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## Reduced effective population size in an overexploited population of the Nile crocodile (*Crocodylus niloticus*)

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## ABSTRACT

Unchecked exploitation of wildlife resources is one of the major factors influencing species persistence throughout the world today. A significant consequence of exploitation is the increasing rate at which genetic diversity is lost as populations decline. Recent studies suggest that life history traits affecting population growth, particularly in long-lived species, may act to moderate the impact of population decline on genetic variation and lead to remnant populations that appear genetically diverse despite having passed through substantial demographic bottlenecks. In this study we show that the retention of genetic variation in a partially recovered population of Nile crocodile is deceptive, as it masks the reality of a significant decline in the population’s effective size ( $N_e$ ). Repeated episodes of unchecked hunting in the mid to late 20th century have today led to a five-fold decrease in the population’s  $N_e$ . Using current census data we estimate the contemporary  $N_e/N$  ratio as  $\sim 0.05$  and, in light of quotas that permit the ongoing removal of adults, simulated the likely effects of genetic drift on extant levels of variation. Results indicate that even if the current effective size is maintained, both allelic diversity and heterozygosity will decline. Our findings have complex implications for long-lived species; an emphasis on the retention of genetic variation alone, whilst disregarding the effects of population decline on effective size, may ultimately obscure the continued decline and extinction of exploited populations.

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### 1. Introduction

Changes in the genetic properties of a population over time is determined by the effective size of the population,  $N_e$ , rather than the actual number of living individuals,  $N$  (Wright, 1931; Crow and Kimura, 1970).  $N_e$  describes the size of an ideal population that has the same rate of genetic change as that of the population under consideration and in so doing governs both the effects of selection on populations and the rate at which diversity is lost via genetic drift (Wright, 1931, 1938). In so doing,  $N_e$  directly influences population viability because the strength of stochastic changes in genetic properties is reduced in populations with larger effective sizes (Frankham et al., 2002; Hedrick, 2005). Since genetic diversity is a fundamental component of adaptive evolution, its loss via drift and/or selection is predicted to decrease the probability of a population persisting through time; accordingly,  $N_e$  is one of the most important parameters in evolutionary and conservation biology

(Frankham, 2005; Waples, 1990a). Processes that rapidly deplete genetic diversity via reductions in  $N_e$  (e.g. population fragmentation and unchecked exploitation) are predicted to increase the chance of short-term inbreeding depression and reduce the ability of a population to respond to novel environmental change (Amos and Balmford, 2001; Frankham, 2005). As a result, declining populations experience increased genetic drift and these effects are magnified through time (Frankham et al., 2002; Hauser et al., 2002; Spielman et al., 2004a), increasing susceptibility to extinction via demographic and environmental stochasticity (e.g. Newman and Pilson, 1997; Saccheri et al., 1998; Westemeier et al., 1998). Notwithstanding these predictions, a number of recent studies propose that the impact of population decline on genetic variation depends considerably on life history traits that affect population growth (Kuo and Janzen, 2004; Hailer et al., 2006; Lippé et al., 2006). Processes driving the loss of genetic diversity may in fact be buffered by intrinsic biological traits, specifically long generation times in age-structured species, and can result in remnant populations that appear genetically diverse despite periods of substantial decline.

We investigate how extant genetic variation of a large, long-lived vertebrate population, occupying an important African wetland, might persist in light of a history characterized by overexploitation and population collapse. The Nile crocodile (*Crocodylus*

