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The effect of bovine tuberculosis and brucellosis on reproduction and survival of wood bison in Wood Buffalo National Park

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Summary

1. Wood bison (*Bison bison athabascae* L.) abundance in Wood Buffalo National Park, Canada, declined from an excess of 10 000 bison in the late 1960s to a low of 2200 bison in the late 1990s.
2. Bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*), were introduced to Wood Buffalo National Park in the late 1920s. As each of these pathogens has the potential to reduce survival and reproduction in bison, they are suspected to have played a role in the decline in bison abundance.
3. We live-captured bison in the winters of 1997–2000, tested for tuberculosis, brucellosis and pregnancy and released animals with radio transmitters to evaluate survival.
4. We found that bison that were positive for both diseases were less likely to be pregnant or to survive the winter than bison positive for one or neither disease. Further, in one population, bison that were tuberculosis-positive had a substantially lower pregnancy probability.
5. Demonstrating a negative effect of diseases on survival and reproduction is a necessary, but not sufficient, test of the role of diseases in bison population decline.

Key-words: *Bison bison*, *Brucella abortus*, disease, epizootiology, *Mycobacterium bovis*.

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Introduction

In recent years, attention paid in the ecological literature to the role of infectious diseases as factors limiting the growth of wildlife populations has increased dramatically. Particular attention has been focused traditionally on those diseases that cause major die-offs (e.g. rinderpest, Prins & Weyerhaeuser 1987). However, we have an incomplete understanding of the limiting effects of diseases with low annual mortality rates or those diseases that mainly affect reproduction (Yuill 1987; Gulland 1997). As a consequence, the role of chronic infectious diseases in conservation is often underestimated.

An example where chronic diseases may have played an important role in conservation is the bovine tuberculosis (*Mycobacterium bovis*), brucellosis (*Brucella abortus*) and wood bison (*Bison bison athabascae* L.) system in Wood Buffalo National Park, Canada (Carbyn, Oosenbrug & Anions 1993; Carbyn, Lunn & Timoney 1998; Fuller 2002). Bison in Wood Buffalo National Park, Canada (WBNP) experienced a sustained decline in numbers, dropping from approximately 11 000 bison in 1970 to 2300 bison in 1997 (Carbyn *et al*. 1993, 1998; WBNP unpublished data). One hypothesis for the decline is that the presence of bovine tuberculosis (*M. bovis*) and brucellosis (*B. abortus*) in the bison population is responsible for the decline. However, others feel that disease could not be responsible for population decline owing to low mortality rates, low levels of observed pathology and the perception that declines within the park are not uniform, despite widespread disease prevalence (Carbyn *et al*. 1993). These diseases were probably introduced in the 1920s (Tessaro 1986), and 70 years later the diseases maintain enzootic proportions in the population (Joly & Messier 2004a).

Both bovine tuberculosis and brucellosis could have potentially dramatic impacts on bison population demography. Bovine brucellosis can cause abortion, stillborn calves and retained placentas (Davis *et al.* 1990; Williams *et al.* 1993; Rhyan *et al.* 1994; Rhyan 2001).

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Tuberculosis may affect reproduction of bison in a direct fashion directly by causing fetal losses (Choquette *et al.* 1961) or indirectly by reducing pregnancy rate. For example, we would expect to see lower pregnancy rates in tuberculosis-positive females if tuberculosis reduces weight gain by diverting energy to immune function or by hindering effective foraging due to debilitation. Further, tuberculosis may increase mortality risk in bison (Fuller 1962; Tessaro, Forbes & Turcotte 1990), especially among older animals; such an effect would translate into a shorter life span and reduced lifetime reproduction (Rodwell, Whyte & Boyce 2001). Brucellosis-induced arthritis could affect bison survival by reducing foraging efficiency and increasing risk of predation by wolves (Tessaro *et al.* 1990).

A necessary, but not sufficient, step in evaluating the role of bovine tuberculosis and brucellosis in the population dynamics of bison in northern Canada is to evaluate the effect of bovine tuberculosis and brucellosis status on bison demography. Specifically, we sought to test whether survival and reproduction rates were lower in bison that tested positive for bovine tuberculosis and/or brucellosis relative to test-negative individuals.

