Mammalian Biology (2021) 101:57–62
https://doi.org/10.1007/s42991-020-00087-x

SHORT COMMUNICATION

Predation of invasive raccoon (*Procyon lotor*) on hibernating bats in the Nietoperek reserve in Poland

Jan Cichocki[1] · Agnieszka Ważna[1] · Anna Bator-Kocoł[1] · Grzegorz Lesiński[2] · Renata Grochowalska[3] · Jacek Bojarski[4]

Received: 10 May 2020 / Accepted: 9 November 2020 / Published online: 9 December 2020

Abstract

The raccoon *Procyon lotor* is an invasive species inhabiting Europe and its impact on local populations, particularly on endangered species, is not well known. The aim of the study was to determine whether raccoons pose a threat to bats wintering in the Nietoperek nature reserve, one of the largest hibernation sites in Central Europe. In the winter of 2016–2017, 67 raccoon scats were collected in one of the main underground corridors in the reserve. It was confirmed, with the use of molecular methods, that the scats belonged to raccoons. Raccoon prey was identified based on the features of skulls, jaws or hair found in the scats. Analysis of the determinable remains indicates that bats (including Daubenton's bat/Natterer's bat/Brandt's bat *M. daubentonii/nattereri/brandtii*, Natterer's bat *Myotis nattereri*, greater mouse-eared bat *Myotis myotis*, brown long-eared bat *Plecotus auritus*) made up the largest percentage of raccoon diet (96%). Remains of other mammal species, lizards *Lacerta* spp., plant materials and anthropogenic food were also identified in raccoon scats. The results of the research indicate that predation by raccoons can constitute a significant factor in the increasing mortality of bats wintering in Nietoperek.

Keywords Raccoon · *Procyon lotor* · Predation · Hibernating bats

One of the key global problems of nature protection is the emergence of invasive predatory species in ecosystems and their negative impact on the local fauna (e.g. Blackburn et al. 2004; O'Donnell et al. 2015; Bellard et al. 2016; Doherty et al. 2016; Mollot et al. 2017; Welch and Leppanen 2017). The three following species of invasive carnivorous mammals have appeared in Central Europe: raccoon dog *Nyctereutes procyonoides*, American mink *Neovison vison* and raccoon *Procyon lotor* (Kauhala 1996; Genovesi et al. 2009). Over the last two decades, the raccoon has been spreading particularly rapidly over the region (Beltrán-Beck et al. 2012; Salgado 2018). Raccoon expansion in Poland is very dynamic and the species is found throughout the country except for the highest parts of the mountains. Its population is particularly numerous in the western part of the country, where it occurs both in urbanized and naturally valuable areas (Bartoszewicz et al. 2008; Gabryś et al. 2014).

The introduction of the raccoon can pose a threat to biodiversity due to both competition and predation on the native fauna (Kauhala 1996). Food opportunism of the raccoon and its high environmental tolerance imply that it is an invasive species that feeds on a number of native animals in new areas (Ikeda et al. 2004). However, there is little direct evidence on the negative impact of the raccoon on valuable and endangered species in Europe (Salgado 2018).

Handling editor: Danilo Russo.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42991-020-00087-x) contains supplementary material, which is available to authorized users.

✉ Agnieszka Ważna
a.wazna@wnb.uz.zgora.pl

[1] Department of Zoology, Institute of Biological Sciences, University of Zielona Góra, Prof. Z. Szafrana 1, 65-516 Zielona Góra, Poland

[2] Institute of Animal Science, Warsaw University of Life Sciences (SGGW), Ciszewskiego 8, 02-787 Warsaw, Poland

[3] Department of Biotechnology, Institute of Biological Sciences, University of Zielona Góra, Prof. Z. Szafrana 1, 65-516 Zielona Góra, Poland

[4] Department of Mathematical Statistics and Econometrics, Institute of Mathematics, University of Zielona Góra, Prof. Z. Szafrana 4a, 65-516 Zielona Góra, Poland

Information about the negative impact of the introduced species on European bats remains poorly described. Records of attacks on Leisler's bat *Nyctalus leisleri* by the rose-ringed parakeets *Psittacula krameri* come from Italy (Menchetti et al. 2014). In Spain, these parrots evicted bats from their hollows and attacked common noctules *Nyctalus noctula* (Hernández-Brito et al. 2018). Bats were also a prey of the domesticated cats (Ancillotto et al. 2013; Khayat et al. 2020).

