

Long-Term Impacts of Poaching on Relatedness, Stress Physiology, and Reproductive Output of Adult Female African Elephants

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Abstract: *Widespread poaching prior to the 1989 ivory ban greatly altered the demographic structure of matrilineal African elephant (*Loxodonta africana*) family groups in many populations by decreasing the number of old, adult females. We assessed the long-term impacts of poaching by investigating genetic, physiological, and reproductive correlates of a disturbed social structure resulting from heavy poaching of an African elephant population in Mikumi National Park, Tanzania, prior to 1989. We examined fecal glucocorticoid levels and reproductive output among 218 adult female elephants from 109 groups differing in size, age structure, and average genetic relatedness over 25 months from 2003 to 2005. The distribution in group size has changed little since 1989, but the number of families with tusked old matriarchs has increased by 14.2%. Females from groups that lacked an old matriarch, first-order adult relatives, and strong social bonds had significantly higher fecal glucocorticoid values than those from groups with these features (all females $R^2 = 0.31$; females in multiadult groups $R^2 = 0.46$). Females that frequented isolated areas with historically high poaching risk had higher fecal glucocorticoid values than those in low poaching risk areas. Females with weak bonds and low group relatedness had significantly lower reproductive output ($R^2[U] = 0.21$). Females from disrupted groups, defined as having observed average group relatedness 1 SD below the expected mean for a simulated unpoached family, had significantly lower reproductive output than females from intact groups, despite many being in their reproductive prime. These results suggest that long-term negative impacts from poaching of old, related matriarchs have persisted among adult female elephants 1.5 decades after the 1989 ivory ban was implemented.*

Keywords: gene-drop analysis, glucocorticoids, *Loxodonta africana*, microsatellite DNA, poaching, relatedness

Impactos a Largo Plazo de la Cacería Furtiva sobre el Grado de Relación, la Fisiología del Estrés y la Productividad de Hembras Adultas de Elefantes Africanos

Resumen: *La cacería furtiva generalizada antes de la prohibición del comercio de marfil en 1989 alteró la estructura demográfica de grupos familiares matrilineales de elefante africano (*Loxodonta africana*) en muchas poblaciones por la disminución del número de hembras adultas, viejas. Evaluamos los impactos a largo plazo de la cacería furtiva investigando correlaciones genéticas, fisiológicas y reproductivas de una estructura social perturbada resultante de la cacería furtiva intensiva de una población de elefante africano en el Parque Nacional Mikumi, Tanzania, antes de 1989. Examinamos los niveles de glucocorticoides fecales y la productividad de 218 hembras adultas de 109 grupos de diferente tamaño, estructura de edades y relación genética promedio durante 25 meses entre 2003 y 2005. La distribución del tamaño de los grupos ha cambiado poco desde 1989, pero el número de familias con matriarcas viejas con colmillos ha incrementado en 14.2%. Las hembras de grupos que carecían de una matriarca vieja, parientes adultos de primer orden y lazos sociales estrechos tenían valores de glucocorticoides significativamente mayores que los grupos con esas características (todas las hembras $R^2 = 0.31$; hembras en grupos multiadultos $R^2 = 0.46$). Las hembras que frecuentaban*

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lugares aislados con riesgo históricamente alto de caza furtiva tuvieron niveles de glucocorticoides más altos que en las áreas con bajo riesgo de caza furtiva. Las hembras con relaciones débiles y un bajo grado de relación entre grupos tuvieron una productividad significativamente menor ($R^2[U] = 0.21$). Las hembras de grupos desestabilizados, definidas con base en un grado observado de relación entre grupos 1 DS debajo de la media esperada para una familia no cazada simulada, tuvieron una productividad significativamente menor que las hembras de grupos intactos, no obstante que muchas estaban en su mejor momento reproductivo. Estos resultados sugieren que los impactos negativos a largo plazo de la cacería de matriarcas viejas emparentadas han persistido entre las hembras adultas jóvenes 1.5 décadas después de la prohibición del comercio de marfil.

Palabras Clave: ADN microsatelital, análisis de declinación genética, cacería furtiva, grado de relación, glucocorticoides, *Loxodonta africana*

Introduction

Demographic disruption associated with poaching of African elephants (*Loxodonta africana*) is potentially long lasting because of the species long generation time. Poaching reduced populations from 1.3 million to fewer than 600,000 elephants in less than a decade (from 1979 to 1987). Poachers first targeted adult male elephants and then large, old females for their tusks. Given their intricate social networks centered on related adult females (males disperse at sexual maturity), this disturbance altered the demographic structure of matrilineal groups in many populations by decreasing the number of old matriarchs (≥ 30 years old) (Moss & Poole 1984; Poole 1989; Barnes & Kapela 1991). We examined the relationship between group structure and physiological condition of 218 adult female elephants from 109 groups in Mikumi National Park, Tanzania, over 25 months (2003–2005) to assess the long-term impacts of poaching 15 years after the ivory ban went into effect.

