

# STUDY AND MONITORING OF BIG CATS IN RUSSIA



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This monograph provides a comprehensive review and analysis of the available literature on the monitoring of big cats. Special attention is paid to the most up-to-date methods based on recent advances in technology, resulting in useful tools to remotely and non-invasively study animals in natural habitats, essential when working with rare species. Existing large- and small-scale approaches to monitoring big cats are described. Methods of monitoring the habitat conditions of the species and their dynamics, as well as the basics of modeling territories with suitable conditions for leopards, are suggested. The whole range of field sampling methods that enable data to be processed using contemporary techniques is described. Moreover, methods of processing collected data (obtained via GPS collars, photo and video recorders, and hormonal and molecular genetic analysis) as well as examples of results are considered.

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## FOREWORD

Over the last decade, populations of rare mammal species, particularly big cats, have been intensively studied and restored in Russia. For this purpose, the Permanent Expedition of Russian Academy of Sciences for study of Russian Red Data Book animals and other key animals of Russian fauna included into A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences was organized. In the framework of the Permanent Expedition, the Severtsov Institute of Ecology and Evolution runs a number of projects on big cats that are rare not only in Russia but also globally, specifically the Amur tiger, the Amur leopard, and the Snow leopard (Rozhnov et al., 2010c, 2012c; Paltsyn et al., 2016). Thanks to efforts to restore the Amur tiger population in the north-west of the Russian part of its range, a method of rehabilitation and releasing orphan tiger cubs back into the wild was developed, and has proved effective (Rozhnov et al., 2011f, 2015b). Female tigers released in the Amur and Jewish autonomous regions of Russia have already produced offspring and have thus formed a new group of this species. The reintroduction of the Caspian tiger in Central Asia and Kazakhstan is currently under discussion (Rozhnov et al., 2012a; Chestin et al., 2017). The program of restoring (reintroducing) the Caucasian leopard in the Caucasus is also in progress (Rozhnov, Lukarevskiy, 2008): the first animals released by the Leopard Recovery Center in the Caucasus have successfully adapted to living in the wild. These studies were published in the book of abstracts of the International Workshop on Rehabilitation and Reintroduction of Large Carnivores, held in Moscow on 25–27 November 2015 (Proceedings..., 2015), and in the monograph on the snow leopard (McCarthy, Mallon, 2016) published in 2016, which paid considerable attention to our work on the rehabilitation and reintroduction of the Amur tiger as a method to restore populations of big cats (Miquellå et al., 2016). Intensive work is ongoing in the Land of the Leopard National Park, where the number of Amur leopards is increasing thanks to enhanced protection measures.

Such works require constant attention to the ongoing processes as well as to the condition of animals released into the wild. Recent technological advances enable the animals to be monitored not only by traditional methods, such as snow tracking, but also by collecting various objective data on rare species' habitat conditions and processes, as well as regarding individual animals and the well-being of restored populations in general. The spread of mobile communications, telephony and the Internet has facilitated new means of collecting data in the field and preparing them for further analysis.

This situation also requires new approaches to organizing the monitoring, requiring highly qualified specialists-researchers as well as technical assistants such as the rangers of nature reserves and hunting management departments.

The present monograph sees these specialists as potential readers.

## INTRODUCTION

The evaluation of the success and possible consequences of reintroducing big cats such as the Amur tiger, Amur leopard and Caucasian leopard requires the regular monitoring of the animals and their natural habitats. Starting at the release sites, its scope should gradually encompass the whole putative habitat of the species being reintroduced.

The post-reintroduction monitoring of big cats during the process of recovering the population is essentially a standardized evaluation system that is used to track possible changes in population parameters, habitat and potential prey base, as well as the condition of individuals and the progress of the species' competitors. It aims to predict their future dynamics and assess the efficiency of particular environmental strategies implemented as part of large-scale conservation and reintroduction projects. Regular monitoring and measuring a set of environmental indicators on the model territories can reveal the ecological mechanisms underlying environmental changes and individual resilience. This can help distinguish the key factors influencing the progress of the reintroduced animals, permitting prediction of both the short- and long-term parameters of population dynamics.

The reintroduction of carnivores should be carefully assessed to confirm its *long-term, short-term and individual success* (Beck et al., 1994; Sarrazin, Barbault, 1996; Hayward et al., 2007a, b). To qualify as a *short-term success*, the reintroduced individuals should be able to hunt natural wild prey effectively, avoid conflicts with humans, establish a home range territory, and breed successfully. Moreover, in the first three to five years after release, the birth rate of the original group and their offspring should exceed the mortality rate. For a *long-term success*, the population should reach the size deemed optimal for the area (based on the required prey base and the ecological capacity of the environment) and remain stable without human intervention. The criteria for a *short-term and individual success*, which for large carnivores is measured during the early stages of reintroduction, are largely the same: sufficient hunting skills; ability to avoid conflict (with humans and conspecific/heterospecific competitors; steady breeding rate and breeding success; and establishing a home range (within the first two years for reintroduced males, which tend to spread sooner after release).

The scale and precision of analysis may range from individual tracks to changes in the local ecosystems in which the animals live.

Regular monitoring is necessary as it provides immediate answers to the following questions:

- What is the current status and distribution of the species under consideration?
- What can be done to ensure and control the preservation of the endangered species?

- What means of population protection and control would demonstrate the greatest efficiency in a particular case, and do the steps currently undertaken to this end actually work?

The monitoring of big cats begins primarily with estimating the size and distribution of the population, which includes the area occupied by the individuals; their actual documented or potential presence (or absence) there; the territories used permanently or temporarily (i.e., important travel routes unsuitable for constant habitation); anthropogenic pressure such as the expansion of highways and livestock grazing; population or group density; age and sex structure of the population; and its abundance and prey distribution. These data indicate the main trends in population conditions, such as temporal changes in site usage and the number of animals sharing it, as well as the frequency of recording. Another important factor to be considered is animal welfare: the presence of pathogens in the area; epizootic spread and dynamics; genetic diversity and genetic drift within the population; and the animals' nutritional status and stress levels.

The present work overviews the contemporary methods used in felid monitoring, and offers guidelines developed at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (IEE RAS), the A.K. Tembotov Institute of Ecology of Mountain Territories of the Russian Academy of Sciences (IEMT RAS), and the Caspian Institute of Biological Resources of Dagestan Scientific Centre of the Russian Academy of Sciences (PIBR DSC RAS). The recommendations are based on studies of big cats and other rare mammals carried out during the Permanent Expedition of Russian Academy of Sciences for study of Russian Red Data Book animals and other key animals of Russian fauna.

The authors would like to thank the citizens of the Republic of Dagestan: the Chunkov family from the Khunzakh village; Gitinov I.K., director of the Urada secondary school; Magomedov B.M., teacher at Kosob secondary school; Nasrulayev N.I. and Magomedov M.M., members of PIBR DSC RAS who helped organize expeditions in the East Caucasus. We would also like to express our gratitude to Gatziyev M.M., Director of "Alaniya National Park"; Dzekoyev A.Y. and Dzhikayev A.M., rangers of the Park; Popov K.P., Weinberg P.I., Komarov Y.E., Dzutsev Z.V., Butayeva F.G., Butayeva L.B., scientists and members of the "North Ossetian Natural Reserve"; senior scientist of IEMT RAS Zhashuyev A.Z.; Sozanov Z.U., Director of North Ossetia Governmental Game Management; Alibekov A.B. and Nemoytina T.V. from PJSC "RusHydro" and Getoyeva Z.K. from the North Ossetian branch of the RusHydro Company for their assistance during our research expeditions in the Republic of North Ossetia-Alania.

## I. APPROACHES TO MONITORING BIG CATS

The monitoring of big cats can be classified into *large-scale* and *small-scale*.

For *large-scale* monitoring, any possible information (such as cases of the animals' visual detection or footprints, scratches, tracks on the snow or wet soil, leopard predation on livestock, the wild prey remains in the forest and roadkills) must be collected and mapped in GIS (see von Arx et al., 2004; von Arx and Zimmerman, 2012; Cullen et al., 2016; Molinari-Jobin et al., 2010; Jêdrzejewski et al., 2017). These data are mostly collected through surveying the members of social groups who frequently interact with wildlife, including shepherds, hunters, tourists and nature reserve rangers. Various types of sources should be analyzed to represent the historical range of the species on a GIS map, which would retrospectively reveal the correlation between human-induced habitat modification and population extinction, and ultimately help predict reintroduction success. The basic data for species habitat modeling can be obtained via remote sensing, which takes into account the topographic and climatic features (such as precipitation, snow accumulation and melting), spatial structure of ecological communities, and spatial assessment of anthropogenic (infrastructural, industrial, recreational and agricultural) load on the territory.

Being more detailed, *small-scale monitoring* is rooted in individual data obtained via the short-term tracking of particular individuals. It includes the following methods: recognition through photos from camera trapping surveys, snow tracking, and podometry (Hernandez-Blanco et al., 2005), VHF and satellite tracking (Zimmerman, 2012), analysis of prey hairs and DNA in scats and individuals' molecular and genetic identification, the animals' physiological status, behavior, and the frequency of preference of particular attractants and concentration spots in the biological signal field.

Satellite tagging has become a widespread means of monitoring migrating vertebrates in poorly accessible territories. In Russia it is used to tracked terrestrial mammals such as some rare carnivores and ungulates species (Duplaa et al., 2011; Rozhnov, Salman, 2011), among them the Amur tiger (Rozhnov et al., 2010a, 2011c, 2014; Hernandez-Blanco et al., 2011, 2015a), the Amur leopard (Rozhnov et al., 2011e, 2015a), the snow leopard (Kuksin et al., 2015; Munkhtsog et al., 2015), the polar bear (Rozhnov et al., 2015d; Platonov et al., 2014), the saiga antelope (Rozhnov et al., 2013a), and the European bison (Chistopolova et al., 2012). A new insight into habitat use can be gained by combining satellite tagging and remote sensing, i.e., the integration of satellite tracking data and satellite imagery to obtained a detailed characteristic of wildlife habitat (Dobrynin et al., 2015, 2017).

The main tool for both *long-term* and *short-term* monitoring is GPS collaring, which offers superior opportunities for the monitoring of the reintroduced individuals than VHF telemetry (Devineau et al., 2010; Rozhnov et al., 2014; Chistopolova et al.,



2015a), which was used until the beginning of the twenty-first century (Breitenmoser et al., 2006; Hayward et al., 2006; Sankar et al., 2010).

Aside from verifying reintroduction success, GPS collars regularly provide unique data on individuals' behavior and movements, enabling researchers to track animals immediately based on the received transmitted location, thus saving time in locating the animal. Tracking also helps establish behavioral patterns that cannot be revealed via telemetry, such as hunting behavior (stalking or running down the prey), distance of attack, preference of prey species, prey age and size of prey population.

As an interesting example, we should mention the role of GPS in confirming how big cats may affect the distribution of wild canids like wolves. In the past, there was no solid evidence to prove this point, although some hypotheses were advanced. In one clear case, the tracking of a reintroduced GPS collared female tiger in the Khingan Nature Reserve yielded positive evidence of accidental wolf kills, followed by regular tiger hunting and a subsequent change in the home range of the wolf pack (Kastrikin et al., 2015). This proved the previously observed yet unverified correlation between the increase of tiger presence and the decrease or even complete relocation of wolves (Miquelle et al., 2005; Yudin, Yudina, 2009), thus corroborating the aforementioned hypothesis. A wolf pursued by a female leopard during 2016 was also observed in the Caucasus Nature Reserve and was confirmed by this observation as well. Leopard male released in North Ossetia in 2018 hunted seven jackals as our monitoring data confirmed.

Moreover, tracking (especially snow tracking) provides an opportunity to reveal an animal's preferred snow depth on the chosen route, as well as characteristic habitat features and macro- and micro-terrain. Comparing the results with data from meteorological stations and multispectral satellite photography, and applying geostatistics and other GIS data to evaluate weather station networks, facilitates the selection of regions with snow depth suitable for future reintroduction. A similar strategy can be applied to reveal habitat-seasonal preferences (Dobrynin et al., 2017).

Camera traps (trailcams) are yet another important monitoring tool, especially for assessment of the physical condition of the reintroduced animals (Carbone et al., 2001; Wegge et al., 2004; Jackson et al., 2005; Karanth et al., 2006; Soisalo, Cavalcanti, 2006; Dillon, Kelly, 2008; Royle et al., 2009). Modern methods of analyzing data from camera traps can advance our understanding regarding the density of potential prey's population (Rowcliffe et al., 2008) and abundance rate (individuals per camera hours; see Naidenko et al., 2011b; Rozhnov et al., 2011e, 2012 b), as well as the abundance of species with similar ecology and patterns of mutual space use (Nagy-Reis et al., 2017). Estimating the population density of any mammal species by using camera traps and capture-recapture sampling can work in combination with GPS radio-telemetry. Even in the case of collar malfunction, it is possible to assess the reintroduced animal's condition and space use via an established matrix of camera traps, this also representing the only way to continue monitoring once a GPS collar has yielded every possible kind of data (O'Connell et al., 2005). Using GPS collars over an extended period of time can also yield information regarding the breeding success of reintroduced animals. For instance, camera traps in the Bastak Nature Reserve

(Jewish Autonomous Region) regularly recorded not only the reintroduced female (*Zolushka*) being in a good condition, but a resident male as well (Kalinin et al., 2015). The interaction analysis in recorded locations confirmed attraction between animals. This took only half a year following her release. Two and a half years later she proved to be the first female Amur tiger to breed successfully after reintroduction. Moreover, the working matrix of camera traps recorded a second breeding two years after the first (and 4.5 years post-release respectively). Similarly, in the Zhuravliny Nature Reserve (Jewish Autonomous Region), the meeting and mating of two released tigers (tiger *Borya* and tigress *Svetlaya*), which resulted in offspring, was recorded by trailcams.

Another tool used to evaluate reintroduction success is noninvasive hormonal monitoring, which can be used at all levels of general monitoring. As a rule, it is based on the levels of cortisol, progesterone and prostaglandin metabolites in the feces, which helps assess stress levels (Fanson et al., 2011) and (for females) the reproductive status. However, this method requires preliminary validation; so far it has been carried out both for the tiger and the leopard (Rozhnov et al., 2010b; Naidenko et al., 2011b; Ivanov et al., 2011, 2014). Furthermore, it reveals those areas with more favorable climatic conditions for reintroduction based on cortisol levels in resident individuals (if they inhabit this area) from different areas (Naidenko et al., 2011b; Ivanov et al., 2017). Reproductive success may be linked to stress levels as well. For instance, when the reintroduced Bengal tigers in Sariska National Park failed to breed, the investigation of possible physiological causes showed significantly increased levels of glucocorticoid metabolites in the feces collected in spots characterized by the proximity of livestock, villages and roads to tigers' home range (especially in females), thereby influencing their reproductive success (Bhattacharjee et al., 2015).

The main advantage of hormonal monitoring is its convenience, because a single female sample can be used to determine stress levels and reproductive status simultaneously. The analysis of prostaglandin metabolites can accurately differentiate pregnancy versus pseudopregnancy, which can last up to 70% of the normal term in large felids (Finkenwirth et al., 2010). Thus, the method is highly suitable for assessing reproductive success in released animals.

Molecular phylogenetic analysis is another noninvasive method used to measure reintroduction success. If the genotypes of reintroduced animals are different from those of resident individuals (based on previously collected samples), the contribution of the former to the genotypes of their offspring can be measured. The method can also be used to establish the size of the population under analysis (Rozhnov et al., 2013b).

Furthermore, molecular phylogeny of rare species is particularly important for measuring the genetic polymorphism of the major histocompatibility system, which largely determines the state of the animals' immune system (Tarasyan et al., 2014, 2015).

Multiple studies demonstrate that infectious diseases significantly affect the viability of reintroduced felids. Their frequency and means of transmission, especially in the Far East of Russia, have been carefully studied (Goncharuk et al., 2010, 2011, 2012a, b; Naidenko et al., 2011a, 2012, 2015 b).

Traditional methods of field zoology such as field tracking also play a role in monitoring reintroduced carnivores. Special attention should be paid to nutrition as a major factor determining reintroduction success. Indeed, when reintroducing the Amur tiger in northwest Russia, we regularly compared the nutrition of the reintroduced young tigers with that of the resident animals (Rozhnov et al., 2010; Miquelle et al., 2015a).

Overall, a number of instrumental and noninvasive monitoring methods beyond traditional tracking have been developed to collect reliable data on the reintroduced animals (where possible; see Rozhnov et al., 2009a). These methods, alongside biological sampling and analysis, are essential in order to adequately assess reintroduction success in big cats such as the Caucasian leopard.

## 2. METHODS OF MONITORING BIG CATS AND THEIR HABITATS, PROCESSING THE COLLECTED DATA AND ANALYZING THE RESULTS

This chapter describes the basic approaches and principles of monitoring big cats, both large-scale and more detailed. Monitoring not only includes the animals themselves, but also their habitats, which experience considerable anthropogenic impact and change in time underlying their suitability for leopards.

Various instrumental and non-invasive methods as well as satellite image analysis are used for monitoring. The use of surveys for local respondents constitutes a crucial component of this work. Processing the data derived from these surveys results in the least detailed but most extensive monitoring of local encounters with leopards.

Advance selecting potential sites for foot monitoring is based on literature. Then this selecting is clarified with the animal's ecology, the peculiarities of its hunting, the habitats and ecology of its prey, as well as data from local residents' surveys and the literature in which such encounters have been documented. This plan is elaborated by means of remote sensing data. These are automatically classified with further calibration of classes according to their suitability for animals or animals' preferences. Classification is based on spatial and statistical approaches. Interpreting and automatically analyzing remote sensing data obtained for the same territory in different seasons enables the sites for further field study to be characterized in detail. During fieldwork, geomorphological peculiarities (the forms of micro- and meso-relief preferred by animals) as well as climatic and meteorological factors (the distribution and duration of snow cover, snow characteristics, snow accumulation and melting) should be specified, because snow is a limiting factor for the distribution of big cats and their prey. The vegetation type (qualitative and quantitative vegetation survey), the distance between study sites and sources of potential danger and anxiety factors, and the degree of anthropogenic transformation of the studied part of the range should also be noted. The sources of potential danger and anxiety factors include transport linear structures (roads, pipelines, and power transmission lines), as well as industrial and agricultural facilities and residential areas.

Terrestrial monitoring includes expeditionary research when the sites where the animals may be observed are tested and their traces are mapped. It is conducted: (1) in winter (whenever possible) and used for the feline species that inhabit places with steady snow cover in winter, such as the Eurasian and Canadian lynxes, the Amur tiger, and the Far Eastern leopard; (2) in summer along the banks of water courses and at sites where the elements of biological signal field are concentrated, and where the identified attractors are present; (3) in autumn or spring when during the rainy

season paws may be printed on wet open ground. In the course of such monitoring, data regarding the relief and ecosystems used by animals, their behavior (social, marking, hunting), prey base, the system of biological signal fields organizing the space that the individuals of the studied species inhabit, and their genetics and physiological state, are all accumulated. This process enables the population size, age, sex and spatial structure to be characterized.

This is the combination of methods that enables vast territories to be explored in a relatively short time, a definite advantage of applying several methods in parallel. Thus, remote sensing data reveal potentially suitable sites for further detailed study, and terrestrial monitoring adjusts and specifies these data.

Consideration of the long-term plans for the social and economic development of the territories, including potentially suitable habitats for the Caucasian leopard, represents a crucial aspect of monitoring a population that is to be restored or has already been formed. To this end, close interaction with the authorities responsible for territorial planning is required.

In order to assess anthropogenic transformation in the region, the large-scale monitoring of vast areas based on remote sensing data should be conducted once every 10–15 years. Selected areas appropriate for regular terrestrial monitoring should be studied every three years to understand how the situation is changing. Choosing a relatively small model area in the region suitable for the study of seasonal processes (as well as individual processes in focal individuals for further extrapolation of results) is advisable.

## 2.1. MONITORING BIG CATS' POTENTIAL HABITATS

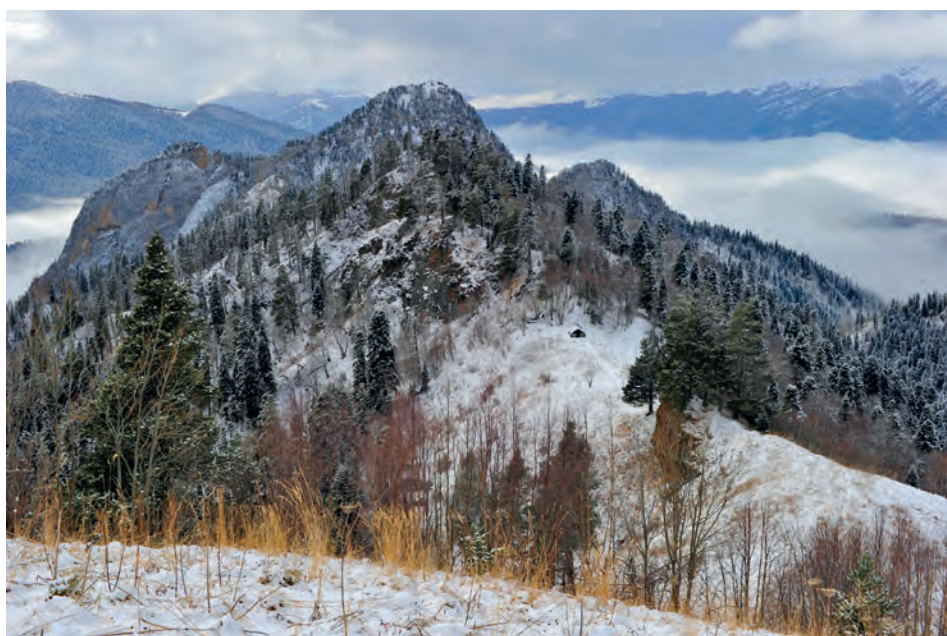
### 2.1.1. MONITORING THE CAUCASIAN LEOPARD'S POTENTIAL HABITATS

In the Caucasus, mountain habitats are typical of the Caucasian leopard (**Figures 1 and 2**). According to zoologists studying the Caucasus fauna (Dinnik, 1914; Geptner, Sludsky, 1972), the leopard inhabits subalpine steppe meadows, deciduous forests, and dense shrublands. It has also been observed in the alpine belt up to heights of 3.000–3.500 m a.s.l. Large numbers of ungulates, namely potential leopard prey (Ibex/Caucasian wild goat, turs, chamois, red deer, roe deer and wild boars) are supposed to be the main requirement for habitat suitability given the high plasticity of the species. In winter, when the snow is deep (up to 1 m), the leopard stays in the highlands (1.400–1.500 m a.s.l.), close to the chamois wintering grounds. On the Black Sea slopes of the Western Caucasus, the leopard inhabits virtually impassable shrublands of cherry laurel, azalea, Caucasian whortleberry, and pontic rhododendron. In the Transcaucasia it prefers almost bare rocky patches, rarely appearing in deciduous forests and riparian woodland along water courses.

The main limiting factor for the species in the Caucasus is the distribution and abundance of the prey base (especially the ungulates), as well as various characteristics of the snow cover, specifically its depth, duration, setting and loss time.



**Figure 1.** The Caucasian leopard's habitat in the Western Caucasus: Caucasus Nature Reserve near the Maliye Balkany Ridge.



**Figure 2.** The Caucasian leopard's habitat in the Western Caucasus: Caucasus Nature Reserve, the Mastakan Ridge (above), the Afon Ridge (below).

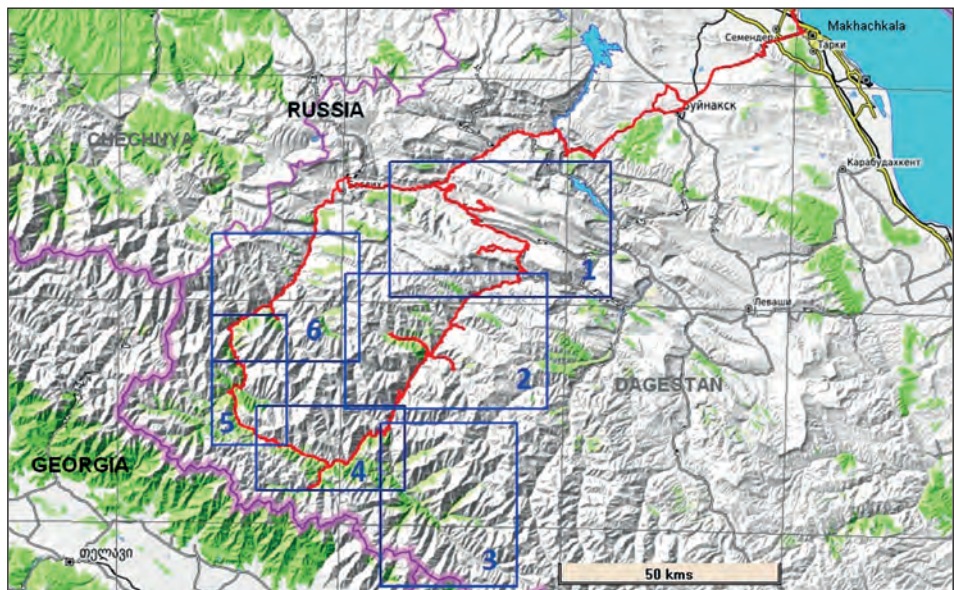
Recent observations of leopards released into the Caucasus Nature Reserve in 2016 have proved their successful adaptation. They have spread across enormous stretches of mountains and foothills in the Maykop Region of the Republic of Adygea, the Mostovsky and Otradnensky Districts of the Krasnodar Krai, and the Urupsky District of the Karachay-Cherkess Republic, hunting wild ungulates such as deer, tur and chamois.

Regular expeditions also play a significant role in monitoring the Caucasian leopard's habitat. The data gathered during the expeditions should be compared with ERS data. Thus, using preliminary ERS evaluation, a set of regions of the Russian part of the Caucasus where expeditions are to be sent has been established (see 2.1.1).

Studying the habitat of the Caucasian leopard in the Eastern and Central Caucasus (the Republic of Dagestan – see Rozhnov et al., 2017; the Republic of North Ossetia-Alania) demonstrates the role of these expeditions. It includes evaluating the state of the prey base and habitats, as well as the extent of anthropogenic impact on them and the whole system of nature reserves. The results have been used for further modeling of the leopard's potential habitats in the Caucasus.

The main focus of study in Dagestan was its southwestern part, known to be the leopard's main habitat (**Figure 3**).

The mountain regions of the Republic of Dagestan are part of the leopard's historical habitat in the North Caucasus. Occasional encounters are still registered in this region, as the areas of suitable habitat have partially been preserved. Moreover, the



**Figure 3.** The route of 2017 habitat inspection expedition into southwestern Dagestan. 1 – the northwestern ledge of the Khunzakh Plateau; 2 – the Avar Koisu Valley; 3 – the Dzhurmut Basin; 4 – the Bezhtin Basin; 5 – the Shaurin Basin; 6 – the Andi Koisu Valley.





**Figure 4.** The Caucasian leopard's historical habitat in Dagestan: the Avar Koisu Valley, the Kosob-Keleb Nature Reserve, near Magit village (above); the Tsuntin Region, valley of the Metlyuta River, flowing into the Andi Koisu Valley (below).



**Figure 5.** The Caucasian leopard’s historical habitat in Dagestan: the Bezhtin Basin, valley of the Simberaz-Khevi River.

mountain territories of Dagestan's borders with Azerbaijan and Georgia also include habitats suitable for big cats.

To facilitate analysis and further comparison, the territory was divided into six highland landscape complexes in various regions of inland and highland Dagestan (**Figure 3**), which belong to the historical area of the Caucasian leopard, with recent habitat changes taken into consideration. The distinction was made according to the actual administrative division and the planned route of the expedition, consisting of several stages.

The route covered more than 3,400 km in total, including the following areas: the northwestern ledge of the Khunzakh Plateau (Khunzakh and Akhvakh districts), the Avar Koisu Valley (Shamil and Tlyaratin regions), the Dzhurmut Basin (Tlyaratin region), the Bezhtin Basin (Tsuntin region, Bezhtin area), the Shaurin Basin (Tsuntin region) and the Andi Koisu Valley (Tsumadin region).

Among the factors taken into account were the type and state of the canopy cover and the distribution of the leopard's major prey: Ibex/Caucasian wild goat, Dagestan tur, red deer, chamois, roe deer and wild boar. For every area on the map, the habitat condition, intactness and connections (the presence of ecological corridors between areas) were investigated. Moreover, local inhabitants were questioned about seasonal snow patterns. Key biotope categories were singled out, and GPS locations recorded; the state of the prey base was evaluated (i.e., species diversity and abundance, and seasonal preference of particular areas). Population density and possible human disturbance were investigated according to the number of settlements and their overall population, as well as farming intensity, including the types of agricultural activity and livestock numbers (sheep, goats, cattle and horses). The presence of nature or game reserves and current conservation progress was taken into consideration too, and the need for biotechnical intervention and increased public outreach was assessed.

The Caucasian leopard's habitats in North Ossetia as well as the adjacent territory of the Kabardino-Balkar Republic (to be used for further modeling) were studied in autumn 2017 and spring 2018 (**Figures 6–8**).

The Republic of North Ossetia-Alania (**Figures 6–8**) is known for its varied terrain, featuring great ranges of absolute and relative altitudes as a result of a long geological history. Its largest orographic elements are the Ciscaucasian plains in the north and the Greater Caucasus in the south. The mountainous part of the Republic consists of the Lesisty, Pastbischny, Skalisty, Vodorazdelny and Bokovoy ridges. During the expedition, the valleys and gorges of major rivers such as the Uruk, Ardon, Fiagdon, Terek, Gizeldon and Genaldon were inspected, as well as the sites of leopard encounters. A GIS mathematical model of local landscapes and biotopes was developed on the basis of field data.

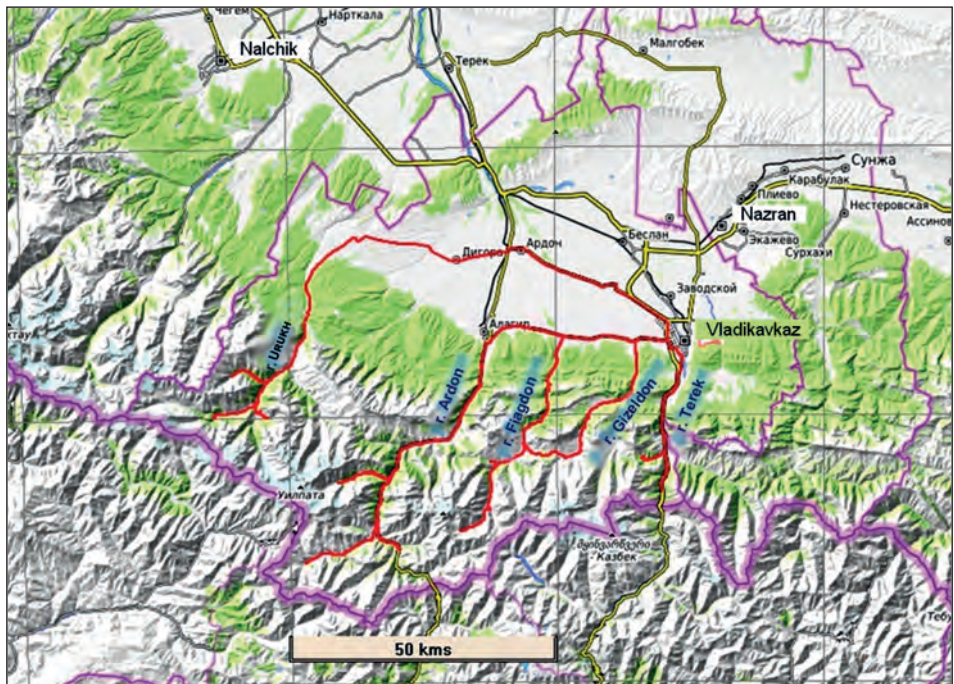
The range of biotope classes was explored during the expedition, and GPS mapping used thereafter to depict the main types of vegetation forming communities on terrains varying in slope, exposition and consequently light. The evidence provided by local zoologists formed the core of a database containing numbers of leopards' potential prey (tur, chamois, red deer, wild boar, roe deer, European bison). The local



**Figure 6.** The Caucasian leopard's historical habitat in North Ossetia: the Zadalessk Basin, near Zadalessk village (above); Alaniya National Park, near Mount Laboda on the edge of the Tchifandzar Swamp (below).



**Figure 7.** The Caucasian leopard's historical habitat in North Ossetia: the Fiagdon Gorge, valley of the Gizeldon River (above); Tsey National Park (protected area), the Lesisty Ridge (below).



**Figure 8.** The route of the 2017 Caucasian leopard habitat inspection expedition into North Ossetia.

inhabitants in turn offered information concerning previous encounter sites, the numbers and seasonal distribution of livestock, and the grazing system. The following parameters were also considered: population density, local wildlife management, and inhabitants' attitudes to leopard reintroduction, as well as their general awareness of leopards' biology, competitor species (wolves, bears, lynx), and prey.

The monitoring of the Caucasian leopard's potential territories is based on its habitat map, including data on habitat conditions; changes in anthropogenic infrastructure and natural complexes, as well as snow cover observations and the animals' dynamic preference of particular areas.

Anthropogenic activity may cause rapid and inevitable changes in landscapes, rendering them inaccessible or dangerous for wild animals. To avoid this, the dynamics of anthropogenic infrastructure should be regularly analyzed. Thus, some predicted changes in the intensity of landscape use could be taken into account, enabling researchers to forecast their impact on both animals and the biotopes on which they depend.

The distribution of animals along the nature complexes varies from season to season. Joint analysis of data obtained from remote sensing and satellite tagging helps establish a retrospective correlation between the seasons and preferred biotopes. Any preferences revealed in the process should be verified and interpreted with reference to field data or high-resolution satellite images.

