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Doctoral Dissertation

Sika deer as an alien species: Perception in scientific literature and ecological traits of non-native populations

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Abstract

The introduction of alien species represents the second most dramatic cause of biodiversity loss all over the world. In the last centuries, sika deer (*Cervus nippon*) have widely spread from their native areal across most continents and they are nowadays considered one of the most invasive existing mammal species. In Europe, sika deer were introduced starting around 150 years ago, mainly for aesthetic reasons, in addition to the native fauna and flora. Currently recognized as a source of damages for the vegetation and as a threat for the native red deer (*Cervus elaphus*) due to hybridization, sika deer have settled in several countries and have widely colonized the area with increasing population numbers. One of the European countries hosting the biggest populations of sika deer is the Czech Republic, where the species was introduced in 1891. In the country, the sika deer represents a threat to the native species and the ecosystem. The situation is different in the neighboring country Austria. There, sika deer are not spread across the majority of the country but are present in only a few local subpopulations. The taxonomic status and history of these subpopulations are still contentious, and still little is known on the effects of the species on native fauna and local flora.

One goal of this thesis is to evaluate the global knowledge and the perception of sika deer in the scientific literature highlighting blind spots and unexplored topics, trends, and highly investigated matters, as well as new issues. Our results showed that native countries (most of all Japan) are the most productive in terms of the number of published documents and that in Europe countries hosting the biggest populations are also the most prolific. Moreover, native literature focuses mostly on the effects of sika deer on vegetation and is also recently opening the discussion to new topics such as the medical use of sika deer parts. Countries hosting alien populations, on the other hand, focus more on the effects of hybridization between sika and native species. The second goal of this thesis is to evaluate the management of sika deer in the Czech Republic by investigating the trend of the number of animals culled in the country between 1994 and 2018. We conclude that a better management strategy, the collection of complementary data, and a specific plan for containing the growing sika population, mostly in the western part of the country, is not only advisable but very much needed. Last, we aim to perform a descriptive and preliminary study on the diet of sika deer evaluating, using and testing on sika deer feces two different methods of analysis; the DNA metabarcoding and the FTIR spectroscopy, the fecal composition of a population in Lower Austria.

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Introduction

1 *Alien species and Biological Invasions: a worldwide issue*

Being many species able to walk, fly, swim, or be transported by wind or water, migration or dispersal often occur in nature (Shigesada & Kawasaki, 1997). Before human contribution, mountains and oceans represented impassable barriers for most species keeping ecosystems relatively isolated and allowing only specific movements such as migrations, which happen periodically and between two already colonized areas (Shigesada & Kawasaki, 1997; Lowe et al., 2000). The human urge to satisfy social and physical needs (e.g., new game species) (Carpio et al., 2016) or medical treatments (Maema et al., 2016) and the interest in exotic species (e.g., as pest control or for aesthetic reasons) (Avery & Tillman, 2005) has brought to the increasing trend of voluntary and involuntary transportation of wildlife. This phenomenon has become more consistent in time, mainly due to the extensive global trade and passenger movements (Lowe et al., 2000). The spreading of alien species is facilitated by globalization, as it eases and encourages the international market and the traveling of humans and products, and it is intensifying in recent times thanks to newer and modern technologies (Meyerson & Mooney, 2007).

History is rich with tales of biological invasions, as they represent a worldwide problem that strongly complicates conservation and management strategies and efforts (Vilà et al., 2010; Cameron et al., 2016). Biological invasions are, in fact,a global, pervasive problem, and they challenge conservation of biodiversity and natural resources (Simberloff et al., 2013), affect the genetic diversity of native species, the functionality of the ecosystems, homogenize biotas (Lodge, 1993), and are the second most affecting cause of biodiversity loss all over the globe after direct habitat destruction (McNeely, 2001; Wittenberg & Cock, 2001). The World Conservation Union (IUCN) cited their impacts as 'immense, insidious, and usually irreversible' (Caffrey et al., 2014). At the end of the last century it was observed that in the United States, for example, 49% of imperiled species were at risk due at least partially to the impacts of invasive alien species (Wilcove et al., 1998). Nowadays, only 1% of imported species ends up being a pest, but at the current rate, this is enough to be substantial (Mooney & Cleland, 2001) with cases such as UK and Ireland whose altogether flora is composed of non-native species for more than 20% or the New Zealand where the number of alien plants equals the number of native ones (Mooney & Cleland, 2001). Ecological invasions represent also an economic and social problem being tightly connected to land use, demographics, market, and agriculture (Perrings et al., 2000). To predict the potential impact of an alien species and, thus, to define preventive measures is, unfortunately, easier said than done. It is, in fact, rather challenging to compare and evaluate damages made by different species, and even more by different taxonomic groups (Nentwig et al., 2010).

1.1 Alien species in Europe

Being a longtime center for trade, Europe has been subject to several introductions and has seen the establishment of several thousand non-native species (Keller et al., 2011). These are currently among the most urgent nature conservation issues that the European Union has to face (Scalera, 2010). Most of the terrestrial non-native species were brought to Europe intentionally and for disparate reasons such as pets trade, live food trade, fur and so on. Many of these pathways have been restricted or modified during time to minimize the risk of invasions but others, one above all the pet trade, are still very active (Keller et al., 2011). Many terrestrial species have been, however, also transported unintentionally but on those less information is available because of them not being recorded until their establishment (Keller et al., 2011).

One example of a detrimental species transferred unintentionally, is the case of the black and the Norway rats (*Rattus rattus, Rattus Norvegicus*), brought all around the world, European continent included, from Asia through commercial ships (King, 2019), Rats colonization of Europe during time has been linked to the development of urbanism and trade networks and their arrival is also linked to the devastating effects of the plague pandemics that interested Europe in the last centuries (Yu et al., 2021).

The high urbanization of Europe has facilitated the spreading and the establishment of alien species, which may become invasive more often than other species (Keller et al., 2011). Apart from rats, other examples are the rose-ringed parakeet (*Psittacula krameri*) and the harlequin ladybird (*Harmonia axyridis*) both thriving in human settlements (Keller et al., 2011).

Even though successful eradications have taken place in Europe, and the positive effects have been observed (e.g., the eradication of rats from the islands of the Mediterranean which cause significant ecological impacts and determined the recovery of many colonial nesting seabirds (e.g., *Hydrobates pelagicus* and *Calonectris diomedea*) (Martín et al., 2000), as well as of several terrestrial bird species (e.g., *Prunella modularis, Troglodytes troglodytes, Anthus* *petrosus*) (Kerbiriou & Pascal, 2004), eradication of invasive species is still not often considered as a strategy (Genovesi, 2005). Many reasons are to be found behind this fact, and they may be represented by i) legal obstacles (animals automatically protected by the law even if alien), ii) bureaucratic difficulties (e.g., unclear lines of authority), iii) scarce awareness on the subject (Genovesi, 2005). In addition, large differences are to be found in how the nations deal with the issue of the alien species. In fact, even though the effects of alien species on the environment get everybody to agree, some countries have detailed lists of alien species and well-established protocols for their trade and control, while others have close to-existent information on the issues (Shirley & Kark, 2006). To address this need across the European countries, a new European Union consortium called DAISIE (Delivering Alien Invasive Inventories for Europe; http: //www.europe-aliens.org) was initiated in order to spread awareness on the alien species and spread knowledge on species traits, management and distribution for terrestrial, marine, and freshwater environments (Shirley & Kark, 2006). Today, even though ecological and economic impacts are still documented only for a small percentage of the c. 12.000 alien species brought to Europe, 11% are estimated to be invasive and it is reasonable to believe that the rate of biological invasions will increase soon (Caffrey et al., 2014).

Alien species represent also an important economic cost for Europe, with an estimated expense of about 12 billion euros for the EU (Kettunen et al., 2008), and about 1.7 billion pounds for the UK, and 262 million euros for Ireland (Kelly et al., 2013).

Even though the species is not mentioned in the "EU Regulation 1143/2014 on Invasive Alien Species" (https://ec.europa.eu/environment/nature/invasivealien/index_en.htm), it is mentioned as one of the most detrimental alien species of Europe in scientific papers such as the one written in 2009 by Hulme. In this study, in fact, sika deer is listed as one of the 15 most invasive terrestrial vertebrates in Europe (Table 1), as the only even-toed ungulatre, along with 4 bird species, 1 reptile species, 1 amphibian species, and other 8 mammal species. (Hulme, 2009).

Table 1 List of the 15 most invasive terrestrial vertebrates of Europe (Hulme, 2009)

Most Invasive alien terrestrial vertebrates of Europe

Canada goose	*(Branta canadensis)*
Sika deer	*(Cervus nippon)*
American bullfrog	*(Lithobates catesbeianus)*
American mink	*(Mustela vison)*
Coypu	*(Myocastor coypus)*
Raccoon dog	*(Nyctereutes procyonoides)*
Muskrat	*(Ondatra zibethicus)*
Ruddy duck	*(Oxyura jamaicensis)*
Raccoon	*(Procyon lotor)*
Rose-ringed parakeet	*(Psittacula krameri)*
Brown rat	*(Rattus norvegicus)*
Eastern grey squirrel	*(Sciurus carolinensis)*
Siberian chipmunk	*(Tamia sibiricus)*
African sacred ibis	*(Threskiornis aethiopicus)*
Pond slider	*(Trachemys scripta)*

Source: Hulme, P. E. (Ed.). (2009). Handbook of alien species in Europe (Vol. 569). Dordrecht, The Netherlands: Springer.

2 Sika deer (*Cervus nippon*)

The sika deer (*Cervus nippon*) (Fig. 1) is a midsized cervid, weighing 70–100 kg in males and 40–70 kg in females (Kobayashi & Takatsuki, 2012) with an average difference between male and female body mass of 8,7% (Feldhamer, 1980; Nowak, 1991). According to the subspecies or theenvironment, however, the size may change substantially (Ohdachi et al., 2009).

Kingdom

Animalia

Phylum

Chordata

Class

Mammalia

Order

Artiodactyla

Family

Cervidae

Genus

Cervus

Species

Cervus nippon

Figure 1 Taxonomy of the sika deer (photo from: Jonathan Travel s.r.o.)

Fourteen subspecies have been recognized for the species (Ohdachi et al., 2009) even though other authors (e.g., Shen-Jin et al., 2014) refer in their studies to 13 subspecies.

Fur color in sika deer ranges from chestnut brown to red olive but can show a wide set of variations going from yellow brown to black according to the subspecies (Feldhamer, 1980; Flerov, 1952; Nowak, 1991; Putman, 1988; Whitehead, 1972). The hairs under the chin, the throat, and the belly are lighter (whitegrey) (Feldhamer, 1980; Nowak, 1991). The species also presents characteristic lighter hairs surrounding the metatarsal (Feldhamer, 1980) and a white rump patch (Feldhamer, 1980, Novak, 1991). Two molts occur each year (Ohdachi et al., 2009), with older individuals molting first (Feldhamer, 1980) and the winter coat is thicker and longer (Putman, 1988). During winter also the neck mane, present in both sexes, darkens ((Feldhamer, 1980; Nowak, 1991). Even though a spotted pattern is usually found only in deer fawns, sika deer keep the white spots during their whole lifetime. The reason behind it is probably to be found in the species native habitat represented by broad leaves forest in which the light coming between the leaves creates a dotted pattern (McCullough et al., 2008). A mid-dorsal darker stripe crossing the whole body from rear to head is also a distinctive character of the species (Feldhamer, 1980, Novak, 1991).

Only male sika deer carry the antlers (Novak, 1991) which grow erect, moderately directed rearward (Feldhamer, 1980; Brown, 1983; and Nowak, 1991), rather short (3066 cm), presenting 25 tines (Feldhamer, 1980; Brown, 1983; Putman, 1988; Nowak, 1991), and a brow tine starting about 2,5 cm from the coronet (Feldhamer, 1980; Brown, 1983). A forked or, rarely, palmate tine tops the antler and faces onward (Brown, 1983 & Feldhamer, 1980). The sika deer antlers are well appreciated in traditional medicines, such as the Chinese one, and today are also well considered as a preciousmodel for the study of the mechanisms of organ regeneration and rapid tissue growth, as well as of bone growth and mineralization in mammals (Zhao et al., 2013).

Sika deer is a polygamous species and the rut occurs, generally, in autumn (Ohdachi et al., 2009). In early Autumn the sika males started to show aggressive behaviors that are triggered by the testosterone and allow the males to compete for the females (Yamauchi et al., 1997). It has been observed that the species shows an uncommon variability when it comes to mating strategy. From the direct defense of the harem by the males as observed in New Zealand and England, to the lek territoriality as observed in the Czech Republic, to the creation of resource-based territories as observed in Russia (Bartoš et al., 1998). During deer reproductive behaviors, vocalizations have a great deal of importance and have several functions such as displaying social dominance (Vannoni & McElligott, 2008), mediation of intraspecific competition (Clutton-Brock & Albon, 1979; Reby et al., 2005), intersexual attraction (McElligott et al., 1999; Charlton et al., 2007a; Charlton et al., 2007b; Reby et al., 2010), location notification (Miura, 1984), and promotion of female estrus (McComb, 1987). During the rut sika deer moan and howl (Minami & Kawamichi, 1992; Reby & McComb, 2003; Yen et al., 2013).

Habitat-wise, the species lives in forests, where they can hide from both predators and adverse climate conditions, just as well as in other habitats represented by different stages of vegetation and disturb where the quality of food is higher (McCullough et al., 2008). It is also able to adapt easily (Ohdachi et al., 2009) and has a long history of, both positive and negative, interactions with humans mostly due to the preferences for agricultural and developmental lands, and to the big amount of damages it can cause to crops (McCullough et al., 2008). Sika are to be found at a variety of elevations, from sea level to about 3,000 m a.s.l (Ohdachi et al., 2009).

Their diet can be extremely varied and include foods such as marsh grasses, fallen leaves, trees, brushy vegetation, herbs, fungi, and more, depending on environmental conditions (Feldhamer 1980). Studies conducted in Japan showed that sika deer relies on some preferred species (one above all the *Sasa nipponica*) (Endo et al., 2017) but, when these are in short supply, they may easily fall back on non-preferred plants, and litterfalls (Nakahama et al., 2021). This makes the sika deer able to thrive also in environments with a highly degraded understory (Nakahama et al., 2021).

Moreover, sika have been observed feeding on the regeneration of pioneer species, severely inhibiting the regeneration of forests (Shimoda et al., 1994). Additionally, the species show a high level of feeding adaptability, and can act as both grazers and browsers (Feldhamer, 1980; Nowak, 1991). For this reason, sika deer are often the cause of severe damages to vegetation through overgrazing (Nakahara et al., 2015) and debarking activity (Akashi & Nakashizuka, 1999; Akashi & Terazawa, 2005) but also due through antlers fraying (Akashi & Terazawa, 2005) (Fig. 2). In addition, hybridization with the red deer occurs, being the two species widely overlapping in Europe (Fig.3), and it was often observed in deer park, but is has also been reported from feral and wild stocks (Lowe & Gardiner, 1975; Goodman et al.,1999; Senn et al., 2010).

Sika deer is a native member of the Asian fauna and during the centuries it has been widely introduced into many other countries around the world (Abe, 2005; McCullough et al., 2008).

Figure 2 Trees damaged by sika deer in the UK
(Image courtesy of Stuart Morrison: https://www.mosswoodland.com)

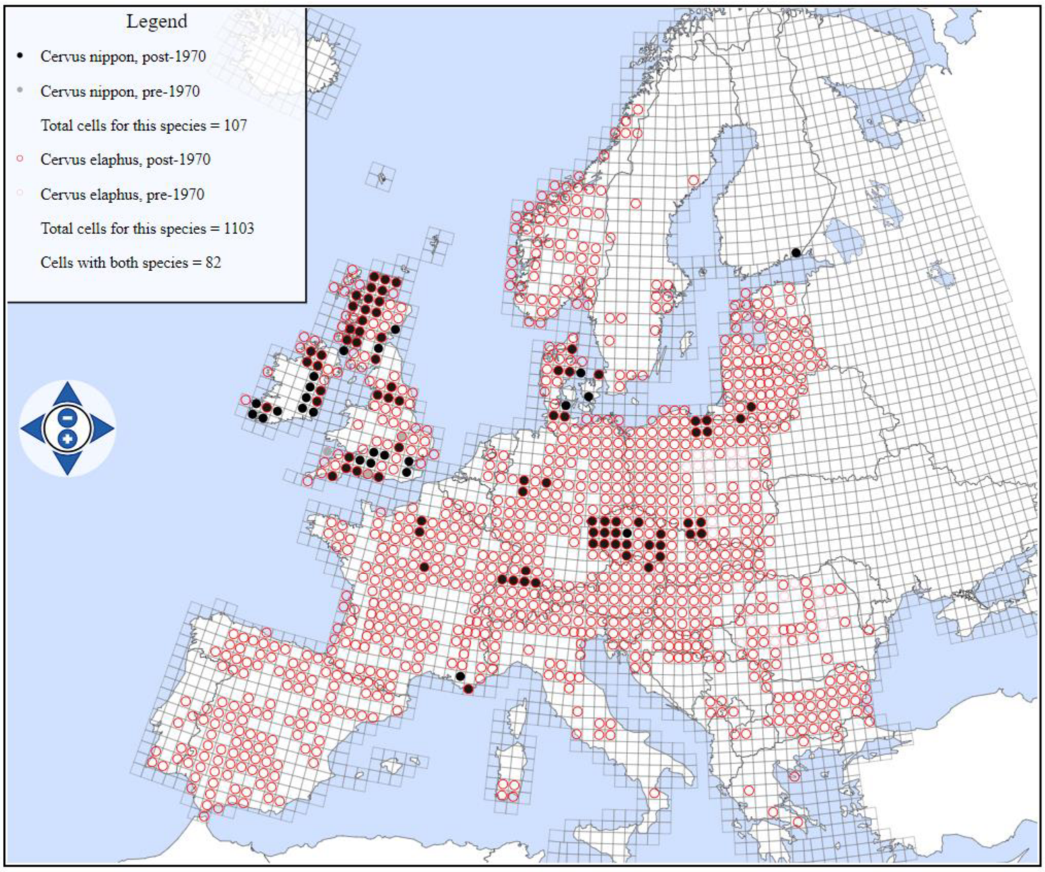


Figure 3 European range of sika deer (black dots) and red deer (red dots) (source: https://www.european-mammals.org)

2.1 Sika deer in native and non-native ranges

2.1.1 Sika deer in the Native Range

This species was originally widespread throughout northeastern Asia, from the Ussuri area (Russian) to northern Vietnam and Taiwan (Ohdachi et al., 2009).

Sika deer in Japan Six of the fourteen subspecies of sika deer are spread across Japan covering about 40% of the country area (Ohdachi et al., 2009). Up to 73% of the distribution has beenobserved to be colonized in the last few decades and, in some areas, density rose to be 20foldhigher in less than 15 years. Several areas that showed no deer presence in 1979, were discovered hosting populations of sika in 2002, and the species numbers are currently still increasing (Ohdachi et al., 2009; Takatsuki, 2009). While many species in the country started to decrease in numbers due to habitat fragmentation and conflict with human activities, sika deer populations, following a severe decline in some areas of Japan at the end of the XIX century (Nagata et al., 1998; Kaji et al., 2010), rose exponentially (Nagata et al., 1998). The reasons behind this growth are still unclear, but bans or restrictions on hunting activities and the establishment of reserves appear as plausible causes (Takatsuki, 1992; Takatsuki & Gorai, 1994). Another possible explanation is represented by the absenceof natural predators, being the Japanese wolf extinct since 1905, and the brown bear, present on the Hokkaido island, feeding mostly on winterkill deer carcasses (Kaji et al., 2004; Takii et al., 2012). In addition, global warming is leading to a decline in the number and intensity of snow events and, therefore, in a reduction of fawns' mortality in recent years. Last, the decline in the number of Japanese hunters together with their poor recruitment rate, human dynamics such as aging and relocation away from the countryside and mountainous areas seem to be beneficial to wildlife including sika deer (Takatsuki, 2009). Japanese sika appear to prefer edges of forests and areas with the presence of understory, nonetheless are easily adaptable to a variety of other habitats (Ohdachi et al., 2009). The species can be found at different elevations according to the season (Maruyama et al., 1976) and from sea level up to 3000 m a.s.l. (Ohdachi, 2009), even though they tend to avoid deep snow (Honda, 2009). All around Japan, sika are involved in traffic incidents (Nagata, 1998) and damages to forests and agricultural lands (Nagata, 1998; Tsujino & Yumoto, 2004). On the Hokkaido island, where sika impacts are rather severe (Akashi et al., 2015), in 1998 a population control management plan was required and put in motion by the Hokkaido government (Uno et al., 2009). Recently, sika caused severe damages also in Western Japan, on Mount Ōdaigahara, and similarly in Central Japan, where, mostly through debarking activity on numerous tree species, they impacted the entire forest dynamic and related economic activities. (Akashi & Nakashizuka, 1999; Sano, 2017). Even though the impact of the Japanese sika deer on vegetation and ecosystems is well documented in the Japanese literature, it is unfortunately not well acknowledged in other countries due to the high amount of reports written in Japanese and to the difficulties in accessing journals and papers (Takatsuki, 2009).

Sika deer in China Once widely distributed in China, the original Pleistocenic range along with Taiwan, sika deer are nowadays a threatened and protected species throughout the country, listed as Category I State Key Protected Wild Animal Species in China. (Wu et al., 2004; McCullough et al., 2008; Li et al., 2019). Starting from the XX century, intense hunting activity and habitat loss caused a severe decline and extinction of most of the sika deer populations across its historical range (Zhang et al., 2018), and the species is now to be found in the wild only in small isolated patches (McCullough et al., 2008). Five subspecies were originally found in the country, but nowadays only 3 are left (McCullough et al., 2008; Li et al., 2019). A reason behind the exploitation of sika by humans is to be found in the traditional Chinese medicine that largely uses sika blood, antlers, and muscles to cure a wide range of diseases (Wu et al., 2004; Wu et al., 2013). Starting from the '70s, to save wild populations of sika, 17 reserves have been established throughout the country (McCullough et al., 2008). Contrarily to Japanese populations, "sika deer in China did not escape the prevalent tragedy of domestication" (Jiang et al., 2016) and the management plans regarding the species have focused mostly on zoos and wildlife parks hosting captive animals (Shen-Jin et al., 2014). Due to the restriction on commercial hunting of wild sika deer decided by China Veterinary Associations, sika deer husbandry rose in the last few years and now China is the country harboring, in the province of Jilin, the largest sika deer farming area of the world (Wu et al., 2013). As stated by McCullogh et al.,in 2009, the number of wild sika deer was approximately 8500 individuals, while the numberof captivated ones increased up to 290.000 (McCullough et al., 2008).

Sika deer in Taiwan Sika deer spread to Taiwan from the mainland of Southeastern Asia (McCullough et al., 2008). According to the IUCN Red List report (Harris, 2015). Free-ranging populations of sika deer (subspecies *C. n. taiouanus*) were extirpated in 1969 but, starting from captive individuals (Smith & Xie, 2008), the species has been subsequently successfully reintroduced to Kenting National Park in 1988 (Green, 1989). Currently, the subpopulation of sika deer residing in Taiwan still endures.