Methods

STUDY AREA

WBNP is approximately 44 000 km^2 in size, and straddles the border between the Province of Alberta and the North-west Territories, Canada (60°N 112°W; Fig. 1). Aspects of the WBNP environment relevant to bison ecology are described in detail in Carbyn *et al*. (1993). Bison in WBNP are distributed spatially as a metapopulation, with five main populations (*sensu* Wells & Richmond 1995): Garden River, Delta, Hay Camp, Nyarling River and Little Buffalo (Joly 2001). Bison abundance and densities at the start of the study varied dramatically among our three study populations: Nyarling River: ~200 bison (0·011 bison km^2); Hay Camp: ~680 bison (0·124 bison km^2); Delta: ~780 bison (0·162 bison km^2; Joly & Messier 2004b).

BISON CAPTURES

Bison were captured for testing in the Nyarling River (females only), Hay Camp and Delta populations in late February and early March of each year (Fig. 1). Bison capture and handling protocols were described in detail in Joly (2001) and Joly & Messier (2004a). Briefly, bison were captured with netguns fired from helicopters or chemically immobilized from the air using carfentanil and xylazine hydrochloride (Haigh & Gates 1995). Bison were held either in captivity or reimmobilized for 72 h, after which the results of tuberculosis testing were determined. Bison were subsequently released with radio-collars to monitor movement and survival (see below).

PREGNANCY, BODY CONDITION AND AGE ASSESSMENT

Pregnancy status of female bison was determined by testing for the presence of pregnancy-specific protein B (PSPB) (Biotracking, Moscow, Idaho, USA; Haigh *et al.* 1991). We used a 93% binding criterion in the PSPB test (Noyes *et al*. 1997). We validated the PSPB test in 2000 by palpating females at the same time as sampling for the PSPB (n = 87). The test correctly identified 69/70 pregnant females (sensitivity = 98·6%) and 11/12 barren females (specificity = 90·5%).

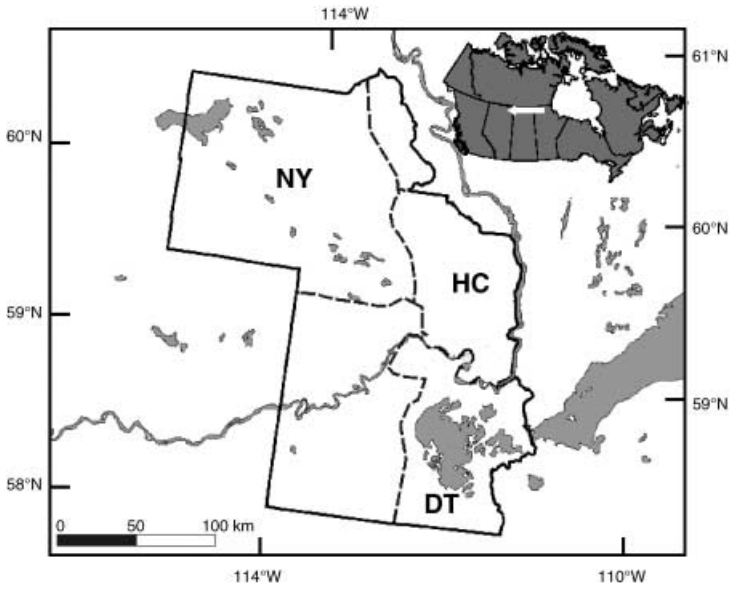

Fig. 1. Wood Buffalo National Park, Canada. Dashed lines indicate the locations of the bison populations in this study (NY: Nyarling River; HC: Hay Camp; DT: Delta; Joly 2001).

Bison were assigned a subjective, relative body condition score for each bison based on criteria in Gerhart *et al*. (1996). For all analyses, we converted this five-category scale to a two category scale: 'poor' (i.e. less than the median score) and 'good' (i.e. greater than the median score). Bison were aged based on tooth-eruption patterns (Fuller 1959) and by counting cementum annuli on the third incisor (Matson's Laboratory, Milltown, MT, USA).