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*niloticus* Laurenti) is a top aquatic predator broadly distributed throughout the freshwater and brackish habitats of sub-Saharan Africa. Overexploitation through the mid to late 20th century caused the decline and extinction of Nile crocodile populations in many parts of its range (de Smet, 1998), and studies indicate that demographic stability in crocodile populations is sensitive even to relatively small perturbations (Games, 1992; Graham et al., 1992). Today, wild populations continue to experience harvesting pressure; the species remains one of the top commercially utilized species of crocodylian worldwide (Ross, 1998). The population of the Okavango Delta, Botswana exemplifies the effects of uncontrolled exploitation; from the mid 20th century onwards the explicit targeting of adult individuals by hide hunters resulted in repeated cycles of population collapse. For example, records estimate that up to 48,000 adult individuals were removed from the Okavango Delta during the period 1957–1968 alone (Pooley, 1982). Following a Convention on International Trade in Endangered Species (CITES) ban in 1973 on the trade of products from wild *C. niloticus* individuals, and together with a decade-long moratorium on the exploitation of the population, commercial ranching was introduced in 1983 as a way of sustainably meeting continued international demands for hides. To populate ranches, farmers removed ~1050 live adults and 14,000 eggs from the Okavango Delta between 1983 and 1988; nest surveys conducted in 1987 indicated that this activity had already led to a 50% reduction in the number of active nests (Simbotwe and Matlhare, 1987). Today the Okavango supports a partially recovered but much depleted population of Nile crocodiles; while the quota-based removal of breeding adults and egg clutches for commercial ranching is ongoing (Dzoma et al., 2008).

In this study we assess whether life history traits inherent to Nile crocodiles have buffered the negative genetic effects expected of repeated population collapse in the Okavango Delta. Microsatellite data for a total of 154 individuals were examined for signatures of repeated population bottlenecks, predicting low genetic diversity and reduced effective size. To determine whether extensive removal of individuals during the recent past has influenced the current effective size, we estimated  $N_e$  for different time periods in the recent history of the population. Given the continued exploitation of this population, we used a contemporary estimate of  $N_e$  to explore the prospect of maintaining extant levels of genetic diversity by simulating the predicted decline in diversity due to random drift. Using census estimates from mark-recapture data for a total of 1084 animals, we also obtained an estimate of the current  $N_e/N$  ratio and discuss our findings relating to current conservation management policy.

## 2. Materials and methods

### 2.1. Sample collection and DNA extraction

Nile crocodiles are long-lived (50–80+ years), reaching sexual maturity between 12 and 15 years (Leslie, 1997). During the wet season individual crocodiles are found throughout the flood plain and delta of the Okavango River; during the dry season animals retreat to the deep-water perennial swamps of the main river channel, known as the Panhandle (Fig. 1). Breeding activity is restricted to the Panhandle, where 99% of all nests occur (Graham et al., 1992). Consequently, exploitation by hide hunters was also concentrated in the Panhandle. From January 2002 to December 2006 we carried out annual capture-mark-recapture (CMR) surveys of the population within the Panhandle from Mohembo (18°15'17"S, 21°46'59"E) to Seronga (18°49'43"S, 22°24'22"E) (Fig. 1). Depending on their size, individuals were either captured by hand (<120 cm), by means of a swiveling noose (Animal Han-

dling Co.) in the river, or within baited box and Pitman traps placed on the river bank. Animals were aged according to their size (Hutton, 1989; Leslie, 1997): hatchlings  $\leq 17$  cm snout-vent length (SVL), yearlings 18–38 cm juveniles, 39–65 cm, sub-adults 66–115 cm, adults  $\geq 116$  cm. Blood was collected from the caudal vein in the tail from each crocodile and stored in lysis buffer at  $-20^\circ\text{C}$ ; all individuals were unambiguously marked by scute clipping (Chabreck, 1963). To account for heterogeneity in capture probability as animals increased in size CMR data was supplemented by nocturnal spotlight count data (Hutton and Woolhouse, 1989; Platt and Thorbjarnarson, 2000), collected during the sampling sessions, to determine the current adult class distribution for the population. DNA was extracted from 154 individuals (80 adults and 74 hatchlings representing the 2004/2005 sampling season) using a DNeasy Tissue Kit (Qiagen) following manufacturer's instructions. Individuals were genotyped at seven microsatellite loci developed for the genus *Crocodylus* (FitzSimmons et al., 2001) (see Supplementary material).

