The impact of a pathogenic bacterium on a social carnivore population

Oliver P. Höner[1]*, Bettina Wachter[1], Katja V. Goller[1], Heribert Hofer[1], Victor Runyoro[2], Dagmar Thierer[1], Robert D. Fyumagwa[3], Thomas Müller[4] and Marion L. East[1]

[1]*Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany;* [2]*Ngorongoro Conservation Area Authority, PO Box 1, Ngorongoro, Tanzania;* [3]*Tanzania Wildlife Research Institute, PO Box 661, Arusha, Tanzania; and* [4]*Friedrich-Loeffler-Institut, Seestraße 55, 16868 Wusterhausen an der Dosse, Germany*

Summary

1. The long-term ecological impact of pathogens on group-living, large mammal populations is largely unknown. We evaluated the impact of a pathogenic bacterium, *Streptococcus equi ruminatorum*, and other key ecological factors on the dynamics of the spotted hyena *Crocuta crocuta* population in the Ngorongoro Crater, Tanzania.

2. We compared key demographic parameters during two years when external signs of bacterial infection were prevalent ('outbreak') and periods of five years before and after the outbreak when such signs were absent or rare. We also tested for density dependence and calculated the basic reproductive rate R_0 of the bacterium.

3. During the five pre-outbreak years, the mean annual hyena mortality rate was 0·088, and annual population growth was relatively high (13·6%). During the outbreak, mortality increased by 78% to a rate of 0·156, resulting in an annual population decline of 4·3%. After the outbreak, population size increased moderately (5·1%) during the first three post-outbreak years before resuming a growth similar to pre-outbreak levels (13·9%). We found no evidence that these demographic changes were driven by density dependence or other ecological factors.

4. Most hyenas showed signs of infection when prey abundance in their territory was low. During the outbreak, mortality increased among adult males and yearlings, but not among adult females – the socially dominant group members. These results suggest that infection and mortality were modulated by factors linked to low social status and poor nutrition. During the outbreak, we estimated R_0 for the bacterium to be 2·7, indicating relatively fast transmission.

5. Our results suggest that the short-term 'top–down' impact of *S. equi ruminatorum* during the outbreak was driven by 'bottom–up' effects on nutritionally disadvantaged age–sex classes, whereas the longer-term post-outbreak reduction in population growth was caused by poor survival of juveniles during the outbreak and subsequent poor recruitment of breeding females. These results suggest synergistic effects of 'bottom–up' and 'top–down' processes on host population dynamics.

Key-words: basic reproductive rate (R_0), *Crocuta crocuta*, disease ecology, Ngorongoro Crater, population dynamics, population regulation, spotted hyena, *Streptococcus*

Introduction

Long-term effects of pathogens on host population dynamics are a key component of population ecology (Begon, Townsend & Harper 2006). Infection may lead to rapid death, reduced survival and a nutritionally demanding immune response that could reduce host fecundity (Hudson, Dobson & Newborn 1998; Burthe *et al.* 2008). These effects may prevail throughout a population or affect specific categories of hosts in terms of age, sex or social status (Sapolsky 2005;

Sahin & DePinho 2010). A host's ability to mount an effective immune response to infection is likely to depend on its body condition (Beldomenico *et al.* 2008). One important factor determining body condition is access to resources (Nelson & Demas 1996; Tompkins & Begon 1999) which will be modified by factors including resource abundance, intra- and interspecific competition and, in most social species, social status (Tompkins & Begon 1999; Hofer & East 2003; Archie *et al.* 2006).

Theoretically, pathogen–host dynamics are affected by factors such as the pathogen's reproductive rate (R_0) and virulence, the proportion of the host population susceptible to

*Correspondence author. E-mail: hoener@izw-berlin.de

infection, and whether the pathogen affects classes of host individuals particularly important for population growth such as juveniles or fertile females (Anderson & May 1991). Experimental studies indicate that pathogens can regulate host populations ('top–down' regulation) by reducing host survival or fecundity (Hudson, Dobson & Newborn 1998; Tompkins & Begon 1999), and observational studies revealed significant host mortalities following pathogen epidemics (Dietz *et al*. 1989; Hawkins *et al*. 2006).

Host population size may also be influenced by resources ('bottom–up' regulation; Begon, Townsend & Harper 2006). Thus, although pathogens can negatively impact host populations, their impact could be modulated by 'bottom–up' processes mediated by resource access. The extent of 'top–down' processes is expected to vary over time because of stochastic fluctuation in pathogen circulation, host immunity and host population structure and size (Lloyd-Smith *et al*. 2005; Martin, Weil & Nelson 2008). Furthermore, when pathogen-mediated mortality sufficiently reduces negative density dependence, thereby causing an increase in fecundity, pathogen-mediated mortality need not necessarily decrease host populations (Forchhammer & Asferg 2000; Begon, Townsend & Harper 2006). A robust and comprehensive assessment of the demographic consequences of pathogens, including their ecological effects and impact on the viability of host populations (Jones *et al*. 2008), is therefore preferably achieved through long-term monitoring of pathogens and their host populations.

Here we use 14 years monitoring of a population of individually known spotted hyenas *Crocuta crocuta* to assess the effect of the bacterium *Streptococcus equi ruminatorum* and other key ecological factors such as density dependence, intra- and interspecific competition for food, prey abundance, other important pathogens and predation on the demography, size and dynamics of the hyena population. Prevalence of external signs of infection with this bacterium sharply increased between September 2002 and February 2003 (Höner *et al*. 2006; hereafter, 2002 and 2003 are termed 'outbreak years'), and 12·5% of hyenas died within days of developing external signs (Höner *et al*. 2006). Because it was proposed that pathological effects of *Streptococcus equi* increase with stressful conditions such as high host density and adverse environmental conditions (Timoney 1999; Robert *et al*. 2005), we predicted that socially subordinate and nutritionally disadvantaged hyenas should be most susceptible to severe infection and death induced by this bacterium.

Materials and methods

STUDY POPULATION

Spotted hyenas live in structured social groups ('clans') in which females socially dominate males and have priority of access to food resources within the clan territory (Kruuk 1972). The resident hyena population on the 250 km² floor of the Ngorongoro Crater in northern Tanzania was monitored between April 1996 and April 2010; it contained eight hyena clans at a mean density of 0·9 adults per km² (Höner *et al*. 2005). Cubs were reared in communal dens inside clan

territories; thus, numerous cubs (mean = 5·9 ± 3·5 cubs per den, *n* = 61 dens from eight clans during the outbreak years) rested together in underground burrows each day. The density of large herbivores on the Crater floor (80 individuals per km²) is high compared with other ecosystems (Runyoro *et al*. 1995). Large herbivores moved seasonally within the Crater, causing substantial changes in prey densities in clan territories (Höner *et al*. 2005). When prey density in a territory was low, hyenas foraged outside their territory (Höner *et al*. 2005). The Crater hyena population size in the 1960s was estimated at 469 individuals (Kruuk 1972). At the start of our study, it had declined to 171 hyenas, most probably because vegetation changes mediated a reduction in the abundance of the main prey (Höner *et al*. 2005). Several hundred domestic stock utilised the Crater floor for grazing and drinking throughout the year.