DISEASE TESTING

Disease-testing procedures were previously reported in Joly & Messier (2004a). Briefly, blood samples were taken from each bison from the caudal or carotid vein during the first handling period. Blood was collected in serum-separator tubes to facilitate clotting, and prevented from freezing. Serum was removed by centrifuge within 12 h, and serum samples were frozen until serological tests were conducted. Brucellosis status was determined using the complement-fixation test (CFT; Nielsen *et al*. 1996; Gall *et al*. 2000). Testing was conducted at the Animal Disease Research Institute, Lethbridge, Alberta (Animal and Plant Health, Canadian Food Inspection Agency). The standard criterion for brucellosis-positive status with the CFT is serum that agglutinates at dilutions of 1 : 5 or greater. However, the immunological response of a bison varies with time since first infection with brucellosis, increasing at the time of first abortion then declining with time (see Cheville, McCullough & Paulson 1998). Roffe *et al*. (1999) found that a complement-fixation titre of 1 : 40 correlated better with culture results (and therefore with a current rather than previous infection), than a threshold of 1 : 5. Therefore, we refer to bison with a complement-fixation titre ≥ 1 : 40 as brucellosis-positive. The sensitivity and specificity of the complement-fixation test has not been evaluated formally at this criterion, but estimates for specificity of 95·5% and sensitivity of 89·5% were evaluated previously for bison at a titre of 1 : 5 (Gall *et al*. 2000). We tested for *M. bovis* using the caudal-fold test with PPD tuberculin (Thoen *et al*. 1988; Monaghan *et al*. 1994) and the fluorescent-polarization assay (Lin *et al*. 1996; Joly & Messier 2004a). Bison that tested positive on either the caudal-fold or the fluorescent-polarization tests were considered positive and are referred to as *M. bovis*-positive. There are no controlled studies evaluating the sensitivity or specificity of the caudal-fold test with PPD tuberculin in bison, although Tessaro (1989) showed the caudal-fold test using old-type (OT) tuberculin has a sensitivity of 66·7% and specificity of 89·6% in bison. Sensitivity and specificity of the fluorescent-polarization assay are unknown for bison (Joly & Messier 2004a).

RADIO-TELEMETRY

We deployed radio-collars on bison in February–March 1997–99 (see chapter 2). Each radio-collar was equipped with a motion sensor to detect mortality, and had a battery life of 24 months. In addition, we deployed ear-tag radio transmitters on calves (< 12 months old) in 1997–99. These transmitters had a battery life of 10 months in 1997 and 15 months in 1998 and 1999. We attempted to relocate bison from an aircraft once every 10 days in the spring and early summer, and approximately every 3 weeks for the remainder of the year.

When mortality was detected during aerial surveys, we investigated the carcass on the ground. When sufficient remains were present a standardized protocol was followed during each mortality investigation, based on methods outlined in Wobeser & Spraker (1980) and Roffe, Friend & Locke (1994). Predation was identified by degree of skeleton disarticulation, evidence of a chase sequence in snow, scattered blood or fur, and location of rumen (in or outside the abdominal cavity). We classified the cause of mortality as 'undetermined' if we could not distinguish predation from scavenging or there was no indication as to the cause of death. Causes of mortalities classified as 'other' include drowning, hunting, and disease.

DATA ANALYSIS

We examined the following variables as predictors of pregnancy rate in bison: body condition, age and population (Delta, Hay Camp and Nyarling River), as well as tuberculosis and brucellosis status.

Annual survival rate was estimated as described by Heisey & Fuller (1985), except that the analysis was based on seasonal, rather than daily, survival probabilities. Winter (7 November–4 May) was defined by the median date of ice freeze-up and break-up reported in Carbyn *et al*. (1993: 60) for the years 1953–81, and divided into two (early, 7 November–1 March; late, 2 March–4 May) to accommodate the addition of new radio-collars in 1998 and 1999. The other seasons were defined by modification of those proposed by Komers, Messier & Gates (1992): spring, 5 May–30 June; summer, 1 July–31 August; and autumn, 1 September–6 November.

We estimated seasonal cause-specific mortality rates as $m_{ij} = y_{ij}/x_i$, where m_{ij} is the probability that a bison dies from cause j during season i, y_{ij} is the number of deaths in season i due to cause j and x_i is the number of bison at risk during the season (Heisey & Fuller 1985). Annual rates were calculated as the product of the seasonal rates. Ninety-five per cent confidence limits were estimated using the Taylor series approximation method in program MICROMORT (Heisey & Fuller 1985).

We censored bison from analysis at time of lost radio-contact except for calves (10–11 months of age at capture) that had a 10-month battery life on their transmitters. We assumed that calves died if we lost contact within 6 months of collar deployment (i.e. start of the autumn season). We excluded those bison that were determined to have died from capture-related causes (described in detail in Joly 2001). The following variables were examined as predictors of seasonal survival probability: year, population (Hay Camp, Delta or Nyarling River), brucellosis status (complement-fixation titre ≥ 1 : 40), tuberculosis

status (caudal-fold or fluorescent-polarization assay positive), age and sex. In addition, all two-way interactions among biological variables were included.