Mikumi is a part of the larger Selous–Mikumi–Udzungwa ecosystem. At 55,000 km², this ecosystem is the largest protected area in East Africa and was arguably one of the most heavily poached areas in the years preceding the 1989 ivory ban (Stephenson 1986; Siege & Balduš 2000). Up to 75% of the Mikumi population may have been eliminated (Balozi 1989; TWCM 1991). A 1987 study of elephant skulls revealed that the majority of poaching in Mikumi occurred in areas burned by fires (started by poachers) that were the farthest from the tourist areas. Eighty percent of the poached skulls were females, averaging 32.4 years old (Ihde 1991). Poaching dropped significantly across Tanzania following the ban; poaching mortality in Mikumi was reduced to a few elephants per year on average (Siege & Balduš 2000; Ereckson 2001; J. Shemkunde, personal communication).

Current group composition in poached populations differs significantly from those of unpoached populations (Foley 2002; Aleper & Moe 2006). Groups in relatively unpoached populations contain many adult females: an old matriarch (≥ 30 years old) and her adult female relatives, commonly spanning several generations (Moss 2001). Poached populations, such as those in

Mikumi, Ruaha, Kikepedo, and Tarangire national parks, contain a sizable portion of elephant groups that conspicuously lack many of these characteristics (e.g., Poole 1989; Barnes & Kapela 1991; Foley 2002; Aleper & Moe 2006).

We conducted a correlational study in an extant elephant population in attempt to deduce long-term impacts of historical poaching on this population. We assumed the structure of the current population was in large part due to past poaching, as evidenced by the large number of poached adult skulls found in the park in the decade prior to the ban and a relative absence of skulls and extreme environmental and human-related pressures (i.e., drought, disease, increased elephant density, or tourism increase) during the subsequent 15 years (Norton et al. 1987; Wasser & Norton 1993; Ereckson 2001). The assumption that poaching shaped group composition in Mikumi in the 1980s, but has since decreased is further substantiated by an observed large increase in the number of family groups with tusked old females since 1989 (Poole 1989).

Relatively few wildlife areas in Africa have been exempt from poaching, hunting, or culling, all of which selectively remove mature elephants. Thus, many elephant populations across Africa likely share group compositions characteristic of a poached population. Recognizing that agents other than legal or illegal off-take (e.g., drought, disease or starvation) can also alter group composition, our results contribute to an overall understanding of the long-term effects of any factor, including poaching, that selectively reduces the number of adult female elephants in a population.

We hypothesized that adult female elephants that lack an old matriarch, close adult female kin, strong adult social bonds, or reside in an area with historically high risk of poaching maintain higher physiological stress and lower reproductive output than females from groups possessing these elements or that reside in areas of low poaching risk. We examined this hypothesis against the alternative that group composition factors do not have long-term downstream effects; rather, females habituate to these conditions and maintain relatively low glucocorticoids and high reproductive success.

An inherent assumption in our hypotheses is that high physiological stress, as reflected by high fecal glucocorticoid measures (cortisol metabolites), indicates a negative physiological state for an elephant. Fecal glucocorticoids reflect adrenal activation in elephants as validated in an adrenocorticotropic hormone challenge and are correlated with the presence of external stressors in several species, including free-ranging African elephants (Wasser et al. 2000; Foley et al. 2001; Creel et al. 2002). Chronically high levels of these hormones are associated with diminished reproductive function, depressed immunity, muscle breakdown, and an increased risk of mortality in many species and therefore are an important measure of physiological condition (Wingfield & Ramenofsky 1999; Sapolsky et al. 2000).

Methods

Study Area

Mikumi (6.9°–7.7°S, 36.9°–37.4°E, area 3230 km²) consists of a diverse mosaic of *Brachystegia* and *Combretum* woodland and long-grass savanna, with grass height and density at its peak in June (Norton et al. 1987; Wasser & Norton 1993). Mikumi is the home of 1140–3100 free-roaming elephants (TWCM 1998; Blanc et al. 2002). The current growth rate of this population is uncertain because survey reliability and migration confounds population estimates (Hofer et al. 2004).

Sample Collection

We conducted surveys of 6 tourist roads (totaling 110 km) by vehicle across the northern third of Mikumi (1000 km²) in 3 seasonal quarters for 15 months in 2003–2005. When an elephant group was encountered, we recorded its latitude and longitude with a global positioning system device. We performed an initial scan from a maximum distance of 100 m, recording the number of individuals, their sex, age class (young adult 10–19 years; middle-aged adult 20–29 years; old adult ≥30 years), and number of visibly pregnant and lactating females (Kangwana 1996). Observation sessions continued off-road if necessary, concluding when the group left our field of view. Thereafter, we attempted to collect feces from observed defecations by adult females. Samples were preserved for subsequent hormone and DNA analyses (see later).