DEMOGRAPHIC DATA

All hyenas were individually identified and their age and sex determined following Höner *et al*. (2005). Hyenas aged < 12 months were classified as cubs, those between 12 and 24 months as yearlings and those ≥ 24 months as adults. Adult rank was based on the outcome of dyadic interactions using submissive responses (Höner *et al*. 2005). Cubs and yearlings were assigned the rank of their mother. Clan and population sizes were determined annually on 1 January unless otherwise stated. Clan size was the total number of members, and population size was the total number of hyenas resident on the Crater floor. Possible density dependence was assessed using annual population sizes and methods refined by Dennis & Taper (1994). The null hypothesis was that the population was undergoing stochastic exponential growth and included random walk as a special case; the alternative hypothesis was that the population was undergoing stochastic, discrete logistic growth, i.e. was subject to density dependence. Demonstrating density dependence is here equivalent to rejecting the null hypothesis of density independence. The distribution of the test statistic under both null and alternative hypothesis was obtained through parametric bootstrapping and tested with a likelihood ratio test (Dennis & Taper 1994). Dennis & Taper (1994) showed that this method of assessing possible density dependence was superior to other methods in terms of both biological realism and statistical properties (power, avoiding excessive type I error). Application of this method showed no evidence of density dependence during the study period (see Results section). This permitted us to directly estimate the growth trajectory in the absence of disease outbreaks and compare it with the observed population growth.

To assess whether infection compromised long-term survivorship of hyenas with external signs that did not die within days, we calculated the period between the date when individuals were first seen with external signs and the date when last seen. We compared this period with the mean survival period, starting from the same date, of individuals (mean number = 2·8, range = 1–8) not observed with external signs matched by sex, clan, social status (above or below median rank) and age (born within 6 months of the birth of the infected individual).

We additionally assessed the impact of the bacterium on hyena survival by analysing age-class and sex specific mortality and survivorship. Annual mortality rates were calculated for adult females, adult males, yearlings and cubs as the proportion of individuals in these age–sex classes that died each year. Individuals were considered dead if their body was found or if they were not seen for one year. Females that cease to be observed were most likely dead because they rarely disperse (Höner *et al*. 2005). Males usually disperse to clans on the Crater floor but a few disperse elsewhere

(Höner *et al.* 2007). Thus, the absence of males from the Crater floor may indicate death or dispersal elsewhere. To assess possible changes in the proportion of males dispersing elsewhere, we calculated the number and proportion of young females of all adult and yearling hyenas for each clan and year following Höner *et al.* (2007) because males disperse to clans that contain the largest number of young females (Höner *et al.* 2007).

We compared the survivorship of cubs and yearlings before, during and after outbreak years, by calculating the nonparametric Kaplan–Meier survivorship function that incorporates the lifespan of animals alive at the end of the study as a minimum estimate (right-censored data; Parmar & Machin 1995). We also compared the survivorship to adulthood of cubs nursed by females with external signs with that of cubs from the same clans nursed by mothers without external signs. One randomly selected cub per female was included in the analysis to avoid pseudo-replication.

We calculated each hyena's annual contribution to population growth following Coulson *et al.* (2006) and summed the results across adult females, adult males, yearlings, and cubs, respectively. Contributions by adult males were based on paternity analyses for 679 offspring (70·2% of all known births) carried out as previously described (Höner *et al.* 2010). Paternities were assigned based on amplification of nine highly polymorphic microsatellite loci using maximum-likelihood methods as implemented in Cervus 3.0 (Kalinowski, Taper & Marshall 2007). All reproductively active male clan members when a litter was conceived were candidate fathers. For 630 offspring (92·8% of all sampled offspring), all candidate males were typed; the mean proportion of typed candidate males was 0·986. For 648 offspring (95·4%), paternity was determined with 95% confidence. Mean expected heterozygosity was 0·829, and total exclusionary power was 0·999.

DISEASE MONITORING AND PREVALENCE OF EXTERNAL SIGNS OF INFECTION

External signs of *S. equi ruminatorum* infection were clearly visible for periods between seven and 24 days (Höner *et al.* 2006). Signs included diffuse, unilateral swelling of the head that became localised at the mandibular angle and developed into a pronounced abscess in most cases (Fig. 1; details see Höner *et al.* 2006). Presence or absence of external signs was recorded whenever individuals were encountered. Prevalence of external

Fig. 1. Spotted hyena with external signs of infection with *Streptococcus equi ruminatorum* in 2002.

signs was exceptionally high between September 2002 and February 2003. We defined 2002 and 2003 as 'outbreak years'; this definition is conservative with respect to our analyses on the impact of infection with the bacterium.

To assess age-class and sex specific susceptibility to external signs of infection during the outbreak years, we calculated selection ratios $\hat{w}_i$ for the age–sex classes adult females, adult males, yearlings and cubs by dividing the proportion with external signs o_i by the proportion available in the population shortly before the outbreak π_i. The standardised selection ratio $\alpha_i = \hat{w}_i / (\sum_{j=1}^{m} \hat{w}_j)$ then estimates the probability that a randomly infected hyena will be in age–sex class i if all age–sex classes are equally frequent in the original population of available units (Chesson 1983; Manly, McDonald & Thomas 1993). Unlike many other measures of preference, α_i does not change with the abundance of each age–sex class (Chesson 1983). Probabilities were calculated using chi-squared statistics developed by Manly, McDonald & Thomas (1993).

Samples from 16 Crater floor and two Crater rim hyenas were screened for the occurrence of *S. equi ruminatorum* following Höner *et al.* (2006). Tissue samples (lung, liver and lymph node) from 15 Crater floor and two Crater rim hyenas were screened for the occurrence of canine distemper virus (CDV) because CDV infection in hyenas can cause death (Haas *et al.* 1996), and CDV outbreaks were proposed for Crater lions *Panthera leo* in both 1997 and 2001 (Kissui & Packer 2004) or only 2001 (Munson *et al.* 2008). Samples were stored at −80 °C or in RNAlater (Sigma-Aldrich Inc., St Louis, MO, USA) at −80 °C and screened following previously described protocols (Barret *et al.* 1993; Goller *et al.* 2010). A hyena tissue sample positive for CDV served as positive control.

We also screened 18 brain and three saliva samples from Crater floor hyenas and four brain samples from Crater rim hyenas for the presence of rabies virus. Rabies is assumed to be a threat to wild carnivores and a rabies epidemic swept through the domestic dogs in the Ngorongoro district surrounding the Crater between 2002 and 2005 (Hampson *et al.* 2008), resulting in the spread of rabies to at least two adult Crater rim hyenas in 2004 (Lembo *et al.* 2007). Samples were stored and transported at −80 °C or in phosphate-buffered 50% glycerol solution and screened by fluorescent antibody test and reverse transcriptase-PCR (Goller *et al.* 2010).

Eighty-six faeces from 82 Crater floor hyenas were screened for the presence of coronavirus and calicivirus, two pathogens of hyenas (East *et al.* 2004; Harrison *et al.* 2004). Samples were stored and transported at −80 °C and screened following Goller *et al.* (2010). Hyenas and other carnivores were also routinely checked by visual means for the presence of external signs suggestive of infection with any other pathogen.

ABUNDANCE OF MAIN PREY AND LIONS

Monthly densities of hyena main prey (wildebeest *Connochaetes taurinus*, plains zebra *Equus burchelli*, Thomson's gazelle *Gazella thomsoni*, Grant's gazelle *Gazella granti*; Höner *et al.* 2002) in clan territories were estimated from bimonthly transect counts and divided into three equal (high, medium and low) categories (Höner *et al.* 2005). We used prey density recorded in an individual's territory during the month in which it was first seen with external signs. To assess the influence of prey abundance on the likelihood of hyenas to express external signs, the occurrence of symptomatic cases during months of high, medium and low prey density was compared with the expected distribution of such cases based on the sum of months with high, medium and low prey density over all clans. To assess the influence of prey abundance on hyena population size, mean annual

prey population sizes were estimated based on total counts following Runyoro *et al.* (1995).

