate oxidation, total and suspended solids, and biochemical oxygen demand (BOD₅₎ were determined in the Laboratory of Chemistry at the University of Forestry. The benthic samples were taken by hand net (500 µm mesh size) in accordance with an adapted version of the multihabitat sampling methodology developed on European AQEM/STAR projects (CHESHMEDJIEV et al., 2011) in accordance with the standards BDS EN ISO 5667-3:2012. The ecological status was assessed by Biotic index (BI), Total number of taxa (TNT) and supporting physicochemical parameters, according to type specific scale developed in Regulation N4/2012. The map of the studied sites was prepared with software product ArcGIS 10.1.

Result and Discussion

Torfeno Branishte Reserve

High status has been achieved based on the results for conductivity, BOD5 and nitrate nitrogen, as well as for the nonclassified in water legislation parameters oxygen saturation, total and suspended solids, permanganate oxidation, chlorides sulphates (Table 2). Relatively and substantial difference was found between the waters of both rivers tested with regard to ammonium nitrogen and dissolved oxygen. According to them, the assessment of the status for Boyanska River indicated presence of nutrient pollution and evaluation was

biological quality element macrozoobenthos, the waters of the Vladayska River was defined within the limits of "high" ecological status for the R2 river type by both regulated indices – BI and TNT. This assessment coincided with the official one presented in the River Basin Management Plans of Danube Region (RBMP) (2016-2021). The waters of the Boyanska River were defined in "good" ecological status by index BI and in "high" by TNT. In this way, the Vladayska River can be characterized as unaffected and optimally functioning aquatic ecosystem, while the Boyanska River was slightly influenced but assessed in the boundaries of the target "good" ecological status.



Fig. 1. Location of Torfeno Branishte and Bistrishko Branishte Reserves on the territory of the Vitosha Nature Park.

Reserve	River site	Coordinates of sampling site	Elevation (m a.s.l)	River type	Water Body Code	Danube River Basin/catchment
Torfeno	Vladayska River	42°53'15,1" N 23°15'40,8" E	1810	R2	BG1IS500R1130	Iskar River
Branishte	Boyanska River	42°36' 09.8''N 24°23'16.2'' E	1800	R2	BG1IS500R1109	Iskar River
Bistrishko	Vitoshka Bistritsa River	42°34'28,8'' N 23°18'37,3'' E	1700	R15	BG1IS700R1107	Iskar River
Branishte	Yanchovska River	42°34'43,1'' N 23°18'33,7'' E	1635	R15	BG1IS700R1107	Iskar River

Fable 1. Descrip	otion of the	sampling	sites.
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Bistirshko Branishte Reserve

The hydrochemical analyzes of the waters showed that both studied sites in BBT are in "high" status for parameters conductivity, dissolved oxygen and ammonium nitrogen. According to Regulation N4/2012, a "good" status was found only for BOD₅ (Vitoshka Bistritsa) and for nitrate nitrogen (Yanchovska River). The additional indicators (oxygen saturation, total and suspended solids, permanganate oxidation, chlorine and sulphate ions) had optimal values which could characterized running ecosystem as clean and undisturbed. The ecological status based on biological indices of the Yanchovska River was determined as "high" with maximum value of the BI and TNT according type specific scale for R15 river type. It could be assumed that there is no anthropogenic impact on the studied water body. The specific characteristics of the bottom substrate (fine sand and forest soil, covered with leaf litter) reflected the relatively poorer macrozoobenthic taxonomic diversity in Vitoshka Bistritsa. This could be the reason for the lower value of the TNT. The values of two biological metrics responded to "good" status for the R15 river type (Table 2).

It should be emphasized that the ecological status of the two rivers (Vitoshka

Bistritsa and Yanchovska) in the reserve is not defined in the RBMP (2016-2021). For comparison, in the RBMP (2010-2015) the ecological status of the Vitoshka Bistritsa and Vladayska River was assessed as "good". Till now, during the implementation of the two RBMP evaluation of the waters of the Boyanska River in the area had not been made. This is the first ecological status assessment of the lotic ecosystem in the studied section.

Conclusions

The present study gave for the first time a complex information of ecological status of the main rivers of two reserves based on physicochemical and biological (benthological) quality elements. The evaluation showed that the waters formed and running through the territory of TBR and BBR can be described as an undisturbed or slightly affected, and characterized with optimally functioning aquatic ecosystems. The results obtained for the representative surveyed river sites, could be utilized in decision-making process to insure the relevant measures for prevention the potential anthropogenic pressure and further maintaining the high quality of the waters in the studied reserves.

	Torfeno Rese	Branishte erve	Bistirshko Res	Bistirshko Branishte Reserve	
Parameters	Vladayska River	Boyanska River	Vitoshka Bistritsa River	Yanchovska River	
T (°C)	8.4	7.8	10	9.7	
pH	6.4	7.5	6.08	6.58	
Conductivity (µS.cm ⁻¹)	46	39.48	45	19.8	
Oxygen saturation (%)	79.7	80.0	92.5	97.1	
Dissolved oxygen (mgO ₂ .dm ⁻³)	7.67	7.17	8.61	8.88	
Total solids (mg.dm ⁻³)	24	24	45	73	
Suspended solids (mg.dm ⁻³)	2	3	4	15	
Permanganate oxidation $(mg O_2.dm^{-3})$	0.69	0.76	0.75	2.63	
$BOD_5(mg O_2.dm^{-3})$	0.39	0.12	1.23	1.18	
$N-NO_3$ (mg.dm ⁻³)	0.11	0.14	0.14	0.66	
$N-NH_4$ (mg.dm ⁻³)	< 0.01	0.08	< 0.01	< 0.01	
Cl^{-} (mg.dm ⁻³)	2.5	< 2.0	< 2.0	3.4	
SO_4^{2-} (mg.dm ⁻³)	2.6	2.6	6.5	14.3	
Physicochemical status	High	Good	Good	High	
TNT	16	15	9	16	
BI	4.5	4	3	4	
Hydrobiological (benthological) status	High	Good	Good	High	
Ecological status	High	Good	Good	High	

Table 2. Classification of ecological status of the studied rivers.

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Breakthrough in Anti-poison Struggle after Introduction of Intensive Satellite Tracking of Griffon Vultures in Balkans

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Abstract. The use of poison baits for controlling predators is illegal practice in Europe nowadays, but it is still in use by some local people as an effective and affordable "solution" for resolving the conflicts with carnivorous mammals and other wildlife species. Many different approaches and activities were implemented in the last two decades to combat poisoning in Balkans, but the problem is still out of control with repeating cases of catastrophic events, also harming seriously the vulture populations in the region. The present research introduce a new strategy for controlling the problem, by using of patagial GPS/GSM transmitters for birds, which in the case of the Griffon vulture (Gyps fulvus), being more exposed to the sun, have an improved charge of the batteries through the solar panel, and thus offer the possibility for intensive tracking and frequent obtaining of detailed data load. A GPS location can be received on every minute or less and data load on every 10 minutes. This intensity of tracking the vultures' movements is a good tool to control their whereabouts in real time, which gives an essential advantage in anti-poison struggle, by fast location of problematic situations. The following work presents the developed approach and the first results, which are the base for proposing of new, early warning system for wildlife poisoning and poaching control and monitoring.

Key words: wildlife poisoning, GPS transmitters, conservation, Gyps fulvus, Vulture safe area.

Introduction

to vultures worldwide and has contributed depletion of the entire species group (BOTHA et al., 2017).

countries even until the 1990s), poisoning was a legal practice, funded and carried out country) after the ratification of the Bern by governmental authorities in order to Convention, which banned this practice control the populations of wild predators (GRUBAČ, 2014; BOTHA et al., 2017).

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(GRUBAČ, 2014). This practice was a severe Poisoning is the most significant threat threat for wildlife and especially for the vulture species, not only in the Balkans but for the regional extinction or severe also across the Mediterranean region (CRAMP & SIMMONS, 1980). The use of poison against wild animals became illegal During the 1950s and 1960s (in some by the end of the 1980s or the beginning of the 1990s (depending on the individual

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control predators is illegal in Europe, including on the Balkans, but it is still in use mainly by local farmers as a quick and affordable "solution" for resolving the conflicts with the carnivore species. The main driver for poison use is the conflict between livestock breeders and mammalian predators (ANDEVSKI, 2013), mainly wolves (Canis lupus) (PARVANOV et al., 2018). In all likelihood, the wider distribution and higher numbers of wolves in the Balkans and the permanent conflict with livestock breeders is one of the main factors behind the great difference in numbers and distributions of the vultures in this region compared to Iberian Peninsula (STOYNOV et al., 2018).

Many different approaches and activities were implemented in the last two decades to combat poisoning in Balkans, e.g. the promotion and provision of guarding compensation prevention dogs, and programmes for livestock to minimize the man/predators conflict and respectively the use of poison (ANDEVSKI, 2013; STOYNOV et al., 2014), public awareness campaigns and most recently the forming of Canine Teams also known as Anti-poison dog units (PANTOVIĆ & ANDEVSKI, 2018). Apart from being a preventive mean, the Canine Teams contribute to the dissemination and increase of awareness regarding this conservation problem and they assist the related authorities in their pre-trial work, collecting findings that can be used as evidence during the investigation and the judicial procedure (PANTOVIĆ & ANDEVSKI, 2018). However, these teams are unable to cover large areas and their effectiveness relies largely on a priori received information of dead animals or poison baits.

Despite the species and habitat protection on government level and the long-term engagement of NGOs in the Balkan region, the threat of poisoning is hardly controlled yet and so far remains misunderstood and underrated among specialized institutions and authorities suspicious unusual behaviour.

At present, the use of poison baits to (PESHEV et al, 2018a; b). This is probably because the practice is illegal, with very dynamic presence in space and time and in most cases remains unidentified. It is occasionally noticed, when some animals are found dead - dogs, cats, wild carnivores, as well as scavengers such as vultures and eagles. However, the observer can hardly relate a located poisoned vulture with the specific site of the poisoning incident. In some poisoning cases, the birds or any other dead animals could be found next to the bait, but in the case of the vultures, they could move to some or even tens of kilometres from the place where they consumed the poisoned meat prior to their death. This was clearly confirmed during the poisoning incident in the Kresna Gorge in Bulgaria from March 2017 (PESHEV et al., 2018a; b). In that case, in two weeks more than thirty Griffon vultures (*Gyps fulvus*) were poisoned in a single poisoning incident - a loss that probably would have been reduced if the bait was discovered and neutralized in time. This recent case confirmed again that the vultures may feed on poisoned carcass, move in large numbers and die up to 20 km away (Fig. 1), some even 60 km away. These specifics make the investigation of the crime almost impossible, regardless the will of the authorities to cope with the problem.

> The case in Kresna Gorge was the final step to trigger the introduction of a new approach for using of transmitter data by tracking intensively the vultures' whereabouts and to use the tracked individuals as "poison *detectives*" in the field, which methodological concept was called "poison aerial control". The already well developed technologies of GPS bird tracking devices (light weight, solar powered, 3D printing of any type and shape of housings, permanent settings control by the customer, etc.) and the affordable prices of data load possibility by GPRS service, gave us a powerful tool to constantly track vultures in real time in the internet and follow them in the field if we made any observations of



Fig. 1. Map of the Kresna Gorge poisoning incident in March 2017. The black circles indicate dead vultures; the green diamonds indicate the traditional roosting sites; the blue circle indicates the project vultures' feeding site; the red circle indicates the poison bait location.

Materials and Methods

The intensive monitoring of vultures' location was conducted by using of *patagial* transmitters OT-P33, developed by our research team in cooperation with Ornitela

UAB, using modern 3D printing technology. Similar transmitter positioning and attachment were used in other raptor research activities, e.g. California condor (Gymnogyps californianus) (POESSEL et al., 2017; STOYNOV et al., 2019). In the case of the Griffon vulture, we expected an improved charge of the batteries, through the solar panel being highly exposed to the sun (Fig. 2), and thus a possibility for frequent and abundant data load - the obtaining of GPS location of the equipped individuals became possible at every minute and data load respectively at every 10 minutes. In contrast to the other transmitter-related studies of vultures, this high frequency of locations identification and data load is of essential importance for the proposed "poison aerial control" and provides a very important advantage, or even a breakthrough in the struggle against poisoning of wildlife.

Griffon vultures were fitted with GPS/GPRS transmitters and their whereabouts were constantly tracked on an internet platform from a project staff member, called "Poison alarmist" (hereafter the Alarmist). The role of the Alarmist was to analyse the received data and to observe for untypical behaviour in vulture's movements. According to the expert's data evaluation, he contacted the local field researchers/rangers in order to check locations of possible incidents.

For a period of 1,5 years, 14 Griffon vultures were racked in this intensive way during a LIFE project, covering almost all of the Balkan Griffon vulture colonies (Vultures back to LIFE 2017; 2018).

Results and Discussion

The proposed "Poison aerial control" system is already confirmed to be effective in real cases. In May 2018, from its office in Bulgaria, c.350 km away from the Agrafa Mountains in Greece, the Alarmist followed a Griffon vulture with a transmitter and identify that it had stopped moving with mortality icon appeared in the internet platform. After analysing the movement of the bird in *Google Earth*, the place where the bird had fallen and its whereabouts prior to the mortality location, the vulture had been its death were researched and revealed that on the ground for c.2 hours, which was in a place located on more than 5 km from probably a sign for a feeding event (Fig.3).



Fig. 2. Patagial GPS/GPRS transmitter OT-P33 mounted on Griffon vulture – left- in flight; right – landed/perched.

The coordinates were sent by the observer, working with the Alarmist to the authorities in Greece and to the Canine Team of Hellenic Ornithological Society (HOS) in the region. The common team visited the site some days later and confirmed the tracked vulture's death, as well as found two more corpses of dead Griffon vultures 5 km from it - at the coordinates reported firstly by the Alarmist. A corpse of a calf (used as a bait) and a plastic bottle of pesticide were also found (Vulture Conservation Foundation, 2018). In this way, although the "poison detective" died 5 km away from the poison bait, it provided the important clue to the distantly situated Alarmist system, which shared the information and thus allowed the case to be detected, recorded, and reacted to.

Although only three Griffon Vultures' corpses were found in that case, it is likely that in the radius of 5 km or more in an

extremely rugged terrain, more dead birds remained hidden. However due to the fast localisation of the poisoning site, a possible catastrophic event, similar in scale to Kresna gorge poisoning, was prevented (PESHEV *et al.*, 2018a; b).

Although more than 50 checks in the field were executed and only one case of poisoning was detected so far, the introduction of this method is a probable breakthrough in the fight against poisoning in the Balkans and should be employed permanently and widely in vultures' conservation practice.

The practical testing of the proposed warning system showed, that its effectiveness of depends mostly on the number of tracked vultures or "poison detectives" which is directly connected with the coverage of the species range in the targeted area. The preliminary verification of the concept, confirmed with identification of real poisoning incidents, showed that a GPS every 4 hours are probably the optimal fix in every 10 minutes and data load in settings for the transmitters power use.



Fig. 3. Map of the case of detected poisoning of Griffon vulture in Agrafa Mountain in Greece in May 2018. Yellow and green circles indicate traditional roosting sites of the species in the region; black circles – indicate the poison bait place and two poisoned Griffon vultures; the red circle indicates the place where the tracked with GPS/GPRS transmitter Griffon vulture was found dead. The lines with different colours indicate the movements of the bird in three consecutive days derived by GPS.

However, in case of emergency - if there is information of potential poisoning incidents in the area, or some specific behaviour of the tracked vulture occurs, i.e. unusual site or position, long staying on the ground etc., the frequency of the GPS fix could be set up on 1-5 min and the data load as intensive as necessary according to the concrete occasion. In such cases, also a team field visit is required to identify every potential problem. If the Alarmist observes that more than one of the tracked vultures go to one location (excluding traditional roosting sites), this is probably due to the presence of food or water source. However, there is also a possibility that vultures are

attracted by a poached wild animal, poison bait or depredated livestock, as in the cases described. All of these cases represent important conservation risks and should be closely monitored by field team, which should check the feeding event. In areas where a Canine Team is operating, it is worth sending it on site to find the potentially poached or poisoned dead animals as soon as possible in order to prevent any feeding from them. The Alarmist (if not going on its own) sends the GPS coordinates to any other project team member or ranger from local NGO/authority who is able to do a direct field check (together or instead of Canine Team, if not available). Despite the check and the work on the case (protocol, monitoring), the presence of the team member at the location where the vultures were/are sets a good example for the local farmers (to whom the dead livestock usually belongs), letting them know that someone is following the vultures in time and space and may notice if they are killed, poisoned etc. In such cases, the witnesses of such interesting phenomena may spread the word in local communities, which raises public awareness on the subject and eventually help prevent criminal attempts.

Conclusion

The described above GPS tracking approach should be widely introduced as poisoning control and prevention measure, especially in marginal (respectively more vulnerable) vulture populations such as those in the Balkans. It will provide fast and accurate detection and thus adequate reaction to poisoning (but also poaching, livestock depredation etc.) cases, even in otherwise remain remote areas that unregistered and could be a powerful tool for prevention of catastrophic mass poisoning events such the one in Kresna gorge in 2017. The analysis of the recent poisoning cases suggested that a minimum of three adult (respectively more territorial) Griffon Vultures should be tracked and used as "poison detectives" at a time per colony. Another two immature birds (more mobile in comparison to adults) per site should be also equipped with transmitters and tracked. Where possible, the poison aerial control should be combined with the work of a Canine Team on the ground. Effectiveness of the system requires not only a monitoring of each colony by at least 3-5 "poison detectives" at a time but also a fast recovery of that number after cases of loss of any transmitter/vulture. The practical testing showed that one Alarmist could follow 30 "poison effectively on-line up to detectives". This approach should be considered as an important conservation

practice, and should be widely introduced by managers and supported in conservation programmes. The large amount of data obtained could be also a base for strictly scientific studies, but the primary goals should be the in time reaction to poisoning incidents, which will prevent losses of threatened species - directly - by rehabilitating poisoned birds that are found still alive, destroying poison baits intime to minimize losses, and indirectly - by increasing the control on poisoning and poaching. This same approach could be introduced in poaching and/or livestock depredation monitoring by managers in protected areas or wherever necessary (in any vulture species range).

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Past and Present State of the Griffon Vulture Gyps fulvus in Rila and Pirin National Parks and Rilski Manastir Nature Park

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Abstract. Widespread in the past, the Griffon Vulture (Gups fulvus) has disappeared in most of Europe (including Bulgaria) in the 20th century. Although this species was reported from Southwestern Bulgaria in the area of Rila and Pirin it became locally extinct. In the end of 20th century it was breeding only in Eastern Rhodopes. With the local reintroduction of the species in Kresna Gorge since 2010, the high mountain pastures and cliffs regained their role of refuge for the Griffon Vulture mainly in summer. Here, we report and analyze the home range and seasonal presence of the species in Rila National Park, Pirin National Park and Rilski Manastir Nature Park. Home range analysis was based on data received from 8 birds tracked by GPS/GSM transmitters in Southwestern Bulgaria. The obtained results (Max. Daily distance = 195.3 ± 51.1 km; Home-range 95% kernel = 238 ± 207.5 km² and core area 50% kernel = 6.5 ± 5.4 km²) indicated that these birds almost exclusively roost and feed in the already established Griffon Vulture colonies or join the seasonal gatherings of conspecifics, which are usually found within a zone of 15 km radius from a complex of feeding site or abundant easy accessible food in close proximity to suitable roosting places. Strategically placed and permanently supplied vulture feeding stations in close proximity of roosting sites, may concentrate large numbers of Griffon Vultures in relatively small and controlled areas. This, in combination with implementation of site based conservation activities such as the creation of Vulture Safe Areas, the impact of any, otherwise, hardly controlled and large-scale threats, such as poisoning and electrocution can be buffered. Rila and Pirin National Parks as well as Rilski Manastir Nature Park are recommended for management, adapted to the Vulture Safe Areas concept.

Key words: local extinction, reintroduction, conservation, Gups fulvus, summer pastures, Vulture Safe Areas.

Introduction

The distribution of the Griffon Vulture (*Gyps fulvus*) in Bulgaria was wide during 19th century, and, although declining, the species was still present until the first half of 20th century (CRAMP & SIMMONS 1980; DEMERDZHIEV et al., 2007; 2014). authors The same compiled numerous observations and information reported in other studies authored by Bulgarian and foreign ornithologists since the end of the 19th century some birds were rarely observed in Southwestern

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about the presence of the species, including in the current studied sites - Rila and Pirin Mountains.

In the 1960s the Griffon Vulture disappeared in most of the country, mainly due to the widespread poisoning campaigns against large predators and was considered extinct until 1978, when a small group of birds was discovered in the Eastern Rhodopes (MICHEV et al., 1980; DEMERDZHIEV et al., 2014). In the following period,

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Bulgaria and were classified as wandering individuals (YANKOV & PROFIROV, 1991).

Since 2010, the Griffon Vulture is a subject of reintroduction efforts in the area of Kresna Gorge, with wide range of related activities, including the release of more than 80 individuals, imported from Western Europe, until the end of 2018. The first breeding attempts with successfully fledged chicks were registered in 2016 (PESHEV *et al.*, 2015; 2017; STOYNOV *et al.*, 2018). The newly formed colony in the Gorge attracted birds from other parts of the Balkans, a fact confirmed with numerous observations of marked vultures. A maximum number of 63 individuals was recorded in November 2016 (PESHEV *et al.*, 2017), with a continuous tendency for increasing in the following years.

During the reintroduction activities, a vulture feeding station was constructed and supplied with 3 to 10 tons of carcass monthly. The dead livestock was delivered to the feeding station from a specialized project team and was obtained by local farmers in variable quantities, due to the different mortality levels during the seasons.

Yet since the summer of 2012, but more profoundly in 2013, the vultures started to migrate vertically to the higher parts of Pirin and, more rarely, Rila Mountains. The birds were obviously attracted by the livestock herds, located there in the summer, and the abundant fresh water sources. In the period 2013-2018, the vultures were observed to use these areas in July-October, and were absent or only rarely used the feeding station in Kresna gorge during that period.

The present study aims to provide new insight for the presence of the Griffon Vulture in Rila National Park (UTM, GM17), Rilski Manastir Nature Park (UTM, FM96) and Pirin National Park (UTM, GM02), which cover most of the Rila and Pirin Mountains and have the statute of protected areas with appointed management administrations and plans. These sites completely overlap with the same named Special protected areas (SPA) from the Natura 2000 network (MOEW, 2013) and the Griffon Vulture is so far not included in the standard Natura 2000 forms of Rila SPA (BG0000495), Rilski Manastir SPA (BG0000496) and its presence in Pirin SPA (BG0000209) is of one individual, according to the related publications (MOEW, 2013).

We, herewith, systematized the data on movements of the equipped with GPS/GSM transmitters Griffon Vultures in the Southwestern Bulgaria and based on most actual data proposing the inclusion and update of the species population parameters in the Standard Natura 2000 forms for the researched here sites. The last is important for the evaluation of importance of the Rila and Pirin National Parks and Rilski Manastir Nature Park as areas in use by the Griffon Vultures and for including of the species in future related strategic documents and for the development and application of certain measures for its conservation including further local population restorations.

The detailed information about the Griffon Vulture's territorial and seasonal movements in the researched area is of significant importance to understand and neutralize major threats for the species like the use of poison baits and the unsecured power lines used for perching, and for management of important factors like the food sources management, breeding and roosting sites protection etc.

Material and Methods

The presented information was obtained through mounting of GPS/GSM transmitters on some of the reintroduced Griffon vultures (*Gyps fulvus*). Eight immature birds, equipped in the period 2012-2017, remained in the area long enough, to allow detailed analysis of their movements.

Vultures were tagged in the adaptation aviary near the Rakitna Village (Kresna Gorge, UTM, FM73) before their releasing. The transmitter weight was 33 g., or 0,3% of the body mass (<3% is recommended for flying birds) and it was also used to hold the wing-tag, mounted on the *patagium* of the vultures to assist the visual recognition. The transmitter was equipped with solar panel and was designed with a weak point in the attachment pin, in order to fall off the bird after few years.

The data download and the reprogramming of transmitters were executed remotely through internet. We estimated the number of days with bird presence in Southwestern Bulgaria, by taking into account all coordinates, closed in the area south from 42°20 N and west from 23°60 E, and by the national borders to the west with North Macedonia and Serbia and to the south with Greece. The days spent in the Rila and Pirin National Parks and Rilski Manastir Nature Park were calculated. The daily distance covered by all tracked vultures were calculated as the average of summed straight-line distances between successive locations on the same day.

We calculated the 50% (core area) and 95% home-range (FIEBERG, 2007) of the vultures by using the classical kernel method (kernelUD, points, extent=0.1) (CALENGE, 2006).

We excluded all coordinate locations acquired between 20:00 and 06:00h local time and they were used only for defining the nocturnal roosting places. The analysis was made using QGIS 3.8.0 (QGIS Development Team, 2009), OpenJUMP HoRAE toolbox (STEINIGER & HUNTER, 2012) for OpenJUMP GIS 1.7.1 (The JUMP Pilot Project, 2008), the package adehabitat for the R software, R package version 3.4.0 (MAECHLER *et al.*, 2013). We estimated the 95% and 50% kernel of all points during birds movement (speed >5km/h), eliminating the impact of the points, where the vultures were resting or roosting, which helped for better understanding of the dynamic parts of their behavior.

We calculated 95% and 50% kernels of the coordinates in the summer months (July – September), when the birds were mainly in the territory of the national parks, and the percent of overlapping of kernel area with the national parks territories was estimated.

To establish the numbers of the Griffon Vultures visiting the Rila and Pirin National Parks and Rilski Manastir Nature Park, we used direct observations either done by our team or reported from other observers in occasional visits. The last were included for analyzing here only if the provided information possessed all required attributes (exact date, place and observer) and was within the scope of the estimated home-range acquired by the GPS/GSM transmitters and/or the observation was proven by photographic evidence.

Results

The seven transmitters sent a total of 80277 GPS coordinate locations (excluding nocturnal and erroneous) in Southwestern Bulgaria. The average number of coordinates sent by a transmitter was 10034.6 \pm 6979.4 (range 2448 – 22211). The tracking period for the birds on average was 152.3 \pm 116.4 days (range 23 – 373). The average number of coordinates per day is 71.8 \pm 17.7 (range 61 – 106.4).

In total the vultures flew over 47081.1 km, mean for vulture 5885.1 ± 4810.9 km (range 1575 – 14737.7) (Table 1).

Daily movement. The average distance of the daily flights was 55.7 ± 36 km (range 22.5 – 143.2), where the maximum distance covered in a single day was 195.3 ± 51.1 km (range 98.1 - 268.4).

Home Ranges Estimation. For all of the tagged birds the total area covered by 95% kernel was 920.3 km², and for 50% kernel was 36.2 km² (Fig. 1).

Table 1. Results from GPS tracking of eight Griffon Vultures (*Gyps fulvus*) in SW Bulgaria.

Tag	Total tracking days in SW Bulgaria	Days with coor- dinates in the Parks	Start - End date	Received GPS coordinates	Coordi- nates per day	Distance traveled, km	Daily distance, mean ± standard deviation	Median daily distance, km	Daily distance (min - max), km
56	280	48	13.07.2017 - 01.05.2018	17651	63.0	14737.70	52.6±47.7	34.60	2.2-231.7
v8	373	33	03.05.2018 - 07.05.2019	22211	59.5	11848.50	32.4 ± 31.0	21.20	0.7-207.8
v3	180	28	24.04.2018 - 24.04.2018	14389	79.9	5324.40	29.6±31.2	18.60	1.8 - 176.4
k9u	23	12	03.08.2017 - 31.03.2018	2448	106.4	1575.00	68.5±57.7	44.30	0.9-238.7
k2m	179	23	03.08.2017 - 01.02.2018	10919	61.0	7489.25	41.8 ± 43.6	23.2	1-195.1
89	53	35	15.07.2016 - 12.09.2016	2455	46.3	1191.03	22.5 ± 26.7	12.00	0-98.1
b70	59	21	18.07.2017 - 14.09.2017	5184	87.7	1002.10	143.2±61. 4	111.80	86-268.4
b71	71	27	03.08.2017 - 14.10.2017	5020	70.7	3913.20	55.1±43.4	46.50	0.1-146.4



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Fig. 1. Total area coverage of home range (95% kernel) and core area (50% kernel) for the eight tracked Griffon Vultures. The generalized map (right) shows the study area location.

Mean area of the home range (95% kernel) and core area (50% kernel) was $238 \pm 207.5 \text{ km}^2$ (range 43.7 – 749) and 6.5 ± 5.4 km² (range 2 – 15.9), respectively.

For all vultures, 32% of the 95% kernel overlaps parts of Rila and Pirin National parks, and for the 50% kernel, the percent is 9. (Table 2).

Kernels for coordinates with speed over 5 *km/h.* Mean area of the home range (95% kernel) and core area (50% kernel) was 882.2 \pm 573.5 km² (range 158.1 – 2219.6) and 57.8 \pm 46.2 km² (range 18 – 135.8), respectively.

Summer coordinates home range estimation. Mean area of the home range (95% kernel) and core area (50% kernel) was $315.9 \pm 189.5 \text{ km}^2$ (range 156.3–749.4) and 9.7 $\pm 4.6 \text{ km}^2$ (range 2.1 – 15.5), respectively.

Direct observations of Griffon Vultures reported for Rila and Pirin National Parks and Rilski Manastir Nature Park. In total 24 observations of Griffon Vultures were collected

and analyzed in this study from 2013 until the end of 2018, although some more for the period 2012-2018 were provided in the technical reports by STOYNOV & PESHEV (2013) and PESHEV et al. (2017; 2018; 2019). The last were not included in the current study due to insufficient precision in data collection and provision. However, this additional data increased the knowledge about the frequency of the presence of Griffon Vulture in high mountains of Southwestern Bulgaria. All reported observations of the species in the target areas were obtained in the period July-October, except one in April 2016. The maximum number of observed together individuals was 32 in Pirin National Park. The maximum seen at once in Rila National Park were 10 individuals, which is the same in Rilski Manastir Nature Park, because the very same observation was in the two sites in the same time (Table 3).

Discussion

The Griffon Vulture was extinct in Southwestern Bulgaria around mid 20th Century (DEMERDZHIEV *et al.*, 2007) and thus was not listed as species of conservation concern in the Rila and Pirin National Parks and Rilski Manastir Nature Park, which were established in their current shape in 1990s (YANKOV & PROFIROV, 1991; MOEW, 2013). The species is not listed in the Standard Natura 2000 forms for Rila National Park and Rilski Manastir Nature Park (MOEW, 2013) and its presence is obviously underestimated in Pirin National Park, where the minimum and maximum population is presented with 1 individual.

Table 2. Home-ranges including Core area - 50% kernel and home-range 95%, Kernels for coordinates with speed over 5 km/h and summer coordinates home range estimation.

Tag	Core areas (within 50% UD isopleths), km ²	Home range area (within 95% UD isopleths), km ²	Speed >5km/h Core areas (within 50% UD isopleths), km ²	Speed >5km/h Home range area (within 95% UD isopleths), km ²	Summer Core areas (within 50% UD isopleths), km ²	Summer Home range area (within 95% UD isopleths), km ²
56	3.23	226.6	15.1	674.4	10.1	448.9
v8	2.00	56.7	18.0	856.6	8.0	163.3
v3	4.50	43.7	25.8	580.0	12.4	156.3
k9u	15.90	749.0	135.8	2219.6	16.1	749.4
k2m	4.00	158.1	15.8	158.1	8.6	341.9
89	15.50	214.2	98.6	750.2	15.5	214.2
b70	4.50	280.7	111.6	1195.6	4.5	280.7
b71	2.60	175.3	42.0	622.9	2.1	172.3

Table 3. Observations of Griffon Vultures (*Gyps fulvus*) in Pirin and Rila National Parks and Rilski Manastir Nature Park for the period 2013-2016.

No	Date	Number of Griffon Vultures observed	National/ Nature Park	Specific site	Observer
1.	20.09.2013	12	Pirin	Spano Pole	Pirin NP ranger
2.	22.09.2014	14	Pirin	Orlite	Todor Georgiev. Pirin NP
3.	23.08.2014	11	Pirin	Koncheto	Margarita Valkova
4.	28.07.2015	7	Pirin	Todorka	Own observation
5.	19.08.2015	6	Pirin	Vihren	Own observation
6.	21.09.2015	16	Pirin	Orlite	Todor Georgiev, Pirin NP
7.	28.04.2016	1	Pirin	Todorka peak	Pepi Sakariev
8.	05.07.2016	5	Pirin	Gorno Vlahinsko ezero	Ivaylo Nikolov
9.	08.07.2016	1	Pirin	Sinanishka porta	Maya Petkova
10.	22.07.2016	5	Pirin	Karaulite	Yordan Pulev
11.	23.07.2016	4	Pirin	Spano pole	Ivaylo Madjarov
12.	23.07.2016	10	Rila & Rilski	Malyovitsa peak	Vera Peltekova
			Manstir		
13.	23.07.2016	28	Pirin	Bashliiska reka	Lyudmil Petrov
14.	30.07.2016	17	Pirin	Chairski ezera	Yordan Kutsarov

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15.	11.08.2016	1	Pirin	Tipitsite peaks	Petko Boyadjiev
16.	11.08.2016	32	Pirin	Bashliiska reka	Own observation
17.	12.08.2016	2	Pirin	Banderishka porta	Own observation
18.	17.08.2016	3	Pirin	Razloshki suhodol	Elena Smilkova
19.	21.08.2016	3	Pirin	Banderishki chukar,	Nikolay Dautov
				Muratov and Karaulite	
20.	28.08.2016	3	Pirin	Todorka peak	Vladimir Milushev
21.	29.08.2016	2	Pirin	Vihren peak	Vladimir Milushev
22.	05.10.2016	1	Rila	Ibar reserve	Yordan Hristov
23.	07.10.2016	7	Pirin	Tevno ezero	Pepi Sakariev
24	26.08.2018	4	Rilski	Kirilova polyana	Nikolay Petkov
			Manastir		



Fig. 2 (left), Fig. 3 (right). Griffon Vultures in high altitude ~2400 m mountain habitat in Pirin National Park (Photo by Hristo Peshev, 11 August 2016).



Fig. 4. The remains of a dead cow eaten by the Griffon Vultures in high altitude ~2200 m mountain pastures in Pirin National Park (note the Griffon Vulture's secondary feather in front) (Photo by Hristo Peshev, 11 August 2016).

The current study provides new insight of Griffon Vultures' movements and whereabouts in Southwestern Bulgaria and some specific data about their seasonal presence and numbers in the targeted Parks' territories.

While the feeding station in Kresna Gorge is definitely the focal point and the center of the core area for the relatively newly formed colony of the species in the study region (STOYNOV *et al.*, 2018), the summer movements of the birds to the higher parts of Rila and Pirin mountains are clearly visible.

For roosting, vultures were using mainly few traditional sites as referred in the study of PESHEV *et al.* (2018), and feeding events outside the specialized feeding stations in Kresna Gorge, Macedonia or Greece were rarely registered, with the exclusion of the regular summer visits to Pirin National Park, where the vultures were feeding on carcasses of free ranging cattle in the period July – October.

Analysis of the points where birds are on the move shows that 95% kernel fully cover Kresna Gorge, about half of the Pirin National Park, parts of Maleshevska Mountain, Rila National Park and Rilski Manastir Nature Park. This falls within the c.15 km radius area around Kresna Gorge, which is perimeter also confirmed in other studies (XIROUCHAKIS & ANDREOU, 2009; GARCÍA-RIPOLES et al., 2011) The 50% kernel for all tracked birds include the feeding station, favorite roosting places in the Kresna Gorge and area of 2-5 km around them. Similar second core that was used exclusively in the summer was observed in Pirin National Park in the areas of Spano pole and Bashliiska reka with the surrounding peaks, where feeding of vultures and roosting sites were only bv the GPS/GSM registered not transmitters, but also through direct observations.

Based on the results of the current study we suggest a summering to be included as a seasonal pattern of presence of the Griffon Vulture in certain Natura 2000 sites which should be listed in the Standard Natura 2000 form for the respective SPAs as follows:

- Pirin National Park (BG0000209) – present in summer, min. 10 ind. – max. 32 individuals (instead of min. 1 and max. 1 individual as it is now).

- Rila National Park (BG0000495) – present in summer, min. 4 ind. – max. 10 individuals (instead of none as it is now). - Rilski Manastir Nature Park (BG0000496) - present in summer, min. 4 ind. – max. 10 individuals (instead of none as it is now).

Conclusions

The results showed that the Griffon Vulture is seasonally present in Rila and Pirin National Parks and Rilski Manastir Nature Park, where the birds are spending the summer months. These territories should be maintained in ways, which could provide safe environment for the vultures preventively addressing threats such as poisoning and electrocution/collision with power lines. The establishment and maintenance of seasonally operated feeding stations, which concentrate the vultures in safe environment in the protected areas in the high mountains should be shortly foreseen and implemented by each of the current study's target Parks' administrations. Also any future management of the livestock grazing and husbandry in the area should acknowledge the vultures' presence and needs. Specific measures for the Griffon Vulture should be incorporated in any future management plans development for the Rila and Pirin National Parks and Rilski Manastir Nature Park. Also the species should be listed accordingly and updated in the Standard Natura 2000 forms.

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Past and Present State of the Cinereous Vulture (Aegypius monachus) and Feasibility Analysis for its Reintroduction in Bulgaria

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Abstract. Widespread in the past, the cinereous vulture has disappeared in most of Europe (including Bulgaria) in the twentieth century. Surviving nucleus remained only in Spain, Greece, Ukraine and the Caucasus. The species is considered extinct as nesting in Bulgaria yet within the Red Data Book in 1985. Despite occasional observations in the country and frequent presence and even sporadic nesting of solitary pairs in Eastern Rhodopes Mts., the cinereous vulture's Balkan Peninsula population (only one colony of 20-30 pairs in NE Greece) shows inability to increase further, disperse and reestablish naturally. This justifies the need to assist creation of new and more nesting sites to ensure long-term conservation of the species, based on local reintroduction in Bulgaria. After the success with the local reintroductions of the griffon vulture since 2010 in the Kresna Gorge and different places along the Balkan Mountains the conservation community in Bulgaria was encouraged to further proceed with the exploration and planning of reintroduction and conservation activities for the cinereous vulture. Herewith assessment of the chosen territories for reintroduction is presented and recent best sites in Bulgaria identified.

Key words: local extinction, reintroduction, conservation, Aegypius monachus, Gyps fulvus.

Introduction

The cinereous vulture monachus (also known as Eurasian black PESHEV, 1985, HRISTOV & STOYNOV, 2002; vulture) - the largest bird of prey in Europe, STOYNOV et al., 2007; GOLEMANSKY et al., obligate scavenger, breeding solitary or in 2015). Surviving nucleus remained only in loose colonies, nesting in trees (CRAMP & Spain, Greece, Ukraine and the Caucasus, SIMMONS, 1980), classified globally as "near while since 1990s small nuclei were threatened" (BirdLife International, 2018) reintroduced in south of France (BOTHA et al., used to be a widespread species in southern 2017). In Spain, from the 1980s to the present Europe, but since late 1800s until mid 1900s day, the species is recovering, which is experienced dramatic decline

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disappeared from most of its range Aegypius (including Bulgaria) (PATEV, 1950; BOTEV & and possible due to the adoption of specific

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strict control on the use of poison baits, the protection of the main nesting colonies and the operation of feeding sites (BOTHA et al., 2017). In Greece - the nearest to Bulgaria breeding colony of the species in Dadia-Soufli-Lefkimi Forest National Park the number ranges between 20-35 pairs in the last three decades (SKARTSI et al., 2003; 2009; POIRAZIDIS *et al.*, 2004; SKARTSI, 2019). Despite the slow and slight increase in the number of the colony in Dadia-Soufli-Lefkimi Forest National Park and subsequently the increase in observations of immature birds roaming across the border in Bulgaria, still no new breeding nuclei or colonies were identified in neither Bulgaria, Greece or Turkey. Recently, increased mortality of young adults and immature birds in the colony was reported reintroduction. especially for birds that are leaving the very central core of the Dadia-Soufli-Lefkimi Forest National Park (SKARTSI, 2019).

The following two facts provide for the need of reintroduction of the cinereous vulture in Bulgaria:

1. The inability of the cinereous vulture's population on the Balkan Peninsula to evaluation increase further and disperse on its own;

2. The need to provide new and more nesting sites to ensure its long-term conservation.

Following the experience of the Foundation for Conservation of the Bearded Vulture (FCBV) in the reintroduction of this species in the Alps and FIR into the reintroduction of the griffon vulture (Gyps fulvus) in France in 1986 and the establishment of the Black Vulture Conservation Foundation (BVCF), today there are enough experience and knowledge to recover the large vultures' populations in Europe (TEWES et al., 2004).

In 2003 the Fund for Wild Flora and Fauna (FWFF), "Green Balkans" and the Birds of Prey Protection Society (BPPS) in Bulgaria launched projects for restoration of the griffon vulture in Kotel Mountain, Sinite Kamani Nature Park, Vrachanski Balkan Nature Park, National Park Central Balkan and the Kresna Gorge. In 2007, the first current suitability derived from experts'

conservation measures, such as the ban and griffon vultures were released in the Kotel Mountain, and in 2010 the same occurred in the Kresna Gorge, the Sinite Kamani Nature Park, the Vrachanski Balkan Nature Park and the Central Balkan National Park. The first results were encouraging with 20-25 breeding pairs and 11 fledged young in 2016 (STOYNOV et al., 2018) and continuing increase along Balkan Mountain sites, after more than half a century of absence from these areas. The initial results give rise to exploration and planning of conservation activities for the rarer cinereous vulture.

> The purpose of this study is to present the past and present state of the cinereous vulture in Bulgaria, to assess the possibility and to rate different sites for their feasibility host colonies of the species upon to

Materials and Methods

The presented past and present state of the species is based on survey of the existing literature and unpublished data on the species and on personal observations.

Feasibility study was made with of the possible sites for reintroduction of the cinereous vulture in Bulgaria. On the basis of a preliminary assessment the following areas have been considered: 1. "Vrachanski Balkan" Nature Park: 2. "Sinite kamani" Nature Park -Kotlenska Planina SPA; 3. Kresna Gorge -Pirin Mts.; 4. Eastern Rhodopes Mts.; 5. Western Rhodopes Mts.; 6. Varbishka Planina Mts.; 7. Kamchiiska Planina Mts. and 8. Provadiya-Royak Plateau.

Under the IUCN Guidelines for reintroductions, each species reintroduction project must be preceded by a feasibility study (IUCN/SSC, 2013). This paper provides the results of such a study for the feasibility for reintroduction of the cinereous vulture in Bulgaria, carried out within the LIFE08NAT/BG/278 project "Vultures' Return in Bulgaria" (2010-2015), supported by the EU LIFE + financial instrument.

Based on data for historical presence and

opinion, a long-list of nine areas as potential Bulgaria was set up (see map Fig. 1 and Table sites for reintroduction of the black vulture in 1).



Fig 1. Map of the studied potential sites for the reintroduction of the black vulture in Bulgaria.
"Dadia" – shows the place, where the last black vulture colony of the Balkans is recently found; 1 – Eastern Rhodopes Mts.; 2 – Western Rhodopes Mts.; 3 – Kresna Gorge/Pirin Mts.; 4 – Vrachanski Balkan Nature Park; 5 – Central Balkan National Park; 6 – Sinite Kamani Nature Park & Kotel Mountain; 7 - Varbishka Mountain; 8 – Kamchiska Mountain; 9 - Provadya- Royak Plateau.

Number on the	Studied area	General value in the concept
map		
Dadia	Dadia in Greece	Last colony in Balkans
1	Eastern Rhodopes	Birds still present, based on the near-by Dadia colony. Occasional breeding. Stronghold of the vultures (griffon, egyptian and black) in Bulgaria and Balkans. Three vulture restaurants. Active NGOs – BSPB - BirdLife Bulgaria and Green Balkans.
2	Western Rhodopes	Occasional visits by birds from Eastern Rhodopes/Dadia. Suitable nesting habitat.

Table 1. List of areas from Fig. 1 with general values on the concept.

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3	Kresna Gorge/Pirin	Occasional visits by birds from Eastern Rhodopes/Dadia. Suitable nesting habitat. Griffon vulture reintroduction since 2010. National park administration. A vulture restaurant. Active NGOs – FWFF, Balkani Wildlife Society, Semperviva.
4	Vrachanski Balkan NP	Occasional visits by birds from Eastern Rhodopes/Dadia. Historical record for cliff nesting black vultures. Griffon vulture reintroduction since 2010. A vulture restaurant.
5	Central Balkan National Park	Griffon vulture reintroduction since 2010. National park administration. A vulture restaurant. Active NGOs – Green Balkans.
6	Sinite kamani NP/ Kotel Mountain	Occasional visits by birds from Eastern Rhodopes/Dadia. Historical record for nesting black vultures. Griffon vulture reintroduction since 2010. Nature park administration. Two vulture restaurants. Active NGOs – Green Balkans (Sinite Kamani) and FWFF (Kotel Mountain).
7	Varbishka Mountain	Potential site with historical importance for the species. Surviving egyptian vulture pairs.
8	Kamchya Mountain	Potential site with historical importance for the species. Surviving egyptian vulture pairs.
9	Provadiya- Royak Plateau	Potential site with historical importance for the species. Surviving egyptian vulture pairs.

The indices of most important belowmentioned factors were averaged and the result was used to rank the site. A short-list from the top three ranked sites was considered for further evaluation and detailed description to each site provided.

The suitability of a site for the reintroduction of the cinereous vulture in Bulgaria was calculated using a rating based on combination of several important factors that were indexed and the score used for the evaluation as follows:

a. Protected area - most of the site falls within a protected area and/or a Natura 2000 protected zone. The best estimate is obtained if the site falls into a protected area that has a management body, especially if it covers the bulk of the suitable nesting habitat: Reserve, national park, nature park -3 points; Partially in a protected area and partly in Natura 2000 – 2 points. Only Natura 2000 zone – 1 point; none – 0.

b. Food base - livestock is calculated conservatively. Only sheep, goats, cattle and

equines (horses and donkeys), mature animals, excluding pigs, chicken and wild ungulates and potential prey species. The cattle and horses are calculated as multiplied by factor of 6 to turn into sheep equivalent. Then this amount is summed with the number of sheep and goats to provide the total sheep equivalent of the area - just to provide possibility to calculate the carrying capacity of the area (based on 20,000 sheep/ equivalent for 100 vultures see bellow TERRASSE & CHOISY, 2007). However the density of livestock heads per square kilometer is taken to rank the area in this study. The bigger the density the higher the score as <30 animals per km² = 1 point, 30-40 animals per $km^2 = 2$ points, >40 animals per $km^2 = 3$ points.

According to (TERRASSE & CHOISY, 2007), at a radius of 30 km from the place of release/nesting in the territory, there must be at least 20,000 sheep or equivalent to expect the survival of a population of 100 griffon vultures. This is calculated for

mortality in livestock of less than or equal to 5%, 150 kg of meat for griffon vulture per year = 300 kg of carcass or 6 000 kg of live animals per bird per year. Of course, it is important to consider the way livestock is farmed and the accessibility of dead corpses to the vultures. In Bulgaria we have to consider also the presence of large predators (wolf, bear and jackal) and very large number of stray dogs, which are in with vultures for competition food resources. See Table 2-5.

c. Breeding habitat - the presence and quality of the breeding habitat is calculated for a minimum of 10 breeding pairs: Suitable forest or trees in rocks at suitable slopes and altitude in remote areas. Subjective assessment based on expert opinion in accordance with the published habitat models.

d. The presence of griffon vulture - the presence of a group or breeding colony in the area or the presence of griffon vultures in separate seasons: Breeding colony – 3 points; frequent seasonal presence – 2 points; occasional presence – 1 point; none – 0.

e. Distance to other colonies of the cinereous vulture - the advantage here is the remoteness of the release site from an active cinereous vulture colony, because the birds released can be attracted and settled permanently in the existing colony. By analogy with the griffon vulture, if the release site is in an area less than 100 km away, almost certainly leads to absorption of the released birds by the existing colony.

f. Active NGOs that are actively engaged in biodiversity conservation and maintain the site and/or have the capacity and could maintain in long-term feeding site for vultures.

g. Poison - here is estimated the danger (potential and current) of poison baits use to control predators and the use of pesticides in intensive farming, which may lead to unintentional poisoning of scavengers. A higher rating means less importance of this factor in the area.

Results and Discussion

Historical distribution in Bulgaria (before 2010).

In Bulgaria, the 1800s and the first decades of the 1900s, the cinereous vulture used to breed in the whole of the country: the Danube plain, the surroundings of Provadia Town, the valley of the Rusenski Lom River, the Danube River Coast, Ludogorie Region, Dobrudja Region, Stara Planina (Balkan Mountain) Mts., Sofia Plain, Vitosha Mts., Rila Mts., Pirin Mts., the Maritsa River Valley, Rhodopes Mts. and Strandzha Mts. At the second half of 20th century, occasional birds or pairs were observed only between Shumen and Preslav, Nevsha, Varna and in the region of the Eastern Rhodopes Mts. - near Borislavtsi, Dolni Glavanak, Kardjali and Kazak (BOEV & MICHEV, 1981; BOTEV & PESHEV, 1985). There is also data for cinereous vulture from the vicinity of Veliko Tarnovo - a stuffed bird, obtained on December 21, 1895, is kept in the National Museum of Natural History. ARABADJIEV (1962) notes that until 1930 -1940 he has observed cinereous vultures relatively often in Strandzha Mts. and Sakar Mts. It is possible that the male and female birds, harvested on 22.02.1933 from the area of Murgash, Western Stara Planina Mts., were a nesting pair (their skins are stored in the NMNH-Sofia). Around 1960 the species is hardly found in Bulgaria. There is reason to believe that some pairs or small nuclei existed until later, possibly until the end of the 1970s. During the period 1960-1970, cinereous vultures were reported only 3 times. In the period 1970-1980 observations were 11, 8 were from the Eastern Rhodopes Mts. and 3 from the Eastern Balkan Mountains (MICHEV, 1985). For the period 1980-1990 the number of observations of cinereous vultures is already 147 (IANKOV et al., 1994). Although this figure is due to the intensification of ornithological observations, the increase of the presence of the species itself is undoubtedly a fact, also with the confirmed breeding of single pair in Eastern Rhodopes Mts. in 1993 (MARIN et al.,

1998), as well as information for possible breeding in the next several years but without finding the nest (STOYNOV *et al.*, 2007). This is also related to the beginning of active conservation of the last colony of the species and the establishment of the Dadia Forest Reserve (later on turned into Dadia-Soufli-Lefkimi Forest National Park) in the Greek part of the Eastern Rhodopes Mts., along with feeding sites operation there, but also in the Bulgarian part of the mountain.

Present state and distribution in Bulgaria (after 2010).

Since 2010 with the reintroduction of the griffon vulture in Kresna Gorge and three sites along Balkan Mountains and intensive feeding site operations in these sites, appearance of cinereous vultures was expected. The frequent presence of food and interspecies attraction provided results in 2013, when the first cinereous vultures were observed in three reintroduction sites with so far established griffon vulture social groups in Kresna Gorge, Sinite Kamani Nature Park and Kotel Mountain. Since then every year observations of one, but up to five (Kresna Gorge) different specimen cinereous vultures were observed in each griffon vulture reintroduction site. The number and presence in time of single individuals gradually increased (STOYNOV & PESHEV, 2011-2014; PESHEV et al., 2015; 2016; 2017; BONCHEV & STOYNOV, 2017) and also single individuals started to appear in Vrachanski Balkan Nature Park in three years in a row since 2017 (George Stoyanov pers. com.). Not all cinereous vultures reported were marked, but all marked ones originated from Dadia-Soufli-Lefkimi Forest National Park's colony in Greece.