The depth of the snow cover is a crucial factor restricting the leopard's distribution. It limits the movement of the predator as well as the spatial distribution of its prey: the ungulates. Given that the depth of the snow cover on the Main Caucasian Ridge is greater than on its spurs and secondary ridges – the Pastbischny and the Skalisty Ridges – the latter once formed the leopard's main habitat. However, the main ridge can only serve as a transit or dispersion zone, and solely in the snowless period. Yet, the intricate pattern of areas with abundant or alternatively little or even no snow in winter renders the mountains a suitable wintering ground for both predators and ungulates, enabling them to avoid a long-distance migration.

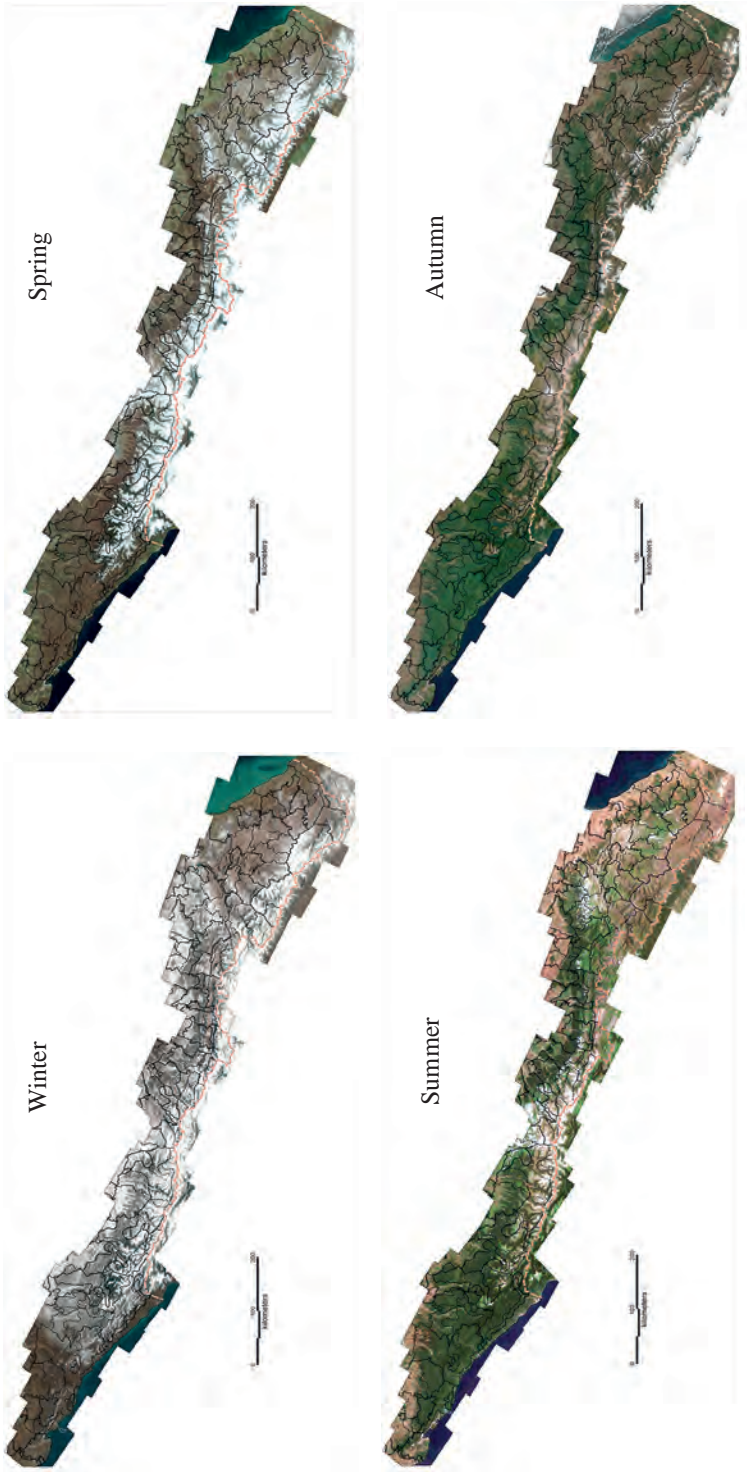
#### 2.1.2. MONITORING HABITAT CURRENT STATUS WITHIN THE CAUCASIAN LEOPARD'S POTENTIAL HABITATS

Historically, the leopard inhabited the highland Caucasus, an area where closely connected elements of the environment follow each other to form systems of vertical zones within particular landscape types (slope – ridge) or their series. The latter is typical of complex terrains, which are characterized by various altitudes, geotectonic and physiographic formative conditions, as well as lithological and age structure. Seven such terrains, known as “geomorphological areas”, have been distinguished in the Western Caucasus (Schiffers, 1946; Sonn, 1946; Chilikina, 1960). These areas can be further divided into natural historical zones characterized by vertical changes of environmental conditions, which can in turn be grouped by similarity. The next unit of spatial categorization is a natural historical area, i.e., a combination of particular climatic, soil and vegetation factors on identical or similar terrains. It is especially important to focus on particular zones when arranging sets of satellite images for modeling. **Figures 9–10** show such a set for the Russian Caucasus.

The first step of the procedure is *selecting* the cloudless mosaics from satellite images in *coarse resolution* (150–250 m spatial resolution). In this case, the whole Russian territory of the North Caucasus is represented in four mosaics in total, one for each season (**Figure 9**). They are further fused into a single *multiseasonal* mosaic (**Figure 10**), which is used to assess the variety and dynamics – seasonal and annual – of the environmental conditions during the final stage of modeling, when preparatory calibration, adjustment and verifying are complete. A proper mosaic provides mass data for future modeling of the leopard's habitat system throughout the potential region. Due to terrain characteristics and heterogeneous climatic conditions, selecting seasonal cloudless fragments is relatively time consuming. Every pixel of the multi-seasonal mosaics contains digital information on the spectral characteristics of each season.

#### *Analysis of anthropogenic pressure within the Caucasian leopard's potential habitats*

Once the comprehensive multiseasonal map is complete, the spatial distribution of anthropogenic pressure on the territory under scrutiny can be assessed and the least encumbered regions identified. The main types of anthropogenic pressure that can be revealed by satellite imaging are as follows: industrial, traffic, agricultural, and rec-



**Figure 9.** Selecting cloudless mosaics from satellite images in survey resolution (150–250 m/pix), four seasons.



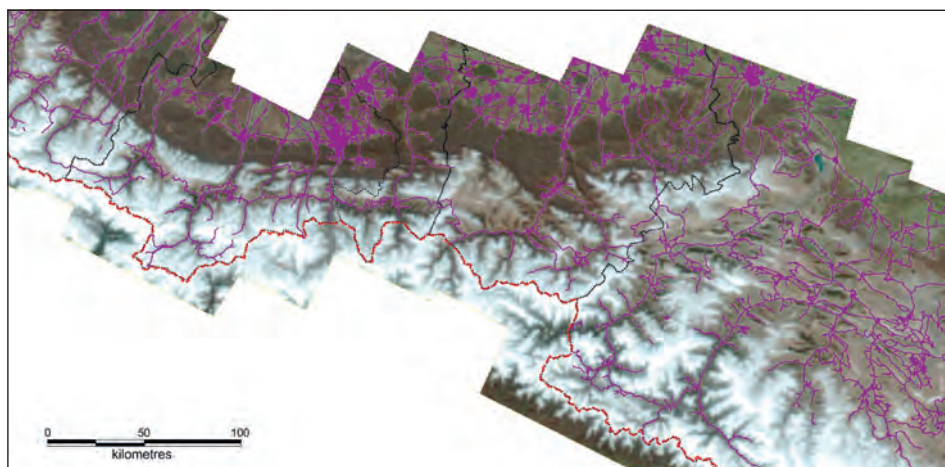


**Figure 10.** Overview of the cloudless mosaic for the Russian Caucasus next to the Main Caucasian Ridge. The mosaic is based on satellite images collected over seven years. A proper mosaic provides mass data for future modeling of the leopard's habitat system.

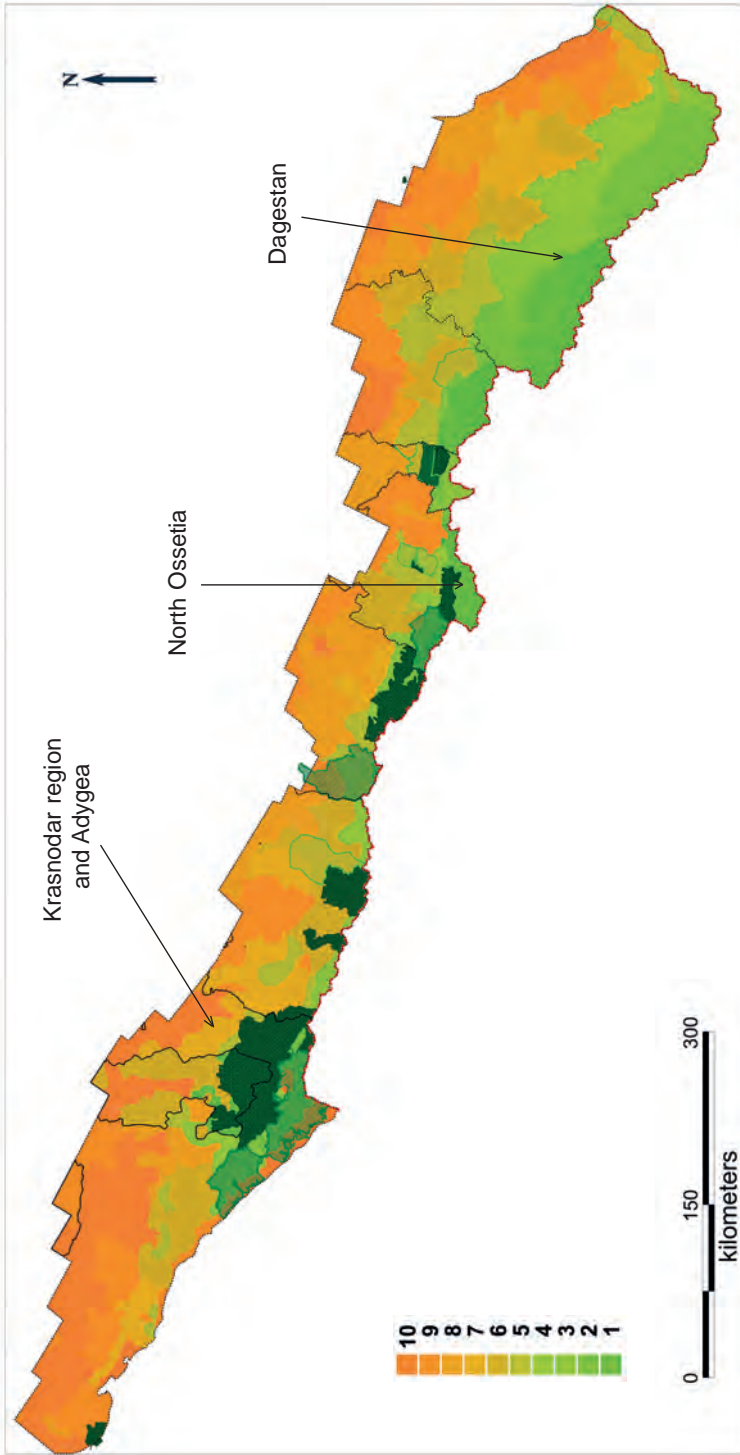
reational. **Figure 11** illustrates an annotated transport network on a satellite image. By mapping the nature reserves on the resulting scheme, a map of territories most/least suitable for leopards can be produced and subsequently converted into a graded scale ranging from the “worst” to the “best” (**Figure 12**). The first stage of processing remote sensing data once completed forms the basis for further steps (**Figure 13**). To model the potential habitat in detail with all possible changes in environmental conditions, some *key areas* least susceptible to anthropogenic pressure can be selected. Similar maps are created for each administrative subject, which subsequently improves logistics in particular regions.

**Figure 12** highlights the least impacted areas in bright green. These are the key areas selected for future work on modeling the leopard’s habitat structure and developing basic methods of projecting the results on the whole territory of the Russian Caucasus. For each key area an orthorectified multiseasonal map is created on the basis of satellite images in *medium* resolution (10-30 m). These maps are used for numerical *modeling* of potential *habitats* within *particular key areas*, relying on spatial statistical analysis of limiting factors and leopards’ habitat preferences. Given that environmental databases contain a huge amount of spatially distributed information, interpretation, analysis and further use should be performed in a systematic way with the utmost care.

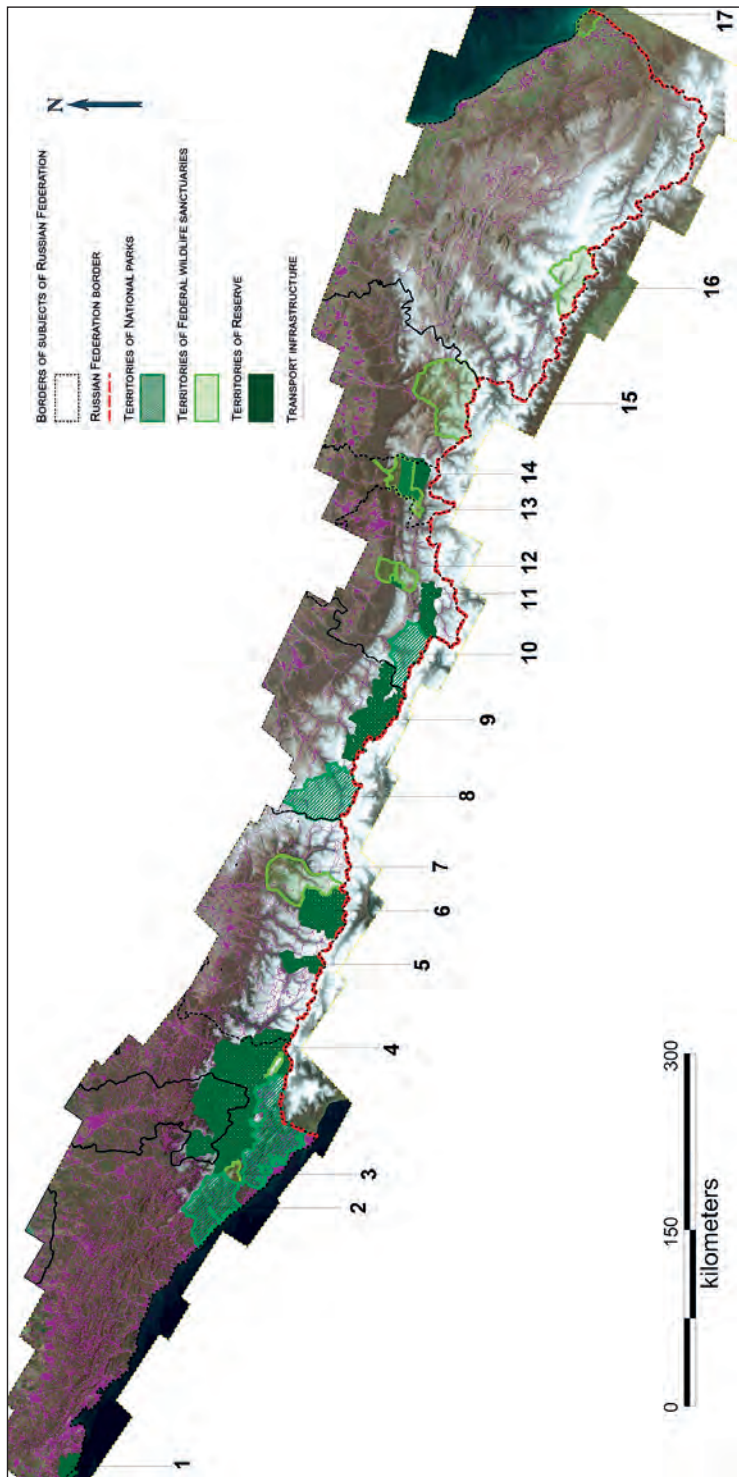
To analyze the dynamics of anthropogenic pressure, relevant satellite images with a high spatial resolution (between 15 and 1.5 m) should be annotated regularly (every 2–3 years). The images are taken in spring, summer and mid-autumn in order to ensure that vegetation (trees and shrubbery) does not shade roads, livestock paths, clearance sites, traces of range or forest fires and possible evidence of illegal recreational and mining activity, forestry practices and hydropower development. The images gathered in summer are used to assess the condition of pastures in view of their possible degradation through overgrazing.



**Figure 11.** A manually annotated transport network on a fragment of the northwestern territory of Russian Caucasus. Bright purple lines represent roads.



**Figure 12.** An anthropogenic pressure map for the Caucasian leopard's potential habitat in the Russian Caucasus (points ranging from 1 to 10).



**Figure 13.** Transport network and nature reserves on the map of the Caucasian leopard's potential habitat in the Russian Caucasus. 1 – the Utrish Nature Reserve; 2 – the Sochi National Park; 3 – the Sochi Preserve; 4 – the Caucasus Nature Reserve; 5, 6 – the Teberda Nature Reserve; 7 – the Daut Preserve; 8 – the Mount Elbrus National Park; 9 – the Kabardino-Balkar Highland Nature Reserve; 10 – Alaniya National Park; 11 – the North Ossetian Nature Reserve; 12 – Tsey Preserve; 13 – the Ingush Nature Reserve; 14 – the Erzi Nature Reserve; 15 – Sovetsky Preserve; 16 – Tlyaratun Preserve; 17 – Samur Preserve.

### *Analysis of non-anthropogenic dynamics of nature complexes within the Caucasian leopard's potential habitat*

This procedure is carried out every 7–10 years by annotating the relevant images with a high spatial resolution between 15 and 0.5 m, taken in spring, summer and mid-autumn to avoid vegetation (trees and shrubbery) concealing hazardous exogenous processes that can seriously interfere with the normal functioning of ecosystems within the leopard's potential habitat in the Caucasus. Such processes include rising water tables and waterlogging, slope erosion and other processes that are uncharacteristic of the area, deflation (wind erosion) and the progressive salinization of the soil. Alongside their activity, non-anthropogenic vegetation dynamics are measured by contrasting the mosaics of satellite images taken at different times and automatically detecting dissimilarities in the spots with corresponding geographical coordinates (“changed detection”). The anomalies revealed via this method are later verified and interpreted on the basis of field data and/or detailed satellite images.

### *Analysis of spatial preferences within the Caucasian leopard's potential habitat*

The procedure is based on annual joint analysis of live data from remote sensing and satellite tracking. This requires satellite images with a spatial resolution between 15 and 0.5 m/pix, supported by large retrospective archives (10 years or more). Images are made in summer, when snow coverage is minimal. Multispectral images are processed with the help of AI algorithms, which allow splitting into more classes (Aksenov et al., 2002, 2003; Dobrynin, Saveliev, 1999a, b, 2012; Karagicheva et al., 2011; Dobrynin et al., 2017). Next, with the help of geoinformatics, the locations of collared animals are processed by neural networks, which statistically estimate the frequency with which animals appear in particular areas of the potential habitat, and define its borders. As a result of digital analysis, statistically established spatial preferences are then verified and interpreted on the basis of field data or super-high-definition (SHD) satellite images.

#### 2.1.3. SNOW COVER MEASUREMENT WITHIN THE BIG CAT'S POTENTIAL HABITAT

Given that snow cover depth is one of the main factors limiting the big cat's distribution in the northern part of its habitat (**Figure 14**) (Sukhova et al., 2015). Snow accumulation and melting depend on various factors, including local vegetation diversity, soil properties, exposition and variously exposed terrains in different biological communities.

### *Stationary observation according to the official guidelines of the Federal Service for Hydrometeorology and Environmental Monitoring (Rushydromet)*

If the hydrometeorological observation network is functional, the data are gathered by collecting historical as well as immediately relevant information from all weather stations of the highland part of the focal cat species habitat. New stations can also be established at the premises of a nature reserve, protected territory or observa-



**Figure 14.** Snow on the slopes of Mount Akhtsarkhva (above); a Caucasian leopard's tracks in the snow, the Dzhuga Mountain Range, the Ozerny Ridge (Western Caucasus, the Caucasus Nature Reserve) (below).

tion site. According to the official guidelines (1985), daily snow observations must be conducted at a site equipped with three stationary snow collectors positioned at the points of a virtual equilateral triangle, one next to the ground thermometer. At observation points the snow collectors must be placed near the snow gauge, the distance between them not exceeding 10 m. Their mutual positions and respective numbers must be maintained. The depth of the snow cover is to be measured daily in the morning.

#### *Snow course surveying*

This procedure is conducted under the same conditions as stationary observation, following the official guidelines (1985). Snow course surveys in the field are held on the 10<sup>th</sup>, 20<sup>th</sup> and final days of each month during snow period; and during the spring when snow melts field surveys are held on the 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup>, 25<sup>th</sup> days and also the last day of every spring month. On woodland trails, snow course surveys are held monthly (on the 20<sup>th</sup>) until January, and then every 10 days and every five days during melting.

#### *Expeditionary observation in biological communities*

Measurements are periodically carried out in the big cat focal species potential habitat and for test purposes while preparing a map of biological communities ranged according to their suitability for the leopard's various activity phases. Snow depth is then measured (**Figure 15**) within the listed communities, partly on a permanent basis (while tracking or servicing camera traps) and partly for map verification purposes.



**Figure 15.** Snow surveys include taking samples with the snow-measuring cylinder, estimating its mass (depending on density and volume) and thickness.

Any irregular distribution of snow arising from a vegetation type should be taken into account as well as exposition variability and the link between precipitation and altitude when selecting a measurement site.

*Analysis of seasonal and annual snow cover dynamics within the Caucasian leopard's potential habitat in the Russian Caucasus*

This procedure is accomplished through annual joint analysis of live data from remote sensing, the data being provided by the Federal Service and field measurements of thickness, humidity and potential crust formation.

## 2.2. FIELD MONITORING BIG CATS AND COLLECTING DATA IN THE FIELD

Full field monitoring integrates the following methodological approaches: remote surveillance tools (GPS collars, trailcams), snow tracking, and collecting biological samples (excrements and various tissues) of the big cats for molecular genetic analysis, excrements for assessing the degree of parasitic invasion and for monitoring animals' stress, checking clusters of locations, collecting samples from big cat's prey for subsequent species/gender identification, and collecting samples of big cat's excrement from clusters for further analysis of animal diet; monitoring the number of potential big cat's prey (ungulates, small and medium carnivores, lagomorphs, rodents, birds, and so forth) and the number of big cat's competitors (bear, wolf, lynx).

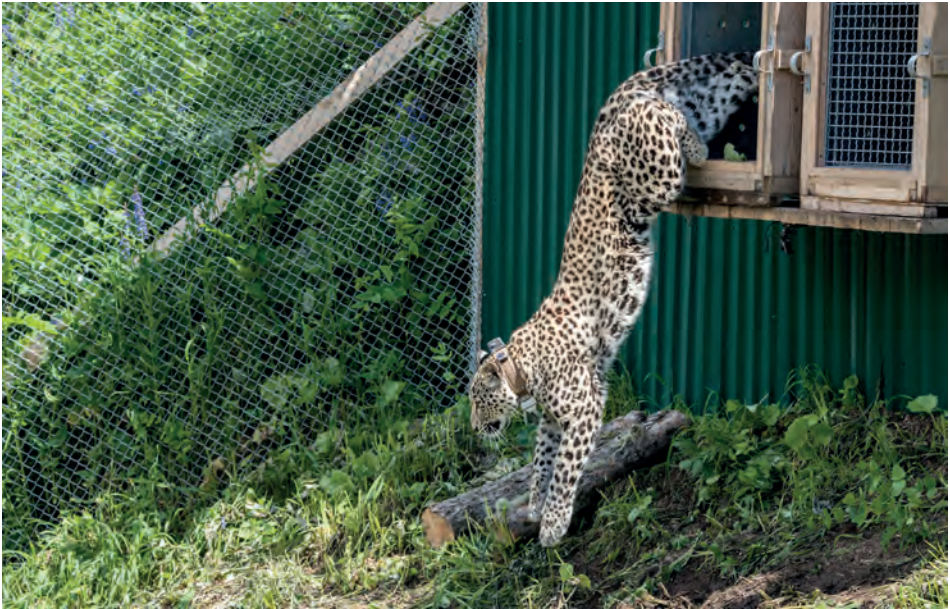
Each separate method upon its regular application forms an independent monitoring area of study focused on one or another aspect.

Before release into nature, all animals ready for reintroduction or caught in the wild should be provided with GPS collars (**Figure 16–18**), which transmit data via the satellite system; in addition, the collar should be equipped with a VHF transmitter. The data received from the collar provide up-to-date information about the animal's location as well as the possible places of prey catching.

Locations corresponding to a prolonged stay of an individual are to be investigated in the field, as such sites may represent places of successful hunting, where the animal remained near the prey for some time. In order to avoid disturbing the animal, such clusters should be checked after the animal had left them, as confirmed by the subsequent coordinates received. Checking clusters of locations allows the remains of the prey caught by a leopard to be detected and used in order to determine the type, age and sex of the prey based on hairs and other remnant in the laboratory (Rozhnov et al., 2011a). This information is utilized to maintain a cumulative database. Bone marrow samples of prey tubular bones may also be collected to determine their fatness (Neiland, 1970).

In case of a collar signal coming from an area near the settlement, the field response team should potentially be ready to travel to the area in order to prevent a possible predator conflict with humans. Importantly, the released animals do not have an individual negative experience of contact with humans, so some time after the release, when the animals begin to breed in nature, the number of registered cases of





**Figure 16.** The leopard equipped with GPS collar is being released to the Caucasian Reserve (July, 2016).

big cats hunting for cattle surges upwards. The same effect was observed when the lynx was restored in the Swiss Alps (IUCN KORA Cat Conservation Source, 2012). This situation should be envisaged in order to provide a rapid response and prevent negative consequences. The most likely explanation is that in the absence of a top-order predator, the wild ungulates are not used to reacting in time, resulting in reduced flight distance (Badridze, 2018). After a while, when the reaction rates of wild ungulates are restored, young leopards can attempt to hunt for an easily attainable prey, namely cattle. Therefore, protecting herds with dogs should be mandatory for shepherds. Over time, as a result of collisions with humans, leopards learn to avoid them through their own experience.

A special protocol permitting us to prove that the leopard was hunting livestock is required. Different predators kill their prey in different ways. The place of the predator's attack on cattle and the corpse of prey should be carefully examined to identify the specific nature of the prey killing and disposal. Whenever possible, the traces of the attacking animal and collected excrements to be genotyped in future should be collected. To this end, a system of interaction with the public should be developed prior to release.

To successfully implement the project of restoring a large predator in nature, the owners of livestock should first be provided with insurance on the one hand, and the experts should be involved in monitoring in the case of human-predator conflict situations on the other.

In those areas where Caucasian leopards are released, trailcams should be installed in advance. They have several purposes. First, obtaining the maximum num-



**Figure 17.** Amur tiger outfitted with a GPS-Iridium collar is being released to the Zhelundinskiy Reserve (May, 2014).



**Figure 18.** A snow leopard equipped with GPS-Argos collar is being released to the Sayano-Shushenskiy Reserve (March, 2011).

ber of images is necessary to periodically assess the physiological status of released leopards, as well as their interaction with other predators and potential prey. Second, the data from the trailcams enables the unified spatially explicit capture-recapture technique to be used to analyze how the population density of leopards is distributed, as well as the total density at the research site and the approximate number. In case the data transfer from collars fails, the array of trailcams will to a certain degree facilitate assessment of the animals' conditions and their distribution across the territory.

It must be considered that the Caucasus is the richest region in Russia in terms of the amount of hydropower resources used and hydroelectric power plant (HPP) in operation. The HPP during the exploitation phase is in fact similar to a natural object that could often be visited by numerous animals. To characterize the status of the populations of these animals, data should also be collected in the areas around the hydroelectric power plants, and any other protected objects should be provided with an extensive video monitoring system (**Figure 19**) and supplemented with photo and video recorders, which are used by researchers (**Figure 20**).

The HPP systems may support biodiversity conservation, being relevant in the policy and programs for the development of the energy sector. The basis for this is the international program, signed by the Russian Federation in 2015, to develop the legal and regulatory framework for conservation and the restoration of biological diversity



**Figure 19.** The snapshot from the DVR of the Zaramag HPP, North Ossetia – Alania with a leopard. The leopard is to the right on the hill.



**Figure 20.** Checking the photo trap in the HPP area (North Ossetia – Alania).

through the optimal technologies. Biodiversity conservation within the energy sector exists in line with the development programs of the United Nations.

The RusHydro company has its own programs aimed at developing and supporting regions, as well as preserving biodiversity. Its cooperation with the A.N. Severtsov Institute of Ecology and Evolution RAS presupposes the support of one of these programs – the Leopard Recovery Program in the Caucasus – as well as participation in basic developments related to the scientific support of this program. Using HPP video system data for analysis significantly expands the range of relevant zoological information regarding the region.

A critical component of monitoring is the collection of biological samples (excrements and various tissues) of the leopard. This is necessary for molecular genetic analysis, assessing the degree of parasitic infections, as well as for monitoring the state of stress in animals, determining their nutrition. The material used for genetic analysis can comprise hair, urine, muscle tissue, a piece of skin, blood, fresh excrements (3–5 days), bones, teeth, fangs, and claws.

While snow-tracking leopards, it is often possible to collect excrements for further analysis of the animals' nutrition. Such analysis enables us to estimate the occurrence and proportion of the main species of prey (for the Caucasus, these are turs, chamois, red deer, roe deer, and wild boar) in leopard nutrition, as well as information on the additional nutritious objects such as rodents, including mice, small predators,

birds, and even plants. Excrements for this kind of analysis are to be collected throughout the year.

Monitoring of the population size of potential leopard prey (such as ungulates, small and medium predators, lagiformes, rodents, birds) as well as other large carnivorous mammal competitors of the leopard (bear, wolf, lynx) can be carried out via the following methods: winter transects census, the installation of an array of trailcams in the census periods, as well as other traditional methods of measurements for zoological studies (lines of traps, grooves, etc.). The use of accounting data from economic entities and regional authorities is also possible.

#### 2.2.1. USING REMOTE SURVEILLANCE TOOLS (GPS COLLARS WITH SATELLITE TRANSMITTERS, PHOTO AND VIDEO RECORDERS)

##### *Using GPS collars with satellite transmitters*

The relevant information concerning the location of a released big cats is crucial for its monitoring. Real-time information is preferable, yet today this task is only feasible if the collar is replaced on a monthly basis. Remote satellite tracking represents a compromise between the weight of the device, the duration of its operation and the amount of incoming data. Recent technological advances are constantly maximizing the duration of work and the number of locations obtained, and minimizing the weight and size of satellite transmitters. The priority that researchers must solve is finding the best solutions when choosing their telemetry equipment (Hernandez-Blanco et al., 2015a).

The main parameters that a collar with a satellite transmitter should have to monitor released animals can be characterized as follows.

1. *12–24 locations per day.* This frequency of locating the animal provides information about its movement, which is the main criterion when searching for places of successful hunting.

2. *1–2 data transfers to the server per day.* These are the minimum requirements that enable a prompt reaction in case of conflict situations.

3. *The availability of an accelerometer to register motor activity.* This ensures the operation of the notification system in case of collar ejection, death of an animal, and so forth. Data on leopard activity enable the determination of behavior, the intensity of movement and the assessment of its changes in time in connection with other parameters, such as distance traveled during the day.

4. *The availability of a VHF transmitter.* This is required for the work of the field team, providing rapid response in case of conflict situations, as it avoids meeting the animal. With the help of the VHF signal, it is easier to search for the collar once it has been discarded, and to find the animal if it needs to be re-caught.

5. *Drop-off.* The drop-off mechanism is necessary to release the animal from a collar that has stopped working; hence when the collar is found, it is possible to obtain accumulated data that have not been transmitted via satellite for various reasons.

As an example, below is the experience with the well-proven Iridium Track M Collar 1D model from Lotek, Canada (**Figure 21**).



**Figure 21.** The collar Iridium Track M Collar 1D from Lotek, Canada with a fastened battery, a GPS block, Iridium and VHF transmitters and a drop-off.

*Technical characteristics of the transmitter for leopards' Iridium Track M Collar 1D from Lotek, Canada.* The instrumental collar case consists of two blocks, as well as a drop-off lock. The total weight of both blocks and the collar is 590 g, the weight of the self-discard lock is 50 g (75 g with remote reset function), the total weight is 640 g, and the maximum allowable weight of the collar should not exceed 2-5% of the animal's weight (White, Garrott, 1990; Kenward, 2001; Millsbaugh, Marzluff, 2001; Silvy, 2012). The weight of the collars provided to the animals prior to release was 1.3% and 1.5% of the weight of the male (49 kg) and female (42 kg) Asiatic leopard, respectively. The lower collar unit contains batteries. The upper one is a 16-channel GPS platform, Iridium satellite data transmission platform, a VHF transmitter and a base board. The upper block is protected from above by a shrouded duralumin ring to protect against bites and bumps. All of the antennae of the transmitter and receiver are integrated into the body of the blocks and the collar itself, and do not protrude outward, which prevents them from being bitten off by animals, this being the main reason for the failure of transmitters of previous generations. The belt of the collar itself is made from a reinforced polymeric material that is moisture resistant.