Extra-territorial foraging is a tactic used most frequently by hyenas of low social status during periods of prey shortage in their territory (Hofer & East 1993; Höner *et al.* 2005). To assess whether adult and yearling hyenas differed in their use of this tactic, we compared the mean proportion of adults and yearlings of both sexes outside their territory following Höner *et al.* (2005).

Lions are the main food competitor and predator of hyenas (Kruuk 1972; Höner *et al.* 2002). To assess the effect of interspecific competition and lion predation on hyena population size, we estimated annual population sizes of adult and subadult lions in the Crater as described by Höner *et al.* (2002).

BASIC REPRODUCTIVE RATE R_0 OF *STREPTOCOCCUS EQUI RUMINATORUM*

To assess whether the bacterium can be maintained within the hyena population, we calculated its basic reproductive rate R_0 using the scenario that a newborn individual's probability of surviving to age t declines exponentially with t (mortality schedule 'II', Anderson & May 1991) for a growing population. Thus, $R_0 = 1 + G/A$, with G being the population size divided by the number of births and A being the average age at infection during the outbreak years. Average age at infection was the sum of infectives divided by the sum of the proportions positive in each age–sex class. Infectives were the proportion positive multiplied by the mean age of the individuals present in the population at the beginning of the outbreak years in each age–sex class. The proportion positive was the excess mortality in the outbreak years in each age–sex class. Estimates calculated with a less appropriate alternative schedule (schedule 'I', Anderson & May 1991) yielded estimates reduced by 37%.

STATISTICS

Statistical analyses were performed using SYSTAT 13.0 (Systat Software Inc., Richmond, USA). The significance of Wilcoxon signed-rank and Mann–Whitney *U*-tests with sample sizes below 30 were based on exact *P*-values. Data used for parametric tests did not deviate from normal distribution (Shapiro–Wilk test). Results are quoted as means ± standard deviations, and probabilities are for two-tailed tests.

Results

Between April 1996 and April 2010, 21 hyenas of a mean total population of 301 hyenas were observed to express external signs of *S. equi ruminatorum* infection. The first hyena with external signs was observed in August 2001 (Fig. 2). Nineteen individuals had external signs on one occasion; one hyena expressed and cleared signs in three different years (2001, 2002, 2004). Hyenas with external signs were mostly observed (in 65% of 23 cases, involving 15 individuals) during a period of six months between September 2002 and February 2003 (Fig. 2). After this outbreak, animals developed less severe swellings and abscesses than during the outbreak and hyenas with signs after the outbreak did not express apathy or marked ataxia.

PREVALENCE OF EXTERNAL SIGNS

The outbreak occurred after an increase in mean clan size from 22 ± 14 members to 42 ± 21 members and in popula-
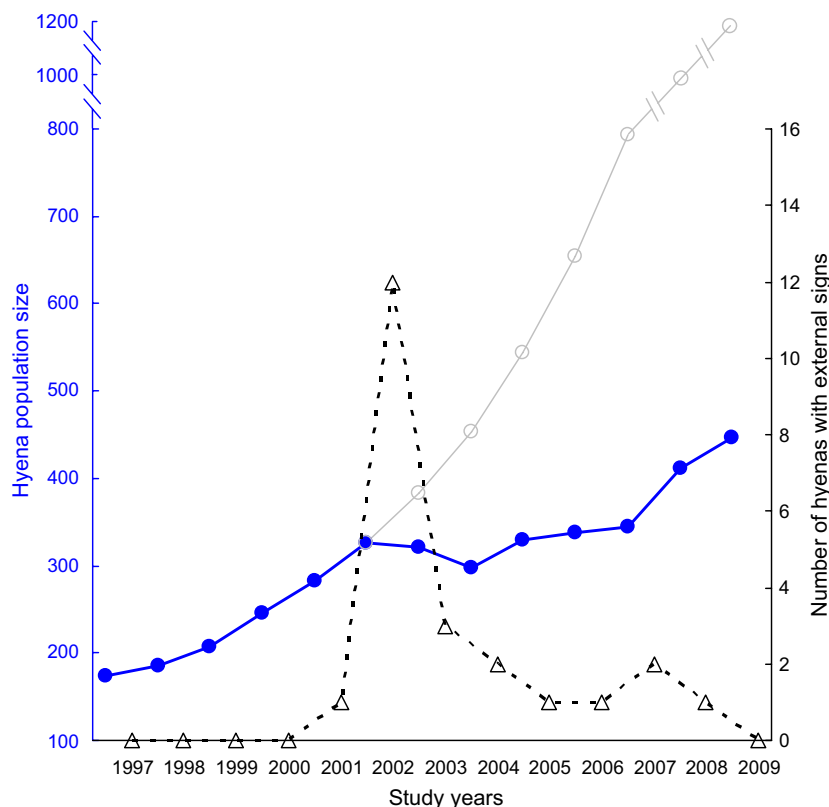
Fig. 2. Annual size of the spotted hyena population in the Ngorongoro Crater (blue circles), population size trajectory in the absence of the disease outbreak (grey circles) and number of hyenas that expressed signs of *Streptococcus equi ruminatorum* infection (black triangles).

Table 1. Prevalence of external signs of *Streptococcus equi ruminatorum* infection among spotted hyenas of different age–sex classes during the outbreak years 2002 and 2003. Included are individuals who died of the disease and individuals who expressed and cleared external signs during this period

	Present shortly before outbreak		With signs of infection during outbreak							
	n	prop (π_i)	n	prop (o_i)	Selection ratio $\hat{w}_i$	Standardised selection ratio α_i	d.f.	χ^2_M	P	Bonferroni corrected[a]
Adult females	109	0·32	9	0·60	1·86 ± 0·37	0·50	1	5·24	0·02	NS
Adult males	110	0·33	3	0·20	0·61 ± 0·37	0·17	1	1·09	0·30	NS
Yearlings	55	0·16	3	0·20	1·23 ± 0·58	0·33	1	0·15	0·70	NS
Cubs	63	0·19	0	0·00	0·00 ± 0·54	0·00	1	3·45	0·06	NS
Total	337	1·00	15	1·00						

[a]Bonferroni adjusted α = 0·0125.

tion size from 173 to 337 between January 1997 and July 2002. Hyenas from five of the eight Crater clans showed external signs; the number of hyenas with such signs in each clan during the outbreak years was positively related to clan size (Spearman's rank correlation, ρ = 0·90, n = 8, P < 0·05). The proportions of adult females, adult males, yearlings and cubs expressing signs during the outbreak were as expected from the proportions of their age–sex classes present on 1 July 2002, shortly before the outbreak (Table 1). Individuals were significantly more likely to show external signs if prey density in their territory was low rather than medium or high (Kolmogorov–Smirnov test, D = 0·49, n = 23, P < 0·01).

SURVIVAL AND MORTALITY

Two of 21 hyenas with external signs died within days of expressing signs and two died when anaesthetised by veterinarians (one while expressing signs, the other after clearing signs). Survival after the first sighting of external signs in 17 hyenas that did not die within days of infection (mean = 42·4 ± 29·6 months) was similar to the mean period of survival of individuals without signs matched for age, sex and clan (mean = 43·2 ± 24·4 months; Wilcoxon signed-rank test, exact P = 0·98). When individuals were additionally matched by rank, the result remained unchanged (mean survival period of hyenas that expressed and cleared signs = 39·1 ± 31·6 months vs. mean survival period of nonsymptomatic hyenas = 50·2 ± 22·1 months; Wilcoxon signed-rank test, n = 8, exact P = 0·44). Nine cubs nursed by females with signs were as likely to survive to adulthood as the cubs from 93 control females from the same clans (Fisher's exact test, P = 0·73).