Based on visual marking method (STOYNOV *et al.*, 2015), four cinereous vultures were identified to have been observed in two different sites after some time as follows: 1. Kresna Gorge and Zlatar in Serbia; 2. Vitachevo in Macedonia and Kresna Gorge; 3. Kresna Gorge and Kotel; 4. Kresna Gorge and Madzharovo (PESHEV *et*

al., 2015). The last shows that the cinereous vultures from Dadia-Soufli-Lefkimi Forest National Park use the existing griffon vulture colonies and related presence of vulture feeding sites in Balkans as stepping stones during their pre-adult roaming movements.

Feasibility study

The cinereous vulture faced local extinction from Bulgaria and despite the close proximity to still existing breeding colony in near-by Greece and ever more intensive vulture conservation activities in the last three decades and increasing number of observed individuals in different parts of the country, the species shows no ability to re-colonize new territories. That is why to restore the local population and to boost the Balkan one release of specimens is foreseen in attempt to reintroduce it in new localities along Bulgaria. Nine sites were studied for feasibility to host cinereous vulture colony.

Among the studied sites, the Sinite Kamani NP - Kotel Mountain and Vrachanski Balkan NP showed the highest values for rank of feasibility (Table 6 and Fig. 2). These two sites received equal rank (2.57) that was higher than the rank of the other seven potential areas.

The Eastern Rhodopes Mts. and Kresna Gorge - Pirin Mts. are the second group of important areas with suitable conditions and relatively high rank of feasibility (2.43). The habitat quality of Eastern Rhodopes Mts. is similar to that of the previous two sites, but the lower score comes from the proximity to the existing colony of the cinereous vulture in Dadia-Soufli-Lefkimi Forest National Park in Greece, that is considered negative factor influencing the establishment of local group/colony of newly released individuals by social attraction and absorption. The Kresna Gorge - Pirin Mts. receives lower score for poisoning threat. Due to the last large poisoning event from March 2017, when more then 40 griffon vultures died in single incident (PESHEV et al., 2018) the site was totally rejected from potential sites for

release, despite it was provisionally selected. However the high rank and the on-going vulture conservation actions in the area provide for a possibility in mid-term the site to be reconsidered for release of cinereous vultures if the illegal poisoning becomes under control.

Despite of the overall high rating, the Kresna Gorge is down-listed, because it receives a low score for the poison, a problem, which is difficult to manage in the short term, as opposed to the low score of Vrachanski Balkan NP for the food base. The latter can be compensated by feeding site and food management and by measures to locally increase the number of livestock and wild ungulates that will result immediately and in the medium term.

Characteristics of the sites with highest rankings is provided in Table 7.

Species	Average annual mortality	Average live weight per individual (kg)	Average amount of meat without bones in a carcass (kg)
Ovine/Caprine	5.0 %	40	34
Bovine	3.1%	250	200
Porcine	4.2%	90	72
Equine	2.3%	120	100

Table 2. Mortality rate of livestock by type and average weight of a carcass.

Species	At temperature °C	Metabolism to maintain life (Kcal/day)	Required energy (Kcal/day)	Feeding per day (in grams)
Camo fulzuro	30°C	531	590-759	472-607
Gyps juious	0°C	549	610-784	488-627
A activity and a characteria	30°C	563	625-804	500-643
Aegyptus monucnus	0°C	572	636-817	509-656
Neophron	30°C	160	178-228	142-182
percnopterus	0°C	236	262-337	209-269
Compative herbetue	30°C	375	417-535	334-428
Gypueius ouroutus	0°C	431	478-615	382-492

Table 3. The daily food requirement in European vultures. Source: DONAZAR (1993).

Table 4. Amount of food needed for potential number of cinereous vultures' local population.

Number of individuals	Required for	ood in kg/year
cinereous vultures	Min.	Max.
1	182.50	239.44
2	365.00	478.88
3	547.50	718.32
4	730.00	957.76
6	1095.00	1436.64
8	1460.00	1915.52
10	1825.00	2394.40
20	3650.00	4788.80
30	5475.00	7183.20
35	6387.50	8380.40
40	7300.00	9577.60
50	9125.00	11972.00

Studied area	Equivalent sheep number	Density of livestock per km ²	Index/rank Food base
Varbishka Mountain	522 932	52.32	3
Eastern Rhodopes Mts.	523 148	49.84	3
Sinite kamani NP/Kotel Mountain	383 469	48.57	3
Kresna Gorge/Pirin Mts.	253 796	35.73	2
Provadiisko - Royak Plateau	294 783	32.22	2
Central Balkan National Park	322 354	31.86	2
Kamchya Mountain	269 085	28.97	1
Western Rhodopes Mts.	234 204	19.44	1
Vrachanski Balkan NP	147 988	5.18	1

Table 5. Numbers of livestock as equivalent sheep heads, density of livestock per km² and ranking of each of the studied sites.

Table 6. Ranking of the evaluated sites for the reintroduction of cinereous vulture in Bulgaria.

Factor	1	2.	3	4.	5. Distance	6		
Area	Protected area	Natural food base	Nesting habitat	Presence of Gyps fulvus	to existing colony of A. <i>monachus</i>	Active NGOs	7. Poison	Rank of feasibility
1. Sinite Kamani NP/Kotel Mountain	2	3	2	3	3	3	2	2.57
2. Vrachanski Balkan	3	1	2	3	3	3	3	2.57
3. Kresna Gorge/Pirin	2	2	3	3	3	3	1	2.43
4. Eastern Rhodopes	2	3	2	3	1	3	3	2.43
5. Central Balkan National Park	3	2	2	2	3	3	2	2.33
6. Varbishka Mountain	1	3	2	1	3	1	3	2.0
7. Western Rhodopes	2	1	3	1	2	2	2	1.86
8. Kamchiiska Mountain	1	1	2	1	3	1	2	1.57
9. Provadiisko- Royak Plateau	1	2	1	1	3	1	1	1.43



Fig. 2. Map of the short-listed potential reintroduction sites: 1 – Sinite Kamani NP & Kotel Mountain; 2. Vrachanski Balkan NP; 3. Kresna Gorge/Pirin Mts. and the location of the last cinereous vulture colony on Balkans – Dadia-Soufli-Lefkimi Forest National Park in Greece.

Table 7. Description of most important features of the shortlisted sites for reintroduction of the cinereous vulture in Bulgaria.

Item	Data
General description	Found in mid eastern Bulgaria, part of the Stara Planina (Balkan
	Mountain) chain. Low-mountainous area (400-1000 m) with
	foothills with transitional Mediterranean and Continental climate.
	Loose populated. Large forest patches are mosaically mixed with
	open pastures and to the north and south with arable land.
Habitat	Heterogeneous. Forest patches of planted Austrian pine are
	found among larger missives of oak and beech forests. In some
	places cliffs and steep slopes tree vegetation provide suitable
	nesting sites.
Food	383 469 equivalent of sheep heads. Livestock density of 48.57
	heads per km ² .

1. Sinite kamani/Kotel Mountain

	Sheep, goats and cattle, as well as free ranging horses are grazing the mountainous pastures. In the southern part near Sliven, large pig farms and slaughterhouses provide large quantities of (potential) food for vultures. The Wolf is present in the mountains, but rarely conflicts with livestock breeders as it feeds mainly on wild boar and red and roe deer, which are abundant in the forests. Two large vulture feeding sites (20 km apart) are supplied with about 15-20 tons per year each, by the two NGOs- Green Balkans and FWFF.
Socio-economic factors	One large city – Sliven (90 000 inhabitants) is found in the southern part of the area. The largest protected area- Sinite Kamani Nature Park is found just above the city. Out of the city agriculture – in the mountain mainly livestock breeding and forestry are the main occupations of the local inhabitants.
Other wildlife and related species	Since 2010 the Griffon Vulture was successfully reintroduced. In 2016 – >50 birds are permanently present, 8-10 pairs bred and 5 chicks were produced and the colony increases. 6-8 pairs of Golden Eagle breed in the area. The Egyptian Vulture tends to recover and 3-5 birds are present each summer. Just recently also the White-tailed Sea Eagle is in attempt to re-colonize the area. Eastern Imperial Eagle and the Lesser Spotted Eagle are breeding and frequently present too. The first black vulture in the area for decades was observed in 2013 at the feeding site and since then every year 1-2 individuals are registered.
Strategic importance	The site is found far from Dadia (200 km) to the north. This is the closest step to extend the Eastern Rhodopean black vulture population to Balkan Mountain. If the reintroduction becomes successful, the distance between the black vulture populations in Rhodopes and Crimea will be with 1/3 shorter.
Score	The site is top rated for start of the reintroduction of the black Vulture in Bulgaria. An excellent combination of suitable habitats, food resources, protected areas, active conservation groups, socio-economic importance and strategic position for future successful reintroduction of the black vulture.

2. Vrachanski Balkan Nature Park

Item	Data
General description	Found in north-western Bulgaria, part of the Stara Planina
_	(Balkan Mountain) chain. Law to Mid-mountainous area (500-
	1500 m) with foothills with Continental climate. Loose populated.
	Large forest patches are mosaically mixed with open mountain
	ridges - pastures and to the north with arable land. Large river
	valley with huge limestone cliffs of up to 400 m walls.
Habitat	Heterogeneous. Forest patches of planted Austrian pine are
	found among larger missives of oak and beech forests. Large
	areas represent cliffs and steep slopes with tree vegetation that
	may provide suitable nesting sites. In some places single pines are
	found in cliffs, providing classic black vulture's nesting habitat.

	In 1931 in this area cliff nesting of black vultures was reported.
Food	148 000 equivalent of sheep heads and density of 5.18 livestock heads per km ² . Sheep, goats and cattle, as well as free ranging horses are grazing the mountainous pastures. In the northern part near Montana, but also Sofia to the south, large pig farms and slaughterhouses provide large quantities of (potential) food for vultures. The Wolf is present in the mountains, but rarely conflicts with livestock breeders as it feeds mainly on wild boar and red and roe deer, which are abundant in the forests. The BPPS in cooperation with the administration of the Nature Park
Socio oconomia	One large city Vrates (54,000 inhabitants) is found in the
factors	northern part of the area. The largest protected area- Vrachanski Balkan Nature Park is found just above the city. Out of the city, agriculture – in the mountain mainly livestock breeding and forestry are the main occupations of the local inhabitants.
Other wildlife and	Since 2010 the Griffon Vulture was successfully reintroduced. In
related species	2016 - >50 birds are permanently present, 8-10 pairs bred and 4 chicks were produced and the colony increases. 5-8 pairs of Golden Eagle breed in the area. The Egyptian Vulture tends to recover and 2-4 birds are present each summer. The first black vulture in the area for decades was observed in 2016 at the feeding site.
Strategic importance	The site is found far from Dadia (330 km) to the northwest towards the direction of the Griffon Vulture colonies in Serbia, Croatia and Italy, where from the connection with France and Spain would be established. If the reintroduction becomes successful, the distance between the black vulture populations in Rhodopes and pre-Alps (France) will be with 1/5 shorter.
Score	The site is second rated for start of the reintroduction of the black vulture in Bulgaria. Very good combination of suitable habitats, (potential) natural and manageable food resources, protected areas, active conservation group, socio-economic importance and strategic position for future successful reintroduction of the black vulture.

3. Kresna Gorge/Pirin

Item	Data
General description	Found in south-western Bulgaria, along the Struma valley,
	between the highest mountains in Bulgaria Rila and Pirin, which
	are also declared National Parks. Law to high-mountainous area
	(300-2900 m) with foothills with Mediterranean and mountainous
	climate. Densely populated valley and loose populated
	mountains. Large forest patches are mosaically mixed with open
	mountain ridges – pastures and rarely arable land.
Habitat	Heterogeneous. Forest patches of autochthonous and planted
	Austrian pine, Scots pine and Bosnian pine are found in the
	mountains - 800- 1800 m. Flat-topped trees are found on high

	altitudes away form people. In the Kresna Gorge a strict reserve preserves Mediterranean forest patches of Juniperus excelsa that forms suitable, but limited in space nesting habitat for black vulture at low altitude.
Food	Sheep, goats and cattle, as well as free ranging horses are grazing the mountainous pastures. The Wolf is present in the mountains and frequently conflicts with livestock breeders, which is a potential threat for illegal use of poisoned baits, especially in the lower parts of the area. The mountain pastures above 2000 m are used for free range pasturing of livestock in summer and the vultures prefer to feed there. The FWFF supply a large feeding site for vultures (>40 tons a year).
Socio-economic factors	One large city – Blagoevgrad (70 000 inhabitants) is found in the northern part of the area. The largest protected areas - Rila National Park, Pirin National Park and the Rila Monastery Nature Park are all found around the city. Out of the city, agriculture – in the mountain mainly livestock breeding, forestry and tourism are the main occupations of the local inhabitants.
Other wildlife and related species	Since 2010 the Griffon Vulture was successfully reintroduced. In 2016 – more than 50 birds are present, 8-10 pairs are formed, 4 bred and 2 chicks were produced and the colony increases. 3-4 pairs of Golden Eagle breed in the area. The Egyptian Vulture tends to recover and 2-3 birds are present each summer as well as black vultures – 5 different individuals in 2015.
Strategic importance	The site is found far from Dadia (280 km) to the west towards the direction of the Griffon Vulture colonies in Serbia, Croatia and Italy, where from the connection with France and Spain would be established. In combination with the potential reintroduction in Vrachanski Balkan, Kresna Gorge will be connection with the Griffon Vultures colonies in Republic of Macedonia and western Greece, providing possibility to be re-occupied by the black vulture in future.
Score	The site is third rated for start of the reintroduction of the black vulture in Bulgaria. Very good combination of suitable habitats, natural food resources, protected areas, active conservation group, socio-economic importance and strategic position for future successful reintroduction of the black vulture. The only concern is the potential use of poisoned baits, which despite of being addressed for years is still a potential threat.

Conclusions

The presence of cinereous vulture significantly decreased on the Balkans from the previous century, as a result of multiple conservation threats. The last remained population in Dadia Forest Reserve (later on turned into Dadia-Soufli-Lefkimi Forest National Park) in Greece is relatively small and cannot expand in the neighboring territories, so reintroduction measures are recommended to support the presence of the species in the region. Three sites in Bulgaria -Sinite kamani NP – Kotel Mts., Vrachanski Balkan NP and Kresna Gorge-Pirin Mts. were selected to start restoration activities since they were rated with a higher score than the others at present, and because the number of individuals available to be released is limited. The import and release of cinereous vultures in Bulgaria itself will be a success. However we will consider success the attachment of the released birds to the reintroduction target areas and increasing of the presence of cinereous vultures there (in number – more than 1 specimen; and presence in time – year-round). The biggest success in short-term will be considered the recording of cinereous vulture breeding attempt in the reintroduction target areas. In mid-term (by 2030) breeding of about 10 pairs in total in the release sites or adjacent areas will be considered as success.

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Phytoplankton and Macrophytes in **Bulgarian Standing Water Bodies**

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Abstract. The current status of a lake can be evaluated via monitoring based on biological quality elements. Reference aquatic flora communities reflect pristine situations that exist or would exist with no or very minor disturbances from anthropogenic pressure. Phytoplankton and macrophytes were studied in 10 national lake types (L1, L3, L4, L5, L7, L8, L11, L12, L13, L17). Type-specific taxa and groups character in reference and near reference conditions were described. Abiotic parameters (water chemistry) were also discussed. Descriptor species from 25 FGs were registered in phytoplankton communities in lakes in reference and near reference conditions. Dinoflagellates (L₀) cryptomonads (Y) and various benthic/periphytic taxa (MP) were distributed in almost all lake types. Character descriptor species and FGs were reported for lake types L1 and L5. Motile mixotrophic dinoflagellates (L₀) and cryptomonads (Y) had highest relative biovolume in ultraoligotrophic alpine lakes (L1) due to their ability to utilize effectively scarce trophic resources. Motile euglenoids (W1, W2), small-celled colonial Cyanobacteria (K), green algae and small cryptomonads (X1, X2), coccal green algae (J) and meroplanctonic diatoms (MP) dominated phytoplankton community in riverine marshes (L5). Recorded FGs from natural lakes were also common in and their analog among heavy modified water bodies: shallow lowland reservoirs L17. Phytoplankton communities of L13 (small and medium-size semi-mountain reservoirs in the Eastern Balkans) were more similar with those of L11 (large deep reservoirs) and mountain L1, L3. Further surveys are needed in order to classify specific features of phytoplankton communities in L3, L4, L7, L11 and L12.

Key words: lakes, reservoirs, phytoplankton, macrophytes, reference conditions.

Introduction

Anthropogenic environmental alterations, 1972; such as nutrient pollution, agricultural run off, HUBENOV, 2005; CHESHMEDJIEV et al., 2010; cage and open water fish farming, as well as STANACHKOVA et al., 2010; PEARL & HUISMAN, changes in the thermal regimes of the water 2008; ELLIOTT et al., 2006; ELLIOTT & MAY, 2008; bodies induce modifications in the physical ELLIOTT, 2012a; 2012b; STOYCHEV & DANOVA, and chemical characteristics of the water, as 2012).

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg well as in the biological communities (YOSHEV, ZHIVKOV & GROUPCHEVA, 1987:

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bioindicators of environmental changes in probability of blooms. Dense submerged aquatic ecosystems as the alterations are vegetation provides refuge for filtering immediately reflected in composition and biovolume (REYNOLDS, 2006). Reference lakes usually have low phytoplankton biovolume and higher species number. Eutrophication in lentic ecosystems is usually assessed by phytoplankton as oligotrophic taxa, e.g. affecting many chrysophyte species prefer lakes with macrophytes in lakes is trophic state (SPENCE, low level of nutrients. Many colonial and cvanobacteria filamentous are developed in nutrient rich habitats and are (KADONO, 1982; HOYER et al., 1996), substrate indicators of eutrophication (REYNOLDS et al., characteristics (PEARSHALL, 1920; BARKO et al., 2002; DOKULIL, 2003).

Central European lowland lakes have 1986) and lake morphology (PEARSHALL, different composition (JÄRVINEN et al., 2013). distributed indicator species in Most alkalinity lakes of Northern Europe are chrysophytes, while diatoms were character in Central European high alkalinity lakes. Despite the above differences, species in reference lakes could be recorded in other lakes but with low relative biovolume. The results suggest that a number of true phytoplankton indicators of near-pristine conditions is rather scarce and that the low proportions of the impacted non-reference taxa in reference conditions may be a more reliable indicator.

The major part of the inland waters in Bulgaria are small and shallow artificial lakes (MICHEV & STOYNEVA, 2007), however, the investigations of lentic water bodies is dominated bv researches in big, multipurpose reservoirs (NAIDENOW, 1970; NAIDENOV & BAEV, 1987; BESHKOVA, 1996; BESHKOVA & BOTEV, 1994; KALCHEV, 1994; 1999; KALCHEV et al., 1996; 2003; 2004; BOUMBAROVA, KALCHEV & 1996; KOZUHAROV, 1994; 1996; 1999; KOZUHAROV et al., 2007; 2009).

Abundance of submerged macrophytes essentially determines the structure and function of shallow waters. In particular, macrophytes have a pronounced positive effect on water clarity: they take up nutrients and store them long term in their biomass, state in Bulgarian water bodies is given in

Phytoplankton species are sensitive thus reducing phytoplankton growth and the zooplankton, which enhances grazing on algae. In addition, shading effects of submerged macrophytes reduce production of phytoplankton (SCHEFFER, 1999).

One of the main environmental factors the development of aquatic 1967; HUTCHINSON, 1975; BEST et al., 1984). well Other factors are general water chemistry 1986), light availability (CANFIELD et al., Reference communities of Northern and 1985), prevailing winds (DUARTE & KALFF, 1917; DUARTE & KALFF, 1986; KALFF, 2002). The effects of these factors can be modified by the water level fluctuation in reservoirs, thus affecting the development of macrophytes. As fluctuations increase in magnitude the macrophyte populations get sparse. This results from the littoral zone of such water bodies being irregularly flooded and dried up, with corresponding changes in basin slope and depth.

> When conditions are stable and the above mentioned factors are favorable for aquatic macrophyte growth, significant relations exist between aquatic macrophyte abundance and lake water chemistry (CANFIELD et al., 1983), phytoplankton abundance (LANDERS, 1982) and many other limnological processes (HUTCHINSON, 1975). highly variable environment The in reservoirs affects the biological diversity, biomass, and community structure of the macrophytes, without corresponding changes in the trophic state of the water bodies TRAYKOV & TOSHEVA (2015).

The main studies on the composition of aquatic macrophytes in Bulgaria are the ground works of YORDANOFF (1931) and KOCHEV & YORDANOFF (1981) among others. The assessment of water quality bv macrophytes and their relation to the main physicochemical parameters and trophic

Ткачкоv (2010, 2012, 2013), Savchovska et al. (2013), TENEVA et al. (2014).

Current study aimed to describe character composition of autotrophic communities in selected water bodies in near-pristine conditions.

Material and Methods

Selected water bodies represented 10 of the national lake types (Fig. 1, Table 1) and were studied in the period 2009-2018. All studied water bodies (n=15) were assessed in high/good status based on phytoplankton and macrophytes.

Total nitrogen and phosphorus were analyzed following adopted standards (EN ISO 11905-1, EN ISO 6878).

Phytoplankton was sampled once to four times during the vegetation season (Aprilfrom the deepest location. October) Phytoplankton sampling and laboratory processing, including chlorophyll-a, followed international standards: ISO 5667-3:2012, EN 15204:2006, ISO 10260:2002. Functional groups (FGs) of the phytoplankton species were determined by their codons following **REYNOLDS** et al. (2002), and PADISÁK et al. (2006; 2009) and assessment was based on Reference Index BORICS et al. (2015). The descriptor species were (GECHEVA et al., 2013a).

GECHEVA et al. (2010; 2011; 2013a; b), TOSHEVA & selected based on their relative biovolume >5% of the total biovolume. Nomenclature of pro- and eukaryotic algae follows LEE (2008). Trophic classification is based on the fixed boundary system (OECD, 1982). According to Regulation H-4 (2013) for ecological status assessment based on phytoplankton were applied Hungarian Lake Phytoplankton Index (HLPI) (BORICS et al., 2018) and Bulgarian method (BELKINOVA et al., 2013).

> The macrophyte surveys were carried out once during the main vegetation period (end of June until September). In each sampling site belt transects of 20-30 m width orthogonal to the shoreline and positioned within an ecologically homogenous section of the littoral were surveyed. The transect numbers were in correlation to the lake size. Species, their abundance and additional relevant parameters were recorded for the defined depth zones (0-1; 1-2; 2-4; and >4 m). The abundance of plant species was estimated on site using a five-scale system (KOHLER, 1978). The nomenclature followed GROLLE & LONG (2000) for liverworts, HILL et al. (2006) for mosses, DELIPAVLOV et al. (2003) for vascular plants. Ecological status



Fig. 1. Map of the studied water bodies. Legend: 1-Bezbog ; 2-Chernoto; 3-Studena; 4-Choklyovo; 5-Velyov vir; 6-Arkutino; 7-Srebarna; 8-Shabla; 9-Alepu; 10-Zhrebchevo; 11-Eleshnitsa; 12-Yasna polyana; 13-Malko Sharkovo; 14-Tsankov kamak; 15-Konush.

Reservoir	Tune	Altitude,	Area	Max
	Type	m a.s.l.	(km²)	depth, m
Bezbog	L1	2240	0.02	7
Chernoto	L1	2302	0.06	15
Studena	L3	846	1.42	16
Choklyovo	L4	940	0.81	3
Velyov vir	L5	8	0.14	1.2
Arkutino	L5	1	0.09	1.1
Srebarna	L5	13.2	10	3.3
Shabla	L7	0	0.64	7.5
Alepu	L8	-0.5	0.14	1.2
Zhrebchevo	L11	273	25.8	52
Eleshnitsa	L12	53	1.18	11.3
Yasna polyana	L12	84	2.32	43
Malko Sharkovo	L13	247	3.9	15
Tsankov kamak	L13	696	200.7	>100
Konush	L17	175	0.38	8

Table 1. List of surveyed water bodies.

Results

According to the measured physicochemical parameters (Table 2) studied water bodies were in high and good status (Regulation H-4, 2013), except for Studena (TP), Shabla (TP), Eleshnitsa (Secchi disk transparency), Malko Sharkovo (TN), Tsankov kamak (TP and TN) and Konush (TP and TN). Values of chlorophyll-a corresponded to all trophic categories: from ultra-oligotrophic до hypertrophic.

Phytoplankton communities

In total descriptor species form 25 FGs were registered (Table 3). The number of FGs increased at lower altitude and higher trophic category. Seven FGs were presented in ultraoligotrophic glacial lakes (L1), while in euhypertrophic lowland lakes (L5) FGs were 20. Despite typological specifics (altitude, area, maximum depth) FG L_0 of dinoflagellates was recorded in all lake types. Cryptomonads from FG Y and various benthic or periphytic diatom taxa from FG MP were wildly represented also. The above confirmed results of PADISÁK et al. (2009) that codons L0 (mostly dinoflagellates) and Y (large size cryptomonads) occur in broad variety of habitats and are able to survive in all lentic ecosystems conditions. Mobility of dinoflagellates and cryptomonads allow effective nutrient uptake.

Table 2. Measured parameters in surveyed water bodies. Legend: * lake-annual values.

Reservoir	Туре	TP [mg L ⁻¹]	TN [mg L ⁻¹]	Secchi [m], mean	Total Biovolume [mg L ⁻¹]*	Chl-a [µg L ⁻¹] *	Trophic Category [chl]
Bezbog	L1	< 0.010	< 0.5	>7	0.3	<1	Ultra-oligo
Chernoto	L1	< 0.010	<0.5	>15	0.026	<1	Ultra-oligo
Studena	L3	0.06	<0.5	2.00	0.83	1.10	Oligo-
Choklyovo	L4	0.018	<0.5	>2.5	0.6725	<1	Ultra-oligo/Oligo
Velyov vir 2012	L5	0.234	1.97	0.3	6.85	17.34	Eu-
Velyov vir 2014	L5	0.096	0.81	0.3	4.14	19.25	Eu-
Arkutino	L5	0.118	1.51	0.3	8.53	24.73	Eu-
Srebarna	L5	0.159	0.881	0.8	13.54	36.00	Hyper-
Shabla	L7	0.125	0.6	1.95	1.22	2.54	Meso-
Alepu	L8	0.055	0.8	1.20	4.55	6.98	Meso-
Zhrebchevo	L11	< 0.010	<0.5	2.20	0.18	3.24	Meso-
Eleshnitsa	L12	0.02	0.378	1.20	0.48	1.0	Oligo-
Yasna polyana	L12	0.025	< 0.5	2.18	1.19	1.94	Oligo-

Reservoir	Туре	TP [mg L ⁻¹]	TN [mg L ⁻¹]	Secchi [m], mean	Total Biovolume [mg L ⁻¹]*	Chl-a [µg L ⁻¹] *	Trophic Category [chl]
Malko Sharkovo	L13	0.051	0.417	3.8	1.1	1.07	Oligo-
Tsankov kamak	L13	0.1	1.694	1.10	3.00	1.48	Oligo-
Konush	L17	0.428	12.14	0.3	25.6	55.0	Hyper-

Table 3. Type-specific taxa and groups - Phytoplankton (FGs) and Macrophytes at least disturbed conditions in studied lake types. Legend: *relative biovolume (%) of phytoplankton species towards total biovolume; / relative biovolume (%) in different samples from one vegetative season.

	Lake Type	1 (Chernoto, Bezbog Lakes)	
	Chernoto Lake	Bezbog Lake	Type-specific taxa and groups – Phytoplankton and Macrophytes
Phytoplankton-	Chlorophyta	Chlorophyta	Phytoplankton FGs: Species
Descriptor	Elakatothrix gelatinosa-*7/6 (F)	Radiococcus nimbatus-5/0 (F)	B: Aulacoseira italica
species	Oocystis apiculata-7/0 (F)	Bacillariophyceae	MP: Gomphonema parvulum,
	Planktosphaeria gelatinosa-6.7/0 (F)	Aulacoseira italica-0/35 (B)	Diatoma mesodon, Pinnularia sp.
	<i>Radiococcus</i> sp 7/6 (F)	Diatoma mesodon-7/0 (MP)	X2: Chromulina sp., Stichococcus
	Stichococcus lacustris-7/0 (X2)	Gomphonema parvulum-0/5 (MP)	lacustris, Rhodomonas lacustris
	Chrysophyceae	Pinnularia sp0/11 (MP)	E: Dinobryon crenulatum,
	<i>Chromulina</i> sp 26.7/0 (X2)	Chrysophyceae	Mallomonas allorgei
	Dinobryon crenulatum-0/6 (E)	Mallomonas allorgei-0/6 (E)	Y: Cryptomonas ovata
	Cryptophyta	Dinophyta	F: Elakatothrix gelatinosa, Radiococcus
	Cryptomonas ovata 0/80 (1)	Gymnoainium paiustre- $74/0$ (L ₀)	sp., R. nimoatus, Occystis apiculata,
	Dipophyta	Periumum inconspicuum-0/35 (L_0)	I • Commodini manahustra Davidini ma
	Peridinium umbonatum $A0/0$ (I.)		inconspict in P imponation
	$1 \text{ ertainium unoonutum} = 070 (L_0)$		псонярсиин, г. иноонишн
Macrophytes	Callitriche palustris	Dicranum bonjeanii	Macrophytes:
	Juncus filiformis	Eleocharis palustris	Spagnum sp.
	Marsupella emarginata	Isoetes lacustris	Sparganium angustifolium
	Spagnum sp.	Juncus filiformis	Subularia aquatica
	Sparganium angustifolium	Polytrichum commune	
	Subularia aquatica	Ranunculus aquatilis	
		Sparganium angustifolium	
		Sphagnum centrale	
	I also Ter	Subularia aquatica	
Dhytoplankton	Lake 1y	pe 5 (Studena Reservoir)	Dhytoplankton EC a Spacios
Descriptor	Cuclotella comta-16 (B)		B : Cuclotella comta C
species	Cyclotella ocellata-76 (B)		ocellata
species	Cryptophyta		Y: Chroomonas sp
	Chroomonas sp -5 (Y)		1. <i>Chrosnionus</i> sp.
Macrophytes	Macrophytes were not recorde	d.	Macrophytes: n.a.
	Lako Turo	4 (Chaklwaya marshland)	
Phytoplankton	Chlorophyta	: 4 (Chokiyovo marshiand)	Phytonlankton FCs: Species
Descriptor	Tetraedron caudatum_0/13 (X1)		C. Cuclotella sp
species	Tetraedron minimum-5/7 (X1)		D: Ulnaria ulna

1 cirucuron cuuuuuni-0/13 (A1)
Tetraedron minimum-5/7 (X1)
Chrysophyceae
Chrysococcus rufescens-5/5 (X3)

D: *Ülnaria ulna* X3: Chrysococcus rufescens

X1: *Tetraedron caudatum,T.*

Phytoplankton and Macrophytes in Bulgarian Standing Water Bodies

Macrophytes	Bacillariophyceae Cyclotella sp0/5 (C) Ulnaria ulna-0/6 (D) Euglenophyta Euglena texta-21/28 (W1) Dinophyta Peridinium cinctum-21/0 (L ₀) Peridinium inconspicuum-11/8 (Ceratophyllum demersum Elodea canadensis Lemna minor Lemna trisulca Myriophyllum spicatum Myriophyllum spicatum Myriophyllum verticillatum Phragmites australis Potamogeton lucens Potamogeton natans Riccia fluitans Scirpus lacustris Sparganium erectum Typha angustifolia Typha latifolia Utricularia minor	L ₀)	minimum L ₀ : Peridinium cinctum, P. inconspicuum W1: Euglena texta Macrophytes: Potamogeton natans Riccia fluitans Utricularia minor Utricularia vulgaris
	Lake Type 5 (Vel	yov vir, Arkutino, Srebarna Lakes)	
	Velyov vir Lake (2012)	Velyov vir Lake (2014)	
Phytoplankton- Descriptor species	Cyanobacteria Aphanocapsa nubilum-0/7 (K) Chlorophyta Micractinium pusillum-0/5 (X1) Chrysophyceae Chrysococcus rufescens-5/10 (X3) Bacillariophyceae Rhopalodia gibba-20/0 (MP) Stauroneis sp13/0 (MP) Stephanodiscus hantzschii-0/7 (D) Ulnaria capitata-5/0 (MP) Cryptophyta Cryptomonas erosa-0/6 (Y) Euglenophyta Euglena hemichromata-30/0 (W1) Trachelomonas armata-0/24 (W2) Trachelomonas bulla-0/6 (W2)	Chlorophyta Chlamydomonas globosa-0/0/9 (X2) Closterium gracile-10/0/0 (P) Sphaerellopsis sp7/0/9 (X3) Chrysophyceae Chrysococcus rufescens-0/5/12 (X3) Bacillariophyceae Stephanodiscus hantzschii-37/0/0 (D) Stephanodiscus minutulus-0/5/0 (D) Euglenophyta Euglena sp 0/11/10 (W1) Lepocinclis ovum-0/11/0 (W1) Trachelomonas manginii-0/0/6 (W2) Dinophyta Peridinium lomnickii-0/8/0 (L ₀) Cryptophyta Cryptomonas marssonii-0/23/11 (Y) Rhodomonas lacustris-0/0/7 (X2)	 Phytoplankton FGs: Species A: Cyclostephanos sp. C: Cyclotella meneghiniana D: Stephanodiscus hantzschii, S. minutulus P: Aulacoseira granulata, Fragilaria crotonensis, Closterium gracile MP: Rhopalodia gibba, Ulnaria capitata, Stauroneis sp., Pinnularia gibba M: Microcystis aeruginosa H1: Anabaena species T: Mougeotia sp. T.: Oscillatoria limosa S1: Leptolyngbya angustissima X3: Chrysococcus rufescens, Sphaerellopsis sp. X2: Chlamydomonas globosa, Rhodomonas lacustris X1: Micractinium pusillum Y: Cryptomonas erosa, C. marssonii F: Dictyosphaerium sp. J: Pediastrum duplex, P. simplex K: Aphanocapsa nubilum, A. greoillei Le: Peridinium lomnickii
Macrophytes	Brachythecium rutabulum Campyliadelphus chrysophyllus Ceratophyllum demersum Ceratophyllum submersum Ditrichum flexicaule Fontinalis antipyretica Nuphar lutea Nymphaea alba Riccia fluitans	Ceratophyllum demersum Fontinalis antipyretica Lemna minor Leptodictium riparium Lysimachia nummularia Nuphar lutea Nymphaea alba Potamogeton pussilus Ranunculus repens	 W1: Euglena sp., E. hemichromata, E. texta, Lepocinclis ovum W2: Trachelomonas armata, T. bulla, T. manginii, T. volvocinopsis Macrophytes: Fontinalis antipyretica Hydrocharis morsus-ranae Riccia fluitans

	Salvinia natans Sparganium erectum	Sparganium erectum Spirodela polurhiza	Stratiotes aloides Utricularia vulgaris
	Spirodella polyrhiza	epinetisi perginitati	
	Typha latifolia		
Phytoplankton-	Arkutino Lake	Srebarna Lake	
Descriptor	Cyanobacteria	Cyanobacteria	
species	Anabaena kisseleviana-7/0/0 (HI)	Anabaena scheremetievii-7/0/0/0	
	Chlorophyta	(HI) Anahama snivoides-8/0/0/0 (H1)	
	Dictuosphaerium sp -0/7/0 (F)	Anhanocansa orezillei-0/8/0/0 (K)	
	Bacillariophyceae	Leptolunghua angustissima-0/0/8/7	
	Rhopalodia gibba-0/0/5 (MP)	(S1)	
	Pinnularia gibba-0/20/0 (MP)	Microcystis aeruginosa-0/0/0/7 (M)	
	Stephanodiscus sp 0/28/0 (D)	Chlorophyta	
	Euglenophyta	<i>Mougeotia</i> sp0/9/0/0 (T)	
	Euglena texta-56/0/0 (W1)	Pediastrum duplex-0/0/0/17 (J)	
	Trachelomonas volvocinopsis-8/0/0	Pediastrum simplex-0/0/0/17 (J)	
	(W2)	Chrysophyceae	
	Cryptophyta	Chrysococcus rufescens- $0/7/0/0$	
	Cryptomonas erosa-0/0/13 (Y)	(A3) Bacillarionhuceae	
		Aulacoseira aranulata-0/71/30/8	
		(P)	
		Fragilaria crotonensis0/19/0/6 (P)	
		Cyclotella meneghiniana-0/16/22/5	
		(Č)	
		Cyclostephanos sp0/12/0/0 (A)	
Macrophytes	Bidens cernua	Alisma plantago-aquatica	
	Carex riparia	Butomus umbellatus	
	Ceratophyllum demersum	Carex riparia	
	Cyperus fuscus	Ceratophyllum demersum	
	Galium palustre	Cyperus lacustris	
	I emna minor	Eneociaris parasiris Enilohium hirsutum	
	Luconus euronaeus	Hudrocharis morsus-ranae	
	Lythrum salicaria	Hydrocharis morsus-ranae	
	Nuphar lutea	Salvinia natans	
	Nymphaea alba	Lemna minor	
	Oenanthe aquatica	Lemna trisulca	
	Polygonum mite	Lemna trisulca	
	Solanum dulcamara	Lycopus europaeu	
	Sparganium erectum	Lythrum salicaria	
	Spiroaela polyrniza	Najas marina Dimensitas quatralia	
	i ypru ungustijotiu	Phrugmites uustruits Salvinia natans	
		Scirnus cf. sulvaticus	
		Spirodela polyrhiza	
		Stratiotes aloides	
		Thelipteris pallustris	
		Typha angustifolia	
		Typha angustifolia	
		Utricularia vulgaris	

Lake Type 7 (Shabla Lake)

Phytoplankto	n- Chlorophyta	Phytoplankton FGs: Species
Descriptor	Oocystis elliptica-6 (F)	P: Aulacoseira granulata
species	Bacillariophyceae	MP : Rhoicosphenia abbreviata
-	Aulacoseira granulata-44 (P)	F: Oocystis elliptica
	Rhoicosphenia abbreviata-5 (MP)	L ₀ : <i>Peridinium</i> sp.
	Dinophyta	-
	Peridinium sp27 (L_0)	

	Lythrum salicaria	
	Lycopus europeus	
	Juncus effusus	
Macrophytes	Cyperus longus	Macrophytes: n.a.
	Cryptomonas marssonii-0/13/13/8 (Y)	
	Cryptophyta	
	Peridinium inconspicuum-0/0/11/7 (L _n)	
	Gumnodinium sp $-0/0/20/9$ (La)	
	Lepocinclis sp5/0/0/0 (W1)	
	Lepocinclis caudata $-5/0/0/16$ (W1)	
	Euglena korshikovii- $0/0/0/12$ (W1)	<i>Lepocinclis caudata, Lepocinclis</i> sp.
	Euglena acus-5/0/0/0 (W1)	W1: Euglena acus, E. korshikovii,
	Euglenophyta	inconspicuum
	Navicula sp6/0/0/0 (MP)	L ₀ : Gymnodinium sp., Peridinium
	Gyrosigma attenuatum-0/0/0/10 (MP)	J: Coelastrum microporum
	Bacillariophyceae	G: Eudorina elegans
	Chrusococcus rufescens-0/15/0/0 (X3)	F: Planktosphaeria gelatinosa
	Chrysophyceae	Y: Cruntomonas marssonii
	Staurastrum oracile-0/0/32/0 (N)	X3 • Chrusococcus rufescens
	Planktosnhaeria celatinosa_63/0/0/0 (F)	Tuvicum sp. T• Mongeotia sp
	Mougeotia sn -0/0/7/0 (T)	Navicula sn
species	Coemsinant microportan-0/0/0/0 (0) Fudoring elegans-0/13/5/0 (C)	MP • Gurosioma attenuatum
Descriptor	Coelastrum microporum 8 /0 / 0 / 0	IN: SUUVASTVUM gracile P: Cloctarium abrandancii
Phytoplankton-	Chorophyta	Phytoplankton FGs: Species
Directory 1 1 1	Lake Type 8 (Alepu Lake)	
	I also Tymo 9 (Along Labo)	
	v unisneria spirans Veronica heccalunga	
	Utricularia minor Vallienaria eniralie	
	1 ypha latifolia Utrigularia minor	
	Sparganium erectum	
	Scyrpus triqueter	
	Scirpus lacustris	
	Potamogeton perfoliatus	
	Potamogeton pectinatus	
	Polygonum lapathifolium	
	Phraomites australis	
	Nuppur luteu	
	Najas marina Numbar lutea	
	Myriophyllum spicatum	
	Lythrum salicaria	
	Lycopus europeus	
	Hydrocharis morsus-ranae	Vallisneria spiralis
	Echinochloa crus-galli	Utricularia minor
	Cyperus longus	Potamogeton perfoliatus
	Calystegia sepium	Potamogeton pectinatus
1 5	Butomus umbellatus	Hydrocharis morsus-ranae
Macrophytes	Bolboschoenus maritimus	Macrophytes:

DescriptorPandorina morum-6 (G)speciesStaurastrum gracile-5 (N)

Phytoplankton FGs: Species B: Cyclotella ocellata N: Staurastrum gracile

Macrophytes	Bacillariophyceae Cyclotella ocellata-51 (B) Fragilaria crotonensis-17 (P) Euglenophyta Euglena sp6 (W1) Bidens tripartita Ceratophyllum demersum Myriophyllum spicatum Najas marina		P: Fragilaria crotonensis G: Pandorina morum W1: Euglena sp. Macrophytes: n.a.
	Lake Type 12 (I	Eleshnitsa, Yasna polyana Reservoirs	
	Eleshnitsa	Yasna polyana	
Phytoplankton- Descriptor species	Chlorophyta Cosmarium sp6 (N) Hariotina polychorda-13 (J) Phacotus coccifer-13 (X _{Ph}) Staurastrum sp10 (N) Stichococcus minutissimus-13 (X2 Dinophyta Ceratium hirundinella-6 (L ₀)	Cyanobacteria Limnothrix redekei-35 (S1) Chrysophyceae Dinobryon divergens-8 (E) Euglenophyta 2) Euglena sp30 (W1)	Phytoplankton FGs: Species N: Cosmarium sp., Staurastrum sp. S1: Limnothrix redekei X2: Stichococcus minutissimus X _{Ph} : Phacotus coccifer E: Dinobryon divergens J: Hariotina polychorda L ₀ : Ceratium hirundinella W1: Euglena sp.
Macrophytes	Macrophytes were not recorded	1. Ranunculus trichophyllus	Macrophytes: n.a.
	Lake Type 13 (Malko	Sharkovo and Tsankov kamak rese	rvoirs)
Phytoplankton- Descriptor species Macrophytes	Malko Sharkovo Cyanobacteria Microcystis flos-aquae 0/13 (M) Chlorophyta Mougeotia sp0/7 (T) Bacillariophyceae Cyclotella ocellata-8/8 (B) Cyclotella comta-83/55 (B) Dinophyta Ceratium furcoides-0/9 (L ₀)	Tsankov kamak Cyanobacteria Snowella fennica-0/7/0 (L ₀) Chlorophyta Eutetramorus planctonicus-0/9/0 (F) Pandorina morum-26/0/0 (G) Bacillariophyceae Fragilaria crotonensis-0/0/31 (P) Navicula tripunctata-0/0/13 (MP) Tabellaria fenestrata-0/0/35 (N) Ulnaria ulna-12/0/0 (D) Dinophyta Ceratium furcoides-12/8/10 (L ₀) Gymnodinium sp7/0/0 (L ₀) Peridinium cinctum-8/0/0 (L ₀) Peridinium sp0/50/0 (L ₀) Cryptophyta Cryptomonas marssonii-25/15/0 (Y) Macrophytes were not recorded.	Phytoplankton FGs: SpeciesB: Cyclotella ocellata, C. comtaD: Ulnaria ulnaN: Tabellaria fenestrataP: Fragilaria crotonensisMP: Navicula tripunctataM: Microcystis flos-aquaeT: Mougeotia sp.Y: Cryptomonas marssoniiF: Eutetramorus planctonicusG: Pandorina morumLo: Ceratium furcoides, Snowellafennica, Gymnodinium sp.,Peridinium cinctum, Peridiniumsp.
	Polygonum hydropiper	17.77 Free 17.77	
Dharto a 1 - a 1 -	Lake	i ype 17 (Konush Reservoir)	
rnytoplankton- Descriptor species	Cyanobacteria Anabaenopsis milleri-0/6/0 (H1) Pseudanabaena limnetica-5/0/0 (Chlorophyta Chlamydomonas sp0/0/18 (X2 Pediastrum simplex-0/21/0 (J) Bacillariophyceae Aulacoseira granulata-0/9/0 (P) Cyclotella meneghiniana-4/3/43 Stephanodiscus hantzschii-7/1/0 Stephanodiscus minutulus-0/0/1 Euglenophyta	S1)) (C) (D) 0 (D)	 Prytoplankton FGs: Species C: Cyclotella meneghiniana D: Stephanodiscus hantzschii, S. minutulus P: Aulacoseira granulate X2: Chlamydomonas sp. S1: Pseudanabaena limnetica H1: Anabaenopsis milleri J: Pediastrum simplex L₀: Ceratium furcoides, Peridinium sp. W1: Euglena eherenbergii, E. texta,

	Euglena eherenbergii-0/0/7 (W1) Euglena texta-0/6/5 (W1) Lepocinclis ovum-0/8/0 (W1) Lepocinclis sp0/8/0 (W1) Dinophyta Ceratium furcoides-26/30/0 (L ₀) Peridinium aciculiferum-1/6/0 (L ₀)	Lepocinclis ovom, Lepocinclis sp.
Macrophytes	Juncus effusus Lycopus europeus Lythrum salicaria Scyrpus maritimus Typha angustifolia Typha latifolia	Macrophytes: n.a.

From lake type L1 (glacial lakes in high mountains) Chernoto and Bezbog lakes were studied. They are small lakes (<0.06 km²) at altitude above 2240 (Table м 1), characterized with high transparency (up to ultra-oligotrophic the bottom) and conditions (Table 2). Descriptor species represented 7 FGs: B, MP, X2, E, Y, F и L₀ (Table 3). Charater dominant species were colonial chlorococcocaleans Radiococcus species, Planktosphaeria gelatinosa, Oocystis apiculata (F). Specific conection with ultraoligotrophic conditions had also centric diatom Aulacoseira italica **(B)**, motile chrysophyceans Dinobryon, Mallomonas (E) and meroplanktonic diatom Diatoma mesodon (MP). Motile mixotrophic dinoflagellates Peridinium, Gynodinium (L₀), Cryptomonas species (Y) were with higher biovolume, which confirmed results of BESHKOVA (2000). In a similar study of phytoplankton assemblages of high mountain lakes and reservoirs in the Rila Mountains, BESHKOVA et al. (2016) also emphasized the importance of motile mixotrophic organisms (Chryso-Dinophyceae) more complete and for utilization of trophic resources. In oligotrophic high altitude lakes diatoms of genus Cyclotella and flagellates the (chrysophytes, dinoflagellates and cryptophytes) are frequently dominant (JÄRVINEN *et al.*, 2013).

Reservoir Studena is from lake type L3 (mountain lakes in the Eastern Balkans). It is a deep mountain oligotrophic reservoir with high transparency (Table 1, 2). Phytoplankton community was dominated totally by centric diatoms *Cyclotella comta* and *C. ocellata* from **B** (Table 3). The low number of descriptor species and FGs is probably due to the insufficient data. Further researches of the type should supply opportunity for more complete description of L3 phytoplankton assemblages in undisturbed conditions.

Choklyovo marschland represents lake type L4 (lowland or semi-mountain natural lakes and swamps). It is shallow, with high transparency and oligotrophic conditions Detailed (Tables 2). previous 1, phytoplankton studies reveled high species richness (STOYNEVA & VALCHANOVA, 1997). Phytoplankton descriptor species belong to FGs connected with shallow environments D (Ulnaria ulna), X1 (Tetraedron caudatum, T. minimum) and W1 (Euglena spp.) - Table 3. Oligotrophic conditions are reflected by codon X3 (Chrysococcus rufescens), which inhabited shallow, well mixed oligotrophic environments (REYNOLDS et al., 2002, PADISÁK et al., 2009).

Velyov vir, Arkutino and Srebarna (L5, riverine marshes in the Pontic Province) were with highest number of FGs c descriptor species (Table 3). Riverine marshes Velyov vir and Arkutino are extremely shallow (Table 1), macrophytedominated lakes with deep layer of silt. Both lakes are not under anthropogenic pressure and phytoplankton dynamic is a result of natural processes (macrophyte decomposition, deposition of phyto- and zooplankton, strong seasonal water level fluctuations). They are naturally eutrophic lakes, characterized by high richness and dynamics of phytoplankton community (GECHEVA et al., 2013b). FGs of the descriptor species were typical for shallow, nutrientrich habitats (PADISÁK et al., 2009): euglenoids from W1 (Euglena spp., Lepocinclis spp.) and W2 (Trachelomonas spp.), small-celled colonial Cyanobacteria from K (Aphanocapsa spp.), green algae and small cryptomonads from X1 (Micractinium) and X2 (Chlamydomonas spp., Rhodomonas), coccal green algae from J (*Pediastrum* spp). The small depth supported the abundance of meroplanktonic diatoms from MP (Rhopalodia, Stauroneis, Pinnularia). For eutrophic standing waters, or slowflowing rivers with emergent macrophytes is caharcter FG T_c (*Oscillatoria limosa*).

Srebarna Lake is eutrophic lake formed on a previous Danube River meander (UZUNOV et al., 2012). It has larger surface area and is deeper in comparison with Velyov vir and Arkutino (Table 2). Multi annual studies of BESHKOVA et al. (2012) revealed that phytoplankton structure in Srebarna is connected with water level fluctuations and connectivity with Danube River. Descriptor species of bloom-forming cyanobacteria H1 (Anabaena spp.), Μ (Microcystis aeruginosa) and high TP (Tables 2, 3) showed that the lake has becomed eutrophic. This is also confirmed by the presence of planctic diatoms from P (Aulacoseira granulata, Cyclotella meneghiniana) (REYNOLDS et al., 2002, PADISÁK et al., 2009).

Shabla Lake belongs to lake type L7 (Black Sea freshwater coastal lakes) and is relatively shallow, meso-eutrophic, with salinity <0.5‰ (Table 1). Phytoplankton was with underlined freshwater character (Table 3). Descriptor species from **P** (*Aulacoseira granulata*) and dinoflagellates from L_0 (*Peridinium* spp.) were with highest biovolume.

Alepu Lake is from type L8 (Black Sea oligohaline coastal lakes) and is shallow, oligotrophic lake with salinity 0.5-5%; typical representative of transitional waters

(Table Mid-seasonal values of 1). chlorophyll-a were in the range of mesotrophic conditions, while TP in eutrophic category (Table 2). Polymictic character of the lakes supported descriptor species with high sinking rate, e.g. chlorococcaleans from J (*Coelastrum* spp.) and F (Planktosphaeria), planktonic desmids from N (Staurastrum) and P (Closterium) and planktonic **T** (*Mougeotia*). Similar with shallow lakes from L5 and L8 typical descriptor species were euglenoids from W1 (Euglena spp., Lepocinclis spp.).

Zhrebchevo Reservoir (L11, large deep reservoirs) is representative of the largest and deepest Bulgarian reservoirs. Midseasonal chlorophyll-a levels reflected mesotrophic category (Table 3). With highest relative biovolume were planctic descriptor species from **B** (Cyclotella ocellata), **P** (Fragilaria crotonensis) and N (Staurastrum spp.), character for the epilimnia of stratified lakes (PADISÁK et al., 2009). Dominance of Cyclotella ocellata and Fragilaria crotonensis in Zhrebchevo Reservoir is confirmed by BESHKOVA et al. (2014) and DOCHIN (2019). Motile volvocaleans or **G** (*Pandorina morum*) are developed in stable phases in larger storage reservoirs (PADISÁK et al., 2009).