The aim of the study was to determine the impact of the raccoon on bats wintering in the Nietoperek nature reserve. We propose the following hypotheses: (1) raccoons penetrate underground corridors, not only to find shelter, but also to feed on wintering bats which can constitute a significant contribution to the predator's food; (2) the presence of specific bat species in the raccoon diet is proportionate to the size of that particular bat species' population within the studied underground corridor.

The research was conducted in the Nietoperek nature reserve in Western Poland (N52.2343 E15.2920). The reserve is located in underground corridors ca. 30 km long, which are part of the fortifications from the Second World War period. The corridors consist of a series of shafts that lead from ground level battle shelters to interconnected underground tunnels, halls and train stations. The corridors are one of the largest bat wintering sites in Central Europe. During the hibernation period, over 38,000 bats, arriving from Poland and Germany, find shelter in the underground corridors. The following species were found in the area: greater mouse-eared bat *Myotis myotis,* pond bat *Myotis dasycneme*, Bechstein's bat *Myotis bechsteinii*, western barbastelle *Barbastella barbastellus,* Daubenton's bat *Myotis daubentonii*, Natterer's bat *Myotis nattereri*, Brandt's bat *Myotis brandtii*, brown long-eared bat *Plecotus auritus* and serotine bat *Eptesicus serotinus* (Cichocki et al. 2015).

The research was carried out in a single, 1500 m long corridor, which serves both as the main entrance to the underground corridors for visitors and one of the most important ways for bats to get into the hibernaculum. The entrance to the corridor starts on the ground level. Scattered light reaches about 500 m inwards, but beyond that the corridor stays in complete darkness (Fig. 1, Online Resource 1).

Raccoon predation on bats was investigated based on the analyses of the composition of predator scats (Online Resource 2). Single scats appeared underground in December 2016, and over time, their numbers increased, indicating a regular penetration of the underground corridor by the predator. We collected material in two rounds, i.e. at the beginning and in the middle of March 2017. A total number of 67 scats were collected and analysed for diet composition.

Predator species were initially identified as raccoon based on the size and shape of scats. At that time, raccoons had only been seen in the ground parts of the fortifications. We

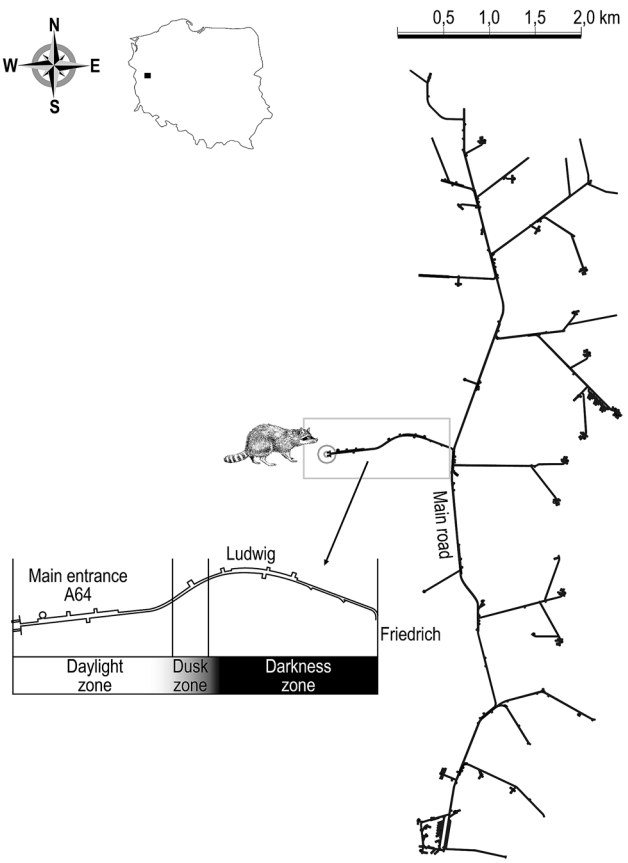

Fig. 1 Location of the corridor penetrated by the raccoon in the winter 2016–2017 in the Nietoperek nature reserve. A-64, Ludwig, Friedrich—names of the underground railway stations

