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BIODIVERSITY ESTIMATES FROM DIFFERENT CAMERA TRAP SURVEYS: A CASE STUDY FROM OSOGOVO MT., BULGARIA

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Inventorying mammal assemblages is vital for their conservation and management, especially when they include rare or endangered species. However, obtaining a correct estimation of the species diversity in a particular area can be challenging due to uncertainties regarding study design and duration. In this paper, we present the biodiversity estimates derived from three unrelated camera trap studies in Osogovo Mt., Bulgaria. They have different duration and positioning schemes of the camera trap locations: Study 1 – grid based, 34 days; Study 2 – random points based, 138 days; Study 3 – locations based on expert opinion, 1437 days. Utilising EstimateS, we compare a number of estimators (Shannon diversity index, Coleman rarefaction curve, ACE (Abundance-based Coverage Estimator), ICE (Incidence-based Coverage Estimator), Chao 1, Chao 2 and Jackknife estimators) to the number of present and confirmed and/or potentially present mammals (excluding bats) in the mountains. A total of 17 mammal species were registered in the three studies, which represents around 76% of the permanently present mammals in the mountain that inhabit its forested area and can be detected by a camera trap. The results point to some guidelines that can aid future camera trap research in temperate forested areas. A grid-based design works best for very short study periods (e.g. 10 days), while the opportunistic expert-based positioning scheme provides good results for longer studies (approx. a month). However, the grid-based design needs to be further tested for longer periods. Generally, the random points approach does not yield satisfactory results. In agreement with other studies, analysis based on the Jackknife procedure (Jack 2) appears to result in the best estimate of species richness. When performing camera trap studies, special care should be taken to minimise the number of unidentifiable photos and to take into account «trap-shy» individuals. The results from this study emphasise the need for careful preliminary planning of camera trap studies depending on aims, duration and target species.

Key words: camera trap, grid, mammals, random points, rare species

Introduction

Camera trapping is nowadays a widely-utilised method to address a variety of biological and ecological questions, especially in mammal research (O'Connell et al., 2011; Caravaggi et al., 2017). Currently, most research employing camera traps is focused on a single taxon, most frequently a species of conservation importance. Such species are often rare, in small numbers or inhabiting large home ranges (Gaston, 1994). This makes obtaining sufficient data for their presence uncertain and effort consuming. Studies typically include some analysis of the total number of registered species, especially if this relates in some way to the focal species (e.g. competitors, predators or prey).

Globally, a limited number of publications address the way mammal registrations accumulate over time (Kauffman et al., 2007; Tobler et al., 2008; Rovero et al., 2010, 2014; Bischof et al., 2014; Si et al., 2014; Kolowski & Forrester, 2017), which are based mainly on tropical species. Furthermore, studies utilise various camera trap models and schemes positioning in the field such as random points, locations based on expert opinion or local knowledge or specifically designed grids (O'Brien, 2008) which is often not further discussed when assessing the results. All of this contributes to a major problem in planning biodiversity research, i.e. determining what should be the number of camera trap locations, their placement and the study period in order to detect even the rarest species (Rovero et al., 2010, 2013). Furthermore, when studying mammals in the temperate zone (where the number of species is much lower than in tropical areas) the direct comparison with the conclusions of the tropical biodiversity studies (where asymptote is often not reached due to frequent discovery of new species (Gotelli & Colwell, 2010)) might be misleading.

In an attempt to aid in the decisions about number and placement of the camera traps in temperate mountainous forests, we present an investigation in the Bulgarian part of Osogovo Mt. as a case study. The mammalian fauna of the mountain is relatively scarcely studied due to the restricted access to this border area (between Bulgaria and Macedonia) in the past. Only a small number of studies have been conducted and published in this area so far (Vasileva et al., 2005; Zlatanova et al., 2005, 2009; Hubancheva, 2009; Racheva et al., 2012; Petrov et al., 2015), dealing with a limited range of species as is the case for most studies in Bulgaria and the Balkan peninsula. Here we present the results from three separate camera trap studies on mammals (excluding bats) with varying field effort, number of camera traps, camera models, and positioning schemes. We emphasise the differences in mammal biodiversity derived from this data and discuss the optimal camera trap placement and study duration to detect rare or elusive mammalian species in forested areas.

Material and Methods

Study area

The Osogovo Mt. is shared between Bulgaria and Macedonia (42°9′30.2868″ N, 22°31′0.3936″ E). Its highest peak is Ruen (2251 m a.s.l.), located at the state border. The mountain's length is approx. 67–68 km and its width is 32–34 km. Osogovo's relief is asymmetric – steep northern slopes and slanted southern slopes towards the valley of the River Eleshnitsa. The climate in the mountain can be classified into two types: transient continental climate (the Rilo-Osogovski low-mountain climatic region) and typical mountainous climate in the highest parts. The Rilo-Osogovski low-mountain climatic region includes the most eastern and northern-eastern parts of the mountain (between 600 and 1000 m a.s.l.), where winters are typically mild (-1 to -2°C mean temperatures in January) and summers not very hot (24°C to 26°C mean temperatures in July). The high altitude areas (above 1000 m a.s.l.) which belong to the mountainous climatic region are characterised by lower winter temperatures (-6°C to -8°C) and long-lasting snow cover. The summers there are short and cool.

Almost 97% of Osogovo is covered by deciduous, coniferous and mixed forests which are relatively unaffected by human influence. Five vertical vegetation belts are represented: 1) xerothermic oak forests (*Quercus* sp.); 2) mesophyllous and xeromesophilic oak forests; 3) common beech forests *Fagus sylvatica* L. (best differentiated and most developed – this belt defines the upper limit of the forest); 4) mixed coniferous-deciduous forests (predominantly common beech and white fir *Abies alba* Mill.); 5) subalpine belt (up to 2200 m), dominated by juniper (*Juniperus* sp.), blueberries (*Vaccinium* sp.) and others. The Bulgarian part of Osogovo is a Natura 2000 site under the Birds Directive and under the Habitats Directive (Birds Protection Directive, 79/409/EEC; Habitats Directive, 92/43/EEC). The only protected area in the mountain is the «Tsarna reka» reserve.