Eleshnitsa and Yasna polyana reservoirs represented L12 (small and medium-size semi-mountain reservoirs in the Pontic Province). These reservoirs are up to 10 km^2 , usually with mid-depth of up to 15 m, but with maximum depth that can reach 40 m (Table 1). Eleshnitsa and Yasna polyana reservoirs are lowland and levels of chlorophyll-a and TP corresponed to oligomesotrophic conditions (Table 2). In comparison with L11, descriptor species were form higher number of FGs: N, S1, X2, $X_{Ph\prime}$ E, J, L₀, W1. Indicators of oligomesotrophic environments are desmids from **N** (*Cosmarium* spp., *Staurastrum* spp.), chrysophyceans from E (Dinobryon spp.) and small green algae from X2 (Stichococcus). Presence of motile flagellats Phacotus coccifer (X_{Ph}) in Eleshnitsa Reservoir probably represented specific physico-chemical characteristics because X_{Ph} inhabits calcium rich, well illuminated, alkaline environments (PADISÁK *et al.*, 2009).

Malko Sharkovo and Tsankov kamak Reservoirs from L13 lake type were studied (small and medium-size semi-mountain reservoirs in the Eastern Balkans). Descriptor species of L13 represented 10 FGs: B, D, N, P, MP, T, Y, F, G, L₀ (Table 3). Despite L12 and L13 are analogical types in the two ecoregions, they had only two common FGs: N и L₀. Lake type 13 was characterized by high relative biovolume of oligomesotrophic descriptor species: centric diatoms from **B** (Cyclotella ocellata, C. comta), pennate diatoms from N (Tabellaria fenestrata) and P (Fragilaria crotonensis), gelatinous chlorococcaleans from **F** (*Eutetramorus planctonicus*), large dinoflagellates from L_0 (*Peridinium* spp.). With respect to the above, phytoplankton community of L13 reveled more similarity with L11 and mountain L1, L3.

Konush Reservoir belongs to lake type L17 (small and medium-size lowland reservoirs in the Eastern Balkans). High phytoplankton diversity was recorded. Descriptor species belonged to 9 FGs, character for eutrophic habitats (Table 3). Comparison with reservoirs from L17 and its analog L5 as shallow natural lakes showed that FGs were comon. With highest relative biovolume were FGs С (Cyclotella meneghiniana), (Pediastrum), L X2 (Chlamydomonas spp.) and L₀ (Peridinium, Ceratium).

Community structure of macrophytes

Seventy-three species were registered, among them nine taxa belonged to bryophytes (Table 3). Bezbog and Chernoto lake (L1) supported well developed macrophyte communities, dominated by Isoetes lacustris at up to 4 m colonization Bezbog Sparganium depth at and angustifolium at Chernoto Lake.

No macrophytes were registered at Studena Resevoir, which as a water body for drinking water supply probably do not present conditions for macrophyte

development. Studied water bodies from types L4, L5 and L7 had species rich macrophyte communities and high abundance of the taxa. Potamogeton natans dominated Choklyovo swamp at up to 4 1.5 m depth. Nymphaea alba, Nyphar lutea and *Ceratophyllum demersum* were the most abundant species in Velyov vir during 2012, while in 2014 Potamogeton pussilus and aquatic moss Fontinalis antipyretica replaced water lilies as dominants. Water lilies dominated Arkutino Lake as well; in Srebarna dominants were C. demersum, Salvinia natans and Uricularia vulgaris. Najas marina and Nuphar lutea were the most abundant macrophytes in Shabla.

Alepu supported abundant bank vegetation, but except *Salvinia natans* no aquatic macrophytes were recorded. The same was registered at Konush Reservoir (*Typha, Juncus, Lythrum, Lycopus, Scirpus*) and could be linked to the fishfarming and low transparency.

Macrophytes were recorded in a single transect in Zhrebchevo Reservoir, as well as in Yasna polyana Reservoir. Tsankov kamak Reservoir did not support macrophytes due slope and to water-level fluctuations, substrate, which affect the distribution of macrophytes communities. Malko Sharkovo had monodominant macrophyte а community. Despite it is from one lake type with Tsankov kamak, the sloping banks and substrate (sand and silt) created suitable conditions - demonstrated by the positive response of submerged macrophytes.

Conclusions

Descriptor species from 25 FGs were registered in phytoplankton communities at undisturbed water bodies from 10 national lake types (L1, L3, L4, L5, L7, L8, L11, L12, L13, L17). The number of FGs increased at lower altitude and higher trophic category: 7 FGs were recorded in ultraoligotrophic glacial lakes L1, while 20 FGs in eutrophic riverine marshes L5. Common for almost all lake types were dinoflagellates (L_0), cryptomonads (Y) and various benthic/periphytic taxa (MP). The most separable were character descriptor species and FGs in lake types L1 and L5. Additional studies are needed for defining specific characteristics of phytoplankton communities in L3, L4, L7, L11 µ L12.

Motile mixotrophic dinoflagellates (L_0) and cryptomonads (Y) had highest relative biovolume in ultra-oligotrophic alpine lakes (L1) due to their ability to utilize effectively scarce trophic resources. Character descriptor species were also gelatinous chlorococcocaleans (F), centric diatom (B)and motile chrysophyceans (E).

Motile euglenoids (W1, W2), smallcelled colonial Cyanobacteria (K), green algae and small cryptomonads (X1, X2), coccal green algae (**J**) and meroplanctonic diatoms (MP) dominated in riverine marshes L5. The presence of bloom-forming cyanobacteria (M, H1) as descriptor species is a sign of anthropogenic eutrophication (Srebarna Lake). Another general finding was that natural lakes (L5) and their analog in the group of shallow lowland reservoirs (L17) have common FGs. Although semimountain reservoirs L12 and L13 are analog in the two ecoregions, they differed in descriptor species and FGs. The phytoplankton communities of L13 were more similar with those of L11 (Large deep reservoirs) and mountain L1, L3.

Rising of the water level in deep regulated lakes (Studena, Zhrebchevo, Yasna polyana, Tsankov kamak) may result in a macrophyte depopulation, due to a compressed vertical niche for macrophytes. Moreover, in such lakes macrophytes in the littoral zones may also suffer from declining water levels. This knowledge could be applied as a management tool to mitigate effects from anthropogenic pressure.

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Quantitive Assessment of the Importance of the Atmospheric **Environment on Air Pollutant Concentrations at Regional and Local Scales in Sofia**

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Abstract. The study was carried out on the territory of the city of Sofia because of its high building density, intensive traffic and specific relief together forming preconditions for high levels of air pollutants. The study comprises data processing of 1-hour concentrations of NO, NO₂, O₃, and PM_{10} , as well as meteorological data as temperature, relative humidity, global solar radiation and wind speed for 2009 - 2015. The results underline the local character of the air pollutants in the area of "Kopitoto" locality which is confirmed by the value of R – coefficient (R = $-0.04 \div 0.12$). For the other observed areas, the regional impact on the occurrence of the air pollutants is stronger or less pronounced R = (0.81 ÷ 0.96). The sole exception is "Druzhba" district where the measured concentrations of PM_{10} expressed strong local influence (R = -0.36) and 3/4 of the ozone concentrations have a regional influence of their occurrence (R = 0.76). The meteorological variables have the strongest impact on the monthly concentrations of NO, NO₂, and PM₁₀, respectively (47.5%), (54.2%) and (49.1%), whereas O₃ concentrations expressed the strongest dependence on meteorological variables at diurnal scale (68.3%).

Key words: NO, NO₂, O₃, PM₁₀, air pollution, meteorological variables.

Introduction

people wellbeing and quality of life. It is well-known air pollution harms human health and environment. Although emissions located in valleys, and air quality problems of many air pollutants decreased, air quality problems persist, especially in cities where particulate matter, nitrogen dioxide, and ground-level ozone are recognized as the pollutants that most significantly affect human health. 80% of cases of premature death from heart disease and strokes are due to air pollution, lung disease and lung cancer according to (WHO, 2014). Polluted air are at present a major issue. PM_{10} and NO_2 increases the incidence of a wide range of effect air quality in Sofia, where 79% of

diseases like respiratory and cardiovascular Air quality is a key element of the diseases and cancer with both long- and short-term health effects (EEA, 2015).

> Many settlements around the world are often occur when pollutant sources are located within or near the valleys. The high concentration of industrial and power plants and the dense road network are responsible for a considerable amount of gases and aerosols released in the atmosphere over Sofia (KOLEV et al., 2000).

Photo-oxidants and particulate matters

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg Union of Scientists in Bulgaria - Plovdiv University of Plovdiv Publishing House inhabitants live in areas with exceedance of with average July temperature of 20.2 °C daily limit value for PM₁₀ and 1% residents (KOLEV *et al.*, 2000). are exposed to high NO_2 concentrations. The health-related threshold of the O_3 was target value was exceeded more than 25 times in 2013 in 18 of the 28 EU members including Bulgaria, in particular, Sofia (EEA, 2015).

Due to the adverse effects of air there is great interest pollution, in improving knowledge (1)on the spatiotemporal evolution of air pollutant concentrations in urban and rural areas and (2) on meteorological influences on the spatiotemporal pattern of air pollutant concentrations. Extended knowledge will help us to develop management options with the aim to reduce air pollution, to prevent adverse impact of air pollution on human health and ecosystems, and to of the measurement stations such as year of mitigate climate change.

Materials and Methods

The study was carried out in the territory of the city of Sofia. The capital has an area of 492 km². It is the largest city in in neighborhoods in different parts in Sofia. Bulgaria and the 15-th in the European Union with more than 1.3 million inhabitants. Sofia is located in the central part of western Bulgaria, in the Sofia Valley that is surrounded by Vitosha Mountain in intensive traffic. All stations record data for South, Lulin Mountain in the west and Balkan Mountain in the north. The average altitude of the valley is 550 a.s.l. Because of its location, Sofia faces air pollution (GSR), relative humidity (RH), and wind problems due to reduced potential of selfcleaning caused by the mountains that surround the valley. The pool thus formed has most often a stable stratification along its depth and is called a valley inversion (WHITEMAN, 1982). The city belongs to the near-mountain and low-mountain climatic region of Western Middle Bulgaria. The orography of the region is characterized by the predominance of sloped and bulging terrain. The winter is cold and dry, with survey period was set from 2009 to 2015. average air temperature in January being -2.4 °C while the summer is relatively cool, monitoring stations.

Meteorological and air pollution data obtained from six Automatic Measurement Stations, part of the Bulgarian Air Quality National Monitoring Network. Three of them are urban stations background and one rural background station. They are situated away from air pollution sources. The rural background station is at considerably high altitude: 1250 m. The other two posts are transport measurement stations located on key roads where the traffic is intensive, and the concentrations of the air pollutants from vehicles are high. The data was provided by Executive Environment Agency.

In Table 1 is given a brief characteristic establishment, territory coverage of the posts, measured variables, and station's type. The main differences between the two station types are their location and their scope. The background stations are located The scope of the background posts is 100 -2000 m while the transport monitoring stations cover a very localized area 10 - 15 m. They are established at crossroads with common pollutants such as NO, NO₂, O₃, and PM_{10} , and meteorological data as air temperature (AT), global solar radiation speed (WS). Only transport station Orlov most does not record ozone data. The above listed variables were used for the spatiotemporal analysis of the air pollutant concentrations in Sofia. The data is recorded as one-hour averaged value for seven years period 2009 - 2015. For the goals of the current study, it was essential all monitoring stations obtain an equal amount of data. By the above statement, the

In Fig.1 are shown the locations of the



Fig. 1. Location of rural background (green arrow), urban background (blue arrow), and transport (red arrow) air quality monitoring sites.

Station ID	Station name	Station type	Measured variables	Scope	Establishment
1	Druzhba	Urban background	NO; NO ₂ ; O ₃ ; PM ₁₀ ; GSR; RH; WS	AT; 100 – 2000 m	2005 -
2	Hypodrum	Urban background	NO; NO ₂ ; O ₃ ; PM ₁₀ ; GSR; RH; WS	AT; 100 – 2000 m	2005 -
3	Nadezhda	Urban background	NO; NO ₂ ; O ₃ ; PM ₁₀ ; GSR; RH; WS	AT; 100 – 2000 m	2005 -
4	Kopito	Rural background	NO; NO ₂ ; O ₃ ; PM ₁₀ ; GSR; RH; WS	AT; 2 – 10 km	2008 -
5	Orlov most	Transport	NO; NO ₂ ; PM ₁₀ ; AT; C RH; WS	GSR; 10 – 15 m	2005 -
6	Pavlovo	Transport	NO; NO ₂ ; O ₃ ; PM ₁₀ ; GSR; RH; WS	AT; 10 – 15 m	2009 -

Table 1.	Characteristic	of the a	automatic	measurement	stations.
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As first step was performed data quality check of meteorological and air pollution data. The data series were checked for abnormal values, gaps, and breakpoints. All unusual values were removed from the datasets. Data availability was calculated for each station by components. As a final stage from this step was performed a gap filling procedure by Matlab software. The aim of the gap filling is to provide complete data series for the further processing of the data.

spatial similarity define То in measurements of the air pollutants and the meteorological variables was used Biorthogonal decomposition (BOD). By BOD the local influence of meteorological variables on air pollution concentrations can be separated from regional. For this purpose, all data series were grouped by components in matrix forms and a sole variance of each component from the general variance of the dataset was determined (AUBRY et al., 1991; HÉMON & SANTI, 2003; VENTURI, 2010). For every component (NO, NO₂, O₃, PM₁₀, air temperature, solar radiation, relative humidity and wind speed) was made a matrix L for example L_{NO}, L_{NO2}, L_{O3} L_{PM10}, L_{AT}, L_{GSR}, L_{RH}, L_{WS}.

$$L_{NO} = \sum_{k=1}^{\infty} \sqrt{\beta_k} \cdot \mu k \cdot \gamma k \qquad (1)$$

Where:

k is an index which defines the BOD components through decomposition

 μk and γk describe component connected parts of inherent temporal and spatial variance of L_{NO}

 $\sqrt{\beta_k}$ is a real weight factor which interprets the variance of the BODcomponents and sorts down the spatial variance of the BOD-components in descending order.

Decomposition of the matrix L_{NO} results in six spatial components. The number of BOD-components corresponds to the number of the stations. Not all BODcomponents consist significant information about the influence of meteorological variables on the air pollutants. Significant BOD-components define the regional contribution to the spatial variance in the studied area. The significance of the BODcomponents was tested by Kaiser criterium KAISER (1960). Then they were used for reconstruction of the regional components of the dataset. The reconstruction of the dataset results in matrix L_{reNO}, L_{reNO2}, L_{reO3}, $L_{rePM10}...$

By the Wavelet coefficient air pollution data was sorted out into 12-time scales. The time-scales are as follow (Table 2):

Table 2. Time-scales, where: Si – time-scale number; h – hours.

S1	2h	S4	12h	S7	168h	S10	2160h
S2	4h	S5	24h	S8	336h	S11	4380h
S3	6h	S6	48h	S9	730h	S12	8760h

Data decomposition was performed by flexible Morlex-Wavelet Function. The data fitting to the different time scales was achieved through compressing and stretching of the data series. Time-scale variance was defined from calculated wavelet coefficients PERCIVAL & WALDEN (2010).

Scaling function:

$$\omega_{n,s}(t) = \omega\left(\frac{t-n}{s}\right) \qquad (2)$$

Where: S – dilation parameter n – displacement parameter

t - time-interval

The Wavelet transformation of data series f(t) represents a folding between f(t) and $\omega_{n,s}(t)$ TORRENCE & COMPO (1998):

$$c_n(s) = \frac{1}{s} \int_{-\infty}^{+\infty} f(t) \omega_{n,s} dt \qquad (3)$$

Where:

 $C_n(s)$ - calculated Wavelet coefficient. By this coefficient data series variance is calculated.

$$W(s) = \int_{-\infty}^{+\infty} |\mathbf{c}_n(s)|^2 dn \qquad (4)$$

W (s) – interpret the explained variance.

Results

Table 3 represent the number of significant BOD components that describe the variance of the data series. Each variable is described by only one significant BOD component. The percentage of the explained variance (EV) differed for the different variables. The explained variance for the air pollutants fluctuates between 84% for L_{O3} and 94% for L_{PM10} . These results mean that significant BOD components could explain a large part of the matrix L variance.

Table 3. An explained variance of the different data series by some significant BOD components.

Data series	Matrix	Some significant components	Explained variance (%)	Reconstructed matrix
NO	L_{NO}	1	87	L _{re,NO}
NO_2	L_{NO2}	1	90	L _{re,NO2}
O_3	L_{O3}	1	84	L _{re,O3}
$PM_{10} \\$	$L_{PM10} \\$	1	94	L _{re,PM10}

Fig. 2 represents he explained variance of NO-, NO₂-, O₃- and PM₁₀ data series for each station. Where correlation coefficient (R) measures the deviation of the stationspecific air pollution data from the whole air pollutant data, which means that the study area has a local influence on air pollutants if the R value is close to null. At the areas of Orlov most and Hipodrum NO concentrations have the most prominent regional influence R-value (0.95 and 0.92), while for Nadezhda, Druzhba, and Pavlovo R-value is 0.82 - 0.83. NO concentrations at the Kopitoto locality demonstrates strong local influence and do not depend on transportation of NO from the other observed areas. NO₂ levels have very strong regional character: R-value (0.90 - 0.96) in Hipodrum, Nadezhda, Pavlovo and Orlov most, while 81 % from NO₂ measured in Druzhba depends on the regional factors for its occuarrence. Kopito station stands out with only 12 % of regional influence. Ozone concentrations have similar regional influence for Hipodrum, Nadezhda, and Pavlovo Rvalue (0.85 - 0.89). Druzhba differ a little from the other areas with regional influence (Rvalue: 0.76). Ozone concentrations presented a strong local character at Kopito locality. Regarding PM₁₀ concentrations, Druzhba and Kopito show a strong local character which is more apparent at Kopito locality R-value (0.04) rather at Druzhba R-value (-0.36). For the rest observed areas, the character of the PM₁₀ concentrations was regional.



Fig. 2. Correlation coefficient (R). Air pollutants explained variance by significant BOD component.

In Fig. 3 are shown the changes in air pollutant concentrations depending on different time scales. Obtained results are for the whole investigation period.

NO concentrations are dominated by (46.1 %) from S3 (12.4 %), S4 (18.3 %), and S5

(15.4 %), which appeared to be the most important time scales for emerging and developing of NO gasses in the atmosphere. Regarding NO₂ concentrations, they had very similar behavior, where 57.2 % from NO₂ concentrations depend on S2 (10.7 %), S3 (13.6 %), S4 (16.8 %), and S5 (16.1 %). Ozone concentrations demonstrate very prominent dependence on S4 (18.8 %) and S5 (25.8 %) which together contain (44.6 %) of explained variance for the whole time scale. About particulate matter, there is no clear dependence on some definite time scale, which is the case with the previous three air pollutants. The explained variance is evenly distributed at the different time scales; it varies from (3.8 %) to (13.4 %).



Fig. 3. Explanied variance of air pollutants by Morlex – Wavelet Function in time scale from S1-S12. Error bars represent the standard deviation.

Results from performed partial leastsquares regression (PLSR) at five different time scales are represented in Table 4.

The values in the cells expressed the dependence of air pollutant concentration on meteorological variables. Meteorological variables have the highest influence on NO concentrations at S7 (41.3 %), S9 (47.5 %), and S12 (40.4 %). Meteorological variables have a little bit more importance for NO₂ concentrations and are reason for the emergence of NO₂ at S5 (48.4 %), at S6 (46.4 %), and at S9 (54.2 %). Ozone expressed the highest dependence on meteorological variables at each time scale among the air pollutants. It is the most influenced by meteorological variables at S5 (68.3 %) and S12 (64.0 %). Dependence on meteorological variables is less prominent for PM10, where S9 (49.1 %) has the highest explained variance.

Table 4. Partial least-squares regression.Explained variance of air pollutants for themost important time scales bymeteorological variables (%).

Air pollutant	S 5	S 7	S 9	S11	S12
NO	36.8	41.3	47.5	36.9	40.4
NO_2	48.4	46.4	54.2	32.8	38.2
O_3	68.3	55.0	55.4	53.8	64.0
PM ₁₀	36.5	35.9	49.1	35.1	38.8

Discussion

Regardless air pollutants emergence at different locations, they are mixed and transported by movement of the air masses above Sofia. The process is deeply explained by (KOLEV et al., 2000). After them, due to the influence of the mountain wind, a gradual destruction of the urban heat island and formation of surface inversion starts, which results in an anti-mountain wind which carrying warm air and aerosol from the central parts of the urban island and thus give rise to the so-called urban plume. Such air quality problems experience many population centers located within or near the valleys, where the specific local relief has a key role in the spatial distribution of air pollutants. The same phenomenon is observed by (GIRI *et al.*, 2008). The topography the Sofia vallev of in combination with local meteorology predetermines the regional character of the air pollutants in Sofia. An exception from

this assertion is Kopitoto locality, where the results prove the local origin of the pollutants. The main reason for this effect is the valley inversion which traps air pollutants above the city, and high altitude of the area that together act as a barrier for transportation of air pollutants from Sofia. These results are quite intriguing because the same factors which define the regional origin of the air pollutants in Sofia determine the local character of pollutants in Kopitoto locality. The outcomes of the current research stressed out the importance of the topography in spatio-temporal evolution and distribution of the air pollutants. Concerning PM₁₀ pollution, it was interesting to observe Druzhba district is a local source of PM₁₀ emissions, whereas it has a regional character for the other study's areas. Moreover, a small scale PM₁₀ transportation was observed from other parts of the city. The predominant local character of PM₁₀ pollution in Druzhba could be addressed to the negative relief, in some cases, denivelation between Druzhba and the surrounded areas reaches more than 80 m and the weight of the particulate matters. This prevents the spread of PM₁₀, originated in Druzhba, to the other parts of the city. Apart from the spatial pattern of air pollution, the current study improves the knowledge about the temporal evolution of the air pollutants in Sofia. Where most important periods for emerging of air pollutants such as NO, NO₂, and O₃ are 12 and 24-hours periods, especially for O₃ 24hours period is most critical for its accumulation in the lower atmosphere. PM₁₀ pollution does not express a strong dependency on any given period, probably because of its nature of origin. As regards the influence of meteorological variables on concentrations of air pollutants such as NO, NO₂, and PM₁₀, they have the strongest impact on their monthly concentrations. The levels of ozone in ambient air mostly depend on meteorological variables at diurnal scale. Likewise, ozone exhibits the strongest dependence on meteorological variables

form the other pollutants because of its nature as secondary photochemical pollutant. The advanced knowledge, as an overall result from the current research, on the spatiotemporal evolution of air pollutant concentrations in urban areas and on meteorological influences on the spatiotemporal pattern of air pollutant concentrations, could be in use of developing management options with the aim to decrease air pollution and to prevent adverse impacts of air pollution on human health and ecosystems.

Conclusions

For most of the observed areas, air pollution concentrations have a regional influence. Exception from this rule is Kopito locality, where air pollutants have a local character. Concentrations of NO, NO₂ and O₃ have clearly marked regional origin in Sofia. It is important to notice that concentration of PM₁₀ has a very strong regional influence in the capital, whereas PM₁₀ could be considered as a local pollutant in "Druzhba" district. The meteorological variables have the strongest impact on the monthly concentrations of NO, NO_2 , and PM_{10} , respectively (47.5%), (54.2%) and (49.1%), whereas O_3 concentrations expressed the strongest dependence on meteorological variables at diurnal scale (68.3%).

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Variations in the Antioxidant Defense System of the Black Sea Mussel, Mytilus galloprovincialis (Lamarck, 1819)

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Abstract. The study aimed at assessing changes in the pro-antioxidant balance in gills, foot and digestive gland, of the mussels, Mytilus galloprovincialis Lam., gathered in June and September in two successive years (2017 - 2018) from 11 different habitats with various environmental conditions of the Bulgarian Black Sea coastal area. The glutathione (GSH) concentration and enzyme activity of catalase (CAT), glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione-S-transferase (GST), superoxide dismutase (SOD), together with the lipid peroxidation (LPO) level and concentrations of selected trace metals (Cd, Cu, Pb, Ni, Zn) were measured in each organ. Significant, although not high, correlations were established between different tissue biomarkers and Cu, Pb and Cd. In gills the highest degree of oxidative stress (OS) was present, compared to the other tissues, as evidenced by the significantly higher LPO levels along with activated antioxidant enzymes CAT, GPX, GST, SOD and extremely high G6PDH. Among the organs, the lowest levels of OS were detected in the foot, indicated by the lowest LPO and the highest GSH concentrations. The digestive gland showed a relatively low degree of OS, indicated by the significantly lower LPO and antioxidant enzyme activities, with exception of the high CAT activity. In conclusion, the established significant variability in the tested OS parameters indicated their different sensitivity towards environmental pro-oxidants and reaction of the antioxidant defense system in different organs of the mussels. The gills seemed to be most suitable for biomonitoring of oxidative stress in the mussels.

Key words: lipid peroxidation, antioxidants, heavy metals, Mytilus galloprovincialis.

Introduction

The Black Sea makes no exception of the increasing anthropogenic pressures on the marine ecosystems due to its geographical position and limited water exchange with other seas and the ocean. In addition, it accepts large amounts of river waters from the territory of due to their toxicity and ability to accumulate or more than 20 countries of Europe and Asia even biomagnify in aquatic organisms.

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Minor, which is added to the local coastal pollution effects (MIRINCHEV et al., 1999; Todorova & Moncheva, 2013; ROBU et al., 2015; BAT et al., 2015; MONCHEVA et al., 2016). One of the dangerous types of water pollution is with trace metals (As, Cu, Cd, Hg, Pb, Zn etc.)

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changes in the Black Sea ecosystem were, until recently, mainly based on physicalchemical analysis of environmental samples, concentrations of xenobiotics in sentinel organisms or shifts in species communities (YANCHEVA et al., 2018) while the individual (G6PDH), glutathione-S-transferase (GST), biological effects and responses of the lipid peroxidation (LPO)). themselves organisms remained underestimated. More recently, multibiomarker approaches (VLAHOGIANNI et al., 2007; SANCHEZ et al., 2013) were used to June and September during two consequent evaluate effects of exposure to contaminants and the responses of marine biota to environmental stress. Being the primary consumers in the water food chain in the Black Sea and filter feeders, the black mussels *Mytilus galloprovincialis* Lam. play a highly important role for the resilience of the whole seawater ecosystem and therefore is among a depth of 1-6 m and from artificial structures the primary objects of research and monitoring.

Numerous studies indicated that a variety of natural factors of the sea water environment, as well as anthropogenic contamination, provoke excess generation of reactive oxygen species (ROS) or decrease of defense antioxidant and thus induce oxidative stress (OS) in bivalves (SOLDATOV et al., 2014; TSANGARIS et al., 2016; MAISANO et al., 2017; GIANNETTO et al., 2017; YANCHEVA et al., 2018). As the environmental effect is multifactorial, different mechanisms may be involved in the production of ROS, and the interactions between them in combination with the antioxidant response rate, contribute to the degree of the oxidative processes in cells. Although OS in marine bivalves is studied and explored in various (biological, ecological, evolutionary) aspects, the response of the different organs (tissues) to the ROS and their contribution to the overall protection and/or adaptation of the whole organism to the adverse effects in the Black Sea environment remains underestimated in Bulgaria.

The present study aims to assess the oxidative stress in different organs (foot, gills and digestive gland) of black mussels from (Sigma-Aldrich Co. LLC, USA): Catalase

The risk assessment of pollution and the Black Sea coastal area of Bulgaria by using multi-biochemical biomarkers (glutathione (GSH), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione reductase (GR), glucose-6-phosphate dehydrogenase

Materials and Methods

The mussel sampling was performed in years (2017 and 2018). The mussels (45-60 mm) were collected from 11 different sites (Fig. 1) along the southern Bulgarian Black Sea coast, including ports, aquaculture and protected areas in order to cover various environmental conditions. Mussels were hand-collected from their natural habitats at in the harbors. They were cleaned on the spot immediately transferred and to the laboratory in clean thermostable containers with sea water.

Measurement of oxidative stress parameters

Tissue preparation. For the biochemical analyses the mussels (n=6 for each site) were immediately dissected on ice and the gills, foot and digestive gland were excised. Each individual organ was frozen in liquid nitrogen and stored at -80°C until the analyses began. Thereafter, the organs were homogenized in 100 mM potassium phosphate buffer (pH 7.4) using Potter Elvehjem homogenizer fitted with a Teflon (Thomas pestle Scientific, USA). The homogenates were centrifuged (Janetzky K24 refrigerated centrifuge, Germany) for 10 min at 3000 g to obtain a post nuclear fraction for determination of lipid peroxidation and glutathione levels. A portion of the fraction was re-centrifuged at 12 000 g for 20 min to obtain a post mitochondrial supernatant used for measurement of the antioxidant enzymes activity. All work was carried out at 4°C.

Biochemical analysis. All tested OS biomarkers were measured spectrophotometrically using commercially available kits dehydrogenase Assay Kit Glutathione Assay Kit CS0260; SOD Assay 19160; Glutathione Peroxidase Kit-WST Cellular Activity Assay CGP1; Glutathione Reductase Assay Kit GRSA; Glutathione-S-Transferase Assay Kit, CS0410; Lipid peroxidation (MDA) assay kit MAK085. The concentrations protein were measured according to LOWRY et al. (1951) by using a standard curve of bovine serum albumin as standard.





Measurement of metal concentration

Tissue preparation. The mussels used for trace metal bioaccumulation analyses were kept alive for 48 hours in sea water, which is considered sufficient to clean their digestive system. The gills, foot and digestive gland (n=35-45 specimens from each sampling site) were excised and washed with distilled water. After drying at 110°C to an air-dry state, weighed and pooled samples of 2 g of comparison to the foot and digestive gland.

Assay Kit CAT100; Glucose-6-phosphate dry weight were prepared and stored in MAK015; desiccator until further analyses.

> *Metal analyses.* The air-dry samples were homogenized in a laboratory mortar and 1 g of each sample was subjected to a wet mineralization with 15 ml of a 2:1 mixture of concentrated perchloric (HClO₄) and nitric (HNO₃) acids. The samples were heated and evaporated on a sand bath to a moist residue. Using the same procedure, a blank sample was prepared with bi-distilled water and a mixture of acids. Tissue concentrations of Cd, Cu, Ni, Pb, and Zn were quantified by using Perkin Elmer Optima 5300 DV/ICP-OES (USA). The data measurements obtained from the analysis were in mg/L, therefore they were recalculated to mg/kg of air-dry sample.

Statistical analyses

Statistical analyses of raw data were carried out using STATISTICA 10 package (StatSoft Inc., 2010).

Results

To analyze the overall patterns of interdependence between the values of the studied OS biomarkers in the different organs we applied factorial ANOVA (Table 1). The analysis indicated that they differed significantly with high degree of reliability. Hence, the reaction of the organism to prooxidative factors appeared to be specific for the target organ and the type of biomarker reactions.

In order to compare the intensity and direction of the pro-oxidative/antioxidative balance in the different studied organs, we compared the mean values of the OS biomarkers among them applying the Student's t-test (Table 2).

In gills the highest concentration of MDA (as LPO indicator) and high activities of the antioxidant enzymes SOD, CAT, GPX and GST were found (Table 2). Although the SOD and GST activities in gills did not differ significantly from those in the foot, they were significantly higher than in the digestive gland. Twice higher activity of G6PDH was measured in the gills in

	SS	Degree of freedom	MS	F	Р
Intercept	58268385	1	58268385	270.21	0.000000
Organ	13323600	2	6661800	30.89	0.000000
Indicator	206860753	4	51715188	239.82	0.000000
Organ*Indicator	85684530	8	10710566	49.66	0.000000
Error	283999213	1317	215641		

Table 1. Analysis of variance (ANOVA – factorial design) of the overall pro/antioxidant biomarker reactions in organs.

Table 2. Mean values of biomarkers (± error of mean) and their significance of difference between organs (Student's t-test; $p \le 0.05$). *Legend:* ⁺- significant difference from foot; ^{*}- significant differences of digestive gland from gills.

Organ\ Indicator	Gills	Foot	Digestive gland	
LPO (nmoles MDA/mg protein)	$5.58 \pm 0.25^{+}$	1.87 ± 0.09	$3.49 \pm 0.16^{*}$	
GSH (ng/mg protein)	999.09±117.8 ⁺	1735.5±125.0	451.41± 46.19**	
SOD (U/mg protein)	18.63±1.70	19.35 ± 1.79	$4.13 \pm 0.39^{+*}$	
CAT (U/mg protein)	2.13±0.43 ⁺	0.86 ± 0.14	$3.90 \pm 0.67^{+*}$	
GPX (U/mg protein)	15.01±2.83+	13.22 ± 1.37	$8.08 \pm 0.69^{+*}$	
GR (U/mg protein)	7.04 ± 0.67	6.93±1.09	$6.23 \pm 1.20^{+*}$	
GST (U/mg protein)	104.23±10.07	112.66±10.22	$48.51 \pm 5.02^{+*}$	
G6PDH (U/mg protein)	40.16±3.39 ⁺	19.58 ± 1.14	$20.94 \pm 1.07*$	

The GSH level, as well as the SOD, GPX and GST activities were found to be lowest in the digestive gland in comparison to that in the other tested organs (Table 2), whereas the CAT activity was the highest. The LPO levels and G6PDH activity did not differ significantly between the digestive gland and the foot.

In the foot the highest GSH level and low MDA concentration were observed. As regards the antioxidant enzymes – the lowest CAT activity among the tested organs was measured in the foot. The SOD and GST activities in the foot did not differ significantly from those in the gills. The GPX activity was significantly lower in the foot than in the gills, but higher than in the digestive gland (Table 2).

The studied OS biomarkers were also analyzed for correlations with the accumulated in the same tissue metals (Table 3). Not all metals studied were found to have significant correlation with one or more biomarkers.

The concentration of Cu in the gills (i.e. branchial tissue) was significantly and positively correlated with the LPO level (r = 0.57) and SOD activity (r = 0.53). Further, a significant although not high, correlation between the Cu concentration and the GSH level was present (r = 0.47) in the digestive gland. The G6PDH activity was correlated significantly with concentrations of Cu (r = 0.51) in the foot.

The concentrations of Pb had significant positive correlations with the LPO level (r = 0.58) and GST activity (r = 0.67) in the foot and a negative correlation

with the GPX activity (r = -0.72) in the digestive gland.

The only significant correlation of Cd concentrations was found to be with the GR activity (r = 0.52) in the gills.

In an attempt to search for relationship between the complex interactions of OS biomarkers in the different organs and metal concentration, principle component analysis (PCA) on correlation matrices was used to reveal the underlying structure of the data (Fig. 2). The studied organs of the mussels were analysed separately.

In the 2D graphical representations (Fig. 2) the underlying variables (PC coordinates) are situated according to their correlation to the two main principle component (PC) axes. Heavy metal concentrations were used as secondary variables and were also visualized on the factor plane.

Tissu	e/	Biomarker response							
	Metal	LPO	SOD	CAT	GSH	GPX	GR	GST	G6PDH
	Cu	<u>0.57</u>	<u>0.53</u>	0.02	-0.19	-0.10	-0.12	-0.00	0.32
	Pb	-0.09	0.09	0.16	-0.11	-0.31	-0.12	0.27	0.22
ills	Zn	0.31	0.35	-0.18	0.03	-0.04	0.11	-0.31	0.05
6	Cd	0.01	0.34	-0.19	-0.33	-0.20	<u>0.52</u>	-0.31	0.16
	Ni	0.18	-0.09	-0.18	-0.26	-0.17	0.05	-0.06	-0.46
	Cu	0.22	0.19	0.21	0.02	0.22	0.06	0.07	<u>0.51</u>
	Pb	<u>0.58</u>	-0.36	0.33	0.10	-0.36	0.29	<u>0.67</u>	0.01
oot	Zn	0.04	0.18	0.13	0.08	0.13	0.09	-0.12	0.31
H	Cd	-0.15	0.28	0.07	0.00	0.16	-0.07	-0.15	0.16
	Ni	0.34	-0.23	-0.04	-0.12	-0.26	-0.03	0.35	-0.22
	Cu	0.33	-0.05	0.12	<u>0.47</u>	-0.07	-0.32	-0.17	-0.21
ive d	Pb	-0.18	-0.26	-0.01	-0.06	<u>-0.72</u>	0.40	0.38	-0.22
Digest glan	Zn	-0.14	0.16	-0.23	0.04	0.32	-0.35	-0.42	-0.03
	Cd	0.09	0.04	-0.31	-0.20	0.29	-0.03	-0.34	-0.21
	Ni	0.33	-0.32	-0.17	0.27	0.23	-0.17	-0.07	-0.40

Table 3. Pearson's Product Moment Correlations between biomarkers and concentrations of trace metals in organs (underlined correlations significant at $p \le 0.05$).



Fig. 2. PCA analysis: projections on factor plane of the first two PC (*circles* - oxidative stress indicators; *triangles* - metal concentrations as supplementary variables) (*Plot 1* – gills; *Plot 2* – foot; *Plot 3* – digestive gland).

The PCA analysis of OS biomarkers data in the gills indicated that the first two extracted PC (factors) explained 63.72% of the total variance. (Fig. 2, Plot 1). The first PC (Factor 1) explained 46.27% of the total variance and showed a gradient from significant negative loads of the activity of SOD, CAT, GPX and GR, towards positive loads of GR activity and to some extend of SOD. The second PC (Factor 2) accounted for 21.17% of the variance with higher loadings of LPO level and G6PDH activity. The Cu concentration showed also significant correlation with the second PC. The Ni concentration was not significantly related to neither of the OS variables and was separated in the positive part of the plot. The

concentration of Cd was related to some extend with the SOD activity.

The PCA analysis of OS biomarkers data in the foot indicated that the first two principle components explain 74.09% of the total variance (Fig. 2, Plot 2). The first PC (Factor 1) explained 55.86% of variance. This PC characterized a gradient of OS biomarkers - from CAT, LPO and GST (with negative loadings) towards SOD, GPX and G6PDH (with positive loadings). The GST activity had lower and similar loadings on Factor 1 and Factor 2. The second PC (Factor 2) explained 18.23% of the total variance and was characterized by relatively lower loads of GSH and GR. The Pb concentration had significant loads both on PC1 and PC2, but having somewhat greater load on the second.

In the digestive gland the first two PC (factors) extracted from the OS biomarkers data explained 59.05% of the total variance (Fig. 2, Plot 3). The first PC (Factor 1) explained 37.32% of variance and indicated a gradient from both GSH and LPO levels and CAT activity (negative loads) towards SOD, although this indicator had a relatively low weight. The second PC (Factor 2) explained 21.73% and was characterized by a gradient from GR towards G6PDH activities. Both indicators however had relatively low loads. The relatively low total variance explained indicated that in the digestive gland more factors affecting the OS biomarkers were present, each with a low individual effect.

Discussion

Our data demonstrated the presence of significant variability in the pro-oxidative processes and antioxidant defense in the different organs of M. galloprovincialis from the Bulgarian costal area of the Black Sea. As far as the studied mussel individuals were variability exposed to the same of environmental conditions during the study, the observed differences in OS biomarkers among the tested organs strongly indicated the presence of specificity in both the reaction towards pro-oxidants and the antioxidant system functioning.

It was found that the gills were the most affected organ by oxidative stress compared to the others, as evidenced by the significantly higher LPO. The gas exchange and filtration function of the gills is a prerequisite to increased intensity of prooxidative pressure, characterized with high background levels of LPO, which was also shown by previous studies (VLAHOGIANNI et al., 2007; FERNÁNDEZ et al., 2010). We observed a high CAT and GPX activity in the gills, probably as a consequence of the effort for eliminating the formed lipid peroxides. The established twofold higher activity of G6PDH in the gills, in comparison to the other organs studied, could explain the relatively high levels of GSH, since G6PDH is involved indirectly in its recovery mechanism (HALLIWELL & GUTTERIDGE, 2015). ROS formation can occur not only by the continuous interaction of gill cells with molecular oxygen in seawater and/or maximal exposure to high dissolved oxygen (SANTOVITO et al., 2005), but also as the result of exposure to different pollutants, including metals. The observed positive correlation between GR induction and Cd concentration in the gills could be the result of overlapping processes related to Cd elimination through GSH utilization and compensatory activation of mechanisms for its recovery. Further, we found a significant positive correlation between LPO and Cu concentrations in gills. In addition, the activity of SOD was also positively correlated with the concentration of Cu in gills. The PCA analysis showed that LPO level in gills, G6PDH and SOD activities together with concentration Cu had significant loads on the second significant factor (PC2) and hence seemed to be involved in the neutralization of pollutants in the gills. This was also in line with findings that SOD in the gills can be induced directly by water pollutants (VLAHOGIANNI et al., 2007; FERNÁNDEZ et al., 2010).

Our results demonstrated that the foot had the lowest levels of pro-oxidants (compared to the other organs) as indicated by the lowest MDA and the highest GSH concentrations, measured. High GST activity in the foot, which is in direct contact with the environment matrixes (water, substrate), is related to the antioxidant defense system of cells eliminating diverse oxidative stress products such as lipid hydroperoxides, quinones, epoxides and α , β -unsaturated aldehydes (Belabed & Soltani, 2013). On the other hand, highest activities of SOD and GPX were also established in the foot. Highest activity of SOD in the foot mussel (compared to other organs) was previously also reported (SOLDATOV et al., 2008a). In general, the activities of the measured antioxidants in the foot were close to those in gills.

In our study the antioxidants' activities in the digestive gland showed the lowest values (in comparison to foot and gills) with the exception of CAT. The lowest activity of GPX and GST, observed in the digestive gland, could be related to the lowest concentration of GSH (which is the co-substrate of the enzymes' action) compared to the values in the foot and gills. Since GSH is involved actively in the neutralization of both the LPO products and ingested toxic substances this could lead to depletion of its resources (GOSTYUKHINA & ANDREENKO, 2015). The high CAT activity in digestive gland was likely to be related to presence of increased H₂O₂ concentrations in this organ (SOLDATOV et al., 2008a). CAT has a leading role in degradation of hydrogen peroxide in high concentrations, because the enzyme has low affinity to H₂O₂ (HALLIWELL & GUTERIDGE, 2015). In the same time high H₂O₂ concentrations inhibit the GPX and make turn it low efficient for hydrogen peroxide neutralization. This seems to be а characteristic of the mollusc digestive gland, which was indicated by the high activity of catalase in this organ (SOLDATOV et al., 2008b).

In our study correlations were present between oxidative stress biomarkers and metal concentrations in the organs. The high activity of CAT and low levels of GSH could be probably due to the level of Cu accumulation. It has been reported, that the cellular levels of glutathione are markedly decreased in Cu-exposed mussels and the accumulated Cu can affect at different extent the GSH metabolism in mussels and it has been also suggested that the rate of bioaccumulation of essential heavy metals is faster than nonessential metals in bivalves (KAMARUZZAAN et al., 2011). Laboratory data have shown that the exposition of bivalves to Cu, Cd and Pb, resulted in a significant increase of CAT activity (RAJKUMAR & MILTON, 2011; BOUDJEMA et al., 2014).

Conclusions

In conclusion, the established significant variability in the tested OS biomarkers among the organs of Black Sea mussel *M*.

galloprovincialis indicated the different sensitivity towards environmental prooxidants and the reaction of the antioxidant defense system. In general, our results strongly indicated that the variation of antioxidant enzymes activities in the mussel foot and gills seemed to be directly related to the presence of different pollutants in the marine environment. The biomarkers of OS in the mussels can present useful bioindication of the Black Sea environmental health. However, the specificity of the proand antioxidant processes in the mussel organs to be used for this purpose should be taken into account. In this respect the gills seemed to be the better bioindicator. Obviously, further studies are needed in develop order to reliable marine environmental assessment scales, based on OS biomarkers in mussel gills.

Acknowledgements

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Variations in the Antioxidant Defense System of the Black Sea Mussel, Mytilus galloprovincialis (Lamarck, 1819)

Complex in Tissues of the Black Sea mollusc *Mytilus galloprovincialis* under Natural Oxidative Stress. - *Journal of Evolutionary Biochemistry and Physiology*, 44(2): 175-182. [DOI]

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Composition and Zoogeographic Features of the Stonefly Fauna (Insecta: Plecoptera) of Mountainous and semi-mountainous streams in Aegean watershed (7th Ecoregion, Eastern Balkans)

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Abstract. This work summarise 23 species and 4 subspecies stoneflies established during sample periods, Autumn 2017 and Spring 2018 at 38 mountainous and semi-mountainous streams from Bulgaria and North Macedonia. The species are relatively estimated by the dominant analysis. The Plecoptera fauna belongs to 7 zoogeographical categories, grouped into 5 complexes. Moreover, eighteen taxa are European species and three (Brachyptera beali beali (Navás, 1923), Leuctra hirsuta Bogoescu & Tabacaru, 1960, Isoperla pesici Murányi, 2011) are Balkan endemic species. Furthermore, Amphinemura sulcicolis (Stephens, 1836) and Isoperla pesici Murányi, 2011 were recorded at North Macedonian fauna only. Among the total of 20 stoneflies species that have been recorded in Bulgaria, 6 taxa are classified as Vulnerable (VU) according to the Red Data Lists of Threatened species.

Key words: stoneflies, distribution, dominant analysis, Bulgaria, North Macedonia.

Introduction

important orders of hemimetabolous aquatic insect group at species and subspecies level. insects being found chiefly in mountain river zone (HYNES, 1970; RESH & ROSENBERG, 1984; CUSHING & ALLAN, 2001). Distributed over all continents (except Antarctica) the stoneflies constitute one of the most hand, North Macedonian stoneflies have significant ecological components of running water ecosystems (ZWICK, 1992).

Established survey (FOCHETTI & TIERNO DE FIGUEROA, 2008) confirmed that, of the 383 European Plecoptera species, 252 (or 66%) are mainly found in crenal of mountainous and semi-mountainous rivers, compared to Trichoptera and Ephemeroptera orders which represents only 12% and 1%, respectively.

In Bulgaria and North Macedonia, Plecoptera presents one of the most Plecoptera presents relatively well-known Concerning Bulgaria, the stonefly fauna comprises 103 species and six subspecies belonging to 23 genera and seven families (TYUFEKCHIEVA et al., 2019). On the other been extensively examined by IKONOMOV (1969; 1970; 1971; 1972; 1974a; b; 1975; 1976a; b; 1977; 1978; 1979; 1980a; b; 1982; 1983a; b; c; 1986a; b), where until mid-1980s, 95 species belonging to 22 genera and seven families were known (IKONOMOV, 1978; 1986b; ZWICK, 1984). After few decades gap, few new records of stonefly fauna enriched with new species followed (GRAF et al., 2012; MURÁNYI

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2014). Up to day, the et al., Macedonian hitherto stonefly fauna comprises 101 species, with so far known few new species that need to be further described (MURÁNYI et al., 2014; 2016).

In the zoogeographical point of view, 64% of the Plecoptera of Bulgaria are determined by Palearctic and European species. The share of endemics is 28.44% **TYUFEKCHIEVA** al., et 2019), which determines its local character. North Macedonian stonefly fauna is also determined by Palearctic and European species (49.1%) followed by 34.4% of endemic taxa (Ікономоу, 1986b).

Nowadays, Plecoptera order is one of the most endangered groups of insects (FOCHETTI & TIERNO DE FIGUEROA, 2006) in Europe. The first data summary of the zoobenthos samplings was applied following conservation status of Bulgarian stoneflies, discussed by TYUFEKCHIEVA et al. (2019) highlights the vulnerability of the species, while the available information on this topic concerning North Macedonia is still scarce (Murányi SLAVEVSKAet al., 2014; STAMENKOVIĆ et al., 2016).

Most of the published papers for Bulgarian stoneflies have emphasized more taxonomical aspects (BRAASCH & JOOST, 1971a; b; c; 1972; 1973; 1975; 1976; 1977; GRAF, 2010). Purposeful studies of the composition and zoogeographic features of the stonefly fauna of mountain streams are few in number (KUMANSKI, 1997; 2004; HUBENOV et al., 2000a; b), with important contributions established recently (MURÁNYI, 2007; TYUFEKCHIEVA et al., 2011; 2013; 2016; subspecies (Table 2) belonging to 12 genera and 2019; VARADINOVA et al., 2013; MURÁNYI et al., 2014; SLAVEVSKA-STAMENKOVIĆ et al., 2016). Nevertheless, data of the precise distribution of stoneflies in mountainous and semimountainous streams from Bulgaria and Macedonia remains incomplete. North Moreover, we give a brief contribution presenting part of the results within this Plecoptera fauna for 14 Bulgarian rivers as paper.

determine species composition, rivers zoogeographic features, endemic

North conservation status, as well as we analysed the frequency of occurrence and dominance of stonefly species in studied mountainous and semi-mountainous stretches of Bulgaria and North Macedonia.

Material and Methods

total 69 samples of bottom In macroinvertebrates were collected in Autumn 2017 (October) and in Spring 2018 (April/May). Twenty-two sites were sampled in the Bulgaria and sixteen sites in North Macedonia (Fig. 1). Data for the altitude and geographical coordinates of each locality, as well as the year(s) of investigation is presented in Table 1. At two sites (marked with "♦" at Table 1) we didn't observe any Plecoptera species.

multi-habitat The approach for CHESHMEDJIEV et al. (2011). The collected material was fixed with 4% formaldehyde in situ. After samples processing in the laboratory, the zoobenthic organisms were sorted by taxonomic groups and stored in 70% ethanol. The stoneflies were identified to the species level. Nomenclature and zoogeographic features for Plecoptera follow MURÁNYI (2008) and DEWALT et al. (2018). Stoneflies are classified according the IUCN categories (TYUFEKCHIEVA et al., 2019). The dominant analysis was made after DE VRIES (1937) and KOJOVA (1970).

Results

Species composition

The study comprises 23 species and 4 six families from 38 mountainous and semimountainous river stretches in Bulgaria and North Macedonia (Table 1). By species numbers, the family Nemouridae dominates. Two species of A. sulcicolis and I. pesici are known only for the fauna of North Macedonia. Herein, for the first time we give original faunistic data for follows: L. hippopus from Draglishka and Thus, the aim of the present work is to Dvorishka River; T. schoenemundi from the Elovitsa, Sushichka, Oshtavska, and Eleshnitsa and Sovolyanska Bistritsa; Α.

triangularis from Dvorishka and Sushichka River; *P. marginata* from the rivers Elovitsa, Bobeshinska and Sovolyanska Bistritsa; *D. megacephala* from Oshtavska, Dragovishtitsa and Eleshnitsa River; *P. intricatus* from Sovolyanska Bistritsa River and *N. flexuosa* from Bobeshinska River. Faunistic data for the stoneflies fauna of the Bachevska, Klinotitsa, Lomishka,

Tsaparevska and Elovitsa rivers are reported for the first time (Table 3, 4).

Zoogeographical characteristics

According to their current distribution the established stoneflies can be assigned to 7 zoogeographical categories, grouped into 5 zoogeographical complexes (Table 2; Figure 2, 3).



Fig. 1. Map of the studied sites.

Table 1. List of the studied rivers and localities from the mountain and semi-mountain streams in Bulgaria and North Macedonia. *Legend:* *Sites collected only at Autumn 2017; **Sites collected only at Spring 2018; ***HPS-Hydroelectric power station.