Sika deer in Viet Nam Historically, the free-ranging population of sika deer (subspecies *C. n. pseudaxis*) in Viet Nam has been recorded in the Northern provinces (Huynh et al., 1990, as appears in Harris, 2015), with sporadic observations also in the Western Nghe Tinh Mountains (Huynh et al., 1990). Nowadays, the species is extinct in the wild (McCullogh et al., 2009) and its presence in the country is mostly due to the farming process. Captive populations are present in two national parks (Huynh et al., 1990) and their number seems to be increasing. Nevertheless, due to the poverty in the country, the possibilityof release of farmed in the wild is equal to zero, as they would easily be poached. As before, due to the paucity of information is complicated to assess the number of individuals with good approximation (McCullough et al., 2008).

Sika deer in Korea According to the IUCN report (Harris, 2015), the wild population of sika deer in Korea appears to be seriously threatened and in danger of extirpation. Considered extinct in North Korea (McCullogh et al., 2009), the species was spread across the whole country and reportedly abundant in North and Central Korea, but its numbers declined dramatically during the Japanese occupation of the country (1910-1945). Following the unsuccessful efforts to reconstitute viable populations from the surviving, dispersed individuals in the NorthWesternProvince, the government of North Korea started a captive breeding program (Won, 1968). Sika (subspecies *C. n. hortulorum*) appears to be either very rare (mainly restricted to one province) or extinct as a wild animal also in South Korea (Won & Smith, 1999) and on Cheju Island. Its survival in the extreme northeast of the country is also doubtful (Won & Smith, 1999). However, it is possible that some movements still occur between Russia and China (McCullogh et al., 2009). Unfortunately, the lack of up-to-date information and reports about the country represents a significant problem in theassessment of the population numbers of this species.

Sika deer in Russia Sika was widespread in far-east Russia, in different types of habitat: coastal environments, in lands, different kinds of forests. In the late nineteenth century, severe overhunting decimated its populations and the species was translocated to other countries and to reserves in European Russia where it adapted quickly to the severe winter conditions (McCullough et al., 2008). About 35 years ago the recovery started and sika are now returning to the original range plus other areas in suitable habitats (McCullough et al., 2008). Sika deer have been farmed in Russia and used for traditional and official medical purposes for centuries (Prokopov et al., 2019)

2.1.2 Sika deer in the Non-native Range

Sika deer have been introduced in many countries across the world and in several of those have established alien populations (Table 2).

Table 2 List of the countries where the sika has been introduced, with the date of the first introduction of the species, the current situation, and relative references.

Country	Date of First Introduction	Current Situation	Bibliography
	EUROPE		
Czech Republic	1891	Most abundant and expanding sika population in continental Europe	Kokeš (1970); Krojerová-Prokešová et al. (2017)
Austria	1907	Two feral populations mainly restricted to lowlands of Lower Austria	Bartoš (2009); Eick et al.(1995(c)); Winter et al. (2018)
Germany	1893	Seven well differentiated population living in the country	Niethammer (1963); Bartoš (2009), based on Eick et al. (1995(a))
France	1890	Found in enclosures for meat. Few populations living in the wild meant to be eradicated	Sand & Klein (1995) ; McCullough et al. (2008); SaintAndrieux et al. (2014).
Switzerland	Unknown. First record 1917	Small, declining population in the wild, very few individuals in farms.	Cailmail (1988) and McCullough et al. (2008); Apollonio et al. (2010)
Denmark	Between 1900 and 1909	Free ranging population	Bartoš (2009); Nielsen et al. (2000); Christian et al. (1960)
UK	1860	Spread across the whole territory, source of severe damages to local fauna and flora	Pérez-Espona et al. (2009); Mann & Putman (1989) ; Uzal Fernandez (2010) ; Mathews et al. (2018).
Ireland	1860	Sika deer represent 50% of all deer present in the country	Diaz et al. (2005); Annett (2015)
Poland	1895	Free ranging population, and increasing farming activity	Matuszewski (1988); Solarz (2008)
Ukraine	Unknown. Estimated 1977	Recent information not available	Dvojnos & Pogrebniak (1977)
Italy	Unknown	Small non-reproductive groups in the Northern part of the country	Presence described by Smith et al. (2018)
Lithuania	1954	Not to be found in the wild, broadly farmed	Pūraitė & Paulauskas (2016); Baleišis et al. (2003)
Armenia	1969	Recent information not available	Khorozyan (1996)
Azerbaijan	No data	IUCN lists Azerbaijan in the non-native range of sika deer but no data are available in literature	IUCN
Finland	No data	IUCN lists Finland in the non-native range of sika deer but no data are available in literature	IUCN
Belgium	Unknown	Spotted but not established	Bartoš (2009)
Netherland	Unknown	Spotted but not established	Bartoš (2009)

	NORTH AMERICA		
Maryland	1916	Biggest population in the USA- Threat for local species	Kalb & Bowman (2017); Feldhamer et al. (1978) ; McCullough et al. (2008)
Texas	Estimated during the ‘30s	Biggest population in the USA- Threat for local species	Traweek & Welch (1992)
	NEW ZEALAND		
New Zealand	1905	Numerous populations. Source of damages	Husheer et al. (2006); Banwell (2009) ; Forsyth et al. (2013); Latham et al. (2015)
	AFRICA		
South Africa	1897	Alien species with special regulations.	Long (2003); Invasives.org.za
Morocco	1951-1953	Non present	Dorst & Giban (1954)
Madagascar	No data	Non present	Whitehead, (1972) ; Grzimek, (1972)
	UNCERTAIN DISTRIBUTIONS		
Papua New Guinea	Probably introduced	Non present	Bentley & Downes (1968)
Philippines	No specific data	Subspecies living in the country: *Cervus nippon soloensis*	Groves & Grubb (1987) ; Wilson & Reeder (1993) ; Long (2003)

Sika deer in The USA Different subspecies of sika deer were introduced in the USA at different times (Feldhamer & Demarais, 2009; Kalb & Bowman, 2017) and it is now considered among the most important domesticated ruminants in all North America (Zhao et al., 2017). In the USA, the management of sika is supported by the requirement of permits, released by local parks and wildlife authorities, for legal hunting activities. This ensures both population control and the complying with laws (Texas Invasive Species Institute). Today, the biggest populations of free-ranging sika deer are located in Maryland and Texas (Feldhamer et al., 1978; McCullough et al., 2008), while smaller populations can be found in other states. Since the spread of exotic species is strongly perceived as a major problem, transportation of sika across states is strictly restricted (McCullough et al., 2008). The introduction of sika deer in the USA has been indeed a serious threat to the white-tailed deer populations, sharing the same habitat and having a big dietary overlapping, which forces a shift in the niche choice by the white-tailed deer that is compelled to consume lower quality food and not accessing supplemental feeding sites (McCullough et al., 2008; Kalb et al., 2018). The first introduction to North America occurred in Maryland in 1916, when five individuals originally from England, previously gifted as pets to a private owner, were released into the wild (Kalb & Bowman, 2017). Since then, the specieshas increased in number and occupied range, across the Delmarva peninsula (Kalb et al., 2018). It is estimated that, in just about 100 years, the sika population of Maryland spread up to 12,000 individuals (Kalb & Bowman, 2017). Today, sika is considered a common game species in the State, and the hunting season 2018/2019 report counted 3,434 sika deer culled (Maryland Annual Deer Report, 2018/2019). Such numbers, also considering the potential impacts of this exotic species, highlight the need for comprehensive management plans (Kalb & Bowman, 2017). As regards Texas, a specific date referring to sika introduction is not available in the literature, but it is reported that the introduction of exotic species in the country began during the '30s, and during the 60' sika were already present in the area (Traweek & Welch, 1992). Data on population size are available for the years 1974 (3,042 animals) (Butts, 1979), 1988 (11,879 animals distributed in 77 counties and 207 ranches) (Traweek & Welch, 1992), and 1992 (over 12,000 animals) (Nelle, 1992). Moreover, in Texas, about 97% of land is private and has developed one of the most efficient systems of pay per hunt and lease (Mozumder et al., 2007).

Sika deer in New Zealand Sika deer were introduced in New Zealand in 1905 (Husheer et al., 2006) and are still present in the country. Sika are, in fact, the second most widespread deer species in North Island, occupying forest and shrub along with a single continuous range in the center of the island (Banwell, 2009; Forsyth et al., 2013). As for other parts of the country, the species spread increasing their number up to high densities and becoming an important big game species for recreational hunters in New Zealand. Sika also have unwanted impacts on local biodiversity, both flora and fauna (Latham et al., 2015), and a play a key role in modifying habitats (Fraser, 1996). Sika deer outcompete red deer and negatively affect seed spreading and seedling regeneration (Husheer et al., 2006; Allen et al., 2015; Latham et al., 2015).

Sika deer in Africa Sika deer have been introduced in 1897 by Cecil Rhodes in his estate in Cape Colony (in present-day South Africa). The species was also introduced in other areas of Africa (e.g., Marocco), but are now present only in the South Africa state (Long, 2003). The species is nowadays considered as Category 2 by the National Environmental Management Biodiversity Act (Tsamaio & Bosetšaba, 2015) and it is so described as an "invasive species, o species deemed to be potentially invasive, in which a permit is required to carry out a restricted activity" (Data from the Department of Environmental Affairs – Republic of South Africa). The main environmental issues are the damages that the local vegetation suffers because of ringbarking activity, the browsing, the antler rubbing, and the trampling. Sika deer have changed the floral and faunal composition of grasslands and wetlands (Invasive animals sika deer (*Cervus nippon*) available at Invasives.org.za. Invasive Species of South Africa).
Sika deer were successfully introduced into the forests of Madagascar (Whitehead, 1972; Grzimek, 1972) along with Fallow deer. The latter along with Timor deer are the only two deer species currently present on the island.
There are few reports of attempts to introduce sika deer in Morocco (Lever, 1985), between 1951 and 1953. The Moroccan introduction took place in three different locations: the forest of Mamora, between 1952 and 1953, Benslimane, and the remoted and reforested mountain area close to the city of Taza (Dorst & Giban, 1954). Nowadays, even though the introduction was successful, no sika population is established there since, within 15 years,all the introduced animals were culled (Benjelloun, 1983; Loggers et al., 1991).

Sika deer in uncertain distributions Sika deer were probably introduced into Papua New Guinea with other deer, but they are not known to be established (Bentley & Downes, 1968) and there have been no further records of them (Long, 2003).
It is also believed that sika deer have been introduced in the south of the Philippines, on Jolo Island, in the past. The subspecies living in the area is known as *Cervus nippon soloensis*. (Groves & Grubb, 1987; Wilson & Reeder 1993; Long, 2003).

Sika deer in Europe In Europe, sika deer were introduced starting around 150 years ago, mainly for aesthetic reasons, in addition to the native fauna and flora. The introductions took place in most of the countries in Western, Central, and Eastern Europe, and some individuals managed to escape from deer parks and collections spreading through the wildlands (Bartoš, 2009; Ratcliffe, 1987). In many European countries, sika deer populations are small and with a rather local distribution. However, this is not the case for Great Britain, Ireland, Germany, and the Czech Republic, where the populations are big and the species is widely spread (Biedrzycka et al., 2012).

Sika deer in Germany. Sika deer were introduced to an enclosed area of Germany in 1836 (Pitra & Lutz, 2005), with the first release in the wild dating back to 1936, when the fence was broken by the snow. After that, several introductions have happened throughout the years (Eick, 1995(a)). Currently, there are seven well differentiated populations of sika deer recognized in Germany (Bartoš, 2009, based on Eick et al., 1995(a)). Even though most of the introductions have been carried out using animals acquired from some private facilities, some of the first introductions were most likely conducted using Dybowski sika deer from Manchuria and from Japan (Pitra et al., 2005). For the rest of the populations it is not possible to track down the origin of the founders.

Sika deer in France. Sika deer arrived in France in 1890 as a gift from the Emperor of Japan (McCullough et al., 2008). Being the given population composed of three hinds and one stag, the deer bred in the enclosure of Rambouillet and reached a total of about 200 individuals (McCullough et al., 2008; Saint-Andrieux et al., 2006). A second location, Forêt de la Harth, hosted sika coming from a zoo and the Huttenaim enclosures and there the animals prospered to the number of about 250 individuals. To reduce the number of animals, about 70 sika were kept while the surplus ones were used to build new herds in other countries, including Switzerland (McCullough et al., 2008; Cailmail, 1988). Today, in France, only a few sika populations are reported to live in the wild (Sand & Klein, 1995; Saint-Andrieux et al., 2014). A part of these populations are not stationary and move across different districts. In addition, populations may appear or disappear in response to accidents, introductions or eradication efforts, and different local management plans (SaintAndrieux et al., 2014). French sika are often to be found in enclosures due to the production of meat and for game purposes. The high risk of escapes due to damages to the fences, human error, and abandonment, along with the ability of wild sika to hide in forest areas and elude hunters, makes its management difficult. National hunting reports (Office National De La Chasse Et De La Faune Sauvage "Lettre Ongulés Sauvages") show, in fact, a progression in number of wild sika deer across the country in the last 20 years. In addition, sika are known to be a possible threat to the native red deer (*Cervus elaphus*) through hybridization, already rarely occurring in France, aa carrier of diseases to other ungulates (Ferté et al., 2000), and as source of damages for vegetation. French management's final goal is the eradication of the species (Saint-Andrieux et al., 2014).

Sika deer in Switzerland. The population of sika deer in Switzerland has been estimated in 2002 to be about 500 individuals but has since then diminished (Apollonio et al., 2010). The source of this population is not univocal but happened through time and with different sources. Some animals were transferred from France (Cailmail, 1988; McCullough et al., 2008), others moved from Germany. The first German sika was recorded in 1917, but the population became to increase only in 1941 when more animals were released close to the Swiss border (Apollonio et al., 2010). The population is strictly under regulation and the sika have no opportunity to spread (Apollonio et al., 2010). A very small percentage (4%) of farmed deer in Switzerland is represented by sika deer (Wyss et al., 2000).

Sika deer in Denmark. Sika deer are present in Denmark in free living populations (Nielsen et al., 2000). They were introduced between 1900 and 1909 with a few individuals coming from Ireland and Germany. A third subpopulation, situated in the center of the country, has an uncertain history but is knownto be the first Danish free-ranging population (Bartoš, 2009). Sika deer is considered a problematic addition to the Danish fauna due to the debarking activity and the habit to feed on young trees (Christian et al., 1960).

Sika deer in the British Islands. Sika deer were introduced in the British Islands starting in 1860 (Lowe & Gardiner, 1975). In the UK two individuals of sika deer were given to the Zoological Society of London (Perez-Espona et al., 2009) and in Ireland, in the same year, a stag and three hinds were added in a mixed deer park in the CentralEast part of the country (Enniskerry) (Diaz et al., 2005). In the following decades, until 1930, due to the aesthetic value of this exotic deer, many other introductions occurred. Few from Asian populations, and others from uncertain sources (Perez-Espona et al., 2009). It appears clear that some of these new introductions involved animals already bred in proximity to red deer and, so, already hybridized. This still makes taxonomic analyses and recognitions difficult (Perez-Espona et al., 2009). Nowadays feral sika populations are to be found in many parts of England, Scotland, and Eire (Lowe & Gardiner, 1975; Perez-Espona et al., 2009) and, even if usually found in woodlands and heathlands, sika have now broad ranges that include various types of habitats (Mann & Putman, 1989; Uzal, 2010; Mathews et al., 2018). In both Great Britain (Diaz et al., 2005) and Northern Ireland, hybridization with red deer has been observed. This issue concerns not only the genetic integrity of the native species, but also its economic value,since sika have a smaller trophy, and so the fertile offspring (Perez-Espona et al., 2009). Apart from hybridization, which represents a great management concern, sika have other impacts on the British and Irish environment; they have become a source of evident ecological and economic impacts. Feral sika populations in Britain have the potential to cause significant damage to forestry, crops, and to the conservation of habitats (Horwood & Masters, 1981; Chadwick et al., 1996; Abernethy, 1998; Putman & Moore, 1998; Putman, 2000; Kelly, 2002; Diaz et al., 2005; Diaz et al., 2006; Hannaford et al., 2006). In England for example, sika have been proved to have a strong negative impact on the saltmarsh flora. Grazing activity, where severe, indeed leads to the almost complete loss of the different types of vegetation cover closeto the shore, and to the development of hypersaline conditions and other cascading effects (Diaz et al., 2005). In Ireland, sika cause severe levels of damage to local vegetation due to its browsing activity, bole scoring, trampling, and bark stripping. Sika can also destroy, being also used to feed on them, crops such as kale, rape, carrot, turnip, and cereals (Purser et al., 2010). The British population has been estimated to be about 103.000 animals (Mathews et al., 2018), and in Ireland sika represent the most numerous deer species in the country, accounting for almost 50% of the total deer population (Annett, 2015).

Sika deer in Poland. Seven sika are believed to arrive in Southern Poland around 1895 from Japan and then, other seven, in Northern Poland in 1910 (Matuszewski, 1988) or, according to other sources, in 1905 from the UK (Matuszewski & Sumiński, 1984; Eick, 1995(b)). The northern population reached, during the time, high numbers and it was estimated, during the ‘60s, to about 250 individuals that spread east and south, reaching the number of 500 animals. The southern population, on the other hand, never reached number higher than 20 individuals. Both populations were first kept enclosed and then released (Matuszewski, 1988). In recent times, the farming of sika deer has become popular across the nation (Solarz, 2008).

Sika deer in Ukraine. According to Dvojnos & Pogrebniak (1977), sika deer were brought to Ukraine from the Far East. The discovery and the registration, in 1977, of the nematode *Ashworthius sidemi* was registered for the first time, and its arrival in the country was, indeed, attributed to the presence of the deer just as in the Czech Republic and France (Ferté & Durette-Deset, 1989; Kotrla & Kotrly, 1973, 1977; Dvojnos & Pogrebniak, 1977; Ovcharenko, 1968). Not much information is available about the Sika deer in Ukraine but it is known that in 1984, sika was present in the country with a population of about 2500 individuals (Khorozyan, 1996).

Sika deer in Italy. In Italy, the sika deer is present in small non-reproductive groups in the Northern part of the country. This small population is a result of individuals escaped or released (Smith et al., 2018).

Sika deer in Lithuania. A herd of 6 male and 18 female sika deer was introduced in Lithuania, from an area in the South of Russia, in 1954 (Pūraitė & Algimantas, 2016). Even though sika were found in different regions and forests, since 1992 animals have not been spotted nor recorded in the wild (Baleišis et al., 2003). There is not clear evidence on why the species disappeared and, based on genetic analysis, one possible reason is the intercrossing with the red deer (Pūraitė & Algimantas, 2016). On the other hand, since 1988, sika are farmed in the country and in about 10 years the number of enclosed sika reached the number of about 750 individuals. The latest information on the number of enclosed sika deer in the country, available from the Lithuanian Ministry of Environment, refers to approximately 2000 animals (Pūraitė & Algimantas, 2016).

Sika deer in Armenia. First data of sika deer in the Armenian Dilijan Reserve refers to a group of 52 deer brought inthe country from Russia in 1969, and of another group of 50 deer brought from the same place in 1970. Two years later, a stag was moved into the reserve from a region in the northern part of the country, and 28 sika arrived, again, from Russia in 1976. Between 1969 and 1981 the population rose from 52 to 230 animals and then slowly kept raising to 292 animals in 1995 (Khorozyan, 1996). In a paper from 1996, Khorozyan discusses how the no longer active status of protected area for the Dilijan Reserve with the following depletion of the forest ecosystem, together with an inadequate culling and exploitation system, put the species in need of an accurate management plan. No following info about the Dilijan Reserve, or other Armenian populations is available.

Sika deer in Finland and Azerbaijan. Even though IUCN includes Finland and Azerbaijan in the not native, resident, range of sika deer, no paper, report, or documents on the local population were found. Finnish hunting services website, however, includes sika deer in the available game species while Azerbaijan do not.

Sika deer in Belgium and Netherland. Even though few sika deer have been spotted in Belgian and Dutch territories, those observations are not enough to consider the sika deer as established in the country (Bartoš, 2009). The risk of assessment does exist and the most likely way for animals to enter the countries and spread, are from feral German populations, or by the escape or release of animals already in the countries, from hobby parks, zoos, or deer farms

(Lammertsma et al., 2012).

Sika deer in the Czech Republic and Austria: the areas of study

Sika deer in the Czech Republic. In 1891, sika deer were first released into the Kluk enclosure near Poděbrady (Kokeš, 1970). The importation for deer parks started indeed around the turn of the nineteenth and twentieth centuries, reportedly from countries such as Japan, eastern China, Korea, and Russia, and later on also from estates in England and Austria (Kokeš, 1970). Maintained in enclosures, sika prospered until the 1940s when, due to the political changes that followed World War II, some deer parks were destroyed allowing sika to escape (Vavrunek & Wolf, 1977). Genetic analyses have recently confirmed the Japanese origin of the sika population in the Czech Republic (Barančeková et al., 2012) where, thanks to the rapid population growth, it successfully expanded within the country (Krojerová-Prokešová et al., 2017). The Czech Republic is currently hosting the most abundant and expanding sika population in continental Europe, whose size has exponentially increased during the last several decades (Krojerová-Prokešová et al., 2017). Nowadays, sika are largely hunted in the country, and the culling of the rutting male deer has deep cultural roots in Central Europe and is a part of the hunting tradition. As a result, the largest numbers of reproductively active deer are shot in October, at the peak of the rut (Balmford et al., 1993; Macháček et al., 2014). In addition to bearing traditional values, game hunting is also used as a management technique to control population size (Hanzal et al., 2017). The hunting pressure has been recorded as the driving force of behavioral changes and, in some areas of the Czech Republic, sika are now strictly nocturnal because of this and other different human activities (e.g., tourism, mushrooms picking) (Bartoš, 1998). Whether hunting is a sufficient strategy of control for larger ungulates in the Northern hemisphere is still debated (Hagen et al., 2018) up to 64% of countries uses data such as the hunting bags as a reliable monitoring methods (Neumann et al., 2020). In the Czech Republic hunt control records are known since half of the 9th century, and hunting statistics are kept since 1966 (Šeplavy et al., 2015). Despite these measures, errors and inaccuracy are common when it comes to hunting plans for Czech large herbivores (Phlal & Kamler, 2013; Dvořak & Palyzová, 2016) due to miscalculations and unfitting methods (Phlal & Kamler, 2013). Real population numbers are probably higher than those estimated and reported each year in hunting statistics. The quick expansion of the Czech population of sika is causing severe problems due to the intense overgrazing, overbrowsing and hybridization with red deer (Krojerová -Prokešová et al., 2017)

Sika deer in Austria. The first record of the occurrence of sika deer in Austria dates back to 1907 (Bartoš, 2009) when it was brought as a gift from the Japanese emperor (Pitra et al., 2005). After being kept in parks, the animals either escaped or were deliberately released, establishing free-living populations in the wild (Pitra et al., 2005). Considered a valuable game species, the sika deer have been successively introduced an unknown number of times for both breeding and hunting purposes. The history of the introductions is, unfortunately, not well documented and only a scarce portion of the introduced individuals came from the native areal (Pitra et al., 2005). As to the last surveys, there seem to be two feral populations of this species (Eick et al., 1995(c)), with a distribution mainly restricted to lowlands of Lower Austria (Winter et al., 2018). The assessment of the geographical origin of the individuals currently living in Austria is a tricky task, as the populations show considerable variability in morphological traits (Pitra et al., 2005). Even though there are several sets of evidence proving the Japanese origin of the founder individuals (e.g., historical sources referring to the Japanese origin of the imported individuals (Eick, 1990 (a),(b) as appears in Pitra et al., 2005), the fact that the most spread phenotype in Europe resembles the smaller sized subspecies of deer from Japan (Eick, 1990 (a),(b) as appears in Pitra et al., 2005) and, last, the presence in the Austrian deer of a parasite which occurs in Japanese sika deer (Rehbein et al., 2003) a more specific determination of the geographical origin and successive translational events appear to be extremely complicated, if not impossible at all. However, Pitra & Lutz's (2005) recent work to infer the origin of the introduced individuals in Austria started from the presumption that, even though several source populations were used for the introductions, the present populations belong to two genetically distinct maternal lineages, native of two differentgeographical areas.