Camera trap studies

The details for the three separate, unrelated camera trap studies in the Osogovo Mt. are presented in Table 1 and Fig. 1. In all cases, the camera traps were located in the forested area of the mountain and they were placed on game trails but not on intensively used dirt roads.

Table 1. Summary of the three analysed studies in the Osogovo Mt.

Study: period; area covered	**Number of camera trap locations**	**Study period (days)**	**Camera trap days per location**	**Camera trap days (total)**	**Positioning scheme**	**Models of the used camera traps**
1. Natura 2000 study: July 2012 – August 2012 area – 173.94 km^2	12	34	32–34, = 32	386	grid 3.5 × 3.5 km	– KeepGuard 680(IR); – Moultrie GameSpy 6Mpx(IR); – ScoutGuard550(IR);
2. Intensive study – 2010*: June 2010 – December 2010 area – 362.58 km^2	40	138	14–35**, = 23	913	random points	– Moultrie GameSpy 6Mpx(IR);
3. Opportunistic study: September 2008 – November 2014 area – 8.37 km^2	19	1437	59–1390, = 421	8036	opportunistic, based on expert opinion	– KG680C(IR); – KG681C(IR); – SG565FV; – Moultrie GameSpy 4Mpx(IR); – Moultrie GameSpy 6Mpx(IR); – Moultrie M100(IR); – SG550(IR); – SG565FV

* – only 6 camera traps were used which were periodically moved to other locations;
** – one camera trap location as an exception was kept for the whole period – 138 trap nights.

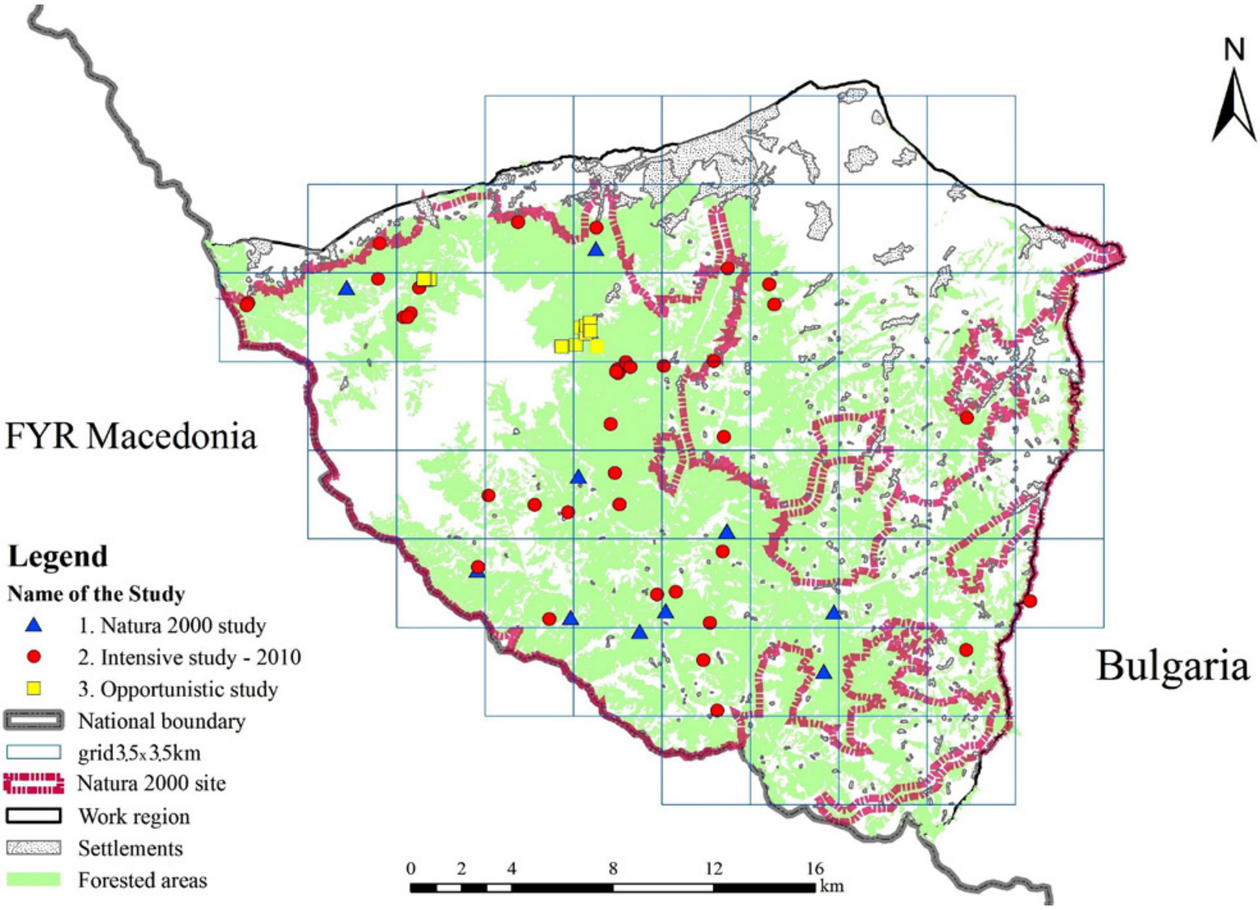

Fig. 1. Map of the camera trap locations in the three studies.

In all three studies, the camera traps were set to record with an interval of 1 minute between consecutive events. In the Natura 2000 study, the cameras were set to take photos while in the Intensive study (2010) and the Opportunistic study some of the cameras took a sequence of 10-second video followed by a photo. The triggering speed of all the camera models was fairly similar – around 1.5 sec.

