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Causes of Mortality of Endangered Garden Dormice *Eliomys quercinus* (Linnaeus, 1766) (Rodentia: Gliridae) from Germany

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Abstract: The underlying reasons for the shrinking distribution of the endangered garden dormouse *Eliomys quercinus* remain largely unclear, making the development of effective conservation measures difficult. In order to understand its biology, habitat requirements and factors threatening the species, the project "In Search of the Garden Dormouse" was launched in Germany (2018–2024). In this context, garden dormice found dead were examined *via* necropsy. Gender distribution was balanced between male (47.4%) and female animals (45.9%). Age distribution was 38.3% juveniles and 58.4% adult animals. Nutritional status was recorded in 40.7% as good but 6.7% of animals were in a severely reduced nutritional status or cachectic. In 7.2% of examined animals, blunt trauma was diagnosed. Internal haemorrhages without fractures or disruptions of the integument were found in 12.4%. In one animal, death related to disease was suspected. In 60.8%, the main finding of necropsy was sharp trauma, most probably caused by predation. Genetic analysis of samples from 15 animals diagnosed with sharp trauma injuries revealed domestic cats were the predator in 80% of cases. In summary, predation is an important mortality factor in garden dormice, with domestic cats playing a major role.

Key words: necropsy, *post mortem*, monitoring, predation, decline

Introduction

One critical component of understanding the driving forces of species decline and the potential for extinction is the knowledge of morbidity and mortality factors and their relative importance and impact on endangered populations (Brand 2014). Increasing human populations, habitat destruction, alien species, pollution, overexploitation and disease were identified as the main drivers (Davies et al. 2006, Wilson 1992). However, the roles of specific factors that threaten species are scarcely substantiated by quantitative studies (Howard et al. 2020, Wilcove et al. 1998). Among endangered

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wildlife, small mammals do not attract as much attention as big charismatic mammals and are often neglected form the conservation point of view (Bertolino et al. 2015). One small mammal species that has been overlooked, despite its strong decline (Lang et al. 2022), is the garden dormouse *Eliomys quercinus* (Linnaeus, 1766), a rodent native to Europe. In 2008, the species was listed as "Near Threatened" by the IUCN (Bertolino et al. 2008) but a change to the status "Vulnerable" has been proposed because of the ongoing range contraction (Bertolino 2017).

In Germany, the garden dormouse was classified as "Endangered" in 2020 (Meinig et al. 2020). As more than 10% of the current range of the garden dormouse is located in Germany, the country has a special responsibility for its conservation (Meinig 2004). As the reasons for the species' shrinking range are poorly understood, effective conservation actions have been lacking so far. Therefore, the project "In Search of the Garden Dormouse" was launched in 2018 in Germany, using different methods to study the behaviour and distribution of this species (e.g., Büchner et al. 2022) as well as to derive concepts and measures to mitigate the decline. Moreover, to obtain information about mortality factors, animals found dead were analysed as part of the project. Besides macroscopic species verification by experts, examination of carcasses offers the opportunity to study biology and morphology in more detail and provides a broad variety of baseline data (e.g., Ryser-Degiorgis et al. 2021, Unterköfler et al. 2022). Information about the population status can be obtained from body measurements, sex determination and assessment of reproductive and nutritional status. Morphometric data for example can be used as an ageing tool (Marti & Ryser-Degiorgis 2018). Necropsies can reveal pathologic alterations and narrow down possible causes of mortality. Additionally, a broad variety of samples can be obtained for subsequent investigations to elucidate impairments such as (sublethal) infectious diseases, including zoonoses (e.g., Fischer et al. 2018, Jeske et al. 2018, 2021, Kinnunen et al. 2011), toxin contamination and intoxication (e.g., Arikan et al. 2018, Buchweitz et al. 2018, Lestrade et al. 2021, Schanzer et al. 2021, Tosh et al. 2012), old injuries and parasitic infections (e.g., Konjević et al. 2007, Makarikov et al. 2018, Makarikov & Georgiev 2020, Ortega Pérez et al. 2020).

In this study, results of gross necropsy are presented identifying factors of mortality in garden dormice.

Materials and Methods

Garden dormice found dead were examined *via* necropsy. Coordinated by employees of the NGO Friends of the Earth – Germany, mainly citizen scientists (volunteers, hereafter referred to as "collectors") sampled garden dormouse carcasses found dead in gardens, on public property, in traps, in nest boxes or brought home by cats. The carcasses were immediately frozen and transferred to the Clinic for Birds, Reptiles, Amphibians and Fish (Giessen, Germany). In total, necropsies of 209 wild living garden dormice were included in this study. The carcasses examined were found dead in 2012 (n = 1) and between 2015 and 2021 (n = 208) and derived from almost the complete known range of the species in Germany (Fig. 1).