The sika deer is fully recognized as a severely harmful species in both its native and non-native range (Genovesi et al., 2009; Takatsuki, 2009; Uno et al., 2009). It has been observed having negative effects on citizens wellbeing, through the involvement in traffic incidents (Bruinderink & Hazebroek, 1996), and though the damages to crops (Honda et al., 2010), as well as on ecosystems, flora, and fauna. One of the main issues linked to the presence of sika deer in the European hosting countries, is the hybridization with the native red deer, which has great consequences on its genetic structure and conservation (Perez-Espona et al., 2009; Krojerová-Prokešová et al., 2017). Additionally, the species has been the source of great damages to European ungulates through the introduction and the spread of the abomasal nematode *Ashwortius sidemi*, which is now found in several other species (e.g., red deer, moose, roe deer) (Kornacka et al., 2020). Due to sika deer highly flexible and varied diet, the species is also able to outcompete overlapping deer. In Europe and in the USA, roe deer (Husheer et al., 2006; Allen et al., 2015; Latham et al., 2015) and white-tailed deer suffer from the presence of sika deer and forced to switch to lower quality food and, often, to also avoid supplementary feeding sites (McCullough et al., 2009; Kalb et al., 2018). Sika deer feeding habits are also source of great damages to vegetation and plant composition. Overbrowsing (Ninomiya, 2003) and overgrazing on vegetation (Nakahara et al., 2015), as well as ringbarking activity (Hino, 2000), have severe consequences on local flora and relative biodiversity. Studies performed in Japanese forests with degraded understories due to the overgrazing process, presented results showing that sika presence increased the soil nitrate output, and led to the eutrophication of aquatic ecosystems (Tsuboike et al., 2021). Sika deer also effects the seedling due to the composition of their pellets which prevents the seeds to germinate (Kanda et al., 2005).

The knowledge on sika deer diet and their pellets composition may represent a key component in the understanding of its role in the hosting ecosystems.

3 *Cervids diet, and methods of analysis*

The knowledge of a species' diet is a fundamental step in the understanding of its role in the ecological community (Bradley et al., 2007). The relationship between herbivores and plants is rather complex because of the animals not being dependent on specific species, and of the responses of vegetation to herbivory being multifold (Howe, 1988). Large herbivores are keystone species in many ecosystems and have, on them, significant impacts (Graham et al., 2010; Czernik et al., 2013). The effects of their feeding, in fact, impact plant communities and productivity, nutrients flow (Danell et al., 1994), and forest regeneration. Consequently, they affect diversity and abundance of other species through their grazing (Trdan & Vidrih, 2007), browsing (van Beeck Calkoen et al., 2019; Parker et al., 2020), and bark stripping (Klich, 2017; Cukor et al., 2019; Krisans et al., 2020).

In the European forests, cervids are very common biotic agents of disturbance (Vasiliauskas et al., 1996) and, in the whole Northern hemisphere, deer populations have experienced a spatial expansion and an increase in numbers in the last decades (Kuiters et al., 1996; Gordon et al., 2004; San Miguel-Ayanz et al., 2010). European populations have increased to the point that many species have become locally overabundant (Valente et al., 2020).

Cervids are ruminants and benefit from a mutualistic interaction with microorganisms and bacteria which, living in their foregut, convert plant cellulose to energy (Hanley, 1997); this system allows them to extract nutrients from a wide variety of vegetation (Shipley, 1999). Among different species of cervids, there are differences in their feeding preferences and they can be classified along a continuum, that goes from grazers to intermediate feeder to browsers (Hofman, 1989; Bodmer, 1990), according to which plant type they prefer from grasses (monocots), or forbs and browse (dicots) which differ in both structure, and chemistry (Shipley, 1999). Feeding damages caused to the ecosystems by deer may range from; a) overbrowsing which may alter the structure of temperate forests by decreasing preferred plants for deer and inhibiting the regeneration of trees, promoting the dominance of those plants which are not preferred by the deer (Nakahama et al., 2021), b) overgrazing that, when sever, may include erosion and subsequent removal of nutrients sometimes causing the ecosystem to reach a stable state with lasting lower productivity (Mysterud, 2006), and c) barkstripping. This latter process may create several ecological damages (e.g., severe infections of multiple trees, trees death) as well as economic ones, due to the spoiling of commercial wood (Czernik et al., 2013; Cukor et al., 2019).

The sika deer represents a rather complex case of study when it comes to its diet. The species is very adaptable and can thrive in different ecosystems adjusting to different types of vegetation. (Zamoto-Niikura et al., 2014).

Even though classified in accordance to the Hofmann's system of ruminants feeding types (Hofmann, 1973) as an intermediate or adaptable mixed feeder, similarly to other species such as the red deer, the sika deer has been observed being more opportunistic, less specialized, and more prone to quickly acclimatize to changes in food resources. (Fraser, 1996). Moreover, it has been described that the particular morphology of sika deer rumen, which ensures a higher digestive capability, also allows the species to outcompete other overlapping deer species in the use of resources (Fraser, 1996).

In Japan, where the species is highly present and is a relevant source of damages (Takatsuki, 2009), the sika deer browsing and grazing activity is a source of a rather high level of stress for the vegetation (Takatsuki, 2009; Uno et al., 2009), as well as great economic losses when the species feeds on pasture plants (Hata et al., 2019). Additionally, sika deer grazing has been described as the starting point for cascading effects involving the decline of small mammals, the increase in invertebrate numbers, and, consequently, the variation of the density of mesopredators such as the red fox (Seki et al., 2020). Also in the non-native countries, the sika deer is known for being a source of damages, but despite the growing interest in its impacts on habitats and natural resources, still not much is known of how the species uses habitats outside of their natural range (Diaz et al., 2017). Studies performed in the UK have shown that, while the alien populations of sika deer may maintain food preferences displayed also in their native habitat (e.g., a preference for grasses), they may as well favor other available sources of food (e.g., heathlands that are less nutrient but offer other rewards such as protection from humans and fibers intake) which complicates the evaluation of the species diet in the non-native range, and the foreseen of possible damages (Diaz et al., 2017).

A successful understanding and the prediction of impact, demography and competition in these ungulates require a better knowledge their foraging behavior (Hanley, 1997). A relevant first step is to determine the range of diets consumed in the wild and the main sources of variations (climate, habitat, season, sex) (Gebert & Verheyden-Tixier, 2001).

Determination of dietary quality for wild ungulates can be both time-consuming and expensive (Leslie & Starkey, 1985; Raye' et al., 2010). The most common methods used to analyze herbivore feces are direct observation and micro-histology (of both feces and stomach content). Both methods present limitations such as mandatory high visibility and proneness to omissions for the former, and inaccuracy and a highly time-consuming nature for the latter (Kartzinel et al., 2015). In the recent past, however a new method has been applied to analyze the dietary composition of animals (Pompanon et al., 2012; Ando et al., 2013; Karzinel et al., 2015;

Nakahara et al., 2015; Komura et al., 2018; Sato et al., 2018, 2019), the DNA barcoding. The DNA barcoding is a system designed to provide rapid and accurate species identifications by using short, standardized gene regions as internal species tags (Hebert & Gregory, 2005). Even though the concept is not new, its widespread application is relatively modern (Valentini et al., 2009). The possibility of using short DNA fragments makes the DNA barcoding an important tool due to the persistence in the environment of such fragments. It may, in fact, allow an assessment of DNA-based diet composition estimated using fecal samples (Valentini et al., 2009). The genetic analysis of samples containing more than one specimen DNA is known as DNA metabarcoding and is a widely tested and validated approach for the analysis of mixed taxon samples (Taberlet et al., 2012). Over the last decade, metabarcoding -which can be used on modern or ancient environmental samples (Taberlet et al., 2012), contributed to the enormous increase in the amount of available genetic data for organisms, communities and habitats (Valentini et al., 2009). Similarly, also spectroscopic methods have been used to estimate diet composition and intake in ruminants (Parveen et al., 2007). In particular, the Fourier Transform Infrared Spectroscopy (FTIR) is an emerging tool for correlating the structure of biomolecules with the vibration of chemical bonds (Xiong et al., 2019). It provides highly detailed chemical information on the sample's composition via the measuring of the fundamental vibration (Parveen et al., 2007). FTIR spectroscopy may be applied to solid, liquid or gas samples and it determines the interaction between those and the IR (infrared) radiation, measuring the intensity of absorption and the frequency at which the sample absorbs (Simonescu, 2012).

The potential value of this method has been the subject of several studies having a wide range of environmental applications (e.g., soil analysis (Raphael, 2011), study of the atmosphere (Simonescu, 2012), study of microorganisms (Santos et al., 2010), detection and identification of pollutants in the atmosphere (Beil et al., 1998)) and it has also the potential to be widely used in forage ecology research, since it provides a low-cost, non-invasive way to analyze, monitor, and eventually manage animal foraging (Nopp-Mayr et al., 2020), and an increasing number of applications in this subject are to be found in the scientific literature.

4 Bibliometric Analysis of Literature

Introduced, simultaneously, by Pritchard and Nalimov & Mulchenko in 1969, the term "Bibliometrics" is defined as "the application of mathematical and statistical methods to books and other communication medium" (Patra, et al., 2006).

Widely recognized as scientific specialty and as an integral part of the research process, bibliometric analysis is now a firmly established and more and more frequently used scientific tool (Ellegaard & Wallin, 2015). Bibliometric analyses allow researchers to explore a scientific discipline, on both prospective and retrospective lines, helping to discover the entire intellectual core of a given field instead of focusing on its specific works (Ali et al., 2019). Bibliometrics allows the measuring of the output side of science (Godin, 2006) and, through the detection of research trends, may help researchers and decision makers to promptly identify and analyze research topics (Lu et al., 2021).

The quantitative analysis of bibliographic materials may provide a comprehensive overview of a given research (Merigó & Yang, 2017) and also allows to investigate in detail; 1) The trend of scientific use of terms (e.g., keywords) (Menichetti et al., 2016), 2) the numerical analysis of publications in a specific field and period of time, 3) citation analysis and relationships between authors or articles , 4) popularity of publications, authors, institutions and countries, 5) international effects of scientific research (Ozsoy & Demir, 2018). Such analysis allows for the construction of a network based on the relationships between items (e.g., countries, journals, organizations, authors and keywords) and the investigated topic (Chen et al., 2016). Network analysis can also help identify and show the clusters of research and researchers (Fahimnia et al., 2015). Several software allows the creation of bibliometric maps. Science mapping, or the depiction of bibliometric networks, is an effective method for analyzing bibliometric data (Van Eck & Waltman, 2014).

Thesis Aims and Goals

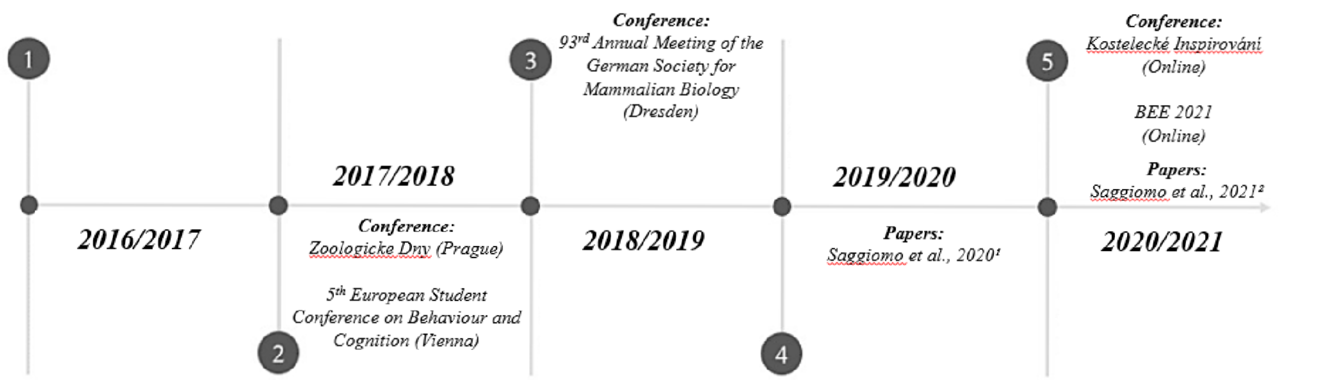
- One goal of this thesis is to evaluate the global knowledge and the perception of sika deer in the scientific literature highlighting blind spots and unexplored topics, trends, and highly investigated matters, as well as new issues.

- The second goal of this thesis is to evaluate the management of sika deer in the Czech Republic by investigating the trend of the number of animals culled in the country between 1994 and 2018.

- Last, we aim to perform a descriptive and preliminary study on the effects of sika deer on the vegetation evaluating, using the DNA metabarcoding on sika deer feces, the diet composition of a population of sika deer in Lower Austria.

Research Timeline

Output Timeline



[1] Saggiomo, Esattore, Picone (2020) *"What are we talking about? Sika deer (Cervus nippon): A bibliometric network analysis"* *Ecological Informatics 60: 101146*

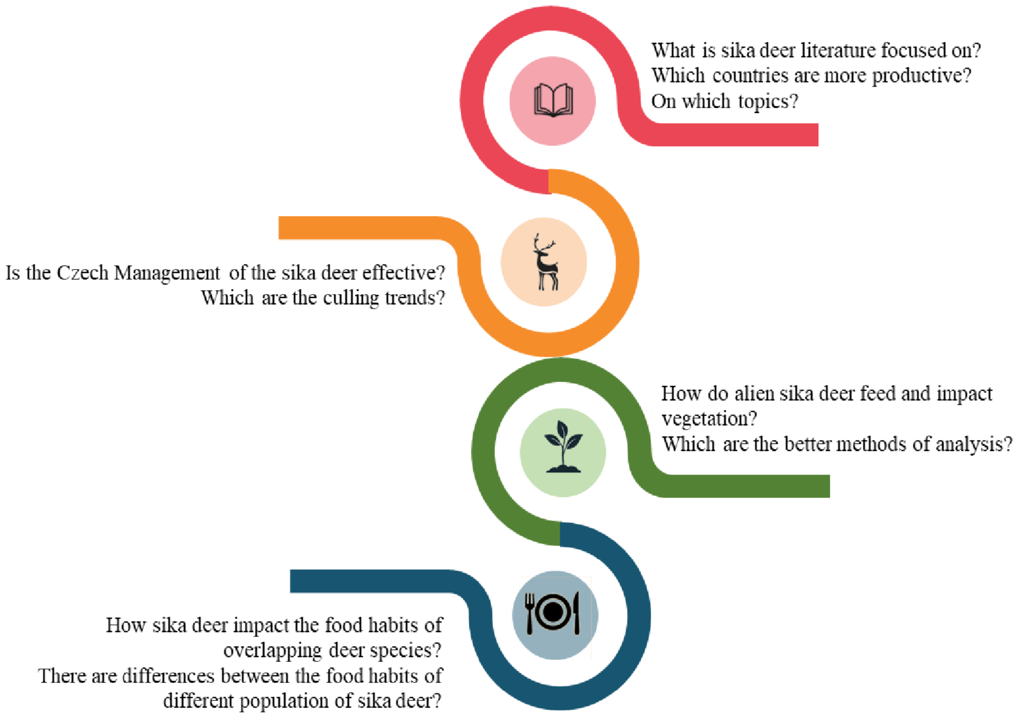
[2] Saggiomo, Esattore, Bartoš (2021) *"Evaluating the management success of an alien species through its hunting bags: the case of the sika deer (Cervus nippon) in the Czech Republic"*
Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis

Conceptual Framework

Sika Deer as an Alien Species:

Perception in Scientific Literature and Ecological Traits of Two Non-native Populations

Questions:



What Are We Talking About? Sika Deer (Cervus Nippon): A Bibliometric Network Analysis

Evaluating the management success of an alien species through its hunting bags: the case of the sika deer in the Czech Republic

DNA metabarcoding and FTIR spectroscopic analysis of Sika deer feces in Lower Austria

Forthcoming projects

Methodology

In this thesis, different methodologies were applied.

Study 1:

What are we talking about? Sika deer (Cervus nippon): A bibliometric network analysis

In this study, two different bibliometric analysis techniques were performed, i.e. the bibliometric network analysis and the citation burst analysis in order to, respectively, (1) quantitatively investigate countries and keywords networks in the scientific literature on the sika deer, and (2) identify fast growing topics over time.

Bibliometric network analysis

The bibliometric network analysis on the sika deer literature was performed using VOSviewer (version 1.6.10) (Van Eck & Waltman, 2019), a software designed to create and visualize maps based on bibliometric data, also allowing the exploration of the output results. The software terminology is explained in Table 3. In a bibliometric network analysis, the objects of interest are referred to as "items" (e.g., keywords, countries, authors) and their size in the resulting network map can be determined by different weight factors depending on the analysis performed. The higher the weight of an item, the higher its importance in the network will be and, consequently, the bigger its size in the map. As weight factors, we chose the number of documents produced and the number of occurrences, respectively, for the co-authorship analysis of countries and for the co-occurrence analysis of keywords. The thickness of the connections between items is based on their "link strength" (Table 3). The resulting network maps display outputs divided into clusters, whose elements are grouped according to their connectedness in the network. In addition to the cluster visualization of network maps, the overlay visualization of VOSviewer delivers a temporal perspective on the network structure. In this case, network items are coloured on the base of their average publication year, providing information on how the network developed over time.

Table 3 Terminology used by VOSviewer software (from Van Eck & Waltman, 2019)

Term	Description
Items	Objects of interest (e.g., publications, researchers, keywords, authors)
Link	Connection or relation between two items (e.g., co-occurrence of key-words)
Link strength	Attribute of each link, expressed by a positive numerical value. In the case of co-authorship links, the higher the value, the higher the number of publications the two researchers have co-authored
Network	Set of items connected by their links
Cluster	Sets of items included in a map. One item can belong only to one cluster
Number of links	The number of links of an item with other items
Total link strength	The cumulative strength of the links of an item with other items

Table 4 VOSviewer analyses performed in this study (from Van Eck & Waltman, 2019)

Analysis	Description
Co-authorship	In co-authorship networks, countries are linked to each other based on the number of publications they have authored jointly
Co-occurrence	The number of co-occurrences of two keywords is the number of publications in which both keywords occur together in the title, abstract or keyword list

In this study, we performed co-authorship and co-occurrence analyses (Table 4) to create network maps of (1) the co-authorship among countries and (2) the co-occurrence of authors' keywords in different literatures as described in section 2.3. Setting details of both analyses are reported in tables 5 and 6.

Table 5 Settings for the different co-authorship analyses of countries performed in this study

Co-authorship analysis of countries				
Area of analysis	global	native	non-native EU	non-native non-EU
Data	bibliographic	bibliographic	bibliographic	bibliographic
Type of counting	fractional	fractional	fractional	fractional
Max number of countries per document	15	15	15	15
Min number of documents per country	2	2	5	3
Weight	number of documents	number of documents	number of documents	number of documents

Table 6 Settings for the different co-occurrence analyses of authors' keywords performed in this study

Co-occurrence analysis of authors' keywords				
Area of analysis	global	native	non-native EU	non-native non-EU
Data	bibliographic	bibliographic	bibliographic	bibliographic
Type of counting	full	full	full	full
Min number of occurrences per keyword	10	10	5	3
Weight	number of occurrences	number of occurrences	number of occurrences	number of occurrences

Citation burst analysis

The citation burst analysis was performed using CiteSpace (Chen, 2006, 2017), a software tool designed to perform scientometric analyses. Among its many features, CiteSpace allows for the analysis of keywords that experienced a citation burst, i.e., an abrupt increase in the number of citations in a specified period of time and in a given research area.

Data collection

To include documents regarding all domains of studies on the sika deer, data were collected using the research string ("Cervus nippon" OR "Sika deer"). The keyword "Sika" was not included in the research string due to the homonymy with an industry present in many territories (e.g., Europe, Americas, Pacific Asia, and Africa). As regards the bibliometric network analysis, we collected the documents on the 7th of January 2020 on the web search engine SCOPUS, selecting a time frame between 1945 and 2020. For this analysis, four different databases were created to investigate literature in native and non-native ranges of distribution. In particular, based on IUCN geographic range (www.iucnredlist.org), the following databases were produced: 1) global literature (all available countries); 2) native range literature (i.e., China, Japan, Korea, Russia, Taiwan, and Viet Nam); 3) non-native European range literature (i.e., Armenia, Austria, Czech Republic, Denmark, France, Germany, Ireland, Lithuania, Poland, Ukraine, UK); 4) non-native non-European (i.e., New Zealand, South Africa, USA). The results of the researches on SCOPUS database were exported as ".csv" files after selecting the "Citation information", "Bibliographical information", and "Abstract & keywords" options. For the citation burst analysis, we collected the documents from the Web of Science database on the 10th of February 2020 over all available years (i.e., 1990-2020). In this case, the results were exported selecting "Full Record and Cited References" as plain text file format.

Additional analyses

In addition to the two abovementioned analyses, the temporal trend of the number of documents published per year (1945-2019) on SCOPUS database was investigated, and information such as year of publication, access type, subject area, document type, and language were also collected and analyzed.

Study 2:

Evaluating the Management Success of an Alien Species Through Its Hunting Bags: The Case of the Sika Deer (Cervus Nippon) in the Czech Republic

In this study, we used the official hunting bag statistics from the Ministry of Agriculture of the Czech Republic from the year 1994 to the year 2018. Our data included the number of female, male and young (up to 1 year of age) sika deer harvested each year in each of the different districts of the country in the totality of hunting areas. Although there are at least two subspecies (i.e., *C. n. hortulorum* and *C. n. nippon*), the statistics available do not distinguish between them. According to Barančeková et al. (2012) there are currently two subpopulations of sika recognised in the Czech Republic, one in the West-Bohemia and one in the Central-Eastern Bouzovsko area. Using GPS telemetry, an investigation of the sika deer's spatial distribution conducted in the country using the minimum convex polygon method revealed that the mean home range size (obtained from subadult and adult males) was 3620 ha (Dvořák et al., 2014). Such a size exceeds the mean area of hunting ground in many parts across the country. Besides, from studies realized elsewhere, we know that some individual sika may be classified as nomadic and have long-distance movements across the landscape associated or un-associated with seasons (Kalb et al., 2013; Takafumi et al., 2017). Thus, we used data from all areas where the sika deer harvest has been recorded. We divided the Czech population of sika deer in three hunting subpopulations according to the area of culling (Fig. 4), taking into consideration both the forest distribution and the inter-district connections, and separating them on the base of districts borders and presence of barriers (e.g., railways, highways, rivers, cities and other large urbanized areas).