A standard form was filled for each camera trap location, containing information about the date, GPS coordinates, serial number of the camera trap, team members setting up the cameras, habitat description (type of forest – deciduous, coniferous or mixed; forest visibility; dominant plant species etc.). The habitat types in each study are represented as follows: **1. Natura 2000 study** – 33% coniferous forests, 17% mixed forests, and 50% deciduous forests; **2. Intensive study 2010** – 18% coniferous forests, 8% scattered low vegetation (shrubs), 20% mixed forests, and 55% deciduous forests; 3. **Opportunistic study:** 25% mixed forests, and 75% deciduous forests.

Biodiversity analysis

The collected camera trap photos and videos were analysed through CameraBase 1.6. (Tobler, 2013), modified for further analyses and translated in Bulgarian for a number of local projects (Zlatanova, unpublished). A series of photos of a prolonged stay of the same species/individuals in front of the camera were regarded as a single independent event (hereafter «registration»), except for cases where it was possible to distinguish that two or more individuals were photographed. There were no cases of individuals returning at the same spot within a period of less than 20 min. This was done to avoid overrepresentation of a species' presence (and number of registrations) due to a continuous activity of a single individual in front of the camera trap.

A specifically designed module of CameraBase was used to export the data for analyses in EstimateS (Colwell, 2013). For these analyses, only the registered wild animals were considered. Pictures of domestic species such as cats, dogs, sheep, and goats were excluded, as well as the pictures of the team members and other people. Cases, where the species could not be identified with high level of certainty, were also excluded. Due to the very similar morphology of the stone marten (*Martes foina* Erx.) and the pine marten (*Martes martes* L.), which are both present and detected during the studies, it is often impossible to distinguish between them on camera trap photos (Petrov et al., 2016). Consequently, for the purposes of this study, the pictures of martens were pooled under the Marten – unidentified (*Martes* spp.) category. The same is applied to the wood mouse (*Apodemus sylvaticus* L.) and yellow-necked wood mouse (*Apodemus flavicollis* Mel.) whose phenotypes are so similar that species discrimination in the field even through live captures is often problematic (Michaux et al., 2005). The species within these two genera (*Martes* and *Apodemus*), due to their similarities, are often considered twin species, so hereafter the term *twin species* is used.

A detection rate index (DR) was used to normalise the number of registrations between the three studies, estimated as number of registrations / 100 camera trap days (Popova, 2017). The DR was mainly used to assess which species was considered rare in the study area.

The following results from the EstimateS output were analysed in the study:

1) Shannon diversity index H (Shannon, 1948), an index indicating the diversity of species registered from all camera traps for each day of the study. The program estimates mean values and exponentially accumulating values for each day, based on the following formula:

$$H' = -\sum p_i \ln p_i$$

Where: H' – Shannon diversity index; p_i – proportion of the registered species from species i.

2) Coleman rarefaction curve (Coleman et al., 1982), indicating the expected number of species registered in one day of the study in mean value for all of the camera traps. This curve assesses the number of species in 1,2,… t number of samples (in our case mean value for every day and every camera trap of the study), based on the assumption that all registered species in all samples are mixed randomly. The following formula was used:

$$E(S_n) = \sum_{i=1}^{S} (1 - p_i) \qquad p_i = \binom{N - x_i}{n} / \binom{N}{n}$$

Where: x_i – the count of species i; (N/n) – binomial coefficient or the number of ways one can choose n from N.

3) Registered rare species – species registered with a single (singletons) or two (doubletons) registrations in each study.

4) Coverage species richness estimators: the coverage of each study is interpreted as the proportion of the total number of registrations that belong to the species represented (Chao & Chiu, 2016). Two coverage estimators were used: ACE (Abundance-based Coverage Estimator) and ICE (Incidence-based Coverage Estimator – presence/absence);

5) Chao 1 and Chao 2 richness estimators (Chao & Chiu, 2016):

Chao 1 estimates true species diversity by deriving the lower bound of undetected species richness in terms of the numbers of singletons (species with only one registration) and doubletons (species with only two registrations). It works with abundance data only;

Chao 2 aggregates occurrence data from multiple samples (camera traps) to estimate the species diversity of the whole study. It works when abundance data on rare species (singletons and doubletons) are not available;

6) Jackknife estimators – reducing the bias of an estimator by removing subsets of the observations and recalculating the estimator (Heltshe & Forrester, 1983; Smith & Van Belle, 2010; Chao & Chiu, 2016). These were found to work well for camera trap data (Tobler et al., 2008).

For Study 1 and 3, Chao 2 was re-computed using Classic, instead of Bias-Corrected Option in the Diversity settings due to Chao's estimated CV for incidence distribution > 0.5.

For the purpose of comparison between the three studies, the EstimateS outputs were rescaled into four time scales: at the 10th day of the studies, the 20th day of the studies, the 30th day of the studies and at the last day of each study.

Results

Study 1 produced a total of 133 independent registrations in which 125 were with identifiable species. 6.02% (n = 8) of all independent registrations could not be identified.

Study 2 produced a total of 165 independent registrations in which 158 were with identifiable species 4.24% (n = 7) of all independent registrations could not be identified.

Study 3 produced a total of 2136 independent registrations in which 2102 were with identifiable species. 1.59% (n = 34) of all independent registrations could not be identified.

The total number of mammal species (excluding bats) present in the study area and capable of triggering a camera trap is 25. Altogether the number of present and confirmed or potentially present mammals (excluding bats) on the mountain is 45 (Appendix).

A total of 17 mammal species (19, if the twin species are considered) were registered in the three studies on Osogovo Mt. (Table 2). This represents around 76% of the permanently present mammals on the mountain that inhabit its forested area and can be detected by a camera trap (Appendix), or around 42% of all present or potentially present species. Additionally, anthropogenic disturbance was observed – presence of humans (including hunters), free-ranging dogs, sheep and domestic cats.

The three studies separately registered the following number of species:

Study 1 (shortest duration, grid placement): 9 species (10, when twin species are considered).

Study 2 (medium duration, random points) – 10 species (11, when twin species are considered).

