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Influence of management and biological factors on parasitic invasions in the wild – Spread of the blood-sucking nematode *Ashworthius sidemi* in European bison (*Bison bonasus*)

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A B S T R A C T

The full course of new parasite introductions in wild animals is difficult to accurately trace. We documented and analysed the invasive blood-sucking nematode *Ashworthius sidemi* (Trichostrongylidae) introduction and spread in European bison (*Bison bonasus*) from the initial phase of its progression. In the Polish part of the Białowieża Primeval Forest (BPF) the parasite was first found in 2000. From 2002 to 2015, 165 culled bison were investigated. The prevalence and intensity of *A. sidemi* Schulz, 1933 infection increased over the following years, reaching 100% of investigated bison four years after introduction and a maximal median intensity of 8200 nematodes per animal in the winter of 2008/2009. Afterwards, a significant decline of median infection intensity was observed to the minimum value of 410 nematodes per animal. Between 2011 and 2014 prevalence varied from 89 to 100%. Among the factors analysed, the number of years since introduction, herd size, age and sex proved to significantly influence infection intensity. A higher infection intensity was recorded in sub-adults compared to juveniles and adults. Males had significantly lower infection intensity than females, but this was the case for adults only. The highest infection intensities were recorded in the biggest bison herds, where the winter supplementary feeding of bison is intense. Moreover, the longer the parasite was present in the host population, the more important herd size became as a factor. Our study indicates that it is not solely biological factors that determine the spread of a newly detected parasite in wildlife, but that management practices can also have a strong influence. This is especially important in endangered species under intensive human care as the management practices may pose a threat to the species.

1. Introduction

Parasitic infections which emerge in wildlife may have a significant effect on individuals and populations (Møller, 2005). This is especially a critical issue in the conservation of threatened species (Thompson et al., 2010). Therefore, understanding the role of infectious and parasitic agents in wildlife populations, such as how they can influence fitness and in severe cases lead to population decline or extinction, requires accurate data on the diversity and load of potential pathogens in natural ecosystems, especially at the local scale (Smith et al., 2009).

The appearance and spread of new parasites in populations of wild animals is not well documented. Most co-evolution studies are conducted with microbial organisms under laboratory conditions, taking advantage of their short generation times (Levin and Lenski, 1983; Buckling and Rainey, 2002; Gandon et al., 2008). However, such simplified artificial models do not reflect the situation in wild populations where infected animals are additionally affected by a number of environmental factors. The difficulty in conducting such studies in wildlife results from both the difficulty in capturing the start point of the introduction of a new pathogen to a population and problems associated with long-term continuous acquisition of material for research.

The European bison (*Bison bonasus* L., 1758) is one of the last mega-herbivores to survive in Europe. Extinct in the wild at the beginning of 20th century, it was restored back to the wild from captive survivors. Nowadays there are over 3500 European bison in

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the wild distributed in 35 isolated populations (Raczyński, 2015). Bison numbers are growing; however, only five populations number more than 200 individuals. Apart from small herd size, isolation and low genetic variation due to a severe genetic bottleneck after extinction in the wild (Tokarska et al., 2011), diseases and parasites (Pucek et al., 2004) are the main threats to bison.

The oldest and largest population of bison is in the Białowieża Primeval Forest (BPF) (984 individuals, in both the Polish and Belarusian parts of BPF), constituting the core of the global population of the species. Since reintroduction to the wild in 1952, this population has been parasitologically investigated and monitored at differing intensities. A total of 88 species of parasites have been discovered in European bison, with an increasing trend in species richness as well as in the prevalence and intensity of infections (Dróżdż, 1995; Karbowiak et al., 2014a,b). This may be a result of transmission from other wild ruminants and cattle, or the increased contact rate between bison in winter due to large aggregations arising from supplementary feeding in fixed locations (Radwan et al., 2010; Pyziel et al., 2011; Karbowiak et al., 2014b). One of the most pathogenic parasites discovered, the blood-sucking nematode *Ashworthius sidemi* Schulz, 1933 was first found in bison in BPF in 2000. The other blood-sucking nematode – *Haemonchus contortus* – was described in bison in BPF in the 1960s, but only in captivity (Dróżdż, 1961, 1967). *A. sidemi* was also found in the neighboring Knyszyn Forest in 2009 (Demiaszkiewicz et al., 2009b). The species is characteristic of Asiatic deer, especially sika deer *Cervus nippon*, and was probably transmitted to Poland from red deer *Cervus elaphus* from Ukraine and Slovak Republic, where sika deer has been previously introduced (Kotrlá and Kotrlý, 1973, 1977; Dróżdż et al., 1998; Demiaszkiewicz et al., 2008). Over the last decade the parasite was found in NE and SE Poland in species such as red deer, roe deer *Capreolus capreolus*, moose *Alces alces*, and European bison (Dróżdż et al., 2003; Demiaszkiewicz et al., 2009a, 2013). *A. sidemi* was also found in deer in France (Ferte et al., 2000). In recent years, the presence of *A. sidemi* was genetically confirmed in cattle, which indicates possible transmission from wildlife to livestock (Moskwa et al., 2015). Previous studies from BPF have shown the influence of winter bison aggregation on *A. sidemi* and coccidia infection (Radwan et al., 2010; Pyziel et al., 2011) and on the seasonal pattern of parasite egg excretion (Kołodziej-Sobocińska et al., 2016a).