The device traces the animal's location using the GPS module system according to a schedule pre-programmed by the researcher. Locations (geographic location coordinates) accumulate in the internal memory of the device and are then sent as a packet of 1–12 (in our case 12) units by one SMS via the Iridium satellite system to the base station number. Subsequently, the data are available to researchers through

the web portal Webservice Lotek. The portal is able to quickly display the location of the collar on Google Maps. By registering the collar in the system, the user receives a login and password, which are used to download data from the aforementioned portal. Together with the location package, the device transmits data regarding the ambient temperature at the time of each location, as well as data on the battery level. If necessary, the GPS operation schedule and the schedule of data transfer via the satellite can be changed remotely after activating the device via the Internet. The duration of the transmitter is contingent on the intensity of battery consumption, which in turn is related to the number of locations per day and the number of messages sent by the device. Thus, the scheme one location per hour and one SMS message every 12 hours provides one year of operation of the transmitter. The scheme one location every two hours enables the life of the device to be extended to about a year and a half. The long stay of the animal in places where communication with the satellite is difficult significantly increases battery power consumption and shortens the life of the collar.

In addition to determining the geographical coordinates, the device records the motor activity of the animal with the built-in accelerometer. Data from the animal motor activity accumulate in the memory of the device; if the collar can be found, these data can be extracted directly from the collar after the self-discard system has operated. An activity sensor in the absence of animal movements for a given time (default 24 hours) transmits a special signal (the so-called “death signal”) along with the location package through the Iridium satellite system.

The self-discard lock installed on the collar is triggered either at a pre-programmed time (maximum 156 weeks after starting the device) or by the user’s decision at any time via a remote radio or Internet command, where this function is available.

The VHF transmitter placed on the collar transmits a radio signal in the background mode, which enables the collar to be found at any time after its launch (the detection range of the collar according to the VHF signal is approximately 6 km in direct line of sight when searching from the ground, and 10 km when searching from the air), as well as in the period between the GPS locations. Furthermore, the presence of a VHF signal allows us to find the collar after the self-discard lock has been triggered in case of a GPS system or satellite data transmission device failure. Thus, the VHF transmitter is an auxiliary system for directing the collar.

*Preparatory work.* After acquiring the collar, it must be adjusted in accordance with the stated purpose of the study and tested in the field. Prior to testing, the specialists of the A.N. Severtsov Institute of Ecology and Evolution RAS contact the Iridium representative to activate the account. To do this, the number of the SIM card (IMEI – International Mobile Hardware Identity) of each device should be known. One should consider that the active Iridium account for data transmission has a subscription fee, so that even with the collar turned off, the funds are spent. Thus, in order to avoid unnecessary financial costs, the Iridium account should be deactivated after testing if the collar is not planned to be put onto the animal in the near future. In case of having a removable power supply, before fastening it to the leopard, it is necessary to ensure the tightness of the seals and to set the internal clock of the

device after adjusting. After setting the collar algorithm, which includes the preparation of files with the schedule of GPS, satellite transmitter and VHF signal, the fixation of GPS locations, the operation of the VHF transmitter, the operation of the system for sending data to the satellite and their tracking through the server should be tested. Before fastening the collar to the animal, it must be turned on. To do so, the magnet has to be removed. Subsequently, the system notifies the correct operation by the code signals of the VHF transmitter.

*Installation of the collar.* When attaching the collar, make sure that an adult's fist can be placed between the collar belt and the neck of the animal, and the collar should not be removed through the head (**Figure 22–24**). It is useful to measure the circumference of the animal's neck and the coverage of its head in the zygomatic arcs in



**Figure 22.** Testing correct collar setting on the Caucasian leopard (above), an immobilized Caucasian leopard with a collar (below).





**Figure 23.** Attaching a GPS-Iridium collar to Amur tiger in the Ussuriyskiy Reserve (May, 2011).



**Figure 24.** Checking the GPS-Argos collar attached to an Far Eastern leopard in the Land of the Leopard National Park (August, 2011).

order to choose the right collar diameter in advance (wear the collar on an adult animal, because a growing animal may experience discomfort). The collar nuts must be tightened with the torque recommended by the manufacturer. A reliability check is carried out by a torque wrench. As a rule, self-locking nuts (usually M8) are used.

*Starting the drop-off activation.* After installing the collar, the additional magnet in the drop-off case should be removed (if any). Next, the program is started, focused on the drop-off of the collar after a certain period of time; some models are launched immediately after the collar is turned on. It is very important not to remove the drop-off magnet before installing the collar.

### *Use of trailcams*

It is recommended to use digital trailcams with infrared flash, equipped with motion sensors, such as Bushnell, Reconyx, Seelock Spromise or their analogs.

*Trailcam array.* In those areas where big cats are released, it is necessary to establish a permanent network of trailcams (**Figure 25**). The aim of the photo trap array is to obtain the maximum number of images of released big cats to assess their physiological state, interaction with other predator species and potential prey. By the array of trailcams the condition of animals and the use of space will be assessed in case the data transfer system from the collars fails.

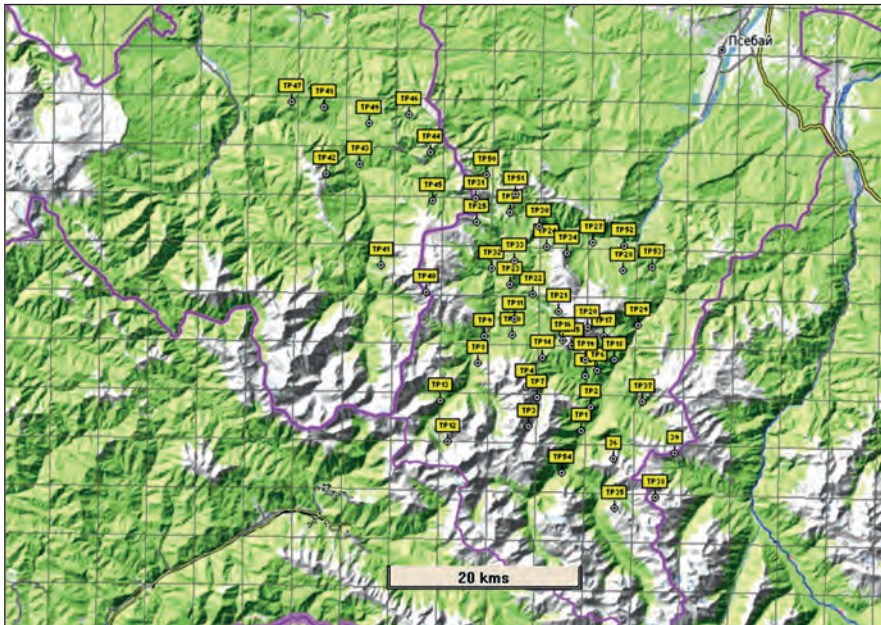
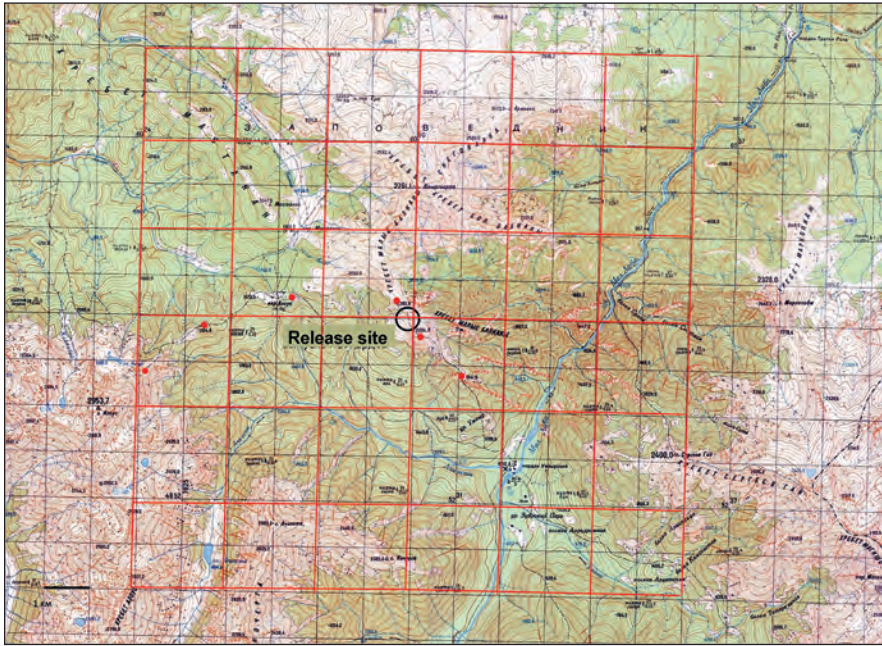
The optimal area of installation of the trailcams array corresponds to the area of an average annual habitat of an adult female, and should not be less than 150 km<sup>2</sup>. Trailcam stations (with up to two cameras in the same place) should be located in such a way as to provide one station every 4 km<sup>2</sup>, avoiding the installation of stations closer than 1.5 km in a straight line from one another.

The placement scheme of trailcams and the selection of optimal locations for their installation should be undertaken with the involvement of regional specialists. The research staff and inspectors of reserves and adjacent territories are well acquainted with the area. The permanent array can be supplemented with trailcams installed in places of special concentration of animals, transitions, trails, and so on. (There can be several places like this within a single square of the original array. Such trailcams can be installed at a short distance from each other.)

The trailcams should be installed with the representatives of the participating field monitoring group, or according to a pre-designed scheme, provided that a group of people setting up trailcams is instructed. All the parameters provided by the protocol must be recorded (**Figure 26**) and transferred to the Center for Data Accumulation and Processing.

To ensure follow-up monitoring, on-line movement through the area under investigation, and transportation of the entire quantity of necessary equipment (which is quite extensive), it is necessary to have an off-road vehicle with a capacious luggage compartment.

The identification of animals is conducted according to an individual pattern on the fur (**Figure 27–29**). The coloring of Caucasian leopards' sides is asymmetrical, hence it is optimal to install two trailcams in each station to accurately identify the



**Figure 25.** An example of a trailcam array setting in the Caucasian Reserve. The red dots depict previously planned trailcam stations (above). The array is designed for 36 trailcam stations. There must be one station in each square. When leopards leave their release spot, the array is translocated to that spot. If leopards stay around the spot, the area covered by the array should be enlarged by 2.5 times.

Trailcam checklist

Trailcam ID (e.g. F1A, F1B): \_\_\_\_\_ Station ID (e.g. F1): \_\_\_\_\_

Amount of trailcams in the station: \_\_\_\_\_ Orientation: (e.g. N, NW, E...) \_\_\_\_\_

Place name: \_\_\_\_\_

Coordinates lat lon (D.ddd) (WGS84): Latitude: \_\_\_\_\_ Longitude: \_\_\_\_\_

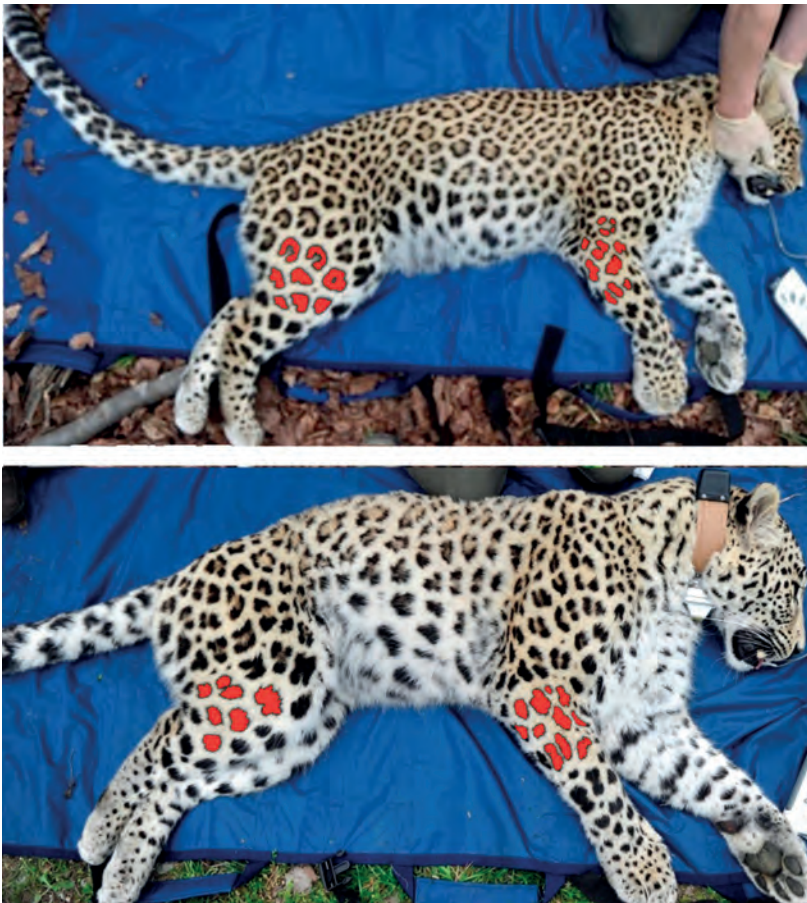
Set date: \_\_\_\_\_ time: \_\_\_\_\_

Type & model: \_\_\_\_\_

Remove date: \_\_\_\_\_

Trailcam settings: (video, photo, hybrid, pics in series, latent period...)  
 \_\_\_\_\_  
 \_\_\_\_\_

**Figure 26.** An example of a trailcam setting protocol card.



**Figure 27.** A comparison of individual patterns belonging to two different Caucasian leopards.



Figure 28. Individual patterns of Amure tiger monitored in Ussuriyskiy Reserve.

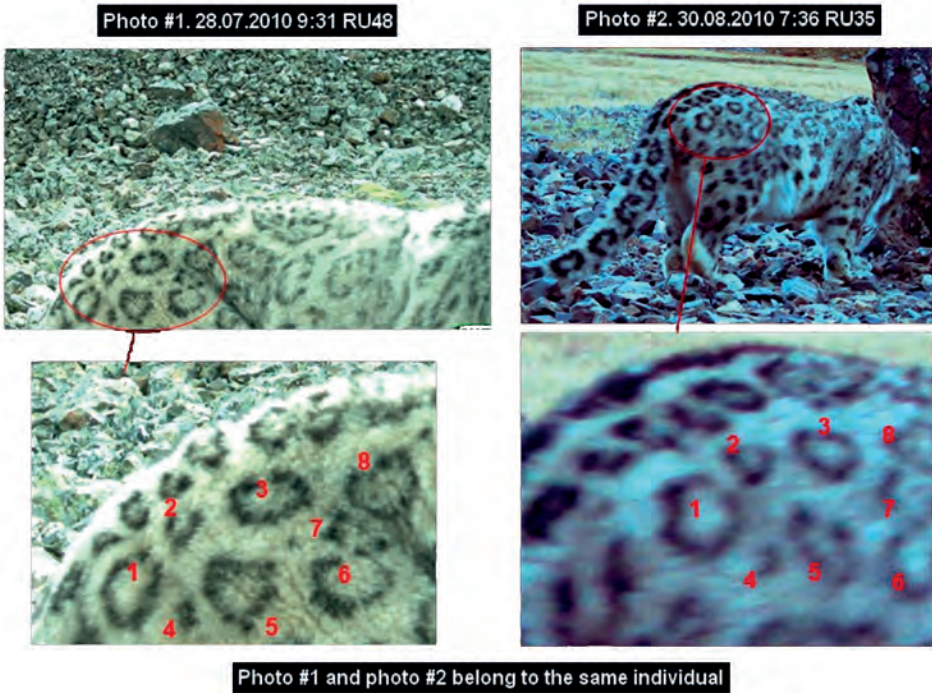


Figure 29. A comparison of individual patterns belonging to the same snow leopard.

individuals from the right and left sides and to increase the information from each recorded passage. If the number of cameras is small, a greater coverage of the study area should be preferred, and one camera in each station should be installed.

Trailcams should be attached to trees (**Figure 30**) or stones so that sensitive parts of infrared sensors are at a height of 50–90 cm above the level of the trail and at a distance of 3.5 to 4 m from the proposed trajectory of the animal's movement. When installing the trailcams in pairs opposite to one another (in order to ensure simultaneous operation), the cameras of the system should be directed approximately to one point, but they must be located at an angle to each other in order to avoid the undesirable potential effect of the flashes on the pictures' exposure.

Avoid direct sunlight falling at the photo trap lens and do not orient the camera east or west when the rising or setting sun illuminates the camera (except for the location of the cameras in the gorges and places with terrain features that obscure the camera). The presence of a moving trap in the field of view of the camera to which the camera sensor can easily respond should also be avoided. For the same reason, do not install cameras on rocking bushes and thin trees. During the growing season, installation sites for trailcams are recommended to be visited at least once in 10 days to remove the herbaceous vegetation growing in front of the cameras.



**Figure 30.** An example of setting trailcams on a tree.

To attract the attention of the animal, a visual or scent bait (feathers of birds, etc.) should be used to increase the time the animal stays in the field of camera view. It is recommended to install the cameras in front of artificial marking points and in places where leopards can leave their scratches.

It is preferred to install trailcams on trails paved by animals on the southern edges of plateau ridges or on pointed ridges and spurs in places where animals cannot bypass the photo trap.

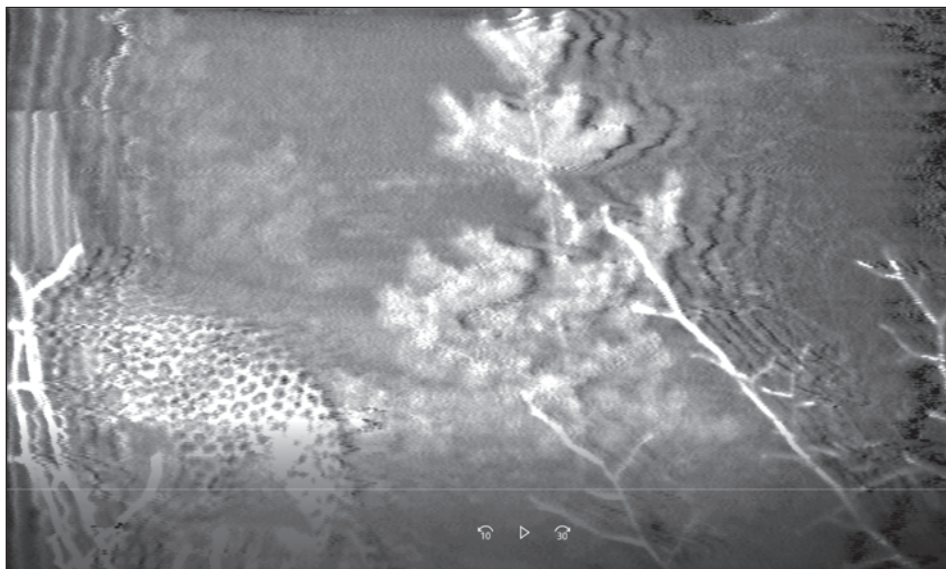
The trailcams set on trails should be set so that after each sensor operation the camera records at least three frames with the smallest latency period (no more than 1 s). Given that the memory size of the cards affects the response rate, memory cards larger than 16 GB and less than 10 GB should not be used. When using trailcams in hybrid or video mode, the duration of the clips should be selected no more than 20–30 seconds, with a latency period of 1 s or less. It should be considered that this mode yields a large number of idle frames, and in this case it is necessary to check the trailcams more frequently.

The trailcams should be checked every 10–15 days.

The issues associated with using trailcams in mountainous conditions (such as in Southern Siberia in the study of the snow leopard) are considered by Karnaukhov et al. (2011a, c, d).

#### *Use of photo and video data of animals of existing special observing systems*

To collect information on large mammals, including leopards, it is possible to use existing space monitoring systems (**Figure 31**). Such systems are installed in loca-



**Figure 31.** A snapshot of a video with Caucasian leopard from the trailcam (North Ossetia – Alania). The video is provided by the border service

tions such as hydroelectric power stations and border zones. To obtain information from such systems, it is necessary to enter into an agreement on cooperation with the owner organizations of systems that have the right to information.

### 2.2.2. COLLECTING BIOLOGICAL SAMPLES FOR LABORATORY STUDIES

Collecting biological samples for laboratory assays is associated with traditional methods of field research, namely animal tracking. One should be familiar with leopard footprints and excrements, given that these are the main sources of information about the animal.

The footprints of the leopard are large, round, relief: the heel is 6×8 cm, the rounded fingers are widely spaced. The overall dimensions of the footprint can reach 11–12 cm in length and 7–8 cm in width. The track is usually straight and clear, and the chain of footprints is even (**Figure 32**). The length of the step of a calmly walking leopard can vary from 40–45 to 45–50 cm.

A fixed protocol is required to measure the prints of the leopard's feet on the substrate (podometry). Analysis of the measurements obtained enables us to determine both the sex and age category of the animal (Hernandez-Blanco et al., 2005). According to the protocol of the large feline podometry, the width of the cushions of the paws should be measured (**Figure 33a**). The paw (i.e., front, back, right or left), type of substrate,



**Figure 32.** Paw footprints and trail of a Caucasian leopard.





**Figure 33.** Measuring footprints of the Caucasian leopard: a – measuring the width of the paw pad (A), b – measuring the step length (H).



**Figure 34.** Snow leopard excrements.

and the depth of sinking in the substrate should all be noted. A photo of the footprint should be taken next to the ruler, indicating the geographical coordinates, date, time and data collector. If one saves a track, he/she must specify the length of the step (**Figure 33b** – the distance between the prints of the front right and front left paws is H).

The big cat's excrements are large and have a typical shape (**Figure 34–36**). Nevertheless, errors are always possible when visually identifying the species to which excrements belong.

Molecular genetic analysis of a snow leopard's (ounce) excrements collected in the field, and ascribed to this species solely by a visual definition, revealed that the error is no less than 60% (Zvychainaya et al., 2011a, b; Rozhnov et al., 2011g). Moreover, almost half of the samples visually described as belonging to the snow leopard were identified fox excrements (48%), while others were attributed to the wolf (6%) and lynx (5%). Therefore, visual identification data are crucial, to be further confirmed through molecular genetic arrays.

The biological samples collected in the field such as excrements and various tissues of animals constitute very important sources of information about individuals. For dif-



**Figure 35.** Caucasian leopard's excrements.



**Figure 36.** Amur tiger excrements.

ferent methods of subsequent laboratory analysis, distinctive methods of sample preservation and storage are required. According to the scheme for organizing the monitoring and interaction of the various participating organizations (see section 3), all collected samples should be transferred to the Severtsov Institute of Ecology and Evolution RAS for further laboratory analysis. Any samples collected without following the rules cannot be analyzed. The rules for collecting and storing samples are given below.

#### *Samples preservation and storage for molecular genetic analysis*

*Sampling of excrements for genetic analysis.* The collection period lasts as follows: for winter, up to 5–7 days from the moment of defecation in the presence of a constant negative temperature; for summer, up to two days in the absence of rainfall during this period. Before collecting the sample, it is necessary to wear rubber or cloth gloves (direct contact between human skin and the sample is unacceptable), and to label the name of the sample, the date and geographical coordinates of the sample site, and the full name of the collector using a special lacquer marker (it should not be washed off with alcohol) on two test tubes, or with a simple pencil on the label (**Figures 37 and 38**). Use a clean scalpel, tweezers or scissors to take a piece not larger than 1.5 cm<sup>3</sup>. Place the obtained material in a 10–25 ml tube with 96% ethyl alcohol. At the end of sampling, the instrument and



**Figure 37.** Excrement collection in the field.



**Figure 38.** An example of a label for the excrement sample.

rubber gloves should be removed in a separate container and not re-used to take other samples. Cleaning of the scalpel, tweezers and scissors should be carried out in the laboratory with a special solution (DNA eraser) and then washed with clean water. Only after the described procedures can the tool be used again. Tubes with samples can be stored at room temperature before analysis. Collecting excrements in separate packages and their subsequent freezing and preservation into test tubes after thawing or storage is not acceptable. Two or more different samples should not be labeled identically.

*Blood samples.* Blood can be collected from an immobilized animal (**Figure 39**) or on traces during snow tracking (**Figure 40**). When collecting the sample from an immobilized animal, rubber gloves should be worn. Sign the name of the sample, the date, type and geographical coordinates of the sample collection site, and the full name of the collector in printed letters with a special lacquer marker (it should not be washed off with alcohol) or with a simple pencil on a test tube or label. Blood is collected in tubes with the addition of K3EDTA with blood volume from 0.1 to 2.5 ml (optimally 1 ml) (**Figure 41**). A blood sample may be stored for up to one month in a refrigerator of +4 °C and further transported without freezing. For longer storage, the test tubes should be stored in the freezer; during transport, a refrigerant should be used. Blood may also be applied to the filter or FTA paper, completely dried and placed in a paper envelope. Two or more different samples should not be labeled in the same way.

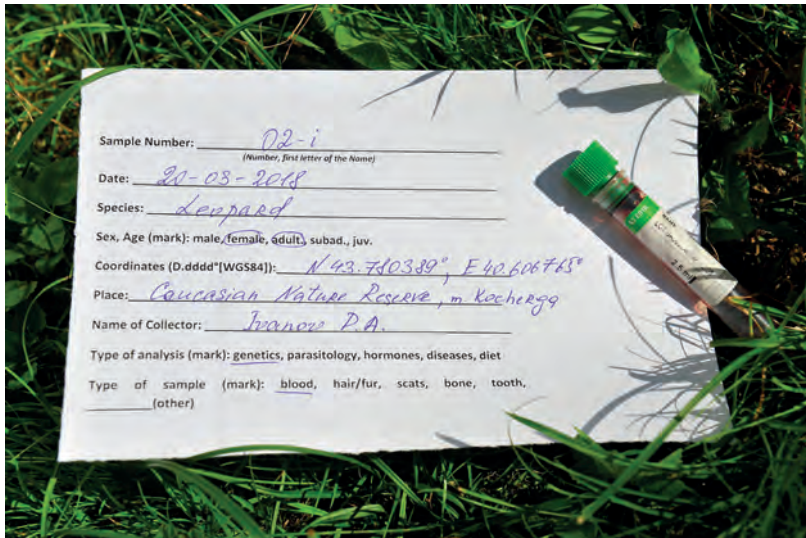
*Hair samples.* When collecting the samples, rubber gloves are necessary (direct contact between human skin and the sample is unacceptable). Label the name of the sample, the type, date and geographical coordinates of the sample collection site, and the full name of the collector in print letters with the special lacquer marker (it should not be washed off with alcohol) or with a simple pencil (by no means sign the sample with a pen) on a paper envelope or test tube. With tempered previously in fire tweezers or haircuts, take a small bundle of hair (the hairs should have hair follicles) and place it in a paper envelope or a tube with alcohol (**Figure 42**). It is unacceptable when sampling to use plastic bags, scotch tape and shearing of hair with scissors. Because in such samples there are no hair bulbs and follicles are damaged. The number of hairs does not matter, but it is better if there are more than 15. For long-term storage, it is better to place the hair in 2 ml tubes with sealed caps with 96% alcohol.



**Figure 39.** Collection of blood samples from an immobilized Far Eastern leopard.



**Figure 40.** Blood spilled on the spot where the big cat stopped (Amur tiger) can be used for molecular genetic analysis.



**Figure 41.** An example of a label for the blood sample collected from the leopard and a K3EDTA test tube.

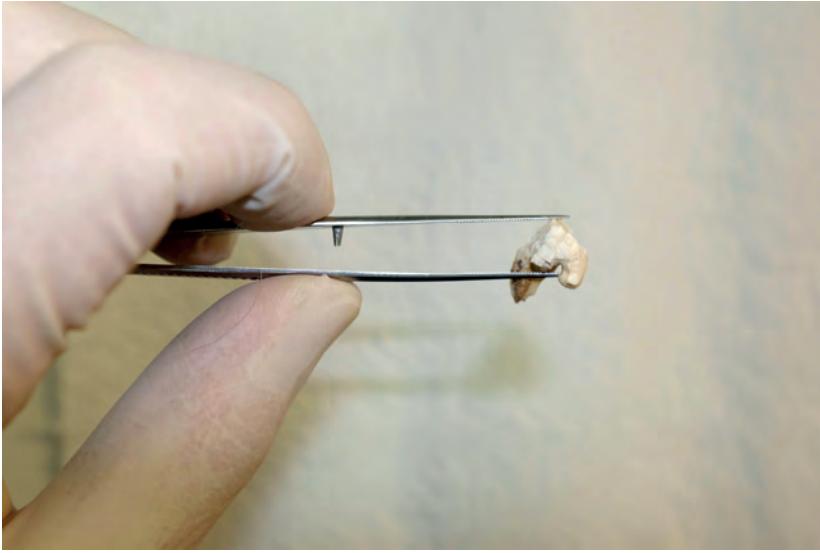


**Figure 42.** Collection of hair samples left on a tree by an animal.

Do not wrap it with adhesive tape. A label written in pencil may be placed directly into a test tube if there is no label on the test tube. Two or more different samples should not be labeled in the same way.

*Samples of muscles or fragments of skin.* When collecting a sample, rubber gloves are to be worn. Mark the name of the sample, the type, date and geographical coordinates of the sample collection site, and the full name of the collector with a special felt pen (not to be washed off with alcohol) on a test tube, or with a simple pencil on the label. Use a clean scalpel, tweezers and scissors to take a piece of skin, muscle or other tissues (it is not advisable to use liver tissue), and place it in a 2 ml or 5 ml test tube with 96% alcohol. Use only leakproof tube types from certain manufacturers, for example SSI, Sardstedt, Eppendorf. Using larger tubes, glass vials and cans is unacceptable, especially if there is the potential for leakage. The sample volume should not exceed 0.5–1 cm<sup>3</sup>; if the amount of alcohol is insufficient, the samples will deteriorate. Wrapping the sample with adhesive tape hinders further work with it. Alcohol may wash off a label written with a pen. A label written in pencil may be placed directly into the tube if there is no label on it, or the tube may be placed in a bag with a label. Two or more different samples should not be labeled in the same way.

*Samples of teeth and bones.* When collecting a sample, rubber gloves should be worn. Sign the name of the sample, the type, date and geographical coordinates of the sample collection site, and the full name of the collector in printed letters with a



**Figure 43.** An exemplary piece of bone.

special felt pen (not to be washed off with alcohol) on a test tube, or with a simple pencil on the label. With clean tweezers take a piece of whole bone, with a volume of at least 5 cm<sup>3</sup>, but no more than 20 cm<sup>3</sup>, or entire tooth or claw (**Figure 43**). A piece of bone or tooth may be placed directly in a bag with a label. Two or more different samples should not be labeled in the same way.

*Preservation and storage of samples of big cat's excrements to monitor stress level*

Excrements for subsequent hormone analysis should not be exposed for more than a few hours to temperatures higher than +10 °C, i.ä., night samples should be collected in the morning. In winter, at temperatures below zero, the samples may be collected from excrements left more than two weeks ago. A sample (weighing 3–5 g or more) is placed in a plastic bag, tied (or zipped), and placed in another sachet and labeled with the species of the animal, the nickname/ID and the number of the animal, the sex, date of collection and geographical coordinates of the site, and the full name of the collector. The sample is put in a household freezer (-18 °C) and stored until transportation. If samples cannot be frozen, they can be collected in a fixed volume (e.g. 5 ml) of 96% ethyl alcohol.

*Preservation and storage of samples of big cat's excrements  
to assess parasitic infections*

The collected excrement samples must be labeled precisely, noting the species and the individual that left them, the date and geographical coordinates of the collection site (recorded using GPS), and the full name of the collector. The samples must

subsequently be put in plastic bags and frozen. To save the labels, they must be placed in a separate plastic bag, which is further put in a bag with excrements. The species to which the excrements belong are determined based on the prints of the animals' paws left near the excrements, and, when necessary, using molecular genetic analysis methods after obtaining nucleotide sequences of the fragment of the cytochrome b gene of mitochondrial DNA (Rozhnov et al., 2009b). Excrements are taken from the rectum of the captured and immobilized predators. Given that identifying the samples as belonging to a particular individual is not always carried out, one cannot rule out the possibility of several collected samples belonging to the same animal. The excrements are frozen in a household freezer or placed in a Barbagallo solution (ratio of sample and solution 1:1), which is prepared as follows: add 1000 g of distilled water to 7.5 g of NaCl and 30 ml of a 3% formalin solution, and the revealed helminths are placed in the same solution. The main approaches to parasites are described in a special manual (Esaulova et al., 2017).

### 2.2.3. COLLECTING BIOLOGICAL SAMPLES FROM LIVE-CAPTURED AND FOUND-DEAD BIG CATS

While monitoring big cats, the researcher should be able to inspect a live animal (for example, caught for changing the collar or in other situations) or the corpse of the deceased animal.

When inspecting a live leopard (as with the immobilization of leopards in the Leopard Recovery Center in the Caucasus, as well as inspecting their cubs by the Center's staff without immobilization), the following samples should be collected for further assays: blood for hematologic, biochemical and hormonal analyses; excrements for parasitological and hormonal analysis; hair for hormonal analysis.

Blood samples for hematologic, biochemical and hormonal analyses are taken from the superficial vein of the shoulder, inguinal vein or jugular vein.

For hematological analysis, 0.25 ml of blood is collected in a test tube with K3EDTA. Cells are counted under a microscope in Goryaev's chamber in a dilution of 200 times (erythrocytes in physiological solution, counting is conducted in five large squares) and 20 times (leukocytes in 3–5% acetic acid solution, counting is carried out in 100 large squares). In addition, a blood smear should be prepared for the subsequent evaluation of the leukocyte ratio. The number of cells is estimated on the same day of sample collection, and the analysis may be carried out on a pre-calibrated automatic blood analyzer.

For a biochemical and hormonal blood test, the samples are collected in an amount of at least 3 ml, then centrifuged at a centrifugal acceleration of 1000 G (which in large centrifuges corresponds to 3000 rpm, and in small ones 6000 rpm) for 20 minutes, or serum is extracted after upholding. Biochemical blood analysis is carried out within 24 hours after sampling, if necessary. Serum can be frozen at -18 °C before analysis. Serum for hormonal analysis is frozen at -18 °C.