Study 3 (longest duration, expert-based locations) – 17 species (19, when twin species are considered).

All the species were registered with a different DRs presented in Table 2. The highest value of the DR is for the European badger (*Meles meles* Linnaeus, 1758) in Study 3 (16.15) due to the presence of a badger sett near one of the cameras.

The three studies registered different number of rare species (species registered with singletons or doubletons) – two in Study 1; one is Study 2; and 6 in Study 3. The only rare species persisting in the three studies is the Grey wolf (*Canis lupus* Linnaeus, 1758), due to its large home range. The Northern white-breasted hedgehog (*Erinaceus roumanicus* Barrett-Hamilton, 1900) is registered in Study 1 and 3 with singletons (Table 2). In Study 3 in addition to the wolf and the hedgehog, four more rare species where registered – Chamois (*Rupicapra rupicapra* Linnaeus, 1758), Least weasel (*Mustela nivalis* Linnaeus, 1766) and Marbled polecat (*Vormela peregusna* Güldenstädt, 1770) with singletons, and the European polecat *Mustela putorius* Linnaeus, 1758 with a doubleton.

The results from EstimateS represent the middle and large mammal biodiversity in the study area (Table 3, Fig. 2).

From all the studies, the mean S_{obs} logically reaches its highest numbers only in Study 3 (n = 18), while the other two studies produce similar results. All results for S_{est} are very close to S_{obs}. The estimation of S_{ind} shows that Study 1 produces much higher expected number of registrations for each sampling level (up to day 30) compared to the other two studies.

The comparison between the studies indicates that the ACE estimator shows the quickest gain in Study 3, being the lowest from all studies at day 10 (5.35) and the highest from all studies at day 30 (10.52). The other studies show slower and similar between the two of them gain. The ICE estimator demonstrates the distinctiveness of Study 3 again, as the values for this estimator are higher that the values of the other two studies at all time intervals.

Table 2. Summary of the observed species in the three studies, including number of independent registrations, number of locations in which the species is observed and DR (detection rate per 100 camera trap nights). The pooled together species which cannot be identified on all photos/videos from camera traps are indicated with an asterisk

Common name	Latin name	Study 1			Study 2			Study 3		
		Number of registrations	Locations	DR	Number of registrations	Locations	DR	Number of registrations	Locations	DR
European badger	*Meles meles*	23	5	5.96	22	7	2.41	1298	10	16.15
Wild boar	*Sus scrofa*	24	6	6.22	40	18	4.38	48	14	0.60
Roe deer	*Capreolus capreolus*	22	6	5.70	24	13	2.63	157	17	1.95
Marten – unidentified*	*Martes* spp.	23	5	5.96	18	13	1.97	69	13	0.86
Red fox	*Vulpes vulpes*	12	4	3.11	15	9	1.64	88	8	1.10
European Hare	*Lepus europaeus*	13	4	3.37	10	5	1.10	108	12	1.34
Red squirrel	*Sciurus vulgaris*	1	1	0.26	17	5	1.86	50	7	0.62
Wildcat	*Felis silvestris*	–	–	0.00	3	2	0.33	58	8	0.72
Wood mice*	*Apodemus* sp.	–	–	0.00	–	–	0.00	77	5	0.96
Eurasian lynx	*Lynx lynx*	–	–	0.00	4	1	0.44	22	5	0.27
Grey wolf	*Canis lupus*	1	1	0.26	1	1	0.11	2	2	0.02
Edible dormouse	*Glis glis*	–	–	0.00	–	–	0.00	25	4	0.31
Northern white-breasted hedgehog	*Erinaceus roumanicus*	1	1	0.26	–	–	0.00	1	1	0.01
European polecat	*Mustela putorius*	–	–	0.00	–	–	0.00	2	2	0.02
Chamois	*Rupicapra rupicapra*	–	–	0.00	–	–	0.00	1	1	0.01
Least weasel	*Mustela nivalis*	–	–	0.00	–	–	0.00	1	1	0.01
Marbled polecat	*Vormela peregusna*	–	–	0.00	–	–	0.00	1	1	0.01

Table 3. Observed and estimated species richness during the three studies in Osogovo Mt.

	Study 1				Study 2				Study 3			
Days	*10*	*20*	*30*	*34*	*10*	*20*	*30*	*138*	*10*	*20*	*30*	*1437*
S_{obs}	6.92	7.74	8.61	9.00	4.26	6.40	7.17	9.00	4.11	6.42	8.05	**18.00**
S_{est}	6.83	7.76	8.65	9.00	4.52	6.41	7.34	9.00	4.10	6.32	7.89	18.00
S_{ind}	113.53	227.06	340.59	386.00	10.51	21.01	31.52	145	22.54	45.08	67.62	3239
ACE	6.93	7.74	8.61	9.00	6.47	7.98	8.08	9.00	5.35	8.48	10.52	22.13
ICE	7.55	8.66	12.55	17.28	9.02	9.79	8.73	9.00	11.32	15.89	17.25	28.00
Chao 1	6.92	7.74	8.61	9.00	5.01	6.99	7.6	9.00	4.84	7.93	10.01	21.00
Chao 2	7.60	8.69	10.79	11.91	6.22	7.7	8.04	9.00	7.95	11.98	13.91	**25.99**
Jack 1	8.05	9.40	11.13	11.91	6.68	8.9	9.11	9.00	6.66	9.98	11.98	22.00
Jack 2	8.56	10.94	13.57	14.74	8.08	9.62	9.54	8.02	8.45	12.13	13.91	**24.99**
Cole Rarefaction	7.80	8.69	8.98	9.00	5.38	7.03	7.77	0	7.37	9.77	11.05	0
H	1.68	1.73	1.76	1.76	1.24	1.62	1.73	**1.93**	0.99	1.29	1.41	1.85

S_{obs} is the mean total number of species observed in each study. S_{est} is the mean estimated number of species in the assemblage represented in the respective studies. S_{ind} – the mean expected number of registrations for all species. ACE (Abundance-based Coverage Estimator of species richness), ICE (Incidence-based Coverage Estimator), Chao 1 and Chao are species richness estimators presented with their means. Jackknife 1 is the first-order jackknife estimator and Jackknife 2 is the second-order jackknife estimator of species richness. Cole (Coleman) Rarefaction is the expected mean number of species registered in one day of study for all of the camera traps. H is the mean Shannon diversity index at the respective day of the studies.