We analyzed the environmental features of the Czech landscape (i.e., water elements, urban fabrics and vegetation, including coniferous, mixed and deciduous forests) during our study timeframe with the support of Google Earth layers (roads and railways), and Corine and Cover (C.L.C.) shape files (Fig. 5). The analysis of the C.L.C. from 2002 and 2012 did not highlight any relevant modification of neither the natural nor the urban environments during the years, therefore we decided to take into consideration only the most recent one (Fig. 5). Using G.I.S. software (QGIS 3.8.1), we estimated the non-artificial vegetation cover (i.e., arable lands, permanent crops, pastures, heterogeneous agricultural crops, forests and semi-natural areas, shrubs and/or herbaceous vegetation association) of the subpopulations ranges in order to highlight similarities and differences among the regions investigated.

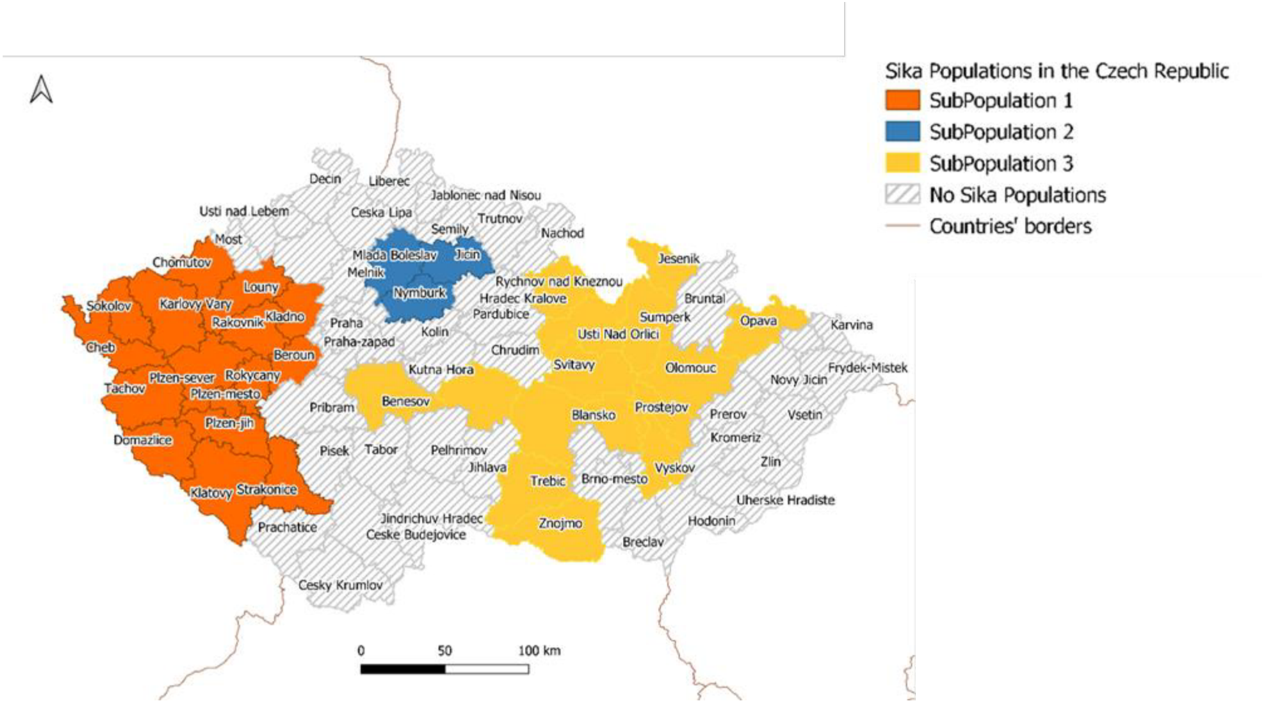


Figure 4 The three subpopulations of sika deer identified for the Czech Republic. Only the districts with at least three culled sika deer for three consecutive years are shown. This measure was taken in order to avoid overestimating the range by including also passages or temporary district

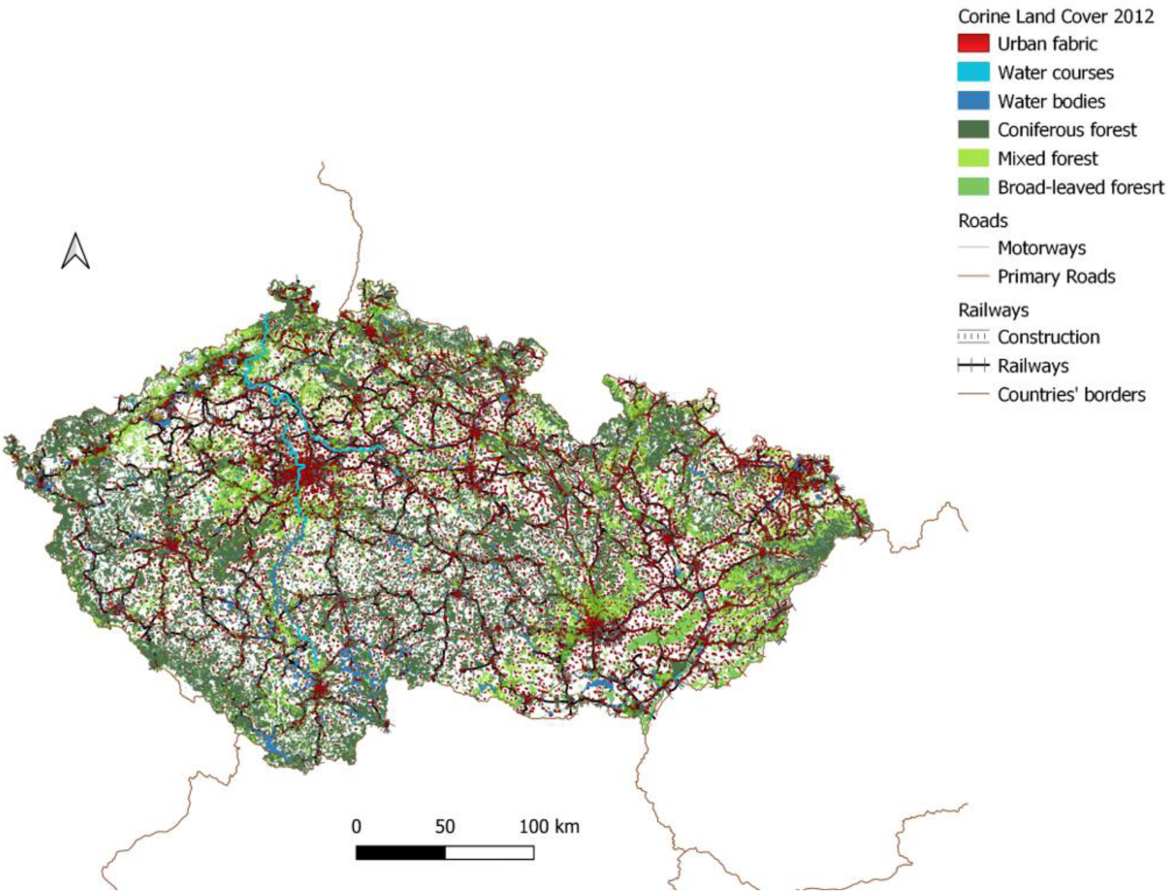


Figure 5 Types of land cover in the Czech Republic considered in this study (data obtained from Corine Land Cover 2012). The map also shows the web of roads and railways of the country (data obtained from Google Earth)

Being our database vast and susceptible to errors during the transcription of data, we checked for extreme values in the database via the identification of outliers. After having organized the values in numerical order, we extrapolated the median and the quartiles. Then, we calculated the interquartile range and multiplied it for 1.5 (inner fence outliers) and 3 (outer fence outliers). The values that were higher or lower than the results of our multiplications were excluded from the analysis (Tab. 7). Furthermore, we estimated the mean annual increase of sika culled in the three different subpopulations according to the equation

$$A.a.i = \left(\frac{x}{y}\right)^{\frac{1}{n}} - 1 \ (1)$$

Where: x = number of the deer culled in that subpopulation in 2018,

y = the number of deer culled in that subpopulation in 1994, and

n = the number of years in the considered timeframe (Tab. I).

Last, we transformed our results in percentage values:

Table 7 : Number of sika deer culled per year in each subpopulation

	SP1	SP2	SP3
1994	2829	60	524
1995	3158	50	627
1996	3087	46	725
1997	-	54	719
1998	5397	75	634
1999	4241	79	620
2000	5053	59	747
2001	5695	75	742
2002	5106	-	680
2003	6390	68	552
2004	6180	72	566
2005	6346	67	563
2006	6123	65	516
2007	7222	82	635
2008	8312	74	695
2009	8686	64	782
2010	10061	69	879
2011	9929	56	884
2012	11301	-	1137
2013	11813	60	966
2014	12959	79	980
2015	13411	60	1070
2016	14876	66	1202
2017	15750	94	1262
2018	16993	76	1299

In order to estimate the role of other sources of mortality on the management of sika deer in the Czech Republic, we searched for data on WVC and predation. We used the Web of Science, Scopus and Google Scholar databases (Keywords: "sika deer AND Czech Republic AND mortality" and "Sika deer AND Czech Republic AND predation" and "Sika deer AND Czech Republic AND traffic collision") but we were not able to find numeric data on these matters. Last, to take into account the presence of wolf populations as a possible source of sika deer mortality, we overlapped the most recent map of wolf packs distribution across the country (2019) with the subpopulations map (Fig. 6) in order to estimate the level of overlapping with every subpopulation and make predictions for the future.

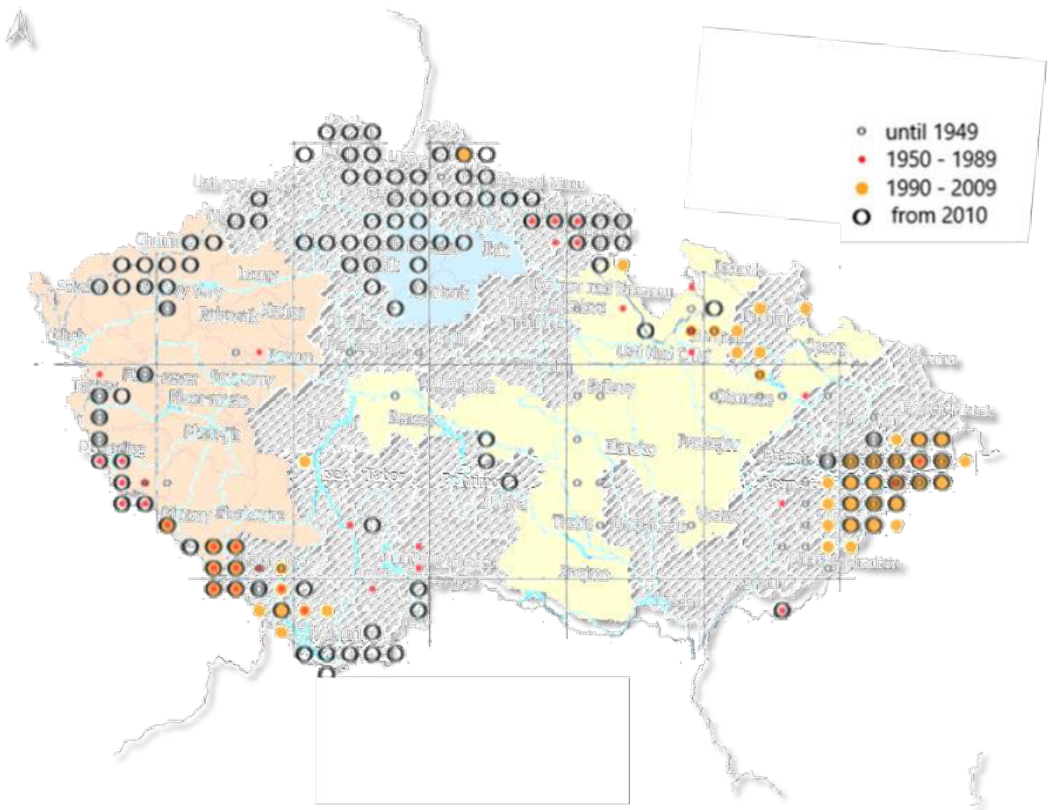


Figure 6 Overlapping map of wolf occurrence and culled sika deer subpopulations. Data on wolf occurrence was obtained from (Lososová et al., 2019)

Study 3:

DNA metabarcoding and FTIR spectroscopic analysis of Sika deer feces in Lower Austria

Sample collection The collection of the samples was performed during April, May, and June 2019 in a hunting area partially fenced, and surrounded by the Danube river, in the area of Zwentendorf (Lower Austria). The sampling area of about 2 km^2 presented both areas with tree cover and a rife understory, and meadows. The hunting area hosted a dense population of sika deer which was reflected in the stressed and highly disturbed vegetation.

Apart from sika deer, two other ungulates, roe deer (*Capreoulus capreolus*) and wild boar (*Sus scrofa*) were present in the area even though in strongly lower numbers.

In order to collect sika feces, we spotted/observed individuals/groups and then we gathered the sample. To reduce the possibility of collecting pellets from the same individuals we collected only piles well distanced (>10 m). The pellets were then stored in plastic bags and the date, the location, and the sample code were written on them. A total of 40 samples was collected.

Sample preparation for DNA Metabarcoding After the collection, samples were stored at -20°C. Successively they were transferred in plastic containers with a screw cap and soaked in 96% ethanol.

DNA Metabarcoding

For the DNA extractions, cell walls were disrupted mechanically by homogenizing the material with zirconium beads and 600 uL of lysis buffer (2% SDS, 2%PVP 40, 250 m MNaCl, 200 M Tris–HCl, 5 mM EDTA, pH8) in a RetschMM200 (Retsch, Haan, Germany) mixer mill. The lysate was then digested with 10 ul of proteinase K (10 mg/ mL) for 30 min followed by a digestion of 10 uL of RNAase A (100 mg/mL) for 15 minutes. Remaining proteins were precipitated by adding 150 uL of potassium acetate (XX M) and incubating the mixture on ice for 30 minutes. Solid particles were then precipitated by centrifuging the lysate in an incrementing way, starting at 1000 rpm for 1 minute and doubling the speed up to 8000 rpm followed by a final centrifugation at 10000 rpm for 10 minutes. 600 uL of supernatant was placed into a new tube and mixed with 600 uL of binding buffer (2 M GuHCl in 95% ethanol). DNA was the bound to silica membranes by pipetting this mixture into Econospin 96 well DNA isolation plates (Epoch Life Science, Texas, USA) and centrifuging them at 6000 rpm for 5 minutes. The silica membrane was washed two times with 600 ul of 70% ethanol solution and centrifuging the plate for 5 minutes at 6000 rpm. A last centrifugation was done to remove residual ethanol. DNA was then eluted two times by adding 50 uL and 100 ul of elution buffer (10 mM Tris, pH 8), respectively and let the plate incubate at room temperature for 5 minutes followed by a centrifugation at 6000 rmp for 5 minutes. DNA quality was evaluated with an agarose gel electrophoreses.

At this point, DNA metabarcoding was applied. Plant taxa present in sika deer scat samples were identified using the trnL-F primers e and f from Taberlet et al. (1991). For that amplicon sequencing libraries for Illumina sequencing were prepared as described in Lanner et al. (2019). Sequencing was outsourced to the Illumina MiSeq at the Genomics Service Unit at Ludwig Maximillian University, Munich, Germany, where the libraries were sequenced in a paired end Illumina MiSeq 300 bp run.

The resulting sequences were quality controlled with cutadapt (Martin, 2011) by selecting reads with amplification primers and trimming them. These reads were then used as input for Dada2 (Calahan et al., 2016) and an amplicon sequence variant (ASV) table was exported. Taxonomic assignment of all ASVs was done by comparing their sequences to the nucleotide database (nt) from GenBank and a custom plant database of Austrian plant done in the scope of the Austrian barcoding of life initiative (ABOL). This was done using blastn (Korf et al., 2003) where only records with a minimum e-value of 1x10-10, identity of 98% and query coverage of 90% were considered. The best match between both databases was taken and since not all possible plant taxa are represented in the database taxonomic assignment was done at the genus level.

Sample preparation for FTIR Spectroscopy After the collection, sample were stored in paper bags and dried up in a low-temperature oven for 7 to 10 days, and then powdered using a steel disc vibratory mill (Fritsch Pulverisette 9) for about 1 minute and a half (two minutes when necessary) and 1000 rotations per minute. Materials were cleaned after every use to avoid contamination of new samples with old ones. Powdered samples were then stored in 1.5 ml Eppendorf tubes.

FTIR Spectroscopy FTIR spectra were recorded with an optical diamond crystal of a Bruker® Helios FTIR micro sampler (Ettlingen, Baden-Württemberg, Germany) (Tensor 27) in the Attenuated Total Refectance (ATR) mode. At a spectral resolution of 4 cm^{-1}, 32 scans per measurement and five to seven replicate measurements per sample were performed. The replications were vector-normalized and averaged with the integrated software OPUS® 7.2. Principal Component Analysis (PCA) based on the full spectral range was performed using The Unscrambler X 10.1© Camo (Oslo, Norway).

Results

What are we talking about? Sika deer (Cervus nippon): A bibliometric network analysis

Global literature The global literature research on the sika deer resulted in 1,374 documents, 71 countries, and 3,262 authors' keywords. The most productive country was Japan (Fig. 7) with 646 documents, indicating that Japanese researchers contributed to the 47% of the existing literature available on the SCOPUS database. Japan resulted to be first also by number of citations and co-authorship with other productive countries such as China and USA that were, respectively, the second and the third by number of documents (Table 8). Taiwan is in the list of the ten most cited countries (9th place, with 289 citations) but absent from the list of top countries for number of documents and total linkstrength (Table 8).

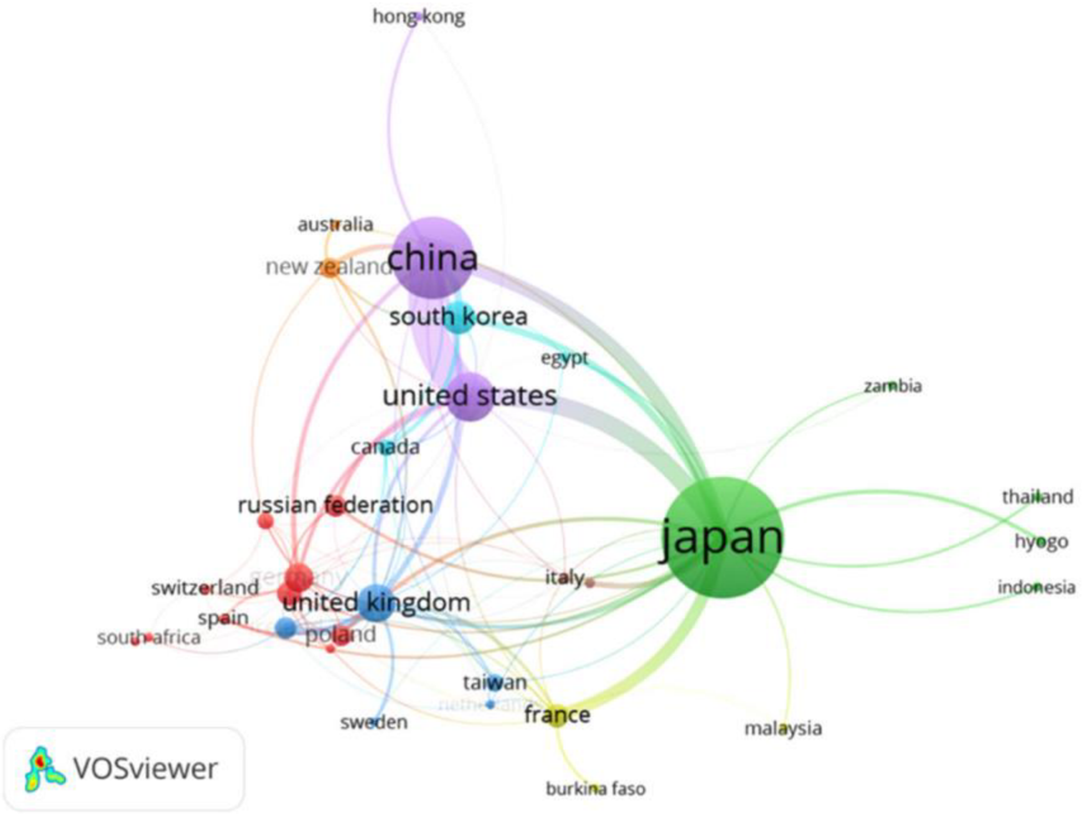


Figure 7 Co-authorship network map of countries publishing on the topic of sika deer from 1945 to January 2020.

Table 8 Top 10 countries publishing on sika deer in the global literature ranked according to the number of documents, number of citations and total link strength.

Country	Documents
Japan	**646**
China	**311**
USA	**119**
UK	**73**
South Korea	**55**
Germany	**43**
Czechia	**33**
France	**31**
Ireland	**27**
Russia	**27**

Country	Citations
Japan	**6812**
UK	**1745**
USA	**1686**
China	**1587**
France	**702**
Ireland	**390**
Germany	**320**
Czechia	**293**
Taiwan	**289**
South Korea	**285**

Country	Total Link Strength
Japan	**69**
China	**56**
USA	**55**
Uk	**36**
France	**21**
South Korea	**17**
Germany	**14**
Ireland	**12**
New Zealand	**12**
Czechia	**11**

The overlay visualization of the co-authorship network map of countries (Fig. 8) showed a different production trend for the three most productive countries over the years. In fact, while

Japan stayed consistent in its scientific production, USA became less productive over the years, and China had, instead, a recent increase of published documents. As regards China, this result was corroborated by the overlay visualization of the co-occurrence network map of authors' keywords (Fig. 9), which showed an increasing use of the keyword "China" in recent years. Other two keywords, "antler" and "velvet antler", appeared to be recently used in the literature.