Metadata such as collection date, geographical location and circumstances of discovery were transmitted to a project database for documentation. The carcasses were provided with a unique identification number, catalogued and stored in a frozen condition at -20 °C until the date of dissection. The animals' origins were classified using the Digital Land Cover Model Germany (LBM-DE2018) (© GeoBasis-DE

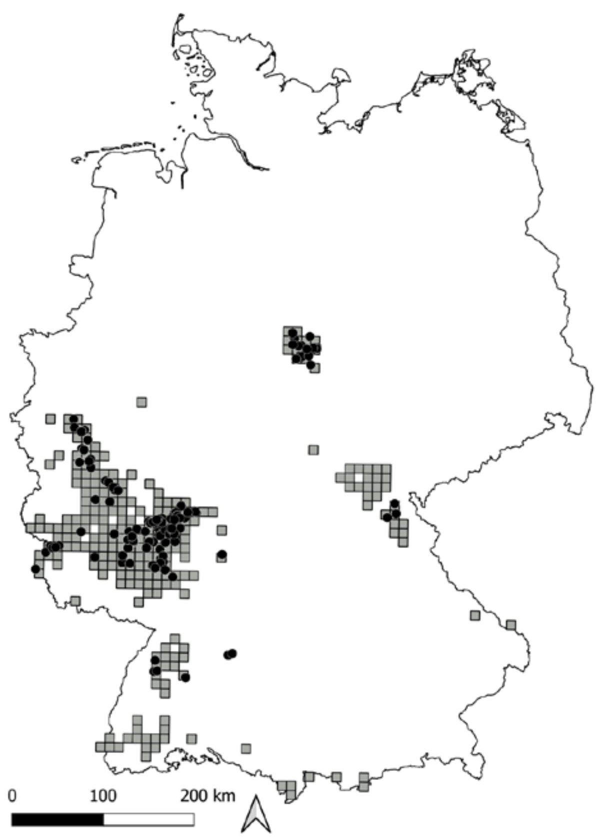

Fig. 1. Distribution range of garden dormice in Germany (grey squares) and origin of the 209 specimens examined post mortem in this study (black dots). © MultiBase CS 2022 and © BKG 2021

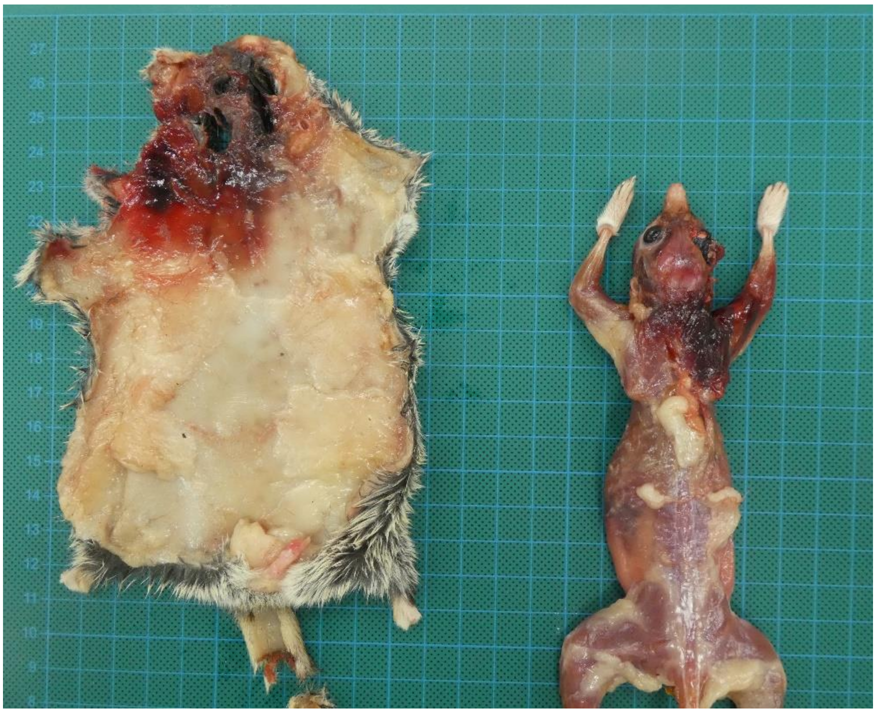

Fig. 2. Garden dormouse after skinning. Note corresponding haemorrhages in subcutaneous tissue and in musculature on the animal's right side.