N⁰	River/Locality	Altitude, m	Ν	Е	2017	2018
1_BG	Cherna Mesta River, upstream HPS***	1125	42.07875	23.72581	x	x
2_BG*	Small brook, tributary to Cherna Mesta, near site 1_BG	1121	42.07776	23.72564	x	
3_BG	Right tributary to Cherna Mesta	1040	42.06104	23.72682	x	x

N⁰	River/Locality	Altitude, m	N	E	2017	2018
4_BG	River, upstream Cherna Mesta v. Mesta River, downstream Yakoruda town	851	42.00163	23.62883	x	x
5_BG	Klinoshtitsa River, upstream Dobarsko v.	1220	41.98504	23.46597	x	x
6_BG	Draglishka River, at Draglishte v.	831	41.93432	23.51248	x	
7_BG	Elovitsa River, at Prevala	1038	41.89799	23.33618	x	x
8_BG	Sushichka River, upstream Sushitsa v.	873	41.80629	23.02944	x	x
9_BG	Sushichka River, downstream Sushitsa v.	780	41.81839	23.07301	x	x
10_BG	Lebnitsa River, after border with R. North Macedonia	789	41.55756	22.99431	x	x
11_BG	Dvorishka (Cironska) River	814	41.56622	22.98840	x	x
12_BG	Tributary to Lebnitsa River, after Dobri Laki v.	797	41.56890	22.99031	x	
13_BG	Tsaparevska River, upstream Tsaparevo v.	622	41.63227	23.07884	x	x
14_BG	Oshtavska River, before its mouth to Struma River	190	41.75985	23.15539	х	x
15_BG	Eleshnitsa River, at Chetirtsi v.	457	42.24273	22.87364	х	х
16_BG	Sovolyanska Bistritsa River, near Kutugertsi v.	797	42.31128	22.50344	x	x
17_BG	Bobeshinska River, before its mouth to Sovolyanska Bistritsa R.	806	42.31732	22.49856	x	x
18_BG*	Sovolyanska Bistritsa River, upstream Gurlyano v.	1090	42.24026	22.55357	x	
19_BG**	Bachevska River, at Bachevo v.	949	41.9290	23.4480		x
20_BG**	Blagoevgradska Bistritsa River, near "Parangalitsa" Reserve	1528	42.0424	23.3643		x
21_BG**	Lomnishka River, at Lomnitsa v.	824	42.3762	22.5482		x
22_BG**	Dragovishtitsa River, downstream Dolno Ujno v.	594	42.4043	22.594		x
1_MKD	Baba River, downstream Koleshinski Waterfall	435	41.3703	22.8078	х	x
2_MKD	Lomnitza River, downstream Smolarski Waterfall	742	41.3709	22.9003	х	x
3_MKD	Drazevska River upstream Drazevo v.	924	41.3715	22.9185	x	x
4_MKD	Star Dol River upstream Staro Konjarevo v.	464	41.3627	22.9529	x	x
5_MKD	River Shtuka, upstream Stuka v.	379	41.479	22.821	х	х
6_MKD	Dvorishka (Prevedenska, Cironska) River, spring region	1138	41.5719	22.8473	x	x
♦7_MKD*	Barlen River, upstream Gabrovo v.	849	41.3764	22.7816		x

Composition and Zoogeographic Features of the Stonefly Fauna (Insecta: Plecoptera)...

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Nio	River/Locality	Altitude,	N	F	2017	2018
1 12	River Locality	m	19	L	2017	2010
8_MKD	Dvorishka (Cironska) River, downstream Dvorishte v.	901	41.5848	22.9336		х
9_MKD	Dvorishka (Cironska) River, upstream Dvorishte v.	944	41.5935	22.9004	х	х
10_MKD	River Ratevska, downstream Rusinovo v.	833	41.6952	22.8325	x	x
11_MKD	River Klepalska Reka, upstream HPS***	1165	41.6666	22.9614	x	x
12_MKD	River Ambarska Reka, upstream HPS***	1165	41.6673	22.9622	x	x
13_MKD	River Klepalska Reka, downstream HPS***	1122	41.6744	22.9428	x	x
14_MKD	River Kriva Reka,upstream Uzem v.	864	42.2152	22.4356	x	x
♦15_MKD	River Luchka Reka, downstream Dobrovitza v.	789	42.2566	22.3492	x	x
16_MKD	River Kriva Reka, upstream Kriva Palanka town	670	42.2122	22.3452	x	x

Table 2. Species composition and zoogeographical characteristics of Plecoptera from the studied mountain and semi-mountainous streams from Bulgaria and North Macedonia. Legend: *Species that refer to Vulnerable (VU) category according to the Red Data Lists of Threatened species of Plecoptera in Bulgaria.

Tava	Abbroviation	Zoogeographical	Zoogeographical
1 axa	Abbreviation	complexes	categories
*Taeniopteryx shoenemundi (Mertens, 1923)	Taensho	European	Mid-European
Brachyptera beali beali (Navás, 1923)	Brachbe	Endemic	Balkan
Brachyptera risi (Morton, 1896)	Brachri	European	Pan-European
Brachyptera seticornis (Klapálek, 1902)	Brachse	European	Mid-and South- European
Leuctra fusca fusca (Linnaeus, 1758)	Leucfus	Holarctic	Palearctic
<i>Leuctra hippopus</i> Kempny, 1899	Leuchip	Holarctic	Palearctic
<i>*Leuctra hirsuta</i> Bogoescu & Tabacaru, 1960	Leuchir	Endemic	Balkan
Leuctra inermis Kempny, 1899	Leucine	European	Pan-European
Leuctra prima Kempny, 1899	Leucpri	European	Mid-and South- European
Leuctra pseudosignifera Aubert, 1954	Leucpsi	European	Mid-and South- European
Amphinemura sulcicolis (Stephens, 1836)	Amphsul	European	Pan-European
Amphinemura standfussi (Ris, 1902)	Amphsta	European	Pan-European
Amphinemura triangularis (Ris, 1902)	Amphtri	European	Mid-and South- European

Tava	Abbroristion	Zoogeographical	Zoogeographical	
Taxa	Abbreviation	complexes	categories	
Protonemura intricata intricata (Ris, 1902)	Protint	European	Mid-European	
Protonemura montana Kimmins, 1941	Protmon	European	Pan-European	
Protonemura praecox praecox (Morton, 1894)	Protpra	European- Mediterranean	European- Anatolian	
Nemoura cinerea cinerea (Retzius, 1783)	Nemocin	Holarctic	Palearctic	
*Nemoura flexuosa Aubert, 1949	Nemofle	European- Mediterranean	European- Anatolian	
*Nemoura subtilis Klapálek, 1895	Nemosub	Mediterranean	Balkan-Anatolian	
Nemoura uncinata Despax, 1934	Nemounc	European	Mid-and South- European	
Perlodes intricatus (Pictet, 1841)	Perlint	European	Mid-and South- European	
Isoperla grammatica (Poda, 1761)	Isopgra	European	Pan-European	
Isoperla pesici Murányi, 2011	Isoppes	Endemic	Balkan	
<i>*Chloroperla tripunctata</i> (Scopoli, 1763)	Chlotri	European	Pan-European	
<i>*Siphonoperla neglecta</i> (Rostock, 1881)	Siphneg	European	Mid-European	
Perla marginata (Panzer, 1799)	Perlmar	European	Mid-and South- European	
Dinocras megacephala (Klapálek, 1907)	Dinmega	European	Mid-and South- European	

Composition and Zoogeographic Features of the Stonefly Fauna (Insecta: Plecoptera)...



Fig. 2. Distribution of stonefly fauna according to zoogeographical complexes.



Fig. 3. Distribution of stonefly fauna according to zoogeographical categories.

The Plecoptera order is defined mainly by Palearctic and European species (78%) and locally with endemic species (11%).

Holarctic species complex includes only one zoogeographical category - Palearctic species represented by one species and two subspecies (11%).

European species complex is best represented and comprises 17 species and one subspecies (67%) from three zoogeographical categories. Mid- and South- European species are dominant (8 taxa, 30%), followed by Pan-European (7 taxa, 26%) and Mid-European (3 taxa, 11%). This complex includes especially widespread species in all Europe.

Mediterranean species complex includes one Balkan-Anatolian species (*N. subtilis*).

European-Mediterranean species complex comprises one species and subspecies, classified into European-Anatolian zoogeographical category.

Endemic species complex includes Balkan endemic species *B. beali beali*, *L. hirsuta* and *I. pesici*. Six species (*T. shoenemundi*, *L. hirsuta*, *N. flexuosa*, *N. subtilis*, *Ch. tripunctata* and *S.neglecta*) refer to Vulnerable (VU) category according to the Red Data Lists of Threatened Species of Plecoptera in Bulgaria (marked with * in Table 2, TYUFEKCHIEVA et al., 2019).

Dominant analysis

Table 3 and 4 give information on the frequency of occurrence (pF%), frequency of dominance (dF%) and the range of dominance (DT) of the stoneflies found during sample periods, Autumn 2017 and Spring 2018. The species with high values of pF and DT (B. seticornis, I. grammatica, N. subtilis, P. marginata and P. intricata intricata) dominate and quantitatively in the composition of the stoneflies complex (Fig. 4, 5). This shows their fundamental importance for establishment of its qualitative and quantitative composition in river zoocenoses. These taxa are also the most spread stoneflies in the investigated semi-mountainous and mountainous river sections.

Composition and Zoogeographic Features of the Stonefly Fauna (Insecta: Plecoptera)...

With a most substantial quantitative part (DT>50%) are eleven taxa (40.7%). The present data establish stenobiotic character of some species as well. These are representative taxa with high values of the range of dominance and low frequency of occurrence (*L. hippous, L. fusca fusca, N. cinerea, A. standfussi* and *Ch. tripunctata*). Their mass development, expressed through numerical domination in the sample, is possible

in relatively narrow living conditions limits only. They do not occur frequently, but once appearing, always dominate in the community. Therefore, apart of the high values of DT, these species could not be specified as dominating. The studied river sections are inhabited by Plecoptera species with different ecological plasticity which is evident from the results of the dominant analysis.

	Table 3. Dominant	analysis of stoneflies	established during sampl	e periods, Autumn 2017.
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Localities/ Taxa	Taensho	Brachri	Leucine	Leuchip	Leucpsi	Leucfus	Leucpri	Amphtri	Protint	Protmon	Protpra	Nemocin	Nemofle	Nemounc	Nemosub	Perlint	Isopgra	Chlotri	Dinomeg	Perlmar
pf	40	3.3	36.7	13.3	3.3	13.3	3.3	6.7	16.7	6.7	6.7	3.3	10	13.3	60	6.7	10	13.3	10.3	50
dF	20		13.3	13.3		13.3		3.3	6.7	3.3			6.7	3.3	43.3	3.3		10		23.3
DT	50		36.4	100		100		50	40	50			66.7	25	72,2	50		75		46.7
1_BG									+						+			+		+
2_BG			+				+							+	+			+		
3_BG			+						+						+					
4_BG			+												+					
5_BG										+					+	+				
6_BG				+																
7_BG	+		+												+					+
8_BG			+							+					+					+
9_BG	+							+									+			+
10_BG	+				+										+					+
11_BG				+				+												+
12_BG				+																
13_BG															+					
14_BG	+					+													+	
15_BG	+					+													+	+
16_BG	+					+									+	+				+
17_BG													+							+
18_BG	+		+								+				+				+	+
1_MK	+														+					+
2_MK	+		+						+						+					+
3_MK	+		+						+						+					+
4_MK			+						+						+		+			
5_MK	+	+	+											+						
6_MK	+												+		+			+		
9_MK			+	+											+					+
10_MK																		+		
11_MK			+								+			+						
12_MK												+	+	+						
14_MK															+		+			
16_MK						+														+

Localities /Taxa	Brachbe	Brachri	Brachse	Leuchip	Leuchir	Leucine	Amphsul	Amphsta	Amphtri	Protint	Protmon	Protpra	Nemocin	Isopgra	Isoppes	Siphneg	Chlotri	Dinomeg	Perlmar
pf	3.1	15.6	50	9.4	9.4	15.6	12.5	3.1	6.25	59.4	3.1	18.8	6.3	40.6	3.1	9.4	6.3	12.5	78.1
dF		3.1	37.5		3.1		6.3	3.1		59.4		9.4	6.3	31.3		3.1		3.1	40.6
DT		20	75		33.3		50	100		100		50	100	76.9		33.3		25	52
1_BG			+	+										+			+		+
3_BG			+	+						+				+					+
4_BG																			+
5_BG			+						+					+					+
7_BG			+		+	+				+				+					+
8_BG						+				+				+					+
9_BG										+				+				+	+
10_BG																			+
11_BG										+									+
13_BG										+		+							+
14_BG										+								+	
15_BG					+									+					+
16_BG																			+
17_BG										+									
19_BG									+	+		+		+					+
20_BG			+							+		+		+					
21_BG					+						+								+
22_BG																		+	+
1_MK										+									+
2_MK			+				+			+									+
3_MK		+	+							+								+	+
4_MK		+	+				+			+									+
5_MK		+	+							+									+
6_MK		+	+							+						+	+		+
8_MK													+						
9_MK	+	+	+			+	+					+	+	+					+
10_MK	+	+	+				+					+	+	+					
11_MK			+			+		+		+		+			+	+			+
12_MK			+			+		+				+			+	+			
13_MK			+	+		+				+				+					
14_MK		+	+											+					+
16_MK																			+

Table 4. Dominant analysis of stoneflies established during sample periods, Spring 2018.



Composition and Zoogeographic Features of the Stonefly Fauna (Insecta: Plecoptera)...

Fig. 3. The frequency of occurrence (pF%) and the range of dominance (DT) of the stoneflies found during sample period, Autumn 2017.



Fig. 4. The frequency of occurrence (pF%) and the range of dominance (DT) of the stoneflies found during sample period, Spring 2018.

Discussion

Available information about the strict requirements of stoneflies to the specific hydromorphological, physical and hydrochemical characteristics of the environment, which they inhabit (ZWICK, 1982; 1992; ZAMORA-MUNOZ *et al.*, 1993) explain the dynamic of the Plecoptera complex in the river coenosis.

AUBERT (1965) and RAVVIZA & RAVVIZA DEMATTEIS (1991) report that the maximum stoneflies species diversity for the Alps is in the lower part of the mountain (circa 1200 m a.s.l.), while for the Pyrenees, BERTHELÉMY (1966) finds this maximum at 900m a.s.l. According KRNO (2003a; b) the maximum species richness is at high-mountain rivers and streams of Slovakia. KAMLER (1965); (1986); SOLDÁN *et al.* (1998); WARD BULANKOVA et al. (2001) and MAVRI et al. (2003) found that in semi-mountainous river stretches occur species Perlodes microcephalus (Pictet, 1933), Leuctra albida Kempny 1899, L. fusca fusca and L. hippopus. According RAVIZZA & GERECKE (1992), RAVIZZA & RAVIZZA DEMATTEIS (1993), KOVÁCS & MURÁNYI (2008) and BOJKOVA et al. (2011) the species B. seticornis, I. grammatica, P. marginata and P. intricata intricata are widely distributed in whole Europe. These taxa are also the most spread stoneflies in the investigated semimountainous and mountainous river sections in North Macedonia and Bulgaria.

Forty one species from the Bulgarian mountain stoneflies fauna are common with the mountain Plecoptera-fauna of Montenegro, 19-with that of Croatia and 26with the mountain zones of Pyrenean Peninsula (VINÇON & PARDO, 2004; MURÁNYI, 2008; POPIJAČ & SIVEC, 2009).

ZWICK (2000) indicates that Plecoptera fauna is result of the paleogeographic and paleoclimatic changes and global distribution patterns suggest that evolution of the extant suborders started with the breakup of Pangaea. We established six stoneflies taxa (*B. seticornis, N. cinerea cinerea, N. flexuosa, L. hippopus, P. intricata intricata* and *P. praecox praecox*) specific for the Hercynian system. In Bulgaria the Struma River valley as a part of Rilo-Rhodope massif and Pirin Mnt. is originated during Herzine epoch (HRISTOVA, 2012). In that order, biogeographical features, climate, geomorphology and paleontological history events in river basins significantly influence diversity of stoneflies at the regional level 2003b). Therefore, (KRNO, in the zoogeographic mountain subregions in which Bulgaria and North Macedonia are located, certain communities, including endemic and rare species, have been established. So far, B. beali beali is reported from Bulgaria (TYUFEKCHIEVA et al., 2019), North Macedonia (IKONOMOV, 1983b; 1986b; MURÁNYI et al., 2014; SLAVEVSKA-Stamenković et al., 2016), Bosnia & (MURIĆ et Herzegovina al., 2011), Montenegro (KACANSKI & BAUMANN, 1981) and Greece (AUBERT, 1963; BERTHÉLEMY, 1971; ZWICK, 1978; KARAOUZAS et al., 2016); L. hirsuta is reported from Bulgaria **TYUFEKCHIEVA** et al., 2019), North Macedonia, Bosnia & Herzegovina, Montenegro, Greece and Romania (KARAOUZAS et al., 2016) and I. pesici – from North Macedonia, Montenegro and Albania (MURÁNYI *et al.*, 2016).

Nowadays, due to the high environmental requirements of stoneflies, many species are reduced to small isolated groups, become vulnerable, threatened by extinction, or are already extinct (RAVIZZA & NICOLAI, 1983; ZWICK, 1992; SANCHEZ-Ortega & Tierno de Figueroa, 1996). Concerning the latitude, Mountains Plecoptera fauna is with less vulnerability compared to the representative one from the lowland rivers. Moreover, especially as result of the environmental changes, the most vulnerable ones are endemic taxa. While the more sustainable the rhithral stoneflies are mainly recent postglacial immigrants to Central Europe from Mediterranean refugia (ZWICK, 1982), the critically endangered and vulnerable Plecoptera are predominantly represented by ancient local fauna (neoendemics) with strong connections to Holarctic taxa, appeared from the Mediterranean and Minor Asia. Twenty one species in South Europe are particularly threatened, where the average annual air temperature is predicted to rise about 4°C and precipitations will decrease up to 0.25 mm/day for the period 2071-2100 (GITAY *et al.*, 2002).

The present work will contribute to incentive further studies of the local and regional biodiversity of Bulgarian and North Macedonian stoneflies fauna as essential element for the environmental classification of water bodies, which up to day is poorly presented and discussed for both countries.

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Ichthyofauna of the Iskar River Section Affected by the Hydropower Cascade "Middle Iskar"

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Abstract. The study aimed to assess the impact of a cascade of 5 small hydropower plants on the composition and structure of the recent fish communities in the affected area of Iskar River. The field surveys cover the river sections up- and downstream the 5 dams, the dam lakes and the fish passes of the dams. Fifteen fish species were recorded in the dam lakes. Limnophylous and eurytopic species predominate the communities. The high species richness and the presence of large-size fishes can be considered as an indirect indication of relatively stable conditions in the dam lakes. Eleven fish species were found in the studied river stretches. The ecological status of these river sections assessed by standardized fish indices were determined as Good and High. Both the abundance and size-age compostion of some type-specific species correspond to "Favorable" conservation status. Totally 8 fish species were recorded to migrate up- and downstream through the fish passess. The most intensive upstream migration was found at the end of May when 2 to 5 species migrated through different dams.

Key words: HPPs, Fish communities, Iskar River, Dams, Fish passes, Ecological state.

Introduction

The effects of construction and operation of the-river hydropower facilities over the fish communities in rivers are widely discussed because of their widespread and increasing numbers as renewables. The main reported impacts are related to the hydro-morphological pressure: barrier effect (river fragmentation and breaking the migration corridors), change of hydrological and morphological features (depths, current velocities, sediment's granulometry etc.), water abstraction (LUCAS & MARMULLA, 2000; LUCAS & BARAS, 2001; STEINMETZ & SUNDQVIST, 2014, VASSILEV et al., 2016; UZUNOVA et al., 2017, etc.). Although the general impacts of the small hydropower plants (HPPs) on the river fish

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typology, fish zone, types of the HPP (run-ofhydroelectrics or diverted), constructive features of the facilities and some further pressures.

The aim of this study was to establish the current state of fish community in a semimountain river zone after construction of a cascade of 5 small run-of-the-river HPPs and to check the use of the fish passes at the dams of HPPs by fishes for upstream migrations. So far no data on these issues have been published for this hydroelectric facility, and for Bulgaria they are too scarce as a whole.

Materal and Methods

Study area. Iskar River is the longest communities are well known the specific effects right Danube tributary on Bulgarian territory are quite variable depending on the river and the only one which crosses the Balkan

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Mountain through a gorge 84 km long. Along the gorge, its middle course is a high-flow, fast-flowing river. The "Middle Iskar" HPP cascade is built in the middle part of the Iskar's gorge. The cascade covers about 30 km along the river including 5 HPPs "Tserovo", (downstream): "Prokopanik", "Lakatnik", "Svrajen" and "Opletnya" (Fig. distance between the 1). The HPPs "Prokopanik" and "Tserovo" is about 6 km, multihabitat fish sampling with electricity those between "Tserovo" and "Lakatnik" about 16 km while the 3 HPPs "Lakatnik", "Svrajen" and "Opletnya" are immediately adjacent consequtively. Two tributaries inflow in the Iskar River close to the cascade area. The Batuliyska River inflows upstream Prokopanik dam lake and the the Gabrovnitsa River inflows downstream the Opletnya HPP.



Fig. 1. Location of the hydropower cascade and the surveyed HPPs.

Study was carried out in 2017 and 2018 comprising spring, summer and autumn seasons. The surveys cover three types of habitats within the studied area: 1) dam lakes of the HPPs; 2) free sections of the Iskar River between the HPPs and these situated immediately upstream and downstream the cascade and 3) fish passes at the dams along with surveys on the passage of fish upstream through the fish passes. Fish were sampled using different methodology and tools relevant to both the concrete task and the habitat features as follows:

In the dam lakes fish were sampled by multimesh gillnets and fish traps which were put during the night time. The processing of fish samples in situ included: species identification of the caught fish, measuring their individual total length (TL) and determination of the number and the total weight of each species;

• In the targeted free river sections was performed by wading according CEN EN 14011 in accessible zones using backpack electrofishing devices. The accessible river section between the HPPs "Lakatnik" and "Svrajen" is quite short and does not allow to find two separate sampling sites (one downstream "Lakatnik" and other upstream "Svrajen") and that's why there is only one site. No sampling sites were also found between the HPPs "Svrajen and "Opletnya" since there is no free river section at all (the dam lake of HPP "Opletnya" begins immediately downstream the fish pass of HPP "Svrajen"). The processing of fish samples in situ included: species identification of the caught fish, measuring their individual total length (TL) and determination of the number and the total weight of each species;

In the fish passes fish were sampled using electrofishing. Fish caught in the different sections of each fish pass (lower, middle and upper) after species identification and determination of the individual length were counted and released in the river or in the dam lake;

• For checking upstream fish migrations through fish passes specially constructed fish trap (Fig. 2A) was put against the upper exit of the fish pass (Fig. 2B). After several trial catches the fish traps were then put only during the night time. The processing of fish samples in situ included: species identification of the caught fish, measuring their individual length (TL) and determination of the number of each species.

The ecological state of the surveyed river sections was assessed by Biological Quality

Element (BQE) Fish using the intercalibrated & PESHEV, 1958). The species composition in (TsBFI) (Adapted method for BQE Fish in rivers of national types R2, R4, R7, R8 Type specific Bulgarian Fish Based Index (TsBFI), 2016) in 5 grades as defined by the Ordinance H-4/2012. Furthermore the status of type specific and indicator species was assessed according the approach used in the National System for Monitoring of Biological Diversity (National System For Monitoring of Biological Diversity, 2014).



Fig. 2. Fish trap for studying the upstream fish migrations through the fish passes. Upper - general view; Lower - working position.

Results and Discussion

Fifteen fish species of 5 familes were found in the dam lakes of the cascade, most of them native for the studied area according the historical data (DRENSKI, 1921; PASPALEV

Type-specific Bulgarian Fish Based Index different dam lakes was almost the same (Table 1). Roach R. rutilus characterizes with higher abundance in the fish communities of dam lakes sharing about 60% of fish caught. It is remarkable however the presence of Vimba bream which is considered as a rheophylous migrating species.

> Four species are considered alien for the studied area: L. gibbosus and P. parva are alien species in Bulgarian ichthyofauna, reported first for the Danube and adjacent wetlands; currently widespread all over the country, manly in standing waters and slow-flowing rivers; C. gibelio is considered native in the Lower Danube region. It was considered an invader upstream along the tributaries although in the middle stream of the Iskar its status (native/alien) is unclear; A. brama is a native species for Bulgaria but it is not typical for the middle stream of the Iskar River. Most likely it was translocated in the Middle Iskar dam lakes. A special case is with the Carp C. carpio, considered a native species in the Danube basin but currently in the Middle Iskar it is probably presented by stocked culture form instead a wild form presented in the past.

> A total of 11 fish species of 3 Families were found in the surveyed *free river sections* (Table 2). The fish communities there were predominated by few rheophylous species eurytopic although some and even limnophylous species are presented.

> The same three alien fish species were found in the river sections but presented with very low abundance (Table 2).

> Ecological state of the surveyed river sections has been determined according the indicative ichthyological parameters as High or Good (Table 3) but the assessment of the section downstream the HPP "Lakatnik" should be considered only as approximate because of the big depth and the fast current the zone where standard electrofishing by wadding could be applied is very restricted. Standard sampling with electricity also inapplicable was downstream the HPP "Svrajen". The highest values of the index TsBFI were calculated for the

longest free river section between the HPPs "Prokopanik" and "Tserovo" (Table 3).

Table 1. Species composition of the fish communities in the dam lakes of surveyed HPPs. Legend: * - alien species for the region.

Family			Dam lakes		
Species	Prokopanik	Tserovo	Lakatnik	Svrajen	Opletnya
Esocidae					
Esox lucius Linnaeus, 1758	+	+	+	+	+
Centrarchidae					
Lepomis gibbosus (Linnaeus, 1758)*	+	+	+	+	+
Cyprinidae					
Abramis brama (Linnaeus, 1758)*		+	+	+	+
Alburnus alburnus (Linnaeus, 1758)	+	+	+	+	+
Barbus petenyi Heckel, 1852	+	+	+	+	+
<i>Carassius gibelio</i> (Bloch, 1782)*	+	+	+	+	+
<i>Cyprinus carpio</i> Linnaeus, 1758	+	+	+	+	+
Gobio gobio (Linnaeus, 1758)	+	+	+	+	+
Pseudorasbora parva	+	+	+	+	+
(Temminck & Schlegel, 1846)*					
Rhodeus amarus (Bloch, 1782)	+	+	+	+	+
Rutilus rutilus (Linnaeus, 1758)	+	+	+	+	+
<i>Squalius cephalus</i> (Linnaeus, 1758)	+	+	+	+	+
Vimba vimba (Linnaeus, 1758)		+	+		+
Percidae					
Perca fluviatilis Linnaeus, 1758	+	+	+	+	+
Siluridae					
Silurus glanis Linnaeus, 1758		+	+	+	

Table 2. Composition of the fish communities in the river sections along the studied area. Legend: Pr – Prokopanik, Ts – Tserovo, La – Lakatnik, Op – Opletnya, DL – dam lake, HPP – Hydropower Plant, (Up) – upstream, (D) – downstream; Ab – abundance: N – ind./ha, B – kg/ha. Legend: * - alien species for the region.

Family	Ab	Pr DL	Pr HPP	Ts DL	Ts HPP	La DL	La HPP	Op HPP
Species		(Up)	(D)	(Up)	(D)	(Up)	(D)	_(D)
Fam. Cyprinidae								
Alburnoides bipunctatus	Ν	860	680	833	3045	2160	1067	3400
(Bloch, 1782)	В	3.900	6.360	6.042	12.727	11.840	4.667	15.000
Alburnus alburnus	Ν		80		750		400	75
	В		0.960		3.545		0.733	0.225
Barbus petenyi	Ν	720	1720	1708	1454	7820	133	2375
	В	9.420	44.400	57.250	19.954	11.160	1.333	24.975
Carassius gibelio*	Ν		120	125	23			
	В		5.880	1.083	0.045			
Gobio gobio	Ν	200	320	167	636	160	600	375
	В	2.200	5.600	2.208	3.590	0.560	2.867	3.700
Pseudorasbora parva*	Ν			42			67	
	В			0.375			0.467	
Rutilus rutilus	Ν		640	750	68			
	В		1.600	13.167	0.068			
Rhodeus amarus	Ν		40				267	
	В		0.120				0.200	

Family	Ab	Pr DL	Pr HPP	Ts DL	Ts HPP	La DL	La HPP	Op HPP
Species		(Up)	(D)	(Up)	(D)	(Up)	(D)	(D)
Squalius cephalus	Ν	520		750	909	880	1200	300
	В	7.900		44.083	10.727	11.040	14.000	9.975
Fam. Centrarchidae								
Lepomis gibbosus*	Ν		80					
, C	В		2.320					
Fam. Cobitidae								
Cobitis elongatoides	Ν							50
Bacescu & Mayer, 1969	В							0.250

Table 3. Ecological state of the surveyed Iskar River sections according TsBFI.

Site - river section	Value of TsBFI	EQR	Ecological state
Upstream DL "Prokopanik"	50	0.71	Good
Downstream HPP "Prokopanik"	74	1	High
Upstream DL "Tserovo"	68	1	High
Downstream HPP "Tserovo"	57	0.83	High
Upstream DL "Lakatnik"	50	0.71	Good
Downstream HPP "Lakatnik"	64	0.97	High
Downstream HPP "Opletnya"	63	0.96	High

In most of the surveyed river sections the numbers, the biomass and the size structure of some common type-specific and indicator fish species, such as: *S. cephalus*, *B. petenyi*, *A. bipunctatus and G. gobio*, correspond to "Favorable" ecological state. High abundance and biomass, as well as the good age structure of *A. bipunctatus* and *B. petenyi* are remarkable as far as they are an indicator species for this river zone and furthermore *B. petenyi* is a species enlisted in the Annex 2 of the Habitat Directive.

The conservation status of *B. petenyi* in the river section downstream the HPPs "Lakatnik" was assessed as "Unfavorable" according the parameters Abundance, Biomass and Age structure. Although as mentioned above the results for this river section are only approximate this is an expected result taking in account the modified habitat of this species there.

The most intensive upstream fish migrations through the *fish passes* were recorded in May-June when as it is well known the most intensive spawning occurs (KARAPETKOVA & ZHIVKOV, 1995) but upstream movements occurred until the end of August even outside the spawning period

(Table 4). During the period 27.05-01.06 upstream fish migrations were recorded through the fish passes of all 5 dams but of different intensity (presented as *fish* specimens per night). As one can see (Table 4), the highest number of migrating species occurred at the both "Lakatnik" and "Opletnya" dams while only one species *B*. *petenyi* was recorded to migrate through the fish pass at the HPP "Tserovo". The highest intensity of migration through the fish pass was recorded at the HPP "Opletnya" and the lowest one - at the HPP "Svrajen. Later the intensity of the upstream migration of fish sharply decreased and only single specimens of 2-3 species were found to move upstream through the fish passes at the three lower dams (Table 4).

The biggest proportion amongst the fishes migrating upstream through all the fish passes shared *B. petenyi* (39 – 100%), *A. bipunctatus* (1,7 – 24%) and *S. cephalus* (1,5 -32%).

Fish were found in the *fish passes* throughout the period from May to August and as the water temperature drops in the autumn all they leaved this habitat. The quantitative features of fish into the fish passes were surveyed during the period of
most intensive upstream fish migration (27.05-01.06.2018 r.). In three out of five surveyed fish passes fish were sampled twice – before and after checking of the upstream migration. In the other 2 of them sampling by different reasons was performed only after finishing the checking and removal of the fish trap. During the sampling some solid wast (plastic and

wooden debris) blocking the fish pass were removed.

The highest species richness was recorded in the fish pass of HPP "Lakatnik" (Table 5) including typical rheophlyous species (i.e., *A. bipunctatus*, *B. petenyi*) but also some limnophylous and eurytopic species, such as: *A. alburnus*, *C. gibelio*, *G. gobio*, *L. gibbosus*, *R. amarus*, *R. rutilus*, *S. cephalus*.

Table 4. Intensity of the upstream movements of fishes through the fish passes as *fish specimens per night* during the studied period.

Dam	Prokopanik	Tserovo	Lakatnik		Svraj	en	Ople	tnya	
		Number	of sp	ecim	ens p	er nigh	t		
Date	27.05-01.06	27.05-01.06	27.05-01.06	10-11.06	29-31.08	27.05-01.06	29-31.08	27.05-01.06	10-11.06
A. bipunctatus	4		1		4		3	91 1	
A. alburnus B. petenyi	47	35	5 23	1	4 6	1	5	1 285	2
B. barbus G. gobio			8					1	
L. gibbosus	1								
S. cephalus	1		19	1	5	1		6	1
V. vimba			3					2	
Total number	53	35	59	2	15	2	8	386	3

Table 5. Number of fishes found in the fish passes. legend: 1 – at the evening (before putting the fish trap); 2 – on the next morning (after the removal of the fish trap).

HPP	Proko	panik	Tser	ovo	Lakatnik		Svrajen	Opletnya
Sampling	1	2	1	2	1	2	2	2
Species				Fi	sh numl	ber		
A. bipunctatus	7		52	98	25	10	199	35
A. alburnus	11	1	10	454	346	69		
B. petenyi	33	4	212	76	42	18	293	26
C. gibelio					30			
C. nasus							1	
G. gobio					16	10		
L. gibbosus		5	12	1				
P. fluviatilis		1						
R. amarus					8			
R. rutilus			4	57	9			
S. cephalus		2	6		77	51	19	1
V. vimba				2	10	8	10	1
Total number	51	13	296	688	563	166	522	62

After the sampling and removing of fish from the fish pass before the fish trap putting, the next morning in the fish pass were recorded 6 species the most abundant being rheophylic and highly mobile species, such as: *A. alburnus* (64%), *A. bipnctatus* (16%) and *B. petenyi* (9%).

Only in the fish pass of HPP "Opletnya" was found the typical migrating species *Chondrostoma nasus* even if presented only with single specimens against with the high proportions of both the *B. petenyi* (65%) and *A. bipunctatus* (28%).

As the water temperature drops in the autumn all the fishes leave the fish passes.

As can be seen by the obtained results, the species typical for standing and/or slow flowing waters predomined in the species composition of fish communities in the dam lakes what is an expected result taking in account the hydrological features. Most of the recorded species are native inhabitants of this part of the Iskar River but the hydromorphological modifications create more favorable conditions for the limnophylous and eurytopic species. The populations of some of these species which are atypical for the Iskar gorge area probably developed after their introduction here for recreational fishing or as a result of unintentional translocation when stocking with game species.

Currently the alien species for the area share about 18% of the total fish community abundance, with Prussian carp sharing the highest propotion (12,7%) and the Stone morocco – the lowest (< 1%) what suggests that the expectations for the rapid development of invasive alien species in the dam lakes of the cascade are not justified so far.

The diversity of the species composition and and the presence of big-size fishes (such as: Carp, Prussian carp, Perch, Pike and Wels catfish) could be cosidered as an indirect sign for stable environmental conditions in the dam lakes.

The fish communities in the surveyed free river sections are predominated by

native species typical for this section of the Iskar River (DRENSKI, 1921; PASPALEV & PESHEV, 1958; KARAPETKOVA, 1994). The comparison with the available historical data (DRENSKI, 1921; PASPALEV & PESHEV, 1958) shows reducing of the species richness of native fish community in the area of the Iskar Gorge along the years together with appearance of some new species. However the absence of recent published data does not allow evaluate the specific impact of the "Middle Iskar" hydropower cascade among the complex of anthropogenic pressures and impacts in this area. Eventually despite the presences of some species indicative for dam influence (such as, R. rutilus) and of 3 alien species in the river fish communities they obviously play negligible role in the community structure because of their very low abundance. This result refutes some predictions for significant increase of the importance of these species in the native river communities within the area of the cascade.

The Good and High ecological state of the surveyed river sections determined by BQE Fish and the Favorable conservation status of the type-specific species suggest relatively little impact of the HPP facilities on the fish communities in the river sections between the dam lakes if these river sections are more than 5 km long as well as on these upstream and downstream the cascade when there are less affected tributaries like the rivers Batuliyska and Gabrovnitsa.

Twelve species occurred in the fish passes in the spring-summer period represent a wide range of ecological guilds from typical rheophylous to more or less eurytopic. Although the obtained results give a reason to conclude that during the night fish from the river actively colonize the fish pass moving upstream only part of them mainly typical rheophylous species realize effective upstream migration passing successfully from the lower to the upper water level of the dams. The rest of species most probably use the fish passes as temporary habitat and stepping stone biocorridor until the end of summer. The seasonal dynamics of the migration obviously is related to the reproductive behavior of river fishes. Although the rheophylous and mobile species predominate in the composition of migrating fishes in all fish passes of the cascade both the species composition and the intensity of migration are quite different in the different fish passes. The available data do not allow us yet to speculate concerning the reasons for the different features of the upstream movements of fish through the fish passes of the "Middle Iskar" HPP cascade. Therefore further investigations on these issues are very recommendable.

Conclusions

Different fish communities composed mainly of native species are formed corresponding to the two main types of habitats (dam lakes and river zones) in the river Iskar section affected by the hydropower cascade "Middle Iskar". The two communities are not strongly isolated but clearly distinguish by the dominating species complexes. The influence of the dam lakes' fish communities over the river one is manifested through spreading of species only in restricted river sections both up-and downstream.

The fish passes are a transient habitat used temporary by river fish species during the spring-summer period when upstream movements of fish occur. Rheophylous and some eurytopic species use successfully the fish passes for upstream migrations which are most intensive during the spawning period but occur also later in summer. Significantly wider range of species (incl. more eurytopic species) uses the fish passes as temporary habitats and stepping stone bio-corridor moving upstream. Impaired function of the fish passes occurs when floating plastic waste and/or wooden debris block the upstream exit or the submerged orifices of the fish pass.

Currently no indications of worsening neither of the ecological state of the "free"

river sections longer than 5 km between the HPPs and both up- and downstream the hydropower "Middle Iskar" cascade determined through standard fish based indices nor of the status of type-specific inhabiting there. certain species А importance for maintaining good ecological state of the Iskar River sections up- and downstream the hydropower cascade probably have the good condition of the tributaries. Negative effect of dam construction over the rheophylous species occurs only in highly modified zones immediately downstream the dams in case of cascade construction where no enough long river sections exist.

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Distribution and Activity of Caspian Whip Snake Dolichophis caspius (Gmelin, 1789) (Reptilia: Colubridae) in South-Western Bulgaria

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Abstract. The purpose of the study is to supplement, summarize and analyse data about the distribution and activity of Dolichophis caspius in south-western Bulgaria. The new data about the species were collected from 1991 to 2019 during herpetological surveys. The total number of records until now is 420: 148 of them can be found in previous publications, and 272 are reported for the first time. There are published data about 10 pieces of shed skins, and other 17 are newly registered. All data available indicate, that D. caspius is widespread in the study area. The species spreads northward throughout Zemen Gorge in the Struma River valley, and northward throughout Momina Klisura Gorge in the Mesta River valley. It is the most common snake in these areas (up to 1000-1100 m a.s.l.) where 12 other snake species also occur. D. caspius is active from the third decade of March till the first decade of November. No winter activity of the species has been recorded, despite the active search in December, January and February in some years. The period of activity can be divided in three - a period of very low, low and high activity. The snake is very warm-loving. It remains active even during the hottest months, and was observed only during the day most often around mid-day and 4 p.m.

Key words: Serpentes, colubrid snakes, ecology, ethology, biogeography, Balkan Peninsula.

Introduction

The Caspian Whip Snake (or the Large Whip Snake), Dolichophis caspius (Gmelin, 1789), is one of the largest, swiftest and strongest European snakes. In the recent past this Whip Snake was regarded as a subspecies of Dolichophis jugularis (Linnaeus, 1758), but today it has a rank of a species. Until recently, in addition to the nominate Europe and the extreme part of western Asia: form (D. caspius caspius) the subspecies D. Hungary, S Romania, E Bosnia-Herzegovina,

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caspius eiselti (Zinner, 1972) was also included (see ZINNER, 1972). It is considered now that the taxon D. caspius eiselti belongs to the species D. jugularis, so D. caspius is a monotypic species (see CATTANEO, 2012; 2018).

The geographical range of the Caspian Whip Snake spreads over south-eastern

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Croatia, Macedonia (now North Macedonia), indicate that in 2018 the species emerged S Montenegro, Serbia, Albania, Bulgaria, from hibernation during the third decade of Greece (including many islands), W Turkey March and the first decade of April. There (including Imroz Island), Moldavia, Ukraine, SW Russia (Dagestan), extreme NW Georgia, extreme NE Turkey and extreme NE Azerbaijan (WALLACH et al., 2014). In Bulgaria the species is widespread in the lower parts, up to about 800-1100 m a.s.l., where the climate is warmer (BESHKOV & NANEV, 2002; BISERKOV et al., 2007; STOJANOV *et al.*, 2011). As an exception, the species was found in Maleshevska Mountains (southwestern Bulgaria) at 1580 m a.s.l. (BESHKOV, 1974). In south-western Bulgaria this snake is distributed in some parts of the valleys of Struma and Mesta Rivers, as well as on the slopes of the (BISERKOV et al., 2007; STOJANOV et al., 2011). The species is a East Sub-Mediterranean faunal element and its significance for the zoogeographical subdivision of Bulgaria was defined by PULEV (2016) to be quite great. D. caspius was defined as an indicator species which range delineates very well the boundaries of the Sub-Mediterranean areas in the country.

The Caspian Whip Snake occurs in natural, but also in anthropogenic habitats. It was registered in some large cities in Bulgaria – Russe (KOVATSCHEFF, 1912), Plovdiv (MOLLOV & VELCHEVA, 2010), Blagoevgrad (PULEV & SAKELARIEVA, 2013), and Burgas (NATCHEV et al., 2016).

Concerning its activity, according to ZINNER (1972) "throughout its distributional the species hibernates range between December and March; the time of highest activity is from April to June; the mating season is between the end of March and the end of May; the eggs are laid about 11/2 to 2 months after mating". In the territory of Bulgaria, the species is active from late March/early April to late October, and the copulation takes place in May/early June (BESHKOV & NANEV, 2002; BISERKOV et al., 2007; STOJANOV et al., 2011). The results from months (including in December, January, and a research conducted in south-western February in some years), and in various Bulgaria by DYUGMEDZHIEV et al. (2019) weather conditions. The specimens killed on

S are also 2 published records of winter activity in Bulgaria (see BURESCH & ZONKOV, 1934; BESHKOV, 1964). In the territory of the country the Whip Snake is active only during the day (BESHKOV & NANEV, 2002; BISERKOV et al., 2007; STOJANOV et al., 2011).

Data about the distribution and activity of the Caspian Whip Snake in south-western Bulgaria are reported by BURESCH & ZONKOV (1934), Beshkov (1964, 1974), Beshkov & NANKINOV (1979), BESHKOV & GERASIMOV (1980), BESHKOV & DUSHKOV (1981), NÖLLERT et al. (1986), KANTARDZHIEV (1992), BISERKOV (1995), PETROV & BESHKOV (2001), PESHEV et surrounding mountains al. (2005), NAUMOV (2005), PETROV et al. (2006), PULEV & SAKELARIEVA (2011, 2013), TZANKOV et al. (2011), DOMOZETSKI (2013), POPGEORGIEV et al. (2016), GROZDANOV et al. (2016), MALAKOVA et al. (2018), MANOLEV et (2019), CAS (2010-2019), BALEJ & al. JABLONSKI (2006-2019), and DYUGMEDZHIEV et al. (2019). Most publications contain the place and date (sometimes the time) of registration, the individuals observed (number, age, condition), the shed skins, and more recent papers include also geographical coordinates of the locations.

> The main purpose of the study is to supplement, summarize and analyze data about the distribution and activity (seasonal and 24-hour) of D. caspius in south-western Bulgaria.

Material and Methods

The new data about the Caspian Whip Snake were collected from 1991 to 2019, more intensively during the last years - 2013-2019 (more than the half of the records), and with single records in some years (1991, 1994, 1999-2001, 2004, 2007). The species has been registered during herpetological surveys (field trips) in various habitats. It has been searched for, day and night, in different the road have been registered both accidentally and as a result of targeted searches. The dead individuals have been defined as "fresh" when their death occurred within 48 hours before their registration.

Unpublished data from the collection of the Regional Historical Museum in the city of Blagoevgrad (RHMB), collected in southwestern Bulgaria in 1978, 1980, 1982, and 1984, were also used for the present research. All published and new data about observed alive and dead individuals, and shed skins have been used to specify the spread of the species in south-western Bulgaria. All data (published and new) about alive, "fresh" road-killed and other found "fresh" dead specimens have been included in the analysis of the seasonal activity pattern. The twentyfour-hour activity pattern has been analysed based on all published and new data about the alive active individuals for which the time of observation was recorded.

The separate locations of the species (both new and published) were grouped according to their affiliations to the squares of the Universal Transverse Mercator (UTM) grid with a resolution of 5×5 km. The gridcells were indicated by the codes of the 10km quadrates of Military Grid Reference System (MGRS; spatially identical with UTM) and capital letters (A-D) were used to denote the separate 5×5 km squares within every 10×10 km square (A indicates the southwestern square, B – the northwestern, C - the southeastern, and D - the northeastern). Mapping and map visualization were done in the projection coordinate system "WGS 84 UTM 35N" by means of ArcGIS v. 10.1 (ESRI, Redlands, CA, USA).

Most of the new records have been collected by the authors of the paper: A. Pulev [AP], G. Manolev [GM], L. Domozetski [LD], B. Naumov [BN], and L. Sakelarieva [LS]. Some of the data have been collected separately or in collaboration with the authors by other colleagues (see the Acknowledgements). The collectors have been noted with their initials in Appendices 3 and 4.

Data which are not included in some previous publications (NAUMOV, 2005; PULEV & SAKELARIEVA, 2011; 2013; DOMOZETSKI, 2013; MALAKOVA *et al.*, 2018) are added in this article and marked with ** in Appendices 1 and 2.

All data of observation (locality, geographic coordinates, altitude, date and time, the number, age and condition of individuals) are summarized in tables and the localities are marked on a map.

Results and Discussion

The total number of records of *Dolichophis caspius* in south-western Bulgaria until now is 420: 148 of them can be found in previous publications, and 272 are reported now for the first time (see Appendices 1 and 3).

Records of more than 168 individuals have been published till now (not always detailed). At least 135 of them were alive at the time of their registration, 11 were found dead (probably killed by people) and 22 were road-killed (15 of them "fresh"). The number of reported adults is the highest (n=55), followed by the number of juveniles (n=13) and subadults (n=8). Exact dates, geographic coordinates or sufficiently precise locations have been specified for most of the records (three of them were during the hibernation period), while the information about the time of observation is extremely scarce (see Appendix 1).

The new data about D. caspius in southwestern Bulgaria include 272 records (263 field observations and 9 museum specimens) of 293 individuals. Most of them (163, 148 of which "fresh") have been registered killed on the road, but the number of the alive individuals was not small as well (127). The dead ones found, without specified cause of death, were only 3. Most of the individuals registered were adult (n=225), and the number of subadults (n=38) and juveniles (n=30) was significantly smaller (see Appendix 3).

There are published data about 10 pieces of shed skins, and other 17 pieces are newly

registered (16 of adult individuals, and 1 of where according to BESHKOV & NANEV (2002) and 4).

The large number of published (102) and new (283) exact locations indicate, that the Caspian Whip Snake is widespread in the study area. The species spreads northward throughout Zemen Gorge in the Struma River valley, and northward throughout Momina Klisura Gorge in the Mesta River valley. It is the most common snake in these areas (up to 1000-1100 m a.s.l.),

juvenile) in 11 localities (see Appendices 2 12 other snake species also occur. The known localities of D. caspius in the research area fall into 94 squares of a 5 km UTM grid. The published localities refer to 43 squares (for 22 of them new data are presented here as well), and the new ones fall in other 51 squares (Fig. 1). The presence of "white spots" on the map in the areas, where the species is likely to occur, as described above, is due to the less explored or unexplored territories.



Fig. 1. Distribution of *Dolichophis caspius* in south-western Bulgaria, based on a 5 km UTM grid.

The species is found mainly in the plainhilly belt. The new records are from areas with altitude up to 1000-1100 m, which corresponds to the publications of **BESHKOV** & NANEV (2002), BISERKOV et al. (2007) and STOJANOV et al. (2011) about the vertical distribution of *D. caspius* in the country. As the altitude increases, the number of registrations sharply decreases. In the range 700-1000 m a.s.l. there are only 11 new records, and other 8 have been reported in previous publications (the highest of which was from 936 m, TZANKOV et al., 2011) (see Appendices 1, 2 and 3). Above 1000 m a.s.l. the species was registered only three times two of the records are reported now (from 1036 and 1095 m a.s.l.) (see Appendix 3), and the third one was published by BESHKOV (1974). It was from 1580 m a.s.l., but against the background of this study such altitude seems rather like an exception.

The species is found at the highest altitudes in the territory of south-western Bulgaria. Reaching altitudes of 1000-1100 m by this plain-hilly species is probably due to the warmer climate, the great difference in altitude, and the suitable habitats in this part of the country. Most of the other reptile species (see PULEV *et al.*, 2018a) also occur at a higher altitude in south-western Bulgaria compared to other parts of the country.

This research confirmed the conclusion made by PULEV (2016) that the Caspian Whip Snake has significant role for the zoogeographical subdivision of Bulgaria, as its range delineates very well the boundaries of the Sub-Mediterranean areas in the country. The species has been indicated as a typical repersentative of Sub-Mediterranean biogeographic space in southern Romania (DRUGESCU & GEACU, 2004).

The distribution of *D. caspius*, as well as of two other reptile *indicator taxa* defined by PULEV (2016) – *Testudo graeca ibera* Pallas, 1814 and *Podarcis tauricus* (Pallas, 1814), show the presence of two Sub-Mediterranean zoogeographical areas in south-western Bulgaria. They cover the valleys of Struma and Mesta Rivers, as well as the slopes of the surrounding mountains, from the boundaries of the Struma and Mesta Mediterranean areas (see PULEV *et al.*, 2018b) to 800-1100 m a.s.l. Struma Sub-Mediterranean area can expand to the north covering the entire Zemen Gorge, and Mesta Sub-Mediterranean area can end northward including the entire Momina Klisura Gorge. The two areas are isolated from each other by Slavyanka, Pirin and Rila Mountains.

The large number of published (76) and new (276) records of active individuals, "fresh" road-killed or other dead ones indicates that D. caspius in south-western Bulgaria is active for most of the year. The species is active from the third decade of March till the first decade of November and most active during the third decade of April, the first decade of May and the first decade of June. It is the least active in the beginning and at the end of the active period (Fig. 2). The annual activity corresponds (generally) to what is reported by BESHKOV & NANEV (2002), BISERKOV et al. (2007) and STOJANOV et al. (2011) for the territory of Bulgaria, as well as to what is published by ZINNER (1972) for the whole range, but the present research offers more details. The activity pattern of the species in south-western Bulgaria is similar to that of another snake - Malpolon insignitus (Geoffroy Saint-Hilaire, 1827) (see PULEV et al., 2018a), although the latest survey was presented by months rather than decades and includes a much smaller number of records.

The period of activity of *D. caspius* in southwestern Bulgaria can be divided in three - a period of very low, low and high activity. The period of very low activity covers the time before and after hibernation (the third decade of March, the third decade of October and the first decade of November). The earliest spring registration of an active individual is from March 26, and the latest autumn one is from November 08 (see Appendix 3). Both individuals were recorded in sunny and warm weather. They were subadults, and were moving very slowly (in semi-torpid state). Probably emerging and entering hibernation depend on the meteorological conditions of the year and on the habitats (type, location, altitude, exposure), i.e.

vary to some extent. Therefore, we assume that in addition to the indicated period of very low activity, emerging from and entering hibernation include the first decade of April and the second decade of October. Thus, in different years, the populations of the species in south-western Bulgaria emerge from hibernation within 2 decades and enter hibernation within three. A recent study by DYUGMEDZHIEV *et al.* (2019) shows the same period of emerging from hibernation (the third decade of March and the first decade of April). The very low activity at the end of the active period (the third decade of October and the first decade of November) was identified in that study, although this activity is not surprising.



months by decades

Fig. 2. Number of observed individuals of *Dolichophis caspius* per decades in south-western Bulgaria.