### 2.2. Population size estimates

We used a sequential Bayesian approach (Gazey and Staley, 1986) based on a non-informative prior distribution (all possible population sizes have equal probability of being true) to derive annual census estimates of the adult  $N_{adult}$  and total population  $\hat{N}$  size. To achieve this we used CMR and spotlight count data for the period September 2002 to December 2006 using the methods of Underhill and Fraser (1989) and Underhill (1990). During iterations individuals were sampled with replacement and the maximum population size tested  $N_{max}$  was set at 5000. The incremental parameter  $N_k$  was set so that all integer sampling population sizes including  $N_{max}$  were sampled; similar estimates of  $\hat{N}$  were obtained for a number of  $N_{max}$  estimates. Following Hutton and Woolhouse (1989) and Kay (2004, 2005) we assumed a closed population given that: (i) immigration from surrounding wet-season floodplains during the study period was effectively zero due to the lack of water in the ephemeral Salinda spillway (Fig. 1), (ii) our data indicate that individual crocodiles did not move significant distances from their original sampling site (average distance moved by marked individuals between years ranged from 2 to 100 m; total river sampled 250 km), and (iii) the population is significantly reduced in the human settled areas of the northern Okavango extending into Namibia and Angola and movement of individual crocodiles into the Panhandle is highly restricted (Bourquin, 2008). Immigration, emigration, and death during the CMR sampling period were likely to be sufficiently small that the assumption of a closed population in this study system is, at least, plausible.

### 2.3. Population genetic analyses

Genetic variability at seven microsatellite loci was quantified using FSTAT v2.9.4 (<http://www2.unil.ch/popgen/softwares/fstat.htm>) as allelic diversity ( $A$ ) and observed ( $H_{obs}$ ) and expected heterozygosity ( $H_{exp}$ ) with respect to Hardy–Weinberg expectations. An absence of linkage was observed among the seven loci (Fisher's exact test;  $p > 0.05$  for all pairwise comparisons across all samples; FSTAT v2.9.4) i.e. allele frequencies at all loci are independent of one another. We tested for the signature of recent and pronounced reductions in population size using two different approaches. Because allelic diversity is lost at a faster rate than heterozygosity in declining populations (Cornuet and Luikart, 1996), we tested for heterozygosity excess ( $H_E$ ) relative to that expected for a population at mutation-drift equilibrium ( $H_{Eq}$ ) in BOTTLENECK v1.2.02 (Piry et al., 1999). We applied the two phase model of microsatellite evolution with 95% of mutations following the