/ BKG [2018]) (Bundesamt für Kartographie und Geodäsie 2020). Land use categories were summarised in three categories with "urban" and "agriculture" representing intensively used habitats and "forests" containing extensively used or unused habitats. Necropsies were conducted following basic rules of wildlife necropsies as described in McAloose et al. (2018). Before starting dissections, a necropsy protocol including a morphometrics' datasheet and a sample checklist was designed in order to ensure consistent data acquisition, uniform documentation of pathological findings and standardised sample gathering (Ryser-Degiorgis 2013). Necropsies were performed in the dissection laboratory of the Clinic for Birds, Reptiles, Amphibians and Fish (Giessen, Germany) to provide a maximum level of personal safety and biosecurity. To exclude cross contamination of, e.g., pathogens, a separate dissection set was used for each animal. The animals were defrosted in the dissection laboratory at room temperature for 2-4 hours or overnight at 4-6 °C. First, an external examination was conducted including documentation of morphometric data: age was estimated (juvenile, adult), sex (male, female, not determined) and several measurements were taken (weight, length of hindfoot, ear length, length from nose-tip to tail, tail length). External orifices were examined, and all animals were checked for ectoparasites and any visible injuries to the exterior. For genetic analysis, a piece of musculature (in most cases the tongue) was removed and stored in 96% ethyl alcohol along with a tuft of hairs. After which, the animals were skinned completely, separating the skin in one piece from the torso. If the animal was in rather good condition, the skin was prepared with special care and conserved in 96% ethyl alcohol for taxidermic preparation later on. In order to investigate bruising and other wounds more clearly, the naked torso as well as the inside out, stretched pelt was examined again (Fig. 2). Afterwards, the animal was put in dorsal recumbency, and the abdomen was opened *via* midline incision (Fig. 3). The state of decomposition was recorded in the categories 'good', 'mildly', 'moderately' or 'severely autolyzed', 'skeletonised' or 'mummified' in order to contextualise pathological findings. Furthermore, the nutritional status of the animals was scored as 'very good', 'good', 'mildly reduced', 'moderately reduced', 'severely reduced' and 'cachectic', taking into account markers such as the body shape, subcutaneous fat and visceral fat deposits. Inner bleedings (haemorrhages) or other internal injuries such as ruptures of the spleen or the diaphragm were documented. Afterwards, the abdominal viscera were removed one by one. Subsequently, the chest was opened by cutting the diaphragm and the ribcage. Bleedings were reported and the organs were assessed for intactness and physiological po-

Table 1. Categorisation of gross necropsy results of garden dormice according to causes of death.

Categories for death causes	Common necropsy results
Sharp trauma	Skin lesions (often accompanied by hair sticking together)
	Disconnection of tissue
	Haemorrhages in the subcutaneous tissue and/ or the musculature
	Bleedings especially in the thoracic region
Blunt trauma	Fractures in absence of stabbing injuries
Internal haemorrhages	Bleedings without any evidence of blunt or sharp trauma (intact body surface and skeleton)
Disease	Macroscopically visible pathology of organs in absence of any signs of trauma
Unclear	Organs not judgeable, e.g. due to advanced autolysis

Fig. 3. A garden dormouse in dorsal recumbency, with opened thoracal and abdominal cavity.

sition. The neck was opened to check especially the trachea for any disruptions or alterations. The skull was left intact for osteological preparation for a morphological study on geographical subpopulations. The brain was removed by syringe-aspiration through the *Foramen magnum*, after detaching the head from the rest of the carcass, preferably at the atlanto-occipital joint. All organs were inspected macroscopically *in situ* as well as after their removal from the body cavity, pathological findings were documented in writing and photographs were taken additionally. In case of macroscopic abnormalities, the affected organ was conserved in 4% formalin. In case they were still identifiable, uterus and ovaries were also stored in formalin. As in the majority of cases the carcass was already in an advanced state of decomposition, no samples for histological examinations were taken routinely. To establish a sample archive for further laboratory investigations various organs, including brain, spleen, liver, lung, kidneys, oesophagus, trachea, urinary and gall bladder, adrenal glands, gonads, heart, a piece of the skeletal musculature, fat (if present) and the right *Os femoris* as well as effusion fluids were refrozen immediately in individual tubes and stored at -20 °C. Furthermore, a triple swab taken from the nose, pharynx and rectum was taken and stored in RNAlater (Thermo Fischer, Germany). The stomach with its contents was preserved for nutritional analysis and the intestines were stored for parasitological investigations. The pelt was refrozen or preserved in ethyl alcohol for subsequent preparation. Fleas and ticks were sent to specialists for species identification and ticks, as well as ear pinnae were stored to test for tick-borne pathogens later on. The main findings of necropsy were summarised in the categories in Table 1.