The aim of our study was the long-term analysis of the invasive *A. sidemi* spread since its appearance in the free ranging population of European bison in BPF and to determine the factors affecting its intensity. We hypothesized that after the new, invasive parasite was introduced to the bison population, a phase of rapid growth in prevalence and intensity of *A. sidemi* was due to the susceptibility of the animals to infection and a higher likelihood of disease transmission. In the bison population, we presume that *A. sidemi* spread is significantly favoured by the high bison densities in large herds which form at fixed locations where supplementary winter feeding is provided, as well as biological factors such as age and sex. Knowledge about the spread of this parasite in wild hosts and particularly about factors which influence its prevalence and intensity may help to model the spread of emerging diseases in wildlife and guide the conservation management of wild endangered fauna.

2. Materials and methods

2.1. Ethics statement

No animals were killed specifically for this study. Bison were culled by rifle by Białowieża National Park staff, as approved by the management plan. The bison population in BPF has been regulated, on average, by approximately 11% annually since the 1970s through culling or translocation (Hayward et al., 2011; Krasińska and Krasiński, 2013). Białowieża National Park staff culled bison under permissions issued each year by the Ministry of Environment and the General Directorate for Environmental Protection (Warsaw, Poland). Tissue samples were collected from culled animals under the permission of the Provincial Wildlife Conservator in the years 1999–2005, and from 2006 under permissions issued each year by the Ministry of Environment, General Directorate for Environmental Protection (Warsaw, Poland) and Regional Directorate for Environmental Protection (Bialystok, Poland). For this research the guidelines of the Polish Nature Conservation Act adopted on October 16, 1991 (Dz.U. Nr 99, poz.1079) and on April 16, 2004 (Dz.U. 2004 Nr 92 poz. 880) were followed.

2.2. Study area

The study was conducted in the Białowieża Primeval Forest (BPF; 52°29′-52°37′N, 23°31′-24°21′E) located on the Polish-Belarusian border. It is one of the best preserved lowland forests in Europe inhabited by well-preserved communities of animals. The Polish part of the forest (600 km^2) is covered mainly by deciduous and mixed tree stands (94%); open habitats constitute the remaining 6% (Sokołowski, 2004).

The climate of BPF is transitional between Atlantic and continental type with clearly marked cold and warm seasons. Mean annual temperature between 2000 and 2015 was +8.0 °C; mean temperature of the coldest month, January, was −3.5 °C, and the warmest, July, was +19.9 °C. The vegetative season lasts, on average, 215 days (range: 198–238) and was calculated according to Tylkowski (2013). Snow cover persisted from 41 to 120 days per year with a maximum recorded depth of 55 cm. Mean annual precipitation was 650 mm.

The studied bison population is widespread in the Polish part of BPF across an area of 800 km^2 (Kowalczyk et al., 2013). The population of 522 individuals, i.e. 15% of all wild living bison (Raczyński, 2015), is isolated from the population in the Belarussian part of the forest by a fence constructed on the border in 1981(Kowalczyk et al., 2012). During snow-free periods bison males live solitarily (62% of males) or in small bull groups (up to 8 ind.), while females with calves and sub-adults roam in mixed groups numbering, on average, 11–15 individuals (Krasińska and Krasiński, 1995; Krasińska et al., 2000). Herd size increases during the rutting season (August–October). In winter, bison are supplementary fed with different intensities and aggregate around seven main feeding sites. Supplementary fed bison stay in a limited area for up to five months creating herds of up to 100 individuals. During the first part of the winter they consume hay stored in roofed haystacks in fixed locations; later in winter, the supplementary food (usually hay) is delivered to the forest floor in these locations. Some bison roam in winter out of the feeding sites, usually utilizing hay left by farmers on mowed meadows out of the forest or grazing on winter crops of rape and cereals (Hofman-Kamińska and Kowalczyk, 2012; Kowalczyk et al., 2011, 2013). There is relatively low number of cattle grazing in neighboring to the Forest pastures, however around 15% of bison, occasionally or seasonally and mainly from autumn until early spring, forage on pastures utilized also by cattle (Kowalczyk et al., 2013). This indicates possibility of interspecies parasite transmission (Moskwa et al., 2015).

2.3. Parasitological examinations

The *A. sidemi* infection was closely monitored from 2000 onwards. Before 2000 no infected animals were recorded (Dróżdż, 1961; Dróżdż et al., 2002). Only from the 1980s to 2000, 41 bison were investigated and no *A. sidemi* was found (Dróżdż et al., 1989, 1994). In 2000–2001, three of the 19 investigated bison were infected with *A. sidemi*, but due to incomplete data on bison sex, age, and body weight or herd size, they were not included in the model, but were used for prevalence calculations. From 2002 to 2015, 165 culled bison were investigated (from 4 to 22 individuals per winter season) (see Table 1). Five individuals which had been released from captivity into the wild were excluded from the analysis due to their infection by a high number of nematodes (max. 77,600) in a short period of time after release. This may have resulted from their different susceptibility to infection, related to their lack of contact with the parasite in captivity as a result of regular deworming.