have made the DNA analysis to confirm the species. Species identification was performed based on sequencing of the mitochondrial COI (mini-barcode) gene fragment from 25 samples. The analysed COI gene segment was 187 bp long (239 bp including primers). The total number of 25 samples, frozen after collection, was subjected to the molecular tests. DNA was isolated with the QIAamp DNA Stool Mini kit (Qiagen) following the manufacturer's protocol. PCRs were performed in thermal cyclers (Sensquest) with the conditions and cycling conditions described by Chaves et al. (2012) as well as primers BC–F3 and BC–R2 (Chaves et al. 2012). Blank PCR controls were used in all reactions to monitor reagent contamination. Amplification products were run on 1.5% agarose/TBE gels stained with MidiGreen. PCR products were purified using EPPiC (A&A Biotechnology) following the manufacturer's protocol. Sequencing was performed in Genomed S.A. (Poland). The obtained sequences were analysed using the DNA Surveillance—Carnivora and a species assay by Chaves et al. (2012). According to the information from this database, the species present in ten samples was confirmed as raccoon.

Raccoon prey was determined based on the analysis of skull features (Ruprecht 1987). Depending on the degree of material preservation, prey was identified in terms of their species, genus or order. In addition, the following features were taken into account: coat colour, length of metacarpal and phalange bones forming bat wings, undigested parts of bat ears. In the absence of bone elements, features of hair structure were analysed (Teerink 1991). Bats that were not identified to species level from the genus *Myotis* were described as: Daubenton's bat/Natterer's bat/Brandt's bat. Other unidentified small bats were assigned to Chiroptera indet., including small *Myotis*, *Plecotus* and *Barbastella.*

The occurrence of species in raccoon scats was calculated as a proportion of samples containing the remains of a given species or group of species to the total number of analyzed samples. The total number of bats included in the tests was determined on the basis of the maximum number of determinable skull elements: tooth series and right and left branches of the jaw. In the absence of skull elements, the sample was assumed to belong to a single individual. This assumption is justified by the composition of scats in which uniform fragments of fur and wing fragments were found. When feeding on greater mouse-eared bats, raccoons often left heads and forearms uneaten. However, this material was not taken into account in the analysis to avoid double-calculation of the same individual (Online Resource 3).

The proportions of scats containing bats (of respective species or group of species) and scats containing other components of raccoon diet, were compared using χ^2 test. The same test was used to assess the difference in the occurrence of two main groups of investigated bats in raccoon diet: the greater mouse-eared bat and Daubenton's bat/Natterer's bat/Brandt's bat.

The number of bats wintering in the underground section penetrated by raccoons was monitored every two weeks, from November 2016 to the end of March 2017. In total, 10 inspections were conducted. To demonstrate which of the species or group of species is most vulnerable to raccoon foraging, the number of individuals from a given group found in scats was compared to the average number of counted individuals hibernating along the investigated section of the underground corridor. The entrance corridor is used by bats dynamically. Therefore, the population inhabiting this section changes over the season. Due to the above reason, the average number of bats of a given species or a group of species in relation to the number of individuals from these groups found in scats was used in the analysis. For all the statistical tests, the significance level $p = 0.05$ was used. All statistical analyses were conducted using R program (R Core Team 2018).

The results of our research describe the first documented case of the negative impact of invasive raccoons on European bat species during their hibernation. Raccoons exploring the underground corridors of the Nietoperek nature reserve prey on hibernating bats during winter. We found 81 individuals in the predator scats out of which 32 were identified to species (20—Natterer's bat, 11—greater mouse-eared bat, 1—brown long-eared bat). Due to material fragmentation, other individuals were identified as Daubenton's bat/Natterer's bat/Brandt's bat (43 individuals) or were included in the group of unidentified small bats Chiroptera indet. (6 individuals).

