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REVIEW ARTICLE

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EVALUATION OF THE EFFECTIVENESS OF THE MONITORING METHODS IN THE ASPECT OF THE POPULATION AND DISTRIBUTION OF THE BROWN BEAR (*URSUS ARCTOS*)

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ABSTRACT

Population monitoring is a key element in research in the field of animal ecology and nature conservation. Proper coordination of the monitoring program is necessary, especially in the case of species which are difficult to observe due to their biology and ecology, such as the brown bear (*Ursus arctos*). This paper discusses several monitoring methods, such as surveys, counting females with cubs, monitoring of dens, tagging and telemetry of individuals, use of camera traps, monitoring of damages caused by bears, mortality monitoring and genetic testing. The combination of several methods, depending on the geographic and economic conditions of the country, allows for their appropriate selection, so that the results obtained are as accurate as possible. In case of the brown bear, monitoring of the population should employ different, complementary methods.

Key words: brown bear, genetic monitoring, monitoring methods

INTRODUCTION

Brown bear monitoring is one of the most difficult in terms of efficiency and applicability of classical methods. Bears activity, limited mostly to spring, summer and early autumn [Selva et al. 2011] together with the lack of strict territoriality and explicit sexual dimorphism, all together make it impossible to rely mainly on direct observations and winter tracking within established area boundaries [Linnell et al. 1998], as it is possible with other large predators – the grey wolf (*Canis lupus*) and Eurasian lynx (*Lynx lynx*). Tracking on surfaces other than snow (mud, sand) is difficult and requires a lot of experience, because the tracks of one individual may vary depending on the actual pace, speed, ground density, as well as the experience of the person performing the measurements [Selva et al. 2011]. It is not possible to unequivocally determine the age or sex of the individual based on the size of the track, with the exception of the youngest individuals [Jakubiec 2010]. According to the Bulgarian research [Spassov et al. 2016], individuals can be determined by their footprints; however, evaluation of their sex and age still leaves some doubts, because tracks left by adult females and sub-adult males can be the same size, like tracks left by young females and young males. On the other side, Danilov [1994] pointed out three age groups of bears that, according to his work, can be determined by footprints: cubs of the year (width of the forepaw track about 6–9 cm), yearlings (1.5-year-old bears with a forepaw width of 9.5–1.5 cm) and 2.5 year and older bears (width of forepaw 12 cm or more). However, it should be emphasized, that unquestionable determination of sex based only by footprints is impossible. Age determination in adults is possible only after laboratory analysis of their teeth [Linnell et al. 1998]. Due to many factors hindering conventional monitoring, several of them are used simultaneously to complement each other as much as possible [Selva et al. 2011]. The aim of this paper is assessment of the methods of brown bear (*Ursus arctos*) monitoring and indication of the most effective of them. In case of species with very specific requirements, like brown bear, the best results of monitoring can be provided with use of few mixed method of monitoring.

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MATERIAL AND METHODS

In this paper we review and compare different methods of brown bear monitoring used in Europe and North America. Some of the presented methods are not common, yet they could provide some valuable data on size and structure of local populations. We also analyse advantages and disadvantages of each method and choose the most effective for a species with such specific requirements as the brown bear is.

RESULTS AND DISCUSSION

Survey research

Survey methods are based on the forms, which are specially prepared for different species of predators. These forms bring the most important information that can be collected during field observation [Breitenmoser et al. 2006]. Once a year survey forms are to be sent to the observers (usually employees of national parks, foresters, members of hunting associations and volunteers), who mark the presence of adult bears as well as young individuals on the form (number, type) . Observations are divided into direct (individuals seen in the field) and indirect i.e. tracks, droppings, resting places, dens, remains of prey and dead individuals [Selva et al. 2011]. Survey forms should be constructed in a simple way, so that filling them in is easy and as fast as possible. To ensure all data will be collected regularly, observers should receive the results of their observations in the form of reports; then their motivation for further data collection increases [Breitenmoser et al. 2006]. Surveys are one of the cheapest and the simplest methods of monitoring [Breitenmoser et al. 2006], but they should be only treated as supplementary to other methods and their results cannot be considered sufficient [Linnell et al. 1998, Breitenmoser et al. 2006]. In this method results are subjective and observations by individual observers differ from each other and cannot be treated completely objectively [Selva et al. 2011]. Proper interpretation of traces left by animals requires experience and knowledge that is difficult to verify in the case of, for example, hunters or volunteers. The social aspect is also important as for all large predators different groups of people (local farmers, hunters, activists) may over- or underestimate the number of traces found in the area, e.g. to stress the damage caused to livestock or the reduction in the number of fallow deer resulting from predators activity. Survey methods can determine the distribution of bears, but they cannot be used to estimate their numbers [Linnell et al. 1998]. On the basis of surveys it is not possible to estimate whether a small number of observations results from behaviour of animals (secretive lifestyle, avoidance of people) or indeed a small population in the area. In addition, the results of surveys are influenced by the frequency of field inspections and the research area – where the field is frequently visited by people or bears are more accustomed to human presence, the number of observations will be automatically higher, but not necessarily indicating the presence of any larger population [Kindberg et al. 2009].

