ГОДИШНИК

НА СОФИЙСКИЯ УНИВЕРСИТЕТ
„СВ. КЛИМЕНТ ОХРИДСКИ“

БИОЛОГИЧЕСКИ ФАКУЛТЕТ

ПЪРВА НАЦИОНАЛНА КОНФЕРЕНЦИЯ ПО
РЕИНТРОДУКЦИЯ НА КОНСЕРВАЦИОННО ЗНАЧИМИ
ВИДОВЕ, СОФИЯ 2015

ANNUAIRE

DE L'UNIVERSITE DE SOFIA
"ST. KLIMENT OHRIDSKI"

FACULTE DE BIOLOGIE

FIRST NATIONAL CONFERENCE OF REINTRODUCTION OF
CONSERVATION-RELIANT SPECIES, SOFIA 2015

УНИВЕРСИТЕТСКО ИЗДАТЕЛСТВО „СВ. КЛИМЕНТ ОХРИДСКИ“
PRESSES UNIVERSITAIRE "ST. KLIMENT OHRIDSKI"
СОФИЯ • 2016 • SOFIA

Prepress
Nikola Atanassov

СУ "СВ. КЛИМЕНТ ОХРИДСКИ"
БИОЛОГИЧЕСКИ ФАКУЛТЕТ

ПЪРВА НАЦИОНАЛНА КОНФЕРЕНЦИЯ
ПО РЕИНТРОДУКЦИЯ НА
КОНСЕРВАЦИОННО ЗНАЧИМИ ВИДОВЕ

19 - 20 НОЕМВРИ 2015

Настоящото издание е отпечатано с финансовата подкрепа на проект „Възстановяване на белошипата ветрушка", LIFE11 NAT/BG/360
Проучванията и представените резултати са дело на авторите на статиите и не са продукт на проекта, финансирал отпечатването на изданието.

ISSN 0204–9902

CONTENTS

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 5-6

THE ROLE OF THE FACULTY OF BIOLOGY, UNIVERSITY OF SOFIA IN THE IMPLEMENTATION OF SPECIES REINTRODUCTION ACTIVITIES IN BULGARIA

ATANAS GROZDANOV[1*], ANITA TOSHEVA[2]

1 – Department of Zoology and anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
2 - Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
**Corresponding author: zootribe@gmail.com*

Keywords: biology, education, reintroduction, nature conservation, Sofia University

Species reintroduction represents an important part of the nature conservation activities worldwide. In Bulgaria many institutions and non-government organizations are involved in different projects, aiming to recover biodiversity by reintroducing species, which are extinct or dramatically decreased in their natural habitats.

The Faculty of biology of University of Sofia is the largest national institution for education of biology specialists in bachelor, master and PhD degrees. The great number of qualified lecturers and motivated students represents a solid basis for supporting the reintroduction processes.

The role of the Faculty could be determined on many levels.

The scientific potential is used for assisting the reintroduction programmes from the initial feasibility studies to the following execution and long-term monitoring activities. The Department of zoology and anthropology was intensively involved in the process of reintroduction of Griffon vulture (Gyps fulvus) in Kresna gorge. The project team from Fund for wild flora and fauna (FWFF) and zoologists from the Department did vast researches of the process and the results were presented and published in conferences and publications (Grozdanov & Stoynov, 2010; 2011). In addition, students from the master programs of zoology were also involved in the reintroduction researches by working on theses, linked with the species.Reintroduction projects were also implemented with the participation of other departments. The Department of general and applied hydrobiology was

part of reintroduction project for the Bullhead (Cottus gobio), where lecturers and students were involved. There are also projects concerning reintroduction of plant species, implemented with the participation of experts from Departments of Botany and Ecology and environmental protection. These include actions for the rare or endangered plants Aldrovanda vesiculosa and Primula deorum. As a part of master of science thesis developed in the Faculty, monitoring of the reintroduced or reinforced species Hottonia palustris, Hippuris vulgaris, Leucojum aestivum, Viola pumila, Fritillaria meleagroides was implemented in the area of Aldomirovtsi marsh (as part of a project of the nature conservation organization Balkani).

On the other hand, the Faculty students from bachelor programs are often taking part in the projects, providing a significant amount of volunteer work. This process includes many benefits not only for the reintroductions, but for education process in the University, which is generally enriched with thematic theoretical and field work. The Students club for education and development (club Skorec), which is based in the Faculty, organised several nature conservation brigades in the area of Kresna gorge. The students were able to observe the projects work directly and took part in important activities like vultures supplementary feeding, preparation of bird aviary for the nesting season, etc. Thus, after such campaigns, the Sofia university students received additional qualification and motivation.

The Faculty of biology was the first institution, which hosted scientific forum, dedicated to the reintroduction of species in Bulgaria. During the First national conference "Reintroduction of conservation-reliant species", most of the ongoing projects and activities were presented and discussed in academic environment, which gave, among the other benefits, a solid basis for a series of new thematic forums, including international ones.

REFERENCES

1. Stoynov E., Grozdanov A. 2010. Re-introduction of Griffon vultures and consequent return of Egyptian vultures in the Kotel Mountains. Global re-introduction perspectives: additional case-studies from around the globe. IUCN Conservation specialists group, 02: 160-163.
2. Stoynov E., Grozdanov A. 2011 First breeding of Griffon vulture (Gyps fulvus) during the reintroduction activities in Kresna gorge. Youth scientific conference "Kliment's days" 22 - 23 November, 2011, Faculty of biology.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 7-17

THE REINTRODUCTIONS OF WILDLIFE SPECIES AS CONSERVATION TOOL – REVIEW OF THE GENERAL ASPECTS, HISTORY, PRESENT STAGE AND FUTURE OF THE REINTRODUCTIONS OF WILDLIFE SPECIES IN BULGARIA

EMILIAN STOYNOV[1,2*] , ATANAS GROZDANOV[1]

1 – Department of zoology and anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
2 – Fund for Wild Flora and Fauna, 2700 Blagoevgrad, P.O. Box 78, 49 Ivan Michaylov Str, room 327, Bulgaria, www.fwff.org
**Corresponding author: pirin@fwff.org, Fund for Wild Flora and Fauna*

Keywords: birds, mammals, game species, crayfishes, fishes, local extinction, translocations, captive breeding, ecological role, habitats management

The concept of saving endangered species by retaining and breeding them in captivity with subsequent release back into the wild or trough direct translocation of wild individuals from one place to another goes back a surprisingly long time. Early animal reintroductions depended on little more than the enthusiasm and whim of a dedicated patron. Today, reintroductions need a formidable amount of planning.

In recent years there has been an increasing recognition of the role that reintroduction programmes can play in species conservation. IUCN has developed international recommendations on the procedures that should be followed before any species reintroduction programmes are planned. These will be reviewed in the Bulgarian nature conservation context.

Bulgaria has traditions in translocations – fishes and game species have been reintroduced officially as State policy since the establishment of the III Bulgarian State in late XIX Century. Some local traditions could be dated back to historical times as for example local freshwater crayfishes reintroductions in Kotlenska Planina, where Stone and Noble crayfishes are moved by local people from one part of the river basin to other, where they were extinct by any reason (overharvesting, drought, pollution etc.) even today. European Souslik has been

moved by transhumant shepherds and nomadic groups of people because it was used for food, but also for traditional medicine. The species was therefore released unintentionally or intentionally to establish local sources to be used later and thus translocation took place.

Game species and fishes were largely (re)introduced to serve as hunting/fishing objects. These include Red deer, Fallow deer, European Bison, Chamois, Brown Bear, Nutria, Alpine Ibex, several species of economically important fishes etc.

Only recently with the new circumstances and realities, species that do not have such obvious use as food source have become objects of reintroduction - more to fulfill aesthetic and recreational needs of people, but also to fill in important ecological gaps. Such are Vultures, Falcons, tortoises, etc.

Nowadays the Bulgarian nature conservation community appears to be the leader in the reintroduction of conservation dependent species in Eastern Europe, with more than 20 recent programmes, involving birds, mammals, fishes, reptiles, different invertebrates and plants. The restoration projects for species such as Griffon Vulture, Lesser Kestrel and Saker Falcon are pioneers in Eastern Europe. The scientific and practical experience should therefore be shared and the recent "First National Conference on Reintroductions of Conservation Dependent Species" is a logical step in promoting this work and providing the much needed know-how exchange.

From 2000 to 2015 all known re-introduction projects in Bulgaria were surveyed. The survey also opportunistically included data from international projects involving reintroduction. In parallel with this, an on-going literature review was set in motion and a total of more than 60 references were collected.

Based on historical data for their presence in the country and later considered locally extinct species are listed in a table describing the possibilities and the need for their reintroduction/restocking.

Some species are considered keystone as their ecological niche has not been filled by any other replacing species. Others may need to be reintroduced in Bulgaria based on habitat suitability and/or existing capacity of the local conservation community, which may benefit the regional or global state of certain species.

Table 1. Species that could be objects of reintroduction/restocking in Bulgaria based on the data for their historical presence. Some are locally extinct, while some are declining fast. Others are still present, but in low numbers or their range is small and thus do not play their ecological role, and some could be introduced in the country to fulfill some ecological niches, or save the species in global aspect.

No	Common name	Scientific name	Conserva-tion state in Bulgaria	Global Conservation state	Ecological importance from restoration of the species and International perspective.
1	2	3	4	5	6
Birds					
	Little Bustard	*Tetrax tetrax*	EX	NT	Bulgaria may play an important role in Global conservation of the species
	Great Bustard	*Otis tarda*	CR (EX)	VU	Bulgaria may play an important role in Global conservation of the species.
	Bearded Vulture	*Gypaetus barbatus*	EX	NT	An important ecological role in removing carcasses from pastures. Fills a bit different niche from Griffon Vulture, able to sustain in alpine zone also consuming bones of dead ungulates. Bulgaria could become safe ground for the species.
	Eurasian Black Vulture	*Aegypius monachus*	EX	NT	An important ecological role in removing carcasses from pastures. Fills a bit different niche from Griffon Vulture, breeding in trees and also able to feed on smaller carcasses. Bulgaria could become safe ground for the species.
	Griffon Vulture	*Gyps fulvus*	EN	LC	An important ecological role in removing carcasses from pastures. Bulgaria could become safe ground for the species.
	Egyptian Vulture	*Neophron percnopterus*	EN (CR)	EN	An important ecological role in removing carcasses from pastures. Bulgaria could become safe ground for the species.
	Saker Falcon	*Falco cherrug*	CR (EX)	EN	Bulgaria may play important role in Global conservation of the species.

1	2	3	4	5	6
Birds					
	Lesser Kestrel	*Falco naumanni*	CR (EX)	LC	Bulgaria may play an important role in Global conservation of the species.
	Red Kite	*Milvus milvus*	CR (EX)	NT	Bulgaria may play an important role in Global conservation of the species.
	Common Crane	*Grus grus*	EX	LC	Bulgaria may play an important role in conservation of the species on European level.
	Demoiselle Crane	*Antropoides virgo*	EX	LC	Bulgaria may play an important role in conservation of the species on European level.
	Great White Pelican	*Pelecanus onocrotalus*	EX	LC	Bulgaria may play an important role in conservation of the species on European level.
	Dalmatian Pelican	*Pelecanus crispus*	CR	VU	Bulgaria may play important role in Global conservation of the species.
	Bald Ibis	*Geronticus eremita*	(EX)	CR	Bulgaria may play an important role in Global conservation of the species.
Mammals					
	Eurasian Ground Squirrel	*Spermophilus cittelus*	VU	VU	An important ecological role of grazer that maintains grasslands, an important food source for several threatened species. Bulgaria may play important role in Global conservation of the species.
	Chamois	*Rupicapra rupicapra*	EN	LC	Grazer maintaining grasslands, food source for several threatened species. Could use alpine pastures. Bulgaria may play an important role for conservation of the local subspecies R. r. balkanica

1	2	3	4	5	6
Mammals					
	Alpine Ibex	*Capra ibex*	(EX)	LC	Grazer maintaining grasslands, food source for several threatened species. Could use alpine pastures.
	Bezoar Goat	*Capra aegagrus*	(EX)	VU	Grazer maintaining grasslands, food source for several threatened species. Could use alpine pastures. Bulgaria may play an important role for conservation of the species.
	Fallow deer	*Dama dama*	(LC)	LC	Grazer maintaining grasslands, food source for several threatened species.
	Red deer	*Cervus elaphus*	(LC)	LC	Grazer maintaining grasslands, food source for several threatened species.
	Konnik/ European Wild horse	*Equus ferus caballus*	(EX)	EX	Grazer maintaining grasslands, food source for several threatened species. Bulgaria may play important role for reestablishment of the species in natural environment.
	Auroch/Taurus	*Bos (taurus) primigenius*	(EX)	EX	Grazer maintaining grasslands, food source for several threatened species. Bulgaria may play important role for reestablishment of the species in natural environment.
	European Bison	*Bison bonasus*	(CR)	VU	Grazer maintaining grasslands, food source for several threatened species. Bulgaria may play important role in Global conservation of the species.
	European Mink	Mustela lutreola	EX	CR	Bulgaria may play an important role in Global conservation of the species.
	Monk seal	*Monachus monachus*	EX	EN	Bulgaria may play an important role in Global conservation of the species.

1	2	3	4	5	6
Reptiles & Amphibians					
	Eastern Hermann's Tortoise	*Eurotestudo hermanni*	EN	NT	Food source for other threatened species and Bulgaria may play important role in Global conservation of the species.
	Spur-thighed Tortoise	*Testudo graeca*	EN	VU	Food source for other threatened species and Bulgaria may play important role in Global conservation of the species.
Fishes					
	Common Sturgeo	*Acipenser sturio*	EX	CR	Bulgaria may play an important role in Global conservation of the species.
	Russian Sturgeon	*Acipenser gueldenstaedtii*	CR	CR	Bulgaria may play an important role in Global conservation of the species.
	Ship Sturgeon	*Acipenser nudiventris*	EX	CR	Bulgaria may play an important role in Global conservation of the species.
	Starry Sturgeon	*Acipenser stellatus*	CR	CR	Bulgaria may play an important role in Global conservation of the species.
	Starlet	*Acipenser ruthenus*	EN	VU	Bulgaria may play an important role in Global conservation of the species.
	Beluga	*Huso huso*	CR	CR	Bulgaria may play an important role in Global conservation of the species.

Crayfishes					
	Stone crayfish	*Austropo-tamobius torrentium*	(VU)	DD	Food source for other threatened species and Bulgaria may play an important role in Global conservation of the species.
	Noble crayfish	*Noble crayfish*	(EN)	VU	Food source for other threatened species and Bulgaria may play an important role in Global conservation of the species.

Legend:
EX – extinct; **CR** – Critically endangered; **EN** – Endanegerd; **VU** – Vulnerable; **NT** – Near threatened; **LC** – Least concern; **DD** – Data deficient (see IUCN Red List of Threatened species).

State given in brackets, means that it is evaluated by the authors for the purpose of this article, based on their knowledge, sometimes in disagreement with the official most current evaluation elsewhere.

With the increasing number of reintroduction/restocking programmes in Bulgaria, there is a need of introducing a standardized approach to measure the reintroduction success. It is recommended to use the Reintroduction standards developed by W.J. Sutherland et al. 2010 as shown in below:

List of actions

Agreeing to comply with the protocol requires completing all except 4c–e and 6. Additions can be stated, for example, "The monitoring protocol was followed including 4c and 6a." Adopting the protocol requires stating the monitoring method used beforehand and then carrying it out after the standard periods.

1. Document the release prior to its taking place. This would require the following information:
a) Species.
b) Location.
c) Proposed year of release.
d) Planned monitoring.
e) Planned documentation.
f) Organization involved.
g) Overseeing body/permitting agency.
And after release
h) State within 6 months of release whether release occurred.

After 5 years, or earlier if all disappear, provide reference to the publication documenting the reintroduction and the results of the monitoring.

2. Consider the monitoring objectives. Consider carrying out the reintroduction as an experiment or part of an experiment to compare different introduction methods.

3. State publishing plan on website or report.

4. When publishing the minimal required information includes:
- Number released (including age and sex where can be determined).
- Date released.
- Whether releases were of captive or of wild-caught population. If captive, then details of origin and time in captivity. If wild, then location of source population and history of source population (e.g., remnant or reintroduced population itself).
- Location released. Distance moved and type of transport.
- Whether any individuals died during capture, holding, movement—if so, how many and why (postmortem reports, observations). Whether pre-release training took place, and if so, what it entailed.

- Whether there was any veterinary screening. What type and what were the results?
- Whether any veterinary treatments or vaccinations were given. Whether genetic screening took place and if so what this entailed. Whether there was any process of acclimatization, including whether it was a hard, soft, or mixed release.
- Whether there was supplementary feeding and, if so, what was fed, how much and how frequently.
- Whether there was any provision of artificial breeding/nesting sites.
- Whether there was any predator or competitor control and, if so, what species and how were they controlled.
- Whether there was any post release monitoring for diseases.
- Whether individuals were marked and, if so, how many and with what sort of mark.

5. Post release monitoring is essential. Our criteria are to carry out a population estimate after:
- One year.
- Five years.
- Optionally, but recommended for long-lived species or lengthy reintroduction programs, 10 years.
- Optionally, but recommended for very long-lived species or programs, 15 years.
- Optionally, but recommended for very long-lived species or programs, 20 years.

6. Ideally document numbers of individuals, where possible classified according to age and sex. In some cases, documenting numbers will be unrealistic and the following may be acceptable alternatives. If these alternatives are to be used then this should be stated from the start:
- Estimate birth and death rates (including the age-specificity of both, if possible), and use this values to calculate expected population growth rate.
- Document breeding by finding nests.
- Document breeding by searching for juveniles.
- Document breeding by color ringing all released birds and recording whether the current birds have rings.
- Or record presence/absence, change in relative abundance or other indices of abundance, e.g., foraging signs, singing males, lek sites etc.
- Where possible also document any of the following:
Breeding success
Survival rate
Dispersal rate
Any causes of death
Any causes of breeding failure

7. Distinguish age-classes and sex, if possible, in monitoring.

8. Where translocation supplements an existing population, distinguish the fate of the restocked and the existing individuals.

9. Document the results after the 5th year (and 10, 15, and 20th year if monitoring is extended) ideally in a journal but otherwise in a report. Add the reference or copy of the report to the website or report used for (1).

REFERENCES

1. Armstrong, D.P., Castro I., Griffiths R. (2007) Using adaptive management to determine requirements of re-introduced populations: the case of the New Zealand hihi. J Appl Ecol 44, 953–962.
2. Armstrong, D.P., Seddon P.J. (2008) Directions in reintroduction biology. Trends Ecol Evol 23, 20–25.
3. Bajomi, B., Pullin A.S., Stewart G.B., Tak˘acs-S˘anta A. (2010) Bias and dispersal in the animal reintroduction literature. Oryx, doi: 10.1017/S0030605310000281.
4. Butchart, S.H.M., Stattersfield A.J., Collar N.J. (2006) How many bird extinctions have we prevented? Oryx 40, 266–278.
5. Ewen, J.G., Armstrong D.P. (2007) Strategic monitoring of reintroductions in ecological restoration programmes. Ecoscience 14, 401–409.
6. Fischer, J., Lindenmayer D.B. (2000) An assessment of the published results of animal relocations. Biol Conserv 96, 1–11.
7. Hoegh-Guldberg, O., Hughes L., McIntyre S. et al. (2008) Assisted colonization and rapid climate change. Science 321, 345–346.
8. IUCN. (1998) Guidelines for re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.
9. IUCN/SSC (2013) Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57 pp.
10. IUCN (2015) The IUCN Red List of Threatened Species. Version 2015-4. <http://www.iucnredlist.org>. Downloaded on 19 November 2015.
11. Kleinman, D.G., Stanley Price M.R., Beck B.B. (1994) Criteria for reintroductions. Pages 288–303 in P.J.S. Olney,
12. Pullin, A.S., Stewart G.B. (2006) Guidelines for systematic review in conservation and environmental management. Conserv Biol 20, 1647–1656.
13. Red Data Book of Peoples Republic of Bulgaria. Vol.2. Animals (1985) Edition of Bulgarian Academy of Sciences. Sofia. pp.183.
14. Red Data Book of Republic of Bulgaria. Vol.2. Animals (2011) Edition of Bulgarian Academy of Sciences. Sofia.
15. Sarrazin, F., Barbault R. (1996) Reintroduction: challenges and lessons for basic ecology. Trends Ecol Evol 11, 474–478.
16. Seddon, P.J. (1999) Persistence without intervention: assessing success in wildlife re-introductions. Trends Ecol Evol 14, 503.
17. Seddon, P.J., Armstrong D.P., Maloney R.F. (2007) Developing the science of reintroduction biology. Conserv. Biol 21, 303–312.
18. Seddon, P.J., Armstrong D.P., Soorae P. et al. (2009) The risks of assisted colonization. Conserv Biol 23, 788–789.

19. Soorae P., editor. (2008) Global re-introduction perspectives: re-introduction case studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Chabi, UAE.
20. Sutherland, WJ., Armstrong D., Butchart SHM., Earnhardt JM., Ewen J., Jamieson I., Jones CG., Lee R., Newbery P., Nicols JD , Parker KA, Sarrazin F., Seddon PJ., Shah N., Tatayah V. (2010) Standards for documenting and monitoring bird reintroduction projects. Conservation Letters 3. 229-235. doi:10.1111/j.1755-263X.2010.00113.x
21. Sutherland, W.J., Pullin A.S., Dolman P.M., Knight T.M. (2004) The need for evidence-based conservation. Trends Ecol Evol 19, 305–308.
22. Wolf, C.M., Garland T. Jr., Griffith B. (1998) Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. Biol Conserv 86, 243–255.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 18-29

IUCN GUIDELINES FOR REINTRODUCTIONS AND CONSERVATION TRANSLOCATIONS OF SPECIES – PROBLEMS AND SOLUTIONS

DIANA ZLATANOVA

Department of Zoology and anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski"
**Corresponding author: zlite2@gmail.com*

Keywords: reintroduction, conservation translocations

Abstract: The current impact of the human civilization on the Earth's biodiversity leads to a rapid population decline and even extinction of many species. Due to this, conservation organizations and institutions undertake efforts for reintroductions of locally extinct species or other conservation translocations. Many of these efforts are without feasibility studies, nor proper assessment of the local situation or a local consent which often is a costly failure. This leads to the clear necessity of setting up rules and identifying potential pitfalls when initiating a reintroduction or other conservation translocation attempt. Such issues are addressed in the IUCN's Guidelines for reintroductions and other conservation translocations developed by the Task Force of the Reintroduction and Invasive Species Specialist Groups (2010 – 2012). The IUCN's view on the definitions and classification of translocations, the needs and objectives, the feasibility studies and planning phase, the design of the translocations, the release and implementation stage, the monitoring and continuing management and the risk assessment on every step of the translocations are presented with a discussion of pitfalls and critical examples of existing translocation projects.

INTRODUCTION

The current impact of the human civilization on the Earth's biodiversity leads to a rapid population decline and even extinction of many species. Due to this, conservation organizations and institutions undertake efforts to recover the locally extinct species by means of reintroduction or other conservation translocations. Many of these efforts are without feasibility studies, nor proper assessment of the local situation or a local consent which often leads to a costly failure. This calls for a clear necessity of setting up rules and identifying potential pitfalls

when initiating a conservation translocation attempt. Such issues are addressed in the IUCN's Guidelines for reintroductions and other conservation translocations (IUCN/SSC, 2013) developed by the Task Force of the Reintroduction and Invasive Species Specialist Groups (2010 – 2012). Down below the IUCN's view on the definitions and classification of translocations, the needs and objectives, the feasibility studies and planning phase, the design of the translocations, the release and implementation stage, the monitoring and continuing management and the risk assessment on every step of the translocations are presented with a discussion of pitfalls and critical examples of existing translocation projects.

History and evolution of definitions

Evolution of RSG Guidelines

The increasing interest in releasing animals in different areas on the Earth led to the IUCN Position Statement on the Translocation of Living Organisms developed in 1987. This was the first attempt to set up some rules for translocations continued by forming a group of specialists known as IUCN Reintroduction Specialist Group. Later on, the IUCN Guidelines for Reintroduction were finalized in 1995 and officially printed in 1998. These guidelines were revised and transformed in 2013 into Guidelines for Re-introduction and other Conservation Translocations (Version 1).

The definitions introduced with these documents are as follows:

- The IUCN Position Statement on the Translocation of Living Organisms (1987):Translocation, Re-introductions & Introductions.
- The IUCN Guidelines for Re-introduction (1995):Re-introductions, Reinforcements & Conservation Introductions.
- The Guidelines for Re-introduction and other Conservation Translocations (2013)Introduced new terminology due to effects of climate change and restoring ecosystem function- Assisted Colonization and Ecological Replacement.

Current definitions

To identify the goals of the recovery of a species first we need to clear up definitions. Very often the word ***reintroduction*** is wrongly used to describe *efforts for reinforcement of the population or even introduction of new species*. Per se, all these recovery actions are conservation translocations.

Translocation *is the human-mediated movement of living organisms from one area, with release in another.* Translocations may move living organisms from the wild or from captive origins. Translocations can be accidental (e.g. stowaways) or intentional. Intentional translocations can address a variety of motivations, including for reducing population size, for welfare, political, commercial or recreational interests, or for conservation objectives. ***Conservation translocation*** on the other hand is the intentional movement and release of a living organism

where the primary objective *is a* ***conservation benefit***. This will usually comprise improving the conservation status of the focal species locally or globally, and/or restoring natural ecosystem functions or processes. Conservation translocation scan entail releases either within or outside the species' *indigenous range* (Fig. 1). **The indigenous range of a species** is the known or inferred distribution generated from historical (written or verbal) records, or physical evidence of the species' occurrence. Where direct evidence is inadequate to confirm previous occupancy, the existence of suitable habitat within ecologically appropriate proximity to proven range may be taken as adequate evidence of previous occupation.

The conservation translocations are divided into two groups: Population restoration and Conservation introduction. Any other translocations with no clear conservation goals should not be treated as conservation translocations.

Population restoration is any conservation translocation within indigenous range and may be expressed with two activities: **Reinforcement** and **Reintroduction.** ***Conservation introductions*** (the intentional movement and release of an organism outside its indigenous range) may be executed via **Assisted**

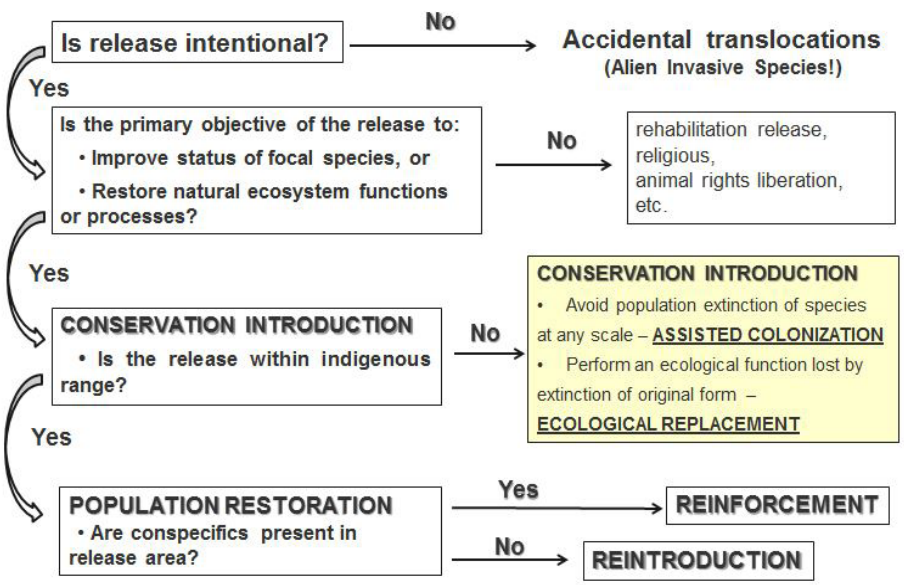


Fig.1 The translocation spectrum according to intentions, objectives and presence of individuals of the species in the release area

Accidental translocations are unintentional releases of animals, which often create catastrophic situation of allowing alien invasive species to severely affect native populations. Islands are the most vulnerable to Accidental translocations – some of the most prominent cases are the grey squirrel (*Sciurus carolinensis*) in Great Britain, rats and cats in Antigua leading almost to total extermination of the Antiguan racer (*Alsophis antiguae*), even the domestic dogs resulting in dingoes in Australia. In many European countries keeping and breeding as pets of the North American red-eared slider (*Trachemys scripta elegans)* led to unintentional local release when people are getting rid of unwanted pets into ponds and rivers around their houses. Because of the high adaptability of this species (allowing it to quickly settle in different places) the red-eared slider is included in the list of the world's 100 most invasive species, published by the IUCN (Lowe et al., 2000).

There are several known cases when animals are intentionally release because of animal rights actions, for religious and other purposes, such as releases of wild animals after treatment for injures and rehabilitation procedures. These intentional releases are not considered Population Restoration actions by the Guidelines.

There are several justifications for conservation introductions of species outside their native range. Usually such actions are not recommended being too the risky. Global evidence shows that introductions of species outside the range can cause extreme negative impacts (long term effect on native populations, risk of creating invasive species, disease transmitting, etc). If a high degree of uncertainty remains then a project should be re-evaluated and alternative conservation options should be sought.

Yet, conservation introductions (via Assisted colonisation or Ecological replacement) might be a last option for saving some species from total extinction. Here are some examples:

- Via ***Assisted colonisation*** (intentional movement and release of an organism outside its indigenous range to avoid the total extinction of populations of the focal species)-translocating of kakapo (or night parrot) *Strigops habroptila* (Gray, 1845) on predator-free offshore islands in New Zealand might save the species from extinction The kakapo is large, flightless, nocturnal, ground-dwelling parrot, endemic to New Zealand and predators pose a significant threat for its survival.

Another example with a different reason for assisted colonisation is the Torreya pine (*Torreya taxifolia*), commonly known as the Florida torreya or gopher wood. This is a rare and endangered tree found in the South-eastern United States, at the state border region of northern Florida. There are 1000 trees left with no recruitment for more than 20 years due to climatic changes. Moving the species to cooler climate in Georgia where it never existed before supports the reproduction of this species.

- Via ***Ecological replacement*** (intentional movement and release of an organism outside its indigenous range to perform a specific ecological function) - a project for introduction of two tortoise species (*Aldabrachelys gigantea* from Seychelles and *Astrochelys radiata* from Madagascar) into Mauritius Island group (Rodriges and Round islands). The primary goal of this project is the replacement of the role of the extinct in 1800s megaherbivore tortoise *Cylindraspis sp.* and to restore its lost ecological functions (Fig. 2).

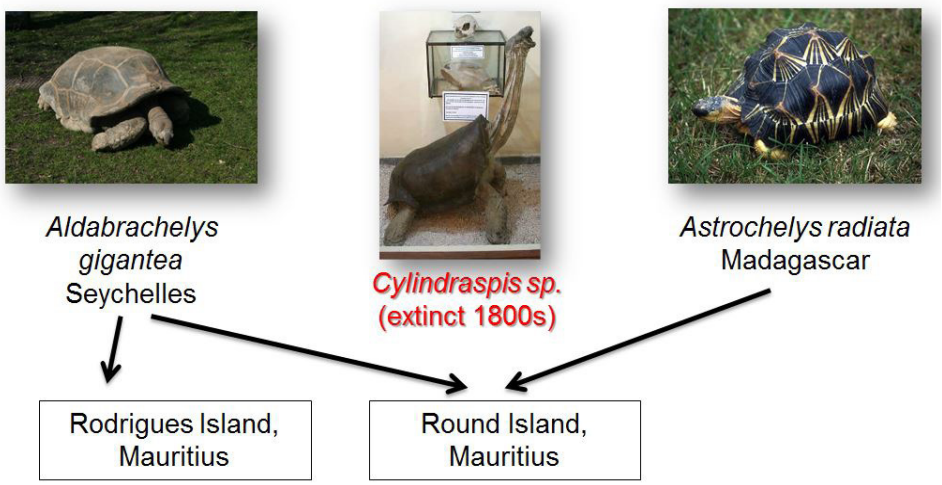


Fig.2 Example of Ecological replacement

The Population Restoration action called ***Reinforcement*** (synonyms: Augmentation; Supplementation; Re-stocking; Enhancement for plants only) is the addition of individuals to an existing population of conspecifics (Fig. 3) to enhance the numbers, to correct skewed sex ratios, improve genetic status, etc. This type of action is **always risky** to the existing wild population, introducing factors such as diseases or genetic pollution by the introduced individuals, which may be of the wrong race and/or subspecies, etc.

There are many examples of such reinforcement projects in Europe and in Bulgaria in particular – for Griffon vultures (*Gyps fulvus*) (Peshev et. al., 2015), European souslik (*Spermophilus citellus*), chamois (*Rupicapra rupicapra*), etc.

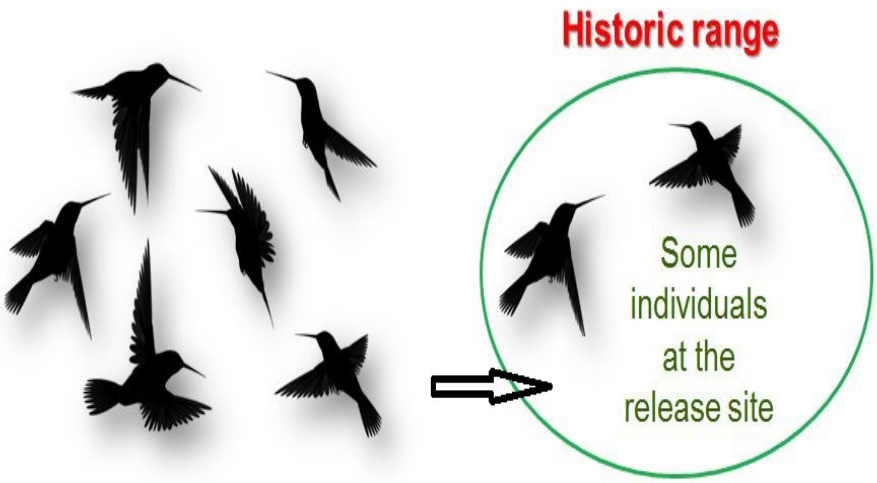


Fig.3 Population Restoration: Reinforcement

The Re-introduction as a Population Restoration is an attempt to establish a species in an area which was once part of its historical range but from which it has been extirpated or become extinct (Fig. 4). For example, the recovery of the Arabian oryx(*Oryx leucoryx*) in the Arabian Peninsula, the Pere-David's deer (*Elaphurus davidianus*) in China, Black-footed ferret (*Mustela nigripes*) in the Canada, United States and Mexico, Lammergeier (*Gypaetus barbatus*) in the Alps and others are success stories for population restoration through reintroduction.

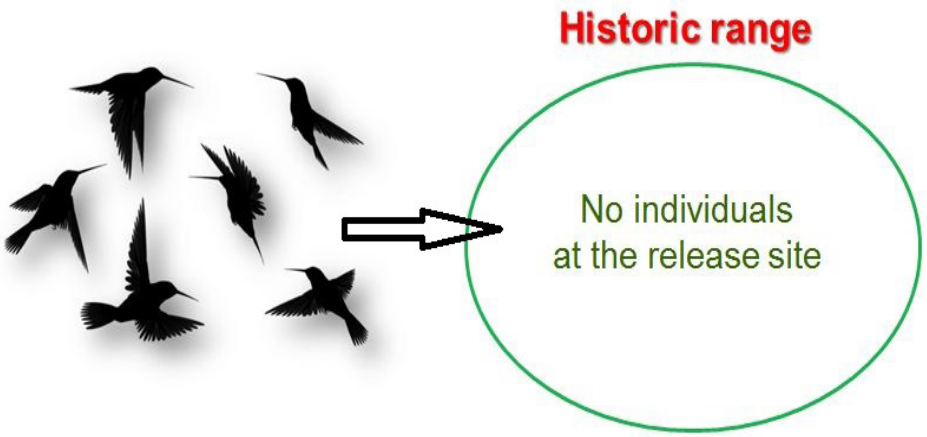


Fig.4 Population Restoration: Re-introduction

Reinforcement or Reintroduction?

There is a room to debate when a project for local translocations such as some of the mentioned species for Bulgaria (Griffon vultures, European souslik, chamois), is a reinforcement or local reintroduction project. The animals are translocated in areas from where they had really extinct, but there is still a population of these species left in the country or the whole population is still intact although in smaller numbers. In most of the cases (in species with intact connectivity in the population such as the griffon vultures) the restoration attempts are in fact reinforcement not reintroduction if they are related to countries and not geographically.

Deciding when translocation is an acceptable option

- There should generally be strong evidence that the threat(s) that caused any previous extinction have been correctly identified and removed or sufficiently reduced. Most of the translocation projects start without a feasibility study and account of the present threats which results in a failure of the species to settle down.
- Assessment of any translocation proposal should include identification of potential benefits and potential negative impacts, covering ecological, social and economic aspects. In any decision on whether to translocate or not, the absolute level of risk must be balanced against the scale of expected benefits.
- Risk analysis around a translocation should be proportional to the presumed risks.
- Justifying a conservation introduction requires an especially high level of confidence over the organisms' performance after release, including over the long-term, with reassurance on its acceptability from the perspective of the release area's ecology, and the social and economic interests of its human communities.

Steps for successful recovery programs based on translocations

Detailed description and discussions on each step's issues of the translocations are provided in the monograph of Ewenet. at. (2012).

Step 1.Planning a translocation

- Clearly defined Goals, Objectives & Actions should be set, discussed and re-evaluate if needed. The translocation project should start with Why (Goals), How (Objectives) and How in details (Actions)– following a logical path without missing a step.
- A clear monitoring program should be designed to follow the progress through all steps.
- Exit strategy should be prepared in advance – in case a project does not go according a "Plan B" should be in place.

Step.2 Feasibility and design of the program

This step comprises 3 important components - Biological feasibility, Social feasibility and Regulatory requirements. Such feasibility studies an example is already done profoundly in Bulgaria by Ragyov et al., 2009.

A. Biological feasibility

- Includes the basic biological knowledge on the species to be translocated. For example if animal species is to be translocated the food preferences, behaviour and sociality, reproduction and dispersal, etc should be well know in advance to predict the performance after release and the success of the project.
- Habitat preferences and based on that - habitat suitability for calculation of caring capacity and selection of appropriate release sites. Very important step to justify the questions Where and How many specimens would be translocated.
- Climatic requirements – there should be an account for the climate change !
- Founders – where to obtain individuals from (source) & availability, from which taxon and taxon substitution (in case of subspecies), genetic considerations of the relatedness of populations or individuals in the founder group
- Animal Welfare – how the animals should be transported, kept before release, capture/tranquilized for capture/release purposes, etc.
- Disease & parasite considerations– there should be a strict control over possible disease spread through translocation: veterinary/phito-sanitary analyses of wild populations before translocation; animals should be kept in quarantine if coming from wild population, etc.

B. Social feasibility

- Any recovery should be linked to legal and policy frameworks, biodiversity action plans or species recovery plans. Non coordinated attempts may have severe consequences.
- Local community and stakeholders should be involved in any step or otherwise their neglect will lead to a failure. The public support prior any release is crucial !
- Evaluate both positive and negative impacts of a translocation to local communities to predict performance of the local community towards the release species.
- Multiple parties involved in most translocations have their own agendas - make sure these are aligned through effective leadership.
- A translocation should not harm other ecosystems, species or human interests, especially in conservation introductions

C. Regulatory requirements

- International movement of species should be conducted according CITES (Washington Convention).
- Legislation of releasing species outside their indigenous range should prevent release of alien invasive species.
- Licenses from relevant authorities to release species should be acquired in advance.
- Cross-border movements should be done according to international, tribal, etc systems.
- Regulatory compliance – veterinary and phyto-sanitary requirements should be met before release.
- Resource availability – funding, specimens for release and all needed equipment should be provided in advance !

Step 3.Release and implementation phase

- Selection of appropriate release site and areas – these sites should be carefully chosen according to the biological (habitat preferences) and social feasibility studies.
- Release strategy – the release should be done according to species dispersion age/season preferences. Age/size/sex composition should be planned in advance, as well as the composition of specimens in multiple releases over time.
- Minimize the stress in animals during capture, transport and pre-release.

Step 4 Monitoring & Information management

Monitoring

- A feedback approach is important lesson learnt. It should lead to better project design and implementation.
- Behavioural monitoring of released individuals can be an early indicator of translocation progress, including ecological monitoring impact on the environment.
- Genetic monitoring where genetic issues (such inbreeding) are critical to the success of a translocation. Most of the translocation projects seriously neglect this step.
- Health and mortality monitoring issues of disease and welfare conditions on a released population.
- Social, cultural and economic monitoring to engage local communities in monitoring exercises can also be used to assess attitudes towards the translocation.

Question to answer before setting a monitoring a translocation progress:

1. What evidence will measure progress towards meeting translocation objectives and, ultimately, success or failure?
2. What data should be collected, where and when, to provide this evidence, and what methods and protocols should be used?
3. Who will collect the data, analyse it and ensure safe keeping?
4. Who will be responsible for disseminating the monitoring information to relevant parties?

Step 5 Dissemination of information – regular reporting in both scientific and grey literature

This step is very important both for supporting the social and funding aspects of the release. Regular reports, leaflets, posters and brochures are integral part of the translocation projects for disseminating the outcome of these projects both in a scientific and popular language.

The conservation translocation cycle

Each conservation translocation should follow the following cycle (Fig. 5) to secure a success.

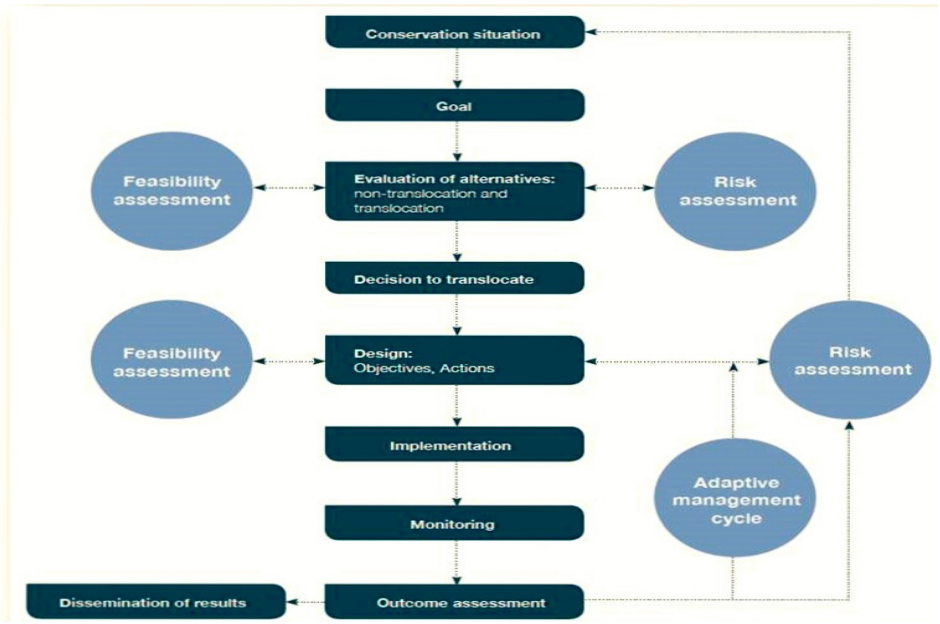


Fig. 5 The conservation translocation steps and cycle

Successful translocation (reintroduction and reinforcement) programs in Europe

Successful and ongoing programs in Europe

- Alpine ibex (*Capra ibex*) in the French, Italian and Swiss Alps (successful)
- Eurasian brown bear (*Ursus arctos arctos*) in the Alps (ongoing)
- Eurasian beaver (*Castor fiber*) in several places in Europe (successful)
- European otter (*Lutra lutra*) in the Netherlands (ongoing)
- Eurasian lynx (*Lynx lynx*) in Switzerland (successful), and other parts of Europe (ongoing)
- European souslik (*Spermophilus citellus*) in Bulgaria (ongoing)
- European black vulture (*Aegypius monachus*) in the Massif Central in France
- Griffon vulture (*Gyps fulvus*) in the Massif Central, France (successful), Central Apennines, Bulgaria, Italy, and Northern and Southern Israel (ongoing)
- Lammergeier(*Gypaetus barbatus*) in the Alps (successful), Switzerland (successful)
- Lesser kestrel (*Falco naumanni*) in Spain (successful), Bulgaria (ongoing)
- Lesser white-fronted goose (*Branta erythropus*) in Sweden and Germany (ongoing)
- Northern bald ibis (*Geronticus eremita*) in Austria and Italy (ongoing)
- Peregrine falcon (*Falco peregrinus*) in Germany, Poland, Sweden and Norway
- Red kite (*Milvus milvus*) in Ireland
- White-tailed eagle (*Haliaeetus albicilla*) in Ireland (ongoing)
- Wisent (*Bison bonasus*) in Poland, Belarus (successful) and other parts of Europe (ongoing)

Successful programs in other places

- South African cheetah (*Acinonyx jubatus*) in Swaziland (successful)
- Arabian oryx (*Oryx leucoryx*) in the Sultanate of Oman (successful), United Arab Emirates (successful), Israel (successful)
- Bornean orangutan (*Pongo pygmaeus*) in East Kalimantan, Indonesia
- North African ostrich (*Struthio camelus*) in Israel and Saudi Arabia (ongoing)
- Nubian ibex (*Capra nubiana*) in Israel (successful)
- Père David's deer (*Elaphurus davidianus*)in China (successful)
- Persian fallow deer (*Dama dama mesopotamica*)in Israel (ongoing)
- Persian onager (*Equus hemionusonager*) in Saudi Arabia (successful)
- Przewalski's horse (*Equus ferus przewalskii*) in Mongolia (ongoing)
- Sudan cheetah (*Acinonyxjubatus*) in United Arab Emirates (ongoing)
- Yarkon Bleak fish (*Acanthobrama telavivensis*) in Israel (successful)
- Black-footed ferret (*Mustela nigripes*) in the Canada, United States and Mexico

- California condor (*Gymnogyps californianus*) in California and Mexico (ongoing)
- Grey wolf (*Canis lupus*) to Yellowstone National Park (successful)
- Musk ox (*Ovibos moschatus*)in Alaska (United States) (successful)

More feedback about current reintroduction case studies are provided by Soorae (2013)

REFERENCES

1. Ewen, J. Armstrong, D., Parker, K., Seddon, Ph. (Eds) 2012. Reintroduction Biology: Integrating Science and Management. Conservation Science and Practice Series. Wiley-Blackwell, UK, 481 pp.
2. IUCN/SSC (2013).Guidelines for Reintroductions and Other Conservation Translocations.Version 1.0. Gland, Switzerland: IUCN Species Survival Commission,
3. viiii + 57 pp.
4. Lowe, S., Browne M., Boudjelas, S. 2000. 100 of the World's Worst Invasive Alien Species. A Selection From the Global Invasive Species Database. IUCN/SSC Invasive Species Specialist Group (ISSG), Auckland, New Zealand.
5. Peshev H., E. Stoynov, A. Grozdanov, N. Vangelova.2015. Reintroduction of the Griffon Vulture Gyps fulvusin Kresna Gorge, Southwestern Bulgaria 2010-2015. Fund for Wild Flora and Fauna, Conservation science Series, Book 3. Blagoevgrad, FWFF, 124 pp
6. Ragyov, D., Kmetova, E., Dixon, A., Franz, K., Koshev, Y. and Nedialkov, N. 2009 Saker Falcon Falco cherrug Reintroduction in Bulgaria: Feasibility Study. SESN. Sofia, 2009.
7. Soorae, P. S. (ed.) 2013. Global Re-introduction Perspectives: 2013. Further case studies from around the globe. Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. xiv + 282 pp

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 30-35

THE ROLE OF ART AS AN INSTRUMENT FOR SCIENTIFIC RESEARCH AND POPULARIZATION OF THE REINTRODUCTION ACTIVITIES IN BULGARIA

HRISTINA GROZDANOVA

Natonal Art Academy, Department of art history, Sofia, Bulgaria
**Corresponding author: hristinart@gmail.com*

Keywords: art, conservation, reintroduction, illustration, photography, sculpture

INTRODUCTION

A variety of activities and branches of science can contribute to the reintroduction efforts for plant and animal species. Art is one of them. This paper aims to present several chief types of art and their vital role in the work of state institutions and nature conservation groups that are involved with restoring certain species' population in Bulgaria. The study has a pioneering nature, as there are no prior efforts in Bulgaria to show the synergies between art and nature conservation.

MATERIALS AND METHODS

The necessary materials and information for this study were collected thanks to collaborative work with nongovernmental organizations and state institutions that actively work toward species' reintroduction in Bulgaria, including the National Museum of Natural Science (NMNS), the Faculty of Biology at Sofia University "St. Kliment Ohridski," the Fund for Wild Flora and Fauna (FWFF), and Green Balkans. In an effort to assess the full impact of artworks in these organizations' activities, the study traces the entire process from the pieces' creation to their practical application. Hence, it was important to establish direct contact with artists working in the field and to synthesize information about specific technological and artistic approaches. This was accomplished through meetings and interviews with a few key contemporary artists – animalists, scientific illustrators, photographers, and sculptors. In addition to atelier and

work place visits, methodology included the field work that is specific to all kinds of art listed in this study with direct applications to natural conservation and reintroduction in particular. Results are presented here through several classic art history methods – biography and formal and comparative analysis of examples.