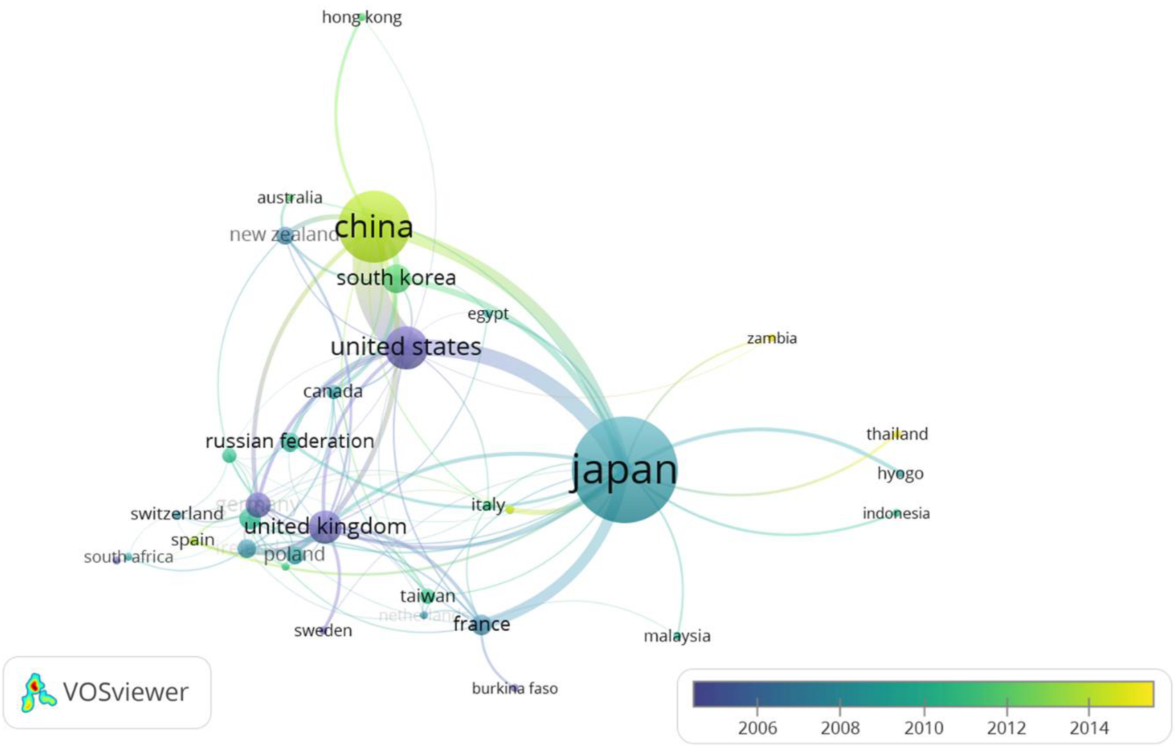


Figure 8 Overlay visualization map of the Co-authorship network of countries publishing on the topic of sika deer from 1945 to January 2020

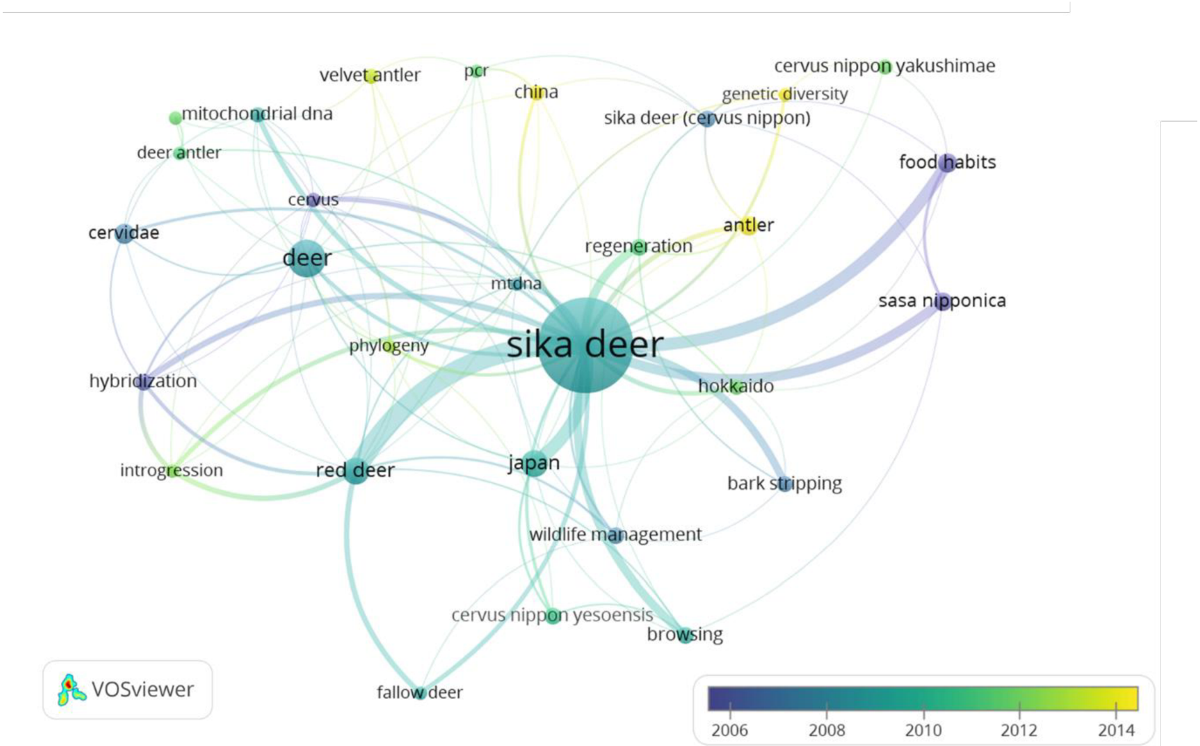


Figure 9 Overlay visualization map of the Co-occurrence network of authors' keywords from articles published on the topic of sika deer from 1945 to January 2020.

In the co-occurrence analysis of authors' keywords (Table 9) the keyword "Japan" ranked 4th by occurrences, and 3rd by total link strength, while "sasa nipponica" ranked high (8th place) in terms of both occurrences and total link strength, along with topics like "browsing" and "regeneration" as well as "food habits. The keyword "red deer" appears in the highest places of both lists, together with topics suchas "hybridization" and "introgression".

Table 9 Top 10 authors' keywords co-occurring in articles published in the global literature on sika deer ranked according to the number of occurrences and total link strength.

Keyword	**Occurrences**
sika deer	**400**
deer	**72**
red deer	**38**
Japan	**37**
cervidae	**22**
food habits	**21**
antler	**21**
Sasa nipponica	**18**
regeneration	**16**
browsing	**16**

Keyword	**Total Link Strength**
sika deer	**187**
red deer	**60**
Japan	**28**
hybridization	**26**
deer	**23**
food habits	**23**
introgression	**22**
Sasa nipponica	**20**
regeneration	**19**
browsing	**18**

Native range literature The research on the scientific literature produced by Japan, China, South Korea, Taiwan, Viet Nam, and Russia provided 1,029 documents, representing the 75% of the total available literature. The majority of these documents (64%) were produced with the co-authorship of Japan, while the 30% is co-authored by China (Table 10).

The co-authorship analysis of countries showed that, among the countries belonging to the original native range of the sika deer, Viet Nam was the least productive one (Table 10), and that collaborations of China and Japan with USA, UK, France, New Zealand, and Germany were common (Fig. 10).

Table 10 Top 10 countries publishing on sika deer in the native range literature ranked according to the number of documents, number of citations and total link strength

Country	Documents	Country	Citations	Country	Total Link Strength
Japan	**645**	Japan	**6812**	Japan	**68**
China	**310**	China	**1587**	China	**56**
South Korea	**55**	USA	**650**	USA	**47**
USA	**47**	UK	**386**	South Korea	**17**
Russia Federation	**27**	Taiwan	**289**	UK	**11**
Taiwan	**18**	South Korea	**285**	France	**9**
UK	**11**	Russian Federation	**199**	Russian Federation	**8**
France	**10**	France	**161**	Germany	**6**
New Zealand	**6**	Poland	**54**	New Zealand	**6**
Germany	**6**	Netherlands	**51**	Canada	**5**

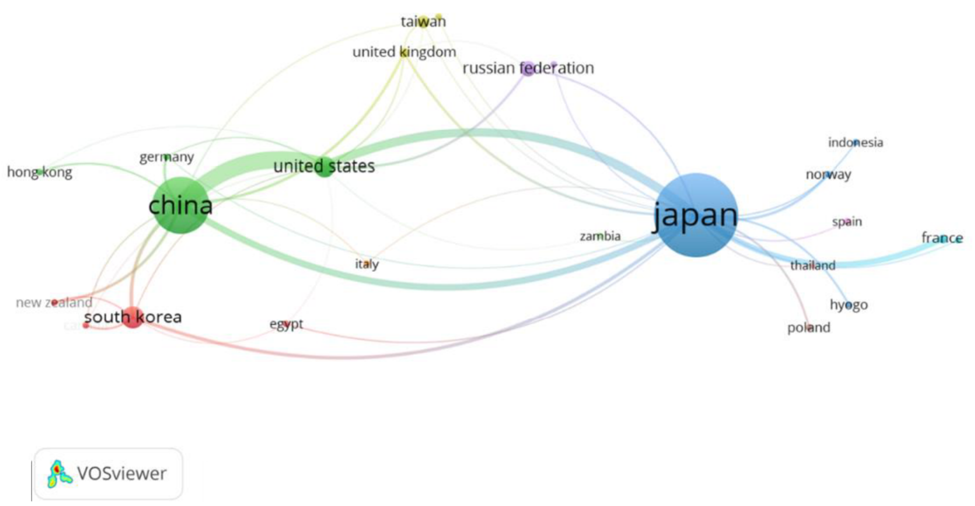


Figure 10 Co-authorship network map of countries belonging to the native range of sika deer publishing on the topic of the species from 1945 to January 2020.

In addition, China and Japan showed a high link strength between them, suggesting a certain degree of collaboration between the two countries. Limiting our database only to countries belonging to the native range produced changes in the keywords analysis results. In the case of native countries scientific research, the topic of hybridization was absent, leaving room to subjects like food habits and vegetation regenerations (Fig. 11). The presence of keywords such as "Cervus nippon yesoensis", along with "Hokkaido" and "Sasa nipponica", proved the high productivity of Japan, whose research focused mainly on Japanese populations.

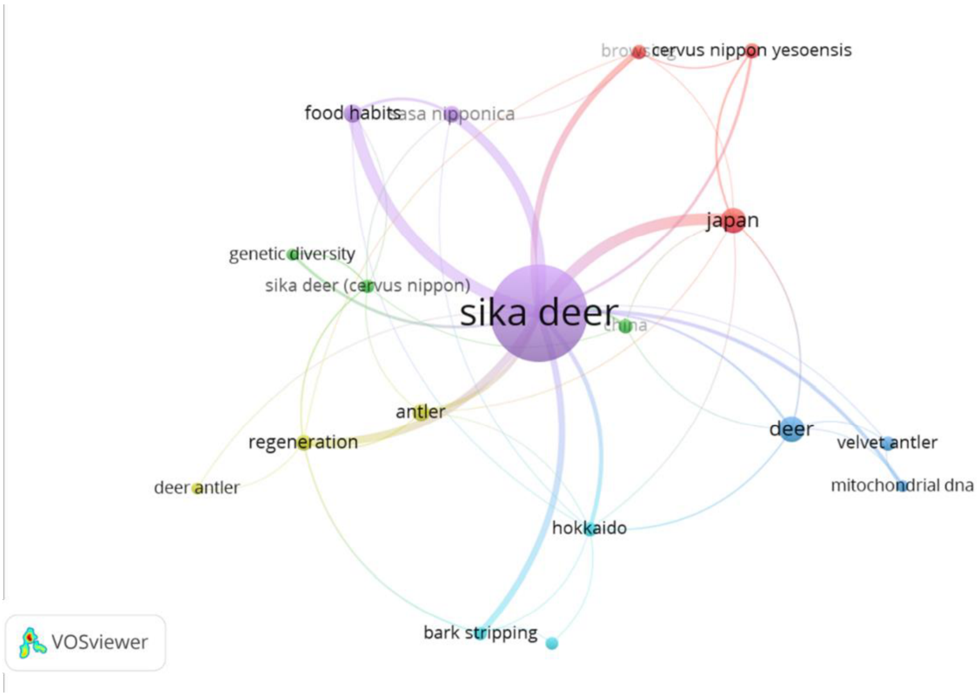


Figure 11 Co-occurrence network map of authors' keywords from articles published by native range countries on the topic of sika deer from 1945 to January 2020

Non-native European literature The bibliographic research of the non-native European literature resulted in 240 documents. The most productive country was the UK with 73 documents, followed by Germany (43) and Czech Republic (33) (Table 11). France, although hosting a rather small population, showed a high productivity (31 documents) (Table 11) and the strongest link with a non-European country (i.e., Japan) (Fig. 12). The overlay visualization of the co-authorship analysis of countries showed that, while Germany and UK published on the issue mostly before 2008, Austria and Czech Republic increased their productivity in the last decade (Fig. 13). The co-occurrence analysis of the authors' keywords showed a focus on genetics research, with topics such as hybridization and introgression (Fig. 14). Moreover, the overlay visualization identifies in phylogeny and parasite studies the newest lines of research (Fig. 15).

Table 11 Top 10 countries publishing on sika deer in the non-native European range literature ranked according to the number of documents, number of citations and total link strength

Country	Documents
UK	**73**
Germany	**43**
Czech Republic	**33**
France	**31**
Ireland	**27**
Poland	**24**
Japan	**21**
Austria	**16**
USA	**13**
China	**10**

Country	Citations
UK	**1745**
France	**702**
Ireland	**390**
Japan	**333**
Germany	**320**
Czech Republic	**293**
Italy	**272**
United States	**267**
Poland	**265**
Switzerland	**172**

Country	Total Link Strength
UK	**28**
Japan	**21**
France	**17**
Germany	**14**
USA	**13**
China	**10**
Ireland	**10**
Czech Republic	**9**
Austria	**6**
Poland	**6**

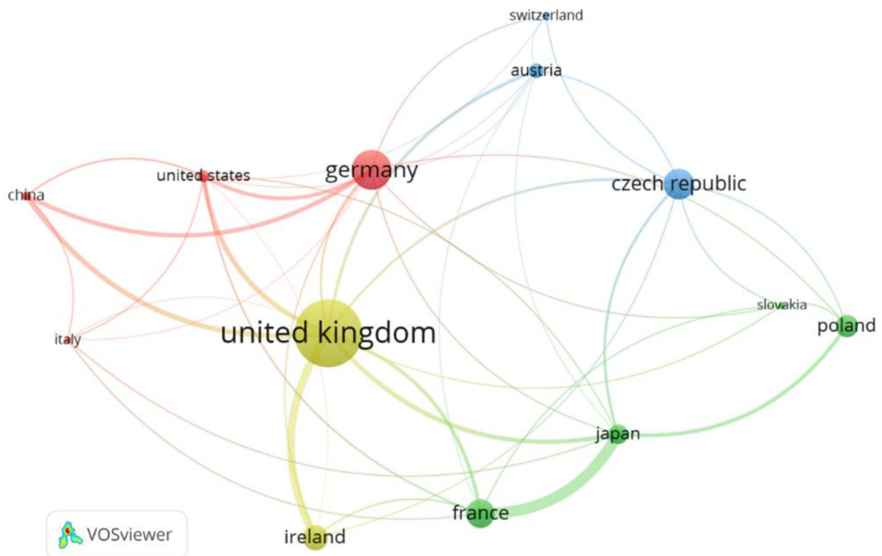


Figure 12 Co-authorship network map of countries belonging to the non-native European range of sika deer publishing on the topic of the species from 1945 to January 2020

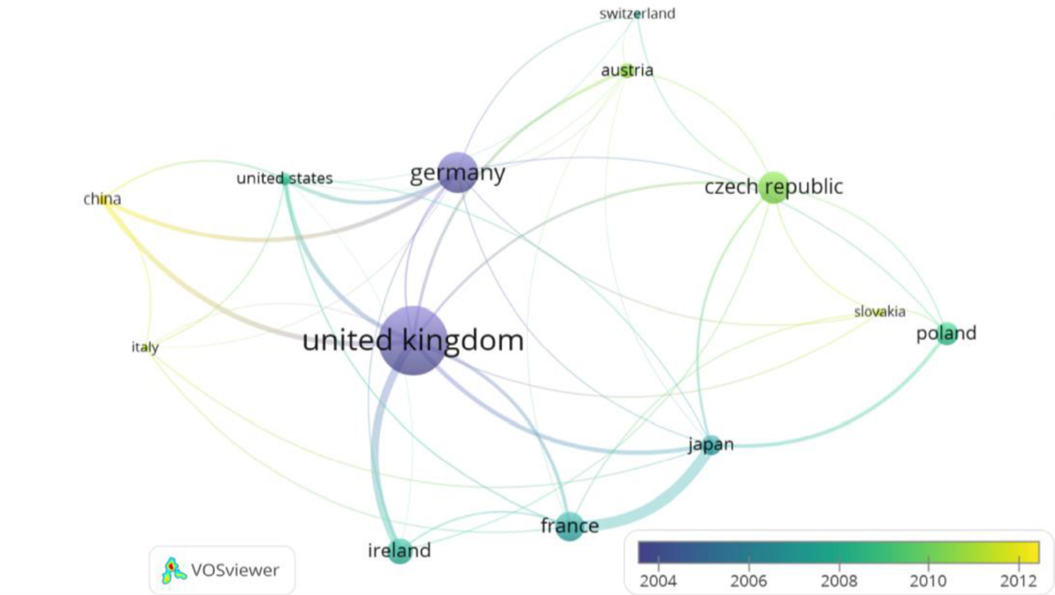


Figure 13 Overlay visualization map of the Co-authorship network of countries belonging to the non-native European range of sika deer publishing on the topic of the species from 1945 to January 2020

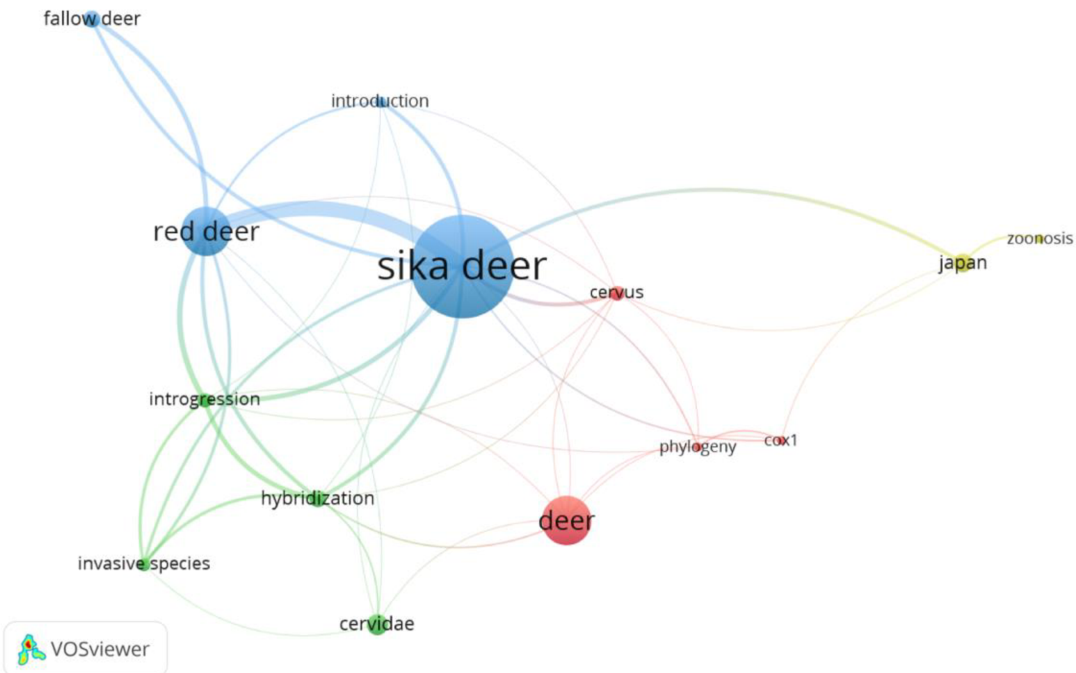


Figure 14 Co-occurrence network map of authors' keywords from articles published by non-native European range countries on the topic of sika deer from 1945 to January 2020

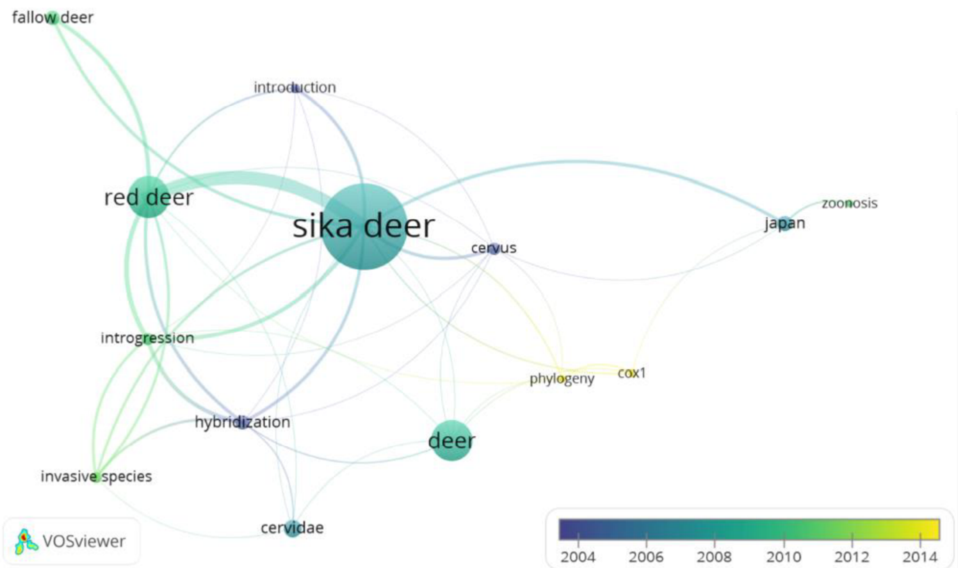


Figure 15 Overlay visualization map of the co-occurrence network of authors' keywords from articles published by non-native European range countries on the topic of sika deer from 1945 to January 2020

Non-native non-European literature The analysis of the non-native and non-European literature resulted in 147 documents, the majority of which (119) produced by the USA (Table 12). The USA shows strong links with China and Japan, while New Zealand, the third most productive country (24 documents) (Table 12) had mainly collaborated with China and UK (Fig. 15). Many European countries were present in this analysis, mostly in connection with the USA (Fig. 16). South Africa was among the least productive countries with only 3 documents (Table 12), not displayed in the map (Fig. 16) because not interacting with the network. The study on the authors' keywords (Fig. 17) showed, in the top 10 most used keyword terms such as "red deer" and "hybridization", "Odocoileus virginianus" and "Cervus nippon yakushimae".