As it was not practical, nor informative, to consider all possible models that could be generated by the above variables and two-way interactions in the reproduction and survival analyses, we made an a priori decision to restrict the number of terms considered in the Akaike information criteria (AICc) analysis. We used backward-elimination, multiple logistic regression (SPSS 10·05, Chicago, IL, USA) to determine which terms (main effects or two-way interactions) had the highest value in predicting pregnancy rate. Independent variables were removed sequentially from the global model at $P > 0{\cdot}10$. Olden & Jackson (2000) showed that with large sample sizes ($n = 60$), backward-elimination multiple regression was among the least biased in selecting models of multiple regression. The bias was to include 'extra' variables rather than miss significant parameters; thus we believe this approach guards effectively against a type I error. We used the small sample-size corrected AICc to rank models of pregnancy rate in bison (STATISTICA 5·5, Tulsa, OK, USA), and calculated model-averaged confidence intervals to estimate the effect of each parameter on pregnancy rate (Anderson *et al*. 2000; Burnham & Anderson 2002). Results are presented as odds ratios and confidence intervals to facilitate interpretation of data. We considered a factor to have an effect if the 95% confidence interval for the model-averaged odds ratio crossed unity (analagous to a type I error rate ≤ 0·05). Akaike weights (ω) estimate the probability that a model is the Kullback–Liebler best model among those considered (Anderson *et al*. 2000). We estimated the relative strength of evidence ($\Sigma\,\omega_i$) for each parameter by summing the Akaike weights among w models:

$$\Sigma\,\omega_i = \sum_{1}^{\omega} \omega_j I_j \qquad \text{eqn 1}$$

where ω_j is the Akaike weight for model j and I is an variable indicating whether parameter I is present in model ω_j (see Anderson *et al*. 2000; Burnham & Anderson 2002). The value $\Sigma\,\omega_i$ can be interpreted as the probability that the Kullback–Liebler best model contains the parameter i. In other words, values of $\Sigma\,\omega_i$ near 1 indicate strong support for the parameter being an important factor.

Results

BISON CAPTURES AND RADIOTELEMTRY

Captures were distributed throughout the park using an approximate 3 : 3 : 1 ratio (Peace-Athabasca Delta : Hay Camp : Nyarling River). No males or females < 2 years of age were captured in the Nyarling River area. We deployed 80, 75 and 72 radio-collars on bison in 1997, 1998 and 1999, respectively. Fifty-four radio-telemetry surveys were conducted between April, 1997 and February, 2000 [mean interval, 20 ± 8 (SD) days].

PREGNANCY TESTING

The overall pregnancy rate of female bison ≥ 2 years was 72·2% ($n = 205$). The factors age and brucellosis status (and their interaction) were excluded by the backward elimination multiple regression ($P > 0{\cdot}10$). The following terms remained: body condition, tuberculosis status, an interaction between tuberculosis and high brucellosis titre and an interaction between population and tuberculosis status. The latter interaction indicated that tuberculosis status might affect pregnancy rate differently among the populations, and further examination of the data indicated that the effect of tuberculosis on pregnancy was probably different in the Nyarling River population from the Hay Camp (Wald statistic 3·30, d.f. = 1, $P = 0{\cdot}07$) and Delta (Wald statistic 3·11, d.f. = 1, $P = 0{\cdot}08$) populations, but the effect was similar in the Delta and Hay Camp (Wald statistic 0·001, d.f. = 1, $P = 0{\cdot}98$). Therefore, we repeated the analysis for Nyarling River separately from a pooled data set involving Delta and Hay Camp bison.

In Delta and Hay Camp bison, those that were in good body condition were 1·7 times more likely to be pregnant as bison in poor body condition (95% confidence interval, 1·2–2·4; Table 1). Odds of pregnancy for tuberculosis-positive bison did not differ from odds for tuberculosis-negative bison (odds ratio 1·0, 95% confidence interval 0·9–1·2), and we were unable to detect a difference in pregnancy rate between bison with low (< 1 : 40) or high complement-fixation titre for brucellosis (Wald Statistic 0·13, d.f. = 1, $P = 0{\cdot}72$). However, bison that tested positive for tuberculosis and had a high titre for brucellosis were 0·7 times as likely to be pregnant (95% confidence interval, 0·6–1·0) as bison with one or neither disease.