The contents of the whole of the abomasa and a 1 m long section of the duodena of culled bison were examined by scraping and sedimentation method (Dróżdż et al., 1998; Demiaszkiewicz et al., 2012). The sediment preserved in 2–3% formalin was diluted with water up to 2 l and thoroughly mixed. A 200 ml aliquot was examined in small portions under a dissecting microscope in order to collect all helminths. Helminths were placed in a mixture of 75% ethanol and 5% glycerol. After evaporation of the alcohol, non-permanent preparations were made from all selected nematodes for species identification. Nematode species were determined morphologically on the basis of Lichtenfels et al. (1994), Jacquiet et al. (1997) and Dróżdż et al. (1998) and the number of *A. sidemi* were counted.

2.4. Statistical analyses

For the analysis of infection intensity we used 160 individuals with a full set of data from 2002 to 2015. To model which factors affected *A. sidemi* infection intensity (actual number of nematodes), we used the following set of independent variables: bison sex, age, body weight, herd size, feeding intensity, and the number of years since parasite introduction. Herd size was determined during the annual inventory of bison in BPF carried out by Białowieża National Park staff. Examined bison were divided into three groups depending on the intensity of supplementary feeding provided in winter: intensively fed bison – food delivered to the feeding sites three to five times a week; less intensively fed bison – food delivered once a week, and non-fed bison – bison not utilizing feeding sites. All culled bison were weighed on a floor scale. Bison age was expressed in three age classes: juveniles (up to 1 year old), sub-adults (2–3 years old) and adults (over 3 years old). We checked for multi-collinearity among explanatory variables, and the following non-correlated ($R < 0.5$) most informative covariates were included in the final model: bison sex, age, body weight, herd size and number of years since introduction. Then, we fitted negative binomial generalized linear models (negative binomial GLM) to count data, which dealt with observed model over-dispersion (Zuur et al., 2009). The number of *A. sidemi* per animal was set as a dependent variable and as main effects, we applied bison age, sex, body mass, and as interactive effects: number of years since introduction with herd size and body mass. The Akaike Information Criterion (AIC) with second-order correction for small sample size (AICc; Burnham and Anderson, 2002) was used for model ranking. Beforehand, out of the set of all possible combinations of models, based on model nesting, we excluded more complex versions of models which had lower AICc scores (Richards, 2008; Richards et al., 2010). The high-ranked top model was chosen as the best model. We checked the normality and homoscedasticity in the distribution of the final model residuals by inspecting the quantile-quantile distribution plot and model residuals against plots of fitted values (estimated responses). All statistical analyses were performed in the R program (version 3.1.2; R Development Core Team, 2012).

Table 1
The number of investigated European bison each year, taking into account the sex and age of the animals.

Winter season	Juv	Subad	Adult females	Adult males	Total
2002/2003	4	0	1	2	7
2003/2004	0	2	2	0	4
2004/2005	10	0	8	2	20
2005/2006	7	6	3	2	18
2006/2007	8	4	9	1	22
2007/2008	7	4	5	1	17
2008/2009	7	5	2	2	16
2009/2010	2	1	2	6	11
2010/2011	3	1	1	2	7
2011/2012	2	0	4	3	9
2012/2013	6	2	4	6	18
2013/2014	1	0	3	3	7
2014/2015	0	0	2	2	4
Total	57	25	46	32	160

3. Results

3.1. *Ashworthius sidemi* infection prevalence and intensity in European bison

Prevalence of *A. sidemi* infection increased rapidly from 0% in 1999, by 9% in 2000, 25% in 2001, reaching 100% in 2004 (Fig. 1). From 2004 to 2010 all investigated bison were infected. Between 2011 and 2015 prevalence varied between 89 and 100% (Fig. 1). *Haemonchus contortus* was not found.

Throughout the *A. sidemi* invasion, the median infection intensity was 2890 and varied from 0 to 44,310 nematodes per animal (Fig. 2). After its introduction in 2000, infection intensity increased, reaching the highest median infection intensity of 8200 *A. sidemi* in the winter of 2008/2009. Afterwards, a significant decline in the median infection intensity was observed to the minimum value of 410 nematodes in the winter of 2014/2015 (nbGLM: slope = −0.22+-0.09, z = −2.45, p = 0.01) (Fig. 2).

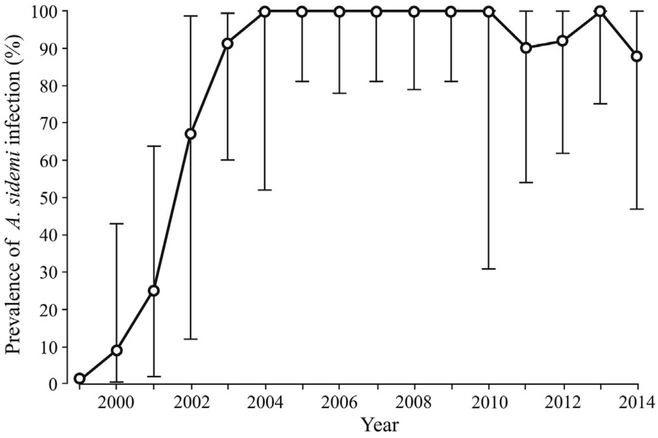

Fig. 1. Prevalence of *Ashworthius sidemi* infection in European bison in the Białowieża Primeval Forest in 1999–2014. Calculation based on material from this study and literature data (Demiaszkiewicz and Pyziel, 2010; Demiaszkiewicz et al., 2009a; Demiaszkiewicz et al., 2008; Dróżdż et al., 2002, 2003). Whiskers denote confidence intervals. Confidence intervals were calculated using the continuity-corrected score method (Blyth and Still, 1983).