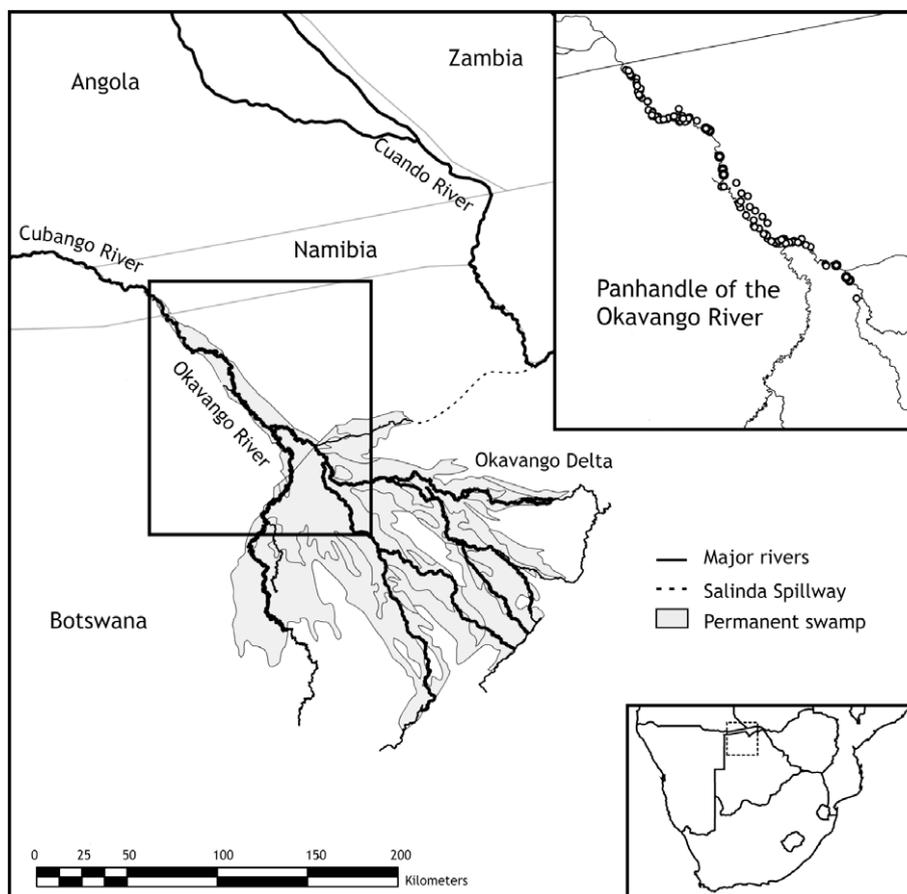


Fig. 1. Map of the Okavango Delta, Botswana. Open circles indicate sampling locations for individual crocodiles used in the genetic study.

step-wise mutation model (SMM) and 5% conforming to the infinite allele model (IAM), and 10% variance of multiple-step mutations. Statistical significance was determined using a Wilcoxon sign-rank test implemented within the program. We also performed Garza and Williamson's (2001)  $M$  ratio test to determine whether repeated cycles of population collapse had resulted in the loss of microsatellite alleles ( $k$ ) at a faster rate than the range in allele size ( $r$ ) thereby reducing the ratio  $M(k/r)$ , as predicted under the SMM. To test the significance of this ratio we estimated the critical ratio ( $M_C$ ) in CRITICAL\_M whereby 95% of 10,000 simulations of an equivalent population at equilibrium had  $M > M_C$ .

### 2.3.1. Estimating effective population size ( $N_e$ )

Because  $\hat{N}_e$  is influenced by changes in  $N_e$  across specific generations we calculated several contemporary estimates of  $N_e$  to match  $\hat{N}_e$  to the appropriate time period (Waples, 2005). We used the linkage disequilibrium method (Hill, 1981) to estimate the parental generation inbreeding effective size ( $N_{eb}$ ; effective number of breeding adults) from a single temporal sample. To explore whether the recent trend of decreasing population size has influenced  $\hat{N}_{eb}$ , we applied this calculation to two data sets, the first representing the current adult generation and the second representing the hatchling cohort using  $N_e$ ESTIMATOR v1.3 (Peel et al., 2004). Populations with reduced breeding pools are expected to accumulate more disequilibrium over time, thereby reducing the frequency of novel gene combinations (Hill, 1981) which are, in turn, expected to be reflected in divergent estimates of  $N_e$ . We also used temporal methods to estimate variance  $N_e$ , representing the interval between the adult and hatchling generations i.e. the harmonic mean of the effective sizes in generations zero through  $t - 1$ . These methods examine temporal changes in allele fre-

quency where the standardized variance in temporal change, a function of drift, is inversely proportional to the  $N_e$  of the population (Nei and Tajima, 1981; Waples, 1989). We estimated variance  $N_e$  using: (i) the moments-based method of Waples (1989), and (ii) the Bayesian coalescent approach of Berthier et al. (2002) which is implemented in TM3.1 (<http://www.rubic.rdg.ac.uk/~mab/software.html>). The posterior distribution of variance  $N_e$  was approximated with *a priori* data estimating the minimum number of breeding females (mean no. of nests from annual nest surveys of the Panhandle 2002–2006). We ran 100,000 replications with a specified maximum  $N_e$  of 500. Increased replications did not improve the accuracy of the estimates as inferred from 95% confidence intervals. Because temporal samples representing adults and hatchlings collected within the same year represent a single season estimate of the variance effective size of breeding individuals ( $N_b$ ) estimates were converted into comparative values by multiplying  $\hat{N}_b$  by the generation time (Waples, 1990a,b). Hatchlings selected for analysis were sampled throughout the 250 km study area to reduce the statistical bias of analyzing individuals from a limited number of clutches. We determined the ratio of effective to census population size  $\hat{N}_e/\hat{N}$ , using the harmonic mean of annual estimates for  $\hat{N}$  (Kalinowski and Waples, 2002).

