FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

DEMOGRAFIA HISTÓRICA E CONTEMPORÂNEA DE GUEPARDOS (*Acinonyx jubatus*) NA NAMÍBIA, ÁFRICA AUSTRAL

Ezequiel Chimbioputo Fabiano

TESE DE DOUTORADO

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

Av. Ipiranga 6681 – Caixa Postal 1429

Fone: (051) 320-3500 – Fax: (051) 339-1564

CEP 90619-900 Porto Alegre – RS

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Orientador: Dr. Eduardo Eizirik

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Sumário

Dedication

This dissertation is dedicated to Almighty God, for giving the opportunity of gaining new knowledge and to Dr. Martin Mbewe who introduced me to the world of conservation earlier in 2000.

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First, I thank God for seeing me through. For being my anchor, source of strength, motivation, wisdom, calmness, provider, indeed "The Lord is ever present".

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I would also like to thank everyone else that in one or another way contributed towards my journey, members of the Central Baptist Church of Porto Alegre, Prayer Partners around the globe, colleagues from the Genoma in particular Fernanda Pedone "for introducing me to world of non-invasive genetics", Ana, Analise, Lucas, Laura; those who took time to help reviewing the dissertation manuscripts in particular Katherine Forsythe, Marina and Alexandro, Amanda Fabiano, Frederico Lemos, Likulela, Carolyn among others....

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Resumo

Contexto: A diversidade genética contemporânea de espécies e populações é resultante da interação entre aspectos ecológicos e biológicos das mesmas em relação aos efeitos de processos históricos naturais, bem como ao efeito atual dos humanos. Essas forças causaram alterações no tamanho efetivo da população de muitos elementos da fauna e flora, afetando não só os seus potenciais evolutivos, mas também suas distribuições geográficas. Conseqüentemente, existe uma necessidade de caracterizar a história demografica de espécies em diferentes níveis.

A baixa diversidade genética contemporânea de guepardos é usualmente considerada como o resultado de um severo gargalo genético em torno do Último Máximo Glacial (8.000 - 20.000 anos atrás), seguido por endogamia, uma expansão em meados do Holoceno (5.000 anos) e finalmente um gargalo durante o ultimo século devido a a uma combinação de fatores humanos e variações climáticas. Hipóteses alternativas incluem uma estrutura de metapopulação e persistência de tamanho efetivo baixo, devido à ocorrência de poliginia, gerando uma alta variância reprodutiva. Apesar dos avanços em ferramentas moleculares nas últimas décadas, estas hipóteses permanecem ainda largamente inexploradas. Da mesma forma, os efeitos de fatores humanos sobre a viabilidade da população, precisam ser quantificados, assim como é necessário determinar as tendências temporais em abundância e densidade utilizando robustas abordagens sistemáticas.

Neste contexto, o objetivo primário deste estudo foi obter novas informações sobre estes aspectos, as quais são consideradas significantes para que medidas de conservação abrangentes sejam colocadas em prática. Especificamente, exploramos a historia demografica da maior população de guepardos ao longo dos últimos 60 mil anos. Segundo, avaliamos a viabilidade genética desta população e sua sensibilidade a perturbações e incertezas sobre o tamanho da população atual, bem como estimativas da sua capacidade suporte. Por fim, avaliamos as tendências em densidade e abundância, assim como certos aspectos ecológicos comportamentais de uma população local.

Ferramentas: Métodos Bayesianos foram aplicados para avaliar e contrastar cenários evolutivos de estabilidade, declínio e de expansão em diferentes períodos nos últimos 60 mil anos. Para estimar o tamanho efetivo contemporâneo da população, foram utilizadas quatro estimativas genéticas e uma baseada em simulações de viabilidade. Simulações foram realizadas para avaliar a sensibilidade da estimativa de tamanho efectivo a perturbações nas taxas vitais, incertezas no tamanho da população e capacidade suporte. Por fim, o tamanho populacional de censo e a densidade populacional foram estimados através de métodos espaciais e não espaciais de captura-recaptura.

Resultados: Primeiro, os cenários demográficos indicaram que a população tem uma história demográfica complexa, caracterizada por períodos de declínio populacional, intercalados por períodos de estabilidade, sem sinal de expansão detectado desde 60.000 mil anos. Um sinal de estabilidade foi detetado para os ultimos 300 anos. Adicionalmente, cenários modelados que assumiram reduções abruptas tiveram taxas baixas de suporte em relação a modelos de redução gradual.

Segundo, estimativas de tamanho efetivo baseadas em simulações indicaram que a população é viável, porém suscetível a perturbações como a proporção de fêmeas reprodutoras, as taxas de sobrevivência de adultos do sexo feminino, e incertezas em estimativas de abundância e de capacidade de suporte. O tamanho de censo da população também foi influenciado por estes parâmetros. No entanto, a influência em ambos os parâmetros é condicionada aos níveis de perturbações.

Terceiro, as estimativas de densidade, principalmente de machos adultos, variaram entre 5 - 20 km^{-3} e foram semelhantes entre os levantamentos realizados no decorrer dos seis anos de amostragem. Os guepardos machos mostraram uma fidelidade de até quatro anos de uso consecutivo de sítios de marcação (*scent-marking sites*) dentro de suas áreas próprias, evidenciando também um padrão de atividade predominantemente noturno.

Discussão: Primeiro, o estudo mostra que a diversidade genética contemporânea da população (e possivelmente de outras populações com as quais está geneticamente ligada) é resultante de um declínio gradual, provavelmente causado por flutuações e reduções de habitat adequado devidas a oscilações climáticas no Pleistoceno e Holoceno, bem como aumentos no nivel de aridez em tempos mais recentes na Namíbia. Segundo, que a viabilidade da população é em grande parte dependente de aspectos relacionados com fêmeas, e que parecem existir valores limiares além dos quais certas perturbações podem ter uma influência negativa sobre a viabilidade. Por último, a densidade de machos parece ser resultado da dinâmica das áreas de vida, visto que a densidade permaneceu semelhante, exceto durante os períodos de instabilidade social causada por áreas vagas. A instabilidade causada por remoções antropogênicas pode, portanto, levar a maior variância reprodutiva.

Conclusões: O estudo indica que uma estimativa realista do risco de extinção desta população requer a integração de resultados obtidos por diversas abordagens analíticas, e que planos de conservação de longo prazo devem incluir tal conjunto de informações. A observação de que a viabilidade é sensível a diferentes fatores biológicos e sociais ressalta a importância desta avaliação, a qual se integra aos demais temas investigados neste estudo. De forma mais ampla, os resultados aqui apresentados são potencialmente relevantes para diversas outras espécies que enfrentam ameaças de extinção semelhantes.

Abstract

Background: The contemporary genetic diversity of species and populations is a product of climatic oscillations over deeper timescales and/or anthropogenic factors over recent times. These forces caused alterations in the effective population size of fauna and flora, thus affecting not only their evolutionary potential but also species spatial distributions. Consequently, a need exists for assessing the historical demography of species at different population levels.

The origin of the contemporary genetic diversity of cheetahs is thought to be the result of a severe decline around the Last Glacium Maximum (8,000 - 20,000 years ago, ya), followed by an expansion around the mid-Holocene (5,000 years) and a subsequent bottleneck within the past century due to a combination of anthropogenic factors and weather variability. Alternative hypotheses include that of a metapopulation structure and the persistence at a low effective size due to a high reproductive variance associated with a polygynous mating system. However, these three remain largely untested despite advances in molecular analytical tools over the past decades. Likewise, the effects of anthropogenic factors on population viability merit quantification as well as trends in abundance and density using robust surveying techniques. This study aims to contribute novel information on these aspects; information deemed of high significance for comprehensive conservation measures that do not underestimate the true risk of extinction the species is facing. First, we explored the historical demography of the largest free-ranging cheetah population over the past 60,000 years. Second, we assessed the population’s genetic viability and its sensitivity to perturbations on vital rates and uncertainties on current population size and carrying capacity estimates. Lastly, we assessed trends in density, abundance, and behavioural ecology aspects of cheetahs.

Methods: To explore the historical demography, we stratified periods during the last 60,000 years and contrasted evolutionary models assuming stability, decline and expansion using approximate Bayesian computation methods. We estimated the population’s contemporary effective size using four genetic estimators and population viability analysis (PVA). Sensitivity analyses of the susceptibility of viability estimates to perturbations were also performed using a PVA approach. To estimate density and abundance, we used a combination of Bayesian spatial capture, recapture and non-spatial methods.

Results: First, demographic scenarios indicated that the population has a complex demographic history, characterised by periods of decline intercalated with periods of stability with no signal of expansion contrived during the past 60,000 ya. The population seems to have been stable over the past 300 years. Additionally, scenarios modeled on abrupt reductions had low levels of support in relation to models assuming gradual reductions. Second, we found the present population to be viable, although susceptible to perturbations such as the proportion of breeding females, adult female survival rates, and uncertainties in current abundance estimates and on carrying capacity. These parameters also influenced the total population size. However, the direction of the impact was related to perturbation levels. Lastly, and mostly applicable for males, we observed density estimates of 5 to 20 km^{-3} that were largely similar across most of the six multi-year surveys. Furthermore, male cheetahs showed high site fidelity, utilising scent-marking locations for up to four consecutive years with possible temporal avoidance. Overall individuals displayed a nocturnal activity pattern.

Discussion: First, the study shows that the population's contemporary genetic diversity (and possibly that of other populations to which our population is genetically connected) is the result of a gradual decline, likely caused by fluctuations and reductions of suitable habitat due to Pleistocene and Holocene climatic oscillations, as well as recent increases in aridification in Namibia. Second, that the population viability is largely dependent on aspects related to females, and that threshold values seem to exist beyond which certain conservation actions may have a negative influence on viability. Lastly, male density seems to be regulated by home range dynamics, as density remained similar across surveys except during periods of social instability caused by vacant home ranges. The instability caused by removals may lead to higher reproductive variance.

Conclusions: Overall, the study shows that a realistic estimate of the risk of extinction faced by this population requires an integration of results obtained with several analytical approached, and that long-term conservation plans should incorporate such a body of information. The observation that viability is susceptible to different biological and social factors highlights the relevance of this assessment, which is integrated to the other themes investigated in this study. In a broader context, the results presented here are potentially relevant for assessments targeting other species facing similar threats of extinction.

Capitulo I

Introdução Geral

1.1 Estrutura da tese

A tese é composta de cinco capítulos, incluindo o capitulo introdutório (Capítulo I), seguido de três capítulos apresentados em formato de artigos científicos, redigidos de acordo com as exigências dos periódicos para os quais se tem interesse em submetê-los, e o capítulo conclusivo (Capitulo VI). Os capítulos I e VI foram redigidos de acordo com as normas da revista científica *Population Ecology*. O título dos três artigos e dos periódicos científicos para os quais serão submetidos são os seguintes:

- Capítulo II: "Inferindo a história demográfica de guepardos da Namibia com base na análise Bayesiana de dados de microssatélites", a ser submetido ao periódico *PLoS ONE*;
- Capítulo III: "Estimativas do tamanho efetivo da população de guepardos (*Acinonyx jubatus*) da Namíbia: implicações para conservação", a ser submetido ao periódico *Population Ecology*;
- Capítulo IV: "Levantamento e monitoramento de tendências em abundância e densidade: um estudo de caso de uma população de guepardos (*Acinonyx jubatus*) no centro-norte da Namíbia", em revisão após a apresentação inicial ao periódico *Ecology and Evolution*; e,
- Capítulo V: " Padrões de atividade temporais de uma população de guepardos, no centro-norte da Namíbia" a ser submetido ao periódico *Oecology*.

A tese termina com o Capítulo VI, que apresenta uma discussão e conclusões gerais do estudo, descreve as implicações conservacionistas dos resultados obtidos, assim como realça áreas importantes para futuras pesquisas. Todas as seções dessa tese contêm suas próprias referências bibliográficas.

A seguir, os aspectos e conceitos centrais desta tese são revisados, incluindo as hipóteses principais propostas acerca dos fatores que influenciaram a diversidade genética contemporânea dos guepardos, e também o conceito do tamanho efetivo de populações. Em seguida são apresentados a relevância do estudo, objetivos gerais e específicos, e métodos utilizados.

1.2. Contexto da tese

As mudanças climáticas e sua variabilidade ao longo dos últimos milhares de anos afetaram a biodiversidade do planeta. É considerada uma força de evolução por induzir mudanças ao ambiente às quais flora e fauna precisam se adaptar (Holt 1990; Ségalen et al. 2007; Weir e Schluter 2007). Alterações frequentemente ocorreram em diferentes ritmos e intensidade, e variaram espacialmente (Hewitt 2000, 2004). Conseqüentemente, a capacidade de uma espécie adaptar-se ou não a novas condições ambientais, ou fugir destas, resultou em alterações de sua distribuição e abundância (Clark et al. 2009; Fraser et al. 2012; Kharouba et al. 2012), enquanto outras se extinguiram, gerando um “turnover” de linhagens (Caughley e Gunn 1996; Reynolds 2007; Faith 2012). Conseqüentemente, a biodiversidade atual, e sua diversidade genética, são o resultado de uma longa história de mudanças climáticas combinadas ou não com fatores antrópicos (Lorenzen et al. 2011; Phillips et al. 2012). Em essência, este é o objetivo geral deste estudo, contribuir precisamente com novos dados acerca dos efeitos das oscilações climáticas e fatores humanos à diversidade genética contemporânea da maior população de guepardos no mundo. Os conceitos centrais elaborados nesta tese e suas relações são apresentados na Figura 1.

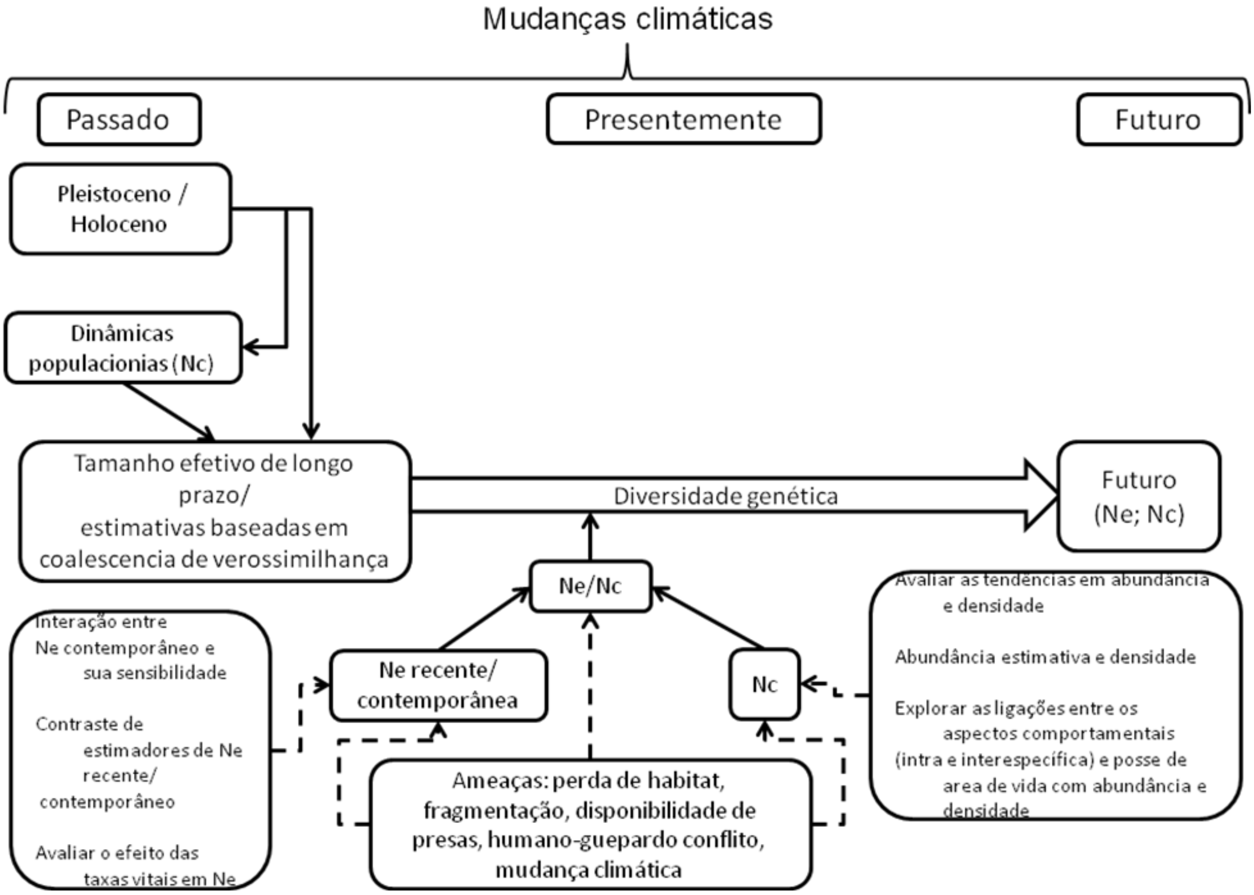

Figura 1. Representação esquemática dos aspectos teóricos e conceituais explorados neste estudo. N_e = effective and N_c = census population sizes.

1.3. Variações da fisionomia vegetacional na África austral durante o Pleistoceno e Holoceno

O clima no continente Africano durante o período Quaternário (2.5 milhões de anos atrás) foi heterogêneo, com o clima na parte norte - ocidental e central do continente tendo sido mais instável do que na região austral (Stokes et al. 1997; Dupont et al. 2008; Maslin et al. 2012). Mesmo considerada como tendo sido mais estável em nível macro, oscilações no clima na África meridional foram notáveis em particular durante o Pleistoceno e Holoceno (Chase et al. 2010; Weldeab et al. 2012). Pelos menos quatro eventos periódicos de significante aridez são conhecidos durante o Pleistoceno nos intervalos 135.000 ou 115.000 - 90.000 anos atrás (aa), 46.000 – 41.000 aa, 26.000 – 20.000 aa e 16.000 – 9.000 aa (Cohen et al. 2007; Stokes et al. 1997). Similarmente, registros indicam que o Último Máximo Glacial (UMG) (~ 26.000 – 14.000 aa) (Feankins e deMenocal 2008; Clark et al. 2009) foi intercalado possivelmente por condições úmidas (~ 27.000 – 22.000 aa e 19.000 – 12.000 aa) (Thomas et al. 2003). Estudos mas recentes indicam pelos menos quatro fases distintas úmidas do sul da África entre 8.500 e 3.500 aa, cada fase durando cerca de 250 anos, e um aumento da aridez, desde então, até 300 anos atrás (Chase et al. 2009). Em geral, o clima do Quaternário tornou-se progressivamente mais frio, mais seco e sazonal (deMenocal 2004) mas às vezes regrediu e manteve-se estável (de Vivo 2008). Associadas a essas alterações, ocorreram mudanças de paisagem que conseqüentemente impactaram as linhagens de fauna e flora.

Durante este período, a vegetação do sul da África mudou, alternando formas, mas progressivamente transicionando de florestas para ambientes abertos (savanas). Estudos indicam um aumento significante de paisagens mais abertas entre 1.8 Ma a 0.6 Ma (Cerling 1992; Bobe e Behrensmeyer 2004), com uma progressão gradual de substituição de plantas adaptadas a condições úmidas por plantas adaptadas a condições áridas (i.e. C4) (Dupont et al. 2008; Feakins e deMenocal 2008). Paisagens dominadas por plantas C4 só foram estabelecidas em torno de 1 Ma (Cerling 1992). Expansões de paisagens abertas ligadas a um aumento da freqüência de secas e aridez também são registradas após 7.000 aa (Dupont et al. 2008) ou 2.300 – 1.200 aa (Jolly et al. 1997). No entanto, há evidências de contrações destas paisagens devido à expansão do deserto ao longo dos últimos 10.000 anos (Hoelzmann et al. 1999; Osmers et al. 2012). Ligadas a estas alterações de paisagens, houve diversas mudanças na composição da fauna.

A expansão de paisagens abertas resultou em uma substituição de comunidades dominadas por herbívoros de grandes tamanhos corporais por ungulados menores de pastagem (Ségalen et al. 2007; de Vivo 2008). O contrário foi observado durante a transição Pleistoceno-Holoceno (18.000 – 12.000 aa), com uma sobreposição de animais florestais e savânicos em vez de uma comunidade primariamente composta de herbívoros de áreas abertas (Reed 1997; de Vivo 2008; Faith 2012). Por exemplo, springbok *Antidorcas* springbok foi extinto no leste da África (~ 400.000 anos atrás), mas não no sul da África, onde divergiram em duas subespécies e recentemente recolonizaram o leste da África (Reynolds 2007). Assim, além de algumas espécies se extinguirem, a distribuição de várias daquelas que persistiram foi alterada. Por sua vez, estas mudanças afetaram os padrões de persistência e distribuição dos carnívoros (Rohland et al. 2005; Cowling et al. 2007). Bertola et al. (2011) indica que leões (*Panthera leo*) recolonizaram a parte oeste-centro da África do Oriente Médio, após a população ter sido extinguida localmente durante períodos de extrema aridez que levaram à redução de presas durante o Pleistoceno.

Em suma, a África austral tem experimentado ciclos úmidos – secos de diferentes durações, acompanhados por mudanças em habitat específicas para cada espécie, cujas conseqüências incluem a extinção local ou regional, mudanças na sua distribuição, e mesmo a formação de subespécies (e.g Johnston e Anthony 2012; Osmers et al. 2012).

1. 4 O *status quo* da história demográfica do guepardo

O guepardo, *Acinonyx jubatus*, é uma espécie ameaçada de extinção e classificada como Vulnerável pela União Internacional para a Conservação da Natureza e Recursos Naturais (IUCN), com menos de 12 mil indivíduos vivos na natureza, distribuídos em 22 países (Durant et al. 2008). Esta distribuição representa 25% da sua ocorrência histórica (Ray et al. 2005). Atualmente, com a exceção de Namíbia e Botswana, as populações restantes são consideradas inviáveis, com populações inferiores a 500 indivíduos (estimativas baseadas em suposições informadas (Marker 1998; Durant et al. 2008). Com base nesta abordagem e em questionários, a população de guepardos na Namibia é estimada de ser de 2.500 indivíduos adultos, com um tamanho populacional (N_c) total estimado de 3.100 a 5.800 indivíduos (Hanssen e Stander 2004; Durant et al. 2008). No entanto, há uma escassez de estimativas derivadas de estudos de médio-longo prazo, usando métodos robustos e sistemáticos (e.g. Durant et al. 2011) Esta falta de estudos de dinâmica populacional é parcialmente devida a aspectos ecológicos e comportamentais da espécie (isto é, inconspícuas, noturnas, ocorrendo em baixa densidade) (Gese 2001), que levam a uma necessidade de esforços de amostragem maiores (Tomas e de Miranda 2003). Entretanto, tendências de abundância com base em registros de animais removidos devido a conflito com humanos indicam um declínio populacional ao longo do século passado (Marker-Kraus et al. 1996; Nowell 1996). Este declínio é resultado de razões ecológicas, incluindo secas, redução de presas, perda e degradação de hábitat, bem como de caça troféus e remoções pelo conflito real ou percebido com humanos (*human-wildlife conflict* -HWC) (O'Brien et al. 1987; Marker Kraus et al. 1996; Nowell 1996; Marker et al. 2007). Na última década, contudo, houve uma redução no número de indivíduos removidos, devido a mudanças de manejo, e a população parece ter se estabilizado (Marker et al. 2007; Castro-Prieto et al. 2011).

A origem da diversidade genética atual da espécie é resultado da combinação de eventos anteriores à civilização moderna, em conjunto com fatores antropogênicos ocorrendo em tempos recentes. Três hipóteses propostas resumem esta combinação de fatores, embora elas ainda careçam de uma avaliação rigorosa utilizando métodos estatísticos modernos. A primeira hipótese sugere um severo gargalo genético em torno do fim do Último Máximo Glacial ou principio do Holocene (12.000 - 8.000 aa), seguido por um período de endogamia intensa, uma expansão em meados do Holoceno (5.000 anos) e finalmente um segundo gargalo durante o ultimo século devido a fatores humanos e variabilidade climática (O'Brien et al. 1985, 1987; Menotti-Raymond e O'Brien 1994; Driscoll et al. 2002). Estas conclusões são baseada no alto nível de homogeneidade detectado com vários tipos de marcadores genéticos (isoenzimas, RFLPs [polimorfismos de comprimento de fragmentos de restrição] de DNA mitocondrial [mtDNA], minissatélites, microssatélites e variabilidade no Complexo Principal de Histocompatibilidade [*MHC*]) em amostras da duas subespécies, *A. j. jubatus* e *A.j. raineyi* da África Austral e Oriental, respectivamente (O'Brien et al. 1985, 1987; Menotti-Raymond e O'Brien 1994). Estudos mas recentes, com maiores amostragens e cobertura geográfica revelam níveis mais altos de diversidade para MHC, mtDNA e microssatélites (Marker et al. 2008; Castro-Prieto et al. 2011; Charruau et al. 2011), ainda que não contradigam claramente as inferências reportadas nos estudos anteriores.

A segunda hipótese sobre a diversidade genética atual dos guepardos descreve a possibilidade da persistência da população com um baixo tamanho efetivo (*Ne*), induzido por uma elevada variação reprodutiva associada com o sistema de acasalamento poligâmico (Pimm et al. 1989). A terceira sugere que a diversidade é resultante de dinâmicas de metapopulações, isto é, ciclos contínuos de extinção de subpopulações e re-colonização de áreas (Pimm et al. 1989; Gilpin 1991; Hedrick 1996). As duas últimas hipóteses foram propostas por razões demográficas, visto que a gravidade do gargalo sugerido como tendo ocorrrido entre 12.000 e 8.000 aa resultaria em uma probabilidade baixa de sobrevivência da espécie como um todo.

Com a exceção da segunda hipótese, as outras são compatíveis e invocam o modelo ambientalmente impulsionado, que prevê fatores ambientais como geradores de evolução (Vrba 1995). O modelo é suportado por estudos em diversas espécies da África (e.g. Lorenzen et al. (2012) apresentam um revisão para ungulados e Teske et al. (2011) para filogeografia marinha) e de outros continentes (e.g. Turchetto-Zolet et al. (2012) apresenta uma revisão para biodiversidade na América do Sul, e Hewitt (2000) uma perspectiva global). Novos estudos sobre guepardos no Serengeti mostram um sistema de acasalamento poligínico/poliândrico, com uma fidelidade baixa de parceiro (Gotelli et al. 2007), sugerindo que a existência em longo prazo com níveis baixos de *Ne* talvez seja improvável. Igualmente, *Ne* pode aumentar sob este sistema de acasalamento devido a uma possível redução na variância reprodutiva masculina (Storz et al. 2001; Pearse and Anderson 2009).

Atualmente, somente dois estudos fornecem informações sobre a viabilidade genética da espécie. Creel (1998) estimou um *Ne* de 207 (*Nc* = 210), assumindo uma proporção sexual desviada pró-fêmeas (0,44: 0,56) e *Ne* 97 (*Nc* = 101) quando incluiu indivíduos sem áreas próprias (‘transients”). Em contraste, Kelly (2001), obteve valores de *Ne* < 50 usando quatro estimadores de *Ne* com diferentes suposições, e não incluiu transientes. Além das diferenças nas estimativas, Creel (1998) observou um efeito mínimo no *Ne* devido a flutuações no tamanho populacional ou proporções sexual desiguais, dado não observado por Kelly (2001). Como as estimativas de abundancia podem afetar o nível de impacto de flutuações demográficas no valor calculado de *Ne (*Vucetich e Waite 1998), este fator pode explicar a falta de influência para o caso de Creel (1998). A exclusão de transientes por Kelly (2001) pode ter introduzido um viés negativo, considerando a infidelidade de fêmeas (Gotelli et al. 2007). Contudo, ambos os estudos mostram que *Ne* é afetado negativamente por sucessos reprodutivos desiguais. Esses dados reforçam a necessidade de estudos semelhantes em outras populações.

1.6. Justificativas, e objetivos gerais e específicos do estudo

O estudo teve como objetivo geral a compreensão da demografia histórica e contemporânea da população de guepardos da Namíbia ao longo dos últimos 60.000 anos, informações estas necessárias para a elaboração de estratégias adequadas para sua conservação em longo prazo por três razões principais. Primeiro, é necessária uma melhor compreensão dos processos históricos e contemporâneos, como por exemplo, o impacto das oscilações climáticas do Quaternário e fatores humanos que moldaram e possivelmente continuam a influenciar a diversidade genética contemporânea desta população.

Segundo, a população de guepardos da África austral, e da Namíbia em particular, representam a maior população natural desta espécie no mundo (Durant et al. 2008; Marker et al. 2010) e tem uma diversidade genética maior do que as outras populações (Charrua et al. 2011). Além disso, aparenta comportar-se como uma população panmítica em escala nacional (Marker et al. 2008). No entanto, *Ne* é frequentemente menor que *Nc* e da proporção de indivíduos reprodutores breeding proportions (*Nb*) (Frankam 1995; Vucetich e Waite 1998; Palstra e Ruzzante 2008; Palstra e Fraser 2012), mesmo em populações grandes (Palstra e Fraser 2012), podendo assim ocasionar uma tendência à perda de variabilidade e menor viabilidade em longo prazo por influência da deriva genética (Hare et al. 2011).

Finalmente, uma compreensão dos processos que regem a dinâmica genética (*Ne*) e demográfica é necessária principalmente para espécies ou populações em conflito com humanos (Lucherini e Merino 2008; Marker et al. 2010). A redução de indivíduos adultos na população afeta o tamanho de censo e tem o potencial de afetar diretamente a diversidade genética da população (Saether et al. 2009; Palstra e Ruzzante 2010; Lee et al. 2011).

De forma geral, a escassez de estudos sobre tamanhos efetivos históricos e contemporâneos, bem como sobre a dinâmica populacional. Isto limita uma compreensão dos fatores afetando a diversidade genética das populações e da espécie.

1.6.1. Objetivos gerais e específicos

Para atingir a meta principal de obter uma compreensão mais ampla sobre os processos que moldam a diversidade genética da espécie, foram delimitados três objetivos específicos, sendo eles: (1) uma avaliação estatística da história demográfica da espécie em relação à variabilidade climática do Quarternário e a fatores antropogênicos; (2) uma investigação da interação entre o seu tamanho populacional efetivo contemporâneoe e suas taxas vitais; e (3) uma avaliação aprofundada de tendências de abundância e densidade.

1.7. Ferramentas de estudo

Para explorar a história demográfica de guepardos durante os últimos 60.000 anos, utilizou-se o método Bayesiano de computação aproximada (ABC) (Storz et al. 2002; Lopes e Beaumont 2010) implementada no pacote DIYABC-FDA (Cornuet et al. 2010, 2008; Estoup et al. 2012). Este período foi estratificado a fim de avaliar correlações entre oscilações climáticas e/ou fatores antropogênicos com mudanças demográficas em escalas menores de tempo. Mudanças demográficas anteriores a 1.000 aa foram interpretadas como sendo relacionadas às oscilações climáticas, e aquelas com idade de 1.000 aa ou menos como sendo um efeito combinado com fatores antropogênicos. Em essência, a análise de ABC utiliza uma matriz coalescente de verossimilhança, gerando um grande número de amostras por meio de simulações de Monte Carlo, e aplica estatísticas sumárias para selecionar os conjuntos de dados mais próximos ao conjunto de dados real) (Excoffier et al. 2005; Lopes e Beaumont 2010). Em seguida, baseando-se nos conjuntos de dados mais próximos, a probabilidade relativa posterior de diferentes modelos é calculada, incluindo inferências de parâmetros demográficos associados.

Para avaliar a sensibilidade da estimativa de viabilidade populacional a perturbações nas taxas vitais, bem como a incertezas no tamanho da população e na capacidade suporte, foram realizadas análises de sensibilidade utilizando-se o programa VORTEX, um *software* de análise de viabilidade que integra vários aspectos da história de vida da população (Miller e Lacy 2005). Três métodos foram utilizados para determinar o tamanho contemporâneo efetivo da população: (i) o método coalescente ABC implementado no programa ONeSAMP (Tallmon et al. 2008); e (ii) método de desequilíbrio de ligação implementado no programa LDNe (Waples 2006; Waples e Do 2010); e (iii) utilizando as simulações demográficas realizadas com o programa VORTEX e a fórmula Ne = ½ (1 - exp $^{(logeHt / t)}$), onde H_t é a heterozigosidade esperada após os processos simulados (Crow e Kimura 1970, ver Eizirik et al. [2002] para uma aplicação).

Por fim, para avaliar as tendências em densidade e abundância em uma população local de guepardos, métodos espaciais de captura-recaptura (Royle et al. 2009) foram aplicados, utilizando o *software* SPACECAP (Gopalaswamy et al. 2012). Estes foram baseados em um conjunto de dados de uma área de tamanho similar amostrada por seis anos com armadilhas fotográficas colocadas, na maior parte, em sítios de marcação. Padrões de utilização de áreas, marcação, e fidelidade em relação a áreas de permanência também foram explorados.

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Capitulo II

Inferring the historical demography of the Namibian cheetah population using Bayesian analysis of microsatellite data

Authors:

Mr. Ezequiel Chimbioputo Fabiano, [a, b]

Dr. Sandro Luis Bonatto, PhD, [a]

Dr. Anne Schmidt-Küntzel, PhD, DMV, [b, c]

Dr. Laurie L. Marker, PhD, [b]

Prof. Eduardo Eizirik, PhD, [a]

Email addresses:

ezequiel.fabiano@pucrs.br, fabianoezekiel@gmail.com

slbonatt@pucrs.br

genetics@cheetah.org

cheetah@iway.na

eduardo.eizirik@pucrs.br

Affiliations:

a. Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil

b. Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia.

c. Life Technologies Conservation Genetics Laboratory, Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia.

Corresponding author:

Mr. Ezequiel Chimbioputo Fabiano, Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil; Cheetah Conservation Fund, Otjiwarongo, Namibia.

Email: ezequiel.fabiano@pucrs.br

Prof. Eduardo Eizirik, Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil

Email: eduardo.eizirik@pucrs.br

Abstract

The contemporary genetic diversity of the cheetah (*Acinonyx jubatus*) has been the focus of several studies over the last 25 years, most of which have revealed low levels of variation at genomic and mitochondrial markers. Such low variation has been suggested to derive from two historical genetic bottlenecks, a severe one at the end of the Last Glacial Maximum (LGM) and a more recent one in the past millennium, with a possible expansion during the mid-Holocene (~ 5000 years ago [ya]). Here, we used approximate Bayesian computation (ABC) methods with temporal stratification to explore the historical demography of the largest free-ranging cheetah population for the past 60,000 years. Results indicate that the population has been declining gradually, interrupted by periods of stability. The timing of the declines coincides with climatic events including over the last 3,500 - 300 ya, throughout the Holocene (~8,000 – 3,500 ya) and the late end of the LGM and early half of the Holocene (~14,400 to 8,000 ya). Prior to 21,000 ya the population appears to have been stable. These results demonstrate the impact of slow contractions likely induced by the direct and indirect effects of climatic oscillations in this population genetic diversity. This phenomenon is also relevant to other species threatened with extinction due to slow loss of habitat ranges.

Introduction

Throughout the Quaternary period (which began 2.5 million years ago [Mya]), the climate was highly heterogeneous in Africa, with western and eastern Africa's climate being more unstable than that of southern Africa [1, 2]. Despite southern Africa's relative climatic stability over the past 3.5 million years [3], notable oscillations have been reported [4]. The climate in this region oscillated between wet and dry periods [5], accompanied by changes on species-specific habitat suitability [1, 2, 6-8]. This heterogeneity in climate has affected the contemporary genetic diversity of many species, with comparative phylogeography across taxa indicating southern Africa as a refugium from which populations recolonized more northerly regions [9-11]. Since responses can be species- or population-specific [12], reconstructing comparative historical patterns requires in-depth studies of many taxa.

One species that seems to have a particularly interesting demographic history is the cheetah (*Acinonyx jubatus*), for which several population genetic studies have revealed remarkably low levels of genetic diversity [13,14]. Namibia has the largest cheetah population, estimated at 2500 adult individuals [15] or a total census population size of 3100 to 5800 individuals [16]. Botswana and South Africa in southern Africa and Kenya and Tanzania in East Africa population sizes range between 500 to 1500 individuals [15]. The remaining 18 population estimates are bbelow 500 individuals [17] and are considered non-viable [18]. Records indicate that the Namibian population has declined over the last century due to various factors, including droughts, reduction in prey availability, habitat loss and degradation, as well as trophy hunting and human-wildlife conflict [19-21]. However, the population is considered to have stabilized in the recent decade [21].

While it is widely recognized that the origin of the cheetah's relatively low extant genetic diversity is the result of events pre-dating modern civilization, possibly in combination with human-related factors, the exact mechanism that led to this observed pattern is unknown. Three hypotheses corresponding to different patterns of reduction in population size have so far been proposed to account for the species' low level of genetic variation. Early population genetic studies using a variety of genetic markers (allozymes, MHC variation, mtDNA restriction fragment length polymorphisms and microsatellites) and samples from the southern and eastern African subspecies, *A. j. jubatus* and *A.j. raineyi*, respectively, revealed high levels of homogeneity [13, 14, 22 -24]. These studies proposed that the low diversity was likely a consequence of a severe bottleneck at the end of the Pleistocene (12,000 - 10,000 years ago [ya]), followed by an expansion *ca.* 5,000 ya and another decline within the past century. Two alternative hypotheses were subsequently proposed for the genetic uniformity. First, that the species persisted at low effective population size (*Ne*) induced by the high reproductive variance observed in species with a polygynous mating system [25]. Second, that populations were subjected to a continuous cycle of extinction of subpopulations followed by re-colonization of the areas,following metapopulation dynamics [25-27]. While additional investigation is required to resolve the debate, all three hypotheses are to some extent non-exclusive, and imply an environmentally driven model [28], which postulates environmental factors as drivers of evolution. This model has been supported in a number of species worldwide [4,6], as well as more specifically in Africa and southern Africa [29–31].

Since the publication of the classical studies on cheetah genetics, and in particular during the past decades, there has been a surge of advances in computational methods for exploring the historical demography of any organism using empirically collected molecular data. Of particular interest is the application of Approximate Bayesian Computation (ABC) approaches to population genetics [32,33]. These methods have now been widely used to investigate the demographic history of many different species, allowing the statistical comparison of contrasting models of past population changes[e.g. 34–37].

Here we explored the demographic history of the Namibian cheetah population using ABC methods, based on a previously published microsatellite data set [37]. This population was considered appropriate for the study due to the availability of a suitable genetic data set, the population having a large census size [17] and being panmictic [37]. The latter is crucial, as it reduces the risk of false signals of bottleneck caused by sub-structuring [38,39] while large sizes reduce the likelihood of the population having experienced high genetic drift in the recent past [40]. We specifically assessed whether the population has remained stable, declined (gradually or severely) or expanded over different historical periods encompassing subspecies divergence times and major climatic events (< 60,000 ya). This study is timely, as a better understanding of this population's demographic history is vital for the development of effective conservation measures on its behalf.

Material and Methods

Data collection

To investigate the demographic history of Namibian cheetahs, we used a previously published data set comprising 90 individuals genotyped for 31 dinucleotide microsatellite loci [37]. This particular subset of the original data was composed only of unrelated cheetahs, determined based on behavioural data, parentage analyses and confirmed with estimates of genetic relatedness. For a full description of data collection, see [37].

Past demographic analysis

We used the coalescence-based approach implemented in the program DIYABC-FDA (hereafter DIYABC) [43-45]. This approach is based on the Wright-Fisher model, and hence assumes that the study population approximates this model [95,96].

DIYABC allows for the simulation, comparison and confidence assessment of model choices considering more complex evolutionary scenarios [44]. It implements a linear discriminant analysis (LDA) on summary statistics (Ss) prior to computing posterior probabilities or evaluating confidence in scenario choice, as a means of reducing computation time [44,45]. Ultimately, this gain in computation time allows for additional simulations, thus partially overcoming a drawback concerning model discrimination [50]. We assessed whether the population was stable, declined (gradually or severely) or expanded over different timeframes that ranged from very recent (120 to 6 ya) to ancestral (60,000 ya) times (Fig. 1, Table 1). The timeframes were set so as to include subspecies divergence time estimates, climatic oscillations and anthropogenic factors (Table S1). We grouped models into three main categories, with each category corresponding to a discrete period (Table 1). Category one encompassed recent (< 1,000 ya) and category 2 ancestral (> 1,000 ya) timeframes, while category 3 encompassed both, recent and ancestral timeframes (Table 1). We attributed any demographic change occurring more than

1,000 ya as linked to climatic oscillations, while more recent changes were attributed to anthropogenic influences with (1,000 ya to 300 ya) or without (300 ya to present) climatic factors. This temporal stratification allowed for an assessment at a finer temporal resolution than DIYABC assuming *Ne* to be constant between time periods [44]. As part of category three we assessed robustness of model inference by performing three additional runs whose times of decline encompassed most of the multiple fine-scale periods (i.e. models 8 - 10, Table 1). In order to convert time of change into years (*T*) and due to uncertainty in the cheetah's mean generation time, we considered estimates of 2.4 years [46,47], 4.05 years (Fabiano et al. unpublished data [Chapter 3]) and 6 years [48]. The first value is based on long-term monitoring data for females, the second derives from VORTEX simulations that incorporate life history parameters and the third from data of captive animals at zoological institutions.

Priors for ancestral effective population size (N_{anc}) were vague due to a lack of records prior to the 1970's [19]. For the recent *Ne* (N_{rec}), we used "uninformative" priors based on our parallel work (Chapter 3), and population size estimates of 2,500 adult individuals [17] or a total population size of 3,138 to 5,775 [37]. Hence, priors for N_{rec} overlapped across scenarios (Table S2). As we were also interested in the magnitude of the decline(s), we confined the upper and lower bound of the N_{rec} and N_{anc} to differ by less than 5% (less severe) and at least by 50% (severe), respectively, for five scenarios (Table 1, S2). Demographic parameters were sampled from a uniform distribution (U). Microsatellite loci were assumed to follow the generalized stepwise mutation model, with mutational parameters kept at the default values (Table S2) [73–75]. Default priors for the mean and individual locus mutation rates encompassed rates previously used for cheetahs (2.05×10^{-3}, 5.6 X

10^{-4}, 2.05 X 10^{-4}) [51]. We also assessed the impact of using a broader prior for the geometric distribution (*P*) U ~ [0.1 - 0.3] to U ~ [0.1 - 0.7] [52] with longer timeframe periods (240,000 ya) on model selection (Table S3). This accounted for the uncertainties in mutation rates of dinucleotide microsatellites in the context of model selection [52,53].

Confidence on model choice: For each model, we performed 5 X 10^5 simulations of which 1% were selected based on the closest Euclidean distance between their Ss and the Ss derived from the actual data for model checking, comparison and parameter estimation [43]. We used as Ss the mean number of alleles, genic diversity, variance in allele size in base pairs and Garza-Williamson's MWG [44,45]. The posterior probabilities of different scenarios were then computed using a polychotomous logistic regression based on (K- 1) discriminant variables determined by applying a linear discriminating analysis on the Ss of the closest simulated data sets [45]. As the K-1 discriminant variables maximize differences among scenarios, they provided an assessment of model discrimination. In addition, following Cornuet et al. (2008) [44], we computed type I and II errors based on 500 simulated data sets, as a means of discriminating among scenarios. Specifically, we estimated type I error as the number of instances a scenario used to generate the data did not exhibit the highest posterior probability (HPP) among the competing scenarios, and type II error (β) as the proportion of times when a scenario had the HPP when the data had actually been simulated under a competing scenario (i.e. statistical power = 1 - β). Additionally, we assessed the predictive power of different scenarios by conducting a principal component analysis (PCA) on Ss derived from 1000 records drawn from the posterior distribution, and visually inspected whether the observed data set fell within the simulated data set with initial priors [45]. The lack of low tail probabilities was also used as evidence of model fit [44].

Parameter estimation: The closest data sets (1% of simulated data sets), were then used to calculate posterior probabilities of each scenario, upon which point estimates with 95% HPD were determined using a logistic regression [44]. Point estimates were present along the 95% HPD, with the relative mean square error (RRMSE), bias and factor 2 used as measures of precision. Additionally, we report the posterior distribution 50% and 95% coverage, and the mean integrated squared error (RRMISE). The RRMISE was used as the optimizer criteria for parameter estimates [54].

RESULTS

Single demographic changes

To assess whether and how the population size has changed over time, we evaluated seven time periods for three to four scenarios each, assuming a single demographic change (stable, gradual or severe declining, or expanding population) (Table 1). The stable scenario unequivocally had the highest posterior probability (HPP) in three timeframes, in three timeframes the stable and declining scenarios were equally probable (their 95% HPP overlapped) and in one timeframe the declining scenario had the HPP. In no instance did a scenario assuming an expansion have a relative HPP, nor was it equally probable to another scenario (Table 1). These findings suggest that, except, for a decline between 14,400 and 300 ya, and possibly between 40,000 and 30,000 ya, the population was more likely to have been stable over most of the timeframes assessed. It is noteworthy that severe declines were the least supported for time periods assessed for this scenario (models 3, 4, 6, 7 in Table 1). Confidence in scenario choices was high, as statistical power among the competing best scenarios was on average 80% (S2 Table 4). Furthermore, none of the test statistics used to assess model misfit had a low tail probability, suggesting that models did fit the data (i.e. probabilities were within 0,05 - 0,95 interval, S2 Table 5).

A congruent interpretation of the population history was also recovered based on simulations characterized by broader priors for the time of change (models 8 - 10, Table 1) (S2 Table 3, 4, 6). Under the broadest prior distribution that encompassed all temporal periods (60,000 to 10 ya), the stable and decline scenarios were equally probable (95% HPP range 0.4233 - 0.4722). A signal of decline was recovered with model nine (*T* set to 12,000 to 3,000 ya) keeping largely in agreement with conclusion of model three and a partly with models two and four (Table 1). Lastly, under model 10, the stable scenario had the HPP in accord with models 7 and 8. Scenarios with broader priors for *P* also supported possible declines around 3,000 to 300 ya and stability from 300 ya to the present (S2 Table 6). Hence, model inference and result interpretations were largely robust to assumptions on the prior distribution of time and *P*.

DISCUSSION

The study shows that the Namibian cheetah population appears to have had a complex demographic history, as evidenced by support for periods of stability intercepted by periods of decline. Based on results from a temporally stratified approach encompassing the past 60,000 years, we failed to recover a signal of expansion, and instead retrieved signals of stability and/or declines. A signal of expansion was detected prior to this period (> 180,000 ya) (*data not shown*), Additionally, the study shows that declines appear to have been gradual rather than drastic. Hence, the population's low neutral genetic diversity seems to result from gradual and continuous decline over evolutionary timescales. The equal probability of certain scenarios such as for declines around the period between 21,000 and 3,000 ya (Table 1) may indicate insufficient power in the data. Nevertheless, simulations using different sampling strategies favouring population expansion and stability using MSVAR1.3 indicated no impact of prior on posterior distributions (S1, S2 Table 7). Furthermore, results based on broader priors for time of change and mutation rate parameters, yielded congruent findings. Overall, based on this study design our results appear to be robust, in support of a gradual decline rather than a severe bottleneck and highlights the importance of temporal stratification for better appreciation of demographic evolution.

The study's primary aim was to contrast evolutionary scenarios that span different periods of environmental change, so as to assess the evolutionary trajectory of the population. Our findings support the hypothesis that the population's contemporary genetic diversity, and possibly that for all of southern Africa (given ongoing gene flow in the region [15]), is the result of multiple gradual reductions interrupted by periods of stability. This conclusion is supported by a number of reasons, including signals for declines that were retrieved for several timeframes, including during the past 3,600 to 300 ya and parts of the late end of the LGM, throughout the Holocene (14,400 - 3,600 ya). Likewise, we recovered signals for periods of stability during the past 300 years and between 30,000 and 21,000 ya and.

Evidence for slow *versus* abrupt declines derives from the lack of support for severe declines relative to less severe competing scenarios, as well as the equal probability between stable and declining scenarios. Recent studies [e.g. 59-62] indicate that slow range contractions may result in lower genetic diversity and higher differentiation than abrupt declines. This pattern is likely to be the case for broadly distributed species whose genetic diversity can only be understood within a metapopulation framework [63,64]. Furthermore, the low neutral genetic diversity can result from a similar effect as allele surfing, the random increase of allele frequencies from low to high during colonization, whose effect may be resistant to selection [59–62].