The high activity period includes the time from the first decade of April to the second decade of July with a peak around the middle (a total of 11 decades). Decreases in values during some of the decades (the second of April, the second of May and the second of June) are probably due to irregular and insufficient observations. The period of high activity can be related to the reproductive behavior – emerging from hibernation (active eating, territorial behavior, finding a partner, copulation) to laying eggs. We do not have data on copulation of this species in the territory of south-western Bulgaria, but we do have one observation from a territory very close to the

one surveyed (in North Macedonia) from the middle of the high activity period. On 27.05.2018, 12:15 *p.m.* 2 copulation individuals were registered near the Bulgarian church in Star Doyran (N41°11'19" E22°43'08", 163 m a.s.l.). A copulation from the same decade of May (22.05.2006) was registered also in Hungary by BELLAAGH *et al.* (2008).

The low activity period starts from the third decade of July and ends by the second decade of October, including a total of 9 decades. It covers the time from laying eggs to preparing for hibernation. Activity is stable during this period with no sharp downturns and peaks that outline any trend. The differences in the values in the separate decades can also be explained by irregular and insufficient number of observations.

No winter activity of the species has been recorded in south-western Bulgaria, despite the active search in December, January and February. The two reported observations are from other parts of the country - in the vicinity of the town of Septemvri on 18.12.1930 (BURESCH & ZONKOV, 1934), and near the village of Lakatnik on 02.02.1955 (BESHKOV, 1964). The winter activity of the species reported by BESHKOV (1977) and BESHKOV & NANEV (2002) probably refers to the one registered in Lakatnik. The lack of such activity in south-western Bulgaria (though it is possible) indicates that this behavior is rather an exception for *D. caspius*.

The diurnal activity of the Caspian Whip Snake reported by BESHKOV & NANEV (2002), BISERKOV et al. (2007) and STOJANOV et al. (2011) in the territory of the country has been confirmed completely. Information is available about 121 live individuals (107 new and 14 published records) with an exact time of registration. The snake was only observed during the day from 7:55 a.m. to 7:05 p.m. (recorded in this study). The highest activity is recorded around mid-day, there is a second peak around 4 p.m. While during the and low seasonal activity high the registrations are at different times of the day, during the very low activity period (emerging from and entering hibernation) the records are only from the warmer part of the day (between 10:40 a.m. and 4:25 p.m., maximum around and after mid-day) (see Appendices 1 and 3, Fig. 3). Similar data about the activity of Vipera ammodytes (Linnaeus, 1758) in Bulgaria were reported by BESHKOV (1993a). No nocturnal activity of the Caspian Whip Snake was recorded during the study, unlike other mainly daily active snake species (Natrix natrix (Linnaeus, 1758), Natrix tessellata (Laurenti, 1768), Zamenis situla (Linnaeus, 1758), Zamenis longissimus (Laurenti, 1768), and Vipera ammodytes) which have been registered in the night during the research period.

During the warmest and driest months of the year (July, August), *D. caspius* has been found throughout the day, including in the hours with the highest temperatures – 1:00-4:00 *p.m.* (see Appendix 3). This shows the great heat resistance of the species reported by other authors too. BESHKOV (1993a) writes that in the southern half of Kresna Gorge and the neighboring parts of the Maleshevska Mountains, in June and July, when daytime temperatures are particularly high (between 1:00 and 2:30 *p.m.*), the largest number of specimens of *D. caspius* was collected (compared to other snakes in the area).



Fig. 3. Number of observed individuals of *Dolichophis caspius* per hours in southwestern Bulgaria.

The activity pattern of the species in south-western Bulgaria shows that it is very warm-loving – it emerges from hibernation relatively late, it is even active during the hottest months as well as in the hours with the highest temperatures.

The species has been found in urbanized habitats in the study area. There are a number of records not only from the city of Blagoevgrad (including from the city center), but also from many smaller settlements (see Appendices 1 and 3). Possible prerequisites for this are the availability of suitable micro-habitats and a good nutritional base. According to its level of synanthropy after the classification given by KLAUSNITZER (1990) *D. caspius* was determined as a *hemerodiaphoric* species for the city of Blagoevgrad (PULEV & SAKELARIEVA, 2013) and for the city of Plovdiv (MOLLOV, 2014; 2019).

Most of the individuals recorded are adults (n=225) and their ratio to the subadults is 6:1. The very small number of juveniles (n=30) is probably due mainly to their small size. It is much more difficult to observe alive juvenile individuals in the wild, as well as to record road-killed ones. Since their small size makes them difficult to kill on the road. Juveniles may be much more cautious and less active than adults and subadults, since they have many natural enemies, have no reproductive behavior. On the other hand, the large number of adult and subadult individuals can be partly explained by the long life of the species. It matures at 3-4 years of age and lives up to 10-15 years (ZINNER, 1972; ARNOLD, 2002).

Different authors (BESHKOV, 1993b; ARNOLD, 2002; NATCHEV et al., 2016; TYTAR & NEKRASOVA, 2016; SPEYBROECK et al., 2016) note that the Caspian Whip Snake is often killed by traffic. Specific cases of road-killed specimens are found in many publications (for example PULEV & SAKELARIEVA, 2011; MALAKOVA et al., 2018; MANOLEV et al., 2019; BALEJ & JABLONSKI, 2006-2019; SAHLEAN et al., 2019). There are even specialized studies addressing the problem of reptiles killed on the road (containing data about *D. caspius*) as those published by TOK et al. (2011), et KAMBOUROVA-IVANOVA al. (2012), COVACIU-MARCOV et al. (2012), MOLLOV et al. (2013). According to ARNOLD (2002) the basks on roads is the main reason for the frequent road killing in its entire range, while SPEYBROECK et al. (2016) indicate the species active foraging strategy. NATCHEV et al. (2016) reported both reasons for the city of Burgas and the surrounding area (southeastern Bulgaria).

The large number of road-killed specimens in south-western Bulgaria (found

in both previous and present studies) could not be related only to the above mentioned reasons. We have data for both cases, but they are rare. For example, the juvenile individual D. caspius registered on 04.06.2017 of (MALAKOVA et al., 2018) pursued subad. Lacerta viridis (Laurenti, 1768) on the road - a case of foraging behavior. The published ad. D. caspius recorded on 13.10.2018 by MANOLEV et al. (2019) was sunbathing on the road. Much larger is the number of registrations at which individuals are observed to cross the road during their daily movements without knowing the exact reason for this. For example, those published by MANOLEV et al. (2019): 1 subad. on 01.07.2018, 1 juv. on 19.09.2018, 1 subad. on 19.09.2018, the records from this study: 1 ad. on 29.04.2012, 1 ad. on 11.06.2016, 1 ad. on 20.05.2017, 1 ad. on 10.06.2017, 1 ad. on 31.05.2018, 1 ad. on 02.06.2018, 1 juv. on 11.05.2019, 1 ad. on 07.07.2019, 1 subad. on 07.07.2019, 1 ad. on 10.08.2019, and others. D.caspius is the most widespread snake up to 1000 m a.s.l. with high population densities in the study area. The large size of the species also makes it very vulnerable to traffic (this is the largest snake in Bulgaria - see BESHKOV, 1964; TELENCHEV et *al.*, 2019). Last but not least, the diurnal activity of the species probably contributes to its killing on the road (traffic is much busier during the day).

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Received: 17.09.2019 Accepted: 20.12.2019 **Appendix 1.** Published data about *Dolichophis caspius* in south-western Bulgaria (data source, locality, geographic coordinates, altitude, date, time, observed individuals) and UTM (when it is possible to be determined) in Fig. 1

BURESCH & ZONKOV (1934): Blagoevgrad vicinity, 1930, 1931, 2 spec. - UTM: FM75A; near Poruchik Minkov railway station (Sandanski vicinity), 11.07.1927, 1 ad. - UTM: FM80D; near Petrich, 25.06.1931, 2 spec. - UTM: FL88B; BESHKOV (1964): above Gorna Breznitsa, 26.06.1964, 8:00 a.m., 1 ad.; BESHKOV (1974): Maleshevska Planina Mts. (1971-1972), 22 spec. -UTM: FM62C (n=1), FM72A (n=2), FM72C (n=16), FM72D (n=3); BESHKOV & NANKINOV (1979), BESHKOV & GERASIMOV (1980), BESHKOV & DUSHKOV (1981): Maleshevska Planina Mts. (1971-1974), April, 1 spec.; May, 10 spec. (incl. 1 spec. 28.05.1974); June, 26 spec. (incl. 1 ad. male 04.06.1972, 1 ad. female 17.06.1974); July, 8 spec. (incl. 1 spec. 19.07.1974); August (26.08.1972, *ca* 6:00 *p.m.*), 1 ad.; September, 1 spec.; October, 1 spec.; BESHKOV & DUSHKOV (1981): Zemen, 1 spec. - UTM: FN40A; Dolna Gradeshnitsa, 1 spec. - UTM: FM81B; NÖLLERT et al. (1986): Melnik, 1 ad. - UTM: FL99D; KANTARDZHIEV (1992): northern part of Kozhuh volcanic ridge – UTM: FL89C; BISERKOV (1995): Petrich – UTM: FL88B; Sandanski – UTM: FM80C; PETROV & BESHKOV (2001): Kresna Gorge; PESHEV et al. (2005): Rilska River basin (FM76;86); NAUMOV (2005): two kilometers SE of Pastuh (FM57), (N42°12'01" E22°54'44")**, 600 m a.s.l. (438 m a.s.l.)**, 02.05.2001, (10:10 a.m.)**, 1 spec. (1 ad.)** - UTM: FM57C; PETROV et al. (2006): Gospodintsi, 575 m a.s.l., 06.06.1999, 1 male RK - UTM: GM21D; S of Ablanitsa, 550 m a.s.l., 30.04.2001, 1 spec. - UTM: GM40A; PULEV & SAKELARIEVA (2011): Blagoevgrad, 10.05.1983, 1 spec. - UTM: FM75A; Blagoevgrad vicinity, 10.05.1994, 1 spec. - UTM: FM75A; Blagoevgrad, Strumsko residential area, 15.04.1975, 1 spec. - UTM: FM75A; Belo pole, 10.08.1985, 1 spec. - UTM: FM65D; at the mouth of the Mishavets River, N42°02'14" E23°08'25", 483 m a.s.l., 1 spec. - UTM: FM75D; at the road fork to Gorno Harsovo, N42°02'06" E23°08'02", 462 m a.s.l., 1 spec. RK - UTM: FM75D; W of Gorno Harsovo, N42°01'00" E23°10'07", 619 m a.s.l., 06.07.2003, (2:35 p.m.)**, 1 spec. RK (1 ad. RK)** - UTM: FM75C; at the mouth of the Sheitanski Andak River, N42°01'55" E23°07'04", 435 m a.s.l., summer 2007, 1 spec. - UTM: FM75D; same locality, N42°01'52" E23°07'04", 424 m a.s.l., spring 2010, 1 dead spec. - UTM: FM75D; the Zoo of Blagoevgrad, N42°00'51" E23°06'08", 425 m a.s.l., 1 spec. - UTM: FM75A; by the road from Blagoevgrad to Delvino, N42°01'23" E23°07'23", 653 m a.s.l., 28.08.2006, 2 juv. (1 dead) - UTM: FM75C; Blagoevgrad, Orlova chuka residential area, on the road to Delvino, N42°00'42" E23°06'30", 493 m a.s.l., 01.06.2004, (5:10 p.m.)**, 1 spec. RK (1 ad. RK)** - UTM: FM75A; Blagoevgrad, the industrial area, N42°00'17" E23°05'14", 351 m a.s.l., 1 dead spec. - UTM: FM75A ; E of Strumsko residential area, N41°59'20" E23°05'50", 414 m a.s.l., 1 dead spec. – UTM: FM75A; the Kaimenska chuka height, S of Blagoevgrad, N41°58'40" E23°05'27", 348 m a.s.l., 1 spec. - UTM: FM74B; S of the Kaimenska chuka height, on the road fork to Izgrev, N41°58'28" E23°05'38", 333 m a.s.l., 1 spec. RK - UTM: FM74B; S of Blagoevgrad, on the road before the road fork to Tserovo, N41°58'23" E23°05'41", 327 m a.s.l., 14.07.2009, (1:10 p.m.)**, 1 spec. RK (1 subad. RK)** -UTM: FM74B; S of Blagoevgrad, on the road after the road fork to Tserovo, N41°58'08" E23°06'05", 426 m a.s.l., 1 spec. RK - UTM: FM74B; S of Blagoevgrad, on the road to Dolno Tserovo after the road fork to Tserovo, N41°57'48" E23°06'06", 319 m a.s.l., 1 spec. RK – UTM: FM74B; N of Dolno Tserovo, N41°57'02" E23°06'08", 335 m a.s.l., 1 spec., 1 spec. RK - UTM: FM74B; E of Riltsi, N42°02'42" E23°04'58", 452 m a.s.l., 04.05.2008, (11:05 a.m.)**, 1 spec. RK (1 ad. RK)** - UTM: FM75B; between Belo pole and Riltsi, N42°02'15" E23°03'04", 390 m a.s.l., 29.06.1996, (3:55 p.m.)**, 1 spec. RK (1 ad. RK)** - UTM: FM65D; between Pokrovnik and the Struma River, N41°59'13" E23°03'41", 326 m a.s.l., 1 spec. - UTM: FM75A; Obel, Zlatkovtsi

neighbourhood, 1 juv. - UTM: FM54D; TZANKOV et al. (2011): Rilska River basin, N42°07'19.0" E23°16'20.2", 936 m a.s.l. - UTM: FM86D; PULEV & SAKELARIEVA (2013): Blagoevgrad, (N42°00'21" E23°05'08")**, (352 m a.s.l.)**, (1 ad.)** - UTM: FM75A; Blagoevgrad, (N42°00'20" E23°06'03")**, (414 m a.s.l.)**, (1 ad.)** - UTM: FM75A; POPGEORGIEV et al. (2016): E/SE of Klyuch, 41.35763 23.03192, 10.08.2011, 1 ad. - UTM: FL68C; SW outskirts of Belasitsa, 41.36423 23.1331, 13.08.2011, 1 ad. - UTM: FL78C; S of Petrich, 41.38357 23.2018, 14.08.2011, 1 ad. - UTM: FL88A; SE outskirts of Petrich, 41.39335 23.22346, 07.04.2012, 1 juv. - UTM: FL88C; NE of Razhdak, 41.40532 23.251, 25.04.2013, 1 ad. -UTM: FL88D; GROZDANOV et al. (2016): SW of Rakitna, 41°50'50"N 23°10'29"E, 697 m a.s.l., 10.05.2015, 1 ad. - UTM: FM83B; SW of Rakitna, 41°50'48"N 23°10'31"E, 678 m a.s.l., 10.05.2015, 1 juv. - UTM: FM83B; Between Poleto and Brezhani, 41°51'50"N 23°09'58"E, 420 m a.s.l., summer 2015, 4 dead ind. - UTM: FM73D; SW of Rakitna, 41°50'48"N 23°10'31"E, 690 m a.s.l., 2011, 1 dead ind. - UTM: FM83B; MALAKOVA et al. (2018): NW of Tserovo, N41°58'17" E23°06'02", 359 m a.s.l., 04.06.2017, (3:50 p.m.)**, 1 juv. - UTM: FM74B; W of Tserovo, N41°57'32" E23°06'16", 320 m a.s.l., 28.07.2017, 1 ad. RK - UTM: FM74B; E of Tserovo, N41°57'35" E23°08'20", 777 m a.s.l., 29.05.2016, (10:30 a.m.)**, 1 ad. - UTM: FM74D; same locality, N41°57'21" E23°08'35", 812 m a.s.l., 11.06.2016, (12:55 p.m.)**, 1 ad. - UTM: FM74D; N/NE of Dolno Tserovo neighbourhood, Tserovo, N41°57'11" E23°06'18", 317 m a.s.l., 27.06.2016, 1 ad. RK* - UTM: FM74B; S of Dolno Tserovo neighbourhood, N41°56'20" E23°06'08", 309 m a.s.l., 10.07.2016, 1 ad. RK* - UTM: FM74B; same locality, N41°56'16" E23°06'09", 322 m a.s.l., 28.04.2016, (11:55 a.m.)**, 1 juv. RK - UTM: FM74B; E of Dzhaleva Mahala neighbourhood, Zheleznitsa, N41°56'07" E23°05'53", 348 m a.s.l., 22.06.2014, 1 dead ad. - UTM: FM74A; W of Zheleznitsa, N41°55'38" E23°05'56", 448 m a.s.l., 19.06.2016, (1:30 p.m.)**, 1 ad. - UTM: FM74A; MANOLEV et al. (2019): SW outskirts of Bogolin, N41°32'19" E23°57'13", 610 m a.s.l., 22.04.2018, 6:00 p.m., 1 ad. RK - UTM: GM40C; E outskirts of Beslen, N41°28'20" E23°58'13", 639 m a.s.l., 13.05.2018, 10:35 a.m., 1 ad. - UTM: GL49D; E of Hadzhidimovo, N41°31'12" E23°53'09", 484 m a.s.l., 20.05.2018, 11:15 a.m., 1 juv. - UTM: GM40A; E/SE of Hadzhidimovo, N41°30'22" E23°54'17", 457 m a.s.l., 02.06.2018, 11:55 a.m., 1 ad. - UTM: GL49B; W outskirts of Slashten, N41°29'55" E24°00'41", 608 m a.s.l., 01.07.2018, 5:25 p.m., 1 subad. - UTM: KF59B; Valkosel, N41°31'49" E23°59'32", 770 m a.s.l., 19.09.2018, 4:30 p.m., 1 juv. - UTM: GM40C; NW of Slashten, N41°30'06" E24°00'34", 612 m a.s.l., 19.09.2018, 3:45 p.m., 1 subad. - UTM: KF59B; N of Godeshevo, N41°29'00" E24°03'03", 788 m a.s.l., 19.09.2018, 4:05 p.m., 1 juv. RK - UTM: KF59B; N of Godeshevo, N41°29'06" E24°03'07", 791 m a.s.l., 13.10.2018, 1:50 p.m., 1 ad. - UTM: KF59B; CAS (2010-2019): Gorna Breznitsa, 30.04.1977, 1 ad. - UTM: FM72C; Strumyani, 200 m a.s.l., 17.05.2000, 1 subad. - UTM: FM81A; BALEJ & JABLONSKI (2006-2019): Bistritsa, Rila Mts, 08.05.2015, 1 ad., 1 subad. - UTM: FM77D; Damyanitsa, 150-200 m a.s.l., 27.03.2005, 1 juv.; Damyanitsa, 120 m a.s.l., 16.04.2006, 1 ad. RK*; Damyanitsa, 150-230 m a.s.l., 16.04.2006, 3 ad.; Damyanitsa, 150 m a.s.l., 02.05.2007, 1 ad.; Damyanitsa, 140 m a.s.l., 21.05.2013, 1 subad. RK*; Damyanitsa, 140 m a.s.l., 22.05.2013, 1 subad.; Damyanitsa, 130 m a.s.l., 22.05.2013, 1 ad. - UTM: FL89D; Kresna, 250 m a.s.l., 20.04.2006, 1 ad.; Kresna, 270 m a.s.l., 01.05.2007, 1 ad.; Kresna, 2001, 1 ad.; Kresna, 400 m a.s.l., 27.06.2007, 1 ad.; Kresna, 01.09.2011, 1 ad. - UTM: FM72C; Leshnitsa, 150 m a.s.l., 16.04.2006, 1 ad. RK* - UTM: FM90A; Melnik, 2001, 1 juv.; Melnik, 450 m a.s.l., 04.05.2011, 1 subad. - UTM: FL99D; Novo Delchevo, 06.05.2015, 1 dead ad. - UTM: FL99B; Sandanski, 140 m a.s.l., 27.07.2011, 1 ad. RK*; Sandanski, 350 m a.s.l., 22.05.2013, 1 juv. RK* - UTM: FM80C; DYUGMEDZHIEV et al. (2019): the vicinity of the town of Kresna, 41°43'N 23°10'E, 180 m a.s.l., 06.04.2013, 4 ad. (in hibernation); 18.03.2014, 2 ad. (in hibernation); 21.03.2017, 2 ad. (in

hibernation); 28.03.2018, 1 ad.; 01.04.2018, 2 ind.; 02.04.2018, 1 ind.; 03.04.2018, 2 ind.; 05.04.2018, 5 ind.; 08.04.2018, 2 ind. –UTM: FM81B.

*individuals killed on the road more than 48 hours prior to the registration

**additional data about the species published by NAUMOV (2005), PULEV & SAKELARIEVA (2011, 2013), and MALAKOVA *et al.* (2018)

Appendix 2. Published data of *Dolichophis caspius* shed skins in south-western Bulgaria (data source, locality, geographic coordinates, altitude, date, observed skins) and UTM (when it is possible to be determined) in Fig. 1

PULEV & SAKELARIEVA (2011): N of Dolno Tserovo, N41°56'56" E23°05'54", 325 m a.s.l., 22.07.1997, 2 shed skins – UTM: FM74B; DOMOZETSKI (2013): NE of Beslen, (N41°28'21" E23°58'15")**, (626 m a.s.l.)**, 07.09.2012, 1 shed skin – UTM: GL49D; MALAKOVA *et al.* (2018): NW of Zheleznitsa, N41°55'49" E23°05'07", 538 m a.s.l., 19.06.2016, 1 shed skin (ad.) – UTM: FM74A; same locality, N41°55'44" E23°04'32", 534 m a.s.l., 10.07.2016, 1 shed skin (ad.) – UTM: FM74A; same locality, N41°55'52" E23°03'45", 698 m a.s.l., 10.07.2016, 1 shed skin (ad.) – UTM: FM74A; S of Gabrovo, N41°53'21" E22°56'48", 799 m a.s.l., 09.10.2016, 1 shed skin (ad.) – UTM: FM63B; W of Leshko, N41°56'04" E22°57'42", 616 m a.s.l., 15.10.2016, 1 shed skin (ad.) – UTM: FM64A; E of Padesh, N41°56'03" E23°02'56", 771 m a.s.l., 29.07.2017, 2 shed skins (ad.) – UTM: FM64C.

**additional data about the species published by DOMOZETSKI (2013)

	Geographic	Altit	Data and time of	Tre direct decelo	ТТТМ
Locality	coordinates	ude	Date and time of	Individuals	
	(N/E)	(m)	observation	observed	5×5 KM
Marino Pole near Struma River,	n/a	n/a	17.07.1978	1 ad.	FL98D
museum number (mn) RHMB 7.3/4.14					
Marino Pole, mn RHMB 7.3/4.35	n/a	n/a	17.07.1978	1 ad.	FL98D
Kresna Gorge, mn RHMB 7.3/4.36	n/a	n/a	20.07.1978	1 subad.	n/a
Kresna Gorge, mn RHMB 7.3/4.47	n/a	n/a	July 1978	1 ad.	n/a
Kolarovo, mn RHMB 7.3/4.33	n/a	n/a	05.04.1980	1 ad.	FL78C
Kolarovo, mn RHMB 7.3/4.28	n/a	n/a	15.04.1980	1 ad.	FL78C
Kolarovo, mn RHMB 7.3/4.41	n/a	n/a	17.06.1980	1 ad.	FL78C
Kolarovo, mn RHMB 7.3/4.34	n/a	n/a	July 1982	1 ad.	FL78C
Kolarovo, mn RHMB 7.3/4.69	n/a	n/a	10.04.1984	1 ad.	FL78C
Kresna Gorge (S) [BN, ZV]	41°45'36" 23°09'21"	221	25.08.1991 n/a	1 ad.	FM72D
Kresna Gorge (N) [BN, IL]	41°46'46" 23°09'14"	240	30.04.1992 3:30 p.m.	1 ad.	FM72D
N of Kresna [BN, MS, IL]	41°43'58" 23°09'26"	197	05.07.1992 n/a	1 ad.	FM72C
Kozhuh volcanic ridge (N) [BN]	41°27'36" 23°15'23"	196	30.04.1993 4:00 p.m.	1 juv.	FL89C
Kozhuh volcanic ridge (N) [BN]	41°27'59" 23°15'32"	93	30.04.1993 7:05 p.m.	1 juv.	FL89C
Kresna Gorge (N) [BN, MS]	41°48'42" 23°09'47"	266	08.05.1993 n/a	1 subad.	FM73C
Kresna Gorge (N) [BN, MS]	41°47'31" 23°09'46"	250	11.05.1994 4:15 p.m.	1 ad.	FM72D
Kresna Gorge (N) [BN, MS]	41°46'27" 23°09'19"	222	09.06.1995 4:25 p.m.	1 ad.	FM72D
Kresna Gorge (S) [BN, MS]	41°45'46" 23°09'20"	198	09.06.1995 6:30 p.m.	1 ad.	FM72D

Appendix 3. New data of Dolichophis caspius individuals in south-western Bulgaria

Kresna Gorge (N) [BN, MS]	41°48'26" 23°09'51"	252	09.06.1995 11:30 a.m.	1 ad.	FM73C
Kresna Gorge (S) [BN, MS]	41°45'55" 23°09'08"	216	10.06.1995 2:00 p.m.	1 ad.	FM72D
Kresna Gorge (S) [BN, MS]	41°45'36" 23°09'20"	220	27.04.1996 10:30 a.m.	1 ad.	FM72D
Kresna Gorge (N) [BN, MS]	41°47'37" 23°09'40"	265	29.04.1996 1:15 p.m.	1 ad.	FM72D
Kresna Gorge (N) [BN, MN, SM]	41°47'30" 23°09'45"	241	30.04.1997 3:30 p.m.	1 ad.	FM72D
Kresna Gorge (S) [BN, MN, SM]	41°45'16" 23°09'03"	195	01.05.1997 11:30 a.m.	1 juv.	FM72C
Kresna Gorge (N) [BN, MN, SM]	41°47'35" 23°09'45"	274	02.05.1997 3:20 p.m.	1 ad.	FM72D
W/SW of Baldevo [AP]	41°37'36" 23°45'39"	547	17.05.1997 2:30 p.m.	1 ad.	GM21C
SE of Borovo [AP]	41°35'28" 23°44'31"	527	17.05.1997	1 ad. RK*	GM20D
W of Sandanski [AP]	41°33'56" 23°14'30"	118	30.07.1997 8:25 a.m.	1 ad. RK	FM80C
S of Dolna Gradeshnitsa [AP]	41°39'26" 23°10'52"	150	30.07.1997 2:40 p.m.	1 ad. RK	FM81A
E of Stob [AP]	42°05'38" 23°07'06"	577	24.08.1997 4:35 p.m.	1 ad.	FM76C
Kresna Gorge (N) [BN]	41°46'52" 23°09'17"	235	07.05.1998 12:50 p.m.	1 ad.	FM72D
Kresna Gorge (S) [BN]	41°46'15" 23°09'23"	225	09.05.1998 3:20 p.m.	1 subad.	FM72D
N of Novo Lyaski [AP]	41°32'53" 23°46'23"	490	15.08.1998 10:45 p.m.	1 ad. RK	GM30A
Kresna Gorge (N) [BN]	41°47'22" 23°09'15"	250	08.05.1999 12:30 p.m.	1 ad.	FM72D
Kresna Gorge (S) [BN, MN, SM]	41°45'52" 23°09'13"	205	01.05.2000 n/a	1 ad.	FM72D
SW outskirts of Gotse Delchev [DC]	41°33'49" 23°43'06"	637	07.07.2001 n/a	1 ad.	GM20C
Kresna Gorge (S) [BN, MN, MS]	41°46'06" 23°09'07"	227	23.04.2002 10:45 a.m.	2 ad.	FM72D
Kresna Gorge (S) [BN, MN]	41°45'58" 23°09'10"	211	10.05.2002 n/a	1 ad.	FM72D
Kresna Gorge (N) [BN, MN, BS]	41°47'18" 23°09'12"	236	24.05.2002 n/a	1 ad. RK	FM72D
NE of Simitli [LD]	41°53'48" 23°07'58"	410	02.06.2002 10:40 a.m.	1 ad.	FM74C
E of Kalimantsi [ML, SL]	41°27'36" 23°29'27"	462	22.06.2002 n/a	1 ad.	GL09C
Domozetska neighb., Debochitsa [LD]	41°51'19" 22°57'21"	1095	18.07.2002 11:15 a.m.	1 subad.	FM63B
Gotse Delchev, the industrial area	41°35'03" 23°44'37"	531	26.04.2003 n/a	1 subad.	GM20D
[AP]				RK	
Kresna Gorge (S) [BN, MN]	41°46'10" 23°09'46"	306	03.05.2003 n/a	1 juv. RK	FM72D
Kresna Gorge (N) [AP, RI]	41°50'05" 23°09'16"	276	05.05.2003 11:10 a.m.	1 ad. RK	FM73C
Kresna Gorge (N) [AP, RI]	41°49'31" 23°09'09"	284	05.05.2003 11:45 a.m.	1 ad. RK,	FM73C
				1 subad.	
				RK	
Kresna Gorge (N) [AP, RI]	41°50'05" 23°09'16"	276	26.05.2003 10:05 a.m.	1 ad. RK	FM73C
Kresna Gorge (N) [AP, RI]	41°48'52" 23°09'28"	264	26.05.2003 11:35 a.m.	1 ad. RK	FM73C
Kresna Gorge (S) [AP]	41°44'56" 23°09'31"	207	27.05.2003 12:15 p.m.	1 subad.	FM72C
Kresna Gorge (N) [AP, RI]	41°47'30" 23°09'42"	236	02.06.2003 3:10 p.m.	1 ad.	FM72D
Kresna Gorge (S) [ML]	41°45'49" 23°10'13"	452	03.06.2003 n/a	1 ad. RK	FM82B
S/SE of Strumsko residential area	41°58'28" 23°05'38"	329	07.09.2003 4:00 p.m.	1 ad. RK	FM74B
[LD]					
Kresna Gorge (N) [AP]	41°47'26" 23°09'28"	266	24.09.2003 4:00 p.m.	1 ad.	FM72D
Kresna Gorge (S) [AP, RI]	41°44'33" 23°09'39"	194	29.09.2003 3:45 p.m.	1 ad. RK	FM72C
N of Kresna [AP, RI]	41°44'03" 23°09'33"	188	29.09.2003 4:30 p.m.	1 juv. RK	FM72C
Kresna Gorge (N) [AP]	41°47'30" 23°09'42"	236	22.10.2003 4:25 p.m.	1 juv.	FM72D
SE of Brestovo [LD]	41°48'56" 23°01'04"	984	05.07.2004 12:10 p.m.	1 ad.	FM63C
N/NE of Simitli [LD]	41°54'06" 23°07'17"	294	28.06.2005 10:55 a.m.	1 ad.	FM74C
Gotse Delchev [AP, RI]	41°34'23" 23°44'02"	544	07.07.2005 9:40 a.m.	1 ad. RK	GM20D

E outskirts of Simitli [LD]	41°53'27" 23°07'39"	335	01.09.2005 4:50 p.m.	1 ad.	FM73D
S of Gospodintsi [ML]	41°38'18" 23°44'17"	554	06.06.2006 n/a	1 ad. RK	GM21C
Kresna Gorge (N) [LD]	41°47'31" 23°09'37"	260	03.07.2006 4:00 p.m.	1 subad.	FM72D
SW outskirts of Katuntsi [BN]	41°26'25" 23°25'13"	176	04.07.2006 n/a	1 subad.	GL09A
				RK	
Kresna Gorge (S) [LD]	41°45'59" 23°10'25"	530	27.07.2006 n/a	1 ad. RK	FM82B
N outskirts of Novo Lyaski [DD]	41°32'13" 23°46'47"	518	05.10.2006 1:55 p.m.	1 ad.	GM30A
Rupite Area [LD]	41°27'31" 23°15'47"	88	24.09.2007 3:00 p.m.	1 juv.	FL89C
N of Mesta [DD]	41°46'28" 23°40'33"	672	13.05.2008 1:25 p.m.	1 ad.	GM22B
E of Domozetska neighb., Debochitsa	41°51'21" 22°57'46"	1036	06.07.2008 11:30 a.m.	1 ad.	FM63B
[LD]					
Kresna Gorge (S) [LD]	41°45'53" 23°10'04"	384	09.08.2008	1 subad.	FM82B
				RK*	
E outskirts of Novo Lyaski [ML]	41°31'53" 23°47'02"	520	12.05.2009 n/a	1 ad.	GM30A
E/NE of Paril [ML]	41°26'27" 23°42'17"	952	06.07.2009 n/a	1 ad.	GL29C
N of Simitli [LD]	41°54'32" 23°07'09"	324	23.09.2009 11:35 a.m.	1 ad.	FM74C
W/SW of Rakitna [AP, GM, ES]	41°50'44" 23°09'50"	719	10.10.2009 11:30 a.m.	1 juv.	FM73C
Rupite Area [LD]	41°27'39" 23°15'47"	90	29.08.2010 11:45 a.m.	1 juv. RK	FL89C
Kozhuh volcanic ridge (N) [LD]	41°27'42" 23°15'28"	220	09.09.2010 6:50 p.m.	1 juv.	FL89C
Rupite Area [LD]	41°27'43" 23°15'42"	96	25.04.2011 5:45 p.m.	1 dead	
				subad.	FL89C
Rupite Area [LD]	41°27'05" 23°16'02"	94	23.05.2011 8:05 a.m.	1 subad.	FL89C
Rupite Area [LD]	41°27'16" 23°15'58"	88	07.06.2011 8:20 a.m.	1 ad.	FL89C
W of Ognyanovo [LS]	41°36'40" 23°46'12"	531	29.04.2012 11:05 a.m.	1 ad.	GM31A
Gotse Delchev [AP]	41°34'15" 23°43'30"	545	03.05.2012 1:35 p.m.	1 ad.	GM20D
N of Kresna [KD]	41°43'52" 23°09'32"	201	01.06.2012 n/a	1 ad. RK	FM72C
SW of Bukovo [LD]	41°42'27" 23°42'30"	639	05.06.2012 2:10 p.m.	1 ad.	GM22C
Dolistovo [AP]	42°18'16" 23°00'33"	521	13.06.2012 2:05 p.m.	1 ad.	FM68D
Dupnitsa [AP]	42°14'56" 23°05'36"	468	10.07.2012	1 ad. RK*	FM77B
Kresna Gorge (S) [LD]	41°45'40" 23°09'21"	216	15.07.2012 12:45 p.m.	1 subad.	FM72D
SE of Petrovo [LD]	41°25'10" 23°32'36"	641	20.07.2012 11:55 a.m.	1 juv.	GL18B
W of Zheleznitsa [LD]	41°55'37" 23°04'57"	420	22.08.2012 12:15 p.m.	1 ad.	FM74A
Kresna Gorge (S) [LD]	41°46'15" 23°09'23"	225	27.04.2013 3:05 p.m.	1 ad.	FM72D
NW of Piperkov Chiflik [GM]	42°16'52" 22°43'27"	507	28.04.2013 12:30 p.m.	1 ad. RK	FM48A
N of Marino Pole [LD]	41°25'20" 23°20'54"	175	02.05.2013 11:35 a.m.	1 ad.	FL98D
N of Nevestino [GM]	42°15'33" 22°51'04"	448	05.05.2013 6:25 p.m.	1 ad. RK	FM58A
Kresna Gorge (S) [NK]	41°45'57" 23°09'13"	205	26.05.2013 n/a	1 ad. RK	FM72D
N of Simitli [GM]	41°53'58" 23°06'56"	302	02.06.2013 10:20 a.m.	1 ad. RK	FM74C
NE of Blagoevgrad [GM]	42°02'06" 23°08'04"	469	19.06.2013 6:35 p.m.	1 ad. RK	FM75D
NE of Blagoevgrad [GM]	42°02'14" 23°08'28"	495	21.06.2013 3:40 p.m.	1 ad. RK	FM75D
Rupite Area [LD]	41°27'25" 23°15'48"	87	25.06.2013 11:15 a.m.	1 subad.	FL89C
"	"	"	26.06.2013 6:15 p.m.	1 ad.	FL89C
"	41°27'03" 23°16'10"	88	18.08.2013 9:40 a.m.	1 ad.	FL89C
N of Golyam Varbovnik [GM]	42°16'24" 22°58'27"	556	26.08.2013 7:25 p.m.	1 ad. RK	FM68A
NE outskirts of Boboshevo [GM]	42°09'13" 23°00'52"	377	03.09.2013 12:05 <i>p.m</i> .	1 ad. RK	FM66D

SE of Dolna Koznitsa [GM]	42°15'49" 22°55'10"	488	14.09.2013 8:30 a.m.	1 ad. RK	FM58C
N of Nevestino [GM]	42°15'27" 22°50'59"	444	29.09.2013 2:40 p.m.	1 subad.	FM58A
				RK	
E of Novi Chiflik [OK]	42°16'03" 22°48'49"	453	29.09.2013 1:10 p.m.	1 ad.	FM48C
NW outskirts of Banichan [AP, GM]	41°37'17" 23°44'05"	545	06.10.2013 3:35 p.m.	1 juv.	GM21C
SE of Kopilovtsi [GM]	42°19'22" 22°45'13"	467	12.10.2013 1:30 p.m.	1 subad.	FM48B
				RK	
W of Konyavo [GM]	42°19'20" 22°45'52"	482	12.10.2013 1:45 p.m.	1 subad.	FM48D
· · ·			,	RK	
W/SW of Kocherinovo [AP]	42°04'51" 23°02'26"	373	07.04.2014	1 ad. RK*	FM66C
Boboshevo [GM]	42°09'14" 23°00'00"	379	09.04.2014 6:20 p.m.	1 ad. RK	FM66D
SW of Lilyanovo [LD]	41°36'36" 23°18'33"	455	07.05.2014 11:20 a.m.	1 juv.	FM90B
NE of Lilyanovo [LD]	41°37'20" 23°19'41"	547	07.05.2014 12:40 p.m.	1 subad.	FM91A
Blagoevgrad [AP]	42°01'10" 23°06'02"	391	10.05.2014 4:10 p.m.	1 dead juv.	FM75A
NE of Blagoevgrad [GM]	42°01'50" 23°07'09"	444	26.05.2014 8:15 a.m.	1 ad. RK	FM75D
S outskirts of Simitli [AP]	41°53'02" 23°07'05"	284	05.06.2014 7:10 p.m.	1 ad. RK	FM73D
N outskirts of Kolarovo [AP]	41°21'58" 23°06'46"	371	09.06.2014 4:25 p.m.	1 ad. RK	FL78C
SW of Ilindentsi [RI]	41°38'40" 23°13'10"	243	, 11.06.2014 7:55 a.m.	1 ad.	FM81A
NW of Golyam Varbovnik [GM]	42°16'05" 22°57'09"	665	22.06.2014 3:25 p.m.	1 ad. RK	FM68A
N/NE of Dolno Tserovo neighb.,	41°57'05" 23°06'14"	319	22.06.2014 12:35 p.m.	1 juv. RK	FM74B
Tserovo [AP, GM]			1	,	
NW of Predel Pass [LD]	41°54'01" 23°18'59"	989	25.06.2014 9:30 a.m.	1 ad. RK	FM94A
SW of Kocherinovo [GM]	42°04'42" 23°02'19"	379	27.06.2014 10:00 a.m.	1 ad. RK	FM66C
S/SE of Strumsko residential area	41°58'30" 23°05'36"	341	27.06.2014 8:15 p.m.	1 ad. RK	FM74B
[GM]			·····		
E of Nevestino [GM]	42°15'24" 22°52'45"	449	28.06.2014 11:00 a.m.	1 ad. RK	FM57D
S/SE of Strumsko residential area	41°58'48" 23°05'23"	331	12.07.2014 7:50 p.m.	1 ad. RK	FM74B
[GM]			·····		
Kresna gorge (S) [AP]	41°45'08" 23°09'09"	198	28.07.2014 7:25 p.m.	1 ad. RK	FM72C
SE of Dobrovo [GM]	42°10'03" 22°59'42"	394	16.08.2014 9:10 <i>a.m.</i>	1 ad. RK	FM67A
SW of Gradevo [GM]	41°54'29" 23°10'40"	429	19.09.2014 10:45 <i>a.m.</i>	1 ad. RK	FM84A
N/NW of Kolarovo [GG]	41°22'20" 23°06'13"	315	20.09.2014 n/a	1 ad.	FL78C
<i>"</i>	" "	"	21.092014 n/a	1 ad	FL78C
E of Novi Chiflik [OK]	42°16'03" 22°48'49"	453	08 11 2014 1.25 n m	1 subad	FM48C
E/NE of Boboshevo [GM]	42°09'20" 23°01'26"	384	13.04.201512.20nm	1 subad	FM66D
	12 07 20 20 01 20	001	10.01.2010 12.20 p.m.	RK	111000
SF outskirts of Levski neighb	42°03'48" 23°02'20"	357	24 04 2015	1 ad RK*	FM65D
Kocherinovo [AP]	12 00 10 20 02 20	001	21.01.2010	i uu. iuv	1111002
N/NW of Pastub [OK]	42°12'57" 22°54'01"	445	27 04 2015 5·40 n m	1 ad RK	FM57D
N of Simitli [GM]	41°54'09" 23°06'49"	312	$27.04.2015 \ 3.40 \ p.m.$	1 ad RK	FM74C
NE of Dobrovo [OK]	41 04 07 20 00 47	307	04.05.2015 12:20 p.m.	1 ad	FM67A
SE of Skring [I D]	42°10'00' 22' 59'00'	577	04.05.2015.11.25 a.m	$1 i_{1137} \mathbb{R}^{1}$	FM67A
F/NE of Novi Chiflik [OK]	$42^{0}16^{1}11^{1}0^{0}18^{1}47^{1}$	<u>1</u> 17	14.05.2015.11.25.0.011	1 ad RV	FM/8C
Blagoevarad [A D]	12 10 11 22 40 47	11/ 27/	14.05.2015.10.25 <i>a.m.</i>	1 iu. IXX	EM75 A
E/CE of Kromon [AD]	42 01 00 23 04 44	047	14.00.2010 10.20 <i>u.m.</i>	1 juv.	CM22A
E/ SE OF Kreihen [AF]	41 44 50 25 59 24"	947	24.05.2015 5:25 p.m.	i du.	GIVIZZA

Distribution and Activity of Caspian Whip Snake Dolichophis caspius (Gmelin, 1789) (Reptilia: Colubridae)...

E outskirts of Simitli [LD]	41°53'27" 23°07'39"	335	24.05.2015 1:20 p.m.	1 ad.	FM73D
E of Ribnik [AP]	41°29'24" 23°15'58"	94	28.05.2015 9:45 a.m.	1 ad. RK	FL89D
N outskirts of Nevestino [OK]	42°15'28" 22°51'04"	444	01.06.2015 2:00 p.m.	1 ad. RK	FM58A
NE of Blagoevgrad [AP, GM]	42°02'08" 23°08'22"	488	06.06.2015 9:50 p.m.	1 ad. RK	FM75D
N/NE of Dobrovo [GM]	42°10'40" 22°58'56"	391	07.06.2015 10:30 a.m.	1 ad. RK	FM67A
SE of Dobrovo [GM]	42°09'54" 22°59'43"	388	07.06.2015 10:25 a.m.	1 ad. RK	FM67A
E/NE of Boboshevo [GM]	42°09'18" 23°01'47"	376	07.06.2015 10:15 a.m.	1 ad. RK	FM66D
Novi Chiflik [OK]	42°16'01" 22°48'26"	459	12.06.2015 4:30 p.m.	1 ad. RK	FM48C
S of Mursalevo [AP]	42°05'59" 23°02'24"	363	14.06.2015	1 ad. RK*	FM66C
NE of Blagoevgrad [GM]	42°02'09" 23°08'10"	462	21.06.2015 3:40 p.m.	1 ad. RK	FM75D
NW outskirts of Dolna Grashtitsa	42°18'07" 22°47'26"	489	30.06.2015 10:15 a.m.	1 ad. RK	FM48C
[OK]					
N of Nevestino [OK]	42°15'37" 22°50'52"	453	17.07.2015 1:35 p.m.	1 ad. RK	FM58A
NE of Blagoevgrad [GM]	42°02'28" 23°09'04"	507	19.07.2015 12:30 p.m.	1 ad. RK	FM75D
Levski neighb., Kocherinovo [AP]	42°03'55" 23°02'10"	367	31.07.2015 2:45 p.m.	1 ad.	FM65D
Blagoevgrad [GM]	42°00'41" 23°05'47"	381	30.08.2015 1:10 p.m.	1 juv. RK	FM75A
E of Dyakovo [GM]	42°20'01" 23°06'01"	630	03.10.2015 9:45 a.m.	1 ad. RK	FM78B
W of Marulevo [GM]	41°59'55" 23°08'51"	625	01.11.2015 12:45 p.m.	1 ad.	FM75C
SW of Moshtanets [GM, MI]	41°57'46" 23°04'02"	522	07.04.2016 4:05 p.m.	2 ad.	FM74B
W outskirts of Blagoevgrad [AP]	42°00'53" 23°04'19"	348	18.04.2016 3:50 p.m.	1 ad.	FM75A
NW of Marulevo [GM]	42°00'30" 23°10'01"	779	20.04.2016 3:15 p.m.	1 ad.	FM75C
NE of Levski neighb., Kocherinovo	42°03'59" 23°02'20"	368	23.04.2016 9:40 a.m.	1 subad.	FM65D
[AP]				RK	
E of Levski neighb., Kocherinovo [AP]	42°03'54" 23°02'25"	362	23.04.2016	1 ad. RK*	FM65D
E/SE of Levski neighb., Kocherinovo	42°03'50" 23°02'29"	359	23.04.2016 9:45 a.m.	3 ad. RK, 1	FM65D
[AP]				subad. RK	
SE of Levski neighb., Kocherinovo	42°03'44" 23°02'34"	358	23.04.2016 9:50 a.m.	3 ad. RK, 1	FM65D
[AP]				ad. RK*	
SE of Levski neighb., Kocherinovo	42°03'33" 23°02'45"	384	23.04.2016 10:05 a.m.	1 subad.	FM65D
[AP]				RK, 2 ad.	
				RK*	
SE of Levski neighb., Kocherinovo	42°03'30" 23°02'47"	392	23.04.2016 10:10 a.m.	5 ad. RK	FM65D
[AP]					
N of Tserovo [AP, GM]	41°58'25" 23°07'24"	468	30.04.2016 5:00 p.m.	1 ad.	FM74D
NW of Pelatikovo [GM]	42°10'58" 22°46'15"	774	01.05.2016 12:15 p.m.	1 subad.	FM47C
NW outskirts of Dzherman [AP]	42°13'44" 23°04'52"	448	14.05.2016 1:15 p.m.	1 ad.	FM77B
SW of Barakovo [AP]	42°03'19" 23°02'53"	404	14.05.2016 1:40 p.m.	1 ad. RK	FM65D
W of Selishte [AP, GM]	41°59'47" 22°58'33"	548	23.05.2016 4:40 p.m.	1 ad.	FM65A
Blagoevgrad [AP]	42°00'39" 23°05'31"	363	29.05.2016	1 ad. RK*	FM75A
Blagoevgrad [AP, MI]	41°59'59" 23°05'22"	358	29.05.2016 2:10 p.m.	1 ad. RK	FM75A
S/SW of Mursalevo [AP]	42°06'08" 23°02'05"	357	02.06.2016 5:30 p.m.	1 ad. RK	FM66C
W of Dupnitsa [AP]	42°15'38" 23°04'20"	543	02.06.2016 5:45 p.m.	1 ad. RK	FM78A
Blagoevgrad, the industrial area [AP,	42°00'15" 23°04'55"	348	05.06.2016 1:15 p.m.	1 ad.	FM75A
KS]					
N outskirts of Blagoevgrad [AP]	42°01'47" 23°05'20"	468	05.06.2016 3:15 p.m.	1 juv. RK	FM75B

W of Barakovo [AP]	42°03'39" 23°02'40"	367	07.06.2016 11:00 a.m.	1 ad. RK	FM65D
SE of Dyakovo [AP]	42°19'25" 23°05'45"	611	10.06.2016	1 ad. RK*	FM78B
W of Dupnitsa [AP]	42°15'38" 23°04'20"	543	10.06.2016	1 ad. RK*	FM78A
E outskirts of Blagoevgrad [AP, MI]	42°00'27" 23°06'39"	458	10.06.2016	1 ad. RK*	FM75A
NE of Levski neighb., Kocherinovo	42°04'08" 23°02'18"	382	11.06.2016 10:50 a.m.	1 ad.	FM65D
[LP]					
Selishte [GM, AP]	41°59'46" 22°59'47"	536	11.06.2016 2:30 p.m.	1 ad. RK	FM65C
Simitli [MI]	41°53'26" 23°07'03"	291	12.06.2016 2:45 p.m.	1 ad. RK	FM73D
N of Mursalevo [LP]	42°07'28" 23°02'22"	375	12.06.2016 5:15 p.m.	1 ad. RK	FM66D
W of Selishte [AP, GM, LP]	41°59'48" 22°59'00"	540	12.06.2016 4:30 p.m.	1 ad. RK	FM65A
NE of Blagoevgrad [MI]	42°02'05" 23°07'59"	463	24.06.2016 11:50 a.m.	1 ad. RK	FM75D
N of Simitli [GM]	41°54'27" 23°06'48"	302	03.07.2016 10:20 a.m.	1 ad.	FM74C
E/NE of Boboshevo [GM]	42°09'19" 23°01'24"	382	03.07.2016 4:20 p.m.	1 ad. RK	FM66D
E of Zheleznitsa [AP]	41°55'24" 23°06'37"	310	09.07.2016	1 ad. RK*	FM74A
Pashovtsi neighb., Delvino [MI]	42°00'43" 23°07'54"	647	25.07.2016 n/a	1 dead ad.	FM75C
N/NE of Blagoevgrad [AP]	42°02'19" 23°05'50"	560	09.08.2016 1:20 p.m.	2 ad. RK	FM75B
Ilindentsi [RI]	41°38'48" 23°13'48"	310	11.08.2016 3:00 p.m.	1 ad. RK	FM81C
W outskirts of Blagoevgrad [GM]	42°00'30" 23°04'33"	348	13.10.2016 11:35 a.m.	4 juv.	FM75A
NE of Riltsi [MI]	42°03'08" 23°04'38"	464	03.11.2016 2:50 p.m.	1 subad.	FM75B
SW of Koprivlen [LD]	41°29'46" 23°45'36"	919	26.03.2017 10:40 a.m.	1 subad.	GL39B
NE outskirts of Kulata [LD]	41°23'33" 23°22'07"	133	07.04.2017 11:20 a.m.	1 ad.	FL98D
SE of Gospodintsi [AP]	41°39'13" 23°44'00"	555	09.04.2017 7:15 p.m.	1 ad. RK	GM21C
Blagoevgrad, the industrial area [AP]	42°00'13" 23°05'36"	373	14.05.2017 2:00 p.m.	1 ad. RK	FM75A
SW of Bukovo [AP, LS]	41°42'22" 23°42'19"	598	20.05.2017 2:55 p.m.	1 ad.	GM22C
S/SW of Logodazh [LD]	41°58'53" 22°55'52"	783	02.06.2017 11:45 a.m.	1 ad.	FM54D
NW outskirts of Izgrev [KS]	41°59'37" 23°06'24"	429	10.06.2017 12:05 p.m.	1 ad., 1 ad.	FM75A
			,	RK	
SE outskirts of Sadovo [AP]	41°30'08" 23°49'31"	524	13.06.2017 2:10 p.m.	1 ad. RK	GL39D
NE outskirts of Balgarchevo [MI]	42°01'44" 23°02'13"	355	13.06.2017 7:30 p.m.	1 ad. RK	FM65C
E/NE of Dobrinishte [AP]	41°49'32" 23°35'49"	779	23.06.2017 5:25 p.m.	1 ad. RK	GM13C
N of Blagoevgrad [MI]	42°01'56" 23°05'16"	471	26.06.2017 4:50 p.m.	1 juv. RK	FM75B
N of Blagoevgrad [MI]	42°02'21" 23°05'07"	477	26.06.2017 4:55 p.m.	1 ad. RK	FM75B
S/SE of Zheleznitsa [GM]	41°54'50" 23°06'48"	311	04.07.2017 10:15 p.m.	1 juv. RK	FM74C
W of Tserovo [GM]	41°57'39" 23°06'13"	321	04.07.2017 11:10 p.m.	1 ad. RK	FM74B
S of Mesta [AP]	41°44'42" 23°40'30"	752	08.07.2017 1:15 p.m.	1 ad. RK	GM22A
N/NW of Cherniche [AP, GM]	41°52'08" 23°07'15"	284	21.07.2017 11:40 p.m.	1 subad.	FM73D
			,	RK	
NE of Boboshevo [MI]	42°09'18" 23°01'13"	390	22.08.2017 6:10 p.m.	1 ad.	FM66D
SE of Koprivlen [AP]	41°30'47" 23°48'33"	506	10.09.2017 3:30 p.m.	1 subad.	GL39B
SW of Blagoevgrad [GM, MI]	41°59'26" 23°04'06"	322	20.10.2017 2:10 p.m.	1 ad.	FM75A
NW of Nevestino [GM]	42°15'38" 22°49'56"	449	09.04.2018 11:10 a.m.	1 ad. RK	FM58A
N/NW of Topolnitsa [LD]	41°24'59" 23°18'57"	92	09.04.2018 1:15 p.m.	1 juv.	FL98B
E of Borovo [GM]	41°35'38" 23°44'31"	529	29.04.2018 4:05 p.m.	1 ad.	GM20D
S/SE of Strumsko residential area	41°58'41" 23°05'24"	331	07.05.2018 5:30 p.m.	1 ad. RK	FM74B
[GM]					

Simitli [GM]	41°53'20" 23°06'54"	288	10.05.2018 3:10 p.m.	1 subad. RK	FM73D
Slokoshtitsa [AP, GM]	42°16'00" 22°42'09"	589	17.05.2018 11:15 a.m.	1 subad. RK	FM48A
W outskirts of Blagoevgrad [LP GM]	42°01'00" 23°04'16"	354	18 05 2018 2.40 n m	1 ad RK	FM75A
Selishte [AP]	41°59'54" 23°00'04"	527	23 05 2018 1:10 <i>p.m.</i>	1 subad	FM65C
NE outskirts of Blagoevgrad [GM]	42°01'37" 23°06'34"	399	25.05.2018 11:45 a m	1 ad	FM75A
N outskirts of Duppitsa [I P]	42°16'06" 23°07'13"	529	26.05.2018 6:15 n m	1 ad RK	FM78A
S of Mursalevo [LP]	42°06'28" 23°02'18"	378	26.05.2018 6:35 <i>p.m.</i>	1 ad RK	FM66C
S of Ribnik [AP]	41°28'30" 23°15'19"	103	27.05.2018 5:30 p.m.	1 ad RK	FI 89C
F of Kruppik [AP]	41°50'53" 23°08'28"	274	27.05.2018 6:45 <i>n m</i>	1 ad RK	FM73D
E/SE of Simitli [GM]	41°52'57" 23°08'47"	335	27.05.2018 6:25 n m	1 ad	FM73D
Kardzhali neighb Gradevo [AP]	41°55'38" 23°12'20"	505	29.05.2018 7:05 <i>a m</i>	1 ad RK	FM84A
SE of Novo I vaski [AP]	41°31'46" 23°47'07"	524	29.05.2018 4:40 n m	1 ad. RK	GM30A
Strumsko residential area [AP]	41°59'46" 23°05'35"	365	27.05.2010 4.40 p.m. 31 05 2018 3:40 p.m.	1 ad	EM75A
E of Zlatarevo [MN]	41°23'51" 22°59'47"	198	02 06 2018 5:35 <i>n m</i>	1 ad RK	FI 68C
N/NE of Levski neighb Kocherinovo	42°04'18" 23°02'20"	401	02.06.2018 5:05 p.m.	1 ad	FM65D
[LP]	12 01 10 25 02 20	101	02.00.2010 0.00 p.m.	i uu.	1111000
NE outskirts of Gospodintsi [AP]	41°39'32" 23°43'57"	560	24 06 2018 5:15 n m	1 ad RK	GM21D
W outskirts of Blagoevgrad [AP, GM]	42°00'30" 23°04'31"	347	08.07.2018 1:25 <i>n.m.</i>	1 ad. RK	EM75A
N of Novo Lvaski [GM]	41°32'24" 23°46'38"	508	22.07.2018 10:15 a.m.	1 ad. RK	GM30A
W outskirts of Koprivlen [AP]	41°31'17" 23°47'24"	531	27.08.2018 2:35 <i>n.m.</i>	1 ad. RK	GM30A
SE of Mesta [AP]	41°45'12" 23°40'46"	656	29.08.2018 3:35 <i>p.m.</i>	1 ad. RK	GM22B
NW outskirts of Blagoevgrad [KS]	42°01'23" 23°04'50"	414	02.09.2018 7:25 <i>p.m.</i>	1 iuv. RK	FM75A
Barakovo [LP]	42°03'51" 23°03'37"	383	03.09.2018 2:15 <i>p.m.</i>	1 juv.	FM75B
N of Blagoevgrad [MI]	42°02'03" 23°05'47"	516	06.09.2018 7:50 p.m.	1 juv. RK	FM75B
Sadovo [AP]	41°30'11" 23°49'27"	518	05.10.2018 11:05 a.m.	, 1 ad. RK	GL39D
Blagoevgrad, the industrial area [AP]	42°00'19" 23°04'56"	349	21.10.2018 3:05 p.m.	1 ad. RK	FM75A
N of Simitli [AP]	41°54'45" 23°06'59"	302	29.10.2018 2:40 p.m.	1 subad.	FM74C
			,	RK	
Rupite Area [LD]	41°26'56" 23°16'00"	90	30.03.2019 12:50 p.m.	1 ad.	FL89C
NE outskirts of Rupite [LD]	41°26'45" 23°14'46"	100	31.03.2019 11:35 a.m.	1 subad.	FL89C
E of Stara Kresna [HP]	41°47'25" 23°12'17"	606	26.04.2019 10:30 a.m.	1 ad., 1	FM82B
				subad.	
S/SE of Strumsko residential area [AP]	41°58'30" 23°05'38"	350	05.05.2019 2:20 p.m.	1 ad.	FM74B
Logodazh [AP]	41°59'37" 22°56'15"	693	11.05.2019 5:25 p.m.	1 juv.	FM65A
NE of Drakata [LD]	41°37'02" 23°13'09"	130	24.05.2019 10:15 a.m.	1 ad.	FM80B
S/SE of Zheleznitsa [AP]	41°54'55" 23°06'49"	310	26.05.2019 3:35 p.m.	1 ad. RK	FM74C
S/SE of Strumsko residential area [AP]	41°58'24" 23°05'35"	326	02.06.2019 4:40 p.m.	1 ad. RK	FM74B
S/SW of Parvomay [GG]	41°23'32" 23°07'41"	171	08.06.2019 9:25 a.m.	1 ad.	FL78C
E of Musomishta [AP]	41°33'10" 23°46'15"	493	08.06.2019 11:20 a.m.	1 ad. RK	GM30A
NE outskirts of Pokrovnik [AP]	41°59'07" 23°03'29"	330	12.06.2019 4:50 p.m.	1 subad.	FM75A
				RK	

Distribution and Activity of Caspian Whip Snake Dolichophis caspius (Gmelin, 1789) (Reptilia: Colubridae)...