Overall mean annual mortality rate during the 5 years preceding the outbreak was 0·088 ± 0·03. During the outbreak years, overall mean annual mortality rate was 0·156 ± 0·01, a rate substantially higher than the mean across all other study years (0·104 ± 0·04; one-sample t-test, t = −4·63, d.f. = 9, P = 0·0012). This significant difference applied to adult males (t = −6·63, d.f. = 9, P = 0·0001, Fig. 3b) and yearling hyenas (yearling females: t = −3·40, d.f. = 9, P = 0·0079, Fig. 3c; yearling males: t = −6·42, d.f. = 9,

P = 0·0001, Fig. 3d) but not to adult females or cubs (adult females: t = −1·45, d.f. = 9, P = 0·18, Fig. 3a; cubs: t = −1·53, d.f. = 9, P = 0·16, Fig. 3e). Hyenas born (n = 73) or those that became yearlings (n = 47) during the outbreak had a significantly lower survivorship than cubs born (n = 685) or yearlings (n = 319) raised before or after this period (Tarone-Ware nonparametric log-rank test, cubs: χ^2 = 6·13, d.f. = 2, P = 0·022, Fig. 4a; yearlings: χ^2 = 5·07, d.f. = 1, P = 0·0011; Fig. 4b). There was no relationship between population size and cub or yearling annual mortality rate (linear regression, cubs: $F_{1,10}$ = 1·02, P = 0·34; yearlings: $F_{1,10}$ = 2·43, P = 0·15).

The annual contribution to population growth by adult females during the outbreak years (mean = 0·054 ± 0·0004) was similar to that during other years (mean = 0·045 ± 0·017; t = −1·61, d.f. = 9, P = 0·14). The annual contribution to population growth by females with external signs was similar during the 5 years preceding infection and following clearance of signs (Wilcoxon signed-rank test, n = 6, exact P = 0·094). In contrast, the annual contribution by adult males was lower during the outbreak years (mean = 0·042 ± 0·0013) than during other years (mean = 0·061 ± 0·01; t = 6·35, d.f. = 9, P = 0·0001). This was unlikely to be caused by increased male dispersal elsewhere because the proportion of young females (of all adult and yearling hyenas) in Crater clans was higher during the outbreak years (mean = 0·225 ± 0·004) than other years (mean = 0·191 ± 0·019; t = −5·84, d.f. = 10, P = 0·002).

POPULATION DYNAMICS

Hyena population size increased at an annual rate of 0·136 ± 0·042 during the 5 years before the outbreak (Fig. 2). During the outbreak years, population size decreased by 4·3 ± 4·0%. Annual population growth rates during the post-outbreak period (mean = 0·086 ± 0·069) were moderate during the first 3 years (mean = 0·051 ± 0·069) before resuming rates similar to those during the pre-outbreak period (mean = 0·139 ± 0·073). Population growth during outbreak years was significantly lower than that of other years (t = 8·17, d.f. = 9, P = 0·0001).
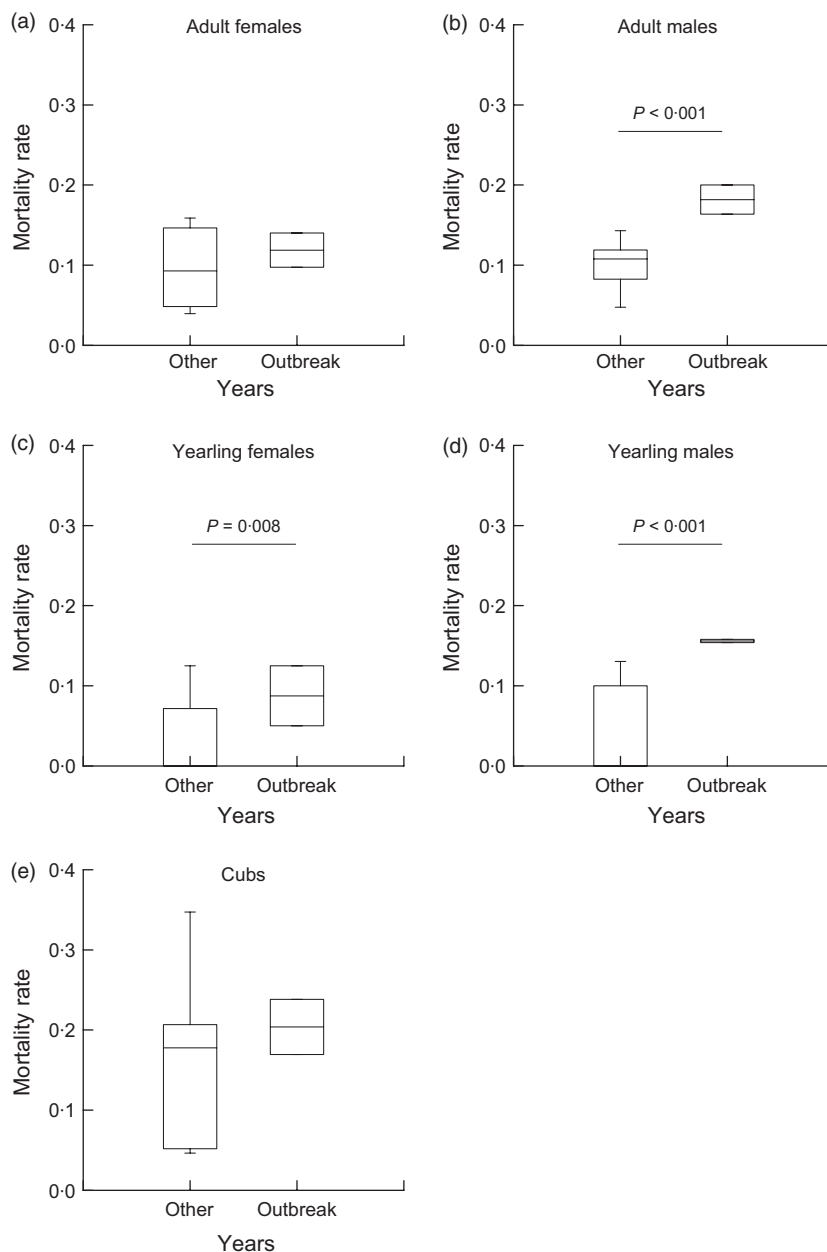
Fig. 3. Annual mortality rates of spotted hyena adult females (a), adult males (b), yearling females (c), yearling males (d), and cubs (e) in the Ngorongoro Crater during outbreak years and other study years. Boxes indicate interquartile ranges around the median (line inside box), and vertical error bars represent values $\pm$ 1·5 times the interquartile range.

The hyena population showed no evidence of density dependence (entire population: likelihood ratio test statistic = $-1\cdot09$, $n = 12$, $P = 0\cdot282$; adults plus yearlings: likelihood ratio test statistic = $-0\cdot66$, $n = 12$, $P = 0\cdot44$; adults only: likelihood ratio test statistic = $-1\cdot30$, $n = 12$, $P = 0\cdot19$). The likely population growth trajectory in the absence of the disease outbreak (Fig. 2) was based on a linear regression of the total number of hyenas in year $i + 1$ on the total number of hyenas in year i during the years before the outbreak ($y = 1\cdot249x + 23\cdot789$, $r^2 = 0\cdot989$, $P < 0\cdot001$).

EXTRATERRITORIAL EXCURSIONS, ABUNDANCE OF MAIN PREY AND LIONS

The mean proportion of sightings outside the territory of all sightings of yearling hyena females (mean = $0\cdot062 \pm 0\cdot15$, $n = 250$) was lower than that of adult females (mean = $0\cdot083 \pm 0\cdot13$; $n = 269$; Mann–Whitney U-test, $U = 43955$, $P < 0\cdot0001$). The same was true for yearling males (mean = $0\cdot049 \pm 0\cdot13$, $n = 248$) compared with adult males (mean = $0\cdot193 \pm 0\cdot20$, $n = 279$; $U = 55815\cdot5$, $P < 0\cdot0001$).