Bats were present in 96% of the analysed scats. In 69% of these single individuals were found, in 28% two individuals were identified, and in 3% three individuals were recorded. Only three of the analysed samples did not contain any bats. Apart from bats, the total number of 15 samples also contained other diet components (Table 1). The fraction test for two groups ($\chi^2 = 71.06$, $df = 1$, $p < 0.001$) indicates a significantly higher contribution of bats in the raccoon diet compared to other food fractions (hypothesis # 1 confirmed).

On average, 3019.9 ± 605.4 individuals (range 2129—4112) of 9 bat species (*M. myotis* 2566.8 ± 804.7, *M. daubentonii* 212.6 ± 145.2, *M. nattereri* 41.8 ± 24.6, *M. brandtii* 34.1 ± 7.8, *M. bechsteinii* 0.3 ± 0.5, *M. dasycneme* 0.9 ± 1.2, *P. auritus* 66.3 ± 41.6, *B. barbastellus* 91.8 ± 62.1, *E. serotinus* 3.0 ± 1.0, Chiroptera indet. 1.3 ± 1.6) wintered in the studied section of the corridor in the period from November 2016 to March 2017 (Fig. 2).

The fraction test for two groups of bats found in raccoon scats, i.e. the greater mouse-eared bat and the Daubenton's bat/Natterer's bat/Brandt's bat ($\chi^2 = 37.45$, $df = 1$, $p < 0.001$), indicates that the dominant group among the diet was Daubenton's bat/Natterer's bat/Brandt's bat (hypothesis #2 rejected). The percentage of individuals found in raccoon scats for the selected species or a group of species is inverse in relation to the average number of individuals hibernating in the examined underground section (Fig. 3).

Although the greater mouse-eared bat is the most abundant group of bats wintering in Nietoperek nature reserve, the main prey of raccoons included different species: Daubenton's bat/Natterer's bat/Brandt's bat. Furthermore, individuals of the greater mouse-eared bat species constitute the largest percentage of dead bats found in the reserve (J. Cichocki unpbl. data). The majority of greater mouse-eared bats (99%) winter in places located more than 2 m above the bottom of the underground corridors. Due to this fact, they are inaccessible to raccoons. Based on that, we conclude that raccoons probably ate dead greater mouse-eared bats that had fallen out of the clusters earlier. In contrast to the greater mouse-eared bats, Natterer's bats and Daubenton's bats often hibernate near the ground (even as low as 1 m), therefore, they stay within the feeding range of raccoons. It is possible that high winter activity and the close-to-the-ground hibernation were the reasons for the

Table 1 Food composition of the raccoon *Procyon lotor* in the Nietoperek reserve and mean number of bats hibernating along the investigated section of the underground during 10 inspections in the winter of 2016–2017

Species/group of species	Mean number of hibernating bats	Frequency of occurrence in raccoon scats ($N=67$)	
		N	%
Greater mouse-eared bat *Myotis myotis*	2231.0	10	14.9
Natterer's bat *Myotis nattereri*	34.8	19	28.4
Daubenton's bat/Natterer's bat/Brandt's bat *M. daubentonii/nattereri/brandtii*	81.1	39	58.2
Brown long-eared bat *Plecotus auritus*	55.2	1	1.5
Bats Chiroptera indet. (small *Myotis*, *Plecotus*, *Barbastella*)	44.3	6	9.0
Vole *Microtus* spp.		2	3.0
House mouse *Mus musculus*		1	1.5
Water vole *Arvicola amphibius*		1	1.5
Weasel *Mustela nivalis*[a]		2	3.0
Domestic cat *Felis catus*[a]		2	3.0
Lizard *Lacerta/Anguis* indet.		1	1.5
Plant material		9	13.4
Anthropogenic food		3	4.5