Samples of excrements (from 1 to 10 g) are collected directly from the rectum of the immobilized animal (it is possible to collect fresh samples from the enclosure), labeled (nickname and animal number, species, sex, age, date and site of collection) and frozen (-18 °C) before the research.





**Figure 44.** The deceased/dead Caucasian leopard and its place of detection.

The hair of the animal for hormonal analysis is cut from the outside of the corpse (as close to the skin as possible) from the area of 2×2 cm, packed in a plastic bag, labeled (nickname and number of the animal, species, sex, age, date and site of collection) and placed in a freezer (-18 °C) before carrying out the research.

The discovered corpse of the deceased leopard (**Figure 44**) should be taken wholly and transferred to the veterinarian for pathoanatomical research.

### 2.3. MONITORING THE CONDITION OF THE PREY BASE OF THE BIG CATS AND ITS COMPETITORS, COLLECTING DATA FOR DIET ASSESSMENT

Ungulates are the main prey of the big cats. For instance, in the Western and Central Caucasus, these include west caucasian tur, chamois, red deer, roe deer, and wild boar; in the Eastern Caucasus, a Ibex/Caucasian goat could be added to the food object range. In addition to these main species constituting the prey base, the Caucasian leopard can prey on other species of mammals as well as birds.

In the Caucasus region the Caucasian leopard experiences competitive pressure from the wolf, jackal, fox, lynx and brown bear. Particular attention should be paid to the relationship between the Caucasian leopard and the wolf, the latter being the main competitor regarding ungulates.

#### 2.3.1. MONITORING THE POPULATION SIZE OF PREY SPECIES AND COMPETITORS OF THE BIG CATS

Monitoring both the population size of prey species and the competitors of the big cats is necessary to assess its involvement in the ecosystem functioning after its appearance in habitats where this species has long been absent. This assessment is based on analysis of the cumulative redistribution of predatory load on wild ungulates, changes in the behavior of wild ungulates, and changes in distribution density and the number of animals such as lynx and wolf where the big cats appears.

The animals representing the prey base for big cats (many species of ungulate, hares, badgers, large birds) or their large competitors (for the leopard in the Caucasus these are the bear, wolf, lynx) are mostly animals for hunting. Thus, various methods of population estimation have already been developed and are traditionally used. The monograph by V.A. Kuzyakin (2017) focuses on these methods and presents a full overview of these questions.

The existing methods used to estimate the number of animals in model areas can be classified into traditional and modern. Traditional methods include the winter transects census method, the method of double encirclement (carried out in the presence of snow cover), and the scaring and run method; visual surveys of ungulates (on rocky slopes, in open spaces) are conducted during periods of ungulate accumulation in aggregations, herds and groups (for example, during a breeding period, migration, calving). For ungulates, which are accounted for by these methods, the breeding period and accordingly the census occur in late autumn and the beginning of winter: for

chamois – October-November; west Caucasian tur – November-January; Ibex/Caucasian wild goat – November-December (Danilkin, 2005).

### *Traditional methods of estimating the number of animals*

The primary goal of any census is to obtain a reliable estimate of the number of individuals (potential prey) living in a given territory or within the area they inhabit. Being one of the most important parameters of the population and an indicator of its state, the estimate of population density reveals the overall picture of the spatial distribution of densities, abundance and dynamics. Data on the distribution of population densities form the basis to plan the protection of both ungulates as food objects and predators that are largely dependent on the state of food resources.

When planning the system of census and monitoring for any population, it is necessary to solve two problems at the same time. Indeed, these problems are tightly intertwined, and solving them can ensure that the information obtained for a given volume is sufficiently accurate. The representative sample for a certain phenological period should be as clear as possible and cover the volume needed, while the costs necessary to obtain information should be minimized to a degree that still allows enough accuracy. The latter should not be lower than that specified for this period. Depending on the degree of scaling of the tasks set and the human and financial resources available, several stages can be distinguished regarding the tactics of conducting the census and assessing the state of the populations analyzed. In particular, these include: determining the limits of the area of the distribution of the species, population, groupings; selecting the method of accounting; selecting the model sites where animals are supposed to be counted; and counting animals in model areas and identifying the structural parameters of population groups in the survey areas. Animal registration itself includes analyzing both the distribution of their densities over a territory and the main structural parameters of population groups in model areas (sex ratio and age structure, herd concentration, incidence) and the main population processes (fertility, mortality, migration activity), determining their status and providing trends in their number in the territory and over time. Also assessing the state of the populations analyzed includes the relevance of comparative analysis of individual populations and their groups subjected to varying degrees of predation and its consequences; determining the optimal value of the annual withdrawal volume and the minimum values of the population structural parameters, which would be able to withstand calculated degree of a long-term pressure from predators and compensate for the damage.

To accomplish this, in each case it is necessary to consider the rate of normal fluctuations purely associated with internal causes, and to establish not just the number and potential level of reduction in the population part being exploited, but also the allowable measure of deviations of the structural-functional organization of the remaining part of the population that could be self-compensated during the annual cycle.

Various methods exist to count ungulates: *complete counts* (in limited areas, with a compact distribution of animals, good detection and visibility conditions, applicable for open plain areas); *selective route counts* (not suitable for mountainous areas and

does not reflect the absolute values of abundance, although it reflects their occurrence well); *areal* (continuous survey on pre-selected limited areas with a known area); *depositing*; *runs*, and so forth. Some of these methods are described below.

Winter transects census are carried out in the presence of stable snow cover by the standard method, with the estimation of the given species daily distance and the description of biotopes where traces on the snow were detected. Winter transects census is an integrated census method: it can simultaneously determine the number of many species of mammals and sedentary birds, and it is used to determine the population density and the number of mammals and birds in large areas. The standard method of accounting in winter transects census is based on the fact that the number of crossings of the trails of the accounted mammal species by the reference route is directly proportional to the population density of this species. At the same time, the number of recorded traces depends on the average length of the daily distance covered by the animals: the increasing of the length of the daily distance increases the probability of their intersection by the reference route. Thus, to determine the population density of animals (the number of individuals per area unit), two indicators need to be defined: 1) the average number of crossings of the daily track of accounted mammal species per 10 km of the route; 2) the average length of the daily distance of each species, on the basis of which the conversion factor is calculated under weather conditions similar to the day of counting. The length of a daily track distance is contingent on snow deepness and structural features of snow coverage.

The method of winter transects census is traditionally used to monitor the number of potential species of prey for predatory mammals, although it is fraught with difficulties. The absence of snow cover or lengthy periods of thaw with melting snow do not allow this method to be applied equally successfully over the territory of the country. Moreover, this method is effective only for certain species and with a high or moderate population density, whereas it does not work for most predators and for some species of ungulates, or in cases of low density.

For areas where there is no snow cover, or its state varies significantly during winter, good results might be obtained by automatically estimating the abundance of ungulates with trailcams without their individual identification (Rowcliffe et al., 2008). This assessment allows for monitoring the relative number of animals and analyzing changes over time.

The method of multiday encirclement can be used to determine the number of deer, roe deer, wild boar, and hare. Multiday or repeated encirclement is a way of counting animals on sample sites consisting of accounting encirclement of the same area, the contours of which are closed by the route. The sites are evenly (or randomly) laid on the habitat area of the animal species counted. The method enables determination of the absolute (not relative) number of active land species of animals in the survey area, following their tracks in the snow for two or more passes of each recording site. Specifically, one must obtain a sample of the actual population density of animals in the studied area, then extrapolate the obtained data over the entire territory and estimate the average population density of animals, as well as their total number with the necessary and sufficient statistical accuracy. Counting is carried out in a

closed route, limiting a predetermined part of the studied area (reference area), and carried out in the presence of snow cover. Counting by the method of multiday encirclement is carried out in the studied area, in which the area of the "forest" is not more than 50000 hectares. The number of animals of each species on each accounting site for each day of the census is calculated based on data from the daily statements by determining the difference between the input and output traces of animals recorded on the accounting route, acknowledging the animals that did not give any traces on the previous day and (or) the next day conducting accounting.

The run method can be used for roe deer, red deer, wild boar, fox, lynx, wolf, and hare. The principle of accounting is as follows: the animals run away from the accounting sites and are recalculated visually or based on their traces. The ratio of the number of animals of a given species driven from the site to its area allows us to determine the indicator of the species population density on the site. If a sufficient number of these accounting sites are laid throughout the entire surveyed area, then the number of animals over the whole territory can be determined by extrapolation. The run method can be used at any time of the year. First, the site is passed around the perimeter and all input and output traces are erased. Then the chain of beaters moves around the site, expelling all animals with cries and noise. Marginal (outside) beaters go along the boundaries of the site, noting fresh entrance traces where they appear after grouting. Although this method is accurate, it is very laborious, especially when visually registering animals. It requires a large number of accountants and is extremely disturbing to animals, and hence is often unacceptable.

*Ungulate accounting peculiarities in mountain conditions.* Making records in the mountains was achieved by accounting from a helicopter, through the registration of ungulates on routes and sites.

Attempts to conduct continuous surveys using a helicopter in various regions of Dagestan showed almost 60–120% error in the direction of undercounting animals, depending on the complexity of the relief, its afforestation, and weather conditions. During these surveys it was absolutely impossible to register the structural parameters of the population and the age composition; the data on the sex ratio in the population were significantly distorted. Registering the animals on routes characterizes only the occurrence of animals at various elements of landscapes, and is not suitable for assessing their absolute density. For example, in the conditions of the Caucasian Reserve, errors during route registration of tours varied in different years from 135% to 250%, with a helicopter reporting the error as an average of 315% (Kotov, 1968).

The site method is effective for the absolute majority of ungulate species occupying, as a rule, a vast area, and aims to estimate the density of their populations at selected sites with a subsequent extrapolation of the results of these counts to identical territories.

The beginning of all counts is preceded by the preliminary work related to the choice of size, shape and number of accounting sites. After reconnaissance acquaintance with the terrain and its orographic features, attention should be paid to the following points: the ability to establish and maintain the number of sites sufficient to ensure a high reliability of counting results in a short period of time (5–7 days); im-

plementation of accounting work organized by the smallest number of researchers and requiring the least amount of time; the correspondence between sample size and the objects' accommodation density to be taken into account, which ensures the highest possible approximation of the empirical distribution of quantities in the statistical population to the normal theoretical distribution (Gaussian distribution). To accomplish this, the most typical areas for the selected region are selected (of an area of 1500–5000 hectares) in different parts of the studied species territory. Within these areas, after binding to the topographic grid and additional inspection of the territory, 5–6 reference sites are selected so that there are at least 4–5 registrations of animals or their groups per site. If possible, it is better to set the limits of reference sites so that they would be limited to ridges and are separated by large slopes or tracts. The whole territory (extended slopes) could also be divided into sectors, the boundaries of which should be consistent with the orographic features of the area, i.e., with highly visible landmarks. The size of accounting sites, as a rule, varies: this does not affect the accuracy of the counts, but affects the labor costs.

The size of each accounting site and the total area where the records are carried out are determined by planning the topographic maps of 1:50 000 and 1:100 000 scales. When estimating the areas (accounting sites, the total area on which the records are made, the territory, etc.), the maps require a vertical projection correction to determine the actual surface area of the slopes (Zotov et al., 1987). For large mountain arrays, it is more convenient to calculate the averaged conversion factor. To assess animals' tendency to elements of the landscape, the same maps can be used to calculate vertical extensions and slope angles in their habitats. The vertical length of the slopes is calculated from the difference in height between the isoline levels of the ridges and river valleys. The slope angles can be determined using a protractor of a geological compass or with the formula  $tg \alpha = a/b$ , where "a" is the vertical extent of the slope, and "b" is the projection of the distance from the base of the slope to the crest on the plane (Magomedov, Akhmedov, 1994).

Counting animals at the sites is carried out for 3–5 clear days, depending on the area (100–250 hectares) and configuration. The whole territory could be successfully scanned by 2–3, or in some cases 5–7 accountants using high resolution spyglasses and field binoculars. Calculations by one researcher result in a significant underestimation of the animals with a large error (30–50%) while significantly reducing the area of each recording site, and their number leads to impeding statistical accuracy. The most favorable periods for recording are early morning and evening, but in order to reduce the possible errors associated with the movement of animals at the same site, reporting should be undertaken several times during full daylight hours. The number of registered animals, their gender-age groups, the places of their detection, the direction of movement and the time of detection are plotted on the large-scale map or aerial photographs of the area, or are entered into the GPS memory. In addition to the sex and age ratio of the groups of registered animals, their number in the group, we should note the presence of animals that have distinctive features (features of the forms and defects of horns, lameness, body color and size, activity, etc.) that can further help recognize the groupings during accounting.

The data on population density obtained at different survey sites within one accounting array are averaged and extrapolated over the entire populated area of the given region with similar natural conditions (for typological groups of natural regions). All initial data are plotted on the map of the area, and the results of the calculations are reduced to the special forms with the indicated dates and time of the survey, the numbers and quantity of sites, the size of each site, the number of animals found at each site, the average population density for a given natural region, the total animals counted at sites, extrapolated number of species for a given region, and the standard error of counts. A special report is compiled for each account, where the date, weather conditions, composition of the accounting areas, amount of time spent and other data are indicated, in addition to the method of recording and describing the characteristics of the area.

When accounting the number of ungulates, particular attention should be paid to the degree and nature of the segregation of herds in different periods of their lives. The study of population structure is feasible for almost all ungulates species without their being removed from nature. Thus, among other benefits, it becomes possible to directly assess the structure of their herds. Evaluation of the spatial and temporal structure of prey populations, besides the need for accounting (optimization of counting time during the day and season and studying the localization of animals in daily and seasonal aspects, etc.), has its own value. For this purpose, material based on the analysis of animal encounters, depending on the nature of their habitat, can be used. Characteristics such as height above sea level, the gradient and exposure of the slope, the ratio of areas and the topography of landscape elements (rocks, talus, pastures) can be taken as the basis for the classification of habitats in mountainous areas. The nature of the activity, the time of registration, weather conditions, and the presence and characteristics of the snow cover should also be noted. When the influence of another factor becomes clear during, it should additionally be considered (the degree of studied factor influence should be amenable to statistical analysis, i.e., it is necessary to choose a parameter to make it metric). In analyzing data on the occurrence of animals depending on any factor, the number of animals according to individual gradations is usually converted to percentages.

When registering the distribution of animals relative to slope exposure, the distribution along the southern and northern slopes is usually considered, as they most strongly differ in terms of the conditions and nature of use by animals in all seasons of the year. To simplify the calculations, in some cases the slopes of the southeastern and southwestern exposures can also be attributed to the south, and the northeastern and northwestern exposures to the north. To analyze the spatial-temporal structure, it is necessary to incorporate the following parameter – the time animals stay in a particular territory – as it is closely related to the nature of daily activity. Therefore, apart from the meeting time and the state of animal activity, one should also note the quantitative and age sex ratio of each group encountered, the meeting place (height above sea level, slope exposure, and landscape elements such as rocks, talus or pastures), weather conditions, accumulated excrements at different elements of the landscape, and so forth. To this end, indirect data based on animal activity traces can also be used, including the presence of food vestiges, hair left by animals on rocks and trees

during shedding, and data on animal tracking after rain or freshly fallen snow. Furthermore, it is desirable to calculate the areas of both summer and winter pastures and their productivity, the areas of rock outcrops and scree and the nature of their distribution in the territory, the area of pastures used for grazing, the density of domestic animals grazed in each territory, and others.

In the context of the objectives of this book and taking into account feeding selectivity and food preference, we should incorporate several parameters to monitor and assess the abundance of prey. The structural parameters of the studied populations include: the herd instances, occurrence, and sex ratio. All data on the sex-age structure of herds are used to characterize the overall sex-age structure of ungulate populations in a given area as a whole. The most important population statistics are based on demographic tables built on these data. A comparative analysis of these tables of different population groups makes it possible to assess which age categories are affected by the influence of the focal predators' species, the role of predation, and other factors. The age structure of populations based on direct observations, and analysis of empirical data can be obtained only for males. Limiting this assay to the remains of hunted animals usually indicates a huge discrepancy with the real demographic picture of their populations (Magomedov, Akhmedov, 2000). The age distribution of females and males can be presented clearly through age pyramids: they demonstrate the differences in the ratio of females and males in populations within different age classes. A comparative analysis of demographic tables of various populations permits determination of the age class most affected by predatory activity in a given territory in the absence of other significant factors of influence (hunting, poaching, diseases, climate, etc.).

Taking into account the characteristics of reproduction and the overall fecundity of the population, the survival of the younglings, and the nature of individuals' mortality during different ages and in different sex groups, all of the data obtained are used to model the population exploited by predators in order to predict their well-being.

*Modern methods for assessing the abundance of the big cat's prey  
and their competitors*

Modern methods used to assess the abundance of animals suggest year-round, non-invasive observations (long-term monitoring) with automatic recorders (trailcams) distributed through the habitat in a special way. Trailcams are organized by researchers into a system: a spatial array, uniformly covering a given accounted area. The results of photo registration, taking into account the spatial distribution of animals (wild boar, red deer, roe deer, bear, badger, wolf, jackal, lynx, tur, chamois), enable the index of the abundance of the leopard's main prey or competitors to be calculated, as well as estimate seasonal, biotopic and relief differences in the space use by different species that can have a significant impact on the diet of big cats (leopards).

In mountainous terrain, the use of an array of trailcams yields good results in assessing the abundance of the prey base of big cats (Karnaukhov et al., 2011b; Rozhnov et al., 2012b). It is advisable to install the array at four terrain stations with four parallel camera trails (10 trailcams in each row installed at an average of 250 m from each other): one row along the ridge separating one valley from the other, the second



along the valley, and the other two in the middle of the northern and southern slopes of the valley. In addition, trailcams should be located in various types of plant associations, enabling a differentiated assessment of the animals' use of different habitats in different periods of the year. For example, when studying the abundance of the prey base of the Amur tiger in the Ussuriyskiy Reserve (Rozhnov et al., 2012b), we identified four types of such habitats: (1) young and middle-aged deciduous forests (mainly located along the river valley), which included tiers of forest canopies consisting of different species of maples, elm, poplar, and ash; such forests were also characterized by the presence of high grass cover with a large number of shrubs; (2) ripe broad-leaved forests, where the dominant species were linden, oak, maples, elm, and ash; these were characterized by a well-developed grass cover and thickets of shrubs; (3) mixed dark-coniferous-deciduous forests, that is, ripe forests where along with deciduous species, Korean pine, fir, white fir and spruce were largely present; (4) dark-coniferous forests, where the aforementioned conifers dominated; here the grass cover is not continuous, and is poorly developed.

When studying the prey base of big cats including the leopard, trailcams are mainly directed to photograph ungulates, and should be set at a height of about 1.2–1.5 m above the ground surface. The number of days spent by each trailcam is recorded as the number of days elapsed from the time the trailcam was installed (or the batteries were replaced) until the last frame shot by this trailcam (when changing batteries, usually about every four months, one should approach the trailcams to confirm that it is in working mode). The number of animals' passes is recorded, counting several photographs taken successively with a trailcam at intervals of up to 3 minutes as a passage (photo location) of one animal.

### 2.3.2. DATA COLLECTION FOR THE BIG CAT'S DIET ASSESSMENT

Evaluation of the diet of any species of big cat's in the wild constitutes one of the most important aspects of its monitoring. It enables the ratio of different types of prey in the big cat's diet to be assessed, and provides data about the optimal natural diet of these rare predators during the seasons. It is possible to evaluate whether natural conditions are suitable for a reintroduced carnivore trained in captivity by the natural diet. The choice of natural prey (wild ungulates) for the wild predator confirms its normal physiological status: only a healthy predator can successfully hunt wild ungulates regularly (**Figure 45**). Indeed, the big cat's nutritional assessment is necessary to understand how this predator affects the food resource (wild ungulates and other animals). Validated data of this kind are important in explaining the role of the big cat's in ecosystems on hunters and villagers, particularly regarding how it influences the number of wild ungulates, as well as its normal lack of interest in grazing livestock owned by humans.

There are two main ways to assess the diet of big cats: to check the clusters of locations obtained from GPS collars (potential hunting places where animals stay close to the carcass for a long time), and analysis of scats collected by field researchers, with further determination of the species being the leopard's prey.



**Figure 45.** Wild Amur tiger male (*Professor*) eating red deer. Ussuriyskiy Reserve.

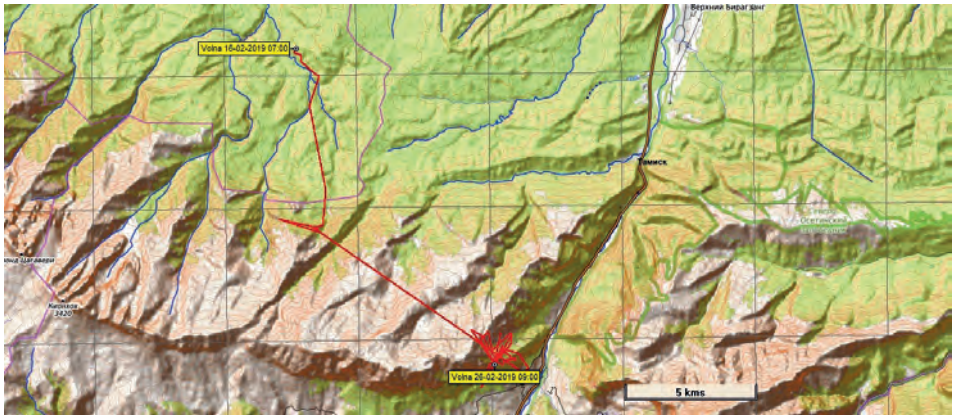
Thus, analysis of the hunting success of the Caucasian leopard female (*Victoria*) from the moment of releasing it on 15 July 2016 to 14 June 2017 – when the GPS-transmitter stopped working (11 months) – revealed 31 clusters of locations, 29 of which were confirmed by field researchers as successful hunts. *Victoria* obtained her first prey in the wild (female of wild boar) in six days after the release (21 July 2016). During the tracking period, the average time *Victoria* remained with the carcass at the kill site was 70 hours (min 14 h, max 172 h, N = 29). At the same time, the period between successful hunting to large animals (“the hungry period”) averaged 171 hours 33 minutes (a little more than seven days) with a maximum value of 578 hours (about 24 days). Analysis of the hunting success revealed a negative correlation between the average air temperature and the duration of the hungry period ( $-0.37$ ,  $p < 0.05$ ). These data indicate that hungry periods lasting more than a week during the cold period of the year are not uncommon: *Victoria* had 10 hungry periods lasting from 9 to 24 days (M = 13), six of which occurred in the winter.

*Checking clusters of locations, collecting samples from the big cat's prey  
for subsequent species/gender identification*

Big cat's hunt various animals with differing degrees of biotope dependence (**Figures 46–49**). Accordingly, the locations of their successful hunts are located from the bottom and the slopes of valleys, where they hunt wild boar, to the rocky projections that are difficult to reach for humans, characterized by turs and chamois. To identify the location of such prey, clusters of consecutive locations (concentration of locations) are detected, where the animal was staying for 12 hours or more. The diameter of the “cluster spot” determines whether the concentration of locations does not exceed 260 m.



**Figure 46.** The cluster of locations of the Caucasian leopard male *Killy* (marked by an arrow) in the valley near Podgornaya Stanitsa in the Otradnensky District of the Krasnodar Krai, and the remains of a roe deer found and any evidence of dragging along (07.06.2017).



**Figure 47.** The cluster of locations of the Caucasian leopard female *Volna* (marked by an arrow) in the North Ossetia and the remains of chamois (20.02.2019).



**Figure 48.** The cluster of locations of the Caucasian leopard female *Victoria* downhill of the Aphonian Range (shown by an arrow and a star), where she spent 68 hours, finding pieces of deer skin and excrement (29.11.2016 – 3.12.2016).



**Figure 49.** The carcass of a female deer hunted by the Caucasian leopard female *Victoria* in the Caucasian Natural Reserve (Umpirskaya Depression). The carcass is being eaten by vultures.

When checking clusters of locations and finding the remains of any big cat's caught prey, the site is carefully photographed with a scale (centimeter tape), the necessary measurements are made, and all possible information about the species, age and sex of the prey is collected. The scheme of clusters organised by reintroduced tiger female is shown on **Figure 50**, with remains of her prey.

Even if this cannot be done on the spot, it is still possible later. In any case, it is necessary to carefully collect samples from the prey remains, label them correctly, and indicate the coordinates. Through fur and other tissues in the laboratory, one can precisely determine the species of the prey and its gender. A cumulative database is formed on the basis of this information. Bone marrow samples should also be collected from prey bones to determine their fatness (Neiland, 1970).

Data collected for the analysis when experts have been checked up prey on the clusters is displayed in **Table 1**.

Nevertheless, situations of predatory mammals attacking livestock are likely to occur, and so this should be carefully considered before any reintroduction project. Such situations are especially likely in regions where livestock graze in the same pastures as wild ungulates, without any protection from shepherds with herding dogs, electric shepherds and sound indicators (bells). All cases of leopards attacking livestock must be registered, and experts should be involved to confirm whether it was indeed a leopard

**Table 1.** An example of a table used to record the diet of released animals based on clusters of locations. The columns refer to the number of killed animals throughout three, six, and 12 months after release respectively

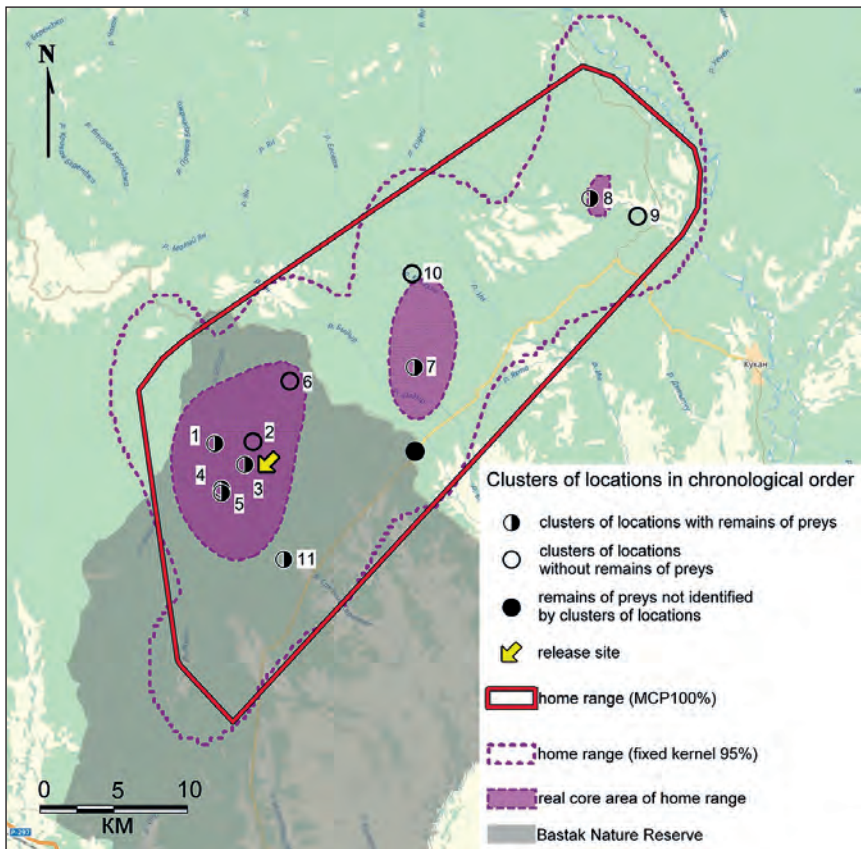
#### Caucasus nature reserve 2016–2017

| <i>Prey type</i>                        | <i>Killy</i> |    |    | <i>Victoria</i> |    |    |
|---|--------------|----|----|-----------------|----|----|
| Wild boar ( <i>Sus scorfa</i> )         | -            | 3  | 4  | 1               | 1  | 1  |
| Tur ( <i>Capra caucasica</i> )          | 1            | 1  | 1  | 3               | 3  | 3  |
| Red deer ( <i>Cervus elaphus</i> )      | 2            | 5  | 5  | 2               | 8  | 9  |
| Chamois ( <i>Rupicapra rupicapra</i> )  | 2            | 3  | 4  | -               | 1  | 4  |
| Roe deer ( <i>Capreólus capreólus</i> ) | -            | -  | 1  | -               | -  | 1  |
| Wolf ( <i>Canis lupus</i> )             | -            | -  | -  | 1               | 1  | 1  |
| <b>Unchecked clusters</b>               | 5            | 10 | 15 | 3               | 5  | 13 |
| <b>Total</b>                            | 10           | 23 | 30 | 10              | 19 | 32 |

*Killy* has more unchecked clusters as it usually hunts in remote and difficult-to-reach areas.

#### North Ossetia 2018–2019

| <i>Prey type</i>                        | <i>Elbrus</i> |  |  | <i>Volna</i> |    |    |
|---|---------------|--|--|--------------|----|----|
| Wild boar ( <i>Sus scorfa</i> )         | -             |  |  | -            | -  | -  |
| Tur ( <i>Capra caucasica</i> )          | -             |  |  | -            | -  | -  |
| Red deer ( <i>Cervus elaphus</i> )      | -             |  |  | -            | -  | -  |
| Chamois ( <i>Rupicapra rupicapra</i> )  | -             |  |  | 2            | 5  | 7  |
| Roe deer ( <i>Capreólus capreólus</i> ) | -             |  |  | -            | -  | 1  |
| Wolf ( <i>Canis lupus</i> )             | -             |  |  | -            | -  | -  |
| Jackal ( <i>Canis aureus</i> )          | 6             |  |  | 1            | 1  | 4  |
| Badger ( <i>Meles meles</i> )           | 2             |  |  | -            | 2  | 5  |
| <b>Unchecked clusters</b>               | 2             |  |  | 1            | 3  | 3  |
| <b>Total</b>                            | 10            |  |  | 4            | 11 | 20 |



**Figure 50.** MCP and Fixed Kernel estimates of home range of a reintroduces Amur tiger female *Zolushka (Cinderella)* 3,5 months after release and clusters of locations (above). The cluster No.7 of reintroduced Amur tiger *Zolushka (Cinderella)* locations in the river *Bydyr* basin in the *Khabarovsky District* of the *Khabarovsky Krai*, and the remains of a wild boar found (belowe).



that killed the prey. Badridze (2018) has demonstrated how as the number of wolves increases in an area from which a different predator has long been absent, the distance of avoiding wild ungulates changes over time (from 30 to 200 m); the reaction of ungulates on the characteristics of the wolf attack alters as a result, and consequently a new balance in interspecies interaction emerges. Similar findings were described for the reintroduction project of the Eurasian lynx in Switzerland, implemented since 1984 (Breitenmoser et al., 1998; Stahl et al., 2001; Breitenmoser et al., 2010), as well as when the wolves were being restored in Yellowstone National Park (Ripple, Beschta, 2003; White, Garrott, 2005). When some time after predator reintroduction passes wild ungulates use to change the distance of avoidance in response to the characteristics of the predator's hunting strategy. Due to this reintroduced predators may have a period when livestock become easier prey than wild ungulates. Before the predators adapt to the new conditions, they can thus attack livestock more often.

Such situations should be considered in advance and be included on a list of the project's putative risks, because they are relevant both for scientific research and for working with people in the local community. Certainly, it is important to develop a system for evaluating each particular occurrence of a predator attacking livestock, enabling confirmation that the prey was hunted by a reintroduced predator. This can be based on an assessment system developed by the International Union for Conservation of Nature (IUCN) for a project of the Eurasian lynx Restoration in Switzerland, and a scheme for identifying prey caught by lynx and other carnivore species (Ryser, Ryser-Degiorgis, 2012). The data description below is adapted for a leopard; the veterinarian must complete an autopsy training course. It includes (1) a description of the kill site and the likely scenario of the incident, (2) the search for evidence of an attack on the ground, (3) the search for evidence of an attack on the carcass. With all of the descriptions, it is important to acknowledge the biology and ecology of the different species of predators inhabiting the region.

1. In the standard questionnaire, it is necessary to note the coordinates (GPS) and information about the kill site (forest, pasture, presence of a number of houses, roads); it is important to indicate whether there was any fence (electric fence or other), whether the cattle were protected by a shepherd, shepherd dogs; whether the cattle were equipped with bells; it is also important to specify the nature of the substrate (snow, grass, wet land, dry fallen leaves); it should be noted how many animals were killed, and whether the prey was alive at the time of the discovery by the person and at the time of arrival of the field team for investigation.

2. It is important to describe the state in which the prey was found, including whether it was covered with leaves and branches, and whether its predator dragged it to a secluded place (thickets, bushes); which parts of the prey's body were already eaten or affected; whether there are any parts of the body missing and if so, which. It is also necessary to carefully examine the surrounding territory. Usually it is possible to find traces of the struggle and, as a result, the predator's hair (this should be collected for species identification via genetic methods); if there were any types of fences, hair can be found on them; if the prey is dragged into the bushes, the predator's hair may be on the branches. If the substrate is snow or wet soil, the predator's foot-

prints may remain clearly. Traces and footprints (and possibly excrements detected) should be photographed with a scale (ruler). Hair and excrements must be fixed as described in section 2.2.2. Collecting biological samples for laboratory research.