E/SE of Ilinden [AP]	41°27'23" 23°49'28"	630	15.06.2019 3:05 p.m.	1 ad. RK	GL39C
E/SE of Musomishta [AP]	41°32'50" 23°46'24"	492	15.06.2019 3:25 p.m.	1 ad. RK	GM30A
Izgrev [KS, AP]	41°59'18" 23°06'37"	385	19.06.2019 10:30 a.m.	1 ad. RK	FM75A
N of Mesta [AP]	41°46'24" 23°40'30"	674	27.06.2019 5:50 p.m.	1 ad.	GM22B
N of Pokrovnik [AP]	41°59'34" 23°03'14"	321	30.06.2019 3:30 p.m.	1 ad. RK	FM75A
N outskirts of Blagoevgrad [HP]	42°01'49" 23°05'19"	467	01.07.2019 1:30 p.m.	1 ad. RK	FM75B
E/SE of Musomishta [AP]	41°32'53" 23°46'23"	490	01.07.2019 3:35 p.m.	1 subad.	GM30A
				RK	
Gradevo [AP, KD]	41°55'30" 23°11'59"	496	06.07.2019 3:40 p.m.	1 ad. RK	FM84A
W outskirts of Selishte [AP]	41°59'48" 22°59'31"	536	07.07.2019 6:05 p.m.	1 ad.	FM65A
E/NE of Eredeltsi neighb., Selishte	42°00'15" 23°01'10"	419	07.07.2019 6:40 p.m.	1 subad.	FM65C
[AP]					
N/NW of Tserovo [AP]	41°58'13" 23°07'03"	513	09.07.2019 3:40 p.m.	1 ad. RK	FM74D
N outskirts of Blagoevgrad [MI]	42°01'37" 23°05'25"	460	13.07.2019 5:50 p.m.	1 ad. RK	FM75A
N of Pokrovnik [AP]	42°00'02" 23°03'26"	323	15.07.2019 2:35 p.m.	1 ad. RK	FM75A
W of Logodazh [AP]	41°59'36" 22°55'43"	672	18.07.2019 5:55 p.m.	1 ad. RK	FM55C
SW of Barakovo [GG, AP]	42°03'00" 23°02'59"	411	19.07.2019 11:00 a.m.	1 ad. RK	FM65D
W outskirts of Blagoevgrad [AP]	42°00'51" 23°04'15"	345	19.07.2019 11:10 a.m.	1 ad. RK	FM75A
Ushite neighb., Padesh [AP]	41°56'54" 23°01'21"	602	20.07.2019 3:30 p.m.	1 subad.	FM64D
				RK	
S of Simitli [AP]	41°52'31" 23°07'17"	301	31.07.2019 10:05 a.m.	1 ad. RK	FM73D
W of Strumsko residential area [AP]	41°59'27" 23°04'22"	324	08.08.2019 1:40 p.m.	1 ad. RK	FM75A
E outskirts of Kamenik [HP]	42°12'59" 23°01'19"	545	10.08.2019 12:05 p.m.	1 ad.	FM67D
سيماء وسوجينا ليروح والرجين الروالالار والمردار تروتك ويرا	10 harris mulanta the	maniat	un time		

*individuals killed on the road more than 48 hours prior to the registration

Locality	Geographic coordinates (N/E)	Altit ude (m)	Date of observation	Shed skins observed	UTM 5×5 km
S outskirts of Banichan [BP]	41°36'55" 23°44'21"	531	01.11.1994	1 shed skin (ad.)	GM21C
SW vicinity of Dolna Gradeshnitsa	41°40'36" 23°11'05"	145	30.07.1997	1 shed skin (ad.)	FM81B
[AP]					
SE of Levski neighbourhood,	42°03'45" 23°02'20"	357	24.08.1997	1 shed skin (ad.)	FM65D
Kocherinovo [AP]					
"	"	"	03.07.1998	3 shed skins (ad.)	FM65D
SW of Chetirtsi [BN]	42°13'49" 22°51'50"	497	03.07.2008	1 shed skin (ad.)	FM57B
Rupite Area [LD]	41°27'22" 23°15'39"	112	21.08.2010	1 shed skin (ad.)	FL89C
NE of Riltsi [AP, GM]	42°02'49" 23°05'06"	440	19.05.2013	1 shed skin (ad.)	FM75B
NW outskirts of Banichan [AP, GM]	41°37'17" 23°44'05"	545	06.10.2013	2 shed skins (ad.)	GM21C
E of Starchevo [GM]	41°28'15" 23°15'26"	132	27.10.2013	2 shed skins (ad.)	FL89C
N/NE of Leshko [GM, MI]	41°56'19" 22°58'44"	639	20.10.2017	1 shed skin (juv.)	FM64A
W of Delvino [AP]	42°01'23" 23°07'19"	631	25.07.2019	1 shed skin (ad.)	FM75C
SE of Kresna [AP]	41°42'30" 23°10'52"	181	08.08.2019	2 shed skin (ad.)	FM81B

Appendix 4. New data of *Dolichophis caspius* shed skins in south-western Bulgaria

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Distribution of European Cat Snake Telescopus fallax (Fleischmann, 1831) (Reptilia: Colubridae) in South-Western Bulgaria

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Abstract. The European Cat Snake is one of the most recently discovered representatives of the Bulgarian herpetofauna, and one of the rarest snakes in Bulgaria. The research presented aims to update the data about the distribution of Telescopus fallax in south-western Bulgaria. Many new records from the Struma River valley are reported. To the north the species reaches the city of Blagoevgrad and its surroundings. Some data on the biology and ecology of the snake in this part of the country are also reported.

Key words: colubrid snakes, new localities, UTM grid, highest altitude, biogeography, nocturnal activity, Balkan Peninsula.

Introduction

The geographical range of *Telescopus* fallax (Fleischmann, 1831) includes southeastern Europe, Middle East and southwestern Asia: extreme NE Italy, Slovenia, most recently discovered representatives of Croatia (including many islands), extreme S Bosnia-Herzegovina, S Montenegro, W Albania, Macedonia (now North Macedonia), Bulgaria, Greece (including many SW islands), Cyprus, Malta, Turkey, N Syria, Lebanon, N Israel, NE Egypt, SW Russia (Dagestan), S Georgia, S Armenia, Azerbaijan, Turkmenistan, Iraq and Iran al., 2011; BALEJ & JABLONSKI, 2006-2019) up to (WALLACH *et al.*, 2014). The distribution in Europe is mainly in the Balkan BESHKOV & NANEV, 2002).

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Peninsula. The species is polytypic, and in Bulgaria the nominate form (T. fallax fallax) occurs.

The European Cat Snake is one of the the Bulgarian herpetofauna and one of the rarest snakes in the country. The first record was in 1958 (BESHKOV, 1959; 1961). The known distribution of the species includes the southern part of the country: the valleys of the Struma and Arda Rivers (BESHKOV & NANEV, 2002; PETROV et al., 2002; STOJANOV et species about 700 m a.s.l. (BESHKOV, 1959; 1985;

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Data about the occurrence of *T. fallax* in south-western Bulgaria are reported by BESHKOV (1959; 1961; 1974; 1981; 1985), BESHKOV & BERON (1964), KANTARDZHIEV (1992), BISERKOV (1995), PETROV & BESHKOV (2001), BESHKOV & NANEV (2002), STOJANOV et al. (2011), CAS (2010-2019), BALEJ & JABLONSKI (2006-2019), and DYUGMEDZHIEV et al. (2019). According to BESHKOV (1981), PETROV & BESHKOV (2001), and BESHKOV & NANEV (2002) the northern boundary of the species range in the Struma River valley spreads to Kresna Inn (in the middle of the south-eastern, and D - the north-eastern). Kresna Gorge).

registered in the period 1958-1981 (BESHKOV, 1981), and till 1985 the number of known records was 12 (BESHKOV, 1985). The species was included in the Red Data Book of Bulgaria - in its first edition (1985) in category Rare species, and in the second one (2015) in category Vulnerable species. The species was included by NANKINOV (2000) in the list of the threatened animals of Bulgaria. According to BESHKOV (2015) 437 individuals belonging to 11 snake species were collected around the village of Gorna Breznitsa and Kresna Gorge in 1971-1975, and T. fallax was presented by 2 specimens only (0.45%); in the 1980s, approximately 15 individuals were registered; and in 2000-2015, several individuals were recorded each year.

about the distribution of T. fallax in southwestern Bulgaria.

Material and Methods

The data about the distribution of *T*. fallax in south-western Bulgaria were gathered from 1988 to 2019. The species has been registered during herpetological field reported for the first time (see Appendix 2). surveys in various habitats and in different seasons. The collection of the Regional Historical Museum in the city of Blagoevgrad (RHMB) was also inspected. All published and new data (alive individuals, dead specimens, shed skins) have been used to specify the distribution of the species in south-western Bulgaria.

The separate locations of the species (both new and published) were grouped according to their affiliations to the squares of the Universal Transverse Mercator (UTM) grid with a resolution of 5×5 km. The gridcells were indicated by the codes of the 10km quadrates of Military Grid Reference System (MGRS; spatially identical with UTM) and capital letters (A-D) were used to denote the separate 5×5 km squares within every 10×10 km square (A indicates the southwestern square, B - the north-western, C -Mapping and map visualization were done Only 8 specimens of *T. fallax* had been in the projection coordinate system "WGS 84 UTM 35N" by means of ArcGIS v. 10.1 (ESRI, Redlands, CA, USA).

> All known data as locality, geographic coordinates, altitude, date and time, number, age and condition of the individuals are included in tables.

> Most of the new records have been made by the authors of the paper: L. Domozetski [LD], A. Pulev [AP], G. Manolev [GM], B. Naumov [BN]. Some of the data have been collected separately or in collaboration with the authors by other colleagues (see the Acknowledgements). The collectors have been noted with their initials in Appendix 2.

Results and Discussion

The total number of records of *Telescopus* The research aims to update the data *fallax* from south-western Bulgaria until now is 78: 19 of them (more than 23 individuals) were published (see Appendix 1) and 59 (55 field observations and 4 museum specimens) are reported now (63 individuals altogether, presented in Appendix 2). A record of 2 pieces of shed skins from one locality (NW outskirts of General Todorov Village) is also

> Among the 55 individuals registered during the field trips, 18 have been roadkilled (all of them "fresh"), and for 2 other specimens the cause of death was unknown. The proportion of the newly registered adults is 46% (n=29), followed by the proportion of juveniles (n=23 or 36%) and subadults (n=11 or 18%).

distribution of T. fallax the lower reaches of some of its tributaries. All new ones fall in other 11 grid squares (Fig. 1).

The published and new data on the known localities of *T. fallax* in the study area fall in south-western into 21 squares of the 5 km UTM grid. The Bulgaria show that the species occur only in part published localities refer to 10 grid squares (for 5 of the Struma River valley and in the valleys in of them new data are presented as well), and the



Fig. 1. Distribution of Telescopus fallax in south-western Bulgaria, based on a 5 km UTM grid.

The new distributional data from the Struma River valley show that the European Cat Snake occur about 25 km north of the middle of Kresna Gorge, which previously was considered to be the northern boundary of its range (BESHKOV, 1981; 1985; PETROV & BESHKOV, 2001;

and BESHKOV & NANEV, 2002). For the first time localities of T. fallax are reported from Oranovo Gorge and its surroundings (17 records). The species spreads northwards to the city of Blagoevgrad (two records) and to the village of Delvino (one record).

Distribution of European Cat Snake Telescopus fallax (Fleischmann, 1831) (Reptilia: Colubridae)...

T. fallax fallax is a good *indicator subspecies* as its range delineates very well the boundaries of the Mediterranean area in the Struma River valley. The species can play a significant role in a future zoogeographical subdivision of Bulgaria as other snake species that are also good indicators – *Xerotyphlops vermicularis* (Merrem, 1820) (see PULEV *et al.*, 2018a) and *Malpolon insignitus* (Geoffroy Saint-Hilaire, 1827) (see PULEV *et al.*, 2018b). PULEV *et al.* (2018a) proposed two Mediterranean areas in south-western Bulgaria (Struma and Mesta) which suggests that *T. fallax* also inhabits the southern part of the Mesta River valley located in the country (Hadzhidimovo Gorge).

Probably, the maximum altitude (700 m) reported after the first species registration (BESHKOV, 1959) was wrong. The location described by BESHKOV (1959) is approximately at about 500 not at 700 m a.s.l. Therefore, the altitude of 656 m recorded from the Pashovtsi neighborhood (the village of Delvino) could be the highest one for the species in Bulgaria so far (see Appendix 2).

The new data show that the species is active from the third decade of March till the second decade of October, which confirms the information published by STOJANOV *et al.* (2011). The earliest spring registration is from March 25, and the latest autumn one is from October 11. The species is most active during the warmest and driest months of the year (July, August). More than the half of the records are from the second decade of July till the third decade of August, with a peak in the first decade of August (see Appendix 2). No winter activity of the species has been recorded, although two such observations were published by STOJANOV *et al.* (2011).

All alive individuals were observed at night (from 9:05 pm to 12:45 am), except one registered during the day at its shelter (under a stone) (see Appendix 2). These data confirm the predominantly nocturnal activity of the species indicated by BESHKOV & NANEV (2002) and STOJANOV *et al.* (2011).

T. fallax was often found in urbanized places in the study area (BESHKOV, 1974;

BISERKOV, 1995; BALEJ & JABLONSKI, 2006-2019). There are some new records from the city of Blagoevgrad, and from many smaller settlements (see Appendix 2). Probably this is greatly facilitated by the species nocturnal activity.

Interesting ecological note is the feeding of *T. fallax* with mainly daily active species of lizards. The observed subadult individual from Kresna Gorge on 03.08.2010 swallowed subadult *Podarcis erhardii* (Bedriaga, 1882), and the observed subadult individual from Rupite Area on 21.08.2010 regurgitated recently eaten juvenile *Lacerta viridis* (Laurenti, 1768). These observations coincide with the indicated by BESHKOV & DUSHKOV (1981) and BESHKOV & NANEV (2002) herpetophagy.

The study confirms the conclusion drawn by BESHKOV (1993) and BESHKOV & NANEV (2002), that the snake is not as rare as previously thought. In the same publications, the authors suggested that the species abundance was increasing along the Struma River valley. In our opinion, the late detection of T. fallax in Bulgaria and the scarce published data can be explained by both the hidden (nocturnal) way of life and the insufficiency of field studies focused specifically on this species. The large number of new observations of the species in its known range, as well as its registration in new places (such as Oranovo Gorge), are probably due not to the actual expansion of the range and increase in numbers, but to the higher intensity of field research in the recent years. In this sense, BESHKOV'S (2015) statement that the "separate populations are completely isolated from one another" does not seem to be true. The spatial distribution of known localities shows rather a continuity of the range of the species, and the lack of data from some UTM grid squares is likely to be filled by future research.

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Distribution of European Cat Snake Telescopus fallax (Fleischmann, 1831) (Reptilia: Colubridae)...

Appendix 1. Published data about *Telescopus fallax* in south-western Bulgaria (data source, locality, geographic coordinates, altitude, date, time, observed individuals) and UTM (when it is possible to be determined) in Fig. 1

BESHKOV (1959): near Vlahi (at 200 m below the village, by the road to the Vlahinska River waterfall), 700 m a.s.l., 21.06.1958, 1 ad. - UTM: FM82C; BESHKOV (1961): same locality, 24.06.1960 - UTM: FM82C; BESHKOV & BERON (1964): Dolno Spanchevo - UTM: FL98D; BESHKOV (1974): Gorna Breznitsa (1971-1972), 200-400 m a.s.l., 2 spec. - UTM: FM72C; BESHKOV (1981): Vlahi - UTM: FM82C, Dolno Spanchevo - UTM: FL98D, Gorna Breznitsa -UTM: FM72C (probably repetition of previously published records; there are no sources cited), Kozhuh volcanic ridge - UTM: FL89C, near Kresna inn - UTM: FM72D; BESHKOV (1985): the gorge of Sheytan Dere (Dyavolska Reka) River, 500 m upstream from its mouth -UTM: FM72D, Kozhuh volcanic ridge - UTM: FL89C (information from an unpublished source was cited for this record); KANTARDZHIEV (1992): northern part of Kozhuh volcanic ridge - UTM: FL89C; BISERKOV (1995): Petrich - UTM: FL88B; PETROV & BESHKOV (2001): Kresna Gorge; BESHKOV & NANEV (2002): Kresna Gorge; STOJANOV et al. (2011): Kresna Gorge, 07.11-10.11.2010, 2 spec., Kozhuh volcanic ridge, 20.12.1980, 13.12.1981, 2 spec. -UTM: FL89C; CAS (2010-2019): Kresna, 21.05.1992, 1 subad. - UTM: FM72C; BALEJ & JABLONSKI (2006-2019): Melnik, 29.08.2013, 1 ad. - UTM: FL99D, Damyanitsa, 150-200 m a.s.l., 28.03.2005, 1 ad., 1 juv., Damyanitsa, 140 m a.s.l., 16.04.2006, 1 subad. - UTM: FL89D, Valkovo, 300 m a.s.l., 15.04.2006, 1 subad. - UTM: FM80D; DYUGMEDZHIEV et al. (2019): the vicinity of the town of Kresna, 41°43'N 23°10'E, 180 m a.s.l., 11.10.2018, 1 juv. - UTM: FM81B.

Appendix 2. New data of *Telescopus fallax* individuals and shed skins in south-western Bulgaria.

	Coorranhia	Altitudo	Data and time of	Individuale	ITM
Locality	Geographic	Annuae			
	coordinates (N/E)	(m)	observation	observed	5x5 km
Dolno Spanchevo, museum number (mn)	n/a	n/a	10.10.1963	1 ad.	FL98D
RHMB 7.3/4.89 (collected by Y. Ganev)					
General Todorov, mn RHMB 7.3/4.92	n/a	n/a	25.03.1986	1 ad.	FL99A
(collected by D. Kantardzhiev)					
General Todorov, mn RHMB 7.3/4.93	n/a	n/a	25.03.1986	1 ad.	FL99A
(collected by D. Kantardzhiev)					
Rupite Area, mn RHMB 7.3/4.94	n/a	n/a	25.07.1986	1 ad.	FL89C
(collected by D. Kantardzhiev)					
Blagoevgrad, the industrial area [LD]	42°00'32" 23°05'30"	360	05.09.2016	1 dead ad.	FM75A
Blagoevgrad, the industrial area [GM]	42°00'00" 23°05'19"	364	16.08.2019 9:40 pm	1 juv.	FM75A
Pashovtsi neighb., Delvino [MI]	42°00'43" 23°07'54"	656	15.08.2015 11:25 pm	1 subad.	FM75C
S/SE of Strumsko residential area [GM]	41°58'35" 23°05'30"	331	02.08.2018 10:50 pm	1 ad.	FM74B
S/SE of Strumsko residential area [AP, GM]	41°58'34" 23°05'32"	339	09.08.2016 10:30 pm	1 ad.	FM74B
NW of Tserovo [GM]	41°58'19" 23°06'06"	347	31.08.2016 11:15 pm	1 subad.	FM74B
NW of Tserovo [GM]	41°58'08" 23°06'05"	426	18.09.2016	1 subad. RK	FM74B
NW of Tserovo [GM]	41°58'18" 23°06'29"	484	06.08.2018 10:55 pm	1 ad.	FM74B
NW of Tserovo [GM]	41°58'19" 23°06'37"	490	25.07.2018 10:20 pm	1 juv.	FM74B
N/NW of Tserovo [GM]	41°58'17" 23°06'49"	500	05.08.2018 12:45 am	1 subad.	FM74D
N/NW of Tserovo [AP, GM]	41°58'13" 23°06'58"	509	10.08.2016 11:10 pm	1 juv.	FM74D

N/NW of Tserovo [GM]	41°58'09" 23°07'13"	548	05.08.2018 12:35 am	1 juv.	FM74D
N of Tserovo [GM]	41°57'56" 23°07'34"	612	15.08.2016 11:05 pm	1 juv.	FM74D
W of Tserovo [AP]	41°57'19" 23°06'16"	317	23.07.2019	1 ad. RK	FM74B
Zheleznitsa [LD]	41°55'37" 23°06'18"	306	15.08.2013	1 ad. RK	FM74A
S/SE of Zheleznitsa [LD]	41°54'58" 23°06'49"	312	28.07.2017 10:35 pm	1 subad.	FM74C
S/SE of Zheleznitsa [LD]	41°54'56" 23°06'48"	316	28.06.2014 10:25 pm	1 juv.	FM74C
S/SE of Zheleznitsa [AP, GM]	41°54'27" 23°06'47"	306	05.08.2013 9:30 pm	1 ad.	FM74C
Oranovo residential area, Simitli [LD]	41°54'09" 23°07'38"	345	12.08.2009 11:05 pm	1 juv.	FM74C
Oranovo residential area, Simitli [LD]	41°53'39" 23°07'31"	306	11.10.2009	1 dead subad.	FM74C
W outskirts of Simitli [LP]	41°53'26" 23°06'12"	331	02.10.2018 9:05 pm	1 juv.	FM73B
Kresna Gorge (North) [AP, RI]	41°49'37" 23°09'06"	279	14.07.2003	1 ad. RK	FM73C
Kresna Gorge [AP, RI]	41°49'31" 23°09'09"	284	07.07.2003	1 ad. RK	FM73C
Kresna Gorge [AP]	41°49'14" 23°09'30"	270	08.08.2019	1 ad. RK	FM73C
Kresna Gorge [BN, MS]	41°48'24" 23°09'23"	483	1988 n/a	1 ad.	FM73C
Kresna Gorge [AP, RI]	41°48'18" 23°09'45"	257	26.05.2003	1 ad. RK	FM73C
Kresna Gorge [AP, RI]	41°47'40" 23°09'32"	251	14.07.2003	1 juv. RK	FM72D
Kresna Gorge [LD]	41°47'05" 23°09'18"	236	18.07.2005 10:45 pm	1 subad.	FM72D
Kresna Gorge [AP, RI]	41°46'39" 23°09'15"	220	02.06.2003	1 ad. RK	FM72D
Kresna Gorge [LD]	41°46'35" 23°09'19"	223	15.05.2011 11:20 pm	1 ad.	FM72D
Kresna Gorge (South) [LD]	41°46'24" 23°09'20"	225	03.08.2010 11:05 pm	1 subad.	FM72D
Kresna Gorge [LD]	41°46'15" 23°09'23"	225	24.08.2012 11:20 pm	1 juv.	FM72D
Kresna Gorge [LD]	41°46'10" 23°09'23"	223	25.06.2016 10:00 pm	1 juv.	FM72D
Kresna Gorge [LD]	41°46'09" 23°09'23"	226	03.08.2010 9:55 pm	1 ad.	FM72D
Kresna Gorge [BN]	41°45'54" 23°09'20"	208	25.08.1991 12:30 am	1 ad.	FM72D
Kresna Gorge [LD]	41°45'25" 23°09'11"	220	06.09.2010 9:55 pm	1 subad.	FM72D
Kresna Gorge [BN, MS]	41°45'12" 23°09'11"	206	09.06.1995 10:30 pm	1 juv.	FM72C
Kresna Gorge [BN, MS]	41°45'06" 23°09'11"	203	08.06.1995 11:30 pm	1 juv.	FM72C
Kresna Gorge [AP, RI]	41°45'02" 23°09'12"	200	28.07.2003	1 ad. RK	FM72C
Kresna Gorge [AP, RI]	41°44'56" 23°09'18"	198	28.07.2003	1 juv. RK	FM72C
Kresna Gorge [BN, MN]	41°44'56" 23°09'28"	207	24.05.2002 11:15 pm	1 juv.	FM72C
Kresna Gorge [AP, RI]	41°44'55" 23°09'31"	201	28.07.2003	1 juv. RK	FM72C
Kresna Gorge [AP, LD]	41°44'51" 23°09'46"	200	14.07.2003	1 juv. RK	FM72C
Kresna Gorge [AP, RI]	41°44'05" 23°09'32"	188	28.07.2003	1 subad. RK	FM72C
Kresna Gorge [AP, RI]	41°44'01" 23°09'33"	186	28.07.2003	1 ad. RK	FM72C
N outskirts of Kresna [AP, RI]	41°43'45" 23°09'12"	186	11.08.2003	1 juv. RK	FM72C
S of Damyanitsa [LD]	41°29'58" 23°15'56"	100	23.04.2011	1 ad., under stone	FL89D
Kozhuh volcanic ridge [LD]	41°27'45" 23°15'41"	100	17.08.2010	1 ad. RK	FL89C
Rupite Area [LD]	41°27'39" 23°15'46"	90	14.07.2013 10:50 pm	1 juv.	FL89C
Rupite Area [LD]	41°27'14" 23°15'59"	88	21.08.2010 12:25 am	1 subad.	FL89C
Rupite Area [LD]	41°27'19" 23°16'04"	89	18.08.2013 11:45 pm	1 ad.	FL89C
Rupite Area [LD]	41°27'23" 23°16'11"	92	18.08.2013 11:30 pm	1 ad.	FL89C
NW outskirts of General Todorov [LD]	41°27'23" 23°16'41"	89	22.08.2010	2 shed skin (ad.)	FL99A
Topolnitsa [AP]	41°24'17" 23°19'26"	91	28.04.2018 9:35 pm	2 ad.	FL98B
Petrovo [AP]	41°26'19" 23°30'09"	442	05.10.2014 n/a	4 juv.	GL09C
SE outskirts of Petrovo [BP]	41°26'13" 23°30'38"	425	12.09.1992	1 ad. RK	GL09C

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Evaluation of the Risk of Pb and Cd Deposition on Bulgarian Forests Using a Critical Load Approach

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Abstract. The current study was designed to calculate the critical loads of lead (Pb) and cadmium (Cd) for forests in different mountainous sites in Bulgaria and to assess a risk of damage caused by these metals for 2017 year. Steady-state mass balance model for input and output metal fluxes from an ecosystem was applied, based on the criterion for ecotoxicological protection. These mass fluxes were calculated based on measured data. The value of critical load for Cd was considerably lower than those for Pb. That means all forest ecosystems were more sensitive to the Cd deposition compared to the Pb one. It was found that the critical load for Pb and Cd for coniferous stands was higher than those for the deciduous one. Exceedances of critical loads of Pb and Cd for four study sites during the 2017 year were calculated and used as a criterion for risk assessment against heavy metal pollution. Exceedances of critical loads for both metals were found, e.g. a real risk of the harmful effect of Pb and Cd for all study sites.

Key words: critical loads, heavy metals, risk assessment, forest ecosystems, lead and cadmium.

Introduction

mercury are extremely toxic in very low concentrations (BRECKLE & KAHILE, 1992; IOBAL & MEHMOOD, 1991; KAHLE & BRECKLE, 1898). In contrast to other metals (Cu, Zn) that are essential at small amount because play role of cofactors of enzymes, Pb, Cd and Hg only cause damage effect. Organisms have not natural biochemical mechanisms for their elimination. That way metals have remained in ecosystems for a long period damaging the most sensitive organisms initially and causing harmful effect of more organisms in the future.

Bv definition critical loads are Some metals such as lead, cadmium and quantitative estimates of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (NILSSON & GRENNFELT, 1988). Critical loads can be used to determine the sensitivity of a given receptor. When the value of the critical load is high, the receptor is more tolerant and less sensitive to the pollutant of concern. In this case the receptor can withstand large amounts of pollutant deposition without any damages to occur. The risk of harmful effect can be assessed using critical load exceedance (AGREN, 1997). That is

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a deposition rate of pollutant, which is more This effect-based approach is very effective because it takes into account all regional characteristics of a specific receptor and that way reveals the present state of the receptor or the whole ecosystem. Assessing the real state of a site of interest, policymakers can plan and predict measures for prevention and emission control.

The aim of this study was to carry out comparative investigations on the sensitivity and risk of damage of heavy metal pollutants on two types of forests (deciduous and coniferous) in four Bulgarian mountains by means of calculation of critical load and their exceedances based only on simultaneous measured data. From this point of view the following tasks were taken into account:

1. Determination of Pb and Cd deposition cadmium rates in the throughfall at the study sites;

2. Collection of measured data needed for the calculation of local critical loads for Pb and Cd for forests in order to assess their sensitivity to heavy metal pollution;

3. Assessing the risk of harmful effects and damages of forests by computing the measured. exceedances of critical loads for Pb and Cd.

Materal and Methods

Sites' location and characteristics

mountains regions of Bulgaria and represent typical coniferous and deciduous forests for the country. Site Petrohan is located at Western Balkan and is a part of an international network for long-range ecosystem research (LTER). Site Plana is a part of the same network and is situated at Plana Mountain not far from the capital city Sofia. Site Beklemeto is located at Central Balkan. Sites Pismenovo is located at Strandja Mountain. Three of the study sites are located in a range between 1245 and 1430 m a.s.l. but the site Pismenovo only is at 65 m a.s.l and situated at Strandja Mountain. All study sites were not under anthropogenic influence. At every study site two plots with one coniferous and one deciduous stand were chosen.

Plots and stands characteristics such as than the acceptable level, e.g. the critical load. altitude, ages, and species are given in Table 1.

Sampling, analysis and database collection

Permanently opened polyethylene plastic collectors were used for collection of throughfall deposition. Each collector had a collection area of 314 cm² and stood approximately 1.5 m above ground level. Three throughfall precipitation collectors were used at each plot. Sample collections were done every month. Water quantity and activity (pH) were measured at each sampling time for each collector at the plots. Mix samples were formed for each plot for deposition and stored at 4 °C. For analyzing dissolved element forms, all samples were filtered through 0.45 µm cellulose filter using membrane filtering system. Lead and measured were by atomic absorbance spectrophotometer.

For an increment estimation a model tree was determined for every sample plot and tree samples were taken. For that purpose heights and diameters of at least 50 trees in every sample plot were

Calculation of an increment, critical loads (CL) of Pb and Cd and their exceedances

effect-based steady-state The mass The study sites are located in different balance model was used to calculate the critical loads of Pb and Cd (POSCH & HETTELINGH, 2004; POSH et al., 2003). The model implies that the critical load equals the net uptake by the forest growth plus an acceptable metal leaching rate, according to the follow equation:

$$CL(M) = Mu + Mle(crit)$$
 (1),

where:

CL(M) = critical load of a heavy metal $(g.ha^{-1}.yr^{-1});$

Mu = Net uptake of metal M in thevegetation under critical load conditions $(g.ha^{-1}.yr^{-1});$

Mle(crit) = Critical leaching of a metal M $(g.ha^{-1}.yr^{-1}).$

The metal net uptake in the vegetation was calculated by multiplying the annual yield by the metal content in the stem of trees as follow:

$$Mu = Yveg [M]veg$$
(2),

where:

Yveg = Net increment of stem (dry weight) (kg.ha⁻¹.yr⁻¹);

[M]veg = Metal content in the stem of trees (g.kg⁻¹ dw).

The critical leaching flux of heavy metals Mle(crit) (g.ha⁻¹.yr⁻¹) was calculated according to the follow equation:

$$Mle(crit) = cle Qle [M]ss(crit)$$
 (3),

where:

Qle = Flux of drainage water (m.yr⁻¹);

[M]ss(crit) = Critical limit for the total concentration of heavy metal.

The exceedances of critical loads of Pb and Cd for forest ecosystems (CL(M)ex) (SCHUTZE & HETTELINGH 2004; HETTELINGH *et al.*, 2008) were calculated by the following equation:

$$CL(M)ex = Dep(M) - CL(M)$$
 (4),

where:

Dep(M) = Trhoughfoll deposition rate (g.ha⁻¹.yr⁻¹).

In the present study, the growth rate has been evaluated for the growing stock as a periodic annual increment over the past 10 years.

It was calculated by the equation:

$$Z_{v}^{mek} = \frac{V_{a} - V_{a-n}}{n}$$
 (5),

where:

 Z_{ν}^{mek} = a periodic annual increment, m³.ha⁻¹.yr⁻¹

 V_a = volume per 1ha at the ending of the growing period, m³.ha⁻¹

 V_{a-n} = volume per 1 ha at the beginning of the growing period, m³.ha⁻¹

n = years of the growing period (10).

The volume at the end of the period V_a is calculated by a method developed by Mihov (MIHOV, 2000) as a function of three variables: an average diameter ($d_{cp.}$), a mean height ($h_{cp.}$) and an average tree spacing ($a_{cp.}$):

$$V_{a} = \left(\frac{d_{cp.}}{a_{cp.}}\right)^{2} \left(b_{1} + b_{2}h_{cp.} + b_{3}h_{cp.}^{2}\right)$$
(6),

where regression coefficients b_1 , b_2 , b_3 are different for each tree species, and an average tree spacing is calculated by the equation (7):

$$a_{cp.} = \sqrt{\frac{10000}{N}}$$
 (7),

where N is the number of trees per 1 ha.

The volume at the beginning of the period was calculated using the abovementioned method. For establishing the d_{cp} values increment cores were taken with increment borer and h_{cp} . To reconstruct height growth a partial stem analysis was performed. In none of the sample plots there are no fellings and dead trees, so the number of trees 10 years ago we assume to be the same as at the present.

Results and Discussion

The results of the periodic annual increment for the all study sample plots are presented in Table 2. The data showed the highest increment for both coniferous and deciduous stands at site Pismenovo. That results were logical because of the fact that both stands were the youngest from all study stands. Very similar values were calculated for the forests at site Plana. The biggest difference in the increment (almost double) was found between stands of Norway spruce and Common beech at site Petrohan with 30 years of age difference. Evaluation of the Risk of Pb and Cd Deposition on Bulgarian Forests Using a Critical Load Approach



Fig. 1. Indicative map of the sampling plots (OpenStreetMap[®], OpenStreetMap Foundation (OSMF)).

Plots	Altitude, m a.s.l.	Age, year	Species
Petrohan 1	1420	84	Norway spruce
Petrohan 2	1430	114	Common beech
Plana 1	1245	37	Scots pine
Plana 2	1250	48	Common beech
Beklemeto 1	1300	108	Austrian pine
Beklemeto 2	1410	124	Common beech
Pismenovo 1	65	26	Austrian pine
Pismenovo 2	65	26	Pedunculate oak

Table 1. Plots' characteristics.

Table 2. Net increment for 2017 year in g.ha⁻¹.yr⁻¹.

Site	Petrohan	Petrohan	Plana 1	Plana 2	Beklemeto	Beklemeto	Pismenovo	Pismenovo
	1	2			1	2	1	2
Increment	8366	4320	12948	10440	3100	2592	13206	10665

Sites	Mu(Pb), g.ha ⁻¹ .yr ⁻¹	Le(Pb), g.ha ⁻¹ .yr ⁻¹	CL(Pb), g.ha ⁻¹ .yr ⁻¹
Petrohan 1	58.56	10.64	69.20
Petrohan 2	25.92	10.64	36.56
Plana 1	90.64	2.84	93.48
Plana 2	62.64	2.84	65.48
Beklemeto 1	21.7	1.26	22.96
Beklemeto 2	15.5	1.26	16.81
Pismenovo 1	92.44	5.96	98.40
Pismenovo 2	63.99	5.96	69.95

Table 3. Pb net uptake in vegetation (M(Pb)), critical leaching of Pb (Le(Pb)) and critical loads of Pb (CL(Pb)) for 2017 in g.ha⁻¹.yr⁻¹.

Using the data obtained for the annual increment and drainage water from the catchment, the mass fluxes for both toxicant leaving the ecosystems as biomass uptake and leaching were calculated. Their values and those for calculated critical loads are presented in Tables 3 and 4.

It can be seen that net uptake by vegetation fluxes were considerably higher than those by leaching for both metals lead and cadmium (Tables 3, 4). The highest biomass uptake corresponded to the highest annual increment. Taking into account both factors influencing on vegetation uptake: increment and metal content in biomass, it could be figure out the higher biomass metal fluxes for coniferous than deciduous stands at every site. Critical leaching fluxes are depended mainly on drainage water fluxes from an every site that depended on catchment characteristics. Hereby site Petrohan characterised by highest critical metal leaching fluxes for both metals: lead and cadmium.

Calculated critical loads for Pb (Table 3) for all study plots varied between 22.96 and 98.40 g.ha⁻¹.yr⁻¹. The comparison between the different receptors indicates that higher values were typical for the coniferous forests, while deciduous species from the same site had lower levels of critical loads. The difference was about 40% but for Petrohan site only it reached

double value. According to the main principle of the theory, the lower the critical load, the more sensitive the receptor is. Therefore, the broadleaves forests were more sensitive than the coniferous for all study sites. The most tolerant to the Pb pollution appeared stands of Austrian pine at site Pismenovo and Scotch pine at site Plana, which were the younger one, despite the difference of about 1200 m above sea level between the two sites. The most vulnerable were both forest species at the site Beklemeto, which oldest of all the studied. were the Moreover, comparison between two beeches stands at Petrohan and Beklemeto with the similar ages, revealed double sensitivity of the stand of Beklemeto. That means the assessment of the sensitivity is very specific and regional distinct factor, which have to be taken into account when an environmental protection measures are planned.

Calculated critical loads for cadmium (Table 4) were considerably lower than those were calculated for the lead. Their values were in the interval 1.16 to 7.07 g.ha⁻¹.yr⁻¹. The same tendency was found for cadmium pollutant in respect of the type of receptor: the coniferous three species had the higher critical loads in every study site than the deciduous. That implies the coniferous forests have a lower sensitivity to the Cd pollution. On the

contrary, the broadleaves stands would be damaged easily by Cd compared to the coniferous at the same site. The oldest stands at site Beklemeto had the lowest critical loads for Cd and appeared to be the least tolerant to this pollutant from all study sites. The most resistant were the stands at site Pismenovo and site Petrohan.

To assess the present risk for the study forests, it is necessary to compare the value of calculated critical loads to the rate of lead and cadmium deposition.

Data in the figures 2 and 3 showed that for 2017 all critical loads for both heavy metals were exceeded. For some plots such as Plana 2 the exceedance of critical load for Pb was only 1.5%, which is negligible and can be considered as the acceptable value of that toxicant for the beech forest. It have to be mentioned that the exceedance of Pb for Scotch pine at Plana 1 was 15% only, which was the lowest value from four study coniferous species. For the most of the study forest, critical loads were exceeded from two to four times of the lead deposition. Even the most tolerant Austrian pine stand could not withstand to the damage influence of Pb pollution because deposition of the metal was one and the half times more than the acceptable level.

In respect to the cadmium was established the exceedances of critical loads such as for the lead (Fig. 3). The difference was in the levels that ranged from 50% to 90% times above the critical loads. The most vulnerable were both types of forests on the Belkmeto sites, where exceedances of critical loads for Cd were about 95% for deciduous and 90% for the coniferous stands. Even the forests at site Plana with the lowest exceedances for Pb, had exceedances for Cd 80% for beech and 55% for black pine stands.

Table 4. Cd net uptake in vegetation (Mu(Cd)), critical leaching of Cd (Le(Cd)) and critical loads of Pb (Cd(Cd)) for 2017 in g.ha⁻¹.yr⁻¹.

Sites	Mu(Cd), g.ha ⁻¹ .yr ⁻¹	Le(Cd), g.ha ⁻¹ .yr ⁻¹	CL(Cd), g.ha ⁻¹ .yr ⁻¹
Petrohan 1	3.35	3.19	6.54
Petrohan 2	1.3	3.19	4.49
Plana 1	5.18	0.85	6.03
Plana 2	3.13	0.85	3.99
Beklemeto 1	1.24	0.38	1.62
Beklemeto 2	0.78	0.38	1.16
Pismenovo 1	5.28	1.79	7.07
Pismenovo 2	3.2	1.79	4.99



Fig 2. Critical loads (CL) and exceedances of critical loads (CLex) for Pb for 2017 in g.ha⁻¹.yr⁻¹.



Fig 3. Critical loads (CL) and exceedances of critical loads (CLex) for Cd for 2017 in g.ha⁻¹.yr⁻¹.

Conclusions

The critical loads for Pb were higher than those for Cd. Critical loads for coniferous species for both metals were higher that means the higher tolerance to the metal influence than these for the broadleaves one. The main component formed critical loads was metal uptake flux by vegetation. The younger stands were more tolerant of both Pb and Cd pollutants than the older ones. Exceedances of critical loads for both pollutants were established. The present risk of damage from Pb and Cd for study forests was found. It is urgent measures for reduction of heavy metals emission to be taken.

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Modelling of the Behavior of Natural Radionuclides and the Environmental Risk in the Sites from the Mining Uranium Ore in Bulgaria

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Abstract. In the article are included some parts of the results of the modelling of the behavior of the natural radionuclides and the risk for the environment for the technogenic soils formed after underground and open uranium ore extraction and zonal soil types in the area of 30 sites in the forests of Bulgaria (Stara Planina massif, Rila-Rhodope massif and Transko Kraishte). The modelling performed is based on the established strong negative linear correlations between the studied radionuclides and the pH. With reducing the acidity with one pH unit - the prognosis is for increasing the content of radionuclides (232Th - section "V shaft" mine, 238U - "Ribaritsa" mine, 40K, ²³²Th - "Yavorovets" mine, ⁴⁰K, ²³²Th, ²²⁶Ra, ²³⁸U - "Selishte" mine, ²²⁶Ra, α-activity - "Zdravetz" mine). The environmental risk is significant due to the stimulation of the processes of weathering and release of pollutants. In conclusion, some good practice examples have been proposed as possibilities for reducing the environmental risk, following the re-cultivation of the "Kurilo" mine -"Iskra" section and the "V shaft" section. The technologies used in the re-cultivation are developed on the basis of the specific activity of ²²⁶Ra in the soil. These technologies can be used in sites with a similar activity of ²²⁶Ra in the soil.

Key words: model, radionuclides, mining uranium ore.

Introduction

uranium mining in Europe, when uranium environmental risk of the formed technogenic production was closed. Unfortunately, a huge part of the 50 uranium deposits have not been reclaimed in the most appropriate way. Complexity is determined by the specificity of the processes, and it depends on the petrographic and mineral differences, climate conditions, technogenic relief features, long exposure on the Earth's surface, the applied 2017) for Serbia (DRAGOVICA et al., 2008; extraction technologies etc. In most of these DRAGOVICA et al., 2014; TANIĆ et al., 2014; sites, there is a proven radiological risk to the environment and human health due to the (TURHAN et al., 2012; AKÖZCAN, 2014; CENGIZ, increased activity of natural radionuclides (²³⁸U, 2017; TURHANA et al., 2018) for Croatia

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²²⁶Ra, ²³²Th, ⁴⁰K, α - and β -activity). All this points By the 1990s Bulgaria ranks fourth in to the necessity for regional assessment of the soils obtained after the uranium mining.

> In recent years, studies of environmental hazards that come from abandoned uranium mines in specific regions of the Balkan Peninsula have been carried out for Bulgaria (BOGOEV et al., 2010; TSEKOVA & BOGOEV, 2010; Petrova, 2012; Tsekova & Lozev, ABDULQADER et al., 2018), for Turkey

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(ŠOŠTARIĆ *et al.*, 2017; PETRINEC *et al.*, 2018; established values at the section "east" where IVANIĆ *et al.*, 2019) and others.

Materal and Methods

The technogenic soils, which are formed after lifting and open extraction of uranium ore and zonal soil types and bottom sludge from adjacent gullies and rivers in the area of the 30 sites in the forests of Bulgaria, were studied. The content of natural radionuclides was determined (²³⁸U, ²²⁶Ra, ²³²Th, ⁴⁰K) in the 157 samples from the Stara Planina massif, in the 92 samples from the Rila-Rhodope massif and in the 38 samples from the Transko Kraishte.

The reaction of the soil solution is defined according to ISO 10390.

Statistics10 and Excel10 were used for Statistical Data Processing and Result Visualization.

Results and Discussion

The main factor, which affects the geochemical reactions associated with radionuclides and explains their mobility in the soil, is the pH. Knowing the relationships between the pH and the contents of major radionuclides is important and it depends on both the technology of uranium mining and on a set of interrelated indicators. This reflects on the assessment of the risk of radioactive contamination of the environment and planned reclamation activities.

The linear model for the object Buhovo mine "V shaft", which describes the activity of ²³²Th depending on the pH, is the most accurate between 320 and 930 Bq.kg-1, therefore the forecast coincides with approximately 16% of the data (Fig. 1). From the analysis of the dependencies between the main radionuclides, it can be seen that in the higher contents of ²³²Th the corresponding content of ⁴⁰K is lower and vice versa. The ²³²Th content from the section "west" is lower, so the prediction by the model is increased, except for a sample of 850 Bq.kg⁻¹ measured in the established lowest pH 4.2. The forecast covers more precisely the experimentally

established values at the section "east" where the content of the radioactive isotope is higher. The exception for the peak values is most likely due to the exported in mining ore nuggets rich(the except for the peak values most likely due to exported in mining ore nuggets rich²³²Th.

The established Relationship between the content of ²³²Th and soil pH is negative (R = -0.40). Therefore, the increase in the acidity results in an increase in the predictive activity of ²³²Th with 104 Bq.kg⁻¹.