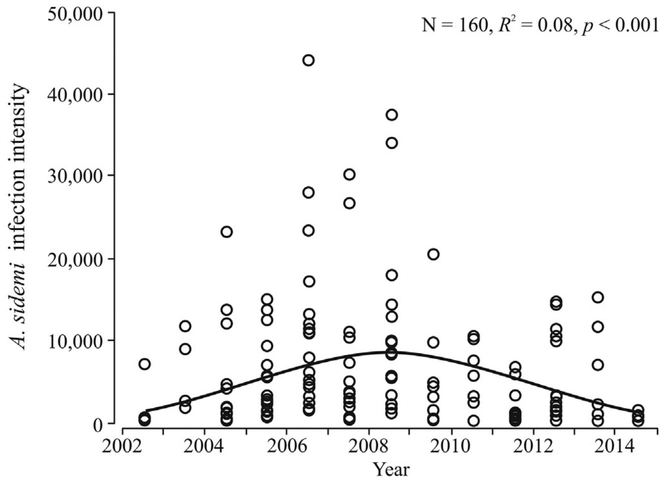

Fig. 2. Dynamics of *Ashworthius sidemi* infection intensity in European bison in the Białowieża Primeval Forest.

3.2. Herd size and the intensity of supplementary feeding of European bison

In 2002–2006, bison herd size, from which studied individuals originated ranged from 4 to 104 individuals and did not differ significantly across years. Then, from 2007, herd size significantly decreased (2007 – max herd size = 75 individuals, 2015 = 31 individuals; $p < 0.001$) (Fig. 3). The size of the bison herds was strongly correlated with the intensity of supplementary winter feeding (Fig. 4). The size of the bison herds utilizing provided forage grew as the frequency and amount of food delivered to feeding sites increased.

3.3. Factors affecting Ashworthius sidemi infection intensity in European bison

Model selection (based on the AICc criteria) for the considered negative binomial generalized linear model (GLM) revealed that changes in *A. sidemi* infection intensity were statistically significantly associated with bison age, and the interactive effects between herd size and the number of years since introduction. (Tables 2 and 3). *A. sidemi* infection intensity was the highest in subadults (median: 10,835 nematodes), medium in adults (median: 2955) and the lowest in juveniles (median: 1700) (Fig. 5). This was also confirmed by a LOESS model (Local Polynomial Regression) – infection intensity grew up to the age of approximately 38–42 months, then it dropped and after 75 months remained at a fairly stable level (Fig. 6). The positive effect of bison herd size on *A. sidemi* infection intensity increased significantly with the number of years since nematode introduction (Table 3, Fig. 7). Though the final model indicated that bison sex did not have a significant effect on *A. sidemi* infection intensity, we found significant differences between sexes when only adult individuals were considered (males – 1785 nematodes, females – 3982) (Fig. 8, Mann-Whitney test, $W = 954$, $p = 0.027$).

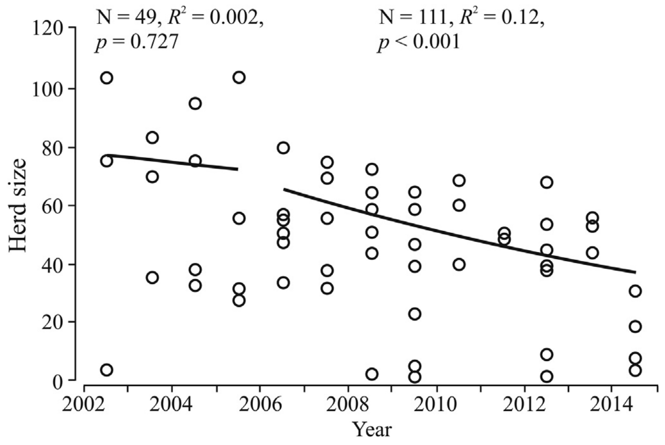

Fig. 3. Changes in the herd size of free-living European bison in the Białowieża Primeval Forest in 2002–2015.