Establishing the presence of females with the new progeny

Counting of females with the new (i.e. born last winter) progeny is the commonly used strategy of brown bear monitoring. Females are more active during the day, they stay longer with their youngsters, their areas are smaller and they are easier to spot than individual animals, so their identification is easier [Ordiz et al. 2007]. In order to obtain correct data, only females with cubs should be counted, so it is best to conduct observations in spring and early summer, when it is possible to distinguish between the cubs and the youngsters from the previous year [Selva et al. 2011]. Observations of different family groups can be determined on the basis of time intervals and distances between observations, as well as the characteristics of the family groups, e.g. the number of progeny [Ordiz et al. 2007].

This method of counting can provide reliable data on parameters and dynamics of the population, provided it is carried out systematically and for a long time [Brodie and Gibeau 2007]. However, it should be remembered that the results obtained in this way differ, depending on factors such as the frequency of observations and the conditions of the habitat in particular season [Linnell et al. 1998]. Counting females with cubs works best in the areas with low numbers of bears, as the risk of counting several groups as one and the same is then lower [Ordiz et al. 2007]. When carried out systematically, this method can provide valuable data on bear reproduction. However, the disadvantage of this method, the same as in the case of survey research, is the human impact and the possibility of making a mistake.

Monitoring of dens and habitats

Monitoring of dens means recording and marking all dens used by bears during winter fast. Finding dens can be difficult due to the diversity of the terrain and places used. In the higher parts of the mountains and in the northern areas of the range it is possible to conduct bear tracking at the turn of winter and spring when the animals start to wake up and leave their dens more often. Due to the remaining layer of snow their trails can be easily seen. [Selva et al. 2011, Zwijacz-Kozica and Zięba 2011]. This method, however, does not work for females with cubs as they usually wake much later, only when the snow cover melts [Haroldson et al. 2002]. An effective method for monitoring is the use of telemetry and monitoring the activity of tagged individuals, determining the location and use of dens [Manchi and Swenson 2005].

Den monitoring, for being effective, must be carried out every season, because bears usually do not use the same place every year [Manchi and Swenson 2005]. Determining the location of dens allows planning appropriate protective measures to reduce the presence of people in the areas and unnecessary disturbance of bears. Identification of these sites also provides valuable data on habitat preferences in terms of den location [Selva et al. 2011]. Monitoring of dens, as every method, has its limitations. Finding a den can be difficult, as often they are located in places which are difficult to reach. Furthermore, we do not know whether the den was used in the previous years. It is always possible that the den found had not been used for long and this can affect and counterfeit the results.

Tagging and telemetry of individuals

The use of telemetry and tagging of individuals with special ear tags remain one of the more difficult and more expensive methods of monitoring [Selva et al. 2011]. Telemetry allows the accurate location of wild animals and the areas of their occurrence, tracking their migration routes and observation of activity, both seasonal and daily [Nowak and Mysłajek 2007].

Initially, animals were put on collars with radio transmitters, and then their location was determined by using a directional antenna and a receiver. Currently, more and more often, transmitters using the GPS system are used. Thanks to the transmission of data over GSM cellular network or via satellite signals they enable tracking of individuals even during very distant migration. Modern transmitters are also equipped with automatic fasteners that cause the collar to unfasten and drop the transmitter when the battery is empty.

The use of telemetry allows estimating the size of bear populations [Linnell et al. 1998], as well as observation and monitoring of problem subjects [Selva et al. 2011]. In order to obtain effective results, a representative group of individuals from the population should be tagged and then the tagged and untagged individuals should be caught regularly.

When the sample is large enough, the proportion of both categories of individuals is similar to that in the whole population [Selva et al. 2011].