RESULTS AND DISCUSSION

As an art form, scientific illustration must combine two very different but compatible functions: the informative as well as the aesthetic. In most cases, it relates to a particular text and aims above all to visualize and show scientific facts presented in the writing. Scientific illustrators are specialized artists working in the field of science. They apply scientific observations and combine them with technical and aesthetic qualities in order to represent their object. Precision and communication are both vital to a scientific illustration (Hodges, 2003.) Despite this, even the images meant for strictly scientific publications can be viewed and analysed with regard to their purely artistic qualities. There are a variety of factors that determine the illustrations' characteristics, such as the nature and purpose of the publication in which they appear or their artist's personal style. These characteristics determine the balance between the maximum precision in representation and the artistic freedom an artist can afford to exhibit.

The art of scientific illustration has a long history. Its roots can be traced back to the Renaissance and even further to ancient art, although of course in these cases it cannot be viewed as a separate art. Its differentiation and development into its modern form corresponded with the separation of sciences and above all the appearance of specialized publications. In Western Europe and the USA, this process occurred significantly earlier than in Bulgaria, where it began only toward the end of the 19th and beginning of 20th centuries. At first, publications in the country featured predominantly translated Western and Russian literature; these publications were also the first influences on scientific illustration in Bulgaria. The earliest Bulgarian editions dedicated to biology and wildlife conservation also contain reproduced foreign engravings and illustrations. Over time, these were replaced by the works of the first Bulgarian artists that helped establish the foundations of scientific illustration in the country. An important point in this process was the establishment of the Bulgarian "Nature" magazine in 1893. Over the next few decades, original artist works in Bulgarian scientific and popular periodicals were scarce. One of the earliest larger specialized publications in Bulgaria was the first volume of *The Fauna of Bulgaria* series, entitled *Birds in Bulgaria*. The volume's illustrations were created by the zoologist Nikolay Boev, who is better known for his contribution to zoology and conservation in Bulgaria.

In the last few decades, artists have shown an increased interest in the themes of nature and its conservation. The Bulgarian field of scientific illustration has

grown rapidly, with certain tendencies in technique, artistic approaches, and artists' personal characteristics.

One tendency is that of scientific illustrators to fall into one of two broad groups. A majority of the artists who publish their artwork in scientific publications have not had a formal training in the arts. Often, they are biologists or other academics, reminiscent of the encyclopaedic personalities from former eras. Much like the New World explorers, they combine their zoological knowledge with drawing, illustrating their materials themselves and acquiring the ability to represent their objects empirically through observation. This group of contemporary Bulgarian scientific illustrators features Georgi Pchelarov, who authored the illustrations in a large array of scientific publications, including foundational editions such as *The Fauna of Bulgaria* or *The Birds on the Balkan Peninsula*. Lately, he emphasizes acrylic paint, which allows him to layer colours to a different degree and place accents in colour, form, or volume. This approach has resulted in particularly expressive artwork (Grozdanova, 2016.)

Asen Ignatov is another artist educated in biology whose works have illustrated a large number of Bulgarian scientific publications such as *The Red Book of the Republic of Bulgaria Vol. II: Owls in Bulgaria* or *Poisonous Plants and Their Effects*. Ignatov prefers to work with water colour and water colour pencils. These materials allow him to work in the field, directly observing and representing plants and animals in nature. This approach provides for the artist's ability to show his objects reliably with their characteristic movement and liveliness.

Another tendency is the differentiation of Bulgarian scientific illustration through specialized education at the National Academy of Art. It is only in the last few years that scientific illustration has found a place at the Academy through a Master's program. This development can be attributed to prof. Viktor Paunov, an experienced artist in the field, whose zoological illustrations have been featured in a number of Bulgarian publications. Paunov also illustrates various publications, including a large range of fictional literature. His works can be characterized by their focus on purely artistic problems, something that is apparent even in strictly representative scientific works.

Denitsa Peeva is yet another Bulgarian artist whose work embodies the role of specialized education in scientific illustration. After graduating from the National Academy of Art, she enrolled in a Masters' program in scientific illustration in the Netherlands. Attending a course that had long and well-established traditions of teaching this field independently, Peeva took Bulgarian scientific illustration to a global level. Her accomplishments were recognized with a 2014 award from the International Contest of Herpetological Illustration. Peeva's contribution is also theoretical, since she has published a short handbook to illustrating field guides (Peneva, 2012.)

Sculpture is another kind of art that significantly impacts reintroduction projects. Some highly specialized sculptors work on modelling certain animal species in plastic. This is important as many wild animals are hard to observe in their natural habitat. At the same time, popularizing the challenges associated with maintaining their populations in certain areas forms an important part of most reintroduction projects in Bulgaria. To that end, in areas where such projects take place, there are often visitors' centres involved with educational activities. Along with natural history museums, these centres are the chief commissioners for sculpted animal replicas.

Animal models can also serve practical purposes. For instance, sculpted replicas of vultures have been utilized to attract and retain the species in a particular area (Peshev, H., E. Stoynov at al, 2015.)

This method has also been applied in projects concerning other animal species.

There are only a few artists in Bulgaria that work on animal replicas. The sculptor Nikolay Todorov trained at the St. Luca Applied Arts School and subsequently the National Academy of Arts. Due to his deep interest in animals and the environment, he specialized in representing nature faithfully. Todorov's pieces can be said to be three dimensional scientific illustrations. He has created many of the sculpted models presented in the NMNS, the History Museum in Panagyurishte, and several visitors' centres, including those in Gabrovo, Belene, and Levka. His ability to combine scientific precision with the plasticity of sculpture was something inherited by his son, Mincho Todorov, also a graduate of the National Academy of Art's sculpture program. Father and son often work together on projects. Yet another alumnus of Bulgaria's most prestigious art institution, Nayden Slavchev, often works on sculpture models commissioned by large non-governmental organizations such as Green Balkans.

Another kind of art that plays an important part in reintroduction projects is photography. On the one hand, it is used for its practical applications, such as documenting or recognizing different species and even individual animals. However, even when used for these purposes, it can exhibit the artistic qualities of art photography. Just like scientific illustration, field photography can fulfil a variety of functions that in the end determine its characteristics. Despite this, documenting plants and animals in nature features as a main genre in art photography and therefore presents a number of purely artistic concerns.

In Bulgaria, Nikolay Raykov was among the first photographers to dedicate his work to wild nature, conservation, and animal pictures. He also wrote the first Bulgarian books on the subject: *Hunting without a Gun* and *Steps in the Sand*. In literature abroad, the concepts of "wildlife art" and "wildlife photography" have existed for a long while, but in Bulgaria they are only now gaining popularity, and so far have not been explored in original research. It could be said that Nikolay Raykov established the field of wildlife photography for the country, not

only through his work behind the lens, but also through his written theoretical contributions.

No less important, Luybomir Andreev has contributed to Bulgarian photography through his active work in the 1990s, when he photographed rare animal species for posters and other materials for popularizing natural conservation. Today, Andreev's focus has shifted to film, but he continues to work with the same themes.

The development of contemporary photography in Bulgaria is fast-paced, with rapid technological improvements offering new opportunities for taking and rendering digital images online. Additionally, images are increasingly available, helping professional and amateur photographers to become engaged and better versed in the artistic problems of wildlife photography – both outdoors and in motion, this kind of photography poses very different challenges than the ones associated with work in a controlled studio.

There are many photographers who are inspired by nature conservation and rewilding and who work in the field. Among them, of particular interest is the artistic perspective of Asen Ignatov, whose work as a conventional artist and illustrator lends a unique approach to his views on photography. Other contemporary photographers include Hristo Peshev of the FWFF and Atanas Grozdanov of the Sofia University Faculty of Biology. Both have developed their artistic approaches to photography as a result of necessity during their experiences in the field.

All of these artists collaborate with state and non-governmental organizations involved in nature conservation and rare species reintroduction. Their photography has been featured in a number of specialized publications, as well as information and popularization materials.

CONCLUSION

Art holds an indisputable role in nature conservation and in projects on the reintroduction of key species in Bulgaria. Some types of art, such as illustration or photography, are indispensable elements of scientific publications. They also find practical applications outside the constraints of science or specialized work, as key means of public communication and conveyors of little known but vitally important issues.

In 2015, an art exhibit entitled "The Great Return" was opened in parallel with the First Bulgarian National Conference on the Reintroduction and Conservation of Species. The exhibit aimed, as has been argued here, to demonstrate that art and science are components of a cohesive whole. More than 50 artists participated, with more than 30 images of animal and plant species featuring in Bulgarian reintroduction projects (Grozdanova, 2015.)

The problems associated with the artistic qualities and aesthetic of different kinds of art as they exist in this narrowly specialized field can be reviewed in much more depth with many more examples. A study like that could help trace the emergence and development of particular tendencies mentioned in this paper. The examples given here have aimed instead to highlight some of the key issues in the main types of art that are applicable to wildlife conservation and species reintroduction in Bulgaria. Future publications will examine the arguments presented here in more depth.

REFERENCES

1. Grozdanova, H., 2015. The Great Return exhibition catalogue. Sofia.
2. Grozdanova, H., 2016. Art in Science. Living Nature – Retrospective Exhibit of Georgi Pchelarov. (in press)
3. Hodges, E.,2003. The Guild Handbook of Scientific Illustration. John Wiley & Sons, USA.
4. Peneva, D., 2012. Illustrating a Field Guide. Field Guide of Amphibians and Reptiles of Cusuco National Park, Honduras. Maastricht.
5. Peshev H., E. Stoynov, A. Grozdanov, N. Vangelova.2015. Reintroduction of the Griffon Vulture Gyps fulvus in Kresna Gorge, Southwestern Bulgaria 2010-2015. Fund for Wild Flora and Fauna, Conservation science Series, Book 3. Blagoevgrad, FWFF, 124 pp Reintroduction of the critically endangered plant Marsh Clubmoss

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 36-42

REINTRODUCTION OF THE CRITICALLY ENDANGERED PLANT MARSH CLUBMOSS (*LYCOPODIELLA INUNDATA*) IN ONE OF ITS HISTORICAL OCCURRENCES

DANIELA IVANOVA*, RAYNA NATCHEVA

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Department of Plant and Fungal Diversity and Resources
**Corresponding author: dani@bio.bas.bg*

Keywords: Central Rhodopi Mts., conservation, *Lycopodiella inundata,* plants, reintroduction

Abstract: Reintroduction of Marsh Clubmoss (*Lycopodiella inundata*) in Chairski lakes, Central Rhodopi Mts. was attempted. The species is one of the rarest in Bulgaria and is Critically Endangered at national level. In many countries of its European range *L. inundata* is rare or threatened. Conservation of *L. inundata* is however difficult since little is known about its ecology, including potential reasons for decline and response to environmental change. The species is a weak competitor. Some of the natural populations of the Marsh Clubmoss are already extinct. Its occurrence has been confirmed at three out of seven localities. Therefore, we attempted to transplant clones at the peat islands in Chairski lakes, Central Rhodopi Mts. The experiment lasted for eight years. During the first three years of the experiment the clones developed relatively well, expansion of the vegetative shoots was observed and abundant sporophytes were produced. After 2010 the plot within *Sphagnum fallax*/*S. flexuosum* was very quickly overgrown by *Carex rostrata* and *Menyanthes trifoliata*. The bare peat area of the plot set within *Sphagnum capillifolium* was maintained easier and remained in a good state for a longer period without human interference. The overall factor important for the occurrence of this species is the presence of microhabitats with low competition. Reintroduction can only be successful if mild disturbance or continuous maintenance is provided. We suggest that the focus for the conservation of *Lycopodiella inundata* should be placed on the protection of its natural habitats instead.

INTRODUCTION

Lycopodiella inundata (L.) Holub is one of the rarest plants in Bulgaria. It is evaluated as Critically Endangered at national level (Ivanova, 2009, 2015) and included in Annex 3 of the Bulgarian Biodiversity Act.

Lycopodiella inundata is a small homosporous perennial clubmoss. The vegetative shoots are horizontal, creeping, and weakly branched. The generative shoots are upright, unbranched, with spore-bearing single cones at the apex. The distribution range of the species includes temperate and cold regions of the Northern Hemisphere – Europe (excluding the Mediterranean and most of Western Russia), the Azores, Asia and North America (Hultén and Fries, 1986). In Europe, the species occurs in 25 countries (Rothmaler and Jermy, 1993) and is Red listed in 12 of them (Byfield and Stewart, 2007). The considerable decline of its populations is due to natural or anthropogenic drying up of the wetlands and direct habitat destruction.

Lycopodiella inundata is strongly heliophylous requiring constantly moist oligotrophic environment. In Bulgaria it grows on bare (or almost so) peat and among peat mosses (*Sphagnum*) in mountainous mires of two types: oligotrophic spring fens (in Milevska Mt.) and on peat islands in distrophic lakes (in the Central Rhodopi Mts.). The habitat belongs to Natura 2000 type 7140 Transition mires and quaking bogs. It is included in Annex 1 of Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora and in Annex 1 of the Bulgarian Biodiversity Act.

Historically, *L. inundata* is known from seven localities in Bulgaria (Jordanoff, 1940; Stanev, 1975; Vodenicharov and Vassilev, 1999; Ivanova & al. 2013, and unpublished herbarium data). At present its occurrence has been confirmed in only three of them: two in the Central Rhodopi Mts. (Smolyanski and Chairski lakes) and one in Znepole floristic region (Milevska Mt.). In the remaining localities the species was not re-found, as far as it was possible to localize the sites.

The recently found locality of *L. inundata* in Milevska Mts. is in good state at present. The situation is however very different in the two other remaining locations in the Central Rhodopi Mts. The population in Chairski lakes is extremely small – only a few individuals (possibly belonging to one clone) on an area of 1-2 m^2 and no suitable unpopulated microhabitats.

The population in Smolyanski lakes is situated on the peat island in lake Lagera. It consists of numerous individuals on an area of ca. 330 m^2. In the past, the lake was transformed into a micro-dam, which has led to elevation of the water level and has had a positive impact on the development of the ecosystem and on *L. inundata* in particular. The population further benefitted from the regular visits of the island by fishermen whose trampling created suitable microsites for the establishment of the species. After 2005 the area around the lake was heavily destroyed due to intensive building of hotel complexes. In 2013 the water level was lowered to less than 1/3 of its original level by human activities. This proved catastrophic to the entire ecosystem causing severe drying out of the peat island, intensive growth of competitive larger vascular plants and decline in the population of *L. inundata* and other typical species of conservation importance.

All these observations led us to consider urgent measures for the conservation of *L. inundata* in Bulgaria and to undertake the current transplant experiment.

MATERIAL AND METHODS

The experiment was performed on the *Sphagnum* islands in two of the Chairski lakes (Central Rhodopi Mts.) – Golemia gyol and Kadirev gyol. It lasted for 8 years. Plant material was taken from the nearest population of the species at lake Lagera (Smolyan lakes). Since the ecology and biology of *L. inundata* in Bulgaria is still poorly understood, we made two transplant plots of ca. 1 m^2. The plots were cleared off vascular plants and 2-3 cm of the peat moss cover was removed (Figs. 1A, 2A). Thus, we created a microhabitat that was similar to the source population. The plot at lake Golemia gyol was made within *Sphagnum fallax*/*S. flexuosum* stand (faster growing at a wetter site). The plot at lake Kadirev gyol was made in a pure *S. capillifolium* mat (slower growing at a relatively drier site). In the middle of each plot, we inserted one ca. 25×25 cm clone of *L. inundata*. The plots were visited in 2008, 2010, 2013-2015. During each visit the size of the clone was measured and the number of fertile shoots was counted. We cleaned also the vascular plants that were growing within the plot area and the clone itself as much as possible, taking care not to disturb *L. inundata*.

A. in 2007

B. in 2015

Fig. 1. Plot at lake Golemia gyol

A. in 2007

B. in 2015

Fig. 2. Plot at lake Kadirev gyol

RESULTS AND DISCUSSION

During the first three years of the experiment the development of *L. inundata* at both sites progressed at comparable rate (Figs. 3, 4). It has to be noted that the clone size at lake Kadirev gyol increased slower than the one at lake Golemia gyol. This could partly be due to the increase in the cover of vascular plants at the latter site and the tendency of *L. inundata* to escape their competition and spread faster sideways at the expense of spore production. This is supported by the lower number of fertile shoots at lake Golemia gyol in the period 2008-2010 as compared to lake Kadirev gyol.

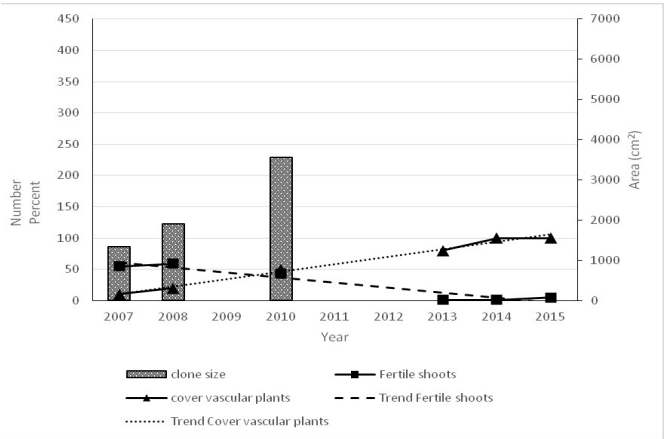


Fig. 3. Development of the transplanted clone of *Lycopodiella inundata* on the *Sphagnum* island in lake Golemia gyol.

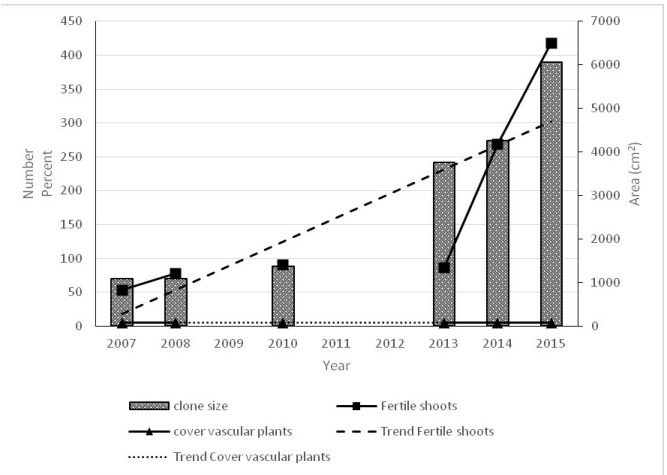


Fig. 4. Development of the transplanted clone of *Lycopodiella inundata* on the *Sphagnum* island in lake Kadirev gyol.

During 2011-2012 no maintenance of the clones was performed. At lake Golemia gyol this led to very intensive overgrowing of the plot by vascular plants and severe decrease in clone size and fertility. In 2014-2015 the cover was 100% and the shoots of *L. inundata* were barely seen with only a few fertile shoots (Fig. 1B). This is in sharp contrast to the situation in lake Kadirev gyol, where the transplanted clone and the cleaned-up area around persisted in a good state even without maintenance for three years. Furthermore, both clone size and spore production increased considerably (Figs. 2B, 4). The number of fertile shoots increased especially after the clearance of the area in 2013.

No spore recruitment was observed within the plots or anywhere in the surroundings within the duration of the experiment.

Lycopodiella inundata is a relatively slow growing pioneer species of open habitats. An important condition for its establishment and growth is moderate disturbance of the vegetation cover. As a weak competitor it requires short and open vegetation that remains in this state for a relatively long time. The overgrowing of open peat by faster growing larger species such as *Molinia coerulea*, *Potentilla palustris*, *Carex rostrata*, etc. leads to suppression of *L. inundata*.

Water availability and quality has an important role for the occurrence of *L. inundata*. Too high water level leads to fast development of more hygrophilous species, such as *Sphagnum flexuosum* and *Menyanthes trifoliata*. Too low water table even for part of the vegetative season leads to increased competition by grasses and sedges and gradual disappearance of *L. inundata*. The process of drying and increased competition is especially well seen at lake Lagera (Smolyan lakes) after 2013.

The low competitive ability of *L. inundata* is well exemplified by the transplant at lake Golemia gyol. At this site, strong competitors like *Menyanthes trifoliata*, *Molinia caerulea*, and *Carex rostrata* quickly overgrew the transplanted clone within three years of lack of maintenance. The water availability is relatively stable and water table is sufficiently high at lake Golemia gyol. It seems that other factors govern vegetation dynamics. One possibility is eutrophication that is caused by the nearby tourist hut. The hut has became actively used for the past 15 years, which has coincided with the intensive development of macrophytes and accelerated siltation of the lake.

CONCLUSION

The major factor for the occurrence of *L. inundata* is the presence of microhabitats with low competition. On the other hand we still know very little about the biology and ecology of the species in Bulgaria. Our transplant experiment demonstrated that reintroduction approach can only be effective if continuous maintenance is provided. The efforts for the conservation of the

species should be directed towards the protection of its natural habitats and ensuring a suitable management regime that will provide moderate disturbance of the vegetation layer.

Acknowledgement: This work was partly supported by grant Life08 NAT/BG/000279

REFERENCES

1. Byfield, A., Stewart, N. 2007. *Lycopodiella inundata* (L.) Holub. http://www.plantlife.org.uk/uploads/documents/Lycopodiella_inundata_dossier.pdf
2. Hultén, E., Fries, M. 1986. Atlas of north European vascular plants north of the Tropic of Cancer. Koeltz Scientific Books, Konigstein.
3. Ivanova, D. 2009. *Lycopodiella inundata* (L.) Holub. In: Petrova, A. and Vladimirov, V. (Eds.). Red List of Bulgarian vascular plants. *Phytologia Balcanica*, 15(1): 63-94.
4. Ivanova, D. 2015. *Lycopodiella inundata* (L.) Holub. In: Peev, D. et al. (Eds.). Red Data Book of the Republic of Bulgaria. Vol. 1. Plants and Fungi. BAS & MoEW, Sofia, p. 148.
5. Ivanova, D., Natcheva, R., Stoyanov, S. 2013. Reports 77-78. In: Vladimirov, V. et al. (comps). New floristic records in the Balkans: 22. *Phytologia Balcanica*, 19(2): 278-279.
6. Jordanoff, D. 1940. Materialen zur Kenntnis der Flora von Bulgarien – 1938–1939. *God. Sofiisk. Univ. Fiz. Mat. Fak*., 36(3): 251-262. (In Bulgarian).
7. Rothmaler, W., Jermy, A.C. 1993. *Lycopodiella* J. Holub. In: Tutin, T. G. & al. (eds), Flora Europaea. Ed. 2, vol. 1. Cambridge Univ. Press. Cambridge, pp. 4-5.
8. Stanev, S. 1975. Materials on Bulgarian flora with critical notes. In: Velchev, V., Kuzmanov, B. and Palamarev, E. (Eds.). In Honour of Academician Daki Jordanov. Publishing House Bulg. Acad. Sci., Sofia, pp. 253-263. (in Bulgarian)
9. Vodenicharov, D., Vassilev, D. 1999. *Lycopodiella inundata* (L.) Holub and *Hammarbia paludosa* (L.) Kuntze in Bulgaria. In: Jubilee Scientific Conference. "25 years Shumen University", 30.10.-1.11.1996. Publishing House Shumen University, Shumen, pp. 100-102. (in Bulgarian).

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 43-53

RESTORATION AND PROTECTION OF THE POPULATION OF RARE AND PROTECTED PLANT SPECIES IN VITOSHA NATURE PARK

DESSISLAVA GYUROVA[1*], ISKRA VARBANOVA-YORDANOVA[2], STEFAN YORDANOV[3]

1 – Vitosha Nature Park Directorate, 1303 Sofia, 17 Antim I Str, Bulgaria
2, 3 – Green Spring Nursery, Negovan, Bulgaria
**Corresponding author: dessybg2001@yahoo.com*

Keywords: plants, restoration, conservation, reproduction, Vitosha Nature Park, *Taxus baccata, Clematis alpina, Acer heldreichii, Menyanthes trifoliata, Drosera rotundifolia*

Abstract: The populations of rare and endangered plant species in Vitosha have been supported in accordance with the conservation program stipulated in the Management Plan of Vitosha Nature Park. *Taxus baccata* L., *Clematis alpina* (L.) Mill, *Acer heldreichii* Orph., *Menyanthes trifoliata* L. and *Drosera rotundifolia* L. are species with conservation importance. *Clematis alpina* and *Menyanthes trifoliata* are considered extinct from the territory of Vitosha due to non-confirmation of their localities for several decades. As a result of changes in the water regime of the peatlands in Vitosha mountains the population of *Drosera rotundifolia* is significantly reduced. *Acer heldreichii* and *Taxus baccata* are poorly presented in the park and with limited regenerative abilities of the individuals.

The present work studies the existing experience in plant reproduction. Collected propagating materials (seeds and cuttings) have been used in an *in vivo* experiment. The appropriate methods and techniques have been selected to ensure the greatest possible success in reproduction.

New individuals of the five species have been produced and planted in their natural habitats in Vitosha. Varying degree of survival of the new individuals has been found by subsequent monitoring.

INTRODUCTION

Among the large diversity of plant species occurring in the territory of Vitosha Nature Park (1489 species, vascular plants) there are some which are rare, vulnerable or with low populations. These species are of special concern under the management actions carried out by the Vitosha Nature Park Directorate through implementing programs for their reintroduction (Management Plan of Vitosha Nature Park, 2005-2014).

The Common Yew (*Taxus baccata*) is an endangered species, according to the Bulgarian Red Data Book (Evstatieva, 2015) and is included in Annex 3 of the Bulgarian Biodiversity Act. The only location of *Taxus baccata* in Vitosha Mountain is above the Boyana district, under the Boyana Waterfalls. It was studied by Arnaudov (1920) and Penev (1940). Information about it is found also in the study of Jordanov (1977), which was confirmed by our observations – many adult trees in bad state and lack of seedlings. Due to the limited distribution and weak regeneration potential of the species, the Vitosha Nature Park Directorate implemented actions for reintroduction and created new localities of the Common Yew in the Northern (under the peak Kopitito) and in the Southern (Zhivata voda locality) part of the mountains (Project for restoration of *Taxus baccata* L. in the territory of Vitosha Nature Park, 2001) in the period 2001-2003. The experience gained motivated the team to continue these actions in the next years. It should be noted that there are attempts to protect the populations of this endangered species through reintroduction carried out by other park administrations – these of Nature Park "Rilski Manastir" and "Strandzha" (Project Life08 NAT/BG/000281).

The Alpine Clematis (*Clematis alpina*) is included in Annex 3 of the Bulgarian Bioiversity Act, as well as in the Red List of Bulgarian vascular plants, classified as „vulnerable" (Petrova and Vladimirov, 2009). In Vitosha Mountain it is reported in the rocky sites of Reznyovete and Kominite localities (Kitanov and Penev, 1963). Recent studies carried out by the Vitosha Nature Park Directorate do not confirm the presence of the species in these localities, so measures for the restoration of its localities in the territory of the Park are required. Recent attempts to produce individuals of Alpine Clematis, carried out in the period 2005-2007 ended without success (Project for restoration of the locality of *Clematis alpina* (L.) Mill. in the territory of Vitosha Nature Park, 2005). The continuation of the activities for the reintroduction of the species is therefore required.

The Mountain Maple (*Acer heldreichii*) is one of the rare species in the flora of the Vitosha Mountains, listed as vulnerable in the Red Data Book of Bulgaria (Dimitrova, 2015) and protected by the Bulgarian Biodiversity Act (Annex 3). A study carried out in the territory of the Park by Dountchev (2005) confirmed six locations of Mountain Maple in Vitosha – Belcheva Skala peak, "Bistrishko branishte" Reserve, Zeleni del locality, Balabana locality, Chernata skala peak and Vladayski Cherni vrah peak. Cavkov carried out an inventory and detailed analysis of these localities in 2011. The weak, mostly coppice natural regeneration of the Mountain Maple and its modest, isolated locations in Vitosha were identified as prominent problems in both reports. The conservation of the species in the territory of the Park should therefore be considered a priority, raising the question of sustaining its populations.

The Buckbean (*Menyanthes trifoliata*) and the Round-leaved Sundew (*Drosera rotundifolia*) are protected by the Bulgarian Biodiversity Act (Annex 3). *Menyanthes trifoliata* is listed in the Red Data Book of Bulgaria as endangered

species (Evstatieva, 2015) and *Drosera rotundifolia* is included in the Red List of Bulgarian vascular plants, classified as „vulnerable“ (Petrova and Vladimirov, 2009). In the past *Menyanthes trifoliata* occurred in the peat areas around the springs near Boeritsa Chalet under the Black Rock Peak and possibly at other locations in Vitosha (Kitanov and Penev, 1963). As the habitats of the Buckbean in Vitosha have not been confirmed for the last decades, the species has been considered extinct from Vitosha. There is a number of data on the prevalence of *Drosera rotundifolia* in Vitosha. More recent studies of Hajek et al. (2005) and Natcheva et al. (2016) of the subalpine peatland in Vitosha Mountain, confirm its limited distribution in the mountains. The Buckbean and the Round-leaved Sundew have specific environmental requirements, and are extremely sensitive to disruption of their natural habitat, namely 7140 Transition mires and quaking bogs (Directive 92/43/EEC). The threats to their existence were associated with tendencies towards reduction of these habitats as a result of climatic and anthropogenic factors - changes in the hydrologic regime, diversion of water for drinking purposes, overgrowth of grasses and so on, and with regards to the Buckbean - collection as a medicinal plant as well. For all these reasons, the two species are also subject to reintroduction carried out by the Vitosha Nature Park Directorate.

The current article presents the results from the attempts to restore the populations of Alpine Clematis and Buckbean, and protect the populations of the Common Yew, Mountain Maple and Round-leaved Sundew in the territory of Vitosha Nature Park.

MATERIAL AND METHODS

In September 2009 two hundred and fifty mature stem cuttings and thirty seeds of adults *Taxus baccata* individuals were collected from the maternal field in the vicinity of the Boyana Waterfalls. The cuttings were selected from both male and female specimens. Reproducing material from Common Yew, as well as other target species mentioned in this article was multiplied using the „Green Spring“ nursery in the village of Negovan. The cuttings collected from *Taxus baccata* were set on a substrate of sand and peat and the rooting of the cuttings took about eight months. Vegetative production of seedlings was preferred as the process of seed germination takes too long (15 months). Rooted cuttings were removed from the greenhouse and planted in a soil mixture in one-liter plastic containers (Figure 1). After a year of growing, they were planted in stages in their natural environment. The necessary sex ratio was observed during the planting process.

Fig. 1. Potted plants of *Taxus baccata*

The location of *Clematis alpina*, used for production of reproductive material is situated along the river Yadenitsa, the Western Rhodopes. The species there develops on steep and rocky terrain beneath the forest canopy of spruce forests. About 200 mature seeds were collected in October 2009 from that location and were immediately planted in a suitable substrate (a mixture of peat and sand) and allowed for natural winter stratification outdoors. We established a high percentage of germination in the spring of 2010 and a relatively fast development of the seedlings (Figure 2). The young plants were planted in one-liter pots (Figure 3).

Fig. 2. *Clematis alpina* seedlings

Fig. 3. Potted plants of *Clematis alpina*

Reproducing material of *Acer heldreichii* was collected from the best preserved and numerous population in Vitosha, in the Balabana locality. It is situated at an altitude of 1700 m, on a Northern slope in mixed forest with dominated by *Fagus sylvatica* and *Picea abies* (L.) Karst. According to Dountchev (2005) and Tsavkov (2015) good fruiting is observed in this location. Despite that, fruit production was not found during a visit to collect seeds carried out in October 2009. Because of that, four hundred and seventy pieces of Mountain Maple soft cuttings were obtained in June 2010 and June 2011, which were set in a plastic greenhouse with the installation of an artificial fog. A mixture of sand and peat was used as substrate. Rooting cuttings started in about 40-60 days. For the purpose of acclimatization and successful hibernation, rooted cuttings were periodically removed from the greenhouse and placed in a cold conservatory. They were potted in containers of one liter in a mixture of peat, soil and manure (Figure 4).

Fig. 4. Potted plants of *Acer heldreichii*

50 small boxes with seeds from *Drosera rotundifolia* were collected in September 2012. To do so, a numerous population was selected in the peatland in the Konyarnika locality, at an altitude of 1800 m. The seeds were planted in pure peat moss at the end of January 2013, soaked with distilled water and were watered throughout the growing season. The sowing was carried out in two batches: one was merged into a common seed tray and the second was planted in a seed board with separate sockets. The seedlings were submerged in containers of distilled water and a soil-heating device was made and equipped with a phyto lamp working around the clock, in order to speed up the sprouting process. The seed germination process started after about 20 days and continued until about 40 days after sowing. Seeds showed high rates of germination. In addition to distilled water young seedlings require a large amount of sunlight and cool

temperature. The sprouting seeds of *Drosera rotundifolia* from the merged seed tray were later potted. It was found that the seedlings easily tolerated potting. The new plants grew relatively slow and the growth could not be accelerated through fertilization due to salt intolerance. Potted individuals of Round-leaved Sundew were prepared for planting in the field in September 2013 (Figure 5).

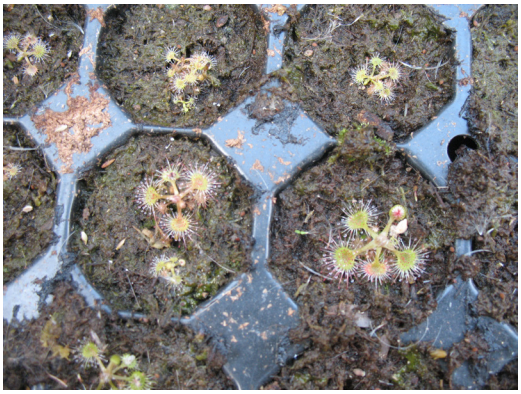


Fig. 5. Potted plants of *Drosera rotundifolia*

Vegetative propagation was applied for *Menyanthes trifoliata*. Rootage from 20 plants were collected in June 2013 from the Smolyan Lakes, Middle Rhodopi Mountains. The collected plants were divided into cuttings and planted directly in one-liter pots in clean sand with controlled release of „Osmocote“ fertilizer. The planted pots were completely immersed in water baths (Figure 6). Rooting plant cuttings took place relatively quickly. The young specimens of *Menyanthes trifoliata* were prepared for planting outdoor in September 2013.

Fig. 6. Potted plants of *Menyanthes trifoliata*

The new individuals from the five species were planted on preliminary chosen locations in the Vitosha Nature Park territory. Ecological conditions of the source population were taken into consideration when selecting the recipient sites. The planting activities were organized by the team of the Vitosha Nature Park Directorate and were supported by many volunteers.

RESULTS AND DISCUSSION

A total of 420 pieces of container seedlings of *Taxus baccata* were produced. Steep terrain (25^0) in beech habitats (natural habitat type 9130 *Asperulo-Fagetum* beech forests), Northern exposition and an altitude of 1300-1400 m were the conditions selected for planting the seedlings, similar to the those of the source population. The saplings were planted in stages (July 2011, May and October 2012) in the Bai Krustyo locality in Northern Vitosha (270 individuals) and above the village of Chuypetlovo, in the Southern Vitosha (150 individuals). One hundred and fifty saplings were planted on a shady place as an undergrowth in the high canopy beech forest, and the remaining 150 saplings were planted in beech forest with lower canopy and participation of *Betula pendula* and *Populus tremula* L. We established a weak interception of *Taxus baccata* seedlings on both sites, but more the attempt in the better lighted area - 30% (Figure 7) was more successful as compared to the shady one (15%). The result is approximately the same above the village of Chuypetlovo, where barely barely 20% of saplings were intercepted.

Fig. 7. *Taxus baccata* sapling

The choice of location for planting *Clematis alpina* was based on literature data and in particular on those outside the reserve "Bistrishko Branishte", namely the Kominite locality. The terrain is steep and stony, with a high percentage of rock cover, vegetation is wood-shrub with prevailing *Betula pendula* (L.) Roth, *Salix caprea* L., *Coryllus avellana* L., etc. The high air humidity in this stretch of the river valley, the Western exposition, and the rocky terrain, represent a combination of appropriate conditions for the development of *Clematis alpina*. There are 70% successfully adapted young individuals from all the one hundred and fifty planted in June 2011 (Figure 8).

Fig. 9. *Acer heldreichii* sapling

Vegetative propagation is an atypical and unreliable method for *Acer heldreichii*, and our experience confirmed that - the low percentage of rooting cuttings produced only 10 pieces of saplings. They were planted in the Cvetna Polyana locality, at an altitude of 1500 m, in the medium forest vegetation belt. There is a rich, fresh spruce habitat, which is the most appropriate type according to Cavkov (2015). The area was affected by windthrow in 2001 and was subsequently afforested with Norway Spruce. All ten saplings successfully developed among the pioneer woods and shrubby vegetation with the participation of *Sorbus aucuparia* (L.) Crantz., *Corylus avellana*, *Rubus idaeus* L., *Sambucus racemosa* L. etc., which was confirmed during the last inventory this year (Fig. 9).

Fig. 10. Newly planted individual of *Drosera rotundifolia*

A total of 70 young specimens of *Menyanthes trifoliata* were planted within the same habitat type in September 2013. A leading criterion in the choice of location was the availability of water level, remaining as long as possible during the growth season. *Sphagnum*-dominated mires around the Boyana River over the building "Kamennoto Zdanie", at an altitude of 1800 m were identified as suitable and 20 new individuals of *Menyanthes trifoliata* were planted. A total of 80% of individuals survived. Other 30 of the young individuals were planted in the area of the Konyarnika locality in peatland along the stream with a constantly running water. This site showed highest interception of individuals - 90%, and flowering individuals were observed in the spring of 2015, which proves the successful adaptation of the species. The remaining 20 new plants from the Buckbean were planted in the peripheries and the shallows of bogs at the foot of the Cherny Vruh peak. The altitude here is bigger (2200 m) and the growing season is shorter, yet the plants adapted well and a high degree of interception was observed (70%) (Figure 11).

Fig. 11. Newly planted individuals of *Menyanthes trifoliata*

CONCLUSION

Taxus baccata has been successfully propagated from mature stem cuttings. The attempt for its reintroduction showed that the limiting factor for the young seedlings is sunlight. They are growing well in places with high air humidity, on moist and rich soils. Partially lighted places in the beech forest communities were found appropriate for their development (natural habitat type 9130 Asperulo-Fagetum beech forests).

The reintroduction of the seed generation of *Clematis alpina* along the Dragalevska River in the Kominite locality has been successful, in conditions of ample air humidity, Western exposition and rocky silicious terrain. The vegetative method for production of *Acer heldreichii* used because of lack of fruit production is atypical and unreliable. The few produced seedlings grew well in fresh and rich spruce habitats.

Suitable locations for the reintroduction of *Drosera rotundifolia* and *Menyanthes trifoliata* are waterlogged areas, representing the natural habitat type 7140 Transition mires and quaking bogs. The seedlings of Round-leaved Sundew are in good condition in areas with preserved hydrologic regime, dominated by peat mosses. They have high requirements of light and cannot tolerate the competition of tall grasses and hedge herbs.

The successful reintroduction and the restoration of extinct species in Vitosha Nature Park are the most valuable results. *Menyanthes trifoliata* has successfully propagated vegetatively. It should be noted that high water level during the active season of vegetation types in the bogs and peatlands is a necessary condition for the successful development of the plants. The presence of individuals with generative organs in the spring of 2015 is a confirmation for its successful adaptation in the Vitosha Mountains (Figure 12).

Fig. 12. *Menyanthes trifoliata* at the time of flowering

Acknowledgment: This work was done within the Projects №58301-33-453 „Implementation of the priority activities of the Management Plan of Vitosha Nature Park" funded by Contract № 58301-C-003 co-financed by the European Regional Development Fund and the Cohesion Fund of the European Union, and LIFE+08 NAT/BG/000281 "Conservation and restoration of 11 Natura 2000 Riparian and Wetland Habitats in 10 SCI's in Bulgarian Forests". The authors are grateful to Associate Professor Rayna Natcheva, PhD from Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, and to the team of the Vitosha Nature Park Directorate for their kind assistance.

REFERENCES

1. Arnaudov, N. 1920. Common Yew in Vitosha. *Ann. Sofia Univ*., *FFM*, 15-16: 1-9.
2. Biological Diversity Act, prom. SG №77/09.8.2002
3. Council Directive 92/43/EEC of 21 May 1992 on the Conservation of natural habitats and of wild fauna and flora.
4. Dimitrova, D. 2015. *Acer heldreichii* Orph. - In: Peev, D. (ed.), Red Data Book of The Republic of Bulgaria, Vol. 1. Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water. Digital edition: http://e-ecodb.bas.bg/rdb/bg/
5. Dountchev, A. 2005. An Investigation of the state of Heldreich's Maple (*Acer heldreichii* Orph. Ex Boiss) on the territory of Vitosha Nature Park, Report to a contract №41/25.07.05 with the Vitosha Nature Park Directorate.
6. Evstatieva, L. 2015. *Taxus baccata* L. - In: Peev, D. (ed.), Red Data Book of The Republic of Bulgaria, Vol. 1. Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water. Digital edition: http://e-ecodb.bas.bg/rdb/bg/
7. Evstatieva, L. 2015. *Menyanthes trifoliata* L. - In: Peev, D. (ed.), Red Data Book of The Republic of Bulgaria, Vol. 1. Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water. Digital edition: http://e-ecodb.bas.bg/rdb/bg/
8. Hajek, M., Tzonev R., Hajkova, P., Ganeva, A., Apostolova I. 2005. Plant communities of the subalpine mires and springs in the Vitosha Mt. *Phytologia Balcanica*, 11 (2): 193-205.
9. Jordanov, L. 1977. Vitosha mountain, Zemizdat, Sofia, pp. 96-98.
10. Management Plan of Vitosha Nature Park (2005-2014). Vitosha Nature Park Directorate, Executive Forest Agency of Ministry of Agriculture and Food.
11. Kitanov, B., Penev, I. 1963. Flora of Vitosha, Nauka and Izkustvo, Sofia, 513.
12. Natcheva, R., Ganeva, A., Dimitrov, M., Gyurova, D. 2016. Plant diversity of Sphagnum-dominated mires in Vitosha Nature Park. *Forest review* (in press).
13. Penev, N. 1940. Common Yew in Vitosha. *Lesovadska missal*, 9: 315-320.
14. Petrova, A., Vladimirov, V. (eds) 2009. Red List of Bulgarian vascular plants. *Phytologia Balcanica*, 15 (1): 63-94.
15. Project Life08 NAT/BG/000281, 2014. Conservation and restoration of 11 Natura 2000 Riparian and Wetland Habitats in 10 SCI's in Bulgarian Forests. Technical report „A description of the activities and lessons learned", WWF, Sofia, 51.
16. Project of resumption of the Common Yew (*Taxus baccata* L.) on the territory of Vitosha Nature Park, 2001. Vitosha Nature Park Directorate and "Home Garden TT" Ltd.
17. Project of resumption of the locality of the Alpine Clematis (*Clematis alpina* (L.) Mill.) on the territory of Vitosha Nature Park, 2005. Vitosha Nature Park Directorate and "Home Garden TT" Ltd.
18. Tsavkov, E. 2011. An investigation on the state of Heldreich's Maple (*Acer heldreichii* Orph. in Boiss) on the territory of Vitosha Nature Park. Report to a contract OPOS №50/01.09.2011 with the Vitosha Nature Park Directorate.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 54-62

RESTORATION AND PROTECTION OF THE POPULATION OF *SALIX PENTANDRA L.* AND *GALANTHUS ELWESII* HOOK IN VITOSHA NATURE PARK

DESSISLAVA GYUROVA[1*], SLAVCHO SAVEV[2]

1 – Vitosha Nature Park Directorate, 1303 Sofia, 17 Antim I Str, Bulgaria
2 – Department of Silviculture, Faculty of Forestry, University of Forestry, Sofia
**Corresponding author: dessybg2001@yahoo.com*

Keywords: plants, restoration, protection, vegetative propagation, Vitosha Nature Park, *Salix pentandra, Galanthus elwesii*

Abstract: One of the objectives of the Management Plan of Vitosha Nature Park is to protect the natural conditions and restore populations of species of conservation significance. Bay Willow (*Salix pentandra* L.) and Giant Snowdrop (*Galanthus elwesii* Hook.) are included in Annex 3 of the Bulgarian Biodiversity Act and are enlisted in the Bulgarian Red Data Book as Critically Endangered (CR) and Endangered (EN) species respectively. Bay Willow in Bulgaria occurs only in the territory of Vitosha Nature Park with limited numbers and Giant Snowdrop is considered extinct from the park territory. Methods of vegetative propagation, suitable substrates and period of collection of vegetative propagules are established through an *in vivo* experiment. New individuals of the two species have been produced and they have been planted in their natural habitats in Vitosha. High degree of survival of new individuals has been found through subsequent monitoring.

INTRODUCTION

One thousand four hundred eighty nine higher plant species are described in the territory of the Vitosha Nature Park. Some of them are under the protection of the environmental legislation in Bulgaria (Management Plan of Vitosha Nature Park, 2005-2014). *Salix pentandra* and *Galanthus elwesii* are protected by the Bulgarian Biodiversity Act (Annex 3) and included in the Bulgarian Red Data Book as critically endangered (Apostolova, 2015) and endangered species (Evstatieva, 2015) respectively. The Management Plan of Vitosha Nature Park prioritized the conservation of the most important plant species by developing

and implementing programs that support their habitats and through reintroduction activities.

Bay Willow (*Salix pentandra*) is a glacial relict and in Bulgaria it only occurs in Vitosha and the Choklyovo swamp (where it is considered extinct). There are some non-published data about attempts for reintroduction of the species along the swamp, carried out in the period 2001-2004. Two specific localities occur in the territory of Vitosha and there are some chorological data about that – the woodlands around the art house of Artists and the peatlands in the area of Ofeliite. The species has weak reproductive potential and it is sensitive to climate changes, forest fires, drainage of habitats etc. (Apostolova, 2015). The inventory carried out by experts of the Vitosha Nature Park Directorate did not confirm the presence of the species in the Ofeliite locality, which encouraged activities for the reintroduction of the species back. These activities carried out in 2001-2003 resulted in the restoration of *Salix pentandra* in this area (Project of restoration of the localities of Bay Willow in Vitosha Nature Park, 2001) and the subsequent introduction of the species along the information alley "Forest peat bogs" (Fig. 1).

Fig. 1. *Salix pentandra* L. in Ofeliite locality, 2013

The reduction of the localities of *Salix pentandra* is also due to ecological succession and habitat changes. The species is a part of habitat type 91D0 Bog Woodland, subtype 44.A412 Spruce and Spruce-pine forests on peat substrates with the participation of *Moneses uniflora* and *Sphagnum girgensohnii* in enlightened areas (Directive 92/43/EEC). The seed propagation of the species is really difficult because of the strong fragmentation of this forest subtype. The

reasons for this fragmentation are mainly anthropogenic. Because of that the reintroduction of vegetatively produced seedlings of the species is needed.

Giant Snowdrop (*Galanthus elwesii*) is rare throughout most of the country, not reported from the Black Sea coast, Belasitsa and Strandzha Mts (Evstatieva, 2015). According to old herbarium materials, the species occurred in Vitosha in the area of Boyana village, on Boyana River and in the area of the Boyana Waterfall. The last chorological data are from 1961 (Sidjimova, 2008), but nowadays this species has nor been confirmed in this area. There are reports for damaging the population of Giant Snowdrop (Stoyanov, 1937) and for collecting it as a decorative and medicinal plant in the past (Urumov, 1930; Jordanov, 1977). Considering this, we can certainly think that *Galanthus elwesii* has gone extinct from the territory of Vitosha.

This article presents the results from the restoration and protection of the population of *Salix pentandra* and *Galanthus elwesii* in the Vitosha Nature Park.

MATERIALS AND METHODS

The natural habitat of *Salix pentandra* in Vitosha occurs around the art house of Artists on the South-western part of the mountain at an altitude of 1470 m and it represents a swamp forest in the community of *Picea abies* L. Reproductive materials were collected from individuals, which grow in this area (Figure 2) - vegetative annual shoots with a length of 30-50 cm (Figure 3). They were collected in two consecutive years – in April 2013 and 2014 before beginning of the vegetation. A total of thirty branches were picked each year. Wet towels were used to retain the moisture during the transport to the nursery. The reproduction of vegetative materials was carried out in the "Gorski dar" nursery in Studena village. The greenhouse was used for this purpose.

Fig. 2. Motherhood locality of *Salix pentandra*

Fig. 3. Vegetative annual shoots from *Salix pentandra*

On the day of the collection, the shoots were cut into cuttings, 7-10 cm long. After that, they were potted in 20 cm deep containers at 50 % of their length. The substrate used was a mixture of sand and perlite at a ratio of 4:1. The water regime was maintained by double-watering through a fogging system which provided a relative humidity of above 80 %. Callus formation began in the third-fourth week after potting. Rooting in the sterile substrate finished in the twelfth-thirteenth week.

The rooted cuttings were potted on the fifteenth week in two-liter containers with peatland soil substrate. Their adaptation lasted for six weeks in the same conditions as in the greenhouse. Their placement occurred in May and September 2014.

The natural habitat of *Galanthus elwesii*, which we used for the collection of reproductive materials, is located on the riverside of Iskar River in the Lozen Mountains, Kokalyane village, under the fortress of Urvich, at an altitude of 650 m (Sidjimova, 2008). The species there is developing in a forest, dominated by *Alnus glutinosa* (L.) Gaertn. on alluvial soils (AlluviiFluvisoils). Bulbs from Giant Snowdrop were collected as reproductive materials. Thirty bulbs were collected in March 2013 (Fig. 4). The transport and reproduction took place under the mentioned conditions and in mixture of sand, perlite and alkaline peat at a ratio of 2:1:4 were used (Fig. 5). The length of the bulbs picked was about 1,5 times their original length.