Our findings are partly in agreement with previous hypotheses proposed as for the cause of the species' low genetic diversity. It corroborates the hypothesis of multiple declines including one around the late end of the LGM and a second, more recent one, but is at odds regarding (i) the timing of for the latter (previously indicated to have occurred within the last century), and (ii) that the decline at the LGM was severe [13,14, 22]. Our results also contrast with a suggested demographic expansion during mid-Holocene [23]. Likewise, support for the metapopulation dynamics hypothesis, which involves cycles of extinction and recolonization [27], is limited by the lack of severe expansion signals in our data. We acknowledge that our study design precludes a direct assessment of the metapopulation hypothesis and this needs thorough assessment. However, the suggested recolonization of Eastern Africa by cheetahs from southern Africa [51] to some extent lends support for this hypothesis, essentially given the high level of climatic heterogeneity and variation in East Africa relative to southern Africa [3]. Kerdelhué *et al*. (2009) [65] has also shown differences in the effect of local variations with populations in sites more affected by glacial cycles differing from those in less affected areas. Future studies should explore this hypothesis further.

We hypothesize the transition from dense/woodlands to open or pure grasslands, availability of suitable habitat, and the speed of alterations between vegetation forms, as the probable causes of demographic reductions during the first change (~ 40,000 ya). Even though often associated with pure/open grasslands, cheetahs prefer a mosaic habitat type [66-70]. In addition, they present habitat utilisation stratification (e.g. use of woodlands and savannah mostly for hunting [66,68] or use of dense habitat after parturition (unpublished data). The increase in southern Africa of grasslands due to drier conditions of the LGM (~ 20 ka) [71] or of denser vegetation because of the humid conditions at the end of the LGM [72], could have resulted in population fluctuations and declines. In addition, these alterations also affected ungulate distributions which in turn would be expected to affect the cheetah. Osmers *et al.*, [73] showed that the oryx (*Oryx gazella*), a desert-adapted species, declined during the late Pleistocene as it took refuge along the Namibian coast due to the increase in humid conditions and a reduction on desert extent. Likewise, it is plausible that interspecific competition heightened due to a reduction of cover as lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*), the cheetah's main competitors [46,74], were still present at the time in present-day farmlands in Namibia, where these species have been extirpated [20, 75]. Altogether, this suggests that the population is likely to have declined due to a combination of low prey availability, interspecific competition and changes in suitable habitat.

The same logic applies to the timing of the second change (3,600 – 300 ya), which coincides with the end of the Africa Humid Period and an increase in aridity in Namibia (~ 3,500 to 300 ya) [4,5]. Despite this trend and stability in vegetation structure over this period [76], the periods such as the Medieval Warm Period (~1,000 - 750 ya) or Little Ice Age (~ 500 - 350 ya) could be responsible for the population decline over this period. Another factor possibly contributing to the decline of cheetahs post 1,000 ya is the interplay between farming intensification and cheetah removals, as evidenced by the large removal rates priors the 1980's [19] and the extirpations of large predators in the area [76, 20]. It should be noted that sophisticated removal techniques (e.g. guns) rather than human density per se (~ 485 ka around 1950, [77]) are more likely to have been the cause of decline, a conclusion shared with Mondol *et al.* (2009) [78] for declines in tiger density over the past ~600 years.

The lack of a signal for decline in the last 300 years, and more so in the last 120 years (*data not shown*), which coincides with a period where records indicate high removal rates and the population considered as having been halved [19,20], is interpreted as the population having been sufficiently large. This is contrary to other carnivores that have declined very recently, such as the African wild dog (*Lycaon pictus*) (1 - 154 95% CI across three stronghold populations) [79] and tigers (~ 200 ya) [78]. Nevertheless, high migration rates and lack of power due to an $N > 1000$ can explain this lack of a declining signal [40]. Likewise, as was the case for elephants (*Loxodonta africana*), in which temporary genetic effects were detectable at the cohort level due to selective poaching [34], N_e is affected differently based on the age classes removed [80–82]. This seems to be the case for the study population, based on sensitivity analyses using simulations (Fabiano et al unpublished data [Chapter 2]).

Our findings are indicative of larger effective populations across different times in the past. Additional analyses using LAMARC 2.0 [56] (unpublished data) also retrieved a similar trajectory using three microsatellite mutation rates previously used in cheetah studies. Precisely, it showed a decline starting around 50,000 to 30,000, followed by a period of stability until 8,000 ya, followed by a rapid decline until 2,000 ya. Likewise, Bayes factor (BF) analyses of converged MSVAR1.3 runs, irrespective of the mutation rate, also favoured ancestral times of decline (> 1,000 ya, BF ≥ 4, S1, Table S7), in particular runs based on slower mutation rates (10^{-3}) (i.e. for the period between 70,000 and 20,000 ya). This suggests, that the population genetic diversity seems not to be a result of long-term low effective population sizes as it was found to be the case for the Amsterdam albatrosses *Diomedea exulans* and *Diomedea amsterdamensis* [57] or the Madagascar bald-eagle *Haliaeetus vociferoides* [58].

Our conclusions regarding periods of decline are congruent with times of decline in other species and with known climatic events. For instance, the African elephants have also declined ~ 2,500 ya, at the end of African Humid Period [88]. Likewise, cheetah diversification times are concordant with those of other species (e.g. [20] for a review on savannah ungulates). This implies that climatic oscillations facilitated the divergence and formation of cheetah subspecies [51, 85], as observed in multiple other African species [9].

CONCLUSION

Overall, our study shows that the low genetic diversity of the Namibian cheetah population is likely to be resultant from multiple demographic changes, with a declining trend, facilitated by changes in habitat suitability (vegetation, prey and interspecific competition) induced by the prevailing climatic conditions of late-

Pleistocene, Holocene and recent aridification. In addition, the study showed that to explore demographic events at deeper timescales, temporal stratification may result in a better understanding of historical events than simple models. Ultimately, the study provides novel insights regarding the consequences of slow range contractions on the genetic diversity of a population. Overall, this study provides useful information for improving our understanding of cheetah biology and long-term demography, especially for populations in southern Africa, and fosters additional investigations targeting this complex species.

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Legends for Figures

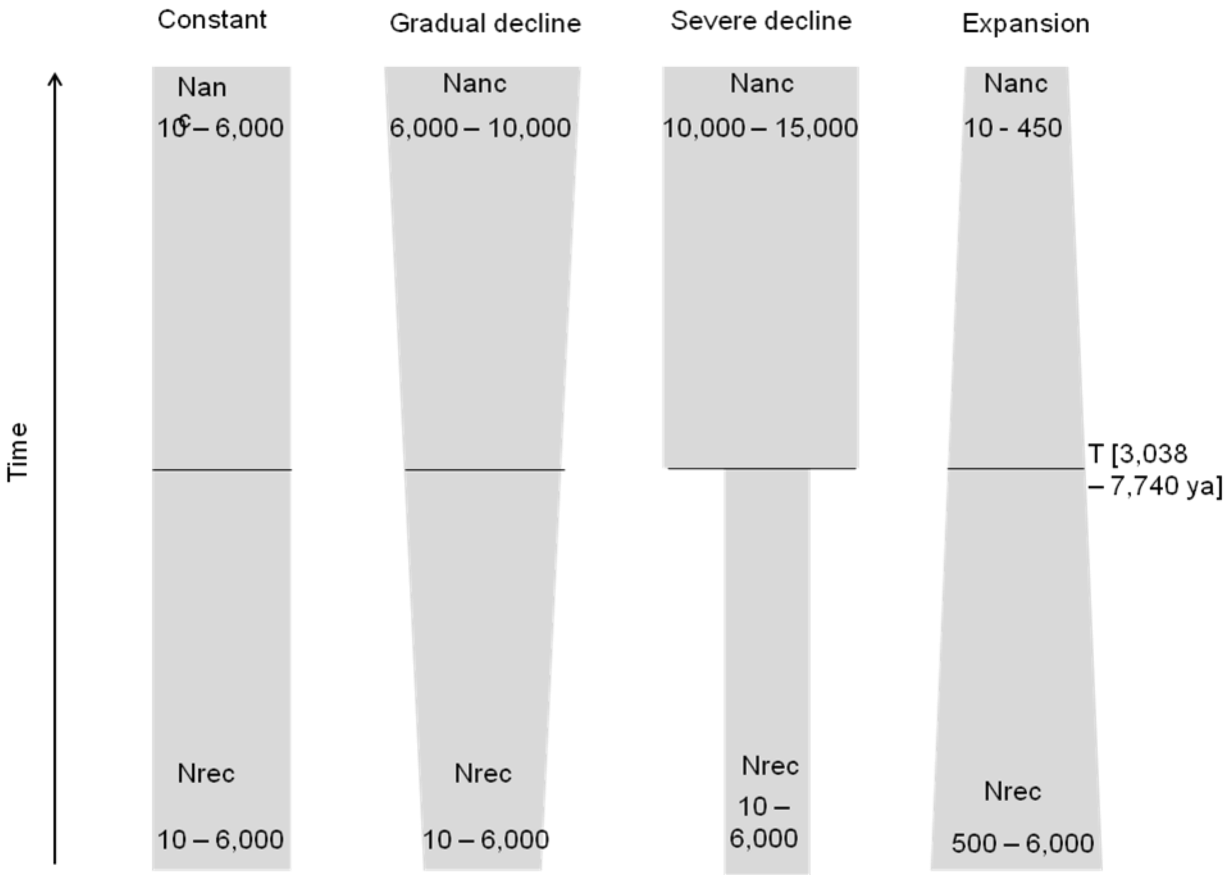
Fig. 1 Alternative demographic scenarios constrated for the Namibia cheetah population. Numbers in brackets indicate prior distributions for recent and ancestral effective population sizes, Nrec and Nanc, respectively and time of decline (*T*). Scenario presented corresponds to the period between 3,000 and 7740 (scenario category 3 in Table 1).

Table 1 Summary of the posterior probabilities of evolutionary model that assumed a single demographic change for different timeframes assessed using DIYABC. Demographic changes within models 1 - 2, 3 - 7 and 8 - 10 were linked to combined effect of anthropogenic and climatic events, climatic events and sensitivity of scenario choice to priors for the time of change distribution.

Models	Climatic oscillation or environmental periods		Assumptions				
	Time period (in years [a])	Description of event	Stable	Decline			Expansion
				Less severe [e]	Severe [f]		
1	300 - 6	Anthropogenic [b]	0,5777 [0,5614-0,5941]	0,3942 [0,3778-0,4106]			0,0281 [0,0239-0,0323]
2	3,600 - 300	Agriculture intensification, high indiscriminate removals and aridification [c]	0,4947 [0,4826-0,5068]	0,4783 [0,4661-0,4906]			0,0269 [0,0239-0,0299]
3	7,740 - 3,000	Holocene specifically the Africa Humid Period [c]	0,3037 [0,2900-0,3173]	0,4296 [0,4149-0,4444]	0,2609 [0,2471-0,2747]		0,0058 [0,0045-0,0071]
4	14,400 - 7,800	Early Holocene and end of the late half of the Last Glacial Maximum (LGM) [c]	0,3024 [0,2870-0,3179]	0,3144 [0,2980-0,3308]	0,2115 [0,1986-0,2244]	0,1717 [0,1572-0,1863] [g]	
5	24,000 - 12,000	End and late end of the LGM [c, d]	0,7158 [0,7058-0,7257]	0,1863 [0,1778-0,1949]			0,0979 [0,0911-0,1047]
6	30,000 - 21,000	Mostly the LGM [d]	0,3607 [0,3443-0,3772]	0,3143 [0,2976-0,3309]	0,1655 [0,1520-0,1790]		0,1595 [0,1476-0,1715]
7	40,000 - 30,000	Prior to the LGM [d]	0,3210 [0,3051-0,3368]	0,2965 [0,2804-0,3127]	0,1787 [0,1652-0,1923]		0,2038 [0,1905-0,2171]
8	60,000 - 60	Sensitivity analyses of prior effects covering a range of climate events throughout the Holocene into the Pleistocene	0,4361 [0,4233-0,4489]	0,4590 [0,4459-0,4722]			0,1049 [0,0972-0,1125]
9	12,600 - 3,000		0,4705 [0,4498-0,4913]	0,5270 [0,5062-0,5479]			0,0024 [0,0016-0,0032]
10	48,000 - 24,000		0,3899 [0,3739-0,4059]	0,3306 [0,3148-0,3465]			0,2795 [0,2651-0,2939]

[a] 6 years generation interval was used [47]; [b][4, 5,16,17,86,87] [c] [1,2,4,5,89,90] [d] [1,2,89-93]; [e] upper and lower priors for the recent and ancestral population size differ by less than 5% (less severe) and [f] by at least 50% (severe), respectively; [g] severe decline from an assumed small ancestral population

Fig. 1.


Constant
Gradual decline
Severe decline
Expansion
Time
Nanc
10 – 6,000
Nanc
6,000 – 10,000
Nanc
10,000 – 15,000
Nanc
10 - 450
T [3,038 – 7,740 ya]
Nrec
10 – 6,000
Nrec
10 – 6,000
Nrec
10 – 6,000
Nrec
500 – 6,000

-

Supplementary information 1

Description and preliminary results using MSVAR1.3

MSVAR assumes that a stable population (N_1) started to change in size, evolving towards its current size (N_0) linearly or exponentially, t_a generations ago (T). The model assumes mutations to follow a Stepwise Mutational Model (SMM) [1]. The program employs a Markov chains Monte Carlo (MCMC) approach to generate posterior probability distributions for N_0, N_1 and T, based on the full allelic distribution contained in the data combined with these parameters' prior (log normal) and hyperprior distributions.

We performed six independent runs of 2 X10^9 iterations, with thinning values of 100,000, and two others of 4 X 10^9 iterations thinned every 200,000 steps, in both cases with 20,000 samples recorded (Table S7). The two longer chains contrasted scenarios assuming stable populations, but differed as one assumed an exponential and the other a linear population growth mode. The remaining four runs assumed an exponential growth mode. The exponential mode was favoured as it is considered more realistic when assessing the impact of more recent events, such as anthropogenic factors [1]. Chains also differed in the mean hyperprior for the mutation rate, which for long chains was set to 10^{-3} versus 10^{-6} for the shorter chains based on preliminary runs (Table S7). We set $N0 = N1$ or $N0 > N1$ (i.e. assessed for stability or expansion, respectively) at different time spans in order to consider scenarios that are alternatives to the classically proposed bottleneck [2,3]. We used different sampling strategies by setting vague priors for *N1* and *T*, and "uninformative priors" for N_0. The use of broad priors was applied to reduce their influence on posterior distributions. The broad parameter space for *T* encompassed both recent

(< 1000 ya) and ancestral (> 1000 ya) timeframes (Table S7). Thus, we attributed any demographic change occurring more than 1000 ya as linked to climatic oscillations, while more recent (1000 ya to the present) due to a combination of anthropogenic and climatic factors. In order to convert time of change into years (*T*) and due to uncertainty in the cheetah's mean generation time, we considered estimates of 2.4 years [4,5], 4.05 years (Fabiano et al. unpublished data [see chapter 3]) and 6 years [6]. The first value is based on long-term monitoring data for females, and the third on data from zoological institutions; the 4.05 estimate derives from VORTEX simulations that incorporate life history parameters.

To check for convergence, we discarded at least the first 50% of the recorded values and applied the Geweke, and Gelman and Rubin diagnostic tests to the individual and combined data sets, respectively, as implemented in the CODA R package [7]. A value of 1.1 - 1.2 for the corrected scale reduction factor served as an indicator of distribution stabilization [1]. CODA was also used to compute the model parameters' marginal posterior distributions, point estimates (mean, median and mode) and 95% highest probability densities (HPD). In order to assess the relative probability of alternative scenarios, i.e. whether demographic changes are recent (*T0*) or ancestral (*T1*), Bayes factors (BF) were computed. This involved counting the number of times the ratio between the posterior distributions of two scenarios were lower, equal or greater than one. For instance, a BF of one (i.e. (*T0* / *T1* > 1) / (*T0* / *T1* < 1) = 1) is indicative that both scenarios are equally probable, whereas BF > 1 or < 1 indicate that a more recent or ancestral time of change was favoured, respectively (*after* [8]). BF values between four and seven and > 7 served as indicators of positive and significant evidence for a scenario, respectively. These comparisons of BF across different time intervals also allowed the assessment of whether the use of an exponential model induced a bias by favouring more recent over ancestral times for population change [8].

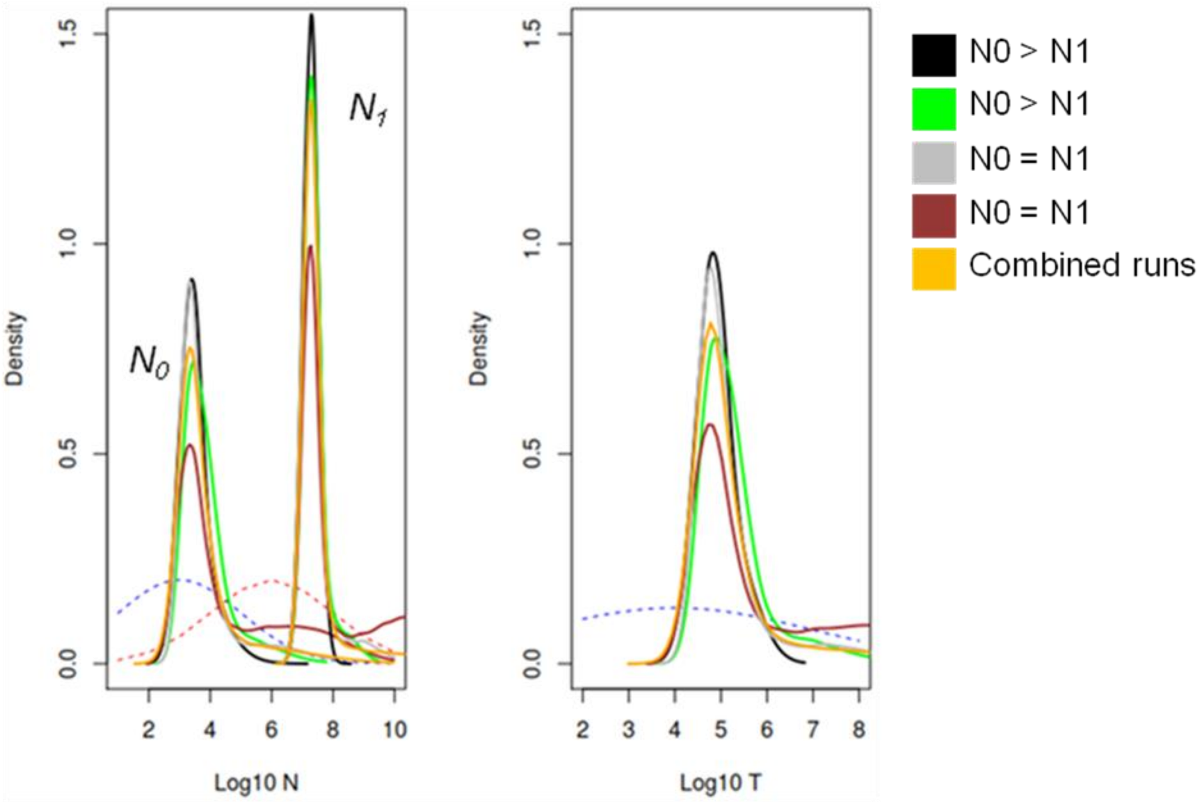


Fig. 1. Posterior distribution of present (N0) and past (N1) effective population sizes and the time decline (T), derived from independent runs using MSVAR1.3. Black and green lines represent runs that assumed the population to have expanded while brown and gray to have remained stable. Orange represents the combined runs.

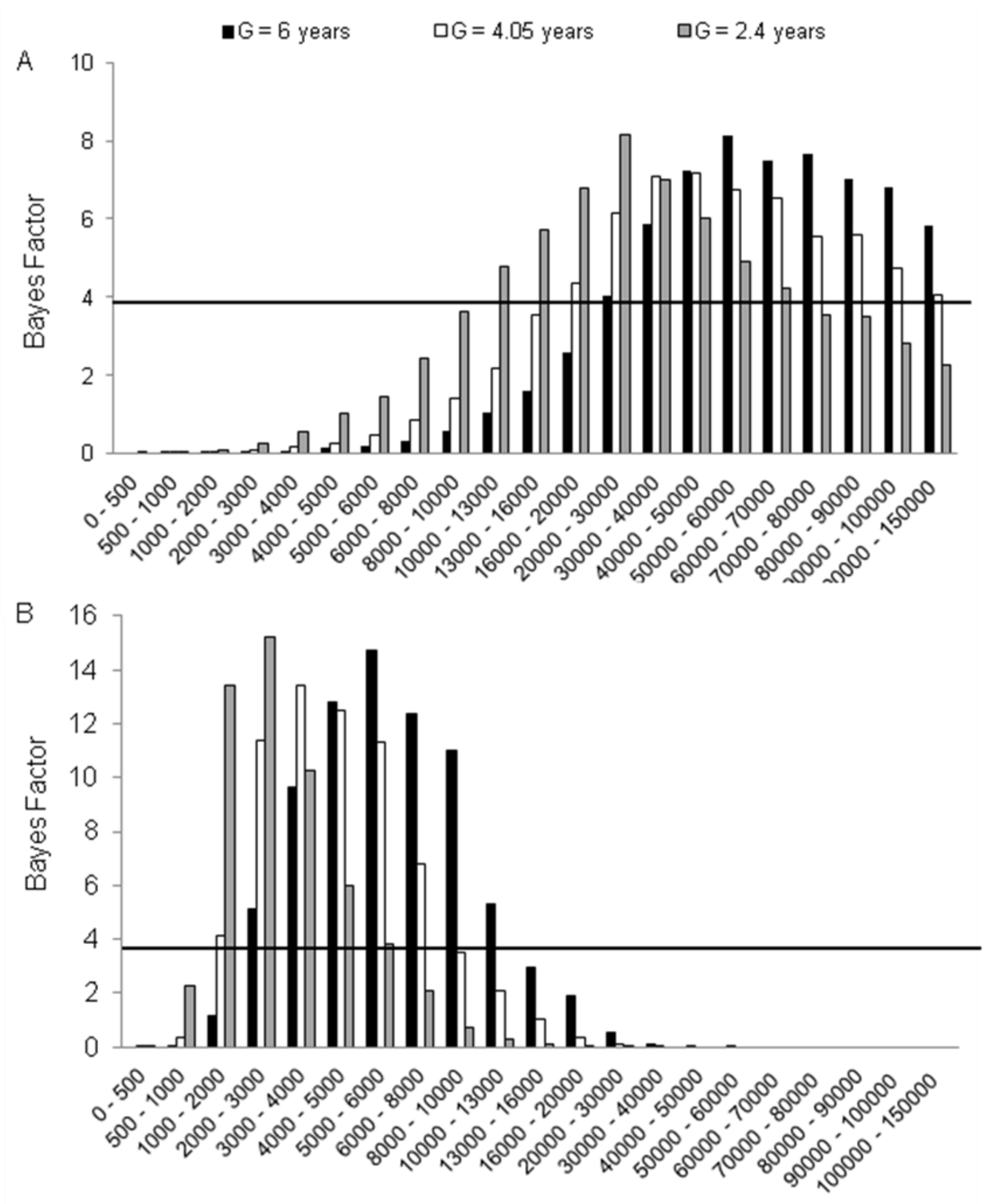


Fig. 2. Probable periods for the cheetah population decline, estimated using MSVAR1.3. Bayes Factors (BF) are based on the combined posterior distribution of the four independent MSVAR1.3 runs (72,000 records out of 8 X 10^{-9} iterations each) with a mean and variance microsatellite rate hyperprior set to **(A)** 10^{-6} and **(B)** 10^{-3}, and 0.25. Values above the solid line indicate positive evidence for times of decline and BF > 7 indicates significant evidence.

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Supporting information 2

Table 1. Estimates of cheetah subspecies divergence times.

Table 2. Prior distributions for models assessed using DIYABC. Models 1 - 10 assessed whether the population had declined, been stable or expanded during different timeframes. In addition, categories 3, 4, 6 and 7 included a scenario of severe decline (upper and lower prior of the recent and ancestral population size differed by at least 50% versus 5%). Categories 8 - 10 assessed the impact of broader prior distributions on model selection.

Table 3. Prior distributions for sensitivity analyses that assessed the impact of a broader prior distribution of the geometric mean governing allele size variation on model selection using DIYABC.

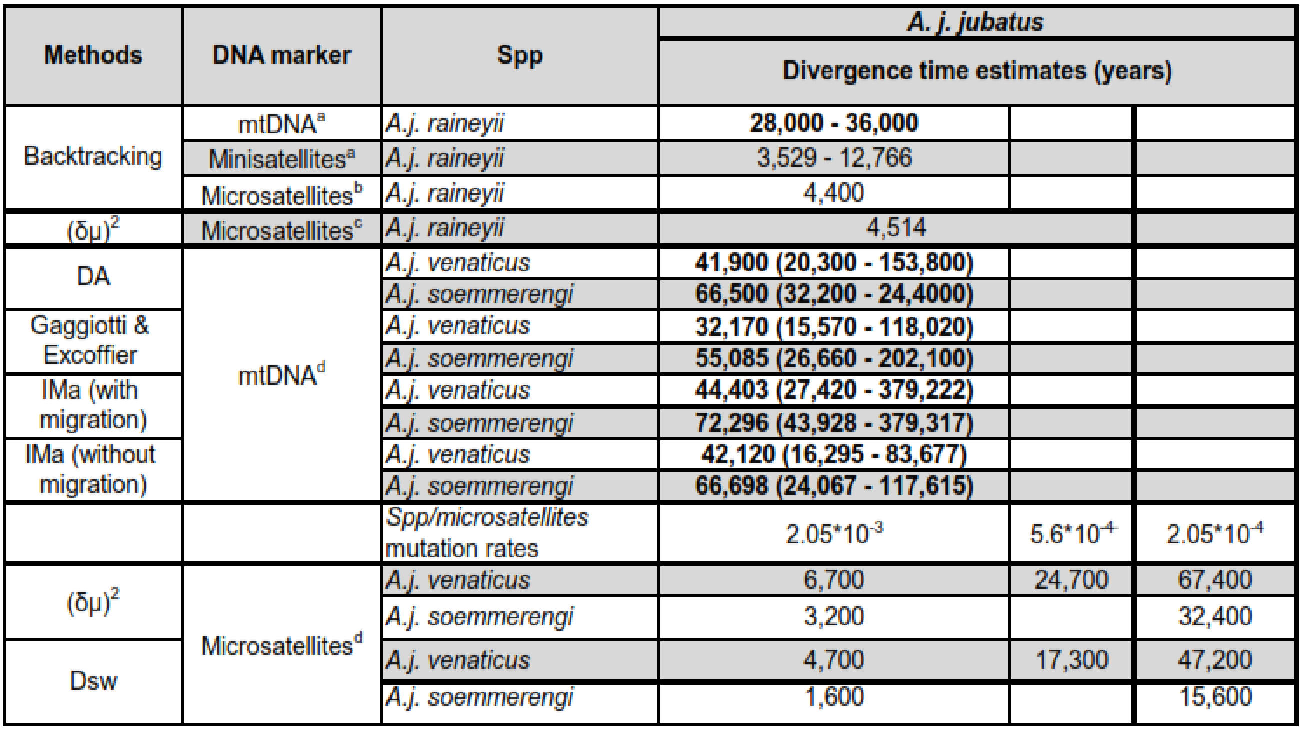
Table 4. Confidence in scenario choices based on type I and type II errors, and statistical power (1 - type II error) for the two scenarios with the highest relative posterior probability of simple scenarios assessed using DIYABC. Type I error was computed as the number of times a "true" scenarios did not have the highest posterior probability (HPP) and II, as the number of times it had the HPP when the data were simulated under a different scenario.

Table 5. Assessment of model fit based on the summary statistics (Ss) ability to recover similar genetic diversity as that observed in the observed data, in the case of simple scenarios assessed using DIYABC. Ss ≤ 0.5 were significant (Moyer et al. 2009). NAL = mean number of alleles and MGW = mean ratio of the number of alleles over the range of allele sizes.

Table 6. Relative posterior probabilities, with 95% credible intervals, of sensitivity analyses of model selection with respect to a broader geometric mean governing allele repeat size performed using DIYABC.

Table 7. Priors, hyperpriors, point estimates and convergence statistics for the combined posterior distribution of the four independent runs performed using MSVAR 1.3.

. **Table 1**



Methods	**DNA marker**	**Spp**	***A. j. jubatus***		
			Divergence time estimates (years)		
Backtracking	mtDNA[a]	*A.j. raineyii*	**28,000 - 36,000**		
	Minisatellites[a]	*A.j. raineyii*	3,529 - 12,766		
	Microsatellites[b]	*A.j. raineyii*	4,400		
$(\delta\mu)^2$	Microsatellites[c]	*A.j. raineyii*	4,514		
DA	mtDNA[d]	*A.j. venaticus*	**41,900 (20,300 - 153,800)**		
		A.j. soemmerengi	**66,500 (32,200 - 24,4000)**		
Gaggiotti & Excoffier		*A.j. venaticus*	**32,170 (15,570 - 118,020)**		
		A.j. soemmerengi	**55,085 (26,660 - 202,100)**		
IMa (with migration)		*A.j. venaticus*	**44,403 (27,420 - 379,222)**		
		A.j. soemmerengi	**72,296 (43,928 - 379,317)**		
IMa (without migration)		*A.j. venaticus*	**42,120 (16,295 - 83,677)**		
		A.j. soemmerengi	**66,698 (24,067 - 117,615)**		
		Spp/microsatellites mutation rates	$2.05*10^{-3}$	$5.6*10^{-4}$	$2.05*10^{-4}$
$(\delta\mu)^2$	Microsatellites[d]	*A.j. venaticus*	6,700	24,700	67,400
		A.j. soemmerengi	3,200		32,400
Dsw		*A.j. venaticus*	4,700	17,300	47,200
		A.j. soemmerengi	1,600		15,600

[a] Menotti-Raymond and O'Brien (1993), [b] Menotti-Raymond & O'Brien (1995), [c] Driscoll et al (2002), [d] Charrua et al (2011)

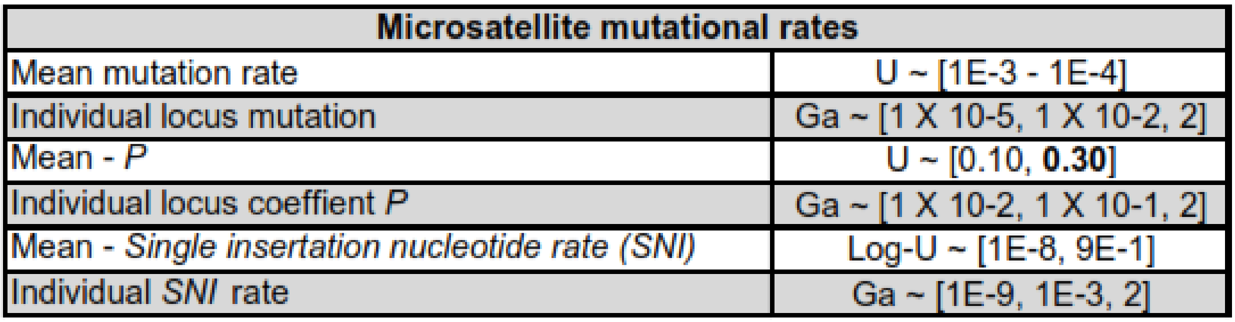
Table 2

Models according to Table 1	Parameters	Sce 1	Sce 2	Sce 3	Sce 4
1	Nanc	**10 - 5,000**	5001 - 10,000	10 - 1,000	
	T (Gen = 6 years)	**1 - 50**			
	Nrec	**10 - 5,000**			
2	Nanc	**10 - 7,000**	**7,001 - 12,000**	10 - 2,000	
	T (Gen = 6 years)	**51 - 600**			
	Nrec	**10 - 7,000**			
3	Nanc	**10 - 6,000**	6,006 - 10,000	10 - 450	10,006 - 15,000
	t	**506 - 1,290**			
	Nrec	**10 - 6,000**		500 - 6,000	10 - 6,000
4	Nanc	**10 - 6,000**	6,001 - 12,000	12,000 - 16,000	1,501 - 6,000
	T (Gen = 6 years)	**1,300 - 2,400**			
	Nrec	**10 - 6,000**			10 - 1,500
5	Nanc	**10 - 5,000**	10,000 - 15,000	10 - 1,000	
	T (Gen = 6 years)	**2,101 - 4,000**			
	Nrec	**10 - 5,000**		2,000 - 5,000	

Table 2 (con't)

Models according to Table 1	**Parameters**	**Sce 1**	**Sce 2**	**Sce 3**	**Sce 4**
6	Nanc	**10 - 7,000**	7100 - 12,000	10 - 390	12,100 - 16,000
	T (Gen = 6 years)	**3500 - 5000**			
	Nrec	**10 - 7,000**		400 - 7000	10 - 7,000
7	Nanc	**10 - 7,000**	**7,100 - 14,000**	10 - 400	14,000 - 17,000
	T (Gen = 6 years)	**5,000 - 8,000**			
	Nrec	**10 - 7,000**		400 - 7,000	10 - 7,000
8	Nanc	10 - 10,000	8001 - 13,000	10 - 1,000	
	T (Gen = 6 years)	10 – 10,000			
	Nrec	10 - 10,000		1500 -13,000	
9	Parameters	Sce 1	Sce 2	Sce 3	
	Nanc	10 - 7,000	**7,001 - 13 ka**	10 - 1,000	
	T (Gen = 6 years)	**601 -2,100**			
	Nrec	**10 - 7,000**			
10	Nanc	**10 - 5,000**	10 - 13,000	10 - 500	
	T (G = 6 years)	**4001 - 8,000**			
	Nrec	**10 - 5,000**		600 - 5,000	

Table 2 (con’t)



Microsatellite mutational rates	
Mean mutation rate	U ~ [1E-3 - 1E-4]
Individual locus mutation	Ga ~ [1 X 10-5, 1 X 10-2, 2]
Mean - *P*	U ~ [0.10, **0.30**]
Individual locus coeffient *P*	Ga ~ [1 X 10-2, 1 X 10-1, 2]
Mean - *Single insertation nucleotide rate (SNI)*	Log-U ~ [1E-8, 9E-1]
Individual *SNI* rate	Ga ~ [1E-9, 1E-3, 2]

Table 3

Parameter	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Nanc	5,000 - 10,000		10,000 - 30,000		
T (Gen = 6 years)	1 - 20	**50 - 300**	5000 - 40,000		
Nrec	10 - 5,000				
Nanc	4,001 - 8,000	6,001 - 10,000	10,000 - 30,000		
T (Gen = 6 years)	**1 – 50**	**51 – 600**	5,000 – 20,000		
Nrec	10 - 4,000	10 - 6,000	10 - 5,000		
Nanc	10 - 10,000			10 - 15,000	
T (Gen = 6 years)	**1 - 50**	51 - 600	601 - 2,100	2,100 - 5,000	5,000 - 40,000
Nrec	10 - 10,000			10 - 15,000	10 - 15,000
Nanc	**8,000 - 30,000**				
T (Gen = 6 years)	1 - 3,000	5,000 - 10,000	**13,000 - 30,000**	1 - 40,000	
Nrec	**10 - 5,000**				

Table 3 (con't)

Mutational rate priors for scenarios	
Mean mutation rate	U ~ [1E-3 - 1E-4]
Individual locus mutation	Ga ~ [1 X 10-5, 1 X 10-2, 2]
Mean - *P*	U ~ [0.10, **0.70**]
Individual locus coeffient *P*	Ga ~ [1 X 10-2, 1 X 10-1, 2]
Mean - *Single insertation nucleotide rate (SNI)*	Log-U ~ [1E-8, 9E-1]
Individual *SNI* rate	Ga ~ [1E-9, 1E-3, 2]

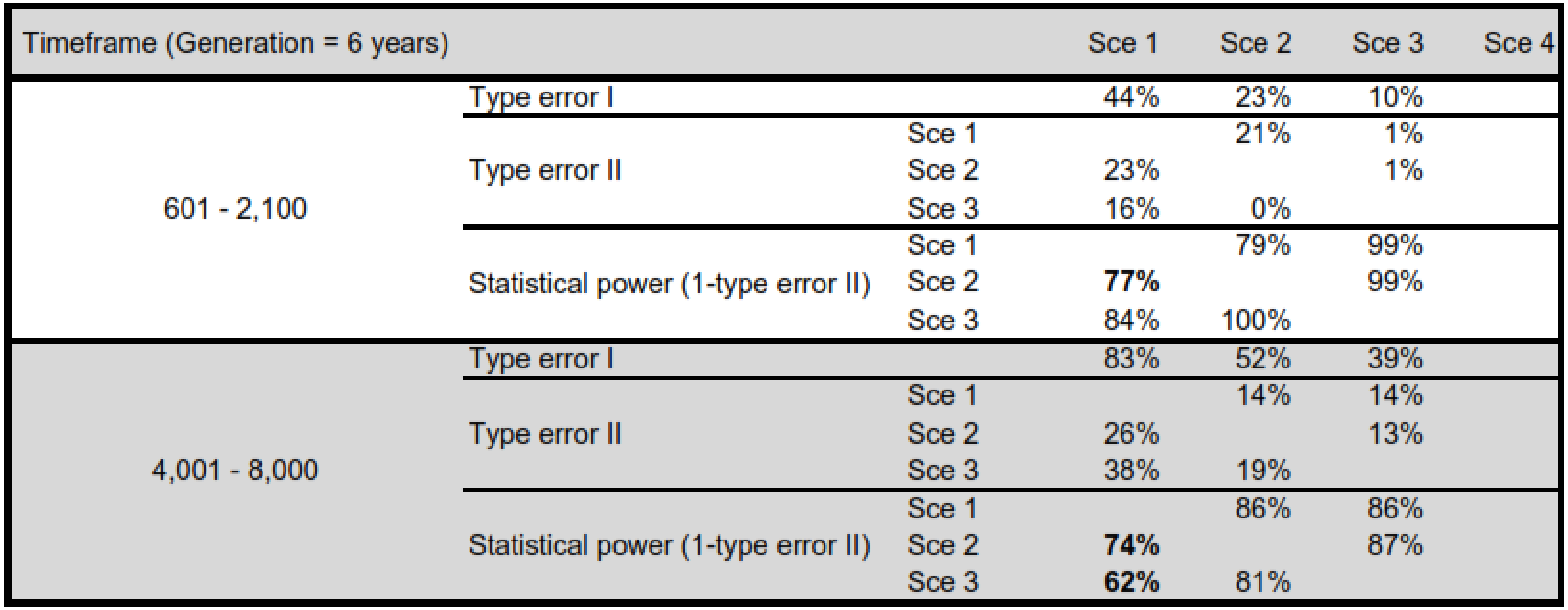
Table 4

Timeframe (Generation = 6 years)			Sce 1	Sce 2	Sce 3	Sce 4
1 - 50	Type error I		53%	27%	10%	
	Type error II	Sce 1		24%	8%	
		Sce 2	20%			
		Sce 3	29%	0%		
	Statistical power (1-type error II)	Sce 1		76%	92%	
		Sce 2	80%		100%	
		Sce 3	**71%**	100%		
51 - 600	Type error I		53%	27%	10%	
	Type error II	Sce 1		24%	8%	
		Sce 2	20%		0%	
		Sce 3	29%	0%		
	Statistical power (1-type error II)	Sce 1		**76%**	92%	
		Sce 2	80%		100%	
		Sce 3	**71%**	100%		
506 - 1,290	Type error I		47%	60%		
	Type error II	Sce 1		20%		
		Sce 2	21%			
	Statistical power (1-type error II)	Sce 1		80%		
		Sce 2	79%			
1,300 - 2,400	Type error I		45%	73%		
	Type error II	Sce 1		15%		
		Sce 2	14%			
	Statistical power (1- type error II)	Sce 1		85%		
		Sce 2	86%			

Table 4 (con't)

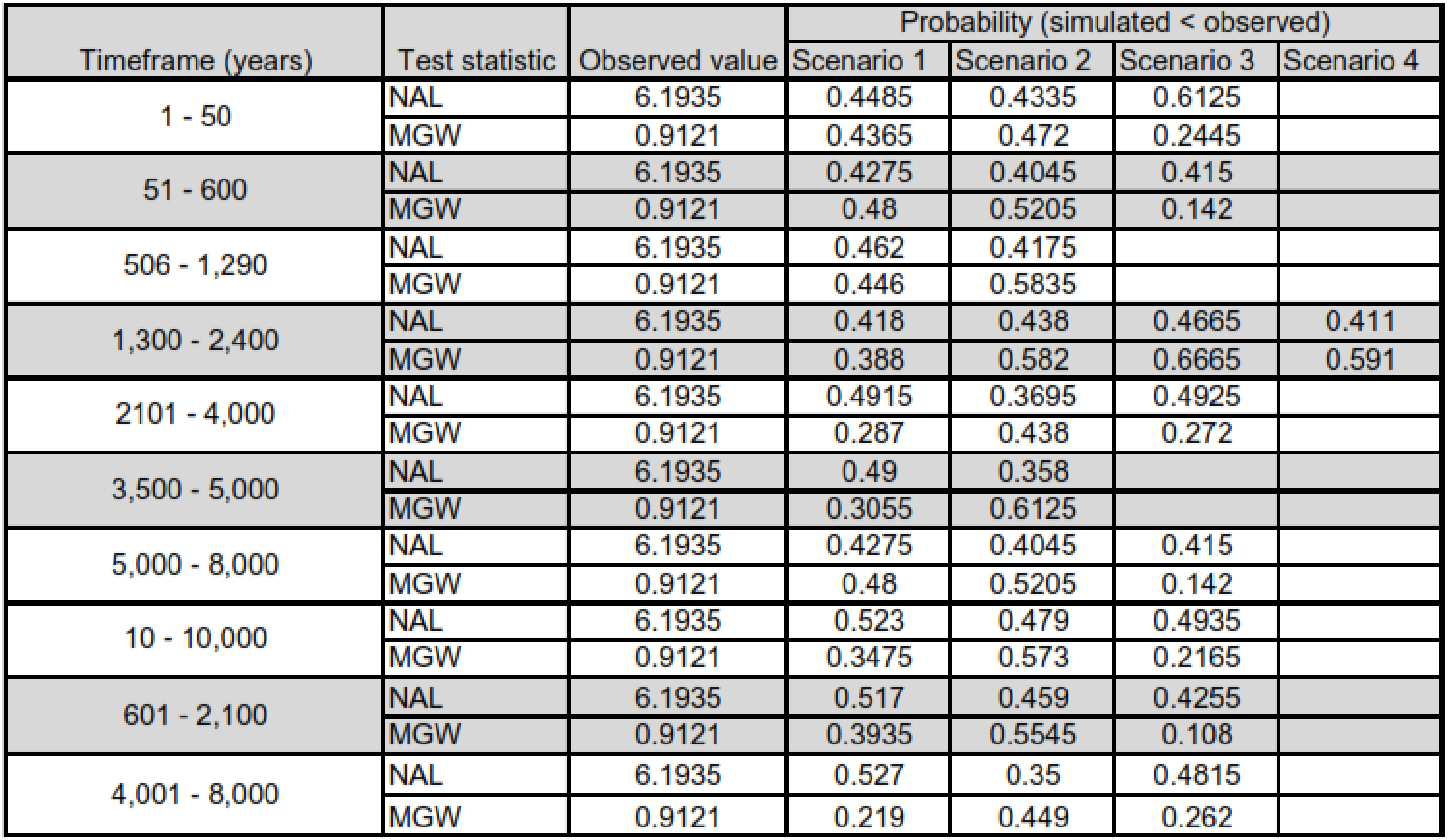
Timeframe (Generation = 6			Sce 1	Sce 2	Sce 3	Sce 4
2,101 - 4,000	Type error I		83%	57%	39%	
	Type error II	Sce 1		16%	14%	
		Sce 2	29%		12%	
		Sce 3	36%	21%		
	Statistical power (1-type error II)	Sce 1		84%	86%	
		Sce 2	**71%**		88%	
		Sce 3	**64%**	**79%**		
3,500 - 5,000	Type error I		68%	76%		
	Type error II	Sce 1		22%		
		Sce 2	15%			
	Statistical power (1-type error II)	Sce 1		78%		
		Sce 2	85%			
5,000 - 8,000	Type error I		76%	82%		
	Type error II	Sce 1		18%		
		Sce 2	14%			
	Statistical power (1-type error II)	Sce 1		82%		
		Sce 2	86%			
10 - 10,000	Type error I		66%	29%	19%	
	Type error II	Sce 1		22%	14%	
		Sce 2	35%		3%	
		Sce 3	23%	3%		
	Statistical power (1-type error II)	Sce 1		**78%**	86%	
		Sce 2	**65%**		97%	
		Sce 3	**77%**	97%		

Table 4 (con't)



Timeframe (Generation = 6 years)			Sce 1	Sce 2	Sce 3	Sce 4
601 - 2,100	Type error I		44%	23%	10%	
	Type error II	Sce 1		21%	1%	
		Sce 2	23%		1%	
		Sce 3	16%	0%		
	Statistical power (1-type error II)	Sce 1		79%	99%	
		Sce 2	**77%**		99%	
		Sce 3	84%	100%		
4,001 - 8,000	Type error I		83%	52%	39%	
	Type error II	Sce 1		14%	14%	
		Sce 2	26%		13%	
		Sce 3	38%	19%		
	Statistical power (1-type error II)	Sce 1		86%	86%	
		Sce 2	**74%**		87%	
		Sce 3	**62%**	81%		

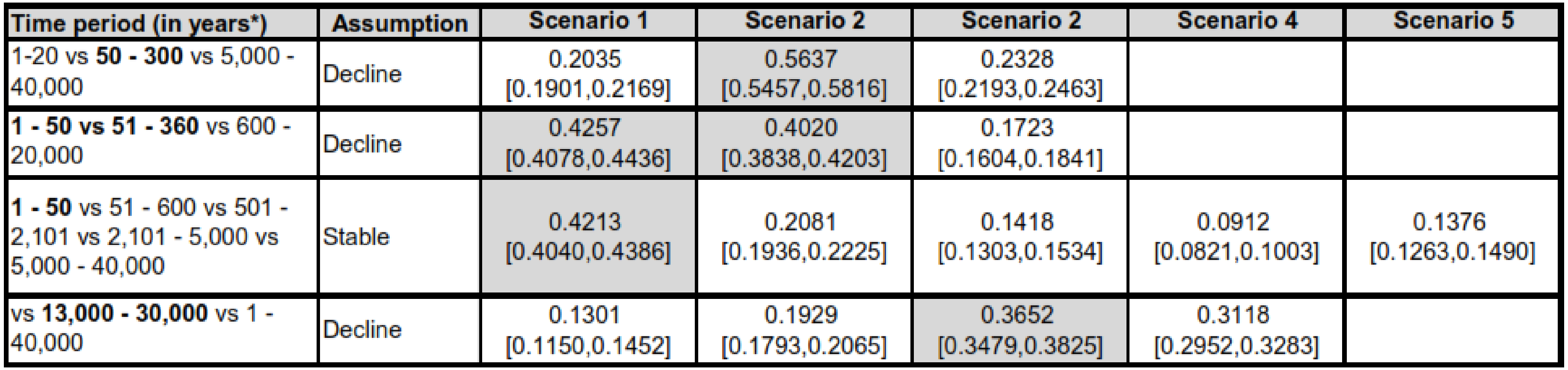
Table 5



Timeframe (years)	Test statistic	Observed value	Probability (simulated < observed)			
			Scenario 1	Scenario 2	Scenario 3	Scenario 4
1 - 50	NAL	6.1935	0.4485	0.4335	0.6125	
	MGW	0.9121	0.4365	0.472	0.2445	
51 - 600	NAL	6.1935	0.4275	0.4045	0.415	
	MGW	0.9121	0.48	0.5205	0.142	
506 - 1,290	NAL	6.1935	0.462	0.4175		
	MGW	0.9121	0.446	0.5835		
1,300 - 2,400	NAL	6.1935	0.418	0.438	0.4665	0.411
	MGW	0.9121	0.388	0.582	0.6665	0.591
2101 - 4,000	NAL	6.1935	0.4915	0.3695	0.4925	
	MGW	0.9121	0.287	0.438	0.272	
3,500 - 5,000	NAL	6.1935	0.49	0.358		
	MGW	0.9121	0.3055	0.6125		
5,000 - 8,000	NAL	6.1935	0.4275	0.4045	0.415	
	MGW	0.9121	0.48	0.5205	0.142	
10 - 10,000	NAL	6.1935	0.523	0.479	0.4935	
	MGW	0.9121	0.3475	0.573	0.2165	
601 - 2,100	NAL	6.1935	0.517	0.459	0.4255	
	MGW	0.9121	0.3935	0.5545	0.108	
4,001 - 8,000	NAL	6.1935	0.527	0.35	0.4815	
	MGW	0.9121	0.219	0.449	0.262	