The mean number of main prey per capita (adult plus yearling hyenas) was similar during outbreak ($55\cdot8 \pm 2\cdot6$) and other years ($69\cdot4 \pm 33\cdot5$; $t = 1\cdot35$, d.f. = 10, $P = 0\cdot21$). There was no relationship between the mean number of main prey in year i and hyena population size in year $i + 1$ ($F_{1,11} = 0\cdot35$, $P = 0\cdot57$) or year $i + 2$ ($F_{1,10} = 0\cdot43$, $P = 0\cdot53$).

Mean lion population size during outbreak years ($35\cdot0 \pm 4\cdot2$) tended to be lower than that during other years ($42\cdot3 \pm 10\cdot9$; $t = 2\cdot17$, d.f. = 10, $P = 0\cdot055$). There was no relationship between lion population size in year i and hyena
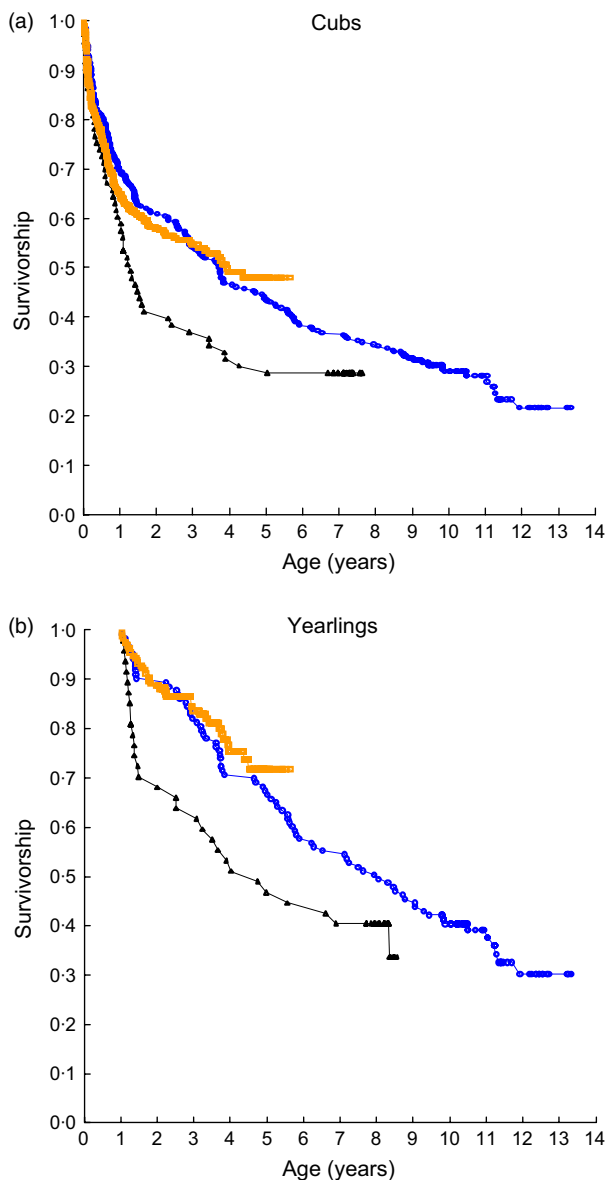
(a) Cubs

(b) Yearlings

Fig. 4. Survivorship of spotted hyenas in the Ngorongoro Crater that were born (a), or became yearlings (b) before (blue circles), during (black triangles) and after (yellow squares) the outbreak of symptomatic *Streptococcus equi ruminatorum* infection.

population size in year i (linear regression, $F_{1,11} = 1·09$, $P = 0·32$) or year $i + 1$ ($F_{1,10} = 1·25$, $P = 0·29$).

BASIC REPRODUCTIVE RATE R_0 AND INTRASPECIFIC TRANSMISSION

During outbreak years, G was 4·1, the sum of infectives was 1·84, and the sum of the proportions positive (the sum of the differences in mortality between outbreak and nonoutbreak years) was 0·77. As a result, the average age at infection A was 2·4, and the basic reproductive rate R_0 of the bacterium in the hyena population was 2·7. This indicates that the bacterium had a significant potential to spread within the population. Clan-specific values for R_0 varied between 1·9 and 4·6

(mean = 3·1 ± 1·0). There was no significant correlation between R_0 in a clan and clan size just before the outbreak ($\rho = -0·26$, $n = 8$, NS). Seven individuals with active infections were observed at feeding sites either biting other hyenas or depositing pus and saliva likely to contain bacteria on a carcass then consumed by other hyenas. One of 39 (2·6%) adult hyenas and zero of five younger individuals that could have been infected at these feeding sites developed external signs.

INTERSPECIFIC TRANSMISSION

No zebra or African buffalo *Syncerus caffer* was observed with external signs suggestive of *S. equi ruminatorum* disease before, during or after the outbreak, even though both species were infected with the bacterium (Höner *et al.* 2006; Speck *et al.* 2008). We therefore were not able to assess whether infection in these prey populations occurred before or increased during outbreak years. Mean numbers were similar during outbreak (zebra: 3753 ± 165; buffalo: 2539 ± 250) and other years (zebra: 4124 ± 1123; $t = 1·09$, d.f. = 10, $P = 0·30$; buffalo: 2643 ± 854; $t = 0·41$, d.f. = 10, $P = 0·69$). The number of zebras and buffaloes consumed by hyenas per monitoring day during outbreak years (zebra: mean = 0·026 ± 0·016; buffalo: mean = 0·030 ± 0·0) was similar to that during other study years (zebra: mean = 0·029 ± 0·018; $t = 0·68$, d.f. = 11, $P = 0·51$; buffalo: mean = 0·044 ± 0·033; $t = 1·51$, d.f. = 11, $P = 0·16$).

OTHER PATHOGENS

All samples from hyenas on the Crater floor and Crater rim tested negative for CDV. Of 1354 Crater floor hyenas monitored between 1996 and 2010, none expressed external signs typical of active CDV infection in hyenas (neurological disorders, ataxia and ocular and nasal discharge; Haas *et al.* 1996). If CDV occurred, it did not decrease hyena population growth in 1997 or 2001. Population size increased during 1997 at 6·9% and during 2001 at 15·2% (Fig. 2). All brain and saliva samples from Crater floor hyenas tested negative for rabies, and none of the 1354 monitored Crater floor hyenas expressed external signs of either dumb or furious rabies. Brain samples from two hyenas on the Crater rim in 2004 tested positive for rabies. There was also no evidence of external signs of infection with other pathogens. None of the faecal samples tested contained coronavirus RNA, whereas 19 of 86 samples (22·1%) from 18 individuals (22·0%) contained calicivirus RNA. The mean annual proportion of calicivirus-positive samples was 0·23 ± 0·15 and varied between 0·0 in 1998 and 0·5 in 1997. The proportion of calicivirus-positive samples during outbreak years (0·20 ± 0·08) was similar to that during other years (0·24 ± 0·17; $U = 7$, exact $P = 1·0$).

Discussion

Between 1996 and 2001, no hyena in the Crater was observed with external signs of *S. equi ruminatorum* infection. Twelve

months after the first observation of external signs in 2001, the prevalence of external signs sharply increased and remained high during a 6-month period between September 2002 and February 2003. After this period, prevalence declined rapidly and remained low (Fig. 2). Because hyenas can be infected with *S. equi ruminatorum* without developing external signs (Höner *et al.* 2006), and several different strains of this bacterium were present in hyenas and prey species shortly after the outbreak (Speck *et al.* 2008), it is possible that individuals were exposed and infected before 2001.