For a subsample of 15 animals diagnosed with sharp trauma probably caused by predation, DNA samples were taken for species identification of predators. For this purpose, swabs soaked with 1xTE buffer were used to collect saliva traces from the carcasses' fur near conspicuous external wounds. DNA was extracted using the QIAamp DNA Investigator Kit (QIAGEN) following the manufacturers' protocols. The cat-specific primers LF4 and CHR were used to amplify a 130 bp fragment of the mitochondrial control region (described in von Thaden et al. 2021).

Results of necropsies were interpreted in context of the animals' history as far as the latter

Table 2. Age and gender distribution, state of decomposition and nutritional status of garden dormice examined *via* necropsy according to land use categories. (n. d. = not determined).

		Urban (*n* = 151)	**Forests** (*n* = 16)	**Agri-culture** (*n* = 19)	**No coor-dinates** (*n* = 45)	**Total** (*n* = 209)
Age distribution	juvenile	60 (28.7%)	3 (1.4%)	4 (1.9%)	13 (6.2%)	80 (38.3%)
	adult	87 (41.6%)	13 (6.2%)	13 (6.2%)	9 (4.3%)	122 (58.4%)
	n. d.	4 (1.9%)	0 (0.0%)	2 (1.0%)	1 (0.5%)	7 (3.3%)
Gender distribution	male	70 (33.5%)	6 (2.9%)	12 (5.7%)	11 (5.3%)	99 (47.4%)
	female	69 (33.0%)	9 (4.3%)	6 (2.9%)	12 (5.7%)	96 (45.9%)
	n. d.	12 (5.7%)	1 (0.5%)	1 (0.5%)	0 (0.0%)	14 (6.7%)
State of decomposition	good	3 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.4%)
	mildly autol-ysed	40 (19.1%)	5 (2.4%)	8 (3.8%)	3 (1.4%)	56 (26.8%)
	moderately autolysed	52 (24.9%)	5 (2.4%)	8 (3.8%)	11 (5.3%)	76 (36.4%)
	severely autolysed	47 (22.5%)	6 (2.9%)	2 (1.0%)	8 (3.8%)	63 (30.1%)
	mummified	2 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
	n. d.	7 (3.3%)	0 (0.0%)	1 (0.5%)	1 (0.5%)	9 (4.3%)
Nutritional status	very good	3 (1.4%)	1 (0.5%)	2 (1.0%)	1 (0.5%)	7 (3.3%)
	good	65 (31.1%)	6 (2.9%)	9 (4.3%)	5 (2.4%)	85 (40.7%)
	mildly re-duced	27 (12.9%)	1 (0.5%)	3 (1.4%)	0 (0.0%)	31 (14.8%)
	moderately reduced	8 (3.8%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	9 (4.3%)
	severely reduced	9 (4.3%)	1 (0.5%)	0 (0.0%)	2 (1.0%)	12 (5.7%)
	cachectic	2 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
	n. d.	37 (17.7%)	7 (3.3%)	4 (1.9%)	15 (7.2%)	63 (30.1%)

was known. Diagnosis of mortality causes such as drowning, traffic accident and intraspecific aggression were dependent on the information provided by the collectors, if there was not any conflicting evidence from necropsy.

Results

Origin and baseline data of the garden dormouse carcasses examined *via* necropsy

Carcasses originated mainly from habitats in urban areas (72.2%). State of decomposition was recorded in most cases as moderately (36.4%) to severely autolysed (30.1%). Gender distribution was balanced between male (47.4%) and female animals (45.9%). Age distribution was 38.3% juveniles and 58.4% adult animals. Nutritional status was recorded in the majority of animals (40.7%) as good. 6.7% of animals were in a severely reduced nutritional status or cachectic. Detailed results are provided in Table 2.