Moyer et al. (2012) Past and Present Processes Influencing Genetic Diversity
and Effective Population Size in a Natural Population
of Atlantic Sturgeon. Transactions of the American Fisheries Society 141:56–67, 2012

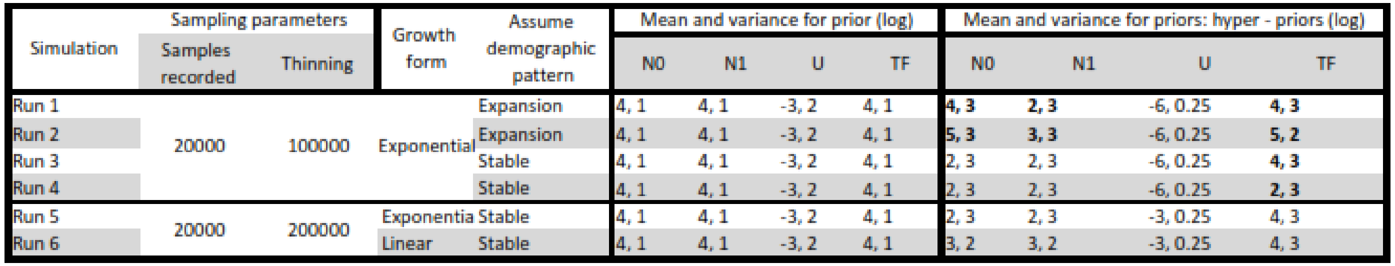
Table 6



Time period (in years*)	Assumption	Scenario 1	Scenario 2	Scenario 2	Scenario 4	Scenario 5
1-20 vs **50 - 300** vs 5,000 - 40,000	Decline	0.2035 [0.1901,0.2169]	0.5637 [0.5457,0.5816]	0.2328 [0.2193,0.2463]		
1 - 50 vs 51 - 360 vs 600 - 20,000	Decline	0.4257 [0.4078,0.4436]	0.4020 [0.3838,0.4203]	0.1723 [0.1604,0.1841]		
1 - 50 vs 51 - 600 vs 501 - 2,101 vs 2,101 - 5,000 vs 5,000 - 40,000	Stable	0.4213 [0.4040,0.4386]	0.2081 [0.1936,0.2225]	0.1418 [0.1303,0.1534]	0.0912 [0.0821,0.1003]	0.1376 [0.1263,0.1490]
vs **13,000 - 30,000** vs 1 - 40,000	Decline	0.1301 [0.1150,0.1452]	0.1929 [0.1793,0.2065]	0.3652 [0.3479,0.3825]	0.3118 [0.2952,0.3283]	

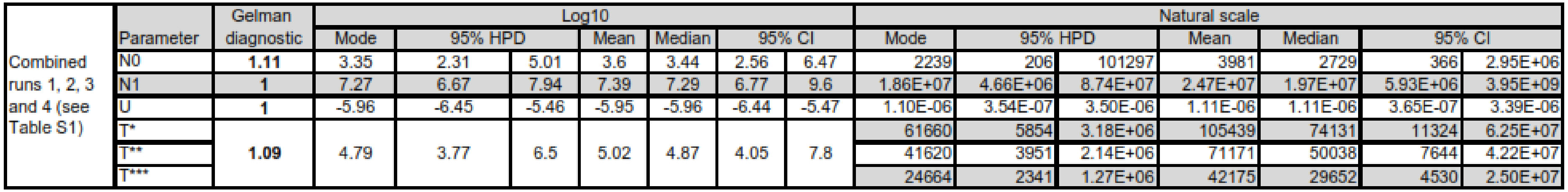
P : U~ [0.1 - 0.7]

Table 7

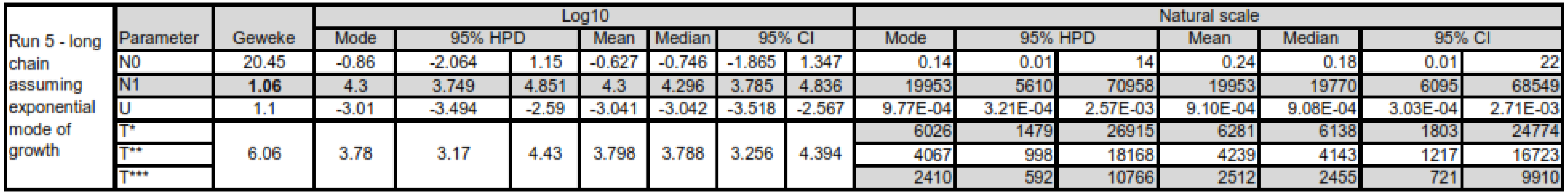


Simulation	Sampling parameters		Growth form	Assume demographic pattern	Mean and variance for prior (log)				Mean and variance for priors: hyper - priors (log)			
	Samples recorded	Thinning			N0	N1	U	TF	N0	N1	U	TF
Run 1	20000	100000	Exponential	Expansion	4, 1	4, 1	-3, 2	4, 1	**4, 3**	**2, 3**	-6, 0.25	**4, 3**
Run 2				Expansion	4, 1	4, 1	-3, 2	4, 1	**5, 3**	**3, 3**	-6, 0.25	**5, 2**
Run 3				Stable	4, 1	4, 1	-3, 2	4, 1	2, 3	2, 3	-6, 0.25	**4, 3**
Run 4				Stable	4, 1	4, 1	-3, 2	4, 1	2, 3	2, 3	-6, 0.25	**2, 3**
Run 5	20000	200000	Exponentia	Stable	4, 1	4, 1	-3, 2	4, 1	2, 3	2, 3	-3, 0.25	4, 3
Run 6			Linear	Stable	4, 1	4, 1	-3, 2	4, 1	3, 2	3, 2	-3, 0.25	4, 3

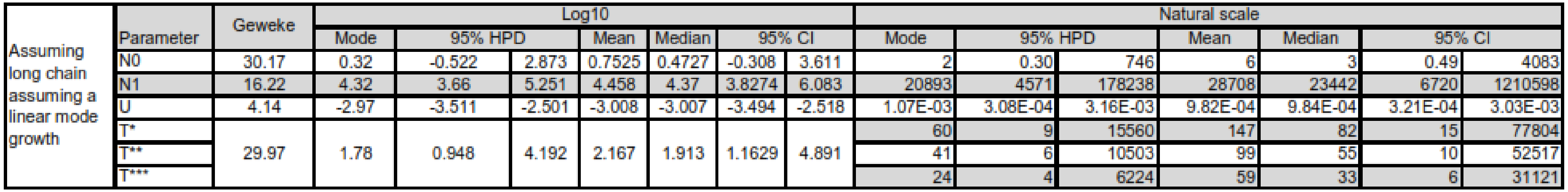
Table S6. Point estimates and convergence statistics for the combined posterior distribution of the four independent runs and two long chains performed using MSVAR 1.3. N0 and N1 represent present



	Parameter	Gelman diagnostic	Log10							Natural scale						
			Mode	95% HPD		Mean	Median	95% CI		Mode	95% HPD		Mean	Median	95% CI	
Combined runs 1, 2, 3 and 4 (see Table S1)	N0	**1.11**	3.35	2.31	5.01	3.6	3.44	2.56	6.47	2239	206	101297	3981	2729	366	2.95E+06
	N1	**1**	7.27	6.67	7.94	7.39	7.29	6.77	9.6	1.86E+07	4.66E+06	8.74E+07	2.47E+07	1.97E+07	5.93E+06	3.95E+09
	U	**1**	-5.96	-6.45	-5.46	-5.95	-5.96	-6.44	-5.47	1.10E-06	3.54E-07	3.50E-06	1.11E-06	1.11E-06	3.65E-07	3.39E-06
	T*	**1.09**	4.79	3.77	6.5	5.02	4.87	4.05	7.8	61660	5854	3.18E+06	105439	74131	11324	6.25E+07
	T**									41620	3951	2.14E+06	71171	50038	7644	4.22E+07
	T***									24664	2341	1.27E+06	42175	29652	4530	2.50E+07



	Parameter	Geweke	Log10							Natural scale						
			Mode	95% HPD		Mean	Median	95% CI		Mode	95% HPD		Mean	Median	95% CI	
Run 5 - long chain assuming exponential mode of growth	N0	20.45	-0.86	-2.064	1.15	-0.627	-0.746	-1.865	1.347	0.14	0.01	14	0.24	0.18	0.01	22
	N1	**1.06**	4.3	3.749	4.851	4.3	4.296	3.785	4.836	19953	5610	70958	19953	19770	6095	68549
	U	1.1	-3.01	-3.494	-2.59	-3.041	-3.042	-3.518	-2.567	9.77E-04	3.21E-04	2.57E-03	9.10E-04	9.08E-04	3.03E-04	2.71E-03
	T*	6.06	3.78	3.17	4.43	3.798	3.788	3.256	4.394	6026	1479	26915	6281	6138	1803	24774
	T**									4067	998	18168	4239	4143	1217	16723
	T***									2410	592	10766	2512	2455	721	9910



	Parameter	Geweke	Log10							Natural scale						
			Mode	95% HPD		Mean	Median	95% CI		Mode	95% HPD		Mean	Median	95% CI	
Assuming long chain assuming a linear mode growth	N0	30.17	0.32	-0.522	2.873	0.7525	0.4727	-0.308	3.611	2	0.30	746	6	3	0.49	4083
	N1	16.22	4.32	3.66	5.251	4.458	4.37	3.8274	6.083	20893	4571	178238	28708	23442	6720	1210598
	U	4.14	-2.97	-3.511	-2.501	-3.008	-3.007	-3.494	-2.518	1.07E-03	3.08E-04	3.16E-03	9.82E-04	9.84E-04	3.21E-04	3.03E-03
	T*	29.97	1.78	0.948	4.192	2.167	1.913	1.1629	4.891	60	9	15560	147	82	15	77804
	T**									41	6	10503	99	55	10	52517
	T***									24	4	6224	59	33	6	31121

CHAPTER III

Estimating the effective population size of the Namibian cheetah *Acinonyx jubatus*: comparison of analytical approaches and assessment of the impact of vital rate variation.

Author list:

Ezequiel Chimbioputo Fabiano [a,b,c]

Laurie Marker [b]

Anne Schmidt-Küntzel [b, c]

Eduardo Eizirik [a]

Affiliations:

a. Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil

b. Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia.

c. Life Technologies Conservation Genetics Laboratory, Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia.

Email addresses:

ezequiel.fabiano@acad.pucrs.br, fabianoezekiel@gmail.com

cheetah@iway.na

genetics@cheetah.org

eduardo.eizirik@pucrs.br

Corresponding authors:

Ezequiel C. Fabiano, Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia. Email: ezequiel.fabiano@acad.pucrs.br, fabianoezekiel@gmail.com

Prof. Eduardo Eizirik, Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil, Email: eduardo.eizirik@pucrs.br

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Abstract

N_e is necessary to assess the genetic viability of large conservation species. The long-term viability of a population is often associated with an effective population size (N_e) threshold value of > 500. N_e is expected to be smaller than the total population size (N_c) and may be influenced by independent factors. We used two one-sample genetic and one demographic estimator to determine the contemporary N_e of the Namibian cheetah population. We also assessed the sensitivity of N_e to perturbations in survival rates, proportion of breeders as well as uncertainties in carrying capacity and N_c. Genetic estimates (134, 95% CI 99 - 224) were significantly less than 500 and demographic estimates, which ranged from 450 to 2500 depending on generation time used (2.4 or 6 years). Demographic N_e estimates were mostly sensitive to perturbations in the proportion of females breeding, adult female survival rates, uncertainties in N_c and the carrying capacity. These same parameters also influenced N_c. We observed contrasting effects due to different perturbations levels of the same parameter, possibly due to non-linear responses by factors affecting N_e and N_c. Overall, our finding suggests that the population is viable but N_e changes according to the contemporary status of demographic parameters. Conservation actions should continue to focus on aspects related to females.

Keywords Population viability • Reproductive variance • Linkage disequilibrium • Survivorship patterns • Simulation modeling

1. Introduction

Population viability is governed by interactions among several demographic parameters, in particular its census (N_c) and effective (N_e) sizes. As a result, conservation actions often aim to maximize both measures. These two measures differ in that N_c represents the total annual population size, while N_e the size of an ideal Wright-Fisher (WF) population that presents the same genetic properties (e.g. drift-induced rate of loss of genetic diversity) as the study population (Palstra and Fraser 2012). An ideal population is characterized by random mating, discrete generations, reproduction success following a Poisson distribution, and even sex ratio, in addition to being closed, having drift as the only source of linkage disequilibrium, and being assessed only for unlinked loci) (Charlesworth 2009). The N_e/N_c ratio varies across species (Nunney 1993; Frankham 1995; Palstra and Ruzzante 2008) and among subpopulations **(**Phillipsen et al. 2011) . N_e is highly susceptible to fluctuations in N_c, among other variables (Frankham 1995; Vucetich and Waite 1998) importantly, populations with large N_c can have low N_e, and hence experience substantial genetic drift (Hedrick 2005; Hare et al. 2011). Consequently, in the context of conservation planning there is a need to determine a population's contemporary N_e, and to assess its sensitivity to varying threat and management scenarios (Andrello et al. 2012; Baalsrud 2011).

The cheetah, *Acinonyx jubatus*, is classified as Vulnerable by IUCN with less than 12,000 living individuals spread across 22 countries (Durant et al. 2008). Currently, only Namibia and Botswana possess adult population sizes above 1000 individuals, with at least 2,000 and 1,800 individuals, respectively (Purchase et al. 2007). Common threats of extinction across the range of this species include habitat loss, fragmentation and degradation, as well as human-wildlife conflict (HWC)

(Sunquist and Sunquist 2002; Purchase et al. 2007; Durant et al. 2008). Moreover, HWC has been deemed the determining factor of Namibia's current and future ecological carrying capacity (Turpie et al. 2010). Population viability analyses, as measured by a positive growth rate ($\lambda > 1$), indicate that the cheetahs are vulnerable to total mortality rate patterns (natural and additive) and reproduction patterns (Berry et al. 1997; Crooks et al. 1998; Kelly and Durant, 2000; Lubben et al. 2008). Consequently, conservation strategies have so far focused on increasing survival rates and limiting the removal of females. However, it remains unknown how these actions and removal of different age classesaffect N_e.

The Namibian cheetah population has fluctuated and declined during the past century due to diverse factors including droughts, prey reduction, trophy hunting and indiscriminate removals resulting from human wildlife conflict (HWC) (O'Brien et al. 1987; Nowell 1996; Marker-Kraus et al. 1996; Marker et al. 2007). These fluctuations and removals certainly have caused N_c to fluctuate, induced differences in survival rate patterns and an unstable age structure, reduced the actual and potential number of breeders (Marker et al. 2003), as well as altered generation times (G). Furthermore, these processes likely caused increases in family size variances (Saether, Engen and Solberg 2009). All these factors affect N_e in concert or independently, as they are violations of some of the WF model assumptions (Nunney and Elam 1994; Waite and Parker 1996). This is an important issue, as N_e estimates are biased if estimators do not account for these violations (Jorde and Ryman 1995, 2007). Therefore, as the degree of robustness of N_e estimators to violation of WF assumptions differ (Luikart et al. 2010) studies assessing N_e should apply multiple estimators.

In this study, we computed inbreeding *Ne* estimates for the Namibian cheetah population, and assessed their variability as a function of uncertainty in the initial population size (N_{init}), carrying capacity (K), and vital rates (mortality and reproduction). We also estimated the impact of these variables on the estimates of N_c, deterministic λ, sex ratio (*SR*) and *G.* To estimate N_e, we applied two different 'one-sample' genetic estimators, one based on linkage disequilibrium (LD) (Hill 1981) and implemented in the program LDNe (Waples 2006; Waples and Do 2008), and the other using Approximate Bayesian computation (ABC) and implemented in the software ONeSAMP (Tallmon et al. 2008). A demographic estimator, based on a strategy that uses heterozygosity levels derived from simulations of population processes, was also computed (Eizirik et al. 2002; Sato and Harada 2008). Genetic and demographic estimators are complementary, as they may reflect different historical time frames and be affected differently by various population processes (Nunney and Elam 1994; Wang 2012, et al. 2010, 2012; Waples and Waples 2011). Ultimately, the study aim was to allow an improved understanding of the processes affecting cheetah population dynamics, and their impact on long-term genetic viability in this population.

2. *Methods*

2.1. Dataset construction and genetic estimates of N_e

To estimate N_e using genetic approaches, we used a set of 90 unrelated cheetah individuals captured by farmers between 1991 and 1999, and previously genotyped for 36 dinucleotide microsatellite repeats (Marker et al. 2008). From this initial panel, however, only 31 loci were used due to the data requirements of some estimators (e.g. ONeSAMP requires that only polymorphic loci with limited missing data are used (Tallmon et al. 2008)).

We obtained N_e estimates using LD, with the approach implemented in LDNe (Waples, 2006; Waples and Do, 2008), and also through the ABC method available in ONeSAMP (Tallmon et al. 2008). LDNe employs the Burrow Δ measure of LD for estimating N_e (Waples and Do 2008). N_e was computed assuming random mating, and using the jacknife model to compute 95% confidence intervals (CI). Preliminary results using the recommended $P_{crit} = 0.02$ for $n > 25$ (Waples and Do 2010) yielded similar results (95% CI overlap) as $P_{crit} = 0.01$ and 0.05, here we report the average of these three estimates.

The ONeSAMP estimate, in contrast, uses seven summary statistics (SS) that are a function of N_e and a *prior* range supplied for N_e when computing the posterior estimate of this variable (Tallmon et al. 2008). We performed three runs, using 20 with 1500 or 2000 as *priors*. N_e estimates are inferred through a weighted local regression based on a subset of the 50,000 posterior records that have the most similar SS to those of the observed data set. Despite some variability in N_e estimates based on different priors, these overlapped (e.g., Phillipsen et al. 2011), thus we report the harmonic mean of N_e estimates across runs. Following Waples and Do (2010), LDNe and ONeSAMP estimates were combined in an attempt to increase overall N_e precision.

2.3. Demographic-based N_e estimation

2.3.1. N_e and N_e/N_c estimation

Demographic N_e estimates were determined using a hybrid strategy based on population simulations. We used the Population Viability Analysis (PVA) software VORTEX (Miller and Lacy 2005) to simulate demographic processes, and then used a genetic approach to calculate N_e based on the observed loss of heterozygosity along the simulations. VORTEX model structure is presented in S1. We used the formula $N_e = ½(1- \exp^{(\mathrm{logeHt})/t})$ (Crow and Kimura 1970; *after* Rieman and Allendorf 2001; Eizirik et al. 2002), where H_t is VORTEX's estimate of heterozygosity and t the number of years (yrs) in a simulation divided by the G. Due to uncertainties in G estimates, N_e was computed using G of 2.4, 5.34 yrs (Kelly 2001), 6 yrs (Marker and O’Brien 1989;) and 4.05 yrs (*this study*). N_e based on these G estimates are referred accordingly to as N_{e5}, N_{e4}, N_{e3} and N_{e1}. To calculate N_e/N_c ratios, we used VORTEX's N_c estimate after the simulations. Overall, 45 different scenarios were simulated including the baseline scenario, and each scenario was replicated three times; we therefore report averages for N_e and N_c across replicates for each scenario.

2.3.2. Baseline scenario input parameters

Input parameters were drawn from the literature (Marker et al. 2003, 2008; Lindsey et al. 2009) and are summarized on Table 1. The scenario based on these values was treated as the baseline scenario, representing a hybrid between the population realistic and biological potential. Appendix S1 presents a detailed rationale for their inclusion, as well as parameter estimation approaches. Below we provide a brief overview, specifically focusing on parameters that were recalculated. Overall, we ran 45 models, each of them comprising 500 iterations spanning 200 yrs and defining extinction as when only one sex remained. The population was assumed to be panmictic (Marker et al. 2008). N_{init} and K were set to 3670 individuals, according to a crude extrapolation of a minimal density of 5.3 individuals (inds.) per 1000 km^2 (Fabiano et al. in revision, Chapter IV) to an area of 692,404 km^2 (*after* Hanssen and Stander 2004). This estimate is conservative, as N_{init} could be > 11,000 inds., if we considered a higher density estimate of 16 inds. / 1000 km^2 (Fabiano et al. in revision, Chapter IV). The proportion of males in the breeding pool was set to 62%, a value estimated by dividing total number of adult males (≥ 3 years) by the total number of males captured by farmers from 1991 to 2011. In addition to only counting the adults, only one male per coalition was counted. This proportion may be overestimated due to sampling bias and if the population is characterized by a polygynous-polyandry mating system as observed for cheetahs in the Serengeti (Gotelli et al. 2007). Harvesting rates were set as the maximum number of individuals handled by the Cheetah Conservation Fund (CCF) per age class between 1991 and 2011 recorded. Mortality rate (q_x) estimates were determined using BaSTA (Bayesian survival trajectory analysis) (Colchero et al. 2012; Colchero and Clark 2012) based on a data set of 90 aged dead cheetahs handled by CCF between 1991 and 2011. The data set included 64 males and 26 females ranging from cubs to 12-year-old animals. Mortality estimates reflect total mortality, including natural and anthropogenic. For the q_x SD, we followed Stacey and Taper (1992) (S1).

2.3.2.1 Sensitivity analyses of viability, and relationship among population parameters

Sensitivity analyses were performed by decreasing baseline mortality rates and the proportion of breeders by 10% (low), 20% (moderate) and ≥ 40% (high), while other parameters were kept constant (S2). For example, to assess the impact of survival rates of active breeding females we increased simultaneously the survival rates for adult female and cubs (age 0 - 1) by 10, 20 or 40% (e.g. F2M0-1F0-1 (10%) in Fig. 1, Table S2). It is northworthy that while some scenarios are of direct relevance to conservation others provide a through understanding of processes affecting N_e (e.g. survival rate of cubs, M0-1). To investigate the effects of these varying vital rates on N_e, N_c, λ, *SR* and *G*, we determined the percentage change in each of these, relative to the baseline estimate of each parameter. The significance of parameter response to perturbation levels was determined through the Kruskal-Wallis test (*KW*). In addition, we used an analysis of variance (ANOVA) to ascertain whether N_e estimates differed among the assumed values of *G*. Pearson's correlation coefficient (*r*) was used to determine the significance of the relationship among N_e estimates, and Spearman's rho to assess it among N_e, N_c, λ, *SR* and *G*. Since preliminary results indicated that N_e based on *G* = 6 (N_{e3}), 5.34 (N_{e4}) and 2.05 yrs (N_{e5}) only differed in their magnitude, we focus mostly on N_{e3} and N_{e5}.

3. Results

3.1. Genetic-based N_e estimates

LDNe yielded a N_e estimate of 119 individuals (95% CI 94 - 156), while that calculated with ONeSAMP was 153 (95% CI 99 - 392), with a combined N_e estimate of 134 (95% CI 99 – 224, coverage 0.933). Despite differences in precision, estimates were not significantly different (t = 2.55, *d.f.* = 4, $p > 0.05$). A noteworthy observation is that they were all significantly lower than 500 (one sample t = - 27.05, *d.f.* = 5, $p < 0.01$), which often used as a threshold value for determining long-term genetic viability (Allendorf and Luikart 2007). When the combined N_e estimate is multiplied by a six years generation time we obtained an approximate generational N_e of 804 (95% CI 594 – 1344). Using VORTEX N_{init} of 3670, we obtain a N_e/N_c ratio of 0.16 (CI 0.22 - 0.37).

3.2 Demographic-based N_e estimates

Final heterozygosity estimates for all 45 scenarios ranged from 0.9601 to 0.9806 (baseline [*b*] of 0.9701) and resulted in N_e estimates of approximately 450 to 2500 (N_{eb} of 550 - 1650), conditioned on *G* (Table S1). These estimates were significantly higher than genetic-based estimates (ANOVA $F = 809.57$, $p < 0.01$). Furthermore, N_e estimates based on different G values differed significantly amongst themselves (ANOVA $F = 529.37$, $p < 0.01$), with smaller G associated with larger N_e estimates (Table S1). This highlights the importance of considering multiple G estimates when estimating N_e even for the same population. Nevertheless, these were highly correlated ($r = 95\%$, $p < 0.01$; Table 2).

3.2.1 Sensitivity of viability analysis to perturbations in vital rates and uncertainties in population parameters

The sensitivity of H_{exp}, N_e, N_c, λ, SR and G to perturbations in survival rates, proportion of breeders, N_{init} and K indicate their susceptibility to various sources of uncertainties and or conservation actions. In general, most perturbations resulted in H_{exp}, N_e and N_c estimates higher by 50% relative to baseline values, except for λ (34%) and G (14%) (Table 2, Fig. 1).

H_{exp} was on average 0.9722 ± 0.0039 with a 40% and 10% decline in the proportion of breeding females determining its range (0.9601 – 0.9805). It decreased with a 40% and 20% reduction in the proportion of males breeding and N_{init}, respectively. H_{exp} increased with declines of at least 40% in K and with a simultaneous decline of 20% in adult female mortality and non-adults (irrespective of gender) (Fig. 1a). In turn, Ne increased in direct proportion to increases on adult survival, particularly that of females (Fig. 1b, c, d; Table S2). For instance, increases of 10%, 20% and 40% in adult female survival resulted in an increase of 8%, 14% and 22% in N_e, respectively, versus increases of only two, three and six percent due to increases on male survival. N_e was also increased with shorter G (i.e. 1810 ± 268 inds, G = 2.4 yrs; 876 ± 112 inds, G = 4.05 yrs; 680 ± 100 inds, G = 6 yrs) (Table S2).

Even though H_{exp} and N_e are directly linked differences in the magnitude of perturbations of three scenarios resulted in contrasting effects (Fig. 1a. b, c, d). First, a reduction in the proportion of breeding females of 10% yielded an increase of 1.07% and 54% on H_{expb} and N_{eb} respectively, while a reduction of 20% caused these values to decrease by at least 0.42 and 14%, respectively. Second, a reduction in K of 40% (K = 2202) increased N_e, while a decline of 20% (K = 2936) deflated N_e. Third, a 20% decrease in N_{init} (N_{init} =2936) increased H_{expb} and N_{eb} by 0.83% or 19%, respectively, while a decrease of 40% (N_{init} = 2202) caused H_{expb} and N_{eb} to decline by -0.9% and -3%, respectively. Overall, H_{exp} and N_e were most sensitive to perturbations on the proportion of breeders, followed by uncertainties in N_{init}, and or K and moderate concurrent increases of adult female survival and that of non-adults.

N_c values ranged from 10 to 3550 individuals (N_{cb} = 790) and it was positively influenced by 10% decreases on the N_{init}, the proportion of female reproducing and the survival rates for all female age classes and that of adult males (Fig. 1e, Table S2). The latter, had a lesser impact than declines on female (Table S2). On the contrary, N_c declined with at least a 20% reduction in the proportion of female breeders and K and with a 40% ≤ reduction in N_{init}, similar to H_{exp} and N_e. Furthermore, increases of 10% to 40% in non-adult male mortality also affected N_c negatively (Fig. 1e).

A severe skewed adult female SR was prevalent in all but two scenarios (0.38 ± 0.06, excluding an outlier of 0.99) (Fig. S1g). Only moderate and or high declines in male cubs and adults, respectively caused *SR* to ~ 1:1 (male: female). Likewise, declines of 20% in the proportion of females breeding or 80% in adult female survival also reduced *SR* to 0.6:1. Otherwise, perturbations in female survival only accentuated this bias whereas those involving male survival had the opposite effect.

The population was self-sustaining λ_b = 1.10 (1.12 ± 0.06, range 0.97 - 1.28) with λ positively influenced by increases in adult female survival rates, concurrently or not with that of non-adults. On the other hand, λ declined with decreases in the proportion of breeding females while 26 scenarios mostly those involving increases on male survival rates, declines on N_{init} or *K* had no influence on λ (i.e. λ_b = 1.10).

G was on average 4.16 ± 0.23 years (G_b = 4.05, range 3.98 - 5.15 yrs) (Fig. 1i). Increases in females' survival rates reduced G_b by -2 to -0.2% but it increased with moderate and high declines in adult mortality by 0.4% to 27.3%. Furthermore, G_b increased with a concurrent moderate decreases in female and male cub (~ 6%) (Fig. 1i). *G* was unaffected by increases in non-adult males, and declines in the proportion of males breeding, N_{init}, *K* and moderate declines in combined adult survival.

N_e/N_c ratio among all N_e's ranged from 0.21 and 3.75, with 64%, 93% and 27% of N_{e1} and N_{e3} (n = 44), and N_5/N_c (n = 43) < 1, respectively (Fig. 2, Table S2). Range bounds were primarily the result of a 20% reduction on the proportion of females breeding and a high (80%) decline of adult male mortality. By excluding these we observed narrower ranges (N_{e1}, N_{e3} and N_5/N_c of 0.27 - 1.21, 0.21 - 0.82 and 0.64 - 2.45, respectively). In addition, to N_e/N_c ratios varying with *G* used to determine N_e with N_{e5} (G = 2.4 yrs)/N_c > N_{e1} (4.05 yrs)/N_c or N_{e3} (6 yrs)/N_c, these also vary based on gender-specific survival rates. Scenarios involving reductions in female survival simultaneously or not with that of cubs were predominantly less than 0.5 while above 0.5 for those involving male survival rates (Fig. 2). It is noted that N_e/N_{adults} had comparable pattern as N_e/N_c (ranged from 0.40 to 7.12 with N_{e1}, N_{e3} and N_5/N_{adults} being ≥ 1 by 70%, 55% and 100%, respectively).

3.2.2 Relationship among N_e, Nc, λ, SR and G

N_e's correlated positively and significantly with H_{exp}, N_c, N_{adult}, G, λ but not with SR (Table 2). Furthermore, correlations between H_{exp} and N_c, N_{adult} or G, G and λ, λ and N_c, were also significant (Table 2). A correlation between SR and λ was negative but significant. In general, even though high N_e estimates were associated with high N_c this was not always when G, SR and λ were their highest (Fig. 2).

4. Discussion

The study findings indicate that the population is likely to be genetically and demographically viable, even though one-sample genetic estimates were below the recommended viability threshold value of 500 (Allendorf and Luikart 2007). Sensitivity analyses indicate that this viability is susceptible to moderate declines in the survival of single adult females or those accompanied by cubs, as well as low declines in the proportion of females breeding. In addition, moderate or high levels in uncertainty in population size (N_{init}) or carrying capacity (K) influence the viability estimate in itself. These factors also influenced the census size (N_c), growth rate (λ) and mean generation time (G), but the magnitude and direction of influence differed among these variables. Hence, even though the influence of certain conservation actions on N_e and N_c or N_{adult} may be positive, others may have an opposite effect, causing N_e/N_c ratio to fluctuate. Nevertheless, in order to maximize the evolutionary potential of this population, conservation actions should concentrate on aspects primarily related to females.

Below, we argue that the population is likely viable, even though genetic N_e estimates were significantly below the often recommended threshold value. Second, that underlying processes and nonlinear response of demographic parameters, along with their interaction with life history traits, variance in family size and longevity, explains the sensitivity of viability estimates to perturbations.

4.1 Genetic and demographic Ne estimates

The observed genetic estimates were significantly lower than demographic ones and than 500, a result that might indicate that the population is not viable in the long term, due to limited evolutionary potential. However, due to the violations of some of the genetic estimators' underlying assumptions, these N_e estimates may be downward biased. First, the discrete generation assumption was violated, as cheetahs have overlapping generations. LDNe is susceptible to this violation (Waples and Do 2010) often introduces a negative bias (Palstra and Ruzzante 2008; Waples 2010). The bias originates from differences in individual and cohort lifetime reproductive success (Jorde and Ryman 1995; Engen et al. 2005; Engen et al. 2007; Waples 2010) that are functions of age-specific survival and birth rates (Jorde and Ryman 1995; Saether et al. 2009). In addition, because generational N_e is conditioned on lifetime reproductive success, N_e estimates may represent the parental cohort(s) of the sample or an intermediate value between this and N_e (Waples 2005; Waples and Do 2010). In this study, the congruence of genetic estimates with demographic estimates, after having been multiplied by generation times, supports this relation. However, this conjecture requires further assessment

(Waples and Do 2010; Skrbinsek et al. 2011). Nevertheless, unless estimators account for these sources of variance, N_e estimates will be biased.

A second possible cause of negative bias is the small sample sizes (*S*), particularly relative to the number of cohorts in the dataset. As alluded, the latter introduces LD which is accentuated if *S* is low, as only small fractions of the progeny of different cohorts are likely to have been sampled (Luikart et al. 2010). Moreover, this violates the assumption that genetic drift was the only cause of allelic variance in the data set (Waples 2006; Tallmon et al. 2008). Furthermore, unequal sample sizes across loci may increase sampling noise causing N_e to deflate as some of this noise is treated as drift *(*Peel et al. 2013). Indeed, the LDNe 95% CI ranged from 624 to infinity based on 88 inds and 36 loci (*data not shown*).

Lastly, LDNe is susceptible to fluctuations in N_c (Vucetich and Waite 1996; Waples 2006; Luikart et al. 2010) and, given that the study population has been neither stable nor closed over ecological timescales, these likely have influenced N_e. The population has declined during the past century due to various reasons including droughts, prey reduction, habitat loss and human wildlife conflict (HWC) (Marker-Kraus et al. 1996; Nowell 1996; Marker et al. 2007). For instance, like selective harvesting (Harris et al. 2002; Milner et al. 2007), HWC possibly affected N_e directly (e.g. removals of adults, primarily males) or indirectly (e.g. removal of females accompanied by cubs) (Marker et al. 2003). Sensitivity analysis results support this conclusion, as N_e was reduced with a 40% decline in N_{init} (2200 vs. 3670) and in the proportion of breeding individuals (Fig. 1a). As LD can persist for a few generations (Luikart et al. 2010; Waples 2006) N_e may reflect periods prior to the 1990's, when N_e could have been lower due consistently high removal rates (Marker-Kraus et al. 1996; Nowell 1996). Noteworthy it is that, if we consider the suggestion that to obtain accurate genetic estimates, at least 10% of the true N_e needs to be sampled so as to maximize S/N_e (Palstra and Ruzzante 2008; Tallmon et al. 2010), we obtain an S/N_e > 10% (i.e. 90/804) based on "corrected" genetic estimates. Hence, even though estimates may be downward biased, these may reflect periods when N_e was lower, as corroborated by sensitivity analyses.

On the contrary, we consider demographic estimates as more accurate, as VORTEX accounts for all WF assumptions except for migration (in the case of our simulated scenarios), when computing heterozygosity. Annual and lifetime mean (and variances) in reproductive success are accounted for through the tracking of each individual's life events, according to probability distributions, parameters that determine generational N_e (Jorde and Ryman 1995). As such, we considered the population to be viable, as supported by sensitivity analyses, which also provided insights into factors influencing this viability as well as N_c, and thus N_e/N_c. It should be noticed that VORTEX assumes that reproductive and survival probabilities of adults are age-independent, likely affecting lifetime reproductive success and demographic variance (Saether et al. 2009; Lee et al. 2011), which have an impact on N_e estimates.

4.2. Sensitivity to perturbations on vital rates

The long-term population viability (as assessed by N_e) appears to be conditioned on the prevailing environmental conditions and levels of perturbations that can either have a positive or negative effect. Factors that influenced Ne positively include those related to females, in particular the proportion of breeders and the survival rates of all age classes. This implies that females represent the main breeding potential of the population, and that removal of males may have limited effect on N_e (Levitan

2005; Desbiez et al. 2012 but see Rankin and Kokko 2007). This is supported by the sensitivity analysis results of self-sustaining populations ($\lambda \geq 1$) as well as a skewed female ratio. Furthermore, the lower impact on N_e of increases in male survival, and the negligible impacts of increases in the proportion of males breeding, supports this conclusion (Fig 1a, b, c). These findings are in agreement with previous PVA studies on the species (Berry et al. 1996; Crooks et al. 1998; Kery and Durant, 2000; Lubben et al. 2008; Lindsey et al. 2009), on other felids such as the *Panthera tigris* (Karanth and Stith 1999; Chapron et al. 2008) and *Panthera onca* (Eizirik et al. 2002; Desbiez et al. 2012). However, mate availability may become a limiting factor due to the apparent female preference for unrelated males (Gotelli et al. 2007; Milner et al. 2007).

The relationship between N_e and females in cheetahs can be attributed to different causes. First, the increase in the proportion of female breeders, or longer female lifespans, may translate into an increase in the number of potential reproductive attempts, which can result on an even or uneven reproductive variance causing N_e to increase or not. This is of significance in cheetahs for their ability to return to an estrous state shortly after losing a litter (Caro 1994), and being reproductively active year-round (Marker et al. 2003; Wachter et al. 2011). Second, N_e increases under a polygynous mating system (Clutton-Brock 1988; Nunney et al. 1993, 1996; Storz et al. 2001a), which is possibly reinforced if combined with polyandry (Pearse and Anderson 2009; Hess et al. 2012) and female selection for unrelated males (Gotelli et al. 2007). These mating systems are prevalent in the Serengeti cheetah population (Gotelli et al. 2007), and we do not expect the Namibian system to be very dissimilar.

In addition to the mating system, the relationship between breeding status and time of removal (e.g. due to HWC) also explains the lesser impact on N_e resultant from increases in male and non-breeder survival rates relative to that of females. First, the gametic pool narrows and broadens accordingly if animals are removed prior or post breeding, a pattern that can increase or reduce the reproductive variance (Nunney 1993). Following this logic, the high adult turnover rates due to indiscriminate removals, or the occupancy of vacant home ranges by relocated (Marker et al. 2008) or transients individuals (Chapter IV) may lead to a reduction in male variance (Storz et al. 2001a; 2001b). Observations that, in the absence of dominant males, non-dominant males breed earlier substantiate this conclusion (Milner et al. 2007; Archie and Chiyo 2012). Thus, continuous removals may buffer N_e from reducing severely across generations provided enough surplus individuals are available. Second, removals of prime adults prior to breeding, as well as that of non-adults, affect N_e indirectly through the loss of unique alleles, by inducing gender-specific survival rates and skewed age-class sex ratios (Nunney 1993; Lynch and Walsh 1998; Sæther et al. 2009). Additionally, a high cub or juvenile mortality affects reproductive success, while adult removals influence the demographic variance, both of which can cause a reduction on N_e (Lee et al. 2011).

The contrasting effects due to perturbation levels in N_e or H_{exp} and N_c suggest the existence of threshold values beyond which perturbations may have no or unintended impacts (Milner et al. 2007). The positive and negative impacts on N_e due to 10% *vs*. 20% decrease in the proportion of breeding females, or 20% *vs.* 40% decline in N_{init}, or 40% *vs.* 20% increase in K, support this conclusion. This suggests that the long-term viability of a population, after accounting for all vital rates, is conditioned on the magnitude of differences between parameters (N_{init} *vs*. K) as well as on the composition of N_c (adults *vs.* cubs or juveniles), when low. For the Namibian cheetah and possibly other populations whose K is determined largely by social tolerance (Turpie et al. 2010), the high reproductive system (e.g. high reproduction and large litter sizes), may be fundamental in increasing or maintaining N_e above 500. Furthermore, these contrasting responses are indicative of non-linear relationships that tend to be prevalent during periods of instability (Stott et al. 2012). In other words, conservation actions may affect multiple demographic parameters concurrently or not in a positive, negative or dissimilar form. Andrello et al. (2012) reached a similar conclusion when assessing the effects of vital rates on population growth and N_e for the *Dracocephalum austriacum* iteroporous plant.

4.2.1 Relationship between N_e and N_{adults}/N_c

As expected, we observed a positive relationship between N_e and N_c, implying higher degree of genetic drift when N_c is low. However, this relationship is not simple and varies as parameters fluctuate (Luikart et al. 2010). Ficetola, Wang, and Garner (2009) showed that in frogs (*Rana latastei*), N_e/N_c decreased as N_c increased due to a concurrent increase in polygyny or a increase in male reproductive success variance. Miller et al. (2009) also showed an increase in loss of genetic diversity due to greater male reproductive skew at low density. This study showed that the magnitude of increase in N_e and N_c due to perturbations in female and male survival rates ratios can also explain variations in N_e/N_c ratio. For instance, the greater percentile impact in N_c than in N_e resulting from increases in female survival maintained the N_e/N_c below 0.5, whereas perturbations in male survival had the opposite effect, $N_e/N_c \geq 0.5$ as N_e increased (Fig. 2). Likewise, as G affects N_e, it also affects this ratio. Contrary to the positive relation, N_{adults}/N_c and N_c were negative related; a finding interpreted as genetic compensation (Palstra and Ruzzante 2008;

Ficetola et al. 2009). However, the validity of this on cheetahs requires further long-term population genetic studies. Moreover, our N_e/N_c (0.21) or N_e/N_{adult} (0.40) ratios were in agreement with the median values of 0.231 and 0.225 (Palstra and Fraser 2012) and theoretical expectations of 0.25 to 0.75 (Nunney 1993, 1996).

4.3. Comparison with previous estimates

The N_e estimates obtained in this study were considerably larger than the demographic-based estimates of the Serengeti cheetah population (Creel 1998; Kelly 2001). These differences can be explained by the Serengeti's low *Nc* (200 to 250), methodological differences and an incomplete account of reproductive variance due to the prevalent polyandry breeding system. Creel (1998) estimated N_e of 96 to 207 accounting for a female biased sex ratio and including transients. On the other hand, Kelly (2001) obtained $N_e < 50$ for all four estimators used with fluctuations in N_c and biased sex ratio deflating N_e. This is in agreement with our findings of N_e increased under scenarios where *SR* ~ 1:1 (e.g., 40% or 80% increase on adult male survival, Fig. S1g) and of simulations with 40% lower N_{init} induced greater variation among iterations. However, the exclusion of transients by Kelly (2001) may have resulted in greater male variance essentially because of females high infidelity (Gotelli et al. 2007) causing the greater impact of *SR* on N_e. The same logic applies to our study, as VORTEX does not model a polygyny polyandry mating system, as it is the case for the Serengeti populations (Gotelli et al. 2007). This suggests that our estimates could be underestimated. Contrary to ours and Kelly (2001), Creel (1998) failed to detect a major effect on N_e due to fluctuations on N_c or *SR*. Both the inclusion of transients and fewer N_c counts can possibly explain differences among the studies. Vucetich and Waite (1998) shows that the number of N_c counts to have a major influence on the impact on N_e. Overall, factors influencing

N_e across studies include variance in reproductive success and fluctuations in Nc and SR.

4.4. Study limitations

Although our findings were consistent with the literature (e.g. Nunney 1993) vital rates standard deviations may be overestimated due to small sample sizes. Furthermore, even though immigration patterns due to connectivity with neighboring populations could have influenced estimates, rates may be bi-directional and likely to be above 5 to 10%, a range to which estimates derived using LDNe and possibly ONeSAMP are robust to (Waples and England 2011). Furthermore, metapopulation studies assessing the influence of immigration on N_e estimates have reached opposing conclusion. For example, while Baalsrud (2011) found immigration to affect N_e estimates the most in a house sparrow, Gomez-Uchida et al. (2013) reached a dissimilar conclusion on salmonids. Despite these caveats, our results seem to be robust to a wide variety of perturbations, and provide a foundation for additional studies that incorporate N_e values and/or further pursue improved estimates of this parameter for cheetahs.

4.4. Conservation implication and conclusion

This study has demonstrated, through extensive sensitivity analyses, that the Namibian cheetah population's N_e is likely to be at least 500 individuals, with N_e/N_c or $N_e\ /N_{adults}$ ratios of at least 0.21 or 0.40, respectively. In addition, it showed that conservation actions might affect genetic and demographic parameters similarly or differently, but that factors increasing N_e also have a positive effect on N_c. These conservation actions should continue to focus primarily on increasing the survival of young and adult females (i.e. release back into the wild of females with cubs caught due to conflict). However, there seem to be threshold values beyond which conservation actions may have adverse impacts on the population genetic diversity and thus long-term evolutionary potential. As the population is part of a broader Southern Africa population, ensuring continuous gene flow among regional populations is vital. Future modeling studies should also incorporate this aspect of metapopulation structure and spatially complex connectivity, which should allow an even more realistic assessment of long-term evolutionary potential, and perhaps a relevant system with which to compare genetic and demographic estimators of N_e.

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Figures legends

Figure. 1 Percentage of change in (a) heterozgosity (Het), (b) effective population size (N_e) based on a mean generation of 4.05 years, (c) 6 years and (d) 2 years, (e) total (N_c) and (f) adult (N_{adults}) population sizes, (g) N_c sex ratio, (h) lambda and (i) mean generation time (4.05, derived by VORTEX) to pertubations in demographic parameters. Pertubations levels are presented in brackets and ranged from 10, 20, 40, 60 and 80%, with M = male and F = Female. Demographic parameters pertubated include: F/M0-1, F/1-2, F2, M2-3, M3 = increases in female cubs, juveniles/adolescents and prime adults survival rates; F2 F0-1 M0-1, F2 F0-1 M0-1 EV, F2 F1-2 M2-3 = concurrent increases in adult female, cubs survival rates and environmental variation or without the latter, and adult females with juveniles/adolescents; M0-1, M1-2, M2-3, M3 = increases in male cub, juveniles, adolescents and adult; F2 M3 = increase in adult survivals; N_{init} or K = reductions in the initial population size and carrying capacity estimates; ReproF/M = reductions in the proportions of breeding females or males. Age classes follow Marker et al. (2003): 0-1 = cubs (< 12 months), 1 = adolescents and newly independent, for females only (12-30 months), 2 = adult for females and newly independent for males, and 3 = adult males. For instance, a simultaneous increase in adult females and cubs survival rates (i.e. F2M0-1F0-1) of 10 or 20% caused Het to increase by 42% and 66% (Fig. 1a) and N_e by 16% and 24% (Fig. 1b - d).

Figure. 2 Ratios between the effective population size (determined using generation times of 4.05 (N_{e1}), 6 (N_{e3}) and 2 (N_{e5}) years) and the total (N_{census}) or adult (N_{adults}) population size estimates based on VORTEX simulations, and their variation as a function of perturbations in input demographic parameters. Legends are as in Table 2 and Figure 1.

Table 1. Summary of baseline scenario input parameters.

Parameter	Estimate (standard deviation)
Initial population and Carrying capacity	3670*
Reproductive system (polygyny)	
Age of first offspring for males	2 years [b]
Age of first offspring for females	3 years [b]
Maximum age of reproduction	12 years [b]
Maximum number of progeny per year	1 litter of 6 cubs [b]
Sex ratio at birth	1:1 [b]
Reproductive rates	
Proportion of breeding females	55.4 (± 11%) [b, *]
Percent males in breeding pool	62*
Distribution of number of cubs per female per litter (1, 2, 3, 4, 5)	6%, 24%, 42%, 24%, 3%*
Annual mortality rates (Males :Females)	
0 – 1 (cubs)	39 (30); 30 (8)*
1 – 2 (large, adolescents)	13 (8); 22 (8)*
2 – 3 (prime and breeding females and prime adults males)	38 (4); 30 (12)*
> 3 (breeding males)	42 (10)*
Harvesting*	
First year of harvest	1
Last year of harvest	200
Interval between harvests	1
Number of males and females of Age 1 harvested	13
Number of males and females of Age 2 harvested	11
Number of adult males and females of Age 3 or more	39:13

* this study; [b] Marker et al. (2003), Bisset and Bernard (2011)

Table 2. Correlation matrix among effective population size (N_{e1}, $N_{e2,3,4}$), expected heterozygosity (H_{exp}), mean generation time (G), lambda and total (N_{census}) and adult (N_{adult}) population sizes and sex ratios ($N_{censusSR}$ and $N_{adultSR}$).