[a]Most likely eaten in the form of carrion

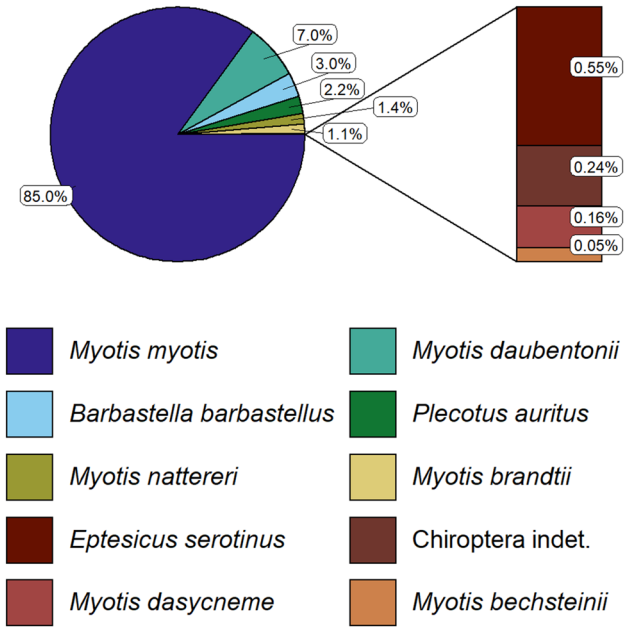

Fig. 2 Percentage of the bats hibernating in corridor penetrated by the raccoon in the winter 2016–2017 in the Nietoperek nature reserve

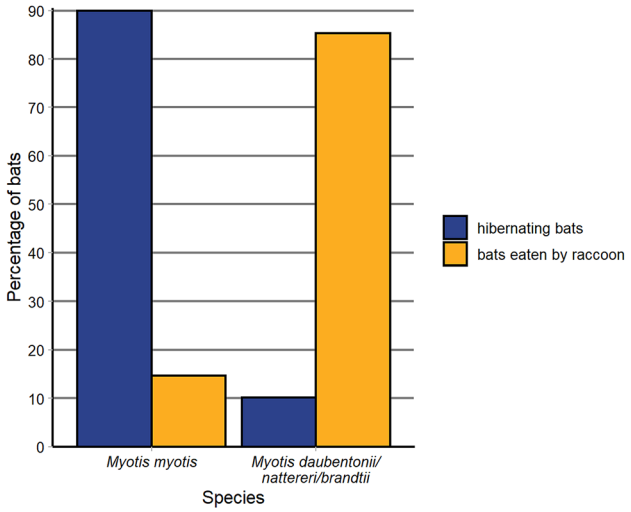

Fig. 3 Percentage of the greater mouse-eared bats and Daubenton's bat/Natterer's bat/Brandt's bat hibernating along the underground section penetrated by the raccoon in the Nietoperek nature reserve in relation to the percentage of individuals eaten by the predator

increased occurrence of this species in raccoon diet (Online Resource 4).

Raccoons are food opportunists (Rulison et al. 2012). In their natural range, raccoon diet is usually dominated by plants and invertebrates, with a significant presence of crustaceans, crabs and crayfish in the areas of their availability (Lotze and Anderson 1979). In Germany, the first European country where raccoons were introduced, the animal diet was dominated by plant food and various species of invertebrates (Engelmann et al. 2011). Consequently, vertebrates accounted only for 15% of biomass eaten by raccoons. However, the occurrence of birds and mammals in the predator diet was increasing in winter (Engelmann et al. 2011). In the floodplain areas of Western Poland, rodents represented the largest share in raccoon diet. However, the phenological period in which the material was obtained was not determined by the authors of the study (Bartoszewicz et al. 2008).

In our research, raccoons used the availability of bats opportunistically, treating them as the main component of their diet. Bats were present in the diet of raccoons inhabiting caves in North America (Munson and Keith 1984; McAlpine et al. 2011). Raccoons also fed on dead individuals with white nose syndrome (McAlpine et al. 2011).

Until now, the only predator recorded to be preying on bats in the underground corridors of the Nietoperek nature reserve, was the stone marten *Martes foina* (Urbańczyk 1981; Lesiński and Romanowski 1988; Tryjanowski 1997). Research on the diet of the stone marten, conducted in the 1980s, revealed that its diet was dominated by Daubenton's bats and brown long-eared bats (Lesiński and Romanowski 1988). However, this fact does not seem to have a significant impact on the number of bats in the reserve. The emergence of a new effective and universal predator, such as raccoons, has increased predation pressure on bats. Consequently, this can lead to an increase of bat mortality, at least in the corridors accessible to raccoons, and become a factor of significant impact on the number of bats in wintering sites.