Six distinct locations of elephant use, each centered around a waterhole, became apparent as sampling progressed. Most groups frequented just one of these locations throughout the entire study. We assumed that at least part of their home range was included in their primary location; female African elephants faithfully maintain core home ranges, although they may expand their range on a seasonal basis beyond that core (Western & Lindsey 1984).

We identified 4 locations as areas of historically high poaching risk and 2 as low poaching risk. High-risk locations were those farthest from centrally located headquarters and tourist tracks but closest to park boundaries and neighboring villages, as observed by Ihde (1991). The poaching risk associated with the 6 areas in our study was also independently ranked on the basis of carcass densities and encounters with poachers by an antipoaching warden who worked in Mikumi prior to the ban (J. Hando, personal communication).

Identification of Elephants and Family Groups

We identified elephants on the basis of unique physical characteristics and a photo-identification file built over repeated sightings of all 109 families. We confirmed identifications by genotyping 2 separate dung samples collected on separate days from 37% of the individuals (100% agreement in Cervus [version 2.0]). Groups were defined as in Moss (2001). Group size was defined as the number of adult females behaving as a group for at least 50% of their sightings.

We derived each female's maximum pairwise association index (AI), representing the strongest social bond she developed with another adult female in the population, with the program SOCPROG (version 1.2; Whitehead 1997). The AI was determined from the number of times 2 elephants were sighted in close proximity behaving as a group divided by the total number of times each was sighted (ranges from 0 to 1, with 1 indicating that the 2 elephants were together every time they were sighted) (Cairns & Schwager 1987; Whitehead 1997). We used a sampling period of 1 day to ensure that behavioral observations were not autocorrelated.

Seasonal Factors

We measured daily rainfall (Novalynx [Grass Valley, California] rain gauge) and partitioned the data into the following seasons: early wet, December–February; late wet, March–May; early dry, June–August; and late dry, September–November, with December 1 defined as the start of the ecological year (Wasser & Norton 1993). The annual mean rainfall was 778 mm and was consistent with past trends observed in Mikumi (Wasser & Norton 1993). We conducted a principal components analysis (PCA) to capture seasonal complexity, reducing data from 5 variables (ecological day, ecological season, daily rainfall, monthly rainfall, and rainfall for the prior season) to 2 components.

A lack of vegetative cover increases vigilance behaviors and decreases foraging in some ungulates and may increase physiological stress (Bowyer et al. 1998). Total rainfall in December in Mikumi is positively correlated with the amount of grass cover for the entire ecological year; the annual growth pattern is set in motion with the first rains after the dry season fires (Wasser & Norton

1993). Thus, we included December rain in our models as a positive correlate of vegetative cover for the ecological year.

Fecal Hormone Metabolites and Reproductive Status

We rapidly dried fecal samples in the field with a custom-made drier. We previously validated this method of fecal hormone preservation (for up to 3 weeks) as comparable to freezing or freeze drying (Gobush 2008). Dried samples were frozen at -20°C within 3 weeks of collection and freeze-dried just prior to hormone extraction. We used the methods of Wasser et al. (1996, 2000) validated for the extraction and assay of elephant fecal glucocorticoids and progestins (interassay CV for glucocorticoids 6%, progestins 10%). We assumed that 1–2 fecal samples reflected an elephant's physiological state for a season, as justified by low CVs for glucocorticoid values from multiple samples per female (combined average CV = 4%, $n = 18$ individual CVs).

Fecal progestin metabolite concentrations, validated for captive and wild African elephants to establish pregnancy, were used to index gestation by trimester (Wasser et al. 1996; Foley et al. 2001). We designated each female's pregnancy status (by trimester) from a combination of the following criteria: progestin metabolite concentration ≥ 1000 ng/g, persistently elevated progestin across multiple samples, engorged mammary glands, distended abdomen walls, and presence of a newborn (Foley et al. 2001). A female's reproductive state, defined as a single component of her log progestin concentration and gestational trimester via PCA, was included in the glucocorticoid models as a covariate. For our reproductive output analyses, we classified females as nonreproductive, pregnant, or having an infant (< 2 years).

Fecal DNA and Relatedness

Fecal samples for DNA analysis were preserved in 20% dimethyl sulfoxide buffer. Fecal DNA was extracted from duplicate dung subsamples with a QIAamp DNA stool kit (QIAGEN, Valencia, California), purified with a gene clean, amplified with polymerase chain reaction at 10 di-nucleotide microsatellite loci, and scored on an Applied Biosystems 3100 Genetic Analyzer utilizing Genotyper (version 3.7; Comstock et al. 2000). All alleles were scored twice by the primary researcher, and 10% were called a third time by a second researcher; 98.1% of the scores were in agreement. To guard against incorrect genotyping due to allelic dropout, we repeatedly amplified samples until heterozygote alleles were observed at least 2 times and homozygote alleles at least 3 times.