Fig. 4. Bulbs of *Galanthus elwesii*

Fig. 5. Placing the bulbs of *Galanthus elwesii* in a suitable substrate

The formation of daughter bulbs started in the fourth-fifth week after the death of vegetative leaves and ended after the fortieth week. The reproduction materials were planted in 0,9 liter containers right after the formation of daughter bulbs. The individuals were adapted for eight weeks. The new individuals were planted in their new place in April 2014 after the generative organs of the plants wither away.

The new individuals were planted in preliminary chosen places. The ecological conditions of the source population were taken under consideration for the right selection of sites. The afforestation activities were organized by the team of the Nature Park Vitosha Directorate and were supported by many volunteers.

RESULTS AND DISCUSSION

The new individuals from both species were ready to be introduced in their natural habitat in the spring of 2014. Two hundred saplings of *Salix pentandra* (Figure 6) and two hundred new plants of *Galanthus elwesii* were produced (Figure 7).

Fig. 6. Saplings of *Salix pentandra*

Fig. 7. New plants of *Galanthus elwesii*

Waterlogged pitches, marshy places, forest woodlands or peatlands in beech and coniferous belt are suitable for the introduction of saplings of Bay Willow. These specific types exist in several places in Vitosha and they are presented in the mentioned habitat 91D0 Bog Woodlands, subtype 44.A412 Spruce forests on peat substrates. The saplings were planted in the spring and autumn of 2014. They were divided in two halves. The first one (100) were planted in the area near the original locality of Bay Willow on waterlogged soils, at an altitude of

1500 m. Plantation took place on three different sites with different lighting. The other half (100) was planted at an altitude of 170 m, in forest peatlands near the "Zvezditsa" hut (Figure 8). The saplings there were divided equally in two adjacent peatlands, all planted in open ground. The subsequent monitoring in the autumn of 2015 showed that there was a greater percentage of interception (90%) on the peatlands in open ground as compared to under the canopy of the bog woodland (30%).

Fig. 8. Newly planted saplings of *Salix pentandra*

We studied some areas around riverside forests on rich alluvial soils, which are close to the original locality in Lozenska Mts for the right place to plant *Galanthus elwesii*. Such places exist along the rivers of Struma and Kladnishka and they are a part of the natural habitat 91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Directive 92/43/CEO). They are characterized by rich alluvial soils, inundated by the river periodically. One hundred individuals of Giant Snowdrop were planted on Kladnishka River, the other sixty plants were planted along the Struma River. One small part of snowdrops (forty) were planted on Boyana River, around the Boyana Village because this site is mentioned as historic locality in the literature. All of the three populations created are at an altitude between 950 m and 1000 m. The conditions along the Boyana River are different from the two other sites, yet this was the place where the saplings had the highest percentage of interception (100%) – on rocky steep terrains in the zone of beech forests (habitat type 9110 *Luzulo-Fagetum* beech forests) - Fig. 9.

Fig. 9. *Galathus elwesii* on Boyana Rive

The success in the other two sites is as follows: we found 60 % interception along the Struma River, and the lowest number of plants was counted along the Kladnishka River – 20 %.

CONCLUSION

Our experience with reintroducing *Salix pentandra* showed that young saplings successfully grew in sparse forest communities, forming the natural habitat 91D0 Bog Woodland, subtype 44. A412 Spruce forests on peatland substrates. The young saplings are extremely photophilic and do not develop normally under forest canopy. The limiting factors are clay soils. The young individuals prefer soil with good aeration and good humidity and the peatlands provide such conditions.

The reintroduction of *Galanthus elwesii* individuals along three Vitosha rivers showed a different rate of success. The young individuals develop the best on steep, rocky along-river terrains, forming habitat type 9110 *Luzulo-Fagetum* beech forests. The illumination of the terrain, the richness and mechanical ingredients of the soil substrate do not matter much for their development. The most important condition is the better aeration of the soil. Waterlogged terrains are bad for them. Because of this reason the interception of snowdrops is smallest on heavy and waterlogged soils along Kladsnishka River.

Acknowledgment: This work was financed through Project N:5103020-11-654 "Implementation of the priority activities of the Management Plan of Vitosha Nature Park - Phase II" funded by Contract N:5103020-C-001 grant under the Operational Program "Environment 2007-2013" co-financed by the European regional Development Fund and the Cohesion Fund of the European Union.

The authors are grateful to Chief Assistant Boryana Sidjimova, PhD from Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, and the team of the Vitosha Nature Park Directorate for their kind assistance.

REFERENCES

1. Apostolova, I. 2015. *Salix pentandra* L. – In: Peev, D. (ed.), Red Data Book of The Republic of Bulgaria, Vol. 1. Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water. Digital edition: http://e-ecodb.bas.bg/rdb/bg/
2. Biological Diversity Act, prom. SG №77/09.8.2002
3. Council Directive 92/43/EEC of 21 May 1992 on the Conservation of natural habitats and of wild fauna and flora.
4. Evstatieva, L. 2015. *Galanthus elwesii* Hook. - In: Peev, D. (ed.), Red Data Book of The Republic of Bulgaria, Vol. 1. Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water. Digital edition: http://e-ecodb.bas.bg/rdb/bg/
5. Jordanov, L. 1977. Vitosha mountain. Zemizdat, Sofia, pp. 103-104.
6. Management Plan of Vitosha Nature Park (2005-2014). Vitosha Nature Park Directorate, Executive Forest Agency of Ministry of Agriculture and Food.
7. Project of restoration of the localities of Bay Willow in Vitosha Nature Park, 2001. Vitosha Nature Park Directorate.
8. Sidjimova, B. 2008. Biological and phytochemical study of species of the genus *Galanthus* L. (Snowdrop) in Bulgaria. Dissertation for obtaining an educational and scientific degree "doctor". Institute of Botany at the Bulgarian Academy of Sciences, Sofia.
9. Stoyanov, N. 1937. Plant relations in Sofia valley. *Ann. Sofia Univ*., 15 (1): 1-37
10. Urumov, I. 1930. The flora of Vitosha mountain. *Collection of Bulgarian Academy of Sciences*, 26 (1): 8 p.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 63-70

PRESERVATION OF THE BULGARIAN ENDEMIC VERBASCUM DAVIDOFFII (*SCROPHULARIACEAE*) BY MEANS OF *IN VITRO* PROPAGATION

MARINA STANILOVA*, STOYAN STOYANOV, BORYANA SIDZHIMOVA, BORYANKA TRAYKOVA

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Department of Plant and Fungal Diversity and Resources, Akad. G. Bonchev Str., bl.23, 1113 Sofia, Bulgaria
**Corresponding author: marina.stanilova@gmail.com*

Keywords: plant biotechnology, seed germination, endangered plants, reintroduction

Abstract: *Verbascum davidoffii* Murb. (*Scrophulariaceae*) is one of the rarest plant species in the Bulgarian flora. It is a Bulgarian endemic, protected by the national Biodiversity Act (2002), included in the Red List of vascular plants in Bulgaria and in the Red Data Book of Bulgaria with conservation status "Critically Endangered". The populations of this biennial herbaceous plant consist of sparse individuals and are severely fragmented; all known localities are within Pirin National Park and in a site of the European ecological network Natura 2000 in Bulgaria, between the valleys of Banderitsa River and Razlozhki Suhodol, at an altitude of 1800 up to 2300 m a.s.l. The distribution of *V. davidoffii* is limited due to its peculiar biology related to specific ecological requirements and low reproductive capability, as well as to the anthropogenic pressure: forest felling, destruction and pollution of the habitats because of tourism and infrastructure development in the region.

In order to preserve this species of conservation value, *ex situ* and *in situ* activities have been designed concerning elaboration of a specific protocol for *in vitro* propagation, followed by establishment of an *ex situ* collection, and strengthening of the wild populations. As a first step, seed germination has been studied. Seeds were gathered in August 2015 from the locality close to the "Banderitsa" rest-house and were successfully disinfected through a standard laboratory procedure. Seed germination was poor on the basal MS medium: only 1 seedling was obtained from 100 seeds for a period of 6 weeks; and no seed germinated on MS medium supplemented with 1 mg/l Kin. The stimulation of the process by seeds soaking in 0,35 % solution of gibberellic acid for 22 hours increased the germination rate up to 61 % and 18 % for the two media, respectively. The effect of the gibberellic acid was strong even if applied for only 2 h, and the concentration of kinetin was better when supplemented in 10-fold less concentration. Seed stratification with low temperature at 6°C for a month prior to cultivation had additional effect on germination which depended on the presence of kinetin in the medium. *In vitro* seedlings with several leaves and roots were potted in soil substrate of soil mixture, sand and coconut fiber (2:1:1), and easily *ex vitro* adapted into the ambience of the laboratory phytotron, under controlled temperature, light, and humidity variations.

INTRODUCTION

Verbascum davidoffii Murb. (Scrophulariaceae) is a biennial herbaceous plant with erect unbranched stem, densely haired leaves, calyx covered with long glandular black hairs, and with golden yellow flowers (Stefanova-Gateva, 1995). It is a Bulgarian endemic (Petrova, 2006), protected by the National Biodiversity Act (2002), included in the Red List of vascular plants in Bulgaria (Assyov & Denchev, 2009) and in the Red Data Book of the Republic of Bulgaria (Assyov & Denchev, 2015) classified as "Critically Endangered" acording to the IUCN criteria. All known localities are within Pirin National Park, some of them in Bayuvi Dupki-Dzhindzhiritsa Strict Nature Reserve, and in a protected site of the European ecological network Natura 2000 in Bulgaria.

The population is represented by several subpopulations, located between the Valley of Banderitsa River and Razlozhki Suhodol peak, at an altitude of 1800 to 2300 m alt. Habitats are calcareous, stony and grassy places in subalpine belts and in open forests of *Pinus heldreichii.* The distribution of *V. davidoffii* is limited due to its peculiar biology (reproduction by seeds), related to specific ecological requirements and low reproductive capability, as well as to the anthropogenic pressure: forest felling, destruction and pollution of the habitats because of tourism and infrastructure development in the region. According to the monitoring of the population in 2014, the species is currently in unfavorable-bad condition. This is due to the small number of individuals, established on an area of 30 hectares.

In order to preserve this species of conservation value, *ex situ* and *in situ* activities have been designed concerning elaboration of a specific protocol for *in vitro* propagation, followed by establishment of *ex situ* collection, and strengthening of the wild populations.

MATERIALS AND METHODS

The monitoring of *V. davidoffii* was held in July 2015 according to the approved monitoring methodology, developed for the needs of NSEM (National System for Environmental Monitoring).

Seeds were gathered in August 2015 from the locality close to the "Banderitsa" rest-house. Their germination capability was tested immediately as well as after one month of stratification with low temperature at 6°C. The two sets consisted of 400 seeds each, divided in four groups, to test additional stimulation with both gibberellic acid (GA_3) and / or Kinetin (Kin). Seeds were soaked in 0,35 % solution of GA_3 for 22 h before sterilizing. Then, they were disinfected through standard laboratory procedure (consecutive soaking in 70 % ethanol for 1 min, and in commercial bleach, chlorine < 5 %, for 10 min, followed by thrice rinses in sterile distilled water, 10 min each). The plant growth regulator Kin was added to the nutrient medium in 2 concentrations: 1 mg/l or 0,1 mg/l (media MS-K1 and

MS-K01). Basal MS medium (Murashige and Skoog, 1962) and seed soaking in tap water instead of gibberellic acid, was used as a control variant. Additionally, the effect of GA_3 was studied for shorter time: 2 h of seed treatment, only for MS basal medium.

The germination rate was calculated as an average percentage of germinated seeds on the basis of two repetitions of 50 seeds per variant, for a period of 4 weeks.

In order to compare the germination under different laboratory conditions, seeds were treated with GA_3 as described above (or water in the control variant) and placed either in petri dishes supplied bilaterally with wet filter paper, 25 seeds per petri dish, 3 replications per variant, or in a special terrine of 96 small pots with soil substrate, covered by polyethylene folio to maintain suitable air humidity.

The bigger 35 seedlings were *ex vitro* adapted in soil substrate consisting of soil mixture, sand, and coconut fibers in proportion 2:1:1, two plantlets per pot with diameter of 9 cm. The first step of the adaptation was conducted in a growth camera (POL-EKO Aparatura) for 4 weeks, under strict control of the important ambient conditions, simulating their natural daily dynamic: 10 h "day" under 2070 lx white light at 23°C, 8 h "night" in a dark, at 18°C, and two intermediate periods of 3 h each, under 1500 lx at 20°C. Plants are currently in the second step of the *ex vitro* adaptation, on the shelves of a room phytotron with a window, with less strict control of the temperature (22 ± 4°C), air humidity (between 35 and 60 %), and light (mixed: daylight and artificial light provided by warm white LED bands 16 h per day).

All the other seedlings were sub-cultured on fresh media supplemented with Kin or 6-benzylaminopurine (BAP) in combination with α-naphthaleneacetate (NAA) or Indole-3-butyric acid (IBA) to stimulate further shoot multiplication.

RESULTS AND DISCUSSION

The results of the monitoring, conducted in July 2015, showed that in an area of about 5 ha, including the transect route Vihren hut-Banderitsa hut-Malkiya Kazan locality, *V. davidoffii* was represented with 52 adult and 16 vegetative individuals. In comparison, the data of NSEM concerning the species in 2014 reported 30 flowering and 15 juvenile plants counted between the huts of Banderitsa and Vihren (Karakiev, unpublished). In view of the size of the inspected area which was about twice larger in 2015, the numbers of the *V. davidoffii* population was similar. The variable correlation between flowering and new individuals along the years depended on seed propagation success of this biannual plant species. Less successful seed germination resulted in less new individuals next year and superiority of the adult flowering plants over the new ones.

Because of the low competitiveness of the species, plants were noticed mainly along the asphalt road between the huts of Banderitsa and Vihren or on other open sites such as stony and erosive terrains on the path connecting the Banderitsa and the Kazan localities.

Non-stimulated seed germination under laboratory conditions was poor: only one seedling was obtained on the control MS medium from 100 seeds for a period of 4 weeks. The stimulation of the process by seeds soaking in 0,35 % solution of gibberellic acid for 22 hours increased the germination rate to 61 % on the basal MS medium (Fig. 1). The effect of the gibberellic acid when applied for 2 h was significant but less strong, in this case 28 % of the seeds germinated (Fig. 2). The stratification slightly enhanced the stimulation effect of GA_3, and seed germination reached 66,1 % on the basal MS medium. In comparison, the seed stratification applied alone was useless for *V. davidoffii* as only 3 % of the seeds soaked in water germinated on MS medium.

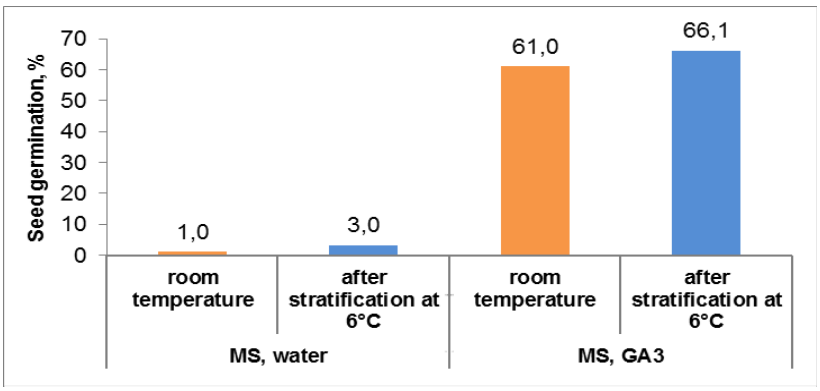


Fig. 1. Germination of *V. davidoffii* seeds on basal MS medium, with or without stratification and pretreatment with GA_3.

Fig. 2. Germination of *V. davidoffii* seeds on basal MS medium after soaking in: **A**) water, for 22 h; **B**) 0,35 % GA_3 for 22 h; **C**) 0,35 % GA_3 for 2 h.

No seed germinated on medium MS-K1 supplemented with 1 mg/l Kin (Fig. 3). Furthermore, the presence of kinetin in the medium inhibited the process as it caused both delayed germination and decreased effect of GA_3, and only 18 % of the seeds germinated on medium MS-K1. To decrease the negative influence of kinetin, the medium supplemented with this plant growth regulator was modified, its concentration being reduced to 0,1 mg/l. The use of Kin in a 10-fold lower concentration in the medium had a significant stimulation effect. Thus, 29,2 % of the seeds soaked in water germinated on medium MS-K01 which was about ten times more compared to that of the basal MS medium (Fig. 3 & Fig. 1). It is possible that the stratification had an additive influence. The combined effect of GA_3 and 0,1 mg/l Kin resulted in additional increase of the germination, up to 63,2 %. The highest germination rate of 66,1 % was however observed in the variant without Kin, when seeds were pretreated with both one-month low temperature at 6°C and soaked in 0,35 % GA_3 for 22 h.

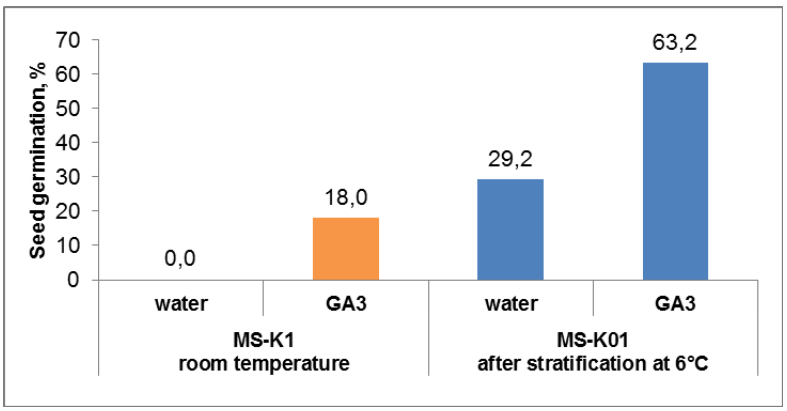


Fig. 3. Germination of *V. davidoffii* seeds on media supplemented with different concentrations of Kin, with or without stratification and pretreatment with GA_3.

It is worth to mention that a part of the seedlings had etiolated and deformed leaves due to the long seed treatment with GA_3 and finally 8,2 % of them were lost. This effect was not observed in the case of the short seeds soaking. In all variants on basal MS medium seed germination began in the first week when stimulated with GA_3 pretreatment and in the second week without its usage. The end of the germination was noticed after 3 weeks. In the variant of medium MS-K1 the beginning and the end of the process were delayed with 2 weeks. The stratification with low temperature enhanced the germination time with about one week in all tested variants.

Seeds placed in the terrine with soil substrate had similar germination rates to those of the *in vitro* germinated seeds. No seed germinated during the first

4 weeks when soaked in water, while 60 % of the seeds pretreated with GA_3 germinated. The highest seed germination rates were noticed in the petri dishes: 72,7 % when pretreated with GA_3 for 22 h and 9,4 % when soaked in water. However, these seeds were tiny, and their survival was problematic.

Low seed germination is a common problem for many endangered species and endemics. There are different approaches for increasing the germination rates or for the seed dormancy break: treatment with plant growth regulators, stratification with high or low temperature, application of red light or different light regime, etc.

The effect of some of these factors was tested on two *Verbascum* species endemic for Turkey: *V. bithynicum* and *V. wiedemannianum* (Senel et al., 2007). Contrariwise to the authors expectations, GA_3 prevented their seed germination even if applied in very low concentration, between 20 and 200 mg/l. To our opinion this was due to the application way: seeds were incubated in petri dishes with the GA_3 solution. We also noticed the inhibitory effect of the gibberellic acid on seed germination of another Bulgarian endemic of this genus, *Verbascum tzar-borisii*, when added in the nutrient medium (unpublished data).

Gibberellic acid is frequently used to pretreat seeds of many species in order to stimulate their germination, by seed soaking for several hours prior to cultivation (Kabar, 1990; Srivastava et al., 2011; Roychowdhury et al., 2012). It was successfully applied in a large concentration range for different time. The usual way of kinetin application is also as solution for seed pretreatment (Kabar, 1990; Roychowdhury et al., 2012).

The results of our study showed that the gibberellic acid was the most important stimulator of the germination of *V. davidoffii* seeds. The effect of Kin was less expressed and ambiguous. The reason for this difference could however be due to the way of the application of the two plant growth regulators: we tested GA_3 only as pretreating solution while Kin was added in the nutrient medium.

Different plant growth regulators: NAA, IAA, GA_3, as well as some simple combinations like KNO_3, HNO_3, HCl, H_2SO_4 were found to have various stimulating effect on seed germination when applied as solutions for seed pretreatment (Srivastava et al., 2011). Their effect depended on the concentration, the time of seed soaking, and the temperature. The seed response differed also from one plant species to the other.

Other authors also tested combinations of GA_3 and Kin on seed germination. The effect depended on the species as well as on the way of application. Thus, the germination of the lettuce seeds was enhanced by the combination of kinetin and suboptimal concentration of gibberellic acid (Ikuma and Thimann, 1963) while the same combination applied for 72 h caused a slight inhibitory effect on dark-grown *Lepidium* seeds (Evans and Fratianne, 1977). Different effects of these two plant growth regulators were noticed when applied to several dicots and monocots under saline conditions. Kinetin was more effective for seed germination

enhancement of most tested dicots, and the combination of GA_3 and Kin did not exceed its effect (Kabar, 1990). Even seeds with relatively high germination rate were influenced by solutions of GA_3, Kin, and IAA (Roychowdhury et al., 2012). Authors found out the optimal concentration of each of these stimulants and revealed their inhibitory effect in higher and lower concentrations.

Seedlings in all the variants were not uniform because seeds needed different time to germinate. The faster growing *in vitro* seedlings with several leaves and roots each were ready to be *ex vitro* adapted 8 weeks after the beginning of the experiment. They were easily adapted to the used soil mixture in the growth chamber with a strict control of temperature, humidity and light regime. The gradual decrease of the air humidity is of crucial importance for most of the *in vitro* obtained plantlets. The drastic difference of the survival rate due to the air humidity was reported in our previous work on other species (Stanilova et al., 2013). In the case of *V. davidoffii* 31 plants of the first 35 survived, strengthened and grew enough to be transferred to the shelves of the room phytotron after 4 weeks (Fig. 4). All of them are growing well and will be further used for establishment of an *ex situ* collection and for reintroduction in their population of origin.

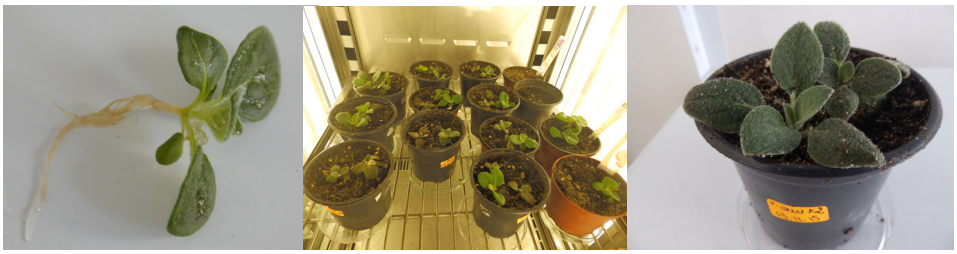


Fig. 4. *Ex vitro* adaptation of *V. davidoffii*: **A**) In vitro obtained 8-week aged seedling; **B**) Potted in vitro plants, in the growth chamber; **C**) Plants on the shelves of the room phytotron

CONCLUSIONS

In vitro cultures of *Verbascum davidoffii* have been successfully initiated. The seed germination rate reached its maximum of 66,1 % when seeds were placed on basal MS medium after being pretreated with both: one month stratification at 6°C, and 22 h soaking in 0,35 % solution of gibberellic acid. The first 31 *ex vitro* adapted plants are currently growing well in the phytotron. They will be used for establishment of an *ex situ* collection and for reintroduction in their population of origin.

The study will continue with sub-cultivation of the seedlings on fresh media with different compositions, supplemented with Kin or BAP in combination with NAA or IBA, to test their effect on shoot multiplication. The reproductive peculiarities of *Verbascum davidoffii* will be clarified, as well as the genetic diversity of the populations and the genetic fidelity of the *in vitro* multiplied plants.

Acknowledgment: This work was supported by grant DFNI-BO2/18 of the Bulgarian National Science Fund.

REFERENCES

1. Assyov, B. & Denchev, C. 2009. *Verbascum davidoffii* Murb. – In: Petrova, A. & Vladimirov, V. (eds). Red List of Bulgarian vascular plants. Phytologia Balcanica, 15(1): 63-94.
2. Assyov, B. & Denchev, C. 2015. *Verbascum davidoffii* Murb. – In: Peev, D. & al. (eds). Red Data Book of the Republic of Bulgaria. Vol. 1. Plants and Fungi, p. 350. IBER-BAS & MOEW, Sofia.
3. Biological Diversity Act. 2002. Official gazette of Republic of Bulgaria 77 (from 09.08.2002): 9-43; amendments: Darzhaven Vestnik, 88 (04.11.2005), 105 (29.12.2005), 29 (07.04.2006), 30 (11.04.2006), 34 (25.04.2006), 52 (29.06.2007), 64 (07.08.2007), 94 (16.11.2007), 43 (29.04.2008), 19 (13.03.2009), 80 (09.10.2009), 103 (29.12.2009), 62 (10.08.2010), 89 (12.11.2010) (in Bulgarian).
4. Evans, R.C., Fratianne, D.G. 1977. Interactions of applied hormones in the germination of *Lepidium virginicum* seeds. Ohio J. of Science, 77 (5): 236-239.
5. Ikuma, H., Thimann, K.V. 1963. Action of kinetin on photosensitive germination of lettuce seed as compared with that of gibberellic acid. Plant Cell Physiol., 4: 113-128.
6. Kabar, K. 1990. Comparison of Kinetin and Gibberellic Acid Effects on Seed Germination under Saline Conditions. Phyton (Horn, Austria) 30 (2): 291-298.
7. Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., 15: 473-497.
8. Petrova, A. (ed.) 2006. Atlas of Bulgarian Endemic Plants, p. 234. Gea-Libris, Sofia.
9. Roychowdhury, R., Mamgain, A., Ray, S., Tah, J. 2012. Effect of Gibberellic Acid, Kinetin and Indole 3-Acetic Acid on Seed Germination Performance of *Dianthus caryophyllus* (Carnation). Agriculturae Conspectus Scientificus, 77(3):157-160.
10. Senel, E., Ozdener, Y., Incedere, D. 2007. Effect of temperature, light, seed weight and GA_3 on the germination of *Verbascum bithynicum, Verbascum wiedemannianum* and *Salvia dicroantha*. Pakistan J. of Biol. Sciences 10 (7): 1118-1121.
11. Srivastava, N., Sharma, V., Dobriyal, A.K., Kamal, B., Gupta, S., Jadom, V.S. 2011. Influence of pre-sowing treatment on in vitro seed germination of ativisha (*Aconitum heterophyllum* Wall) of Uttarakhand. Biotechnology, 10 (2): 2015-2019.
12. Stanilova M, Gorgorov R, Beeva Y, Vitkova A. 2013. Acclimation of in vitro produced *Alchemilla* plants. Proceedings of the Seminar of Ecology, p. 89-95.
13. Stefanova-Gateva, B. 1995. *Verbascum*. In: Kožuharov, S. (ed.), Fl. Republ. Bulgaricae. Vol. 10, 26-100. Editio Acad. "Prof. Marin Drinov", Serdicae (in Bulgarian).

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 71-78

INTER-SIMPLE SEQUENCE REPEAT ANALYSIS OF GENETIC FIDELITY IN *IN VITRO* MICROPROPAGATED PLANTS OF THE ENDANGERED PLANT SPECIES *CENTAUREA DAVIDOVII* URUM. (ASTERACEAE)

GALYA PETROVA[1*], MARINA STANILOVA[2],
SVETLANA BANCHEVA[2], STEFAN PETROV[3]

1 - Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria
2 - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
3 - Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria
**Corresponding author: galiaty@abv.bg*

Keywords: Centaurea, *Ex situ* conservation, Balkan Mountains, ISSR, Genetic fidelity

Abstract: The genus *Centaurea* s. l. (Asteraceae) is one of the richest in endemics in the Bulgarian flora, represented by more than 70 species. One of them, the endemic *Centaurea davidovii* Urum., occurs in several very fragmented populations in the floristic region of Balkan Mountains. Habitat destruction and low reproductive capacity of this species, mainly due to weak seed germination and damage of seeds by insects, necessitate its conservation both *in situ* and *ex situ*. An *ex situ* collection of *Centaurea davidovii* individuals has been established recently at the experimental field of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. Plants had been obtained from a single seed using *in vitro* multiplication techniques. They were successfully acclimatized to the open-air field plot, bloomed and formed fertile seeds. True to type clonal fidelity is one of the most important prerequisites in the micropropagation of any plant species. A major problem encountered with the *in vitro* culture is the potential presence of somaclonal variation. Various factors, such as the *in vitro* process and its duration, nutritional conditions, *in vitro* stress, etc. occasionally appear to induce somaclonal variation. Hence, it becomes imperative to regularly check the genetic integrity of the *in vitro* regenerated plants in order to produce clonally uniform progeny. In the present study, the genetic stability of *in vitro* propagated *Centaurea davidovii* plants was tested for the first time using Inter Simple Sequence Repeat (ISSR) markers. The amplification products were monomorphic and no polymorphism was detected. Furthermore, ISSR analysis indicated the genetic integrity of *in vitro* propagated plants, as well as an identical banding pattern after more than ten passages of subculture. Our results showed that the regenerated plants are similar and therefore the developed micropropagation protocol for *in vitro* multiplication of *C. davidovii* is reliable and

represents a safe mode for production of true-to-type plants. Besides the establishment of the *ex situ* collection, planting of *in vitro* obtained plants in the wild populations has been designed aiming at their strengthening. This *in situ* measure should be conducted with strict respect to the genetic diversity conservation, which requires improvement of the seed germination rate and regeneration of plants of numerous normal genotypes.

INTRODUCTION

Centaurea davidovii is a Bulgarian endemic, represented nowadays by seven or eight very fragmented subpopulations, each with an area of occupancy of about 1-2 decares. The total number of individuals of this species does not exceed 1000. The plant populations are decreasing in size and the species is considered critically endangered, according to the IUCN criteria (Bancheva and Gorgorov, 2010).

The limited natural resources of *Centaurea davidovii*, as well as its low reproductive capacity require an urgent development of a long-term conservation program with multidisciplinary investigations on preservation and sustainable use of available genetic resources of this endangered plant species.

The use of biotechnological tools, such as *in situ* and *ex situ* conservation is a key strategy for long term conservation of endangered plant species (Henry, 2006). An *ex situ* collection of *Centaurea davidovii* individuals has been established recently at the Institute of Biodiversity and Ecosystem Research, BAS. The regenerated plants had normal vegetative and reproductive organs and intensive growth on the open experimental field. Their fertility has been manifested by mass flowering (**Fig. 1**) and formation of seeds which were able to germinate and to give rise to *in vitro* flowering plantlets (Gorgorov et al., 2015).

Fig. 1. *Ex situ* collection of *C. davidovii* established from *in vitro* multiplied plants. **A.** Acclimatized plants on the open field (May, 2014); **B**. Flowering stage (June, 2015)

True to type clonal fidelity is however one of the most important prerequisites in the micropropagation of any plant species (Chandrika et al., 2010). It is well known that somaclonal variation occurs ocasionally, depending on different factors, such as the species and the *in vitro* conditions, especially in case of callus derived plantlets (Nwauzoma and Jaja, 2013). On the other hand, the different methods of clonal propagation increase the genetic fidelity of the propagated plants, but also provoke genetic instability, stimulating DNA mutations of the regenerants (Bairu et al., 2006).

The present study was undertaken as a rapid screening for evaluation of the genetic stability of mature plants obtained by *in vitro* micropropagation of *Centaurea davidovii*, using the Inter Simple Sequence Repeat (ISSR) technique. This is the first molecular assessment of *C. davidovii*, as the first attempt for *in vitro* cultivation of this endemic plant species has been published recently. Molecular analyses of genetic diversity and population structure of this species are also contemplated as a part of other particular study.

MATERIALS AND METHODS

Plant material

An *ex situ* collection of *Centaurea davidovii* individuals has been established recently at the experimental field of the Institute of Biodiversity and Ecosystem Research, BAS. Plants were obtained from a single seed using *in vitro* multiplication techniques for direct and indirect regeneration, as previously described (Gorgorov et al., 2015). They were successfully acclimatized to the open-air field plot, bloomed and formed fertile seeds.

DNA extraction

Genomic DNA was extracted from leaf tissues (50 mg) of nine *Centaurea davidovii* plants from the *ex situ* collection, following the procedure described by Doyle and Doyle (1987). All DNA samples were quantified using NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

Inter Simple Sequence Repeat Analysis

The following ten ISSR primers (Microsynth, Balgach, Switzerland) were used in the analysis of genetic integrity of nine randomly chosen *in vitro* propagated *Centaurea davidovii* plantlets:

ISSR 1: 5' - GAG AGA GAG AGA GAG AT - 3'
ISSR 2: 5' - GAG AGA GAG AGA GAG AA - 3'
ISSR 3: 5' - CAC ACA CAC ACA CAC AG - 3'
ISSR 4: 5' - ACA CAC ACA CAC ACA CT - 3'
ISSR 5: 5' - ACA CAC ACA CAC ACA CC - 3'
ISSR 6: 5' - ACA CAC ACA CAC ACA CG - 3'
ISSR 7: 5' - AGA GAG AGA GAG AGAGYT - 3'
ISSR 8: 5' - AGA GAG AGA GAG AGA GYC - 3'
ISSR 9: 5' - GAG AGA GAG AGA GAG AYG - 3'
ISSR 10: 5' - ACA CAC ACA CAC ACA CYT - 3'

The ISSR-PCR analyses followed Petrova et al. (2014) with minor modifications. All PCR reactions were performed in a volume of 25 µl (1X PCR buffer (GenetBio, Korea), 1U Taq DNA polymerase (GenetBio, Korea), 100 µM of each dNTP, 1 µM of each primer and 50 ng of extracted DNA). The PCR experiments in the present study were performed using a a Techne TC-5000 Thermal cycler at the following cycling conditions: initial denaturation at 95ºC for 5 min, followed by 35 cycles of amplification [45 s at 94ºC, 1 min at the annealing temperature (T_a is adjusted according to the T_m of each ISSR primer being utilized in the reaction), elongation at 72ºC for 2 min] and a final elongation at 72ºC for 5 min. The PCR products were analyzed on 2% agarose gel electrophoresis in 0.5X TBE buffer (1.5 h at 120 V). The gels were stained by Ethidium bromide (0.5 mg/ml in TBE). The DNA - profiles were visualized with a UV - transilluminator and further analyzed along with 100 bp DNA ladder size (Thermo Scientific, Vilnius, Lithuania) using a video image analyzer (BioImaging Systems, Cambridge, UK). The well resolved and consistently reproducible amplified DNA fragments as bands were scored with regards to their presence (1) or absence (0).

RESULTS AND DISCUSSION

True-to-type clonal fidelity is one of the most important prerequisites in the micropropagation of any plant species. Many studies have however shown that the major problems in the protocols for *in vitro* cultures are the potential presence of somaclonal variation among sub-clones of one parental line, as well as the loss of genetic stability caused by genetic and epigenetic variations in regenerants (Tiwari et al., 2010). High concentrations of different plant growth regulators may induce somaclonal variation in different development stages (Bairu et al., 2006). The genetic instability is however highly unacceptable, especially in cases, where the *in vitro* collection is directed to the conservation of germplasm of endangered and rare species (Mallón et al., 2008).

The molecular approaches are being used extensively for scrutinizing the genetic stability of *in vitro* raised plants. As DNA based markers are not influenced by environmental factors, they present the most effective way to screen the tissue culture induced variations (Peredo et al., 2009). Nowadays, DNA markers are particularly useful for assessing genetic stability in many micropropagated plants ranging from

economically valuable clones (Valladares et al., 2006) to endangered plant species (Guo et al., 2007). As such, they are very important when there is no prior knowledge of the genome under consideration (Agarwal et al., 2008).

Among the PCR - based marker technique, ISSR molecular markers offer an advantage of being considerably less expensive and less time-consuming, because of the reduced number of protocol steps required and the smaller amounts of DNA consumed. Furthermore, no prior genomic information is required for their use and they can detect a greater number of polymorphisms than RFLP or RAPD (Bornet et al., 2002; Ye et al., 2005; Seyedimoradi and Talebi, 2014; etc.). ISSR - system is considered suitable to detect variations among micropropagated plants since a simple sequence repeat targets the fast evolving hypervariable sequences (Parida et al., 2011).

So far, ISSR analysis has been successfully applied to study the genetic stability of micropropagated plantlets of different crops (Martins et al., 2004; Alizadeh et al., 2008; 2009; Tsvetkov et al., 2014; etc.). Due to the clonal origin of the plants from our *C. davidovii ex situ* collection, they have been expected to share identical genotypes with that of the initial seed.

In the present molecular study with nine randomly chosen micropropagated plants *C. davidovii*, along with the donor mother plant, all selected ISSR primers generated a unique set of amplification products ranging in size between 200 and 3000 bp, total of 115 bands within 200 and 3000 bp. The number of formed bands in each primer ranged from 8 to 15 (**Table 1**).

Table 1. Number and molecular weight range of bands generated as products of ISSR -PCR amplification with selected primers and DNA from *C. davidovii* individuals regenerated by *in vitro* culture.

ISSR primer	Annealing temperature (°C)	Total number of bands	Size range (bp)
ISSR 1	60	8	200-1000
ISSR 2	60	14	250-2650
ISSR 3	60	10	300-2500
ISSR 4	60	12	370-2750
ISSR 5	60	10	200-2500
ISSR 6	60	12	200-2500
ISSR 7	55	13	350-1550
ISSR 8	55	12	220-3000
ISSR 9	55	9	300-2700
ISSR 10	55	15	250-3000

The ISSR-profile of all analyzed regenerants was compared with that of the original mother plant of 'Kozyata Stena' chalet (**Fig. 2**).

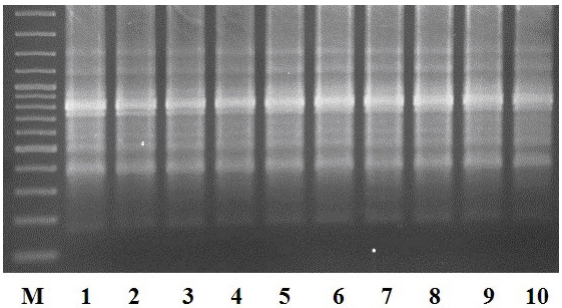


Fig. 2. Inter Simple Sequence Repeat (ISSR) amplification pattern obtained for mother plant (**10**) and daughter/micropropagated *C. davidovii* individuals (**1-9**) using primer ISSR7. **M**: 100bp DNA ladder

The obtained results showed that the ISSR-profile of all analyzed regenerants matches the profile of the original mother plant. The genetic stability of another species of genus *Centaurea, C. ragusina,* has been studied under the conditions of long-term *in vitro* culture (Radić et al., 2005). Authors reported polyploidy and aneuploidy concerning only single cells which did not affect the cytogenetic stability of the whole plants. The genetic integrity of the regenerated plants from the critically endangered Spain endemic *Centaurea ultreiae* has been demonstrated using Random Amplified Polymorphic DNA (RAPD) analysis, which did not reveal genomic alterations in any of the regenerated plants (Mallón et al., 2010). Here, we present that none of selected ISSR-primers showed polymorphism in the analyzed *Centaurea davidovii* plants which indicated the fidelity of regenerated plantlet population to the genotype of mother plant. Based on our findings, we suggest that the *in vitro* propagation did not cause variation in the genetic make-up of regenerated *C. davidovii* plantlets.

CONCLUSION

Since all ISSR-based bands were monomorphic and there were no variations detected in the micropropagated *C. davidovii* plants compared with the mother plant, indicating genetic stability among the clones, the previously developed micropropagation protocol could be carried out for a considerable length of time without much risk of genetic instability. However, no technique alone can completely guarantee the genetic fidelity of regenerated plants. Therefore, even if ISSR analysis showed no genomic alterations, as in the present study, this does not necessarily mean that there are none. Our finding has important implications

for „true-to-type“ micropropagation protocol for *C. davidovii,* which is crucial for urgent conservation of the species. However, further research needs to be undertaken to investigate the ploidy evaluation of *in vitro* regenerated plants in order to assure trueness-to-type.

Acknowledgment: This work was supported by grant DFNI-BO2/18 of the Bulgarian National Science Fund.

REFERENCES

1. Agarwal, M., Shrivastava, N., Padh, H. 2008. Advances in molecular marker techniques and their applications in plant science. *Plant Cell Reports*, 27(4): 617-613.
2. Alizadeh, M., Singh, S.K. 2009. Molecular assessment of clonal fidelity in micropropagated grape (*Vitis* spp.) rootstock genotypes using RAPD and ISSR markers. *Iranian Journal of Biotechnology*, 7(1): 37-44.
3. Alizadeh, M., Singh, S.K., Tripta, J., Sharma, T.R. 2008. Inter simple sequence repeat analysis to confirm genetic stability of micropropagated plantlets in three grape (*Vitis* spp.) rootstock genotypes. *Journal of Plant Biochemistry and Biotechnology*, 17(1): 77-80.
4. Bairu, M.W., Fennell, C.W., van Staden, J. 2006. The effect of plant growth regulators on somaclonal variation in *Cavendish banana (Musa* AAA *cv. 'Zelig'*). *Scientia Horticulturae*, 108(4): 347-351.
5. Bancheva, S., Gorgorov, R. 2010. Taxonomic revision and conservation status of *Centaurea davidovii* (sect. Lepteranthus, Asteraceae). *Phytologia Balcanica*, 16(2): 255-261.
6. Bornet, B.C., Muller, F.P., Branchard, M. 2002. Highly informative nature of inter simple sequence repeat (ISSR) sequences amplified using tri- and tetra-nucleotide primers from DNA of cauliflower (*Brassica oleracea* var. 'botrytus' L.). *Genome*, 45(5): 890-896.
7. Chandrika, M., Rai, V.R., Thoyajaksha. 2010. ISSR marker based analysis of micropropagated plantlets of *Nothapodytes foetida*. *Biologia Plantarum*, 54(3): 561-565.
8. Doyle, J.J., Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19(1): 11-15.
9. Gorgorov, R., Traykova, B., Stanilova, M. 2015. *Ex Situ* Conservation of *Centaurea davidovii* Urum.: an in Vitro Approach. *Comptes Rendus de l'Académie bulgare des Sciences*, 68(10): 1265-1270.
10. Guo, H.B., Lu, B.R., Wu, Q.H., Chen, J.K., Zhou, T.S. 2007. Abundant genetic diversity in cultivated *Codonopsis pilosula* populations revealed by RAPD polymorphisms. *Genetic* Resources and Crop Evolution*,* 54(5): 917-924.
11. Henry, R.J. 2006. 'Plant conservation genetics: importance, options and opportunities', in RJ Henry (ed.), *Plant conservation genetics*, Haworth Press Inc, Binghamton, NY, pp. 1-6.
12. Mallón, R., Bunn, E., Turner, S.R., González, M.L. 2008. Cryopreservation of *Centaurea ultreiae* (Compositae) a critically endangered species from Galicia (Spain). *Cryo Letters,* 29(5): 363-370.

13. Mallón, R., Rodriguez-Oubina, J., Gonzalez, M.L. 2010. In vitro propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. *Plant Cell, Tissue and Organ Culture,* 101(1): 31-39.
14. Martins, M., Sarmento, D., Oliveira, M.M. 2004. Genetic stability of micropropagated almond plantlets as assessed by RAPD and ISSR markers. *Plant Cell Reports*, 23(7): 492-496.
15. Nwauzoma, A.B., Jaja, E.T. 2013. A review of somaclonal variation in plantain (*Musa spp.*) mechanisms and applications. *Journal of Applied Biosciences*, 67: 5252-5260.
16. Parida, R., Mohanty, S., Nayak, S. 2011. Evaluation of genetic fidelity of *in vitro* propagated greater galangal (*Alpinia galangal* L.) using DNA based markers. *International Journal of Plant, Animal and Environmental Sciences*, 1(3): 123-133.
17. Peredo, E.L., Folgado, R., Revilla, M.A., Arroyo-García, R. 2009. Genetic and epigenetic stability of *Humulus Lupulus* after *in vitro* procedures. *Acta Hortic*, 848: 115-124.
18. Petrova, G., Dzhambazova, T., Moyankova, D., Georgieva, D., Michova, A., Djilianov, D., Möller, M. 2014. Morphological variation, genetic diversity and genome size of critically endangered *Haberlea* (Gesneriaceae) populations in Bulgaria do not support the recognition of two different species. *Plant Systematics and Evolution*, 300(1): 29-41.
19. Radić, S., Prolić, M., Pavlica, M., Pevalek-Kozlina, B. 2005. Cytogenetic stability of *Centaurea ragusina* long-term culture. *Plant Cell, Tissue and Organ Culture*, 82(3): 343-348.
20. Seyedimoradi, H., Talebi, R. 2014. Detecting DNA polymorphism and genetic diversity in Lentil (*Lens culinaris* Medik.) germplasm: comparison of ISSR and DAMD marker. *Physiology and Molecular Biology of Plants*, 20(4): 495-500.
21. Tiwari, J.K., Poonam, Sarkar, D., Pandey, S.K., Gopal, J., Kumar, S.R. 2010. Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. and S. *etuberosum* Lindl. *Plant Cell Tissue Organ Culture* 103(2): 175-187.
22. Tsvetkov, I., Dzhambazova, T., Kondakova, V., Batchvarova, R. 2014. *In vitro* long-term storage and regeneration of Bulgarian grapevine variety "Velika" via repetitive somatic embryogenesis. *Universal Journal of Plant Science*, 2(2): 48-51.
23. Valladares, S., Sánchez, C., Martínez, M.T., Ballester, A., Vieitez, A.M. 2006. Plant regeneration through somatic embryogenesis from tissues of mature oak trees: true-to-type conformity of plantlets by RAPD analysis. *Plant Cell Reports*, 25(9): 879-86.
24. Ye, C., Yu, Z., Kong, F., Wu, S., Wang, B. 2005. R-ISSR as a new tool for genomic fingerprinting, mapping, and gene tagging. *Plant Molecular Biology Reporter,* 23(2): 167-177.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 79-84

CAN ARTIFICIALLY BREED STURGEONS SURVIVE IN LOWER DANUBE?

APOSTOLOU A[1]., STEFANOV T[2]., TSEKOV A[3]., VELKOV B[1]., KOEV V[4]., MARGARITOVA B[5]., MIHOV S[5].

1. Institute for Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences
2. National Museum of Natural History, Bulgarian Academy of Sciences
3. University of Plovdiv "Paisii Hilendarski", department of Ecology
4. Persina Nature Park
5. WWF-Bulgaria
**Corresponding author: apostolosfish@abv.bg*

Keywords: conservation species, sturgeons, stocking, recapture, lower Danube

Abstract: Black Sea sturgeon stocks are rapidly decreasing during the last decades; this decline resulted from over exploitation and environmental degradation. Mainly because of their high economic value, they were and are fishes of high public interest. Their long and complex life cycles (in freshwater, brackish, and marine habitats) require global conservation programs spanning across several ecosystems of different nations. The protective measures for the conservation of the most endangered species include mainly fishing regulations, habitat restoration, juvenile stocking. Recently, registrations of reintroduced sturgeons in lower Danube and Black Sea prove that introductions are effective, at least partially.

INTRODUCTION

Most of the 27 recognized extant species of sturgeons and paddlefish, all characterized by limited adult abundance, are threatened in their entire range of distribution (Billard and Lecointre, 2001). Black Sea sturgeon stocks are rapidly decreasing during the last decades; this decline resulted from over exploitation and environmental degradation. Mainly because of their high economic value, they were and are fishes of high public interest. Their long and complex life cycles (in freshwater, brackish, and marine habitats) require global conservation programs spanning across several ecosystems of different nations (Ludwig, 2006). Only a combination of alternatives integrating habitat protection and recovery with harvest restrictions and supplementation can be expected to sustain sturgeon populations (Birnstein et al*.,* 1997a). Several authors consider

that stocking of juveniles contributes significantly to sustained populations. Birstein, 1993 recommended immediate breeding of some threatened species. Ex situ conservation (keeping and reproducing live sturgeon in fish farms as a mean of conservation) is carried out in several countries. Collection of rare and endangered sturgeon species is established in some places: the Moscow region, the Far East, Krasnodar, and the Federal Genetic Collection of the Black and Azov Seas (Chebanov et al., 1998; Artyukin et al., 1999). Breeders of several sturgeon species are presently reared in fish farms or in experimental facilities in some other European countries.

Lower Danube below the Iron Gates represents the last breeding refuge for Black Sea sturgeons after the damming of Dnieper and Dniester Rivers, during 50's (Ambrose, 1964; Vassilev, 2006). The protective measures for their conservation include mainly fishing regulations, habitat restoration, juvenile stocking. Such stockings have been executed in Bulgaria since 1998 (Tsekov, 2007), mainly by the government and during 2014-2015 by an NGO. The survival of stocked sturgeons has been estimated in some cases (Chebanov et al*.,* 2002), but about lower Danube there are no data concerning the status of such fish: the aim of the current study is to bring first evidence about registrations and growth of artificial breed sturgeons introduced in the Bulgarian Danube sector during the last years, and compare the results with other cases of known recaptures.

MATERIAL AND METHODS

The strategy for registration of previously tagged fish was based on three aspects:

1. Continuous support of a circumpontic scientific web of sturgeon specialists, which exchange information about tagged specimens' captures, to establish easily their origin.
2. Additionally information from questionnaires, periodically given to local fishermen about sturgeon registrations and
3. Direct sampling before Vetren Island (Danube 356 to 355 km) during summer – from 2013 to 2015, where there have been used drifting gill nets with 20mm eye diameter, height 2m and length100m. Sampling depth varied from 4 to 14m. Length and weight were measured *in situ* and then the fishes were released immediately, tag also was described.