THE IMPACT OF THE PATHOGEN ON THE SPOTTED HYENA POPULATION

The rapid increase in the prevalence of signs of infection (Fig. 1) was concurrent with a substantial increase in the overall mortality rate and a short-term decline in population size (Fig. 2). The proportion of hyenas observed to have died of infection of 12·5% (Höner *et al.* 2006) was similar to the proportion of domestic horses that died of infection with *Streptococcus equi equi* of 8–10% (Sweeney *et al.* 1989). After the outbreak, mortality rates declined to pre-outbreak levels and hyena population size once more increased. However, owing to the reduced survivorship of cubs and yearlings (Fig. 4) and the reduced number of yearling females surviving to reproductive age during the outbreak years (Fig. 3c), population growth rates remained modest during 3 years after the outbreak before increasing to pre-outbreak rates (Fig. 2). The population decline during the outbreak and the modest growth in the three following years resulted in a significant difference between observed and projected population growth in the absence of infection (Fig. 2). Thus, the outbreak of *S. equi ruminatorum* infection had substantial long-term consequences for the dynamics of the hyena population.

We found no evidence that the short-term decline in hyena population size in 2002 and 2003 and the subsequent low rate of increase could be ascribed to any of the following factors: density dependence, a change in the number of male hyenas dispersing to areas outside the Crater, a change in overall prey abundance, changes in the number of lions and thus changes in the level of predation by and competition for food with lions.

We also have no evidence that CDV and rabies had any negative impact on the population dynamics of hyenas living on the Crater floor. During a CDV epidemic that caused mortality among lions in the adjacent Serengeti in 1994, sympatric juvenile hyenas infected with a highly homologous CDV strain also sustained increased mortality (Haas *et al.* 1996). Thus, die-offs in Crater lions in 1997 and/or 2001 thought to be associated with outbreaks of CDV (Kissui & Packer 2004; Munson *et al.* 2008) might be expected to decrease hyena cub survival and hyena population size in 1997 and 2001 and decrease population growth in these years and the 2 years that followed because of subsequent low recruitment. Our results do not conform to these expectations, and we found no evidence that the Crater hyena population sustained a CDV outbreak in 1997 and/or 2001. Furthermore, clinical signs and pathologies caused by severe *S. equi ruminatorum* infection (Höner *et al.* 2006) are inconsistent with those described for CDV infection in hyenas (Haas *et al.* 1996).

The two Crater rim hyenas positive for rabies were sampled in 2004 during an epidemic of rabies in domestic dogs in areas surrounding the Crater (Hampson *et al.* 2008). Lembo *et al.* (2007) also reported two rabies-positive hyenas on the Crater rim in 2004 infected with strains identical to those from domestic dogs in Tanzania. It is possible that one or both cases of Lembo *et al.* (2007) are the same as those reported in this study. We have no evidence that rabies spread from the Crater rim to the hyena population on the Crater floor, and no evidence that rabies increased mortality in the hyena population on the Crater floor between 2003 and 2005.

During a phocine distemper outbreak in harbour seals *Phoca vitulina*, a concurrent infection with *Streptococcus equi zooepidemicus* may have increased the severity of phocine distemper (Akineden *et al.* 2005), and the severity of CDV infection in a pack of African wild dogs *Lycaon pictus* may have been increased by a concurrent *S. equi ruminatorum* infection (Goller *et al.* 2010). We cannot entirely exclude the possibility that the outbreak of *S. equi ruminatorum* in the Crater hyena population was associated with a concurrent infection with another as yet undetected pathogen, but as we have no evidence to suggest this, we consider the observed population changes were most likely induced by infection with *Streptococcus*.

STRESS, EXPOSURE AND SUSCEPTIBILITY

During the outbreak, hyena mortality was significantly increased in yearlings and adult males but not in adult females or cubs (Fig. 3). Because females are socially dominant and have priority of access to food in their territory, they are likely to maintain a better nutritional and immune status than immigrant males. Recently independent yearlings are inexperienced at foraging and rarely undertook extra-territorial foraging excursions to escape periods of low prey abundance in their own territory. Adult males are the most subordinate class in hyena clans and usually feed only after adult females and their offspring have left a carcass (Kruuk 1972), and although they frequently undertake extra-territorial foraging excursions (Höner *et al.* 2007), they are subordinate to all territory owners during these excursions (Hofer & East 1993). Our results suggest that adult females are less compromised by *S. equi ruminatorum* infection because of lower levels of nutritional stress than yearlings and adult males. As we currently know little about routes of transmission between hyenas and immune responses to contain infection, we cannot exclude the possibility of differences between these classes of hyenas in either exposure to loads of infectious particles or the efficacy of immune responses. A possible link between poor nutritional state and susceptibility to infections is supported by the fact that external signs

appeared in hyenas when prey abundance in an infected individual's territory was low.

Adult females tended to be more frequently observed with external signs, yet showed lower mortality and higher disease-related survival during the outbreak than yearlings and adult males (Table 1). Lower mortality in adult females is unlikely to be a consequence of reduced exposure to bacteria in prey because rank-related access to large carcasses results in adult females feeding first and therefore consuming more body parts commonly infected with *S. equi ruminatorum* such as tonsils, the throat, skin and digestive organs (Timoney 1999; Speck *et al.* 2008) than yearlings and adult males. This suggests that yearlings and adult males more rapidly succumbed to the disease and were thus less often observed with external signs than adult females. The likelihood of observing a lactating female with external signs may be high because they visit communal dens more frequently than yearlings or adult males.

In domestic horses, the severity of *S. equi* infection is related to the number of bacteria to which horses have been exposed (Timoney 1999), and outbreaks are likely to occur more often in stables with more rather than fewer horses because of a greater chance of contact with animals shedding bacteria (Jorm 1990). Between 1996 and 2002, Crater hyena clan and population sizes increased by a factor of almost two (Fig. 2). In hyenas, individual corticosteroid concentrations induced by social stress are elevated in large clans (Goymann *et al.* 2003). The increase in clan sizes between 1996 and 2002 is likely to have increased contact rates among clan members and competition for food, thereby elevating levels of social and nutritional stress. It is possible that by 2002, contact rates between hyenas, number of individuals shedding bacteria, and levels of stress were sufficient to precipitate an outbreak. There was no evidence that the outbreak was the result of an increase in consumption of zebras and buffaloes, two prey species in the Crater known to be infected with *S. equi ruminatorum*, because hyenas consumed zebras and buffaloes at similar rates and densities of zebras and buffaloes were similar during outbreak and nonoutbreak years.

As (i) mortality rate during the outbreak years was higher among individuals disadvantaged in terms of access to food resources (subordinate males and inexperienced yearlings), (ii) external signs of infections mainly occurred when prey abundance within the territory was low, and the sharp increase in external signs occurred after (iii) a substantial increase in hyena clan and population sizes and (iv) a decrease in prey availability per capita, our study provides evidence that 'bottom–up' processes triggered the sharp increase in infections which is a 'top–down' process. This is consistent with the idea that carnivore populations may be regulated by a combination of 'top–down' and 'bottom–up' processes (Beldomenico *et al.* 2008).