The differences found in the forecasts for both sections (west and east) are the result of differences in the technology, the timing and the duration of uranium mining. The ground relief of the technogenic forms resulting from human activities facilitates the translocation of the radioactive pollutants through the location and the manner of landfilling of mining waste (pic. 1 and pic. 2).

The analysis showed specific differences between the two areas, which again stressed the need for micro-locally study and modelling of the ongoing processes.

These processes are related to the behavior of the natural radionuclides and the environmental risk in the object Buhovo - mine "V shaft" (Photo 1 & 2), which is located in the east-northeast direction about 25 km by air from the central parts of Sofia.

The "Ribaritsa" mine is located near the village Ribaritsa (just over 10 km as the crow flies in the southern direction) to the border of the National Park "Central Balkan". The landfilled mining waste falls within the borders of the site "Tsentralen Balkan bufer" and that includes two protected areas from the European ecological network Natura 2000 (the Habitats Directive BG0001493 Natura 2000 and The Birds Directive BG0002128).

The function that describes the ²³⁸U content depending on the soil pH represents the most accurate prediction in the range of 100 to 1100 Bq.kg⁻¹ and the experimental data match is about 23% (Fig. 2). The Fig. shows that the samples with higher content of the model radionuclide are in the site No 1. Conversely, the values of site No 2 are lower,

so the model's predictions are too high. The varies in the acidic range of 4.8 to 5.4, which forecast covers more precisely experimentally established values at the site No 1, except for one peak value (1941 Bq.kg⁻¹). This is most likely due to the exported and deposited uranium-rich ore fragments at the sampling site.

= -0.48) between ²³⁸U and the soil pH results Bq.kg⁻¹. This increase in quantities may be in the predicted increase in acidity to reflect on an increase in the predicted levels of ²³⁸U with 760 Bq.kg⁻¹. This forecast shows a high environmental risk is significant as the site is risk of uranium contamination in the in close proximity to a river. environment.

The study presented differences between the two sites due to the various stages of mining waste disposal from uranium mining. The established lower uranium concentrations are the result of processes ongoing active surface of weathering and allowing (without transport) the disaggregation products of weathering in place.

From natural point of view, the knowledge of the behavior of the natural radionuclides contained in the uranium mined waste discharged after is important because of the significance of the territory.

The object "Ribaritsa" also falls into the Central Balkan Biosphere Reserve, and under the Seville strategy these sites are an iconic place, an example of a harmonious coexistence between man and nature and they demonstrate good practices and policies. They are part of the UNESCO World Wide Web Reserve. All this calls for measures to reduce the risk to the environment in the Ribaritsa mine.

Yavorovets mine is located in about 1 km southwest of the village Yavorovets, Maglizh Municipality.

The analysis of site data indicates that the function describing the ⁴⁰K content in relation to soil pH (R = -0.86) is the most accurate in the range of 190 to 920 Bq.kg⁻¹ on the site No 2 content of ⁴⁰K is higher than the content of ⁴⁰K on the site No 1 (Fig. 3). The active soil reaction is neutral to slightly alkaline on site No 1, and on site No 2 the pH data. Acidification of the environment with one pH

the also determines the higher values of ⁴⁰K. In general, the model shows that 75% of experimental data are the same as predicted. In this case, the forecast is over 95% correct on site 2.

The predicted acidification of the The determined negative dependence (R environment leads to an increase of 195 due to the extraction of the radioactive isotope from the sand fraction. The

> The modelling of the dependence significant between the ²³²Th content and the pH of the environment found the most accurate estimate in the range of 14 to 51 Bq.kg⁻¹, and the match to the experimental data is 57%. The determined negative linear regression is represented by the equation: 232 Th = 97.797 - $9.8126 \times pH.$

> > Similarly to the content of ⁴⁰K and here concentrations of ²³²Th are on the site No 2 are higher than those on the site No 1. At site No 1, the model's forecast is higher, with the exception of the three samples with a lower forecast, as two of which are in the 0 - 20 cm layer.

> > negative The observed correlation ²³²Th and pH (R = -0.76) between predetermines the increase in the predicted ²³²Th by about 10 Bq.kg⁻¹ in acidification of the environment by one pH unit. The environmental risk is similar to the one with 40 K.

"Selishte" mine is located in about 2 km south-southeast of the town of Lucky and falls into two protected areas of the Natura 2000 network (BG0002073 Dobrostan under The Birds Directive and the Habitats Directive BG0001031 Rodopi - Sredni).

The analysis of the regression linear model, which describes the content of ⁴⁰K in relation to pH, exhibited the greatest match in the range of 480 to 950 Bq.kg⁻¹ (Fig. 4). The established dependence is negative (R=-0.65). The large variation of the values determines the 42% match of the forecasts with the observed

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unit results in an estimated increase in the content of the radioactive isotope by about 290 Bq.kg⁻¹.

Fig. 1. Modelling the activity of ²³²Th depending on the soil pH for the object Buhovo (site west – No 1; site east – No 2).



Photo. 1. Object Buhovo - section "V shaft" mine, site west (Photo: Rossitsa Petrova).



Photo 2. Object Buhovo - section "V shaft" mine, site east (Photo: Rossitsa Petrova).



Fig. 2. Modelling the content of ²³⁸U depending on the soil pH for the object Ribaritsa (site No 1, site No 2).



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Fig. 3. Modelling the content of ⁴⁰K depending on the soil pH for the object Yavorovets (site No 1, site No 2).



Fig. 4. Modelling the content of ⁴⁰K depending on the soil pH for the object Selishte (site No 1, site No 5, site No 6, site No 7).

For an object known as Settlement mine, the model established in the ²²⁶Ra content, which depends on the pH, is most resistant in the range of 1000 to 5700 Bq.kg⁻¹. The model is used at pH up to 5.53 unit. Amounts of the radioactive isotope in site No 1 are significantly higher than those in other sites. The large variation in data leads to the model describing approximately 37% of the values.

The negative relationship between the natural radionuclide content and the pH of the

environment (R = -0.61) is the reason for the predicted acidification by one pH unit and it is expected to increase the amount of ²²⁶Ra by about 2610 Bq.kg⁻¹ (Fig. 5). Acidification of the environment will lead to the danger of extracting the radioactive isotope from the surface of the ore.The model for a Selishte mine which presents the predicted ²³²Th content, depending on the, pH, is the most stable in the range of 26 to 97 Bq.kg⁻¹. Variation of the data means that the model describes approximately

34% of the values. The determined linear regression is represented by the equation: 232 Th = 284.3 - 43.724 × pH.

The established negative relationship (R = -0.58), is the reason for in case of acidification by one pH unit that there might be an increase to the contents of 232 Th to about 44 Bq.kg⁻¹. The presented forecast shows the risks of extracting the natural radionuclide from ore exported to the surface.

The estimated content of ²³⁸U is in range of 260 to 6510 Bq.kg⁻¹. But for one point, the contents of the radioactive isotope on site No 1 exceed those on the other sites. The model describes approximately 34% of the values resulting from the large variation in isotope content. The determined negative linear dependence (R = -0.58) is represented by the equation: ²³⁸U = 28708 - 5162.7 × pH.

Predicting the contents of the model is in a pH range of not less than 5.53 unit.

With the predicted acidification of the environment, the ²³⁸U content is expected to increase by about 3253 Bq.kg⁻¹. The acidification of the environment leads to the extraction of the studied radionuclides from surface ore particles and to the pollution of the environment.

The "Zdravetz" mine is situated in about 4 km west of the village Dobralak, Kuklen Municipality, Plovdiv district. The object falls into protected areas according to the two directives for the Natura 2000 network (BG0002073 Dobrostan under the Birds Directive and the Habitats Directive BG0001031 Rodopi – Sredni).

The model of the content of alpha specific activity in relation to pH, operates within a range of 1525 to 23882 Bq.kg⁻¹ (Fig. 6). A strong negative relationship is established (R = -0.70). Similarly to the model describing the content of ²²⁶Ra and in this case two very high levels of alpha specific activity on site No 1 were established. As a result of this variation, the calculated model describes about 49% of the values. Again, the prognosis is higher at the low contents on the site No 4.

The predicted acidification of the environment with one pH unit predicts an

increase in the specific activity level of about 5200 Bq.kg⁻¹.

The estimated content ²²⁶Ra. of depending on the pH for the Zdravetz object, indicates that the model can be used in an interval between 122 and 3403 Bq.kg⁻¹. The determined linear relationship is represented by the equation: ²²⁶Ra = 5234 -763.03 \times pH. The two very high contents found on site No 1 lead to the model describing about 47% of the observed values. The forecast is increased with low content in the site No 4.

The predicted acidification of the environment results in an increase in the content of ²²⁶Ra by 763 Bq.kg⁻¹, due to a negative relationship between the radionuclide content and the environment pH (R = -0.69).

The higher environmental risk must be reduced to acceptable levels by applying a set of measures for reclamation of the sites. In the case of higher contents of ²²⁶Ra in the soil ("Selishte" mine and "Zdravetz" mine), the instance of the re-cultivation of the "Kurilo" mine - section "Iskra" and the section "V shaft" can be used.

Conclusions

The modelling of the specific activity of the studied natural radionuclides as a function of pH has once again demonstrated the complexity of ongoing processes in systems influenced by human activity. The models specific to each object were identified. They are particularly important for assessing the degree of contamination and the risk to human health and to the environment from the geological exploration and mining of uranium ore which were carried out in the past.

The petrographic and the mineral differences, the climate conditions, the specific features of the technogenic relief, and the extended period of exposure to the Earth's surface are the main factors that influence the modelling of natural radionuclide behavior.

The analysis found out that the risk to the environment was significant, due to the stimulation of the processes of weathering and release of pollutants into the environment. Modelling of the Behavior of Natural Radionuclide sand the Environmental Risk in the Sites...

All of the presented results show increased risks of radiation as a result of the

anthropogenic impact and the extraction of rock pieces uranium ore.



Fig. 5. Modelling the content of ²²⁶Ra depending on the soil pH for the object Selishte (site No 1, site No 5, site No 6, site No 7).



Fig. 6. Modelling the content of Total alpha activity depending on the soil pH for the object Zdravetz (site No 1, site No 4).

In conclusion, all of the studied objects must be reclaimed with a purpose to reduce the risk to the environment and human health. This conclusion is also supported by the importance of sites from a natural point of view, as most of them fall within the European ecological network Natura 2000. All sites fall into forest areas, some of which are in close proximity to large urban areas or health resort villages and are of interest to tourism. Not a small part of the objects are in dangerous proximity to surface water bodies, and all are potentially dangerous to

underground water bodies with which they can come into contact when releasing pollutants into the environment.

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Species Structure of the Earthworm Communities (Lumbricidae) in the Grounds of Two Liquidated Uranium Mines (Senokos and Eleshnitsa) in Bulgaria

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Abstract. The soil earthworm communities (Lumbricidae) in the abandoned uranium mines Eleshnitsa and Senokos, located in the southwestern part of Bulgaria, were studied in 2011 - 2016. The sampling areas have been polluted to varying degrees with heavy metals and radionuclides and disturbed by different mining and reclaiming activities. The species structure of the communities has been determined by the species composition, the number of species, the total abundance, and some diversity indices (species richness, evenness, dominance, total diversity). The number of species identified in the sampling sites is equal (Eleshnitsa mine) or higher (Senokos mine) than the one in the control sites. The total abundance of the earthworm communities is higher in the control areas and in the autumn samples. The number of immature worms is higher than that of the mature ones and more abundant in the autumn. Higher earthworm diversity is evaluated for Senokos mine and it seems that it is under the influence of many factors (environmental characteristics, mining and reclaiming activities), not only the pollution.

Key words: earthworms, diversity, uranium mines, Bulgaria.

Introduction

that determine biodiversity in terrestrial LAVELLE et al., 1997). Invertebrates living in ecosystems. Soil has abiotic components, and soil have often been considered as indicators live elements that altogether create the soil for soil condition (status) due to their environment (COLEMAN et al., 2004). As soil ecological demands (LAVELLE & SPAIN, 2001; organisms, the earthworms (Lumbricidae) GARCIA-RUIZ et al., 2009) and the earthworms take part in mixing the humus with the are among the invertebrates used most often mineral soil and provide the soil fertility by (FRAGOSO et al., 1999; HOLE et al., 2005). Their enhancing macroporosity, humidification, popularity as indicator organisms is based on and mineralization of the organic matter their close connection to the land, limited (FRANCIS & FRASER, 1998). Soil earthworm locomotion, ease communities encompass a wide range of sensitivity to the chemical and physical organisms performing various functions that characteristics of

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physical properties regulate soil and Soil and its characteristics are key factors chemical processes (STINNER & HOUSE, 1990; of determination and the soil environment

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different human activities, including the anthropogenic pollution (CHRISTENSEN et al., 1987; PAOLETTI, 1999; GARBEVA et al., 2004). Changes in abundance, biomass, or species richness of natural communities are common ecological endpoints to identify point-sources of soil pollution in the field monitoring Spurgeon & Hopkin, 1999; Nahmani & LAVELLE, 2002; DUNGER & VOIGTLÄNDER, 2005; VANDECASTEELE et al., 2004). The tolerance of the earthworms to highly metal contaminated soils and the capacity to accumulate elevated concentrations of heavy metals in their tissues led to their use as sentinel species (LUKKARI et al., 2004).

Uranium (U) and heavy metals pollution (HMs) of the ecosystems resulting from mine activities affect the individuals, populations, biotic communities and ecosystems as a whole. Bulgaria was one of the biggest producers of U in Europe till 1992 when the U production and milling was ceased by a decree of the Bulgarian government.

The important role of earthworms as bioindicators in a polluted with heavy metals soil is mainly proved in a laboratory setting and for the aim of this investigation, any related reports are missing. There are some data referring the effects and consequences of the two abandoned U mines Eleshnitsa mine and Senokos mine, but they regard the soil the plant and bacterial characteristics, communities (BOGOEV et al., 2010; KENAROVA et al., 2010; 2014; RADEVA et al., 2013; BOTEVA et al., 2015) and the water condition in the impacted rivers around the mines (STOYANOVA et al., 2014, KOLEV et al., 2014). The purpose of this study is to assess the of species structure the earthworm communities exposed to long-term U and HMs contamination in the territory of Eleshnitsa and Senokos mines.

Material and Methods

Study areas. The abandoned uranium mines Eleshnitsa (41°51'18.0" N; 23°38'13.7" E) and Senokos (41°49'53.0" N; 23°13' 11.8" E) are located in the southwestern part of

(PAOLETTI, 1999; TISCHER, 2009) and to Bulgaria. The mining operations in Eleshnitsa had been conducted in a conventional underground manner (Fig. 1), while Senokos was an open-cast mine (Fig. 2). Nevertheless, during the operation of the mines and later (since 1992) as a result of the compromised rehabilitation large amounts of mine wastes have been dispersed in the surrounding areas by both surface erosion and wind action (Senokos mine) and by water effluents draining the mine galleries (Eleshnitsa mine).

> Experimental design, Sampling and *Identification.* The sampling took place in May and October from 2011 till 2016. The specimens were collected by digging and hand sorting the 0.25x0.25 m blocks, as well as by turning over rocks, debris, and logs. The sampling sites were located in front of some of the the entrances of the galleries of Eleshnitsa mine and at different spots in the territory of Senokos mine. The control sites were chosen to be far enough from the mines. The Eleshnitsa sampling area contains 3 sampling sites in the territory of the mine (mine gallery 31 Sps, mine gallery 53 Sps and Sps WT close to the Waste water treatment plant) and one control site SpsBE. The Senokos sampling area contains 4 sampling sites (Sps 1, Sps 2, Sps 3 and Sps 4) and a control sampling site Sps 22. In order to get a better idea of a sampling site, 5 subsamples were collected while walking in a zigzag pattern across the area of the field. The approximate depth of 25 cm was chosen to reach all the important soil layers. The worm materials from all the subsamples collected from one site were mixed and filled in sample bags (collectors). The collectors from the different sampling sites were placed separately, labeled to identify and transported to the laboratory. There the earthworms were cleaned, then killed in 70% ethanol, fixed in 4% formalin solution, and stored in 90% ethanol. The identification and nomenclature of taxa were made in the laboratories of Sofia University and University of Kragujevac (Serbia), by R. Tsekova and M. Stojanovic, according to BLAKEMORE (2008), MRŠIĆ (1991), ZICSI (1982), ŠAPKAREV (1978), and CSUZDI & ZICSI (2003).

The species structure of the earthworm higher in the mine sampling sites than in the communities has been determined by the species composition, the number of taxa (S), the frequency of occurrence (pF%), the Sorenson's similarity index (QS%) (SORENSON, 1948), the total abundance (N), and some diversity indices: Margalef index of species richness (d) (MARGALEF, 1958), Simpson index of dominance (c) (SIMPSON, 1949), Pielou index of evenness (e) (PIELOU, 1966), Shannon index (H) of total diversity (SHANNON & WEAVER, 1963). The seasonal distribution of the mature and immature earthworms has also been determined.

Results and Discussion

Species composition and number of species (S) Eight earthworm species (Allolobophora chlorotica, Aporrectodea caliginosa, A. trapesoides, A. rosea, Bimastos rubidus, Eisenia fetida, Octolasion lacteum, Lumbricus rubellus) belonging to six genera of the Lumbricidae family were identified during the study. The genus Aporrectodea is represented by the highest number of species three. The number of species found in all the sampling and control sites for the whole period of study varied between 2 and 7 (on average 4), which corresponds with the statement of EDWARDS & BOHLEN (1996), that the earthworm diversity of all the habitat types ranges from 1 to 15 species (Fig. 3).

One and the same species were found in the territory of Elesnitsa mine and in the control site - A. rosea, E. fetida, O. lacteum, L. rubellus. The number of species (S = 4) remained constant for the whole research period both in the spring and autumn samples with two exceptions only (May 2013 and May 2015) (Fig. 3).

All eight species identified during the study were observed in the Senokos mine sampling sites, and two of them: Al. chlorotica and B. rubidus were found only there. The number of species ranged - between 3 (May 2014) and 7 (October 2012), and in all the years (except in 2011) it was larger in the autumn samples. The number of species from the Senokos control site was six (A. caliginosa, A. trapesoides, A. rosea, E. fetida, O. lacteum, L. rubellus), and it ranged between 2 and 5. In all cases except in May 2013 the species richness is rarest species (pF = 8,3%).

control ones (Fig. 3). The reason may due to the reclamation activities following the liquidation of the mines, done by spreading a humus layer, sands and natural fertilizers on the surface to create conditions for the normal development of the plant and soil species.

For the whole period of study E. fetida is the most numerous species in the area of Eleshnitsa mine as well as in the control site both in the spring and autumn samples (Fig. 4).

Two species dominated in numbers in the area of Senokos mine and the control site: A. rosea and O. lacteum. They were more abundant in the control site both in the spring samples and especially in the autumn ones (almost twice) (Fig. 5). Two other species were present in large numbers in the Senokos control sampling site: E. *fetida* in the spring and *L. rubellus* in the autumn. *B. rubidus* was presented by 2 adults only (Fig. 5). The species is an epigeic species which inhabits and feeds in the litter and organically enriched surface layers of soil (HENDRIX, 1995). The reason for the low number of individuals of the species is its preferences to substrates rich in organic material, such as rotting wood and other plant matter, compost, peat, and manure, which are absent on the territory of the mines.

The values of the frequency of occurrence index show that in Eleshnitsa sampling area all the species identified were constant components of the earthworm communities with very high frequency of occurrence (pF = 100% or pF = 91,6%) both in the mine and control sampling sites.

In Senokos sampling area two species occurred very often both in the mine and control sampling sites: A. rosea (pF = 100%) and O. lacteum (pF = 75%). E. fetida and L. rubellus have also high values of pF, varying between 58% and 67% in the mine and control sites. The species A. caliginosa (pF = 57%) and A. trapesoides (pF = 100%) were constant in the mine samples, but quite rare in the control ones (pF = 25%). The two species that were found only in the territory of the mine have different values of the frequency of occurrence index: pF = 58% for Al. chlorotica and B. rubidus was the Species Structure of the Earthworm Communities (Lumbricidae)....



Fig. 1. View from Eleshnitsa mine.



Fig. 2. View from Senokos mine.



Fig. 3. Number of species collected from all the sampling sites and for the whole period of study (2011-2016).



Fig. 4. Number of the adult individuals of the earthworm species collected from the Eleshnitsa mine and the control site (2011-2016).

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Fig. 5. Number of the adult individuals of the earthworm species collected from the Senokos mine and the control site (2011-2016).

From all the species found A. rosea, O. lacteum, L. rubellus and E. fetida were the most numerous and with the highest values of the frequency of occurrence index. They belong to four widespread genera of earthworms which are numerically dominants in arable land, agro-ecosystems and other ecosystems with considerable anthropogenic impacts on soils. The taxa are cosmopolitan around the world, because of their high adaptability and wide tolerance to many of the environmental factors.

There is complete similarity of 100% between the species composition of the earthworm communities from the Eleshnitsa mine and control sites and very high similarity (QS = 85%) between the species composition of the communities from Senokos mine and control sites. The similarity between the earthworm communities from both sampling areas (Eleshnitsa and Senokos) is also comparatively high (QS = 66%) although they are situated at a distance and the environmental conditions are different.

Total abundance

A total of 971 individuals were collected from Eleshnitsa sampling area, 462 from the territory of the mine and 509 from the control site, 458 of all individuals were mature and 513 immatures. The abundance of the earthworm communities from the mine and control sites was equal (2013, 2014, 2015) or larger in the control site (2011, 2012, 2016) (Fig. 6).

The number of the individuals collected from Senokos sampling area was 902, 432 from the territory of the mine and 470 from the control site. The number of mature and immature individuals was equal – 451. The abundance of the communities from the mine sampling site was equal (2011), or higher (2012, 2014), or lower (2013, 2015, 2016) than that of the communities from the control site. (Fig. 7) The total abundance of the earthworm communities from both sampling areas had the highest values in the last 2 years (2015, 2016) (Fig. 6 and 7).

Mature earthworms were more abundant in the autumn samples (Fig. 8). The number of immature worms was more abundant in the autumn and the values increase with 25 to 50 % (Fig. 9). Changes in the number of immature earthworms over the time seemed to be in agreement with WATANABE & TSUKAMOTO (1976) who found that immature worms and cocoons were recorded mainly in autumn, being not affected by the high and low temperatures in the summer and winter, respectively. Juveniles made up the majority of individuals from most of samples and their proportional the abundance (relative to adults) was similar across the sampling sites.

Diversity indices

The values of Margalef index of species richness from Eleshnitsa sampling area (mine and control) are relatively close, which corresponds to the one and the same number of species found in the whole investigated area.

Analysis of data revealed that maximum species richness in term of Margalef index was found in Senokos mine. Senokos mine is the sampling area where all the eight identified in the study earthworm species occurred. The values of the index range from 0.26 (Senokos control site, May 2011) to 1.39 (Senokos mine, Oct. 2012) (Fig. 10). The lower values at the Senokos control site may due to different agricultural human activities, located far enough from the mine, where the control site is situated.

Dominance is inversely proportional with diversity. The Simpson index varies from 0.18 (Senokos mine, May 2011) to 0.82 (Senokos control site, Oct. 2012, where from 22 individuals collected, 17 were belonging to two species A. rosea - 8 ind. and O. lacteum - 9 ind.). Most of the values are lower or about 0.4 for all the sampling sites in both mines (Fig. 11). The only exceptions are from Senokos control site in May 2011, Eleshnitsa mine and Senokos control site in October 2012, Senokos control site in May and October 2014 and Elesnitsa mine in May 2015. The highest values of the Simpson index correspond to numerically close values of the Pielou index (Fig. 12). This is relatively rare and happens in cases of reduced number of taxa, represented by single individuals. The Pielou index varies between 0.62 (Eleshnitsa mine, October 2012) and 1 (Senokos control site, May 2014). The earthworm communities in Eleshnitsa control sampling site have the lowest variation in the values of both Simpson and Pielou indices, which stable corresponds with the more environmental conditions there.

Shannon diversity index reports the species richness and evenness, that's why it may varies in extended ranges. The calculated values of the diversity index lie between 0.94 (Senokos control site, May 2011 and October 2014, when the species richness was very low (S = 2) and 2.5 (Senokos mine, May 2011 and October 2016), when the number of species was very high (S = 7), and the species were represented by almost equal number of individuals (Fig. 13). The only exception with short range of the diversity values is evaluated for Eleshnitsa control site –

from 1.5 to 1.98, which again confirms the stable environmental conditions on this sampling area. Soil properties are likely to be verv influential in determining the earthworm activities. The results are consistent with the findings of HOLLAND (2004) who showed that there is relationship between the environmental conditions, especially the structure (texture) soil and the earthworm biodiversity. The variations of the values of Shannon index are probably result of levels of HMs and the soil texture (sandy soils). Sandy soils are often dry, nutrient deficient and fastdraining. They have little (or no) ability to transport water from deeper layers through capillary transport, that makes

the earthworms less numerous in sandy soils.

Conclusion

The earthworm species, identified in the study, were variable presented. The highest number of species were reported for one of the mine areas - Senokos. The abundance of both groups - mature and immature was higher in the autumn samples. The total abundance was higher in the control areas but there was still no clear trend in the number of species, their seasonal abundance or diversity pattern. That is probably due to the different sensitivity of the species to the environmental characteristics of the sampling areas.



Fig. 6. Total abundance of the earthworm communities from the Eleshnitsa mine and control sampling sites in 2011-2016.



Fig. 7. Total abundance of the earthworm communities from the Senokos mine and control sampling sites in 2011-2016.





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Fig. 9. Total number of immature individuals collected from all the sampling sites and the whole period of study (2011-2016).



Fig. 10. Values of Margalef index from all the investigated areas and the whole period of study (2011-2016).



Fig. 11. Values of Simpson index from all the investigated areas and the whole period of study (2011-2016).



Fig. 12. Values of Pielou index from all the investigated areas and whole period of study (2011-2016).

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Fig. 13. Values of Shannon-Weaver index from all the investigated areas and the whole period of study (2011-2016).

This is confirmed in the study of LAVELLE & SPAIN (2001) where the response of earthworms is likely to depend mostly on environmental conditions in the field, such as site latitude, soil type, soil texture, sampling season, sampling method, etc. The soils of the investigated samplings from U areas Eleshnitsa and Senokos are heterogeneously loaded from slight to moderate levels of contamination with U and HMs (BOTEVA, et al., 2015). Higher earthworm diversity is evaluated for Senokos mine (in terms of higher number of species represented by almost equal number of individuals) and lower diversity - for Senokos control site. For Eleshnitsa sampling area is opposite, higher value of diversity in general is evaluated for Eleshnitsa control site and lower diversity - for the sampling territory of the mine. The diversity seems to be under the influence of many factors as environmental characteristics, mining and reclaiming activities also, not only from the

pollution with varying degrees of heavy metals and radionuclides.

Regarding all these, a clear general response of the earthworm community to the set of uranium mining activities cannot be clarified. However, the study provided some new insights about the existing effects of the anthropogenic mining activities on the soil diversity and the distribution of the earthworm species.

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Waste-cluster Relationship on the Example of Regional Waste Landfill in Blagoevgrad

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Abstract. Bulgaria is facing serious challenges about environmentally friendly way of waste management. Commissioning of the Regional Waste Management System - Blagoevgrad is going to ensure their environmentally friendly utilization and disposal, which is in close relation with the defined hierarchy in Bulgarian Waste management act - prevention, utilization, final disposal. The attempt to be created relation between regional waste management and cluster policy is related to the development of a cluster model, named by authors "Waste utilization and mitigation of climate change". The implementation of the model will contribute to sustainable and efficient waste management in Blagoevgrad region, as well as in the territory of Bulgaria, in relation with the requirements of European legislation. In other side this contributing to the realization of the main Community priorities for Cohesion Policy: sustainable development and increasing the attractiveness of regions, by improving accessibility, ensuring adequate quality and level of services and preserving their environmental potential.

Key words: regional waste management, cluster model, resource utilization, climate change, mitigation, Blagoevgrad, municipality.

Introduction

Environmentally friendly management is a priority for the European countries in transition, especially for the last 10-15 years. Following the waste management model, applied in some European countries, the Bulgarian Ministry of environment and water has adopted the approach for building of regional landfills for waste disposal with pre-treatment. In order to implement the recommendations of Ministry of environment and water in 2011 has been formed "Regional Waste Management Association _ Blagoevgrad". The association is independent, landfill under the project ""Regional Waste voluntary, non-government

which includes municipalities from waste Blagoevgrad and Kyustendil province. (Ministry of environment and water, List of Regional associations of waste management in Bulgaria).

The participants in this association are the following municipalities: Municipality of Blagoevgrad and Municipality of Simitly, both from Blagoevgrad district and Municipality of Rila, Municipality of Boboshevo and Municipality of Kocherinovo, from Kyustendil district. In September 2019 has finished the first stage of construction of the regional organization, Management System, Blagoevgrad Region,

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg Union of Scientists in Bulgaria - Plovdiv University of Plovdiv Publishing House serving the municipalities of Blagoevgrad, Simitly, Rila, Kocherinovo and Boboshevo -Phase I". The implementation of projects for municipal the construction of waste management systems, of which regional landfills are part, is regulated by Directive 1999/31/EC on the landfill of waste. In accordance with its requirements, it is planned to build regional systems to cover waste management in the country. (Ministry of environment and water, Waste management Blagoevgrad).

In this paper the authors are facing the challenge to represent a relationship between wastes and clusters. Clusters, according to Porter (PORTER, 2003) "are geographically proximate group interconnected of companies, suppliers, service providers and associated institutions in a particular field, linked by externalities of various types.".

Material and Methods

For the purpose of the present study the authors have been investigated some characteristics of the clusters and the structure of the regional waste management association. The authors present an example of a model, which is focused to waste-cluster relationship on the example of the regional landfill in Blagoevgrad.

Results and Discussion

The different definitions for clusters are focusing on different aspects. The concept of clusters usually consists of three important dimensions (SLAVOVA & BANKOVA, 2016). According to the same authors the first dimension is related to considering clusters as a geographic concentration of specialized firms, advanced skills and competences of the workforce, as well as the support of institutions that increase knowledge flows and their dissemination as a result of their grouping of different proximity. This strengths is often referred to as a promising strategy for maintaining global competitiveness. Second - Clusters include a network of joint / cooperative enterprises (companies that have formal, social and waste management processes.

economic links between). It is thought that geographical proximity facilitates planned interactions, which are important elements of the innovation process. Third - Clusters are characterized by a certain dynamic social and organizational element, the so-called "institutional attachment fixation" / attracting various interconnected innovation partners, thus facilitating intensive interaction and cooperation between them. H. Rocha and R. Sternberg (ROCHA & STERNBERG, 2005) call the third dimension of clusters a network of cooperative organizations.

The structure Regional of waste management association is consisted of assembly Chairman General and of administrative board. The administrative board includes the mayors of the five municipalities (Blagoevgrad, Simitly, Rila, Boboshevo and Kocherinovo) and the Chairman of the Administrative board is the mayor of Blagoevgrad municipality.

Each municipality participates with different percent in the activities for construction of the regional landfill and putting it into operation; construction of waste treatment facilities by making cash (property) contributions; providing project financing for the different stages of construction of the regional landfill and / or other waste treatment facilities; the amount of the contribution of the regional association to the realization of the project.

The participation of each municipality as a member of the association is shown on the diagram showed on Fig. 1.

The main objectives of the Regional Management Waste Association Blagoevgrad are the joint construction, management and operation of the regional landfill for non-hazardous wastes, situated on a property, owned by the Blagoevgrad Municipality. The property falls within the boundaries of the existing municipal landfill of Blagoevgrad Municipality; management of widespread and specific waste streams; efficient resources utilization, resulting from



Fig.1. Participation of each member (municipality) of the Regional Association (in %) (after ATANASOVA, 2017).

In order to be achieved the above mentioned objectives it is upcoming for all municipalities to:

Select company/companies for:

- Collecting of municipal waste from the containers according to appropriate developed transport schedules;

- Operation of the regional landfill on the territory of Blagoevgrad Municipality.

Select company/companies for:

- Collecting of widespread and specific waste streams and their transportation to the installation and places for pre-treatment, preparing for recycling and composting.

- Collecting of hazardous household waste by announcing schedules for each municipality;

- Collecting of bulky waste by announcing schedules for each municipality;

Select company/companies for:

- Operation of waste pre-treatment/ separation installations for waste and their future recycling; anaerobic disposal of biowaste and composting of green waste;

- Operation of installations for storage of hazardous and bulky waste.

The regional waste management cluster policy relationship is consisted of the potential benefits for the above mentioned future companies. Taking into consideration all above mentioned the authors have developed an example for acting regional cluster, which can be used for waste management. The cluster in this case is on the example of regional waste landfill in Blagoevgrad.

The activities are separated in different groups - A, B, C, D.

A. Collecting and transportation of total municipal waste stream, bio-waste and green waste.

One company (**A**) can be responsible for operation of the regional landfill and also for collecting the household waste from Blagoevgrad and Simitly.

One company (**B**) can be responsible for collecting of household waste from Kocherinovo, Rila and Boboshevo. It is necessary to be done on a set schedule.

B. Collection of widespread waste - packaging waste and transfer to the pre-treatment (separation) installation located in the industrial zone of Blagoevgrad;

The implementation of this activity can be done from one Packaging Waste Recovery Organization (**C**), licensed by the Ministry of environment and water of Bulgaria. The Organization can operate on the territory of all five municipalities on a set schedule.

C. Collection, transportation and operation of sites for hazardous and bulky waste
Waste-cluster relationship on the example of regional waste landfill in Blagoevgrad

The activity, for all five municipalities, can be implemented by one company (**D**), licensed by the Ministry of environment and water of Bulgaria for working with hazardous waste.

D. Operation of pre-treatment installation

The activity can be done from one company (E) with good experience.

All companies are interconnected due to the fact that the waste management processes are consistent and continuous. The Regional Waste Management Association should Blagoevgrad oversee the implementation of all waste activities and financial dimensions. The proposed example for cluster organization can be defined as an organized effort to maximize the benefits of waste treatment, growth and competitiveness of clusters in the Blagoevgrad region.

Municipality	Collection and transportatio n of household bio-waste and green waste	Collection of packaging waste including widespread waste	Collection, transport and operation of sites and installation for hazardous and bulky waste	Collection, transport and operation of sites and installation for pre-treatment of waste
Blagoevgrad	Company A	Recovery organization C	Company D	Company E
Simitly	Company A	Recovery organization C	Company D	Company E
Rila	Company B	Recovery organization C	Company D	Company E
Kocherinovo	Company B	Recovery organization C	Company D	Company E
Boboshevo	Company B	Recovery organization C	Company D	Company E

Table 1. Proposal for participation of the companies included in the regional system.

Conclusions

The study represents the aim of the authors to research and create a connection between waste and clusters on the example of the regional landfill in Blagoevgrad. This type of differentiation and organization of the activities between the separate companies can be used in other regional landfills. The implementation of the proposed model cluster could have multiplier effect - on future regional waste management and disposal systems; economy, activities related to mitigate the climate change.

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Remote Sensing Based Vegetation Analysis in Parangalitsa Reserved Area

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Abstract. In the last decade the remote sensing and the Unmanned Aerial Vehicles (UAV) become a very popular technology for observing the spatial distribution of different objects and processes. Generating a point cloud, extracting DSM and DTM from a photogrammetric mosaic and analyze the change of the canopy are the main features of the remote sensing ground monitoring applications. This study is focused on the feasibility and adaptability analysis of the UAV techniques and the satellite images processing software as instrument for interpretation of the vegetation health in Parangalitsa reserve area. The area of interest that is subject of investigation in this research is situated in Rila National Park, South-West Bulgaria. To analyze the canopy in the reserve have been used two types of remote sensing information the first one is a rapid eye satellite picture with a 5 m spatial resolution as the second one is a photogrammetry mosaic extracted with a fix wing E-bee UAV equipped with a high resolution 20 MP S.O.D.A and multispectral Parrot Sequoia cameras. The main idea of this study was to use the high resolution images captured by the UAV as a benchmark and to extract the NDVI values of the pixels that represent vegetation in very bad shape and after that to search for pixels with the same NDVI values on the low resolution satellite images in order to find areas on the satellite pictures with dead or dying vegetation and also to analyze the dynamics in the health status of the forest vegetation inside the reserve for 8th Years period between 2009 and 2017.

Key words: remote sensing, satellite images, NDVI, forest ecosystems.

Introduction

oldest reserve in Bulgaria. It is known with its etalon forests of *Picea abies* L./H, *Abies alba* M., Fagus sylvatica L. and has an enormous influence over the biodiversity preservation in Bulgaria and Europe as well. But recently due to the whirlwinds activity and vermin infections the old forests in the reserve are constantly dying. Because of that, the dynamic changes in the health status of the forest vegetation inside the boundaries of the reserve area, represent a big scientific interest.

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The main goals of this research are to be Parangalitsa reserve area is the second analyzed the changes in the vegetation health on the territory of the Parangalitsa reserve area between 2009 and 2017 by remote sensing methods including satellite images and UAV data. As a bench mark area for more detail observation has been chosen the North-Western corner of the reserve between Blagoevgradska Bistritsa River and Haidushka River (Fig. 1) which will be referred bellow as a small area of interest. Thirty years ago this particular part of the reserve had been known with its large volume of wood growing stock which according some estimations were more than

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1000 m³/ha. Today the forest ecosystems in this part and in the reserve as a whole are in very bad condition and huge numbers of ill, dry and fallen trees can be observed. The main reasons for this change probably are the large numbers of fallen trees due to the whirlwind activity in the area. These whirlwind spots and the fallen trees that were left inside, served as a food base for vermin which consequently affected the living trees around. The same methodology after that was applied to the bigger area of interest which coincide with that area of the reserve which is covered by forest vegetation.

Materials and Methods

To achieve the main goal of this research have been used two sources of remote sensing information - multi spectral satellite images captured by Rapid Aye satellites with spatial resolution of 5 m and a high resolution, multi spectral orthophoto mosaic with a spatial resolution of 11 cm.

The satellite images are captured on 13.07.2009 and on 20.07.2017 by Rapid aye satellites that are operated by Planet Labs Inc.. These satellites are equipped with a multi-spectral push broom imager sensors that are capable of capturing the spectral bands shown in Table 1. The satellite images where processed with an image processing software Erdas Imagine 2018.

 Table 1. Spectral resolution of Rapid Aye satellites.

Туре	Wavelength (nm)
Blue	440 - 510
Green	520 - 590
Red	630 - 685
Red Edge	690 - 730
NIR	760 - 850

The orthophoto mosaic has been assembled from 365 pictures captured by E-Bee UAV equipped with a multispectral Parrot Sequoia camera, the technical specification of the last are presented in Table 2. All the UAV pictures were process by an image processing software - Pix4D.

Based on the spectral information that is incorporated inside the satellite images and the orthophoto mosaic a NDVI index for each image has been built. The Normalized Difference Vegetation Index (NDVI) quantifies vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs). The mathematical formula that stands behind the NDVI index is (NIR-Red)/(NIR+Red). The values of the NDVI can be between -1 and +1, but in the scientific literature there is no consensus about the boundary between the values of unhealthy or dead vegetation and healthy vegetation. Because of that in order to achieve reliable values of NDVI for the forest vegetation in the reserve area and also to distinguish dry or unhealthy from healthy vegetation, in this research we used a combination of high resolution RGB orthophoto mosaic and NDVI of the same mosaic. This approach is very useful when the precise location of the dry wood stock (in White on the Fig.2-left side) have to be determined and more over the values of the NDVI that represent a Dry wood stocks (in Black on Fig. 2- right side) can be extracted (Fig.2.).

NDVI values from the high resolution orthophoto were extracted in an image processing Erdas Imagine 2018 Software. Additional unsupervised classification has been executed and these values after that where separated in 36 classes. This unsupervised classification was used as an instrument which helped to distinguish a healthy from unhealthy and dry vegetation. Classes with the same values of the NDVI were also determined for the same area of interest on the two satellite images (Fig. 3).

This approach to extract NDVI values from the high resolution images and compared these values with the NDVI values extracted from the satellite images leads to precise determination of the areas occupied with dry woods and healthy or unhealthy vegetation even on the satellite pictures with a spatial resolution of 5 m. Taking in consideration the works of EIGEMEIER *et al.* (2012), GOSPODINOVA & KANDILAROV (2018) and XIAO & MCPHERSON (2005) the defined 36 classes were additionally combined in 10 classes (Appendix 1, 2, 3, 4). This is more suitable for the purposes of this research due to

the resolution of the satellite pictures and the size of vegetation was divided on five categories - dead the Parangalitsa reserve area.

on colored maps (Fig. 4) with the health status of the forest vegetation in the small area of interest and the whole forest area of the reserve as well. Based on the NDVI values the forest above 0.9.

or dry with NDVI values under 0.1; vegetation The values of these 10 classes are presented in bad shape, with values between 0,1 and 0.41; in generally good condition, between 0.42 and 0.69; in very good condition, between 0.7 and 0.89 and in excellent health with NDVI values



Fig. 1. Map of Parangalitsa reserve (above) and Orthophoto mosaic with NDVI (bellow) of the western corner of the Parangalitsa reserve with a pixel size of 11 cm.

Sensor	Multispectral sensor + RGB camera
Multispectral sensor	4-band
RGB resolution	16 MP, 4608 x 3456 px
Single-band resolution	1.2 MP, 1280 x 960 px
Multispectral bands	Green (550nm ± 40 nm) Red (660nm ± 40 nm) Red edge (735nm ± 10 nm) Near infrared (790nm ± 40 nm)
Single-band shutter	Global
RGB Shutter	Rolling
RGB FOV	HFOV: 64° VFOV: 50° DFOV: 74°
Single-band FOV	HFOV: 62° VFOV: 49° DFOV: 74°
Calibration	Automatic radiometric calibration
Support RTK/PPK	Yes

Table 2. Technical specifications of Parrot Sequoia multi-spectral camera.



Fig. 2. Part of the Orthophoto mosaic with a spatial resolution of 11cm in RGB - on the left and NDVI - on the right.



Fig. 3. 36 classes of the NDVI values (13.07.2009 on the left and 20.07.2017 on the right) along the small Area of interest – northwestern corner of the Parangalitsa reserve. White colors represent a vegetation in good health, the dark colors represent dry or vegetation in bad health. The table with the NDVI values can be found in appendix 1 and 3.



Fig.4. Colored map with the health status of the vegetation in the small area of interest - 2009 on the left and 2017 on the right.

The same methodology was applied to generate the maps (Fig. 5) with the health status of the vegetation, but this time the NDVI values were extracted from satellite images of the whole area (270 ha from which 240 ha are broad leaf and coniferous forest and 30 ha are meadow) occupied by forest inside Parangalitsa reserve area.

Results and Discussion

After thorough investigation of NDVI values generated from the high resolution

orthophoto mosaic and the satellite images some general relations were detected. From one side in the NDVI values based on the satellite images in the small area of interest, some single standing dry trees due to the pixel size cannot be detected but their presents generally leads to decrease in the value of the index in this areas. The forests in this area, even though there are some heathy trees, generally are classified as forests in bad condition. Because of this, based on the data from the satellite pictures the dry wood stocks are decreasing between 2009 and 2017 but the area with forest vegetation in bad health is bigger in 2017 than in 2009 (Table 3). This confirms the negative trend in the health status of the forest vegetation and more over it can be used as an evidence that the NDVI build on the satellite images with 5m resolution is not suitable for inventory analyzes of a single dry trees, but is a reliable instrument for evaluation of the general health status of bigger areas.

On the other side on a higher altitudes in the areas occupied with coniferous forest the NDVI values indicate that these forests are in a bad health and more over the areas with a dry trees are also increasing in the research period. This increase in the areas with a dry vegetation is mainly due to the presents of large numbers of dry trees which are evenly distributed along that part of the reserve and more over large number of trees are affected by moss and lichen which decrease the quantities of the leafs (needles) on the coniferous trees. All of the above leads to decrease in the values of the NDVI along the East and South-East Part of Parangalitsa reserve area and confirm the negative trend in the vegetation health (Fig. 5).

After thorough investigation of the NDVI values in the small area of interest (24 ha) have been noticed that between 2009- 2017 the area occupied with dry trees increase with 0.49 ha (Table 3). This area in the past was occupied by the most productive forest with large presents of *Picea abies* L./H, *Abies alba* M., *Fagus sylvatica* L. Based on the data presented in Fig, 4, was concluded that the new dry trees

in the small area of interest are distirubuted west of the whirlwinds spots that occurred between 1966 and 2013 (PANAYOTOV *et al.*, 2015). Because Parangalitsa reserve is an area with a maximum protection the fallen trees were left intact inside this whirlwind spots and served as food base for different vermin which consequently affected the living trees around these whirlwind spots.

Furthermore it was noticed that in some of the areas where in 2009 were observed pixels with dry trees in 2017 the same areas are occupied with new broad leaf tree types as Salix sp., Populus tremula L., Sotbus aucuparia L. Acer sp., Cr and etc. The reflectance values of NIR light for these broad leaf tree types are bigger than the values of the coniferous trees and this leads to generally higher values of the NDVI in this areas. Because of this in the small area of interest between 2009 and 2017, a decrease in the size of the areas classified as forest in bad health and increase of the size of the area that are in good health with 0.98 ha can be observed.

On the Fig 4. The areas occupied with forests in good health completely much the location of the whirlwinds spots mapped by PANAYOTOV et al. (2015). These areas are presented with green and light green colors. Even though the good health areas and the whirlwinds share the same locations there are substantial differences in their size as the area affected by the whirlwinds activity is almost two times smaller than the one that is occupied with vegetation in good health. This can be an evidence that the old coniferous trees around whirlwinds spots have been affected by the vermin activity and because of that, the size of the areas occupied by the new broadleaf tree types are growing constantly.

Because of the bad health status and the loss of old coniferous trees the density of the forest around the whirlwinds spots has been decreased and due to this reason the spatial distribution of new whirlwinds coincide with the borders of the old ones (PANAYOTOV *et al.* 2015).



Fig. 5. Colored map with the health status of the forest vegetation inside Parangalitsa reserve based on the NDVI values (above – 13.07.2009, bellow – 20.07.2017).

NDVI values combined in five groups	2009 in ha	2017 in ha	Difference in ha	Correction due to the 5 m resolution in ha				
Small area of interest								
under 0.1	0.5825	1.0725	+0.49					
$0.1 \div 0.4$	8.29	7.31	- 0.98					
0.41÷0.69	9.12	9.67	+ 0.55					
0.7÷0.89	1.74	1.93	+ 0.19					
above 0.9	4.67	4.42	- 0.25					
Т	he area occup	ied by forest	in the reserve					
under 0.1	15.7	19.95	+ 4.25	+ 4.2				
$0.1 \div 0.4$	95.4	101.92	+ 6.52	+ 6.2				
0.41÷0.69	104.32	88.13	- 16.19	- 16.4				
0.7÷0.89	34.025	34.415	+ 0.39	+ 0.3				
above 0.9	20.65	26.4275	+ 5.78	+ 5.7				

Table 3. Areas (in ha) in Parangalitsa reserve classified by the NDVI values.

The forest vegetation in the small area of interest possess a complicated, broad leaf and coniferous, mosaic shaped structure and because of that the vermin activity is not so intensive as it would be if the forests were homogeneously coniferous, but the dynamics of the negative changes in their health status is much faster than the processes of natural drying due to mature age of the trees. The values of the NDVI indicated that in the western part of the small area of interest (Fig. 4) the old forest vegetation that consist of Picea abies L./H, Abies alba M., Fagus sylvatica L. are retreating and they have been substituted by predominantly broad leaf tree types. From one side this new broad leaf vegetation possess grater absorption capacity of CO₂ and it is very useful as a measure to mitigate the climate changes but on the other side this also means a loss of biodiversity and unique forests without equivalent inside the country.

Opposite to the small area of interest the NDVI values of the bigger forest part of the reserve indicated that there is a substantial decrease (16.4 ha which is 6.8% of the total area covered with forest) in the size of the areas that are classified as forest in normal health. Such a high percent for a short period indicate that the changes in the canopy occur with a high intensity.

In the East and the South part of the Parangalitsa reserve, where the dominant species are *Picea abies* L./H, *Abies alba* M., the

values of the NDVI indicated that this part of the reserve is occupied by vegetation in a bad shape or already dry trees. Furthermore the analysis of the NDVI shows that between 2009 and 2017 the pixels that indicate dry or vegetation in bad health increase with 10.4 ha and that the most of the pixels that are affected by this change are distributed in these parts of the reserve (Table 3.). The total size of the classes with NDVI values between 0 and 0.40 in 2009 are 111.1 ha which is 46 % of the total forest area, that may be an evidence that negative trends in the vegetation health in Parangalitsa reserve are older than the time span of this research. Higher values of NDVI are also associated with the meadows and sparse, new broad leaf forests which can be observed at the whirlwinds spots. Some of this whirlwinds are older than 100 years and even this long period has not been enough for the restoration of the old coniferous forests.

The increase with 5.7 ha of the area with values of the NDVI higher than 0.9 is very well correlated with the increase in the areas whit NDVI values below 0.4 and this increase is mainly due to new broad leaf tree species inside the whirlwind activity. Evaluation of the changes in the vegetation health inside the big whirlwind spot that occurred between 1962-1965 along Haidushka river which marked the west border of the reserve represent a substantial scientific interest. Between 2009 and 2017 there is

no change in the size of this whirlwind spot, but there are some changes in the NDVI values. The number of pixels with NDVI values above 0.9 are smaller in 2017 than in the 2009 but also the number of pixels with NDVI values between 0.5 and 0.89 (good and very good health) are higher. This can be used as an evidence that in this whirlwind spot are running processes of restoration of the normal functionality of the forest vegetation. May be the reason for that is the fact that the fallen wood stock were extracted and the infected standing trees by Ips typographus also were fallen down. Probably because of that there are no increase in the numbers of the pixels with NDVI values that indicate dry or vegetation in bad health, inside the neighboring areas. This and the negative changes in the health status of the forest vegetation around the rest of the whirlwind spots that was disclosed in the previous chapter, can be used as an evidence that a new approach for biodiversity preservation inside protected areas is needed. This approach has to be aligned with the modern tendency of non-equilibrium perspective in the management of protected areas and the biodiversity preservation.

Conclusions

Based on the evidences disclosed in the previous chapters the following conclusions can be made:

• Five m. resolution satellite images even though are not good instrument for inventory of single dry trees, can be reliable source for analysis of the health status of forest ecosystems.

• Based on the NDVI values extracted from the satellite image captured in 2009 was concluded that the negative trend in the health status of the forests ecosystems inside Parangalitsa reserve started before the time span of this research.

• Between 2009 and 2017 the negative dynamic in the shape of the forest vegetation inside the boundaries of the reserve is still in tack. In 2017 the area with NDVI values that indicated vegetation in bad health is bigger with 10.4 ha than in the 2009.

• Due to the whirlwind activity the old forest of *Picea abies* L./H, *Abies alba* M., *Fagus sylvatica* L. are substituted with a new broad leaf vegetation.

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• The new whirlwind spots are distributed around the old ones and fallen trees inside are very good food based for vermin activity.

• The natural process of restoration of the forest vegetation in the old whirlwind spots which are situated between 1750 and 1900 m.a.b.s.l. is very slow and contemporary meadows and sparse forest are observed inside this spots.

• The forest vegetation inside the big whirlwind spot (occurred in the period 1962-1965) that is situated along the west border of the reserve is in very good health. The good health of the last is due to the fact that all fallen and infected trees were extracted in the past.

• The presented evidences confirmed the need to impose the modern approach of nonequilibrium perspective in the management of protected areas and biodiversity protection.

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Appendix 1. NDVI in 36 classes of the small area of interest based on the satellite image from 13.07.2009.