In Nyarling River bison tuberculosis status was a strong predictor of pregnancy, where positive bison were 0·26 times as likely to be pregnant as negative bison (95% confidence interval, 0·08–0·82; Table 2). Bison that tested positive for both diseases also were less likely to be pregnant although the odds ratio did not differ from one (odds ratio 0·61, 95% confidence interval, 0·31–1·23). This seemingly contradictory result is an artefact of

Table 1. Factors affecting pregnancy rate in female bison in the Hay Camp and Delta populations of WBNP ($n = 167$). The odds ratio refers to the effect of each parameter on odds of being pregnant. The Σ ω column indicates the sum of the Akaike weights for models that contained each parameter, and gives a relative measure of support for that parameter (see Anderson *et al*. 2000)

Parameter	Odds ratio	95% CI	Σ ω
Good vs. poor body condition	1·69	1·17–2·44	0·96
TB positive vs. negative[a]	1·04	0·90–1·20	0·32
TB and brucellosis-positive vs. negative on one or both diseases[a]	0·74	0·55–1·00	0·73

[a]Brucellosis-positive: complement-fixation titre ≥ 1 : 40; tuberculosis-positive: FP ≥ 174 mp and/or caudal-fold test positive. CI: confidence interval.

Table 2. Factors affecting pregnancy rate in female bison in the Nyarling River population of WBNP (n = 36). The odds ratio refers to the effect of each parameter on odds of being pregnant. The Σ ω column indicates the sum of the Akaike weights for models that contained each parameter, and gives a relative measure of support for that parameter (see Anderson *et al*. 2000)

Parameter	Odds ratio	95% CI	Σ ω
Good vs. poor body condition	1·63	0·80–3·35	0·58
TB positive vs. negative[a]	0·26	0·08–0·82	0·95
TB and brucellosis-positive vs. negative on one or both diseases[a]	0·61	0·31–1·23	0·51

[a]Brucellosis-positive: complement-fixation titre ≥ 1 : 40; tuberculosis-positive: FP ≥ 174 mp and/or caudal-fold test positive. CI: confidence interval.

sample size and the older age distribution of female bison in the Nyarling River population sample.

ANNUAL SURVIVAL AND CAUSE-SPECIFIC MORTALITY

Annual survival of bison varied from a low of 0·77 in the Delta population (1998/99) to a high of 1 in the Nyarling River population (1997/98 and 1999/00; Table 3). Mortality from all causes was highest in the Delta population, particularly wolf (*Canis lupus* L.) predation. Wolf predation was a minor source of mortality in the Hay Camp and Nyarling River populations (mean annual probability, < 0·01). Two bison from the Hay Camp population were shot outside the WBNP boundary near Mission Farms and the Salt River area. Drowning was a minor source of mortality among radio-collared bison (Table 3). One bison died of generalized tuberculosis in the Delta area.

SEASONAL SURVIVAL PROBABILITIES

In spring (5 May–30 June) and summer (1 July and 31 August) survival probabilities for bison > 1 year of age were high, exceeding 0·97 in all populations. This high survival probability prevented analysis of factors associated with mortality. Further, we could not assess factors associated with survival in the Nyarling River population owing to the very high survival rate there (Table 3). Therefore, we focus on autumn, early and winter survival in the Hay Camp and Delta populations.

Autumn (1 September–6 November) survival for bison ≥ 1 year was high in the Delta, Hay Camp and Nyarling River populations. We excluded all but the following factors associated with autumn survival probability with the backward elimination multiple regression: tuberculosis status, sex, an interaction between sex and age and an interaction between tuberculosis status and age (remaining variables and interaction terms, P > 0·10). However, the 95% confidence intervals for the odds ratios overlapped one for all these terms (Table 4). Neither tuberculosis nor brucellosis status were significant main effects.

Survival in the early winter season (7 November–1 March) was lower in 1997 than 1999 (odds ratio 2·0, 95% confidence interval, 1·0–3·8), but there was no difference between 1998 and 1999 (odds ratio 1·0, 95% confidence interval, 0·6–2·03; Table 5). Bison that tested positive for tuberculosis and had a high titre for brucellosis were 2·5 times more likely to die during this season than bison that tested positive for one or neither disease (95% confidence interval, 1·0–6·1). All other factors were excluded by the elimination procedure (including tuberculosis and brucellosis-status as main effects).

One adult female bison was killed by wolves in late winter (2 March–4 May) of 1999, 2 days after capture (see Joly 2001). Although this mortality was unrelated to capture, no other mortalities occurred during this season. Therefore, we restricted the analysis to late winter of 1997 and 1998. Five terms were not excluded by the backward elimination as predictors of late winter survival: sex, population, year, an interaction between tuberculosis status and age and an interaction between tuberculosis and a high brucellosis titre (Table 6). Brucellosis and tuberculosis as main effects were excluded.