TRANSMISSION, IMMUNITY AND MAINTENANCE

Why was there no *Streptococcus* outbreak after 2004, when hyena population size exceeded pre-outbreak levels, causing similar or higher levels of nutritional challenge and social contact rates than during the outbreak? Disease outbreaks can be eliminated or prevented when there is sufficient immunity against the disease in a population. This 'critical percentage' of immunity can be calculated as $100(1 - 1/R_0)$ (Coleman & Dye 1996). Using the estimated R_0 for *S. equi ruminatorum* of 2·7 during the outbreak, the critical percentage of immune hyenas required to prevent another outbreak would be 63%. Several results suggest that many hyenas mounted a successful immune response to infection during the outbreak and that this herd immunity may have been sufficient to prevent further outbreaks before the end of the study. These include (i) a low prevalence of external signs and apparent lack of decrease in survivorship of hyenas exposed to individuals likely to be actively shedding bacteria, (ii) a lack of decrease in survivorship of individuals that cleared signs of infection, (iii) a reduction in the severity of signs expressed by hyenas after the outbreak years and (iv) the fact that only one of the 14 hyenas surviving symptomatic infection expressed external signs on other occasions.

Increased levels of immunity may decrease pathogen virulence in at least two ways. First, a primed immune system will more speedily tackle invading bacteria. Frequent exposure to the bacterium and consistently high rates of transmission can stimulate high levels of immunity within host populations, thereby reducing the number of susceptible individuals (Anderson & May 1985) and limiting the impact of a pathogen on a host population (Guiserix *et al.* 2007). The estimates of R_0 suggest that during the outbreak, the bacterium could potentially be transmitted within each clan without transmission from other clans, populations or species. Owing to the highly social nature of hyenas, there is extensive contact between clan members, often involving transfer of body fluids such as saliva, nasal discharge, wound secretion or bacterial particles expelled during coughing. Furthermore, when feeding, particularly when consuming bones, hyenas extensively salivate, and at large carcasses many hyenas, including members of other clans (Höner *et al.* 2005), that gather to feed. *Streptococcus equi* can persist for prolonged periods on substrates such as wood (up to 3 days under natural conditions in temperate environments; Weese, Jarlot & Morley 2008) and be transmitted indirectly via contaminated water or food (Reif 1979; Timoney 1999). If *S. equi ruminatorum* is present in saliva and can persist in water or carcasses of infected prey or hyenas, social interactions and feeding sites provide considerable potential for intraspecific transmission within and between clans and prolonged maintenance of, and immunity against, these bacteria. Furthermore, the carcasses of hyenas that died of infection would presumably hold high levels of bacterial particles that would be transmitted to hyenas scavenging from such carcasses.

Second, increased host immunity may stimulate escape mutations in genes encoding proteins of the bacterium important for pathogenesis. Evidence for such a process comes from studies of *S. equi equi* infections of horses in which immunity against further infection occurred during recovery (Timoney 1999) and M-like protein genes of strains

recovered during symptomatic infection differed from those obtained during nonsymptomatic infection (Chanter *et al.* 2000). The M-like protein gene of the *S. equi ruminatorum* isolate from one Crater hyena that died of infection differed from isolates of nonsymptomatic hyenas (Speck *et al.* 2008), suggesting that escape mutations occurred in strains infecting Crater hyenas.

We currently cannot exclude the possibility that nonvirulent strains of *S. equi ruminatorum* were present in the Crater hyena population before the outbreak and that the outbreak was caused by the introduction of a more virulent strain that lost virulence over time. Previously, we demonstrated that zebras have a high prevalence of apparently nonsymptomatic infection and that direct transmission of *S. equi ruminatorum* is likely to occur from zebras to hyenas (Speck *et al.* 2008). More recently we discovered that buffaloes in the Crater, wild dogs in an area northwest of the Crater and camels *Camelus dromedarius* kept by Maasai are infected with similar strains (Goller *et al.* 2010; O.P. Höner, B. Wachter, S. Speck, M. Grobbel, unpublished data). However, because sequence data for *S. equi ruminatorum* are only available from one virulent hyena strain, it is difficult to establish the source of infection among the Crater hyenas, and whether the lack of additional outbreaks was because of increased immunity or decreased pathogen virulence.

Acknowledgements

References

Akineden, Ö., Hassan, A.A., Alber, J., El-Sayed, A., Estoepangestie, A.T.S., Lämmler, C., Weiss, R. & Siebert, U. (2005) Phenotypic and genotypic properties of *Streptococcus equi* subsp. *zooepidemicus* isolated from harbour seals (*Phoca vitulina*) from the German North Sea during the phocine distemper outbreak in 2002. *Veterinary Microbiology*, **110**, 147–152.

Anderson, R.M. & May, R.M. (1985) Vaccination and herd immunity to infectious diseases. *Nature*, **318**, 323–329.

Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.

Archie, E.A., Morrison, T.A., Foley, C.A.H., Moss, C.J. & Alberts, S.C. (2006) Dominance rank relationships among wild female African elephants, *Loxodonta africana*. *Animal Behaviour*, **71**, 117–127.

Barret, T., Visser, I.K.G., Mamaev, L., Goatley, L., Van Bressem, M.F. & Osterhaus, A.D.M.E. (1993) Dolphin and porpoise morbilliviruses are genetically distinct from Phocine distemper virus. *Virology*, **193**, 1010–1012.

Begon, M., Townsend, C.R. & Harper, J.L. (2006) *Ecology: From Individuals to Ecosystems*. Blackwell Publishing, Oxford.

Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M. & Begon, M. (2008) Poor condition and infection: a vicious circle in natural populations. *Proceedings of the Royal Society of London B*, **275**, 1753–1759.

Burthe, S., Telfer, S., Begon, M., Bennett, M., Smith, A. & Lambin, X. (2008) Cowpox virus infection in natural field vole *Microtus agrestis* populations: significant negative impacts on survival. *Journal of Animal Ecology*, **77**, 110–119.

Chanter, N., Talbot, N.C., Newton, J.R., Hewson, D. & Verheyen, K. (2000) *Streptococcus equi* with truncated M-proteins isolated from outwardly healthy horses. *Microbiology*, **146**, 1361–1369.

Chesson, J. (1983) The estimation and analysis of preference and its relationship to foraging models. *Ecology*, **64**, 1297–1304.

Coleman, P.G. & Dye, C. (1996) Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine*, **14**, 185–186.

Coulson, T., Benton, T.G., Lundberg, P., Dall, S.R.X., Kendall, B.E. & Gaillard, J.-M. (2006) Estimating individual contributions to population growth: evolutionary fitness in ecological time. *Proceedings of the Royal Society of London B*, **273**, 547–555.

Dennis, B. & Taper, M.L. (1994) Density dependence in time-series observations of natural populations: estimation and testing. *Ecological Monographs*, **64**, 205–224.

Dietz, R., Ansen, C.T., Have, P. & Heide-Jørgensen, M.-P. (1989) Clue to seal epizootic? *Nature*, **338**, 627.

East, M.L., Moestl, K., Benetka, V., Pitra, C., Höner, O.P., Wachter, B. & Hofer, H. (2004) Coronavirus infection of spotted hyenas in the Serengeti ecosystem. *Veterinary Microbiology*, **102**, 1–9.

Forchhammer, M.C. & Asferg, T. (2000) Invading parasites cause a structural shift in red fox dynamics. *Proceedings of the Royal Society of London B*, **267**, 779–786.

Goller, K.V., Fyumagwa, R.D., Nikolin, V., East, M.L., Kilewo, M., Speck, S., Müller, T., Matzke, M. & Wibbelt, G. (2010) Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. *Veterinary Microbiology*, **146**, 245–252.

Goymann, W., East, M.L., Wachter, B., Höner, O.P., Möstl, E. & Hofer, H. (2003) Social status does not predict corticosteroid levels in post-dispersal male spotted hyenas. *Hormones and Behavior*, **43**, 474–479.

Guiserix, M., Bahi-Jaber, N., Fouchet, D., Sauvage, F. & Pontier, D. (2007) The canine distemper epidemic in the Serengeti: are lions victims of a new highly virulent canine distemper virus strain, or is pathogen circulation stochasticity to blame? *Journal of the Royal Society Interface*, **17**, 1127–1134.