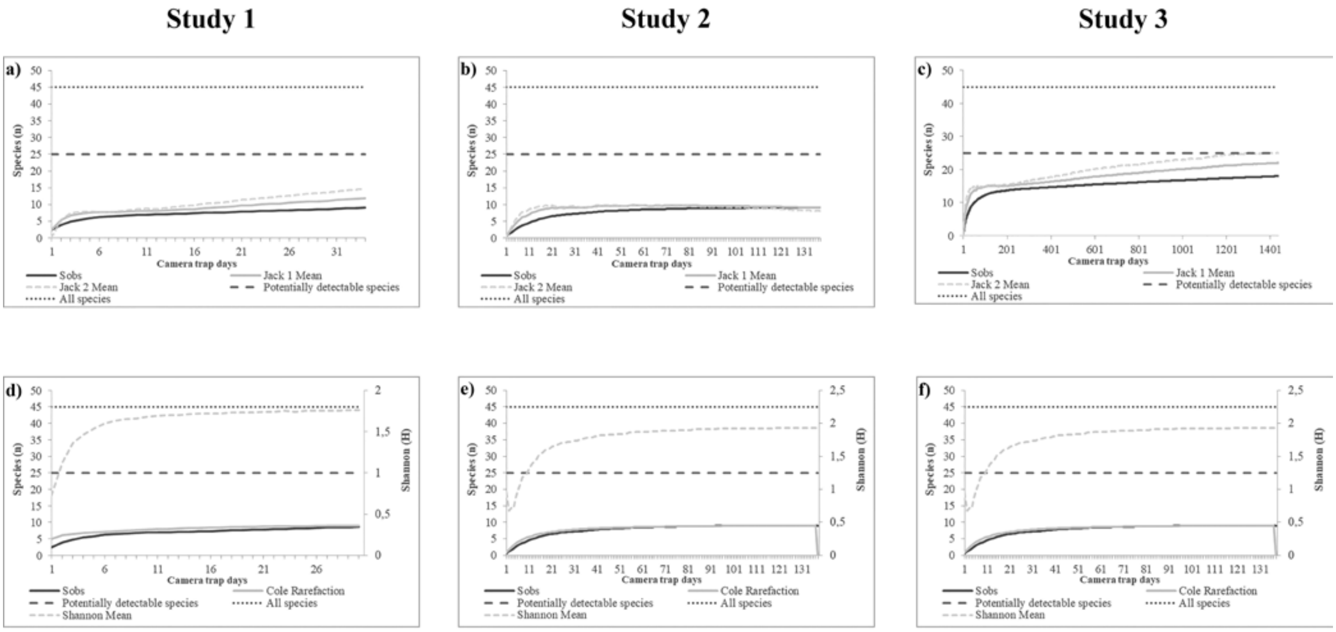

Fig. 2. Comparison of different species estimators for the three studies in Osogovo Mt.: Study 1(a,d) – grid; Study 2 (b,e) – random points; Study 3 (c,f) – opportunistic, based on expert opinion. Potentially detectable species – the number of mammals (except bats) with confirmed permanent presence in the mountain, inhabiting forested habitats and big enough to trigger a camera trap. All species – the total number of species of mammals (excluding bats) potentially present in the mountain, including permanently present, those with unconfirmed presence and species that are present, but not in the study area (forested part of the mountain) or too small to trigger a camera trap (Appendix).

The Chao 1 and 2 estimators in Study 1 are showing different gain in relation to the gain of the Shannon index H when the three studies are compared.

In Study 1 the Shannon index (H) reaches its maximum value in the shortest number of camera trap days (n = 10), compared to the other two studies. This is confirmed by Chao 1 but not by Chao 2 for this study. In Study 2 the H values reach their maximum values at a slower pace, although at the end of the study period (138 days) it produces the highest H of all studies (1.93). Around day 30 the H values are very similar in Studies 1 and 2 (1.76 and 1.73 respectively). In contrast, on this day the index in Study 3 still has a lower value (1.41). This is not confirmed by the estimate for Chao 2 in the Study 3 which is showing even higher value (~ 26) than the number of species appointed by us as capable of triggering the camera (25). Only Jackknife 2 in Study 3 reaches the number of potentially detectable species.

The Coleman rarefaction curve (Fig. 2d,e,f) indicates that the highest number of species is registered in Study 1 in the first 10 days, followed by Study 3. After this period retardation in the accumulation of new species is observed in Study 1, whereas this is not the case in study 3. Study 2 has the slowest gain of species registration accumulation.

Discussion

Many studies point out the importance of camera trapping for inventorying the community of medium to large terrestrial mammals (Silveira et al., 2003; O'Connell et al., 2011; Rovero et al., 2014), even for the discovery of new mammal species (Rovero et al., 2008). During one of the analysed studies (Study 3) on Osogovo Mt. the first hard evidence of Eurasian lynx re-establishment in Bulgaria was collected after the species extinction from the country approximately 60 years ago (Zlatanova et al., 2009). During the same survey, a marbled polecat was registered for the first time on this mountain, residing in atypical for the species high mountainous forest habitat (unpublished). The chamois had never been reported on this mountain before – the only registration was of a young specimen in dispersing age probably seeking an area to settle. In another *species oriented* camera trap survey (when specific species are targeted) the pine marten and the brown bear (*Ursus arctos* L.) (believed to inhabit the mountain but no hard evidence had been collected before) were also confirmed for the first time.