Table 3. Annual survival and cause-specific mortality rates for bison (≥ 1 year old) in WBNP, 1997–2000. 'Pop' refers to population. Ranges in parentheses are 95% confidence intervals

				Cause-specific mortality rates		
Year	Pop	n	Annual survival	Undetermined[a]	Wolf predation	Other
1997/98[b]	DT[c]	40	0·81 (0·68–0·96)	0·07 (0–0·15)	0·10 (0–0·21)	0·02[d] (0–0·07)
	HC	24	0·96 (0·89–1)	0 (–)	0 (–)	0·04[e] (0–0·11)
	NY	10	1 (–)	0 (–)	0 (–)	0 (–)
1998/99	DT	41	0·77 (0·64–0·91)	0·06 (0–0·14)	0·12 (0·02–0·21)	0·05[f] (0–0·12)
	HC	44	0·98 (0·93–1)	0·02 (0–0·07)	0 (–)	0 (–)
	NY	21	0·99 (0·77–1)	0·01 (0–0·23)	0 (–)	0 (–)
1999/2000	DT	30	0·89 (0·79–1)	0 (–)	0·09 (0–0·18)	0·03[d] (0–0·08)
	HC	62	0·92 (0·86–1)	0·04 (0–0·09)	0·01 (0–0·04)	0·02[e] (0–0·06)
	NY	20	1 (–)	0 (–)	0 (–)	0 (–)

[a]Includes mortalities where wolf predation could not be excluded; [b]year assumed to begin on 1 March; [c]Delta, DT; Hay Camp, HC; Nyarling River, NY; [d]drowning (n = 1 in 1997/98 and 1999/2000); [e]hunting (n = 1 in each year); [f]drowning (n = 1), generalized tuberculosis (n = 1).

Table 4. Factors affecting autumn (1 September–7 November) mortality probability for bison in the Hay Camp and Delta populations of WBNP (n = 222). The odds ratio refers to the effect of each parameter on odds of dying during the autumn. The Σ ω column indicates the sum of the Akaike weights for models that contained each parameter, and gives a relative measure of support for that parameter (see Anderson *et al.* 2000)

Parameter	Odds ratio	95% CI	Σ ω
Male vs. female	1·0	0·5–1·7	0·37
TB positive vs. negative[a]	1·3	0·7–2·6	0·44
Sex × age interaction	1·0	0·9–1·1	0·37
TB × age interaction	1·0	0·9–1·1	0·40

[a]Tuberculosis-positive: FP ≥ 174 mp and/or caudal-fold test positive. CI: confidence interval.

Table 5. Factors affecting early winter (7 November–1 March) mortality probability for bison in the Hay Camp and Delta populations of WBNP (n = 210). The odds ratio refers to the effect of each parameter on odds of dying during early winter. The Σ ω column indicates the sum of the Akaike weights for models that contained each parameter, and gives a relative measure of support for that parameter (see Anderson *et al.* 2000)

Parameter	Odds ratio	95% CI	Σ ω
Year			
1997 vs. 1999	2·0	1·0–3·8	0·61
1998 vs. 1999	0·96	0·5–2·0	
Age	0·98	0·92–1·04	0·33
TB × age interaction	1·0	0·96–1·03	0·33
TB and brucellosis-positive vs. negative on one or both diseases[a]	2·5	1·0–6·6	0·86

[a]Brucellosis-positive: complement-fixation titre ≥ 1 : 40; tuberculosis-positive: FP ≥ 174 mp and/or caudal-fold test positive. CI: confidence interval.

An interaction term between sex and tuberculosis was significant in the multiple regression, although we did not consider this term, as it indicated unrealistically that tuberculosis increased survival. Bison in the Delta population were three times more likely to die during this season than bison in the Hay Camp population (95% confidence limit, 1·0–9·0). Tuberculosis-positive bison with high brucellosis titre were 3·7 times more likely to die than bison with one or neither disease (95% confidence limit 1·3–11·1; Table 6). There was some indication that survival in this season was lower in 1998 than 1997, although the confidence limits surpassed one (Table 6).

Discussion

A difficult aspect of evaluating the role of disease in wildlife demographic studies is that errors in disease testing result in an underestimation of the effect size, reducing our ability to detect this effect (Greiner & Gardner 2000; Joly 2001). True effect size is the difference between demographic rates for known infected and non-infected individuals. Effect size is underestimated when there are errors in disease testing as some individuals are misclassified. This results in an overestimate of the true demographic rate for infected individuals and an underestimate of the demographic rate of non-infected individuals. The sum of these biases reduces the ability of a statistical test to detect this difference (e.g. Peterman 1990). Therefore, estimates of the effect of disease on a demographic parameter in which disease testing is used to identify infected individuals should be viewed as conservative estimates.