Table 12 Top 11 countries publishing on sika deer in the non-native and non-European range literature ranked according to the number of documents, number of citations and total link strength

Country	Documents
USA	**119**
China	**31**
New Zealand	**24**
Japan	**17**
UK	**9**
Russia Federation	**5**
Germany	**5**
South Korea	**4**
South Africa	**3**
Austria	**3**
France	**3**

Country	Citations
USA	**1688**
China	**295**
New Zealand	**276**
UK	**268**
Japan	**238**
Russian Federation	**124**
South Africa	**41**
Austria	**37**
Germany	**33**
France	**22**
South Korea	**1**

Country	Total Link Strength
USA	**53**
China	**31**
Japan	**17**
New Zealand	**12**
UK	**9**
Germany	**5**
Russian Federation	**5**
South Korea	**4**
Austria	**3**
France	**3**
South Africa	**0**

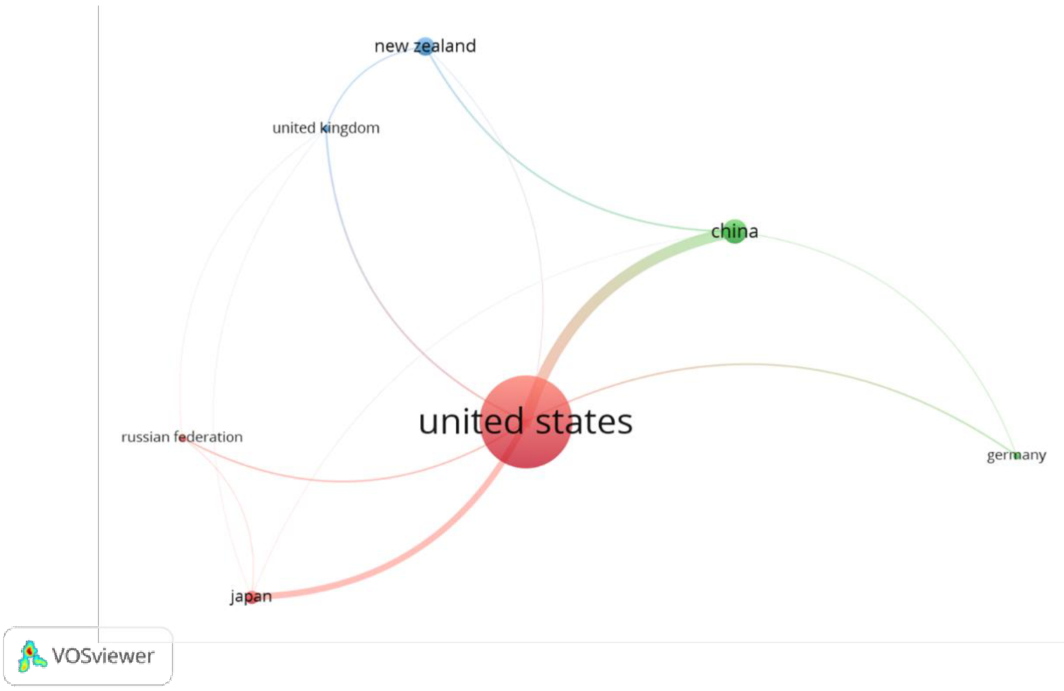


Figure 16 Co-authorship network map of non-native and non-European range countries publishing on the topic of the sika deer from 1945 to January 2020.

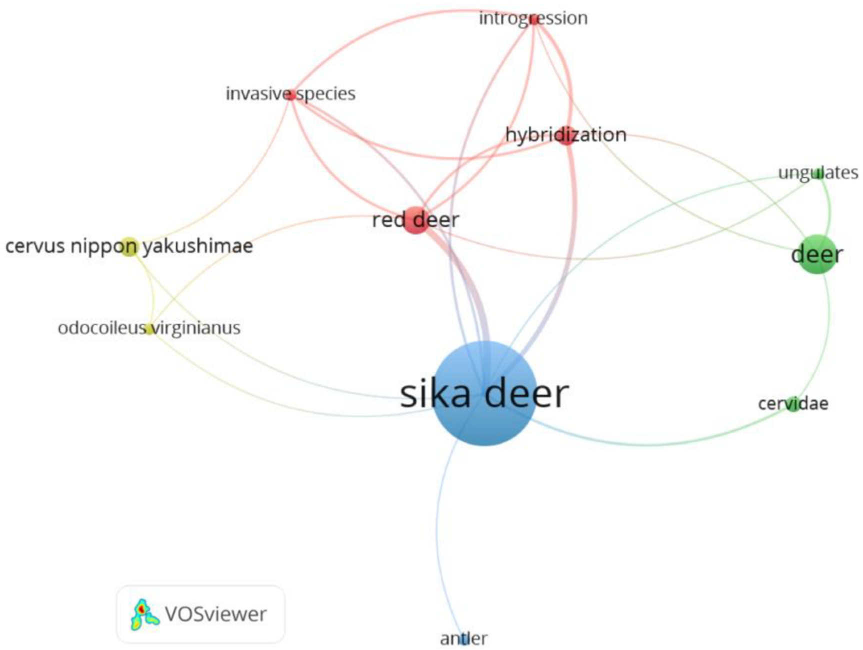


Figure 17 Co-occurrence network map of authors' keywords from articles published by non-native and non-European range countries on the topic of sika deer from 1945 to January 2020

Citation burst analysis The citation burst analysis showed that 32 keywords experienced an abrupt increase in citations in the time range from 1990 to 2020 (Fig. 18). Burst keywords such as "mitochondrial DNA", "sequence", "expression", and "genetic diversity" were indeed found across the years of investigation. Since 2001 to2005, the keyword "red deer" experienced a citation burst, while keywords such as "velevet antler", "regeneration", and "antler" are experiencing a burst in citations in the very recent years.

Last, the keyword "management" appears to have been widely used only in the last 4 years.

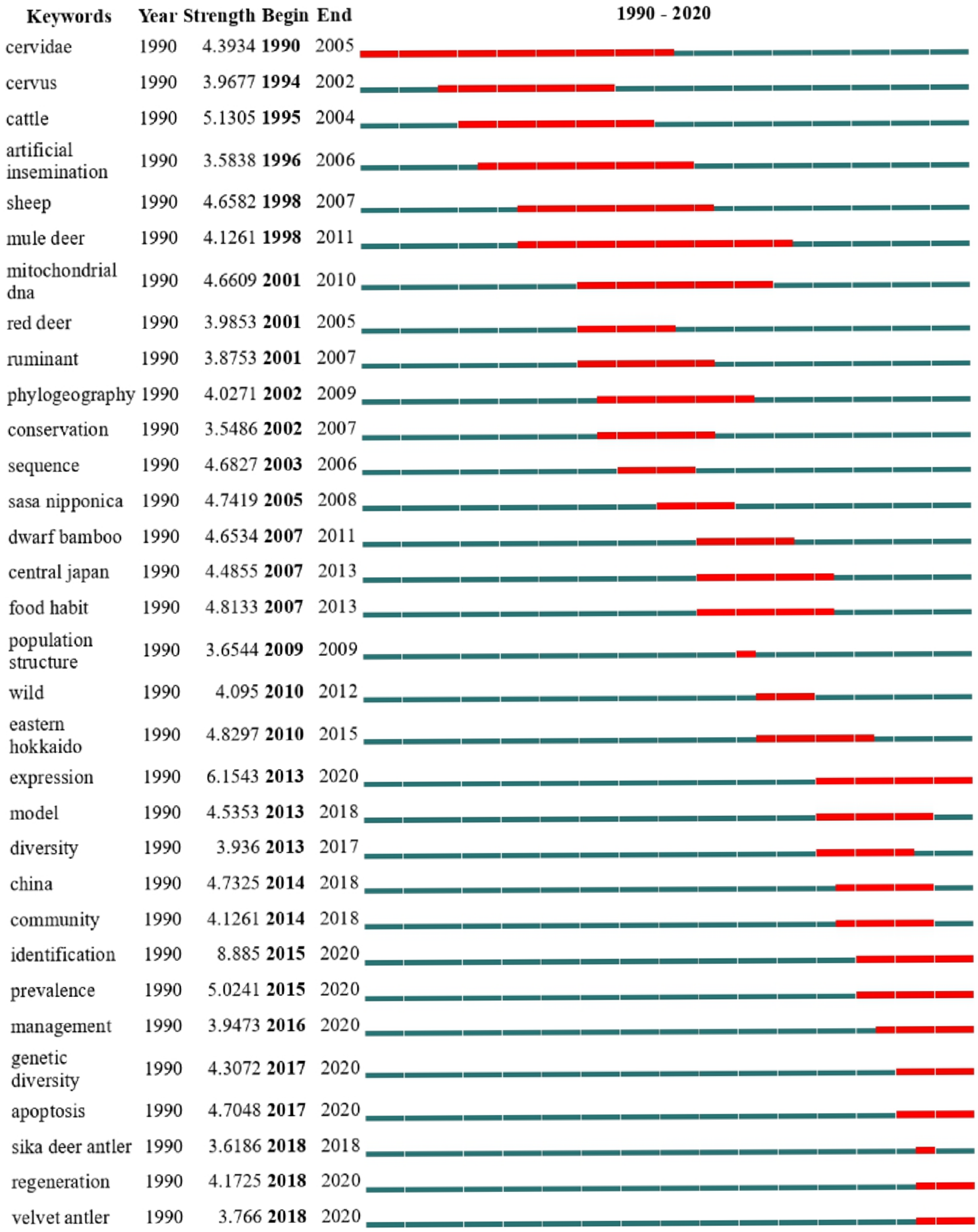


Figure 18 Citation burst analysis of keywords in sika deer related literature. For each keyword the strength of the burst (min. 1 year) and the time spawn are showed.

Additional analyses Analyzing the main subject areas of the collected documents, “Agricultural and Biological Sciences” resulted the most common across all the different literatures. Apart from few small discrepancies, the results from all the literatures appeared rather homogeneous (Fig. 19). Similarly, the main type of access to literature was not “Open access” (Fig. 20), and the most utilized language was English, even though Chinese and Japanese were the chosen languages for 11% of the available literature (Fig. 21).

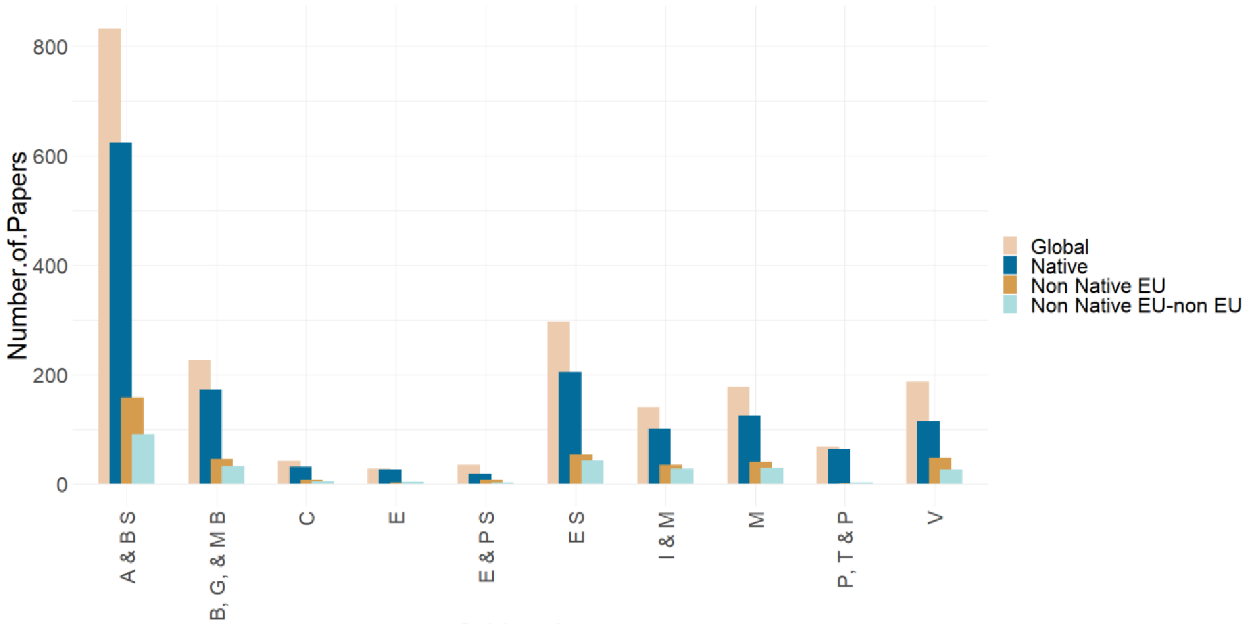


Figure 19 Number of publications for subject area for each of the analyzed literatures (A&BS= Agricultural and Biological Sciences; ES=Environmental Science; B,G, &M B=Biochemistry, Genetics and Molecular Biology; V=Veterinary; M=Medicine; I&M=Immunology and Microbiology; P,T&P=Pharmacology, Toxicology and Pharmaceutics; C=Chemistry; E&PS=Earth and Planetary Sciences; E=Engineering)

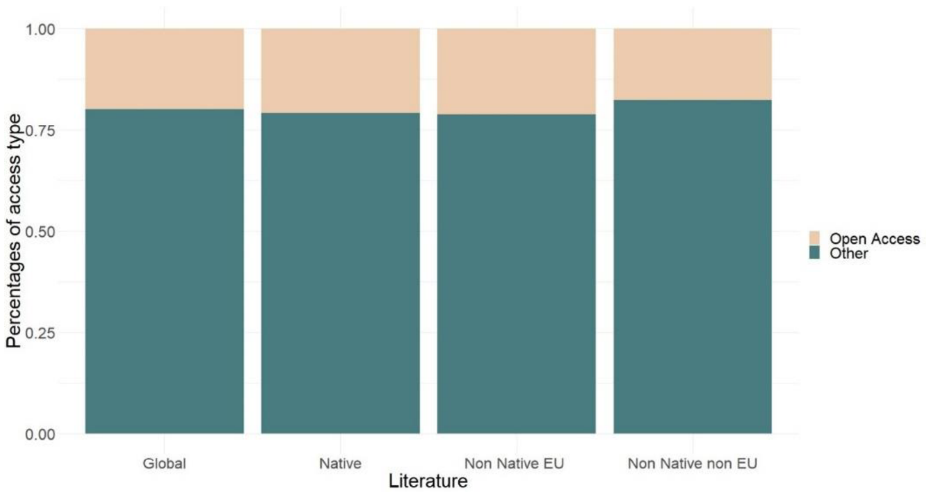


Figure 20 Percentage of publications open access or not open access for each of the analyzed literatures

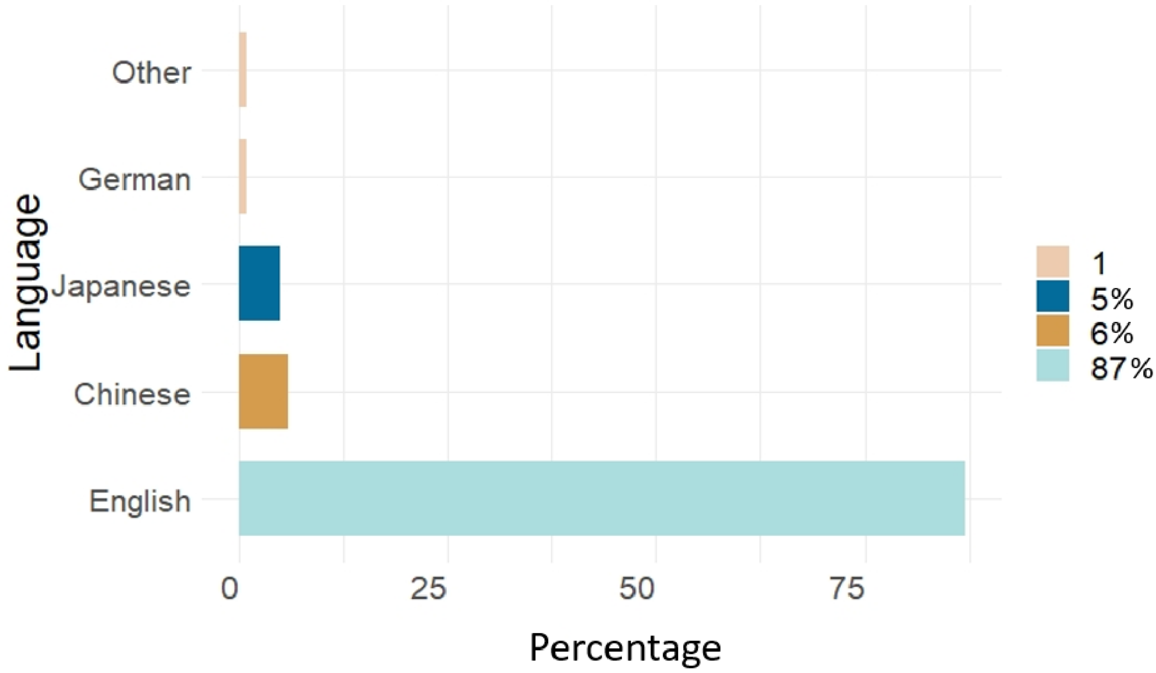


Figure 21 Percentage of the language of publication in the available literature on sika deer.

All literatures investigated in this study experienced an increase in the number of publications. In particular, the global literature showed a growing exponential trend of publication starting from the half of the 1990s. Instead, both non-native literatures saw a rather small growth in the number of documents produced in the last 20 years (Fig. 22).

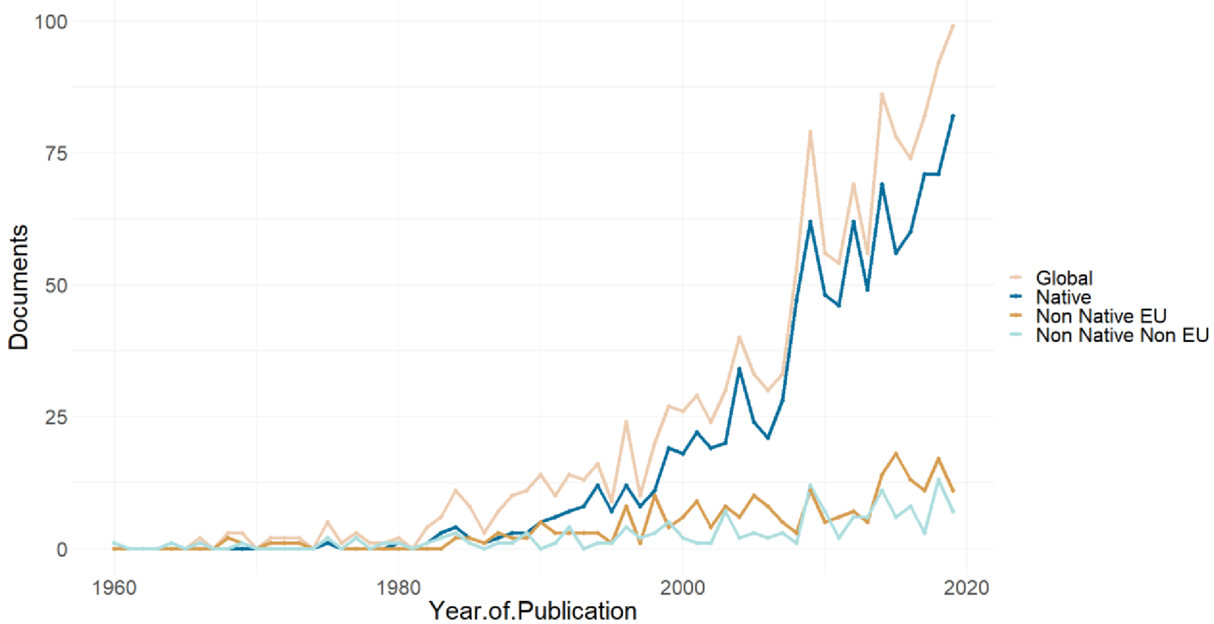


Figure 22 Temporal trend of publication of documents on sika deer in analyzed literatures

Evaluating the Management Success of an Alien Species Through Its Hunting Bags: The Case of the Sika Deer (Cervus Nippon) in the Czech Republic

The analyses of the sika deer culled in the three culling subpopulations showed very different trends (Fig. 23). Over the period between 1994 and 2018, the average annual percentages of culling were of 7.4. The SP1, located in the West of the country, resulted to be the second biggest subpopulation in terms of spatial extension (occupying an area of 16.243 km2) and the first in terms of the number of culled deer. Almost the totality of the sika harvested during the years in the country was, in fact, represented by animals culled in these districts (Fig. 24). The SP1 was also the subpopulation in which the culled deer incremented the most. The SP2, which occupied a small number of districts in the Northern part of the country (covering an area of about 2.760 km2) had, instead, the lowest increase. The third and last subpopulation SP3, situated in the Eastern part of the Czech Republic (covering an area of about 18.279 km2), ranked second for the total number of overall culled deer. Even though all three areas were covered by vegetation for the almost totality, the vegetal composition was different among the ranges (Fig. 25). The SP2, specifically, was the one with the highest level of arable lands and the one with the smallest percentage of forest cover (Fig. 24), while the SP1 was the one with the highest presence of forested lands and the smallest of arable ones. Even though all the hunting subpopulations presented a certain degree of overlapping or proximity with observations of wolves, the SP2 was the one with the most overlapping range, while SP3 had the least, with only few wolf observations (wolfoccurrence: Lososová et al. 2019) (Fig. 6).

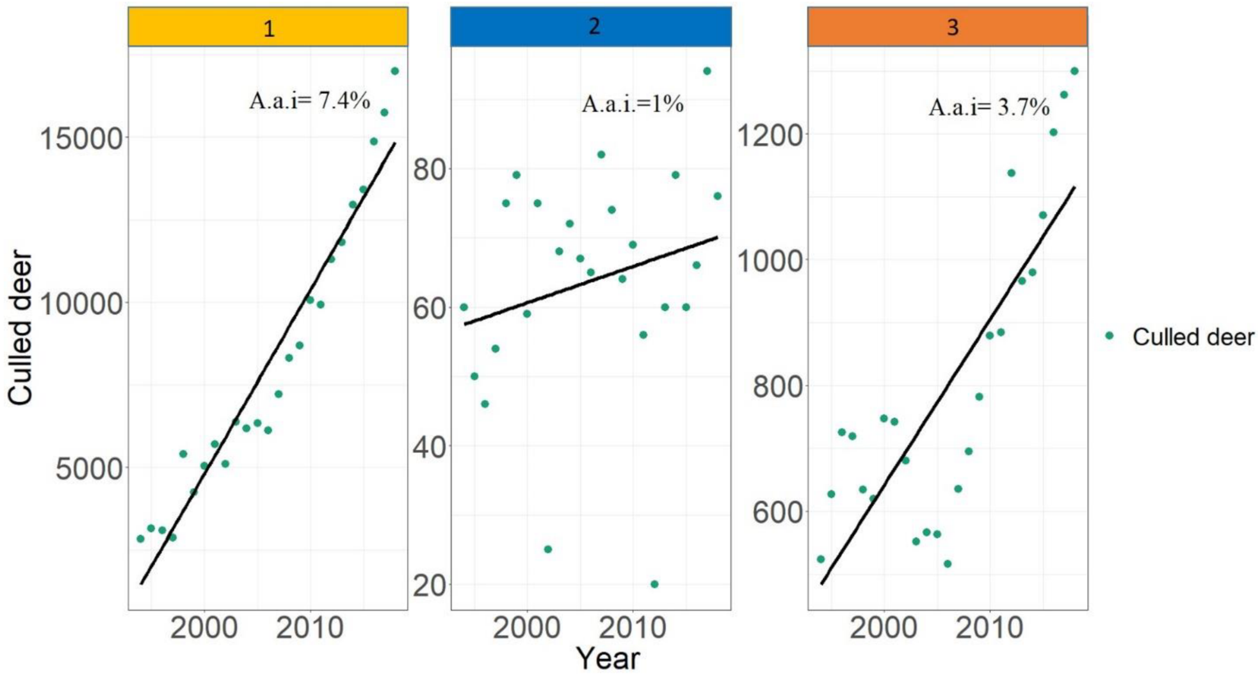


Figure 23 Trends of sika deer culled in the Czech Republic in the three subpopulations (1994-2018) and annual average percentage of increase (A.a.i)

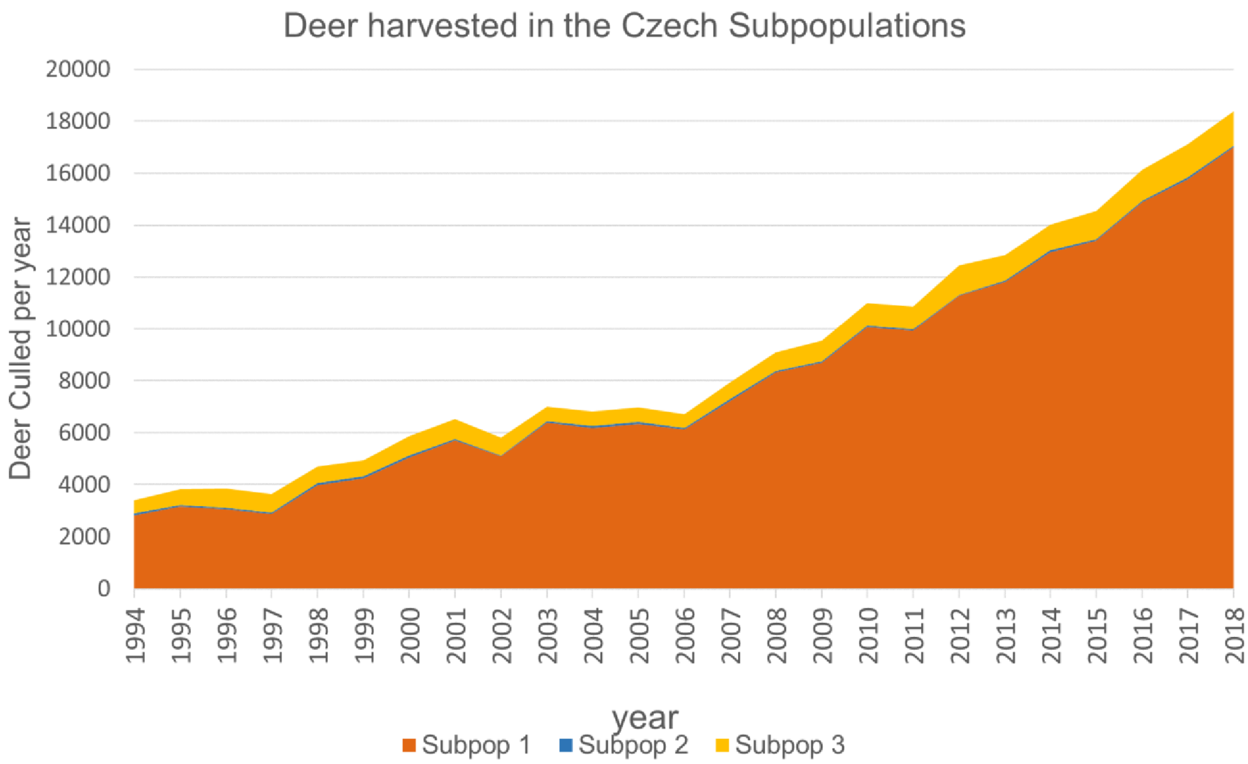


Figure 24 Sika deer culled in the Czech Republic by year and by subpopulation (1994-2018).