In North American caves, raccoons usually penetrated relatively small entrance sections but were also found in the deeper parts (Munson and Keith 1984; McAlpine et al. 2011). Some caves are used by raccoons as hideouts and resting place (Levis 2002; Moseley et al. 2013; Montalvo et al. 2017). In the Nietoperek nature reserve, raccoons penetrated the corridor section to a distance of about 900 m from the entrance. Scats were also found 1300 m from the entrance indicating that the predator was moving in complete darkness.

There are several dozen entrances in the reserve but only these accessible at ground level can be used by terrestrial animals. Therefore, only a few entrances are available to raccoons. The animal may probably use entrances to vertical shafts of staircases as a shelter, but this does not interfere with bats' wintering zone. However, the range of predation shown in this study indicates the need to monitor the situation and perhaps take more radical measures to protect bats.

Acknowledgements We are grateful to Marcin Grzelczak and Robert M. Jurga for providing an underground documentation, Mateusz Ciepliński for creating graphics and Maurycy Ignaczak, Katarzyna Janik-Superson and Grzegorz Błachowski for providing photos. We also thank Paulo B. Chaves for sharing the DNA sequences and consultations and Emiliano Mori and two anonymous reviewers for helpful comments on the manuscript.

Compliance with ethical standard

Conflict of interest The authors declare that they do not have any conflict of interest.

References

Ancillotto L, Serangeli TM, Russo D (2013) Curiosity killed the bat: domestic cats as bat predators. Mamm Biol 78:369–373. https://doi.org/10.1016/j.mambio.2013.01.003

Bartoszewicz M, Okarma H, Zalewski A, Szczęsna J (2008) Ecology of the raccoon (*Procyon lotor*) from western Poland. Ann Zool Fenn 45:291–298. https://doi.org/10.5735/086.045.0409

Bellard C, Genovesi P, Jeschke JM (2016) Global patterns in threats to vertebrates by biological invasions. Proc R Soc B 283:20152454. https://doi.org/10.1098/rspb.2015.2454

Beltrán-Beck B, García FJ, Gortázar C (2012) Raccoons in Europe: disease hazards due to the establishment of an invasive species. Eur J Wildl Res 58:5–15. https://doi.org/10.1007/s10344-011-0600-4

Blackburn TM, Cassey P, Duncan RP, Evans KL, Gaston KJ (2004) Avian extinction and mammalian introductions on oceanic islands. Science 305(5692):1955–1958. https://doi.org/10.1126/science.1101617

Chaves PB, Graeff VG, Lion MB, Oliveira LR, Eizirik E (2012) DNA barcoding meets molecular scatology: short mtDNA sequences for standardized species assignment of carnivore noninvasive samples. Mol Ecol Resour 12:18–35. https://doi.org/10.1111/j.1755-0998.2011.03056.x

Cichocki J, Stopczyński M, Bator A, Grzywiński W, Ignaczak M, Ignaszak K, Jaros R, Kowalski M, Łochyński M, Postawa T, Warchałowski M, Węgiel A, Wojtaszyn G (2015) The number of bats wintering in the Nietoperek bat reserve in 2015. In: 24 National bat conference, 13–15 Nov 2015. Kazimierz Dolny (In Polish, English summary). https://www.researchgate.net/publication/285236621

Doherty TS, Glen AS, Nimmo DG, Ritchie EG, Dickman CR (2016) Invasive predators and global biodiversity loss. Proc Natl Acad Sci USA 113:11261–11265. https://doi.org/10.1073/pnas.1602480113

Engelmann A, Köhnemann BA, Michler FU (2011) Nahrungsökologische Analyse von Exkrementen gefangener Waschbären (*Procyon lotor* L., 1758) aus dem Müritz-Nationalpark (Mecklenburg-Vorpommern) unter Berücksichtigung individueller Parameter. Beitr Jagd Wildforsch 36:587–602 (**In German**)

Gabryś G, Nowaczyk J, Ważna A, Kościelska A, Nowakowski K, Cichocki J (2014) Expansion of the raccoon *Procyon lotor* in Poland. Acta Biol 21:169–181

Genovesi P, Bacher S, Kobelt M, Pascal M, Scalera R (2009) Alien mammals of Europe. In: Drake JA (ed) Handbook of alien species in Europe. Invading nature: Springer series in invasion ecology. Springer, Dordrecht, pp 119–128