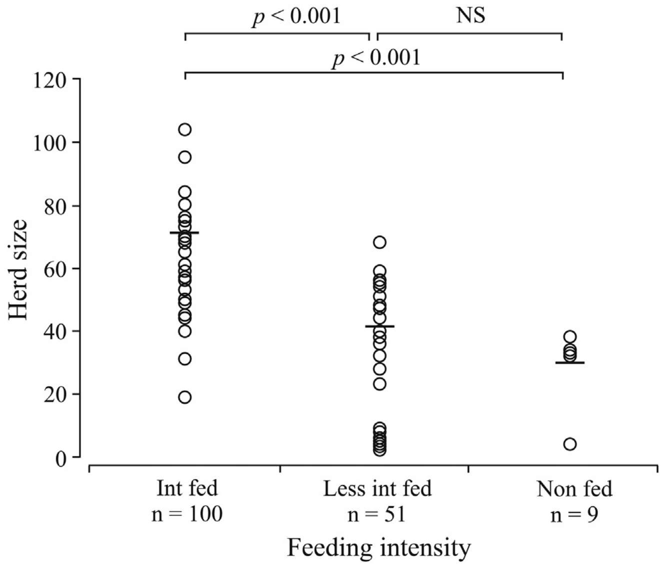

Fig. 4. The relationship between intensity of supplementary feeding and the size of bison herds in the Białowieża Primeval Forest. Bison groups: intensively fed bison – bison provided with food 3–5 times a week, less intensively fed bison – fed usually once a week, non-fed bison – bison did not aggregate at feeding sites. Horizontal lines denote mean values. *P*- values were calculated with Tukey's HSD test.

4. Discussion

In this paper we analysed the spread of a pathogenic parasite in a wild ungulate from the initial phase of its progression. This was possible due to the long-term parasitological studies conducted on European bison in BPF; this enabled the capture of the initial phase of the invasive blood-sucking nematode introduction, and the

Table 2
Model selection (based on the AICc criteria) for the considered (non nested) negative binomial generalized linear models ($\Delta AIC_c \leq 10$). The models aimed to assess the effect of bison age, body weight, bison sex, herd size, and number of years since *A. sidemi* introduction, on the intensity of *A. sidemi* infection in the bison population in BPF (2002–2015). The top model on the list was chosen as the best model.

Model	*K*	R^2	AIC_c	ΔAIC_c
Age + Herd size + Year + Herd size × Year	7	0.17	2989.9	0
Age + Herd size + Weight	6	0.16	2991.1	1.25
Age + Herd size	5	0.14	2991.4	1.46
Age + Weight	5	0.12	2996.2	6.31
Age + Sex	5	0.11	2998.3	8.37
Age	4	0.09	2998.5	8.60

K number of estimated parameters; AIC_c – Akaike's information criterion with a second order correction for small sample sizes; ΔAIC_c – difference in AIC_c between the given model and the most parsimonious model. Parameter estimates for the top model are presented in Table 3.

Table 3
Parameter estimates for the negative binomial generalized linear model (GLM) describing the effects of bison age, herd size and the number of years since *A. sidemi* introduction, on the intensity of *A. sidemi* infection in the bison population in BPF in 2002–2015.

Variables	Estimate	SE	*z* value	*P* Value
Age				
Juv (ad)	−0.756	0.250	−3.025	0.002
Subad (ad)	0.635	0.325	1.954	0.051
Subad (juv)	1.391	0.330	4.214	<0.001
Year	−0.154	0.084	−1.830	0.067
Herd size	−0.014	0.013	−1.098	0.272
Year × Herd size	0.004	0.001	2.664	0.008

Age – adult (ad), sub-adult (subad), juvenile (juv). Reference levels for analysed factors are presented in parenthesis.

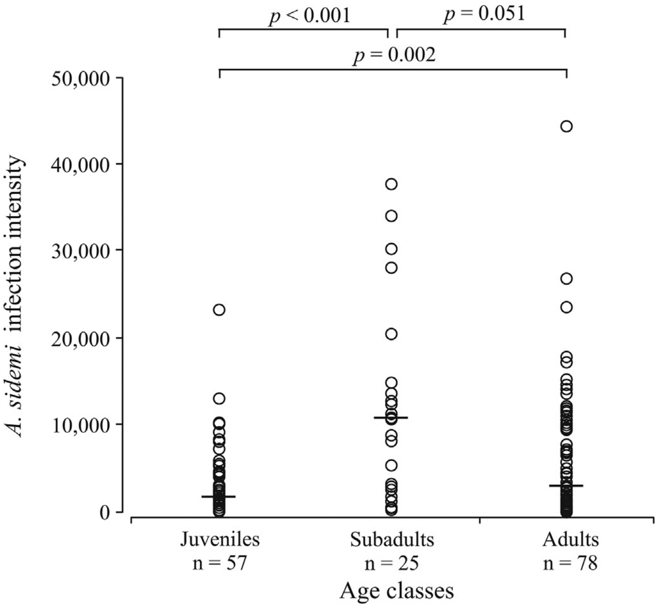

Fig. 5. Influence of bison age on *Ashworthius sidemi* infection intensity in the Białowieża Primeval Forest. Bison age classes: juveniles – 0–12 months; sub-adults – 13–36 months; adults – > 36 months. Horizontal lines denote median infection intensities. *P*-values are based on the results of the negative binomial generalized linear model (Table 3).