The relative average fish weight gain (RGR, in $\%d^{-1}$) was determined according to Pyka and Kolman, 2003.

RESULTS

Four artificially breed and tagged specimens have been captured in Black Sea from 2002 to 2012, other eight in Danube River from 13th of June to 26th of July, this year (table 1). A Russian sturgeon released in Danube and captured near Trabzon (fig. 1), showed remarkable growth rate – about 1.4 kg for a year. From three individuals introduced in Sakkarya R. estuary, a stellate sturgeon (fig. 2) and a Russian sturgeon have not showed growth at all during their capture, whereas calculations for another stellate sturgeon registered near Kiten could not be performed, since the only evidence is a photo, send by a local fisherman. From the eight sterlets captured 1-42 days after their introduction, the latest registered specimen showed clearly weight increase. RGR for the sterlets caught only few days after stocking was not calculated, because individual weight fluctuations could be higher than final weight, and thus give false estimations.

Table 1. Recent captures of artificially breed and tagged sturgeons in Black Sea and Danube River.

Species	*A. gueldenstaedti*			*A. stellatus*	*A. ruthenus*	
Number of tagged fish	30	No data	No data	No data	2000	
Parental origin	No data	Russia, not Danubian	Russia, not Danubian	Russia, not Danubian	Danubian	
Place of introduction	Russe Danube BG	Sakkarya R. estuary TR	Sakkarya R. estuary TR	Sakkarya R. estuary TR	Vetren Danube BG	
Initial weight kg	0.3	1.7	0.1	0.1	**Average** 0.008	
Date	1.10.2001	6.2006	11.2011	11.2011	12.6.2015	
Place of capture	Trabzon Black Sea TR	Galata/ Varna Black Sea BG	Burgas Black Sea BG	Kiten Black Sea BG	Vetren Danube BG	Vetren Danube BG
Captured fish	1	1	1	1	7	1
Final weight kg	1.7	1.7	No data	0.1	**Average** 0.018	0.048
Date	10.2002	11.2006	3.2012	14.4.2012	13-26.06.2015	23.7.2015
RGR	0.225	0	No data	0	**Calculation innacurate**	1.98

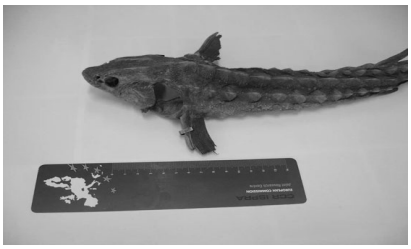


Fig. 1. Russian sturgeon released in Bulgarian sector of Danube in 2001 and captured near Trabzon (Turkey) in 2002.

Fig. 2. Stellate sturgeon released in Sakarya R. estuary-Turkey and captured near Varna/Galata-Bulgaria in 2012.

The survivorship of stocked sturgeons has been calculated in some cases (table 2). On the basis of these data, 20-40 tagged sterlets from the specimens introduced during 2014-2015 could survive, and potentially recaptured. Although the success of stocking efforts is difficult to assess and requires marking techniques that are not widely available, previous works on population dynamics showed that in Volga River the proportion of hatchery propagated beluga in the captures was 9.3% in 1971–1975, 77% in 1981–1985 and 96% in 1991–1995 (Khoderevskaya et al., 1999).

Table 2. Correlation between weight in gr. and survivorship in % for some sturgeon species according to Chebanov *et al.,* 2002.

Species	**0,5-1,5**	**2-7**	**8-10**	**11-20**	**21-30**	**31-40**	**41-50**	**> 50**
Ac. gueldenstaedti	0,4	0,6-1,3	2,5	5,0	20	32	51,6	100
Ac. stellatus	0,4	0,6-1,3	2,5	5,0	20	32	51,2	100
Huso huso	0,4	0,6-1,3	2,5	5,0	20	32	51,2	100
Ac. ruthenus	0,2	0,4-00,9	2,1	4,2	16,8	26,9	43,0	100

Considering the outcome of sturgeon translocation efforts, it is strongly recommended to use only native specimens for stocking programs, if available (Ludwig 2006). The proper introduction scheme is given by Billard and Lecointre, 2001 (fig. 3). This has been taken in mind during the introductions 2014-2015, whereas for older ones, no concrete data could be obtained.

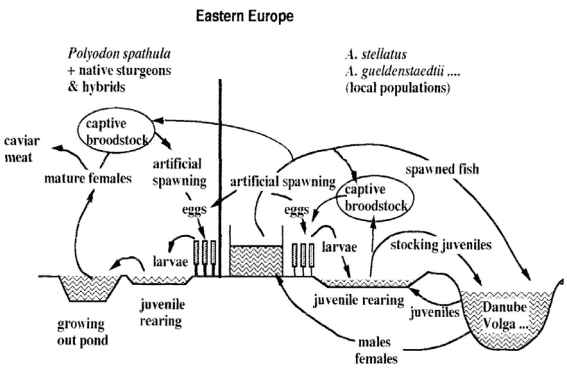


Fig. 3. Schematic representation of two systems of sturgeon culture according to Billard, ecointre 2001. One (right) is for the production of juveniles of local populations for stocking; The other system (left) is for the production of sturgeons (local or exotic species and hybrids) for meat and caviar. Production is extensive (polyculture more common in Eastern Europe). These cultured sturgeons should not escape into the wild.

The reason for zero growth of the Turkish specimens could be alien origin; another hypothesis could be releasing near estuary, but not upstream. Notwithstanding, no solid evidence can be redounded from the material. As poikilothermic animals, the body temperature, metabolic rate and growth of sturgeons are affected by water temperature. Therefore, their optimum feeding rates reared at different water temperatures are different. Moreover, in aquaculture the size and shape of rearing tanks, age and size of fish, stocking densities, water flow rate, feed placement and feeding strategy are also factors that have some effect on the optimum feeding rate of sturgeons (Hung and Lutes, 1987). These facts – as well as the sturgeon RGR in controlled conditions which was determined as minimum 0.2 (Pyka and Kolman, 2003), point out that the established growth rates of the recaptured, tagged and released in Danube specimens can be determined as satisfactory.

As a whole, the sturgeon stocking for conservation purposes arises two questions: about survivorship and returning. Additional risk for release programs arrives from the homing of anadromous species. In contrast to salmon, only limited data is available for sturgeons (for example Schram et al., 1999). Nevertheless, the released for the purpose specimens should be stocked as young as possible (fertilized eggs, fingerlings) to increase return rates (Ludwig 2006). This is controversial to table 2; the best size for the conditions of the Lower Danube has not been estimated yet. From the same point of view, sterlet introductions could be more successful than these of other species, since it is potamodromous, but not migratory as beluga, stellate or Russian sturgeon – but such conclusions could be made after multiannual stocking in Danube with various age groups.

CONCLUSIONS

Artificially breed and stocked sturgeons can survive in lower Danube; all specimens released in the river showed growth when recaptured, if calculations possible. Survivorship is difficult to be established; the returning rate is much more difficult and is resource-consuming. In any case, sturgeon reintroduction must be performed only with proved local parental origin.

Acknowledgements: The preparation of the manuscript has been performed under the support of the project No 5103020-C-012 with beneficiary WWF-Bulgaria.

REFERENCES

1. Ambrose A. 1964. Sturgeons of North-western Black Sea. *Proc. VNIRO*, 52: 287-347 (in Russian).
2. Artyukhin, E.N., Vecsei, P. 1999. On the status of Atlantic sturgeon: conspecificity of European Acipenser sturio and North American Acipenser oxyrinchus. *J. Appl. Ichthyol*., 15: 35–37.
3. Billard R., Lecointre G. 2001. Biology and conservation of sturgeon and paddlefish. *Reviews in Fish Biology and Fisheries*, 10: 355–392.
4. Birstein, V.J. 1993. Sturgeons and paddlefishes: threatened fishes in need of conservation. *Conserv. Biol,*. 7: 773–787.
5. Birstein, V.J., Waldman, J.R., Bemis, W.E. (eds.) 1997a. Sturgeon biodiversity and conservation. Kluwer Academic Publisher Dordrecht, 444 pp.
6. Chebanov M.S., Savelyeva E.A., Galich E.V., Chmyr Yu.N. 1998. Reproduction of sturgeons under the conditions of the flow regulation of rivers. In: Rauta M., Bacalbasa-Dobrovici N., Vasilescu G., Oprea L. (eds.) Fisheries Management in the River Danube Basin Galati, Romania, pp. 58–59.
7. Chebanov M.S, Karnaukhov G.I., Galich E.V., Chmyr Yu.N. 2002. Hatchery stock enhancement and conservation of sturgeon, with an emphaseis on the Azov Sea population// *J. Appl. Ichthyol*., 18: 463 469.)
8. Hung S., Lutes P. 1987. Optimum feeding rate of hatchery produced juvenile white sturgeon (Acipenser transmontanus) at 20 °C. *Aquaculture*, 65: 307-317.
9. Khodorevskaya R.P., Krasikov Ye.V. 1999. Sturgeon abundance and distribution in the Caspian Sea. *J. Appl. Ichthyol*., 15: 106–113.
10. Ludwig A 2006. A sturgeon view on conservation genetics. *Eur. J. Wildl. Res*., 52: 3–8.
11. Julian P., Kolman R. 2003. Feeding intensity and growth of Siberian sturgeon *Acipenser baeri* Brandt in pond cultivation. *Arch. Pol. Fish*., 11: 287-294.
12. Schram S.T., Lindgren J., Evrard L.M. 1999. Reintroduction of lake sturgeon in the St. Louis River, western Lake Superior. *North Am J Fish Manage.,* 19: 815–823.
13. Tsekov A. 2007. Natural water bodies restocked with farmed sturgeon in Bulgaria. *Eurofish*, 3: 51.
14. Vassilev M. 2006. Lower Danube – the last refuge for surviving of sturgeon fishes in the Black Sea Region. In: Hubert P (ed). Water observation and information system for decision support. Conference Proceedings, Balwois, Ohrid, Macedonia.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 85-106

RESULTS OF THE REINTRODUCTION OF GRIFFON VULTURE (*GYPS FULVUS*) IN VRACHANSKI BALKAN NATURE PARK, NW BULGARIA

GEORGI STOYANOV[1]*, EMILIAN STOYNOV[2], ELENA KMETOVA-BIRO[3], IVELIN IVANOV[3], NADYA VANGELOVA[2], ZLATKA NIKOLOVA[3], TAMARA LAZAROVA[1]

1 – Birds of Prey Protection Society, 23 Golyam Bratan Str., 1618 Sofia, Bulgaria, www.bpps.org; bpps@abv.bg
2 – Fund for Wild Flora and Fauna – 49 Ivan Michaylov str, office 327, P.O. Box 78, 2700 Blagoevgrad, BULGARIA, www.fwff.org ; pirin@fwff.org
3- Green Balkans – Stara Zagora – 9 Stara planina Str., Stara Zagora 6000, BULGARIA, www.greenbalkans.org, officesz@greenbalkans.org
**Corresponding author: bpps@abv.bg*

Keywords: *Gyps fulvus***,** Griffon Vulture, conservation, *Neophron percnopterus*, adaptation, release, feeding site, reintroduction

Abstract: In 2003 a project for the reintroduction of Griffon Vulture started in Vrachanski Balkan with its preparation phase, when a feasibility study was elaborated. At 2015, the project is still on-going. The programme is led by the Birds of Prey Protection Society (BPPS) together with the Green Balkans - Stara Zagora NGO and the Fund for Wild Flora and Fauna, supported by the Vrachanski Balkan Nature Park Directorate as a local partner.

The first supplementary feeding site for vultures in the area was established in 2004 in an attempt to attract Griffon Vultures that migrate through the area. A specialized adaptation aviary and a second supplementary feeding site nearby were built in 2008.

In April 2009 the first group of 8 Griffon Vultures imported from Spain was accommodated into the adaptation aviary. The first release of 8 birds took place in October 2010. Groups of Griffon Vultures have been released every year since then and at 2015 the total number of birds released is 43.

The largest numbers of Griffon Vultures identified and present in the area of release through the years have been as follows: 2010 – 7 ind.; 2011 – 12 ind.; 2012 – 13 ind.; 2013 – 29 ind.; 2014 – 46 ind.; 2015 – 53 ind. The largest number of simultaneously observed Griffon Vultures in the area is 53, counted in September 2015.

The first breeding attempts of two pairs were reported in 2014, but they were unsuccessful. In 2015 a total of five breeding pairs were observed of which one pair successfully raised an offspring. This has been the very first successful reproduction and the first young Griffon Vultures fledged into the wild registered in the Balkan Mountains of Bulgaria for more than 60 years.

Mortality caused by electrocution on a 20 kV aerial power line was found out as the main negative factor impacting the Griffon Vultures released in the area. A total of four individuals were found electrocuted out of the 43 Griffon Vultures released in the area.

INTRODUCTION

Griffon Vulture (*Gyps fulvus*) was a numerous and widespread breeding species in Bulgaria to 1940s (Patev 1950). After the middle of the last century the species got rarely observed in various parts of the country (Boev, Mitchev. 1981). A new breeding locality of the species was discovered in 1978 in the Eastern Rhodopes with an estimated number of nesting pairs between one (Mitchev et al. 1980) or 1-4 (Yankov and Profirov 1991). Thanks to a variety of conservation measures the species slowly recovered to 70 breeding pairs in 2014 (Dobrev and Stoychev, 2014). The Eastern Rhodopes appeared to be the only place in Bulgaria, where the speceis bred at the beginning of the XXIst century, until 2010, when several reintroduction projects were launched in the Balkan Mountains (Stoev *et al*., Yankov *et al.* this volume) and Kresna Gorge (Peshev et al. 2015).

There is specific data on the nesting of the species in Vrachanski Balkan. A total of 7 areas where the species can be assumed with high reliability to have bred until about 1950s have been identified based on information collected by interviews of elderly local residents (Stoyanov, 2010 a; Stoyanov, 2010 b).

The work on the restoration of Griffon Vulture in Vrachanski Balkan went through several stages, as follows:

1. Feasibility study and preparation for the start of the main actions: 2003.

2. Initiation of field work, preliminary studies carried out on test feedings, field inventories of suitable territories, construction of the first supplementary feeding site, preparation of a Viability Study for the reintroduction of Griffon Vulture in Vrachanski Balkan (Stoyanov *et al*. 2006), establishment of contacts with various authorities, feeding, monitoring, etc.: 2004 – 2008.

3. Construction of a second supplementary feeding site and an adaptation aviary, feeding, monitoring, etc. - 2008 – 2009.

4. Start of the Vultures Return in Bulgaria LIFE10 NAT/BG/278 Project and the first practical steps towards vulture reintroduction: import of Griffon Vultures from Spain and France, keeping them in the adaptation aviary and periodic release into the wild; feeding, monitoring and others - 2010 – 2015.

The Griffon Vulture restoration activities in the Vrachanski Balkan area are an integral part of a large international project - the Action Plan for the Recovery and Conservation of Vultures on the Balkan Peninsula and Adjacent Regions (BVAP). In Bulgaria it is implemented along the entire Balkan Mountain chain (Stara Planina) in four target sites as follows: Vrachanski Balkan (UTM FN99), Central Balkan (UTM LH32), Sinite Kamani - Grebents (UTM MH43) and Kotlenska Planina (UTM MH65). The project is implemented by Green Balkans

- Stara Zagora NGO, the Fund for Wild Flora and Fauna (FWFF) and the Birds of Prey Protection Society (BPPS). In the area of Vrachanski Balkan the main local partner is the Vrachanski Balkan Nature Park Directorate (VBNPD). The Bulgarian Environmental Partnership Foundation was also a partner in the period 2004 to 2009. Foreign partners supporting the programme are the Frankfurt Zoological Society, the Deutche Bundesstiftung Umwelt (DBU), the Black Vulture Conservation Foundation (BVCF) and later the Vulture Conservation Foundation (VCF). In 2010 the practical reintroduction was carried out through the Vultures Return in Bulgaria project LIFE08 NAT/BG/278, co-funded by the financial instrument of LIFE+ of EC.

MATERIALS AND METHODS

The release methodology chosen was the successfully used for the restoration of Griffon Vulture in the France, published by Choisy and Henriquet (1992), Terrasse (2006) and Terrasse and Choisy (2007).

One of the important activities started from the very beggining and developed over the years is the establishment of a system for supplying food for vultures. The food comprises mostly carcasses of domestic animals submitted by local farmers or shipped from more distant commercial farms. The feeding of Griffon Vultures is carried out on a specificall built supplementary feeding site, which is securely fenced and certified by the veterinary services in Bulgaria (NVS), as required by the EC (Commission Regulation (EC) № 1774/2002), (Regulation (EC) № 1069 / 2009). Regular feeding has been provided, to secure permanent food availability on site, accessible for both the released and the wild Griffon Vultures.

The Griffon Vultures were reared in an aviary built on the territory of NPVB, 4 km from Dolno Ozirovo village (municipality Varshets, Montana). It has a solid metal supporting structure spouse of concrete bricks and wooden roof. The cage is surrounded by a wire mesh that the roof has a large “eye” (up to 20x20 cm) not to hold a lot of snow. The cage is divided into two cells: large dimensions 16 m × 10 m and a small 6 m x 10 m. The height of the two cells at the front end is 4 m, and the rear end 2 m. In both cells are constructed concrete basins of 150 liters of water for drinking and bathing vultures during the warmer months of the year. At the feeding site outside the cages, in moveable containers water was also supplied to released and wild vultures coming to eat here.

Periodically, groups of Griffon Vultures from Spain and France were transferred to the adaptation aviary. They were kept about one year in captivity and then were released into the wild. The first group of 6 birds was set up in April 2009. In October 2010, the first release of eight birds together took place. By the end of 2015 in Vrachanski Balkan release site were released 43 Griffon Vultures.

Observations of Griffon Vultures were carried out mainly in the area of the feeding site and the adaptation aviary in the vicinity of Dolno Ozirovo village. In addition to the standard optics for observations – powerful telescope and binoculars, since 2013 a motion detection camera is in use. Periodic observations for the presence of vultures have been conducted in other important sites of Vrachanski Balkan - especially large rocks. Besides personally collected data, in certain cases also information from other sources was also collected and analized – e.g. by Directorate of the National Park Vrachanski Balkan, tourists, locals, etc.

Description of the area

Geographic situation

Vrachanski Balkan Nature Park is situated in North-Western Bulgaria (see Figure 1), to the North of the main Balkan Mountains range (Stara Planina) and to the West of the town of Vratsa. (altitude between 43° and 44° N and longitude between 28° and 24° W).

Fig. 1. Vrachanski Balkan Nature Park

The mountain has Northwestern and Southeastern exposure. It is about 30 km long and some 15 km wide. It comprises a total territory of 355 km². The territory of the Vrachanski Balkan Nature Park covers a total of 288, 5 km². The lowest part is at 350 m a.s.l., while the highest reaches 1482 m a.s.l. – peak "Beglichka mogila".

The entire massif of Vrachanski Balkan has a karst geological structure and features a large number of vertical rock formations, cliffs, single rocks, screes, wreaths, clint fields. Many of the rock massifs are difficult to reach due to their

natural protection by steep screens and vegetation formed by tree and bush species. The ridge areas are covered by large grasslands.

The area comprises the Vratsata Gorge, which has a karst structure and features a vertical cliff, which is more than 400 m high. These are the highest limestone verticals in the Balkan Peninsula, and at the same time the highest pure verticals at such an altitude in Europe. Additionally, there are plenty of cliffs, single rocks and rocky areas. Here is the longest mountainous cliff massif in Bulgaria – about 6 km long cliff, with some parts exceeding 100 m in hight.

Climate

The climate in the mountain features big diversity and quick changes. The climate is a mixture between sub-continental and mountain climate zones. The average annual temperature is +7°C. The average for January is +1.6° C. Temperatures lower than – 10° are registered very rare. During winter there is a striking difference between the temperatures and the snow coverage because of the exposition of the hills. At lower altitudes the snow cover stays about 50 – 60 days, while at the higher altitudes it lasts some 80 – 100 days.

Spring comes relatively later and is cold. Summer is hot, especially below 1 000 m a.s.l. Long-lasting thick fogs are characteristic for the Vratsa valley (East of the mountain), but they are rarer and short lasting in the mountains.

The winds in the mountain are mainly (65%) with NW – SE direction. "Feuhn" winds occasionally occur in early spring and late autumn. The peak of the rainfalls is May – June, the driest periods being February and August. The average amount of the rainfall is 1 000 mm.

Suitable habitats for reintroduction

Vrachanski Balkan Mountains is one of the amplest regions in Bulgaria with respect to suitable rock massifs for Griffon Vulture. The reason is the presence of vertical cliffs with height ranging from tens of meters to 100-200 m. The highest can reach up to 400 m sheer precipice. A big part of these rock habitats are situated in the altitude range of 100-400 m, and many of the cliff walls have Southern and Eastern expositions.

The Karst structure of the rocks determines the abundance of holes, caves, rock cornices on the territory of Vrachanski Balkan and makes the rock massifs very suitable as nesting habitats for Griffon Vulture.

Protected areas

The protected areas located in the region are as follows: Vrachanski Balkan Nature Park with a total areaof 28 844,8 ha. (of which 20 733,4 ha are forests).

This is the second biggest nature park in Bulgaria. Within its territory is situated Vrachanski Karst Reserve at a total area of 1 409 ha, and a buffer zone of 623 ha.

There is a proposal for extending the reserve.

In addition to the reserve, there are some more protected territories in the park, mainly rock formations comprising some 15% of the area of the park, such as the Lakatnishki Skali and Vezhdata Protected Areas, the Nature Landmarks Ritlite, the Ledenika Cave, the Vratzata Gorge.

VBNP was designated a protected natural area with international significance for protection of biodiversity and the richness and beauty of nature in 1997.

On 04.11.2008 NPVB was designated a Natura 2000 protected site - SPA BG0002053. It is also a SCI

RESULTS AND DISCUSSION

In the period January 2010 - June 2015, a total of 44 Griffon Vultures imported from Spain and France were released from the Vrachanski Balkan release site (see Table 1). Out of these 41 were immatures (2-3 years old), 1 subad (4 years) and 1 ad (>5 years). Most vultures were released in the period March–November (See Table 2). Sixteen were males, 21 females and 7 unknown.

Table 1. Released Griffon Vultures in Vrachanski Balkan by year

Year	Number of released birds
2010	8
2011	7
2012	5
2013	8
2014	12
2015	4
Total	**44**

Table 2. Released Griffon Vultures in Vrachanski Balkan by month

Month	Number of released birds
Jan	0
Feb	0
Mar	7
Apr	6
May	6
Jun	4
Jul	2
Aug	4
Sep	4
Oct	9
Nov	2
Dec	0
Total	**44**

The maximum number of observed Griffon Vultures in Vrachanski Balkan release site by year and months is presented on Table 3 and Figure 2:

Table 3. Maximum number of observed Griffon Vultures in Vrachanski Balkan release site by year and months

Total number of Griffon Vultures by months and years	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
2010								2	6	13	8	7
2011	6	7	8	8	9	9	9	10	12	12	11	13
2012	9	6	9	8	10	11	12	13	13	14	12	11
2013	12	12	7	24	21	28	26	23	29	19	20	19
2014	23	20	21	26	30	50	44	44	35	45	40	40
2015	40	40	40	37	43	40	50	48	53	47	43	46

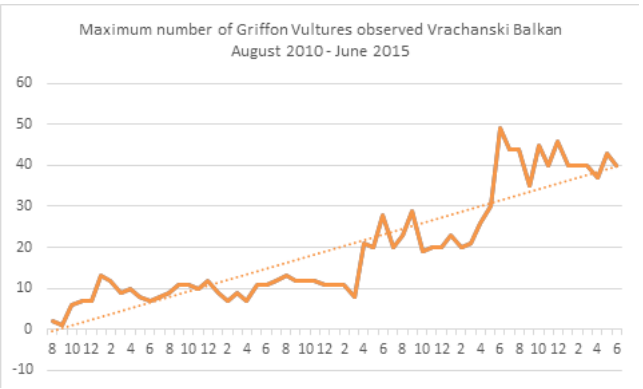


Fig. 2. Maximum number of Griffon Vultures observed in Vrachanski Balkan, August 2010 - June 2015

There is a very big difference in the presence of Griffon Vultures in the period 2003- 2009 (Stoyanov 2010 a; Stoyanov b), compared to the data from 2010 to 2015. The first period covers the time when only soft measures were implemented in Vrachanski Balkan – supplementary feeding, hoping that vultures would be attracted to the area. In the second period, an adaptation aviary was constructed and release of vultures took place in the area in addition to the supplementary feeding carried out. In the first period, only single Griffon Vultures were very rarely observed. These birds only briefly stayed in the Vrachanski Balkan area, in spite of the feeding present and the abundance of suitable nesting cliffs. At the same time, monitoring data from 2010 to 2015 shows long-lasting presence of the species in the area and a progressive increase of the number of vultures each year, with not only released within the project, but also with wild, non marked ones, or such marked elsewhere. This is due to several factors. On one hand, this is related to the fact, that the released vultures formed a "nucleus" of birds that permanently stayed in the area and attracted conspecifics passing through the Vrachanski Balkan are. Observations show that the adaptation aviary and the birds inside also play a key role in attracting exogenous vultures on passage. Such were seen perching on the roof of the aviary and getting food at the feeding site, which is just in front of the aviary. The increased amount and frequency of food provision at the feeding site (See Table 7 and Table 8) also had a great importance for increasing the number and the continued detention of Griffon Vultures in the area all-year-round.

The areas where vultures are tagged and released from adaptation aviaries in Bulagria are as follows: Vrachanski Balkan, Central Balkan, Sinite Kamani - Grebenets, Kotlenska Planina (the release sites of Green Balkans, FWFF and BPPS along the Balkan Mountains) and Kresna Gorge (release site of FWFF). The Griffon Vulture "Michelle" is a non-tagged juvenile Griffon Vulture successfully fledged from a nest in Vrachanskia Balkan in 2015. The vultures from Serbia and Croatia have been marked as fledglings in the nests, while those marked in Israel are trapped on field. A total of 103 different Griffon Vultures with tags were observed and identified in Vrachanski Balkan between 2010 - 2015. A total of 44 of them had been released in Vrachanski Balkan, 20 vultures originated from the Central Balkan release site, 8 vultures had been released in Sinite Kamani, 4 vultures in Kotlenska Planina, 9 vultures originated from Kresna Gorge, 5 vultures from Serbia, 3 vultures from Croatia, 7 vultures had been tagged in Israel and three vultures were carrying unreadable markings and thus their origin remained unknown (See Table 4).

Table 4. Number of marked Griffon Vultures observed in Vrachanski Balkan by years

Area of origin of the marked Griffon Vulture	2010	2011	2012	2013	2014	2015	Total
Vrachanski Balkan	8	14	15	20	26	28	44
Central Balkan	0	0	0	8	11	12	20
Sinite Kamani	0	0	1	7	3	2	8
Kotlenska Planina	0	0	0	0	3	3	4
Kresna Gorge	0	0	0	2	6	6	9
Serbia	0	0	0	2	1	4	5
Croatia	0	0	0	1	2	1	3
Israel	2	0	1	2	2	3	7
Unknown	0	1	0	3	1	1	3

Imigration

Between 1 and 10 non-marked individuals were observed for some time throughout the year in the reporting period. The number of these birds is very likely greater, but their individual identification is difficult. A dedicated study carried out in Kresna Gorge, where visual marking by photographing of each individual was applied, the number of non-marked Griffons was found out to be up to 3 times bigger than the locally released birds present in the area (Peshev et al. 2015).

Dispersal and movements

Griffon Vultures released in Vrachanski Balkan have been observed in different parts of the Balkan Peninsula – Northern Serbia on the border with Hungary, Eastren Serbia, Eastren Rodopes (Bulgaria), Sinite Kamani/ Kotlenska Planina (Bulgaria), Dadia (Greece), Romania, Kresna Gorge (Bulgaria), etc.

Adaptation of the released Griffon Vultures

The first group of 8 Griffon Vultures was released on 27.10.2010. It is often rainy, cold and foggy in this time of the year. The weather had a very negative impact on the released vultures. The birds were observed moving on foot around the release site, because it was often wet, the visibility was poor, and the area was still unfamiliar to them. These vultures perched and kept roosting mainly on the ground or stones protruding above the ground, very rarely on trees. In the winter of 2010-2011 individual birds were seen perching on electricity pylons. Single birds were seen landing on roofs of houses in and around settlements. During this period, the vultures flew infrequently and mostly over short distances. These first flights of the birds exploring the release area revealed several problems. Birds perched on low sites, overgrown with vegetation, so they could not take off and had to move to higher sites walking. Thus vultures were observed to walk from a few dozen to more than 200 meters on foot in some cases.

The releases of Griffon Vultures in 2011- 2015 took place mainly in warmer months of the year – a total of 32 individuals were released in the period from March to September. These birds experienced faster adaptation than those released in autumn and winter (from October to November). This is due to the more favorable weather conditions and the longer daylight in the period from March to September. The already formed nucleus of previously released birds also played a great role, so that the newly released ones followed them during their adaptation period and thus made it shorter.

Wintering

In low temperatures (-27° C) and at the presence of permanent snow cover the Griffon Vultures reintroduced in Vrachanski Balkan, stayed mostly in the lower parts of the mountain and the adjacent lowland areas. A vulture perching on rocks in higher sites was a very rare sight in case the presence of snow in this period. In winter, the vultures were seen perching / roosting in trees, such as deciduous and coniferous trees, electricity pylons, the roof of the aviary, roofs of different types of buildings around settlements and rarely in villages. Large metal electricity pylons (along 400 kV power lines), which are located about 4 km away from the supplementary feeding site form an important place for roosting of the Griffon Vultures in winter, but also in bad weather conditions in late autumn and early spring. On overall, vultures were much less mobile during the cold months of the year. Their main flights were between the roosting place and the supplementary feeding site. The greatest distances between these two points are up to about 5 km.

Food for vultures

The establishment of a network for supplying food is of key importance for the feeding of the vultures. Numerous contacts with veterinarians, mayors, farmers, etc. were established in the target area, through a long-term awareness raising campaign. It was implemented through dissemination of leaflets/brochures, radio- and TV broadcasts, multiple meetings with vetertinarians, mayors, local farmers, etc. held in the target areas. In the period 2003-2010, this network mostly comprised small-scale farmers in a 30 km radius from the supplementary feeding site. The network secured mainly goats and sheep, less often donkeys and horses and very rarely cows. In occasional cases, road kills were also used – dogs, jackals, foxes and badgers. In 2011 the team also started obtaining food from big-scale pig farms, located up to 130 km away from the supplementary feeding site. This has become necessary following the arrival and first release of Griffon Vultures in October 2010.

Yet another important reason to use pig farms is the progressively decreasing number of livestock of the small-scale farmers in the districts of Montana and Vratsa. This negative trend has been observed for decades and is still valid in the years of vulture restoration efforts (Table 5 and Table 6) Collective (1956), Collective (1960), Collective (1969), Collective (1978), Collective (1981), Collective (1987), Collective (1995), Collective (1996 a), Collective (1996 b); unpublished data of the Regional Food Safety Agency of Montana and Vratsa for 2015.

The use of carcasses from big-scale pig-breeding farms for supplementary feeding proved to be of extreme importance for the successful work with Griffon Vultures in Vrachanski Balkan. Between 50 to 90 % of the food for Griffon Vultures in the area in various periods (months) from 2011 to 2015 was secured this way. In isolated cases, when carcasses were not available, offal and other slaughter waste was used instead. Griffon Vultures do not prefer such food, except for large pieces of offal, such as lungs, liver and heart. Furthermore, it attracts an unwanted number of Ravens. A significant step for securing waste carcasses for supplementary feeding is the obtaining of the necessary legal permits. BPPS obtained such permits from the veterinary authorities at regional and national level in 2005 and 2011. This process was significantly eased by the ratification of EC - Regulation 1774 / 2002 and EC Regulation 1069 / 2009.

Table 5. Number of livestock in the district of Montana between 1961 - 2015

Year	1961	1965	1976	1985	1990	1995	2001	2015
Sheep	295773	327890	325197	318554	226000	118000	67213	36804
Cattle	67854	60202	70064	61336	48000	12000	10000	14329
Pigs	110863	112205	218513	165463	153000	93000	17258	3971
Goats	18356	12250	29411	30000	35000	38584	33277	16053
Buffalo	5387	2857	2557	1313	no data	no data	412	416
Horses	7837	7196	4498	4073	no data	no data	5457	2273
Donkeys and mules	no data	4920	8795	13036	no data	no data	8384	1482
Total for the year	**506070**	**527520**	**659035**	**593775**	**462000**	**261584**	**142001**	**75328**

Table 6. Number of livestock in the district of Vratsa between 1956 - 2015

Year	1956	1965	1976	1985	1990	1995	2001	2015
Sheep	1061973	159889	168746	319062	281022	136945	100032	48015
Cattle	295707	52117	66823	64515	64874	16955	22449	22486
Pigs	268433	123103	148829	210072	229872	48461	49275	2490
Goats	62144	no data	no data	32146	31105	39797	40888	17460
Buffalo	23308	no data	400	2203	2272	1538	832	1179
Horses	53978	10670	4477	6373	no data	no data	7968	4315
Donkeys and mules	10072	no data	no data	17829	no data	no data	9072	1664
Total for the year	**1775615**	**345779**	**389275**	**652200**	**609145**	**243696**	**230516**	**97609**

The first supplementary feeding site was constructed in 2004 above the village of Dolno Ozirovo at 430 m above sea level, on a high, open area (43º 14′ 38. 62″ N, 23º 22′ 20. 49″ EO). It covers an area of 2 dka and is surrounded with a metal fence, carried by concrete poles, 150 cm above the ground. The entire length of the fence is concreted so no predators can dig under and enter the fenced area. The closest rocks are located some 5 km line of sight. We consider this distance as the main reason why Griffon Vultures were never observed to use the site. This supplementary feeding site was maintained until 2008 and after that only the second one was used.

The second supplementary feeding site was constructed in 2009, in direct proximity to the adaptation aviary. The overall area surrounded by fence is 900 square meters, at 375 m a.s.l., GPS 43º 15′. 261″ N 23º 22′. 522″EO. There are several small vertical cliffs up to 20 m high, located some 250 m from the line of sight. We consider that the proximity of these cliffs turned out to be of key importance for the acceptance of this feeding site. Vultures almost always land on these cliffs to check the area before landing on the feeding site. The cliffs are also used for roosting overnight. Yet another advantage of the terrain is that the slope is steep, what allows the Griffon Vultures to take off easily, gaining height. Two other factors had additional positive impact on the acceptance and use of the feeding site. One of them is the presence of Griffon Vultures in the adaptation aviary, as they attracted the vultures already released, as well as additional exogenous birds. On the other hand, wooden perches were mounted on the roof of the aviary and they were often used by the vultures, before landing for food.

The initial feeding of the vultures was done outside a specific weekly schedule, disposing food as soon as it was available. Later on, thanks to the connections established with local livestock owners and farms, the supplementary feeding was carried out twice or four times a week. Our experience shows that the most effective schedule for the area is carrying out supplementary feeding twice a week. The main aim is to secure greater amount of fresh food on a regular basis. On the other hand, such feeding considers the rough terrain, the difficult access and the distance to the supplementary feeding site, as well as the transport costs and the depreciation costs of the vehicles. The supplementary feedings were carried out within an interval of about 3 days. Following this schedule, it was always attempted to provide a greater amount of food - from 150-300 kg to about 500 kg at a single feeding event. Mainly carcasses of farm animals were used. The skin of the carcasses, especially that of the greater animals, was partially cut to ease the access of the vultures.

At first a single, but later two freezers (500 l) were used for storing food for the vultures. This food was used for feeding during the summer months, when there are usually fewer calls for dead livestock. In the winter, when there is food in excess, the carcasses were safely stored at the very supplementary feeding site. This way, there is abundant food at the site, which can be naturally stored by the

low temperatures in winter. This reserve on the spot was used multiple times in severe winter conditions, when even off-road vehicle cannot access the site. In the winter of January - February 2012 the food for the vultures was deposited with a rented motor sledge and an ATV Track. In case of permanent snow cover, the snow was periodically shovelled to ease the access of the vultures to the food.

The total amount of food disposed for the vultures between 2004 – 2015 is shown on Table 7 and Table 8 below.

Table 7. Number of livestock in the district of Montana between 1961 - 2015

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total kg	3900	7985	5510	6680	1500	6748	6175	12905	15670	22265	30120	46845
Average kg/ month	325	665	459	556	125	562	514	1075	1305	1855	2510	3903

Table 8. Table 8. Quantities of food (kg) for supplementary feeding of the Griffon Vultures at the supplementary feeding site and adaptation aviary of the village of Dolno Ozirovo by months and years for the period of releasing Griffon Vultures, October 2010 - 2015

Month/ year	1	2	3	4	5	6	7	8	9	10	11	12
2010										890	1020	765
2011	980	860	600	845	365	1290	1080	1095	1670	1250	1530	1340
2012	1450	300	1000	1490	1420	1060	1110	1760	1100	1935	1025	2020
2013	1525	1240	1025	1660	1580	2005	1630	3140	1960	1800	2780	1920
2014	2150	2860	1040	2360	2330	1250	4310	2970	2740	2780	3030	2300
2015	3720	2660	2490	4020	4560	3680	4700	3800	4740	4870	3885	3720

Water sources, used by the Griffon Vultures

There are very few surface water sources, suitable for use by Griffon Vultures in Vrachanski Balkan, due to the karst character of the terrain, the steep slopes, the narrow gorges and overgrown river valleys. The first groups of vultures released in 2010-2011 were observed drinking from open areas of the river-beds of Botunya and Cherna reka rivers. Since mid-2012 the Griffon Vultures released in Vrachanski Balkan have started using small (several tens of square meters) endorheic lakes, located along the ridge of the mountain. The vultures prefer these small wetlands and they currently have a key role for the birds. This is probably due to the fact that these lakes are located at high open areas, where heavy birds, such as Griffon Vultures can easily land and take off. Furthermore, these sites are seldom visited by people or vehicles, which could potentially disturb the birds.

The observations of the Griffon Vultrues kept at the adaptation aviary show

that they need water very much during summer and they would also eagerly use it for bathing. This is why, the adaptation aviary is regularly provided with water through a system for collecting rainwater installed on site or through transporting water tanks from the village of Dolno Ozirovo.

Breeding

Two pairs of immature birds were first observed demonstrating breeding behaviour on suitable nesting sites on cliffs to the East of the village of Dolno Ozirovo in March 2013. The rock massif there consists of vertical limestone cliffs at an overall length of 6 km and predominant height of some 30-100 m, reaching up to 100-200 m at sites. The main exposure of the massif is South-West, while the altitude is about 800-1000 m a.s.l. In April 2013 individual adults of the group were observed landing in suitable nesting sites on that massif.

On 14.02.2015 the photo-trap set on the roof of the adaptation aviary photographed copulations of K54 (male) and K41 (female). Towards the end of February 2015, five pairs of Griffon Vultures were displaying nesting behaviour on suitable sites in the cliff above the village of Dolno Ozirovo.

Five pairs were once again observed displaying nesting behaviour on the cliff above the village of Dolno Ozirovo on 2.03.2015. Around 5-8.03.2015 there was a heavy snow in the area. On 16.03.2015 the photo-trap photographed two immature individuals copulating. The male was possibly K4M. The Griffon Vultures also regularly visited the cliffs / roosting site close to the town of Vratsa throughout March 2015.

At April 2015 there was no information on the nesting success of the nesting Griffon Vultures above the village of Dolno Ozirovo. At the same time Griffon Vultures (up to 15 individuals) were often seen on the cliffs near the town of Vratsa.

Two incubating birds were located in May 2015 on two of the five sites where nesting activity had been detected earlier in March 2015.

The very first pullus of Griffon Vulture was observed in a nest on the cliff above the village of Dolno Ozirovo on 9.06.2015. The hatching of that chick had most probably happened towards the end of May 2015. The other pair of vultures seen incubating in May 2015 was not found on 9.06.2015 so we believe that its attempt was not successful. In June 2015 about 5 Griffon Vultures spent time and roosted on the cliff near the town of Vratsa.

At July 2015 the young Griffon Vulture was developing well in the nest above the village of Dolno Ozirovo. It was already quite big, with good plumage, flapping wings and movind along the edge of the nest. At the same time, some 4-5 Griffon Vultures were seen at the roosting site to the South-West of the town of Vratsa, above the village of Zgorigrad. Another 6 Griffon Vultures were seen on a different rock massif to the East of the town of Vratsa. Two of them were reported to have landed in an old nest of Black Stork (*C. nigra*) and were seen arranging the nesting material. Data shows that a second roosting site for Griffon Vultures

formed in the area near the town of Vratsa in July 2015.

This new site was confirmed by the data sent by the transmitter of Griffon Vulture tagged "3", which roosted on these rocks in July, but also used a rock massif to the East of the village of Opletnya, high above the Iskar Gorge (pers. comm. E. Stoynov, FWFF).

At August 2015 the juvenile Griffon Vulture was still in its nest. It had already reached the size of a full-grown adult. The parents of the juvenile were identified as 837, male, released from Vrachanski Balkan and K56, female, released from the Sinite kamani release site.

On 3.10.2015 the juvenile was seen leaving the nest. It flew quite well, following one of its parents and calling for food. At the same date, a climber reached the nest and found out that it had no nesting material. The rock ledge, where the nest was located, is some 70-100 cm wide and some 3 m long. The nest was at about 80 meters above the base of the cliff and some 55 m from the top, the exposure was South-West. The juvenile had probably fledged on 1-2.10.2015. Until the end of October 2015, the young vulture was often seen to return to the nest for roosting during the day or the night. The two parents were seen still feeding the juvenile either in the nest or close to it. This first successfully fledged Griffon Vulture was called Michel, after Michel Terrasse, who is among the leading French experts carried out the first successful reintroductions of Griffon Vulture in the 70s of the XXth century in France. Mr. Terrasse is among the experts who consulted the current restoration project in the Balkan Mountains and has had a significant impact for its successful implementation.

The juvenile fledged in 2015 is the first successfully fledged wild-born Griffon Vulture among all sites for reintroducting the species in Bulgaria.

Other species, observed at the supplementary feeding sites above the village of Dolno Ozirovo

Egyptian vulture (*N. percnopterus*)

Between June - August 2005, the first supplementary feeding site was regularly visited by single adult birds from the pair nesting in the Northern parts of the Vrachanski Balkan Nature Park.

Between April - August 2006 and 2007 single adult birds from the same pair also regularly visited the site.

From May to Jule 2009, a single or two adult birds from the pair were observed feeding together at the second supplementary feeding site multiple times.

A single adult Egyptian Vulture periodically used the supplementary feeding site between June - August 2012.

A single adult Egyptian Vulture was observed at the supplementary feeding site of Dolno Ozirovo in the end of May and the entire June until 6.07.2014. This bird carried a metal ring and a highly discoloured plastic yellow ring on its left foot. The bird was therefore identified as a juvenile, ringed in the nest by

our colleagues from BSPS BirdLife Bulgaria sometime between in 2008-2010 in another region of Bulgaria.

After mid-June 2014, a single, non-marked immature (1-2 years old) was registered at the supplementary feeding site. In some cases immature and adult Egyptian Vultures were seen to use the feeding site together.

Raven (*C. corax*)

The most common and abundant species, using the supplementary feeding sites all-year-round. Most numerous in the cold period of the year, when there is a great quanitity of easily accessible food, such as offal and segmented carcasses. Maximum concentrations of up to 150-200 Ravens at a single feeding are seen then.

Eurasian Magpie (*P. pica*)

Very rare - single observations, most probably vagrant individuals, passing through the area.

Hooded Crow (*C. cornix*)

Very rare - single observations, most probably vagrant individuals, passing through the area.

Rook (*C. frugilegus*)

A single individual voluntarily entered the aviary through the netting of the roof and was trapped on 20.03.2010.

Golden Eagle (*A. chrysaetos*)

Observed multiple times at the first supplementary feeding site above the village of Dolno Ozirovo during the cold months of the year. This was especially true for periods with permanent snow cover. Single immature and adult birds have been seen, as well as a pair with a juvenile, which had not left its parents.

No foraging birds have been seen using the second supplementary feeding site, even though individuals have been seen landing nearby. This is probably due to the fact that the Golden Eagles are intimidated by the construction of the aviary, located right next to the supplementary feeding site.

White-tailed Eagle (*H. albicilla*)

A single immature White-tailed Eagle was observed at the supplementary feeding site in the period October-November 2013. This bird was regularly foraging here, perching on the aviary. Initially the group of Griffon vultures got scared when the White-tailed Eagle appeared. They however soon got used to one another and were seen foraging or roosting together.

On 29-30.12.2014 a subadult White-tailed Eagle was reported foraging at the supplementary feeding site and once it appeared, the Girffons vacated the area.

Common Buzzard (*B. buteo*)

Single individuals often seen foraging at the supplementary feeding sites during the winter period.

Rough-legged Buzzard (*B. lagopus*)

Very rarely single individuals seen foraging on the first supplementary feeding site in the winter period.

Goshawk (*A. gentilis*)

Single individuals seen foraging on the first supplementary feeding site above the village of Dolno Ozirovo in the winter period.

Red Kite (*M. milvus*)

A single individual reported and trapped on 18.04.2011. The bird had entered through the coarse netting of the roof of the vulture adaptation aviary.

Black Kite (*M. migrans*)

One or two Kites have been reported foraging at the second supplementary feeding site above the village of Dolno Ozirovo in separate years. Such observations are usually done in spring or at the start of summer - April, the beginning of June, less often in autumn - September, October. The species is also reported at the waste-disposal sites in nearby areas, close to the town of Montana.

Black Stork (*C. nigra*)

Single individuals observed landing on the supplementary feeding site in front of the adaptation aviary a number of times in May 2009. Black Storks were probably foraging on rodents or large insects abundant on the animal carcasses.

The following insectivore species of birds have been reported at the supplementary feeding site or in the air above, attracted by the great number of insects: Common Starling (*St. vulgaris*), Great Tit (*P. major*), Chaffinch (*Fr. coelebs*), Yellowhammer (*E. citrinella*), Corn Bunting (*E. calandra*), Rock Bunting (*E. cia*), Black Redstart (*P. ochruros*), Robbin (*E. rubecula*), White Wagtail (*M. alba*), Red-backed shrike (*L. collurio*), Barn Swallow (*H. rustica*), Red-rumped Swallow (*H. daurica*), Common house martin (*D. urbica*), Crag martin (*P. rupestris*), Alpine Swift (*P. melba*).

Direct negative factors impacting Griffon Vultures in the area

- Electrocution and collision with 20 kV power lines. This is the main factor causing mortality of the Griffon Vultures released. It has been proven to have caused the death of at least four Griffon Vultures (K1N, K15, KOW, K37) from the ones released from Vrachanski Balkan. It is very likely that there are more casualties which have not been found. In 2013 and 2014 CEZ Ltd (the local electicity provider) isolated 20 of the most dangerous pylons along a 20 kV powerline in the area of the village of Dolno Ozirovo.
- Predators. A single, almost entirely eaten Griffon Vulture (tagged K11) was found on the ground, close to the supplementary feeding site, eaten by predators - foxes, jackals, etc. in January 2012. An additional negative factor which has most probably contributed to the death of this bird was the severe winter with over 1 meter of snow cover and temperatures of -20 ^{0}C. Furthermore, the bird probably lacked experience, released towards the end of August 2011 and spending its first winter out. There are several other documented cases of

jackals or stray dogs stalking and attacking Griffon Vultures on the ground without success even in warmer seasons. This threat is greater for recently released Griffon Vultures; vultures, which are roosting on the ground; wet vultures on the ground or vultures resting in the shade of trees or bushes on the ground, where they can be easily surprised by predators.

- Stray dogs. Despite the fences, dogs sometimes manage to penetrate the vulture supplementary feeding sites. They can then disturb or attack the vultures on the site, as well as eat a significant quantity of the food or permanently damage the integrity of the netting.
- Ravens. Numerous species seen at the supplementary feeding sites all-year-round, reaching up to 150-200 individuals in winter. Ravens eat a hige quantity of food being active throughout the day and being unsusceptible to poor weather conditions. Ravens have also been reported to directly persecute or disturb vultures on numerous occasions. In some of the cases Ravens systematically chased a particular vulture in the aviary or outside it, until the disturbed bird threw up the content of its crop, which got immediately eaten. On other cases, Ravens learned quickly the location of the incubating Griffon Vultures on the cliffs. In 2014 - 2015 there were numerous observations of a single, two or even three Ravens simultaneously attacking a laying Griffon Vulture in vain, in attempt to chase off the incubating bird and eat the egg.
- Poison. One of the Griffon Vultures (K1N) found dead under an electricity pylon tested positive for organic pesticides. Because of the location of the carcass and the signs of electrocution, the identified traces of pesticides could not be confirmed to have been the main cause of death. There is no data for the use of strychnine, luminal and other toxic substances in Vrachanski Balkan Nature Park for the period 2003-2015.
- Poaching. We have data for two non-tagged Griffon Vultures shot by poachers, but this information has not been confirmed.

Problems and challenges

The following chapter briefly presents the main difficulties challenging the restoration of Griffon Vultrue in the Vrachanski Balkan area. This information is important to better understand the local environment, as well as consider when planning future efforts in the area.