We used genotypes to generate a pairwise coefficient of relatedness (r , ranging from -1 to 1) between all adult female pairs with KINSHIP (version 1.2), with an $r = 0$ representing the population average (Queller & Goodnight 1989). The r values were calibrated by computing

the relatedness of 7 known mother–infant pairs. Their average r was 0.41 (SE 0.05) (determined by jackknifing across all loci). We derived an average r for each group (assigned as zero when only 1 adult female was in the group) and the number of first-order relatives each elephant had in her group (minimum r for inclusion as a first-order relative was 0.37, consistent with our calibration).

Simulated Unpoached Genotypic Data Set

We categorized each observed Mikumi group as deviating from an unpoached elephant family with a simulated unpoached genotypic data set created through a gene-drop analysis program written in C++ (version 6.0) (Gobush 2008). This program is similar to MORGAN (version 2.8, Thompson 2005), but we simplified it for use with our data set. Gene-drop analysis is a Monte Carlo Markov chain simulation technique that successively drops alleles through a pedigree from founders to descendants. To accomplish this, the analysis randomly assigned genotypes to the pedigree's founders, the matriarch, and all fathers on the basis of allele frequencies derived from the Mikumi matriarchs (i.e., the eldest female per group born prior to peak poaching [1973] from 94 observed families, $n = 50$). We assumed Mikumi founders were an unrelated, random sample of the population; any alleles they shared would represent the background relatedness level of the population. Descendants' genotypes were then produced by simulating meiosis, recombination (genes were unlinked), and mating in chronological order.

We modeled the unpoached pedigree for gene-drop analysis on elephant family structure as described in Moss (2001). We independently repeated the gene-drop 1000 times for parsimonious pedigrees of 2–6 (i.e., no missing females, so the shortest branch lengths possible between females in the pedigree) and derived a mean group r for each. For example, a group of 3 with no missing adult females could be a mother and 2 adult daughters (average $r = 0.53$) or 3 half-sisters (average $r = 0.26$). The minimum average r for parsimonious pedigrees with 2–6 adult females was 0.26 (i.e., families of all maternal half-sisters). Groups were classified as disrupted if their observed average r was ≤ 1 SD below the expected average (simulated average r [SD] = 0.26 [0.18]); groups with $r < 0.08$ were designated as disrupted). Otherwise, groups were considered intact (i.e., likely missing no adult females for their group size).

Statistical Analyses

We conducted statistical analyses in SPSS (version 11.5, SPSS, Chicago, Illinois) and JMP (version 6.0, SAS, Cary, North Carolina). We conducted Kolmogorov–Smirnov (K-S) tests, one-way analysis of variance, and chi-squared analyses to examine distributions and relationships among variables. To test the hypothesis that the lack of an old matriarch, close adult relatives, and a strong

social bond negatively influenced adult female elephant stress physiology, we used a backward stepwise method to build a reduced general linear mixed model (GLMM) to predict glucocorticoids. Glucocorticoid data were log transformed before statistical analysis to normalize the data. We included individual identity as a random variable in these models to reduce autocorrelation. We included the following factors in our models: rainfall, December rain, reproductive state, age, location, group size, maximum AI and presence of old matriarch, and first-order adult relative and infant. We removed all nonsignificant factors from the final models.

Analyses were confined to dry-season data because wet-season flooding prevented sampling in 3 locations. This approach was further justified after preliminary data inspection revealed that location significantly influenced glucocorticoid levels. Seasonal bias in sampling is believed to be minimal because 93% of females were sampled in both seasons. Tourist presence and distance to villages were not included in the model per se, but were incorporated in the historic poaching-risk designation of each location.

Maximum AI, old matriarch, and first-order relative presence were significantly related, so we conducted a PCA creating 3 independent components (1 factor loaded at >0.90 and 2 factors loaded at <0.28 for all 3 components). We also built a GLMM to predict maximum AI to understand what factors influenced this variable. We analyzed the glucocorticoid data with and without solitary female groups to better explore fine-scale patterns among the larger groups (69.6% of the population). All solitary females had extremely low AIs, no first-order relatives, and only 6% were in the eldest age class. In combination, these attributes had the potential to mask the more complicated data patterns found among larger groups.

We initially looked at reproductive output according to age class. Young adults were omitted from subsequent modeling because they were more likely to be nulliparous, but possibly pregnant, than older females. We built an ordinal logistic fit model predicting reproductive output to test the hypotheses that the lack of an old matriarch, first-order relatives, and low bond strength have negative impacts on reproduction.

To test the hypothesis that a lower-than-expected group relatedness was associated with low reproductive output, we compared the reproductive output of groups identified as disrupted or intact (through the gene-drop analysis) with chi-squared analysis.

Results

Group Size and Age Distribution

We sampled 218 adult female elephants from 109 groups, 2–8 times each, successfully genotyping 93% of them.

Group sizes ranged from 2 to 19 individuals; 30.3% contained only 1 adult female. The Mikumi female elephants consisted of 28.4% young adults, 44.2% middle-aged adults, and 27.4% old adults. Old matriarchs (≥ 30 years) were present in 58.9% of the families, markedly increased from the 14.5% reported by Poole in 1989. Tuskeness in this old adult cohort dropped from 43.0% in 1989 to 28.8% presently.