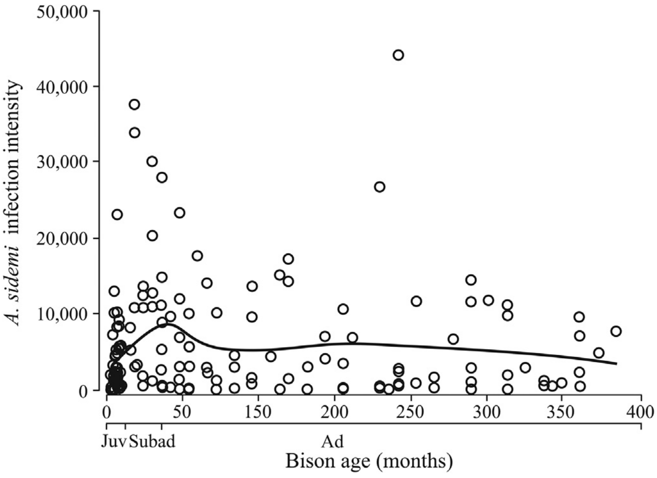

Fig. 6. Age-related changes in *Ashworthius sidemi* infection intensity in free-living European bison in the Białowieża Primeval Forest. The solid line shows locally weighted scatterplot smoothing (Local Polynomial Regression – LOESS).

investigation of its spread over the following years. Such studies are of great importance because they allow for modelling the spread of a new parasite in a population, and its impact on the host population (Anderson and May 1978; Hudson et al., 1998) and ecological processes (Thomas et al., 1999; Mouritsen and Poulin, 2005). The study reinforces the importance of the analysis of factors influencing the infection.

Long term field studies of host and parasite populations represent the ideal approach to analyse the dynamics and consequences of co-evolution because they allow the direct detection of host and parasite reciprocal influences (Decaestecker et al., 2007). However, the full course of new parasite introduction and spread in wild vertebrates is difficult to accurately trace. Recently, host-parasite interactions in the extreme environment of the Arctic have been reviewed by Kutz et al. (2014). The authors, based on studies of parasites of Holarctic ruminants such as moose (*Alces alces*), muskoxen (*Ovibos moschatus*) and caribou (*Rangifer tarandus*), concluded that insights gained from studying the history and ecology of host–parasite systems in the Arctic will be central to understanding the role that climate change is playing in these complex systems (Hoberg et al., 1999; Kutz et al., 2001; Kutz et al., 2007, 2012; Hoberg et al., 2012; Steele et al., 2013; Kutz et al., 2014). It can also guide wildlife management and conservation throughout the Arctic, and be generalized to provide insights into host–parasite interactions globally (Kutz et al., 2014).

Parasites influence host populations causing deleterious effects on their hosts (Anderson and May 1979; Prado et al., 2009), and so they are constantly forced to adapt to one another (Schmid-Hempel, 2011). Short phylogenetic parasite-host relationships are known to be more pathogenic for the host than longer co-existing relationships (Gandon et al., 2008). Our study revealed that ten years after *A. sidemi* introduction, its infection intensity dropped significantly and prevalence varied between 89 and 100%. The question is whether several years of interaction with the pathogen adaptive mechanisms to limit the number of parasites was developed? Most co-evolution studies are conducted with microbial organisms under laboratory conditions taking advantage of their short generation times (Levin and Lenski, 1983; Buckling and Rainey, 2002; Gandon et al., 2008), and these studies aren't comparable with mega-herbivore species such as European bison. Long-term co-evolution sometimes leads to a relatively stable relationship tending towards commensalism or mutualism and the establishment of parasite-host balance, as it is in the evolutionary interest of the parasite that its host thrives (Rook, 2007). Although we have not investigated the immunological parameters, it could be expected, based on other host-parasite interaction studies, that co-evolution could have appeared, or will appear in *A. sidemi*-bison interactions and affect the course of invasion. Due to the lack of data on this topic further studies are required to unequivocally confirm this hypothesis.

European bison are characterized by very low genetic variation (the lowland line of the European bison originates from only seven founders); this raises the questions about the relationship of genetic diversity to the infection of the species by numerous new parasites and the increasing prevalence and intensity of these infections (Tokarska et al., 2009, 2011). There is considerable evidence that at least part of the natural variation in resistance to nematode infection is under genetic control (Wakelin, 1985; Barger, 1989; Stear et al., 1999). However, analysis of the impact of genetic factors (the Major Histocompatibility Complex) on the intensity of *A. sidemi* infection in bison has shown that despite high sequence divergence, neither the alleles nor DRB heterozygosity were significantly associated with infection intensity (Radwan et al., 2007, 2010). Despite this, the risk associated with the occurrence of any new pathogen in a population of a species with such a