Parameter	$N_{e2,3,4}$	H_{exp}	G	$N_{adultSR}$	*Lambda*	N_{census}	N_{adult}	$N_{censusSR}$
N_{e1}	0.977**	0.719**	0.270**	-0.09	0.296**	0.769**	0.810**	-0.075
$N_{e2,3,4}$		0.737**	0.414**	-0.04	0.279**	0.730**	0.781**	-0.029
Hexp			0.332**	0.08	0.120	0.493**	0.539**	0.100
Mean G				0.00	.295**	0.093	0.141	0.043
$N_{adultSR}$					-.790**	-0.437	-0.413	0.919**
Lambda						0.479**	0.481**	-0.743
N_{census}							0.991**	-0.435
N_{adult}								-0.413

**correlation is significant at the 0.01 level (2-tailed)

Fig. 1a

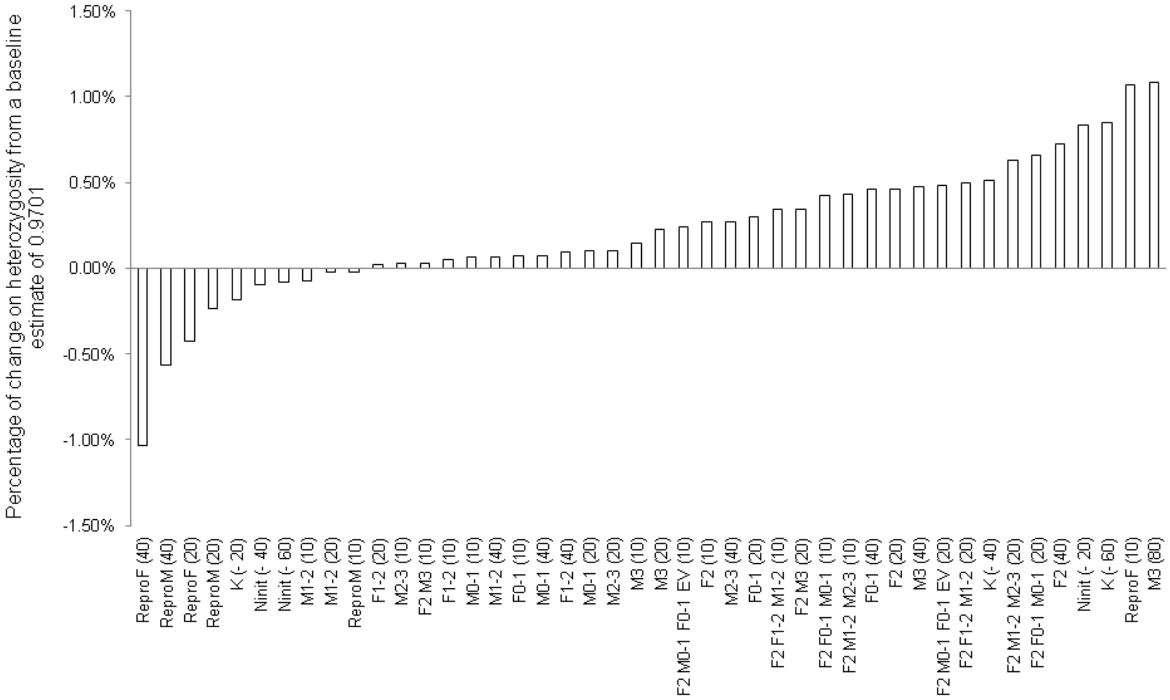

Percentage of change on heterozygosity from a baseline estimate of 0.9701
1.50%
1.00%
0.50%
0.00%
-0.50%
-1.00%
-1.50%
ReproF (40)
ReproM (40)
ReproF (20)
ReproM (20)
K (- 20)
Ninit (- 40)
Ninit (- 60)
M1-2 (10)
M1-2 (20)
ReproM (10)
F1-2 (20)
M2-3 (10)
F2 M3 (10)
F1-2 (10)
M0-1 (10)
M1-2 (40)
F0-1 (10)
M0-1 (40)
F1-2 (40)
M0-1 (20)
M2-3 (20)
M3 (10)
M3 (20)
F2 M0-1 F0-1 EV (10)
F2 (10)
M2-3 (40)
F0-1 (20)
F2 F1-2 M1-2 (10)
F2 M3 (20)
F2 F0-1 M0-1 (10)
F2 M1-2 M2-3 (10)
F0-1 (40)
F2 (20)
M3 (40)
F2 M0-1 F0-1 EV (20)
F2 F1-2 M1-2 (20)
K (- 40)
F2 M1-2 M2-3 (20)
F2 F0-1 M0-1 (20)
F2 (40)
Ninit (- 20)
K (- 60)
ReproF (10)
M3 (80)

Fig. 1b

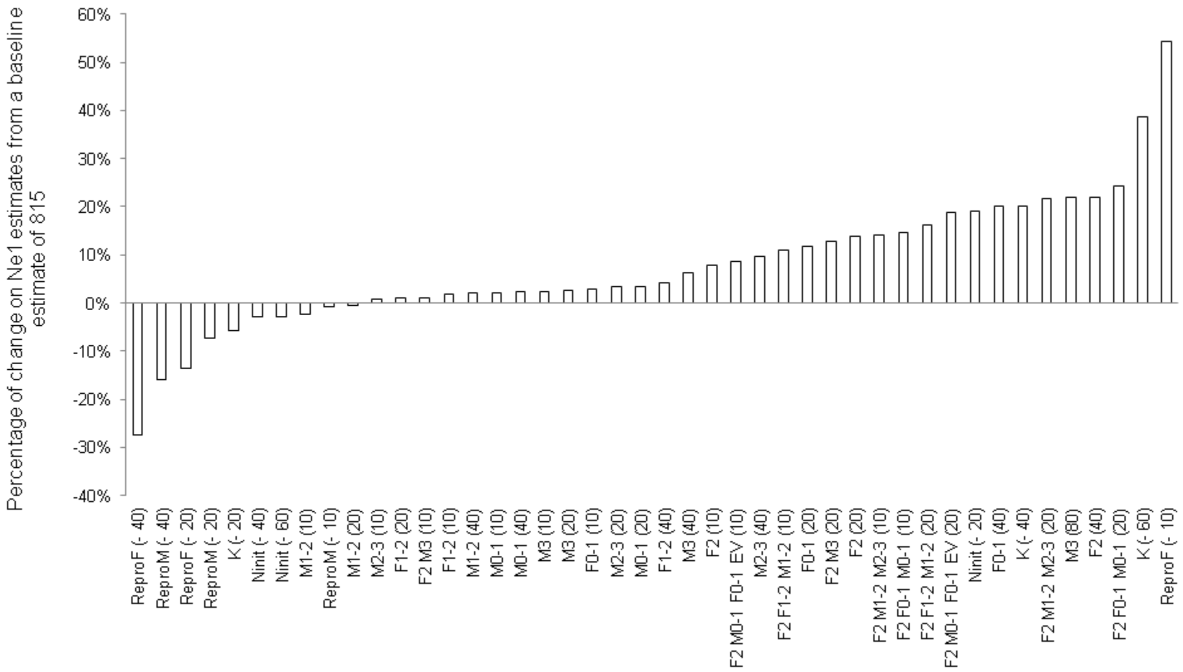

Percentage of change on Ne1 estimates from a baseline estimate of 815
60%
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
-40%
ReproF (- 40)
ReproM (- 40)
ReproF (- 20)
ReproM (- 20)
K (- 20)
Ninit (- 40)
Ninit (- 60)
M1-2 (10)
ReproM (- 10)
M1-2 (20)
M2-3 (10)
F1-2 (20)
F2 M3 (10)
F1-2 (10)
M1-2 (40)
M0-1 (10)
M0-1 (40)
M3 (10)
M3 (20)
F0-1 (10)
M2-3 (20)
M0-1 (20)
F1-2 (40)
M3 (40)
F2 (10)
F2 M0-1 F0-1 EV (10)
M2-3 (40)
F2 F1-2 M1-2 (10)
F0-1 (20)
F2 M3 (20)
F2 (20)
F2 M1-2 M2-3 (10)
F2 F0-1 M0-1 (10)
F2 F1-2 M1-2 (20)
F2 M0-1 F0-1 EV (20)
Ninit (- 20)
F0-1 (40)
K (- 40)
F2 M1-2 M2-3 (20)
M3 (80)
F2 (40)
F2 F0-1 M0-1 (20)
K (- 60)
ReproF (- 10)

Fig. 1c

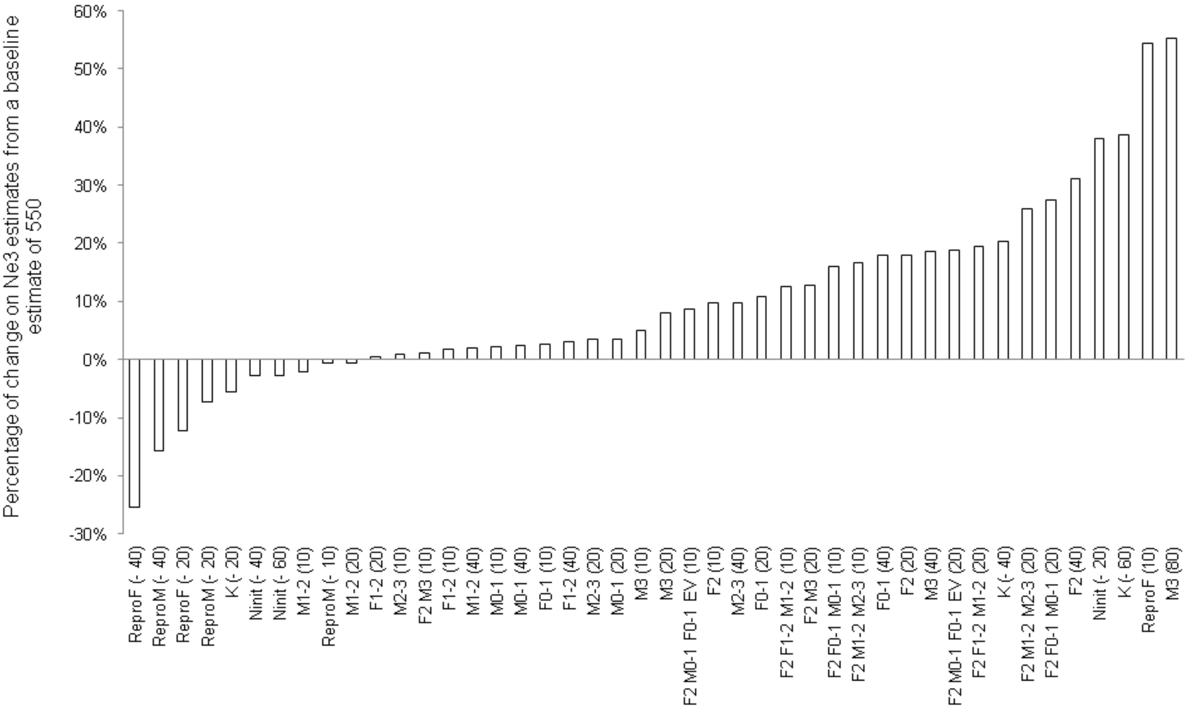

Percentage of change on Ne3 estimates from a baseline estimate of 550
60%
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
ReproF (- 40)
ReproM (- 40)
ReproF (- 20)
ReproM (- 20)
K (- 20)
Ninit (- 40)
Ninit (- 60)
M1-2 (10)
ReproM (- 10)
M1-2 (20)
F1-2 (20)
M2-3 (10)
F2 M3 (10)
F1-2 (10)
M1-2 (40)
M0-1 (10)
M0-1 (40)
F0-1 (10)
F1-2 (40)
M2-3 (20)
M0-1 (20)
M3 (10)
M3 (20)
F2 M0-1 F0-1 EV (10)
F2 (10)
M2-3 (40)
F0-1 (20)
F2 F1-2 M1-2 (10)
F2 M3 (20)
F2 F0-1 M0-1 (10)
F2 M1-2 M2-3 (10)
F0-1 (40)
F2 (20)
M3 (40)
F2 M0-1 F0-1 EV (20)
F2 F1-2 M1-2 (20)
K (- 40)
F2 M1-2 M2-3 (20)
F2 F0-1 M0-1 (20)
F2 (40)
Ninit (- 20)
K (- 60)
ReproF (10)
M3 (80)

Fig. 1d

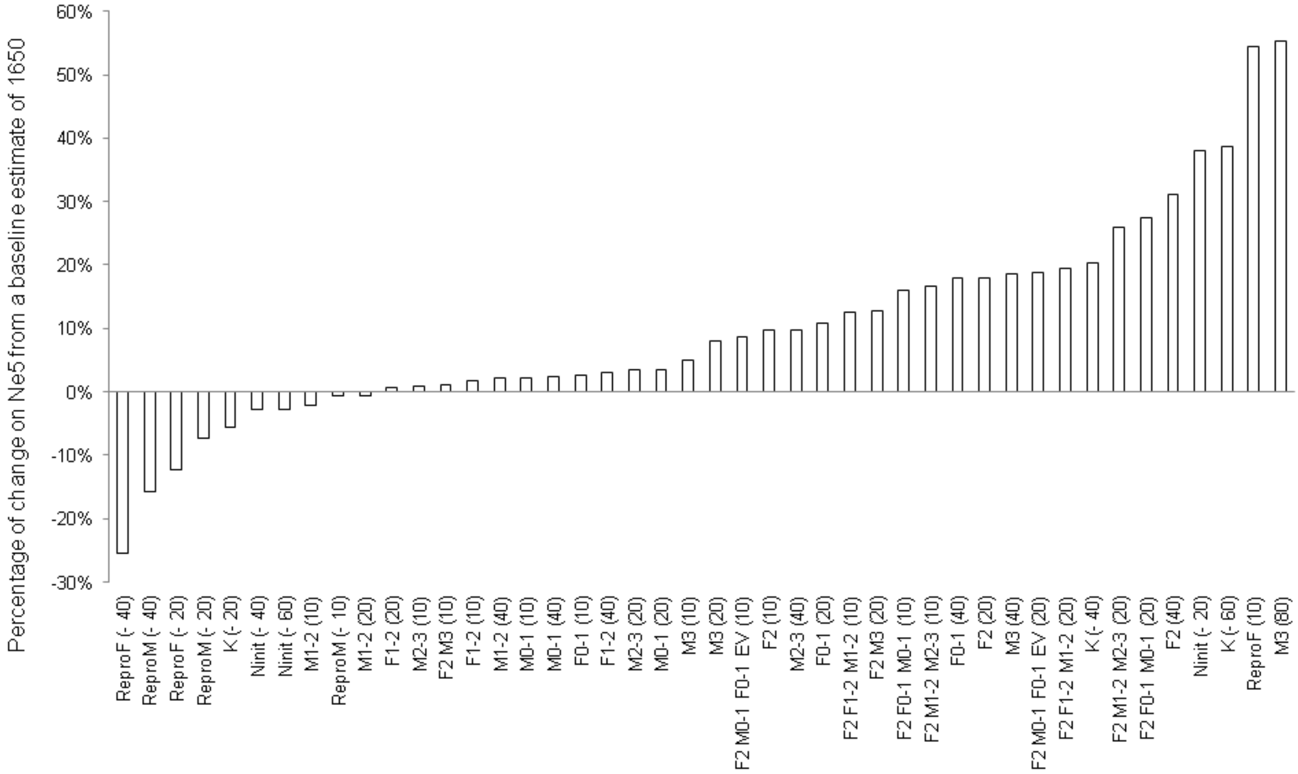

Percentage of change on Ne5 from a baseline estimate of 1650
60%
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
ReproF (- 40)
ReproM (- 40)
ReproF (- 20)
ReproM (- 20)
K (- 20)
Ninit (- 40)
Ninit (- 60)
M1-2 (10)
ReproM (- 10)
M1-2 (20)
F1-2 (20)
M2-3 (10)
F2 M3 (10)
F1-2 (10)
M1-2 (40)
M0-1 (10)
M0-1 (40)
F0-1 (10)
F1-2 (40)
M2-3 (20)
M0-1 (20)
M3 (10)
M3 (20)
F2 M0-1 F0-1 EV (10)
F2 (10)
M2-3 (40)
F0-1 (20)
F2 F1-2 M1-2 (10)
F2 M3 (20)
F2 F0-1 M0-1 (10)
F2 M1-2 M2-3 (10)
F0-1 (40)
F2 (20)
M3 (40)
F2 M0-1 F0-1 EV (20)
F2 F1-2 M1-2 (20)
K (- 40)
F2 M1-2 M2-3 (20)
F2 F0-1 M0-1 (20)
F2 (40)
Ninit (- 20)
K (- 60)
ReproF (10)
M3 (80)

Fig. 1e

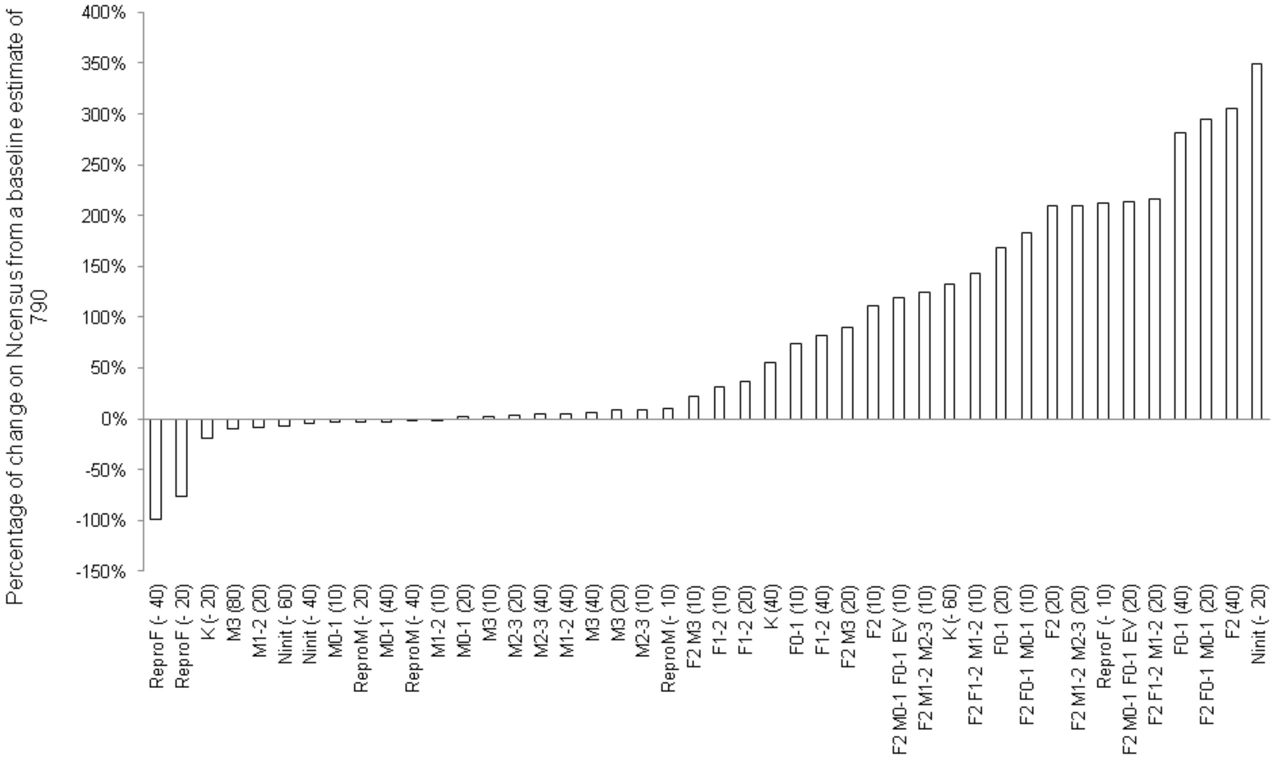

Percentage of change on Ncensus from a baseline estimate of 790
400%
350%
300%
250%
200%
150%
100%
50%
0%
-50%
-100%
-150%
ReproF (- 40)
ReproF (- 20)
K (- 20)
M3 (80)
M1-2 (20)
Ninit (- 60)
Ninit (- 40)
M0-1 (10)
ReproM (- 20)
M0-1 (40)
ReproM (- 40)
M1-2 (10)
M0-1 (20)
M3 (10)
M2-3 (20)
M2-3 (40)
M1-2 (40)
M3 (40)
M3 (20)
M2-3 (10)
ReproM (- 10)
F2 M3 (10)
F1-2 (10)
F1-2 (20)
K (40)
F0-1 (10)
F1-2 (40)
F2 M3 (20)
F2 (10)
F2 M0-1 F0-1 EV (10)
F2 M1-2 M2-3 (10)
K (- 60)
F2 F1-2 M1-2 (10)
F0-1 (20)
F2 F0-1 M0-1 (10)
F2 (20)
F2 M1-2 M2-3 (20)
ReproF (- 10)
F2 M0-1 F0-1 EV (20)
F2 F1-2 M1-2 (20)
F0-1 (40)
F2 F0-1 M0-1 (20)
F2 (40)
Ninit (- 20)

Fig. 1f

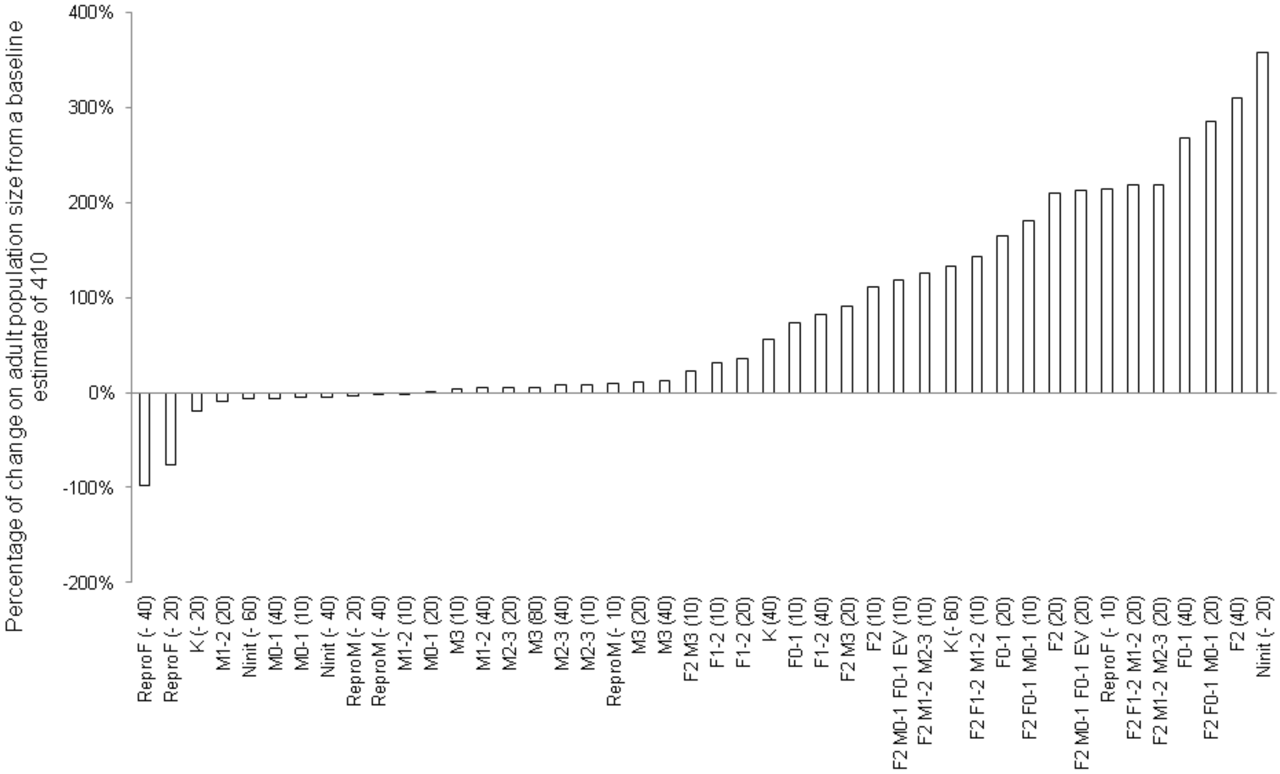

Percentage of change on adult population size from a baseline estimate of 410
400%
300%
200%
100%
0%
-100%
-200%
ReproF (- 40)
ReproF (- 20)
K (- 20)
M1-2 (20)
Ninit (- 60)
M0-1 (40)
M0-1 (10)
Ninit (- 40)
ReproM (- 20)
ReproM (- 40)
M1-2 (10)
M0-1 (20)
M3 (10)
M1-2 (40)
M2-3 (20)
M3 (80)
M2-3 (40)
M2-3 (10)
ReproM (- 10)
M3 (20)
M3 (40)
F2 M3 (10)
F1-2 (10)
F1-2 (20)
K (40)
F0-1 (10)
F1-2 (40)
F2 M3 (20)
F2 (10)
F2 M0-1 F0-1 EV (10)
F2 M1-2 M2-3 (10)
K (- 60)
F2 F1-2 M1-2 (10)
F0-1 (20)
F2 F0-1 M0-1 (10)
F2 (20)
F2 M0-1 F0-1 EV (20)
ReproF (- 10)
F2 F1-2 M1-2 (20)
F2 M1-2 M2-3 (20)
F0-1 (40)
F2 F0-1 M0-1 (20)
F2 (40)
Ninit (- 20)

Fig. 1g

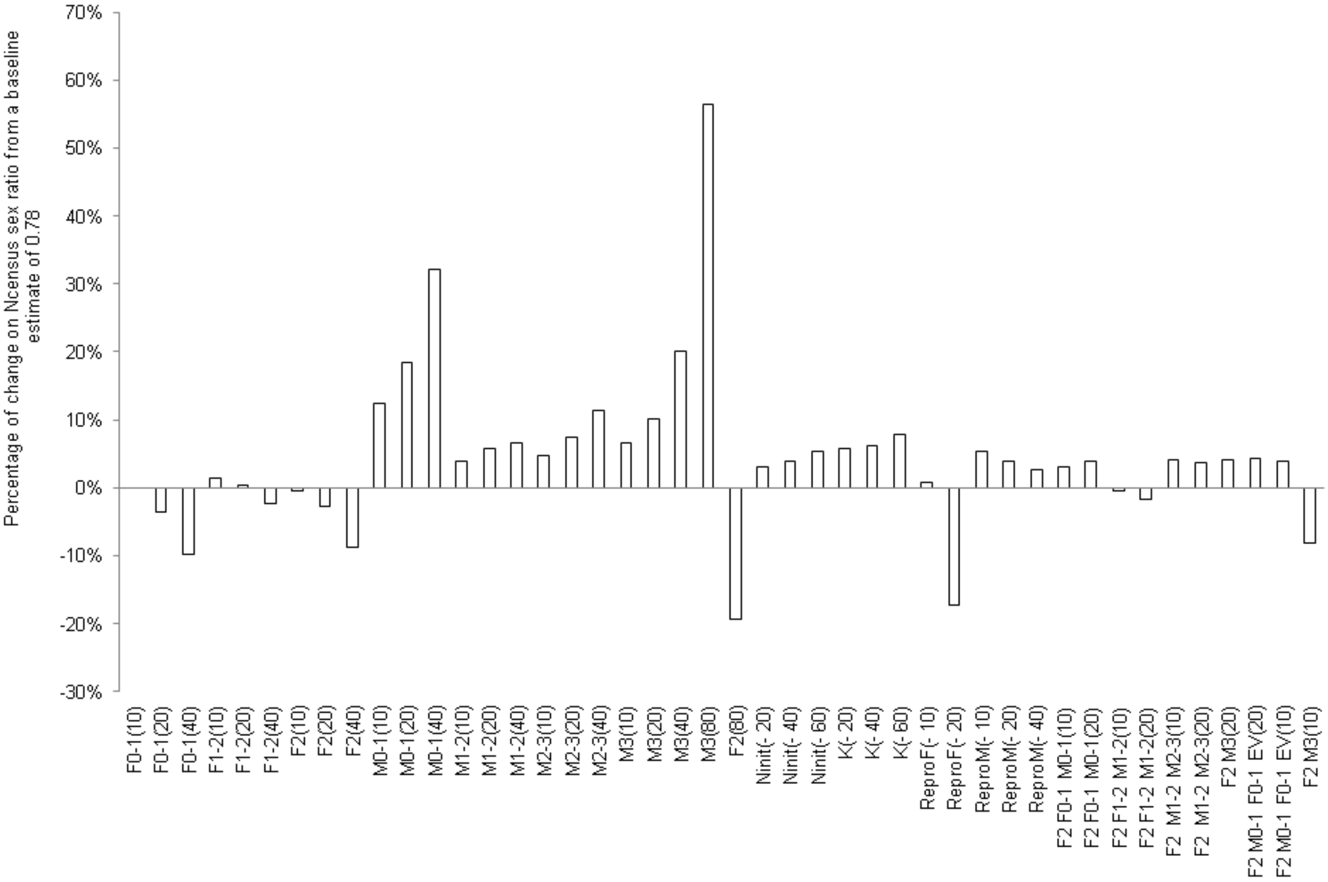

Percentage of change on Ncensus sex ratio from a baseline estimate of 0.78
70%
60%
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
F0-1(10)
F0-1(20)
F0-1(40)
F1-2(10)
F1-2(20)
F1-2(40)
F2(10)
F2(20)
F2(40)
M0-1(10)
M0-1(20)
M0-1(40)
M1-2(10)
M1-2(20)
M1-2(40)
M2-3(10)
M2-3(20)
M2-3(40)
M3(10)
M3(20)
M3(40)
M3(80)
F2(80)
Ninit(- 20)
Ninit(- 40)
Ninit(- 60)
K(- 20)
K(- 40)
K(- 60)
ReproF(- 10)
ReproF(- 20)
ReproM(- 10)
ReproM(- 20)
ReproM(- 40)
F2 F0-1 M0-1(10)
F2 F0-1 M0-1(20)
F2 F1-2 M1-2(10)
F2 F1-2 M1-2(20)
F2 M1-2 M2-3(10)
F2 M1-2 M2-3(20)
F2 M3(20)
F2 M0-1 F0-1 EV(20)
F2 M0-1 F0-1 EV(10)
F2 M3(10)

Fig. 1h

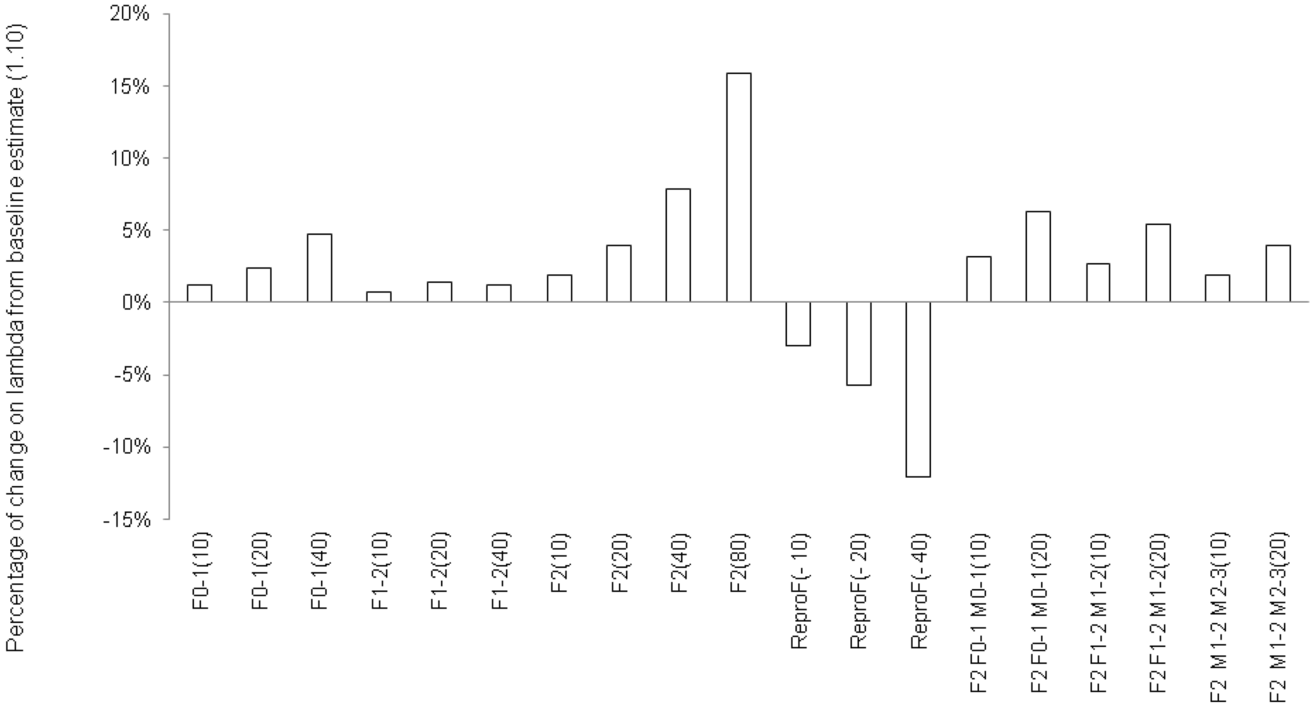

Percentage of change on lambda from baseline estimate (1.10)
20%
15%
10%
5%
0%
-5%
-10%
-15%
F0-1(10)
F0-1(20)
F0-1(40)
F1-2(10)
F1-2(20)
F1-2(40)
F2(10)
F2(20)
F2(40)
F2(80)
ReproF(- 10)
ReproF(- 20)
ReproF(- 40)
F2 F0-1 M0-1(10)
F2 F0-1 M0-1(20)
F2 F1-2 M1-2(10)
F2 F1-2 M1-2(20)
F2 M1-2 M2-3(10)
F2 M1-2 M2-3(20)

Fig. 1i

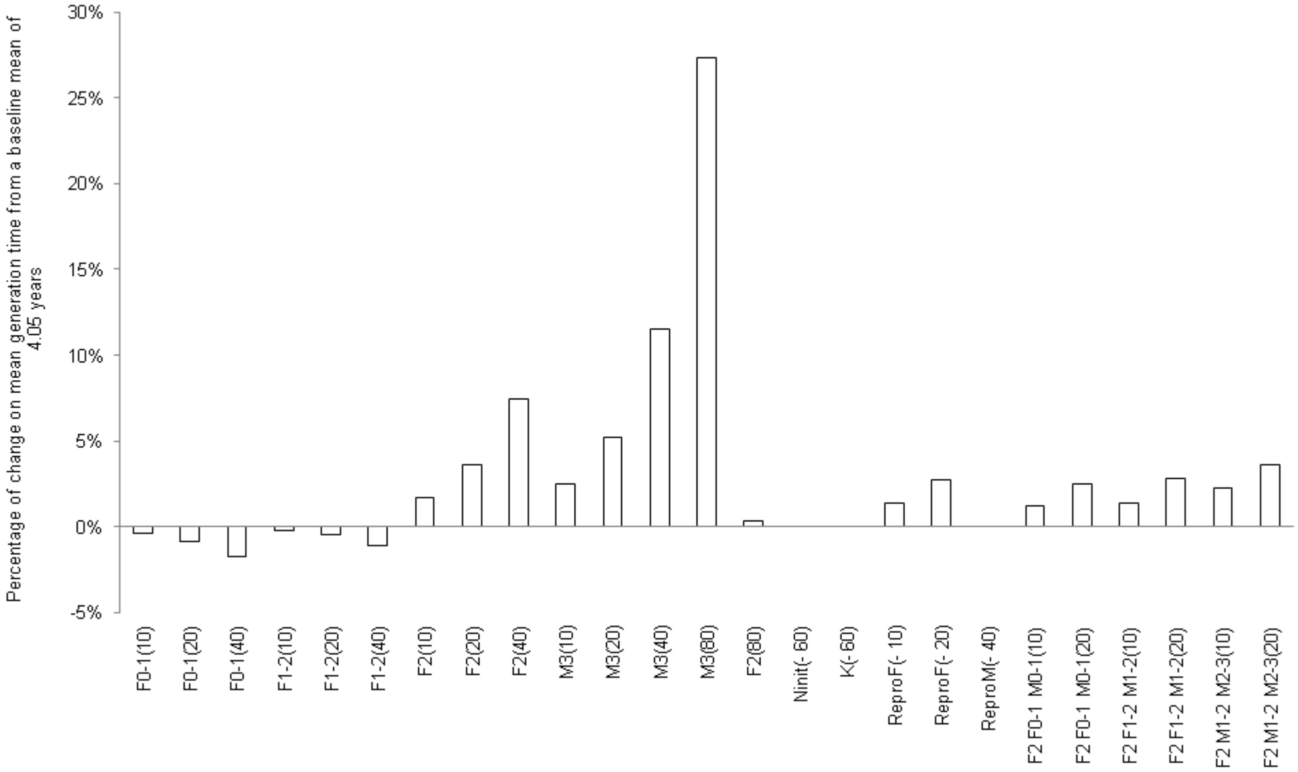
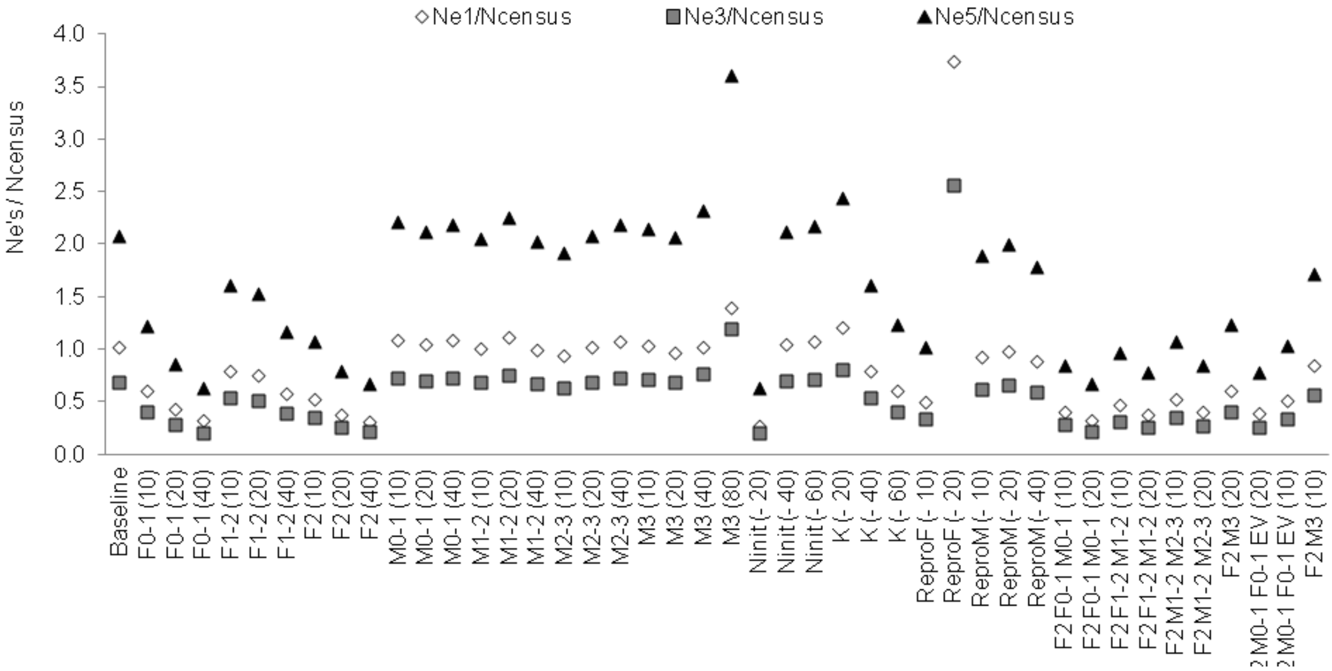

Percentage of change on mean generation time from a baseline mean of 4.05 years
30%
25%
20%
15%
10%
5%
0%
-5%
F0-1(10)
F0-1(20)
F0-1(40)
F1-2(10)
F1-2(20)
F1-2(40)
F2(10)
F2(20)
F2(40)
M3(10)
M3(20)
M3(40)
M3(80)
F2(80)
Ninit(- 60)
K(- 60)
ReproF(- 10)
ReproF(- 20)
ReproM(- 40)
F2 F0-1 M0-1(10)
F2 F0-1 M0-1(20)
F2 F1-2 M1-2(10)
F2 F1-2 M1-2(20)
F2 M1-2 M2-3(10)
F2 M1-2 M2-3(20)

Fig. 2


◇Ne1/Ncensus
■Ne3/Ncensus
▲Ne5/Ncensus
Ne's / Ncensus
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Baseline
F0-1 (10)
F0-1 (20)
F0-1 (40)
F1-2 (10)
F1-2 (20)
F1-2 (40)
F2 (10)
F2 (20)
F2 (40)
M0-1 (10)
M0-1 (20)
M0-1 (40)
M1-2 (10)
M1-2 (20)
M1-2 (40)
M2-3 (10)
M2-3 (20)
M2-3 (40)
M3 (10)
M3 (20)
M3 (40)
M3 (80)
Ninit (- 20)
Ninit (- 40)
Ninit (- 60)
K (- 20)
K (- 40)
K (- 60)
ReproF (- 10)
ReproF (- 20)
ReproM (- 10)
ReproM (- 20)
ReproM (- 40)
F2 F0-1 M0-1 (10)
F2 F0-1 M0-1 (20)
F2 F1-2 M1-2 (10)
F2 F1-2 M1-2 (20)
F2 M1-2 M2-3 (10)
F2 M1-2 M2-3 (20)
F2 M3 (20)
2 M0-1 F0-1 EV (20)
2 M0-1 F0-1 EV (10)
F2 M3 (10)

SUPPLEMENTARY: S1

1.1 Rationale for baseline scenario input parameters

Overall, we ran 45 models each for 500 iterations over 200 years. We assumed that extinction occurred when one sex remained and that the population is panmitic (Marker et al. 2008). We excluded *EV* concordance of reproduction and survival, as well as the occurrence of catastrophes, due to lack of information. This implies that decay in H_{exp} was mainly due to drift. A polygynous mating system was assumed as this parameter is unknown for this population and because VORTEX does not model polyandry, the mating system inferred to be most applicable to the Serengeti cheetah population (Gottelli et al. 2007). Moreover, polygyny is a highly prevalent mating system in carnivore species (Wolff and Macdonald 2004). Even though both genders are physiologically able to breed at 2 years of age (Bissett and Bernard 2011; Marker et al. 2003, CCF 2012, *unpublished data*), this was delayed to 3 years for males due to social constrains (Marker et al. 2003a). We assumed that genders remain reproductively active until the age of 12 years (Berry et al. 1997). The proportion of females breeding was set to 55%, with a female giving birth annually to a litter of 1 to 6 cubs, with 50% sex ratio (Lindsey et al. 2009). The distribution of litter sizes per dam was determined based on the number of female cheetahs accompanied by cubs captured by farmers during the 1991 to 2011 period (CCF 2012, *unpublished data*). In general, proportions of breeders are conservative and only approximations. Models assumed a stable population distribution and density-dependence was modelled as $N_{census} \leq K$. We excluded *EV* in *K* as we considered harvesting as a form of *EV* in *K* because *K* is largely determined by societal needs (Turpie et al. 2010).

1.2. Determination of environmental variation for mortality rates using the Stacey and Taper (1992) approach

We used a binomial distribution to determine environmental variation using p_{iy} (1-p_{iy})/N_{iy}, where $p_{iy} = m_{iy}/N_{iy}$ (m is the number of deaths, N the total number of individuals reported captured, i age class and y year). Thus, p is equivalent to annual mortality rates (these estimates were similar to those estimated through the BaSTA life table function). A weighted survival average was then determined as $X = \sum m_i/\sum N_i$, the total weighted variance as $\sigma_{tot} = \sum(N_i*(p_i-X)^2)/N_i$, demographic weighted variance as $\sigma_{dem} = \sum(p_{iy}(1-p_{iy})/N_{iy})/N_i$, the environmental variance as $\sigma_{env} = \sigma_{tot} - \sigma_{dem}$, and the standard deviation for environmental variation of survival as $SD\ env = \sqrt{\sigma_{env}}$. Because cheetahs breed between 2 and 12 years of age (Kelly et al. 1998, Marker et al. 2003, Bisset and Bernard 2011), a single SD_{env} was computed for all these age classes. Even though 87% of the 104 deaths were human-related, we considered the SD_{env} to be representative, as the interaction between the cheetah population and farmers has lasted for several generations.

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SUPPLEMENTARY S2

Table S1 Demographic-based estimates of cheetah effective population size (N_e) based on VORTEX simulations. The table includes the output of the heterozygosity (H_{et}) retained after 200 years (including its standard deviation [SD]), with N_e based on the different generation time applied: N_{e1} = 4.05 years VORTEX-derived, N_{e3} = 6 years (Marker and O'Brien 1989), and N_{e4} = 5.34 and N_{e5} = 2 years (Kelly, 2001). We also show estimates for the adult proportion (N_{adult}) and total population (N_c) after the simulations for the 45 modelled scenarios (500 iterations per scenario). Pertubations levels are presented in brackets and ranged from 10, 20, 40, 60 and 80%, with M = male and F = Female. Demographic parameters pertubated include: F/M0-1, F/M1-2, F2, M2-3, M3 = increases in female/male cubs, juveniles/adolescents and prime adults survival rates, respectively; F2 F0-1 M0-1 = concurrent increases in adult female and cubs survival rates; F2 F0-1 M0-1 EV = as before but includes environmental variation (EV); F2 F1-2 M2-3 = increase on adult females with juveniles/adolescents; M0-1, M1-2, M2-3, M3 = increases in male cub, juveniles, adolescents and adult, respectively; F2 M3 = increase in adult survivals; N_{init} or K = reductions in initial population size and carrying capacity estimates; reproduction females/males = reductions in the proportions of breeding females or males. Age classes follow Marker et al. (2003): 0-1 = cubs (< 12 months), 1 = adolescents and newly independent, for females only (12-30 months), 2 = adult for females and newly independent for males, and 3 = adult males. For example, a M3 (80) implies an increase in adult male survival of 80%; a N_{init} (-20) and K (-40) a 20% and 40% reduction in the initial population size and carrying capacity parameters, respectively.