### 2.3.2. Demographic simulations

In light of CITES quotas that permit the ongoing removal of both egg clutches and breeding females to stock crocodile farms, we explored the prospect of maintaining extant levels of genetic diversity by means of simulation using the software BOTTLESIM v2.6 (Kuo and Janzen, 2003) (<http://chkuo.name/software/BottleSim.html>). With different  $N_e$  values temporally stable over a period of 200 years, we estimated the sustainable effective population

size required to meet the recommended conservation objective of maintaining 90% of initial genetic diversity for a minimum period of 100 years (Frankham et al., 2002). Simulations were performed with the following life history parameters remaining constant: longevity = 50, age at sexual maturity = 13.5 (est. 12–15), overlapping generations, random mating, sex ratio ( $M:F$ ) = 1:1.3, 1000 iterations. Adult sex ratio data was determined from our CMR study.

### 3. Results

#### 3.1. Population size estimates

A total of 1084 crocodiles were marked in the study. The mean annual Bayesian estimate of the yearling census population size  $\pm$  s.e.m. was  $768.8 \pm 89.8$ . The harmonic mean of the total population census size  $\pm$  s.e.m. was estimated at  $\tilde{N} = 2502.4 \pm 198.5$ , whilst the harmonic mean of the adult population  $\pm$  s.e.m. was estimated at  $\tilde{N}_{adult} = 663.1 \pm 50.3$ .

#### 3.2. Detecting bottlenecks

Levels of genetic diversity in the Okavango Nile crocodile population were moderate; allelic diversity ranged from 3 to 11 alleles per locus (mean = 6.7) and observed heterozygosity ( $H_{obs}$ ) ranged from 0.18 to 0.79 (overall  $H_{obs} = 0.51$ ) across the seven loci (see Table S1; Supplementary material). Overall, the population was found to deviate significantly from Hardy–Weinberg expected proportions (overall  $H_{exp} = 0.72$ ) with a significant heterozygote deficit (Chi<sup>2</sup> test;  $p < 0.01$ ). Tests for the effects of population bottleneck events produced differing results that reflect the time period to which the methods apply (discussed below). A significant test for heterozygosity excess in the population supports the recent deviation from mutation–drift equilibrium in response to repeated cycles of population decline (Wilcoxon sign-rank test;  $p = 0.003$ ). Nonetheless, evidence for population decline as assessed by the ratio of allele number to allele size could not be inferred from the  $M$  ratio test; average ratio over seven loci = 0.87, greater than the critical ratio  $M_C$  of 0.68.

#### 3.3. Estimates of $N_e$ and demographic simulations

We identified significant disparity between estimates of  $N_e$  for contemporary adult and hatchling crocodiles based on the inbreeding effective size of pre-exploitation and current parental generations (Table 1). This surprising find reveals that despite partial demographic recovery of the population, the effective size has decreased by approximately five-fold  $\tilde{N}_e$   $t - 1$  to  $\sim t - 5 = 477$ ; 95% C.I. = 160–5534;  $\tilde{N}_e$  current parental generation = 89.2; 95% C.I. = 52–235). Methods based on the temporal fluctuations in allele frequencies yielded comparable  $\tilde{N}_b$  estimates with similar confidence intervals (moments-based  $\tilde{N}_b$  17.3; 95% C.I. = 8.6–35.6; Bayesian  $\tilde{N}_b$  18.5; 95% C.I. = 11.5–42.3). The  $\tilde{N}_e$  estimates ( $Gen^n * N_b$ ) from these two methods ( $\tilde{N}_e$  range 233.6–249.8) fell within the range of the parental generation  $\tilde{N}_e$  values estimated by the linkage disequilibrium method, and represent estimates of the harmonic mean  $N_e$  for the period covered by the two temporal sam-

ples. The ratio of effective size to census size  $\tilde{N}_e/\tilde{N}$  ranged from 0.04 (current parental generation  $N_e$ ) to 0.1 (harmonic mean  $N_e$ ).