- Constant on-going rapid decline in livestock (see Tables 5 and 6). This situation has not changed following the accession of Bulgarai in EU in 2007 and the issue is worsening in many aspects.
- Almost total lack of large wild animals, suitable and accessible as food for the vultures in the wild;
- Poverty, which prevents people from submitting sick / weak domestic animals, as they are used for human consumption;
- Inquirying payment for submitted carcasses due to poverty or lack of interest for cooperation;

- Periodical full bans for disposing particular livestock, imposed by the veterinarian authorities due to the occurrence of diseases, such as: swine fever, bluetongue disease, bovine spongiform encephalopathy, anthrax, brucellosis, etc.
- The dead livestock is used by the local farmers for feeding domestic dogs;
- Competition with groups of people, who illegally collect waste meat for human consumption, commercial dog breeding or attracting wolves on hunting hides.
- Severe depopulation in the area - fewer people maintaining livestock;
- Very limited choice of sites suitable for constructing supplementary feeding sites or adaptation aviaries;
- Difficult or impossible access to the supplementary feeding sites and the adaptation aviary for long periods of time due to poor roads and deep snow cover in winter;
- Very common problems with the vehicles used;
- Many challenges in recruiting suitable local people to directly involve in the vulture restoration activities;
- A huge proportion of the food disposed for the vultures being eaten by great concentrations of Ravens;
- Re-occurring problems with stray dogs and foxes damaging the integrity of the fence surrounding the supplementary feeding site. These animals not only eat a lot of the food, but also chase and disturb the Griffon Vultures visiting the supplementary feeding site.

CONCLUSION

There are several key aspects in the efforts aiming at restoring Griffon Vulture as a nesting species in the Vrachanski Balkan Nature Park.

The activities implemented between 2004 - 2009 were focused on providing supplementary feeding only. Despite these efforts, no vultures were permanently attracted or started nesting in the region in this period. Between 2010 - 2015 a total of 43 Griffon Vultures were gradually released from the adaptation aviary, while the efforts on securing supplementary food continued. A permanent group of constantly present Griffon Vultures was established as a result. The first two unsuccessful breeding attempts were registered as early as 2014, only four years after the first releases in the area. Already five pairs nested in 2015 and one of them successfully raised a chick from its second clutch. These results show that the restoration of Griffon Vulture in the Vrachanski Balkan Nature Park only succeeded following the start of the reintroduction efforts in 2010 - 2015.

We consider that at the current stage, this is the only possible way to achieve such considerable success in Vrachanski Balkan or any other area with similar characteristics in Bulgaria.

Based on experience, there are several key points which need to be considered when planning the future Griffon Vulture related work in Vrachanski Balkan. The regular provision of huge quantities of food is crucial for maintaining and increasing the results achieved by now. The food needed to maintain a group of some 50 Griffon Vultures present in the area is about 2000 - 2500 kg or more per month. These quantities are necessary as it has been proven that some 50 - 70 % of the food disposed does not reach vultures but is eaten by Ravens or decays in the hot summer months. It should also be considered that the meat and offal of a carcass is below 50 % of its total weight while the other parts (skin, bones, etc.) cannot be utilized by Griffon Vultures. The supplementary feeding shall continue at the already established feeding site above the village of Dolno Ozirovo, as well as one extra site. We therefore recommend the construction of an additional supplementary feeding site in the Eastern parts of the Vrachansi Balkan Nature Park. This area is very close to suitable nesting massifs near the town of Vratsa and the Iskar Gorge. It is very important that this feeding site is located so that it is well-accepted by the Griffon Vultures, is supported by the local population so no disturbance occurs and is easily accessible throughout the year.

Further data analysis should show whether it would be necessary to continue the vulture releases. In case sufficient food is available, releasing additional Griffon Vultures in the area could be beneficial for the restoration activities in the area.

Continuing the already established collaboration is of crucial importance for the future vulture restoration activities. Key partners are the Vrachanski Balkan Nature Park Directorate, the large-scale commercial farms, the structures of the Bulgarian food Safety Agency, the Ministry of Environment and Waters and the Regional Inspectorates of Environment and Waters, the Regional Forestry Managment of Berkovitsa and the local Forestry Boards, municipalities and town halls in various settlements. The wide-scale awareness raising campain should also continue, involving various actors, such as hunters, farmers, climbers, etc.

The current results show that the restoration of Griffon Vulture in Vrachanski Balkan as a nesting species is completely feasible in the contemporary environment. Considering our current experience, there is a great potential in elaborating these efforts, achieving even better results.

Acknowledgements: We are extremely grateful to all, who have contributed to our efforts over the years:

Colleagues from various organizations: Ivo Danchev (BPPS), Dobromir Domuschiev (BPPS), Stoyan Yotov (BPPS), Krum Kosharevski (BPPS), Iliyan Stoev (Green Balkans), Lachezar Bonchev (FWFF), Hristo Peshev (FWFF), Marin Kurtev, Stefan Avramov (BBF), Hristo Hristov („Edelvays“ Club), Boris Nikolov (BAS), Lyubomir Andreev and many others.

Staff of the Vrachanski Balkan Nature Park Direcorate: Mr. Nikoly Nenchev, Director, as well as the following experts: Eng. Daniela Borisova, Eng. Georgi Antonov, Eng.

Tsvetan Tsvetkov, Olya Genova, Dimitar Torbov, Ivo Ivanov and others. Cavers and rock climbers: Plamen Yotov, Tsvetomir Velkovski, Nikolay Vanyov and others. Veterinarians: Dr. Gergo Gergov, Dr. Lyudmil Antov, Dr. Asparuh Tsenev, Dr. Miroslava Zdravkova, Dr. Lyubomir Plamenov, Dr. Valentin Lilov and others. Local people, who have helped our work: Ivaylo Tsvetanov, Georgi N. Grozdanov, Yotso Petrov, Dimitar Hristov and others. Mayors of settlements from the Municipality of Varshets: Angel Petrov, Ivaylo Yordanov, Petar Stefanov, Ivan Lazarov and others.

Colleagues from various countires: Ohad Hatzofe (Israel), Dora Skartsi (Greece), Goran Sushic (Croatia), Bratislav Grubac (Serbia), Sasa Marinkovic (Serbia), Branko Bjelic (Serbia), Goran Sekulic (Serbia), Fulvio Genero (Italy).

The team from the Reintroduction Committee, which developed the vulture restoration project: Michel Terrasse (France), Wolfgang Fremuth (Germany), Hans Frey (Austria), Evelyn Tewes (Spain), Juan Jose Sanchez Artes (Spain), Alvaro Camina (Spain), as well as Jovan Andevski (Macedonia).

To the Frankfurt Zoological Society, the Deutche Bundesstiftung Umwelt (DBU), the Black Vulture Conservation Foundation (BVCF), the Vulture Conservation Foundation (VCF), the Bulgarian Partnership Foundation.

The Ministry of Environment and Waters, the Regional Inspectorate of Environment and Waters Montana and Vratsa, the Bulgarian food Safety Agency and the Regional Food Safety Agency of Montana, the Regional Forest Management of Berkovitsa, the State Forestry Board of Berkovitsa, the Municipality of Varshets and others.

CEZ Ltd and special thanks to Eng. Rosen Katerinov.

REFERENCES

1. Archives of RAF Berkovitza 2005. Data for the quantity of game after the collecting and processing species' lists.
2. Archives of the administration "Agriculture and Forests" – region of Montana. 2005. Data for the number of domestic animals bred.
3. Archives of the administration "Agriculture and Forests", region of Vratza, 2005 – Data for the number of domestic animals bred.
4. Boev, N., T. Mitchev. 1981. Past and Present Distribution Of The Vultures in Bulgaria. Sofia, BAS, Â: Regional Symposium Collection Project ¹ 8 – MAB, UNESCO, 20-24. X. 1980, 566-575 p. (in Bulgarian)
5. BSPB. 2014. Vultures are about to get extinct in Europe and Africa in our Lifetime. News up-date on BSPB website. http://bspb.org/bg/news/Leshoqdite-v-Evropa-i-Afrika-na-pyt-da-izcheznat-v-ramkite-na-nashiq-zhivot.html
6. Choisy, J.-P., Tesseir, C., Henriquet. 1992. Reintroduction of the Griffon Vulture Gyps fulvus – Current state of the method in France. p. 1-7.
7. Collective, 1956. Statistical annual book of the region of Vratza, page 195.
8. Collective, 1960. Statistical manual of the Region of Mihaylovgrad (Montana), p. 120
9. Collective, 1969. Statistical manual – Mihaylovgrad region, p. 230
10. Collective. 1969. Statistical manual – region Vratza. Page 450.
11. Collective. 1978. Statistical manual – the region of Vratza, p. 250
12. Collective. 1981. Statistical manual – region of Mihaylovgrad, p. 210
13. Collective. 1987. Statistical manual – region of Vratza, p. 280
14. Collective. 1995. Periodical of the National Institute of Statistics – Agriculture animals in Bulgaria up to 01.01.1995, page 155.

15. Collective. 1996 a. Statistical manual – Montana. 130 p.
16. Collective. 1996 b. Statistical manual – Vratza, p. 155.
17. Georgiev, G. 2000. Biogeographycal Characteristic of Bulgaria. Part 2, University press, Blagoevgrad,311p.
18. Grubach, B. 2014. Beloglavi sup Gyps fulvus. Zavod za zashtitu prirode Srbije, Beograd, Scanner Studio, 256 s.
19. Marinkovic, S., B. Karadzic. 2008. Griffon vulture (*Gyps fulvus*). Belgrad. 73 p.
20. Michev, T., V. Pomakov, V. Stefanov, P. Yankov.1980. Koloniya na beloglaviya leshoyad (Gyps fulvus Hablizl) v Iztochni Rodopi. Ekologiya, 6. s. 74-79.
21. Patev, P. 1950. Ptitsite v Balgariya, Sofiya. BAN. 364 s.
22. Peshev, H., E. Stoynov, A. Grozdanov, N. Vangelova. 2015. Reintroduktsiya na beloglaviya leshoyad (Gyps fulvus) v Kresnenskiya prolom, Yugozapadna Balgariya 2010-2015. Fond za divata flora i fauna. Prorodozashtitno-nauchna poreditsa, Kniga 3. Blagoevgrad, 112 s.
23. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation)
24. Regulation (EC) No 1774/2002 Of The European Parliament And Of The Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption
25. Simeonov, S., T. Mitchev, D. Nankinov. 1990. The Fauna of Bulgaria, Aves, BAS, Sofia. Vol. 20, Part I, 350 p.
26. Slotta-Bachmayr, L., Bogel, R., Camina Cardenal A. 2004. The Eiroasian Griffon Vulture (*Gyps fulvus*) in Europa and Mediterranean- Status report and Action plan. 98 p.
27. Stoyanov, G. 2010 a. Fruhere und jetzige Verbreitung des Gansegeiers *Gyps fulvus* in West-Bulgarien. Teil 1, Ornithologishe Mitteilungen. 62 (7): 235-239.
28. Stoyanov, G. 2010 b. Fruhere und jetzige Verbreitung des Gansegeiers *Gyps fulvus* in West-Bulgarien. Teil 2, Ornithologishe Mitteilungen. 62 (8): 277-281.
29. Stoyanov, G., Lazarova, T., Danchev, I., Domuschiev, D., Borisova, D. 2006. Viability study of the potential for reintroduction of the Griffon Vulture (*Gyps fulvus*) in the Vrachanska Mountain (Vrachanski Balkan Nature Park), Bulgaria. Sofia. Birds of Prey Protection Society. Manuscript. 62 p.
30. Tåwes, E., M. Terrasse, H. Frey, J. J. Sanchesse. W. Fremuth. 2002. Action Plan for Recovery and Conservation of Vultures on the Balkan Peninsula. Draft for the planning Workshop. Sofia. October 2002. 47 p.
31. Vaỉtkov, I. 1987. Vratza Mountain – guide-book. Sofia. 56 p.
32. Vrachanski Balkan Nature Park Directorate. 2011. Management Plan for Vrachanski Balkan Nature Park. Available at: http://www.vr-balkan.net/bg/plan-za-upravlenie-na-dpp-vrachanski-balkan/
33. Yankov, P., L. Profirov. 1991. Present condition of Griffon vulture (Gyps fulvus Hablizl) population in Bulgaria. BAS. Ecology, 24: 44-51 p.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 107-115

ACTIONS FOR RESTORATION OF GRIFFON VULTURE (*GYPS FULVUS*) IN CENTRAL BALKAN, BULGARIA

LYUBOMIR YANKOV[1], GEORGI STOYANOV[2], ELENA KMETOVA-BIRO[1]*, IVAN RADUNCHEV[2], IVELIN IVANOV[1], ILIYAN STOEV[1], GRADIMIR GRADEV[1]

1- Green Balkans – Stara Zagora – 9 Stara planina str, Stara Zagora 6000, BULGARIA, www.greenbalkans.org, officesz@greenbalkans.org
2- Birds of Prey Protection Society - 23 Golyam Bratan str., Sofia 1618, BULGARIA, www.bpps.org , bpps@abv.bg
**Corresponding author: officesz@greenbalkans.org*

Keywords: Griffon Vulture, *Gyps fulvus,* Central Balkan Mountains, Bulgaria, reintroduction.

Abstract: The current work presents the methodology and results following five years of attempts for the re-introduction of Griffon Vulture (*Gyps fulvus*) in the Central Balkan Mountains of Bulgaria, practically started in 2010 within the Vultures Return in Bulgaria LIFE08 NAT/BG/278 Project.

Between January 2010 - June 2015 a total of 59 Griffon Vultures were released from the Central Balkan adaptation aviary, found at the foot of the Central Balkan National Park in the territory of the Manolovo / Tuzha Villages (UTM LH32).

At June 2015 there is a fixed group of some 7-9 Griffon Vultures constantly present in the area of release. Up to 24 Griffon Vultures have been seen at a single feeding event (10.06.2013), among them 6 wild, non-tagged ones. The feeding site is also used by Golden Eagle (*Aquila chrysaetus*), Raven (*Corvus corax*), Common Buzzard (*Buteo buteo*), Rough-legged Buzzard (*Buteo rufinus*), Magpie (*Pica pica*) and Goshawk (*Accipiter gentilis*).

INTRODUCTION

In 2003, following over 20 years of local conservation efforts, a long-term International Action Plan for the Recovery and Conservation of Vultures on the Balkan Peninsula and Adjacent Regions (BVAP) was initiated. It provides for a step-by-step recovery of the species, starting with Griffon and finishing with Bearded Vultures (Tewes et al. 2004). As part of the strategy for restoration of the Griffon Vulture along Balkan Mountain, Central Balkan was chosen as one of

the most prospective release sites. The Birds of Prey Protection Society (BPPS) and the Green Balkans NGO joined efforts for establishing an adaptation aviary, feeding site and a working team in the area. A specialized project "Recovery of the Populations of Large European Vultures in Bulgaria" LIFE08 NAT/BG/278 was therefore triggered in 2010. The project is carried out by Green Balkans - Stara Zagora NGO in partnership with the Fund for Wild Flora and Fauna (FWFF) and the Birds of Prey Protection Society (BPPS).

The overall aim of the project was restoring the populations of the three large vulture species in Bulgaria through conservation measures and increasing the institutional capacity for their conservation. The current article describes the results of Griffon Vulture releases in one of the four chosen release sites along the Balkan Mountain (Stara Planina) within the project, namely the Central Balkan National Park.

MATERIALS AND METHODS

Geographical area

Central Balkan NP is one of the largest and most valuable European protected areas. It was founded in 1991 in order to preserve forever the unique nature of Central Balkan Mountain and the traditions and livelihood related to it, in benefit of the society.

This is one of the most significant ecological corridors in Bulgaria, contributing to the genetic exchange, the free movement and natural links between species in the Carpathians and other European mountains and the southern regions of the Balkan Peninsula and Asia Minor (Danchev et al. 2007).

The Central Balkan covers the Northern and Southern slopes of the highest part of the Balkan Mountain chain from 500 to 2,376 m altitude. The base rock is silicate and limestone, with canyons, caves, precipices, waterfalls, mighty massifs and cliffs. The bigger part of the territory is covered by forests, while the high-mountain zone consists of open areas. The vegetation cover is mixed broadleaved forests of *Fagus sylvatica* L. subsp. *moesiaca*, *Carpinus betulus*, *Quercus dalechampii*, etc. (Kostadinova and Gramatikov, 2007).

Data for historical presence of the species exists in a number of scientific articles (Demerdjiev, 2007 and the citations therein). Till the late 40s of 20th century the Griffon Vultures were numerous in the region and inhabited all appropriate habitats (Danchev et al. 2007). The most recent records of breeding date back to 1941, when 60 Griffons were found poisoned on a cow carcass, near the village of Anton, located on the South-Eastern edge of the National Park. The nesting cliff inhabited by these birds has reportedly been on a nearby cliff, which currently belongs to the National Park territory. Yet other 10 Griffon vultures inhabited the "Kuru Dere" gorge (located on the South-Eastern edge of Triglav rang, photo 3) for some years before they were poisoned with strychnine

sometime in the 1960s (Danchev et al. 2007). Since then there have been isolated observations of single individuals or small passing groups but no nesting has been confirmed (Danchev et al. 2007). In 2007 a "Viability Study on the reintroduction of the Griffon Vulture (*Gyps fulvus*) in Stara planina Mountain, Bulgaria" (Danchev et al. 2007) was elaborated by the team of BPPS in accordance with the IUCN Guidelines for reintroduction (IUCN, 1998) to justify the selection of the Central Balkan area as among the most prospective for the start of a full-scale reintroduction programme.

The release methodology chosen was the successfully used for the restoration of Griffon Vulture in the France, published by Choisy and Henriquet (1992), Terrasse (2006) and Terrasse and Choisy (2007).

An adaptation aviary with the following dimensions 25*10*3 meters, was established at the foot of the Central Balkan National Park in the territory of the Manolovo / Tuzha Villages (UTM LH32) in 2009. It was centered in a fenced area of 35*25 meters that served both: 1. to prevent the access of wild predators, dogs, livestock and people to the aviary and thus avoid disturbance of vultures, and 2. as supplementary feeding site for the released birds.

A total of 69 Griffon Vultures were shipped and accommodated at the Central Balkan adaptation aviary between January 2010 - July 2015. Fifty six of those birds originate from rehabilitation centres in Spain, other 6 birds came from rehabilitation centres in France, while the rest were captive bred in several European Zoos.

In most cases Griffon Vultures were admitted at the Wildlife Rescue Centre of Green Balkans – Stara Zagora for initial examination and quarantine. Birds were all treated against internal and external parasites and weighted. Following an average of a month of quarantine, as required by the Bulgarian veterinary regulations, vultures were individually captured, re-examined, ringed, wing-tagged and disseminated among the four adaptation aviaries in the Balkan Mountains (Vrachanski Balkan, Central Balkan, Sinite kamani - Grebenets and Kotlenska planina). In several cases, when birds originated from controlled environment, such as zoos, vultures were directly accommodated at the adaptation aviaries, skipping the quarantine at the Rescue Centre.

All vultures, released in the Central Balkan area have been tagged with yellow PVC rings and matching wing tags on both wings with vertical three-alpha numeric code, starting with K. A total of 46 out of all vultures were fitted with standard metal W-size rings of the Bulgarian ornithological central, while the rest arrived with metal rings from the country of origin.

Food (meat) was provided to the birds in the aviary, but also at the feeding site, outside the cage for the released vultures, as well as to attract any passing birds through the area.

Food was provided on a regular basis (once or twice per week) upon availability of livestock carcasses in the local villages and farms, or was intentionally transported from slaughterhouses (offal).

The vultures were frequently (every 2 to 4 days) observed by binoculars and spotting scopes at the feeding site and the known roosting sites. Local people and tourist reports on seen vultures and identified tags were used, as well as photo-traps in order to record and identify the birds present at the supplementary feeding site, adjacent to the release aviary. Initially the project employed a Microsoft Office Excel spread-sheet with individual data on every bird imported within the Vultures Return in Bulgaria Project.

An interactive web-database was later developed within the Vultures Return in Bulgaria Project and tested for optimizing the storing and processing of information.

The database currently stores individual information on every bird, supplementary feeding site and observation. It is also compatible with the data sent by the GPS/GSM transmitters and it is able to visualize the points of observation of a given bird in chronological order, thus tracking its overall movements and behavior following release.

RESULTS AND DISCUSSION

A total of 59 Griffon Vultures were released from the Central Balkan adaptation aviary between January 2010 - July 2015 (see Table 1).

Table 1. Number of Griffon Vultures (Gyps fulvus) released in Central Balkan area

	Central Balkan
2009	0
2010	8
2011	0
2012	15
2013	10
2014	19
2015	7
Average/year	10
Total	**59**

A total of 4 different birds were tagged with VHF radio transmitters. One of these birds was confirmed to have been electrocuted. One additional bird was tagged with a GPS/GSM transmitter.

Most of the birds stayed in the adaptation aviaries between 7-12 months (53 %) before release. Of them 2 (6 %) were confirmed to have later died. Other 23 (39 %) of the birds were kept between 1- 3 months before release. Of them 5 (22 %) were confirmed to have later died. A single bird stayed in the adaptation aviary for less than a month.

Vultures were released generally between March – October (See Table 2, Figure 1). Two main strategies were employed, focusing on early spring and autumn releases. The idea behind the spring releases was to coincide with the improvement of the soaring flight conditions with the increase of the temperatures and to avoid release of inexperienced birds in or just prior to the winter.

The autumn releases were intended to provide sufficient time for the vultures to explore the areas and locate the best roosting and feeding sites before the start of the winter.

Table 2. Number of birds released in the various months of the year and their survival rate

	Birds released	Survived	Died	Mortality %
January				
February				
March	10	10		0
April	1		1	100
May	19		3	16
June				
July	8		3	38
August	12	12		0
September	1	1		0
October	8		2	25
November				
December				
Total	59	23	9	15

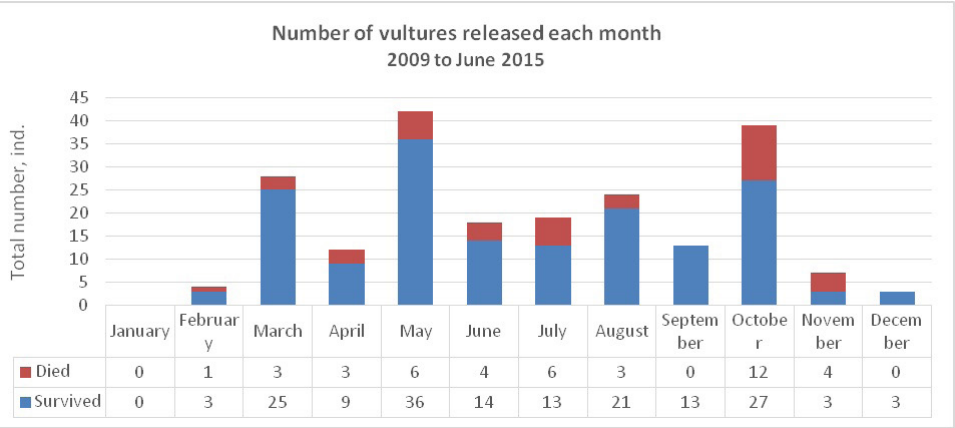


Fig. 1. Number of vultures released each month and their survival rate

Birds were released depending on the particular weather conditions, preferably at the presence of experienced vultures outside the aviaries at the time of release.

A total of 9 of the 59 Griffon Vultures released in the area were eventually found dead between January 2010 - December 2015, which means a total mortality of 15 %. Mostly body remains and wing tags were found by local citizens or tourists so cause of death could not be determined. Two of the cases were suspected cases of electrocution, since the remains were found under electric pylons, but carcasses were not fit for necropsy. A total of 8 out of the 9 of the dead birds were reported between November - January, which turns out to be critical for the birds post-release adaptation and survival.

Following the start of the releases, there is a clear positive trend in the number of free-flying Griffon Vultures observed in the area. The highest number of feeding birds was reported in June 2013 - a total of 18 individuals at a single feeding event, among them wild, non-tagged individuals (see Figure 2). The released vultures obviously followed them and vacated the area, what explains the sharp decline of the number of vultures observed after that. There is still a clear positive trend, indicated with the spotted line presented in Figure 2. Figure 2 is based on the maximum number of Griffon Vultures present in the Central Balkan feeding site at a single observation. It does not consider the aggregated number of different birds observed in the given month, but represents the maximum concentration of birds counted at a single feeding event:

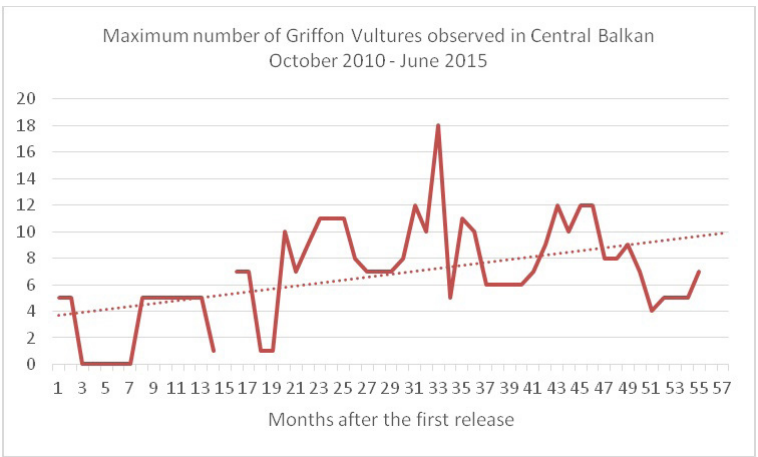


Fig. 2. Maximum number of Griffon Vultures observed at a single feeding event at the Central Balkan supplementary feeding site (October 2010 - June 2015)

At June 2015 there is a fixed group of some 7-9 Griffon Vultures constantly present in the area of release. At the same time, up to 24 Griffon Vultures have been seen in the area (10.06.2013), among them 6 wild, non-tagged ones. Usually following such a visit of exogenous birds, a great part of the group follows and disperses.

No breeding attempts have been reported in the area. However, a bird, originating from the Central Balkan release site tagged K4M has formed a breeding pair with a bird tagged K41 in the area of Vrachanski Balkan in 2014.

Feeding of vultures in Central Balkan was provided within 630 feeding events for 6 years in the period 2010 - June 2015, with total amount of 25 680 kg carcasses, and/or slaughter offal disposed on site. This makes on average of 4310 kg per year and 2.01 feedings per week.

Compared with other successful release sites (e.g. Vrachanski Balkan, Eastern Balkans, and Kresna Gorge) the amount of provided food, and the frequency of the feedings is lower (Stoev *et al.*; Stoyanov *et al.;* this volume; Peshev *et al.,* 2015). This fact, together with the delayed release of the first group of Griffon Vultures, as compared to the neighboring sites of Vrachanski Balkan and the Eastern Balkan Mountains, may explain the lower success in settling of the birds and establishing a colony of the species observed.

In addition to the vultures, the supplementary feeding site is also used by Golden Eagle (*Aquila chrysaetus*), Raven (*Corvus corax*), Common Buzzard (*Buteo buteo*), Rough-legged Buzzard (*Buteo rufinus*), Magpie (*Pica pica*) and Goshawk (*Accipiter gentilis*). Accompanying species were also observed on other feeding sites like Kresna gorge (Stoynov et al. 2015).

CONCLUSION

Even though a large number of birds have been released from the Central Balkan release site, the area sustains the lowest number of free-flying Griffon Vultures from all four release areas maintained in the Balkan Mountains of Bulgaria (Vrachanski Balkan, Central Balkan, Eastren Balkan Mountains - Sinite Kamani /Kotlenska Planina). Birds from Central Balkans are known to have settled in a number of other release sites: Vrachanski Balkan (at least 7 individuals), Eastern Balkan Mountains (at least 7 individuals), Eastern Rhodopes (between 7 to 10 individuals).

The insufficient and/or infrequent food supply, inaccessibility of the food at the feeding site (flat instead of steep terrain and a relatively small fenced area) may be the reason for emigration of the released birds.

However, observations of non-tagged (wild) Griffon Vultures, as well as the coordinates from the GPS/GSM tagged vultures elsewhere clearly show the importance of the area and the need to be further developed as a link between the Western Balkan Mountains (Vrachanski Balkan) and the Eastern Balkan

Mountains (Sinite kamani /Kotlenska Planina). Last, but not least, the area is also the closest to the Eastern Rodopes and may play a key role as a stepping stone for the vultures on passage between the Balkan Mountains and the Rhodopes.

Acknowledgements: At the pre-release stage the project was supported by the Deutche Bundesstiftung Umwelt (DBU), Frankfurt Zoological Society (FZS), Black Vulture Conservation Foundation (BVCF), Rufford Small Grants (RSG) and others. Since 2010 the vulture restoration programme in the Balkan Mountains of Bulgaria has been supported by the LIFE+ financial instrument of EU within the project "Vultures Return in Bulgaria" LIFE08 NAT/BG/278, and is co-financed by the Deutche Bundesstiftung Umwelt (DBU) and the Frankfurt Zoological Society (FZS).

Special thanks to all experts and state institutions, who helped and assisted us throughout the project implementation.

The actions will continue through the Vultures Back to LIFE14 NAT/BG/649 Project, coordinated by Green Balkans, with the participation of Fund for Wild Flora and Fauna, Vulture Conservation Foundation, junta de Extremadura and EuroNatur.

Additional information:For more information of the project and the releases, please visit: www.greenbalkans.org/birdsofprey/life

REFERENCES

1. Дончев, С. 1974. Птиците на Средна и Източна Стара планина. Изв. на Зоол. Инст. с Музей - БАН, 41, 33-63.
2. Костадинова, И., М. Граматиков (ред.). 2007. Орнитологично важните места н България и НАТУРА 2000. БДЗП, Природозащитна поредица, Книга 11. София, БДЗП.
3. Симеонов, С., Т. Мичев, Д. Нанкинов. 1990. Фауна на България. Птици. Част I., София, БАН, 350 с.
4. Спиридонов, 1987. Оазиси на дивата природа. София, Земиздат, 191 с.
5. Янков, П., Л. Профиров. 1991. Съвременно състояние на популацията на белоглавия лешояд (Gyps fulvus, Hablizl) в България. – Екология, 24, 44-52.
6. Baumgart, W. 1974. Wie steht es um Europas Geier? – Der Falke, 8, 258-267.
7. Choisy, J.-P., Tesseir, C., Henriquet. 1992. Reintroduction of the Griffon Vulture Gyps fulvus – Current state of the method in France. pp. 1-7
8. Danchev, I., D. Domuschiev, A. Stanchev, G. P. Stoyanov, T. Lazarova, Lubomira Klcheva. 2007. Viability study on the potential for reintroduction of the Griffon Vulture (Gyps fulvus) in Central Balkan National Park, Bulgaria. Birds of Prey Protection Society.
9. Demerdzhiev D., E. Stoynov, M. Kurtev, P. Yankov 2007. Griffon Vulture (Gyps fulvus). – In: Yankov, P. (ed.). Atlas of Breeding Birds in Bulgaria. Bulgarian Society for the Protection of Birds, Conservation Series, Book 10. Sofia, BSPB, p 134-135.
10. Farman, C. 1869. On some of the birds of prey in Central Bulgaria. Ibis, 2 (5), p. 199.
11. IUCN/SSC. 1998. IUCN Guidelines for Re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN. Gland, Switzerland and Cambridge, UK, 10 p.
12. Peshev H., Stoynov E., Grozdanov A., Vangelova N. 2015. Reintroduction of the Griffon Vulture Gyps fulvus in Kresna Gorge, Southwest Bulgaria 2010-2015. Conservation Sience Series, Book 3. Fund for Wild Flora and Fauna, Blagoevgrad.

13. Stoyanov, G. 2010 a. Fruhere und jetzige Verbreitung des Gansegeiers Gyps fulvus in West-Bulgarien. Teil 1, Ornithologishe Mitteilungen. 62 (7): 235-239
14. Stoyanov, G. 2010 b. Fruhere und jetzige Verbreitung des Gansegeiers Gyps fulvus in West-Bulgarien. Teil 2, Ornithologishe Mitteilungen. 62 (8): 277-281
15. Stoynov E., L. Bonchev. 2012. Reintroduction of Griffon Vulture Gyps fulvus in Kotel Mountain, Bulgaria, Overview 2007-2011, Fund for Wild Flora and Fauna, Blagoevgrad.
16. Stoynov E., Peshev H., Grozdanov A., Delov V., Vangelova N., Peshev D. 2015. New data for the presence and numbers of some conservation dependent birds in Kresna Gorge with proposal of original method for individual identification of vultures. Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte De Biologie 2015, volume, livre 4, pp. First National Conference of Biotechnology, Sofia 2014
17. Terrasse M. 2006. Long-term Reintroduction Projects of Griffon Vulture Gyps fulvus and Black Vulture Aegypius monachus in France. In: Houston & Piper 2006. Proceedings of the International Conference on Conservation and Management of Vulture Populations. 14-16 November 2005, Thessaloniki, Greece. Natural History Museum of Crete & WWF Greece. pp. 98-107.
18. Terrasse M., J-P. Choisy. 2007. Reintroduction of the Griffon Vulture- technical guideline. LPO Mission Rapaces, PN des Cevennes, PN du Vercors.
19. Tewes, E., M. Terrasse, J. J. Sanchez Artés, W. Fremuth, H. Frey. 2004. Action Plan for the Recovery and Conservation of Vultures on the Balkan Peninsula: activities and projects during 2002 and 2003. Raptors Worldwide. WWGBP/MME.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 116-129

RESTORATION OF GRIFFON VULTURE (*GYPS FULVUS*) IN EASTERN BALKAN MOUNTAINS, BULGARIA

ILIYAN STOEV[1], LUCHEZAR BONCHEV[2], EMILIAN STOYNOV[2], ELENA KMETOVA-BIRO[1], IVELIN IVANOV[1], LYUBOMIR YANKOV[1], YAVOR ILIEV[2], ZLATKA NIKOLOVA[1], NADYA VANGELOVA[2], ATANAS GROZDANOV[2]

1 - Green Balkans – Stara Zagora – 9 Stara planina str, Stara Zagora 6000, BULGARIA, www.greenbalkans.org, officesz@greenbalkans.org
2 - Fund for Wild Flora and Fauna – 49 Ivan Michaylov str, office 327, P.O. Box 78, 2700 Blagoevgrad, BULGARIA, www.fwff.org , pirin@fwff.org
**Corresponding author: pirin@fwff.org*

Keywords: Griffon Vulture, *Gyps fulvus*, Eastern Balkan Mountains, Bulgaria, Kotlenska Planina, Sinite Kamani Nature Park, reintroduction.

Abstract: The current work presents the methodology and results following six year of the re-introduction of Griffon Vultures (*Gyps fulvus*) in the Eastern Balkan Mountains of Bulgaria, started in 2009 with pilot release and continued within the Vultures Return in Bulgaria LIFE08 NAT/BG/278 Project.

The practical releases started with 3 Griffon Vultures, released from the Kotel adaptation aviary found in the Kotlenska Planina (Kotel Mountain) in 2007. In the next two years 2008 and 2009 five more birds were released. In the period January 2010 - June 2015, a total of 44 more birds were released. At the same time (2010 – June 2015), a total of 65 Griffon Vultures were released from a nearby adaptation aviary found in the Sinite Kamani Nature Park.

Due to the relatively small distance between the Sinite Kamani Nature Park and the Kotlenska Planina sites, the Griffon Vultures released from the two sites have formed a joint group, regularly visiting both sites, depending on the presence of food and the weather conditions. At October 2015 there was a fixed group of some 25-35 Griffon Vultures constantly present in the areas of release. A minimum of 85 different Griffon Vultures were identified to have visited the feeding sites in 2015, and many non-marked birds also are frequently present. Up to 37 Griffon Vultures have been seen together at the roosting sites near Kotel in October 2015.

First breeding attempt of Griffon Vultures, released within the restoration programme was recorded in 2012 at about 60 km to the East of the release sites. All known breeding attempts from 2013 to 2015 were recorded in the vicinity of Kotel. In the breeding season 2014-2015 there were minimum three territorial pairs of Griffon Vultures in the Eastern Balkan Mountains.

INTRODUCTION

A total of four species of vultures were known to nest in Bulgaria: Eurasian Griffon Vulture (*Gyps fulvus*), Black Vulture (*Aegypius monachus*), Egyptian Vulture (*Neophron perconpterus*) and Bearded Vulture (*Gypaetus barbatus*) (Patev 1950, Simeonov and Michev 1990).

In the beginning of XXI century only two of the species remained as nesting in the country - Griffon and Egyptian Vulture. The last breeding of Black Vulture was confirmed in 1992 in the Eastern Rhodopes (Marin et al. 1998, Stoynov et al. 2007) after a gap of more than thirty years, while Bearded Vulture has been considered extinct since the 70s of the XX century, despite the occasional sightings of individual birds (Donchev 1974, Stoynov 2007).

In 2003, following over 20 years of local conservation efforts, a long-term International Action Plan for the Recovery and Conservation of Vultures on the Balkan Peninsula and Adjacent Regions (BVAP) was initiated. It provides for a step-by-step recovery of the species, starting with Griffon Vulture in some strategic historical breeding sites and finishing with the reintroduction of Bearded Vulture. As an initial step, a Griffon Vulture adaptation aviary was constructed in the area of Kotlenska Planina (UTM MH65), near the town of Kotel, by the Fund for Wild Flora and Fauna (FWFF) in 2003 and 3 vultures were released in 2007 (Stoynov and Bonchev, 2012). In 2008 an additional adaptation aviary was also constructed by Green Balkans – Stara Zagora NGO in the territory of the Sinite kamani Nature Park (UTM MH43), 25 km away from the initial release site near the town of Kotel. Considering the proximity of the two adaptation aviaries and the behavior of the birds released from the two release sites, the current publication considers them as a common, Eastern Balkan Mountains release site. Both sites are known to be historical breeding area for the Griffon Vulture (Demerdjiev, 2007; Patev, 1950; Simeonov et al. 1990, Slotta-Bachmayr et al. 2004, Spiridonov, 1987).

Site description

Kotlenska Planina

Kotlenska Planina is located in the Central part of the Eastern Balkan Mountains chain. Many clearly formed secondary ridges with steep slopes and deeply cutting gorges and ravines descend from the main ridge of the mountain to the Ticha and the Luda Kamchia river valleys. The terrain is steep and heavily indented. The region is sparsely populated, mainly in its periphery and along the river valleys. About 23 % of the mountain territory is occupied by primary broad-leaved forests mainly of European beech *Fagus sylvatica subsp. moesiaca*, at places mixed with European Hornbeam *Carpinus betulus*. The forests of Oak *Quercus dalechampii* are more limited, sometimes mixed with European Hornbeam *C. betulus* and Oriental Hornbeam *Carpinus orientalis*, and mixed forests of Turkey Oak *Quercus cerris* and Hungarian Oak *Quercus frainetto*. A

patches of natural mixed woods of White Fir *Abies alba* and Beech *Fagus sylvatica* have been established in the Western part of the mountain. The secondary forests and shrubs of Oriental Hornbeam have a very limited distribution in the region. The rest of the territory is occupied by open grasslands, used as pastures and farmland. A considerable part of the open areas, including the agricultural plots, have a secondary origin, i.e. they spread on territory previously occupied by old forests. The wet areas and the river valleys cover a comparatively small part of the mountain territory. Limestone rocks and karst formations are dispersed everywhere in the high mountain areas (Kostadinova & Gramatikov 2007). The mean altitude of the site is about 500 m above sea level and the highest peak is a little over 1000 m.

Sinite Kamani Nature Park

The area is located in the Sliven Mountains, a part of the Southern slopes of the Eastern Balkan Mountains (part of Balkan Mountain chain). The Western part and the Southwestern limit of the area is defined by the Asenovets Reservoir and the Asenovska River. On the North it passes along a watershed ridge, which is practically the highest part of the area at over 1000 m above sea level. The Northern slopes are steep, covered by Beech forest. The Southern border of the area passes along the Southern slopes of the mountain just next to the town of Sliven. A considerable part of the area is covered by broadleaved forests. The mixed oak forests of *Quercus delechampii*, *Q. cerris*, *Q. frainetto* and *Q. pubescens* prevail. Forests of Crimean Beech *Fagus moesiaca* and European Hornbeam *Carpinus betulus*, as well as Silver Linden *Tilia tomentosa* and Oriental Hornbeam *Carpinus orientalis* are also represented. Shrub and grass associations on silicate base occupy 2 % of the area, while about 6% is covered by secondary steppe and dry calciphile grass communities. The grass formations have a secondary origin. The rock formations, which have given the name of the site, cover some 7 % of its total area.

As part of the strategy for restoration of the Griffon Vulture along Balkan Mountains, the Eastern Balkan Mountains were chosen as one of the most prospective release sites. In 2007 a "Viability Study on the reintroduction of the Griffon Vulture (*Gyps fulvus*) in Kotlenska planina, Bulgaria" (Stoynov et al. 2007) was elaborated by the team of the Fund for Wild Flora and Fauna in accordance with the IUCN Guidelines for reintroduction (IUCN, 1998) to justify the selection of the Central Balkan area as among the most prospective for the start of a full-scale reintroduction programme.

Three years later, a specialized project "Recovery of the Populations of Large European Vultures in Bulgaria" LIFE08 NAT/BG/278 was triggered in 2010. The project was carried out by Green Balkans - Stara Zagora NGO in partnership with the Fund for Wild Flora and Fauna (FWFF) and the Birds of Prey Protection Society (BPPS).

The overall aim of the project was restoring the populations of the three large vulture species in Bulgaria through reintroduction of the Griffon Vulture along the whole Balkan Mountain chain (four releases sites – from West to East – Vrachanski Balkan, Central Balkan, Sinite Kamani - Grebents and Kotlenska Planina). A complex of preparatory and direct conservation measures was applied and the capacity of NGOs and local institutions was increased for conservation action. The current article reports the results of the Griffon Vulture reintroduction in the Eastern Balkan Mountains for the period 2010-2015.

MATERIALS AND METHODS

The original plan was to follow the release methodology successfully used for the restoration of Griffon Vulture in the Alps Choisy and Henriquet (1992), Terrasse (2006) and Terrasse and Choisy (2007). Their methodology provides for soft release (open the cage and wait for the birds to go out on their own) of subadult individuals (more than 3 years old) that have stayed some 2-3 years in the aviary (Sarrazin and Legendre 2000). The releases take place in autumn, as to avoid dispersal and migration (Terrasse and Choisy, 2007). Based on some experiments with the timing of releases in Bulgaria, we adapted the method as follows: first of all we also practiced hard release (release from hand) of immature birds (1-2 years old), which had stayed in the adaptation aviary for a period of a single month up to 1-2 years. We released most of the birds in late winter, spring and summer as to use the good flight conditions (warm weather), and to give more time to the vultures to adapt to the wild and store fat prior to winter.

A total of 100 Griffon Vultures were shipped and accommodated in the Sinite kamani adaptation aviary between January 2010 - July 2015. Another 50 were shipped and accommodated at the Kotlenska Planina adaptation aviary for between 2007 – July 2015, making a total of 150 birds secured for the area of the Eastern Balkan Mountains. A total of 123 of those birds originate from rehabilitation centres in Spain (which means that they were wild birds with previous flying experience, but had suffered some kind of injuries to be then collected and rehabilitated by those centres), other 10 birds came from rehabilitation centres in France, while the rest were captive bred in several European Zoos. It should however be noted that later, in the course of the project implementation, some of these birds were transferred to other release sites (such as Kresna Gorge release site), so when analyzing the results, the number of released vultures should be considered rather than the number of initially accommodated.

In most cases Griffon Vultures were admitted at the Wildlife Rescue Centre of Green Balkans – Stara Zagora for initial examination and quarantine. Birds were all treated against internal and external parasites and weighted. Following an average of a month, vultures were individually captured, re-examined, ringed, wing-tagged and forwarded to the adaptation aviaries in the Balkan Mountains

(Vrachanski Balkan, Central Balkan, Sinite kamani - Grebenets and Kotlenska planina). In several cases, when birds originated from controlled environment, such as zoos, vultures were directly accommodated at the adaptation aviaries, skipping the quarantine at the Rescue Centre.

A total of 100 of the vultures, released in the Eastern Balkan Mountains area were tagged with yellow PVC rings and matching wing tags on both wings with vertical three-alpha numeric code, starting with K. Two more Griffons, which voluntarily entered the adaptation aviaries were tagged and released with blue wing-tags with yellow inscriptions (B64 and B67). A single bird was released with a blue wing-tag with a horizontal orange inscription "10", two birds were released with green rings with white inscriptions (B18 and B20) and three birds were released with metal rings only.

Fenced supplementary feeding sites (vulture restaurants) were established right next to the adaptation aviaries and food (carcasses of livestock and/ or slaughter offal were provided on regular basis). It appears that not only the amount, but mostly the frequency of food provision is of great importance for the settlement of a nucleus of birds, for the survival of the releases ones and thus for the establishment of a colony.

The vultures were frequently (every 2 to 4 days) observed by binoculars and spotting scopes at the feeding site and the known roosting sites. Local people and tourist reports on seen vultures and identified tags were used, as well as photo-traps in order to record and identify the birds present at the supplementary feeding site, adjacent to the release aviary. Initially the project employed a Microsoft Office Excel spread-sheet with individual data on every bird imported within the Vultures Return in Bulgaria Project.

An interactive web-database was later developed within the Vultures Return in Bulgaria Project and tested for optimizing the storing and processing of information.

The database currently stores individual information on every bird, supplementary feeding site and observation. It is also compatible with the data sent by the GPS/GSM transmitters and it is able to visualize the points of observation of a given bird in chronological order, thus tracking its overall movements and behavior following release.

RESULTS AND DISCUSSION

The practical releases started with 3 Griffon Vultures, released from the Kotel adaptation aviary located in the Kotlenska planina in September 2007. In the next two years 2008 and 2009 five more birds were released. These first releases were used to try out and practise the release methods and to adapt them to the local conditions, which proved to be useful later on when additional groups of birds were released.

In the period January 2010 - June 2015, a total of 44 more birds were released from Kotlenska planina and additional 65 were released from the nearby Sinite kamani release site. A total of 109 Griffon Vultures were therefore released from the Eastern Balkans release site between 2009 - July 2015.

Table 1. Number of Griffon Vultures (Gyps fulvus) released in Eastern Balkan area

	Sliven	**Kotel**	**Total**
2009	0	5	***5***
2010	7	7	***14***
2011	12	11	***23***
2012	19	7	***26***
2013	8	0	***8***
2014	16	10	***26***
2015	3	4	*7*
Average	9	6	***16***
Total	**65**	**44**	**109**

Four different birds were tagged with VHF radio transmitters, to follow them during the adaptation period. One of these birds was later found dead. A total of 21 birds were tagged with prototypes of GPS/GSM transmitters.

Most of the birds released between 2010 – June 2015 stayed in the adaptation aviaries between 7-12 months (over 26 %) before release. Of them 7 (25 %) were confirmed to have later died. Other 24 (23 %) of the birds were kept between 4 - 6 months before release. Of them 7 (29 %) were confirmed to have later died. A total of 8 birds stayed in the adaptation aviary for less than a month, just one of them has later been confirmed to have died:

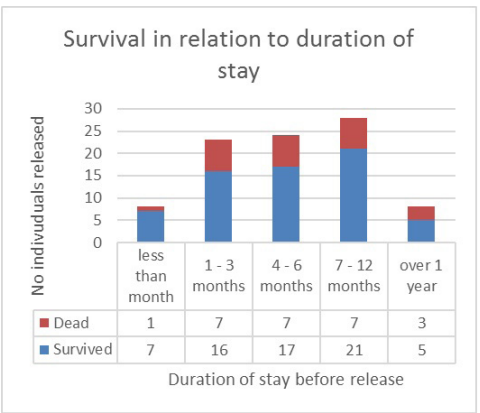


Fig. 1. Confirmed mortality of the Griffon Vultures in relation to their duration of stay in the adaptation aviaries prior their release

This data may be biased by the fact that a batch of adult, wild-caught Griffon Vultures was released from the Sinite Kamani site – all of them experienced birds, that quickly dispersed and discovered the natural vulture colonies on the Balkans and/or the other feeding sites and thus survived, but did not settle in the release area.

Vultures were released generally between March – October. Two main strategies were employed, focusing on early spring and autumn releases. The idea behind the spring releases was to coincide the start of the breeding season and force birds to quickly look for partners and nesting sites in close proximity to the release sites (Terrasse & Choisy 2007).

The autumn releases were intended to provide sufficient time for the vultures to explore the areas and locate the best roosting and feeding sites before the start of the winter (2009 – June 2015).

Table 2. Number of birds released in the various months of the year and their survival rate

	Birds released	Survived	Died	Mortality %
January	0	0	0	0
February	4	3	1	25
March	11	8	3	27
April	6	4	3	50
May	17	15	6	35
June	14	10	4	29
July	11	9	6	55
August	8	6	3	38
September	8	8	0	0
October	23	15	12	52
November	6	2	4	67
December	3	3	0	0
Total	111	83	42	38

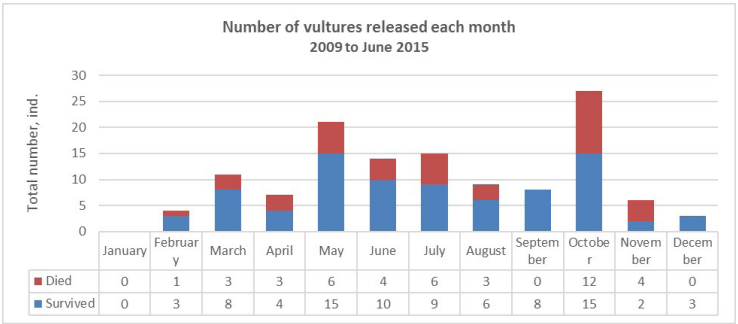


Fig. 2. Number of vultures released each month and their survival rate

Birds were released depending on the particular weather conditions, preferably at the presence of experienced vultures outside the aviaries at the time of release.