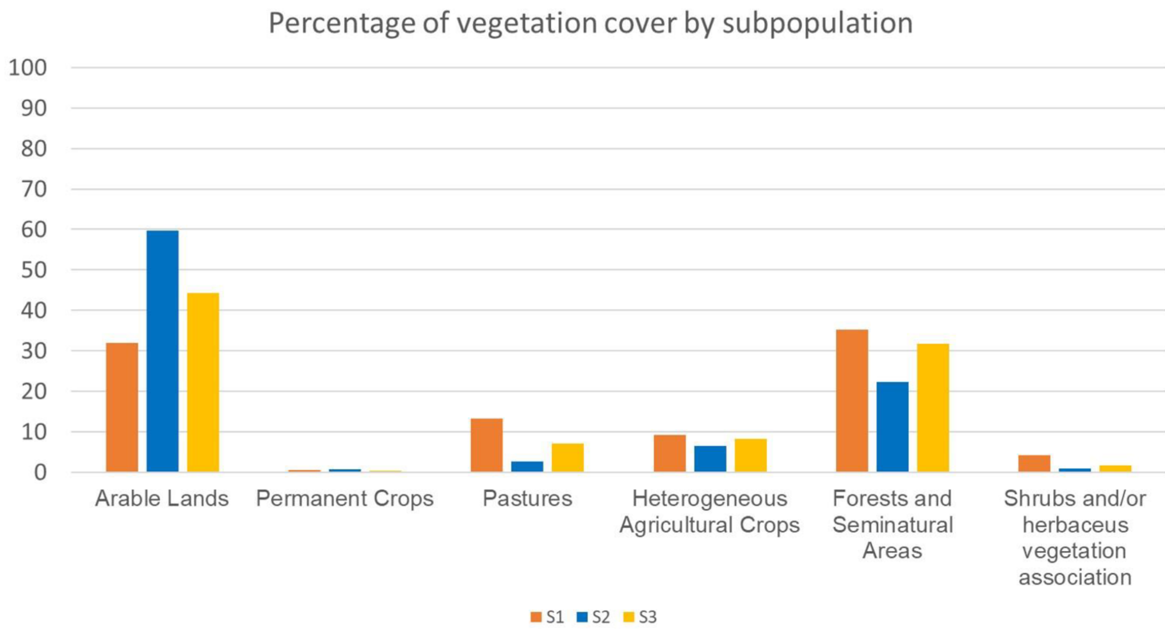


Figure 25 Percentage of land covered by different types of non-artificial vegetation in the three subpopulations areas

DNA metabarcoding and FTIR spectroscopic analysis of Sika deer feces in Lower Austria

DNA Metabarcoding

Out of the 40 samples analyzed 23 contained sequences attributable to known taxa. Our results consisted of 55.493 reads and 28 taxa identified, 10 dicotyledons, 5 monocotyledons, and 13 moss belonging to 21 genera, 4 families, 2 tribes, 1 order (Fig. 26 and 27).

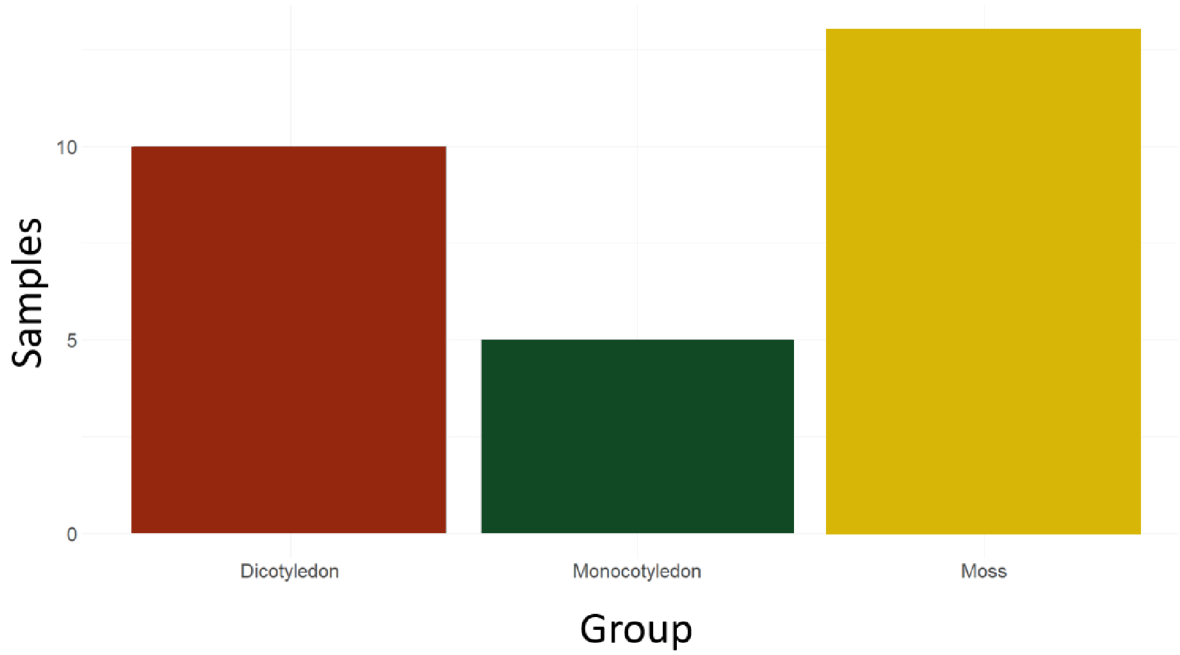


Figure 26 number of flowering plants (monocotyledon and dicotyledon) and mosses found in the sika deer fecal sample using the DNA metabarcoding analysis.

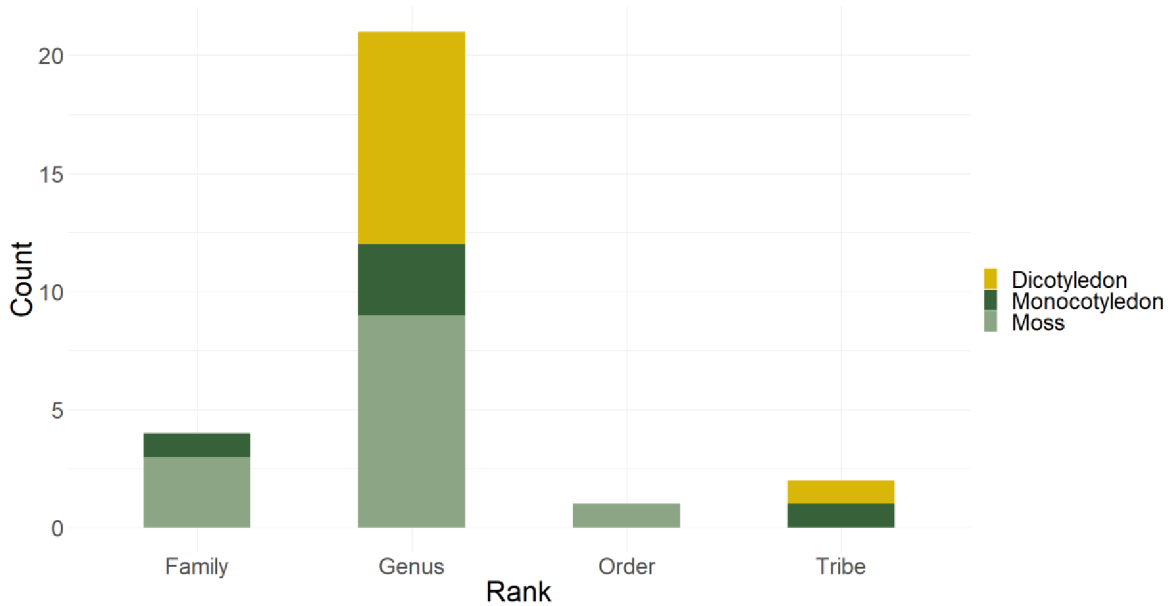


Figure 27 taxa found in the fecal samples of sika deer through the DNA barcoding analysis divided by type.

The most present taxon is "*Stellaria*" (Fig. 28), a cosmopolitan genus of herbaceous plants (Sharples & Tripp, 2019) found in 18 samples with 27592 reads (49%). Following the genus "*Clematis*", a predominantly climbing taxon with also shrubby or herbaceous species (Miikeda et al., 2006), (14 samples) and the genus "*Cornus*" consisting mainly of shrubs and small trees (Xiang et al., 2006), (12 samples) can be found (Fig. 28). Most present moss genus is "*Abietinella*", present in 5 samples (Fig. 28).

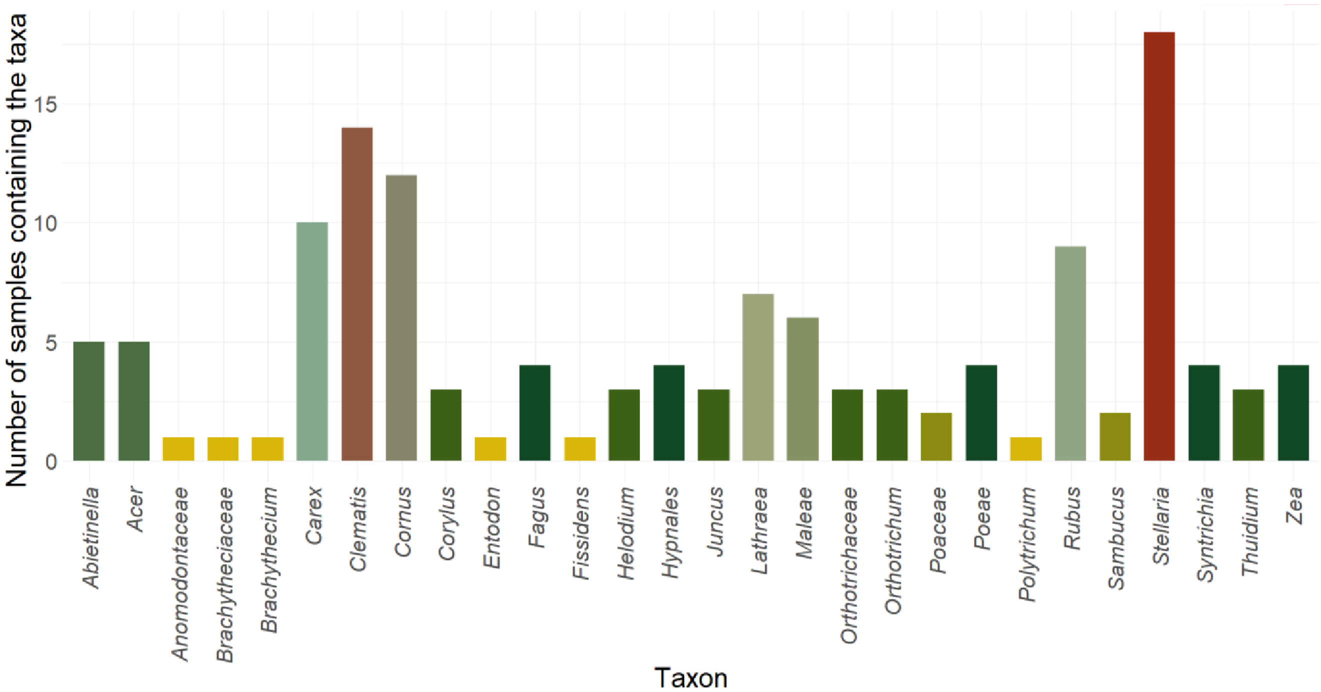


Figure 28 taxa found in the fecal samples of sika deer through the DNA barcoding analysis

Spectroscopy

The FTIR spectroscopy of sika deer fecal samples collected in the area of Zwentendorf (lower Austria) showed absorption peaks at 3391 nm, 2918 nm, 1618 nm, 1543 nm, 1427 nm, 1315 nm, 1313 nm, 999 nm, 872 nm, and 453 nm. (Fig. 29, 30, and 31). Our results show presence of C=O stretches, both symmetrical and asymmetrical, deriving from both oxalates and carboxylates (peaks 1618; 1543; 1315; 1313) (Table 13). Asymmetric and symmetric stretch of methylene are also present (peak 2918), as well as amino acids' carboxylates (peak 1427). The 999 peak may suggest C-O stretching of primary alcohol. Clay or mineral residues are also showed, which may indicate presence of soil's remains on the surface of our samples (peaks 3391; 872; 453).

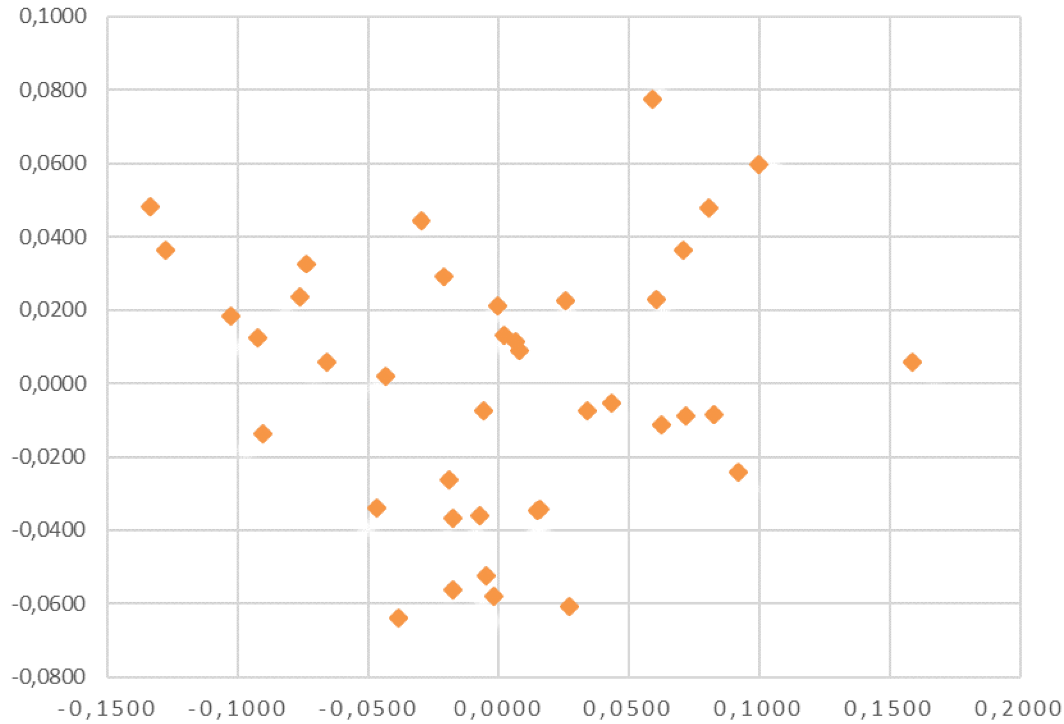


Figure 29 Scores' plot of a PCA based on FTIR spectra of sika deer droppings

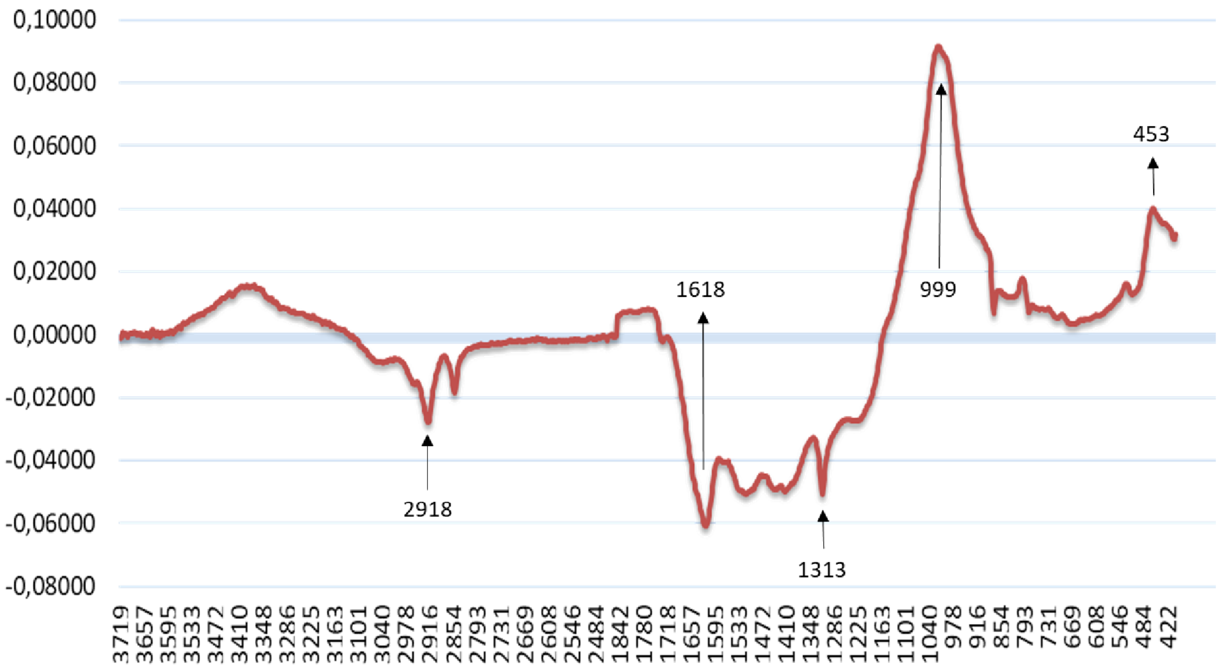


Figure 30 Spectral characteristics sika deer samples (PC2 62%)

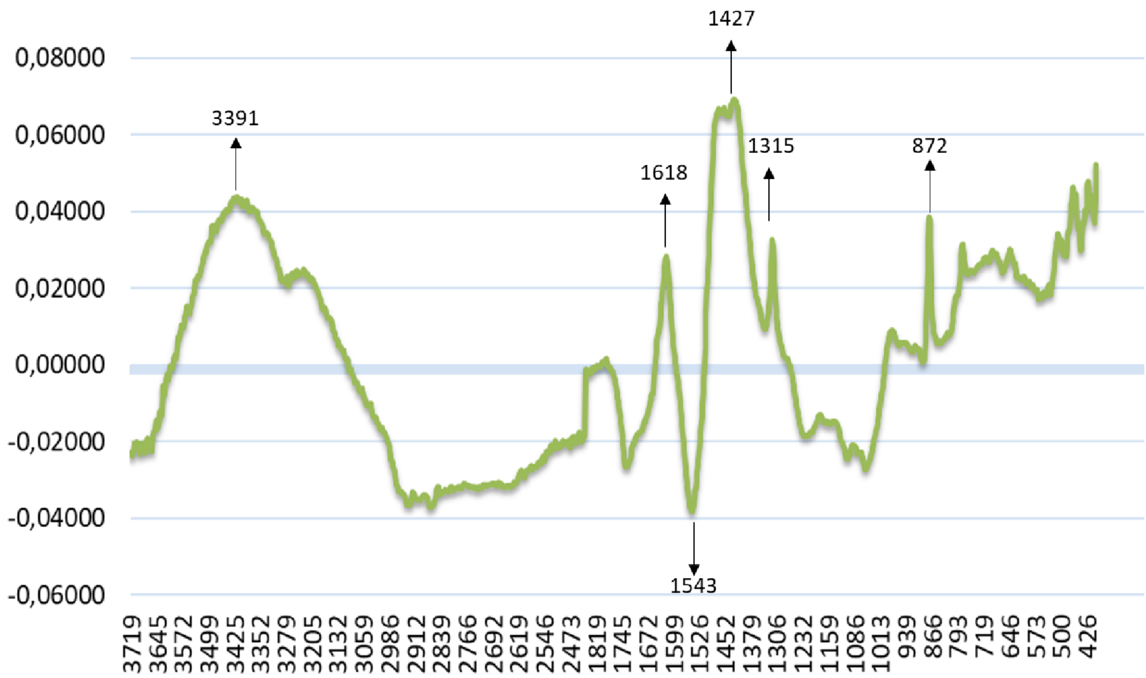


Figure 31 Spectral characteristics sika deer samples (PC2 18%)

Table 13 Assignment of band maxima to plausible origin according to literature

Band maxima in presented spectra	Plausible vibrational origin	Wavenumber range	Reference
3391	e.g. OH stretch in clay minerals (montmorillonite)	3600-3000	Madejová, 2003
2918	Symmetric and Asymmetric stretch of methylene groups	3000-2800	Meissl et al., 2008
1618	Asymmetric COO stretch (oxalate)	1622-1620	Piro et al., 2018 Tintner et al., 2018
1543	Asymmetric CO_2 stretch of carboxylates	1650-1540	Smith, 1998
1427	Carboxylates in amino acids	1420-1360	Mihoubi et al., 2017 Kumar et al., 2014
1315	Symmetric stretch COO (oxalate)	1320-1319	Piro et al., 2018 Tintner et al., 2018
1313	Symmetric stretch COO (oxalate)	1320-1319	Piro et al., 2018 Tintner et al., 2018
999	C-O stretching of primary alcohol	1047-900	Faix, 1992
872	calcite	875-870	Smidt et al., 2010; Bruckman & Wriessnig, 2013
453	e.g. clay minerals	470-420	Tinti et al., 2015

Discussions

What are we talking about? Sika deer (Cervus nippon): A bibliometric network analysis

The analysis of the available scientific literature on the sika deer showed that the most productive countries, apart from China and South Korea (i.e., UK, USA, Japan, Germany and the Czech Republic), are also the ones hosting the biggest and, in the case of non-Asian countries, the most invasive populations. China, on the other hand, ranked second even though it hosts a rather small, threatened, population of sika deer, and the majority of animals in the country lives in fenced areas, zoos, or deer parks. The results of the overlay maps and the keywords network maps show also that China is increasing the number of publications on the topic, while Japan stays consistent and the USA are becoming less productive. China, as it appears, is also opening the discussion to new medical topics. A rather recent topic discussed now also by Chinese researchers is, for example, the use of the antler to investigate the organ regeneration in mammals (Akhtar et al., 2019; Su et al., 2019), to develop new systems of dental implantation (He et al., 2018)., and for other pharmaceutical purposes (e.g., in Yang et al., 2017). To confirm the increasing relevance of Chinese literature, the keyword “China” is being used now way more than before, and it is often linked to other keywords such as “antler” and “velvet antler”, “pcr”, and “regenerations” also recently used in the literature. This result is particularly interesting because it goes in a new direction compared to the ancient Chinese medical tradition of utilizing sika deer part to cure several diseases, that largely contributed to the decreasing number of free ranging sika deer in the country. Anyhow, China is also linked to topics such as “mitochondrial DNA” suggesting studies focusing on analyses of the velvet antler that are a practical method to distinguish real velvet from counterfeit (Gu et al., 2013). Such practice has, as well, a relevant use in the pharmaceutical sector, even though, more related to ancient and traditional one (Gao et al., 2016).