Table S1

Scenario description: Gender, age class and % of change	*Het* ± *SD*	N_{e1} ± *SD*	N_{e3} ± *SD*	N_{e4} ± *SD*	N_{e5} ± *SD*	N_{adult} ± *SD*	N_{census} ± *SD*
Baseline	0.9701 ± 0.0004	814.65 ± 9.93	549.29 ± 6.69	617.15 ± 7.52	1647.36 ± 20.07	409.74 ± 9.35	789.53 ± 23.68
F0-1 (10)	0.9708 ± 0.0006	838.54 ± 17.53	563.3 ± 11.78	632.9 ± 13.23	1689.41 ± 35.33	708.8 ± 21.63	1373.24 ± 26.93
F0-1 (20)	0.973 ± 0.0003	910.2 ± 9.91	608.4 ± 6.63	683.56 ± 7.44	1824.7 ± 19.88	1082.63 ± 45.48	2115.08 ± 81.86
F0-1 (40)	0.9746 ± 0.0002	978.06 ± 7.8	648.05 ± 5.17	728.11 ± 5.81	1943.65 ± 15.5	1509.51 ± 34.15	3016.34 ± 59.23
F1-2 (10)	0.9706 ± 0.0007	830.76 ± 19.83	558.77 ± 13.34	627.8 ± 14.99	1675.8 ± 40.01	535.73 ± 35.59	1038.51 ± 74.16
F1-2 (20)	0.9703 ± 0.0003	823.35 ± 7.06	552.41 ± 4.74	620.66 ± 5.32	1656.74 ± 14.21	558.52 ± 39.52	1077.59 ± 70.86
F1-2 (40)	0.971 ± 0.0004	849.76 ± 10.67	566.59 ± 7.11	636.59 ± 7.99	1699.26 ± 21.34	745.27 ± 9.75	1440.1 ± 19.12
F2 (10)	0.9727 ± 0.0006	878.2 ± 20.17	602.38 ± 13.83	676.8 ± 15.55	1806.63 ± 41.51	862.72 ± 24.14	1672.41 ± 33.63
F2 (20)	0.9746 ± 0.0003	927.88 ± 9.74	648.05 ± 6.8	728.12 ± 7.64	1943.65 ± 20.4	1271.86 ± 32.09	2447.09 ± 69.29
F2 (40)	0.9771 ± 0.0002	993.72 ± 7.57	719.69 ± 5.48	808.61 ± 6.15	2158.56 ± 16.45	1677.65 ± 12.48	3198.23 ± 31.34
M0-1 (10)	0.9707 ± 0.0005	832.54 ± 14.91	561.35 ± 10.06	630.7 ± 11.3	1683.55 ± 30.16	388.16 ± 37.68	760.35 ± 80.3
M0-1 (20)	0.9711 ± 0.0001	843.26 ± 2.96	568.58 ± 2	638.82 ± 2.24	1705.23 ± 5.99	412.31 ± 18.95	802.29 ± 34.46
M0-1 (40)	0.9708 ± 0.0007	833.5 ± 20.4	562 ± 13.76	631.43 ± 15.46	1685.49 ± 41.26	384.5 ± 24.29	766.57 ± 48.23
M1-2 (10)	0.9694 ± 0.0011	796.6 ± 27.86	537.12 ± 18.79	603.47 ± 21.1	1610.87 ± 56.35	408.13 ± 46.94	784.08 ± 85.34
M1-2 (20)	0.9699 ± 0.0009	810.06 ± 25.87	546.19 ± 17.44	613.67 ± 19.6	1638.08 ± 52.32	373.32 ± 52.6	723.57 ± 89.14
M1-2 (40)	0.9707 ± 0.0003	831.58 ± 7.65	560.71 ± 5.16	629.97 ± 5.8	1681.61 ± 15.48	430.15 ± 50.76	827.64 ± 95.1
M2-3 (10)	0.9704 ± 0.0005	822.09 ± 13.12	554.3 ± 8.85	622.79 ± 9.94	1662.41 ± 26.55	445.61 ± 19.17	863.23 ± 37.54
M2-3 (20)	0.9711 ± 0.0008	842.26 ± 23.79	567.91 ± 16.04	638.07 ± 18.02	1703.23 ± 48.11	430.75 ± 33.26	816.7 ± 53.13
M2-3 (40)	0.9727 ± 0.0004	894.5 ± 11.7	603.12 ± 7.89	677.64 ± 8.87	1808.87 ± 23.66	444.6 ± 54.5	825.94 ± 95.41
M3 (10)	0.9715 ± 0.0003	834.64 ± 7.82	576.67 ± 5.4	647.92 ± 6.07	1729.51 ± 16.21	424.33 ± 32.05	805.95 ± 54.51
M3 (20)	0.9723 ± 0.0004	835.86 ± 10.72	592.84 ± 7.6	666.08 ± 8.54	1778.01 ± 22.79	455.02 ± 23.53	860.01 ± 32.98
M3 (40)	0.9747 ± 0.0012	866.66 ± 43.77	651.5 ± 32.9	731.99 ± 36.97	1954 ± 98.7	461.28 ± 12.51	840.75 ± 27.81
M3 (80)	0.9806 ± 0.0002	993.14 ± 10.73	852.48 ± 9.21	957.81 ± 10.35	2556.92 ± 27.63	431.67 ± 14.31	708.42 ± 27.55

Table S1 (continued)

Scenario description: Gender, age class and % of change	*Het* ± *SD*	N_{e1} ± *SD*	N_{e2} ± *SD*	N_{e3} ± *SD*	N_{e4} ± *SD*	N_{adult} ± *SD*	N_{census} ± *SD*
N_{init} (-20)	0.9782 ± 0.0001	969.12 ± 2.6	757.58 ± 2.04	851.19 ± 2.29	2272.25 ± 6.1	1878.38 ± 8.95	3542.93 ± 13.46
N_{init} (-40)	0.9692 ± 0.0019	791.32 ± 48.16	533.56 ± 32.46	599.48 ± 36.48	1600.19 ± 97.4	391.43 ± 13.71	752.08 ± 28.98
N_{init} (-60)	0.9693 ± 0.0008	792.22 ± 21.64	534.17 ± 14.59	600.16 ± 16.39	1602.01 ± 43.77	384.04 ± 4.82	736.96 ± 8.71
K (-20)	0.9683 ± 0.0004	768.51 ± 8.67	518.18 ± 5.85	582.2 ± 6.57	1554.06 ± 17.54	327.58 ± 14.45	635.54 ± 20.03
K (-40)	0.9751 ± 0.0006	979.35 ± 22.83	660.33 ± 15.39	741.91 ± 17.3	1980.49 ± 46.18	641.57 ± 33.18	1225.11 ± 60.44
K (-60)	0.9783 ± 0.0003	1128.85 ± 17.06	761.11 ± 11.5	855.16 ± 12.93	2282.85 ± 34.51	955.34 ± 14.16	1833.29 ± 22.71
Reproduction females (10)	0.9805 ± 0.0008	1257.79 ± 50.13	848.05 ± 33.8	952.83 ± 37.97	2543.64 ± 101.39	1288.08 ± 76.62	2468.04 ± 165.84
Reproduction females (20)	0.966 ± 0.0013	704.63 ± 27.7	481.58 ± 18.93	541.07 ± 21.27	1444.23 ± 56.8	98.94 ± 29.13	188 ± 55.48
Reproduction females (40)	0.9601 ± 0.011	591.21 ± 210.22	409.5 ± 145.57	460.07 ± 163.57	1227.96 ± 436.73	5.89 ± 4.49	9.96 ± 7.61
Reproduction males (10)	0.9699 ± 0	809.15 ± 0	545.58 ± 0	612.98 ± 0	1636.25 ± 0	446.49 ± 28.02	864.75 ± 43.88
Reproduction males (20)	0.9678 ± 0.0011	754.78 ± 26.53	508.93 ± 17.89	571.8 ± 20.1	1526.28 ± 53.66	394.39 ± 24.18	761.37 ± 52.5
Reproduction males (40)	0.9646 ± 0.0004	686.17 ± 7.08	462.68 ± 4.77	519.83 ± 5.37	1387.53 ± 14.33	399.8 ± 38.73	773.75 ± 69.58
F2 F0-1 M0-1 (10)	0.9742 ± 0.0003	933.28 ± 9.19	637.04 ± 6.27	715.74 ± 7.05	1910.63 ± 18.82	1147.65 ± 35.94	2239.36 ± 81.23
F2 F0-1 M0-1 (20)	0.9765 ± 0.0002	1013.3 ± 6.64	700.1 ± 4.58	786.6 ± 5.16	2099.79 ± 13.77	1579.48 ± 25.95	3113.22 ± 63.38
F2 F1-2 M1-2 (10)	0.9734 ± 0.0002	904.93 ± 5.95	618.45 ± 4.06	694.85 ± 4.57	1854.84 ± 12.19	997.57 ± 31.3	1918.16 ± 53.67
F2 F1-2 M1-2 (20)	0.9749 ± 0.0001	945.89 ± 3.82	655.89 ± 2.65	736.93 ± 2.97	1967.18 ± 7.94	1303.61 ± 53.72	2497.09 ± 90.24
F2 M1-2 M2-3 (10)	0.9743 ± 0.0015	929.73 ± 48.34	640.37 ± 39.41	719.49 ± 44.29	1920.6 ± 118.24	923.81 ± 223.59	1768.78 ± 421.53
F2 M1-2 M2-3 (20)	0.9762 ± 0.0004	991.05 ± 17.88	692.16 ± 12.48	777.68 ± 14.03	2075.99 ± 37.46	1305.19 ± 1.52	2449.84 ± 5.04
F2 M3 (10)	0.9704 ± 0.0004	823.97 ± 11.52	555.57 ± 7.77	624.21 ± 8.73	1666.22 ± 23.3	502.29 ± 8.55	968.89 ± 18.52
F2 M3 (20)	0.9734 ± 0.0027	918.52 ± 99.58	619.32 ± 67.13	695.84 ± 75.44	1857.45 ± 201.41	784.08 ± 384.16	1503.76 ± 709.56
F2 M0-1 F0-1 EV (10)	0.9724 ± 0.0002	884.63 ± 4.98	596.47 ± 3.36	670.16 ± 3.78	1788.92 ± 10.07	894.05 ± 30.02	1728.09 ± 61.13
F2 M0-1 F0-1 EV (20)	0.9748 ± 0.0001	967.57 ± 4.49	652.38 ± 3.03	732.99 ± 3.41	1956.65 ± 9.09	1282.29 ± 16.34	2480.33 ± 46.19

Chapter IV

Using remote camera traps for surveying and monitoring trends in abundance and density: a case study of a cheetah population in north-central Namibia

Author list:

Ezequiel Chimbioputo Fabiano [1, 2]

Matti Nghikembua [2]

Eduardo Eizirik [1]

Laurie Marker [2]

Affiliations:

1. Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil

2. Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia. Fax: 264 67 306247

Email addresses:
ezequiel.fabiano@acad.pucrs.br, fabianoezekiel@gmail.com
ccfmatti@iway.na
eduardo.eizirik@pucrs.br

cheetah@iway.na

Corresponding authors:

Ezequiel C. Fabiano

Cheetah Conservation Fund,

P.O. Box 1755, Otjiwarongo, Namibia.

Fax: 264 67 306247

Running title:

Cheetah population dynamics assessed with remote camera traps.

SUMMARY

1. The ability to monitor population abundance and density is necessary in assessing trends, while providing vital information for designing of conservation actions. This is a complex task, especially for species such as the cheetah *Acinonyx jubatus* due to behavioural and ecological aspects. Consequently, current population estimates are often based on methods that do not account for sampling biases such as imperfect detection. We investigated the use of remote camera trapping (RCT) with classical and spatial capture recapture (SCR) models for monitoring the abundance and density of a cheetah population while exploring the utilisation patterns of scent marking sites in north-central Namibia.

2. We analysed RCT data collected mostly at scent marking sites for 10 surveys conducted between 2005 and 2011, each lasting three months. In addition, four camera-detected male cheetahs were fitted with GSM collars to assess home range overlap in relation to the camera trap polygon.

3. Overall, 54 unique cheetahs were identified (8 ± 4 individuals per survey), which were predominantly nocturnal with some crepuscular behaviour. We observed high fidelity to scent stations for up to 4 years by resident cheetahs, with new recruits displaying spatial and temporal patterns that were similar to their predecessors'.

4. Density estimates based on effective sampling areas were determined using classical methods such as the full Mean Maximum Distance Moved and the home range radius or sum of home range sizes, which were similar to SCR-based estimates. However, SCR multi-survey density estimates were more

consistent and ranged between 5 and 16 km^{-3}, with a coefficient of variation of 11.7%. Further, our data provide some evidence suggesting that male density may be regulated by home range dynamics, as it remained similar only in periods of social stability.

5. In addition to reporting robust longitudinal density estimates for cheetahs using RCT with SCR methods, this study provides information on behavioural aspects such as spatial overlap and utilisation of scent marking sites. Overall, we found that the techniques applied here hold great promise for surveying cheetah populations in general, and highlight the need for sampling large areas in order to obtain reliable estimates.

Key-words: camera trapping, population dynamics, scent marking sites, spatial capture recapture, spatial and temporal activity patterns.

Introduction

The cheetah (*Acinonyx jubatus jubatus,* Schreber, 1775) is classified as a vulnerable species by the IUCN (Durant *et al*. 2008). Currently, Namibia holds the largest free-ranging population of cheetahs (Durant *et al.* 2008), of which 90% occurs on livestock and game farmlands where cheetah dominant competitors such as lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) have been extirpated (Marker-Kraus *et al.* 1996; Durant 2000). Thus, unlike in protected areas, where cheetahs live at low density due to interspecific competition (Caro 1994; Durant 2000), indiscriminate removals are considered a major factor affecting the population dynamics in areas where interspecific competition is minimal due to the absence of lions and spotted hyenas (Marker *et al.* 2003, 2007). In addition, anthropogenic factors tend to reduce and slow down growth rate of carnivores (Creel and Rotella 2010; Sparkman, Waits and Murray 2011; Wich *et al*. 2011). Other factors influencing population dynamics include habitat, prey availability, droughts, social organization (Packer *et al.* 2005), home range or territoriality dynamics, spatial use patterns and dispersal (Odum 2004; Gorman and Trowbridge 1989). These mechanisms affect the reproduction patterns, hence the population effective size (*Ne*) (Mayer and Pasinelli 2013). The extent to which these mechanisms are prevalent in the Namibian population is largely unknown.

RCT has been used as a means of effectively estimating abundance and density of a variety of cryptic species including tigers (*Panthera tigris*) (Karanth *et al*. 2006), leopards (*P. pardus*), brown hyena (*Hyaena brunnea*), aardwolf (*Proteles cristata*) (O'Brien and Kinnaird 2011) and cheetahs (Marker, Fabiano and Nhikembua 2008). However, in a few cases, RCT has been utilized to determine long-term population trends (Karanth *et al.* 2006; Barlow *et al*. 2009). Only a single study has assessed long-term trends on cheetah through distance sampling (Durant *et al*. 2011). However, distance sampling may be unsuitable for surveying cheetahs in areas outside protected area (e.g. in Namibia) due to the species ecological behaviour (e.g. roam across vast home ranges, be elusive, skittish) (Gese 2001) or due to limited visibility.

Density-related RCT studies often estimate effective sampling areas (ESA) using a variety of *ad hoc* buffer estimates, determined using the half and full mean maximum distance moved by all animals recaptured at multiple stations and the home range (HR) radius of individuals radio collared simultaneously as RCT surveys are ongoing or based on the literature (Soisalo and Calvacanti 2006; Marker, Fabiano and Nghikembua 2008). The application of these approaches is problematic as these buffer estimates may lack a formal relationship to HRs or territories of the sampled individuals (Gardner *et al.* 2010a). Furthermore, these approaches fail to capture the heterogeneity in the probability of detection caused by populations being geographically open as sampling areas are often smaller than camera-detected individual's HRs (White *et al.* 1982). Consequently, these shortfalls often yield positively biased estimates as the movements patterns of individuals in relation to camera trap layout are underestimated (Marker, Fabiano and Nghikembua 2008; Gerber, Karpanty and Kelly 2012 *but see* Balme, Hunter and Slotow 2009). Other surveying techniques previously used to estimate cheetah abundance and density in Namibia are also affected by these sources of error and others (e.g. double counting, imperfect detection). Techniques include radio tracking (Morbasch 1975; Marker 2002), spoor tracking (Fabiano 2007) and questionnaires, interviews and sighting reports (Myers 1975; Hanssen and Stander (2004). Ultimately, the impossibility of estimating population trends in Namibia is caused by the lack of a reliable, precise estimate of abundance and density.

In order to circumvent these and other issues that may affect the reliability of density estimations, spatial capture recapture models (SCR) have been developed and continue to be refined (Efford 2004; Royle and Young 2008; Borchers and Efford 2008; Royle *et al*. 2009; Efford and Fewster 2012; Royle *et al.* 2013). SCR approaches account for individual heterogeneity by incorporating the auxiliary spatial information about where individuals are captured when constructing capture histories, thus making full use of the information from camera data sets (Royle *et al*. 2009). As such, detection rates are a function of the distance between the camera trap locations and the centre of an individual's HR (Efford 2004). Consequently, this parameterization allows for a direct determination of the ESA thus obtaining less biased estimates. Overall, we compared non-spatial and SCR approaches on cheetahs.

Cheetahs scent marks terminate mounds and certain trees with urine and less often with faeces (Eaton 1970; Marker-Kraus *et al*. 1996). Eaton (1970) suggested that for cheetahs in the Serengeti, scent marking is a "time-plan" by which conspecifics individuals with overlapping HR coexist with minimal risk of direct encounter, through intensive marking, as it is unviable for them to establish exclusive territories given their extensive HR. This time plan to be effective requires at higher scent marking frequency (Eaton 1970) and at strategic sites in order to maximize encounter rates (Gorman and Trowbridge 1989). Male cheetahs in Namibia visit a scent-marking site on average every six (± 9) days (Marnewick, Funston and Karanth 2006; Marker, Fabiano and Nhikembua 2008). Scent marking is also involved on advertisement of reproductive status (Smith *et al*. 1989), territory demarcation and defence (Gese and Ruff 1997; Sillero-Zubiri and Macdonald 1998), foraging orientation including food resources, maintaining social structure (Revilla and Palomares 2002; Gorman and Trowbridge 1989), HR familiarity to cubs by mothers (Seidensticker *et al.* 1973). However, the distribution and visitation pattern (utilization pattern) of scent marking sites in relation to individual HR is largely unknown for the Namibian cheetah population.

Here, we used a combination of a six years remote camera trap (RCT) placed mostly at scent marking sites and radio telemetry data sets, to provide new insights into processes affecting a cheetah population (fine scale level) while assessing trends in abundance and density based on a robust systematic approach. Specific objectives were to (i) determine the population structure over time, (ii) describe the spatial utilisation patterns of scent marking sites that are important for ecological and behavioural purposes, and (iii) compare cheetah densities estimated using non-spatial and SCR methods. Furthermore, we discuss the pros and cons of using spatial vs. non-spatial methods for density estimation.

Materials and methods

STUDY AREA

We conducted this study primarily on the Cheetah Conservation Fund property, located in the Waterberg Conservancy in north-central Namibia (20°28'56''S 17°2'24''E, Fig. 1). The area is semi-arid and falls between 400 mm and 500 mm rainfall isopleths (Barnard 1998). Namibia is considered to have three seasons: summer, intermediate and winter (Berry 1980). The area falls within the thornbush, tree and woodland savanna vegetation zone (Geiss 1971) with prevalent occurrence of bush encroachment (Barnard 1998). Livestock (primarily cattle and smallstock) and wildlife, including fenced game farms, eco-tourism and trophy hunting are the primary forms of land use (Marker-Kraus *et al*. 1996). The area harbours a diversity of ungulates including the oryx (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*), eland (*Taurotragus oryx*), springbok (*Antidorcas marsupialis*), and steenbok (*Raphicerus campestris*) among others, and sympatric carnivores including brown hyena, caracal (*Caracal caracal*), black-backed jackal (*Canis mesomelas*), and leopard (nomenclature for species names follows Estes 1991).

STUDY DESIGN AND DATA COLLECTION

RCT surveys were conducted annually for three months from 2005 to 2009, and then continuously between July 2010 and December 2011, encompassing an average area of 377 km^2 (SD ± 41.15 km^2) (Fig. 1, S1, Table 1). Although Namibia is considered to have three seasons, for the purpose of this study, surveys were grouped into two main seasons, Summer (Nov – May) and Winter (June – Oct). Overall, five surveys each were conducted during the summer and winter seasons. During the course of the study, 32 different sampling stations were deployed (15 to 19 per survey, Table 1). Sampling effort was similar across surveys despite relocating or terminating stations that failed to detect a cheetah in a survey in order to increase detection probabilities (as suggested by Karanth and Nichols [2002]). Sixty-three percent (*n* = 20) of the stations were located at scent marking posts and 19% (*n* = 6) either next to roads/fence lines and in close proximity to cheetah captive facilities. Inter-camera distances were on average 17 km (± 9.24 km), a distance that falls within the HR radius (i) of four radio collared individuals (3 to 10 km) (*this study*) and (ii) of 22.95 km (n = 41, 26 males and 15 females) from a previous long-term telemetry study conducted in the area (Marker *et al.* 2008). This spacing and geographical distribution of cameras ensured that no animal HR was unsampled

(Karanth and Nichols 2002). We used DeerCamTM DC200 (DeerCam, Park Falls, WI, USA) and Bushnell Trophy (Trophy Cam 2009, Kansas, USA) cameras from 2005 to 2009 and 2010 through to 2011, respectively. In 2008, one station had a Moultrie M60 digital camera (Georgia, USA). We placed two cameras per station, mounted *ca.* 75 cm above the ground and 5 m apart. Cameras sites were checked for functionality, as well as to change film/memory card and/or batteries on average once a week (range of 2 to 7 days). For data management, cheetah pictures from developed films were logged into an Excel database, while an automatic storage software was used for memory cards (Harris *et al*. 2010).

One of the non-spatial buffer estimates used to determine ESA was the radius of a 95% minimum convex polygon (MCP) home range, as determined by radio telemetry. To allow this assessment, four RCT-detected male cheetahs (two single males and two males belonging to two coalitions of two males each) were captured, immobilized and fitted with Global System for Mobile communications (GSM) collars in 2007. Collars took three to five Global Positioning System (GPS) readings per day, lasting for a maximum of nine months. Immobilization procedures followed those of Marker *et al*. (2003). Cheetahs were released shortly after capture as close as possible to the capture site, in order to minimize unavailability for detection by cameras. These cheetahs are referred to by unique numbers (i.e. AJU#, Table 2).

Data analysis

CAPTURE SUCCESS, DEMOGRAPHIC STRUCTURE AND SPATIAL USE PATTERNS OF SCENT MARKING SITES

To be consistent with our previous work and adhere to recommendations of sampling periods to uphold the closure assumption (Karanth and Nichols 2002; Marker, Fabiano and Nghikembua 2008), we stratified the continuous effort (i.e. 2010 through 2011 data set) into six surveys, of 90 days each. We identified individual cheetahs manually based on unique spot patterns, which remain unaltered throughout their lifetime (Caro and Durant 1991). Gender was determined using cues such as the presence of visible genitals, ear tag position (i.e. right for males and left for females) or accompanying cubs. A cheetah was classified as either a cub (≤ 1 years old) or adult (> 1 year old); male or female; or as single, member of male coalition or breeding female (i.e. with accompanying cubs). Individuals were also classified as resident if they were captured during at least two consecutive inter-annual surveys (i.e. the six surveys spaced equally apart) or during two consecutive stratified surveys within the 2010 - 2011 continuous survey (Caro and Collins 1987; Barlow *et al*. 2009). All other individuals were treated as non-resident as non-resident cheetahs tend to remain no more than a few days in the same area (Bothma and Walker 1999). Likewise, we considered a camera site to be an integral component of an individual's HR if it was visited continuously on all surveys in which the individual was detected. This consideration was limited to residents.

We explored the distribution and density of camera traps sites in relation to GSM HR. HR were calculated as core (45% Kernel), intermediate (80%) and border (> 80%) (Castillo, Lucherini and Casanave 2011).

POPULATION ESTIMATION: NON-SPATIAL CAPTURE-RECAPTURE

We used data on identified individuals for estimating and assessing trends in abundance and density. The programs CloseTest and CAPTURE (Otis *et al.* 1978; Rexstad and Burnham 1991) were used to test the closure assumption, with the latter also being employed to estimate abundance (*N*). For individual capture histories, we set an occasion to equal six sampling days. Evidence for the presence of transients was determined through program U-CARE2.2 (Choquet *et al.* 2005). Non-spatial density estimates were computed by dividing abundance by the size of the four ESAs. ESAs were determined by buffering the camera trap MCP with the: (i) full mean maximum distance traversed by individuals captured on multiple stations (FMMDM); (ii) mean radius of the 95% MCP HRs of four GSM collared males (GSM-ESA); (iii) mean radius of the 95% MCP HRs of 41 individuals (26 males and 15 females) VHF collared reported by Marker *et al.* (2008) (VHF-ESA). The FMMDM was used as opposed to the half MMDM, as our previous work showed the latter to be underestimated resulting in unrealistic density estimates (Marker, Fabiano and Nhikembua 2008 see also Soisolo and Calvacanti 2006). Lastly, we also used the size of the 95% MCP for the GSM collar-derived HRs as ESA (GSM-polygon). The latter was considered a minimum density estimate. The 95% MCP, Kernel HR sizes and range overlaps were computed using the HR and Spatial Analysis extension of ArcView 3.2 (version 3.2) and ArcGis 9.3 (ESRI, Redlands, CA, USA) and HR radius as Area = πr^2, with r being the radius. ESA computations were also conducted using this software. HR asymptotes were determined using ABODE (Laver 2005). For the FMMDM, a single distance value was used for coalitions, as no evidence supporting spatio-temporal separation of male coalitions was found for the study area in

Namibia. Naive standard errors for *D* were calculated by dividing standard error (SE) of *N* by the ESA.

POPULATION ESTIMATION: BAYESIAN SPATIAL CAPTURE-RECAPTURE

We also used the Bayesian hierarchical R package SPACECAP 1.0.5 for SCR analysis that accounts for imperfect detection and makes use of the geographic locations of where individuals are captured to infer detection probabilities (Gopalaswamy *et al*. 2012). We set state-space *S* to 6158 km^2 (i.e. the area of the camera trap array buffered with 22.95 km, the HR radius from Marker *et al.* 2008) with a 3 x 3 km grid resolution. Both the grid resolution and *S* size are based on prior knowledge of animal movement ranges and HR sizes (Marker *et al.* 2008; *this study*). Density results were not affected by *S* specification, as observed on trials with smaller polygons of similar or finer grid resolutions (e.g. 1430 km^2, 2848 km^2, grids of 0.5 km, 1.5 km).

We used a Markov Chain Monte Carlo (MCMC) of 100,000 iterations with a burn-in of 10% of the total samples, and thinned every 10 steps, as well as incorporating the following features: data augmentation to 60 or 90 (*M*); trap response; half-normal detection function; and Bernoulli's encounter model. We used the default uninformative priors for all model parameters (i.e. σ, λ_0, psi, and gamma) and standardized density estimates to 1000 km^2. We converted sigma (σ, animal range) and lambda (λ_0, baseline encounter rate) into an equivalent 95% home-range radius and individual capture probability, respectively, through $qchisq\ ((0.95,2)^{0.5})*(\boldsymbol{\sigma})$ and $1 - \exp^{(-\boldsymbol{\lambda 0})}$ (Noss *et al.* 2012). Underlying this mathematical conversion is the assumption that HRs follows a bivariate normal distribution (Royle *et al.* 2009).

Estimates were considered to be significantly different at $\alpha = 0.05$, if their 95% CIs did not overlap by more than half (Cumming and Finch 2005; Noss *et al*. 2012). Means are presented with standard deviation unless stated otherwise.

Results

CAPTURE SUCCESS, DEMOGRAPHIC STRUCTURE AND PATTERNS

Table 1 presents a summary of the sampling effort per survey for the 10 surveys conducted between 2005 and 2011. Up to 32 stations were active at some stage during the study (mode of 15 per survey) with 22% being active during all 10 surveys. This sampling effort resulted in 13770 (1377 ± 120) trap-nights and 2596 (260 ± 78) usable cheetah photographs per survey. While this estimate of sampling effort does not account for cameras failures, seldom were both cameras on a site non-functional. Fifty-four unique individuals were identified across the 10 surveys, including two females released into the study area as part of CCF's management actions (Table 1). Two potentially additional individuals were detected on different surveys, but discarded as it was not possible to ascertain their identity. Of the 54 individuals, 39 were adults and 15 were cubs. The majority of adult were males, 32 versus 7 females, with a consistent biased male sex ratio across surveys (Fig. 2b). There was only one individual for which the sex could not be determined. Captured adult males were mostly single (68%) whereas 32% occurred in coalitions. For three males, it was possible to infer the age of death: the members of a coalition of two (AJU1459 and 1460) died at the age of 5 and 6 years and a member of another coalition (AJU1542) at 6 years (Table 2). The AJU1542 coalition member was still alive at the end of 2011. While AJU1542 died in the midst of the fifth survey, AJU1459 and 1460 died outside of any sampling period and a year apart (i.e. 2006 and 2007, respectively). As for females, all but one had accompanying cubs, with a mean litter size of 3 ± 1.2. With respect to seasonal patterns, males were captured throughout the surveys (usually including multiple recaptures), while females showed a trend for increased capture probability in the summer (10 out of 15 capture/recapture events). Females tended to be captured only once, with only five cases of recapturing.

On average 8 ± 4 (coefficient of variation 15%) individuals were captured per survey (Table 1), with capture saturation occurring on average 7 (± 3) occasions (approximately 44 days as) after the start of the survey (Fig. 3). Although saturation occurred on average earlier in winter (7 ± 2 occasions) than in summer (8 ± 4 occasions), this difference was not statistically significant (t = 0.47, *d.f.* = 8, P = 0.65). Overall, capture rates declined as the study progressed, with non-resident adults and cubs driving fluctuations in abundance.

TEMPORAL AND SPATIAL UTILISATION PATTERNS OF CHEETAHS BASED ON RCT DATA

Temporal and spatial patterns of use of areas surveyed by camera traps by resident cheetahs are presented in Table 2 and Fig. 4. The majority of detected individuals were non-residents (77% of 39 adults). On average a cheetah was captured every 5 days (± 8), with residents (n = 7) being captured significantly more frequently than non-residents (U = 14202, P < 0.01). When seasons were assessed separately, the median time between captures was significantly different between residents and non-residents in the summer (2 and 7 days, respectively; U = 4652, P < 0.01), and in winter (2 and 6 days, respectively; U =*2559* P < 0.01). When resident and non-resident cheetahs were assessed separately, no significant difference was observed in the summer capture interval (resident: 3.6 ± 6.5 days in summer and 2.5 ± 4.3

days in winter< *U* = 30598, *P* > 0.05; non-residents: 9.6 ± 11 days in summer and 15.6 ± 17 days in winter; *U* = 376, *P* > 0.05).

A certain level of spatial organization was deduced based on the distribution of resident cheetah detections (Table 2). Clusters based on captures occurred around stations 3 - 5, stations 10-12, and stations 14, 15 and 17 (Table 2). These stations were in closer proximity to one another (Fig. 1). While some stations were used within and across surveys by different individuals (e.g. stations 12, 14, 15, 17), other were used either once or infrequently by different social groups (e.g. station 11). Utilisation patterns remained similar even after a turnover in resident individuals occurred. For example, the activity centres for AJU1537 and 1538 were very similar to those of their predecessors (i.e. AJU1459 and 1460), with these social groups 95% MCP overlapping by at least 35% (Table 2, Fig. 4). Likewise, utilisation patterns of AJU1533 were similar to those of AJU1542 and 1543, with an overlap of two years in their presence (Table 2). On the other hand, no new individuals were detected at stations visited by M1 from 2007 until 2010-2 and 2011-2 when AJU1543 was detected once at stations 3 and 5, respectively (Table 2). Overall, multi-year detections per station ranged from six to 48 months (31 ± 16.3 months).

SPATIAL UTILISATION PATTERNS OF CHEETAHS BASED ON TELEMETRY DATA

The spatial organization based on the GSM data was in agreement with that retrieved by RCT data, whereby HRs for AJU1533, 1537 and 1459 encompassed the camera sites where these individuals where detected (Fig. 1, Table 2). On average 741 fixes (± 695) were recorded over a period of 70 to 226 days (129 ± 68). The high variation in fixes was a result of AJU1533 having 1763 fixes while the remaining individual fixes ≤ 510 (TABLE S2). Despite the limited monitoring period all individuals HRs reached asymptotes (AJU 1537 at 66 pts, AJU 1533 at 121 pts, AJU 1459 at 129 pts, and AJU 1536 at 144 pts) corroborating their status of residents.

The minimum average 95% MCP and Kernel HR size for the four individuals were 269 ± 238.4 km^2 and 126.5 ± 170.4 km^2 respectively, indicating consistency between these two methods ($t = 0.97$, *d.f.* = 6, $P > 0.05$). The extent of overlap between each individual's 95% MCP and the 2007 - 2008 RCT study area ranged from 3% to 19% (7.7 ± 7.2%), in a descending order of overlap for AJUs 1459, 1537, 1536 and 1533 (Fig. 1). Despite our small sample size, these findings are consistent with a previous long-term telemetry study, whose study area overlapped our sampling area (Marker *et al*. 2008).

Based on kernel HR categories, collared individuals except for AJU1536, as it was not captured on camera, utilized stations outside their HR or within their core area (Fig. 4). AJU1537 which occupied the vacant HRs previously occupied by AJU1459 overlapped (50 ± 21.60 95% MCP HR overlap), with the former having a single and smaller core HR while AJU1537 had two and larger core HRs (Fig. 4, Table S2). Nevertheless, both were captured on stations outside their HRs. Likewise AJU1533 also utilized two stations outside its HR but mostly used stations within its 80% HR (Fig. 4).

POPULATION ABUNDANCE AND DENSITY ESTIMATES

Analyses revealed that the population should be considered open in the case of several surveys (Table 3). Moreover, evidence for the presence of transients was provided by the transient test in the program U-CARE2 ($\chi^2 = 2.09$, *d.f.* = 7, $P < 0.05$). Models including heterogeneity and or time, as sources of variation, were often preferred except on surveys seven and nine (Table 3). Individual probabilities of capture (*p*) reported by CAPTURE ranged from 0.12 to 0.78 (0.34 ± 0.21 per survey) (Fig. S2A). This resulted in negligible probabilities of not detecting an individual that was in the study area during a survey (Table 3). Overlapping estimates of *p* were obtained using the SCR approach (0.52 ± 0.40, ranging from 0.13 to 1.24) (Fig. S2A). Individual capture probabilities tended to be higher for recaptures (0.65 ± 0.26, ranging from 0.28 to 0.91) relative to initial captures (0.29 ± 0.25, ranging from 0.03 to 0.79) (Fig. S2B).

CAPTURE revealed an average abundance (*N*) of nine (± 7) cheetahs per survey with *N* estimates for the first two and the sixth survey being significantly higher in relation to the other surveys based on 95% CI ranges (Fig. S2C). In addition, surveys 1, 2, 6 and 10 were the least precise (95% CI coverage > 1). An average abundance of seven (± 3.5) is observed if survey six is excluded (*N* = 25 ± 9.74). Although not directly comparable, these trends differed from the SCR-based number of activity centres (*N''*), which were more similar across surveys except for those of survey 2 (23 ± 6.29) and 10 (20 ± 7.61) (Fig. S2C). If the latter are excluded, *N''* is on average of 10 ± 1.68 (range 3 to 21) but slightly higher and less precise if included (12 ± 5.17, range 3 to 35). *N''* estimates appear not to have been affected by the prior data augmentation as *N''* was far below 100. Further, psi, the probability of an augmented individual being part of the surveyed population, was below unity for all surveys ranging from 0.12 to 0.33 (0.20 ± 0.07) (Fig. S5D).

With respect to individual movements, collared individuals traversed daily distances ranging from 3.1 to 10.4 km (6.49 ± 3.03), and had an average HR radius of 9.25 km for the GSM collared individuals which was smaller than the radius from a previous study based on VHF and a larger sample size (22.95 km, *n* = 41) (Table S2, Fig. S2E). These estimates overlapped with the FMMDM buffer width estimates

(9.67 ± 4.7 km; 3.8 to 20.2 km), whose 95% CIs were broad and overlapped for most surveys (i.e. 1, 3, 4, 6, 9 and 10). The resulting ESA based on FMMDM, HR radius from this study (GSM-ESA) and that based on the VHF data collected by Marker *et al.* (2008b) (VHF-ESA), as well as the GSM MCP polygon (1430 km^2), overlapped with the camera trapping area by 13 - 30%, i.e. they excluded the trapping polygon by 70 - 87%. In turn, the SCR animal movement parameter (σ) and its equivalent HR radius were consistently lower and higher, respectively, for most surveys, relative to the FMMDM and GSM-based animal movement estimates (Fig. S2E). Animal movement parameters and their equivalent HR radius were on average 4.34 ± 2.09 km (1.5 to 6.9) and 10.63 ± 5.11 km (3.66 to 16.91), respectively.

Density estimates obtained by dividing *N* by the ESA determined by conventional methods (FMMDM, VHF, GSM, GSM-polygon ESA) were generally similar across surveys based on degree of overlap of 95% CI and detected similar trends as the SCR-based estimates (Fig. 5, Table S1). Both approaches depicted a slight decrease in density after the first two surveys after which density seemed to remain constant, except for surveys 6 and 10. Density estimates based on conventional methods were similar, except for VHF-ESA and SCR estimates (Bonferroni $P < 0.05$). Density estimates based on FMMDM ranged from 2 - 18 individuals km^{-3}, from 1 - 6 individuals km^{-3} for VHF-ESA, from 2 - 17 individuals km^{-3} for GSM-ESA, and 4 - 20 individuals km^{-3} for GSM-polygon (Fig. 5). These estimates overlapped considerably with SCR density estimates, which ranged from 5 - 20 individuals km^{-3}. Overall, the VHF-ESA density estimates were consistently lower than those based on other ESA, with densities becoming more similar as the study progressed. The high imprecision of certain surveys (especially 6 or 2 and 10) could potentially be indicative of some non-detected temporal variation.

Discussion

This study shows that RCT can and preferably when combined with other non-invasive techniques can provide relevant information regarding trends in cheetah abundance and density, while simultaneously assessing other aspects of this species' behaviour. Moreover, we were able to show the population structure of a local resident population, that male density may be linked to social stability and that the utilization of scent marking sites by both genders seems not to be related to reproductive status. Overall, and despite large confidence intervals, the study shows potential of using SCR techniques further understand ecological and demographic processes at a fine scale level.

Capture success, demographic structure and spatial use of scent marking

The study design was effective in capturing individual cheetahs, as demonstrated by the observed relative high capture probabilities (> 0.20 *as per* Otis *et al.* [1978]) and by the capturing of different social groups which varied per survey. These capture probabilities are similar but mostly higher than that reported for other large carnivores (e.g. for snow leopards (*Uncia uncia*) of 0.33 - 0.46 [Jackson et al. 2005], and cheetahs 0.17 [Marnewick et al. 2006] and 0.14 leopards in South Africa [Balme, Hunter and Slotow 2009]). This capture success is a result of: (i) high quality photos that in most cases enabled the identification of individuals; (ii) high camera density in the study area; and (iii) placement of cameras at strategic sites (i.e. scent marking trees). The latter explains the higher encounter rate after initial capture due to resident fidelity in the use of scent marking trees. Olfactory and visual cues aid with orienting individuals towards marking sites as scent chemicals remain effective for extended periods of time (Eisenberg and Kleiman 1972; Potts, Harris and Giuggioli

2012) and are placed non-randomly at strategic sites that maximize their encounter by individuals (Gorman and Trowbridge 1989). This phenomenon could help explain the high detection rate of non-residents. Furthermore, edge effects as demonstrated by the larger HR of the GSM radio collared individuals in relation to the camera-trapping polygon can also explain the latter.

The population structure varied per survey with a consistent male bias and females accompanied by cubs captured the most. This may be a result of edge effect but also due to the placement of cameras at scent marking sites and the ecological and behavioural role of these sites to both genders. Nevertheless, the observed sex bias observed is not unique to this study (e.g. Maffei *et al.* 2011) and it is agreement with sex ratio estimates (and demographic structure) based on cheetahs captured by farmers (e.g. 2.9: 1 male: female [Marker *et al.* 2003]). This similarity is due to the placement of capture cages by farmers or RC at scent marking sites (Marker *et al*. 2003). Marnewick et al. (2006) also observed a similar sex bias in South Africa. This sex ratio bias however differs from that of the Serengeti cheetah population, which tends to be female biased (Kelly 2001). Similarly, Barlow *et al*. (2009) observed a higher female to male ratio of resident individuals (11:4), a finding possible related to the smaller HR sizes of tigresses in relation to the study area. Nevertheless, Barlow *et al*. (2009) as it is the case for our study, also recorded that out of 91 that captured in a 100 km^2 monitored over 7 years using RCT and radio tracking, 62% and 22% of individuals observed were cubs and transients, respectively. Overall, the demographic structure and abundace at fine scale levels for cheetah and tigers appear to be affected by patterns in cubs and transients.

Differences in the ecological role of scent marking (i.e. most camera sites) hence discrepancies in their utilisation patterns by both gender may explain the observed differences in captures between genders. For example, in Namibia, female cheetahs exhibit reproductive activity throughout the year (Wachter *et al*. 2011) with peaks in parturition rates occurring in March, June and July, thus limiting female movements during these time periods (Marker *et al.* 2008; Houser, Somers and Boast 2009). However, because female movement increases as cubs' mature, higher capture rates can be expected in summer. Despite the small sample size, four of the six females accompanied by cubs in this study were detected in summer. Furthermore, the lack of an increase in captures during the two years of continuous sampling (e.g. 2010-2011 in Table 2) suggests that utilization of scent marking sites by cheetahs in particular females in the study areas appears not to be related to reproductive status as observed on tigers and snow leopards (Smith *et al.* 1989; Jackson 1996). On the other hand, male cheetahs, particularly resident, were captured more often than non-resident individuals irrespective of the season. Non-residents cheetahs often remain in an area only a few days (Bothma and Walker 1999). These results add to our knowledge of cheetah behavioural ecology, especially with respect to males. As for females, their behaviour remains more elusive due to the small sample size recorded in this and other studies. Overall, there is a need for behavioural studies regarding the significance and use of scent marking posts by females for all free-ranging felids in order to improve current surveying designs, particularly those relying on animals signs (Sharma *et al.* 2006).

Spatial utilisation of scent marking sites

The spatial distribution of scent marking sites in relation to radio-collared individuals indicates their use of stations mostly located outside/border and within the core area.

However, due to a small sample size no conclusive inferences can be made regarding the role of scent marking based on this utilization pattern in relation to territory formation, demarcation and defence, foraging orientation (Revilla and Palomares 2002; Giuggioli, Potts, and Harris 2011). Nevertheless, this utilization pattern possible ensures a high detection rate of scent by intruders while scent stations within the core may be link to resource use or defence (Gorman and Trowbridge 1989). Bothma and Walker (1999) indicate that cheetah select scent marking areas to minimize encounter by conspecifics. Cheetahs in the study area tend to select for sites with high visibility but sufficient cover (Muntifering et al. 2006). This is partly supported by the observation of the stations that were located outside the collared individual's HR been nearby open fields. This aggregation implies non-random distribution of cheetahs, high-density zones. In the Serengeti, cheetah competition for territories often results on death of individuals either inside or at the border of territories (Caro and Kelly 2001). This possible could be the case here. Although due to body decomposition the cause of death for AJU1459 could not be ascertain, remains were located inside its HR. However, further studies with larger sample sizes are required, as it is likely that we did not identified all scent-marking stations within individuals HRs.

The different levels of fidelity by male cheetahs to different camera sites and new recruits displaying similar usage patterns as their predecessors, provides insights into home range tenure. The observed home range tenure length, with an upper bound of 4 years during the length of the study (with three animals remaining in their areas after the end of the present surveys), falls within that reported for cheetahs (4 months to 6 years) (Caro and Collins 1987). This period of tenure is higher than that of tigers (2.8 years) (Smith and McDougal 1991) and of lions (2.75

years) (Packer *et al.* 2001). Barlow et al. (2009) also observed residency of up to seven years on tigers. The fact that fidelity to sites can be retained for up to 6 years implies that HR tenure may last until after prime age.

Furthermore, this long-tenure and the continuous utilization of scent marking trees by males may reflect HR quality. Marker *et al.* (2008) has shown cheetahs in the study area as having relatively stable HR year-round. In addition, longevity in tenure in cheetahs has been linked to social group structure and animal health (Caro and Collins 1987). Our data seem to support at least the first component of this hypothesis, given the inferred displacement of a single male by a coalition of two individuals. This numerical advantage of coalitions renders them a higher likelihood of holding a territory, displacing or killing resident singletons as observed in the Serengeti (Caro and Kelly 2001; Durant, Kelly and Caro 2004). These findings are of relevance for their influence on lifetime reproductive success.

Comparison of buffering estimates

In this study, conventional and SCR based density estimates were congruent. However, the latter are considered more reliable SCR despite their high higher variances. SCR reliability rests on a number of factors including the relaxation of the geographical closure assumption, providing valid inferences for small sample sizes and by modelling individuals' movements explicitly (Efford 2004; Kéry *et al.* 2011; Royle *et al.* 2011). Furthermore, it accounts for spatial effects (the heterogeneity resultant from the spatial organization and habitat use patterns of individuals in relation to a sampling area) and edge effects (e.g. when the HR of camera detected individual is larger than the camera trapping polygon) through the inclusion of a state-space or area of integration (Gerber, Karpanty and Kelly 2012; Efford and

Fewster 2012). The explicit inclusion of movements and trap response are of high relevance as sampling designs often select sites to maximize captures, as was the case in this study.

Density estimates low precision is linked to the process of estimating the sampling variance that may not affect the point estimate itself (Efford and Fewster 2012). In other words, it is due to the inclusion of sources of uncertainties (e.g. in area estimation) combined with data inadequacies (low sample sizes with no or few recaptures) (Gerber, Karpanty and Kelly 2012). These conditions result in model parameters often being biased and unreliable (e.g. Geweke diagnostic > 1.1 for σ for surveys 6, 8, 9 and 10) (O'Brien and Kinnaird 2011; Noss *et al.* 2012). While this may be the case for our study, the congruence among movement parameters (SCR-based and radio telemetry) suggests that our density estimates may not be highly overestimated. Other studies have reported similar low precision estimates (e.g. O'Brien and Kinnaird 2011; Pesenti and Zimmermann 2013). On the contrary, conventional methods are susceptible to edge effect and by the spacing of the cameras, which needs to be sufficient large to avoid underestimating true movement. Additionally, they fail to account for spatial heterogeneity. Hence, CAPTURE abundance and subsequent density estimates based on the different ESA could be biased and precision is unreliable.

Nevertheless, the degree of bias in density estimates based on conventional methods should be lower if intra-camera spacing is large enough and multiple individuals are recaptured at different stations or by using the HR radius of individuals that were also camera detected. Our data support this assumption, as all, but the VHF- based densities, were similar (Fig. 5). Sharma *et al.* (2009) reached a similar conclusion (but see Obbard, Howe and Kyle 2010; Reppucci, Gardner and

Lucherini 2011; Noss *et al*. 2012 for diverging views). Furthermore, overlap between density estimates across methods was the result of SCR sigma (movement parameter) and the overlap of converted 95% HR radius estimates with the daily distance ranges of the collared cheetahs, their HR radius, and FMMDM estimates. This reiterates that the distance between camera stations was similar to or larger than the HR radius of the study population and corroborates that the grid resolution used for SCR was appropriate. It should be noted that if the half MMDM was used higher densities would be expected (Marker, Fabiano and Nighkembua 2008). FMMDM estimates may be negatively biased as they fail to account for as much variation as GSM or SCR movement estimates.

As such, we are of the opinion that while similarities between density estimates based on conventional ESA and SCR approaches may be accidental or reflect camera spacing (Obbard, Howe and Kyle 2010; Gerber, Karpanty and Kelly 2012), this seems not to having been the case here. Overall, as Noss *et al.* (2012) and Gardner *et al*. (2010a, b), we recommend the use of SCR approaches whenever possible, and if not, attempts to acquire HR radius while RCT is ongoing should be made. We also discourage the use of HR radius as buffer estimates based on other studies outside or for the same study area but different time as these may introduce additional level of heterogeneity (e.g. intraspecific heterogeneity due differences in spatial utilisation patterns, e.g. AJU1459 vs. 1537). This was likely the case for the significantly low VHF-based estimates in this study.

It should be noted that detection probabilities were not adjusted for varying sampling effort (e.g. due to camera failure, memory cards/films overexposed, knocked down). Hence, trends in density may be confounded by trends in sampling effort as detection probability was not consistent with sampling effort (e.g. 1 day sampling occasion) (Efford, Borchers and Mowat 2013). Nevertheless, as alluded earlier, seldom was a station non-functional (i.e. both cameras down). Furthermore, we are confident that this bias is minimal based on similar trends in the detection of individuals (resident and non-residents) among surveys (actual CR data sets). This together with data aggregation (e.g. 6 sampling days = 1 occasion) may have reduced further this effect of varying effort on biasing estimators. Density estimates are often robust to the loss of ability in modelling detection probability at finer scales (Efford, Borchers and Mowat 2013).

Density estimates

SCR density estimates were similar across most surveys and ranged from 5 to 20 km^{-3}. A possible reason for this similarity is that this population was close to maximum carrying capacity. Our data support this hypothesis, as HR tenure changes only occurred after a HR became vacant and through the displacement of a single male by a coalition of two. Caro and Collins (1987) and Durant, Kelly and Caro (2004), have previously shown that male cheetahs in groups have a higher probability of acquiring and retaining a territory or HR (including cases of displacement of residents) than single males. This finding implies that HR dynamics (due to social stability) may govern cheetah density especially for males (López-Sepulcre and Kokko 2005; Wang and Grimm 2007). Seidensttoker *et al*. (1973) made similar observations for pumas although a more recent study suggests prey availability and not land tenure control puma density (Pierce, Bleich and Bowyer 2000). The regulatory impact of prey on cheetah density requires further assessment as the cheetah-prey relationship in areas characterized by minimal interspecific competition needs proper assessment.

Apart from the present study, only one other study assessed trends in cheetah density for the same population (Durant *et al*. 2011). However, studies differ as Durant *et al.* (2011) assessed the applicability of distance sampling over three time periods, 10 and 20 years apart (i.e. 1975, 1985 and 2005) for cheetahs in the Serengeti whereas here we focused on RCT and on inter-annual variation. In addition, in the Serengeti, interspecific competition and not anthropogenic factors influence cheetah dynamics the most (Durant *et al*. 2000; Marker *et al.* 2003; Bisset and Bernard 2012) with shifts in density and distribution varying across seasons and vegetation forms (Durant *et al*. 2011). As highlighted earlier cheetahs in our study are stable year-around possible due to suitable habitat (Marker *et al.* 2008b). Despite, these differences, no significant change were detected for cheetahs in the Serengeti (Durant *et al.* 2011). Altogether, both techniques show potential with non-invasive techniques such as RCT possible being more applicable for areas where individuals are more elusive, as in Namibia. Given the lack of significant differences between surveys (and seasons) surveys we recommend surveys at similar spatial scales as for this study to be conducted every 1-3 years (see also Durant *et al.* 2011).