Hernández-Brito D, Carrete M, Ibáñez C, Juste J, Tella JL (2018) Nest-site competition and killing by invasive parakeets cause the decline of a threatened bat population. R Soc Open Sci 5:172477. https://doi.org/10.1098/rsos.172477

Ikeda T, Asano M, Matoba Y, Abe G (2004) Present status of invasive alien raccoon and its impact in Japan. Glob Environ Res 8:125–131

Kauhala K (1996) Introduced carnivores in Europe with special reference to central and northern Europe. Wildl Biol 2:197–204. https://doi.org/10.2981/wlb.1996.019

Khayat RO, Grant RA, Ryan H, Melling LM, Dougill G, Killick DR, Shaw KJ (2020) Investigating cat predation as the cause of bat wing tears using forensic DNA analysis. Ecol Evol 10:8368–8378

Lesiński G, Romanowski J (1988) Martens hunting on bats Wszechświat 89:210 (**In Polish**)

Levis JJ (2002) Community conservation assessment for cave guano habitats and associated rare animal species. USDA Forest Service, Eastern Region, Hoosier National Forest

Lotze J-H, Anderson S (1979) *Procyon lotor*. Mamm Species 19:1–8

McAlpine DF, Vanderwolf KJ, Forbes GJ, Malloch D (2011) Consumption of bats (*Myotis* spp.) by raccoons (*Procyon lotor*) during an outbreak of white-nose syndrome in New Brunswick, Canada: implications for estimates of bat mortality. Can Field Nat 125:257–260. https://doi.org/10.22621/cfn.v125i3.1231

Menchetti M, Scalera R, Mori E (2014) First record of a possibly overlooked impact by alien parrots on a bat (*Nyctalus leisleri*). Hystrix Ital J Mamm 25:61–62. https://doi.org/10.4404/hystrix-25.1-9989

Mollot G, Pantel JH, Romanuk TN (2017) Chapter two-the effects of invasive species on the decline in species richness: a global meta-analysis. Adv Ecol Res 56:61–83. https://doi.org/10.1016/bs.aecr.2016.10.002

Montalvo AE, Lopez RR, Parker ID, Silvy NJ, Cooper SM, Feagin RA (2017) Quantifying meso-mammal cave use in central Texas. Wildl Biol. https://doi.org/10.2981/wlb.00320

Moseley M, Smith M, Broders M, Burns L (2013) Ecology of Wisqoq cave, a raccoon—inhabited cave in Nova Scotia. Speleobiol Notes 5:66–73

Munson PJ, Keith HJ (1984) Prehistoric raccoon predation on hibernating *Myotis*, Wayndotte cave, Indiana. J Mammal 65:152–155. https://doi.org/10.2307/1381219

O'Donnell CFJ, Clapperton BK, Monks JM (2015) Impacts of introduced mammalian predators on indigenous birds of freshwater wetlands in New Zealand. New Zeal J Ecol 39:19–33

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing (Vienna). http://www.R–project.org.

Rulison EL, Luiselli L, Burke RL (2012) Relative impacts of habitat and geography on raccoon diets. Am Midl Nat 168:231–246. https://doi.org/10.1674/0003-0031-168.2.231

Ruprecht AL (1987) A key for mandible identification of Polish bats. Przegl Zool 31(1):89–105 (**In Polish**)

Salgado I (2018) Is the raccoon (*Procyon lotor*) out of control in Europe? Biodivers Conserv 27:2243–2256. https://doi.org/10.1007/s10531-018-1535-9

Teerink BJ (1991) Atlas and identification key hair of Westeuropean mammals. Cambridge University Press, Cambridge

Tryjanowski P (1997) Food of the stone marten (*Martes foina*) in Nietoperek bat reserve. Zeitschr Säugetier 68:318–320

Urbańczyk Z (1981) Fledermäuse (Chiroptera) in der Nahrung der Marders (*Martes* sp.). Säugetierkund Mitt 29:77–79 (**In German**)

Welch JN, Leppanen C (2017) The threat of invasive species to bats: a review. Mammal Rev 47:277–290. https://doi.org/10.1111/mam.12099