Table 6. Factors affecting late winter (1 March–4 May) mortality probability for bison in the Hay Camp and Delta populations of WBNP (n = 163). The odds ratio refers to the effect of each parameter on odds of dying during late winter. The Σ ω column indicates the sum of the Akaike weights for models that contained each parameter, and gives a relative measure of support for that parameter (see Anderson *et al.* 2000)

Parameter	Odds ratio	95% CI	Σ ω
Year			
1997 vs. 1998	0·6	0·36–1·04	0·69
Pop	3·0	1·02–8·96	0·92
Male vs. female	0·7	0·47–1·07	0·57
TB × age interaction[a]	1·0	0·96–1·03	0·28
TB and brucellosis-positive vs. negative on one or both diseases[a]	3·7	1·27–11·05	0·98

[a]Brucellosis-positive: complement-fixation titre ≥ 1 : 40; tuberculosis-positive: FP ≥ 174 mp and/or caudal-fold test positive. CI: confidence interval.

The effect of tuberculosis on pregnancy rate was stronger in the Nyarling River population than in the Hay Camp and Delta populations, after controlling for body condition. We hypothesize that this was a consequence of the lower autumn and winter survival rates in the Hay Camp and Delta populations. Reduced survival in autumn and winter associated with tuberculosis would result in a decrease in the proportion of bison that have generalized tuberculosis over the winter, relative to those that test positive but have not yet developed clinical signs. If bison with generalized tuberculosis have a lower pregnancy rate, the average pregnancy rate for surviving test-positive bison would increase over the winter, thus decreasing the ability to detect an effect. In contrast, the effect of disease on pregnancy may have been more evident in the Nyarling River area as more bison in later stages of infection survived the winter.

Previous surveys in WBNP have failed to detect an effect of tuberculosis on pregnancy in bison (e.g. Fuller 1962). It is difficult to explain why these results conflict with ours. The simplest explanation is a spurious correlation with a variable we have not controlled for. The sum of the Akaike weights for models including tuberculosis is 0·95, indicating very strong support for inclusion of this factor among the models examined, but this does not preclude existence of a lurking variable. Methodological differences may also be responsible for the

conflicting results. Tuberculosis induces abortion in cattle in late gestation (Plum 1924, 1937). Fuller (1962) conducted his examinations during December and January slaughters. If the effect of tuberculosis on bison reproduction mirrors that in cattle as it does in other aspects (e.g. Tessaro *et al*. 1990), then the timing of Fuller's study may have been premature to detect an effect. Experimental studies of the effect of tuberculosis on reproduction of bison would aid in clarifying this relationship.

We did not detect a main effect of brucellosis on pregnancy. This result is not surprising as these data reflect pregnancy rates before the period in which *Brucella*-induced abortions are expected to occur in bison (Williams *et al*. 1993; Rhyan *et al*. 1994), although some abortions do occur earlier (Rhyan 2001). Further, we determined pregnancy by measuring levels of pregnancy-specific protein B, which has been shown to have a half-life postcalving or abortion of 7–8 days in dairy cattle (Semambo *et al*. 1992; Kiracofe *et al*. 1993). Bison that aborted shortly before capture may have been misclassified as pregnant. Although we did not find a main effect of brucellosis, bison in the Hay Camp and Delta populations who had a high brucellosis titre and tuberculosis were less likely to be pregnant. Perhaps bison with tuberculosis infection are more likely to suffer brucellosis-induced abortion during winter as a result of overall weakened immune function. Alternatively, as the effect of tuberculosis on pregnancy in the Nyarling River population exceeded the effect of brucellosis, perhaps the presence of brucellosis increases the risk of tuberculosis-induced reproductive failure. Further research may be necessary to elucidate the potential for an interactive effect of tuberculosis and brucellosis on reproductive success of bison.

Annual wolf-predation rates varied from 0·09 to 0·10 in the Delta population, high relative to predation rates elsewhere in the park. The low rate of wolf predation observed in the Hay Camp and Nyarling River populations was not expected. The main difference between these latter populations and the Delta is in bison density. Perhaps reduced bison density leads to reduced wolf density (Joly & Messier 2000) and/or prey-switching by wolves to moose (*Alces alces* L.; Larter *et al*. 1994), as might be expected when two species with differential vulnerabilities are sympatric (e.g. Joly & Patterson 2003). Alternatively, perhaps wolves target mainly juvenile bison in the Hay Camp and Nyarling River populations. We have no data on annual survival of bison < 1 years of age, and only limited data on bison 1–2 years of age, and so cannot address this question directly.