Conclusion and conservation implications

Our findings not only provide the first robust longitudinal cheetah density estimates using RCT and spatial capture-recaptures, but also contribute relevant information on the spatial and temporal patterns of utilisation of scent marking sites by Namibian cheetahs. Results support characteristics of a resident male and possible breeding population (females with cubs) whose density was similar across surveys (5 - 20 km^{-3}). Furthermore, land tenure may play role in regulating male density, affect lifetime reproductive success as lasts within breeding age, and resident cheetahs' exhibit spatial overlap in the utilization of scent marking sites, some of which are outside their home ranges. Our findings further demonstrate the utility RCT for monitoring programs of short to medium-term duration to understand the ecological and behavioural aspects regulating cheetah populations and the inconsistencies of using conventional methods for estimating density. For future studies, we recommend that similar sampling designs are used but most importantly, that sampled areas encompass several home ranges, as this is likely to improve the quality and reliability of the inferred demographic parameters. This should preferably be in combination with genetic based surveys. The role of avoidance mechanisms and role of land tenure system on population dynamics including breeding density merits further investigation.

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Figure legends:

Fig. 1. Location of the study area in Namibia with annual trapping areas for 2007 and 2008, the home ranges of four collared male cheetahs (95% minimum convex polygons; labelled as GSM MCP) and the camera trap stations where all cheetahs (*n* = 54) were detected during the study (labelled 1 through 17). Collared males are identified by AJU (*Acinonyx jubatus* ID#).

Fig. 2a. Demographic structure of cheetahs captured per survey in Namibia between 2005 and 2011 using remote camera traps as a proportion per social group (S = summer and W = winter).

Fig. 2b. Sex ratio of adult cheetahs captured per survey in Namibia between 2005 and 2011 using remote camera traps (S = summer and W = winter).

Fig. 3. Cumulative numbers of unique cheetah individuals captured in Namibia during the 10 camera trapping surveys (S= Summer and W = Winter) and used for abundance and density estimations.

Fig. 4. Spatial distribution of scent marking sites in relation to collared individuals kernel home ranges (AJU = *Acinonyx jubatus* ID#). Kernel home ranges are

categorized into core (45%), intermediate (80%) and border (99%) regions. Circled and not circle black squares indicate stations utilized or not by individuals, respectively.

Fig. 5. Cheetah density estimates per 1000 km^2 in Namibia based non-spatial and spatial methods (SRC). Non-spatial include estimating the effective sampled area by buffering the camera trapping polygon with the full mean maximum distance moved (FMMDM), the 95% home range radius from four radio collared cheetahs in this study (GSM) and that from a previous study (VHF) as well as a minimum density based on the 95% minimum convex polygon (GSM polygon).

Table 1. Surveying effort for the 10 camera trap surveys (Namibia, 2005 – 2011) including the season and months when sampling occurred, the total number of days between surveys, the area surveyed, the number of stations, the number of usable cheetah photographs, the capture rates (number of photos/100 trap nights) , and the absolute and cumulative number of unique adult and total (i.e. including cubs) individuals detected per survey.

Table 2. Trends of visitation rates to camera-trap stations by resident cheetahs captured on multiple surveys with station numbers corresponding to those presented in Fig. 1.

Table 3. Tests for population closure using programs CloseTest and CAPTURE, CAPTURE best (second best) abundance model selected, number of adult individuals captured and the probability of capturing an individual across the survey, for the 10 surveys (2005 - 2011).

Table 4.

Survey	Season	Days between consecutive surveys	Trap array size (km^2)	No. of stations	No. of trap nights[a]	No. of cheetah photographs	No. cheetah photos/ 100 trap nights	No. of new unique adult cheetahs caught	Cumulative No. unique adult cheetahs	Total No. of unique cheetahs caught	Cumulative No. total cheetahs detected
Survey 1	Summer[1]		277	15	1260	420	33	9	9	11	11
Survey 2	Winter[2]	507	477	19	1710	175	10	9	18	9	20
Survey 3	Winter[2]	274	370	15	1350	210	16	8	26	11	31
Survey 4	Winter[2]	272	379	16	1350	177	13	1	27	4	35
Survey 5	Winter[2]	271	384	15	1350	279	21	2	29	2	37
Survey 6	Summer[3]	0	384	15	1350	357	26	4	33	9	46
Survey 7	Summer[4]	0	384	15	1350	256	19	0	33	0	46
Survey 8	Summer[5]	0	384	15	1350	259	19	2	35	2	48
Survey 9	Winter[3]	0	384	15	1350	207	15	2	37	2	50
Survey 10	Summer[4]	0	384	15	1350	256	19	2	39	4	54

[1] November - February, [2] July - October, [3] July - September, [4] October-December, [5] January - March; [6] March - June , [a]assumes sampling effort was constant between camera stations or sampling occasions or between surveys (*see discussion*)

Table 5.

Year	Stations/ Individuals ID	3	4	5	6	9	10	11	12	13	14	15	17	18	23	24	25	26
2005-06	AJU1459** and 1460**					1	5	45	3	3								
2007						X	5	6										
2005-06	M1		1	6														
2007		3	+	2														
2007	AJU1533								6		6	2	17	1				
2008									4		5	7	12		1			
2009								2					1					
2008	AJU1542** and 1543										3				1	2		
2009									2		24		12				11	
2010-1					4						14	2	10					
2010-2		1									34		17					
2010-3											21	1	10					
2011-1											29		11					
2011-2				3	1				2		24		23					
2011-3											15		13					
2008	AJU1537 and 1538						2	8	1									
2009								8										5
2010-1							2	13	1		1		2					4
2010-2							1	16	2		3	1	2					
2010-3								14										2
2011-1								10	1			1						2
2011-2								4										1
2011-3								4										
2011-1	M2										3							
2011-3											1		1					

X indicates that the station was discontinue, + that individual was not detected on a station where it was previously captured, and ** that individual died (AJU1459, AJU1460 and AJU1542 died in 2006, 2007 and 2010-1, respectively).

Table 6.

Survey	CloseTest (χ^2, *d.f.*, *p*)	CAPTURE			
		Closure test (*Z*, *p*)	Best *N′* model	M_{t+1}	$1-(1-\hat{p})^{15}$
Survey 1	**476.42, 13, <0.001**	0.85, 0.80	M_h 1 (M_o 0.96)	9	0.96
Survey 2	18.11, 13, 0.5	2.44, 0.99	M_h 1 (M_o 0.95)	11	0.85
Survey 3	**25.87, 13, 0.02**	-0.36, 0.36	M_{th} 1 (M_t 0.99)	9	0.96
Survey 4	**1120.00, 13, <0.001**	0.78, 0.78	M_h 1 (M_o 0.95)	5	1
Survey 5	**17.69, 4, 0.001**	**-2.17, 0.02**	M_{th} 1 (M_t 0.73)	5	1
Survey 6	**76.52, 13, <0.001**	**-1.77, 0.04**	M_h 1 (M_{tbh} 0.98)	8	0.86
Survey 7	n/a	**-1.97, 0.03**	M_o 1 (M_h 0.94)	3	1
Survey 8	n/a	0.17, 0.57	M_o 1 (M_h 0.95)	5	1
Survey 9	**96.49, 13, <0.001**	-0.24, 0.41	M_o 1 (M_h 0.93)	5	1
Survey 10	n/a	-1.23, 0.11	M_h 1 (M_o 0.99)	5	0.98

Bold indicates test for closure is significant. Abundance (*N′*) models include the M_o = null model, M_h = jacknife heterogeneity model, M_{th} = Chao's time and heterogeneity model, M_t = time model, and M_{tbh} = time, behaviour and heterogeneity model (Otis *et al.* 1978).

Fig. 1

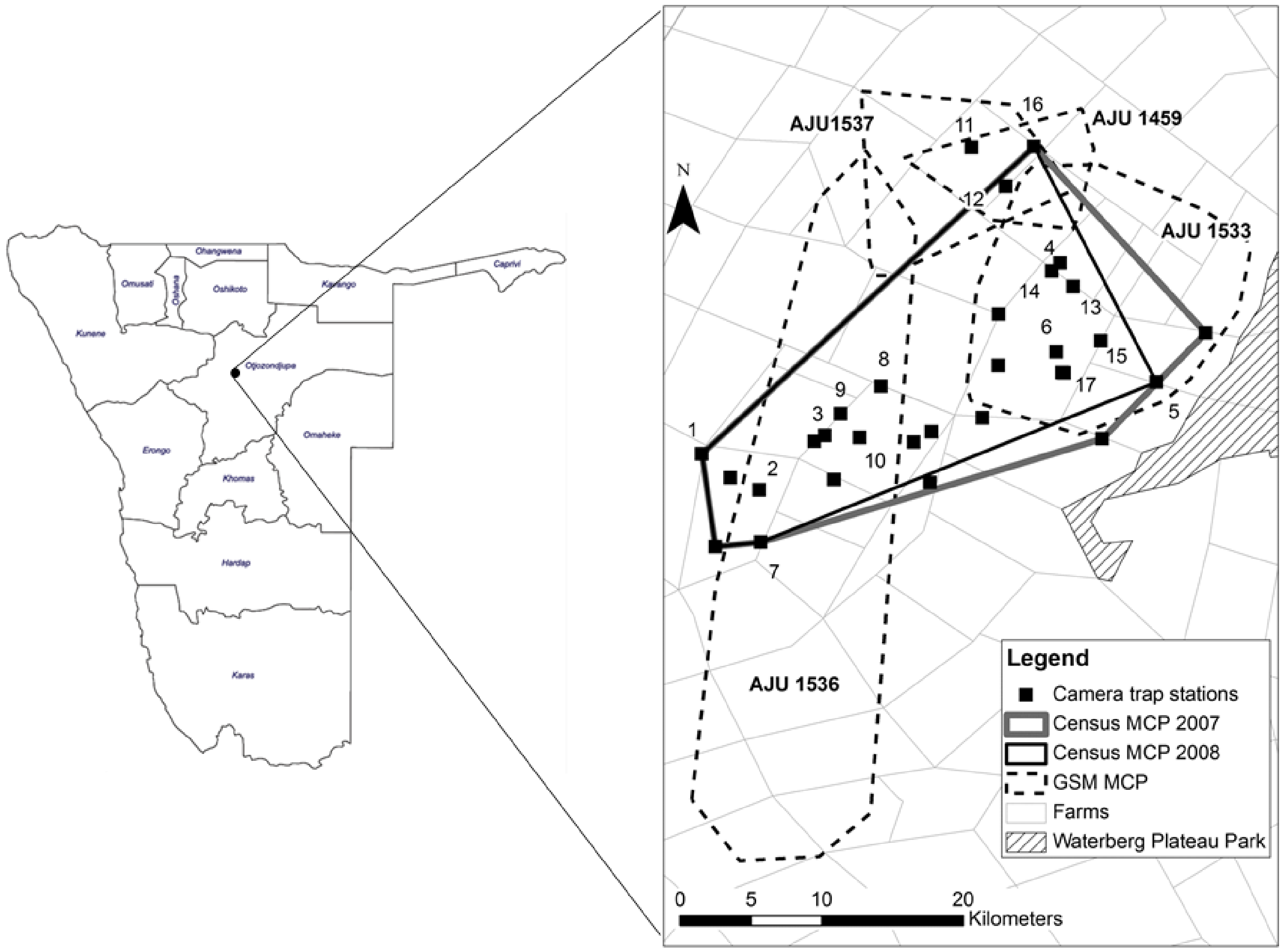
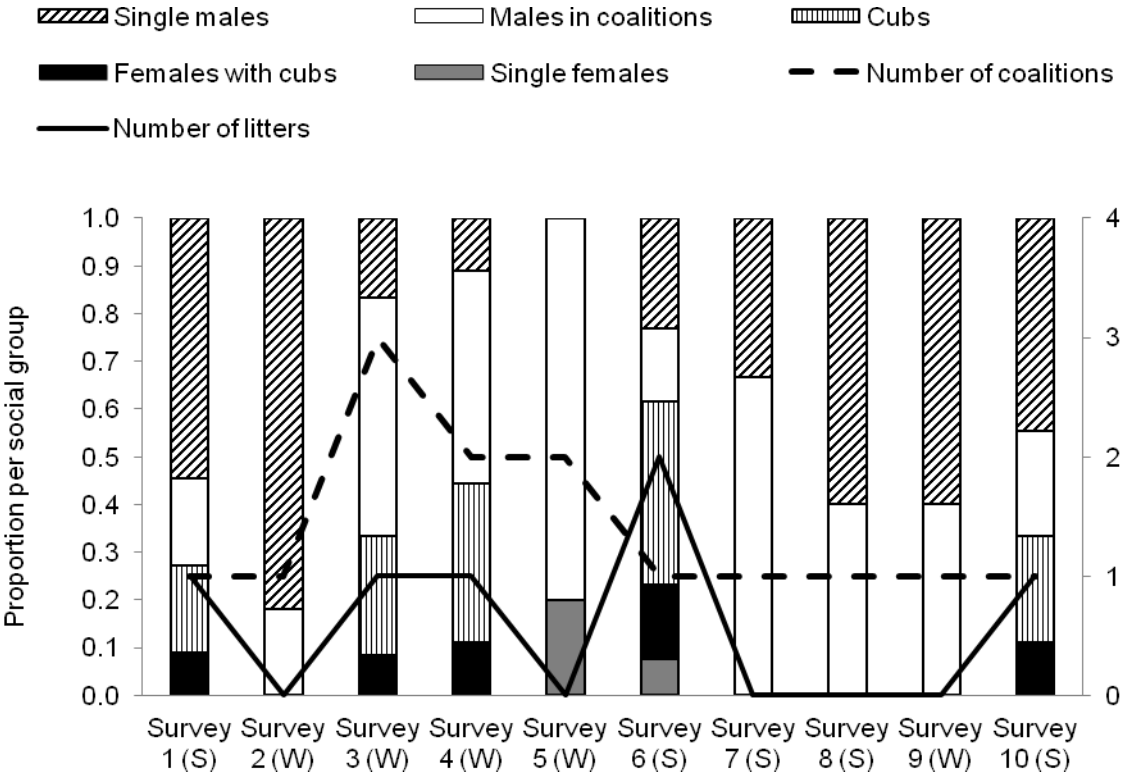

Ohangwena
Omusati
Oshana
Oshikoto
Kavango
Caprivi
Kunene
Otjozondjupa
Omaheke
Erongo
Khomas
Hardap
Karas
N
AJU1537
AJU 1459
AJU 1533
AJU 1536
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Legend
■ Camera trap stations
Census MCP 2007
Census MCP 2008
GSM MCP
Farms
Waterberg Plateau Park
0
5
10
20
Kilometers

Fig. 2a



Single males
Males in coalitions
Cubs
Females with cubs
Single females
Number of coalitions
Number of litters
Proportion per social group
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Number of coalitions or litters
4
3
2
1
0
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)

Fig. 2b

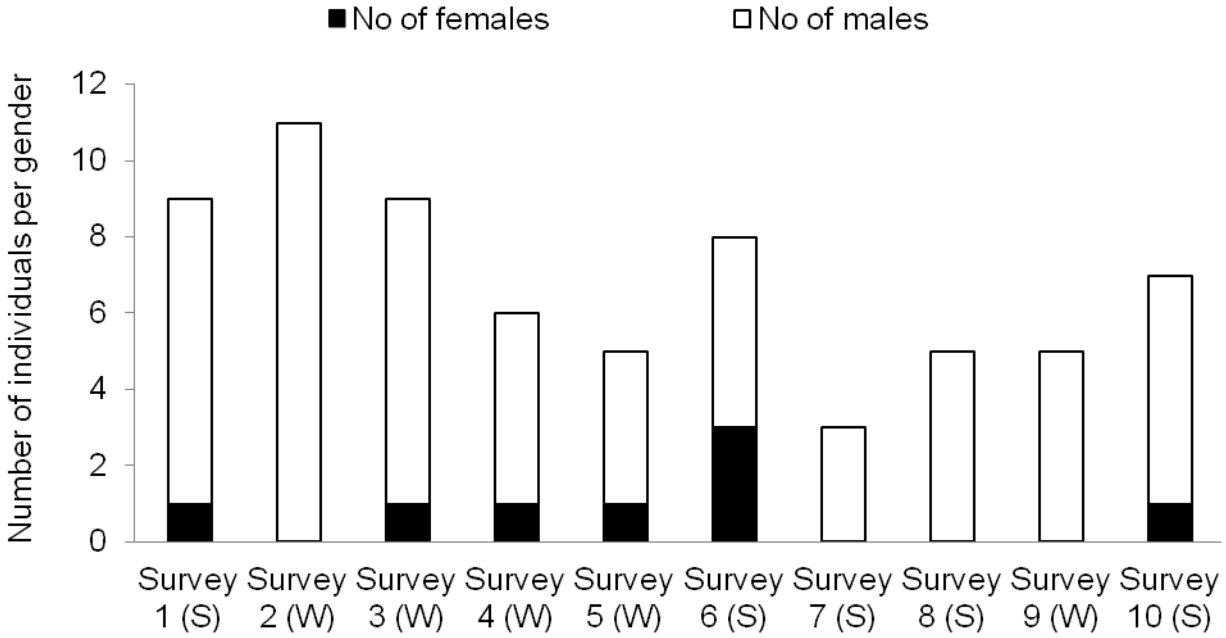

■ No of females
□ No of males
Number of individuals per gender
0
2
4
6
8
10
12
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)

Fig. 3

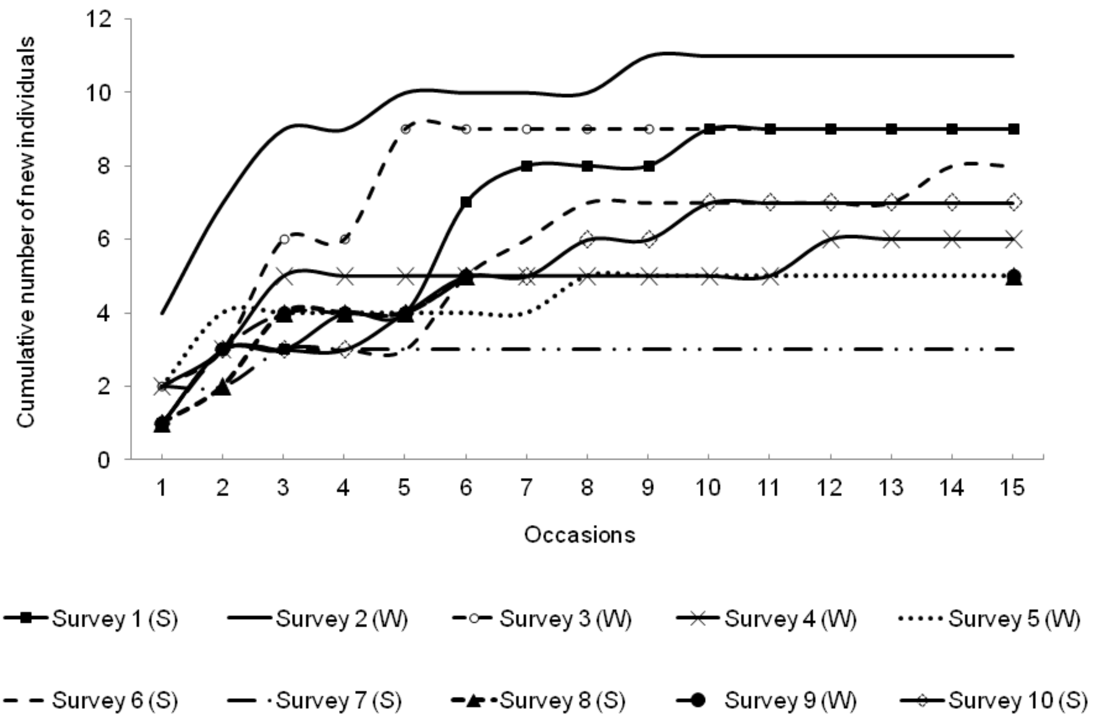

Cumulative number of new individuals
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Occasions
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)

Fig. 4

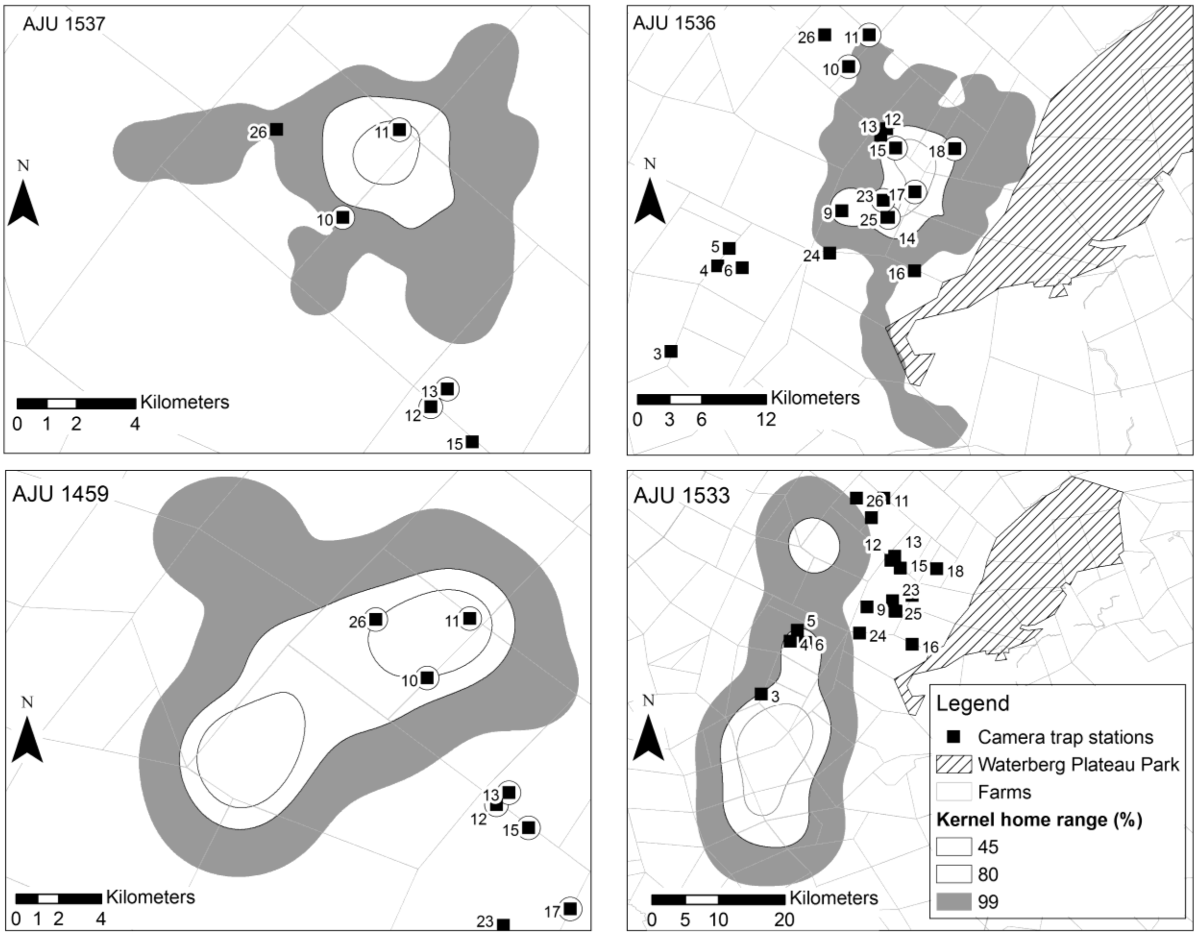
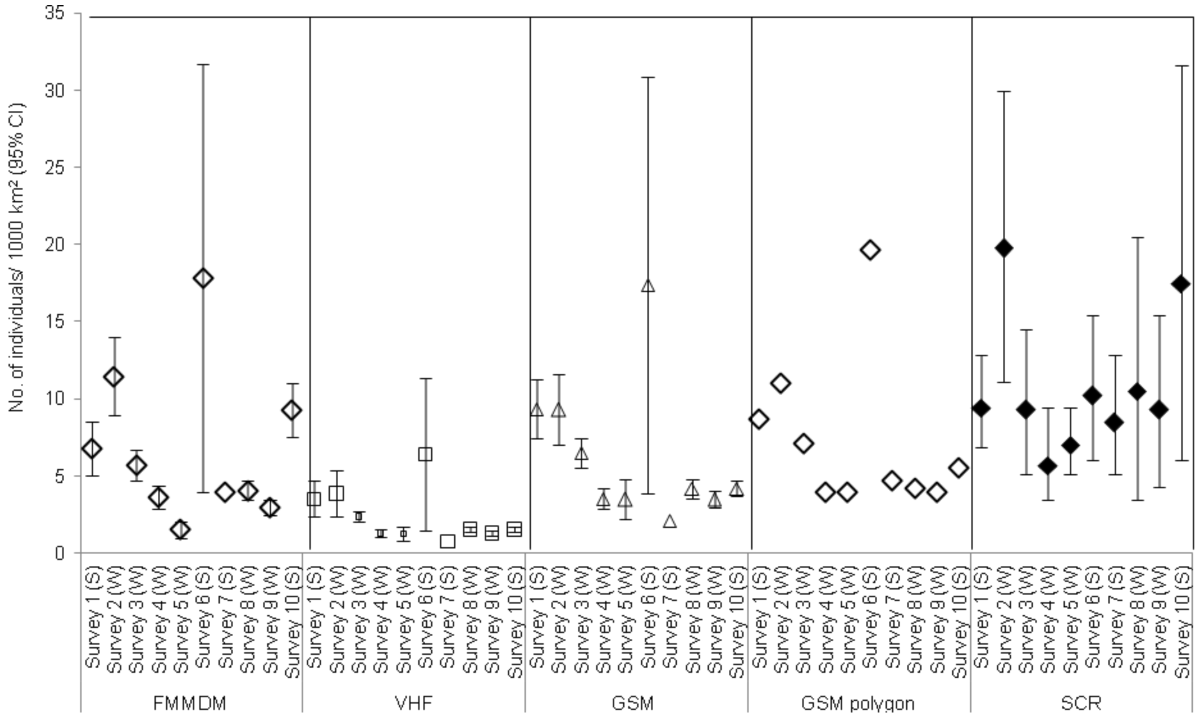

AJU 1537
26
11
10
13
12
15
N
Kilometers
0 1 2 4
AJU 1536
26
11
10
13
12
15
18
23
17
9
25
14
5
4
6
24
16
3
N
Kilometers
0 3 6 12
AJU 1459
26
11
10
13
12
15
17
23
N
Kilometers
0 1 2 4
AJU 1533
26
11
12
13
15
18
23
9
25
5
4
6
24
16
3
N
Kilometers
0 5 10 20
Legend
■ Camera trap stations
▨ Waterberg Plateau Park
☐ Farms
Kernel home range (%)
☐ 45
☐ 80
■ 99

Fig. 5


No. of individuals/ 1000 km² (95% CI)
0
5
10
15
20
25
30
35
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (W)
Survey 9 (W)
Survey 10 (S)
FMMDM
VHF
GSM
GSM polygon
SCR

SUPPLEMENATRY INFORMATION S1

Figure S1. Location of the study area in Namibia with annual trapping areas 2005 through 2011, with the minimum convex polygons of the four collared male cheetahs and the stations where different cheetahs were detected during the study (labelled 1 through 17).

Figure S2 Estimates and posterior summary for cheetah capture probability, population size estimate and activity centers, buffer width and range movement parameter, and density estimates for the 10 three-month surveys (Namibia: 2005 - 2011). Precision for CAPTURE and spatial capture recapture estimates are presented with 95% confidence intervals determined using standard error and deviation, respectively. Estimates were considered to be statistically significant at $\alpha = 0.05$ if 95% high posterior distribution (HPD) or confidence intervals (CI) did not overlap by more than half. **(A)** Capture probabilities derived using the CAPTURE (*p-hat*) and SRC parameter that indicates the mean posterior distribution if the camera trap is located an activity center (λ), which was converted into a capture probability using 1-exp(-λ). **(B)** Capture probability prior and post initial encounter. **(C)** Abundance estimates based on CAPTURE abundance best models (Mh, Mth or Mtbh, see Table 3) and the SRC posterior estimate of N, the number of activity centers in region S. **(D)** PSI, the probability of an individual in the augmented population being part of the sampled population. **(E)** Buffer width estimates for the: Global System for Mobile (GSM), Very High Frequency (VHF), full mean maximum distance moved (FMMDM), SRC animal movement parameter which approximates a home range radius (s ~ HR) and actual SRC s posterior mean. The solid line represents the average 95% GSM HR radius.

Figure S1

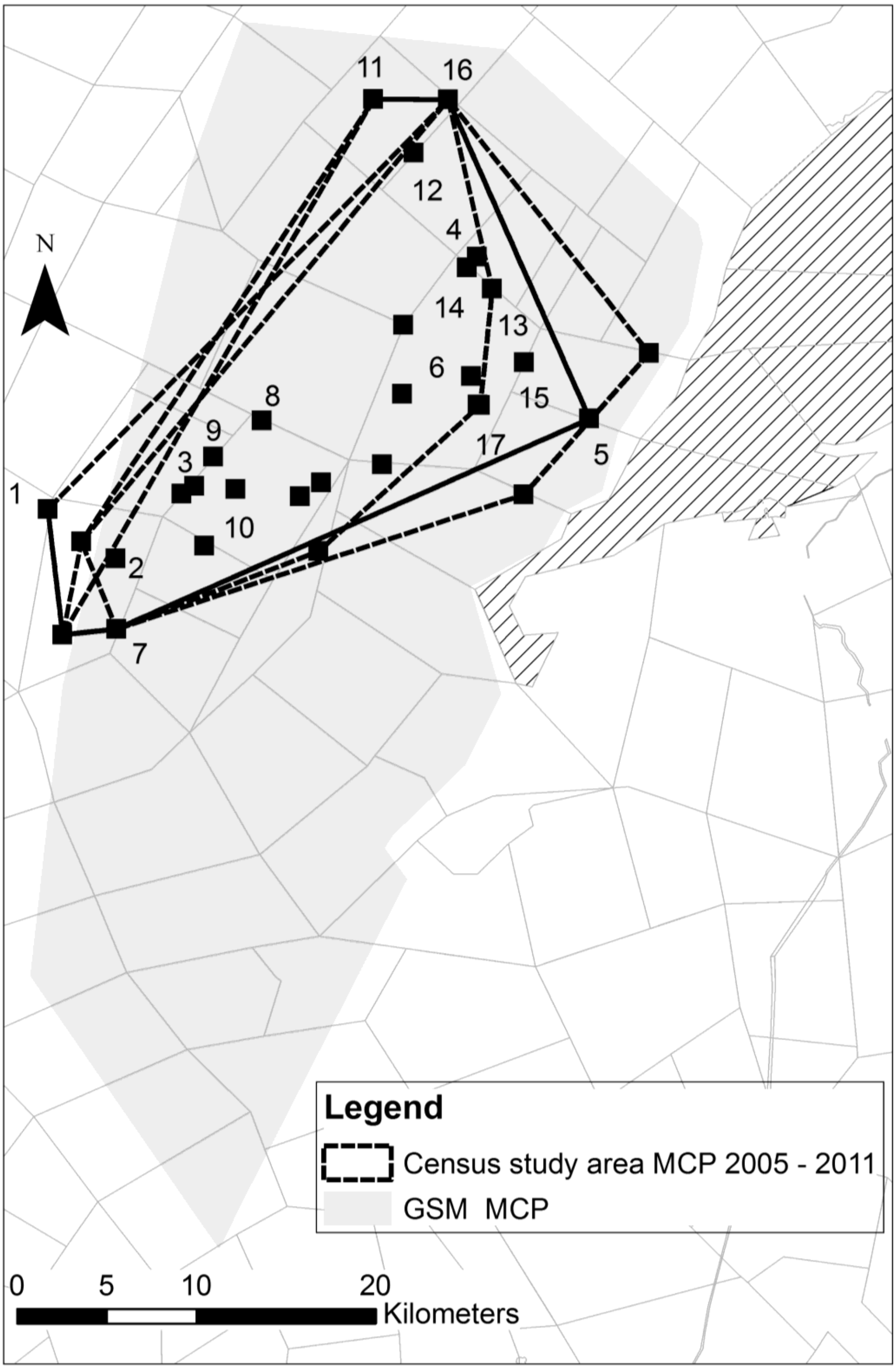

11
16
12
4
14
13
6
15
8
9
17
5
3
1
10
2
7
N
Legend
Census study area MCP 2005 - 2011
GSM MCP
0
5
10
20
Kilometers

Figure S2A

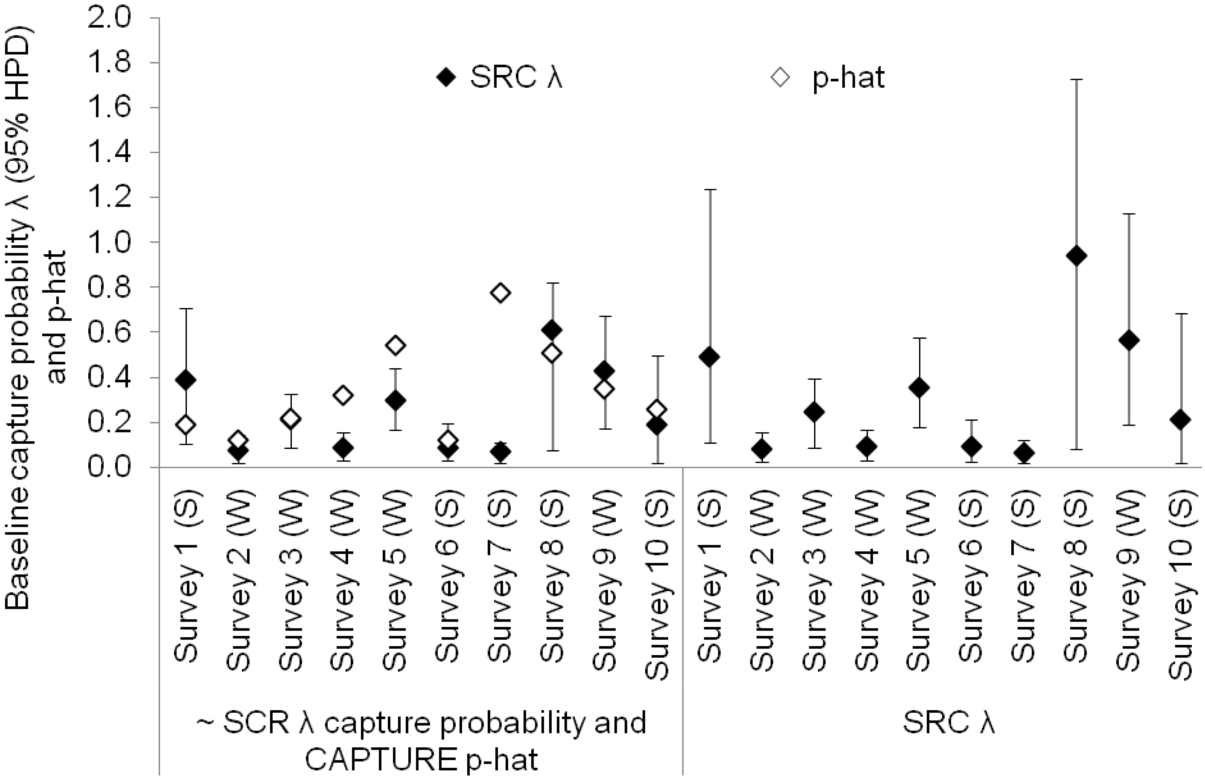

Baseline capture probability λ (95% HPD) and p-hat
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
◆ SRC λ
◇ p-hat
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)
~ SCR λ capture probability and CAPTURE p-hat
SRC λ

Figure S2B

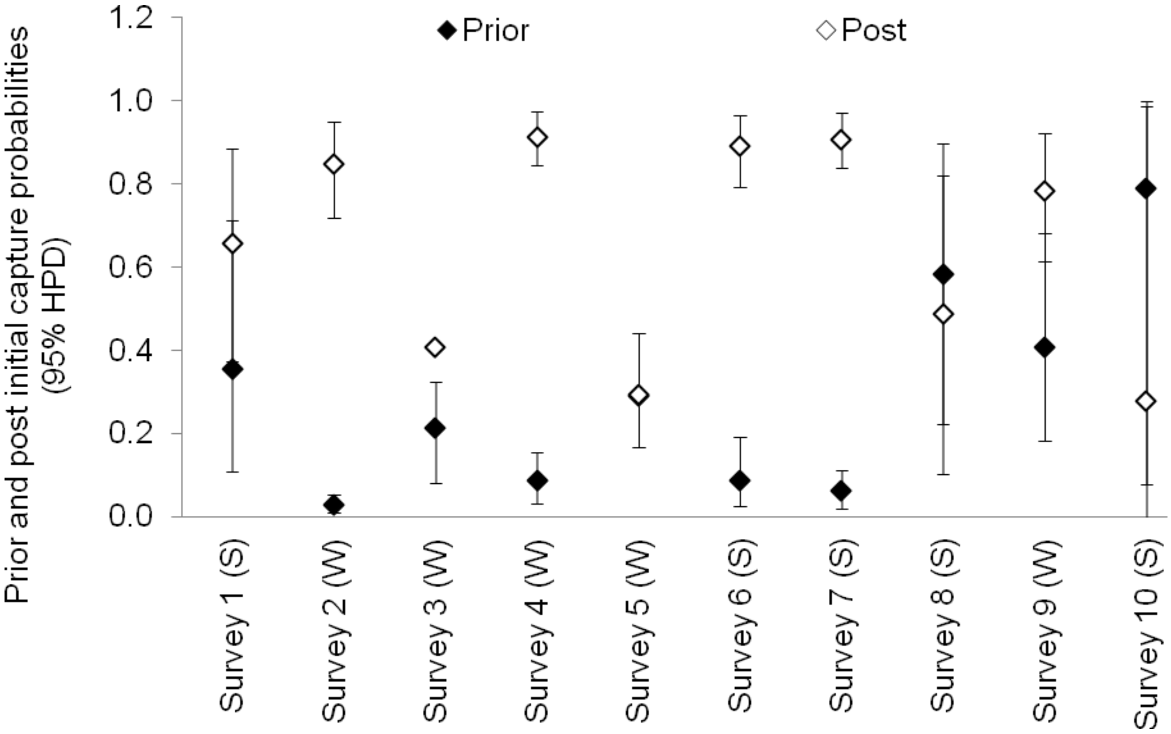

Prior and post initial capture probabilities (95% HPD)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
◆Prior
◇Post
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)

Figure S2C

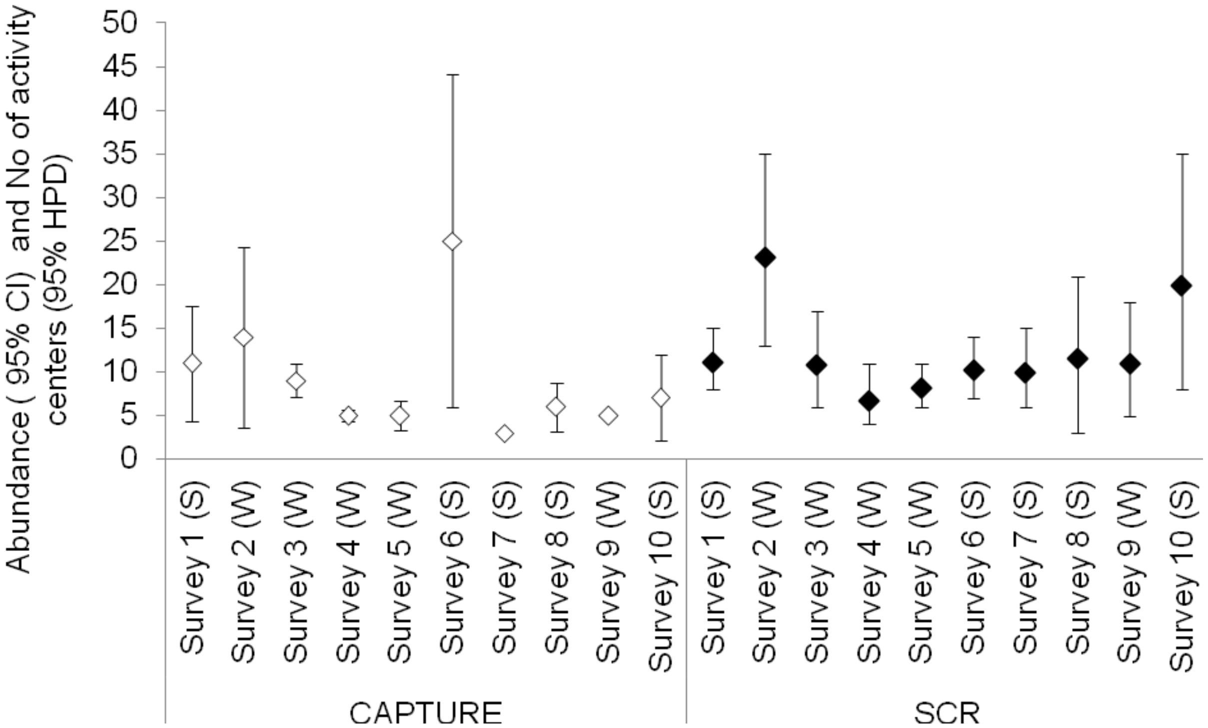

Abundance (95% CI) and No of activity centers (95% HPD)
50
45
40
35
30
25
20
15
10
5
0
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)
CAPTURE
SCR

Figure S2D

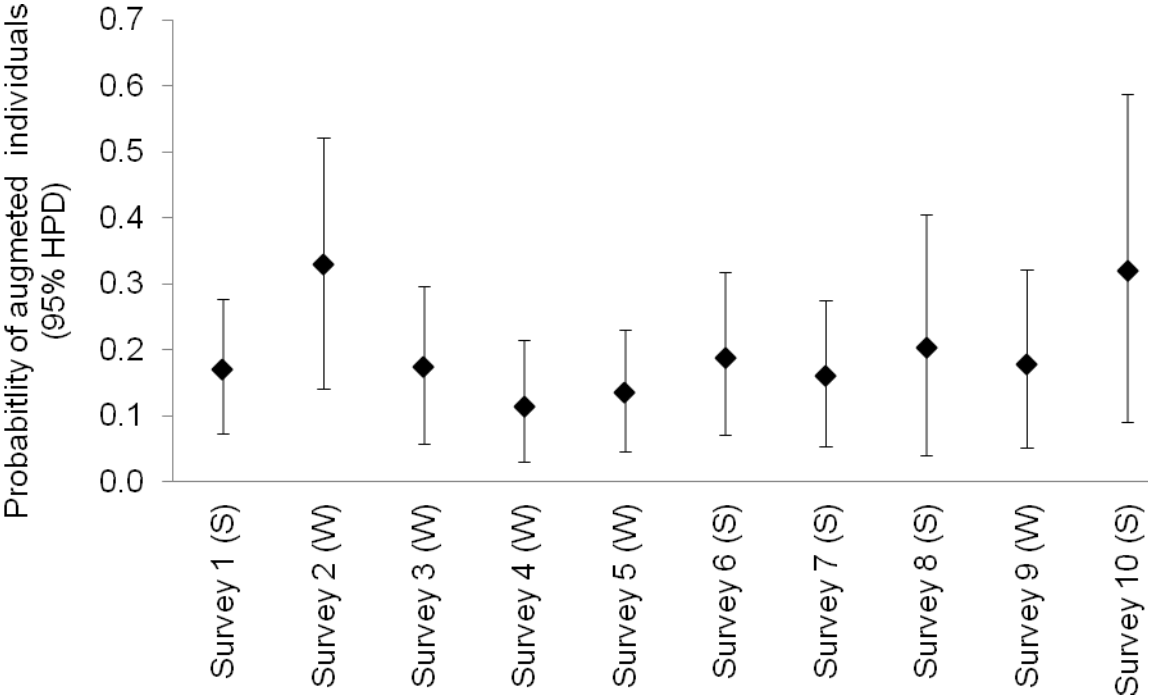
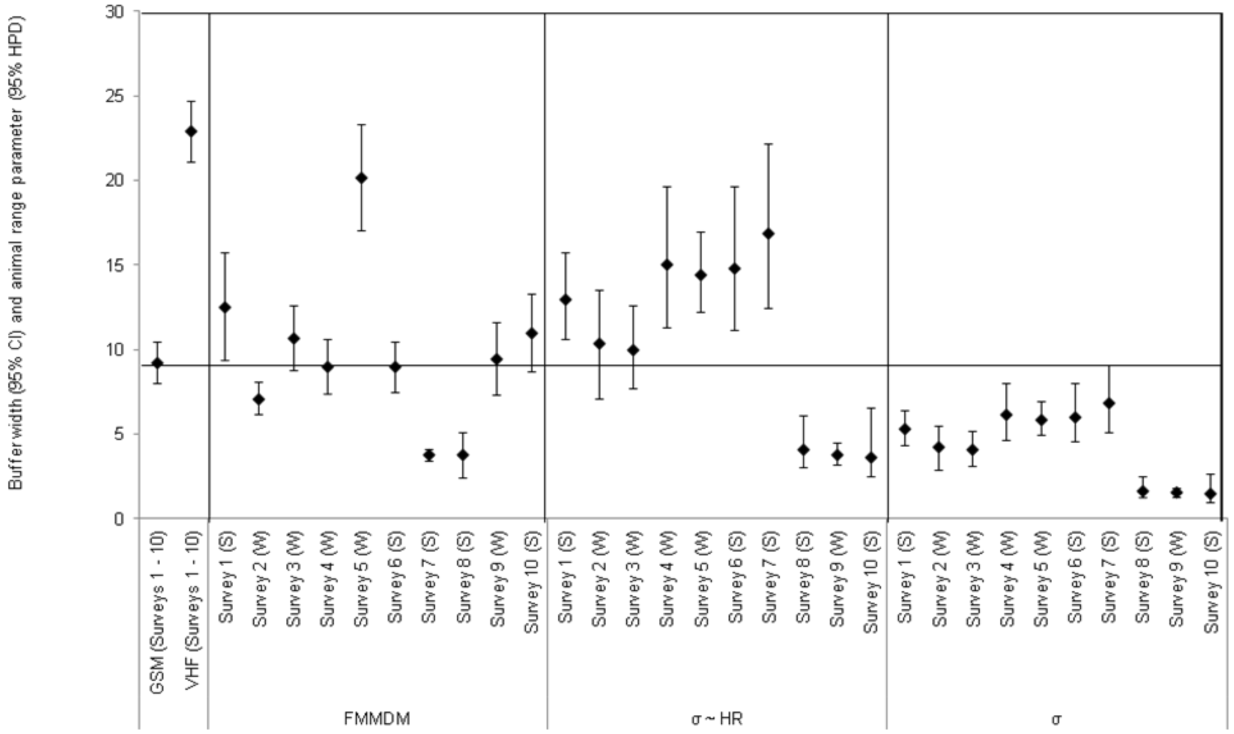

Probabitlity of augmeted individuals
(95% HPD)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)

Figure S3E


Buffer width (95% CI) and animal range parameter (95% HPD)
30
25
20
15
10
5
0
GSM (Surveys 1 - 10)
VHF (Surveys 1 - 10)
Survey 1 (S)
Survey 2 (W)
Survey 3 (W)
Survey 4 (W)
Survey 5 (W)
Survey 6 (S)
Survey 7 (S)
Survey 8 (S)
Survey 9 (W)
Survey 10 (S)
FMMDM
σ ~ HR
σ

Captions for Tables

Table S1 Cheetah density estimates per 1000 km^2 in Namibia based non-spatial and spatial methods (SRC). Non-spatial include estimating the effective sampled area by buffering the camera trapping polygon with the full mean maximum distance moved (FMMDM), the 95% home range radius from four radio collared cheetahs in this study (GSM) and that from a previous study (VHF) as well as a minimum density based on the 95% minimum convex polygon (GSM polygon).

Table S2 The 95% minimum convex polygon (MCP) and kernel home range estimates of the four GSM collared with period tracked and GPS fixes.