A total of 42 of the 111 Griffon Vultures released in the area were eventually found dead between January 2010 - April 2015, which means a mortality of 38 %. A total of 16 of the cases were proven or suspected cases of electrocution, since the remains were found under electric pylons, or the cause of death was proven with necropsy. Three of the cases (7 %) tested positive for pesticides (organo-phosphates/carbamates). Sixteen out of 42 of the dead birds were reported between October - November, which turns out to be a critical period for the adaptation and survival of the vultures.

Following the start of the releases, there is a clear positive trend in the number of free-flying Griffon Vultures observed in the area. Due to the relatively small distance between the Sinite kamani and the Kotlesnka Planina sites, the Griffon Vultures released from the two sites have formed a joint group, regularly visiting both sites, depending on the presence of food and the weather conditions. The birds generally roost on top of the Sinite kamani adaptation aviary or on a rock massif in direct proximity to the Kotlenska Planina release and feeding site.

The maximum number of birds recorded within a single month is 29 different Griffon Vultures (June 2014). In 2014 alone, a minimum of 52 different Griffon Vultures were identified to have visited the Kotlenska Planina feeding site, of which minimum 10 non –marked individuals of different age. The numbers reached 85 different Griffon Vultures in 2015 (Peshev et al. 2015).

The figures 2 and 3 below are based on the maximum number of Griffon Vultures present in the Eastern Balkan Mountain feeding sites at a single observation. They do not consider the aggregated number of different birds observed in the given month, but represents the maximum concentration of birds counted at a single feeding event.

The Eastern Balkan Mountains release sites have been visited by birds released from Kresna Gorge, Serbia, Israel, Croatia and Eastern Rhodopi.

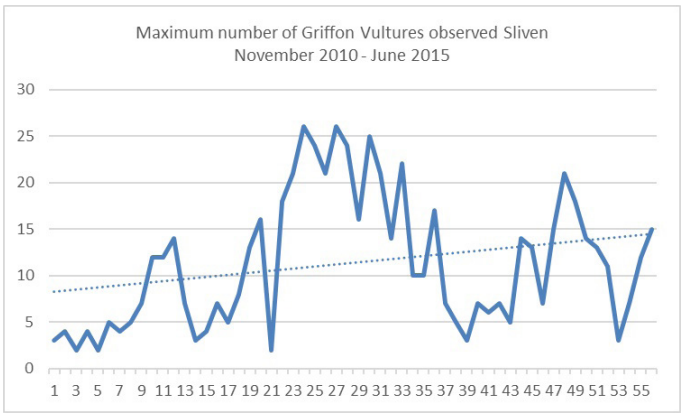


Fig. 3. Maximum number of Griffon Vultures observed at a single feeding event at the Sinite kamani supplementary feeding site (October 2010 - June 2015)

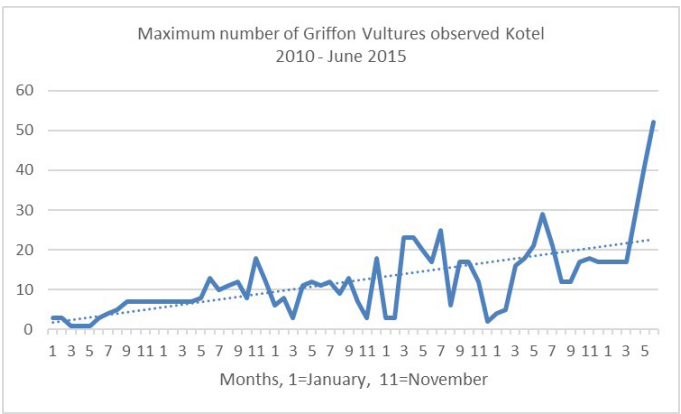


Fig. 4. Maximum number of Griffon Vultures observed at a single feeding event at the Kotlenska Planina supplementary feeding site (October 2010 - June 2015)

The first breeding attempt of Griffon Vultures released within the Balkan Mountains restoration programme was reported in May 2012, when a pair nested some 60 km to the East of the release site of Kotel – in the lower mountain area of Kamchiysika Planina. This may be attributed to the harsh winter conditions in the time of egg laying, because the birds were frequently observed at the feeding site in Kotel (flying over the 60 km distance every time). They returned to the release site in Kotel after the failure of the incubation that year. Next year the same pair laid on a cliff not far from the release site. The number of confirmed breeding pairs in the period 2012 – 2015 has developed as follows:

Table 3. Number of confirmed breeding pairs of Griffon Vultures in the Eastern Balkan Mountains (2012-2015)

Year	Number of confirmed pairs	Number of fledged young
2012	1	0
2013	1	0
2014	2	0
2015	3 (5)	0

Table 4. Detailed history of breeding pairs of Griffon Vultures in the Eastern Balkan Mountains (2012 - 2015)

Pair		Behavior observed	Period of the year	Notes
Male (year)	**Female (year)**			
7G Released 2009 Kotel	B18 Released 2009 Kotel	Display flights, nest located through transmitter	April – May 2012	Nest visited, eggshells found, reasons for failure unknown
7G Released 2009 Kotel	B18 Released 2009 Kotel	Copula, nest located	Nest abandoned end of March 2013	Nest location changed, new nest visited, eggshells found, reasons for failure unknown
7G Released 2009 Kotel	B18 Released 2009 Kotel	Copula, nest unknown	February - March 2014	Nest location changed
Tagged	Tagged	Display flights	February - March 2014	
7G Released 2009 Kotel	B18 Released 2009 Kotel	Copula, display flights	February - March 2015	Egg seen in nest, failure because of heavy snow in march 2015
K1U (born 2011) Released 2012 CB	K2N Released 2012 Sliven	K1U carrying grass, constructing a nest, copula	February - March 2015	Egg seen in nest, failure because of heavy snow in march 2015
K0H	K34	Flight displays+ Keeping territory, nest site selection	February - March 2015	Mating and nest building
K3W	K5M	Flight displays+ Keeping territory, nest site selection	February - March 2015	Mating and nest building
K0M	K4P	Flight displays+ Keeping territory, nest site selection	February - March 2015	Mating and nest building

Attracted other species.

The feeding sites and the presence of Griffon Vultures has attracted conspecifics, but also other species, that have been extinct from the area or have never been reported.

A young Eurasian Black Vulture (*Aegypius monachus*) has been attracted to the area of the Eastern Balkan Mountains and it has been documented to use the feeding sites in the area for a first time in the past thirty years.

The species was observed in three serial years as follows: first observation on the supplementary feeding site in Sliven in April 2013, photographed by the photo-trap set on site. Second observation of a single individual on September 25th 2014. The bird was roaming above the supplementary feeding site but feeding was not confirmed. Third observation of an individual in June 2015, the bird stayed for over a month in the area of Sliven – Kotel in a group with Griffon Vultures, using the same roosting sites.

In addition to that, the two feeding sites in the Eastern Balkan Mountains are often used by Egyptian Vultures (*Neophron percnopterus*). Two Egyptian Vultures – an immature and an adult individual were attracted to the area and stayed for nearly a month In July 2010. A juvenile Egyptian Vulture was observed at the feeding site of Kotlenska Planina in May 2012, while in 2014 three different Egyptian Vultures, one Imperial Eagle (*Aquila heliaca*) and one White-tailed Sea Eagle (*Haliaeetus albicilla*) were observed. In 2015 two or three different Egyptian vultures were observed for several times in the period May-July, once again in Kotel.

There are several well-documented visits of young Imperial Eagles (*Aquila heliaca*) to the feeding site in Sliven. A single bird has been observed every year between 2012-2015, most commonly in September – October. It is usually a second- or third year old bird, in several cases also with green rings.

A single juvenile White-tailed Eagle (*Haliaeetus albicilla*) has been reported at both release sites in the Eastern Balkan Mountains. Between June-August a single individual was seen at the feeding site of Sliven. A juvenile individual was also reported at the feeding site of Kotel on 02.12.2011, and 23.03.2013.

There are known breeding pairs of Golden Eagles (*Aquila chrysaetus*) in the areas of both the Sliven (2.5 km away) and the Kotel feeding sites (5 km away). The species can be seen at both sites all-year-round. The pair breeding near the Kotel feeding site, has been particularly aggressive towards the Griffon Vultures in the area and has managed to actively chase them off suitable roosting sites.

Black kites (*Milvus migrans*) are frequently observed at feed at the feeding site in Kotel.

CONCLUSION

The first breeding attempt of Griffon Vultures has been reported in the Eastern Balkan Mountains after over 50 years of absence. At 2015 a small breeding colony has been formed and since mostly juvenile birds have been released, it is expected that the number of nesting pairs will gradually increase with time. The Griffon Vultures released from the adaptation aviaries of Sinite kamani Nature Park (Sliven) and Kotlenska Planina (Kotel Mountain) have formed a common group and are visiting both sites and using common roosting sites, regardless of their original release site.

There is a well-documented movement of Griffon Vultures between the Eastern Balkan Mountains and the two natural colonies of Griffon Vultures in the area of Studen kladenets (UTM LG80) and Madjarovo (UTM MG01), Bulgaria and Dadia (UTM MF35), Greece in the Eastern Rhodopes. This movement is valid for both birds released within the Balkan restoration programme, which move to the South and then back North, as well as birds originating from the natural colonies visiting the release sites to their North. The latter movement is confirmed with the tagging and post-release tracking of non-marked juvenile Griffon Vultures, which have accidentally entered the adaptation aviaries. The Griffon Vultures released in the Eastern Balkan Mountains tend to join the natural colonies to the South especially during harsh winters, yet they do return to the release sites in spring. This movement matches the expected natural movement of Griffon Vultures in the early XX century, which followed the transhumance of sheep from their summer pastures in the Eastern Balkan Mountains to their winter grounds in the South Bulgaria and back. Birds released from the Eastern Balkans Mountains are also known to have settled in a number of other release sites: Vrachanski Balkan (an average of 7 individuals), Eastern Rhodopes (a minimum of 20 identified individuals). Griffon Vultures released from the two sites in the Eastern Balkan Mountains have been observed in the following countries: Bulgaria, Greece, Serbia, Macedonia, Turkey, Italy, Hungary, France, Poland, Jordan, thus interactions and connections with the wild populations have been successfully established.

At 2015 there is a fixed group of some 25-35 Griffon Vultures constantly present in the area of release. A minimum of 85 different Griffon Vultures were identified to have visited the feeding sites in 2015 and up to 37 Griffon Vultures have been seen at the roosting sites near Kotel in October 2015.

The feeding sites are also used by Black Vulture, Egyptian Vulture, Golden Eagle, Imperial Eagle, White-tailed Eagle, Black Kite, Raven etc.

During the preparation of this article, in the early 2016, 8-10 pairs of Griffon Vultures formed pairs in two distinct colonies in Kotlenska Planina. The field teams have confirmed the laying of 6 eggs and the hatching of three chicks (by 20 April 2016). This turns out to be the milestone for the return of the species in the area, proving that the conditions are good and the species has been successfully restored in the Eastern Balkan Mountains.

Acknowledgements: At the pre-release stage the project was supported by the Deutche Bundesstiftung Umwelt (DBU), Frankfurt Zoological Society (FZS), Black Vulture Conservation Foundation (BVCF), Rufford Small Grants (RSG) and others. Since 2010 the vulture restoration programme in the Balkan Mountains of Bulgaria has been supported by the LIFE+ financial instrument of EU within the project "Vultures Return in Bulgaria" LIFE08 NAT/BG/278, and is co-financed by the Deutche Bundesstiftung Umwelt (DBU) and the Frankfurt Zoological Society (FZS). Birds for release were kindly provided by BVCF, VCF, GREFA, LPO Grand Causes, Association Hegalida, Zoo de Doue, Parc Animalier de Sainte-Croix, Parc Zoologique et Botanique de Mulhouse, Allwetter Zoo, Burger's Zoo, Ostrava Zoo and the Green Balkans Wildlife Rescue Centre.

Special thanks to all experts and state institutions, who helped and assisted us throughout the project implementation, especially the Sinite kamani Nature Park Direcorate.

The actions will continue through the Vultures Back to LIFE14 NAT/BG/649 Project, coordinated by Green Balkans, with the participation of Fund for Wild Flora and Fauna, Vulture Conservation Foundation, Junta de Extremadura and EuroNatur.

Additional information: For more information of the project and the releases, please visit:
www.greenbalkans.org/birdsofprey/life
http://fwff.org/BG/griffon-vulture-reintroduction-in-kotel-mountain/
www.greenbalkans.org/vulturesback

REFERENCES

1. Choisy, J.-P., Tesseir, C., Henriquet. 1992. Reintroduction of the Griffon Vulture Gyps fulvus – Current state of the method in France. pp. 1-7
2. Demerdzhiev D., E. Stoynov, M. Kurtev, P. Yankov 2007. Griffon Vulture (Gyps fulvus). – In: Yankov, P. (ed.). Atlas of Breeding Birds in Bulgaria. Bulgarian Society for the Protection of Birds, Conservation Series, Book 10. Sofia, BSPB, p 134-135.
3. Donchev S., 1974, The Birds of Central and Eastern Balkan Mountains. Buletin De L'institut De Zoologie Et Musee, Vol. XLI, October 1974, Sofia.
4. IUCN/SSC. 1998. IUCN Guidelines for Re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN. Gland, Switzerland and Cambridge, UK, 10 p.
5. Kostadinova, I, M. Gramatikov (eds.) 2007. Important bird areas in Bulgaria and Natura 2000. Bulgarian Society for the Protection of Birds, Conservation Series, Book 11.Sofia, BSPB, 639 p.

6. Marin, S., A. Rogev, et al. (1998). New observations and nesting of the Black Vulture (Aegypius monachus. L. 1766) in Bulgaria. International symposium on the Black Vulture in South-Eastern Europe and adjacent regions, Daida, Greece, BVCF - FZS.
7. Patev, P. 1950. Ptitsite v Balgariya. BAN, Sofiya, 364 s.
8. Peshev H., Stoynov E., Grozdanov A., Vangelova N. 2015. Reintroduction of the Griffon Vulture Gyps fulvus in Kresna Gorge, Southwest Bulgaria 2010-2015. Conservation Sience Serries, Book 3. Fund for Wild Flora and Fauna, Blagoevgrad.
9. Sarrazin, F., S. Legendre 2000, Demographic Approach to Releasing Adults versus Young in Reintroductions. Conservation Biology, Volume 14.No 2, April 2000.
10. Simeonov, S., T. Michev, D. Nankinov. 1990. Fauna na Balgariya. Ptitsi. Chast I., Sofiya, BAN, 350 s. /in Bulgarian/.
11. Slotta-Bachmayr, R. Bögel, and A. Camiña. 2004. Status report and action plan: the Eurasian Griffon Vulture (*Gyps fulvus*) in Europe and the Mediterranean. East European/ Mediterranean Griffon Vulture Working Group (EGVWG), Zoo Salzburg, Salzburg, Germany. 100 pp.
12. Spiridonov, 1987. Oazisi na divata priroda. Sofiya, Zemizdat, 191 s.
13. Stoynov E., L. Bonchev. 2012. Reintroduction of Griffon Vulture *Gyps fulvus* in Kotel Mountain, Bulgaria, Overview 2007-2011, *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/BG/griffon-vulture-reintroduction-in-kotel-mountain/
14. Stoynov, E., M. Kurtev, et al. (2007). Cheren leshoyad. Atlas na gnezdeshtite ptitsi v Balgariya. P. Yankov. Sofiya, Balgarsko druzhestvo za zashtita na ptitsite. Prirodozashtitna poreditsa, Kniga 10: 136-137.
15. Terrasse M. 2006. Long-term Reintroduction Projects of Griffon Vulture Gyps fulvus and Black Vulture Aegypius monachus in France. In: Houston & Piper 2006. Proceedings of the International Conference on Conservation and Management of Vulture Populations. 14-16 November 2005, Thessaloniki, Greece. Natural History Museum of Crete & WWF Greece. pp. 98-107.
16. Terrasse M., J-P. Choisy. 2007. Reintroduction of the Griffon Vulture- technical guideline. LPO Mission Rapaces, PN des Cevennes, PN du Vercors.
17. Terrasse, M., C. Bagnolini, J.L. Pinna, F. Sarrazin 1994. Reintroduction of the Griffon Vulture Gyps fulvus in the Massif Central, France. Raptor Conservation Today, WWGBP/The Pica Press: 479-491.
18. Tewes, E., M. Terrasse, J. J. Sanchez Artés, W. Fremuth, H. Frey. 2004. Action Plan for the Recovery and Conservation of Vultures on the Balkan Peninsula: activities and projects during 2002 and 2003. Raptors Worldwide. WWGBP/MME.
19. Yankov, P., L. Profirov. 1991. Savremenno sastoyanie na populatsiyata na beloglaviya leshoyad (Gyps fulvus, Hablizl) v Balgariya. – Ekologiya, 24, 44-52.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 130-135

REINTRODUCTION OF THE GRIFFON VULTURE (*GYPS FULVUS*) IN KRESNA GORGE, SOUTH-WEST BULGARIA IN THE PERIOD 2010-2015

EMILIAN STOYNOV[1,2*], HRISTO PESHEV [2],
ATANAS GROZDANOV[1], NADYA VANGELOVA[2]

1 – Department of Zoology and anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
2 – Fund for Wild Flora and Fauna, 2700 Blagoevgrad, P.O. Box 78, 49 Ivan Michaylov Str, room 327, Bulgaria
**Corresponding author: pirin@fwff.org*

Keywords: birds, conservation, reintroduction, Kresna gorge, *Gyps fulvus*

Abstract: The following research presents the activities and results of the reintroduction of Griffon Vulture (*Gyps fulvus*) in Kresna gorge, Southwest Bulgaria. The preparations for this conservation initiative started in 2000, after the foundation of the non-government organization Fund for wild flora and fauna, dedicated to conservation of wildlife, nature habitats and sustainable land management. The first 26 Griffon Vultures for Kresna gorge were provided by GREFA foundation, Spain and arrived in the area on 18.02.2010. The main idea of the following activities was the re-establishment of a Griffon Vulture colony in Southwestern Bulgaria, to play the role of a „connecting bridge" between the species populations in the Eastern Rhodopes and Macedonia. As a part of the process, a feeding site for vultures was established in the area. Both the future new colony and the feeding site were planned to play an important role of safe ground for the vultures, migrating from the Central and Western Balkans (Croatia, Serbia and Macedonia) to the Rhodopi Mountains, Middle East, Africa and back. The reintroduction of Griffon Vultures was expected to support in natural way the return of Egyptian vultures (*Neophron percnopterus*), extinct from the area in the last decade, but also to provide basis for future reintroductions of Egyptian, Black and Bearded Vulture.

For the acclimatization of birds, an adaptation aviary was build in the vicinity of Rakitna village, municipality of Simitli. After some technical and methodological problems in the beginning, the following years showed success of the activities and the number of observed birds in the area progressively increased: 16 ind. observed at once in 2012, 28 in 2014 and 47 in 2015. In 2014 the presence of over 100 different birds was proven in the area, with the assistance of original method of photographic identification of the individuals.

During the 5 year period we observed marked individuals from Israel, Greece, Serbia, Croatia and birds from other reintroduction sites in Bulgaria. On the other hand, vultures released in Kresna gorge were identified in Serbia, Italy, Macedonia, Hungary and some parts of Bulgaria (Western and Eastern Balkan Mountains, Eastern Rhodopes).

In 2012 the reintroduction activities were reinforced through the "Life for Kresna gorge" project, supported by the LIFE financial instrument of EC and co-funded by private donors, such as Friends of Vienna Zoo, Austria and Zoo de Doue, France.
During the research period the vulture feeding site also attracted many other rare raptor species: Black vulture (*Aegipius monahus*), Golden Eagle (*Aquila chrysaetos*), Imperial Eagle (*Aquila heliaca*), Steppe Eagle (*Aquila nipalensis*), White-tailed Eahle (*Haliaeetus albicilla*), Greater Spotted Eagle (*Aquila clanga*), Red Kite (*Milvus milvus*) etc.

INTRODUCTION

The Kresna Gorge of Struma River is found in Southwestern Bulgaria, between Pirin Mountains to the East and Maleshevska Mountain to the West (UTM, FM73). It comprises rough terrain of silicate rocky habitats and degraded deciduous forests with Mediterranean climate influence. It is in close proximity to the Bulgarian border with Greece and FYR of Macedonia, where suitable vulture habitats and small populations are still present.

Griffon Vulture used to breed in the area until the end of 1950s, when it was extirpated by massive, long-lasting and well organized state campaign for the use of poisonous baits for eradication of terrestrial predators – mainly Wolf (*Canis lupus*).

On 18.02.2010 following several years of preparation, the first group of 26 Griffon Vultures arrived to Kresna Gorge with the support of the Spanish GREFA. This marked the start of the main activities of the species reintroduction in the area.

The current study presents the main results from the reintroduction activities carried out between 2010 - 2015 and includes a list of publications, issued during the period, where additional detailed information can be found.

Results and discussion

Two days after the arrival of the first group of vultures, a strong wind partly opened the cage and 15 birds escaped. Intensive food provision let about 10 of the birds remain close to the release site. Three days latter the first exogenous immature Griffon Vulture was recorded, which was later followed by permanent visits from migrating, wintering, vagrant and summering individuals native to the Balkans.

In June 2010 there was a poisoning accident, related to the use of a poisoned wild boar as food for the vultures at the feeding site, which killed two birds and one was observed to be sick, but later managed to recover. The other birds also fed there, but with no damage.

Until the end of the year we observed 8 Griffon Vultures which overwintered and 2-3 pairs formed. The nest of one pair was documented, where an egg was laid on 17.02.2011 and a chick hatched in mid-April 2011. The chick was observed in the nest until mid-June 2011, when it disappeared. The reason remained unknown.

In 2012 the project almost started from the beginning, as the first group of

birds dispersed and a second one was prepared for release. The same year the LIFE for Kresna Gorge project started and additional funding was provided for the reintroduction activities. The next release of 12 immature birds took place in July-August 2012 and they managed to form a stable nucleus. In the same year we observed the first Egyptian Vultures in the area. Probably the release of Griffon Vulture in combination with the maintenance of the feeding site are a good tool for attracting roaming Egyptian Vultures, which was also confirmed in Kotel Mountain, Bulgaria (Stoynov & Grozdanov 2010).

In 2013 a total of 15 birds were released (escaped) from the aviary in a storm accident. These Griffon Vultures had no problems, since they had already spent several months there for acclimatization. Thus the local group in Kresna Gorge reached more than 20 birds. Two Black Vultures, three Egyptian Vultures and more than 70 different Griffon Vultures were attracted in the area and identified and monitored thanks to newly developed visual marking method (Hristov & Stoynov 2002, Stoynov & Peshev 2014, Stoynov et.al. 2015). The method includes constant photographing of the vultures in flight and individual identification, based on individual plumage characteristics (scratches, missing feathers etc.). This visual practice seems to be a very important tool for small populations of vultures, where all individuals could be photographed and visually marked. This way maximum numbers, dynamics and individual presence could be monitored.

In 2012 and 2013 four vultures got electrocuted on 20 kV power lines, two of which close to the release site in Kresna gorge. We immediately initiated mounting of perch discouragers to eight most important pylons and the problem was solved.

In 2014 the Griffon Vulture presence in the area continued to increase with record numbers of simultaneously present individuals at the roosting site - 28 on 02 October 2014 and more than 70 exogenous individuals present for some time in different time of the year. Thus in total nearly 100 different Griffon Vultures were observed in Kresna Gorge in 2014 comprising birds released within the project, but also migrating, summering, wintering and vagrant vultures form other parts of the Balkan Peninsula. Marked birds from Israel, Greece, Serbia, Croatia and other parts of Bulgaria were observed again. Birds released in Kresna Gorge were observed in Serbia, Italy, Greece, and FYR of Macedonia, as well as other parts of Bulgaria (Vrachanski Balkan, Sinite Kamani, Central Balkan, Kotel, and Eastern Rodopi). This year the Griffon Vultures spent even more time in the National Parks of Rila and Pirin (at altitude above 2500 m) during the hot summer months, where they were recorded by the transmitters they carried, but also they were directly observed and photographed by tourists and park staff in the area of Vihren and Todorka peaks, Koncheto, Orlite and Spano Pole in Pirin National Park.

This year for second year in a row, Black Vultures (*Aegypius monachus*) were observed in the area of Kresna Gorge. Two different birds were photographed and their presence was well documented.

For the first time an Imperial Eagle (*Aquila heliaca*) was documented for more than 15 days at the feeding site in Kresna Gorge and the adjacent area.

No mortality cases of vultures were recorded in 2014 in Kresna Gorge, nor for birds released within the project.

Conservation measures for improving the habitat for vultures in Kresna Gorge are still underway – providing food for the vultures, anti-poison activities, compensation for farmers and prevention program against livestock depredation, eco-tourism promotion, insulation of dangerous power-lines, introduction of rare breeds of cattle, Fallow deer etc.

In 2015 the colony reached 22-25 birds including wintering Griffon Vultures from Serbia and Croatia and at the start of the breeding season a total of six pairs were formed, four of which built nests and two laid eggs. One of the pairs failed in incubation just ten days after the laying, while the other left the non-hatched egg 72 days after laying. An unsuccessful attempt again, but we hope that this is due to the fact that most of the birds in the pairs are still young – 4-5 years old.

CONCLUSION

For the period 2010-2015 the area of Kresna Gorge turned to be one of the most significant places for vultures in Bulgaria. It is now an important stepping stone for the migrating, wintering, summering and vagrant Griffon Vultures on the Balkans, as well as a safe refuge for non breeding Egyptian and Black Vultures. Such stepping stones are extremely important on the Balkans now, because of the recovery of Wolf population and the increasing conflict with the farmers which rises the threat of use of poisonous baits.

FWFF continues to work in the frame of the "Conservation of birds of prey in Kresna Gorge, Bulgaria" project supported by the LIFE financial instrument of EC and co-funded by private donors as Friends of Vienna Zoo, Austria and Bioparc Zoo de Doue, France.

List of the released publications for the Griffon Vulture reintroduction and the related results in Kresna gorge (2010 - 2015)

1. **Stoynov E., Peshev H.** 2011. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2010**,** *Fund for Wild Flora and Fauna, Blagoevgrad. http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/*.

2. **Stoynov E., Peshev H.** 2012. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2011**,** *Fund for Wild Flora and Fauna, Blagoevgrad. http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/*.

3. **Stoynov E., Peshev H.** 2013. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2012**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/.
4. **Stoynov E., Peshev H.** 2014. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2013**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/.
5. **Peshev H., Stoynov E.** 2015. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2014**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/.
6. **Stoynov E., Grozdanov A., Peshev D.** 2011 First breeding of Griffon Vulture (*Gyps fulvus*) during the reintroduction activities in Kresna gorge. Youth scientific conference "Kliment's Days", November 2011, Conference proceedings book 2: 104-106.
7. **Stoynov E., Peshev H., Grozdanov A., Delov V., Vangelova N., Peshev D.** 2015. New data for the presence and numbers of some conservation dependent birds in Kresna Gorge with proposal of original method for individual identification of vultures. Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte De Biologie 2015, volume, livre 4, pp. First National Conference of Biotechnology, Sofia 2014
8. **Stoynov E., Grozdanov A., Peshev H., Peshev D.** 2013. Present distribution and conservation specifics of the Egyptian vulture (*Neophron percnopterus* Linnaeus, 1758) in Southwest Bulgaria. Bulg. J. Agric. Sci., Supplement 2, 19: 259-261.
9. **Stoynov E., Peshev H., Grozdanov A.** 2014. Rare birds of prey observations in Kresna Gorge in Bulgaria. Vulture news, 66: 56 - 59.
10. **Stoynov E., Grozdanov A., Stanchev S., Peshev H., Vangelova N., Peshev D.** 2014. How to avoid depredation on livestock by wolf - theories and tests. Bulg. J. Agric. Sci., Supplement 1, 20: 129-134.
11. **Stoynov E., Peshev H., Grozdanov A., Vangelova N.** 2015. Five years overview of the reintroduction of Griffon Vulture Gyps fulvus in Kresna gorge, Bulgaria. Vulture News, 69: 33-39.

Since June 2012 the action is supported by the LIFE+ financial instrument of EU within the project "Conservation of Birds of Prey in Kresna Gorge" LIFE11 NAT /BG/363, and is co-financed by Friends of Vienna Zoo, Austria and Bioparc Zoo de Doue, France.

At the pre-release stage the project was financially supported by:

Deutche Bundesstiftung Umwelt (DBU), Frankfurt Zoological Society (FZS), Foundation Ensemble, Rufford Small Grants (RSG), Whitley Fund for Nature (WFN), Black Vulture Conservation Foundation (BVCF), EAZA Carnivore initiative; Zoo Sainte de la Croix, and others.

We are grateful to GREFA (Ernesto Xsusto and Manuel Crespo) and Spanish Government for the Griffon Vultures provided for release.

We are also grateful to all colleagues and nature lovers that provided information for observed Griffon Vultures: Sergey Panayotov; Stefan Kostov – Mayor of Rakitna; Blagoi Stefanov- Mayor of Brezhani; Hristo Milev- Mayor of Mechkul; Stefan Avramov- BBF; George Stoyanov - BPPS; Elena Kmetova- Green Balkans; Lachezar Bonchev – FWFF; Blagoi Simov- Pirin National Park authority; Theodora Skartsi – WWF Greece, Dadia Project; Marin Kurtev – BSPB; Rosen Aleksov – RIEW Blagoevgrad; Goran Susic – Caput Insulae, Beli, Croatia; Sasha Marinkovic and Irena Hrisbek – Birds of Prey Protection Fund, Serbia, Ohad Hatzofe – NPA, Israel and others.

REFERENCES

1. Hristov, H., Stoynov, E. 2002. National Action Plan for the Conservation of the Eurasian Black Vulture (Aegipius monachus) in Bulgaria 2002-2006. – In: Yankov, P. (ed.). Globally threatened species of birds in Bulgaria. National Action Plans for conservation, Part I. BSPB- MOEW, Conservation series, Book 4, BSPB, Sofia, pp. 106-132. (*in Bulgarian*)
2. Peshev, H., Stoynov, E. 2015. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2014**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/
3. Stoynov, E., Grozdanov, A. 2010. Re-introduction of Griffon Vultures and consequent return of Egyptian vultures in the Kotel Mountains in Bulgaria. In: Soorae, P.S. (ed.) 2010. GLOBAL RE-INTRODUCTION PERSPECTIVES: Additional case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE, pp. 147-150.
4. Stoynov, E., Peshev, H. 2011. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2010**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/
5. Stoynov, E., Peshev, H. 2012. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2011**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/
6. Stoynov, E., Peshev, H. 2013. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2012**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/
7. Stoynov, E., Peshev, H. 2014. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2013**,** *Fund for Wild Flora and Fauna, Blagoevgrad.* http://fwff.org/griffon-vulture-reintroduction-in-kresna-gorge/
8. Stoynov, E., Peshev, H., Grozdanov, A., Delov, V., Vangelova, N., Peshev, D. 2015. New data for the presence and numbers of some conservation dependent birds in Kresna Gorge with proposal of original method for individual identification of vultures. Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte De Biologie 2015, volume, livre 4, pp. First National Conference of Biotechnology, Sofia 2014.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 136-144

RECOVERING THE LESSER KESTREL (*FALCO NAUMANNI*) AS A BREEDER IN BULGARIA

GRADIMIR GRADEV[1*], SIMEON MARIN[1],
PAVLIN ZHELEV[1], JOSÉ ANTOLÍN[2]

1 - Green Balkans-Stara Zagora NGO, 9 Stara Planina Str., 6000 Stara Zagora, Bulgaria
2 - DEMA, Defensa y Estudio del Medio Ambiente, Ctra. Fuente del Maestre, km 17, 06200 Almendralejo, Spain
**Corresponding author: ggradev@greenbalkans.org*

Keywords: Lesser Kestrel, *Falco naumanni*, reinforcement, re-stocking, recovery, SPA, Natura 2000

Abstract: The Lesser Kestrel (*Falco naumanni*) is one of the rarest raptors in Bulgaria. Although once a common species, Lesser Kestrel has not been confirmed as a breeder in the country for the past few decades. Having implemented feasibility studies, Green Balkans launched the implementation of direct actions aimed at restoring the species as a breeder in Sakar SPA, a NATURA 2000 site in Bulgaria, through release of juveniles into the wild. With regard to this approach, based on a special methodology designed by DEMA called "Ambiente de Colonia" - a combination of the so called "hacking" and "foster parenting" methods, a Lesser Kestrel Release and Adaptation Module has been established. Lesser Kestrel chicks translocated from Spain are released into the wild. The juveniles are bred in captivity at DEMA's breeding center. A breeding stock has been established at Green Balkans' Wildlife Rehabilitation and Breeding Center (WRBC), consisting of wild birds, which have undergone rehabilitation at rescue centers in Spain and were ceded by the Government of Extremadura (Spain). This breeding stock is also providing offspring to be released.

A total of 286 juvenile Lesser Kestrels were released through the Module as follows – 90 individuals in 2013, 114 individuals in 2014, 82 individuals in 2015. As a result of these actions, the species has been restored as a breeder in Bulgaria. In 2014, there were 8 newly formed pairs, while in 2015 the number of breeding pairs was 9. The number of chicks that fledged in the colony in Levka village, Sakar SPA, was as follows – 15 individuals in 2014 and 17 individuals in 2015.

INTRODUCTION

The Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) is one of the nine representatives of the genus *Falco*, found in the territory of Bulgaria (Simeonov *et al*., 1990). This small, mainly insectivorous falcon inhabits open steppe

habitats, forming breeding colonies (Cramp & Simmons 1987). The species is distributed across the Palaearctic region, with populations breeding from the Iberian Peninsula to China (Negro 1997), as both European and Asian populations migrate to overwinter in Africa (Negro 1997, Rodríguez *et al.* 2009, Catry *et al*. 2011, Rodríguez *et al*. 2011).

By the mid-20th C, the Lesser Kestrel was considered to be one of the most common birds of prey in Europe (Bijleveld 1974). Like with other species using arable areas, the abundance of the Lesser Kestrel has decreased (Hagemeijer, Iankov, 1997). Probably due to the intensification of agriculture, in the past few decades the European population of the species has marked a severe decline (Tella *et al*, 1998; Birdlife International, 2004).

In Bulgaria, at the close of the 19th C the Lesser Kestrel was reported as "nesting everywhere" (Radakoff, 1879), and in the mid-20th C as "fairly common" and widely distributed (Patev, 1950, Arabadzhiev, 1962). This was probably followed by a considerable decline in the species' abundance and Michev (1982) reported a change in the status of the Lesser Kestrel in the period 1950-1982 – from "breeder" to "rare breeder". In 1985, the species was listed in Bulgaria's Red Data Book under the "threatened" category (Botev 1985) and later on described as "obscure" (Cramp and Simmons, 1987). In the period 1990-1995, the population was estimated at 10-100 pairs (Biber 1996); Iankov, *et al*. 1994 reported not more than 4 colonies. The abundance of the species was estimated at 0 to 5 breeding pairs in the period 1995-2000 (BirdLife International 2004, Barov 2002). In 2000-2010, there were no breeding birds reported (Iñigo, Barov 2011), i.e. no confirmed breeding of the species (Barov *et al*. 2007). Later, according to the updated edition of the Red Data Book of Bulgaria, the species was announced critically endangered (CR) (Barov *et al*. 2011).

According to the implemented feasibility study and the developed habitat model, although the neighboring countries (Greece and Turkey) still harbor breeding colonies of the species, natural re-colonization of the Lesser Kestrel in Bulgaria is deemed impossible (Kmetova, 2010, and Kmetova *et al*. 2012). Therefore, in 2013 Green Balkans launched the implementation of direct activities to recover the Lesser Kestrel as a breeder in Bulgaria, which, according to IUCN/SSC (2013), are considered reinforcement of the species.

MATERIALS AND METHODS

The recovery of the Lesser Kestrel in Bulgaria was done in line with the reintroduction guidelines of IUCN/SSC (2013).

A habitat model for the restoration of the Lesser Kestrel in Bulgaria (Kmetova, 2010) identified the area of the village of Levka, Sakar SPA (BG0002021), a NATURA 2000 site in Bulgaria, as one of the most suitable regions for the recovery of the species as a breeder.

Also, the Lesser Kestrel was identified as a target species subject to protection in Sakar SPA BG0002021 (MOEW, 2015).

The design of the Lesser Kestrel Release and Adaptation Module (LKRAM) established in Bulgaria was developed by DEMA, following the methodology *Ambiente de colonia* (Colony environment) (Antolín, 2001). This method is based on the combination of the so called "hacking" (Sherrod, 1987) and "foster parenting" (Jones, 1996) methods with some additions and improvements. However, the design was further enhanced when applied in Levka, Sakar SPA.

This method provides for accommodation of juvenile, non-fledged individuals in release boxes equipped with an opening enabling direct access to the outer area. The juveniles are free to leave the facility once they feel confident and ready. Outside the release boxes, the chicks walk along ledges to strengthen their confidence before their first attempts to fly.

Adult individuals, used as foster parents, are placed in a special aviary mounted in front of the release boxes. The chicks in the release boxes are separated from the adults with a mesh net, providing visibility of the landscape in the vicinity of the Module. Urged by their parental instinct, the adults feed the chicks through the mesh, which further strengthens the process of imprinting to the release site.

Artificial nest boxes are placed next to the release boxes and the aviary with the foster parents, which are then occupied by the birds released in previous years after their return from migration. The Module is equipped with windows enabling individual identification of the birds in the colony. In addition, a video monitoring and surveillance system is installed to provide a general picture of the situation in the different sections of the Module.

Four adult birds are placed in a suspended cage, attached by its back side to the wall of the building where the Module is established. The outer walls of this cage are covered with wire-net of 1.5 x 1.5 cm mesh. Thus, the birds in the cage have visibility of the entire vicinity and possibility to communicate with the birds flying in the area. The main purpose of the birds in the cage is to be used as foster parents of the chicks on one hand, and, on the other, to attract and strengthen the attachment of the released juveniles and other wild Lesser Kestrels to the area. However, two nest boxes have also been provided for these pairs used as decoys, to secure suitable nesting conditions, if needed, and shelter in case of bad weather or predator attacks.

The juvenile individuals are transported and placed in the Release and Adaptation Module at the age of about 20 days, each duly marked and banded.

Specialized food (mice, insects, and day old chicken chick) is provided on a daily basis both for the juveniles and the adult Lesser Kestrels. The food is served twice a day (morning and evening) through special pipes so that the birds have no direct visual contact with the keepers.

Once the juvenile Lesser Kestrels leave the release boxes, the food is provided on the roof of the aviary with the adult birds. The feeding time is a

suitable moment to monitor the abundance and presence of individuals, their behavior, relations, etc. Direct observation through a one-way window enables precise identification of individuals through their color rings. Also, through these observations the keepers can easily detect the presence of wild, non-banded birds from other colonies. In parallel, when needed, the team implements monitoring of individuals, pair formation, nest box occupancy, etc. from observation points outside the Module, using suitable equipment such as binoculars and spotting scopes. Such data is also gathered through the video surveillance system comprising 15 cameras and DV-R providing an important advantage, namely storing records that can be checked later.

Juvenile birds from Spain were released at the Module. Using MHC markers, it has been established that there is no genetic differentiation among western European birds (from Spain, France, Italy or Greece). However, these European birds differ genetically from those sampled in Israel and Kazahstan (Rodriguez *et al.* 2011). Therefore, the launch of the reinforcement of the Lesser Kestrel in the territory of SPA was absolutely possible and scientifically grounded.

The released birds were banded with metal ornithological rings, and the separate batches were backed up with the necessary CITES certificates.

The individual identification of birds was secured through a color-ring scheme (orange ring with a black three alpha code starting with the letter 'B', and orange ring with a black two symbol alpha-numeric code) coordinated with EURING.

Ring recording was done on a daily basis – 2 to 6 hours a day, from March to October, implemented by one or two members of the team, depending on the number of birds. Rings were read as follows: from inside, through the window, from a distance of up to 2 m, and/or from outside, through a 60x spotting scope, from a distance of not more than 50 m.

In accordance with Cheylan's (1981) classification, the definition of the breeding parameters used in this study was: (1) phenology or date of egg laying, which was assimilated with the beginning of incubation; (2) clutch size, the number of eggs in the entire clutch; (3) hatching success, the percentage of eggs that hatched in relation to the total number laid; (4) productive pairs, the number of pairs that laid eggs; (5) productivity, the number of chicks fledged in relation to the number of monitored territories; (6) breeding success, the number of chicks fledged in relation to the number of nests in which eggs were laid; (7) fledging rate, the number of chicks fledged compared to the number of nests with chicks.

RESULTS AND DISCUSSION

Provision of birds for the recovery of the species in Bulgaria:

Lesser Kestrel chicks translocated from Spain were released into the wild in Bulgaria. The juveniles were bred in captivity at DEMA's breeding center.

A breeding stock has been established at Green Balkans' Wildlife Rehabilitation and Breeding Center, consisting of 40 wild birds, which have undergone rehabilitation at rescue centers in Spain and were ceded by the government of Extremadura, Spain.

This breeding stock also provided offspring to be released. When needed, eggs and abandoned chicks from the colony in Levka were also hatched and reared at Green Balkans' Wildlife Rehabilitation and Breeding Center.

A total of 286 juvenile Lesser Kestrels were released through the Module as follows – 90 individuals in 2013, 114 individuals in 2014, 82 individuals in 2015 (see Table 1).

In addition, already in the first year, the Module was visited by two juvenile birds. In the following years, the number of birds (of different sex and age) from wild populations (non-banded) that visited the Module was bigger: 8 individuals in 2014 and 9 individuals in 2015.

Table 1. Origin of the 286 Lesser Kestrels released/fledged at the LKRAM in the village of Levka (2013-2015)

Source of chicks released/fledged at the LKRAM Levka:	**2013**	**2014**	**2015**
DEMA Breeding Center	90	60	45
Green Balkans WRBC	-	29	20
Levka breeding colony	-	16	17
Green Balkans WRBC - resqued eggs/chicks from the breeding colony in Levka	-	9	0
TOTAL	**90**	**114**	**82**

Breeding parameters

Following the release of individuals that took place in the first year (2013), a relatively stable group of 8 juveniles remained in the area of the Module. In the following years, the number of resident birds in the area of the colony continued to increase – 20 Lesser Kestrels in 2014 and 40 in 2015, most of them already mature individuals.

Based on the behavior and plumage (brood patches) of the Lesser Kestrels recorded at the LKRAM in Levka, the number of pairs breeding in the area of Levka was estimated at 8-9 (2014) and 9-13 (2015) respectively. Despite our efforts, we could not identify the location of all breeding pairs.

A particularly interesting observation was the fact that the breeding pairs in the two breeding seasons of the colony established in Levka included birds from the wild population. In 2014 this was an adult, more than 2 years old male, and in 2015 – an adult female individual.

Of all confirmed pairs in 2014, there were 8 newly formed pairs, while in 2015 the number of breeding pairs was 9. The number of chicks that fledged in the colony in Levka village, Sakar SPA, was as follows – 15 individuals in 2014 and 17 individuals in 2015.

Breeding parameters were calculated only for confirmed and controlled breeding pairs. The breeding parameters are presented in Table 2.

Table 2. Breeding parameters of the Lesser Kestrel colony at the LKRAM Levka, Bulgaria

Year	Released juveniles	Registered sexually matured	Resident in the home range	Established pairs	Confirmed pairs and laid eggs	Incubating pairs	Pairs with hatched chicks	Pairs with a reared chick
2013	90	0	8	0	0	0	0	0
2014	114	46	20	8 to 9	8	7	5	5
2015	82	59	40	9 to 13	9	9	5	5
Year	Eggs laid	Chicks hatched	Clutch size	Brood size	Success Rate	Productivity	Breeding Success	Fledging Success
2013	0	0	0	0	0	0	0	0
2014	37	16	4,6 (n=8)	4 (n=5)	0,62 (n=8)	2 (n=8)	2,28 (n=7)	3,2 (n=5)
2015	41	17	4,5 (n=9)	3,4 (n=5)	0,55 (n=9)	1,88 (n=9)	1,88 (n=9)	3,4 (n=5)

An year after the launch of the release of Lesser Kestrels in the village of Levka (2013), on June 23rd, 2014 a male individual in distress was received at Green Balkans' Wildlife Rehabilitation and Breeding Center. The bird was found in the territory of Lukoil Neftochim Burgas AD.

The immediate on-site inspection (Konstantin Popov, pers. comm.) established the presence of at least 2 pairs.

In late May 2015, in cooperation with Lukoil Neftochim Burgas AD, we estimated the population and confirmed at least 3 breeding pairs. The Lesser Kestrels nested on the technological facilities in the area of Lukoil Neftochim Burgas AD, breeding in former nests of Jackdaws (*Corvus monedula*), as the abundance of the latter amounted to more than 30-40 pairs. Some 50 pairs of Common Kestrels (*Falco tinnunculus*) were also breeding in that territory.

The identification of this breeding locality of Lesser Kestrel near the town of Burgas, situated on the Black Sea coast, at a distance of 117 km from Levka, which

coincided with the launch of the species restoration activities implemented there, gives us a reason to believe that there is a connection between these two events. Moreover, already in the year following the establishment of the LKRAM, the team reported a fourfold increase in the number of birds from the wild population recorded in the area of the colony in Levka. The existence of the colony in Levka resulted in an evident flow of wild individuals toward the territory of Bulgaria.

CONCLUSIONS

1. The methodology *Ambiente de colonia* was applied for the first time in Bulgaria, being further enhanced, adjusted, and adapted to the specific environment.
2. The applied methodology proved to be successful and can be used in other regions of Bulgaria.
3. The functioning of the LKRAM established in the village of Levka as a facility for release of juvenile Lesser Kestrels can continue in the future, in order to secure natural re-colonization of habitats suitable for the species.
4. The Lesser Kestrel breeding stock established at Green Balkans' WRBC can be considered a national source of juvenile individuals needed for other projects for the recovery and restoration of the species in Bulgaria.
5. As a result of the actions implemented, the species has been restored as a breeder in Bulgaria.
6. The LKRAM in Levka, Sakar SPA, attracts birds from the wild population to the interior of the country, thus contributing to the re-colonization of the species in Bulgaria.

Acknowledgements: These results were achieved through Lesser Kestrel Recovery, LIFE11 NAT/BG/360 project funded by the LIFE program of the European Union.

The Government of Extremadura (Spain) provided 40 specimens rescued from the wild populations or hatched in captive breeding centers for establishing a Bulgarian captive breeding stock and another 195 young birds from DEMA Breeding Center translocated to be released into the wild in Bulgaria, and secured the required CITES certificates.

Our thanks also go to Lukoil Neftochim Burgas AD for their assistance and the provided access to the company's territory for the implementation of surveys.

REFERENCES

1. Antolín, J. 2001. Liberación de cernícalos primilla (Falco naumanni) nacidos en cautividad, creando ambiente de colonia: Método DEMA. Pages 372–376 in J.F.G. Toledano and M.C. Matesanz [Eds.], Biología y conservación del cernícalo primilla: Actas del IV Congreso Nacional sobre el Cernícalo Primilla. Consejería de Medio Ambiente, Dirección General Promoción y Disciplina Ambiental/GREFA, Madrid, Spain.