Haas, L., Hofer, H., East, M., Wohlsein, P., Liess, B. & Barrett, T. (1996) Canine distemper virus infection in Serengeti spotted hyaenas. *Veterinary Microbiology*, **49**, 147–152.

Hampson, K., Dobson, A., Kaare, M., Dushoff, J., Magoto, M., Sindoya, E. & Cleaveland, S. (2008) Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. *PLoS Neglected Tropical Diseases*, **2**, e339.

Harrison, T.M., Mazet, J.K., Holekamp, K.E., Dubovi, E., Engh, A.E., Nelson, K., Van Horn, R.C. & Munson, L. (2004) Antibodies to canine and feline viruses in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve. *Journal of Wildlife Diseases*, **40**, 1–10.

Hawkins, C.E., Baars, C., Hesterman, H., Hocking, G.J., Jones, M.E., Lazenby, B., Mann, D., Mooney, N., Pemberton, D., Pyecroft, S., Restani, M. & Wiersma, J. (2006) Emerging disease and population decline of an island endemic, the Tasmanian devil *Sarcophilus harrisii*. *Biological Conservation*, **131**, 307–324.

Hofer, H. & East, M.L. (1993) The commuting system of Serengeti spotted hyaenas: how a predator copes with migratory prey. II. Intrusion pressure and commuters' space use. *Animal Behaviour*, **46**, 559–574.

Hofer, H. & East, M.L. (2003) Behavioral processes and costs of co-existence in female spotted hyenas: a life history perspective. *Evolutionary Ecology*, **17**, 315–331.

Höner, O.P., Wachter, B., East, M.L. & Hofer, H. (2002) The response of spotted hyaenas to long-term changes in prey populations: functional response and interspecific kleptoparasitism. *Journal of Animal Ecology*, **71**, 236–246.

Höner, O.P., Wachter, B., East, M.L., Runyoro, V.A. & Hofer, H. (2005) The effect of prey abundance and foraging tactics on the population dynamics of a social, territorial carnivore, the spotted hyena. *Oikos*, **108**, 544–554.

Höner, O.P., Wachter, B., Speck, S., Wibbelt, G., Ludwig, A., Fyumagwa, R.D., Wohlsein, P., Lieckfeldt, D., Hofer, H. & East, M.L. (2006) Severe *Streptococcus* infection in spotted hyenas in the Ngorongoro Crater, Tanzania. *Veterinary Microbiology*, **115**, 223–228.

Höner, O.P., Wachter, B., East, M.L., Streich, W.J., Wilhelm, K., Burke, T. & Hofer, H. (2007) Female mate-choice drives the evolution of male-biased dispersal in a social mammal. *Nature*, **448**, 798–801.

Höner, O.P., Wachter, B., Hofer, H., Wilhelm, K., Thierer, D., Trillmich, F., Burke, T. & East, M.L. (2010) The fitness of dispersing spotted hyaena sons is influenced by maternal social status. *Nature Communications*, **1**, 60.

Hudson, P.J., Dobson, A.P. & Newborn, D. (1998) Prevention of population cycles by parasite removal. *Science*, **282**, 2256–2258.

Jones, M.E., Cockburn, A., Hamede, R., Hawkins, C., Hesterman, H., Lachish, S., Mann, D., McCallum, H. & Pemberton, D. (2008) Life-history

change in disease-ravaged Tasmanian devil populations. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10023–10027.

Jorm, L.R. (1990) Strangles in horse studs: incidence, risk factors and effect of vaccination. *Australian Veterinary Journal*, **67**, 436–439.

Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.

Kissui, B.M. & Packer, C. (2004) Top–down population regulation of a top predator: lions in the Ngorongoro Crater. *Proceedings of the Royal Society of London B*, **271**, 1867–1874.

Kruuk, H. (1972) *The Spotted Hyena. A Study of Predation and Social Behavior.* University of Chicago Press, Chicago, IL.

Lembo, T., Haydon, D.T., Velasco-Villa, A., Rupprecht, C.E., Packer, C., Brandão, P.E., Kuzmin, I.V., Fooks, A.R., Barrat, J. & Cleaveland, S. (2007) Molecular epidemiology identifies only a single rabies virus variant circulating in complex carnivore communities of the Serengeti. *Proceedings of the Royal Society of London B*, **274**, 2123–2130.

Lloyd-Smith, J.O., Cross, P.C., Briggs, C.J., Daughterty, M., Getz, W.M., Latto, J., Sanchez, M.S., Smith, A.B. & Swei, A. (2005) Should we expect population thresholds for wildlife disease? *Trends in Ecology and Evolution*, **20**, 511–519.

Manly, B.F.J., McDonald, L.L. & Thomas, D.L. (1993) *Resource Selection by Animals. Statistical Design and Analysis for Field Studies.* Chapman and Hall, London.

Martin, L.B., Weil, Z.M. & Nelson, R.J. (2008) Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **363**, 321–339.

Munson, L., Terio, K.A., Kock, R., Mlengeya, T., Roelke, M.E., Dubovi, E., Summers, B., Sinclair, A.R.E. & Packer, C. (2008) Climate extremes promote fatal co-infections during canine distemper epidemics in African lions. *PLoS ONE*, **3**, e2545.

Nelson, R.J. & Demas, G.E. (1996) Seasonal changes in immune function. *Quarterly Review of Biology*, **71**, 511–548.

Parmar, M.K.B. & Machin, D. (1995) *Survival Analysis: A Practical Approach.* John Wiley and Sons, New York, NY.

Reif, J.S. (1979) Epidemiology of equine infectious respiratory disease. *Veterinary Clinics of North America Large Animal Practice*, **1**, 3–15.

Robert, N., Walzer, C., Ruegg, S.R., Kaczensky, P., Ganbaatar, O. & Stauffer, C. (2005) Pathologic findings in reintroduced Przewalski's horses (*Equus caballus przewalskii*) in southwestern Mongolia. *Journal of Zoo and Wildlife Medicine*, **36**, 273–285.

Runyoro, V.A., Hofer, H., Chausi, E.B. & Moehlman, P.D. (1995) Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. *Serengeti II: Dynamics, Management, and Conservation of an Ecosystem* (eds A.R.E. Sinclair & P. Arcese), pp. 146–168. University of Chicago Press, Chicago, IL.

Sahin, E. & DePinho, R.A. (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*, **464**, 520–528.

Sapolsky, R.M. (2005) The influence of social hierarchy on primate health. *Science*, **308**, 648–652.

Speck, S., Höner, O.P., Wachter, B. & Fickel, J. (2008) Characterization of *Streptococcus equi* subsp. *ruminatorum* isolated from spotted hyenas (*Crocuta crocuta*) and plains zebras (*Equus burchelli*), and identification of a M-like protein (SrM) encoding gene. *Veterinary Microbiology*, **128**, 148–159.

Sweeney, C.R., Benson, C.E., Whitlock, R.H., Meirs, D.A., Barningham, S.O., Whitehead, S.C. & Cohen, D. (1989) Description of an epizootic and persistence of *Streptococcus equi* infections in horses. *Journal of the American Veterinary Medical Association*, **194**, 1281–1286.

Timoney, J.F. (1999) Equine strangles: 1999. *Proceedings of the American Association of Equine Practitioners*, **45**, 31–37.

Tompkins, D.M. & Begon, M. (1999) Parasites can regulate wildlife populations. *Parasitology Today*, **15**, 311–313.

Weese, J.S., Jarlot, C. & Morley, P. (2008) Survival of *Streptococcus equi* in an outdoor environment. *Journal of Veterinary Internal Medicine*, **22**, 819.