Trapping and tagging of individuals is difficult in terms of logistics of the entire project, but also when the area of catches is to be determined. So that the results obtained reflect the population size and distribution as accurately as possible, the research area should be defined accordingly, with respect to the degree of the isolation of the population. Migrations of individuals should be taken into account and it should be assessed whether the animals can stay outside the testing area during the next trapping [Linnell et al. 1998]. One should also remember about the proper training of persons who perform all procedures related to immobilization and tagging of bears.

Usually, in order to put collars and transmitters on, bears are put to sleep with the use of specific substances (e.g. tiletamine and medetomidine hydrochloride) [Sundell et al. 2006]. Additionally, animals are tagged with identification tags and fragments of tissues are collected for DNA testing [Selva et al. 2011, Crupi et al. 2017]. Despite its high financial costs and logistical difficulties, not only does telemetry allow determining the areas and migration routes of individuals, but also collecting samples for genetic testing and accurate identification of animals [Selva et al. 2011]. However, the biggest disadvantage of this method is the acute stress involved. After waking up animals are confused and experience stress.

Monitoring with camera traps

The advantage of using camera traps, apart from them being non-invasive is the cost, which is lower than the ones of genetic studies or telemetry. Camera traps make possible to study species found in small densities, timid and difficult to observe or catch [O'Connell et al. 2011]. Data in the form of films or photos are saved on storage media and can be played and analysed repeatedly to avoid mistakes made by observers, as it happens in surveys [Breitenmoser et al. 2006].

Camera traps also provide information on animal behaviour, which is practically impossible to collect with other methods. Additionally, camera traps, when used in combination with tagging with collars or ear tags in different colours can supplement population size monitoring [Selva et al. 2011]. They are also useful in monitoring feeding places or damage caused in livestock and apiaries. Camera traps are a very effective method of monitoring, provided they are properly used. It is important to set up the areas of the highest bear activity or their frequent migration path before arranging the cameras.

Monitoring of damage caused by bears

Collecting data on damage caused by predators (not only bears, but also wolves) allows finding the so-called problem subjects, as well as improving existing systems for preventing damage and compensation payments [Selva et al. 2011]. This information is useful for determining distribution and migration patterns of bears.

This data should be collected every year, as the factors influencing the number of damage caused by predators are variable and may vary in subsequent years [Mysłajek and Nowak 2014]. Many factors affect the presence of bears in the areas of human activity, including access to the natural food base, season, number of attractive food sources (e.g. apiaries), habituation, anthropogenic sources of food, access to farm animals, hunting, preventive measures applied, population size in the area, as well as age and sex of the individual [Majić et al. 2015].

It should be remembered, however, that data on damage has a relatively large margin of error – it cannot be determined with certainty whether the increase in the number of damages indicates the increase in the number of bears, changes in habits or the presence of so-called "problem individuals" [Linnell et al. 1998], which are responsible for the disproportionate number of damage. Therefore, estimates based on the number of damage should be verified by other methods so that incidental cases can be identified and distinguished from those caused by problem subjects.

Mortality monitoring

Data on any dead individuals should be part of any species monitoring program. Each hunted bear – (in the countries where bear hunting is legal) should be accurately measured and weighed, and biometric data together with samples of genetic material delivered to the relevant administrative bodies or scientific units [Selva et al. 2011]. Wherever the populations of bears are small, each case of a dead predator should be accurately described on a special form and forwarded to the relevant authorities responsible for monitoring and managing the population. Data on dead specimens are necessary to determine the causes of death and to take appropriate preventive measures in the event of a disease factor [Jakubiec 2010]. In mortality monitoring it is also important to collect genetic material from dead individuals for further testing. Data obtained from genetic analyses of samples taken from dead specimens provide valuable information and are complementary to genetic monitoring in analysing population trends. Correctly prepared documentation in turn provides information which is collected in databases and indicates places and periods of increased mortality [Selva et al. 2011]. Mortality monitoring is effective only when each hunted bear is measured and tissue samples are taken for further testing. It is also important to notice each case of a dead bear in the field, to determine the cause of its death and to take necessary steps if needed.