The analysis of the most commonly used keywords also confirms the leadership of Japan, which ranked first not only for productivity, but also for degree of collaboration with other countries, and for the number of citations. In the top ten most used keywords we can find terms such as

“japan” and “sasa nipponica”, which refers to a low growing, evergreen dwarf bamboo found in the understory layer of broad-leaved forests of Japan (Takatsuki, 1983; Takatsuki, 1986).
Also worth noticing is the concurrent presence of Taiwan in the list of the ten most cited countries (9th place, with 289 citations) and its absence in the list of top countries for number of documents and total link strength. Such result suggest that Taiwan’s scientific production is not particularly wide, but it is highly quoted. Relevant is also the presence of “red deer” in the highest places of both occurrence and total link strength lists, together with topics such as “hybridization” and “introgression”, suggesting genetics as an important field of research in the sika deer scientific literature. The analysis of the global literature pointed out two main currents of research on the sika deer, one investigating the impacts of the species’ browsing activity on vegetation (mostly on the Sasa nipponica), and another one focusing on the issue of the hybridization with red deer.

Our analyses showed that, even being the sika deer known for its detrimental nature, the non-native literature on the species is still not sufficient, mostly when it comes to studies that focus on, and describe, the species’ impacts on the local vegetation, its diet composition or its food habits. Most, and sometimes, only, studied topics are in fact the hybridization with the red deer and, in the case of the USA the competition between sika and white-tailed deer, and the demographic history of sika populations in Delmarva Peninsula (East USA).

Even though non- native countries, and especially European ones, are recently opening the literature to new topics, mostly parasites and phylogeny, the role of the sika deer and its impacts on the ecosystems remains underestimated as it is confirmed by the keyword “management” that is not often found in the literature, and is not largely used if not in the very last years.

Evaluating the Management Success of an Alien Species Through Its Hunting Bags: The Case of the Sika Deer (Cervus Nippon) in the Czech Republic

In this study, we wanted to estimate the growth trends of the number of culled sika deer in the culling subpopulations present in the Czech Republic. To do so, we took into account the hunting bags and the environmental features of the areas occupied by the different subpopulations, to remark existing limits to a successful management of the species. Also, we investigated other sources of mortality and future perspectives for the management of this species. As Apollonio et al. (2010) have thoroughly discussed, the management of ungulates is a complex matter which should cover several issues, such as the damages that these species can cause to the environment, the role of ungulates as driver of habitat change, and the occurrence of road traffic incidents involving ungulates (Apollonio et al., 2010). Being the sika deer also present as an alien species in the Czech Republic, their monitoring, management, and hunting season and strategy should be faultlessly planned and carried out. In the last five decades, the number of sika deer in the Czech Republic has increased exponentially, which seems to suggest that the numbers are underestimated and, hence, the number of culled deer is not enough to maintain the population stable and safeguard the native species and the local vegetation. Thus, over the whole time interval (i.e., 25 years), the number of culled sika deer in the different areas of occupancyrevealed an alarming mean annual increase between 1 and 7.4. Reasons for the different harvesting trends shown in the three areas and for the different success of the management strategies could be found in the characteristics of the occupied areas, mostly with regards to parameters of largeness and vegetation cover. Among the three subpopulations, in fact, SP2 seems to be the easiest to manage in terms of counting and monitoring of deer (having a rather smaller area of occupancy) and, consequently, in planning hunting seasons. The scarcity of forested area in favor of an abundance of arable lands (Fig. 24) may also make it more difficult for the deer to hide, both during the operations of counting and during the hunting season. Conversely, SP1 and SP3 are spread across relatively larger areas hosting, as suggested by the high number of culled deer, a significantly denser deer population, which may make the monitoring process more susceptible to errors and miscounting. SP1 also presents a higher percentage of forested areas (Fig. 24) and close proximity to the Šumava National Park. Moreover, a denser population may lessen the effects of other sources of mortality such as traffic collisions and railway incidents. Unfortunately, for these two factors, it is not possible to estimate their impact on sikadeer due to the lack of data on the species. Even though data suitable to estimate the effects ofwolf predation on the species are not available, we consider it to be an important asset for the future management due to the wolves spreading across the country in recent times (Lososová et al., 2019). Considering the relatively small extension of the area and the limited number of sika deer deduced by the stable hunting bags, the presence of the wolf packs may represent, in the future, an additional factor to keep the deer population under control in this area and help making up for underestimated culling rates (Ripple & Beschta, 2012; Ripple et al., 2014). Nevertheless, the need of properly planned counts and of a better aimed management strategy, including the collection of complementary data (e.g., predation, WVC) has been proven to be essential and unavoidable.

DNA metabarcoding and FTIR spectroscopic analysis of Sika deer feces in Lower Austria

The most common methods used to analyze herbivore feces are direct observation, micro-histology. Both methods present limitations such as; mandatory high visibility and proneness to omissions for the former, and inaccuracy and a highly time-consuming nature for the latter (Kartzinel et al., 2015). DNA metabarcoding bests those methods in terms of practicability and is at least just as efficient (Kartzinel et al., 2015). Our study shows that sika deer have a rather diversified diet feeding on several taxa, and that different individuals may choose different plants belonging to several families and genera. Out of 28 taxa, in fact, only *Stellaria* and *Clematis* were present in more than half of the samples, while the other 26 were identified in only a few. Our study also confirms that FTIR, which showed several absorption peaks may represent a high-potential, non-invasive method of investigation to analyze cervid diet. Our spectroscopic analysis showed presence of oxalates, commonly found in plants (crops and pasture weeds (Libert & Franceschi, 1987)), and methylene (CH2) bands with maxima at 2918 cm−1 which represent the backbone of many macromolecules. This band indicate degradation and stabilization of organic matter during microbial transformation processes (Smidt et al., 2005). The presence of peaks linked to clay and minerals, also shows the need to clean the sample or scratch the surface before the pulverization.

To achieve better, more comprehensive, and clearer results however, a higher number of samples is advised, as well as an aimed collection and analysis of the eaten plants, in order to correlate the spectra of undigested (fresh) and digested (fecal) samples and perform a more detailed analysis of the quality of the animals' diet.

Conclusions

What are we talking about? Sika deer (Cervus nippon): A bibliometric network analysis

In this study, we explored the global scientific literature produced on sika deer by performing bibliometric analyses. Our research pointed out that sika deer is a widely studied species as countries in Asia, Europe, Africa, and America have, during the years, produced scientific documents on the species. Our results showed that the literature of different areas of investigations (i.e., native range area and non-native range areas) explored different main research topics, andthat these were well defined and consistent in time. It appeared clear that non-native literature has its main area of interest in the role that sika plays as an alien species, and the great deal of damages that it causes to the native species such as red deer. Even though it is known that sika also damages other parts of the natural patrimony such as vegetation and ecosystems,these effects did not seem to have been extensively explored in the non-native range. Native literature was, instead, primarily interested in the effects of sika (mostly of the growing and abundant population of Japan) on the vegetation, which included also specific cases of study, asthe *Sasa nipponica*, the dwarf bamboo. Our study also allowed us to inspect the countries and keywords which had an increase in production or occurrence in recent years. As confirmed byboth our analyses, it is clear that China is increasing the number of publications on sika deer, also opening the literature to new lines of research that connect the species with medical and pharmaceutical topics. Surprisingly, even though the explored literatures based the majority of their documents on damages and issues related to the species, the keyword "management" started being widely used only four years ago. Our investigation on the occurrence of keywords in Scopus and VOSviewer, and on their burst, using Web of Science and CiteSpace, produced rather homogeneous results, pointing out the usefulness of integrating different methodologies and approaches, through the support of informatics tools, to investigate and corroborate the patterns and trends in scientific literature.

Evaluating the Management Success of an Alien Species Through Its Hunting Bags: The Case of the Sika Deer (Cervus Nippon) in the Czech Republic

The Czech Republic hosts a high, permanently increasing, number of sika deer. The hunting pressure and the hunting control exerted following the current system and practice are not sufficient to keep the population stable and contained, especially in the Western part of the country. Sika are well known for the significant impacts that their browsing, debarking, and grazing activities have on ecosystems and for the effects that the hybridization with red deer has on the latter species' genetics and conservation. Moreover, large herbivores, when in high density, can also become a threat to the safety of citizen due to the increasing number of traffic-related accidents. Considering the well documented rising number of sika deer in the country and the damages to both flora and fauna, and taking into account both the low efficiency of the management strategies so far carried out and the low impact of the exiguous number of predators currently living in the country, we conclude that investing more resources to improve the quality of hunting plans, as well as to implement the management strategies, seems to be an impelling need for the Czech Republic, especially in the Western part of the country.

DNA metabarcoding and FTIR spectroscopic analysis of Sika deer feces in Lower Austria

In this study we aimed to investigate the feeding behavior of sika deer, analyzing fecal content and chemical structure. Sika are highly adaptable, have a various diet, and feed on a rather wide range of taxa. In Japan, sika have been observed to rely mostly on preferred plants (one above the others the *Sasa nipponica*) during the spring and winter but, they can easily rely on other sources of food. Due to the adaptability of the species and to its ability to thrive in different ecosystems and stressed environments, *ad hoc* studies are necessary when analyzing its feeding behavior and diet composition. DNA metabarcoding represents an adequate method for the analysis of deer' diet but, since it is also prone to errors a large sample number is highly advised. Similarly, FTIR spectroscopy constitutes a rapid and non-invasive method of investigation for the chemical composition of the fecal matter but, to obtain clear results, an adequate planning of the sampling activity is mandatory and it should also include the collection of plant samples, to perform a comparison between fresh and digested ones and achieve comprehensive results.

A deeper knowledge of sika deer diet may represent an important step in evaluating the impact of this alien species in the host environments, and the analysis of the chemical matter of feces may help explore the adaptability of the species, its spreading in various habitats, and the competition with other ungulates.

General Discussion

The sika deer is a member of the Asian fauna, but it can be found in many other countries. In several of those, it established invasive populations which are severely detrimental for the local flora and fauna. Hybridization with native species such as the red deer, and loss of types of vegetation due to the overbrowsing and bark stripping are, in fact, examples of described effects of sika deer populations. As documented in the native countries, and especially in Japan, sika deer are the cause of numerous damages to the vegetation and are also source of human-wildlife conflicts due to their preference for crops and agricultural land. Sika deer effects on native flora and fauna have also been described in non-native environments such as, the UK where their grazing activity led to the loss of different types of vegetation, or Ukraine, France, and Czech Republic where the species brought a parasite, the *Ashworthius sidemi* which spread and infected other ungulates.

In this thesis we investigated patterns and trends in the global scientific literature on the sika deer carrying out a bibliometric analysis, a technique increasingly used to quantitatively review the scientific literature across various disciplines. To achieve our goal, we jointly used two software tools (VOSviewer and CiteSpace). It did not surprise that, as our results showed, the most productive country on sika deer was Japan, and that the keyword "Japan" ranked 4th among the most used ones. In the country, in fact, the deer is largely present and, in the last decades, their number rapidly rose also possibly thanks to the absence of natural predator and the climate change which reduced snow and fawn mortality, as well as ban on the hunting of the species. What was surprising, is that Japanese researcher authored the 47% of the total number of documents available on the Scopus database. The presence of "*Sasa nipponica*" (a dwarf bamboo found in Japan) in the top ten of the most used terms (8th place) seems to corroborate the country undeniable leading role in the scientific production on the species, and the large number of studies focused on the feeding behavior of the deer and its impact on Japanese flora. Japan also resulted to be the first authoring country by number of citations and co-authorship with other productive countries such as China and USA, respectively, the second and the third by number of documents published. It is worth noticing, analyzing the temporal trend of productivity, that while Japan stayed stable and the USA lowered the amount of work, China showed an increase in the number of published documents. The analysis of the author keywords confirmed this trend and showed “China” as a recently used keyword. The situation of the sika deer in China is rather complex, being the species threatened and protected throughout the country, and largely farmed. The utilization of sika deer part in the traditional medicine took a huge toll on the wild population and the institution of reserves was necessary. Today, our study shows that China is opening the literature on sika deer to new medical topics, mostly focusing the attention on the use of the sika deer antler to investigate the organ regeneration in mammals, and to develop new systems of dental implantation.

The analysis of the global literature showed also the presence in the list of the most used keywords, of terms such as “red deer”, “hybridization” and “introgression”. This suggests the importance of topics related to the relationship between the two species and the genetic and ecological consequences of their overlapping. Our study highlighted that two main currents of research on the sika deer, one investigating the impacts of the species’ browsing activity on vegetation (mostly on the *Sasa nipponica*), and another one focusing on the issue of the hybridization with red deer.

Isolating the scientific literature produced by sika deer native countries, which represents the 75% of the total available production, Viet Nam appeared to be the least productive one, probably also due to the extinct state of the species in the country, while Japan and China were not only the most productive, but also the most prone to collaborations among themselves and with European countries, USA, and New Zealand. Limiting our database only to countries belonging to the native range of the sika deer, the topic of hybridization is absent, leaving room to subjects like food habits and vegetation regenerations with relative damages. Unfortunately, part of this literature, being in the local language, is not available to everybody.

Despite its harmful nature, the non-native literature on the species is still defective and insufficient lacking, apart from a few cases, studies that focus on the impacts of the species on the local vegetation, on its diet composition or food habits, and showing a rather limited range of topics focusing mostly, and in some cases solely, on the hybridization with local red deer. The USA focus mostly on the competition between sika and white-tailed deer, the most occurring deer of North America which also represents an economic asset for the country. According to the temporal trend analysis of keywords, European countries are opening the literature to new topics, mostly parasites and phylogeny. This seems to be a necessary step due to the severe impacts that sika deer diseases had on the local fauna and the livestock, as well as the uncertainty that is still related to the origin of European populations and to the subspecies that are present in the continent.

It does not surprise that in Europe the three most prolific countries, UK, Germany and the Czech Republic are also the ones hosting the largest and most numerous populations of sika deer but it surprises that the keyword “management” does not show often in their publications, and has not been largely used if not in the very last years. This, in the case of the Czech Republic, reflects just accurately the practical management of the species which has been, in the last decades, faulty. Sika deer have, in fact, spread across the country reaching alarming high numbers. In this thesis, we identified three areas in the Czech Republic, separated by natural or urban features, in which sika deer are annually or regularly shot, and we estimated the trends of the number of culled sika deer in these three areas. They showed a rather inhomogeneous pattern. The Western and Easter culling subpopulations, in fact, were showed to be experiencing a dramatic increase in the number of shot animals, while the central one resulted to be rather stable with a small increment.

Among the biggest limits to a successful management of the species, we identified, first, a non-adequately carried out spring count. The number of sika deer living in the country appears, in fact, to be severely underestimated and, therefore, the number of the deer culled every year is not enough to maintain the population stable and safeguard the native species and the local vegetation. This is manly true in the Easter and Western part of the country probably due to the sika deer living there in bigger, denser subpopulations and to the higher presence of forests. Sika deer may hide very well in forested areas and escape to both the counting and the hunting process. The central area, being be smaller, hosts less animals, and presents less forested areas, in favor of a higher percentage of arable lands which may easier the identification, the counting, and the culling of sika.

Second obstacle to a proper management, according to our research, is the lack of data on the species mortality for causes others than the hunting activity, for example traffic incidents and predation. Even though data suitable to estimate the effects of wolf predation on the species are not available, we consider it to be an important asset for the future management due to the wolves spreading across the country in recent times (Lososová et al., 2019). In the smallest culling subpopulation, in particular, considering its relatively small extension and the limited number of sika deer deduced by the stable hunting bags, the presence of the wolf packs may represent, in the future, an additional factor to keep the deer population under control in this area and help making up for underestimated culling rates (Ripple & Beschta, 2012; Ripple et al., 2014). Similarly, the collection of complementary data such as rate of collision with vehicles may represent an asset for a better management.

As Apollonio et al. (2010) have thoroughly discussed, in fact, the management of ungulates is a complex matter which should cover several issues, such as the damages that these species can cause to the environment, the role of ungulates as driver of habitat change, and the occurrence of road traffic incidents involving ungulates (Apollonio et al., 2010). Being the sika deer also present as an alien species in the Czech Republic, and being the country hosting one of the three biggest population in Europe, their monitoring, management, and hunting season and strategy should be faultlessly planned and carried out for the safeguard of both the native species and the local vegetation. In Europe sika deer have been, by now, widely recognized as a serious threat for the populations of red deer (also specifically in the Czech Republic) and have also been identified as a source of severe damages to ecosystems, and to citizens through their involvement in traffic incidents. A better and more efficient management is, then, an impelling, essential and, unavoidable need for the country, and may be achieved with a fitter designed management plan, a more precise and better structured spring counting, and with the collection of supplementary data.

Also Austria hosts free ranging populations of sika, but not much is known about them. Austria also hosts populations of other deer species, with which the sika overlaps and may hybridize and compete, and is present in subpopulations that are expanding. Still, the subject is rather underestimated in the scientific literature with only 16 papers mentioning the sika deer in 75 years. In our study we tried to investigate the diet of a population of sika deer in Lower Austria living in a rather small and dense area with a rather stressed vegetation. We tested two methods of analysis, DNA metabarcoding to explore the species ingested, and FTIR spectroscopy to inspect the chemical content of the deer fecal matter, on sika deer fecal samples. Even though the methods have been already tested on cervid species, to our knowledge this was not done on sika deer in Europe. The knowledge of the diet of a species is crucial to understand its role in the ecosystem, and being the sika deer highly adaptable and having a great local variation in food habits, proper study on different populations are necessary.

The results of our preliminary study seemed to suggest that sika deer living in a rather stressed environment have a varied diet and do not show a specific preference for any taxa. The sample presented, in fact, traces of both flowering plants and mosses, and different families and genera. Also the FTIR gave rather clear results showing several peaks of absorption. Anyhow, even though our study may represent a good starting point for future studies, to achieve better, more comprehensive and clearer results, a higher number of samples is advised, as well as an aimed collection and analysis of the eaten plants, in order to correlate the spectra of undigested (fresh) and digested (fecal) samples and perform a more detailed analysis of the quality of the animals' diet. Last, FTIR spectroscopy showed clay or mineral residues, which may indicate presence of soil's remains on the surface of our samples and, therefore, the need to scratch the sample surface during their preparation.

Sika deer have been present in several countries of Europe for a long time, and their detrimental role has been widely discussed but, still, many ecological and behavioral aspects of the species remain unexplored or understudied. The underestimation of the sika deer effects and role in damaging the hosting ecosystems is also shown in the fact that the species is known to be a detrimental invasive species, but it is not mentioned in the EU Regulation 1143/2014 on Invasive Alien Species. Moreover, their diet, which may represent a great tool to better understand their role in the host ecosystems and in the food webs, is rarely investigated, and their management is still faulty. We believe that, given the alien nature of the species, its adaptability, and its ability to survive and thrive in unfavorable environments, as well as the well-known hybridization occurring with the native red deer, a deeper knowledge and a better attention are crucial points for the future.

General Conclusions

The findings of the presented dissertation thesis contribute to the scientific knowledge on sika deer assessing the perception of the species in the available literature, its role as an alien species, and overlooked ecological traits of alien populations.
Particularly, the thesis provides emphasis on: *i)* research trends and discussed topic,
ii) blind spots and unexplored issues related to the species,
iii) the management of one of the biggest and most invasive populations of Europe,
iv) the diet of a severely unobserved alien population,
v) the testing of methods of analysis for future studies,
and *vi)* possible future perspectives.

The sika deer is a highly adaptable, likely to became invasive species which is able to compete with other ungulates, even bigger in size, and hybridize with the red deer. Ongoing climate change, and consequent lower snow rates, may easily increase the invasiveness of the species in the near future mitigating the fawn death rate due to high snow and let it thrive in warmest environments. Similarly, the adaptability of sika and their elusive nature may pose a challenge for the management, mostly in dense and big populations, and their flexibility when it comes to feeding may facilitate the establishment and the prosper of invasive nuclei. Being the species highly versatile and able to adapt to different environments and habitats, the study of the ecological characteristics requires local and ad hoc planned studies. Nonetheless, the threat that the species poses on ecosystems, local fauna, and local economies, calls for a higher management and research effort.
The results of this thesis emphasize the importance of planning better managements actions, design more adequate hunting seasons, and increase the knowledge on the sika deer ecological traits, one above all, its diet and food habits. The latter goal may be achieved by using modern, non-invasive methods but a properly designed study is necessary. Our last study, in fact, proved the feasibility of the general methodology, however, we suggest to collect not only fecal matter, but also the raw plant material in order to compare the spectra and make clearer assumptions and reach clearer results. We also suggest to collect more samples due to the non-infallibility of the methods and the sample preparation process. Moreover, in this multimethod and multidisciplinary thesis we pointed out the usefulness of a bibliometric analysis which may help

to understand the full range of scientific output on a specific topic and plan future studies and research. Additionally, we highlighted the usefulness of integrating different methodologies and approaches, through the support of informatics tools, to investigate and corroborate the patterns and trends in scientific literature.

This thesis provided a new understanding of the global situation of the species, and new insights of the effect of sika deer on ecosystems and of overlooked populations, and proposed a new spatial approach to support the spatial management of the species through the hunting bags. In conclusion, even advising further studies on the topic, we consider the aims of our study reached.

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