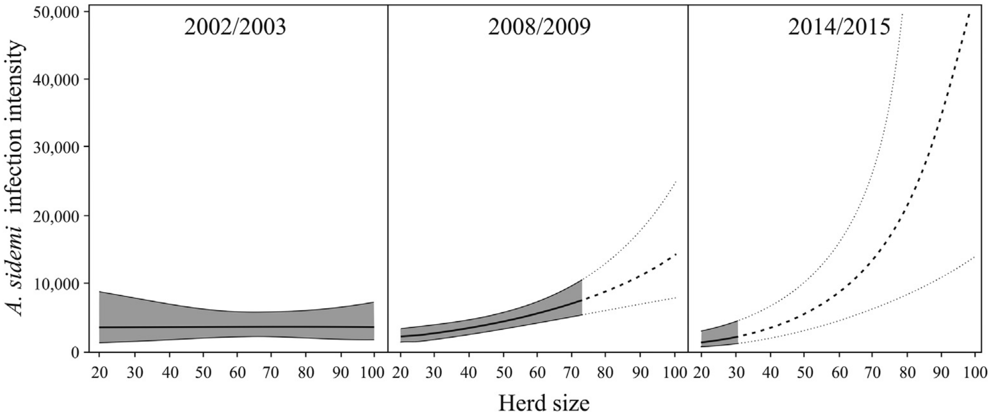

Fig. 7. *Ashworthius sidemi* infection intensity in bison inhabiting the Białowieża Primeval Forest, in relation to the interactive effect between the number of years since parasite introduction and bison herd size (year x herd size). N = 160. Model estimates predicted by the final negative binomial generalized linear model (Table 3). Solid lines and grey confidence intervals present model predictions for the herd size range observed during the study in a given year. Only the three most representative seasons are presented (2002/2003 – beginning of infection, 2007/2008 – intermediate phase, 2014/2015 – declining phase.

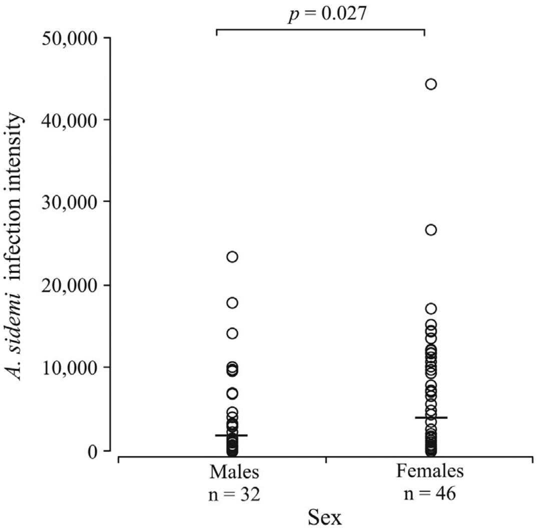

Fig. 8. *Ashworthius sidemi* infection intensity in adult European bison males and females in the Białowieża Primeval Forest. The horizontal lines denote median infection intensities. *P*- values were calculated with the Mann-Whitney test.

limited gene pool should be taken into account.

Blood-sucking parasites, such as *A. sidemi*, are strongly pathogenic (Osińska et al., 2010), especially as a cause of anaemia (Stefański, 1968; Gatongi et al., 1998). A study by Boughton and Hardy (1935) revealed that 2000 *H. contortus* females can suck 30 ml of blood per day; thus the high infection intensity of *A. sidemi* (reaching 44,300 nematodes) observed in bison in BPF may cause significant blood loss resulting in a serious impact on host condition (Kołodziej-Sobocińska et al., 2016b). Moreover, the histopathological examination of tissue from infected bison showed infiltrations of inflammatory cells in the walls of the abomasa and duodena (mainly lymphoid cells and eosinophils, as well as hyperaemia, oedema and lesions of mucosa and proliferation of lymphatic follicles) at various levels of intensity (Osińska et al., 2010). Such serious histopathological lesions connected with a deterioration in blood parameters in infected animals (Kołodziej-Sobocińska et al., 2016b) confirm the parasite potentially has a strong impact on the bison population.

There are many mechanisms and factors involved in parasitism: the immune system, toxins, behavioural traits, population density and others (Solomon et al., 2015). One of the major host defence mechanisms is immunity, which is influenced by factors such as age, sex and nutritional status (Bush et al., 2001; Peña et al., 2004; Cornell et al., 2008). The higher *A. sidemi* infection intensity in sub-adult bison compared to juveniles could be due to longer exposure to the parasite. Difference between results obtained from young animals to these from adults is probably connected to an immature immune system. Young animals, with immature immune systems, are the most susceptible to infection and disease and they spread a high number of infective eggs into the environment in their faeces (Woolhouse, 1998; Cornell et al., 2008; Treboganova, 2010). Neonates and juvenile ruminants are very susceptible to paratuberculosis infection, and that this is related to a high degree of exposure from their mothers and an immature immune system (Thakur et al., 2013). Also in captive bison, it was found that young bison are usually characterized by the heaviest infection levels and suffer the most severe consequences (Treboganova, 2010). In BPF, the youngest bison (up to one year old) have a lower parasitic load than sub-adults. We hypothesize that this could be a result of both the short exposure time of the host to the parasite, and maternal antibodies ingested by bison calves which provide immunological protection (Jackson and Nazar, 2006; Hurley and Theil, 2011). The suckling period in bison lasts up to one year (Krasińska and Krasiński, 2013). However, most calves are born in May–July, and by winter are already a few months old and eat hay provided at feeding sites (Krasińska and Krasiński, 2013) which exposes them to infectious agents in the contaminated environment (Radwan et al., 2010; Pyziel et al., 2011). Our detailed analysis of infection dynamics in relation to age showed that infection intensity grew with age up to approximately 38–42 months, then dropped, and after 75 months, when bison are fully mature, remained at a fairly stable level. By adulthood animals have developed a stronger immunity and usually harbour lower infection levels (Brass and Stevens, 1982; Kethineni et al., 2006).