Nevertheless, the Mikumi population remained extremely skewed toward smaller group sizes with a mean group size (2.2 [SE 0.11]) and distribution significantly less than that reported for the unpoached Amboseli population (6.76 [SE 0.58] [Archie et al 2006]; K-S test $p < 0.0001$; Fig. 1). Distribution of Mikumi group sizes

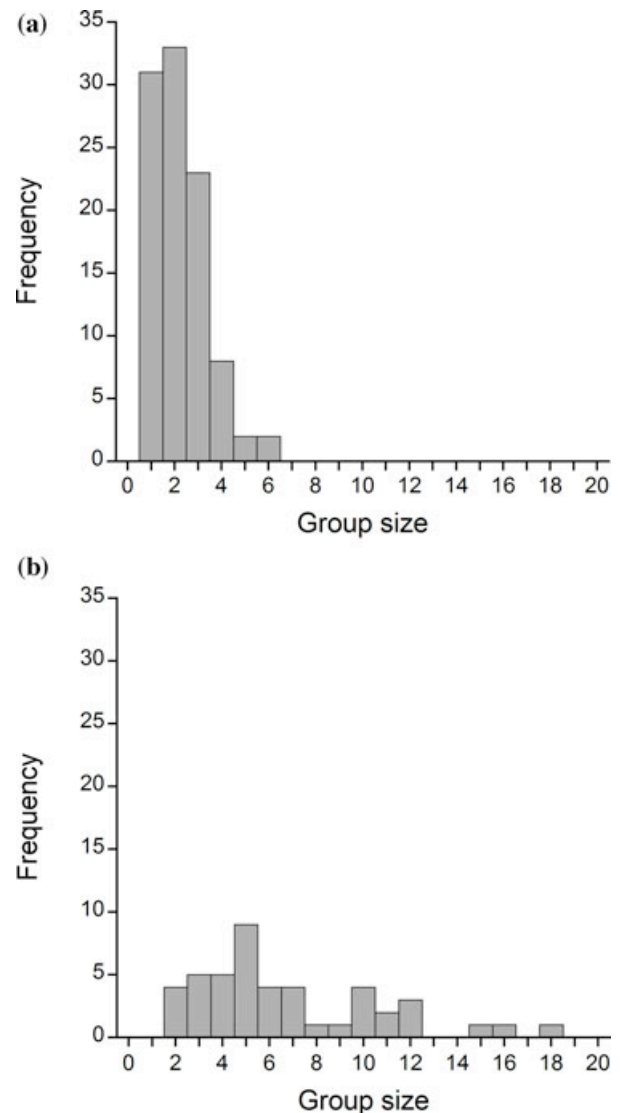


Figure 1. Group size distributions (number of adult females per group) for (a) Mikumi ($n = 189$ adult females in 102 groups [2003–2005]) and (b) Amboseli elephant populations ($n = 304$ adult females in 45 groups [data from Archie et al. 2006]).

in this study did not differ from data on the Mikumi population in 1989 ($n = 466$ elephants in 69 groups; Poole 1989).

Maximum Association Index

Females were sighted an average of 6 times each. Maximum AI among group members averaged 0.67 (0.03 SE) (range 0.5–1.0); 42% of individuals had an AI ≥ 0.9 . The number of sightings was uncorrelated with maximum AI ($R^2 = 0.01$, $F_{1,195} = 1.2$, $p = 0.28$). Maximum AI and age were significantly related: young adults and old adults had nearly equal values, whereas middle-aged adults' AIs were significantly lower ($R^2 = 0.04$, $F_{1,195} = 12.03$, $p = 0.03$).

The GLMM for maximum AI (adjusted $R^2 = 0.23$) showed that the lack of an old matriarch predicted a lower AI ($F_{1,170} = 17.2$, $p < 0.0001$). Location significantly influenced maximum AI ($F_{4,167} = 3.2$, $p = 0.01$), with the lowest mean in an area of high poaching risk. An interaction of these 2 factors was also significant ($F_{4,167} = 8.1$, $p \leq 0.0001$): missing an old matriarch had particularly strong impacts on AI in 2 of the 4 areas of high poaching risk. Group size, pregnancy, first-order relative, and infant presence were not significant.

Relatedness

Average group relatedness in the population was 0.13 (SE 0.01) (range -0.22 to 0.67); 48.6% of females had no first-order adult relatives in their group, 35.2% had 1, and 16.2% had 2. Having an old matriarch significantly predicted whether a female had a first-order relative in her group ($R^2 [U] = 0.04$, Pearson's $\chi^2_{2,158} = 7.98$, $p = 0.02$). Only 12 females of 77 had first-order relatives and no old matriarch.