2. Arabadzhiev, I., 1962, Raptors of Bulgaria, Science and Art Publishing House, Sofia, 25-26 (In Bulgarian).
3. Barov B. 2002. National Action Plan for the Conservation of the Lesser Kestrel (Falco naumanni) in Bulgaria, 2002-2006. – In: Petar Yankov (Ed.): Globally threatened bird species in Bulgaria. Action Plans.Sofia. BSPB – MOSV, 161-183. (In Bulgarian).
4. Barov B., Marin S. & Ivanov I. 2011 : Lesser kestrel Falco naumanni Fleischer 1 81 8, 83. In: Golemanski V(ed.), 2011. Red data book of Republic of Bulgaria, Bulgarian Academy of Science-MOEW, Sofia. 372.
5. Barov, B., P. Iankov, Ts. Petrov, S. Stoychev, E. Stoynov 2007. Lesser Kestrel Falco naumanni. - In: Iankov. P. (ed.) Atlas of the breeding birds in Bulgaria. 2007, BSPB, Conservation Series, Book 10, Sofia. (In Bulgarian).
6. Biber J.-P. 1996. International Action Plan for the lesser kestrel (Falco naumanni). In: Globally threatened birds in Europe: action plans (eds. B. Heredia, L. Rose & M Painter), pp. 191-203. Strasbourg. BirdLife International
7. Bijleveld M. 1974. Birds of Prey in Europe.London, UK: McMillan Press.
8. BirdLife International 2004. Falco naumanni Lesser Kestrel. In: Birds in Europe: Population Estimates, Trends and Conservation Status. BirdLife International, Cambridge, UK.
9. Botev B., Tz. Peshev (eds.). 1985. Red Data Book of the People's Republic of Bulgaria. Volume 2. Animals. Sofia (Publishing House of the Bulgarian Academy of Sciences). 183 p. (In Bulgarian).
10. Catry, I., Dias, M.P., Catry, T., Afanasyev, V., Fox, J., Franco, M.A. & Sutherland, W.J. 2011. Individual variation in migratory movements and winter behaviour of Iberian Lesser Kestrels Falco naumanni revealed by geolocators. Ibis 153: 154–164.
11. Cheylan, G. 1981. Sur le rôle déterminant de l´abondance des ressources dans le succès de reproduction de l´aigle de Bonelli Hieraaetus fasciatus en Provence. Rapaces Mediterranéens. PNRC et Annales du CROP 1: 95–99 (in French)
12. Cramp S. and Simmons K. E. L. 1987. The Birds of the Western Palearctic. Volume II. Hawks to Bustards.Oxford: Oxford University Press,
13. Hagemeijer W. J. M. and P. Iankov. 1997. Lesser Kestrel Falco naumanni. In: EBCC Atlas of European breeding birds: their distribution and abundance (eds. W. J. M. Hagemeijer & M. J. Blair). London, U.K.: T. and A.D. Poyser.
14. Iankov, P., T. Petrov, T. Michev & L. Profirov, 1994 Past and present Status of the Lesser Kestrel Falco naumanni in Bulgaria, Raptor Conservation Today, WWGBR/ The Pica Press, 133-137
15. Iñigo, A. & Barov, B. (2011). Action Plan for the lesser kestrel Falco naumanni in the European Union. Madrid: SEO BirdLife & BirdLife International for the European Commission.
16. IUCN/SSC (2013). Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57 pp.\
17. Jones C.G., Heck W. & Lewis R.E. (1996) The restoration of the Mauritius kestrel Falco punctatus population. Ibis, 137, S173-S180
18. Kmetova, E. 2010. Habitat model for the restoration of Lesser Kestrel (Falco naumanni) in Bulgaria. Master of Science thesis, Central European University, Budapest

19. Kmetova, E., P. Zhelev, A. Mechev, G. Gradev, I. Ivanov. 2012. Natural Colonies of Lesser Kestrel (Falco naumanni) in European Turkey and Discussion on the Chances of Natural Re-colonization of the Species in Bulgaria. Acta zool. bulg., Suppl. 4, 2012:47-54.
20. Michev, T. 1982, Status and conservation of diurnal birds of pray in Buldaria. National theoretical conference on protection and restoration of the environment. V 1, T1, 329 (In Bulgarian).
21. Negro, J.J. 1997. Falco naumanni Lesser Kestrel. Birds of Western Palearctic. Update 1: 49–56.
22. Patev P. 1950. Birds in Bulgaria.Sofia: Bulgarian Academy of Science Press (In Bulgarian).
23. Radakoff W. 1879. Ornitologische Bemerkungen uber Bessarabien, Moldau, Walachei, Bulgarien und Ostrumelien. Bull. Soc. Natur. 53: 150-178.
24. Rodriguez, A., Alcaide, M., Negro, J.J. & Pilard, P. 2011. Using major histocompatibility complex markers to assign the geographic origin of migratory birds: examples from the threatened Lesser Kestrel. Anim. Conserv. 14: 306–313.
25. Rodriguez, A., Negro, J.J., Bustamante, J., Fox, J.W. & Afanasyev, V. 2009. Geolocators map the wintering grounds of threatened Lesser Kestrels in Africa. Divers. Distrib. 15: 1010–1016.
26. Sakar SPA BG0002021. Information system of Natura 2000 sites in Bulgaria. Bulgarian Ministry of Environment and Waters, 2015. http://natura2000.moew.government.bg/Home/ProtectedSite/?code=BG0002021&layerId=3
27. Sherrod, S.K., W.R. Heinrich, W.A. Burnham, J.H. Barclay, and T.J. Cade. 1987. Hacking: a method for releasing Peregrine Falcons and other birds of prey. 3rd ed. The Peregrine Fund, Boise, Idaho, USA.
28. Simeonov S., T. Michev and N. Nankinov 1990. Fauna of Bulgaria. Aves I. Part I. Sofia: BAS, 206-208. (In Bulgarian).
29. Tella JL, Forero MG, Hiraldo F, Donázar JA. 1998. Conflicts between lesser kestrel conservation and European agricultural policies as identified by habitat use analyses. Conservation Biology, 12: 593-604.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 145-152

RADIO-TELEMETRY OF LESSER KESTREL *(FALCO NAUMANNI)* IN THE COURSE OF REINFORCEMENT OF THE SPECIES IN BULGARIA

PAVLIN ZHELEV[1*], GRADIMIR GRADEV[1], SIMEON MARIN[1]

1 - Green Balkans- Stara Zagora NGOs, 9 Stara Planina Str., 6000 Stara Zagora, Bulgaria
**Corresponding author: pzhelev@greenbalkans.org*

Keywords: Lesser Kestrel, *Falco naumanni*, Radiotelemetry, Foraging area, SPA, Natura 2000

Abstract: Despite the fact that Lesser Kestrel (*Falco naumanni*) was a common species in Bulgaria, there are no detailed surveys on its biology and ecology like the ones implemented in countries still harboring abundant populations, such as Spain, Italy, Greece, etc. After Green Balkans launched the reinforcement of the species in Bulgaria and the establishment of a new breeding colony in the village of Levka, Sakar SPA, it is now possible to study the species using modern technologies and methods. In order to identify the foraging grounds, dispersal areas, and roosting sites of the birds from the newly established colony, in the period 2014-2015 radio transmitters of 2.38 gr were mounted on 6 birds of different age and sex. Two of these birds were male individuals from pairs breeding in 2014, while the rest of the tagged birds were juvenile and non-breeding birds. The hunting grounds of the two breeding Lesser Kestrels were identified, covering areas of 29.70 and 46.80 sq.km. respectively, and almost overlapping in the field. The habitats these birds used to forage during the study period were cereal fields at harvest-time and the stubbles left after the crops have been harvested. The remotest recorded location of a tagged bird was at a distance of 7.08 km from the colony. Two roosting sites and pre-migration gathering areas of the birds from the colony were identified, located at 5.00 km (2014) and 4.30 km (2015) from their nesting sites.

INTRODUCTION

Although the Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) was a widespread species in Bulgaria (Patev, 1950, Arabadzhiev 1962), there are no detailed surveys on its biology and ecology like the ones implemented in countries still harboring abundant populations, such as Spain, Italy, Greece, etc. After Green Balkans launched the reinforcement of the species in Bulgaria and

its recovery as a breeder in the country (Gradev *et al*., in press), it is now possible to study the species using modern technologies and methods.

Similar Lesser Kestrel research has been implemented through various types of transmitters – radio transmitters in Greece (Vlachos *et al*, 2014), PTT satellite transmitters in Spain (Liminana *et al,* 2012), GiPSy-4 data-loggers (Gustin *et al*, 2014) in Italy, etc., as well as many other surveys in these and other countries. Since this is one of the smallest species of the genus Falco found in the territory of Bulgaria (Simeonov *et al*., 1990), migrating to Africa (Negro 1997, Rodríguez *et al*. 2009, Catry *et al.* 2011), the size of the tracking devices should not impede the birds' long distance migration nor affect their behavior. Therefore, the team launched the first tracking of Lesser Kestrels in the country tagging the birds with radio transmitters. This enabled the identification of their hunting grounds, roosting sites, and pre-migration aggregation areas.

MATERIALS AND METHODS

The survey was carried out in the area of the village of Levka, Sakar SPA (BG0002021), in a low-mountain and hilly region harboring alternating arable and non-arable open areas. The region is situated in southern Bulgaria, near the national borders with Turkey and Greece.

The survey involved birds from the newly established Lesser Kestrel colony. The establishment of the colony began in 2013 through the construction of a Lesser Kestrel Release and Adaptation module intended for juvenile individuals hatched in captivity (Gradev *et al*. in press).

In the period 2014-2015, 6 birds of different age and sex were tagged with radio transmitters, in order to identify their hunting grounds, dispersal areas, and roosting sites. Two of these birds were male individuals from pairs breeding in 2014, while the rest of the tagged birds were juvenile and non-breeding birds.

Detailed description of all tagged birds and the numbers of the transmitters is presented in *Table 1*. Once removed from the initially tagged birds, two of the transmitters (Radio 1 and Radio 4) were attached to other individuals.

Table 1. Detailed description of all tagged birds and the numbers of the transmitters

Individual PVC ring	Date of transmitter tagging	Number of transmitter / frequency MHz	Description of the bird tagged
BCH	12.6.2014	Radio 1 150.049	Sub-adult (1st calendar year) male individual released within the project in 2013, tagged when returned to the colony after overwintering for the first time.
BTD	13.6.2014	Radio 2 150.082	Adult male individual; a bird from the wild population (not released within the project), caught in the colony, banded with metal and PVC rings and tagged with a radio transmitter.
BJD	20.6.2014	Radio 3 150.133	Juvenile male individual tagged with a radio transmitter during its adaptation at the Release and Adaptation Module.
BKA	21.6.2014	Radio 4 150.159	Juvenile individual of unknown sex tagged during its adaptation at the Release and Adaptation Module.
BAD	13.7.2014	Radio 4 150.159	Sub-adult (1st calendar year) female individual released at the Release and Adaptation Module in 2013, tagged when returned to the colony after overwintering for the first time.
BLF	07.7.2015	Radio 1 150.049	Male sub-adult individual (1st calendar year). Released from the Release and Adaptation Module in 2014, tagged when returned to the colony after overwintering for the first time..

The birds were tagged with four 2.38 gr radio transmitters - PIP Ag393 Tag produced by Biotrack. The battery lifetime estimated by the manufacturer was 16 weeks. The devices belonged to the "backpack" type – i.e. attached to the back of the bird (Garcelón 1985). The transmitters were attached through a 4 mm Teflon ribbon with two straps crossed on the breastbone and stitched through the rear loop of the device with polyamide surgical suture. Each stitch knot was fixed with a small amount of super glue. Thus, the weight of the Teflon ribbons reached approximately 1 gram. The total weight of the transmitter along with the entire harness (Teflon ribbons, stitches, glue) reached some 3.4 gr, accounting for 2.8-2.4% of the bird's body weight (varying from 120 to 140 gr.). This was less

than the maximum possible 4% that does not affect the behavior of the migrating diurnal birds of prey (Sergio, 2015). To track to signal in the field, the team used ICOM radio receivers - models IC-R5 and IC-R6, and aligned TVP Y-4FL antennas of 150-152 MHz frequency range. While in the field, the bird tracking teams communicated through YAESU FT-60 radio stations and mobile phones. In order to locate the current position of the bird tracked, the signal direction had to be caught from two or three different positions at the same time, i.e. by two or three teams positioned on elevated places in the region. This simultaneous location of the signal is called bi-angulation or tri-angulation. To communicate to each other, the teams used radio communication and mobile phones. The position of each team was located through the geographic coordinate system WGS84 and target coordinates X and У. GARMIN Montana™ 650 GPS devices were used to determine the geographical location of the teams. The data gathered through the telemetry was filled in a specific field data form. Then, calculations were made based on the location of each team and the angle of the direction from which the signal was received, in order to locate the position of the bird. These calculations and the location of the bird's position were done through the specialized LOAS V 4 software.

The radio tracking of the individuals tagged with transmitters took place in the period from 12.06.2014 to 25.07.2014 and from 07.07.2015 to 22.07.2015. During these two periods, 3 separate teams spent a total of 26 days implementing field surveys.

The team implemented a total of 356 successful recordings of signal from the transmitters mounted on the tagged individuals, most of which sent by the devices attached to BCH, BTD, and BLF.

RESULTS AND DISCUSSION

This was the first Lesser Kestrel survey of this type implemented in Bulgaria with the smallest birds ever tagged with radio transmitters in the country. Such precise data about the species' hunting grounds and pre-migration aggregation sites has never been gathered before.

Two of these tagged birds were male individuals from pairs breeding in 2014, while the rest of the tagged birds were juvenile and non-breeding birds. The hunting grounds of the two breeding Lesser Kestrels were identified, covering areas of 46.80 (bird banded with ring BCH – Fig. 1) and 29.70 sq.km (bird banded with ring BTD - Fig. 2) respectively, and almost overlapping in the field.

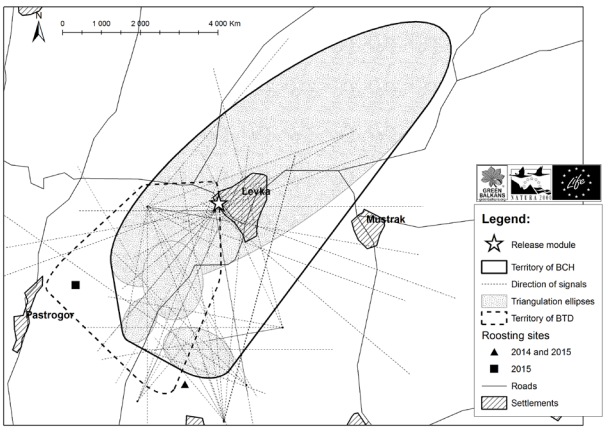


Fig. 1. Hunting ground of BCH with highlighted directions of the caught signal and calculated ellipses of possible triangulation deviation. Overlapping with the territory of BTD

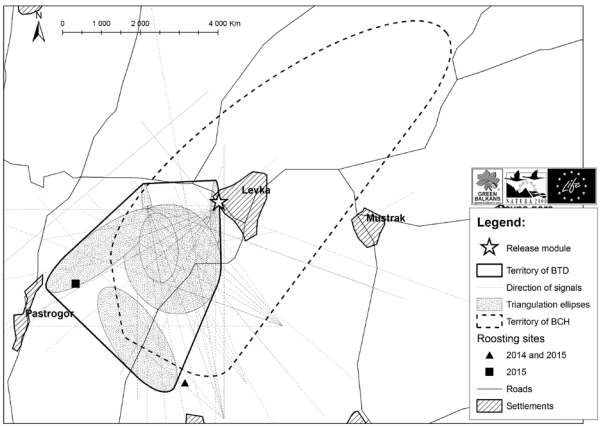


Fig. 2. Hunting ground of BTD with highlighted directions of the caught signal and calculated ellipses of possible triangulation deviation. Overlapping with the territory of BCH

The habitats these birds used to forage during the study period were cereal fields at harvest-time and the stubbles left after the crops have been harvested. The remotest recorded location of a tagged bird was at a distance of 7.08 km from the colony. According to surveys carried out in Italy, the mean distance males strayed from the colony was 6,209 km, while the maximum recorded distance was 18,702 km (Gustin *et al* 2014). Two roosting sites and pre-migration gathering areas of the birds from the colony were identified, located at 5.00 km (2014) and 4.30 km (2015) from their nesting sites.

Data gathered through the tracking of the bird banded with ring BLF was used to identify the location of the Lesser Kestrel pre-migration aggregation site in 2015. Yet, the information gathered through this individual was scarce; hence no other results were recorded. In these two years, the Lesser Kestrels recorded in the pre-migration gathering areas roosted on metal pylons of the high-voltage grid. These electric pylons were also used by Eurasian Hobby (*Falco subbuteo*) and Common Kestrel (*Falco tinnunculus*). Other rare birds of prey were also observed in these areas, including Imperial Eagle (*Aquila heliaca*), Long-legged Buzzard (*Buteo rufinus*), Eurasian Sparrowhawk (*Accipiter nisus*), Common Raven (*Corvus corax*), Common Buzzard (*Buteo buteo*), etc. The identification of the pre-migration aggregation areas enabled the implementation of regular monitoring and observation of other individuals from the colony not tagged with transmitters. Thus, the team recorded the latest dates when Lesser Kestrels were still present in the pre-migration aggregation area, which falls within the territory of the colony; namely, October 10th in 2014 and October 8th in 2015. During this monitoring, the team recorded Lesser Kestrels that did not belong to the newly established colony in Levka, but used the same pre-migration gathering sites. The highest number of such birds recorded at the roosting site was 7 individuals observed on September 1st, 2015. Since all birds released or hatched in the territory of the newly established colony were banded with standard ornithological and color PVC rings, the team could easily identify the different origin of the individuals. The presence of non-ringed birds showed that most probably these individuals originated from colonies in the nearby neighboring countries – Greece and/or Turkey, or even more distant colonies in FYROM.

Summary of the bird tagging results:

BCH: Following the end of the breeding season in 2014 when it was tagged and tracked, the bird left the area of the colony and the pre-migration aggregations. In the spring of 2015, having overwintered, this individual returned to the colony with the transmitter still on its back. The device was in good condition and when the bird was caught the transmitter was removed, equipped with a new battery, and then mounted on another individual in 2015. This proved that Lesser Kestrels could migrate successfully when tagged with this type of transmitters.

BTD: No sightings of this bird have been recorded in the area of the colony since it left the area of the colony and the pre-migration aggregations in 2014.

BJD and BKA: These two juveniles were tagged before fledging. The tracking of the individuals was unsuccessful. **BKA** fell victim to a Northern Goshawk (*Accipiter gentilis*) soon after its first flying attempts. Most probably the same thing happened to the other Lesser Kestrel.

BAD: Once tagged, the bird disappeared from the area of the colony. No data was gathered about this individual.

BLF: The only data gathered through this bird was related to the identification of the pre-migration aggregations and roosting sites in 2015.

The calculations of the hunting grounds of BCH show an area 17,1 sq.km bigger than that of BTD. Fig. 1. clearly illustrates that the territory of BCH includes an ellipse of possible deviation of huge area, shifted from the main concentration of locations of the bird. In our opinion, this ellipse is a result of deviation in the calculation of the signal angles and the real area of the hunting grounds of both birds is similar in size and location.

CONCLUSIONS

In this early stage of the recovery of the Lesser Kestrel as a breeder in Bulgaria, the implemented study proved to be successful, and the acquired information extremely useful for the further implementation of the project activities. The following conclusions can be drawn:

Several individuals should be tagged and tracked at a time to secure more efficient data gathering. Thus, the teams implementing radio telemetry in the field will be able to acquire information about more individuals at the same time;

Lesser Kestrels can successfully migrate tagged with this type of radio transmitters;

Tagging juvenile individuals under this method should be avoided.

In the implementation of such surveys, positions of uncertain quality should be excluded from the general calculations.

Acknowledgements: These results were achieved through Lesser Kestrel Recovery, LIFE11 NAT/BG/360 project funded by the LIFE program of the European Union

REFERENCES

1. Arabadzhiev, I., 1962, Raptors of Bulgaria, Science and Art Publishing House, Sofia, 25-26 (In Bulgarian)
2. Catry, I., Dias, M.P., Catry, T., Afanasyev, V., Fox, J., Franco, M.A. & Sutherland, W.J. 2011. Individual variation in migratory movements and winter behaviour of Iberian Lesser Kestrels *Falco naumanni* revealed by geolocators. Ibis 153: 154–164.
3. Garcelon, D.K. 1985. Mounting backpack telemetry packages on bald eagles. Institute for Wildlife Studies, Arcata, California. 2 pp.
4. Gradev, G., Marin, S., Zhelev, P., Antolín, J. in pres. Recovering the Lesser Kestrel (*Falco naumanni*) as a breeder in Bulgaria.
5. Gustin M., A. Ferrarini, G. Giglio, S. C. Pellegrino, A. Frassanito. 2014. First evidences of sexual divergences in flight behaviour and space use of Lesser Kestrel *Falco naumanni*. Environmental Skeptics and Critics, 2014, 3(1): 1-7
6. Liminana, R., Romero, M., Mellone, U., Urios, V. 2012. Mapping the migratory routes and wintering areas of Lesser Kestrels (*Falco naumanni*): new insights from satellite telemetry. Ibis. British Ornithologists' Union, 154, 389–399
7. Negro, J.J. 1997. *Falco naumanni* Lesser Kestrel. Birds of Western Palearctic. Update 1: 49–56.

8. Patev P. 1950. Birds in Bulgaria.Sofia: Bulgarian Academy of Science Press (In Bulgarian)
9. Rodriguez, A., Negro, J.J., Bustamante, J., Fox, J.W. & Afanasyev, V. 2009. Geolocators map the wintering grounds of threatened Lesser Kestrels in Africa. Divers. Distrib. 15: 1010–1016.
10. Sergio, F., Tavecchia, G., Tanferna, A., Jiménez, L., Blas, J., Stephanis, R., Marchant, T., Kumar, N., Fernando Hiraldo, F. 2015. No effect of satellite tagging on survival, recruitment, longevity, productivity and social dominance of a raptor, and the provisioning and condition of its offspring. Journal of applied Ecology
11. Vlachos, Ch., Bakaloudis, D., Kitikidou, K., Goutner, V., Bontzorlos, V., Papakosta, M., Chatzinikos, E. 2014. Home range and foraging habitat selection by breeding Lesser Kestrels (*Falco naumanni*) in Greece. Journal of Natural History.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 153-165

SUCCESSFUL REINFORCEMENT OF THE EUROPEAN SOUSLIK BY GREEN BALKANS NGO IN "SINITE KAMANI" NATURE PARK, BULGARIA

ELENA STOEVA[1], IVELIN IVANOV[1], ILIYAN STOEV[1], LYUBOMIR YANKOV[1], ALEXANDER MECHEV[1], YORDAN KOSHEV[2]

1 – "Green Balkans – Stara Zagora" NGO, 9 Stara Planina Str., 6000 Stara Zagora, Bulgaria, e-mail: officesz@greenbalkans.org
2 – Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria; e-mail: bgsouslik@gmail.com
**Corresponding author: etilova@greenbalkans.org*

Keywords: European Souslik, *Spermophilus citellus*, conservation, re-stocking, growing density

Abstract: The European Souslik is an important component of the diet of protected bird and mammal species. This is a threatened species whose population and distribution area have been shrinking in its entire range. Translocation is often used to restore the species' distribution and abundance. Unlike Central Europe, there is only scarce experience in the implementation of Souslik translocation activities in SE Europe and the Balkans.

The current work presents the results of the Souslik reinforcement program implemented by Green Balkans in Sinite Kamani Nature Park in the period 2010-2014. The process of reinforcement took part in the Karakyutyuk area within a total area of 43,71 ha. The Souslik colony in this area should therefore be considered critically endangered. The natural habitats of the area were restored, clearing off the shrubs and enhancing the vegetation cover. The pastures were maintained through extensive sheep grazing. Thus, through these direct reinforcement activities, a total of 292 individuals were released during the period 2010-2014. The animals were trapped in threatened or deteriorated habitats, outside the territory of the Nature Park. As a result of the successful activities, the colony expanded from 215 holes of Sousliks, only 65 of which active in 2010, to a total of 1120 holes (879 active) in 2014.

The future maintenance of the habitats would guarantee the sustainability of the Souslik populations in Sinite Kamani NP. The reinforcement method could also be applied in other suitable territories, being further enhanced to secure greater efficiency in the specific environment.

INTRODUCTION

The European Souslik (*Spermophilus citellus*) is found in poorly structured populations (often called "colonies" in Bulgaria) in open, uncultivated woodless habitats in Central Europe and the Balkans (Koshev, 2012*c*). Typical of this species is the annual cycle including a long hibernation period lasting for some 6-7 months.

In the second half of the 20th C, the European Souslik was reported to be a pest animal, which marked the beginning of a systematic campaign to combat this species (Koshev, 2008; Coroiu *et al*., 2008). This was also a period of intensification of agriculture and stock breeding. Thus, its abundance and range in Europe has suffered a severe decline. The European Souslik has gone extinct in Germany and Poland. Its abundance has drastically decreased in the Czech Republic, Slovakia, Moldova, Northern Greece, and Macedonia (Koshev – unpublished data; Coroiu *et al*., 2008). There is a clear range shrinking trend in Bulgaria (Koshev, 2009; Koshev, 2012*c*; Stefanov and Markova, 2009; Stefanov, 2015), as most probably the species has gone extinct in the southwest part of the country (Koshev, 2008).

Usually, the European Souslik (*Spermophilus citellus*) inhabits pastures (72%) and the major threats to the population in Bulgaria include pasture degradation, overbuilding, intensification of agriculture, interruption of biological corridors, and flooding (Koshev, 2008; Koshev, 2009).

The European Souslik is a major food resource for a number of endangered raptors and mammals such as Eastern Imperial Eagle (*Aquila heliaca*), Saker Falcon (*Falco cherrug*), Golden Eagle (*Aquila chrysaetos*), Steppe Polecat (*Mustela eversmanni*), Marbled Polecat (*Vormela peregusna*), etc.

For all these reasons, the European Souslik is declared a protected species, being listed in: IUCN Red List as "vulnerable"; Convention on the Conservation of European Wildlife and Natural Habitat (Bern Convention) - Appendix II – Strictly protected fauna species; Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora – Annex 2 and 4. The species is also listed in Annex 2 to the Biological Diversity Act of 2002 and the Red Data Book of Bulgaria in the "vulnerable" category (Stefanov, 2015).

The territory of Sinite Kamani NP is partially or entirely overlapping with Kamenets-Grebenets SPA (BG0002058) and Sinite Kamani SCI (BG0000164). The species is determined as being of unfavorable conservation status in Sinite Kamani SCI (BG0000164) (Koshev, 2013), which calls for the implementation of urgent conservation measures.

In the territory of Sinite Kamani NP, the Souslik went extinct in the late 1980s and early 1990s. This extinction was mostly due to the abandonment of extensive farming, hence the disappearance of the grass habitats suitable for the species. At the same time, the Souslik is relatively abundant in the flat vicinities of the park,

where the species inhabits mainly abandoned arable areas. Within these habitats the species is particularly vulnerable because of the risk of plowing, which would destroy the Souslik colonies.

The various methods of translocation (introduction, reintroduction, re-stocking, reinforcement, etc.) of Sousliks and Prairie Dogs are often used to preserve such rodent species in Europe and North America (Dullum *et al*., 2006; Balaz *et al*., 2008; Matějů *et al*., 2010; Matějů *et al*., 2012; Tokaji *et al*., 2012; Brenner and Millesi, 2014; Próchnicki, 2012; Lobbova and Hapl, 2014), as these practices have been further enhanced (Gedeon *et al*., 2011*a,b*).

Several Souslik reintroduction projects have been taking place in Bulgaria, being implemented in the Kotel Mountain and "Vitosha", "Balgarka", and "Vrachansky Balkan" Nature Parks (Koshev, Arangelov – unpublished data).

In 2010, a program for the restoration of the European Souslik was launched in the territory of Sinite Kamani Nature Park, aimed at recovering the species as a valuable representative of the Park's fauna (also providing food resources for the rare birds of prey inhabiting the Park), and, at the same time, preventing the mortality of individuals inhabiting "habitats at risk", situated beyond the boundaries of the nature park.

MATERIALS AND METHODS

Brief description of the physico-geographical features of Sinite Kamani Nature Park

Sinite Kamani Nature Park, covering an area of 11 380 ha, is situated in the vicinities of the town of Sliven, falling within the territory of the Eastern Balkan Mountain. The Park's altitude varies from 300 to 1181 m a.s.l. (Balgarka Mount). The weather typical of the area is temperate continental climate with prevailing transfer of air masses from the Atlantic Ocean and frequent distant continental and Mediterranean incursions, also influenced by the Black Sea. The estimated absolute temperatures range from +41° C to -21° C. The mean maximum was recorded in July (15,1 ° C), while the mean minumum was in January (-2,9 ° C). The earliest snow cover date is around November 2nd, while the latest date when snow cover disappears is in early March. The average precipitation is about 600 mm. The maximum precipitation is in May, and the minimum - in August. The area is characterized with NW and N winds. The local wind "Bora", also known as "the Sliven wind", is typical of this region. In the summer, the local mountain-plain wind ("night breeze") blows at the foothills of the mountain and along the river valleys, changing its direction – oriented toward the mountain slopes during the daytime, and toward the valley at night.

The northern slopes of the Park are covered with vast Beech forests, while the southern ones are furrowed by ravines and gorges. The vegetation in this part of the park is scarce, mainly represented by single trees - Sessile oak, Beech, etc (Anonymous, 2003).

Reinforcement of the European Souslik in the Karakyutyuk area, Sinite Kamani Nature Park

The activities envisaged within the program were implemented for a period of 5 years - from 2010 to 2014, as the individuals were captured in the vicinities of Sliven (at about 200 m a.s.l.) and released in three target habitats in Sinite Kamani Nature Park.

This paper presents the reinforcement in the Karakyutyuk site (N 42° 44' 15,11''; E0 26° 18' 17,53'', about 930 m a.s.l.) implemented in a total area of 43,71 ha. The reinforcement of the European Souslik was carried out in line with the regulations of IUCN/SSC (2013) and the guidelines by Hapl *et al*. (2006).

The preliminary genetic and cytogenetic analyses show that the source and release sites belong to the same genetic line (Říčanová *et al*., 2013; Chassovnikarova *et al*., 2015)

Selection of suitable catching localities

The selection of suitable catching localities was based on the identification of suitable donor colonies to be used as a source of individuals. The team surveyed the Souslik colonies within a perimeter of about 30 km to the south of Sliven – in the area of the villages of Zlati Voyvoda, Sotirya, Krushare, Samuilovo, Topolchane, Kamen, Gergevets, and Rechitsa district. Preference was given to areas harboring huge Souslik densities, as well as areas "at risk" – e.g. situated along busy roads (with confirmed high mortality of Sousliks); abandoned uncultivated areas facing the risk of being ploughed up or deteriorated in terms of habitat quality.

The relative population abundance of donor colonies was identified through the linear transect method (Koshev, 2012*a, b*), recording active Souslik holes. Following the extrapolation of the data over the entire area of the colonies, their approximate density and the number of individuals that could be caught without causing any negative effect on the population were estimated.

After the initial selection, a final survey was carried out on May 2nd-3rd, 2010. Based on this survey, the following donor Souslik colonies were identified:

а) Pastures situated in the vicinities of Rechitsa district (town of Sliven);

б) A golf-course near the pastures of Rechitsa district. Being abandoned in 2013, the golf-course is getting overgrown with shrub vegetation. Hence, the Souslik density in this habitat is decreasing, which calls for the implementation of urgent measures to preserve the species.

в) Pastures near the village of Topolchane (used in 2014). These pastures harbor some of the highest densities of Sousliks in the country; yet, they are situated near a main road with heavy traffic causing high mortality of Sousliks. Souslik catching took place in the areas along this busy road (Fig. 1. *c*).

Capturing, measuring, marking, transportation, and release of individuals

Two types of live traps were used: live traps for rats with a bait (e.g. an apple) and live trap "Donsky type" (Fig. 1. *d, h*). The capturing of individuals was done in a way to secure heterogeneity of the group in terms of age and sex, in order to achieve successful and sustainable survivorship of the Sousliks in the new territories. Juveniles, underweight individuals, as well as animals with external parasites and skin disorders were released back into their colonies.

Field data forms were filled in for every individual, including date, time, donor colony, release site, weather conditions, and somatometric indicators, such as body measurements, weight, sex, and estimated age in four classes. The age classes were split in four major groups: Juv. – juveniles, Ad. – adults, and Subadults, as the latter included Juv./1st year and Ad./1st year individuals. The Sousliks were marked with standard *Felixcan* microchip transporders. The temporary marking was done by dying the fur with a hair colorant – females on their backs and males on their heads and necks. Thus, the released Sousliks could be distinguished from the individuals in the colony.

Captured individuals were placed in transportation boxes (Fig. 1. *h*) and transported to the release sites on the day they were caught to minimize any impact and stress. All related activities and manipulations were performed in the presence of a veterinarian, observing the antiseptics and disinfection rules and requirements.

Preparing the reinforcement locality

The selected release area – the Karakyutyuk site, harbors a small Souslik population of almost critically low abundance. In order to restore grass habitats suitable for Sousliks, the shrub vegetation in the selected reinforcement locality was cleared off through trimmers and brush cutters (Fig. 1. *a, b*). The excessive vegetation was removed outside the restored territory. Thus, the shrub and tree overgrowth of more than 60% coverage was reduced to less than 5% with only single shrubs left. No plants of conservation significance and shrubs and young trees with bird nests were removed during the implementation of this activity. The cleared territories are managed through extensive sheep grazing and mechanical maintenance (Fig. 1. *g, f*). Seeds of leguminous plants (mostly clover) were spread to improve the nutritional value of the habitat.

Artificial 80 cm deep holes were dug at an angle of 45° in the release sites through a motor drill (Fig. 1. *a*). These holes provide shelter for the Sousliks, thus reducing stress levels and possible loss of individuals caused by predator attacks. After a certain period of time, Sousliks turn these holes into real burrows.

Piles of seeds were left at certain places near these holes, in order to provide food for Sousliks in the early days of their adaptation period.

The individuals were released at dusk, on the same day they were caught, in small groups of about 5-6 individuals, placing an individual in each preliminary

dug hole. The entrance to the hole was closed with a big tuft of grass to prevent the escape of animals during the night hours. The next morning, the entrance of each hole was re-opened and the Sousliks were put to constant observation.

To survey the presence of potential predators, camera traps were set up in key locations, recording feral dogs, foxes, etc. Supplementary feeding sites were established to divert the predators' interest from the Sousliks.

Guards were provided in the reinforcement area to monitor the behavior of the individuals, chase predators away, etc. After a certain period of time, monitoring was implemented once a week.

The results of the reinforcement were evaluated by counting active (inhabited) and inactive Souslik holes, which can be used as an indirect indicator of the relative abundance of Sousliks (Koshev, 2012*a, b*).

RESULTS AND DISCUSSION

At the launch of this activity in the spring of 2009 and 2010, the relative population abundance was estimated based on the number of Souslik holes in the Karakyutyuk site. In 2010, some 215 holes were recorded as many of them were inactive. Observations from the previous year (2009) showed that only 30 to 40 holes were active during the last season. Not more than 3-4 individuals used to be recorded in the territory during binocular observations.

There has been no survey on the number of holes used by a single individual. There are cases when several individuals share the same burrow to hibernate. According to a survey carried out by Straka (1961), the average number of holes used by a single individual is 13. Assuming these figures reliable, it could be concluded that the Souslik colony in the Karakyutyuk site consists of only 10 to 20 individuals and is therefore critically endangered.

Fig. 1. Preparing the reinforcement locality, capture and release of individuals. *Further details are provided in the text.*

Fig. 1. Preparing the reinforcement locality, capture and release of individuals. *Further details are provided in the text.*

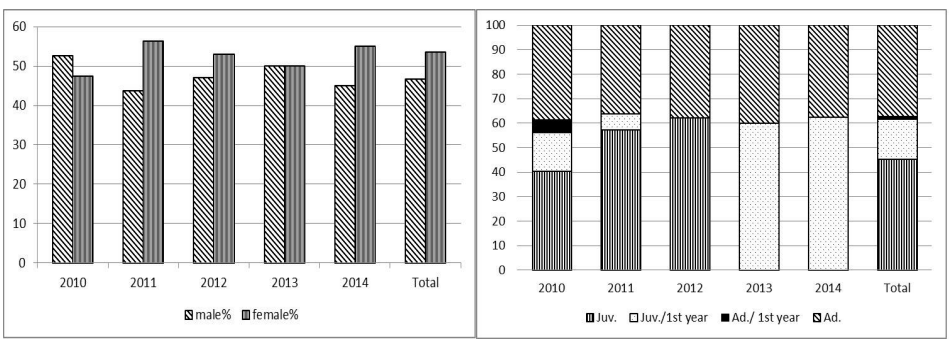
Demographic structure of released individuals during the five years of reinforcement

During the five-year period of the program, a total of 292 individuals were caught and selected for reinforcement. Most of the Sousliks were captured in the first three years - 242 individuals. After the initially recorded success of the reinforcement, the release of individuals continued in the following years (Table 1).

Table 1. Description of the Sousliks released during the five years of reinforcement in the Karakyutyuk site, Sinite Kamani Nature Park. *Further details are provided in the text.*

Year	Periods of releasing	Male	Female	Juv.	Juv./ 1st year	Ad./1st year	Ad.	Total
2010	7 – 22.07.	30	27	23	9	3	22	57
2011	30.06. - 23.08.	52	67	68	8		43	119
2012	25.06.-25.07.	31	35	41	-	-	25	66
2013	19.08.	5	5	-	6	-	4	10
2014	28.6.- 22.7.	18	22	-	25	-	15	40
Total	**28.6-23.08**	**136**	**156**	**132**	**48**	**3**	**109**	**292**

The estimation of the sex index for the entire period shows that females prevailed, accounting for 53,4% of the individuals, compared to males - 46,6%. Male prevalence was recorded only in the first year, while in 2013 both sexes were equally represented (Fig. 2. a).



a **b**

Fig. 2. Sex (*a*) and age (*b*) ratio (in %) of the Sousliks released in the Karakyutyuk site (Sinite Kamani Nature Park)

Most of the released individuals were juveniles (45,2%) and adults (37,3%). The other group - Subadults (Ad./juv/1st year), accounted for only 17,4% of the total number of Sousliks (Fig. 2. *b*).

The implemented activities resulted in a considerable increase in the relative population density of the Souslik colony in the Karakyutyuk site (Fig. 3).

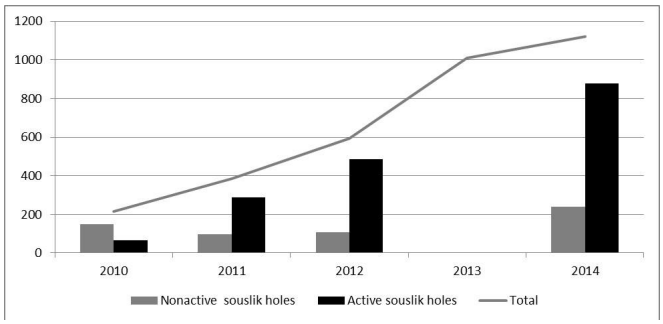


Fig. 3. Number of Souslik holes (active and inactive) in the Karakyutyuk site during the five-year reinforcement period (2010-2014)

Prior to the European Souslik reinforcement activity, in 2010 a total of 215 Souslik holes (65 active and 150 inactive) were recorded in the Karakyutyuk site. In 2011, as a result of the implemented activities, the number of holes was 385 (288 active and 97 inactive), in 2012 - 594 holes (486 active and 108 inactive), in 2013 - 1008 holes, and in 2014 - 1120 Souslik holes in total (879 active and 241 inactive).

In 2014, the number of Souslik holes increased by some 520% compared to 2010. With the active holes, providing a more precise picture of the relevant population abundance of the species, there was an impressive increase by 1352% compared to the numbers recorded in 2010 prior to the release of the first individuals.

The Souslik population in the region has considerably increased its area, occupying successfully more territories (Fig. 4).

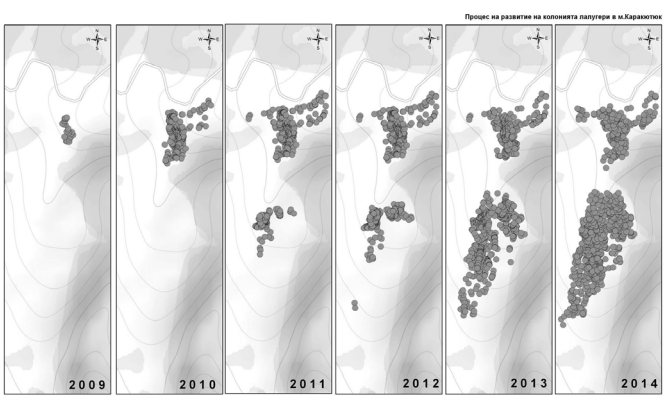


Fig. 4. Spatial growth of the Souslik colony in the Karakyutyuk site (Sinite Kamani Nature Park) achieved through the release of individuals

Reintroduction biology is a rapidly growing but relatively young discipline (Tokaji *et al*., 2012). Translocating European Sousliks has become a popular conservation tool (Gedeon et al., 2011*b*), as in the past 35 years significant experience in European Souslik reintroduction was acquired in Central Europe. Thus, for example, during the European Souslik reintroduction projects carried out in the Czech Republic, Slovakia and Poland since 1989, more than 3,200 European Sousliks were reintroduced at 15 sites or used for reinforcement of 5 populations. Reintroductions can be considered successful at 7 sites, where settlement and reproduction of the released individuals were observed. At other 7 sites reintroductions failed and the result of reintroduction is still unknown at one. Results of reinforcements are unclear at all 5 sites (Matějů *et al*., 2010; Matějů *et al*., 2012).

The first trial to capture, transport and release a number of individuals of European Sousliks (*Spermophilus citellus*) in Hungary was carried out in the '80s. According of Tokaji *et al*. (2012) since that time, more than 250 translocation actions have been organized with different aims. During these decades methods have been developed in many ways from simple impressions to scientific experiments (Tokaji *et al*., 2012).

At the same time, many reintroduction programs did not work successfully in Central Europe. Lobbová *et al*., (2012) show examples of dissimilarity in reintroductions implemented at present and in the past. Unlike Central Europe, there is no experience in the implementation of Souslik translocation projects in Southeast Europe and the Balkans. In Bulgaria, several projects have been implemented, translocating European Sousliks in "Vitosha", "Bulgarka", and "Vratchansky Balkan" Nature Parks, as the results of the translocations are still being processed (Koshev, Arangelov – unpublished data). The successfully implemented reinforcement program can be the basis for the realization of similar activities aimed at preserving this species of conservation significance.

CONCLUSIONS

In the Southeastern part of the species' range there is not enough methodical experience in successful translocation of Sousliks. This article presents the results of successful reinforcement of European Sousliks in the Karakyutyuk site, Sinite Kamani Nature Park, with the following major conclusions:

1. The materials and methods used during the implementation of the activities were in conformity with the international and European standards applied in the translocation of Sousliks.
2. Within a five-year period (2010-2014), a total of 292 individuals were captured, measured, and released. The individuals were caught in areas at risk and released within the boundaries of a protected area. The gender index for the entire period was in favor of the female Sousliks. Most of the released animals were juveniles and adults.

3. The Souslik colony expanded and the number of holes in general, including that of the active ones (occupied by Sousliks), increased from 65 in 2010 to 879 in 2014.

Therefore, the reinforcement activity carried out in the territory of the Park can be considered successful. The method can also be applied in other suitable areas being further enhanced to secure greater effectiveness in the specific environment.

Acknowledgments: The Souslik reinforcement programme was implemented within two main projects funded by Sinite Kamani Nature Park Directorate: "Reinforcement of the populations of Souslik (*Spermophilus citellus*) as a key element in maintaining the favorable conservation status of grasslands and populations of birds of prey in Sinite Kamani Nature Park" and public tender "Measures for restoration and maintenance of biotic components in the territory of Sinite Kamani Nature Park, Sliven" – Action 1.4. Supporting activities for the reinforcement of the populations of European Souslik (*Spermophilus citellus*) in the areas of Karakyutyuk and Lokvata.

REFERENCES

1. Anonymous. 2003. Management plan of Sinite Kamani Nature Park. Ministry of Agriculture and Forestry of Republic Bulgaria, Agrolesproject Ltd., Sofia, Bulgaria, 350pp.
2. Balaz, I., Jancova, A., Ambros, M. 2008. Restitution of the European Ground Squirrel (*Spermophilus citellus*) in Slovakia. *Lynx*, n. s., 39: 235–240.
3. Brenner, M., Millesi, E. 2014. Reintroducing European ground squirrels: Stress coping in a soft release enclosure. In: Millesi, E. and Hoffmann, I. E. (Eds.). Abstracts from 5th European Ground Squirrel Meeting: Perspectives on an endangered species, 02-05 October 2014, Rust, Burgenland, Austria, pp. 37.
4. Chassovnikarova, Ts., Rovatsos, M., Atanasov, N., Koshev, Y. 2015. Sex chromosome variability of *Spermophilus citellus* (Linnaeus, 1766) in the Southeastern part of the Balkan Peninsula. *Mammalian Biology*, 80 (4): 365-371.
5. Coroiu, C., Kryštufek, B., Vohralík, V., Zagorodnyuk, I. 2008. *Spermophilus citellus*. The IUCN Red List of Threatened Species 2008: e.T20472A9204055. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T20472A9204055.en. Downloaded on 28 November 2015.
6. Dullum, J. L. D., Foresman, K. R.,Matchett, M. R. 2006. Efficacy of Translocations for Restoring Populations of Black-Tailed Prairie Dogs" (2005). *US Fish & Wildlife Publications*. Paper 30. http://digitalcommons.unl.edu/usfwspubs/30
7. Gedeon, C. I., Váczi, O., Koósz, B., Altbäcker, V. 2011*a*. Morning release into artificial burrows with retention caps facilitates success of European ground squirrel (*Spermophilus citellus*) translocations. *European Journal of Wildlife Research*, 57 (5): 1101-1105.
8. Gedeon, C. I., Boross, G., Nemeth, A., Altbäcker, V. 2011*b*. Release site manipulation to favour European ground squirrel *Spermophilus citellus* translocations: translocation and habitat manipulation. *Wildlife Biology*, 17: 97-104.
9. Hapl, E., Ambros, M., Olekšák, M., Adamec, M. 2006. Suslik (*Spermophilus citellus*) reintroduction in Slovakia. Guidelines. State Nature Conservancy of the Slovak Republic, Banská Bystrica, 28 pp.

10. IUCN/SSC. 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, 57 pp.
11. Koshev, Y. 2008. Distribution and status of European ground squirrel (*Spermophilus citellus*) in Bulgaria. *Lynx* (Praha), n.s., 39(2): 251–261.
12. Koshev, Y. 2009. Distribution, isolation and recent status of European ground squirrel (*Spermophilus citellus* L.) in Pazardzhik district, Bulgaria. *Annual of Shumen University "Konstantin Preslavsky", Faculty of Natural Sciences*, Vol. XIX B6: 97–109.
13. Koshev, Y. 2012*a*. Methods for determining conservation status of European ground squirrel (*Spermophilus citellus*). Project: "Mapping and determining conservation status of mammals in NATURA 2000 network in Bulgaria 2011-2013". Founded by MOEW-Bulgaria and Operational Programme Environment 2007 – 2013. 24pp.
14. Koshev, Y. 2012*b*. Methods for mapping of European ground squirrel (*Spermophilus citellus*). Project "Mapping and determining conservation status of mammals in NATURA 2000 network in Bulgaria 2011-2013". Founded by MOEW-Bulgaria and Operational Programme Environment 2007 – 2013. 8pp.
15. Koshev, Y. 2012*c*.Ecological and ethological characterization of European ground squirrel (*Spermophilus citellus* L.) in model colonies in Bulgaria. PhD thesis summary, IBER-BAS, Sofia, 30pp.
16. Koshev, Y. 2013. Distribution and determining conservation status of European ground squirrel (*Spermophilus citellus*) in Nature 2000 site BG0000164 „Sinite kamani". Project "Mapping and determining conservation status of mammals in NATURA 2000 network in Bulgaria 2011-2013". Founded by MOEW-Bulgaria and Operational Programme Environment 2007 – 2013.
17. Lobbová, D., Hapl, E., Ambros, M. 2012. Are there any efficient methods of ground-squirrel reintroduction programs? Experiences from field work in Slovakia. In: Kepel, A., Konczak, J. (Eds.). IV European ground squirrel meeting. Programme, Abstracts, Participants. 5-7 September 2012, Kamien Slaski, Poland. Polish Society for Nature Conservation „Salamandra", 18pp.
18. Lobbova, D., Hapl, E. 2014. Conservation of European ground squirrel (Mammalia: Rodentia) in Slovakia: Results of current reintroduction programme. *Slovak Raptor Journal*, 8(2): 105–11 2.
19. Matějů, J., Říčanová, Š., Ambros, M., Kala, B., Hapl, E., Matějů, K. 2010. Reintroductions of the European Ground Squirrel (*Spermophilus citellus*) in Central Europe (Rodentia: Sciuridae). *Lynx*, n.s. 41: 175–191.
20. Matějů, J., Říčanová, Š., Poláková, S., Ambros, M., Kala, B., Matějů, K., Kratochvíl, L. 2012. Method of releasing and number of animals are determinants for the success of European ground squirrel (*Spermophilus citellus*) reintroductions. *European Journal of Wildlife Research,* 58 (2): 473-482.
21. Próchnicki, K. 2012. Conservation and reintroduction of the selected compact colonies of the spotted souslik (*Spermophilus suslicus*): an ongoing project and plans for future. In: Kepel, A. and Konczak, J. (Eds.). 4th European ground squirrel meeting. Programme, Abstracts, Participants. 5-7 September 2012, Kamien Slaski, Poland. Polish Society for Nature Conservation „Salamandra", 20pp.
22. Říčanová, Š., Koshev, Y., Říčan, O., Ćosić, N., Ćirović D., Sedláček, F., Bryja, J. 2013. Multilocus phylogeography of the European ground squirrel: cryptic interglacial refugia of continental climate in Europe. Molecular Ecology, 22: 4256–4269.

23. Stefanov, V. 2015. *Spermophilus citellus* (Linnaeus, 1766). In: Golemansky V. (Ed). The Red Data Book of Bulgaria, Volume 2*,* Animals. In internet: http://e-ecodb.bas.bg/rdb/en/vol2/2editorial-board.html
24. Stefanov, V., Markova, E. 2009. Distribution and current status of the European souslik (*Spermophilus citellus* L.) in the Sofia valley and the adjacent areas. Biotechnology & Biotechnological Equipment, 23(2) Special edition: 381–384.
25. Tokaji, K., Váczi, O., Bakó, B., Gedeon, C. 2012. 25 years of translocation programmes on EGS in Hungary. In: Kepel, A. and Konczak, J. (Eds.). 4th European ground squirrel meeting. Programme, Abstracts, Participants. 5-7 September 2012, Kamien Slaski, Poland. Polish Society for Nature Conservation „Salamandra", 17pp.