Table S1

Survey (Season)	Density (± SE) /1000km^2 (95% CI)				
	FMMDM	VHF	GSM	GSM Polygon	SCR*
Survey 1 (S)	6.77 ± 1.72 (5.05, 8.49)	3.51 ± 1.15 (2.36, 4.66)	9.32 ± 1.9 (7.42, 11.22)	8.65	9.425 ± 1.733 (6.838, 12.821)
Survey 2 (W)	11.15 ± 2.54 (8.61, 13.69)	3.87 ± 1.47 (2.4, 5.34)	9.27 ± 2.29 (6.98, 11.56)	11.01	19.772 ± 5.372 (11.111, 29.915)
Survey 3 (W)	5.67 ± 0.98 (4.69, 6.65)	2.37 ± 0.29 (2.08, 2.66)	6.5 ± 0.97 (5.53, 7.47)	7.08	9.263 ± 2.626 (5.128, 14.53)
Survey 4 (W)	3.6 ± 0.75 (2.85, 4.35)	1.29 ± 0.22 (1.07, 1.51)	3.53 ± 0.67 (2.86, 4.2)	3.93	5.658 ± 1.881 (3.419, 9.402)
Survey 5 (W)	1.5 ± 0.56 (0.94, 2.06)	1.28 ± 0.47 (0.81, 1.75)	3.47 ± 1.3 (2.17, 4.77)	3.93	7.016 ± 1.484 (5.128, 9.402)
Survey 6 (S)	17.82 ± 13.9 (3.92, 31.72)	6.38 ± 4.93 (1.45, 11.31)	17.36 ± 13.49 (3.87, 30.85)	19.67	10.221 ± 2.764 (5.983, 15.385)
Survey 7 (S)	3.97	0.77	2.08	4.72	8.47 ± 2.344 (5.128, 12.821)
Survey 8 (S)	4.05 ± 0.63 (3.42, 4.68)	1.53 ± 0.19 (1.34, 1.72)	4.17 ± 0.62 (3.55, 4.79)	4.2	10.505 ± 4.989 (3.419, 20.513)
Survey 9 (W)	2.94 ± 0.53 (2.41, 3.47)	1.28 ± 0.16 (1.12, 1.44)	3.47 ± 0.52 (2.95, 3.99)	3.93	9.301 ± 3.196 (4.274, 15.385)
Survey 10 (S)	9.26 ± 1.72 (7.54, 10.98)	1.53 ± 0.2 (1.33, 1.73)	4.17 ± 0.51 (3.66, 4.68)	5.51	17.454 ± 7.19 (5.983, 31.624)

*Density (± SD)/ 1000 km^2 (95% CI)

Table S2

Cheetah ID	Days tracked	Fixes	MCP (km^2)				Kernel (km^2)			
			95%	≥ 99%	80%	45%	95%	≥ 99%	80%	45%
AJU 1537	115	203	129	122	54	42	90.05	255	100	38
AJU 1533	226	1763	278	367	77	14	29.4	359	72	18
AJU 1459	70	510	68	63	10	2	9.7	75	16	4
AJU 1536	103	489	601	457	210	66	376.98	1104	414	132
Position of camera stations used by GSM collared individuals per home range category										
AJU 1537				5		2		4		3
AJU 1533				1	5	1		1	4	2
AJU 1459								2		1
AJU 1536				2	2				4	

Chapter V

Insights of temporal activity patterns of resident individual cheetahs, in north-central Namibia

Author list:

Ezequiel Chimbioputo Fabiano [1, 2]

Matti Nghikembua [2]

Eduardo Eizirik [1]

Laurie Marker [2]

Affiliations:

1. Laboratório de Biologia Genômica e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS 90619-900, Brazil

2. Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia. Fax: 264 67 306247

Email addresses:
ezequiel.fabiano@acad.pucrs.br, fabianoezekiel@gmail.com
ccfmatti@iway.na
eduardo.eizirik@pucrs.br
cheetah@iway.na

Corresponding authors:

Ezequiel C. Fabiano

Cheetah Conservation Fund,

P.O. Box 1755, Otjiwarongo, Namibia.

Fax: 264 67 306247

Author Contributions: ECF conceived, designed, performed data analyses, and wrote the manuscript; EE, LM and MN provided editorial advice.

Abstract

The activity patterns of cheetahs are largely unknown for populations where it coexists only with part of the large carnivore guilds. This is the case for the Namibian cheetah population residing on farmlands, where dominant predators, lions and spotted hyenas, are absent, but leopards and brown hyenas persist. In this study, we used photographic captures of 10 three months surveys conducted between 2005 and 2011 (five in winter and summer), to describe the temporal activity patterns of cheetahs across seasons at least to scent marking sites. This is the first insight into inter-individual interactions. A strong nocturnal and crepuscular activity pattern was retrieved which was consistent across seasons essentially for males as females had a low sample size. At the individual level, activities patterns between a resident single male and a coalition of two male, overlapped but differed in the time of activity peak, with the latter increasing in activity at times when the former declines. This suggests that movements by males and at least in respect towards scent marking sites occurs during nighttime. Ultimately, these findings contribute to the growing evidence of cheetahs being nocturnal across its range.

Introduction

Activity patterns represent periods of time when living organisms engage on different daily functions aimed at meeting their biological needs but fine-tuned to allow for flexible responses (Halle 2000). Different forms on temporal niche include diurnality (e.g. Andean cats *Leopardus jacobita* [Lucherini et al. 2009]), nocturnality (e.g. lions *Panthera leo* [Hayward and Slotow 2009]), crepuscularity (e.g. tiger *Cryptoprocta ferox* [Gerber et al. 2012]) or a combination of these as well as cathermal (e.g. leopards *P. pardus*) (see Hayward and Slotow 2009 for a meta-analysis on African large carnivores). Linkie and Ridout (2011) found tiger *Panthera tigris* crepuscular behaviour to overlap with that of its prey. This diversity within the temporal dimension alongside the spatial and food dimensions of the ecological niche provides a framework for understanding resource partitioning among sympatric species and individuals within a population (Schoener 1974).

The cheetah alongside wild dogs and brown hyena are sub-dominant to lions and spotted hyenas that exert an influence on the ecology of the formers (Mills 2005). In the Serengeti, cheetahs configure their spatial distribution as to minimize intraspecific competition with dominant large predators (Durant 2000). In turn, findings are less consistent regarding activity patterns. For example, based on observations (movements, kills) when coexisting with dominant predators cheetahs tend to exhibit a diurnal crepuscular behaviour (Durant 1998; Bothma and Walker 1999) with a daily peak activity occurring in late evening/afternoon (Hayward and Slotow 2009). This pattern was attributed to top-down effects (Hayward and Slotow 2009). In turn, in the Sahara Mountains, cheetahs increase activity during the cooler time of the day (nighttime) (Bothma and Walker 1999) as it is the case of cheetahs in the Okavango Delta (Cozzi et al. 2012). Cozi et al. (2012) also indicates cheetah activity to be influence by lunar cycles.

In Namibia, limited observations also indicate a nocturnal cheetah activity pattern (McVittie 1979; Marker et al. 2008a). However, the temporal activity patterns at the inter- or intra-sexual levels, remains largely unknown. Furthermore, cheetahs in Namibia farmlands only coexist with leopards and brown hyenas, as dominant predators are absent (Marker-Kraus et al. 1996). These ecological setting differs from that of most antecedent studies (e.g. Durant 2000; Hayward and Slotow 2009; Cozzi et al. 2012). This nocturnal activity pattern is considered to be a behavioural response to competitor release (McVittie 1979) or to minimize overlap with human activities (Hayward and Slotow 2009) and need to extend their temporal niche to meet their biological needs (starvation driven) (Cozzi et al. 2012). At the individual level, heterogeneity is has been linked to ecological factors such as patrolling territories boundaries by males at night (Grünewalder et al. 2012). Altogether, intraguild relationships are variable and influenced by ecological conditions (Hallew 2000; Mills 2005). To ascertain that this nocturnal pattern is ubiquitous additional studies are required, under similar or different ecological settings, which is the aim of this study.

Here, we provide insights on the temporal activity patterns of a cheetah population that coexist with an imcomplete large carnivore guild based on a six years remote camera trap dataset, 10 surveys five per summer and winter, respectivley. Specific objectives were (1) to quantify activity patterns per season, (2) compare activity patterns between seasons and (3) provide insights into inter-individual temporal activity patterns. While the study extends and complements antecedent studies (e.g. Durant 2000; Marker et al. 2008a; Hayward and Slotow 2009; Cozzi et al. 2012) it differs in a number of aspects including methodology, sample size, sampling duration, and ecological setting, but mostly by basing activity patterns on photo captures of individuals at scent marking posts. Understanding activity patterns in Namibia is of significance as it provides information regarding the species ecological niche information that can aid when devising conservation measures related to depredation.

Methods

STUDY DESIGN AND DATA COLLECTION

To assess species and individual cheetah temporal activity patterns, we used a remote camera trap (RCT) dataset of a 10 three months cheetah surveys conducted in north-central Namibia. The study area and design are fully described in Chapter IV (but also Marker et al. [2008a, b]). However, we provide a description of the study design that is of relevance for exploring activity patterns.

RCT surveys were conducted annually for three months from 2005 to 2009, and then continuously between July 2010 and December 2011, encompassing an average area of 377 km^2 (SD ± 41.15 km^2) (Fig. 1). To be consistent with our previous work (Marker, Fabiano and Nghikembua 2008), we stratified the continuous effort (i.e. 2010 through 2011 data set) into six surveys, of 90 days each. Although Namibia is considered to have three seasons, for the purpose of this study, surveys were grouped into two main seasons, Summer (Nov – May) and Winter (June – Oct). Overall, five surveys each were conducted during the summer and winter seasons. During the course of the study, 32 different sampling stations were deployed (15 to 19 per annum). Sixty-three percent (*n* = 20) of the stations were located at scent marking posts and 19% (*n* = 6) either next to roads/fence lines and in close proximity to cheetah captive facilities. Inter-camera distances were on average 17 km (± 9.24 km). We used DeerCamTM DC200 (DeerCam, Park Falls, WI, USA) and Bushnell Trophy (Trophy Cam 2009, Kansas, USA) cameras from 2005 to 2009 and 2010 through to 2011, respectively. In 2008, one station had a Moultrie M60 digital camera (Georgia, USA). We placed two cameras per station, mounted *ca.* 75 cm above the ground and 5 m apart. We checked camera sites every 2 to 5 days to ensure functionality, change film/memory card and/or batteries. For data management, pictures from developed films were logged into an Excel database, while CamTrap software (Harris *et al*. 2010) was used for memory cards.

Cheetahs individuals were identified manually based on unique spot patterns, which remain unaltered throughout their lifetime (Caro and Durant 1991). Gender was determined using cues such as the presence of visible genitals, ear tag position for individuals previously physically handled by CFF (i.e. right for males and left for females) or accompanying cubs. A cheetah was classified as either a cub (≤ 1 years old) or adult (> 1 year old); male or female; or as single, member of male coalition or breeding female (i.e. with accompanying cubs). We also classified individuals as resident if they were captured during at least two consecutive inter-annual surveys (i.e. the six surveys spaced equally apart) or during two consecutive stratified surveys within the 2010 - 2011 continuous survey (Caro and Collins 1987; Barlow et al. 2009). All other individuals were treated as non-resident. Likewise, we considered a site an integral component of an individual’s HR if it was visited continuously on all surveys in which the individual was detected. This consideration was limited to residents.

TEMPORAL AND SPATIAL UTILISATION PATTERNS OF CHEETAHS BASED ON RCT DATA

Temporal patterns for all detected cheetahs were determined as the percentage of photos taken per hour in a 24-hour cycle using time and date tags on photographs. Only photos taken more than a minute apart were included. While we recognize that this approach provides only preliminary findings as a one-minute difference between consecutives photos may introduce autocorrelation, this may not constitute a major concern when exploring activity patterns (Jim Anderson pers. comm.). The Kuiper's test was applied to test whether the distribution of photos throughout the day was uniform per season as implemented in the Circular R package (Agostinelli and Lund 2012). Furthermore, we subdivided the data into dawn (04:00 - 07:59), day (08:00 - 15:59), dusk (16:00 - 19:59) and night (20:00 - 03:59), and assessed for differences in proportion of visitation among these periods using a likelihood-ratio chi-square test in a contingency table (after Gerber et al. 2012). Duration of diel periods followed mainly Haywared and Slotow (2009). Conditioned on significance a partial chi-square was used to assess significant contribution of each diel period. Activity peaks were determined as hours with the highest proportion of photographs. In addition to activity patterns at population level, we also assessed interspecific overlap.

Results

TEMPORAL AND SPATIAL UTILISATION PATTERNS OF CHEETAHS BASED ON RCT DATA

The temporal activity patterns of resident cheetahs are presented in Fig. 2. Based on a total 1813 photos taken, 13%, 16%, 42% and 30% were at daylight, dusk, nocturnal and dawn (Fig. 2A). Hence, 46%, 42% and only 16% of the photos were taken during crepuscular, nocturnal and daylight periods, respectively. When considering across seasons even though fewer pictures were obtained in summer than in winter this difference was insignificant (n = 849 and 970, respectively; U = 0.293, $P > 0.05$). However, activity patterns were the same across seasons with cheetahs showing predominantly a nocturnal and crepuscular pattern ($\chi^2 = 12.26$, *d.f.* = 3, $P > 0.05$) (Fig. 2B, C). We noticed an opposite trend in the number of photos taken between 03:00 and 07:00, with an increase in winter and reduction in summer (Fig. 2D). Kuiper's test provided evidence that this distribution pattern of photos throughout the day (08:00 - 16:00) was nearly significant in summer (K = 1.75, $0.05 < P < 0.10$).

The few recaptures by non-residents limits the identification of temporal overlap at individual level (Fig S1). However, restricted to resident individuals there appears to be a certain degree of temporal overlap with differences in the time of peaks (Fig. S1A, D, F, G). For example, resident individual M13 shows a decline in the number of photos between mid-night and 08:00 (Fig S1G). Conversely, M17 and M18 two peaks only occurred at 08:00 (overlapping with that of M13) and at dusk (~ 18:00 - 19:00), an hour later than M13 peak.

Discussion

Cheetahs were found to exhibit a predominant nocturnal and crepuscular activity patterns throughout the year, a finding that corroborates previous studies (McVittie 1979; Bissett and Bernard 2006; Marnewick et al. 2008; Cozzi et al. 2012). Supporting evidence for this is the significant proportion of detections at camera sites during crepuscular and nighttime across surveys and seasons (Fig. 2). Peaks of activities across season were largely between 16:00 to 20:00, 20:00 to 00:00 and

00:00 to 08:00. This peak of activity in the late afternoon is similar to that of other studies (e.g. 18:00 - 21:00, 16:00 - 17:00) (Hayward and Slotow 2009). Hence, the crepuscular activity highlights the importance of this period possible for hunting while avoidance during hot periods for physiological reasons. However, our findings run counter the notion of cheetahs being predominantly diurnal.

Due to the biased male sex ratio, this activity pattern may pertain only to males and at least with respect to scent marking sites. This would imply that retrieved activity patterns might not to be representative of overall activity patterns of cheetahs (i.e. detected, undetected, females). However, the timing of the few photos for females were consistent with this pattern. Specifically these occured early morning (05:00 - 09:00, *n* = 7) but also once at 10:00 and twice in the early evening (19:00) (Fig. S1). Bissett and Bernard (2006) noted a similar pattern whereby the activity pattern of a cheetah male coalition but not of females overlapped with that of lions. Additionally, photos from RCT placed at waterholes indicate that visitation to waterholes also follow a nocturnal crepescular pattern (out of 156 only one photo was at between 08:00 and 16:00, unpublished data). Speed of four GSM collared males cheetah also indicated nearly no movement during daytime. Altogether, this indicates that cheetahs exhibit a nocturnal crepuscular pattern. Future studies with larger datasets should explore this further.

The strictly nocturnal pattern runs counter previous studies (but see Cozzi et al. 2012 for a similar conclusion). Discrepancies of conclusions could be due to ecological settings of the different studies. Unlike is the case in our study area, other studies were mosly based on data from protected areas where intraspecific competition is intense due to the presence of dominant predators such as lions and spotted hyenas (Eaton 1970; Durant 2000; Hayward and Slotow 2009). Hence, this activity pattern could be due to competitor release. Nevertheless, limited evidence is found on the literature supporting this hypothesis as it draws mostly on the observation of dominant competitors (lions, leopards, spotted and brown hyenas) being nocturnal (Hawyard and Slotow 2009). Furthermore, a recent study from a study area where cheetahs coexist with its main predators also retrieved a nocturnal (Cozzi et al. 2012). Their study also indicated that lunar cycles influence cheetah activity pattern and that this behaviour allows cheetahs to maximize hunting opportunities, thus the "starvation driven" hypothesis. Our study extends this conclusion, to include ecological activities related to scent marking as being mostly nocturnal. Cheetah photo-captures at waterholes also indicate nocturnal activity (unpublished data). These studies re-iterate that large carnivore nocturnal traits are deep embedded phylogenetically (Holt 2009) a case similar to rodents (Roll et al. 2006).

Alternatives hypothesis that could explain this activity pattern include behavioural responses to adaptation to local prey species or human activity patterns (foragining hypothesis) (Hayward and Slotow 2009). The foraging success hypothesis postulates that predators tend to be more active at times that coincide with their prey activity patterns (Hayward and Slotow 2009). Our data supports this hypothesis, as cheetahs activity pattern overlapped largely with that of its prey (e.g., oryx, kudu, eland, red hartebeest, warthog) based on photo-captures at cheetah scent-marking sites (Fig S2). Nevertheless, it also overlapped with that of other carnivores such as leopard and jackals (Fig S2). This overlap may be facilitated by the poor detection of cheetah scent by prey species and other carnivores due to the negligible amount of sulphur on cheetah urine that makes their scent odourless (unnoticeable) (Burger et al. 2006; Apps et al. 2012). Future studies, should explore the relation between lunar cycle and species activity patterns (as per Cozzi et al. 2012).

Lastly, this shift could be an adaptation to minimize overlap with human activity patterns. Such adjustments has been reported for other species such as lions in the Makgadikgadi Pans National Park, Botswana (Valeix et al. 2012) or tigers in the Chitwan National Park in Nepal (Carter et al. 2012) areas also characterized by human wildlife conflict. Brown bears (*Ursus arctos*) also adjust on a seasonal and daily basis to human activities (Ordiz et al. 2011). However, the low human density and distribution in Namibia renders direct levels of disturbance to be the unlikely sole cause of this shift as appears to be the case for tigers (Carter et al. 2012; Athreya et al*.* 2013). Nevertheless, further understanding of the role of anthropogenic factors as a causative factor requires a comparative study within protected areas Namibia (e.g. adjacent Waterberg Plateau). Overall, we argue that this temporal activity pattern is likely to be the result of interactions between carnivore guild composition, cheetah social structure, prey availability, human-induced factors underlined by evolutionary plasticity.

The differences in peak of activity among the different resident social groups, single versus male coalitions, with the latter exhibiting fewer captures than the former, suggests a possible role of social group on activity patterns. Eaton (1970) hypothesized that scent marking on cheetah served as a "time-plan", aimed at minimizing encounter rates among conspecifics. While differences on peak periods at individual level partly support this hypothesis, in our study, this is counfounded by the resident individuals' spatial organization. In other words, this temporal overlap was due to the simultaneous temporal utilization of different scent marking sites by resident individuals. We recommend future to explore further this aspect of temporal segregation based on a larger sample.

Overall, this study shows that the temporal activity pattern of cheetahs in north-central Namibia where it coexist with other large sympatric carnivores (leopard and brown hyena) to be largely nocturnal and crepuscular. Coexistence with conspecifics seems facilitated by temporal and spatial segregation, suggesting that scent marking may play a significant role in minimizing potential direct contact, the "time-plan" hypothesis of Eaton's (1970). The similarity in visitation rate to scent marking sites highlights the significance of these sites possible for resource defence or home range. Furthermore, the nocturnal activity part observed may exemplify niche conservatism (Holt 2009). The findings invoke the inclusion of temporal activity patterns in the conservation efforts essentially when devising human-wildlife conflict mitigation. We recommend future studies to explore simultaneously the spatial temporal activity patterns of sympatric predators and their prey, within and outside protected areas in Namibia.

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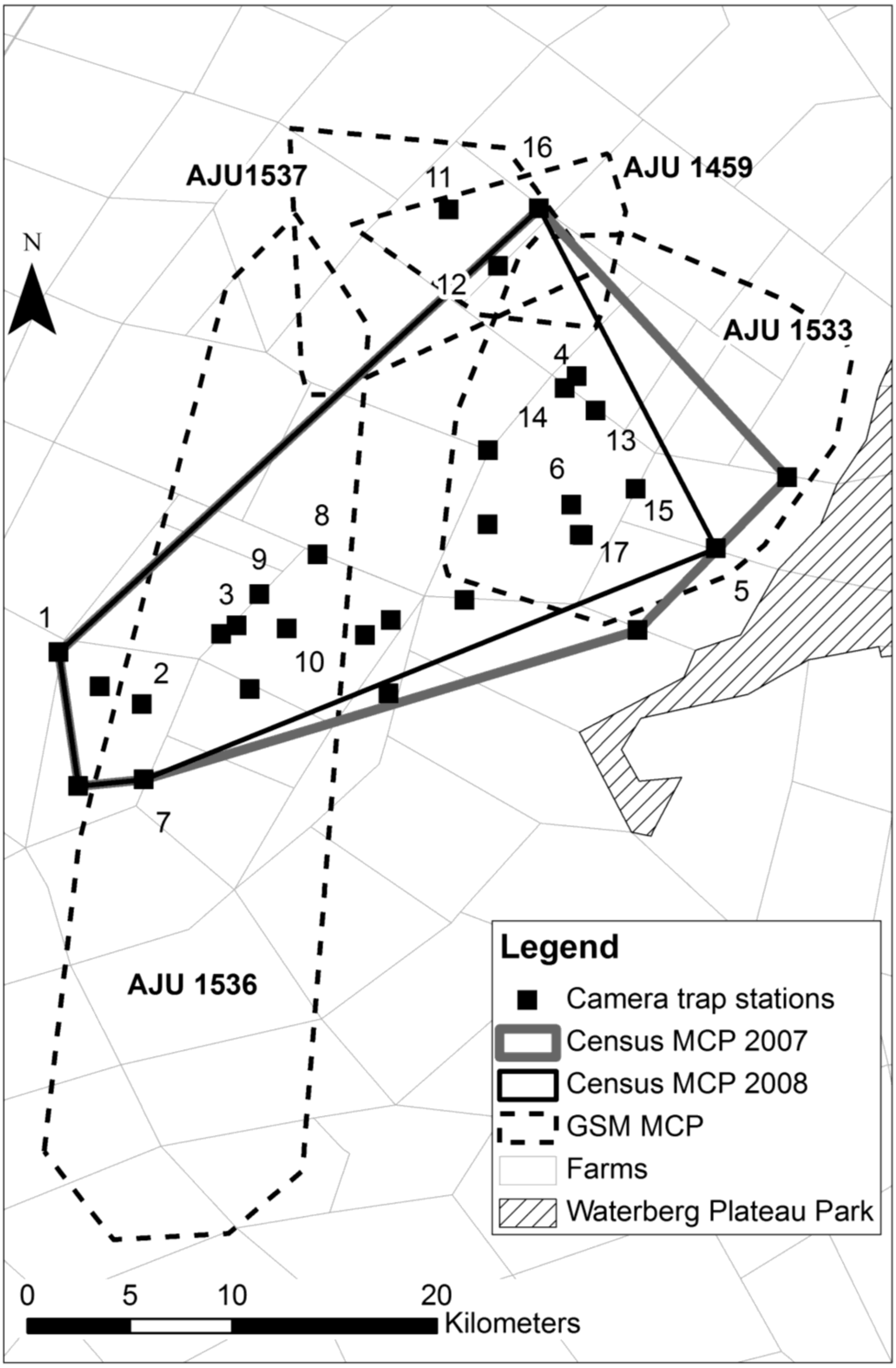
Figure legends:

Fig. 1. Location of the study area in Namibia with annual trapping areas for 2007 and 2008, the home ranges of four collared male cheetahs (95% minimum convex polygons; labelled as GSM MCP) and the camera trap stations where all cheetahs (n = 54) were detected during the study (labelled 1 through 17). Collared males are identified by AJU (*Acinonyx jubatus* ID#).

Fig. 2 Number of cheetah photographs per diel period per season (A), trends in the number of photos per hour for the 5 winter (B) and 5 summer surveys (C) and overall across seasons (D). Surveys were conducted between 2005 - 2011.


AJU1537
AJU 1459
AJU 1533
AJU 1536
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3
4
5
6
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9
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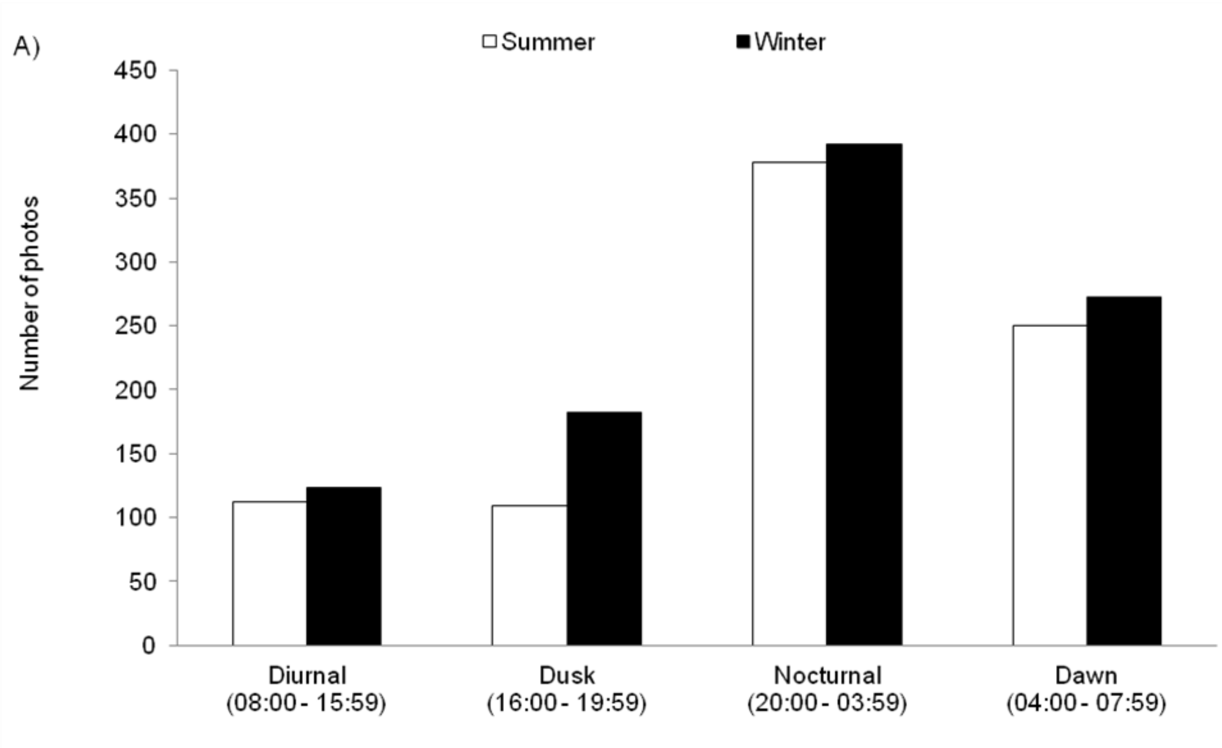
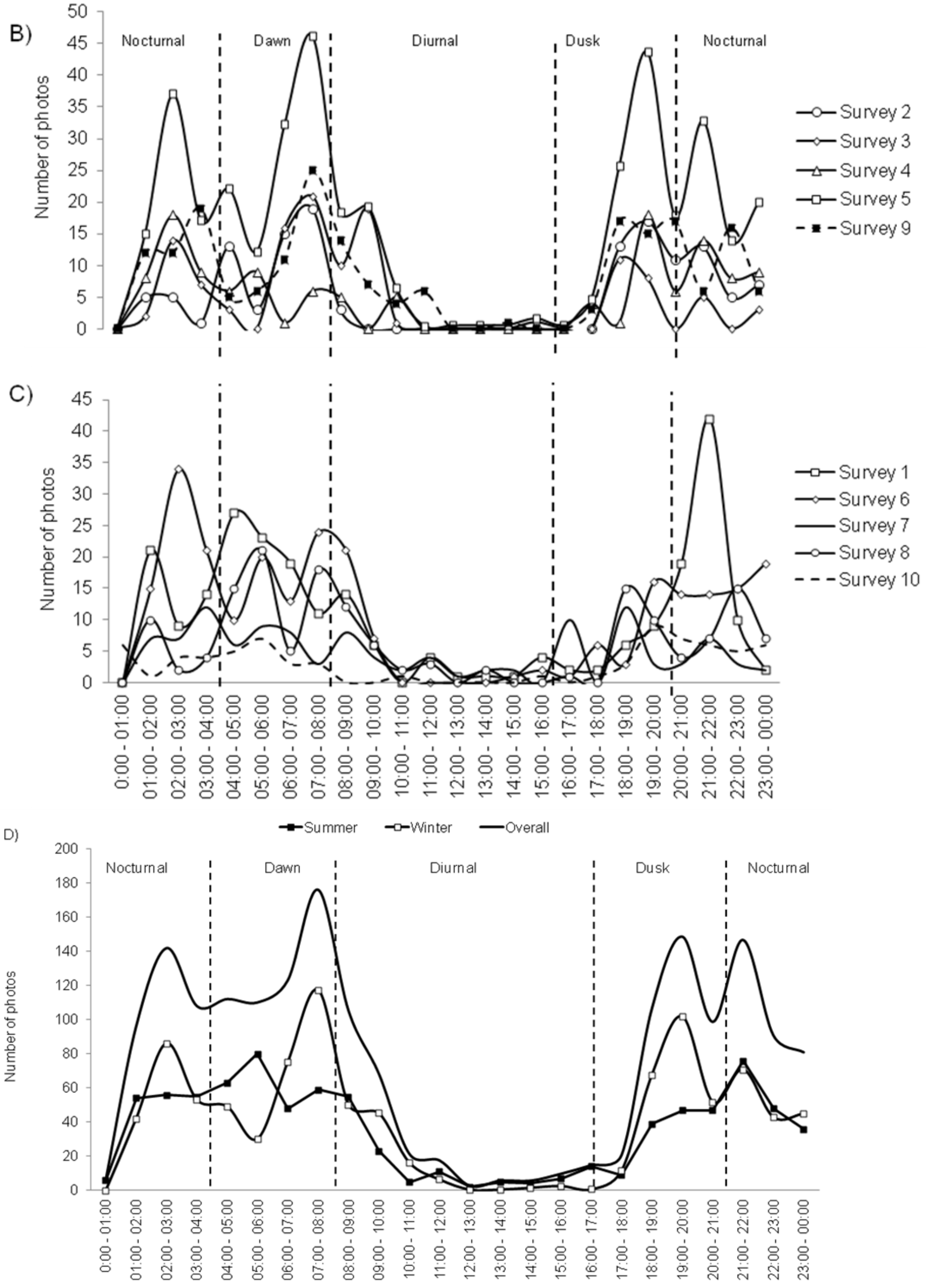
11
12
13
14
15
16
17
Legend
Camera trap stations
Census MCP 2007
Census MCP 2008
GSM MCP
Farms
Waterberg Plateau Park
0
5
10
20
Kilometers


A)
□ Summer
■ Winter
Number of photos
450
400
350
300
250
200
150
100
50
0
Diurnal
(08:00 - 15:59)
Dusk
(16:00 - 19:59)
Nocturnal
(20:00 - 03:59)
Dawn
(04:00 - 07:59)

B)
Number of photos
Nocturnal
Dawn
Diurnal
Dusk
Nocturnal
Survey 2
Survey 3
Survey 4
Survey 5
Survey 9
0
5
10
15
20
25
30
35
40
45
50
C)
Number of photos
Survey 1
Survey 6
Survey 7
Survey 8
Survey 10
0
5
10
15
20
25
30
35
40
45
0:00 - 01:00
01:00 - 02:00
02:00 - 03:00
03:00 - 04:00
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19:00 - 20:00
20:00 - 21:00
21:00 - 22:00
22:00 - 23:00
23:00 - 00:00
D)
Summer
Winter
Overall
Number of photos
Nocturnal
Dawn
Diurnal
Dusk
Nocturnal
0
20
40
60
80
100
120
140
160
180
200
0:00 - 01:00
01:00 - 02:00
02:00 - 03:00
03:00 - 04:00
04:00 - 05:00
05:00 - 06:00
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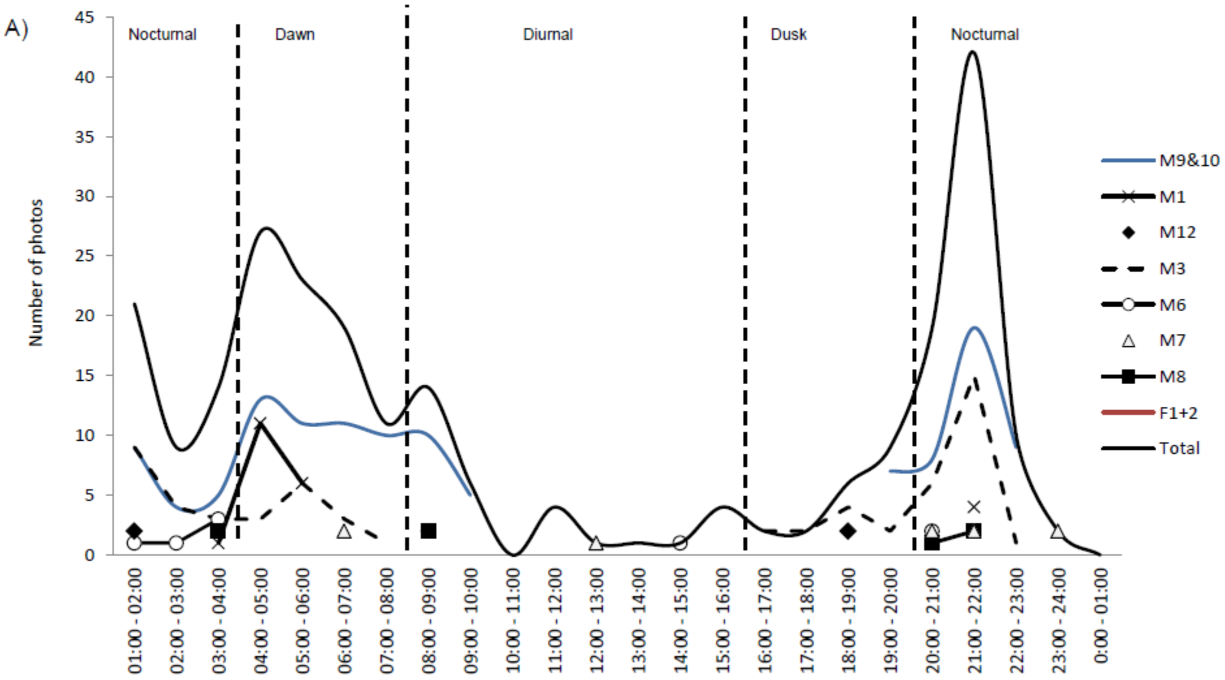
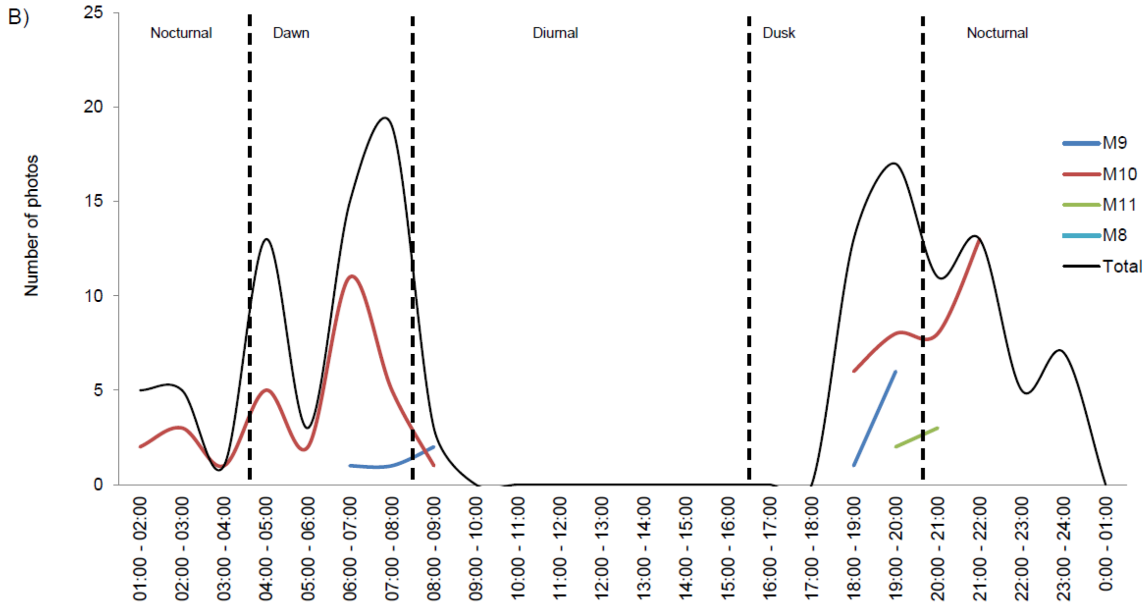
23:00 - 00:00

SUPPLEMENTARY

Fig. S1 Trends in the number of photos per hour per season/survey for individual cheetahs identified across the 10 surveys (2005 - 2011). A) Survey 1 (S), B) Survey 2 (W), C) Survey 3 (W), D) Survey 4 (W), E) Survey 5 (W), F) Survey 6 (S), G) Survey 7 (S), H) Survey 8 (S), I) Survey 9 (W), J) Survey 10 (S). S = Summer and W = winter.




B)
Number of photos
25
20
15
10
5
0
Nocturnal
Dawn
Diurnal
Dusk
Nocturnal
M9
M10
M11
M8
Total
01:00 - 02:00
02:00 - 03:00
03:00 - 04:00
04:00 - 05:00
05:00 - 06:00
06:00 - 07:00
07:00 - 08:00
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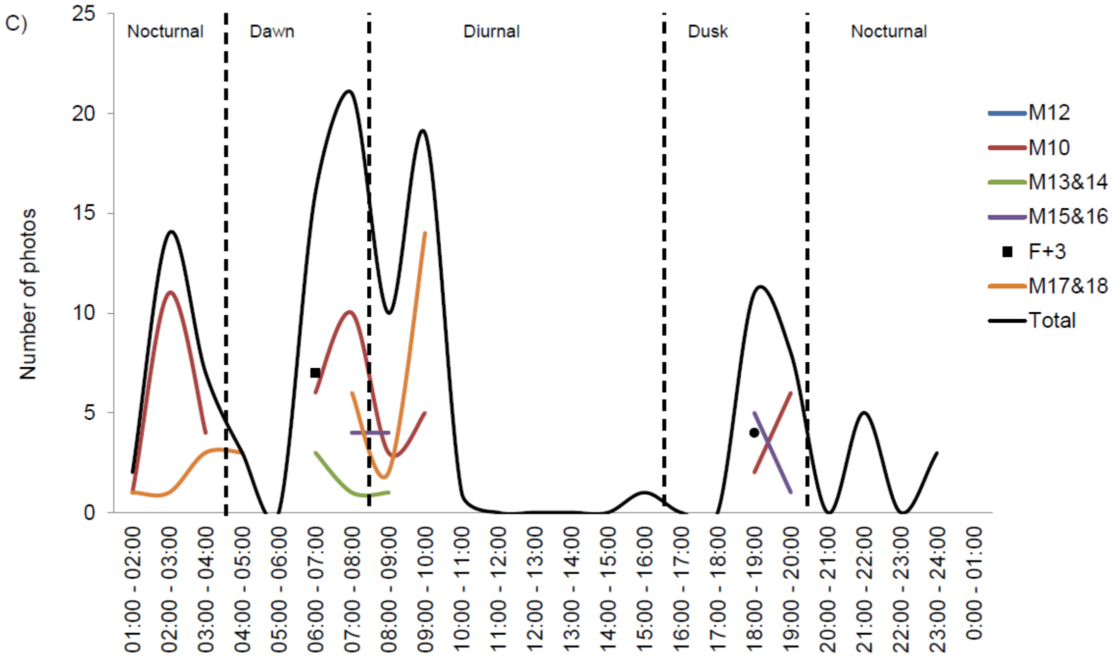
0:00 - 01:00


C)
Number of photos
25
20
15
10
5
0
Nocturnal
Dawn
Diurnal
Dusk
Nocturnal
M12
M10
M13&14
M15&16
F+3
M17&18
Total
01:00 - 02:00
02:00 - 03:00
03:00 - 04:00
04:00 - 05:00
05:00 - 06:00
06:00 - 07:00
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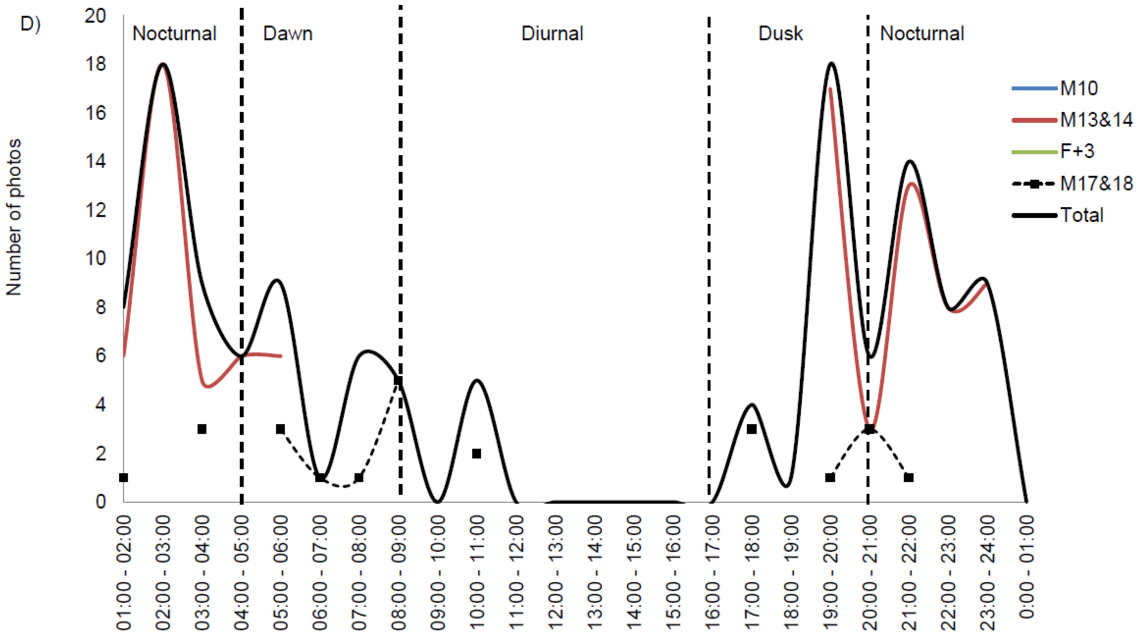
D)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2
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16
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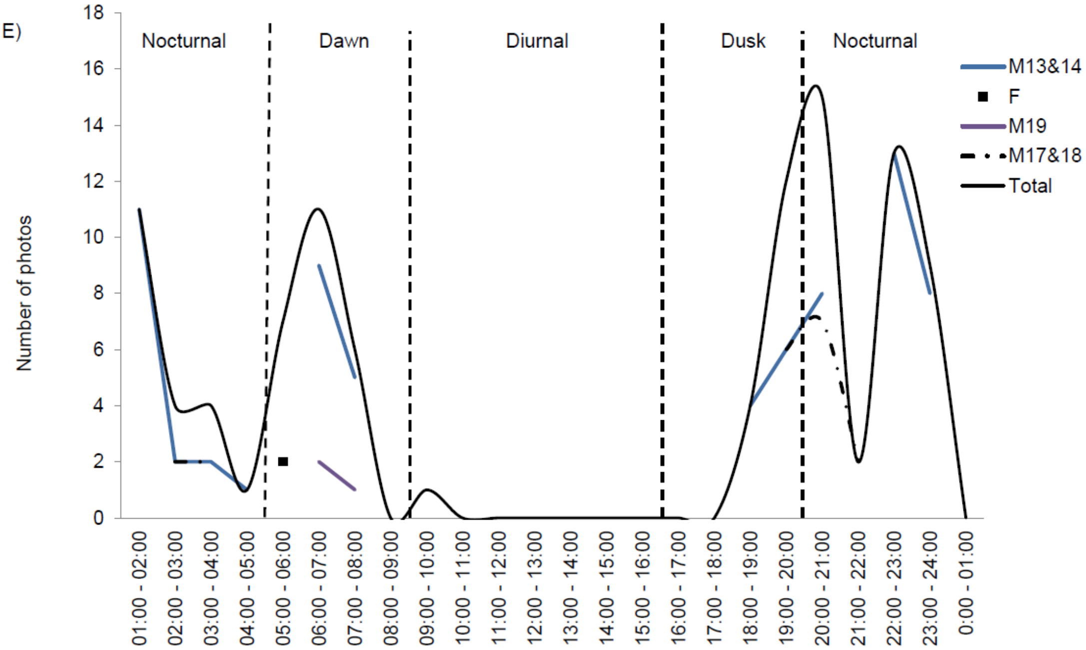
Number of photos
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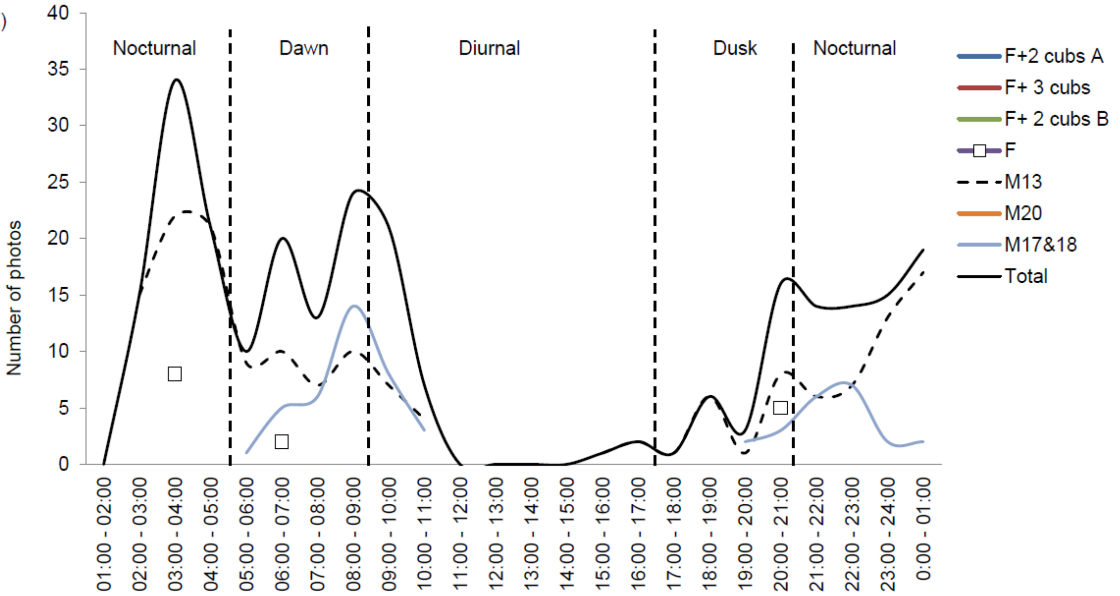
F)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35
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Nocturnal
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F+2 cubs A
F+ 3 cubs
F+ 2 cubs B
F
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M20
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Total
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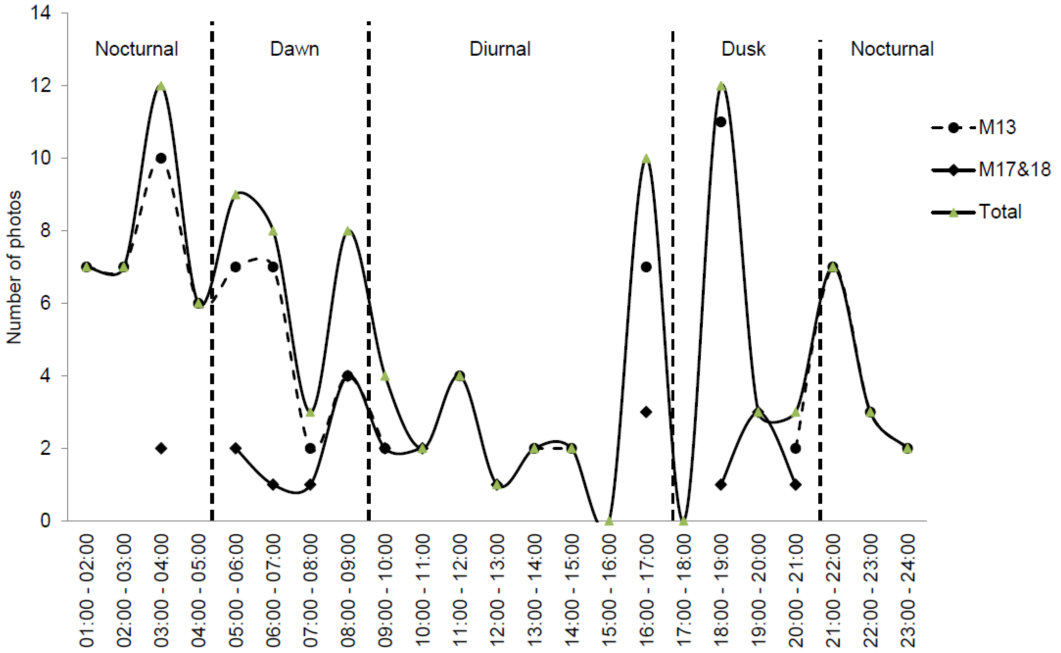
G)