Row	Histogram	Color	Red	Green	Blue	Opacity	Class_Names	Area
0	4739		0	0	0	0	Unclassified	11.8475
1	233		0	0	0	1	Class 1	0.5825
2	630		0.106	0.106	0.106	1	Class 2	1.575
3	698		0.208	0.208	0.208	1	Class 3	1.745
4	529		0.267	0.267	0.267	1	Class 4	1.3225
5	313		0.298	0.298	0.298	1	Class 5	0.7825
e	238		0.322	0.322	0.322	1	Class 6	0.595
7	180		0.337	0.337	0.337	1	Class 7	0.45
8	179		0.353	0.353	0.353	1	Class 8	0.4475
9	175		0.365	0.365	0.365	1	Class 9	0.4375
10	194		0.38	0.38	0.38	1	Class 10	0.485
11	180		0.392	0.392	0.392	1	Class 11	0.45
12	192		0.408	0.408	0.408	1	Class 12	0.48
13	189		0.424	0.424	0.424	1	Class 13	0.4725
14	179		0.435	0.435	0.435	1	Class 14	0.4475
15	5 197		0.451	0.451	0.451	1	Class 15	0.4925
16	199		0.467	0.467	0.467	1	Class 16	0.4975
17	197		0.478	0.478	0.478	1	Class 17	0.4925
18	183	- 0	0.494	0.494	0.494	1	Class 18	0.4575
19	181		0.506	0.506	0.506	1	Class 19	0.4525
20	180		0.522	0.522	0.522	1	Class 20	0.45
21	193		0.537	0.537	0.537	1	Class 21	0.4825
22	2 179		0.549	0.549	0.549	1	Class 22	0.4475
23	174		0.565	0.565	0.565	1	Class 23	0.435
24	173		0.58	0.58	0.58	1	Class 24	0.4325
25	i 197		0.592	0.592	0.592	1	Class 25	0.4925
26	165		0.608	0.608	0.608	1	Class 26	0.4125
27	183		0.62	0.62	0.62	1	Class 27	0.4575
28	177		0.635	0.635	0.635	1	Class 28	0.4425
29	178		0.651	0.651	0.651	1	Class 29	0.445
30	150		0.663	0.663	0.663	1	Class 30	0.375
31	183		0.678	0.678	0.678	1	Class 31	0.4575
32	290		0.698	0.698	0.698	1	Class 32	0.725
33	407		0.729	0.729	0.729	1	Class 33	1.0175
34	602		0.773	0.773	0.773	1	Class 34	1.505
35	5 765		0.843	0.843	0.843	1	Class 35	1.9125
36	499		0.945	0.945	0.945	1	Class 36	1.2475

Appendix 2. NDVI in 10 classes of the small area of interest based on the satellite image from 13.07.2009.

lass10_ndvi_2009_large.img : Layer_1								
Row	Histogram	Color	Red	Green	Blue	Opacity	Class_Names	Area
0	209546		0	0	0	0	Unclassified	523.865
1	6280		0.004	0.004	0.004	1	Class 1	15.7
2	12510		0.192	0.192	0.192	1	Class 2	31.275
3	13506		0.314	0.314	0.314	1	Class 3	33.765
4	12139		0.4	0.4	0.4	1	Class 4	30.3475
5	10399		0.467	0.467	0.467	1	Class 5	25.9975
6	9683		0.529	0.529	0.529	1	Class 6	24.2075
7	9986		0.6	0.6	0.6	1	Class 7	24.965
8	11661		0.682	0.682	0.682	1	Class 8	29.1525
9	13610		0.792	0.792	0.792	1	Class 9	34.025
10	8260		0.941	0.941	0.941	1	Class 10	20.65

Appendix 3. NDVI in 36 classes of the small area of interest based on the satellite image from 20.07.2017.

class36_ndvi_small_2017.img : Layer_1								
Row	Histogram	Color	Red	Green	Blue	Opacity	Class_Names	Area
0	4739		0	0	0	0	Unclassified	11.8475
1	429		0	0	0	1	Class 1	1.0725
2	730		0.122	0.122	0.122	1	Class 2	1.825
3	592		0.208	0.208	0.208	1	Class 3	1.48
4	383		0.263	0.263	0.263	1	Class 4	0.9575
5	243		0.298	0.298	0.298	1	Class 5	0.6075
6	178		0.318	0.318	0.318	1	Class 6	0.445
7	134		0.333	0.333	0.333	1	Class 7	0.335
8	156		0.349	0.349	0.349	1	Class 8	0.39
9	185		0.365	0.365	0.365	1	Class 9	0.4625
10	164		0.376	0.376	0.376	1	Class 10	0.41
11	157		0.392	0.392	0.392	1	Class 11	0.3925
12	179		0.408	0.408	0.408	1	Class 12	0.4475
13	170		0.42	0.42	0.42	1	Class 13	0.425
14	174		0.435	0.435	0.435	1	Class 14	0.435
15	167		0.451	0.451	0.451	1	Class 15	0.4175
16	209		0.467	0.467	0.467	1	Class 16	0.5225
17	226		0.478	0.478	0.478	1	Class 17	0.565
18	188		0.494	0.494	0.494	1	Class 18	0.47
19	178		0.506	0.506	0.506	1	Class 19	0.445
20	177		0.522	0.522	0.522	1	Class 20	0.4425
21	214		0.537	0.537	0.537	1	Class 21	0.535
22	203		0.553	0.553	0.553	1	Class 22	0.5075
23	183		0.565	0.565	0.565	1	Class 23	0.4575
24	193		0.576	0.576	0.576	1	Class 24	0.4825
25	188		0.592	0.592	0.592	1	Class 25	0.47
26	182		0.608	0.608	0.608	1	Class 26	0.455
27	192		0.62	0.62	0.62	1	Class 27	0.48
28	206		0.635	0.635	0.635	1	Class 28	0.515
29	202		0.647	0.647	0.647	1	Class 29	0.505
30	216		0.663	0.663	0.663	1	Class 30	0.54
31	221		0.678	0.678	0.678	1	Class 31	0.5525
32	330		0.698	0.698	0.698	1	Class 32	0.825
33	443		0.729	0.729	0.729	1	Class 33	1.1075
34	615		0.773	0.773	0.773	1	Class 34	1.5375
35	756		0.839	0.839	0.839	1	Class 35	1.89
36	398		0.949	0.949	0.949	1	Class 36	0.995

Appendix 4. NDVI in 10 classes of the small area of interest based on the satellite image from 20.07.2017.

class10_ndvi_2017_large.img : Layer_1								
Row	Histogram	Color	Red	Green	Blue	Opacity	Class_Names	Area
0	209546		0	0	0	0	Unclassified	523.865
1	7678		0.071	0.071	0.071	1	Class 1	19.195
2	15517		0.227	0.227	0.227	1	Class 2	38.7925
3	14377		0.325	0.325	0.325	1	Class 3	35.9425
4	10872		0.4	0.4	0.4	1	Class 4	27.18
5	8593		0.467	0.467	0.467	1	Class 5	21.4825
6	7853		0.529	0.529	0.529	1	Class 6	19.6325
7	8206		0.596	0.596	0.596	1	Class 7	20.515
8	10601		0.682	0.682	0.682	1	Class 8	26.5025
9	13766		0.788	0.788	0.788	1	Class 9	34.415
10	10571		0.925	0.925	0.925	1	Class 10	26.4275

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The Demographic Structure of the Blagoevgrad District and the Challenges to the Protection of the Environment

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Abstract. Demographic problems are at the root of the various challenges in terms of the environmental protection. That is why the prospect of developing all kinds of activities in this direction requires much more serious analysis and evaluation. According to these reasons, an analysis of the available information on the demographic structure of the Blagoevgrad region, it isoffers a specific commentary and forecast of the prospects for the future activities for of the environmental protection.

Key words: demographic problems, environmental protection, demographics, Blagoevgrad.

Introduction

On the eve of 2020, it is important to pay more attention to the increasing issues of our environment. Looking at the situation more accurately, even with the naked eye, it is found that it is already 1/5 years (i.e. 20%) of the 21st century. Society is developing at an unknown pace, compared to the previous XIX and XX centuries, and quite different in the Middle Ages, and so on. In such a context, new objectives and tasks need to be set in front of the environmental protection activities and the forms and ways of their implementation need to be updated. It is no longer sufficient to take into account, as successes in this respect, the commitments for the construction of municipal waste water treatment plants, as these are the main commitments of the structures of urbanization and regional development. And if these commitments were not implemented or realized in the last twentieth century, for

some reason, this should not reassure us that adequate measures are taken to protect our environment in modern society.

In the context described, it is interesting to discuss a different treatment of the situation. We believe it would be useful to interpret the phrase of France's first female cosmonaut, Clonie Enire, that we no longer have to ask ourselves "how will we act", but just "by whom we will act"? what will society be to respond to the challenges of protecting our environment in the future.

In connection with the above, the question of the influence of the Man, incl. and the Society. And while this issue of climate change is still debating, there is enough argument for the role of Man and Society in our environment.

That is why we propose to make more specific analysis and comment on the information on population growth and the development of the Society (Fig. 1).

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Regarding the presented figure (Fig. 1) population (prepared from a number of sources, such as (WPS, 2019), and tailored to the topic discussed), it is necessary to note that studies on population growth trends in the world are quite popular in the world and in our country. But usually they are aimed at different demographic problems and those related to economies, the labor market and more. In this case, this type of information is discussed from the point of view of environmental protection activities and approaches to assessing anthropogenic impact on it.

It is noteworthy that the two-fold increase in population has committed periods of a very different duration - between more than 1000 years to 40 years. Over a long historical period (before the New Age and around the 17th Century) there was a twofold increase in population in 10-20-40 and more generations, whereas in the nineteenth century and then, the double increase in population was recorded for much shorter period (less than 100 years) - that is, from 1 to 5 generations.

Information on the double increase in population can also be an additional criterion for the role of Man in the environment in which he lives and the Society in which he develops. The amendment to this criterion, incl. and the dynamics with which it changes can be used as an additional indicator for assessing the impact of different human activities on environmental components (Fig. 2).

If we introduce the term "growth rate," we will find a very interesting trend.

For example, it is remarkable that the rate of population growth is very different and that environmental impact can be assessed through it.

Three very different periods can be distinguished.

One, very long, up to the 17th century - with a very small, almost invisible, rate of population growth (below 5 people/year).

The other, in the seventeenth century and i and early twentieth century - with a small them.

population growth rate (below 20 people/year).

The third, after the second half of the twentieth century - with a high rate of population growth (more than 60 people/year) and the beginning of the 21st century (more than 240 people/year).

The comparison between the first and third periods makes it possible to draw some important conclusions. For thousands of years, one has been able to live and develop in harmony with nature. Modernity puts a very interesting task to us - one must protect the surrounding environment (ie nature) in order to live and develop.

Material and Methods

The present study used (collected, processed and analyzed) data (Administrative Atlas of the Republic of Bulgaria, 2002; NSI, 2019) for the territory of the country (including Blagoevgrad region), presenting obtained and the results making recommendations to draw the public's attention to the need for its involvement in the idea of improving environmental activities.

Therefore, it is necessary to analyze in more detail the work on impact assessments on our environment. It should be noted that there are several "discrepancies" in the legal framework regarding the term "impact" in the field of our environment.

The information presented above shows that after the second half of the twentieth century it is naïve to expect that the various impacts on our environment may be "insignificant". Even the simple actions of ordinary people to meet their everyday needs, with the current number of people in the world, can have a "significant" impact on the components of the environment (depending on the specifics of the region).

Therefore, it should be noted that not complicating the procedures for environmental assessments (MICHAILOV, 2002) and the search for and application of quantitative measures will increase the role and importance of the overall activity on them.



Fig. 1. World population growth tendencies (data obtained from WPS, 2019).



Population of Blagoevgrad region by year 2011 (by age groups)

Fig. 2. Age structure of Blagoevgrad region.



Demographic structure of some villages in Blagoevgrad region

Fig. 3. Demographic structure of some villages in Blagoevgrad region.

Based on the comment made, we conducted a more specialized study on the future initiatives in the field of environmental protection at a relatively low regional level (Ordinance #256, 2010).

From the attached figure (Fig. 2), it is clear that the majority of the population of the Blagoevgrad region (in 2011) is up to 59 years of age, ie. young people, and in active age, and a much smaller proportion - aged 60 to 69, and even fewer - over 80 years of age.

These values are quite different for individual settlements, especially for some villages in the region, which can be illustrated by the data on some villages (Fig. 3). The structure of the population in this locality is completely different - the relative share of the young population (up to 29 years) is significantly smaller than the proportion of the adult population (over 60).

The above gives us reason to propose for a more serious discussion the subject of the meaning of the different activities for preservation of the components of our environment.

In this regard, as an indicator, we propose to use the so-called the *Anthropogenic Load Index* (C_{antrop}), which takes into account the impact of the different

municipalities, by processing the information on the number of inhabitants in the settlements and the activities they carry out in the construction of the various sites of the transport network, in the construction of infrastructure sites in settlements, as well as mining sites and landfills, and not least through agricultural activities, for manifestations of the population through which an activity of anthropogenic character is carried out.

$$C_{anthrop} = \frac{F_{agr.ar.} + F_{set.ur.} + F_{tt.inf.} + F_{m.w.}}{N_{mum}}$$

(ha/inhabitant),

where:

 $C_{anthrop}$ - anthropogenic load factor (index); $F_{agr.ar.}$ - agricultural areas in the municipalities; $F_{sctur.}$ - area of settlements and other urbanized areas; $F_{tt.inf.}$ - territory for transport and infrastructure; $F_{mw.}$ - area for minerals and landfills for waste; N_{mun} - residents of the municipality.

Results and Discussion

The results obtained are presented graphically (Fig. 4).

It is noteworthy that two distinctly different areas are differentiated, one

focusing more on the abscissa axis and the other one more on the ordinate axis.

This, on the abscissa axis, refers to the municipalities of $C_{antrop} < 10$ and the other, developed more by the ordinate axis, for the municipalities with $C_{antrop} > 10$ th.

The group with $C_{antrop} < 5$ until $C_{antrop} < 10$ includes 67 municipalities (about 25% of the municipalities in Bulgaria), of which 9 municipalities in Plovdiv region (11 municipalities), 8 municipalities in the Pazardzhik region (11 municipalities), 7 municipalities in the Blagoevgrad region (14 municipalities), 6 municipalities in the Sofia region (22 municipalities), 6 municipalities in the Smolyan region (10 municipalities) and 1-2 municipalities - in the other districts (usually the municipality of the district town).

According to the proposed criterion, Cantrop, located mostly on the ordinate axis, is about 75% of the municipalities in Bulgaria. They include Treklyano municipality with C_{antrov} = 130.37 i.e. the anthropogenic activities of the population of the municipality are quite modest compared to the territory of the municipality. Compared to the other group, for Varna municipality, this coefficient is the opposite, = 0.51 i.e. with verv distinct Cantrop anthropogenic interference.

Curious results are obtained using this coefficient, also determined by population data by the end of 2017 (Ordinance #256, 2010). The following (more interesting) classification may be offered.

Extreme values for the level of anthropogenic interference are only available for three municipalities. These are the municipalities of Plovdiv, Varna and Sofia-city, for which the coefficient (index), C_{antrop} , is less than 1.

It is worth noting that with the increase and decrease of the population in the different types of urbanized territories (irrespective of whether they are cities or villages) there are problems in the management, development and protection of the surrounding environment.

The different values obtained by the proposed criterion (coefficient, index), *C*_{antrop},

are quite varied, which is an indication of the necessity of changes in regional policy for all activities, including these for protection each local environment.

In the near and far-off perspective, we will have much more serious and farreaching tasks that will require much more serious attitude towards the education of specialists, which will be able to solve the problems of Society related to them – organizational (Environmental Protection Law, 2002), legal (Administrative Atlas of the Republic of Bulgaria, 2002; MICHAILOV, 2002; NSI, 2019), technical, social (NSI, 2019; Ordinance #53, 2003), etc.

Due to the above findings and conclusions, it is necessary to take into the specific position account of the Blagoevgrad region (Fig. 5, Table 1) in relation to the discussed issues. This area is one of the most distinctive areas with more significant anthropogenesis of the individual municipalities, which will (inevitably) affect the environmental protection activities in the future. It is therefore appropriate to note the need for a deeper study of the processes on especially territory, given its the demographic projections for a population decline in the area in 2080 by about 45%.

The situation described here is quite intriguing. On the one hand are the problems of the Society - demographic, economic, educational, social and others. and on the other hand, the challenges of the environment. Regardless of the different types of forecasts, we cannot expect significant changes in the trends in the movement of the population towards the larger cities and economically developed territories, including the depopulation of other settlements and territories, but at the same time, the challenges facing the different activities they will become increasingly important in protecting our environment.

In this regard, it is imperative to declare that it is necessary to envisage (forecast), plan, and implement activities in the field of environmental protection in all spheres of the Company's activities - administrative,

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educational, production, social, etc. Because the basic rule is - the best means of conservation is the rational use of raw materials and resources.



Fig. 4. Anthropogenic Load Index (*C*_{antrop}) – according by municipalities populations (2017).



Fig. 5. Levels of anthropogenic impact of the municipalities in the Blagoevgrad region.

Municipality	C_{antrop}
Bansko	14.11
Belitsa	7.37
Blagoevgrad	4.48
Gotse Delchev	4.58
Garmen	7.75
Kresna	15.42
Petrich	5.92
Razlog	8.74
Sandanski	10.33
Satovcha	8.70
Simitli	12.50
Strumiani	20.44
Hadjidimovo	16.25
Iakoruda	11.50

Table. 1. Levels of anthropogenic impact ofthe municipalities in the Blagoevgrad region.

Conclusions

The following conclusions and recommendations can be proposed:

1. The current attitude towards demographic problems is not in line with the challenges of the various activities and the problems they raise with regard to the maintenance of life and the need to preserve our environment.

2. It is imperative to update some subnormal documents (ordinances, guidelines, etc.) related to environmental protection activities due to significant discrepancies with the requirements for these activities.

3. In relation to the issues under consideration these forecasts are not very encouraging.

4. It is desirable to continue the analyzes and the above considerations for the other municipalities in Bulgaria, in order to obtain more detailed information on the level of environmental protection activities, as well as the expectations of the Society for achieving a favorable environment for life.

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Synopsis

Modelling of Water Systems in a Convenient Way

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Abstract. Protection of water resources requires many decisions, which, in turn, can be aided by a model of the respective water system. Such a model then can be used, for example, for the analysis of various scenarios (of external forces) and various action options (which can be influenced). On the other hand, modelling often is perceived as something hard or laborious to do (and often, it is). This paper presents some examples of modelling concepts and of their implementation in an easy-to-use simulator, focusing on the combination of dynamic process models with water-balance models, assisting in the management of water resources. The contribution presents examples from various countries.

Key words: biochemical processes, modelling, river water quality, Water-Energy-Food Nexus, water systems, wastewater treatment plant.

Introduction

Management of water resources and water systems becomes ever more important. This holds true in particular in times of climate change and increased pollution of water bodies. Among the core tools supporting sustainable water management, mathematical models have emerged and are used for a wide range of tasks (BACH et al., 2014). Models are considered as simplified representations of reality, often in a simplified way, aiming at assisting in certain tasks. Such tasks include not only design and operation of water systems, but also the visualisation (also to the nonexpert) of relevant processes and interactions thus models can play an important role in stakeholder participation and decision making processes. Furthermore models are useful for education and system understanding.

On the other hand, also a certain reluctance about the use and application of models can be

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg observed. Notwithstanding the fact that modelling indeed involves a number of challenges to be addressed in any given modelling task, this paper aims to motivate the application of modelling in water management, illustrating the use of models for a number of examples. Model application is also greatly enhanced when a user-friendly and easy-to-use simulation system can be applied. The present paper illustrates modelling applications using the simulation framework Simba#, which emerged from 25 years' experience from research and application projects and now forms one of the key simulation systems in water management applied in research and practice all over the world.

Why modelling water systems?

When buying a car, it is generally accepted to be prudent to do a test drive before taking the final purchase decision. Such a test drive can

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assist to identify strong and weak characteristics of the car and, together with a large number of criteria usually considered (e.g. purchase price, operational costs, demand on petrol, space, colour, ...), forms an important base for the decision of whether to buy the car or not. When decisions on water systems, for example, construction or extensions of wastewater treatment plants are to be made - wouldn't it also be desirable to do a "test drive" first. But how to do that, before the plant has been built? Here, modelling can assist - models, even if not calibrated and validated against data, can provide an estimate on the behaviour of the plant to be built. Design and operational options can be tested using the model, often leading to a better design of the plant and/or its better operation, resulting in considerable cost savings or reduction of energy consumption.

Obstacles to modelling of water systems

Whilst dynamic modelling of the physical, biological and chemical processes in wastewater treatment is quite widespread, using, for example the Activated Sludge Models of the International Water Association (HENZE *et al.*, 2000) and their implementation in various computer simulation programs, and, whilst also river water quality modelling is advocated, using, for example, the River Water Quality Model of the International Water Association (SHANAHAN *et al.*, 2001), or less complex models, such as those of the QUAL2 family, there is also some reluctance in uptake and use of modelling when addressing practical issues of water management.

Some obstacles to application of modelling are based on the perception that always a calibration-validation detailed process is necessary prior to any model application. For many applications this is true, and any step taken to increase confidence that the model is able to represent reality to a sufficiently accurate degree for the study under question is appreciated; however, for many applications modelling approaches these days have been established to such a degree that, at least for typical conditions (e.g. wastewater treatment modelling for typical domestic wastewater and

common plant layouts), models can well be applied using default parameters. For example, DZUBUR *et al.* (2019) discuss a methodology for estimating wastewater treatment plant influent characteristics under data-scarce conditions.

Another obstacle to practical application of models is seen in the lack of user-friendliness of simulation software. This indeed can be quite a limiting constraint, as in daily practice – such as often in engineering consultancies and water authorities - not everyone is a modelling specialist as many other tasks are also to be carried out in daily routine. Based on this experience and on over two decades experience in cooperation with consultancies and water authorities and companies, the dynamic simulation environment Simba# has emerged, which meets the needs of practice - easy-to-use and quick-to-comprehend simulator, but also provides sufficient flexibility to the researcher and experienced modeller (see, for example, the feature to modify the pre-defined biochemical process models, such as those by HENZE et al., 2000, or to define and solve the user's own sets of differential equations for biochemical transformations in a user-friendly way) (ALEX et al., 2013, IFAK, 2018). The present paper illustrates some examples of modelling applications of the Simba# simulator and its extensions for different areas of water management.

Dynamic modelling of wastewater treatment plants

As an example, Fig. 1 illustrates a simple dynamic model for wastewater treatment plants, which also can be used to model (and to optimise) operation of the plant. The various components of the model (clarification and aeration tanks) simulate the physical and biochemical transformation processes, using, in this example, the Activated Sludge Model No. 3 (HENZE *et al.*, 2000).

Assessing of impacts of urban discharges on river water quality

River water quality in urban areas is often affected by discharges from sewer systems (e.g. combined sewer overflows), from wastewater

treatment plants (treated effluents) and M3/M7 guidelines in Germany (BWK, 2004). industrial discharges. These discharges are of quite different nature (e.g. treatment plant effluents have usually lower contents of readily biodegradable organic matter (SS fraction of Chemical Oxygen Demand) than combined sewer overflows) and their impacts are overlapping – thus making simple mixing calculations inappropriate. In order to assess the impacts of such discharges on river water quality - after cooperation projects between biologists and engineers - criteria related to duration and frequency of exposure to critical is combined, within the same simulation concentrations of Dissolved Oxygen and ammonium/ammonia (with their balance affected by temperature and pH of the water body) have been established in the British Urban Pollution Management Manual (FWR, 1998) and, in a similar manner, in the BWK- SCHÜTZE et al., 2017) (Fig. 2).

Driven by the request of the Ministry of the Environment of the German Federal State of Hesse to develop an easy-to-apply river model, the Simple Water Quality Model (SWQM), considering the main processes related to Dissolved Oxygen and ammoniacal nitrogen (reaeration, decay, nitrification, ...) has been developed (SCHÜTZE et al., 2011, HMUELV, 2012) and implemented in the Simba# simulation framework.

The treatment plant model shown above environment, with a river model using the Simple Water Quality Model and with a hydrological sewer system model, thus forming an integrated wastewater model of a typical city in Germany (Astlingen, see



Fig. 1. Example model of an activated sludge treatment plant.



Fig. 2. Integrated model of the Astlingen wastewater system.

Modelling of Water Systems in a convenient way

Such an integrated modelling setup then allows to analyse the impact of discharges and of operational measures on the river. For example, Fig. 3 and Fig. 4 compare the effects on Dissolved Oxygen of (a) limiting the influent to the wastewater treatment plant to its standard setting of 330 l/s and (b) permitting the inflow to the WWTP to be increased for one hour. MUSCHALLA *et al.* (2009) provide useful guidance to carrying out such integrated modelling studies.

Visualisation of resource fluxes in sanitation systems

Currently, there is a significant amount of discussion in Germany - and elsewhere about new and alternative sanitation systems. Attempts are called for to reduce water demand for flushing toilets and to make better use of the resource "wastewater", calling also for increased recovery of its nutrients contents. In order to aid such discussions, a simple simulator ("SAmpSONS"), modelling and visualising resource fluxes of user-defined sanitation systems has been developed (SCHÜTZE et al., 2019a), based earlier on work by ORMANDZHIEVA et al. (2014). Fig. 5 illustrates a simple example of a sanitation system with separate greywater treatment and codigestion of kitchen waste and blackwater.

Combining system modelling with Life Cycle Analysis

When discussing upgrades or changes (waste)water systems, a number of to different criteria are to be considered. These often include, besides capital and operational expenditure, criteria such as energy consumption/generation, emissions greenhouse gases, eutrophication of potential, among others. Traditionally these are estimated in separate calculations, sometimes involving additional, often costintensive, software. Within the SAmpSONS simulator (a freely available subset of the Simba# simulation system, ifak.eu), these calculations - often forming part of separate

Life Cycle Assessment (LCA) studies – are integrated with the process simulation modules. Therefore the mentioned criteria are calculated – once input data, such as unit process costs, have been provided – as a byproduct of the process simulation.

Holistic system modelling

Extending the modelling scope of urban wastewater modelling to a wider system perspective, also Integrated Urban Water System models (according to the classification by BACH et al., 2014) can be implemented. Such models, covering the water supply system _ including groundwater resources, albeit in a rather simplified way - can assist in overall water management, in particular in water-scarce regions. In a cooperation with researchers from Israel, a (simplified) overall model of a general urban water system has been set up (see Fig. 6). This is currently being adapted to selected case study cities in Israel and in Germany. The modelling modules implemented here allow the special consideration of water demand (communicated "from right to the left") and water availability (communicated "from left to the right"), attempting to balance availability with demand of water resources. Stochastic modelling of rainwater tank utilisation allows to find optimum tank sizes and operational strategies of rainwater tank usage (SNIR et al., 2019, SCHÜTZE et al., strategies 2019b). Reuse of (treated) greywater and wastewater streams provide important feedback loops in the system and could be shown to alleviate pressure on scarce water resources.

Modelling the Water-Energy-Food-Waste Nexus

Considering that the water and wastewater system constitutes just one element of the urban infrastructure system or "urban metabolism", prudent use of the urban infrastructure would also consider the interactions and potential synergies of the water, energy, solid waste and urban food systems. Whilst some interactions between are these subsystems obvious (e.g. demand electricity for pumping and treating of water), others might appear, whilst equally important, less obvious. For example, energy generation from waste incineration or from co-digestion of wastewater sludge and kitchen waste, production of fertilizer from waste composting and others) constitute relevant transsectoral interactions. Modelling and visualising related resource fluxes and, thus, increasing system awareness among stakeholders, also might assist in strategic pre-planning of urban infrastructure systems, considering water, energy, food and waste in a joint manner.

Within the "Rapid Planning" project (rapid-planning.net), currently models of the cities of Da Nang (Vietnam) and Kigali (Rwanda) are being built, which assist in this preplanning process (Fig. 7). These models assist in the analysis of various scenarios of future developments of these cities and their resource and infrastructure needs. The underlying modelling principles are described by RAMÍREZ CALDERÓN et al. (2015) and ROBLETO (2019). This work, thus, has a wider scope than other Nexus-related studies such as, for example, the study by LANDA CONSIGNO et al. (2019), who focussed mainly on the water system.

Fig. 7 shows the model setup of the infrastructure systems of the rapidly growing city of Da Nang. Fig. 8 provides a concise summary of the main resource fluxes obtained from the detailed simulation of the "transsectoral" future scenario of the city of Da Nang. This diagram representing some of

the material flows in the system is a byproduct of the detailed simulations carried out. Whilst it is indeed a challenge to select and to represent the "most interesting" simulation results for the decision maker and end-user, such visualisations can help to increase the understanding of the importance joint planning of of infrastructure systems.

Conclusions

This has illustrated paper some applications of modelling in water system management in different contexts. Modelling examples range from detailed biochemical process kinetics modelling in wastewater treatment and river water quality over modelling and visualisation of nutrient fluxes in sanitation systems to modelling of the entire Water-Energy-Food-Waste Nexus of agglomerations. Such large modelling applications appear now to be more feasible than ever before as simulation systems have gained considerably in user-friendliness both for the novice user as well as for the simulation expert in research. It is believed that model building and application could be useful complements to field-work based ecological studies such as those carried out by IHTIMANSKA *et al.* (2014). Also novel modelling concepts, such the linkage of modelling with process LCA-type calculations within the same modelling framework and the integration of availabilitydemand balancing with process modelling have been demonstrated. It is hoped that this paper stimulates additional modelling applications, motivated by the statement that "all models are wrong, but some are useful."



Fig. 3. Dissolved Oxygen results in selected river sections of the Astlingen system – Inflow to WWTP restricted to default.

Modelling of Water Systems in a convenient way



Fig. 4. Dissolved Oxygen results in selected river sections of the Astlingen system – Inflow to WWTP temporarily increased.



Fig. 5. Example of a sanitation system modelled in the SAmpSONS simulator.



Fig. 6. Model of a generalised water system, including rainwater harvesting, groundwater abstraction, greywater and wastewater reuse.

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Fig. 7. Model setup of Da Nang (Vietnam) (SCHÜTZE & ROBLETO, 2019).



Fig. 8. Summary of main resource fluxes in Da Nang (Vietnam) in a transsectoral scenario (SCHÜTZE & ROBLETO, 2019).

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Synopsis

Monitoring Ozone Effects on Vegetation: A Review

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Abstract. One of the most phytotoxic air pollutants is ozone, which can cause considerable damage to vegetation: visible leaf injury, reduction in yield quantity and quality and reduction in photosynthesis, alterations to carbon allocation, and in the sensitivity to biotic and abiotic stresses. Ozone is a secondary pollutant and prevailed at high concentrations over rural regions. Moreover, ozone concentrations are expected to increase in the future and will continue to be a serious threat to crop productivity. This paper presents a comprehensive review the undertaken studies of ozone's impacts on crops and natural ecosystems.

Keywords: Ozone, Monitoring, Plants, Crops.

Introduction

Ozone is a naturally occurring chemical that can be found in both the stratosphere (in the 'ozone layer', 10 – 40 km above the earth) and the troposphere (0 - 10 km above the earth). Ground level ozone (O_3) is the atmospheric pollutant that is most likely to threaten food production across the globe due to its phytotoxicity and prevalence over important regions of Europe, North America and Asia (FUHRER & BOOKER, 2003; ROYAL SOCIETY, 2008). There is always a background concentration of ozone at ground level resulting from natural sources of the precursors and stratospheric incursions. The formation of ozone is due to a large number of photochemical reactions taking place in the atmosphere and depends on the temperature,

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg humidity and solar radiation as well as the primary emissions of nitrogen oxides and volatile organic compounds - VOCs (ASHMORE, 2005; BYTNEROWICZ et al., 2007). Tropospheric (ground-level) O_3 is a secondary pollutant, which is not directly emitted into the atmosphere, but is formed from chemical reactions in the presence of sunlight, and natural and anthropogenic precursor gases (mainly NOx and VOCs). Tropospheric O3 is characterised by complex formation mechanisms based on the photooxidation of VOCs in the presence of NOx, following non-linear formation pathways: NOx are involved in O₃ formation but also removal through titration (the reaction of O₃ with NO to form NO_2 and O_2) (MONKS *et al.*, 2015). Together with the non-linear

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relationships between the primary emissions and the ozone formation, these effects complicates the abatement strategies for ground-level ozone and makes photochemical models crucial in addition to (EMEP, the monitoring data 2016). photochemical Additional reactions involving NOx, carbon monoxide and nonmethane volatile organic compounds (NMVOCs) released due to anthropogenic emissions increase the concentration of ozone in the troposphere (JENKIN & CLEMITSHAW, 2000). Ozone is the most pervasive phytotoxic air pollutant affecting natural ecosystems, forest health and an important air pollutant that affects both vegetation and human health (U.S. EPA, 1997, SKARBY et al., 1998, FINLAYSON-PITTS & PITTS, 2000, AINSWORTH *et al.*, 2012).

Additional tropospheric ozone is formed from complex photochemical reactions from fossil fuel burning in industrial and transport activities. These emissions have caused a steady rise in the background ozone concentrations in Europe and the USA since the 1950s (ROYAL SOCIETY, 2008). Ozone episodes can cause short-term responses in plants such as the development of visible leaf injury (fine bronze or pale yellow specks on the upper surface of leaves) or reductions in photosynthesis (FINLAYSON-PITTS & PITTS, 2000). Frequent episodes can occur longerterm responses such as reductions in growth and yield and early die-back (EMBERSON et al., 2000; PLEIJEL et al., 2007). An important sink for the greenhouse gases carbon dioxide (CO_2) and ozone is terrestrial vegetation. The air pollutant ozone has a negative impact on cell metabolism and growth of ozonesensitive plant species. Hence, this will result in a positive feed-back to global warming as less CO_2 and ozone will be sequestered by vegetation, resulting in a further rise of their concentrations in the atmosphere (SITCH et al., 2007).

The definitions "injury" and "damage" are important in evaluating the vegetation response to a pollutant (GUDERIAN, 1977). Injury can be damage, or the consequences of injury can become damage if the injury can cause a subsequent impairment of the intended use of the Damage plant. to vegetation from pollutants can occur without visible symptoms of injury, but damage cannot occur without some form of injury preceding damage (GUDERIAN, 1977). There is an urgent need to be able to assess the current and future risks from O3 exposure to crops and natural ecosystems.

Ozone impacts on crops and natural ecosystems

Ecosystems complex systems are comprising animal, plant and microorganism communities, together with the non-living environment (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005), these systems are dynamic whilst maintaining some essential resilience to natural disturbances. Earth's ecosystems provide an array of services upon which humans depend for food, fresh water, disease management, climate regulation, aesthetic spiritual enjoyment fulfilment and (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005). The role of biodiversity in ecosystem services is often rather unclearly stated - biodiversity is sometimes considered as a separate service and vet is implicit in most ecosystem services.

Indirect drivers of ecosystem change are associated with demographic, economic, socio-political and cultural or religious changes, and advancements in science and technology. Stressed or degraded ecosystems do not have the resilience or rebound capacity of pristine/unstressed systems (RAPPORT & MAFFI, 2009).

Human influence extends into even the remotest landscapes and more often than not has a pervasive influence on the ecosystems they support, frequently irreversibly changing biodiversity. Extinction rates of species are now estimated to be 1,000 times greater than historical background levels (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005; MANTYKA-PRINGLE et al., 2012), Recent studies have identified linkages between changes in ecosystem

functioning and biodiversity, highlighting the higher concentrations) can cause stomata importance of adopting a multi-sectoral (MAESTRE et al., 2012; MACE et al., 2012). Ecosystem services can be classified into provisioning, regulating, supporting and cultural services (MACE et al., 2012). Metaanalyses of published data on effects of species loss on the key ecosystem processes of productivity and decomposition have shown how important species loss is in ecosystem service delivery (HOOPER et al., 2012). Species losses of 21 - 40% reduced plant productivity by 5 – 10%, an equivalent amount of reduction as that estimated for effects of UV light and global warming. The study also indicated that species losses of 41 - 60%, as projected for global extinctions by the end of this century, is predicted to result in a 13% biomass loss, a similar amount to that predicted for ozone effects alone. In a similar study, MANTYKA-PRINGLE *et al.* (2012), investigated the synergies between climate change and habitat loss for explaining biodiversity loss.

reduces Ozone whole plant photosynthesis by directly impacting on the photosynthetic machinery (Rubisco and industrial revolution has been responsible for chlorophyll content), reducing leaf area by senescence promoting early and abscission, diverting carbon (C) use into detoxification and/or repair metabolism, changing stomatal conductance (both increases and decreases have been noted, and altering C allocation in favour of the above ground parts rather than below ground parts. Carbon flux to and from the soil is also altered by changes in leaf litter quality, altered rhizodeposition of C, changes in soil microbial community composition, and altered soil processes (AINSWORTH, 2017). Tropospheric ozone has the capacity to impact on nutrient cycling by both direct and indirect mechanisms: All of these have the capacity either, independently or in concert, to ultimately reduce the long-term sustainability of ecosystems (LINDROTH et al., 2001, SUN et al., 2012). Tropospheric ozone is known to alter stomatal responses to environmental and in the short term (at gas causing global warming (IPCC, 2007).

(leaf pores) to close, however, under approach to policy and decision making prolonged chronic exposure (at lower concentrations) many reports document ozone-induced stomatal opening or loss of stomatal sensitivity to closing stimuli, such as drought, light and humidity (MILLS et al., 2013). Ozone damages crop plants by, for example, reducing photosynthesis, causing a vellowing of leaves and leaf loss, decreased seed production and reduced root growth, in turn resulting in reduced yield quantity and/ or quality and reduced resilience to other stress such as drought. As a consequence, the key components of the food system that ozone interferes with are the productivity of crops, the nutritional value and the stability of food supplies as ozone concentrations and therefore impacts vary from year to year. Some of the world's most important food crops are sensitive (wheat, soybean and other pulses) or moderately sensitive (maize, rice, potato) to ozone and effects on the yield of these crops are of global significance (MILLS & HARMENS, 2011). A recent analysis has suggested that the increase in ozone since the reduction а in photosynthesis of leaf approximately 11% in trees (WITTIG et al., 2007), which may have reduced tree productivity by approximately 7% (WITTIG et al, 2009). In general, deciduous trees tend to be more sensitive to ozone than coniferous trees, with ozone sensitive species present across most of Europe (WITTIG et al., 2009). If ozone concentrations are high enough to reduce photosynthesis and/or above-ground plant growth, then less CO₂ and ozone will be absorbed by the leaves of vegetation, leading to a positive feedback to atmospheric CO₂ and ozone concentrations and therefore more global warming (SITCH et al., 2007). It has been estimated that ozone deposition to vegetation reduces tropospheric ozone concentrations by as much 20% (ROYAL SOCIETY, 2008). This is an especially significant function of vegetation given that ozone is the third most important greenhouse
Typical effects of ozone on sensitive species include: accelerated aging and changes in biomass, resource allocation and/ or seed production. Each of these can impact on the vitality of component species of plant communities, potentially altering plant biodiversity as well as that of the animals, fungi, bacteria and insects that live in close association with plants or in nearby soils. Ozone-induced changes in species diversity or shifts in species balance will impact on many ecological processes, thereby impacting on ecosystem services, flows, goods and values. Effects on species balance have been widely reported from controlled exposure experiments, but a less clear picture emerges studies from field-based with long established communities and from field surveys. Although more studies are needed, it is clear that impacts of ozone are of particular concern for global biodiversity hotspots (MILLS et al., 2013). Importantly, field validation of effects observed under experimental conditions is still lacking for many species and plant communities. Indirect effects remain mostly unknown, despite the fact that they are probably of great importance in terms of assessing ozone effects on ecosystem biodiversity.

Ozone biomonitoring

Ozone biomonitoring is a detection and monitoring technique that includes documenting ozone-induced visible injury to known ozone-sensitive species under conditions of ambient exposure. Ozone is routinely monitored throughout the world and data are mostly recorded as hourly or half-hourly averages.

The Swiss chemist Schönbein in the mid-1800s made the earliest measurements of ground-level ozone, using passive exposure of "test papers" impregnated with potassium iodide (cited in LONDON, 1985).

Tropospheric ozone can be monitored with mechanical monitors, or passive, cumulative, total exposure samplers (MANNING *et al.*, 1996; BYTNEROWICZ *et al.*, 2002; HUNOVA *et al.*, 2003). Mechanical

expensive and require monitors are electricity and a safe climate-controlled shelter for effective operation. Passive samplers are easy to use, inexpensive and require no electricity and have been used in forested, wilderness and remote region, especially in Europe, to identify locations where and areas ambient ozone concentrations exceed normal background levels and resulting pollution levels of ozone may injure sensitive plants. They can be colocated with mechanical monitors. Combining cumulative monitoring data accurate with GIS techniques allows depiction of air quality for large geographic regions (BYTNEROWICZ et al., 2002).

The dose-response relationships have been used to assess risk, either by quantifying yield losses for economic crop loss estimates (ADAMS et al., 1989) or by critical levels mapping exceedance 2007). Dose-response (SIMPSON et al., relationships are central to risk assessments since they provide the link between a pollutant dose and a plant response of concern (EMBERSON et al., 2003). Such relationships would be derived from coordinated standardised experimental campaigns assessing crop response to a range pollutant concentrations of (UNSWORTH & GEISSLER, 1992). China, India, Japan and Pakistan have investigated a wide range of crop species and cultivars using a variety of experimental methods and design (EMBERSON et al., 2001, 2003; MAUZERALL & WANG, 2001).

Open-top chamber have been used in multi-year studies to estimate the long-term impacts of ozone on growth of tree seedlings or saplings. (HEAGLE et al., 1973). Dose/response studies can be done by adding ozone to ambient air and results have been used to determine air quality standards for ambient ozone (US EPA, 1996). Typically, cylindrical open-top chambers are placed over field plots of soilgrown plants and supplied with filtered air, non-filtered air, or non-filtered air with ozone added. Such chambers provide climatic conditions that are similar, but not identical, to those outside (COLLS *et al.*, 1993), and thus some reservations about extrapolation to field conditions remain. This would suggest that open-top chamber data would tend to overestimate the adverse effects of a given ozone concentration (PLEIJEL *et al.*, 1994; FUHRER, 1994).

Ozone phytomonitoring

Ozone pollution has been shown to have an adverse effect on tree growth and alter tree succession, species composition, and pest interactions (FOREST HEALTH AND OZONE, 1987; MILLER & MILLECAN, 1971; SMITH, 1974). In addition, we know that ozone causes direct foliar injury to many species (SKELLY et al., 1987; TRESHOW & STEWART, 1973). We can use this visible injury response to detect and monitor ozone stress in the forest environment. Bioindicators are plants that exhibit typical ozone injury symptoms in the field. Many have been identified and verified and good methodology is available for field surveys in conjunction with active ozone monitors or passive ozone samplers (MANNING et al., 1990, 2002; KRUPA et al., 1998; INNES et al., 2001). A useful bioindicator plant may be a tree, a woody shrub, or a nonwoody herb species. The essential characteristic is that the species respond to ambient levels of ozone pollution with distinct visible foliar symptoms that are easy to diagnose. Field studies and experiments have identified ozone sensitive species and characterized the ozone specific foliar response for bioindicators (DAVIS & UMBACH 1981; DUCHELLE & SKELLY 1981; KRUPA & MANNING, 1988; MAVITY et al., 1995; BRACE, 1996).

During the years 1994, 1995 and 1996 participants of the ICP Vegetation conducted studies in ambient air using the ozone-protectant ethylenediurea (EDU) at experimental sites and/or in commercial fields (BALL *et al.*, 1998). Species tested in this way included subterranean clover (Trifolium subterranean), bean (Phaseolus vulgaris), radish (Raphanus sativus), white clover (Trifolium repens), red clover (Trifolium pratense), tomato (Lycopersicon soybean esculentum), (Glycine max), watermelon (Citrullus lanatus) and tobacco (Nicotiana tabacum). Between 1996 and 2006, ICP Vegetation biomonitoring the programme has involved exposure of an ozone sensitive biotype of white clover (Trifolium repens Regal, NC-S) to ambient air (HAYES et al., 2007; MILLS et al., 2011). Since 2008, participants of the ICP Vegetation have been conducting biomonitoring campaigns using ozonesensitive (S156) and ozone-resistant (R123) genotypes of Phaseolus vulgaris (Bush bean, French Dwarf bean). The bean genotypes were develop (REINERT & EASON, 2000) and tested as a bioindicator system (BURKEY et al., 2005) in the USA. In 2012, experiments were conducted with ozone-sensitive and ozone-resistant bean at nine sites across Europe and one in the USA.

Sixteen species of native detector plants for ambient ozone have been identified for use in Central and Eastern Europe. They include the forbs *Alchemilla sp., Astrantia major, Centuarea nigra, Centauria scabiosa, Impatiens parviflora, Lapsana communis, Rumex acetosa* and *Senecio subalpinus;* the shrubs *Corylus avellana, Cornus sanguinea* and *Sambucus racemosa;* the trees *Alnus incana, Pinus cembra* and *Sorbus aucuparia;*. and the vines *Humulus lupulus* and *Parthenocissus quinquefolia* (MANNING *et al.,* 2002).

Some of the most important native (or naturalized) tree and shrub species that symptoms showed ozone-like in Mediterranean countries (Spain, Italy, Israel) are Abies cephalonica, Acer platanoides, Acer pseudoplatanus, Ailanthus altissima, Arbutus unedo, Alnus incana, Betula pendula, Carpinus betulus, Cornus spp., Corylus avellana, Crataegus spp., Fagus sylvatica, Frangula alnus, Fraxinus excelsior, Fraxinus ornus, Juglans regia, Morus spp, Myrtus communis, Pinus halepensis, Pinus pinea, Pistacia lentiscus,

Pistacia terebintus, Populus spp., Prunus amygdalus, Prunus avium, Prunus serotine, Prunus spinose, Robinia pseudoacacia, Rhamnus spp., Rosa spp., Salix spp., Sambucus spp., Tilia spp., Ulmus glabra, and Viburnum spp. (BUSSOTTI & GEROSA, 2002).

Similar studies were conducted in Asia and North America for wheat (Triticum aestivum L.) and rice (Oryza sativa L.), and group of crop species (legumes: soybean (Glycine max L.) and mung bean (Vigna radiate (L.) R. Wilczek) (EMBERSON, 2009) and Fagus sylvatica in Swistzerland (CLARK et al., 2000). Maize (Zea mays L.) is the receptor of interest in the main maize producing countries, i.e. South Africa, Zambia and Zimbabwe (VAN TIENHOVEN et al., 2006). Phytotoxic ozone effect at urban and rural sites in Bulgaria were assessed with seedlings of ozone-sensitive (Quercus robur L.), (Fraxinus excelsior L.,) and ozone-tolerant (Quercus rubra Michx.) (PARVANOVA et al, 2003; 2009).

Conclusions

Based on the reviewed literature data we conclude that:

A combination of scientific experiments, mathematical models and predictions of pollutant emissions revealed that ambient ozone concentrations are sufficient to cause impact on vegetation.

Planting ozone sensitive species is a useful tool for demonstrating the occurrence of visible leaf injury in ambient conditions.

Trees, shrubs, forbs and vines could be apply to assess the symptoms of probable ozone injury in the vicinity of passive ozone samplers or active ozone monitors in forest condition networks in mostly mountainous regions.

Ozone-sensitive plant species include crops such as wheat, soybean, potato, tomato beans and pulses; trees such as beech, birch, Norway spruce, poplar, oak; and (semi) natural vegetation (represented by *Trifolium* spp. (clover family) and provisionally *Viola* spp.

Ozone concentrations in forest areas appear to be high enough and of sufficient duration to cause foliar injury on a wide variety of native plants.

Ozone remains an important phytotoxic air pollutant. Economic impacts are also possible if growth rates of commercially important tree species are reduced. There is a need to quantify the effects of anthropogenically induced ozone on forest growth in order to establish the economic consequences for the wood industry.

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ECOLOGIA BALKANICA - INSTRUCTIONS FOR AUTHORS (2020)

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In the *Acknowledgements* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

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Example: Management plan for the protected area for birds BG 0002086, "Rice Fields Tsalapitsa". (2013). Retrieved from https://plovdiv.riosv.com (In Bulgarian)

Proceedings or book chapter:

Author, A. (Year of Publication). Title of work. In A. Author (Ed.). *Title of the book or proceedings*. (Edition, pp. XX-XX). Publisher City, Country: Publisher.

Author, A., & Author, B. (Year of Publication). Title of work. In A. Author, & B. Author (Eds.). *Title of the book or proceedings.* (Edition, pp. XX-XX). Publisher City, Country: Publisher.

Author, A., Author, B., & Author, C. (Year of Publication). Title of work. In A. Author, B. Author, & C. Author (Eds.). *Title of the book or proceedings.* (Edition, pp. XX-XX). Publisher City, Country: Publisher.

Software:

Author, A. (Year of Publication). *Name of software*. Vers. XX. Retrieved from http://xxxx

Example:

StatSoft Inc. (2004). *STATISTICA* (*Data analysis software system*), Vers. 7. Retrieved from http://www.statsoft.com

Website:

Author, A. (Year of Publication). *Title of page*. Retrieved from http://xxxx *In case of citing website with unknown author: "Title of page"*. (Year of Publication). Retrieved from http://xxxx

European Directive:

Official European directives, issued from the European parliament and of the Council (EC) should be cited as follows (example):

EC. (2010). Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. *Official Journal of the European Union*, L276, 33-79. Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do? uri=OJ:L:2010:276:0033:0079:en:PDF

Legislation:

Official laws, orders etc. should be cited as follows (see examples). Biological Diversity Act. (2002). *State Gazzette*, 77, 09.08.2002. (In Bulgarian). Medicinal Plants Act. (2000). *State Gazette, 29,* 07.04.2000. (In Bulgarian). Protected Areas Act. (1998). *State Gazette, 133,* 11.11.1998 (In Bulgarian).

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it is recommended to be used. If there is not such a summary, the author's names must be transcribed and the title of the paper must be translated into English. If the name of the journal is also not in Latin letters it also should be transcribed (not translated). This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian, English summary).

Examples:

- Angelov, P. (1960). Communications entomologiques. I. Recherches sur la nourriture de certaines espèces de grenouilles. *Godishnik na muzeite v grad Plovdiv, 3,* 333-337. (In Bulgarian, Russian and French summary).
- Korovin, V. (2004). Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural. Ekaterinburg, Russia: Published by Ural University. (In Russian).

Names of persons who provided unpublished information should be cited as follows: "(Andersson, 2005, Stockholm, pers. comm.)".

<u>Unpublished theses (BSc, MSc, PhD, DSc) are not considered officially published</u> <u>scientific literary sources, therefore from January 2015, "Ecologia Balkanica" no</u> <u>longer allows citations of such references.</u>

<u>Citing references that are still "in press" is also considered frown upon, but not forbidden. If possible, please avoid using such references.</u>

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For special symbols (Greek letters, symbols for male and female etc.) use the Symbol list on the Insert menu in Microsoft Word with the following preferable fonts: Symbol, Webdings, Wingdings, Wingdings 2 and Wingdings 3. Degree symbols (°) must be used (from the Symbol list) and not superscript letter "o" or number "0". Multiplication symbols must be used (×) and not small "x" letters. Spaces must be inserted between numbers and units (e.g., 3 kg) and between numbers and mathematical symbols (+, -, ×, =, <, >), but not between numbers and percent symbols (e.g., 45%).

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Mean values should always be accompanied by some measure of variation. If the goal is to describe variation among individuals that contribute to the mean standard deviation (SD) must be used. When the aim is to illustrate the precision of the mean standard errors (SE) should be given. The last paragraph of Materials and Methods section should briefly present the significance test used. Quote when possible the used software. Real *p* values must be quoted both at significance or non-significance. The use of the sign is acceptable only at low values of *p* (e.g. *p*<0.0001).

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The authors of articles that are based on experiments that caused injuries or death of animals should explain and justify the grounds of the study and state that the scientific results of the study is at least in trade-off with the sufferings caused. In the Materials and Methods section of the manuscript, the authors should explain in detail and as precisely as possible the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not invasive. When alternative non-harming techniques exist, but were not used, the manuscripts may not be considered for publication.

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