Glucocorticoids

The GLMM that included all female elephants (adjusted $R^2 = 0.31$) showed that low monthly rainfall ($F_{1,187} = 21.7$, $p < 0.0001$), low December rainfall ($F_{1,187} = 8.6$, $p = 0.004$), and advancing pregnancy ($F_{1,187} = 4.4$, $p = 0.04$) all predicted high glucocorticoids. Elephants in areas of high poaching risk had higher glucocorticoids than those in low-risk areas ($F_{1,187} = 7.7$, $p = 0.006$). The interaction between poaching risk and December rain was significant ($F_{1,187} = 6.7$, $p = 0.01$). Females in the high-risk locations had significantly higher glucocorticoids during years with low vegetative cover as reflected by low December rainfall. The interaction between maximum AI and location was significant ($F_{1,187} = 3.9$, $p = 0.05$). Females with weaker social bonds, which goes along with lack of close adult kin and an old matriarch, had higher glucocorticoid levels in areas of higher poaching risk. Group size, old matriarch, first-order relative, and infant presence were not significant.

Among females in multiadult groups (adjusted $R^2 = 0.46$), low monthly rainfall ($F_{1,157} = 23.6$, $p < 0.0001$), low December rain ($F_{1,157} = 5.9$, $p = 0.02$), and advancing pregnancy ($F_{1,157} = 4.7$, $p = 0.03$) again all predicted high glucocorticoid levels. Females had higher glucocorticoids in the high-risk locations during years with low vegetative cover (as reflected by low December rain) ($F_{1,157} = 4.9$, $p = 0.03$). Females that lacked close adult kin in an area of high poaching risk had the most elevated glucocorticoids ($F_{1,157} = 4.3$, $p = 0.04$). Females with close adult kin, 84% of which were a part of an old matriarch–adult daughter pair, fared better overall; elephants living in areas of high poaching risk with their adult kin were especially buffered from stress impacts (Fig. 2). Group size, maximum AI, and infant presence were not significant.

Reproductive Output

Thirty-three percent of Mikumi females were classified as nonreproductive, 28% were pregnant, and 48% had an infant during the course of this study. The distribution of our progesterone data (Fig. 3), used to assign pregnancy trimester, was similar to distribution of progesterone data of 16 Tarangire elephants whose entire gestations were followed (Foley et al. 2001). Middle-aged females were more likely to be nonreproductive (37%) than old adults (17%) ($R^2 [U] = 0.04$; Pearson's $\chi^2_{3,215} = 15.961$, $p = 0.014$).

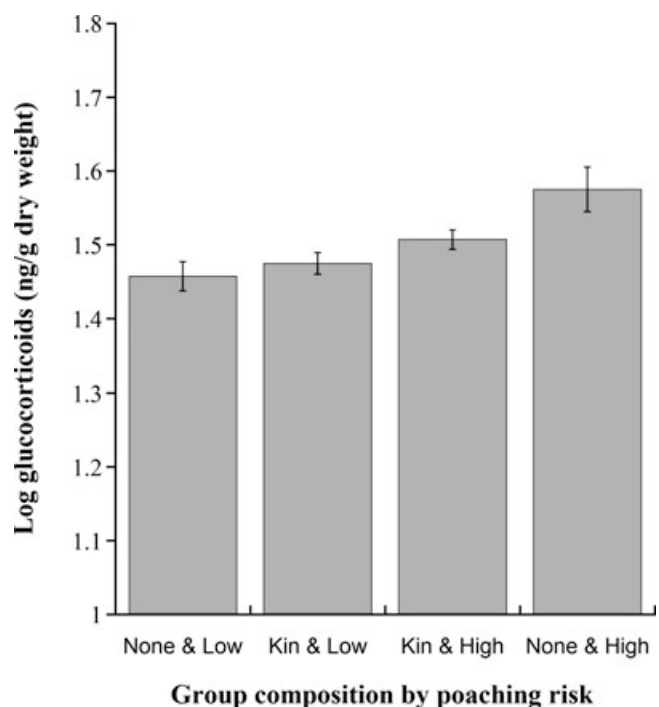


Figure 2. Log fecal glucocorticoid levels for female elephants according to first-order adult relative presence (kin) or absence (none) and poaching risk of home-range location (low and high) (means and SE).

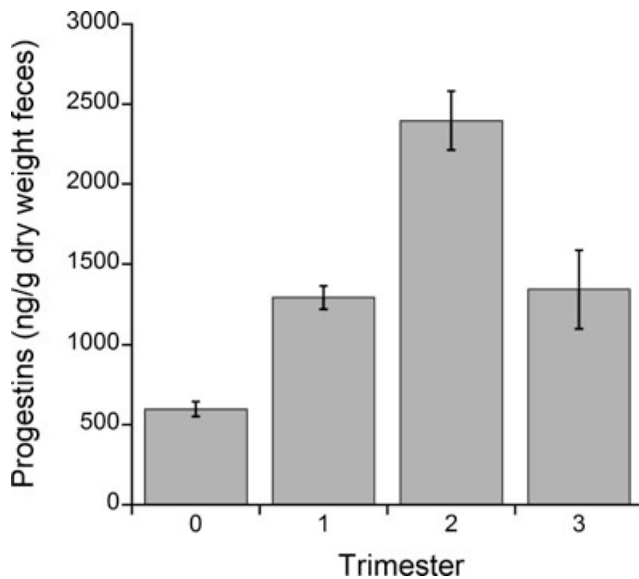


Figure 3. Distribution of fecal progesterin metabolites by pregnancy trimester for Mikumi elephants (means and SE; 0, not pregnant).