The lower infection intensity that we observed in bison bulls stands in opposition to the widely observed patterns of the higher susceptibility of males to parasitosis (Addis, 1946; Solomon, 1966; Alexander and Stimson, 1988; Zuk and McKean, 1996). This phenomenon is explained by the association between testosterone and the immune system; thus, sexually mature male vertebrates are often more susceptible to infection and carry higher parasite burdens in the wild (Zuk and McKean, 1996). Recent studies have revealed that male-biased parasitism is not universal and that there are many other factors that can influence parasite infection such as the age of individuals, sexual size dimorphism, hormone levels, individual host variability (e.g. behavioural, physiological), and immunocompetence (Kiffner et al., 2013). We suggest that the behavioural traits may be responsible for the lower parasitic load in bulls. Bison males live solitarily (62% of males) or in small bull groups (up to 8 individuals), while females with calves and subadults roam in groups numbering, on average, 11–15 individuals (Krasińska and Krasiński, 1995; Krasińska et al., 2000). This leads to sexual segregation and much lower contact rates between males and other bison, which decreases the risk of parasite transmission. Additionally, pregnant and lactating females are immunosuppressed and therefore more susceptible to infection (Lloyd, 1983; Krishnan et al., 1996). European bison have an average gestation period of 264 (range 254–270) days (Krasińska and Krasiński, 2013) and similarly to closely related species such as American bison (*Bison bison*), can give birth each year (Wilson et al., 2002). Also, during a four year study 60% of cows gave birth to at least 3 calves. Moreover, the infection intensity is not an effect of just one season, but may be influenced by pregnancies in previous years. These data support the higher *A. sidemi* infection intensity observed in bison females in BPF.

We found that herd size, as a factor which significantly shapes *A. sidemi* infection intensity in bison, became increasingly important the longer the parasite was present in the host population. This means that the highest infection intensities were recorded in the biggest bison herds, where the winter supplementary feeding of bison was intensive. The role of herd size increased, the longer the time since the first detection of the parasite. This indicates that during the initial phase, the new parasite spread more successfully even in small herds, probably due to the high susceptibility of the host to the new pathogen. After the host population became saturated with the parasite, other factors such as herd size increasingly shaped the dynamics of the disease.

In large herbivore management, supplementary feeding is mainly intended to reduce herbivore impact on agriculture, to enhance body condition and reproductive performance (Kozak et al., 1995), or to supply endangered animals with food or water in crucial periods of their annual life cycle (Loarie et al., 2009). Most of the free-ranging bison populations inhabiting forests are supplementary fed in winter to mitigate migrations and reduce farm crop depredation (Kerley et al., 2012). However, research conducted in the last few years has showed that supplementary feeding may have negative long-term effects on bison and increase parasitic load (Radwan et al., 2010; Pyziel et al., 2011). This is a consequence of bison aggregation in fixed locations for several months of the year and may lead to environmental contamination by parasites and their more efficient transmission (Radwan et al., 2010). Increasing intensity of supplementary feeding leads to higher aggregation and larger herds and reduces bison mobility and ranging (Schneider, 2008). This may be more severe during cold winters when bison occupy much smaller winter ranges (Krasińska et al., 2000). An annual survey of parasite excretion in bison dung showed an increase in their prevalence and number during winter months (Kołodziej-Sobocińska et al., 2016a). Increased winter densities of bison at feeding sites influences the parasitic load of this herbivore, especially in intensively fed herds (Radwan et al., 2010; Pyziel et al., 2011). Our results have shown that the level of *A. sidemi* infection is dependent on the herd size and that the herd size is strongly correlated with the intensity of supplementary feeding. This in turn may shape the demographic composition and the overall fitness of the European bison populations (Hayward et al., 2011). Management practices also influence the quick spread and growth of infection intensity in bison, especially at the end of winter when bison are in a poorer condition (Hayward et al., 2015; Kołodziej-Sobocińska et al., 2016b). The results of this study indicate a strong need to modify supplementary feeding in order to scatter the herds, increase bison ranges and as a consequence reduce parasite transmission. Actions implemented in BPF during the bison conservation program in 2006–2010 aimed to split large winter aggregations of bison (numbering initially up to 104 individuals), which resulted in an increase in the number of winter herds and a reduction in their size. This most probably caused decline of *A. sidemi* infection intensity. Without this, we would predict increased parasitic load strongly related to the herd size and supplementary feeding intensity.

Our study, for the first time, has allowed for the long-term analysis of parasite invasion in a large herbivore in the wild: from the appearance of the parasite, through the period of rapid increase in prevalence and intensity of the infection, to the stabilization phase in the host-parasite system. The spread of animals into new areas, due to climate change and environmental modification, can affect the occurrence and spread of parasitic infections to new areas and new species. Knowledge about the course of biological invasions and the factors influencing them in wildlife can help in the modelling, prediction, prevention, or even reduction of the emergent diseases. This is especially important in the conservation management of rare and endangered species. In recent years, bison have been introduced to new places in Europe; therefore, knowledge about the processes of emergent diseases and the determination of factors influencing the severity of pathogen and parasite infections can help to design conservation management that reduces the risk of infection spread and therefore the consequences for populations of this unique and other wild species.

Conflict of interest

The authors confirm that there is no conflict of interest.

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