Globally, in the published camera trap inventory surveys many different and unstandardised approaches for inventoring the mammal diversity have been employed. Some studies are using the stratified approach with a grid (Tobler et al., 2008), others apply random points or game-trail approach (Cusack et al., 2015). In some of the published papers the sampling approach was not reported (Carvalho et al., 2013).

According to Rovero et al. (2010), the various studies show that camera traps efficiently capture between 57% and 86% of the medium to large terrestrial mammal species during inventory surveys, regardless of the survey design.. These authors state in their paper that 1035 to 3400 camera trap days are needed to obtain such a high species capture rate. During the three studies in our research, the species capture rate was ranging from 40% to 76% for all species that can be registered with a camera trap and from 22% to 42% from all the species believed to inhabit the mountain. The highest species capture rate (76%) is observed in Study 3 (with duration of 1437 camera trap days), which is in accordance with Rovero et al. (2010). This study is also the one that registers the highest numbers of rare species – 3 to 6 times more than the other studies with shorter duration. An interesting finding is the fact that in spite of the shortest duration of the Study 1, it registered more rare species than Study 2 which is based on three times more locations (where the cameras were placed for almost the same duration as in Study 1) and two times bigger area.

Another important issue, when an inventory of animal species is attempted, is the interpretation of rarity. According to Gaston (1994), rare species are regarded as those having low abundance and/or small ranges. Quantifying rarity (i.e. «how many registrations per species on the camera traps define the species as rare?») is a very arbitrary issue as it requires the so-called cut-off points (Gaston, 1994; Chao & Chiu, 2016). Chao & Chiu (2016) point out that the cut-off values of $\kappa = 10$ work well for many empirical data sets and this rarity value is used to estimate the species richness in the sense of the sample coverage. Yet, for practical reasons this value may not be applicable in most of the studies due to conservation or management considerations. In our three analysed studies the rare species are presented with singletons or doubletons, even for species like the Northern white-breasted hedgehog which is believed to be widespread in the mountain. Another questionable species is the grey wolf. A study based on grid and conducted immediately the following year after Study 3 (with no changes in the management of the species or any stochastic events that can lead to the rapid development of the population) produced much more wolf registrations for a shorter period of time (around 3 months), taking the species off the rare list. In this case the camera traps were set up on dirt roads. This shows that the study design can be very species-specific and inventory surveys often overestimate rarity.

The observed number of species recorded in camera trap studies is very sensitive also to the number of registrations, which in turn is influenced by the effective area that is sampled, the replication of these registrations and by the spatial arrangement of the camera traps (camera trap study design). Thus, in its intrinsic essence the ‘species richness’ is in fact also a measure of species density: the number of species collected in a particular total area (Gotelli & Colwell, 2010). For most of the camera trapping studies which involve an inventory of the mammal species the survey duration (for practical or other reasons) often does not allow long-lasting efforts to capture rare species. Some of the methodological papers (Rovero et al., 2013; Trolliet et al., 2014) published recently give only general recommendations about the duration of the study and numbers of trap sites. For example, in Rovero et al. (2013) no data is provided about the needed effort in terms of numbers of trap sites, neither for the design of the studies. Recommendations for the duration in this paper are given only for tropical areas, which might be unsuitable for temperate forested areas where the number of species and their abundance is different. So employing species richness estimators based on sampling data can be very beneficial. Usually most of the inventory studies make use of Shannon’s H index, based on the observed species, but the Chao 1 and 2 estimators are preferable showing the true diversity estimates by accounting for the undetectable or «invisible» species in a highly-diverse assemblage (Chao & Chiu, 2016). In our studies, the H index gives much lower values of species richness than the Chao values, based on the rarity of the registered species. Additionally, the ACE and ICE estimators provide valuable input about the differences in species richness coverage (Chao & Chiu, 2016), using the proportion of the total number of registrations belonging to the species represented.

The above mentioned estimators are also very useful in defining the effort needed in terms of survey duration and survey design. In our study we found that although the study with the longest duration and based on expert selection of trap sites (Study 3) shows the lowest Shannon diversity index at day 30, its true species diversity (Chao 2 estimator) shows the highest value, which accounts for 56% of the species that can be registered with a camera trap on this mountain. For Study 3 the maximum H values are approached in day 360 and after that the gain is negligible. For the Chao 2 values to be equal to the number of species detectable with a camera trap with opportunistic approach (Study 3), about 1250 days (3.5 years) will be needed. These results are in agreement with the findings of Si et al. (2014) for China. According to their analysis of the Coleman rarefaction curve, at least 900 camera trap days are needed to register enough species representative for the area, while 8000 camera trap days are needed for detecting almost all local species. However, generally the rarefaction curve appears not to be the best performing estimator, since it closely follows the observed number of species in all three studies. Applying the Jackknife procedure proves to be more successful in assessing the accumulation of species (Tobler et al., 2008). This is especially valid for Study 3, where Jackknife 2 reaches the number of potentially detectable species.

Based on our results we can conclude that if only a shorter duration is feasible for a mammal inventory study (i.e. a month), then a study design based on opportunistic expert-based approach (even if it covers a small area) is preferable. Yet, if the duration of the study should be as short as 10 days, then a grid-based design is preferred, due to the needed compromise of the relatively bigger area covered and the structured design to avoid bias. This is also supported by the results of the Coleman rarefaction curve for this study. Yet, further analyses are needed to incorporate the probability of detection for the species which can be analysed as detection history (Trolliet et al., 2014). Another important issue in short-term studies is the season when they are conducted. If they are done when mobility of the animals is impaired (e.g. in deep snow winters), then it should be expected that lower number of species would be detected. Short-term studies should be conducted only when researchers are certain that all the potential species expected to be recorded have an equal opportunity to be registered. Additionally, dispersion periods of most of the mammals (which in Europe's latitudes is most intensive during spring and summer) can shorten the time needed for registering them in species inventories where these months are targeted.