Our logistic model showed that females with higher group relatedness and a higher maximum AI had higher reproductive output (Table 1). Both of these factors had significant interactions with group size. Reproductive output increased as group relatedness or maximum AI increased, and this increment was greatest with increasing group size.

Reproductive Output of Disrupted Groups

Forty-one observed groups, representing 118 females, were compared with the simulated unpoached group data. Overall, 33.9% of the adult females came from groups classified as disrupted (average group r range of -0.22 to 0.07). We conducted analyses with and without young adults to examine the relationship between a female's reproductive output and her group status (i.e., disrupted or intact). Both analyses produced the same significance patterns: females from disrupted groups were significantly less likely to be reproductive than females from intact groups ($R^2 [U] = 0.04$, Pearson's $\chi^2_{5,78} =$

12.9 , $p = 0.005$) (Fig. 4a). This difference occurred because fewer females from disrupted groups had infants than those from intact groups ($R^2 [U] = 0.06$, Pearson's $\chi^2_{1,82} = 9.7$, $p = 0.002$) (Fig. 4b).

Discussion

Our results suggest there have been multiple negative impacts of poaching on surviving adult female elephants and that these impacts have persisted as long as 15 years. If a female was an old matriarch or had an old matriarch in her group, she was more likely to have a close relative in her group and she formed stronger bonds than other elephants. This suite of group characteristics decreased a female's stress response and increased her reproductive output.

Mikumi lost over half its elephants to poaching in the decade leading up to the ivory ban. After the ban, poaching mortality dropped to just a few individuals per year in the park, indicating that the disrupted demographic patterns we report arose before the ban. Salient patterns of group size persisted as evidenced by the current percentage of solitary females (30.1%), which is only slightly less than what was reported just prior to the institution of the ivory ban (33.9%) (Poole 1989). Tarangire National Park had a similar poaching history as Mikumi, and 20% of that population still consists of solitary females and their offspring (Foley 2002). Other group composition factors were less static in Mikumi. The number of families with tusked old matriarchs has increased by 14.2% in the last 15 years, further substantiating that poaching mortality of this cohort for its tusks has receded since 1989.

Glucocorticoids

Our predictive models revealed that social bonds, group composition, and perceived poaching risk (i.e., location in park and amount of cover) significantly influenced a female elephant's stress physiology. When a female lacked strong social bonds and had a home range in an area of high poaching risk, she had significantly higher

Table 1. Ordinal logistic-fit model for reproductive output of Mikumi middle-aged and old adult female elephants.*

Response (n)	Total model	Source	df	χ^2	p ($\alpha = 0.05$)
Reproductive output (114)	$R^2(U) = 0.21$	age class	1	2.73	0.099
		group size	4	8.28	0.082
		group relatedness	1	7.56	0.006
		maximum association index	1	6.71	0.010
		group relatedness \times group size	4	10.02	0.040
		maximum association index \times group size	4	9.70	0.046

*Nonsignificant variables tested: home-range location, first-order adult relative, and old matriarch presence.