Causes of death suspected by the collectors

For 77 animals, the collector of the carcass mentioned some suspicion regarding the cause of death. In 55 cases predation was suspected, mainly by domestic cats (*n* = 47), because the carcass was brought home by a cat or was found in the same place the cat usually left mice. Other suspected predators were martens and birds of prey. In seven animals, intoxication was mentioned as the possible cause of death. Trapping was reported in six garden dormice. In three juveniles, death was suspected in context with the death of the mother. Two animals were suspected to be roadkill and there were individual examples where an animal was suspected to have died for the following reasons: cleaning of nest boxes, some kind of infectious disease, drowning in a water barrel or intraspecific aggression. For the remaining 132 animals, no information about a suspected cause of death was available.

Table 3. Causes of death diagnosed by gross necropsy in garden dormice according to land use categories. (n. d. = not determined).

Cause of death	Urban (*n* = 151)	Forests (*n* = 16)	Agriculture (*n* = 19)	No coordinates (*n* = 45)	Total (*n* = 209)
Sharp trauma	105 (50.2%)	10 (4.8%)	10 (4.8%)	2 (1.0%)	127 (60.8%)
Blunt trauma	11 (5.3%)	0 (0.0%)	2 (1.0%)	2 (1.0%)	15 (7.2%)
Internal haemor-rhages	17 (8.1%)	1 (0.5%)	3 (1.4%)	5 (2.4%)	26 (12.4%)
Disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	1 (0.5%)
n. d.	18 (8.6%)	5 (2.4%)	4 (1.9%)	13 (6.2%)	40 (19.1%)

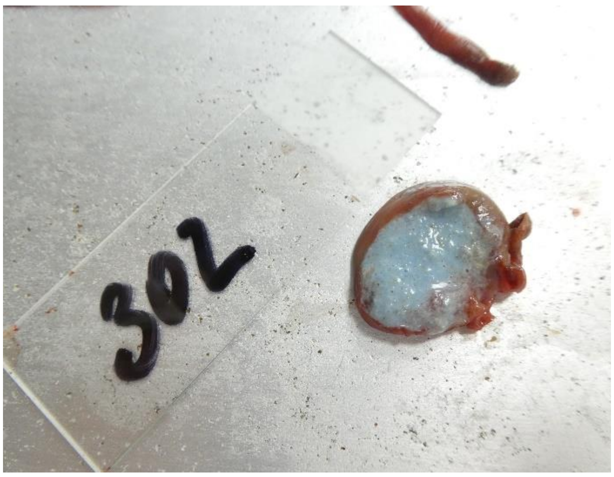

Fig. 4. Stomach (cut open, with bluish content) of a garden dormouse showing haemothorax, probably caused by poisoning.

Causes of death diagnosed by gross necropsy

Quantitative results of gross necropsy categorising the causes of death in sharp trauma, blunt trauma, internal haemorrhages, disease and "not determined" are shown in Table 3. Necropsy revealed in 127 cases (60.8%) that death was associated with sharp trauma. Predation was highly suspected to have caused those injuries with the body cavity being opened and organs missing in some cases. In other cases, the carcass was still complete but pinpoint to pinhead-sized lesions in the skin and sometimes perforations of the body cavity were detected. The thoracic region was particularly affected and hair surrounding the wounds was found stuck together in several cases, possibly representing traces of saliva. In order to test whether DNA isolation was possible from these traces, samples from those regions were genetically analysed for cat DNA in 15 animals. In 12 cases (80%), domestic cat DNA was found. In two of the remaining samples, no cat DNA was found and, in one sample, DNA seemed to be completely degraded, as there was no garden dormouse DNA isolated either. Sharp trauma in context with the genetic results and considering the collector's history led to 42 cases (20%), in which a cat was suspected to have killed the garden dormouse. In one case of suspected trapping, necropsy revealed sharp trauma, hair stuck together and cat DNA was identified genetically, making predation more likely as cause of death. For 84 animals, the predator was not identified. In 15 cases (7.2%), blunt trauma was diagnosed. Together with the animals' history, the aetiological diagnosis of blunt trauma was trapping in five animals and traffic accidents in two garden dormice. For the remaining eight animals, aetiology of trauma was not determined. In four animals reported by the collector as trapped, blunt trauma with fractures of the spinal column were confirmed by necropsy. In another case, trapping was suspected but, except for a fractured forearm, no further information could be obtained because of advanced autolysis. Two animals with blunt trauma were presumed as being killed by traffic because of multiple fractures in the absence of stabbing injuries. Another animal was suspected to have been killed by traffic by the finder but, due to advanced autolysis, it remained unclear if it was fatally hit by a vehicle or if it was rolled flat *post mortem*, so was therefore categorised as 'not determined'. Internal haemorrhages were found in 26 animals (12.4%) without fractures or disruptions of the integument. Those animals were suspected to have died due to intoxication, e.g., with anticoagulant rodenticides, although only in three of those animals the collector suspected intoxication. One animal showing haemothorax without macroscopically visible injuries contained bluish material in its stomach (Fig. 4). In six more cases with internal haemorrhages, domestic cats were suspected as cause of death by the collector, which could not be proven by necropsy because of the absence of bite wounds. Another animal was reported as having drowned in a water barrel. In this case, the main findings were haemorrhagic lungs and frothy fluid in the trachea. In combination with the absence of external injuries, drowning was not rejected as cause of death in this animal. In one animal suspected by the collector to have been killed during nest box cleaning no specific signs were detected other than haemorrhagic lungs and non-coagulated blood in the heart, so cause of death remained unclear. In one animal, death related to disease was suspected based on necropsy as it had multiple pinhead-sized disseminated whitish spots in the liver and on the renal capsule. Furthermore, intrathoracic adhesions between lung, pericardium and diaphragm were found leading to the suspected diagnosis of chronic inflammation. The number of animals in which the reason of death remained unclear amounted to 40 (19.1%).