Another major issue in inventory surveys based on camera trapping is the identification of the species with similar morphology. This issue is of concern since a lot of the collected images (depending on the camera model) can be blurred or showing only a fragment of the animal (due to low triggering speed or other circumstances), resulting in a sizeable amount of unidentified registrations that can belong to any species. The situation is further complicated by employing cameras with infrared flash which produce black and white night photos. In the case of the martens' identification, this can be partially compensated by longer study periods in order to obtain enough daylight photos, helping the identification of the species. However, this is not possible for micro mammalian species, where it is often difficult to identify the animal even to the genus level. This is one of the disadvantages of the conventional camera trap studies, although some efforts are made recently to propose a new camera trap study design for small mammals (Glen et al., 2013; Mccleery et al., 2014; Soininen et al., 2015).

The presence of unidentifiable photos also has an impact on the inventory of mammals. In our studies, the highest percentage of registrations with unidentified species is observed in the study with the shortest duration (Study 1), and all the unidentified registrations were after day 26. The impact of this non-identification can be the reason for the obvious «flat line» of the Shannon's index curve for this period, but the Chao estimators compensate for this setback. The effect of this non-identification on the other two studies is negligible.

All the species in our studies are registered with different DRs which can be used as a rough estimate of their abundance, yet the detection rate can be biased by ecological and sampling-related factors (Sollmann et al., 2013). That is why we use coverage species richness estimators as a comparison approach which provides another valuable insight. Our results suggest that the study with the expert choice for camera trap sites (Study 3) is providing the best results around day 30 for the ACE (abundance) and ICE (presence of species) indices. This is again due to the positioning scheme, taking into account previously observed animal activity – animal trails, tracks, excrements, resting sites etc. These places are more easily accessible or more attractive for wildlife, thus the detection probability is expectedly higher (Foster & Harmsen, 2012).

The increased number of camera trap sites is not necessarily contributing to an increase in the number of species registrations. It was expected that the doubled in numbers camera trap locations in Study 2 in combination with a longer duration will lead to a quicker and better coverage of species richness. Instead, this study generally showed the weakest results of all three. As suggested by Bengsen et al. (2011) that «placing camera traps in a non-random way is not necessarily an issue as it is the animal population within an area that is the subject of sampling by observation stations, not the area itself».

Another issue that might affect species registration is that some of the animals can be «trap shy» when incandescent flash at night is used. This flash can easily scare the target animals and negatively influence future visitation rates in the vicinity of the camera (Séquin et al., 2003; Wegge et al., 2004). In our studies, we did not use cameras with incandescent flash.

Last but not least the accumulation of species in inventory studies can be affected by the use of different camera trap models, as it was in Study 1 and 2 here and how it is very often a common practice due to financial restraints. Many authors warn about the various technical limitations and setbacks in studies which use of mixture of camera models (O'Connell et al., 2011; Rovero et al., 2013; Trolliet et al., 2014) although currently the real impact in the sense of how much species they miss in the field is not studied yet. In our research, the mixed model studies provided better results than the only study (Study 1) with only one model but this is due more to the study design and duration than to the camera model.

Conclusions

The results from the current study point to some guidelines that can aid future camera trap research in temperate forested areas. A grid-based design works best for very short study periods (e.g. 10 days), while the opportunistic expert-based positioning scheme provides good results for longer studies (approx. a month). However, the grid-based design needs to be further tested for longer periods. Generally, the random points approach does not yield satisfactory results. In agreement with other studies, analyses based on the Jackknife procedure (Jack 2) appear to result in the best estimate of species richness. When performing camera trap studies, special care should be taken to minimise the number of unidentifiable photos and to take into account «trap-shy» individuals. Thus the results from our study emphasise the need for careful preliminary planning of camera trap studies depending on aims, duration and target species.

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Appendix. List of the species reported for the Osogovo Mt. (Zlatanova et al., 2005 with additions) with details of the certainty of their presence. They were classified based on the probability for detection in the current study (yes/no) based on their presence in the mountain, preferences to forested areas (the study area is forested) and their size (very small animals cannot trigger the camera traps reliably)