3. It is necessary to describe the nature of the damage to the prey's skin: it is species-specific for predators and permits determination of the cause of the prey's death. On the carcass, first the external features are described after opening (internal). The parts of the prey's body that are subject to mandatory examination and dissection comprise: head and neck (projection from above); head and throat/pharynx (bottom projection); and skin surface, both external and internal (subcutaneous). In addition to the detailed numbered photos, it is necessary to record and note in the protocol: the general condition of the prey's body; the presence of bleeding; whether only the skin or part of the carcass is damaged (and where); condition of the skin surface (damage, lesions, parasites); perforation of the skin (place on the body, the nature of the perforations, number, depth); distribution of damage on the surface of the carcass; missing or eaten body parts; general health status of the animal at the time of death; and circumstances surrounding the carcass and location of the carcass. It should be noted that a number of signs can only be seen subcutaneously, hence it is necessary to open the carcass and check the condition of the organs. It is very important to describe the state of the lungs (to accurately determine the cause of death due to asphyxiation or not), given the main pathological signs of asphyxiation. First, cyanosis of the mucous membranes of the natural openings of the head occurs: the mouth and nose, tongue, lips, conjunctiva; dilated pupils; very pronounced, abundant, dark red with a bluish tinge, especially in the front part of the body, corpse spots and places of bruises of the prey during beating; very bloody jugular veins. Asphyxia is frequently accompanied by hemorrhages on the conjunctiva under the pulmonary pleura. The blood is not coagulated, dark red with a bluish tinge, quickly glowing in the air. The right half of the heart is dilated, filled with blood. The lungs are full-blooded, sometimes with emphysema, but dilated, in some cases swollen. Asphyxia is also accompanied by small superficial hemorrhages, which are often found under the epicardium and on the surface of the lungs (they are especially easy to notice).

The following characteristics serve as confirmation that the prey was attacked by a leopard: a bitten throat, pharynx or muzzle of the prey (soft tissue of the nasolabial part of the facial area of the head); few but clear perforations of the skin on the throat (internal autopsy also shows that the larynx is perforated, unlike for canine hunting); the prey was obtained at the border of the forest part (thickets) and in open space at the border of light and shadow; the prey is dragged into the bushes, hidden among the branches, does not lie openly; predator eats prey from the back of the body, moving to the front for a few days; if an ungulate from the ruminantia taxon is killed, big cats usually do not eat the paunch (unlike canines); big cats eat the whole carcass at the kill site very carefully and do not pull apart the parts of the body (i.e., no parts of the body are displaced relative to each other no matter how long ago the prey was killed and how much meat was eaten); felids eat carefully, often purging the meat from the skin (the eaten prey has the skin just turned inside out); there may occasionally be deep marks of claws on the outer surface of the prey skin. It is also important to

remember one rule: if the prey looks atypical for a large felid, then the prey was not caught by the felid.

It is recommended to install trailcams around the hunting site. In this case, there is a possibility that the predator returning to the prey will be caught “red-handed” and identified. However, it should also be acknowledged that any other predators that live in a given territory may approach an abandoned corpse.

#### *Collection and storage of big cat’s scats for diet analysis*

When snow tracking and performing field routes in big cat’s habitats, it is often possible to collect their scats for the purpose of subsequent diet analysis. Such analysis yields a more complete palette of a leopard’s nutrition than description of the prey on clusters of locations, although as a rule it is not tied to specific individuals. Using the analysis of scats, it is also possible to identify small food items (like medium and small carnivores, rodents, and birds), which cannot be detected by checking clusters because the leopard recycles such prey in a matter of hours or even minutes. Scats for such analysis can be collected at any time of the year. It is recommended that sampling be conducted with latex gloves. Each sample collected in a separate Ziploc bag must be provided with a paper label in a separate package to prevent contact with the sample, on which the individual sample code, date, geographical coordinates of the collection and the full name of the collector should be indicated. Samples can be stored in a freezer or refrigerator or in a cool dry place; indeed, samples should not be stored in warm and humid places.

## 2.4. SERVER (GEO-INFORMATION) SYSTEM FOR COLLECTING, STORING AND VISUALIZING DATA IN THE FIELD

When conducting monitoring and collecting data in the field, it is necessary to find a balance between obtaining the maximum amount of required information, the convenience and utility of its registration, and the provision of operational analysis and visualization.

The availability of various modern technologies (GPS loggers, digital photo and video recorders, thermal sensors, etc.) for the collection of precise empirical data in the field, as necessary for environmental studies, enables monitoring to be organized and conducted in a new way, with computer technologies quickly processing these data to identify cause-effect relationships. Nevertheless, even today the preparation of field-collected data for subsequent analysis (situational linking of information obtained from various sensors with complementary diary entries) is usually undertaken manually, and is thus a process that utilizes a significant portion of the researcher’s time.

Below is an example of an optimized data collection system (representation of the data collected in the field suitable for a computer), allowing the researcher to spend more time on comprehensive analysis of the data set collected in the field, and less on their preparation.

Advances in mobile technologies have engendered significant opportunities for the organization of environmental research (Snaddon et al., 2013; Teacher et al., 2013; Kuntsche, Labhart, 2014). Smartphones used by many field zoologists are actually available portable computers with numerous functions (microphone, accelerometer, gyroscope, light sensor, magnetometer, etc.), being capable of going online and remotely transmitting large amounts of data. They also have geolocation (GPS, GLO-NASS) and access to geo-information data, make and process high-resolution photo and video images, read and generate QR and barcodes, and much more.

Operative analysis and visualization of data collected in the field can be divided into two stages. The first is to adjust smartphones to collect data in the field in a format ready for sequential analysis. The second is to create a server-type database, accessible to all monitoring participants, to which all smartphones will send the collected information. Two-way communication enables the synchronization of all mobile devices involved in data collection.

*Development.* Few systems render it possible to collect data electronically and systematically send them to the server (although one of the first appropriate systems is Cybertracker). We opted for the Russian system NextGIS, which has various auxiliary applications that allow data to be adapted for specific zoological tasks.

As an example, below is a database we developed, located in the NextGIS Web cloud server, which we use in our projects. NextGIS Web is a server-side Web geo-information system designed for storing, visualizing and organizing multi-user access to geodata (<http://nextgis.ru/nextgis-web>). The initial database consists of 14 tables located in the form of 14 layers of a geo-information system (**Table 2**).

Each entry in each layer includes a non-repeating identification number, geographical coordinates, date, time (these three parameters automatically come from the smartphone), and a specific set of parameters for each layer from which the researcher can choose. Each value is set by the researcher or is selected from the proposed pre-defined list. In some layers (for example, footprints, dens, rendezvous-sites, etc.) photos can be attached to records (up to five images) (**Figure 51**).

To facilitate the database entries, interactive computer forms have been developed using NextGIS FormBuilder v2.1 for the NextGIS Mobile application (app), which works on smartphones with the Android operating system. These forms are created for each layer of the database and individually for each smartphone of the field group of zoologists carrying out monitoring in natural conditions. The individuality of the form for each smartphone is enabled by the fact that researchers work in the field largely without access to the Internet (offline). To avoid the same identification numbers coming from devices that are not synchronized with each other offline, the smartphone app adds the identification letter automatically. This letter is assigned to each identification number of any record made personally by each researcher/smartphone during data collection. Each form of this app is programmed for “intuitive” work in the field (for example, for accompanying information the form automatically suggests the final value so that the researcher does not have to mark frequently repeated parameters while working in the same place, such as the place of study, habitat, or the name of the researcher). The structure of the data collection form is de-

**Table 2.** Layers of geo-information system database for collecting, storing and visualizing server-type data

| NN | Layer name                 | Description   |
|----|----------------------------|---|
| 1  | Livestock                  | Spatial and temporal characteristics of recording flocks of livestock, their composition, and other characteristics   |
| 2  | Ungulates Visually         | Spatial and temporal characteristics of visually recording the red deer, Siberian musk deer, bezoar goat, west Caucasian tur, chamois, wild boar, European bison, with description of their groups and behavior |
| 3  | Trailcams                  | Geolocation and monitoring of automatic digital photo and video recorders   |
| 4  | Leopard Acoustics          | Spatial and temporal characteristics of leopard sound signals   |
| 5  | Leopard Places for Resting | Spatial and temporal characteristics and structure of leopard places for resting  |
| 6  | Leopard Scratches          | Spatial and temporal characteristics and structure of leopard scratches   |
| 7  | Leopard Visually           | Spatial and temporal characteristics of visually recording the leopard  |
| 8  | Leopard Dens               | Geolocation and structure of leopard dens   |
| 9  | Leopard Footprints         | Spatial and temporal characteristics and podometry of leopard footprints  |
| 10 | Leopard Excrements         | Spatial and temporal characteristics and distribution across different ways of analyzing leopard excrements   |
| 11 | Leopard Prey               | Geolocation and characteristics of using the carcasses of leopard prey  |
| 12 | Predators                  | Spatial and temporal characteristics of tracing the activity of the wolf, brown bear, lynx, and other carnivores  |
| 13 | Leopard Clusters           | Monitoring the clusters of telemetry locations  |
| 14 | General Zoological         | Geolocation and description of activity traces and visual records of different animal species not covered by other layers   |

signed to minimize data entry errors when the researcher is tired or when field conditions are complicated and so require considerable attention.

To work with forms directly, a mobile application for Android was devised: “NextGIS Mobile.” We use this in the field because it allows us to work with many open-source geoservices both online and offline. After installing the application on the device, tiles (set of images or maps of different scale) are selected and loaded.

Simultaneously with the georegistration process of point data, NextGIS Mobile allows the researcher to record one’s tracks (route movements, polylines). To record



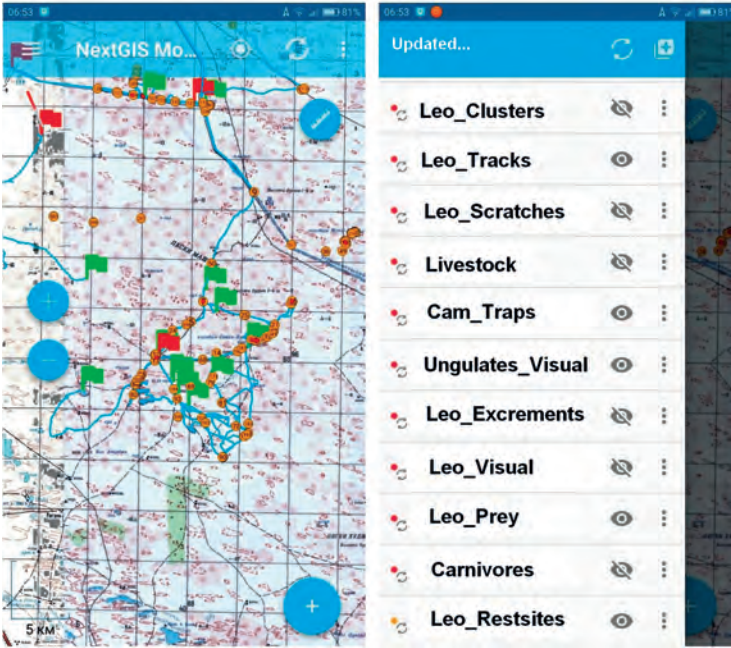
**Figure 51.** An example of a wolf's footprint taken by smartphone and uploaded to the database through NextGIS Mobile form.

animal footprints or tracks following the route of the animal, NextGIS Logger is used in parallel with NextGIS Mobile in the background. All recordings collected using NextGIS Mobile as well as all tracks recorded on NextGIS Mobile and NextGIS Logger are automatically sent to the server having synchronized via the Internet.

*The process of collecting, synchronizing and visualizing data.* The data collection process comprises the following steps. Having opened NextGIS on a smartphone with



**Figure 52.** Mapping and collecting data on the leopard's competitors using NextGIS Mobile.



**Figure 53.** An example of the windows of Next GIS app. The spots and track of several layers are on the left. Choosing the layer during data registration is on the right.

**Figure 54.** Exemplar form to register the excrements of a leopard based on NextGIS Form-builder for NextGIS Mobile.

a working GPS, the researcher puts a point on the map according to his/her location (or on any other place on the map at his/her discretion) (**Figures 52 and 53**). Subsequently, NextGIS suggests choosing one layer from the previously loaded ones, to which the new record should be attributed. After selecting a layer, the form of this layer is opened for filling (**Figure 54**). The researcher saves a record after filling the layer. While the researcher works without access to the Internet, records are stored only in the memory of the smartphone. After returning to the base (where there is often access to the Internet), all previously collected records are synchronized with the server. Furthermore, the database of all other smartphones used in the project is synchronized. To plan data collection and monitoring, any researcher in a city with access to the Internet can for example visualize the information collected by the field team every day using Web NextGIS.

*Testing and debugging.* The development of such a complex and highly technological system as NextGIS at the first stage requires constant monitoring and improvement. The operation of the system during its development and subsequent use requires mandatory testing and debugging in the field. Later, debugging is performed on the forms of the main database on the go. In case of conflict between forms, mobile application or server error, it is important to describe the problem and if possible fix it in the field camp. If necessary, NextGIS specialists working in real time are connected.

Although problems and difficulties may constantly arise, most are solved during the course of the work. Those that cannot be resolved in a short time are recorded as problematic for further improvement of NextGIS. Nevertheless, applying a system that remains in progress and is thus not perfect clearly demonstrates the high efficiency of the data acquisition system based on NextGIS technology. The process of registering traces of the vital activity of animals from various categories is accelerated hundreds of times, and due to this system, it can be carried out in just a few minutes, whereas previously it took days and months of hard work.

The process of collecting and analyzing data based on NextGIS is constantly being tested, improved and discussed with NextGIS specialists to refine the basic structure of the database (by researchers collecting material in the field) and software (by NextGIS).

## 2.5. ANALYZING AND PROCESSING THE COLLECTED DATA AND SAMPLES

All data collected in the field (leopards' locations from satellite transmitters, photos from trailcams, biological samples, registered animal footprints and traces) should be subjected to thorough analysis. Data is further analyzed in the laboratory and subsequently processed using statistical analysis, and could be used for modeling.

Modern research in behavioral ecology benefits considerably from recent advances in new technologies, especially those connected with space industry development. These methods not only include satellite telemetry, but also traditional methods (route



census, tracking) supplemented with GLONASS/GPS navigation, as well as remote sensing data analysis. These methods enable us to obtain abundant material for geo-information analysis and spatial modeling. The digital data collected with the help of these techniques and automatic spatial reference allow us to obtain reliable information and to create automated technologies on this basis.

Existing methods of analyzing the results of satellite tagging differ in terms of levels of complexity with additional geospatial data. Today there is growing interest in analyzing the regularities of the spatial position of satellite location points in relation to ranges on maps (Caroll, Miquelle, 2006). Topographic and thematic maps are traditionally utilized as sources of information. Such research aims to reveal how territories with different characteristics are visited by animals. The results provide new insights into how frequently different habitat types are visited by the tagged animals, as well as enabling us to identify and describe the preferred sites. Apart from describing the ecology of tagged animals, this approach provides an opportunity to assess particular features of their behavior when selecting places for resting, feeding, moving and hunting. Analyzing whether the sites of satellite tagging belong to the outlines on digital thematic maps provides valuable insights into the conditions where the reintroduced animals dwell and the way they select particular biotopes. A full cartographic base characterizing habitat conditions of each animal is derived from identifying multispectral satellite imagery. Being small in size, yet diverse, disintegrated in terms of typology and classification, as well as adapted to particular factors determining the habitat features of the studied animals, these patches serve as the methodological basis for combining the results of satellite tagging and imagery. The cartographic results based on satellite data feature details that can be extrapolated to more extensive territory (the whole chosen geographic range). Using satellite images of different seasons enables us to consider the seasonal features of how animals select particular ecosystems, as well as to perform regular terrestrial monitoring according to the satellite tagging data.

## 2.5.1. HABITAT ANALYSIS

### *#1 Neural networks method*

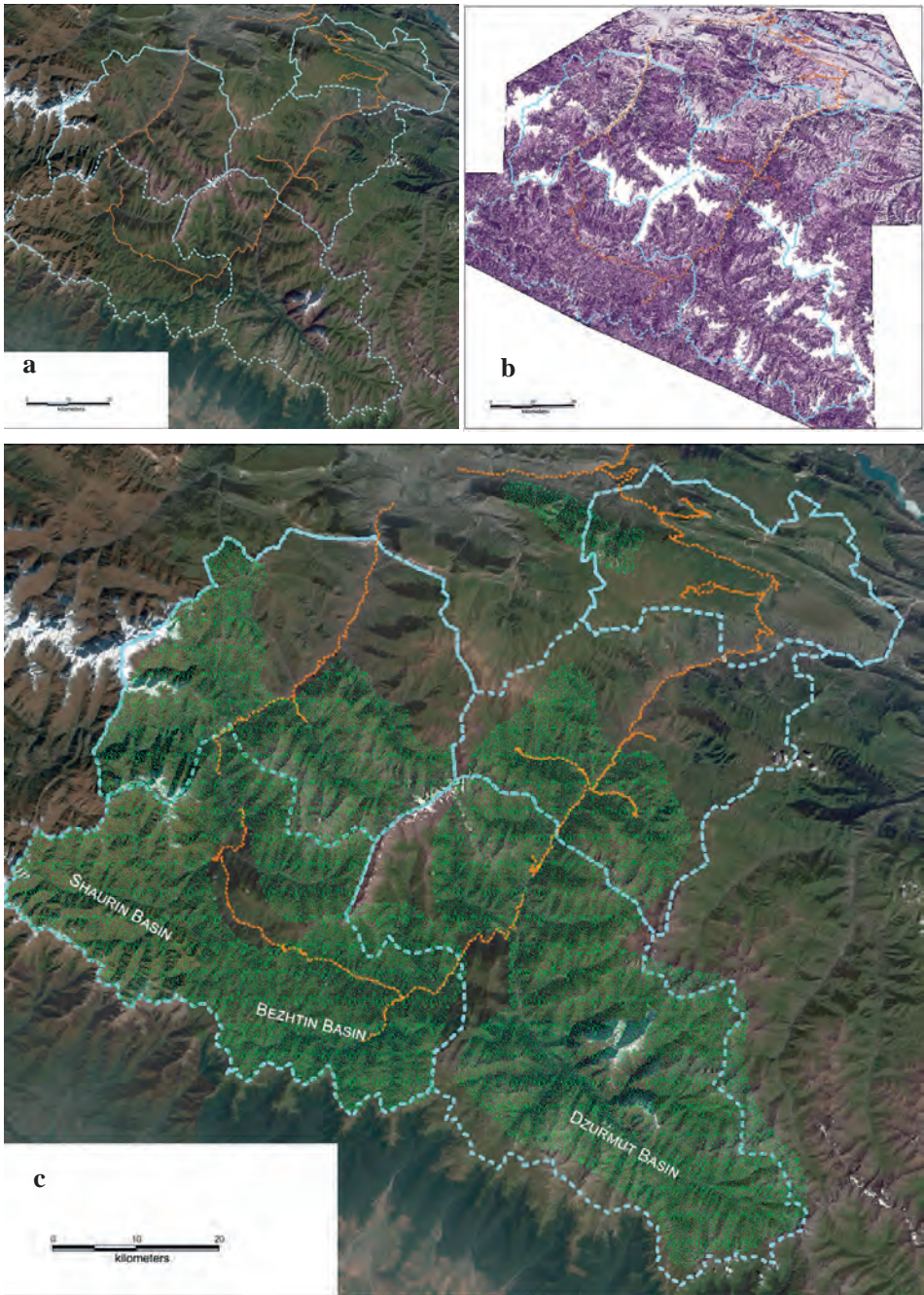
Complexing (integrating for simultaneously using combination) the results of satellite tagging data with multispectral satellite imagery is based on the classification of the latter on a vast number (more than 200) of classes (T-MAP), permitting us to precisely estimate whether the location points of animals belong to the particular biotope types marked on a high-resolution map of natural complexes, a result of interpreting satellite images. To integrate and combine the mass data, they should be divided into seasons and periods associated with cyclical rhythms, behavior and other features of the species ecology under study. This method enables identification of the part of the study area used by animals when satellite tagging data are accumulated, and calculation of their precise characteristics via mathematical methods. The method of thematic calibration T-MAP by GPS location points may be applied not only to a probability mapping of animal distribution throughout their range, but also to reveal the key factors that limit their migration. These

data combined with long-term arrays of location points allow researchers to monitor the geographic range restoration, considering such global factors as climate change and increased anthropogenic impact (Dobrynin et al., 2017).

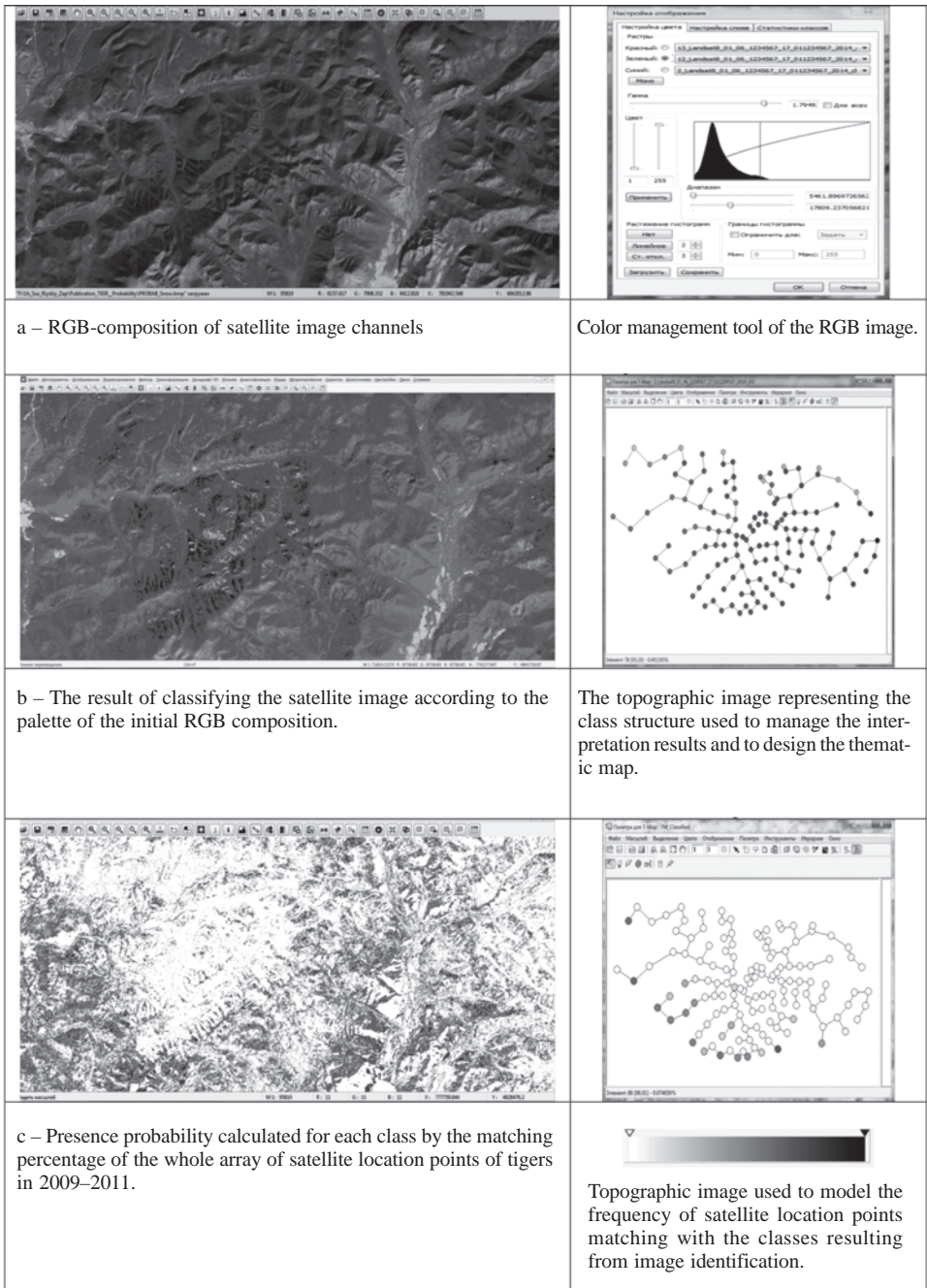
Analyzing the study sites in Dagestan indicates that the leopard may use any of them. However, mathematical modeling of animals' presence probability on the territory of Dagestan based on a multiseasonal mosaic of satellite images (remote sensing data) demonstrated that not all of the sites are suitable for leopards to live and form self-sustaining groups over a long period of time based on the system of biotope and landscape features. The probability characteristics of the leopard site selection derived from satellite image modeling demonstrate that site selection is independent of administrative boundaries. On the probability map of the leopard's presence, a whole ecoregion partly including three adjacent regions can be identified: Dzurmut (Tlyaratskiy region), Bezhtin (Tsuntinsky region, Bezhtin site) and Shaurin (Tsuntinsky region) basins, together forming Dido-Djurmut Basin (**Figure 55**). A continuous forest belt spread for more than 100 km southeast was confirmed as a "corridor" for migrating animals. The width of this "corridor" ranges from 15 to 30 km. The whole historically formed complex of mountain ungulates of the Eastern Caucasus dwells on the whole territory within the ecosystem on the Northern macroslope of the Greater Caucasus Mountain system and its branches, forming the three aforementioned basins. The lowest population density is 13 people per km<sup>2</sup>, identified for the central part of the studied ecosystem (Bezhtin Basin). This area practically lacks roads, there being only 12 km of them. The total area suitable for leopard habitation is approximately 780000 hectares here, consisting of 550000 hectares in Bezhtin basin, 90000 hectares in Dzharmut Basin, and 140000 hectares in Shaurin Basin. Elevation difference ranges from 1600 to 3400 m a.s.l.

To apply this method, satellite images with a high spatial resolution (15–0.5 m) with considerable retrospective archives (25 years and more) are required. The imaging seasons are autumn-winter (period of snow-coverage establishment), winter-spring (maximum snow coverage), and spring-early summer (snow melting period). Multi-spectral images processed via artificial intelligence algorithms (Dobrynin et al., 2017) enable us to divide them into a vast number of classes (**Figure 56**). By means of geospatial methods, data on the thickness of the snow coverage, its density, and the presence of snow crust are analyzed by neural networks. Then the statistical criteria of snow coverage meanings that are critical to the leopard and its potential prey are calculated. The spatial patterns of the anomalous level of snow accumulation revealed as a result of digital analysis are matched with the boundaries of the potential leopard range and locations previously obtained via satellite tagging of control animals. Based on this matching through geospatial methods, the environmental conditions of leopard habitation in winter are annually forecasted. The results of geospatial modeling are validated and interpreted based on the field data (measuring the footprint depth while tracking) or satellite imaging series during snow melting.

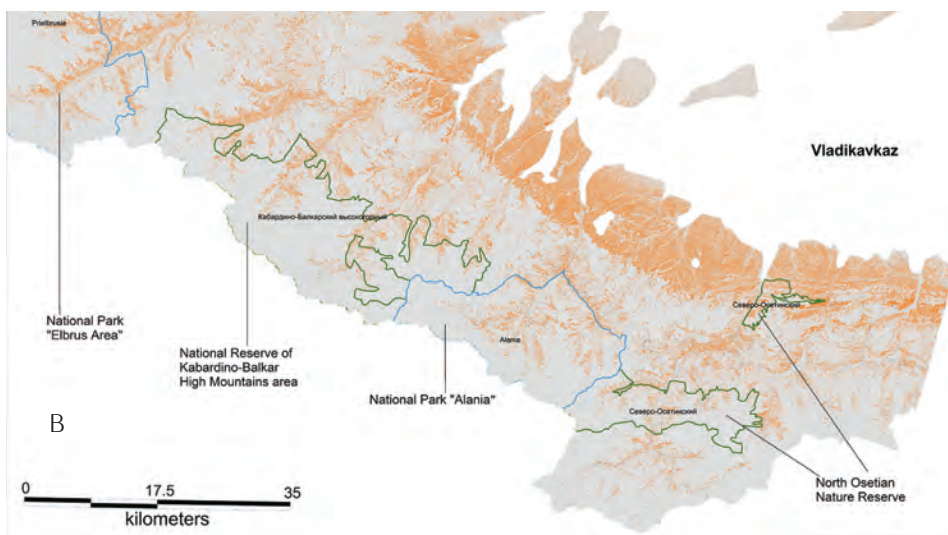
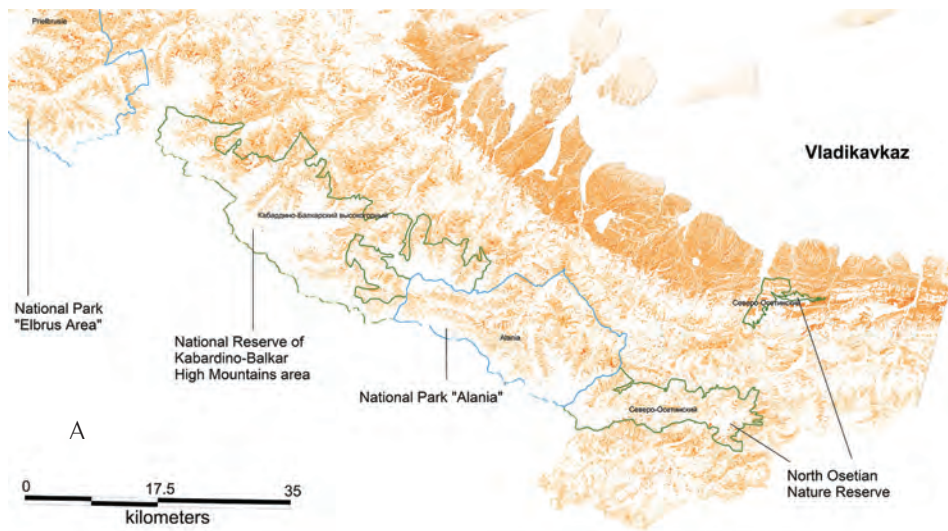
As an example, **Figure 57** illustrates the results of modeling the leopard habitats in the Republic of North Ossetia, Alania and the adjacent territory of the Kabardino-Balkar Republic, considering the distribution of snow coverage.



**Figure 55.** Didoy-Dzharmut Basin: a – the satellite image, b – interpretation result, c – the most suitable sites for the leopard. Color intensity in the interpreted image indicates the probability that the site is used by the leopard. The blue dotted line denotes the administrative boundaries; the orange line denotes the expedition route in 2017.



**Figure 56.** The technology of complexing (integrating for simultaneously using combination) the points of satellite tagging and the results of classifying the satellite imaging data (after Dobrynin et al., 2017). Comments are provided in the text.

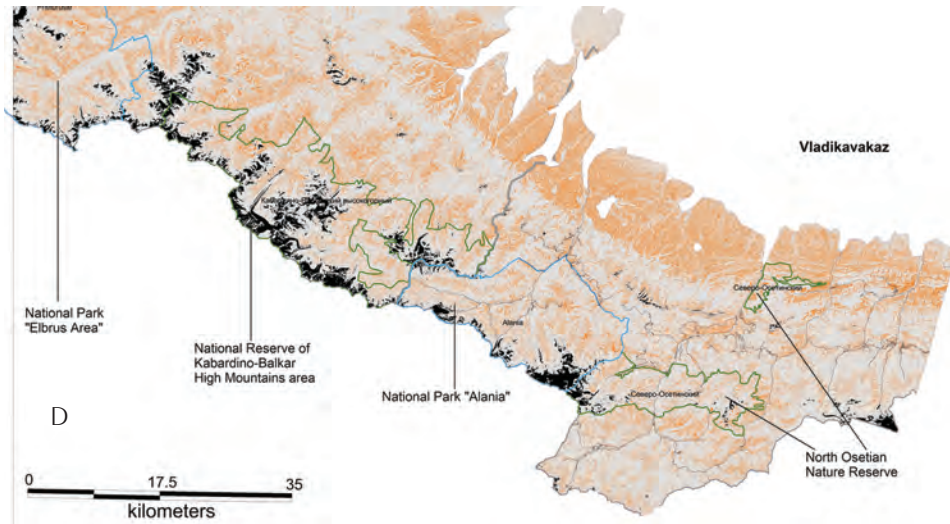
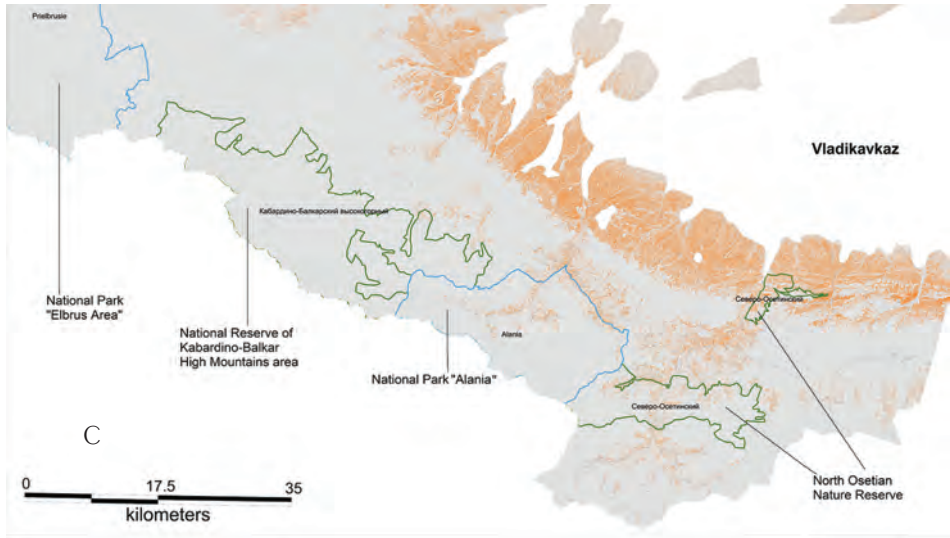


Low suitability for leopards



Good suitability for leopards

**Figure 57.** Map of the Caucasian leopard potential range structure in the Republic of North Ossetia and the Kabardino-Balkar Republic in different seasons of the year: A – the maximum range in summer-autumn; B – the range in the period of average snow cover in the mountains.



Low suitability for leopards



Good suitability for leopards

**Figure 57 (continued).** Map of the potential range structure of the Caucasian leopard in the Republic of North Ossetia and the Kabardino-Balkar Republic in different seasons of the year: C – the range in the period of maximum snow cover in the mountains; D – the structure of the leopard’s range considering its preferences in all the seasons; black spots denote glaciers and snow patches.