Number of photos
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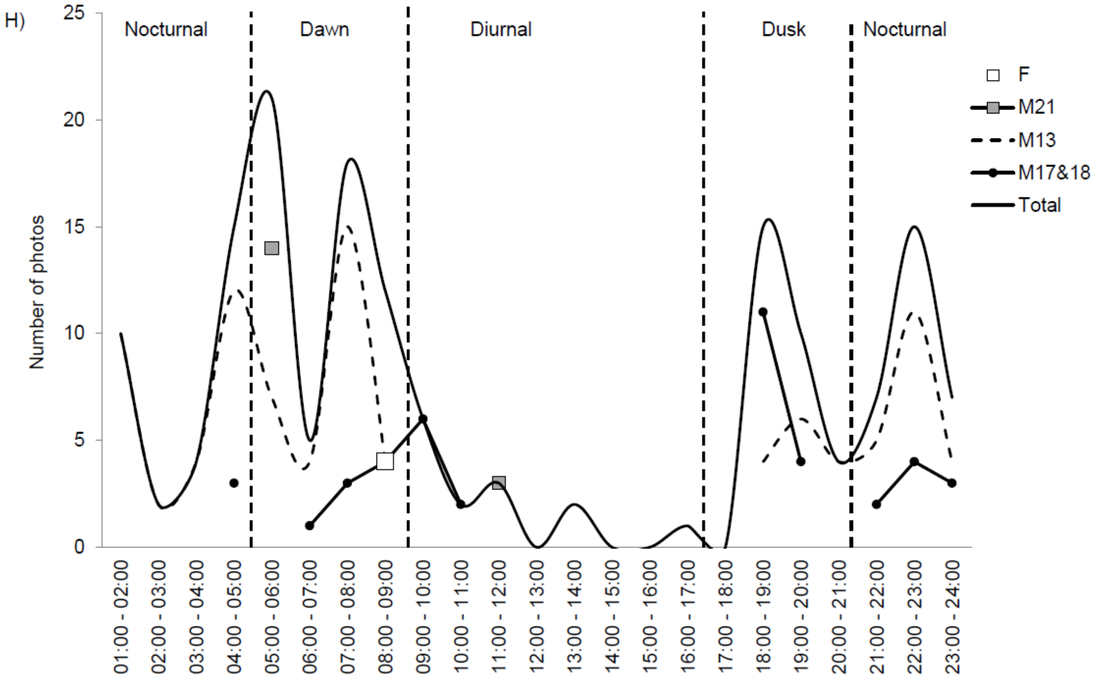
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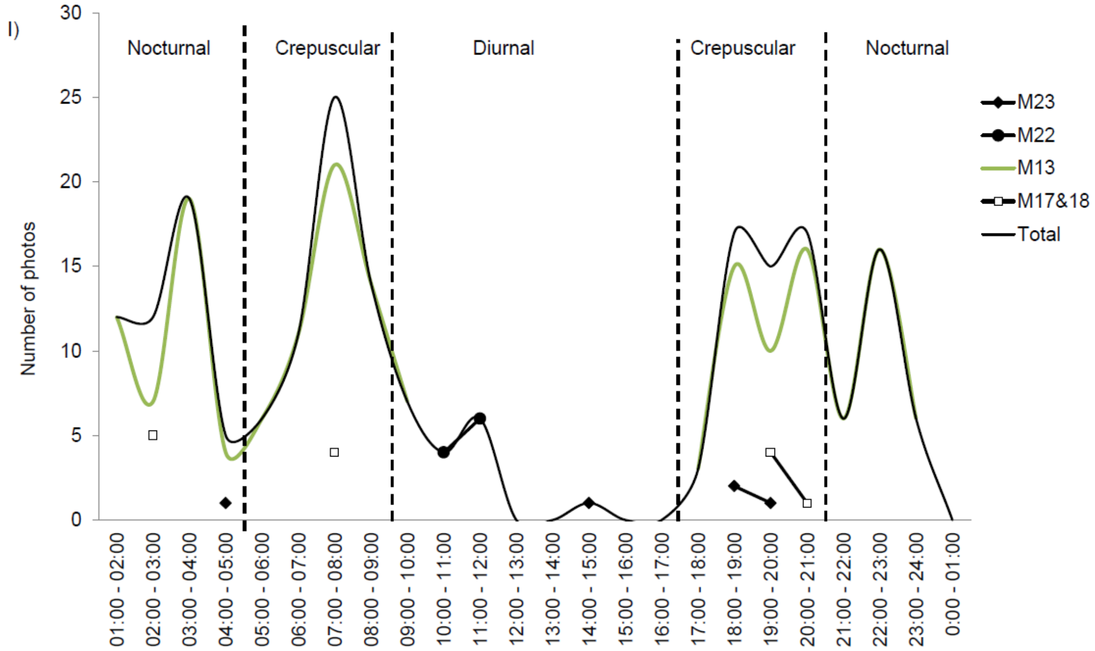
I)
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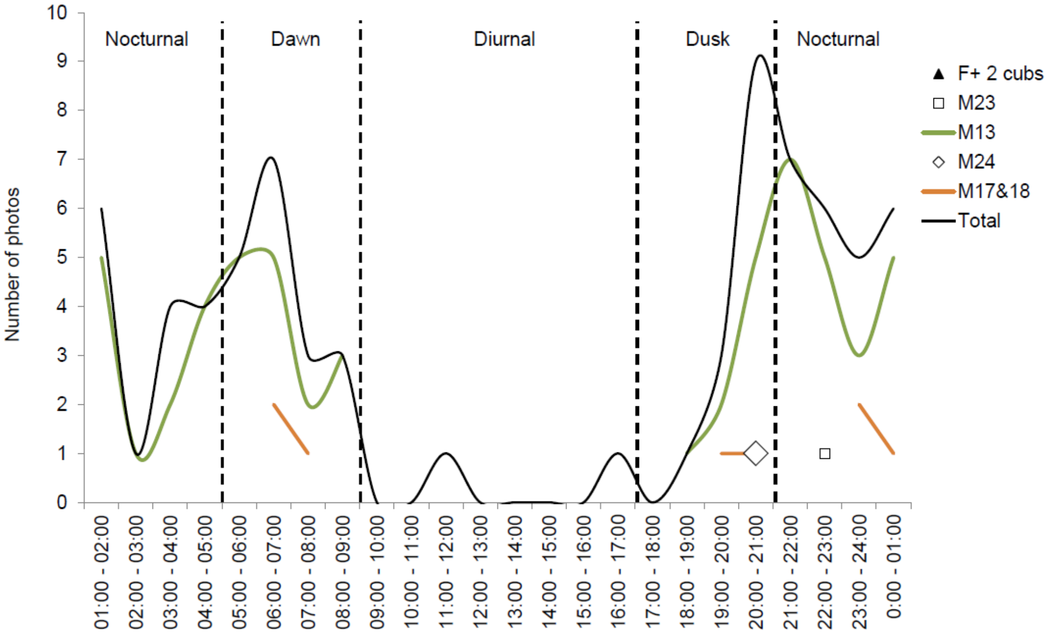
Total

J)



Number of photos
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Dawn
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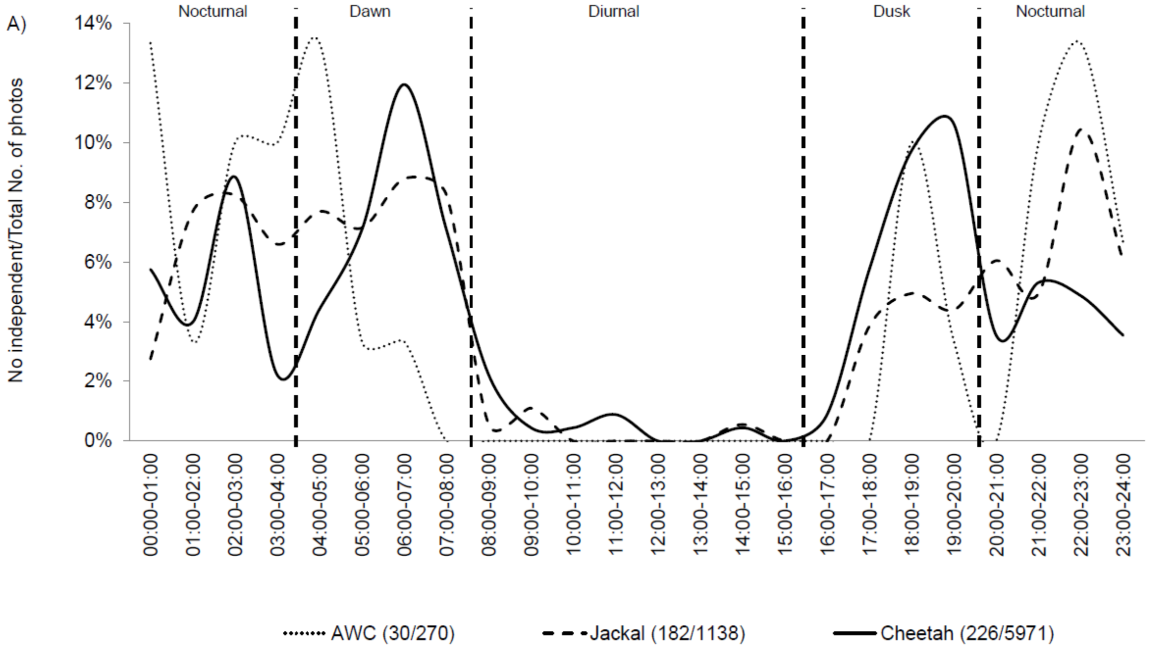
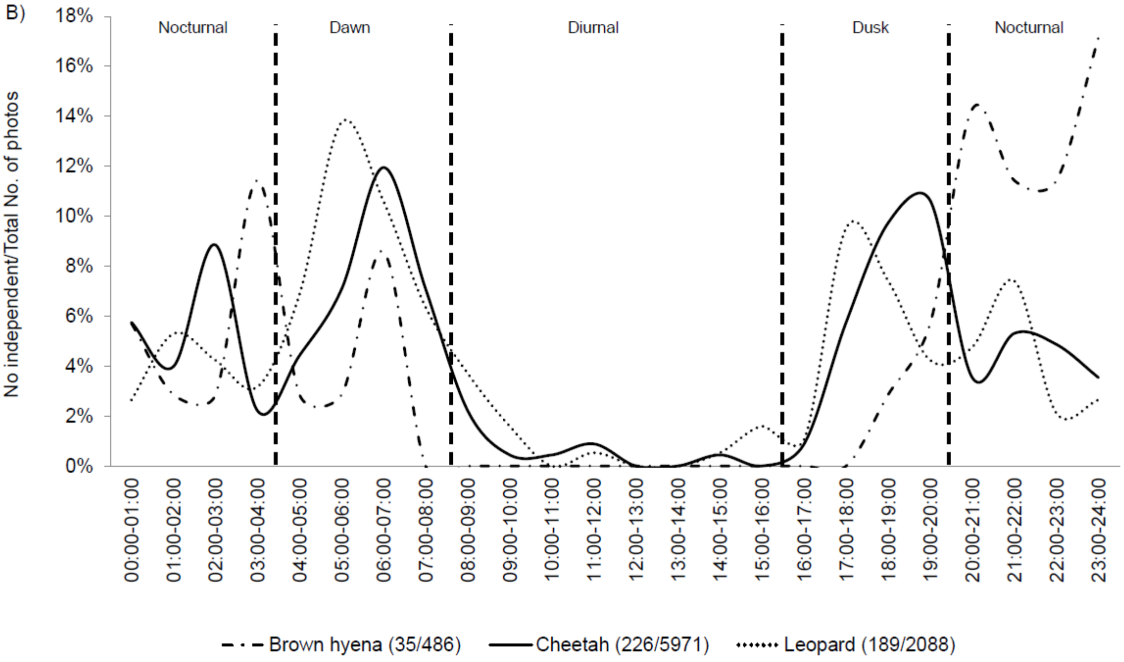
Fig. S2 Activity patterns in one-hour segments of cheetah versus A) small carnivores (Winter), B) large carnivores (Winter), C) small ungulates (Winter), D) large ungulates (Winter), E) small carnivores (Summer), F) large carnivores (Summer), G) small ungulates (Summer) and D) large ungulates (Summer). Number in brackets represents number independent of photos and the total number of photos per species. For winter data was grouped based on two 90 days surveys: 1st July – 28th September 2010 and 26th June – 23rd September 2011; For summer data was grouped over five 90 days surveys 29th September – 27th December 2010, 28th December 2010 – 27th March 2011, 28th March – 25th June 2011, 24th September – 22nd December 2011 and 23rd December 2011 – 21st March 2012. AWC = African Wild Cat.



B)


18%
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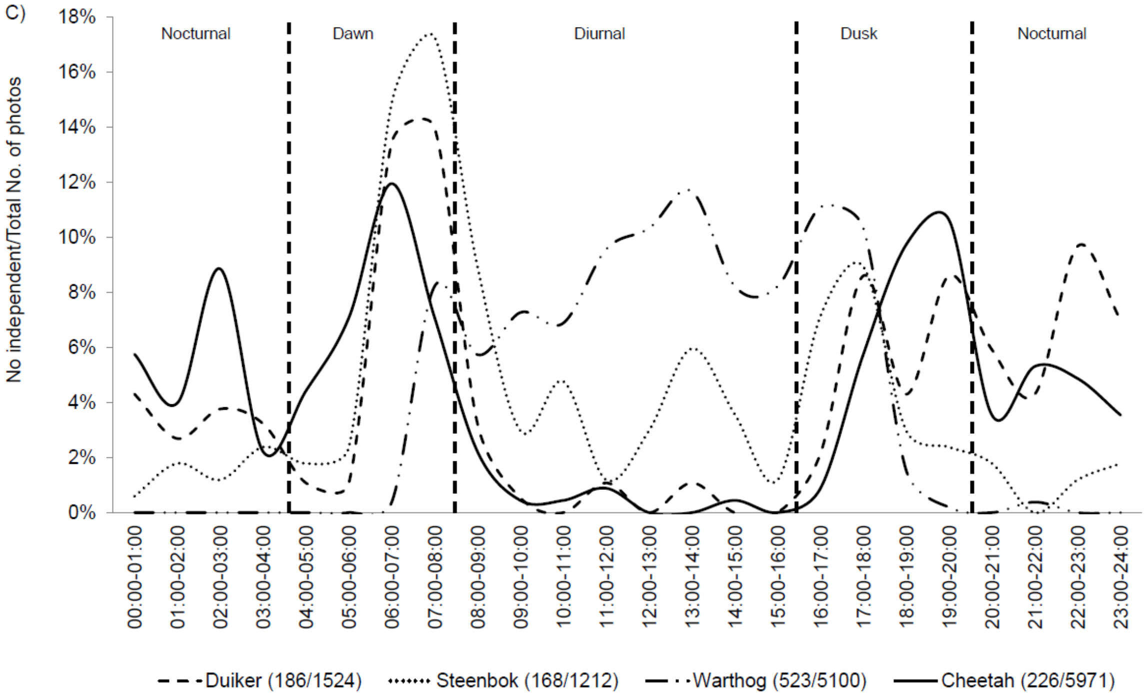
20:00-21:00
21:00-22:00
22:00-23:00
23:00-24:00
Brown hyena (35/486)
Cheetah (226/5971)
Leopard (189/2088)

C)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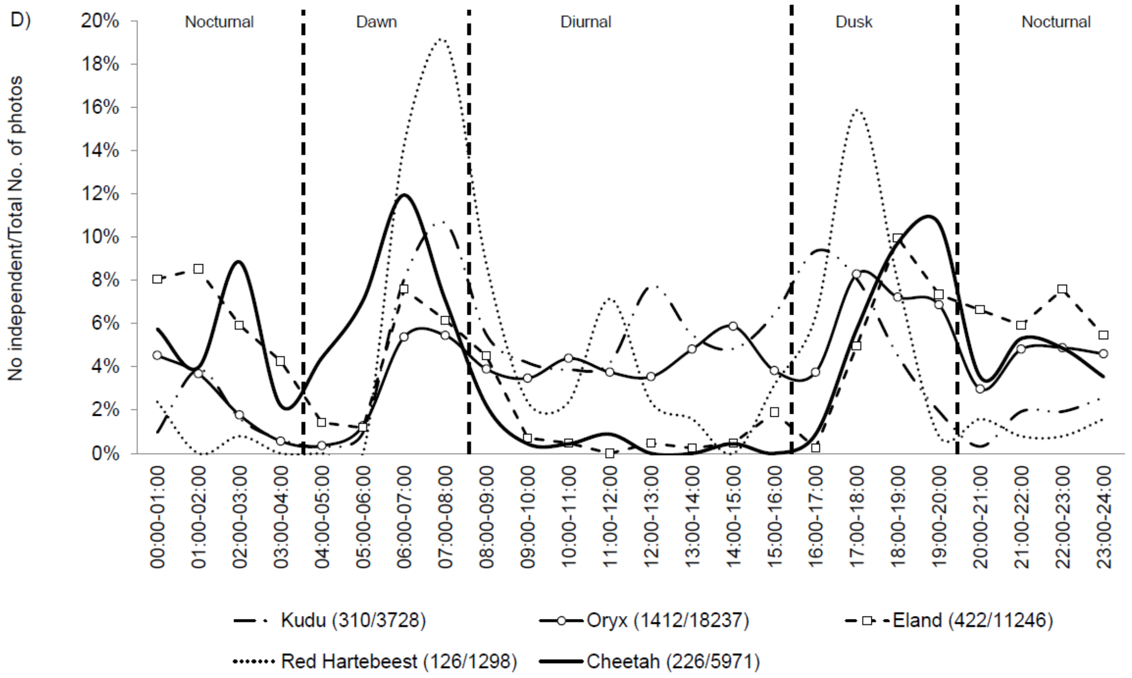
22:00-23:00
23:00-24:00
Duiker (186/1524)
Steenbok (168/1212)
Warthog (523/5100)
Cheetah (226/5971)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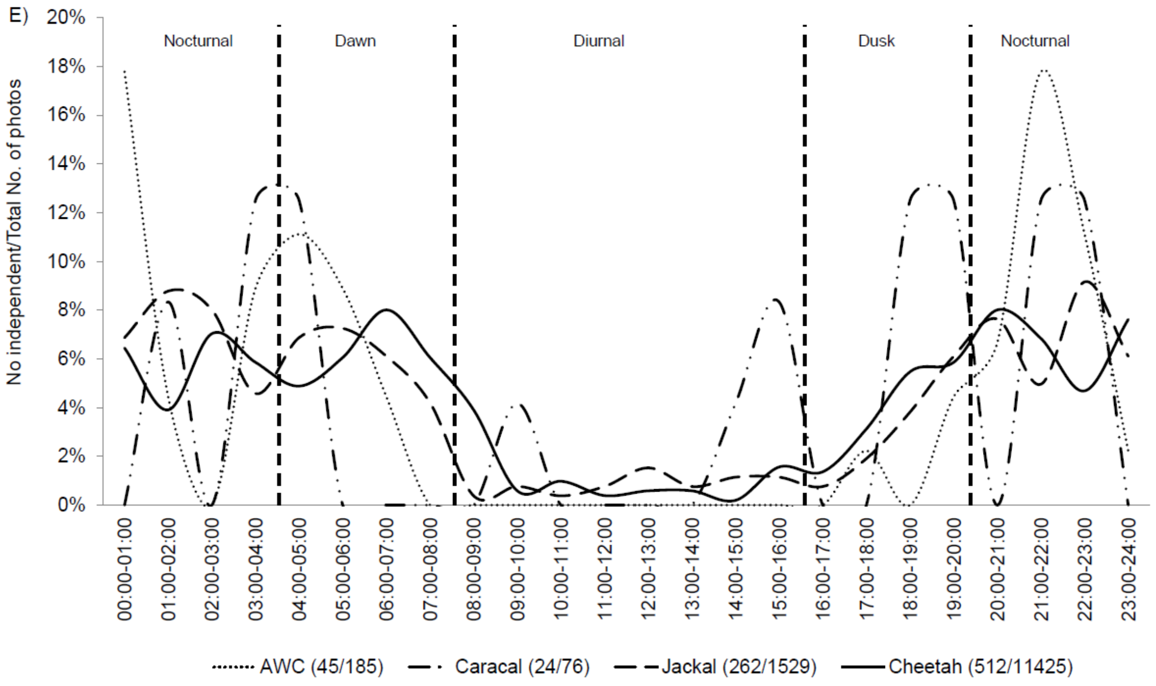
22:00-23:00
23:00-24:00
Kudu (310/3728)
Oryx (1412/18237)
Eland (422/11246)
Red Hartebeest (126/1298)
Cheetah (226/5971)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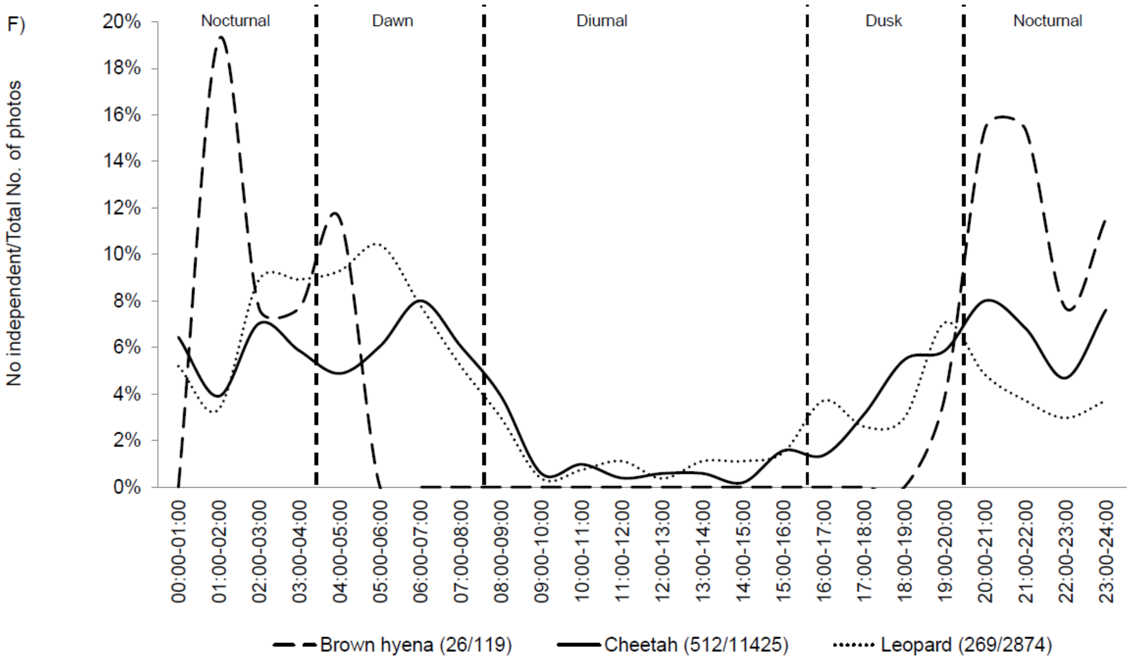
AWC (45/185)
Caracal (24/76)
Jackal (262/1529)
Cheetah (512/11425)

F)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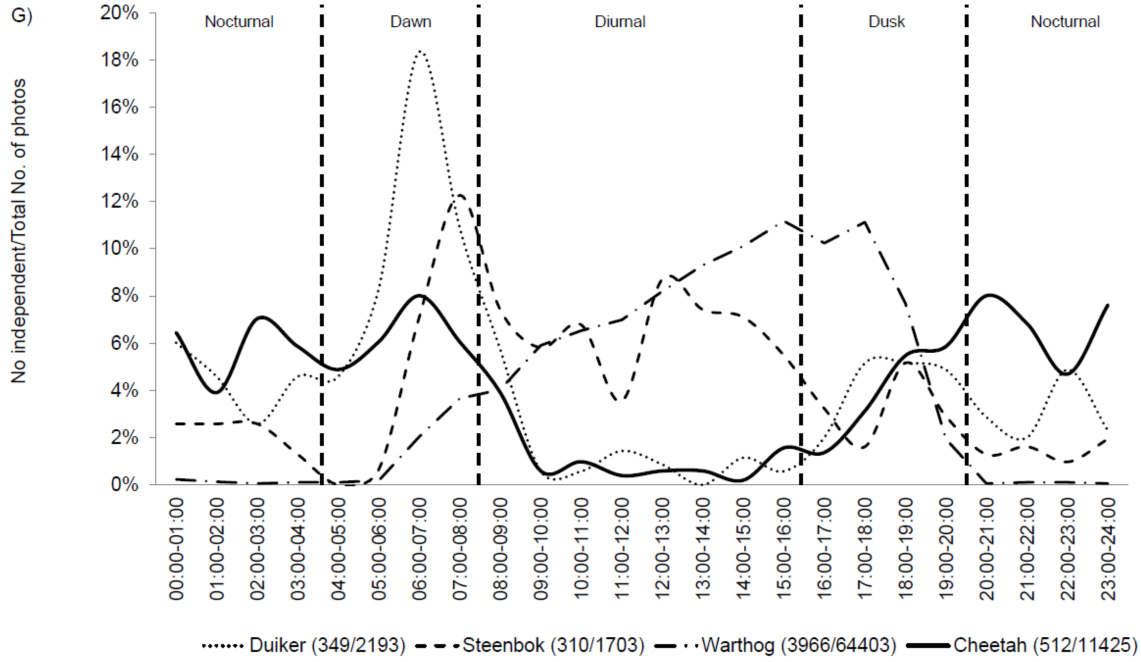
21:00-22:00
22:00-23:00
23:00-24:00
Brown hyena (26/119)
Cheetah (512/11425)
Leopard (269/2874)

G)


No independent/Total No. of photos
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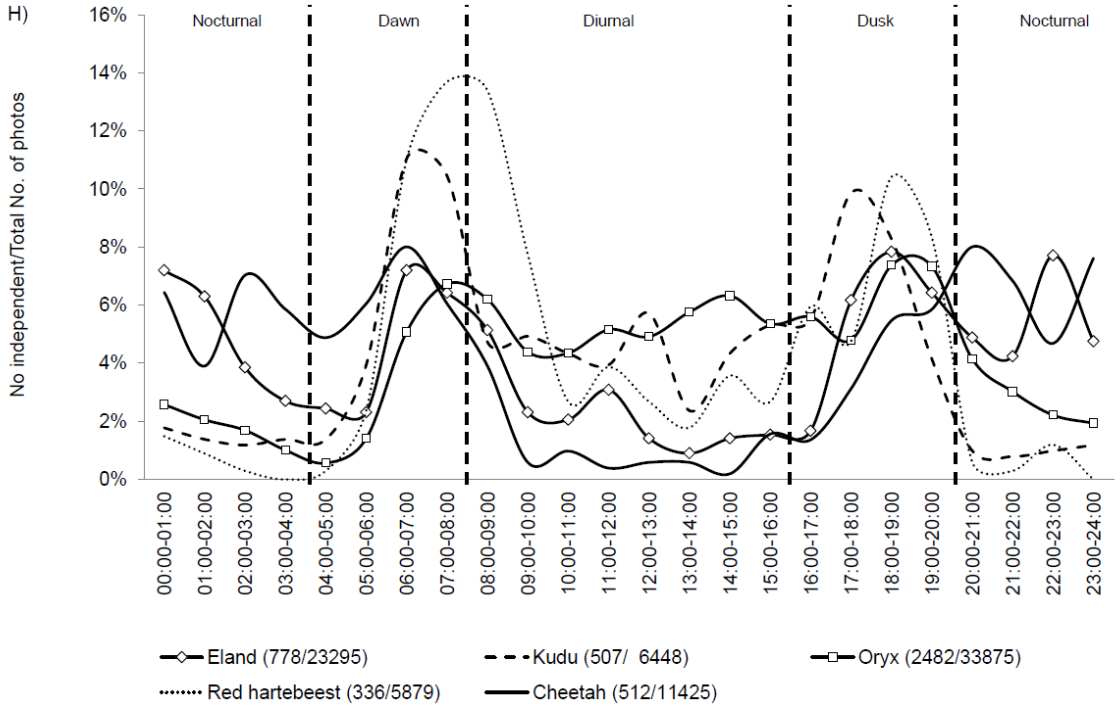
19:00-20:00
20:00-21:00
21:00-22:00
22:00-23:00
23:00-24:00
Duiker (349/2193)
Steenbok (310/1703)
Warthog (3966/64403)
Cheetah (512/11425)

H)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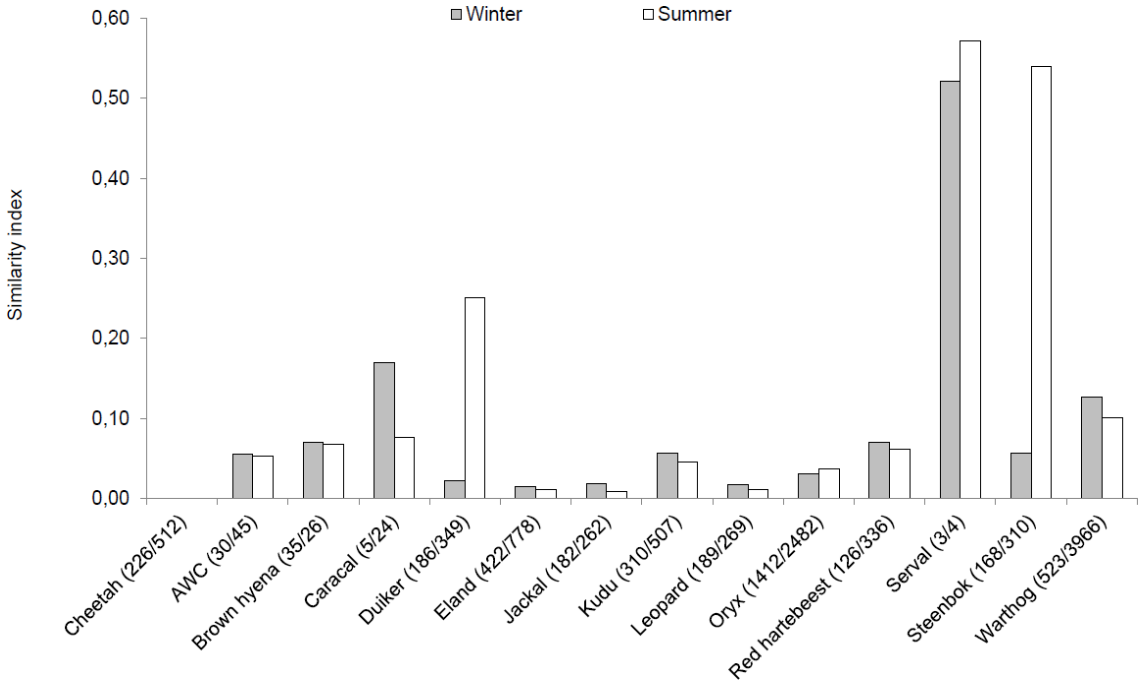
22:00-23:00
23:00-24:00
Eland (778/23295)
Kudu (507/ 6448)
Oryx (2482/33875)
Red hartebeest (336/5879)
Cheetah (512/11425)

Fig. S3 Species activity similarity index (the lower the more similar). Number in brackets represents number of indepedent photos per species for the winter and summer data sets. For winter data was grouped based on two 90 days surveys: 1st July – 28th September 2010 and 26th June – 23rd September 2011; For summer data was grouped over five 90 days surveys 29th September – 27th December 2010, 28th December 2010 – 27th March 2011, 28th March – 25th June 2011, 24th September – 22nd December 2011 and 23rd December 2011 – 21st March 2012. AWC = African Wild Cat.



Capítulo VI

5.1 Discussão geral

O estudo concentrou-se na população de guepardos da Namibia, tendo como objetivo geral a compreensão da demografia histórica e contemporânea desta população e da espécie em geral, visando ao desenvolvimento de ações eficazes para sua conservação e manejo, tendo em vista a existência de diversas ameaças à sua sobrevivência, incluindo efeitos de mudancas climáticas globais. Para isto, foram identificados três objetivos específicos, sendo eles: (1) uma avaliação estatística da história demográfica desta população, em relação à variabilidade climática do Quartenário e fatores antropogênicos; (2) uma estimativa e investigação da interação entre o seu tamanho efetivo contemporâneo (N_e), taxas vitais e viabilidade populacional de longo prazo; e (3) uma avaliação de tendências em abundância e densidade.

Inicialmente, o estudo mostrou que a população tem uma história demográfica complexa, com pelo menos três eventos de redução nos últimos 240 mil anos (kya), corroborando sugestões anteriores de declínio no passado (O’Brien et al. 1985, 1987; Pimm et al. 1989; Menotti-Raymond e O'Brien 1994; Hedrick 1996; Driscoll et al. 2002). Entretanto, cenários assumindo declínios e expansões severos não tiveram probabilidades posteriores mais elevadas em relação aos cenários postulando declínios graduais ou estabilidade em longo prazo. Os períodos de modificação coincidem com os principais eventos climáticos ao longo do tempo investigado, como os episódios periódicos de aridez, que ocorreram entre 135 kya - 90 kya (Cohen et al. 2007) condições de aridez pós LGM (26.5 a 19 kya) (Clark et al. 2009) e aumento de aridez particularmente na Namíbia durante o período de 3500 a 300 anos atrás (Chase et al. 2010) . Tais eventos resultaram em alternância das formas vegetacionais, conversão de florestas em pastagens e vice-versa (Gil-

Romera et al. 2007; Chase et al. 2009; Turpie et al. 2010) e redução ou extinção de espécies de presas no sudoeste da África (Faith 2012; Osmers et al. 2012) e no continente africano como um todo (Reed 1997; deMenocal 2004). A partir disso, postulamos como causas prováveis de declínio, no caso das mudanças demográficas ancestrais (i.e. no final da LGM e Holoceno inicial), a redução de habitats adequados (i.e. disponibilidade de presas e estrutura da vegetação), intensificação da competição interespecífica , periodicidade de mudanças na vegetação e clima, ou uma combinação destes fatores. Situação semelhante parece ter acontecido com a zebra-da-montanha do Cabo, na África do Sul (*Equus zebra zebra*) (Faith 2012), cuja população pode não ter recuperado os níveis demográficos anteriores a ~ 3600 anos atrás.

O efeito de fatores antropogênicos foi considerado mínimo até recentemente (~ 1000-300 anos) devido à baixa população humana na Namibia (i.e. < 500.000 em 1950) (Wikipedia 2013). O setor agrícola na região só se intensificou durante os últimos 1000 anos (Araki 2005), quando a paisagem se degradou (Mendelsohn 2006), as terras de cultivo tornaram-se mais áridas (Araki 2005) e alguns carnívoros (i.e. leões *Panthera leo* e hienas *Crocuta crocuta*) foram reduzidos severamente ou eliminados (Marker Kraus et al. 1996; Nowell 1996; Werner 1993),sugerindo a influência dos fatores antropogênicos. Uma conclusão semelhante foi obtida para os tigres na Índia (~ 600 anos) (Mondol et al. 2009). Adicionalmente, a falta de sinais de declínio nos últimos 300 anos não descarta os impactos genéticos devido a causas antropogênicas. De fato, durante o século passado, a caça furtiva provocou períodos de acentuada deriva genética em elefantes (Okello et al. 2008) e a caça descontrolada reduziuem em cinco vezes o tamanho efetivo (N_e) da população de crocodilos (*Crocodylus niloticus*) (Bishop et al. 2009). Este é provavelmente o caso da população de estudo, a qual diminuiu (população de censo N_c) durante o século passado devido a fatores diversos (e.g. secas, reduções de presas devido a epidemias de raiva e conflitos com os humanos) (Marker-Kraus et al. 1996; Marker et al. 2010).

Posteriormente, o estudo demonstrou que a população parece ser viável em longo prazo (N_e de 450 - 2500), com base em simulações que integram traços da história de vida. Estimativas baseadas em estimadores genéticos (LDNe, ONeSAMP, DIYABC, e MSVAR1.3) foram menores. Para approximar N_e a o tamanho geracional, a estimativa combinada de LDNe e de ONeSAMP foi multiplicada por tempos de geração (G) (*como em* Hare et al. 2010). Utilizando a proporção observada mínima de 0.21 (N_e/N_c) isso seria equivalente a um N_c de 2143 - 11905 indivíduos (ou 2686 - 6314 95% CI baseado na estimativa combinada de LDNe e ONeSAMP). Valores maiores podem ser esperados se $N_e/N_{adultos}$ mínima de 0,40 for considerada. Portanto, em seu tamanho atual a população não está em risco severo de extinção devido a um baixo potencial evolutivo, visto que *Ne* é maior que o valor teórico de 500 frequentemente considerado na literatura (Allendorf e Luikart 2007). No entanto, essa viabilidade é suscetível a diferentes fatores.

Observamos que *Ne* foi influenciada positivamente por uma série de fatores, incluindo o *G* utilizado e conseqüentemente fatores que influenciam a sua média (por exemplo, diminuição de mortalidade de machos adultos). Os fatores incluem diminuições baixas (~ 10%) na proporção de fêmeas reprodutoras e decréscimos moderados (~20%) na taxa de mortalidade de fêmeas adultas acompanhadas ou não de filhotes e/ou juvenis. Reduções no tamanho inicial da população (N_{init}) também influenciaram positivamente o N_e. Estes fatores também influenciaram positivamente N_c ou $N_{adultos}$ e o tempo médio de *G*, mas se diferenciaram em ordem de impacto e magnitude. Por exemplo, declínios de 10% na proporção de fêmeas reprodutoras e 20% em N_{init} afetaram N_e e N_c positivamente, mas o impacto do primeiro foi maior em N_e do que em N_c, e vice-versa para N_{init}. Em geral, alguns destes resultados são suportados pela literatura.

Primeiramente, embora os N_c inferidos não devam ser tomados pelo valor nominal, as estimativas estão de acordo com a literatura (Hanssen & Stander 2004; Marker et al. 2007; Purchase et al. 2007; o que é sugestivo de uma estimativa consistente deste parâmetro. Segundo, assim como Palstra and Palstra (2010) e Andrello et al. (2012), ações de conservação influenciam positivamente e por vezes concomitantemente, a diversidade genética da população (heterozigosidade), N_c, taxa de crescimento e G. Terceiro, observou-se um aumento no N_e devido a uma redução na mortalidade de filhotes e juvenis (i.e. maior sucesso reprodutivo) combinada a um aumento em G (devido aos aumentos moderados na sobrevivência de adultos) da mesma forma que o observado em bisões (*Bison bison*) (Pérez-Figueroa et al. 2012). Nossos resultados, como os de Peréz-Figueroa et al. (2012), diferenciam-se dos de Saether et al. (2009), que observaram um aumento na deriva genética anual de alces americanos (*Alces alces*) com aumentos na sobrevivência de filhotes (N_e diminui). Quarto, N_e/N_c (0,21) ou N_{adulto} (0,40) estão dentro do âmbito empírico de valores medianos de 0.231 e 0,225 (Palstra e Fraser 2012) e estimativas teóricas de 0.5 ou 0.25 e 0.75 (Nunney 1991; Nunney 1993; Nunney e Elam 1994). Esta proporção tambem foi influenciada pelo tipo de N utilizado e G (Waite e Parker 1996; Lee et al. 2011; Palstra e Fraser 2012). Quinto, os padrões observados nas taxas de sobrevivência de machos parecem resultar em um número maior gamético, cuja influência final no N_e é condicionada à distribuição de remoções em relação ao status de reprodução ou classe de idade (Storz et al.

2001). Por fim, reduções moderadas (~20%) na proporção de fêmeas reproduzindo podem também reduzir N_e (Saether et al. 2009). Enfim, as diferenças observadas em N_e devido a níveis de perturbações diferentes em taxas vitais indicam que os impactos não são lineares (Stott et al. 2012), mas que ações de conservação orientadas para N_c também podem influenciar N_e positivamente.

O estudo mostrou que as estimativas de densidade de adultos no centro-norte da Namíbia variaram entre 5 - 20 km^{-2} e foram significativamente semelhantes entre diferentes estações. Esta semelhança parece ser resultado da dinâmica das áreas de vida (devido à estabilidade social) associada à capacidade-suporte na escala amostrada. A abundância variou em uma média de 9 ± 7 indivíduos adultos por amostragem, com flutuações guiadas por indivíduos não residentes (30 de um total de 39 indivíduos adultos). Animais residentes (n = 9) tiveram a posse das áreas de vida dos três aos seis ou mais anos (i.e. idade adulta), com base em sua utilização das estações de marcação olfativa de 6 a 48 meses (31 ± 17), com uma visita de guepardo por estação a cada 5 ± 8 dias em média. Os padrões de atividade mostraram um comportamento noturno que coincide com o padrão observado em espécies de ungulados e carnívoros simpátricos, mas com menos capturas durante o inverno.

Indivíduos residentes foram capturados mais frequentemente em estações localizadas no centro de suas áreas de vida, visitando outras áreas com menor freqüência. Novos recrutas mostraram padrões similares de utilização do espaço em relação a seus antecessores (e.g. um único macho com uma coalizão de dois machos durante dois anos). Os padrões observados foram semelhantes entre indivíduos monitorados por radiotelemetria e entre indivíduos monitorados por armadilhas fotográficas. Novos recrutamentos ocorrerm em duas oportunidades:

uma devido a um território vago pela morte de uma coalizão no ano anterior, e a outra devido ao possível deslocamento de um macho solitário por uma coalizão de dois indivíduos após dois anos de sobreposição espacial. Observou-se uma razão sexual desigual de 4:1 em favor dos machos. Fêmeas acompanhadas de filhotes (2.5 ± 0.57, *n* = 15) foram mais frequentemente capturadas do que fêmeas solitárias (*n* = 1), sendo as capturas mais frequentes durante o verão. Alguns dos resultados ecológicos e comportamentais para essa população estão sendo reportados pela primeira vez, mas são condizentes com as informações encontradas na literatura para a espécie.

As estimativas de densidade deste estudo foram semelhantes às de estudos que utilizaram questionários (Hanssen e Stander 2004). A estrutura demográfica limitada na escala amostrada não é exclusiva deste estudo (O'Connell et al. 2011), embora o nosso período de amostragem seja superior ao de outros trabalhos utilizando armadilhas fotográficas (e.g. Maffei 2011). O período de posse de uma área própria é semelhante à relatada em guepardos no Serengeti, baseado em estudos comportamentais de longo prazo, de 4 a 36 meses (Caro e Collins 1987; Caro e Kelly 2001). Esta fidelidade ao longo das estações é apoiada pela falta de efeito sazonal sobre o tamanho das áreas de vida de guepardos na área de estudo (Marker et al. 2008). Embora esteja baseado em uma amostra pequena, os efeitos da estrutura social de grupo e da saúde dos animais como mecanismos de retenção de áreas próprias de vida estão relacionados, através do possível deslocamento de um macho solitário por uma coalizão e do afastamento dos não-residentes por machos solitários (> 3 anos). Finalmente, houve semelhanças nos padrões de uso espacial por novos recrutas e antecessores (em tempos sem sobreposição) e frequências maiores de visitação em estações localizadas ao centro de areas de vida por indivíduos monitorados por radiotelemetria, assim como em hienas no Kalahari (Mills et al. 1980). Isto sugere o papel de pistas olfativas na demarcação, forrageamento ou orientação de indivíduos aos locais de marcação, bem como de familiarização de habitat (Eisenberg & Kleiman, 1972; Gorman e Trowbridge 1989; Potts et al. 2012). Estes aspectos são englobados pela teoria de utilização dos recursos (Gittleman 1989). Eaton (1970) indica que o sistema de marcação de guepardos é primariamente um mecanismo que permite a coexistência de vários indivíduos dentro da mesma área ("time-plan").

O padrão temporal noturno e crepescular está de acordo com estudos prévios (Marker, Fabiano e Nghikembua 2008; Cozzi et al. 2012) mas diferencia-se do geralmente considerado para a espécie, diurno (Durant 2000; Hayward and Slotow 2009). Cozzi et al. (2012) indica que este movementos de guepardos é influenciado por ciclos lunares. Fundamentalmente, estes dados suportam que esta característica é filogenetica (Holt 2009) visto que outros grande carnívores como o leão, leopardo, hienas e wild dog tambem apresentão um padrão semelhante (Hayward & Slotow 2009; Cozzi et al. 2012).

5.2 Implicações para conservação e manejo

Os resultados do estudo têm diversas implicações para a conservação. Primeiro: os efeitos ilustrados da variabilidade climática e instabilidade ambiental (e.g. aridificação) sobre a demografia desta população e possivelmente de outras populações do sul da África durante o Pleistoceno e Holoceno sugerem que a viabilidade e persistência desta população está condicionada às potenciais ameaças impostas por alterações climáticas futuras e as suas interações com as práticas de uso da terra (Turpie et al. 2010). Portanto, existe uma necessidade de compreensão mais profunda do impacto das alterações climáticas essencialmente em relação à segurança alimentar, visto que estudos sugerem uma perda potencial de produtividade, podendo fomentar uma reversão para níveis menores de tolerância aos carnívoros (Marker et al. 2010; Turpie et al. 2010). Tal situação é de grande importância dado que não detectamos nenhum sinal de expansão dentro do período avaliado.

Segundo: as ações de conservação deveriam concentrar-se particularmente em aspectos relacionados a fêmeas, especialmente aquelas em idade reprodutiva, para maximizar o potencial evolutivo populacional. Além disso, como N_e foi sensível a reduções na proporção de fêmeas reprodutoras e na capacidade suporte, respectivamente, esforços devem ser orientados para monitorar tendências destas variáveis.

Finalmente: os nossos resultados reforçam as ações de conservação atuais de soltura de indivíduos nos locais de captura (Marker et al. 2003; Marker et al. 2008) sempre que possível, e logo após a remoção. Isto se deve à alta fidelidade territorial, ao fluxo de indivíduos não residentes e possívies reduções na variância reprodutiva masculina que podem afetar N_e e, portanto, a viabilidade genética da população.

5.3 Concluções e recomendações

Em resumo, o estudo constatou que a população de guepardos da Namíbia é provavelmente viável em longo prazo, e as análises de sensibilidade sugerem que os esforços de conservação devem continuar a se concentrar principalmente em aspectos relacionados à sobrevivência das fêmeas, seguidos pela dos machose pelos juvenis/não-reprodutivos. Um efeito colateral destas medidas é a estabilidade social e conseqüentemente, a dinâmica das áreas de vida que parece regular a densidade, pelo menos dos machos. Isto é importante à medida que a instabilidade social pode modificar os padrões de acasalamento, afetando o tamanho efetivo populacional ou sua razão com o tamanho total do censo. A diversidade genética da população estudada, e possivelmente a da África austral como um todo,resultam de uma complexa história caracterizada por fases de estabilidade intercaladas por períodos de declínio, com a contribuição de variações climáticas e instabilidade ambiental, juntamente com a competição interespecífica. Quanto ao papel dos fatores antropogênicos, especialmente durante o período estável (300 aa até o presente), são necessárias avaliações adicionais, como a detecção de possíveis sinais transitórios ao nível de coorte. De um modo geral, a persistência e viabilidade da população parece estar relacionada predominantemente a efeitos diretos e indiretos das mudanças climáticas.

Estudos futuros devem explorar a conexão entre a ocupação e distribuição da população e como elas serão provavelmente afetadas pelas tendências projetadas de mudanças climáticas. Isto não deve restringir-se a uma espécie única, mas deve incorporar múltiplas espécies de carnívoros e a sua base de presas. Há também a necessidade urgente de estudos para elucidar o sistema de acasalamento desta população (e.g. Gotelli et al. 2007) bem como estudos comportamentais das fêmeas, os quais.forneceriam uma nova compreensão dos processos que afetam *Ne* e *Nc* (dinâmica populacional). Por fim, há necessidade de estudos de longo prazo abrangendo áreas maiores, utilizando métodos sistemáticos de levantamento, e de uma continuada integração deste tipo de informação com aquelas geradas por análises genéticas detalhadas e simulações computacionais de cenários cada vez mais realistas envolvendo esta e outras populações da espécie.

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