№	Species	Presence in Osogovo Mt.	Potential for registration by camera traps in the study area
1	Northern white-breasted hedgehog (*Erinaceus roumanicus* Barrett-Hamilton, 1900)	confirmed, permanent	yes
2	European hare (*Lepus europaeus* Pallas, 1778)	confirmed, permanent	yes
3	Red squirrel (*Sciurus vulgaris* Linnaeus, 1758)	confirmed, permanent	yes
4	Edible dormouse (*Glis glis* Linnaeus, 1758)	confirmed, permanent	yes
5	Common dormouse (*Muscardinus avellanarius* Linnaeus, 1758)	confirmed, permanent	yes
6	Yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834)	confirmed, permanent	yes*
7	Long-tailed Field Mouse (*Apodemus sylvaticus* Linnaeus, 1758)	confirmed, permanent	yes*
8	Bank Vole (*Myodes glareolus* Schreber, 1780)	confirmed, permanent	yes*
9	Common pine vole (*Microtus subterraneus* de Selys-Longchamps, 1836)	confirmed, permanent	yes*
10	Grey wolf (*Canis lupus* Linnaeus, 1758)	confirmed, permanent	yes
11	Golden jackal (*Canis aureus* Linnaeus, 1758)	confirmed, permanent	yes
12	Red fox (*Vulpes vulpes* Linnaeus, 1758)	confirmed, permanent	yes
13	Least weasel (*Mustela nivalis* Linnaeus, 1758)	confirmed, permanent	yes
14	European polecat (*Mustela putorius* Linnaeus, 1758)	confirmed, permanent	yes
15	Marbled polecat (*Vormela peregusna* Gьldenstдdt, 1770)	confirmed, permanent	yes
16	Stone marten (*Martes foina* Erxleben, 1777)	confirmed, permanent	yes*
17	Pine marten (*Martes martes* Linnaeus, 1758)	confirmed, permanent	yes*
18	European badger (*Meles meles* Linnaeus, 1758)	confirmed, permanent	yes
19	Wildcat (*Felis silvestris* Schreber, 1775)	confirmed, permanent	yes
20	Eurasian lynx (*Lynx lynx* Linnaeus, 1758)	confirmed, permanent	yes
21	Brown bear (*Ursus arctos* Linnaeus, 1758)	confirmed, permanent	yes
22	Wild boar (*Sus scrofa* Linnaeus, 1758)	confirmed, permanent	yes
23	Roe deer (*Capreolus capreolus* Linnaeus, 1758)	confirmed, permanent	yes
24	Red deer (*Cervus elaphus* Linnaeus, 1758)	confirmed, permanent	yes
25	Fallow deer (*Dama dama* Linnaeus, 1758) - introduced	confirmed, permanent	yes**
26	European water mole (*Arvicola terrestris* Linnaeus, 1758)	confirmed, permanent	no***
27	Common otter (*Lutra lutra* Linnaeus, 1758)	confirmed, permanent	no***
28	European mole (*Talpa europaea* Linnaeus, 1758)	confirmed, permanent	no
29	Eurasian Water Shrew (*Neomys fodiens* Pennant, 1771)	potential /unconfirmed	no
30	Mediterranean water shrew (*Neomys anomalus* Cabrera, 1907)	potential /unconfirmed	no
31	Common shrew (*Sorex araneus* Linnaeus, 1758)	confirmed, permanent	no
32	Eurasian pygmy shrew (*Sorex minutus* Linnaeus, 1758)	confirmed, permanent	no
33	Lesser shrew (*Crocidura suaveolens* Pallas, 1811)	potential /unconfirmed	no
34	Bicolored shrew (*Crocidura leucodon* Hermann, 1780)	potential /unconfirmed	no
35	Lesser blind mole rat (*Nannospalax leucodon* Nordmann, 1840)	confirmed, permanent	no
36	Forest dormouse (*Dryomys nitedula* Pallas, 1778)	potential /unconfirmed	no
37	Broad-toothed Field Mouse (*Apodemus mystacinus* Danford & Alston, 1877)	potential /unconfirmed	no
38	Eurasian harvest mouse (*Micromys minutus* Pallas, 1771)	potential /unconfirmed	no
39	Striped field mouse (*Apodemus agrarius* Pallas, 1771)	potential /unconfirmed	no
40	Black rat (*Rattus rattus* Linnaeus, 1758)	confirmed, permanent	no
41	Brown rat (*Rattus norvegicus* Berkenhout, 1769)	confirmed, permanent	no
42	House mouse (*Mus musculus* Linnaeus, 1758)	confirmed, permanent	no
43	Common vole (*Microtus arvalis* Pallas, 1778)	confirmed, permanent	no
44	East European Vole (*Microtus levis* Miller, 1908)	potential /unconfirmed	no
45	European Snow Vole (*Chionomys nivalis* Martins, 1842)	confirmed, permanent	no

* species that cannot be identified reliably from camera trap photos due to similarities with other species;

** introduced in confined game areas, but also present in the wild;

*** species that can inhabit forest habitats. However, the camera traps in the current studies were not located near water bodies, which are mainly preferred by these species.

ОЦЕНКИ БИОРАЗНООБРАЗИЯ ИЗ РАЗЛИЧНЫХ ИССЛЕДОВАНИЙ С ИСПОЛЬЗОВАНИЕМ ФОТОЛОВУШЕК: ПРИМЕР ИЗ ГОРНОГО МАССИВА ОСОГОВО, БОЛГАРИЯ

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Инвентаризация сообществ млекопитающих имеет жизненно важное значение для их сохранения и управления, особенно когда они включают редкие или исчезающие виды. Однако получение правильной оценки видового разнообразия в конкретной области может быть сложным из-за неопределенностей в отношении дизайна и продолжительности исследования. В этой статье мы представляем оценки биоразнообразия, полученные из трех не связанных исследований с использованием фотоловушек в горном массиве Осогово, Болгария. Они имеют разные продолжительность и схемы расположения фотоловушек: исследование 1 – на сеточной основе, 34 дня; исследование 2 – случайное расположение – 138 дней; исследование 3 – расположение на основании мнений экспертов – 1437 дней. Используя программу EstimateS, мы сравниваем ряд оценок (индекс разнообразия Шеннона, кривую случайного размещения Колемана, оценку охвата на основании численности (ACE) и встречаемости (ICE) видов, оценки Chao 1, Chao 2 «складного ножа» (Jackknife)) и количество представленных и подтвержденных и / или потенциально представленных млекопитающих (за исключением рукокрылых) в горном массиве. В трех исследованиях было зарегистрировано в общей сложности 17 видов млекопитающих, что составляет около 76% млекопитающих, постоянно представленных в горном массиве, которые обитают в его лесной зоне и могут быть обнаружены фотоловушкой. Полученные результаты могут служить рекомендациями при проведении будущих исследований с использованием фотоловушек в лесных районах умеренного климата. Дизайн работ на основе сетки лучше всего подходит для очень коротких периодов исследования (например, 10 дней), в то время как оппортунистическая схема позиционирования на основании мнений экспертов дает хорошие результаты для более длительных исследований (около месяца). Однако дизайн на основе сетки нуждается в дополнительном тестировании с более длительными периодами исследования. Как правило, метод случайного расположения фотоловушек не дает удовлетворительных результатов. В соответствии с другими исследованиями, анализ, основанный на методе «складного ножа» второго уровня (Jack 2), по-видимому, приводит к лучшей оценке видового богатства. При проведении исследований с использованием фотоловушек необходимо проявлять особую осторожность, чтобы свести к минимуму количество неидентифицируемых фотографий и учесть «застенчивых» особей. Результаты этого исследования подчеркивают необходимость тщательного предварительного планирования исследований с использованием фотоловушек в зависимости от целей, продолжительности и целевых видов.

Ключевые слова: млекопитающие, редкие виды, случайные точки, фотоловушка, ячейка