Using geographic information systems enables us to identify the places to design ecological corridors for large carnivores, as also demonstrated for the Amur leopard and the Amur tiger in the Russian Far East (Miquelle et al., 2014, 2015b).

## *#2 MaxEnt method*

Today, numerous modeling algorithms for the spatial distribution of species forecasting exist (Stockwell, Peters, 1999; Guisan and Zimmermann, 2000; Austin, 2002; Guisan et al., 2007; Guo, Liu, 2010; Hijmans et al., 2012; Stigall, 2012). One of most popular is the maximum entropy algorithm (Phillips et al., 2006; Phillips, Dudik, 2008; Elith et al., 2011), embedded in MaxEnt software (Maximum Entropy Species Distribution Modelling). Such a model is based on the presence-only data for species whose habitats must be estimated. The working scheme of this algorithm is built on the thesis that approximation should satisfy all limitations known to the researcher. However, at the same time, the resultant distribution has its maximum entropy (Phillips et al., 2006). The result of this algorithm work normally looks like a map with forecasts of the probability of species presence in each cell/pixel raster. Thus, the logical and mathematical basis of Maxent software for species habitat modeling elucidates those habitats, which are the same as those where species were registered in natural conditions. This result is based on the distribution of values of measured environmental features. The hypothesis of balance between species presence and amount of exact characteristics of environmental locations is under the test. Different MAXENT methods yield estimations of habitat results with different degrees of "stringency". Each method during analysis estimates the probability of meeting with an object in each point/location of area. Habitats with maximum probability which were found by several methods are considered as the most favorable. All habitats could be described with number of probabilities for species. These probabilities depend from the set of characteristics were previously chosen. According to logic of the MAX-ENT method, the software algorithm calculates the extrapolation of its forecast overlapping the limits of multidimensional subdomain where species inhabit. The method determines the potential species distribution but does not define the real implemented distribution. By using standard non-parametric significance criteria and standard statistical procedures, it is possible to define the state of environmental properties that influence species distribution. In general, the calculated models permit determination of the parameters of the species connection with environment, and the location of individuals to be predicted.

### *Caucasian leopard habitat modeling*

We also organized modeling of the habitats potentially suitable for the Caucasian leopard in North and South Osetia using MaxEnt. Inaccessibility for direct evaluation considerably limits the study of animal ecology in mountainous regions. To overcome this constraint, remote sensing methods should be broadly applied, and the results obtained for a limited area covered by field routes interpolated for the whole study area.

As basic information for modeling we used the results of satellite tracking of the released Caucasian leopards. These data were modeled and interpolated via the max-



**Figure 58.** Modeling area covered the Caucasus mountain country. Region are: 1 – Krasnodarskiy Krai, 2 – Adygeya Republic, 3 – Karachaevo–Cherkesskaya Republic, 4 – Stavropol'skiy Krai, 5 – Kabardino–Balkarskaya Republic, 6 – Republic of North Ossetia – Alania, 7 – Ingushetiya Republic, 8 – Chechen Republic, 9 – Republic of Dagestan.

imum entropy approach, MAXENT (Baldwin, 2009; Elith et al., 2006, 2011). The modeling area (**Figure 58**) covered the Caucasus mountain country within the following physiographic boundaries: the Caspian Sea in the west, from the Caspian Sea along the Kuma–Manych Depression and further along the border of Krasnodarskiy Krai to the Black Sea in the north, along the coast of the Black Sea in the east, across the Kura–Aras Lowland and along the borders of Azerbaijan, Armenia and Georgia. Digital elevation model (DEM) based on Global Multi-resolution Terrain Elevation Data 2010 (GMTED\_2010) with a resolution of 250 m including morphometric characteristics of the relief (**Table 3**) provided the basis for interpolation. Climatic variables downloaded from global climatic database (Worldclim2) included the monthly mean, minimum and maximum temperatures, precipitation, solar radiation, water vapor pressure, and wind speed, as well as 19 bioclimatic variables.

**Table 3.** Characteristics of variables used for modeling

| II. Relief parameters (from Shuttle radar topographic mission – SRTM) |   |                          |
|---|---|--------------------------|
| Alt   | Altitude above sea level, GMTED2010 values                  | Warmth                   |
| aspect  | Slope exposure (algorithm by Zevenbergen and Thorne, 1987)  | Illumination, humidity   |
| slope   | Slope curvature (algorithm by Zevenbergen and Thorne, 1987) | Humidity, soil thickness |
| curv  | Total slope curvature (Buckley, 2010)                       | Precipitation            |
| pl_curv   | Plain curvature (Moore et al., 1991)                        | Soil moisture            |
| pr_curv   | Profile curvature (Moore et al., 1991)                      | Soil erosion intensity   |



**Table 4.** The number of discrete points used for modeling

| Name                   | N      |
|------------------------|--------|
| <i>Panthera pardus</i> | 264703 |
| Male                   | 133454 |
| Female                 | 131249 |
| <i>Akhun</i>           | 2844   |
| <i>Artek</i>           | 17230  |
| <i>Elbrus</i>          | 1954   |
| <i>Killy</i>           | 111426 |
| <i>Victoria</i>        | 129463 |
| <i>Volna</i>           | 1786   |

To assess the spatial distribution of animals across the Caucasus, the potential habitats were analyzed for the species in general, as well as for males, females and each individual separately. According to the data from GPS collars, the migration of the Caucasian leopards was estimated both in the Caucasus Nature Reserve and in North Ossetia, where the animals were reintroduced. The number of discrete points used in the analysis is listed in **Table 4**.

To obtain a reliable final model, 15 repeated models were run in 10000 iterations in each pixel of the study area, and 25% of the discrete points in each analysis were randomly selected to test the resulting model.

The output algorithm estimated the probability of species occurrence in a range from 0 to 1. Areas with probability of species occurrence of 0.8 and above were considered optimal (in these areas the species can be detected with a probability of 80% and higher). Areas with probability of species occurrence of 0.5 to 0.8 were considered potential, i.e., the species may occupy these areas under particular conditions or use them for migration.

For the species in general, the modeling results demonstrated a high level of accuracy of defining the predicted points confirmed by statistical analysis. To estimate standard error, the Area Under the Curve was calculated (mean AUC value for the 15 repeated models equaled 0.935, and standard deviation was 0.003 (**Figure 59**)).

Out of 111 factors of the interpolation model, 13 contributed more than 1% to modeling the potentially suitable habitats (**Table 6 and 7**). The major factors deter-

**Table 5.** Area of potentially suitable habitats

| Parameter (leopard) | Area, sq. km | % of the total area |
|---------------------|--------------|---------------------|
| All                 | 5152.75      | 1.31                |
| Female              | 1033.88      | 0.26                |
| Male                | 5235.46      | 1.33                |
| <i>Akhun</i>        | 52.63        | 0.01                |
| <i>Artek</i>        | 1315.85      | 0.33                |
| <i>Elbrus</i>       | 1247.64      | 0.32                |
| <i>Killy</i>        | 2364.76      | 0.60                |
| <i>Victoria</i>     | 732.04       | 0.19                |
| <i>Volna</i>        | 375.96       | 0.10                |
| Total               | 394562.06    | 100                 |

**Table 6.** Contribution of different parameters to forming potentially suitable habitats for Caucasian leopards in the Caucasus

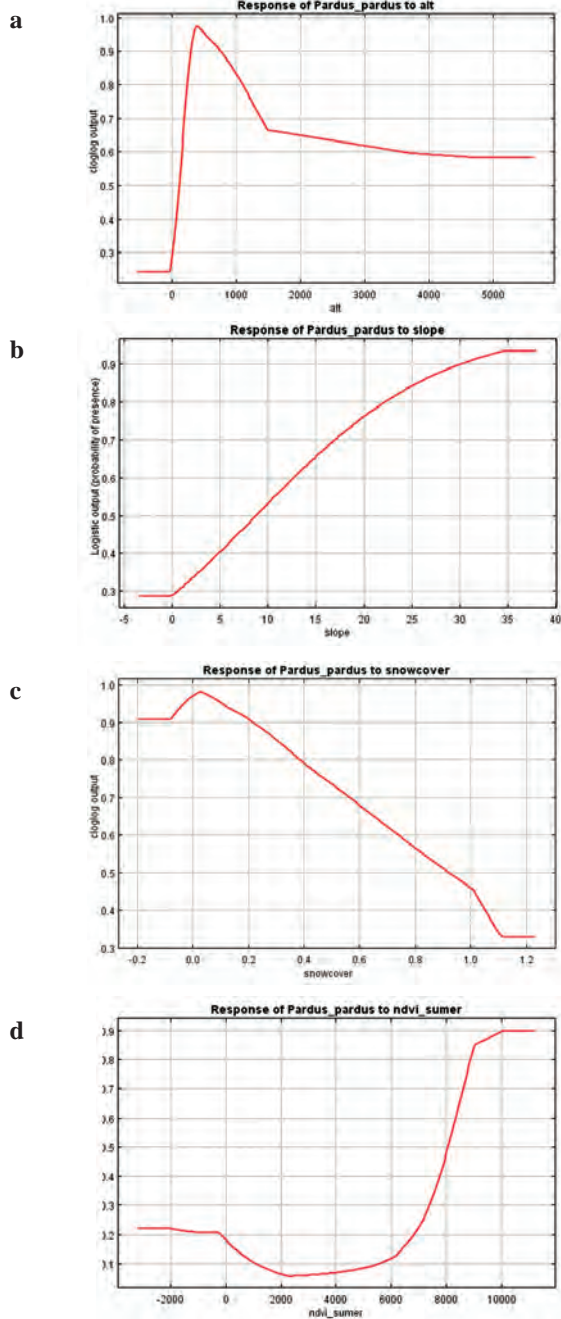
| All      |                      | Female   |                      | Male     |                      | Akhun    |                      | Artek    |                      | Elbrus   |                      | Killy    |                      | Victoria |                      | Volna    |                      |
|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|
| Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution | Variable | Percent contribution |
| bio18    | 45.2                 | bio18    | 22.2                 | bio18    | 38.6                 | srad4    | 15.1                 | prec11   | 22.1                 | bio18    | 21.4                 | bio18    | 31.9                 | prec5    | 14.3                 | bio18    | 15.7                 |
| tmin9    | 11.8                 | prec5    | 16.3                 | wind3    | 12.1                 | tavg4    | 11.9                 | srad6    | 14.2                 | srad4    | 14.3                 | srad4    | 31.5                 | bio18    | 11.3                 | prec10   | 12.2                 |
| wind3    | 7.4                  | prec10   | 7.3                  | tmin9    | 11                   | prec4    | 11.6                 | srad4    | 10.8                 | bio14    | 12.3                 | tmin10   | 9.6                  | srad4    | 9.9                  | srad4    | 11.3                 |
| prec6    | 4.7                  | prec6    | 6.1                  | prec6    | 9.3                  | bio15    | 10.9                 | prec10   | 8.9                  | prec5    | 7.7                  | prec9    | 3.5                  | prec9    | 9.4                  | prec6    | 11.1                 |
| prec9    | 4.2                  | prec8    | 5.2                  | bio12    | 4.1                  | tavg5    | 10.5                 | srad5    | 6.5                  | prec11   | 7.6                  | bio12    | 3.3                  | prec11   | 9.2                  | prec9    | 6.5                  |
| bio12    | 3.3                  | srad12   | 4.9                  | srad9    | 3.2                  | sh_r_s   | 5.8                  | bio15    | 6.4                  | wind3    | 6.8                  | tmin8    | 3.2                  | tmin4    | 8.7                  | prec5    | 6.5                  |
| srad9    | 2.6                  | prec9    | 4.9                  | prec9    | 2.8                  | bio14    | 4.3                  | prec8    | 5.8                  | tmin9    | 4.1                  | tmin7    | 3.2                  | srad5    | 8.6                  | srad7    | 6.4                  |
| prec12   | 2.5                  | bio17    | 4.1                  | prec10   | 2.3                  | sh_r_e   | 3.5                  | prec3    | 5.4                  | prec12   | 3.7                  | prec8    | 3                    | srad6    | 7.4                  | prec11   | 4.1                  |
| srad12   | 2.3                  | srad1    | 3.8                  | prec12   | 1.7                  | tmin5    | 3                    | bio17    | 4.2                  | bio15    | 3.4                  | tmin9    | 2.4                  | bio12    | 5.5                  | bio12    | 3.1                  |
| srad7    | 1.9                  | prec2    | 3.2                  | prec8    | 1.3                  | prec10   | 2.4                  | tmin8    | 3.4                  | prec9    | 2.3                  | prec6    | 1.1                  | tmin5    | 2.6                  | srad12   | 3.1                  |
| prec10   | 1.8                  | srad7    | 2.9                  | srad7    | 1.2                  | srad5    | 2.4                  | tmin6    | 2.7                  | bio4     | 2.2                  | srad10   | 1                    | prec10   | 1.8                  | prec12   | 2.6                  |
| prec8    | 1.7                  | srad3    | 2.4                  | prec1    | 1.2                  | srad6    | 2                    | bio18    | 2                    | bio17    | 1.3                  |          |                      | tmin3    | 1.4                  | bio17    | 1.8                  |
| tmin8    | 1.1                  | bio9     | 1.9                  | tmin8    | 1                    | tmax4    | 1.8                  | srad7    | 1.6                  | srad7    | 1.3                  |          |                      | tmin1    | 1.1                  | prec2    | 1.7                  |
|          |                      | srad11   | 1.5                  |          |                      | bio13    | 1.3                  |          |                      | prec1    | 1.3                  |          |                      | srad10   | 1                    | prec1    | 1.5                  |
|          |                      | tmin1    | 1.5                  |          |                      | tmin10   | 1.2                  |          |                      | wind6    | 1.2                  |          |                      | bio19    | 1                    | prec3    | 1.3                  |
|          |                      | srad5    | 1.4                  |          |                      | bio1     | 1.1                  |          |                      | srad10   | 1.2                  |          |                      |          |                      | srad5    | 1.1                  |
|          |                      | srad2    | 1.3                  |          |                      |          |                      |          |                      | srad9    | 1.1                  |          |                      |          |                      | tmin1    | 1                    |
|          |                      | wind7    | 1                    |          |                      |          |                      |          |                      |          |                      |          |                      |          |                      | slope    | 1                    |

**Table 7.** Assessing the importance of permutation of variables in the formation of a model of potentially suitable habitats of the Caucasian leopard in the Caucasus

| All      |                        | Female   |                        | Male     |                        | Akhun    |                        | Artek    |                        | Elbrus   |                        | Killy    |                        | Victoria |                        | Volna    |                        |
|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|----------|------------------------|
| Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance | Variable | Permutation importance |
| alt      | 14.9                   | prec5    | 18.3                   | alt      | 16.5                   | bio15    | 61.6                   | prec5    | 11                     | wind12   | 29                     | srad4    | 17.6                   | prec11   | 19.3                   | wind12   | 69                     |
| prec11   | 9                      | wind12   | 13.1                   | bio12    | 7.2                    | tavg4    | 14.6                   | bio15    | 10.9                   | bio15    | 26.8                   | bio12    | 13                     | srad10   | 8.7                    | prec5    | 14.4                   |
| wind12   | 8.1                    | bio15    | 10.4                   | wind12   | 6.6                    | srad4    | 7.7                    | srad7    | 6                      | alt      | 5.1                    | prec11   | 11.6                   | bio19    | 8                      | wind1    | 3.3                    |
| bio12    | 7                      | vapr3    | 8.5                    | prec11   | 6.4                    | tmin12   | 7.5                    | srad4    | 5.9                    | srad4    | 4                      | tavg11   | 4                      | tmin3    | 7.1                    | bio15    | 2.3                    |
| wind3    | 6.4                    | prec11   | 6.1                    | wind3    | 6                      | prec11   | 5.3                    | bio17    | 5.1                    | srad3    | 3.2                    | bio15    | 3.8                    | prec5    | 4.6                    | vapr9    | 2                      |
| bio15    | 5.5                    | bio14    | 5.9                    | bio15    | 5.3                    | bio14    | 1.8                    | tmin5    | 4.4                    | bio18    | 2.6                    | alt      | 3.7                    | prec12   | 4.3                    | bio8     | 1.5                    |
| prec2    | 5.5                    | srad1    | 5.8                    | prec2    | 4.3                    |          |                        | tmin12   | 4.4                    | bio7     | 2.2                    | wind3    | 3                      | prec10   | 3.8                    | slope    | 1.2                    |
| srad9    | 3                      | srad7    | 4.1                    | srad7    | 3.5                    |          |                        | tmin8    | 3.2                    | prec1    | 1.8                    | prec9    | 2.8                    | bio2     | 3.7                    |          |                        |
| tmax11   | 2.7                    | tmin12   | 2.7                    | tmax11   | 3.3                    |          |                        | alt      | 3.1                    | bio4     | 1.7                    | prec5    | 2.6                    | srad4    | 3.4                    |          |                        |
| tmin10   | 2.5                    | srad10   | 2.3                    | tmin10   | 3.3                    |          |                        | srad5    | 2.6                    | srad1    | 1.7                    | srad3    | 2.5                    | srad6    | 3.2                    |          |                        |
| prec3    | 2.1                    | wind7    | 2.2                    | slope    | 3                      |          |                        | prec11   | 2.5                    | tmin4    | 1.3                    | srad7    | 2.4                    | bio12    | 3.1                    |          |                        |
| tmin7    | 1.9                    | prec10   | 2.1                    | bio18    | 2.9                    |          |                        | bio9     | 2.3                    | wind6    | 1.2                    | srad1    | 2.4                    | bio8     | 3.1                    |          |                        |
| prec9    | 1.8                    | alt      | 1.9                    | prec7    | 2                      |          |                        | srad10   | 2.2                    | bio8     | 1.2                    | srad5    | 2.3                    | srad1    | 2.3                    |          |                        |
| srad10   | 1.7                    | srad3    | 1.8                    | srad9    | 1.9                    |          |                        | bio4     | 2.2                    | prec5    | 1.1                    | srad10   | 2.2                    | vapr4    | 2.1                    |          |                        |
| slope    | 1.7                    | bio9     | 1.4                    | tmin9    | 1.8                    |          |                        | tavg8    | 2.1                    | srad9    | 1.1                    | prec10   | 2                      | tmin4    | 1.8                    |          |                        |
| bio18    | 1.6                    | slope    | 1.4                    | srad1    | 1.7                    |          |                        | bio19    | 2.1                    | prec3    | 1.1                    | srad6    | 1.9                    | srad12   | 1.6                    |          |                        |
| srad1    | 1.6                    | prec8    | 1.2                    | srad4    | 1.4                    |          |                        | slope    | 1.9                    | tmin7    | 1                      | tmin1    | 1.8                    | prec1    | 1.5                    |          |                        |
| prec10   | 1.5                    | srad9    | 1.2                    | srad3    | 1.4                    |          |                        | srad6    | 1.8                    |          |                        | wind2    | 1.6                    | bio17    | 1.4                    |          |                        |
| tmax10   | 1.5                    | srad12   | 1                      | prec9    | 1.3                    |          |                        | bio16    | 1.8                    |          |                        | bio2     | 1.5                    | srad2    | 1.4                    |          |                        |
| tavg8    | 1.5                    | prec4    | 1                      | srad5    | 1.3                    |          |                        | tmax11   | 1.8                    |          |                        | prec3    | 1.3                    | prec2    | 1.3                    |          |                        |
| prec5    | 1.5                    |          |                        | prec3    | 1.3                    |          |                        | tavg5    | 1.7                    |          |                        | tmin3    | 1.3                    | srad9    | 1.3                    |          |                        |

|        |     |        |     |        |     |        |     |         |     |
|--------|-----|--------|-----|--------|-----|--------|-----|---------|-----|
| srad4  | 1.3 | prec10 | 1.2 | bio3   | 1.6 | bio6   | 1.2 | vapr5   | 1.3 |
| prec7  | 1.3 | srad10 | 1.2 | srad11 | 1.4 | tmin11 | 1.1 | srad11  | 1.2 |
| tmin2  | 1.3 | bio4   | 1.1 | srad9  | 1.4 | srad8  | 1   | srad8   | 1.1 |
| srad7  | 1.2 | tavg8  | 1.1 | tmin7  | 1.2 |        |     | tmin1   | 1   |
| tavg11 | 1.2 |        |     | tavg11 | 1.1 |        |     | pr_curv | 1   |
| tmin8  | 1   |        |     | tmax10 | 1   |        |     | srad3   | 1   |

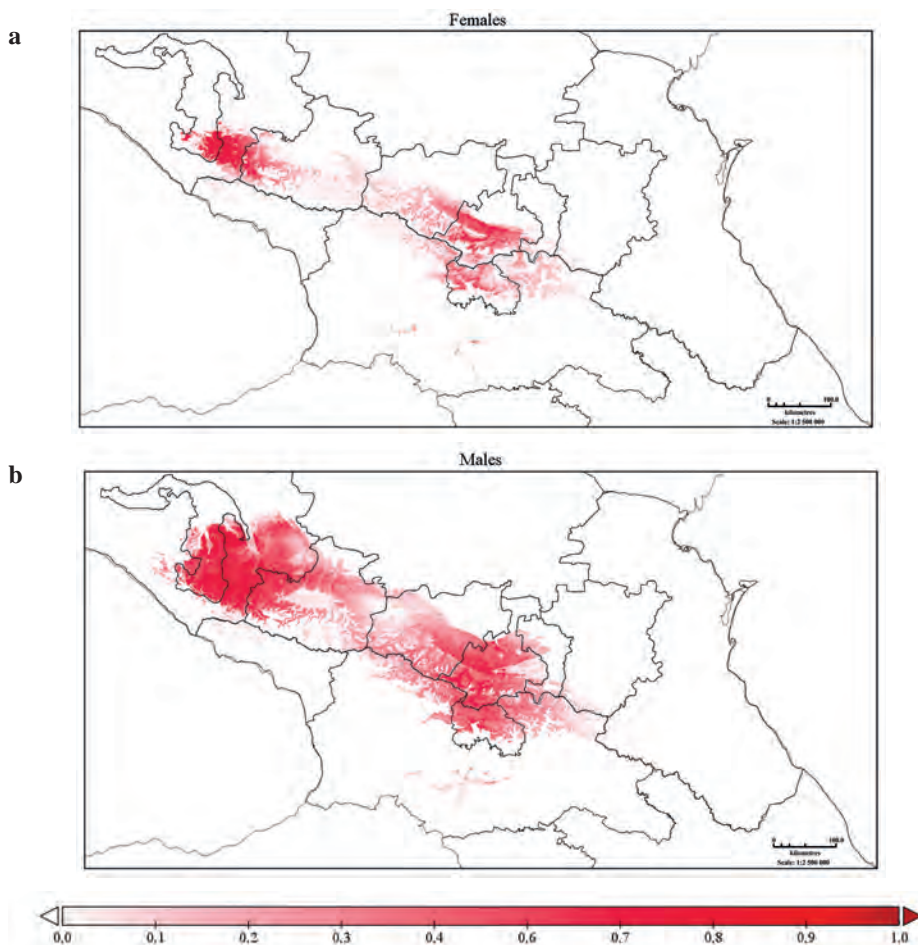
Data used: Brown and Brasnett, 2010, updated annually. *Canadian Meteorological Centre (CMC) Daily Snow Depth Analysis Data, Version 1*. [Indicate subset used]. Boulder, Colorado USA. NASA National Snow and Ice Data Center Distributed Active Archive Center. doi: <https://doi.org/10.5067/W9FOYW0HQEQZ3>.



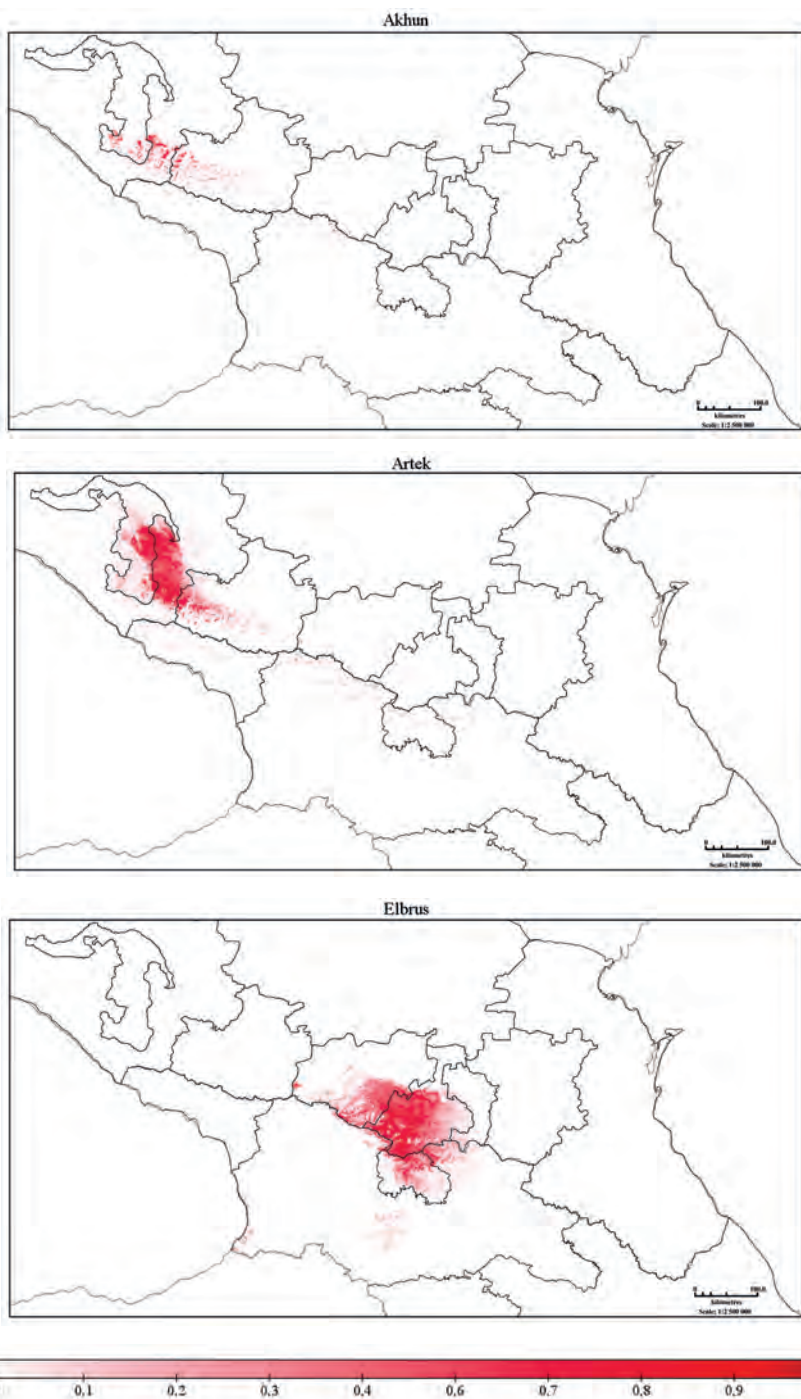
**Figure 59.** The model of potentially suitable habitats of Caucasian leopards in the Caucasus, calculated based on the tracks of released leopards (factors influenced the model mainly). a – height (m); b – slope, degrees; c – the index of snow cover in winter; d – vegetation index.

mining the areas potentially suitable for the leopard included precipitation of the warmest quarter; altitude above sea level (**Figure 59a**); slope (slope steepness) (**Figure 59b**); NDVI (vegetation index) – (**Figure 59d**), and two indices reflecting the depth of the snow cover calculated based on winter images (**Figure 59c**). The snow cover indices did not significantly contribute to the model but exhibited a high permutation coefficient. Notably, the total percentage contribution of the aforementioned factors equaled 44.3%, being relatively low for each of them.

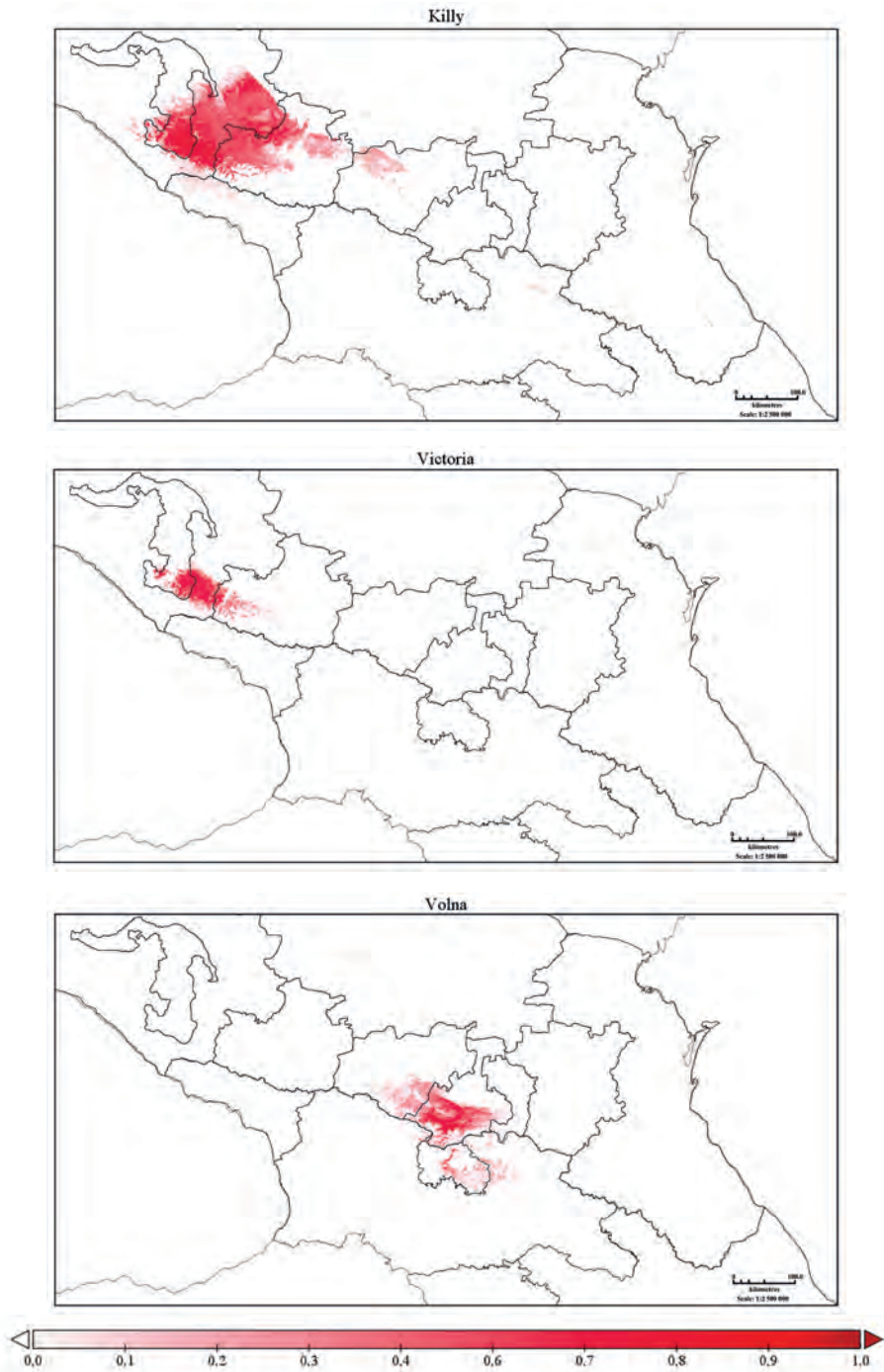
The graphs of leopard occurrence probability contingent on the environmental factors are interpreted in **Figure 59**. The graphs of altitude above sea level, slope steepness, aspect and normalized vegetation index enable the impact of these factors on modeling the potential habitats to be determined. The interpretation demonstrates that the most suitable habitats are confined to rather steep slopes with herbaceous vegetation at altitudes of 1200 m and higher, independent of the aspect with average values of snow cover indices.



**Figure 60.** The model of potentially suitable habitats of Caucasian leopards in the Caucasus, calculated based on the tracks of released leopards (a – females and b – males).



**Figure 61.** The model of potentially suitable habitats of Caucasian leopards in the Caucasus, calculated based on the tracks of released leopards.

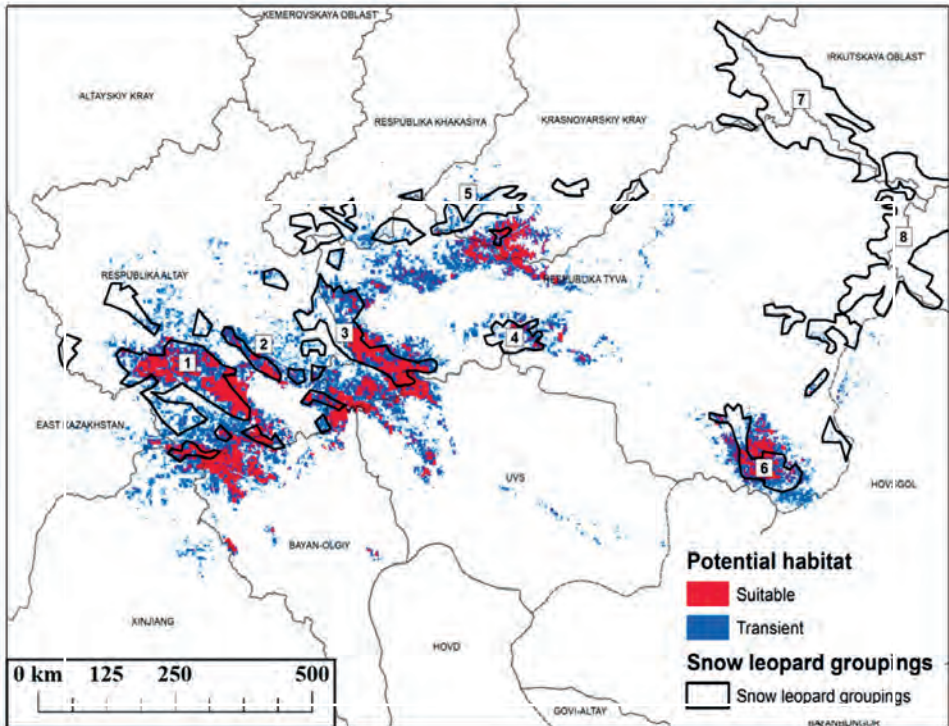


**Figure 61 (continued).** The model of potentially suitable habitats of Caucasian leopards in the Caucasus, calculated based on the tracks of released leopards.