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 166-170

RESTORATION OF THE EUROPEAN GROUND SQUIRREL IN KOTLENSKA PLANINA

VLADIMIR STEFANOV[1*], EMILIAN STOYNOV[1,2], LACHEZAR BONCHEV[2], STAMEN STANCHEV[2]

1 – Department of Ecology and Environmental Protection, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
2 – Fund for Wild Flora and Fauna, 2700 Blagoevgrad, P.O. Box 78, 49 Ivan Michaylov Str, room 327, Bulgaria
**Corresponding author: vladstef@abv.bg*

Keywords: population, reintroduction, restoration, European ground squirrel, Kotlenska Mountain

Abstract: Based on questionnaire data, European Ground Squirrel is considered extinct in the region of Kotel town (Natura sites Kotlenska planina BG0000117 and BG 0002029) since 1990. In 2011, the Fund for Wild Flora and Fauna started a program aiming at restoring the European Ground Squirrel. The program implementation is based on assessment of the regional conditions (physical-geographical mosaicity of the landscape and habitats' conditions incl. dependence on the degree of development of pasture livestock breeding) and, on this basis, a reintroduction of the European Ground Squirrel in territories found suitable for its existence. In 2011 and 2012, a reintroduction of European Ground Squirrels (64 and 56 individuals, respectively) was carried out in two adjacent localities around acting shepherds and a dairy farm. The data obtained in the subsequent monitoring of these places showed that the animals successfully bred and, generally, in both localities a colony was formed inhabiting a total area of 3 ha. In the period 2013-2014, the boundaries of the inhabited territory were relatively constant, while in 2015 settlement and breeding has expanded to a new territory, covering an area of about 1 ha.

In the same year, the assessment of habitats showed significant improvement of the existing conditions for European Ground Squirrel on a large area of pastures as a result of increased number of grazing livestock. The change of the conditions and the success of the initial experiment justified the implementation of a second reintroduction into a new habitat being relatively distant from the first settlement. A total of 44 animals were reintroduced during the second experiment. If the repeated reintroduction is successful, it can be argued with a high degree of certainty that the core for restoration of a viable population of European Ground Squirrel in the region has been successfully established.

INTRODUCTION

During the last decades, the distribution and abundance of European Ground Squirrel (*Spermophilus citellus)* has significantly decreased, mainly due to loss and fragmentation of habitats and, according to the IUCN criteria, the species has been categorized as "vulnerable" (Coroiu et al., 2008; Stefanov, 2015). Habitat management and reintroduction in areas where the species has disappeared have been indicated of basic significance among the conservation activities in Action Plan for the Conservation of the European Ground Squirrel *Spermophilus citellus* in the European Union (Janák et al., 2013), as far as these are associated with the development of cattle pasture and the conservation status of semi-natural grasslands and meadows.

Based on questionnaire data, European Ground Squirrel has been considered extinct in the region of Kotel town (Natura sites Kotlenska planina BG0000117 and BG 0002029) since 1990. This was obviously due to the drastic reduction of grazing cattle and the subsequent successional changes in the habitats. Since 2000, the Fund for Wild Flora and Fauna has launched a complex of activities including traditional ways of cattle grazing, protection and restoration of habitats and species diversity in semi-natural grassland systems, where one of the priority species is European Ground Squirrel.

The region is mountainous and includes significant areas occupied by grasslands, but is relatively isolated from similar areas covered by forest massifs. For this reason, it is not expected that a natural colonization of the territory by European Ground Squirrel will take place in the foreseeable future. Even under favorable conditions, the existence and restoration of the species is only possible through reintroduction. This is why a program aiming at restoring European Ground Squirrel populations in the region started in 2011 (Stoynov et al, 2013).

The present publication summarizes the results of the program implementation achieved to date.

MATERIAL AND METHODS

Reintroduction was carried out following the general approach (Brandler et al. 2012; Dejkin and Tihonov, 1987) using the following scheme:

- exploration of the area and assessment of the habitats condition;
- choice of habitats with conditions suitable for the species;
- translocation;
- subsequent assessment (monitoring) of the habitats condition and the reintroduced animals.

The ultimate goal of the restoration was the creation of several spatially distributed local colonies, so that the future expansion and colonization of new areas could ensure the exchange of individuals and, accordingly, the formation of a sustainable and viable population.

The assessment of habitats was mainly based on the degree of development of grass cover (height and projective cover of the grass stand) as a factor being a subject to management and directly dependent on the degree of development of the cattle pasture. Along with this, the characteristics of the terrain, slope, water regime, and soil layer depth were taken into account.

Areas featuring relatively weak slope, depth of soil layer 60-80 cm, height of grass cover under 15-20 cm. and projective cover below 80% were accepted as appropriate habitats.

The reintroduction was carried out according to the methodology and practical experience described by Hapl et al. (2006) and Matějů et al. (2010), at a time when individuals born in the current year switched to independent living, and adults had not yet entered hibernation. Animals were trapped alive and released in new habitats, with pre-digged holes with tunnel length of 60-100 cm and a diameter of 5 cm.

On overall, three reintroduction sessions were carried out in 2011, 2012 and 2015, respectively, with 164 animals trapped and released in total (Table 1).

Table 1. Number, gender and age composition of the introduced European Ground Squirrels

Reintroduction		juv			ad			Σ
Year	Coordinates (WGS 84)	♀	♂	Σ	♀	♂	Σ	
2011	35 T 456246 4752643	24	19	43	12	9	21	64
2012	35 T 456081 4752391	18	19	37	9	10	19	56
2015	35 T 456818 4751358	22	17	39	3	2	5	44

The assessment of the number and territory inhabited by European Ground Squirrel was based on the number and location of inhabited holes. The populated area was determined by the aggregation of final inhabited holes located in less than 50 meters apart.

RESULTS AND DISCUSSION

For the reintroduction taking place in 2011-2012, a region of a functioning sheep farm with an area of 30 hectares was chosen, where regular livestock grazing is carried out to guarantee the maintenance of the necessary grass cover conditions.

Observations of the European Ground Squirrels reintroduced in the abovementioned period showed that animals reproduced successfully, but mainly

inhabited the areas where they were initially placed (Stoynov et al., 2013). The presence of single holes and observation of single animals showed that individuals were displaced outside the colonies but their number was insufficient to form a colony on the new territories until 2014. The maximum recorded length of displacement was about 500 m from the observed colonies.

Observations conducted in 2015 determined that two colonies have been formed in the sites of release with an area of 0.5 and 2.4 ha, respectively. The distance between the borders of the two colonies was about 200 m, as they were separated by dense fern overgrowth. Nevertheless, there was an apparent exchange of individuals between the two colonies through the dirt road connecting them.

Based on the number of occupied holes, the relative number of the European Ground Squirrels in both colonies showed no significant differences through the years. In 2015, in the period immediately after hibernation, 68 and 15 holes were reported in the two colonies, respectively, which did not practically differ from the data obtained in 2013. The density was estimated at no more than 5-10 ind/ha. Despite the relatively moderate density of the colonies in 2015, it was established that a resettlement of a new territory has taken place in May, with an area of about 1 ha, where seven individuals were observed. Considering the location of the temporary single hole outside the permanently inhabited areas, the animals had moved from two areas. On the newly settled area, young animals born in July of the current year were also observed, suggesting that a new colony has formed in this area.

In general, the reintroduction of European Ground Squirrel can be considered successful at this stage. It allows to proceed, insofar as possible, with the establishment of colonies in new areas. In this regard, favorable conditions for the period 2011-2015 increased the number of grazing livestock: sheep, goats, horses and cattle. In 2011 the pasture load was 0.5 animal units/ha, while in 2015 it already reached 3.2 livestock units/ha. In 2015 respectively, the investigation showed significant changes in the grass cover on large areas: in average, the grass height for the spring-summer period was 10-15 cm (60-80 cm in 2011) and the projective cover was about 80% (up to 100% in 2011). Seven habitats suitable for reintroduction of European Ground Squirrel were identified in these areas, at an average distance of 500 meters from one anther and from the already formed colonies.

In 2015, European Ground Squirrels were introduced over an area of about 1 ha in a habitat with coordinates 35 T 456818 475135844. Subsequent observations have shown that practically no animals have settled permanently in the artificial holes available. Animals have scattered across the surrounding areas, and the maximum recorded distance was about 100 m away.

The initial leaving of the artificial holes is a natural phenomenon and is attributed to the research activity of the animals, unfamiliarity with the territory and relocation stress. Nevertheless, during the investigation done within a month

after the reintroduction, new holes were found (inclined and vertical). It was generally estimated that at least 4% of the animals have settled in a region within a 100 m radius of the center of reintroduction. The final effect of the reintroduction will be clear only after the hibernation period in the spring of 2016.

CONCLUSION

On overall, it can be noted that at this stage the recovery program of European Ground Squirrel in the region of the town of Kotel has allowed the successful establishment of local colonies and conditions for extending the reintroduction efforts have been created, with a real prospect for recovery of a viable population of this species. Achieving the ultimate goal will provide a food base for a number of species whose recovery is linked to trophic relations with European Ground Squirrel, thus supporting the restoration of the biodiversity of the region. Meanwhile, the experience gained so far allows deriving conclusions regarding the methodology of the reintroduction of European Ground Squirrel.

REFERENCES

1. Брандлер, О.В., Власова, О.П., Власов, Е.А., 2012. Реинтродукция степного сурка в Центрально-Черноземном заповеднике. Степной бюллетень, 2012, № 35, 50-55 (Brandler, O.V., Vlasova, O.P., Vlasov, E.A., 2012. Marmot reintroduction in the Central – Chernozem reserve. Stepnoi buleten, 2012, № 35, 50-55)
2. Дежкин А.В., Тихонов А.А. 1987. Методические рекомендации по расселению степного сурка в РСФСР. М.: ЦНИЛ Главохоты. 15 с.(Dejkin A.V., Tihonov A.A. 1987. Methodical recommendations on resettlement of the marmot in RSFSR, M.: CNIL. Glavohotoi. 15 p.)
3. Coroiu, C., Kryštufek, B., Vohralík, V. & Zagorodnyuk, I. 2008. *Spermophilus citellus*. The IUCN Red List of Threatened Species 2008: e.T20472A9204055.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T20472A9204055.en. Downloaded on 29 November 2015
4. Hapl, E., Ambros M., Olekšak M., Adamec M. 2006. Suslik (*Spermophilus citellus*) reintroduction in Slovakia. Guidelines. State Nature Conservancy of the Slovak Republic, Banská Bystrica, 28 pp.
5. Janák M., Marhoul P., Matějů J. 2013. Action Plan for the Conservation of the European Ground Squirrel *Spermophilus citellus* in the European Union. European Commission. http://ec.europa.eu/environment/nature/conservation/species/action_plans/pdf/EUSAP_EuropeanGround%20Squirrel_Final.pdf
6. Matějů, J., Řičanova, Š., Ambros, M., Kala., B, Hapl, E., Matějů, K. 2010: Reintroduction of the European Ground Squirrel (*Spermophilus citellus*) in Central Europe (Rodenia: Sciuridae). *Lynx* (Praha) 41: 175-191.
7. Stefanov, V.S. 2015. European Souslik *Spermophilus citellus* (Linnaeus,1766). In: Golemansky,V. et al.(Eds)2015. Red Data Book of the Republic of Bulgaria.Volume 2.Animals. BAS&MoEW, Sofia, pp. 232
8. Stoynov, E., Bonchev, L., Stanchev, S. 2013. European Ground Squirrel (*Spermophilus citellus*) Re-introduction in Kotel Mountain, Bulgaria. Overview 2011-2013. Fund for Wild Flora and Fauna. Blagoevgrad. Published online on Researchgate, December 2013. DOI: 10.13140/RG.2.1.1987.7609

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 171-175

BEZOAR WILD GOAT (*CAPRA AEGAGRUS* ERXLEBEN, 1777) – HISTORY AND OPPORTUNITIES FOR DEVELOPMENT OF THE SPECIES IN BULGARIA

ATIDZHE AHMED[1*], ALBENA VLASSEVA[1],
STEFKA KITANOVA[2], PETER GENOV[1]

1 – Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria
2 – Forest Research Institute, Bulgarian Academy of Sciences, 132, St. Kliment Ohridski Blvd.,1756 Sofia, Bulgaria
**Corresponding author: atidje_dj@abv.bg*

Keywords: Bezoar wild goat, *Capra aegagrus*, reintroduction, Bulgaria

Abstract: The Bezoar wild goat (*Capra aegagrus* Erxleben, 1777) is assumed that in the past was a natural inhabitant of our mountains, but subsequently disappeared. The aim of this article is to confirm or refute this assumption on the basis of literature data and to answer the question is there a future for the development of the *Capra aegagrus* in Bulgaria? If the answer is „yes", then where are suitable habitats for it?

Paleontological research proves that the *C. aegagrus* is not aboriginal in the European continent and in Bulgaria, but was brought by the people as a live stock at first on islands and then in the mountains in Northern Greece. The Bezoar wild goat has been most probably living in Bulgarian southern mountains, for a short period though, which explains the lack of species' fossil remains and other proof for its dwelling in Bulgaria as well.

The species' low nutritional requirements and successful adaptation are the prerequisites for its development and displacement in the country. C. aegagrus could and should be part of Bulgarian fauna, because the mountains of Bulgaria are beautiful, but will be even more beautiful if they are inhabited by this majestic goat – the Bezoar wild goat.

INTRODUCTION

Taxonomically the Bezoar wild goat (*Capra aegagrus* Erxleben, 1777) refers to the family Bovidae, genus Capra which includes 8 species and 11 subspecies. The *C. aegagrus* has 4 subspecies – *C. a. aegagrus* (distributed in Afghanistan, Armenia, Azerbaijan, Lebanon (extinct), Russia (Eastern Caucasus), Turkey,

Georgia, Iran); *C. a. blithy* (Pakistan, Iran, Irac, Turkmenistan); *C. a. chialtanensis* (Pakistan); *C. a. cretica* (Greece), (Pidancier et al., 2006). It is considered that *C. aegagrus* is one of the ancestors of the domestic goat (*Capra hircus*) (Clutton-Brock J., 2001). The species inhabits mountainous areas where there is a mixture of rocky hills and shrub thickets or coniferous forests. It feeds on grasses, herbaceous plants and shrubs. Although it inhabits rocky dry areas in the Caucasus, it is described as one of the leading forest species by adhering more often to the middle mountain heights (Weinberg et al., 2008; Spasov, 1982).

C. aegagrus is assumed that in the past was a natural inhabitant of our mountains, but subsequently disappeared. The aim of this article is to confirm or refute this assumption on the basis of literature data and to answer the question is there a future for the development of the *C. aegagrus* in Bulgaria? If the answer is „yes", then where are suitable habitats for it?

RESULTS AND DISCUSSION

Short description of the Bezoar Wild Goat

In comparison with other species of the genus Capra, *C. aegagrus* and *C. pyrenaica* are the smallest of all species of the genus. The Bezoar legs are relatively short. Males have massive back-curved saber-like horns. Females have short horns. Coloration is rusty-red in summer and grayish-brown in winter. Throat and chest are almost black. Neck and sides of the body are gray. Belly and feet are gray-white. Their body length is 120-160 cm and the height at the withers 70-100 cm. Both sexes have a distinctive black beard that in the males reache 20 cm, which is why the species is known as the bearded goat (Mihailov & Stoyanov, 2001; Genov et al., 2009).

The breeding period is from mid-December to late January. Females give birth from mid-June to mid-July very often to two offspring (Gundogdu and Ogurlu, 2009). In many countries where the bezoar dwells it is a game species, but is included in the IUCN red list as a vulnerable species (Weinberg et al., 2008).

Historical Data

It is assumed that in the past the bezoar inhabited our mountains as well. Data which gives grounds for this is a capricorn skull excavated in a cave in Troyan, which was defined by Popov (1934) as *C. aegagrus*, (in Spasov, 1982). Later, however, Spasov (1982) proved that this skull is ibex (*C. ibex*), as well as the other remains of legs that were also found. Since then there are 9 Pleistocene deposits of C. ibex in Bulgaria found (Georgiev and Stoicheva, 2010). *C. aegagrus* however, has no traces amongst them.

According to different authors the bezoar occurred in the mountains of Albania, Greece, Crete, Asia Minor until Persia, and some say even in Macedonia (Belon du Mans 1953; Brenties, 1981; Konsulov, 1926). In 1916 a group of soldiers

went out hunting for wild boar in the mountains Parnar dag (Greece) and killed an adult Bezoar Ibex, whose horns are still kept in the Natural History Museum in Sofia (Konsulov, 1926). According to the author, this individual was not a single one, because the horns show visible traces of fights with other males. This is actually the closest known habitat of bezoars to the territory of Bulgaria. Petrov (1986) wrote that in Europe only wild bezoar goat *C. aegagrus* was distributed, and its population was destroyed by hunters during the Middle Ages.

According to Couturier (1962) (in Spasov, 1982) in the recent past wild goats have been displaced from the Greek islands in the mountains of northern Greece, where they gradually disappeared. A similar hypothesis is supported by other authors. Horwitz & Bar-Gal (2006) made a genetic analysis of the Cretan wild goat (*C. a. cretica*), which, although morphologically resembles *C. aegagrus*, its mitochondrial DNA shows affinity to the domestic goats. The authors explain that most likely *C. a. cretica* was imported to the island as a wild primitive form during the sixth millennium BC as a food source. In subsequent interbreeding with domestic goat the species has kept its wild morphotype but has undergone significant genetic change. The authors say that these findings are applicable to other free-living goats and sheep as the mouflon for example. Masseti (2009) noted that there was no evidence of fossils of *C. aegagrus* found on the continent of Europe. Originating from the Middle East, which is its natural habitat, the species was introduced by people of Mediterranean islands from the Pre-Pottery Neolithic period. On the other hand, Van den Brink (1967), (in Geskos, 2013) mentions that *C. aegagrus* once inhabited the European continent occurring in Bulgaria until 1891. According Hadzissarantos & Kanellis (1955), (in Geskos, 2013) however, some naturalists of the nineteenth century and earlier have mistaken *C. aegagrus* with the wild goat *Rupicapra rupicapra*. Brehm (1963) writes that the bezoar occurred in Central and Western Asia, singly on the island of Crete, but also it was dwelling in the Balkans.

A definite proof that *C. aegagrus* was brought to the European continent is described by Konsulov (1926) with a case of a male killed in 1916. It is possible that some animals have come to our southern mountains (Pirin, the Western Rhodopes). Mihailov Stoyanov (2001) writes that the species has disappeared from Bulgaria in the early 20th century and the last habitats were in Slavyanka and Alibotush mountains and the southern units of the Rhodopes. According to L. Harizanov (personal message) the last bezoar in the country was killed in 1939 on the territory of present State Hunting Enterprise „Kormisosh".

Status and development of the species in Bulgaria

Suitable habitats for *C. aegagrus* in the country are found in all of our larger mountains (Stara Planina, Rila, Pirin, the Rhodopes mountain). That's why in 2012 introduction of bezoars from the Czech Republicwas undertaken, where the species was introduction in 1953-1967 (Masseti, 2012). 20 young individuals equal

in gender (10 males and 10 females) were brought in a fenced area in Zabardo, the Rhodopes. The altitude of the place chosen for the introduction is 1200-1300 m. Tree species occurring around are Scots pine (*Pinus sylvestris*) and deciduous species, some of which are remnants of orchards. The animals were brought in December. Due to the harsh climatic conditions three of them died. In the spring of 2013 one of the females gave birth to a lamb. The animals quickly adapted to the local conditions. They had no problems with regard to food, none was hurt and in 2014, eight females have given birth to nine cubs. It is noteworthy that the cubs grew very quickly, especially their horns. In the spring of 2015 5 lambs were born. The same fenced area is also inhabited by a mouflon (*Ovis orientalis musimon* Schreber, 1782) and until one year ago there was a Himalayan Thar (*Hemitragus jemlahicus* H. Smith, 1826). Due to the pronounced aggressiveness of the latter species, they are separated. So now within the fenced area without competitive relationships only Bezoar goat and mouflon live together. This may be due to the common origin of both species from the region of Western Asia. The successful development of these Bezoar goats showed that they could easily be released and settle rocky habitats in our mountains, especially in places where there is no wild goat. In its natural habitat, such as the Caucasus, the Bezoar goat inhabits together with the wild goat and ram without any problems.

CONCLUSION

Paleontological studies have shown that *C. aegagrus* is not aboriginal species to the continent of Europe and our country, but was transported by people as human food stocks first on the islands, and later in the mountains of northern Greece. Most likely the bezoar inhabited our southern mountains, although for a short period of time, which explains the lack of its fossils, and no other evidence of its existence on the territory of Bulgaria

Nevertheless, the low nutritional requirements of the species and its successful adaptation are the prerequisites for future development and displacement. *C. aegagrus* could and should become part of the Bulgarian fauna, because the mountains of Bulgaria are beautiful, but will be even more beautiful if they are inhabited by this majestic goat – Bezoar wild goat.

REFERENCES

1. Belon du Man, P. 1953. Observations of many rare and wonderful things seen in
2. Greece, Asia, Judea, Egypt, Arabia and other foreign countries by Pierre Belon du Man. BAS, Sofia, 308 pp. (in Bulgarian)
3. Brehm A. 1963. Życie zwirząt. Ssaki. Państwowe Wydawnictwo Naukowe, Warszawa, 519 pp.
4. Brenties, B. 1981. „Discovery“ of domestic animals. Zemizdat, Sofia, 159 p. (in Bulgarian)

5. Clutton-Brock, J. 2001. Storia naturale della domesticazione dei mammiferi. Bollati Boringhieri, Torino, 279 pp.
6. Genov, P., Georgiev, G., Georgiev, V. 2009. Persian wild goat (*Capra aegagrus* Erxleben) – biology, ecology and possibilities for its re-introduction in Bulgaria. *Biotechnology & Biotechnological Equipment*, Special Edition, 23 (1): 341-342.
7. Georgiev, D., Stoicheva, S. 2010. New Late Pleistocene habitat of ibex (*Capra ibex* L.), (Mammalia: Bovidae) in Bulgaria. ZooNotes, 14: 1-4. (in Bulgarian)
8. Geskos, A. 2013. Past and present distribution of the genus Capra in Greece. *Acta Theriologica,* 58:1-11.
9. Gundogdu, E., Ogurlu, I. 2009. The distribution of wild goat *Capra aegagrus* Erxleben 1877 and population characteristics in Isparta, Turkey. *Journal of Animal and Veterinary Advances*, 8 (11): 2318-2324.
10. Horwitz, L. K., Bar-Gal, G. K. 2006. The origin and genetic status of insular caprines in the Eastern Mediterranean: a case study of free-ranging goats (*Capra aegagrus cretica*) on Crete. Human. *Evolution*, 21: 123–138.
11. Konsulov, St. 1926. Bezoar goat (*Capra aegagrus*) on the Balkan Peninsula. Hunter, 8/9: 7-8. (in Bulgarian)
12. Masseti, M. 2009. The wild goats *Capra aegagrus* Erxleben, 1777 of the Mediterranean Sea and the Eastern Atlantic Ocean islands. *Mammal Review*, 39 (2): 141–157.
13. Masseti, M. 2012. Atlas of terrestrial mammals of the Ionian and Aegean islands. De Gruyter, Berlin/Boston. 302 pp.
14. Mihailov, Hr. Stoyanov, St. 2001. Hunting birds and mammals in Bulgaria. Practical guide. Pensoft. Sofia. 208 p. (in Bulgarian)
15. Petrov, A. 1986. Early history and evolution of domestic animals. BAS, Sofia, 262 pp. (in Bulgarian)
16. Pidancier, N., Jordan, S., Luikart, G., Taberlet, P. 2006. Evolutionary history of the genus *Capra* (Mammalia, Artiodactyla): Discordance between mitochondrial DNA and Y-chromosome phylogenies. *Molecular Phylogenetics and Evolution,* 40: 739–749.
17. Spassov, N. 1982. Fossils of the Alpine Ibex and the Giant Deer in Bulgaria and role of the horns of the Giant Deer. *Priroda*, BAS, Sofia, 5, 21-27. (in Bulgarian)
18. Weinberg, P., Jdeidi, T., Masseti, M., Nader, I., de Smet, K., Cuzin, F. 2008. *Capra aegagrus. The IUCN Red List of Threatened Species 2008*: e.T3786A10076632. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T3786A10076632.en

Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
First National Conference of
Reintroduction of Conservation-reliant Species, Sofia 2015
University Press 2016, pp. 176-187

NOTES ON BEHAVIOR OF THE GRIFFON VULTURES (*GYPS FULVUS*) DURING RECOVERY OF THE SPECIES IN KRESNA GORGE AND KOTLENSKA PLANINA

VENCESLAV DELOV

Department of Zoology and anthropology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov str., 1421 Sofia, Bulgaria
**Corresponding author: vencidelov@abv.bg*

Keywords: *Gyps fulvus*, behavior, recovery in Bulgaria, release, adaptation

Abstract: Restoring populations of Griffon Vulture in Bulgaria is a complex process, which includes importing of birds from Spain. They are later released in the Kotlenska Planina (near the town of Kotel) and in Kresna Gorge. The birds were preliminary hold in aviary, then released into the wild. The data on bird's behavior was collected by direct observations and by analyzing videotapes. The method used is free description of the behavior "*ad libitum*". In the present work are described mainly forms of social and agonistic behavior from interspecific and intraspecific points of view, nesting behavior, incubation behavior. Specific behavioral strategies were analyzed - associated with feeding behavior and diurnal activity.

INTRODUCTION

Griffon vultures are cliff-nesting raptors and are the most social raptors in Europe, because they breed colonially and feed gregariously in large groups. Their species-specific communication system for exchange of information between individuals and groups is based on simultaneously watching each other's behaviour. It optimises food finding species strategy. Another highly social European raptors that breed colonially such as Lesser kestrel *Falco naumanni* and Montagu's harrier *Circus pygargus* are not so social in searching of food as griffons are. This social lifestyle is regarded as an indirect consequence of their clumped and irregular food supply (Houston, 1979; Donázar, 1993). As they are almost exclusively scavengers they have to compete with time, another birds and mammalian competitors. The vultures forage predominately over open areas with rocks. Their cooperation and information exchange are important in order to forage successfully. The

birds modify their foraging behaviour according to the spatial and temporal distribution of the animal carcases.

Foraging techniques and foraging time budget are dependent of seasonal food availability and population density. The intra-specific competition is important factor for population regulation (Sillett et al., 2004; Dobbs et al., 2007; Bosé and Sarazzin 2007; Hutto 1990; Lovette, Holmes 1995, Hiraldo and Donázar, 1990.

Reintroductions of Griffon Vultures have been implemented in several European countries. Moreover, almost everywhere in the species range is practiced to support populations by artificial feeding. All this makes the species especially appropriate for behavioral research. Summary data on the behavior of species, including a catalog of behavior and the description of the basic species adaptive strategies is represented by Glutz Von Blotzheim *et al*., (1971); Cramp and Simmons (1980). Intraspecific competitive behaviours in relation to understand the potential consequences of food management on competition were studied in a reintroduced population of Griffon Vultures by M. Bose and F. Sarrazin (2007). GPS satellite telemetry was used to assess the home-ranges of non-breeding Eurasian Griffon Vultures in Spain (García-Ripollés et al., 2011). Xirouchakis and Andreou (2009) studied the foraging behavior of Eurasian griffons on the island of Crete during 1997-2005 by direct observations in four colonies and by monitoring the movements of seven radio-equipped individuals.

Despite longstanding protection and maintenance of Griffon vulture in Bulgaria, as well as new activities for the reintroduction of the species in some of its traditional localities in the past, specialized studies on behavior in Bulgaria at the time were not performed. At present, the situation related to the reintroduction of the species is represented in several articles and annual reports (Stoynov and Peshev, 2011; 2012; 2013, Stoynov *et al*., 2011; 2013; 2014; 2015). Stoynov et al. (2014) also commented some related topics as the human-predator conflict in the area, which directly influenced the prosperity of the Griffon vultures.

The reintroduction activities for the Griffon Vulture (*Gyps fulvus*) began in 2010 and the following establishment of a vulture feeding stations occur in Kresna Gorge and Kotlenska Planina – near the town of Kotel.

For the restoration of griffon vulture in the region of Kresna Gorge and Kotlenska Planina were used immature birds imported mainly from Spain. Birds spend adaptation period in the aviary in the area and then are released.

After the release in the most general two categories of birds are formed. The first category brings together individuals with high adaptability of behavior. This are successful individuals who quickly acquire the necessary skills to use the resources available in the region - the platform for eating, opening places for roosting, effective use of low air flow in the area. The other category are birds with low adaptive behavior. They difficult learning new habits necessary for survival. A major problem is that they are not capable to learn to use platform for eating, so exhausted and if special measures are not taken they perish. Naturally

between these two extreme categories there are intermediate ones. They are not subject to detailed analysis in this work because of difficulties in finding connections between behavior during different phases of the recovery process and the effectiveness of behavioral adaptation to the individual level. Intermediate categories of birds is expected to be analyzed at a later stage, after a preliminary study analyzing the working hypotheses and research questions with the help of statistical methods.

Aims

The purpose of this work is to make a preliminary behavioral analysis of based on free description of behavior and expert assessment in the light of conservation ethology of the species, by seeking links between the effectiveness of behavioral adaptation and specific behavior in different stages of the recovery process at individual level. Categories of behavior were used, that suggests at expert level that they have predictive value in terms of the success of behavioral adaptation of birds in the recovery process. An important aspect in this regard is the formation of research questions and hypotheses in the field of conservation ethology for more detailed follow-up analysis.

METHODS

In this study functional classification of behavior was used, according Dewsbury (1985). The behavior is divided into individual, social and reproductive in intraspecific aspect. As a independent category is differentiated behavior in relation to other species. Here as an object of analysis are mostly antagonistic relationships.

Bird's behavior is described by the method of free description of the behavior "*ad libitum*". Data are collected on the basis of immediate observation and description of behavior, and behavioral analysis of the behavior of photos and videos. The analyses were carried out at expert level and are based on Animal psychology approach. The approach was used to analyze the consequences of the behavior. The description of the behavior itself is not the subject of this work.

RESULTS AND DISCUSSION

The results of this work have a value of preliminary experiment, in accordance with the concept of Tinbergen (1963). It will serve as a basis for forming research questions and working hypotheses for more detailed analysis of the behavior, including appropriate statistical analysis. In this study are analyzed only well differentiated visible categories of behavior.

Behavior before releasing in the wild

Individual behavior

Each of the below described forms of individual behavior show its final and intermediate categories. Object for description and analysis are only the final behavioral categories.

1. Behavior during transportation of the vultures

The birds are transported in transport chests. When transporting the two categories of birds are formed. One category are birds is with a strong reflex for freedom, which is intensively trying to leave the chest and the other category are birds with low intensive reflex for freedom. They are kept relatively quiet. On the basis of an expert assessment, the birds from the first category with a low threshold at the reflex are more successful individuals for the recovery in nature. They are easier to adapt in terms of nutrition and easier learning necessary skills to use the nutrition site.

2. Behaviour during the release from the transport box

Upon the releasation two categories of birds are formed - birds that immediately leave the chest and birds that do not leave immediately or they unwilling to leave. Part of the second category includes birds that are oriented with head at the opposite direction in the terms of output. They try to get out in the wrong direction in terms of the actual output. These two main categories of birds correspond to the categories of items 1.

3. Behavior after the releasing in the aviary

We can distinguish two clearly differentiated categories of individuals. The first category includes birds with low threshold levels of anxiety response and good level of orientation behavior. They depart immediately while trying to leave the aviary showing strong reflex of freedom. The other category combines the birds that away slowly from the humans - usually by walking. Some individuals in this category exhibit quite prolonged orientation response before to perform motor activity. These two main categories of birds correspond to the categories of items 1 and 2.

4. Motor behavior in the aviary and reflex for freedom

Motor behavior in the aviary originally is motivated by the desire of birds to leave the enclosure. It is manifested by motion and flying activity varying in degrees and intensity. The behavior represents overflights and landing on perches, hunging on the walls and ceiling of the aviary by trapping with the legs on the net. In this case are also are formed two diferent categories of birds the individuals with high and low intensity of the reflex of freedom. These two main categories of birds correspond to the categories of items 1, 2 and 3.

5. Defensive behavior - defensive reaction to the man

Fears response to human is expressed in motion activity similar to the reflex of freedom and grouping of birds at the opposite end of the aviary in terms of man.

The reaction weakens during the halituation. The habituation is strongly influenced by the emulation element of behavior. The presence of already accustomed or inprinted to human birds in the aviary, greatly helps to accelerate habituation to humans of the newly-arrived vultures. The differentiateion between categories in this behavior are largely influenced by the specific individual experience, which is why there is no clear boundary between them.

6. Feeding behavior

Nutrition and feading behavior are a key element in the process of re-acclimatization. Like the previous category, feading behavior is largely influenced by the emulation element of behavior. If there are already accustomed group of birds in the aviary, feeding can begin immidiately, while in the absence of such birds, the first sign of feeding behavior could be deferred by approximately 10 days of accommodation in the aviary. Feading behavior has pronounced social aspects that will be described in social behavior section.

7. Roosting behavior

The roost at griffons is associated with a certain posture and exact place. In this species the roosting behavior perform specific social patterns that will be addressed in the social behavior part.

Social behavior

1. Social interactions during feeding

Foraging behaviour and feeding food searching techniques in relation to the habitat and breeding strategies have been described by Houston (1974); Pennycuick (1972, 1983, 1989); Prinzinger et al. (2002); Ruxton and Houston (2002); Bögel (1999), König (1974), Bahat and Kaplan (1995).

During feeding in the aviary prevail agonistic interactions including threats and skirmishes. The hierarchy is as situational dominance correlated with motivation level at the particular individual. It is slightly influenced by the age group and gender of the individuals.

2. Social aspects of Roosting

The griffons congregate in communal roosts where they probably very important to exchange information on good feeding grounds (Ward and Zahari, 1973). According our observations the collective roosting behaviour structure is predominated by cohesive behaviour and agonistic interactions. During the preparation for spending the night, often was seen competition between birds to appropriate roosting place. Agonistic interactions lead to apparent differentiation of fully subordinated individuals who can not win their place. The rank of these individuals is unstable and situational. In various nights hierarchical status of the same individual may vary from complete domination to complete submission at constant composition of the individuals in the group.

Behavior after the releasing in the wild

Individual behavior

1. Motor behavior

In releasing the birds stay in the area, as usually spend some time perched on the aviary. During this period it is especially important the effective utilization of flight corridors to natural substrates suitable for recreation, roosting and nesting. In this connection, it is especially important for the effective use of air thermals. Non-adaptive behavior in this respect is causing loss of height, landing at inappropriate and dangerous substrates - for example, electric poles, ineffective diets and weight loss. Ultimately, this can lead to exhaustion and death of individuals. For these individuals sometimes are needed special rescue actions.

2. Defensive behavior.

The species is with long standing cohabitation with man in Europe as it is depending on livestock carcasses for thousands of years. In a group of young birds was seen vomiting of food ingested, resulting in fear of approaching people. This is an adaptive response to weight reduction and restoration of the ability to fly. Later a young bird with a high threshold of fear responses began to eat the vomit food. This behavior is obviously beneficial to the individual and is adaptive. High threshold levels of fear responses in some individuals give them an advantage in the use of food playground and recreation areas near the aviary. Habituation to humans, however, is associated with a specific place - in this case the aviary. The process of generalization probably are not so strong to severe as elsewhere the same individuals showed low threshold levels of fear in terms of human, similar to those in wild birds, unaccustomed to human subjects.

4. Migratory behavior

In the area of studies has both migratory and permanent populations. The population of the recovered birds is permanent. A case was observed of joining of the recently released bird at natural conditions to a group of migrating vultures. This individual was permanently assigned to the migrating population. Vultures migrate singly or in different size groups within the species. mixed flocks with other soaring migrants were not observed. The final points of migratory route are not precisely defined. The birds remain to winter in various appropriate places acording to their judgment along the route.

Social behavior

1. Social behavior during feeding

Similar to the situation in the aviary conditions here are also prevailing the agonistic relations between individuals. It was found, however, that the vultures move the feeder at separate distinct groups. Within these groups the individuals probably know each other personally, based on common places for rest and sleep. Formed pairs of birds move together to the food site within the default group, as

during feeding have not been observed agonistic interactions between partners in the pair. Perhaps a couple of individuals are tolerant to each other in a competitive environment.

Reproductive behavior

1. Courtship

One of the clearly visible forms of courtship are synchronous marriage demonstration flights. Partners of the pair are flying synchronized at the same height. Other elements of courship behavior are dive flights accompanied with placing the legs. They were observed also and group demonstration flights in which the behavior of the pair initiated a similar behavior with other individuals. They join to the demonstration. In a group demonstration up to 5-6 participants were observed. During the incubation were observed courship flights of males with unpaired females, at a time when their female partner incubates.

2. Formation of the pair and maintaining the pair bond

The formation of the pair begins in many cases before the reaching of sexual maturity and the appearance of characteristic plumage of adult individuals. Couples are constant, the intensity of contacts between partners increases with the onset of the breeding season. It is possible that males to be banished from a stronger competitor that he takes female and nesting area - seen in one couple. A case was observed in which adult female in reproductive status and strong reproductive motivation for seeking a partner and nesting site, became a major faktor for formation and retention in the region of a group of vultures.

3. Copulation

Copulation in the species is stereotyped behavior occurring mainly between partners in the pair. For the species is characteristic tendency for extrapair copulations. Then some of the males and immature birds are entering in courship interactions with unpaired females. In zoo-garden conditions was observed a case of bigamy in presence of an adult single female in addition to a formed pair, graduated with a failed nesting of the additional female. Feeding of the female as a form of courtship was not observed. It is not known to the moment whether the main female partner in the couple also tends to extrapair copulation like the male.

3. Construction of the nest

The nest is built mainly by the male. Construction material is transferred with the beak. There are significant differences between different couples in terms of size and shape of the nest. Also, a couple was observed which did not build their oun nests and occupied already available nests of other bird species.

4. Incubation and care for young

Caring for the offspring is relatively evenly distributed between the partners, yet differences were observed in some couples. During incubation, the bird is located in the socket, heading towards the inside of the nesting niche. There is currently not enough data on the care for the young.

5. Relationships with other species

Relationships with other species are mostly agonist, resulting from competition for food resources, nesting niches and places for roosting.

Description of the competitive relationship with Raven and Golden Eagle in the Kresna Gorge is provided by Peshev et al. (2015). Most important is the relationship with Golden Eagle *Aquilla chrysaetos*, which may have a significant negative effect on the adaptation process of the vultures released. Golden Eagle is a highly territorial species which fully dominates in agonistic encounters with Griffon Vulture. Griffon Vultures have a strong fear reaction towards this species. It resembles the defensive reaction of the species - victims against their natural enemy. In young and inexperienced birds, the reaction can lead to loss of height during flight and they may fall in an unfavorable location, especially when the chasing happens before the night or in early morning. Golden Eagle avoids direct encounters with large groups of adult vultures at feeding. In some cases Griffon Vultures with a strong motivation can even win the clashes. There is an observation on the expulsion of a young Golden Eagle perched near the nest of brooding vultures, by the incubating female bird. The female Griffon Vulture left the nest and demonstrated threatening behavior. It ruffled plumage and spread its wings towards the Golden Eagle perched nearby forcing it to leave the place. As a result of this threatening behavior the Golden Eagle flew off.

Agonistic encounters with Ravens *Corvus corax* may hinder young and inexperienced birds during feeding, initial attempts at nesting and roosting. The negative impact of Ravens is reduced with the age and by the accumulation of individual experience in young vultures.

Griffon Vultures usually dominate Egyptian Vulture *Neophron percnopterus* in competition for food. When Griffon Vultures are young and inexperienced, it is however possible for Egyptian Vultures to dominate. Domination of Egyptian Vulture over young Griffon Vultures during the feeding has been reported. The situation changed in favor of Griffon Vultures with advancing of the age of the young birds. Unlike Golden Eagle, Eurasian Black Vulture *Aegypios monachus* is not perceived as a natural enemy, but as a member of the group. Because of its size it dominates in direct confrontations over the Griffon Vultures. The opinion of experts who deal with supplementary feeding of vultures is that Eurasian Black Vulture is the only dignified competitor of Golden Eagle in direct clashes around the carcass. The outcome of the clash depends on the individual characteristics of the birds. Agonistic relationships between Peregrine Falcon *Falco peregrinus* and Griffon Vultures have been observed in flight when vultures are passing falcon breeding territories. According to Peshev, et all (2015), Peregrine falcon displays high aggression against Ravens. It therefore reduces the pressure of Ravens on Griffon Vulture and favors the reintroduction of the species. Unlike Peregrine Falcon, Lanner Falcon *Falco biarmicus* (Feldegg‘s falcon *Falco*

biarmicus feldeggi), which appeared in the region of Kresna Gorge and took a nesting territory, showed great tolerance to Griffon Vultures and even perceived their presence as a positive factor. However, general conclusions of this species cannot be made because the observations are just over one female that occupied a territory but failed to form a pair and raise offspring in the area.

The following birds of prey were identified also in the region:

Buzzard *Buteo buteo*, Long-legged Buzzard *Buteo rufinus*, Black Kite *Milvus migrans*, Goshawk *Accipiter gentilis*, Sparrowhawk *Accipiter nisus*, Levant Sparrowhawk *Accipiter brevipes*, harriers, Imperial Eagle *Aqullla heliaca*, Booted Eagle *Hierraetus pennatus* , Short-toed Eagle *Circaetus gallicus* and Lesser Spotted Eagle *Aquilla pomarina*. No interactions were observed between these species and the Griffons. It is interesting to note that the Imperial eagle has used the same feeding place as the Griffons. As opposed to Golden Eagle, Imperial Eagle did not cause defensive reaction. This is an interesting fact, taking into account the significant morphological similarity between Golden and Imperial eagles. Griffon vultures are probably capable of very precise differentiation and assessment of their natural enemies and competitors.

There is not enough information on the interactions with carnivores.

Guidelines for behavior management of the Griffons

The behavior of the species and its competitors can successfully be managed through the use of dummies. Experimental application of **artificial plastic dummies of Griffon Vultures** placed on suitable rocks attracts the birds. At the same time the dummies have a habitational and repellent effect on Golden Eagle, which after repeated unsuccessful attacks on them habituated to their presence and stoped attacking vultures or left the area.

Power lines have been proven to have a significant negative impact on the vultures released. Electrocution resulting from landing on electricity pylons proved to be a serious problem. We therefore recommend the development of a methodology for pre-release training of the vultures in the aviary before releasing them into the wild. This approach is enshrined in the Conservation ethology and has given good results in other species.

CONCLUSION

Based on the preliminary analyzes conducted in the present study we can say that conservation ethology seems to be a valuable tool assisting the reintroduction of Griffon Vultures. Griffon Vulture is a long-lived species with low productivity. This means that the single individual and its adaptive strategies are of great importance for the survival of the population of the entire species. The relationship between the different stages of the reintroduction process and the predictive importance of the early stages for the success of the release are valuable areas

for future studies. The terminal stages are representing the real reintroduction success. Conservation ethology methods may prove to be an important tool for planning specific individual activities for particular birds. They probably are a valuable tool for managing the behavior of the species through conservation methods of ethology.

Acknowledgements: The research team would like to thank all who contributed to the success of reintroduction of the Griffon Vultures in Bulgaria.

REFFERENCES

1. Bahat, O. A. Kaplan. 1995. Foraging behaviour in Griffon vultures. Torgos 25. 18–26.
2. Bögel, R. 1999: Studies on flight biology and habitat selection of Eurasian Griffon Vultures *Gyps fulvus*, Hablizl (1783) as measured by telemetry techniques. - Vulture News 41. 49–51.
3. Bosé, M., Sarazzin, F. 2007. Competitive behaviour and feeding rate in a reintroduced population of Griffon Vultures *Gyps fulvus*. Ibis 149: 490–501.
4. C. García-Ripollés, P. López-López, V. Urios 2011. Ranging Behaviour of Non-Breeding Eurasian Griffon Vultures *Gyps fulvus*. A GPS-Telemetry Study. Acta Ornithologica Dec 2011 : Vol. 46, Issue 2, 127-134 p.
5. Cramp, S. and Simmons, K.E.L. 1980. The birds of Western Palearctic. Oxford University Press, Volume II, 695 pp.
6. Dewsbury, D. 1985 (ed). Studying Animal Behavior. Univ. of Chicago Press. 512 p
7. Dobbs, R.C., Sillett, T.S., Rodenhouse, N.L., Holmes, R. 2007. Population density affects foraging behaviour of male Black-throated Blue Warblers during the breeding season. Journal of Field Ornithology 78, 133–139.
8. Donázar, J.A. 1993. Los Buitres Ibericos, Biologia y Conservacion. In: Reyero, J.M. (Ed), Madrid, 256 pp. (In Spanish).
9. Glutz Von Blotzheim, U., Bauer, K.M. & Bezzel, E. 1971. Handbuch Der Voegel Mitteleuropas. Volume 4. Falconiformes. - Akademische Verlagsgesellschaft. Frankfurt am Main, 943 pp. (In German).
10. Hiraldo, F., J.A. Donázar. 1990. Foraging time in the Cinereous Vulture *Aegypius monachus*: seasonal and local variations and influence of weather. Bird Study 37, 128–132.
11. Houston, D. 1974. Food searching in griffon vultures. East African Wildlife Journal 12. 63–77.
12. Houston, D. 1979. The adaptation of scavengers. In: Singlair, A.R.E. and Norton-Griffiths, M. (Eds.); Serengeti: Dynamics of an Ecosystem Chicago University Press, Chicago, pp. 263–286.
13. Hutto, R.L. 1990. Measuring availability of resources. Studies in Avian Biology 13, 20–28.
14. König, C. 1974. Zum verhalten spanischer Geier an Kadavern. - Journal of Ornithology 115: 289–320.

15. Lovette, I.J., Holmes, R.T. 1995. Foraging behaviour of American Redstarts in breeding and wintering habitats: implications for relative food availability. Condor 97: 782–791.
16. M. Bose, F. Sarrazin , 2007. Competitive behaviour and feeding rate in a reintroduced population of Griffon Vultures *Gyps fulvus,* Ibis, Vol. 149, Issue 3, 453–669 p.
17. Pennycuick, C. 1972. Soaring behaviour and performance of some East-African birds, observed from a motor-glider. Ibis 114. 178–218.
18. Pennycuick, C.J. 1983. Effective nest density Rüppell's Griffon Vulture in the Serengeti Rift Valley area of Northern Tanzania. In: Wilbur, S.R. and Jackson, J. A. (Eds.). Vulture Biology and management. University of California Press, Berkley, pp. 172–184.
19. Pennycuick, C.J. 1989: Bird Flight Performance. A practical Calculation Manual. Oxford University Press, Oxford, 153 pp.
20. Peshev, H. E. Stoynov, A. Grozdanov, N.Vangelova. 2015. Reintriduction of the Eurasian Griffon Vulture Gyps fulvus in Kresna Gorge, Southwest Bulgaria, 2010-2015. FWFF Conservation science series. Book 3, 110 p.
21. Prinzinger, R., Nagel, B., Bahat, O., Bögel, R., Karl, E., Weihs, D. & Walzer, C. 2002. Energy metabolism and body temperature in the Griffon Vulture (*Gyps fulvus*) with comparative data on the Hooded Vulture (*Necrosyrtes monachus*) and the White-backed Vulture (*Gyps africanus*). Journal of Ornithology 143. 456–467.
22. Ruxton, G.D. , D.C. Houston, 2002. Modelling the energy budget of a colonial bird of prey, the Rüppell's griffon vulture, and consequences for its breeding ecology. African Journal of Ecology 40. 260–266.
23. S. Xirouchakis, G. Andreou. 2009. Foraging behaviour and flight characteristics of Eurasian griffons Gyps fulvus in the island of Crete, Greece. Wildl. Biol. 15. 37-52
24. Sillett, T.S., Rodenhouse, N.L., Holmes, R.T. 2004. Experimentally reducing neighbour density affects reproduction and behaviour of a migratory songbird. Ecology 85, 2467–2477.
25. Stoynov E., A. Grozdanov, D. Peshev 2011. First breeding of Griffon vulture (*Gyps fulvus*) during reintroduction activities in Kresna gorge. Youth scientific conference "Kliment's Days", November 2011. Conference proceedings: 104-106.
26. Stoynov E., H. Peshev 2011. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2010, Fund for Wild Flora and Fauna, Blagoevgrad.
27. Stoynov E., H. Peshev 2012. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2011, Fund for Wild Flora and Fauna, Blagoevgrad.
28. Stoynov E., H. Peshev 2013. Re-introduction of Griffon Vulture (*Gyps fulvus*) in Kresna Gorge of Struma River, Bulgaria, Annual Report 2012, Fund for Wild Flora and Fauna, Blagoevgrad.
29. Stoynov, E., A. Grozdanov, H. Peshev, D. Peshev. 2013. Present distribution and conservation specifics of the Egyptian vulture (*Neophron percnopterus* Linnaeus, 1758) in Southwest Bulgaria. Bulg. J. Agric. Sci., Supplement 2, 19: 259–261.
30. Stoynov E., A. Grozdanov, S. Stanchev, H. Peshev, N. Vangelova, D. Peshev 2014. How to avoid depredation in livestock - theories and tests. Bulg. J. Agric. Sci., Supplement 1, 20: 129–134.
31. Stoynov E., H. Peshev, A. Grozdanov 2014. Rare birds of prey observations in Kresna gorge in Bulgaria. *Vulture news*: 66: 56-59.

32. Stoynov, E., H. Peshev, A. Grozdanov, V. Delov, N. Vangelova, D. Peshev. 2015. New data for the presence and numbers of some conservation dependent birds in Kresna gorge with proposal of original method for individual identification of vultures. First National Conference of Biotechnology, Sofia 2014. Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie, vol. 100, livre 4, pp. 320-331.
33. Tinbergen, N. 1963. On aims and methods of Ethology. Zeitschrift für Tierpsychologie **20** (4): 410–433.
34. Ward, P., A. Zahavi. 1973. The importance of certain assemblages of birds as 'information centres' for food-finding. Ibis 115, 517–534.