Discussion

In general, field studies dealing with diseases in wildlife have to face the difficulty that small mammal and bird carcasses disappear quickly, due to decomposition or consumption by predators. Furthermore, discovery of carcasses is often hampered because sick wild animals tend to hide and die in inconspicuous locations (Pimentel & Burgess 2005). Nonetheless, a large amount of garden dormouse carcasses was collected and provided for necropsy thanks to the cooperation of numerous citizen scientists in the project "In Search of the Garden Dormouse". Necropsy proved to be a suitable method to determine cause of death in more than 80% of all carcasses and to obtain plenty of samples for subsequent analyses. However, it should be kept in mind that the carcasses received in this study probably do not represent a random cross-section of the German garden dormouse population. Instead, a bias towards those forms of mortality most easily detected by the collectors is likely and the bulk of carcasses were derived from urban habitats. Nonetheless, causes of death directly linked to humans including trapping, drowning, poisoning or roadkill were rare, except for domestic cats.

Monitoring causes of mortality can play an outstanding role in setting a basis for protective measures. Drowning, for example, can be avoided easily by covering rainwater barrels. Other causes of mortality are more difficult to address, e.g. poisoning with anticoagulant rodenticides as garden dormice are non-target species for rat poisoning. However, recommendations for the reduction of rat poison should be considered, especially in cities with garden dormouse populations (Smith & Shore 2015) and restricting the application to the hibernating season of garden dormice should be taken into account. In general, pest control should be carried out only after positive identification of the pest species (e.g., by means of wildlife cameras).

The main cause of death was predation, which was identified in 60.8% of all animals. Background data and results of genetic analyses confirmed domestic cats as predators in 26.8% of these cases. Considering that the animals, which were selected for genetic verification of cats as predators, are representative of the remaining animals diagnosed with sharp trauma, cats would be responsible for more than 3/4 of predation-related mortality cases. Cats are opportunistic hunters and their prey includes a wide variety of animals, including small to medium-sized mammals (Mori et al. 2019, Woods et al. 2003). In the US, domestic cats are the top source of human-related mortality in birds and small mammals, easily eclipsing other sources such as mortality from poisons, pesticides and collisions with structures and vehicles (Loss et al. 2013). Due to the high number and density of cats in many urban areas and their hunting instinct, which can be strong even in well-fed pet cats, predation by domestic cats can also be the main cause of mortality in Europe for prey animals living in close proximity to humans, such as garden birds (Pavisse et al. 2019). It cannot be concluded from our data whether predation of garden dormice is not an important cause of death only in urban habitats or it has also an influence as a cause of decline at the population level. Nevertheless, policies should be adopted and implemented to reduce or eliminate the impact of free-ranging domestic cats on biodiversity in general and on garden dormice in particular (Trouwborst et al. 2020, Trouwborst & Somsen 2020). To raise awareness among people living in dormouse habitats, information about the species and its biology is fundamental. In any case, improved knowledge of the distribution of garden dormice and information for the public about its presence as well as increasing sympathy for this endangered and charismatic wildlife species are all key factors to help to its further conservation.

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