Genetic monitoring

Analysis of genetic material originating from fur and droppings found in the field or blood and tissues collected from dead or caught individuals [Aarnes et al. 2013] is a very effective way to assess the size and structure of brown bear populations. The main advantage is its low invasiveness – taking samples from droppings or fur does not require trapping animals and can be done either opportunistically or during planned field inspections. Apart from shreds of fur, which can be occasionally found, special hair traps are also used to collect hair samples. These are constructions made of barbed wire wrapped around tree trunks, which pull hair with the bulbs while the bear is rubbing against the trunk. They are mounted in places where natural rubbing of individuals was observed or bears are lured there with beech tar, which attracts animals [Berezowska-Cnota et al. 2017]. Other hair traps are made from barbed wire hanging around 4–6 trees at two heights: 20–30 cms and 60–70 cms above the ground, forming a net, in the middle of which certain attractants (salmon oil, fish entrails, etc.) are placed [Crupi et al. 2017].

In genetic monitoring, the age of collected samples and the manner of their storage are extremely important to make them suitable for further analysis. The shorter the time after defecation till collection, the greater probability of successful DNA amplification. The highest amplification success was recorded with one-day samples [Murphy et al. 2007]; for samples of 2 or 3 days the success rate was about 58%, while for samples of 7 days and older it was only 41% [Bellemain et al. 2007].

Over time, the risk of genotyping errors also increases [Selva et al. 2011]. The best conditions for collecting material for genetic testing are in the period when the temperatures are below zero and the air is dry [Murphy et al. 2007].

Stool samples are collected into plastic vials or bags and stored at –20°C, after writing the date and place of collecting the sample, while hair should be stored in paper envelopes in a dry and cool place [Aarnes et al. 2013]. When collecting samples, disposable gloves are to be used to minimize the risk of DNA contamination [Nowak and Gruszczyńska 2007].

It is also of great importance to plan the sampling organization correctly. The most important assumption to be made when designing a study is that the likelihood of individual genotyping should be the same [Selva et al. 2011]. For this purpose, three different methods can be used: opportunistic gathering of material by foresters, hunters and volunteers throughout the analysed area [Bellemain et al. 2005], regular crossing of designated transects [Bellemain et al. 2007] or searching for samples in each area of the previously designed network with the same intensity [Boulanger et al. 2008]. In order to estimate the population size as accurately as possible, approximately 2.5–3 times more samples should be collected than it was initially assumed [Solberg et al. 2006].

As well as other methods, genetic monitoring also has its limitations, such as price and difficulties in analysing genetic material. A single sample analysis costs about PLN 400 (approximately 100 EUR) [Selva et al. 2011], however the cost of testing is lower when it is possible to analyse several / several dozen samples at the same time. There is also a risk of DNA contamination during sample collection, and that is why not all analyses are successful. Genotyping errors may also occur, depending on the methods and equipment used as well as the experience of those who collect biological material and carry out the analysis [Roon et al. 2005].

Brown bear genetic monitoring employs species-specific microsatellite sequences. On this basis, individual specimens can be genotyped, and their kinship and genetic variability of the whole population studied can be determined. In Norway, in the years 2006–2008, the following sequences were used for this purpose: G1D, G10B, UarMU05, UarMU09, UarMU15 and UazMU26 [Eiken et al. 2007, Bjervamoen et al. 2008, Wartiainen et al. 2009]; however, most of them (with the exception of UarMU05 and UarMU09) was characterized by a low polymorphism and it was not possible to effectively assess the genetic variability of the population on the basis of the obtained results. In the following years (2009–2012) the G10L, UarMU10, UarMU23, UarMU50, UarMU51, UarMU59 sequences began to be used [Wartiainen et al. 2010, Tobiassen et al. 2011, Tobiassen et al. 2012, Aarnes et al. 2013]. Pilot studies using microsatellite sequences were also performed in Poland. 12 microsatellite *loci* were selected (G10J, G10C, G10B, G10M, G10X MU09, MU10, MU11, MU51, MU59, MU61 and CXX20) and genetic analyses were carried out [Śmietana et al. 2012]. Reliable results were obtained only for the G10J, G10M, G10B, G10C, MU09, MU11, MU59, MU61 loci. The remaining sequences were rejected in further studies due to inconclusive results. Still, reliable results were obtained only for 49.8% of the samples [Śmietana et al. 2012].

Studies using microsatellite *loci* were performed in 2012 in the United States and 21 different sequences were used for analysis (MU59, G10B, G1D, G10M, MU50, G10U, G1A, G10C, CXX110, CXX20, G10L, G10H, G10P, G10X, MU23, REN145, P07, MSUT2, MU51, CPH9, MU26) [Crupi et al. 2017]. Amplification was successful for 67% of all hair samples. In other studies [Morton et al. 2016], G10J *loci* were used to dispose of contaminated samples and to separate material from the black bear. Then samples from brown bears were analysed using the G1A, G10H, G10J, G1D, G10B, MU50 and MU23 *loci* to identify individual specimens.