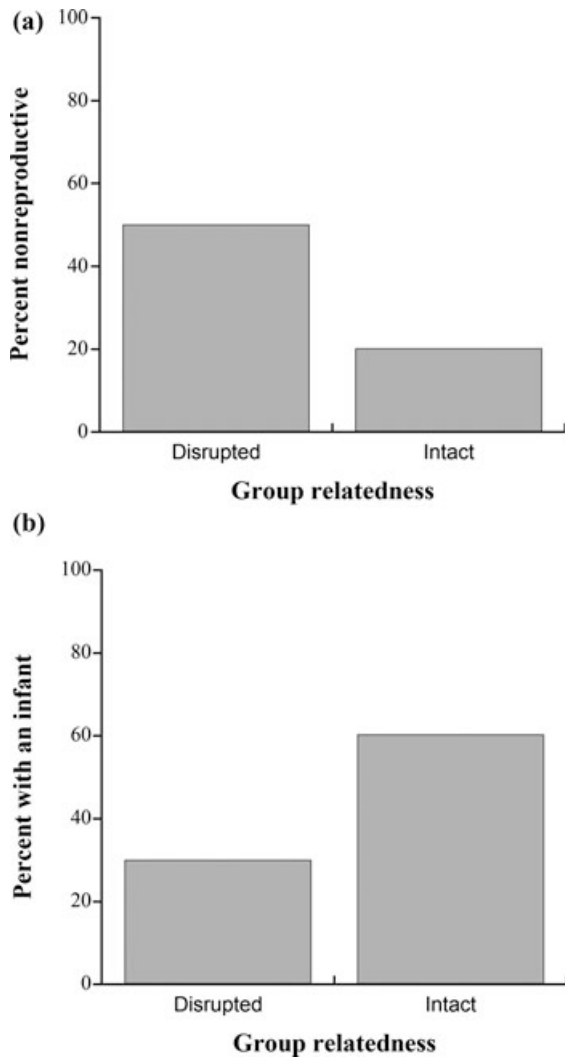


Figure 4. Percentage of female elephants in disrupted and intact groups that (a) were nonreproductive and (b) had an infant < 2 years old.

glucocorticoids than other elephants. Lack of cover, reflected by low December rain exacerbated the poaching-risk effect. For elephants that resided in a high-risk area and also had at least one other adult female in their group, their stress physiology was improved if one of these adults was a close relative. In 1989 the Mikumi elephants were compressed around the 10-km radius of Park Headquarters (Balozi 1989). This area continues to function as a safe haven for elephants, despite the absence of an extreme poaching threat for 15 years. Nevertheless, as elephant families have repopulated the once heavily poached areas, those in groups with close kin, typically strong-bonded, old matriarch-adult daughter pairs, were the ones able to maintain a low stress response.

Our results suggest that a disrupted family group is a chronic stress condition for African elephants. Chronically high glucocorticoids have inhibitory effects on

growth, immunity, and reproduction (Romero 2004). Because chronic stress influences an individual's acute response to stressors, females in these disrupted families may be even more vulnerable to nonpoaching-related disturbances, such as severe drought, climate change, land compression, and disease (Wingfield et al. 1994).

Reproductive Output

Sixty-six percent of the adult females in Mikumi were either pregnant and/or had an infant < 2 years old during our study, and the most fecund individuals were over the age of 30. Average group relatedness, bond strength, and disrupted social structure were important factors in predicting an adult female's reproductive success. Relatively high relatedness and high bond strength were associated with high reproductive output, whereas females with a group relatedness of one or more standard deviations below that of a simulated unpoached group were less likely to have an infant.

In a recent study of an unpoached population of elephants in Addo (South Africa), 95% of the adult females under the age of 49 were pregnant or lactating, and the most fecund individuals were between the ages of 25–29 (Whitehouse & Hall-Martin 2000). Likewise, the most fecund age for adult females in Amboseli was 20–29 years (Lee 1987). Females in this age class in Mikumi had significantly lower reproductive output than their older counterparts. Our results point to a disrupted social structure, associated low kinship, and weak bonding as the culprits.

An Overarching Age Pattern

Middle-aged adults comprised the highest proportion of the elephants that lived alone, lacking all of the healthy group composition factors studied. These females would have been 5–15 years old during the peak poaching era, the minimum age of likely survival without their mothers (Poole 1989). Even when we excluded single-adult groups, we discovered that middle-aged females still formed weaker bonds on average than the other 2 age classes, which tended to associate with each other. Young adult females were more likely to have an old matriarch and a close relative in their group than middle-aged females. Our simulation analysis confirmed this: significantly more middle-aged adults came from disrupted families with a low degree of relatedness compared with the other 2 age classes. These middle-aged adults also had poorer reproductive output than older adults. All of these findings reveal that middle-aged adults in Mikumi, without their mothers, have been at a significant disadvantage compared with the other elephants in this population.

Our findings reflect a transgenerational effect of poaching. Behavioral patterns and social hierarchies may be altered in cases of severe poaching. Older, larger females consistently dominate smaller, younger females in the

unpoached Amboseli population (Archie et al. 2006). When an old matriarch is removed from her family, her influence on other elephants and groups is also removed. For example, more effective competitive and defense behaviors occur if an old matriarch is in the group, conferring greater sensitivity to distinguishing known elephants from intruders (McComb et al. 2001). If a daughter's present and future social status and behavior is influenced by her mother, the loss of an old matriarch could lower her status, altering her social alliances and tolerances. In poached populations, females presently aged 10–19 years may have unusual social, physiological, and reproductive advantages over females aged 20–29 years because they are more likely to have an old, related matriarch in their group.

Old female elephants hold unique social positions in their families. As the first females targeted in heavily poached populations, their loss impairs group social functioning, elevating physiological stress and reducing reproductive output among the females left behind. The consequences of disrupting group composition in this way may persist until sufficient time has elapsed for a new mother–adult daughter pair to form, which could be upwards of 20 years for this long-lived species.

Elephants are a highly intelligent, socially complex, matriarchal species. It should thus come as little surprise that legal or illegal selective removal of old related matriarchs from elephant groups has long-lasting negative consequences for the survivors. Wildlife authorities and managers should give strong consideration to these long-term health impacts when deciding whether to trade or cull this and other highly intelligent, socially complex species.

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