The distribution of potentially suitable habitats by leopards' gender (**Figure 60**) and by individuals (**Figure 61**) shows a possibility of wider usage of the territory by males than by females (except of *Akhun*, his number of locations is poorer than for other individuals and they all points are localized in a small area).

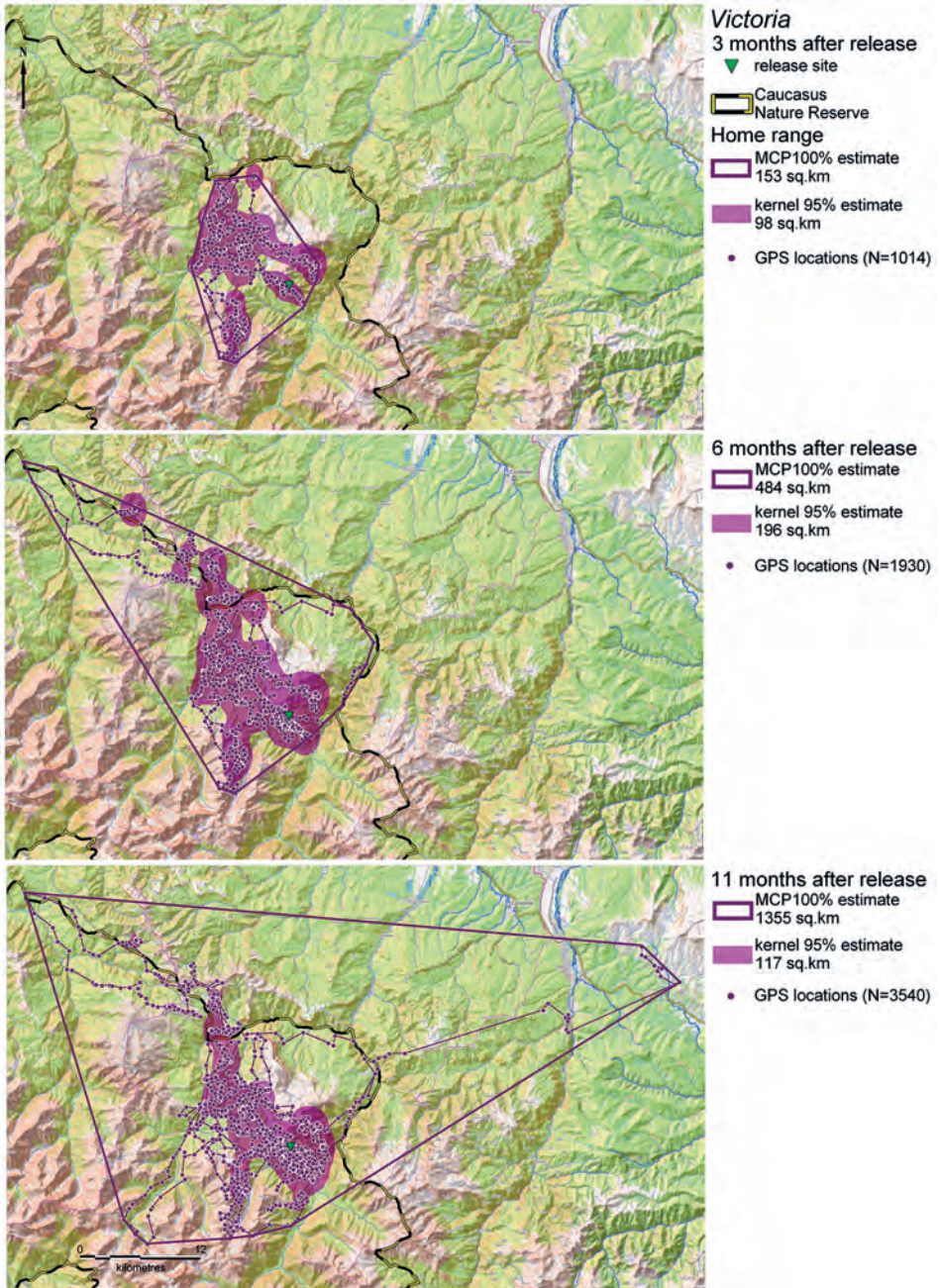
### *Snow leopard habitat modeling*

We also proposed results of modeling via MaxEnt method the suitable habitats for the snow leopard during implementation of our projects for the development of scientific base for work on the big cats conservation (Kalashnikova et. al., 2019). The snow leopard is an endangered large felid inhabiting highlands of 12 Asian countries. It is distributed across vast territories and adequate modern methods are required for mapping its potential habitats. Model of snow leopard potential habitat was developed by us and created a precise map of potentially suitable and transient habitats for the snow leopard within the northern part of its range (Russia and the adjacent regions of Mongolia, China and Kazakhstan) and we compared the existing potential suitability map with the current PA network; determined the real and exact borders of snow leopard optimal



**Figure 62.** Map of snow leopard potential habitat nuclei overlapped with previously indicated existing groupings (marked by numbers) according Poyarkov et al., 2002 with same changing. The numbers of groupings: 1 – Argut river and Yuzhno-Chuyskiy ridge, 2 – Kurayskiy ridge, 3 – Shapshal and Tsagaan-Shibetu mounting, 4 – Big Mongun-Tayga mounting, 5 – Chikhacheva ridge, 6 – Tannu-Ola ridge, 7 – West Sayan ridge (Sayano-Shushenskiy Reserve), 8 – Sangylen mounting.





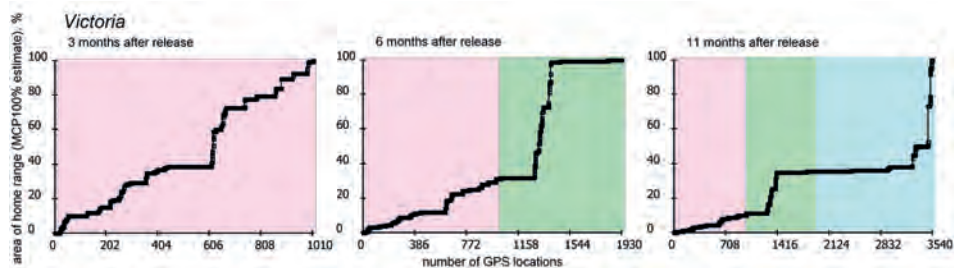
**Figure 63.** MCP and Fixed Kernel estimates of home range of a female Caucasian leopard (*Victoria*) three, six and 11 months after release.

habitats in various places of its western part across the territory of Russia; analyzing the correspondence of PA to the optimal habitat structure, contributing to an enhancement of snow leopard conservation efforts. For that there were used more than 5 years of observations (total number of snow leopard presence points is 449). The resulting map (**Figure 62**) demonstrates that a suitable habitat (probability of the animal's presence between 0.5 and 1) of the northern population of snow leopard in Russia occupies 16 500 km<sup>2</sup> with a buffer of transient territories (probability between 0.25 and 0.49) covering 32 800 km<sup>2</sup>. Most of a suitable habitat within the study area is associated with the Altai Mountains, Western Sayan Mountains, Sangilen Plateau, Tsagan-Shibetu and Shapshal. One third of the suitable habitat lies within areas of a varying protection status. The results of modeling are of importance both for scientists and conservation managers, as they allow for leopard occurrence to be predicted, supporting research on and the conservation of the species.

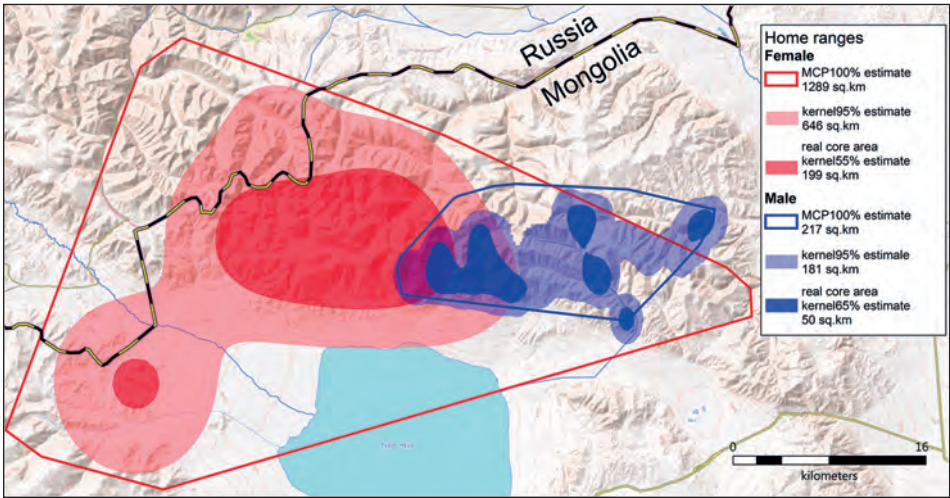
### 2.5.2. ANALYZING THE POPULATION SPATIAL STRUCTURE

Based on the locations obtained for each leopard from a GPS collar, its individual home range is estimated (**Figure 63**) and its area is calculated. Judging by the way in which the area is used by an animal and how it approaches the asymptote, we can draw a conclusion regarding the degree of individual home range-forming completeness (**Figure 64**). Tracking leopards may provide useful information as to how they use space. Information obtained by tracking identifies the behavioral features of an animal (hunting behavior, marking behavior, path selection in different habitats). Furthermore, tracking may provide additional information for the home range area calculation of a released individual in case of collar malfunction. Another important type of data that can be obtained via tracking is a real daily-route length of an animal, which is associated with a set of external conditions, if an animal is tracked from a location with the known time to a location after a 24-hour period.

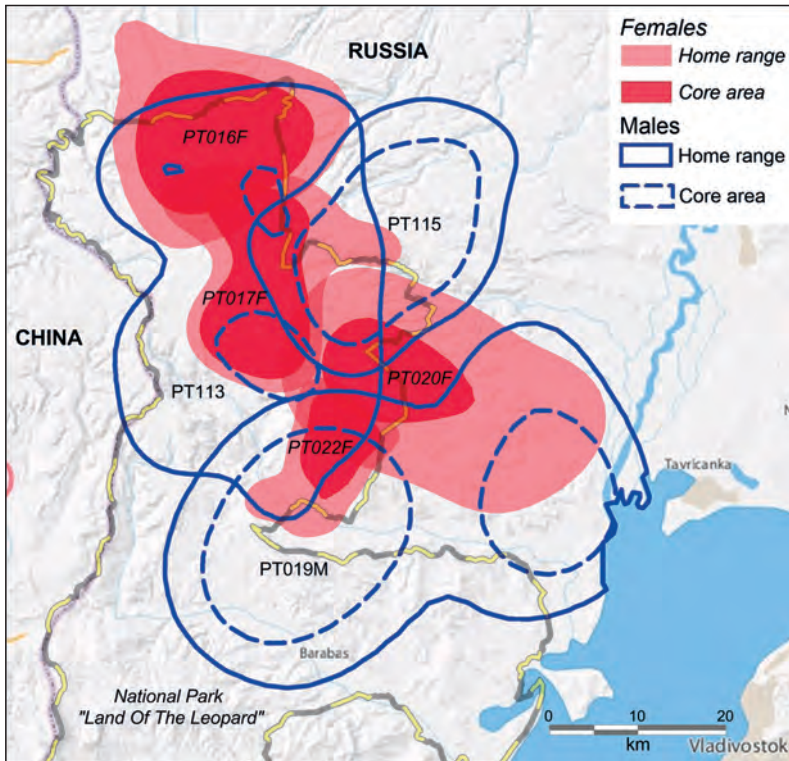
Examples of analyzing data on the use of space by big cats can be found in a number of studies (Rozhnov et al., 2010b, 2011c, e, 2014a, 2015c; Hernandez-Blanco et al., 2011, 2015b; Chistopolova et al., 2015a, b, c). Examples are shown at **Figure 65** – for Snow leopard and **Figure 66** – for the Amur tigers.



**Figure 64.** MCP estimates of home range used by a female Caucasian leopard (*Victoria*) released in Caucasus Nature Reserve in July 2016, reaching an asymptote within different periods.



**Figure 65.** MCP and Fixed Kernel estimates of home ranges and core areas of a snow leopard female (*Tsagana*) and male (*Orgil*) in the Russian-Mongolian border area (after Poyarkov et al., 2018).



**Figure 66.** Fixed kernel home ranges (95%) and real core areas of Amur tigers (3 males and 4 females) in Southwest Primorye, 2010–2011 (after Hernandez-Blanco et al., 2015b).

### 2.5.3. ANALYZING DYNAMIC INTERACTION OF INDIVIDUALS USING SIMULTANEOUS GPS-TELEMETRY DATA

The advent of GPS tracking technology in the late XX<sup>th</sup> century made a substantial contribution to spatial ecology research (Rozhnov et al., 2010a, 2011c, e). A significant increase in the sampling resolution and accuracy of the locations compared to previous methods, such as snow tracking and VHF radio tracking, allowed us to estimate more accurately the size and inner structure of the home range of elusive species, many of them rare and in danger of extinction as big cats are. In addition, a uniform distribution in time of simultaneous samples of locations makes it possible to reveal the presence of interactions between individuals in a population based on home ranges' overlaps (Hernandez-Blanco et al., 2015b). The degree of overlap of the seasonal home ranges of an individual is used to estimate whether the home range has experienced changes over time or not (Garshelis, Pelton, 1981). The degree of overlap of neighboring home ranges gives an idea about the nature of the interaction between neighbors. Macdonald et al. (1980) called the analysis of overlapping range outlines "static interaction". However, animals who share large areas as pure data shown, in overlap analysis may rarely encounter each other because they seldom visit the same place at the same time. In order to explore the dynamic interaction between two individuals it is necessary to analyze their simultaneous locations. Hence, this provides a test of whether two individuals show attraction, indifference or avoidance to each other. As described in Kenward et al. (1993), the observed and possible distances between animals are compared. The mean, geometric mean and median distances are estimated between the  $n$  observed pairs of simultaneous locations for individuals  $A$  and  $B$ . Then the equivalent values are estimated for the  $n \times n$  possible distances if animal  $B$  could be at any of its  $n$  used positions when animal  $A$  was at each of its used positions. The observed and possible distances are compared using Jacob's Index (Jacobs, 1974). This returns a value of 0 if the observed and possible distances were the same (showing indifference), rising up to +1 if observed distances were small relative to possible distances (because the animals were usually together, that means attraction) or falling down to -1 if animals tended to avoid each other. This gives a single index for each dyad of individuals, which tends to be most consistent if based on the geometric mean distances (Walls, Kenward, 2001).

The development of telemetry has led to the improvement of the mathematical approach for analyzing such interactions along with the study of animal trajectories and how independent they are from each other (Long et al., 2014). In modern studies of dynamic interactions based on telemetry data, you can find a wide range of fields: sociality and group behavior (Karlin, Chadwick, 2011), interspecific interactions (Eriksen et al., 2008), interaction within family members (Bandeira de Melo et al., 2007), habitat relations (Atwood, Weeks, 2003), mating behavior (Long et al., 2013) and others.

Indices of dynamic interaction use points (GPS fixes) or paths of connected vectors, depending on how they represent telemetry data. Points-indices estimate levels of attraction/avoidance behavior, while path-indices examine cohesive movement behavior. Among all indices of dynamic interactions based on simultaneous GPS

telemetry data, nine best-reflecting interaction patterns were selected (Long et al., 2014): proximity analysis (Prox), coefficient of association (Ca), Doncaster's non-parametric test (Don), Minta's test for spatial and temporal interaction ( $L_{ixn}$ ), coefficient of sociality (Cs), half-weight association index (HAI), correlation coefficient (Cr), dynamic interaction index (DI) and interaction statistic (IAB).

Before calculating most of these indices, it is worthwhile to understand that two locations are assumed simultaneous based on a time threshold ( $t_c$ ) that the researcher determines on an *ad hoc* basis. In the same way, two fixes are assumed spatially proximal based on a distance threshold ( $d_c$ ).

All these analyzes can be performed by using package *WildlifeDI* (Long et al., 2014) based on package *Adehabitat* (Calenge, 2006), both of them were previously created at R language and environment for statistical computing and graphics (R Core Team, 2018).

#### *Proximity analysis (Prox)*

One of the easiest ways to examine attraction in wildlife telemetry studies is to measure the distance between two individuals (Bertrand et al., 1996).

Proximity reflects the frequency at which two animals are near each other using GPS fixes (**Figure 67**).

#### *Coefficient of association (Ca)*

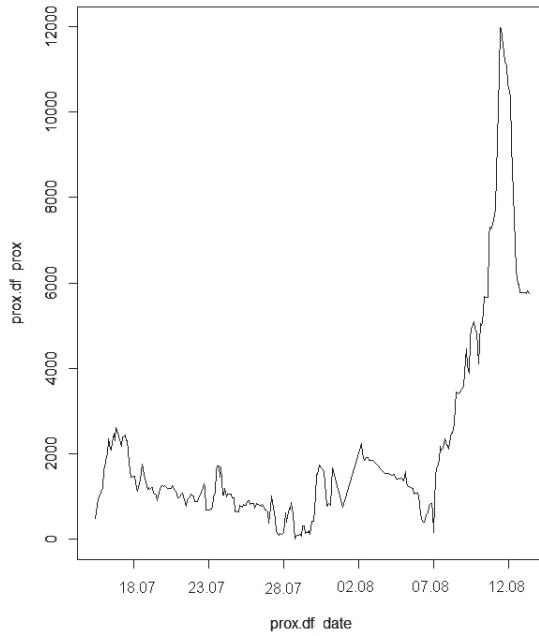
It was originally proposed to assess interspecific interactions (Cole, 1949). Coefficient of association is similar to proximity analysis, but it measures the proportion of simultaneous proximal fixes based on the given distance threshold. As suggested by Bauman (1998),  $Ca > 0.5$  can be interpreted as attractions, while  $Ca < 0.5$  indicates avoidance.

#### *Doncaster's nonparametric test (Don)*

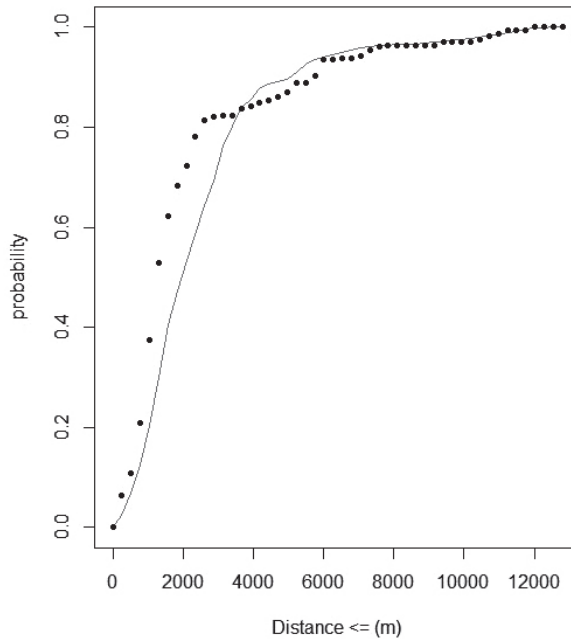
Doncaster (1990) proposed a nonparametric test for interaction within the dyad by considering the distance between the number of simultaneous fixes and their unpaired  $n^2-n$  permutations. The cumulative distribution of the simultaneous fixes' distances can be visually compared with the cumulative distribution of the  $n^2-n$  permuted distances using a graphic (**Figure 68**). Hence, Don is useful for estimating a suitable distance threshold by establishing where the simultaneous fixes plot is above the expected line based on the permutations. Upon choosing a suitable distance threshold value, a contingency table can be built, identifying the number of simultaneous and non-simultaneous fixes' distances that are above and below the distance threshold. In order to check the statistical significance of the counts of simultaneous and non-simultaneous fixes' distances above and below distance threshold, a  $\chi^2$  test (with 1 d.f.) or a binomial test can be used.

#### *Minta's test for spatial and temporal interaction ( $L_{ixn}$ )*

Minta's test uses home range polygons based on telemetry locations of two individuals. A necessary requirement for this test is the presence of an area of overlap



**Figure 67.** Proximity analysis of GPS telemetry data of two collared Caucasian leopards after release.



**Figure 68.** The graph of the count of observed (black dots) vs. expected (grey line) fix distances according to Don's test of GPS telemetry data of two collared Caucasian leopards after release.

between home ranges. However, it is not possible to perform the test if one home range is completely enclosed in another.

If the conditions above are observed we obtain an area of exclusive use for individual *A*, an area of exclusive use for individual *B* and an area of overlap used by both individuals *A* and *B*. Minta (1992) introduced three indices ( $L_{AA}$ ,  $L_{BB}$ ,  $L_{ixn}$ ) for studying spatial and temporal interactions between individuals.

The first two indices ( $L_{AA}$  and  $L_{BB}$ ) reflect how these individuals *A* and *B*, use the overlap and their exclusive areas, respectively. When  $L_{AA} \approx 0$ , *A* uses the area of overlap randomly, while  $L_{AA} > 0$  indicates that *A* is attracted by the shared area and  $L_{AA} < 0$  means that *A* avoid it. The same applies to the  $L_{BB}$  index in relation to *B*.

The index  $L_{ixn}$  is a measure of the simultaneous use of the shared area by individuals *A* and *B*. When  $L_{ixn} \approx 0$ , it imply that both individuals use the area of overlap randomly.  $L_{ixn} > 0$  indicates simultaneous use of the shared area, supposed attraction, while.  $L_{ixn} < 0$  shows solitary use the shared area, supposed avoidance. These statistics result from observed and expected values from a  $2 \times 2$  contingency table. Thus, this allows us to perform a  $\chi^2$  test with 1 degree of freedom in order to check the level of statistical confidence of the  $L_{AA}$ ,  $L_{BB}$ , and  $L_{ixn}$  values.

#### *Coefficient of sociality (Cs)*

The coefficient of sociality was originally proposed by Kenward et al. (1993) as a variation of the Jacobsr index (Jacobs, 1974) to assess the nature of social interactions. It incorporates the mean distances of simultaneous locations and the mean distances of the  $n^2$  permutations of all locations into a single analysis.

As mentioned earlier, this test returns a value from -1 to +1, from avoidance to attraction through indifference. Given that the observed values are paired, it is possible to use a Wilcoxon signed-rank test to determine the statistical significance of the test. One-sided p-values are calculated separately for avoidance and attraction.

#### *Half-weight association index (HAI)*

The half-weight associative index finds application in the analysis of those locations of individuals that fall on the overlapping area (Atwood, Weeks, 2003). HAI is calculated in a similar way to the coefficient of association, but with a different spatial approach aimed at the shared area. Therefore,  $HAI > 0,5$  means attraction, while  $HAI < 0,5$  indicates avoidance.

#### *Correlation coefficient (Cr)*

While to perform all the previous tests we use points (with or without polygons), and the correlation coefficient (Shirabe, 2006) uses paths of vectors. Shirabe proposed this test to measure the level of correlation in animal movements represented as trajectories (a set of segments between locations). The test takes the form of a multivariate Pearson product-moment correlation coefficient.

Basically, Shirabe's correlation coefficient calculates the difference in synchronous paths of individuals *A* and *B*. The differences in the simultaneous trajectories are

defined as deviations from the respective trajectory mean vectors. As well as a typical correlation coefficient  $Cr \approx 1$  indicates a correlation of trajectories,  $Cr \approx -1$  means repulsion and  $Cr \approx 0$  shows random movement, with respect to the other individual.

It is important to note that this test does not take into account the distance between individuals at any point of its deviation. It is a task for the researcher to conclude whether the value of the correlation is significant or not.

### *Dynamic interaction index (DI)*

The dynamic interaction index (Long, Nelson, 2013) also needs telemetry data represented as trajectories to be calculated. **DI** reflects the cohesiveness in two independent components of movements: direction and speed. The main difference from the Shirabe's correlation coefficient is that **DI** does not depend on the respective trajectory mean vectors and **DI** can disentangle the independent effects of correlation in direction and speed. Moreover, **DI** test provides a spatially and temporally local alternative (*di*) that can be computed for each pair of simultaneous movement segments. The *di* index gives an opportunity to explore the spatial and temporal dynamics of the dynamic interaction behavior, through a graph of differences over time to understand when this behavior happened, or through *di* maps to identify the location of this behavior. To analyze the local level of dynamics in the cohesion of the movement, you can create a graph of *di* over time, which will help identify time trends. Note that **DI** and *di* do not consider the distance between fixes, so it is up to the specialist to decide whether interactive behavior occurs or not.

### *Interaction statistic (IAB)*

This approach shows an alternative view on testing of dynamic interaction from telemetry data represented as simultaneous locations (Benhamou et al., 2014).

Instead of the threshold distance, the IAB test uses the parameter (**D**) that represents the point maximum slope of the distance effect function that measures the potential influence domain between the observed animals. This analysis perfectly complements the previous, confirmed or disproving their results and the adequacy of the choice for them of the threshold values of time and distance.

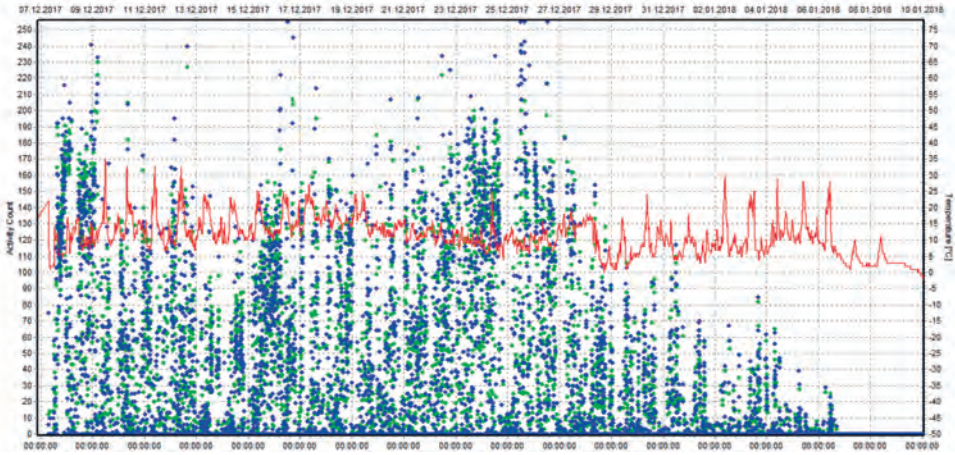
## 2.5.4. ANALYZING ACTIVITY DATA

Each modern collar is equipped with an activity sensor represented by an accelerometer with two or three axes. As a rule, the device shows the percentage of active seconds for a given period of time. A second is considered active if the accelerometer detects an acceleration in a given axis higher than the threshold.

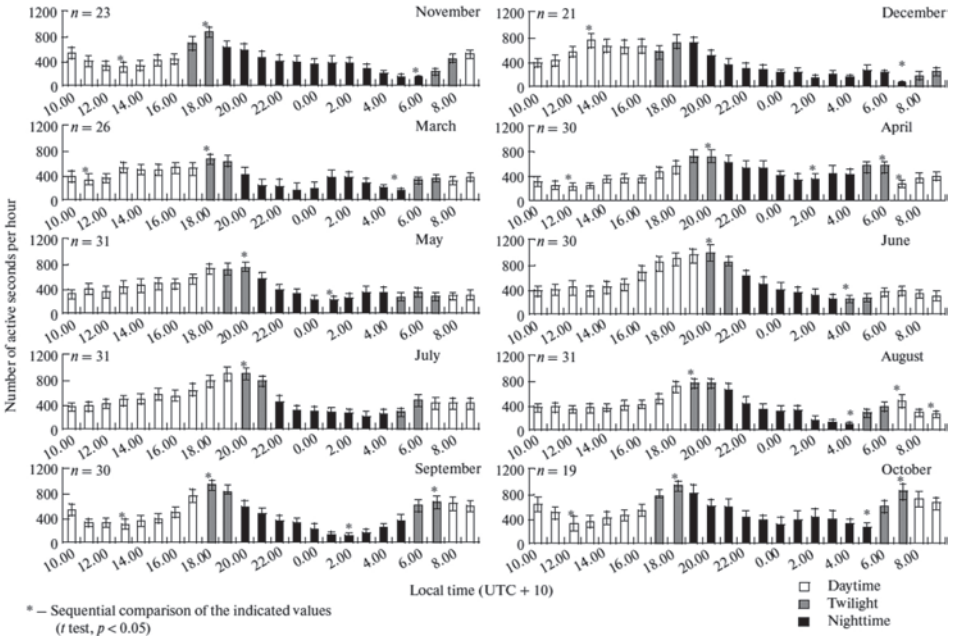
Analyzing the data on activity enables us to determine the circadian rhythm of an animal, its dependence on external factors and seasons, as well as its deviation from the norm. The results obtained for the Caucasian leopard and the Amur tiger can serve as examples of analyzing data on the activity of large felines.

The activity of a female Caucasian leopard (*Victoria*) was gradually declining after she had been released on 28 December 2017 compared with her activity registered in the





**Figure 69.** Activity records of a Caucasian leopard (female *Victoria* after she was re-collared) using an accelerometer. The red line denotes temperature (according to the collar), blue spots denote the number of active seconds in every five minute period (maximum 300 seconds) along the X axis, green spots denote the same along the Y axis. On 7 December 2017 the collar was put on *Victoria*; on 28 December 2017 she was released in Caucasus Nature Reserve.



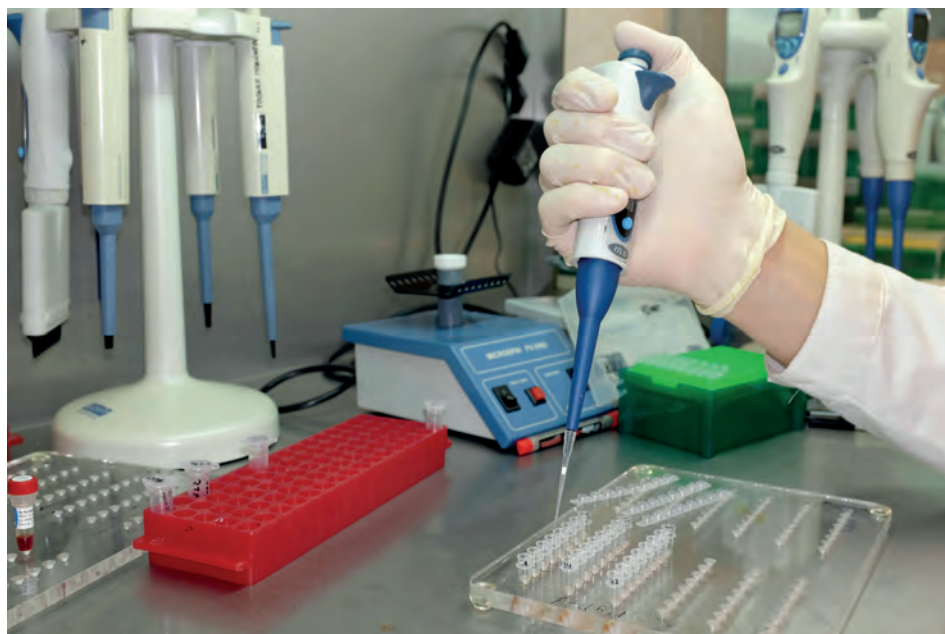
**Figure 70.** Activity records of a wild female Amur tiger in different months by hours (after Rozhnov et al., 2011c).

Leopard Recovery Center in the Caucasus (**Figure 69**); after 4 January 2018 her activity plummeted even further. She died on 6 January 2018 after 4 p.m. [Moscow time].

The activity of a female Amur tiger in different months of the year varies by hours (**Figure 70**). The accelerometer data revealed two peaks in her activity in autumn and spring months, and a single peak in winter and summer, when the air temperature reached its extremes (Rozhnov et al., 2011c).

#### 2.5.5. MOLECULAR GENETIC ANALYSIS TO DETERMINE SPECIES, SEX AND INDIVIDUAL STATUS OF THE LEOPARD AND ITS PREY

To identify the species and subspecies of vertebrate animals, several fragments of mitochondrial DNA are analyzed. For this purpose, DNA is extracted from the studied sample by different methods, and then polymerase chain reaction (PCR) with specific primers (short nucleic acid fragments – oligonucleotides – complementary to the target DNA serve as primers to initiate complementary chain synthesis in PCR) is performed and the DNA sequence determined using capillary sequencer. Furthermore, the species and subspecies to which the DNA sequence belongs are identified by comparing it with the NCBI database (National Center for Biotechnology Information, the central institution for storing and analyzing molecular biology data) and the original database of the Molecular Diagnostics Center of A.N. Severtsov Institute of Ecology and Evolution RAS (**Figure 71**).



**Figure 71.** Preparing polymerase chain reaction (PCR) in a laminar box with a sampler and appropriate tubes and reagents.