Microsatellite sequences were also used in the study of the Cantabrian brown bear population, where samples were analysed for 18 microsatellite *loci* (G1A, G1D, G10B, G10C, G10J, G10L, G10O, G10P, G10X, MU05, MU09, MU10, MU23, MU50, MU51, MU59, MU61, MU64), but in this case only 44.6% of samples were successfully genotyped [Pérez et al. 2014].

Genetic analyses also include identification of sex based on specific amelogenin sequences. This method has been successfully used in several countries, including Norway [Eiken et al. 2007, Bjervamoen et al. 2008, Wartiainen et al. 2009, Wartiainen et al. 2010, Tobiassen et al. 2011, Tobiassen et al. 2012, Aarnes et al. 2013] and USA [Crupi et al. 2017, Morton et al. 2016], whereas in studies of the Cantabrian population the SRY gene was used in sex identification [Pérez et al. 2014].

Despite some limitations, DNA testing is currently the most effective method of genetic monitoring, which also allows us identify individuals. It allows not only estimating the population size with the greatest accuracy, but also obtaining information on sex distribution, degree of kinship, migration, reproduction rate, mortality rate and habitat use. What is more, with genetic analyses it is possible to determine the level of inbreeding and genetic diversity, which is particularly important in small and endangered populations [Selva et al. 2011].

CONCLUSIONS

Monitoring of the brown bear population should be carried out using different, complementary methods. The highest effectiveness of research will be ensured by selection of various monitoring methods, which will bring satisfactory results both in terms of assessing the size and distribution of the population, as well as its structure. The best method for estimating both the size and distribution of the population as well as its structure is genetic monitoring. By marking the location of individual samples, information on the distribution and migration of individuals are obtained, while genetic analysis can determine sex structure of the population. Its results indicate the condition of the population and the degree of kinship between individuals.

Supplementing genetic monitoring with other methods (e.g. questionnaire surveys, tagging and camera traps) increases the chances of correctly estimating the size and distribution of the population in a given area, estimating individual areas, as well as verifying results obtained with other monitoring methods.

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OCENA SKUTECZNOŚCI METOD MONITORINGU W ASPEKCIE OCENY LICZEBNOŚCI POPULACJI I ROZMIESZCZENIA NIEDŹWIEDZIA BRUNATNEGO (*URSUS ARTCTOS*).

STRESZCZENIE

Monitoring populacji jest kluczowym elementem badań w dziedzinie ekologii zwierząt i ochrony przyrody, niezbędnym w celu umożliwienia ustalenia liczebności oraz rozmieszczenia populacji danego gatunku, a następnie podjęcia odpowiednich działań w zakresie zarządzania populacją lub ochrony gatunkowej [Selva et al. 2011]. Odpowiednia koordynacja programu monitoringu jest konieczna, szczególnie w przypadku gatunków, których obserwacje są utrudnione ze względu na ich biologię i ekologię, jak w przypadku niedźwiedzia brunatnego (*Ursus arctos*). W niniejszej pracy omówiono kilka metod monitoringu niedźwiedzia brunatnego, takich jak badania ankietowe, liczenie samic z tegorocznymi młodymi, monitoring gawr, telemetria i znakowanie osobników, wykorzystanie fotopułapek, monitoring szkód wyrządzanych przez niedźwiedzie, monitoring śmiertelności oraz badania genetyczne, w aspekcie ich przydatności do oceny liczebności i rozmieszczenia gatunku. Połączenie kilku metod, w zależności od uwarunkowań geograficznych i ekonomicznych danego państwa, pozwoli na ich dobór, by otrzymane wyniki były jak najdokładniejsze. W przypadku niedźwiedzia brunatnego monitoring populacji powinien się składać z kilku różnych, uzupełniających się metod. Najbardziej efektywne metody badań powinny być zapewnione poprzez taki dobór metod monitoringu, który przyniesie efektywne wyniki zarówno w ocenie wielkości i rozmieszczenia populacji, jak i jej struktury. W celu osiągnięcia założonych efektów i właściwego określenia struktury oraz kondycji populacji niedźwiedzia brunatnego (*Ursus arctos*) najlepszą metodą jest monitoring genetyczny uzupełniony innymi metodami.

Słowa kluczowe: niedźwiedź brunatny, monitoring genetyczny, metody monitoringu

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