We did not detect a main effect of tuberculosis or brucellosis on survival of bison. There is little dispute that tuberculosis causes an increase in mortality in a range of species including bison (e.g. Tessaro 1986; Tessaro *et al*. 1990; O'Reilly & Daborn 1995), and as we observed at least one bison that died of generalized tuberculosis we reject the view that tuberculosis causes *no* mortality in bison. Previous estimates of tuberculosis-induced mortality range from 5 to 6% (Fuller 1962; Tessaro *et al*. 1990). We were unlikely to detect an effect of this magnitude, given the reduction in statistical power associated with errors in disease testing (see Joly 2001). However, we did observe an interactive effect between tuberculosis and brucellosis on winter survival of bison.

The physiological mechanism for an interaction between tuberculosis and brucellosis to affect reproduction and survival of bison is not known. Brucellosis-induced arthritis has been reported in bison in WBNP (e.g. Tessaro *et al*. 1990) and in cattle; however, we were unable to find a published account for brucellar arthritis in bison in Yellowstone National Park, where brucellosis is also endemic yet tuberculosis is not present (Cheville *et al*. 1998). The lack of a published report does not necessarily mean that this aspect of brucellosis pathology does not occur, but it must be rare as there are no reports of these lesions among ~1000 bison slaughtered as they left Yellowstone National Park in the winter of 1996/97. However, these brucellar lesions are common among WBNP bison (e.g. Tessaro *et al*. 1990), where tuberculosis is present. If there is an interaction between pathology *B. abortus* and *M. bovis* in bison, it is not clear whether the presence of *B. abortus* enhances the pathological effects of *M. bovis* or vice versa. It is interesting that macrophage activity and macrophage-derived products are important determinants of host responses to both pathogens. For example, *Brucella* sp. interfere with phagosome–lysosome fusion and function of oxidative metabolites (Köhler *et al*. 2002), and both these processes are important in intracellular establishment and survival of *M. bovis* (see Cross *et al*. 1996 and references therein). The potential for interaction between these diseases on bison demography warrants experimental research, particularly as the consequences of infection with multiple diseases are not well understood for wildlife.

Daszak, Cunningham & Hyatt (2003) argued that, among other criteria, 'pathological evidence that the disease caused death in a significant number of cases within these mortality events' is required to demonstrate that a disease is causing a population decline. Although Daszak *et al*. (2003) framed these criteria in the context of amphibian declines, they are applicable to examining the broader role of wildlife disease in conservation. However, we argue that for a number of reasons it is not necessary to show that an infectious pathogen actually caused the mortality, but rather that those that are infected experience reduced survival or reproduction rates. First, in many, if not most, wildlife populations actual deaths attributed to the pathogen will not often be observed unless there is a systematic, long-term carcass collection mechanism. For example, treatment for gastrointestinal nematodes was shown to increase survival of Soay sheep (*Ovis aries* L.) during a population crash, but the cause of death in all 320 animals that died was 'protein-energy malnutrition' (Gulland 1992). Secondly, for some infectious diseases the probability of observing the event may be related to the cause of death. For example, in situations of trophic transmission

(Lafferty 1999) where the presence of a parasite increases probability of predation, there is little likelihood of being able to determine whether parasitism actually caused the death (e.g. Joly & Messier 2004c). Thirdly, both the previous examples highlight that unequivocal demonstration that disease caused the death of an animal becomes difficult when diseases act in concert with other factors, in particular if it predisposes an animal to die from another cause. Fourthly, it is unlikely that the event of a disease causing a failure to reproduce will be observed, except in a few cases such as disease-induced abortion (e.g. brucellosis); for most cases only a reduction in reproduction rate can be observed.

Our study has focused on examining if bovine tuberculosis and brucellosis have detectable negative consequences for bison survival and reproduction. Although we did not observe any brucellosis-induced abortions, and observed only one animal actually dying of tuberculosis, we did show reduced survival and reproduction rates for bison that tested positive for both tuberculosis and brucellosis. However, this is not a sufficient test of the role of disease in bison-population decline in Wood Buffalo National Park. In a concurrent computer modelling study, we found the extent of reduction in bison productivity demonstrated herein was sufficient to cause a shift from a high density, food-regulated equilibrium to a low density equilibrium regulated by predation by wolves (Joly & Messier 2004b).

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