**Table 8.** The length of nuclear DNA microsatellite loci in nucleotide pairs used for subspecies and individual identification of the leopard

| Sample       | Used loci |     |        |     |       |     |      |     |      |     |       |     |       |     |       |     |        |     |        |     |       |     |
|--------------|-----------|-----|--------|-----|-------|-----|------|-----|------|-----|-------|-----|-------|-----|-------|-----|--------|-----|--------|-----|-------|-----|
|              | E7        |     | Fca304 |     | Fca43 |     | 3e6f |     | E21b |     | Fca77 |     | Fca90 |     | Fca96 |     | Fca310 |     | Fca441 |     | Fca97 |     |
| Blood sample | 154       | 178 | 108    | 120 | 113   | 115 | 156  | 159 | 160  | 160 | 133   | 143 | 106   | 116 | 204   | 204 | 126    | 130 | 144    | 144 | 140   | 150 |
| Victoria*    | 154       | 178 | 108    | 120 | 113   | 115 | 156  | 159 | 160  | 160 | 133   | 143 | 106   | 116 | 204   | 204 | 126    | 130 | 144    | 144 | 140   | 150 |

To identify the subspecies and determine the individual identity, sex and family relationships, microsatellite analysis of nine loci (chromosome regions each containing a single gene; a DNA sequence variant in a given locus is referred to as ‘allele’) and one sex marker for the Amur tiger and of 11 microsatellite loci and one sex marker for the Amur leopard is used. For this purpose, DNA is extracted from the object of study using different methods, polymerase chain reaction with specifically labeled primers is performed, and the allele composition of the studied loci is determined using a capillary sequencer. Next the subspecies and individual identity of the studied DNA specimen is determined through comparison with the original information database of Molecular Diagnostics Center of A.N. Severtsov Institute of Ecology and Evolution RAS. This type of analysis is time-consuming and takes 10 days or more depending on the maintenance and number of specimens.

The data obtained through the molecular genetic analysis are stored in the form of a table (**Table 8**). The example of the expertise number 2 dated 21 April 2014 performed at A.N. Severtsov Institute of Ecology and Evolution RAS using the results of microsatellite analysis of nuclear DNA fragments in nucleotide pares (genetic profiles of an animal according to 11 microsatellite loci and 22 alleles) to determine subspecies and individual identity of the blood sample demonstrated that they are identical to the genotype of the leopard named Victoria.

Methods of molecular genetic analysis of large felines such as the Amur tiger, the Caucasian and Amur leopards, and the snow leopard are described in a number of studies (Rozhnov et al., 2009b, 2011b, g, 2013; Sorokin et al., 2010a, b, 2011, 2015, 2016; Zvychaynaya et al., 2011a, b; Korablev et al., 2016).

#### 2.5.6. ANALYSING HORMONAL STATUS BASED ON COLLECTED EXCREMENTS OF ANIMALS TO ASSESS STRESS LEVEL

In recent years, non-invasive methods of monitoring hormonal status are being rapidly developed and are increasingly used to assess animal well-being/welfare in natural populations. The glucocorticoid level of wild animals is usually assessed non-invasively, without direct contact with the animal. Interspecific differences in hormone metabolism should be validated for each species. This approach has been efficiently applied to assess stress/well-being in a number of species; Amur leopard cat,

Pallas's cat, Amur tiger, domestic cat (Pavlova, Naidenko, 2008; Naidenko et al., 2010, 2011b; Pavlova et al., 2011, 2014).

Once collected, the aliquots of all excrement samples were frozen at  $-18^{\circ}\text{C}$  and delivered to the laboratory at A.N. Severtsov Institute of Ecology and Evolution RAS, where they were stored at  $-18^{\circ}\text{C}$  until hormone extraction. The resulting extract was stored at  $-18^{\circ}\text{C}$  until analyzing. The concentration of immunoreactive substances binding to cortisol antibodies is determined by heterogeneous enzyme-linked immunosorbent assay. For this purpose, an Imuno-FA Cortisol kit of Immunotech (Moscow, Russia) and a Multiscan EX plate plate-reader (ThermoScientific, Finland) are used. All procedures are performed according to the manufacturer's instructions. The concentration of immunoreactive substances is calculated per 1 g of dry excrements. For this purpose, the excrement aliquots with a mass of 0.5–2 g are weighed with an accuracy of 0.1 g using Ohaus electronic balances (Ohaus Corporation, USA) and then dried at  $80^{\circ}\text{C}$  until reaching constant mass. The sample moisture is subsequently determined, and the cortisol concentration is calculated per 1 g of dry excrements.

Methods of hormonal analysis of large felines are described in a number of studies (Pavlova, Naidenko, 2008; Naidenko et al., 2010, 2011b, 2015a; Rozhnov et al., 2010b; Pavlova et al., 2011, 2014, 2015).

#### 2.5.7. ANALYZING THE DIET OF LARGE FELINES BASED ON EXCREMENTS

The diet is analyzed according to the technique applied to reveal the food composition of large felines (Chistopolova et al., 2010; Rozhnov et al., 2011).

The collected scats should be removed from Ziploc bags (sealed packages), the data from the labels should be organized in a table, and the samples should be placed in order to correspond with the data from the labels. During the laboratory analysis, the scats are disinfected in 70% ethanol for 15 min to destroy the parasites capable of infecting humans. Next each sample is filled with water in an individual container and allowed to completely soak (usually for about three days). Then the scats are washed through a fine sieve under running water (**Figure 72**) and allowed to dry completely at room temperature (for about two days). The dried individual hairs of prey washed out of scats are analyzed under a light microscope.

The species of the prey is identified under a light microscope by comparing the hairs from the scats with reference hair samples of different mammal species that inhabit the study area and are the potential prey of the leopard (**Figure 73**). A microscope slide for the analysis can be produced by two techniques: a whole mount preparation of a hair in liquid under a cover glass, or a print of a hair cuticle in varnish (colorless nail polish). The latter is prepared in the following manner. Hairs taken separately from the scats are washed with any detergent, and are then placed in 70% ethanol to remove water from the hairs, before being allowed to dry completely. Next a thin layer of transparent nail polish is placed on a slide, is half-dried, and a prepared hair under tension is placed on the slide with nail polish. Once the polish is dry, the hairs are removed from the slide with a sharp movement.



**Figure 72.** Washing excrement samples of the leopard through a fine sieve to obtain hairs of its prey.

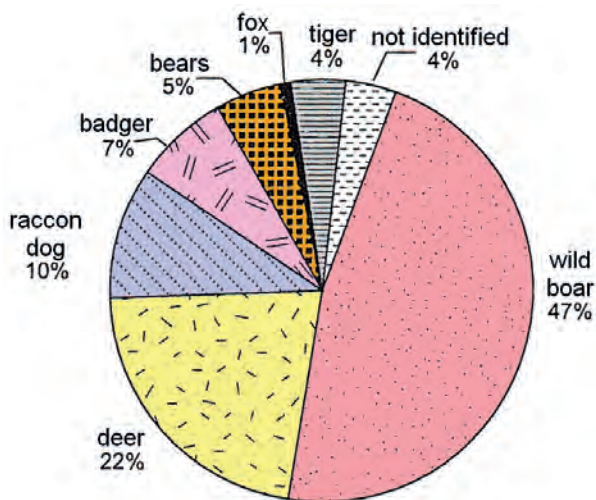
In analyzing scats, the frequency of occurrence of a particular prey species is calculated according to the formula:

$$FA = N_i \cdot 100 / N,$$

where N is the number of scats analyzed, and N<sub>i</sub> is the number of scats containing hairs of a particular species of prey.



**Figure 73.** Analyzing the hair samples under a light microscope to identify the species of prey according to the cuticle structure.



**Figure 74.** The ratio of different prey species in the diet of the Amur tiger (after Chistopolova et al., 2010).

If one excrement contains the remains of more than one species of prey, the FA index is not reliable, as the sum of occurrences of all prey exceeds 100%. In this case another index that adequately estimates the occurrence of all prey species in all analyzed excrements should be applied:

$$PA = Ni * 100 / Nm,$$

where Nm is the sum of all the occurrences of the hairs of a particular prey type in all the analyzed scats.

The results are represented by a diagram (**Figure 74**).

The methods of analyzing excrements to study the diet of large felines were described earlier (Chistopolova et al., 2010; Rozhnov et al., 2011).

#### 2.5.8. ANALYZING EXCREMENTS OF BIG CATS TO DETECT HELMINTHS

Helminths (their eggs and larvae) are detected by flotation using the saturated solution of ammonium nitrate (Vasilevich et al., 2010). To identify the proportion of excrement samples in which helminth eggs and larvae were detected (**Figure 75**) and to determine the dominant species, statistical methods are applied. Helminth species are identified using morphological and molecular genetic methods.

Methods of analyzing helminths in large felids are described in a number of studies (Esaulova et al., 2009a, b, 2010a, b, 2015, 2016), including special guidelines for diagnosing invasive diseases of wild carnivores (Esaulova et al., 2017).

Within the framework of cooperation of A.N. Severtsov Institute of Ecology and Evolution RAS with other institutions participating in the conservation projects of the Permanent Expedition of Russian Academy of Sciences for study of Red Data Book animals and other key animals of the Russian fauna, helminthological studies are per-



**Figure 75.** Confirming the helminth invasion: an egg of *Taxascaris leonine* from an excrement sample of a Amur tiger (ocular x10, objective x10) (after Esaulova et al., 2017).

formed at the Department of Parasitology and Invasive Diseases of Moscow State Academy of Veterinary Medicine and Biotechnology, named after K.I. Skryabin.

#### 2.59. ANALYZING DATA FROM PHOTO TRAPS

The photos obtained by photo traps enable us not only to identify individual animals, but also to calculate their population density and spatial patterns (Karanth, Nichols, 1998; Jackson et al., 2005, 2006; Hernandez-Blanco et al., 2013), as well as to estimate the prey base of carnivores (Rozhnov et al., 2012b).

To estimate the population density of the Amur tiger, we tested the method based on the principle of capture-recapture, considering spatial relationships between the recordings of individually identified animals (Space Capture-Recapture, SECR) using the SPACECAP software in R environment as tested (Hernandez-Blanco et al., 2013). Spatially explicit models of capture-recapture implemented in SPACECAP detect animal population density directly, uniting the history of captures with information concerning the spatial placement of photo traps. All these data are treated through Bayesian models. The main assumption of the method is that each animal has its center of activity with a fixed location. The probability of animal detection is assumed to be inversely proportional to the distance to this center of activity, and each capture is regarded as an independent event.

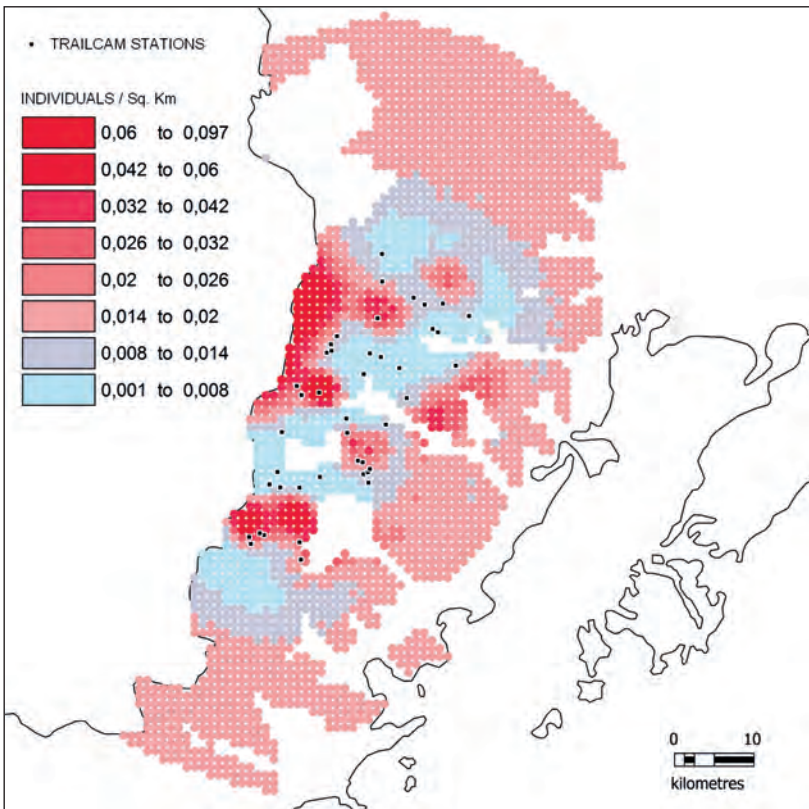
The data obtained by photo traps should be organized in a table, such as that provided in **Figure 76**.

| Date of checking | Date of the latest shot | Total number of shots | Battery replacement (yes/no) | Number of shots with the leopard | Notes | Ranger's name |
|------------------|-------------------------|-----------------------|------------------------------|----------------------------------|-------|---------------|
|                  |                         |                       |                              |                                  |       |               |

**Figure 76.** An example of a table to organize data from trailcams.

*Assessing the population density of the Caucasian leopard.* The population density of the leopard should be assessed once the new population is formed. The assessment technique is described below.

The data obtained by trailcams to be analyzed using spatially explicit capture-recapture software (SPACECAP package for R) are organized into three tables: (1) data on the capture indicating captured individuals, places and period of their capture; (2) data on the capture matrix, where geographical coordinates of the stations and periods of the given capture season (when stations were operating) are indicated; (3) the network of potential centers of home sites, the so-called state space (a network of equidistant points large enough for the probability of photographing an individual, the center of whose outside home range is close to zero). In order to create the state space in a geo-information system (GIS) software (MapInfo, ArcGIS, QGIS or so), we selected a square cell with a side of 1.5 km and a 50 km buffer around the photo trap matrix, which was approximately twice the diameter of a female carnivore home range. The state space obtained in this way (**Figure 77**) is a network of cells each



**Figure 77.** The scheme of spatial distribution of relative population density of the Far Eastern leopard within the “state space” in the “Land of the Leopard” National Park in 2012, modeled by SPACECAP R-package. For each cell with an area of 2.25 km<sup>2</sup>, the density value (specimens per km<sup>2</sup>) is provided; cells unsuitable for leopards are denoted in white.



having an area of 2.25 km<sup>2</sup>. A cell located within a place unsuitable for leopard habitation (human settlements, large basins, and highways) was assigned a value of 0; other cells – 1.

After completing the analysis, the SPACECAP software outputs the following parameters, which are used to assess population density:

$$\text{row for R: } (y) * (qchisq(0.95, 2)^{0.5});$$

where  $y$  is the parameter of individual mobility of the species studied, which can be converted into the radius of 95% of the home range; P1 is the probability of finding an individual before the first detection; P2 is the probability of finding an individual after the first detection; D is the population density per 100 km<sup>2</sup>.

To calculate these parameters, the program uses the Monte Carlo method and the Metropolis algorithm.

We have previously thoroughly described working with photo traps, analyzing the data obtained and possible calculations (Hernandez-Blanco et al., 2010, 2013; Rozhnov et al., 2012b).

*Individual identification of animals.* Photos obtained by photo traps enable us to individually identify animals. The coloration features of leopards serve as individual markers that are routinely noted in the passport of an animal (**Figure 78**).

*Assessing the abundance of the prey base of the big cats.* The installed matrix of photo traps enables us to assess the abundance of the prey base and to obtain data on how other animals use leopard prey when the photo traps are installed nearby. With the help of the photo trap matrix installed on the territory of Ussuriiskii Nature Reserve (Far East Branch of the Russian Academy of Sciences), the spatial distribution of the wild boar, red deer, spotted deer, roe deer and brown bear during the year in different relief types and plant associations (**Figure 79**) was analyzed, and the abundance index of the major prey species of the Amur tiger in different seasons was calculated (Rozhnov et al., 2012b). When this method was applied, the animals were not individually identified. The number of photo locations of potential tiger prey including the red deer, spotted deer, wild boar, roe deer, musk deer, brown and Asian black bears, badger, hare and lynx was analyzed. The photo locations of the tiger, squirrel, chipmunk, sable and sparrows were not taken into account when calculating the tiger prey abundance. For each photo location, the date and time were noted, and the species of potential tiger prey was identified. In cases when species identification based on the photo was impossible, the prey was divided into the following groups: “deer” (red and spotted deer), ungulates (all deer), and “bears” (brown and Asian black bears). The data analysis was performed using the  $\chi^2$  criterion when the distribution of the potential tiger prey was compared with the theoretically expected uniform figure, taking into account the number of trailcam days for each biotope in each season. For every species or group of species of potential tiger prey, the abundance index was calculated as the number of photo locations of this animal group per 100 trailcam days.

This approach allows us to identify seasonal, relief and habitat differences in the use of space by various species, which may constitute a substantial part of the carnivore diet.

## THE PASSPORT OF FEMALE LEOPARD VICTORIA

Look of the head – Front view



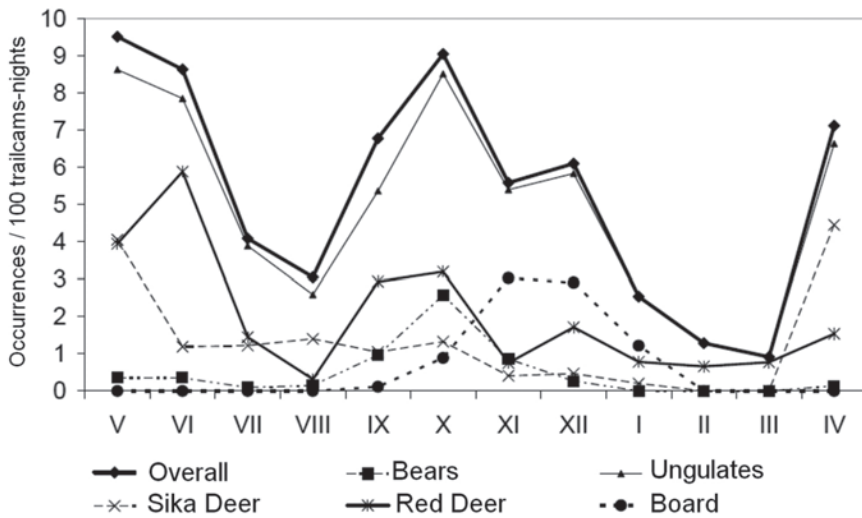
Side view - Left

Side view - Right



| <b>Species</b>                   | the Persian leopard – <i>Panthera pardus saxicolor (ciscaucasica)</i>   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------------------------------|---|-----------|------|------|-------|-------|-------|--------|--------|-------|-----|-----|-----|-----|--------|-------|------|------|-------|-------|-------|--------|--------|-------|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <b>Name</b>                      | Victoria  |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Sex</b>                       | fem   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Date of Birth / Capturing</b> | 12 July 2013  |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Parents</b>                   | Zadig (m), Andrea (fem) – EAZA Zoo, Lisbon (Portugal)   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Genetic Profile</b>           | <table border="1" style="width: 100%; text-align: center;"> <thead> <tr> <th colspan="12">Used loci</th> </tr> <tr> <th>E7</th> <th>Fca304</th> <th>Fca43</th> <th>3e6f</th> <th>E21b</th> <th>Fca77</th> <th>Fca90</th> <th>Fca96</th> <th>Fca310</th> <th>Fca441</th> <th>Fca97</th> <th></th> </tr> </thead> <tbody> <tr> <td>154</td> <td>178</td> <td>108</td> <td>120</td> <td>113</td> <td>115</td> <td>156</td> <td>159</td> <td>160</td> <td>160</td> <td>133</td> <td>143</td> <td>106</td> <td>116</td> <td>204</td> <td>204</td> <td>126</td> <td>130</td> <td>144</td> <td>144</td> <td>140</td> <td>150</td> </tr> <tr> <td>154</td> <td>178</td> <td>108</td> <td>120</td> <td>113</td> <td>115</td> <td>156</td> <td>159</td> <td>160</td> <td>160</td> <td>133</td> <td>143</td> <td>106</td> <td>116</td> <td>204</td> <td>204</td> <td>126</td> <td>130</td> <td>144</td> <td>144</td> <td>140</td> <td>150</td> </tr> </tbody> </table> | Used loci |      |      |       |       |       |        |        |       |     |     |     | E7  | Fca304 | Fca43 | 3e6f | E21b | Fca77 | Fca90 | Fca96 | Fca310 | Fca441 | Fca97 |  | 154 | 178 | 108 | 120 | 113 | 115 | 156 | 159 | 160 | 160 | 133 | 143 | 106 | 116 | 204 | 204 | 126 | 130 | 144 | 144 | 140 | 150 | 154 | 178 | 108 | 120 | 113 | 115 | 156 | 159 | 160 | 160 | 133 | 143 | 106 | 116 | 204 | 204 | 126 | 130 | 144 | 144 | 140 | 150 |
| Used loci                        |   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| E7                               | Fca304  | Fca43     | 3e6f | E21b | Fca77 | Fca90 | Fca96 | Fca310 | Fca441 | Fca97 |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 154                              | 178   | 108       | 120  | 113  | 115   | 156   | 159   | 160    | 160    | 133   | 143 | 106 | 116 | 204 | 204    | 126   | 130  | 144  | 144   | 140   | 150   |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 154                              | 178   | 108       | 120  | 113  | 115   | 156   | 159   | 160    | 160    | 133   | 143 | 106 | 116 | 204 | 204    | 126   | 130  | 144  | 144   | 140   | 150   |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Duration of captivity</b>     | 3 years   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Date of release</b>           | 15 July 2016, 28 Dec 2017   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <b>Place of release</b>          | Caucasus Nature Reserve   |           |      |      |       |       |       |        |        |       |     |     |     |     |        |       |      |      |       |       |       |        |        |       |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Figure 78. The passport of a Caucasian leopard female *Victoria* as an example.



**Figure 79.** The abundance index of potential tiger prey in different seasons in the Suvorovka River valley in Ussuriiskii Nature Reserve (Far East Branch of the Russian Academy of Sciences). The months of the studies in 2009 and 2011 are denoted on the X axis (after Rozhnov et al., 2012b).

## 2.6. DEVELOPING AND MAINTAINING THE BIG CATS DATABASE

All data collected during the monitoring of animals and their habitats are to be consolidated in a unified database. The database is a multifunctional GIS that contains layers and could be transformed to a system of interconnected tables.

For instance, in the program of the Caucasian leopard restoration before leopards are released back into the wild, each animal must have a unique “ID” that provides all the information about it, including the genetic profile.

*Preparing individual passports for animals.* A big cat’s “ID” must include the following information:

- Name;
- Sex;
- Date and place of birth;
- Age and place of releasing;
- Plantar and palmar pads measurement (an identifying attribute);
- Clear photos of each body side, muzzle in full face, and back view of the tail for leopards to be released (it is also necessary to add these photos to the base to facilitate the possibility of comparing them with pictures taken by trailcams in future);
- Unique identification information about genetics: mitochondrial DNA fragment sequence and composition of the alleles by 11 microsatellite loci;
- Unique identification information about the age and measurement at this age (body and tail length, length of legs, neck size, measurements of head, teeth and claws, spacing between canine tooth);

- Unique identification information about collars and an individual satellite code;
- Identification information of microchip implants, if an animal is microchipped.

*GIS processing of data, integration of various types of the data to carry out general analysis, preparing thematic maps, organizing a geoportal, satellite images.* All data obtained from monitoring should be consolidated into the attached thematic and vector layers, which may visualize all data on a specialized online GIS cloud i.e., geoportal. The geoportal is a comprehensive information system that jointly contains the spatial data, results of their processing and analysis, additional descriptions (texts and photos) and so forth, and is based on web technologies. The geoportal is optimized to allow a large number of users to obtain access to the data simultaneously. It is scalable and designed to be used via the Internet or within local networks with remote distributed access, and ensures the simultaneous work of a large number of users without slowing down the operation of the entire system. Geoportals allow optimizing work with large volumes of geotagged data that accumulate over time: for instance, pictures of an area taken by satellites, navigation tracks, meteorological data, etc.; bitmap catalogs are created within the geoportal system (many bitmap layers are automatically consolidated into a catalog based on metadata and attributes). Viewing and searching through a multiple time layer interface is also possible. Development of the geoportal with restricted access that permits following the progress of the project on the restoration of the Caucasian leopard population in the Caucasus (and its habitats in Russia) is currently logistically necessary, as there are many organizations and people directly involved in the project and located in different places at the same time. The online geoportal with restricted access (access is mutually agreed) is based on the GIS support system, including space imagery of the necessary resolution obtained by means of the remote sensing of the Earth. The tasks for developing this geoportal include: (1) visualizing the results of a comprehensive survey on monitoring the released Caucasian leopards with GPS collars, assessing the suitability of habitats, the results of monitoring the leopards during fieldwork recorded in the multi-layer GIS system; (2) visualizing the results of the integrative analysis of the data on Caucasian leopard habitats in the Russian part of the Caucasus, obtained by consolidating information interpreted from high-resolution space imagery and information on various environmental factors, in order to determine the areas inhabited by Caucasian leopards and their number, obtained/verified from ground-based observations.

The geoportal designed is an information geosystem to be supported and developed. All information uploaded to this geoportal can only be confirmed through the research of scientists from A.N. Severtsov Institute of Ecology and Evolution RAS (IEE RAS), who provide a number of vector maps that could be open layer by layer and categorized according to the information they contain. They have been based on the field research data obtained in the potential habitats of leopards in the Krasnodar-skiy Kray, the Kabardino-Balkar Republic, the Karachay-Cherkess Republic, North and South Ossetia, Ingushetia, Dagestan, Azerbaijan, Armenia, Georgia, Turkey, Turkmenistan, and Iran, as well as with information based on the analysis of space imagery (remote sensing of the Earth).

*Keeping a database based on GIS technologies.* All data obtained from monitoring should be consolidated in the corresponding thematic layers, which may be used with the special geoportal as described above.

The integration of satellite tracking data and multispectral remote-sensing data on the basis of SOM permits a more precise assessment of the distribution of animals' locations within the contours of biotopes of certain types, due to a detailed mapping through interpreting satellite images. When large amounts of satellite tracking data are integrated, it is reasonable to group them together by season and period pertaining to the behavior and ecology of the species. This method enables the identification and quantitative assessment of the part of the area being studied, which the animals inhabit when satellite tracking data are accumulated. Thematic SOM calibration by satellite location points may be used to both develop the probability maps of the distribution of leopards in the home range and identify the main factors limiting their movement. Based on these data, and if a location point sequence is accumulated for many years, it is possible to monitor how the home range is inhabited, taking into account the impact of global factors such as climate change and increased anthropogenic load. Such maps may also be added to the geoportal in a special forecast and theoretical modeling section. The information based on real monitoring results, which gradually adds to the geoportal, will facilitate a consistent comparison of the newly obtained results with the preliminary forecasts.

*Questionnaires for local respondents.* The questionnaires should contain the fields with the name of an inhabited locality where the survey was conducted, the social category of a respondent (hunter, fisher, shepherd, ranger, tourist, etc.), and the category of data on a leopard: (a) seeing an animal; (b) seeing traces/footprints; (c) death of an animal on a road; (d) attacks on cattle (**Figure 80**). Each category should include notes on whether the information is confirmed by a document (video/photos) or not.

| A questionnaire for local respondents |                                     |                                  |           |                                 |                          |                     |                  |
|---------------------------------------|-------------------------------------|----------------------------------|-----------|---------------------------------|--------------------------|---------------------|------------------|
| No.                                   | Inhabited locality (name, district) | Inhabited locality (coordinates) | Full name | Social category of a respondent | Information on a leopard | Documented (yes/no) | Type of evidence |
|                                       |                                     |                                  |           | Hunter                          | Seeing a leopard         | Yes                 | Photo            |
|                                       |                                     |                                  |           | Shepherd                        | Traces/footprints        | No                  | Video            |
|                                       |                                     |                                  |           | Ranger                          | Feces                    |                     | Scalable image   |
|                                       |                                     |                                  |           | Tourist                         | Prey                     |                     | Other witnesses  |
|                                       |                                     |                                  |           | Schoolchild                     | Sound (roar)             |                     | No data          |
|                                       |                                     |                                  |           | ...                             | Death on a road          |                     |                  |
|                                       |                                     |                                  |           |                                 | Attack on livestock      |                     |                  |

**Figure 80.** A questionnaire for local respondents.

Tables with survey results (which may also be linked to GIS systems) are thus compiled. As information is accumulated, the GIS system allows conclusions to be made about how leopards use space, as they are rarely distributed in an area.

## 2.7 ANALYSIS OF CURRENT SITUATION AND FUTURE PLANS FOR SOCIAL AND ECONOMIC DEVELOPMENT OF AREAS WITHIN POTENTIAL BIG CATS HABITATS

It is important to consider future regional plans for the social and economic development of a region within potential big cats habitats, existing as part of monitoring a fully fledged big cats populations. This can be achieved through close cooperation with both the federal and regional government authorities responsible for spatial planning in these regions.

### 3. LOGISTICS AND COOPERATION BETWEEN VARIOUS ORGANIZATIONS DURING THE MONITORING BIG CATS

Specialists of various organizations are usually involved in monitoring the released big cats. Thus, it is important to organize their cooperation appropriately.

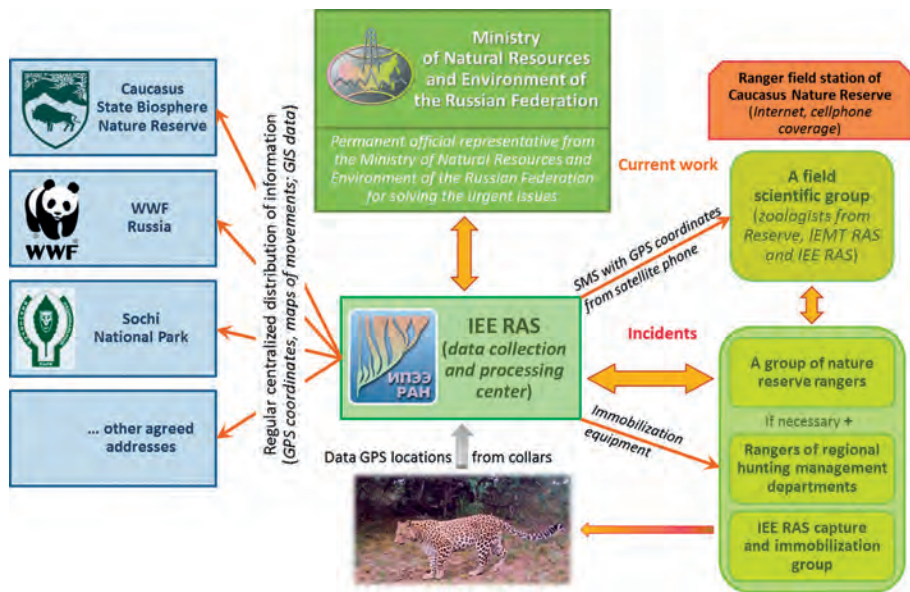
The system of cooperation between various organizations during monitoring includes the following: (1) collection of information and data/samples in the field (tracking, examining clusters of locations, collecting biological samples, recording the prey captured by a predator, installing a matrix of trailcams and receiving information from it); (2) implementation in the laboratory stage (laboratory analysis of samples collected during fieldwork, molecular genetic analysis, hormonal analysis, analysis of diet based on feces consistency, analysis to reveal helminth invasion), analyzing, integrating and summarizing all information (from collar-fitted animals, from the matrix of trailcams, laboratory analyses, remote sensing data got from the satellite images); (3) transferring the necessary information to a team in the location and responsible for rapid operational reaction, transferring the processed information to participants of the program of recovering of Caucasian leopard in the Caucasus, and sharing with open public via the mass media.

Monitoring of the released animals together with a well-formed Caucasian leopard population requires clearly orchestrated cooperation between the various organizations involved in collecting the data. This cooperation scheme was created when leopards were first released in the Caucasus Nature Reserve (Dronova et al., 2015) (**Figure 81**) and has already confirmed its effectiveness.

The following organizations undertake the monitoring of released leopards and collection of scientific information: A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences (IEE RAS), research institutes in the Caucasus (mainly A.K. Tembotov Institute of Ecology of Mountain Territories RAS and Caspian Institute of Biological Resources of Dagestan Scientific Center, the Russian Academy of Sciences (CIBR DSC RAS), and staff and rangers of protected areas like nature reserves, national parks and other organizations. All obtained information is transferred to the database and processed by an informational center at IEE RAS, before being sent to the agreed addresses.

The results of processing the information are also regularly transferred to a field group of zoologists for continued ground-based monitoring of animals, and (if necessary) to the rapid response team to prevent potential incidents.

In December 2016, IEE RAS concluded a cooperation agreement with the North-Ossetia Department of RusHydro (Hydroelectric Power Station Business Company). The agreement provides for the installation of an additional system (matrix) of trail-



**Figure 81.** Cooperation in the process of monitoring Caucasian leopards released in the Caucasus Nature Reserve.

cams in areas surrounding HPPs, which are based on schemes' HPPs developed by IEE RAS, focused on monitoring animal species in the surrounding areas.

Agreements on cooperation during the study and conservation of the Caucasian leopard have been concluded with the Ministry of Natural Resources of the Republic of North Ossetia-Alania, Alania National Park, the North-Ossetian Nature Reserve, and the Ministry of Internal Affairs of the Republic of North Ossetia-Alania, volunteers' organization. Cooperation between these organizations within the framework of agreements ensures the achievement of objectives in the shortest time possible.

The results of satellite imagery interpretation are used for geographic information analysis of changes and assessment of the state of Caucasian leopard habitats, along with the start of field work and social work in a particular region. All maps are prepared to answer exact questions based on these results. These maps show the resulted assessment of natural and anthropogenic threats, as well as year-on-year changes in the area of potential Caucasian leopards' areas, risk winters having a negative impact on leopard populations, and also animals' migration process could be probably reflected on these maps if to combine them.

The agreed system of cooperation between numerous organizations involved in the implementation of the reintroduction program of the Caucasian leopard in the Caucasus ecoregion enables the swift resolution of many of the problems associated with monitoring released animals, their habitats and a new population of animals being formed.

This system of cooperation between organizations may also be used to monitor other species of big cats, taking into account the features of a region and the biological features of species.



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## STUDY AND MONITORING OF BIG CATS IN RUSSIA