Projections of the levels of genetic diversity that would be retained in the Okavango population after both 100 and 200 years ( $\sim 13$ – $16$  generations) reveal that even with a stable population size over two centuries, existing levels of genetic diversity will decrease. These simulations predict allelic diversity will be lost at a significantly faster rate than associated levels of heterozygosity, irrespective of the starting  $N_e$  value (Fig. 2). Based on observed allele frequencies, the population is predicted to retain  $\sim 76\%$  of the observed number of alleles and  $\sim 93\%$  of observed heterozygosity if the effective size of the population remained constant for the next 200 years/ $\sim 15$  generations ( $N_e$  estimated by linkage disequilibrium method). In order to retain 90% of the existing genetic diversity, the effective size would need to persist at a value  $\geq 250$ ; this would result in the preservation of  $\sim 91\%$  of  $A_0$  and 98% of  $H_0$ .

### 4. Discussion

Analysis of our data within a population genetic framework reveals evidence of declining  $N_e$  in a heavily exploited population of crocodiles. This result is unexpected given the partial demographic recovery of the population and the retention of moderate levels of genetic variation. The chronic disturbance to genetic variation, as predicted from the explicit targeting of adult individuals, appears to have been buffered by a combination of the species' longevity together with the delayed sexual maturity of juvenile individuals within the population. Whilst these life history traits appear to have curbed the loss of genetic diversity, they have not prevented the ensuing reduction in effective population size; thereby increasing population susceptibility to the accelerated effects of genetic drift and the potentially negative effects of selection. It follows that current levels of allelic diversity are unlikely to be maintained without considerable immigration and/or gene flow.

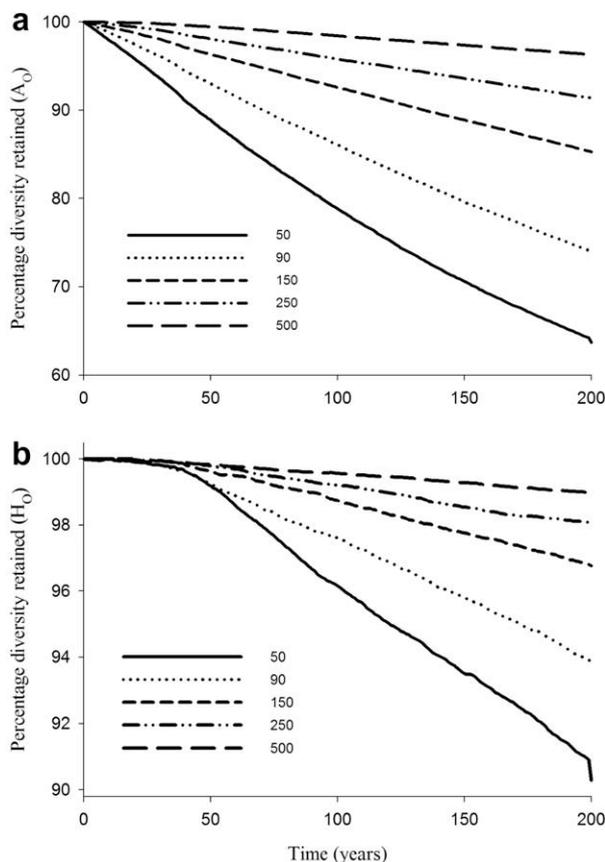
#### 4.1. Population decline and genetic variation

Despite a reduced contemporary adult population  $\tilde{N}_{adult} \sim 660$ , our study suggests that the Okavango Nile crocodile population continues to support moderate levels of neutral genetic variation (mean  $H_{obs} = 0.51$ ) comparable to values reported for a number of crocodylians that have experienced significant population decline (all studies based on microsatellite data) e.g. American alligator *Alligator mississippiensis*  $H_{obs} = 0.46$  (Glenn et al., 1998); Morelet's crocodile *Crocodylus moreletii*  $H_{obs} = 0.49$  (Dever et al., 2002); black caiman *Melanosuchus niger*  $H_{obs} = 0.61$  (de Thoisy et al., 2006). Tests for genetic signatures of recent population decline produced divergent results. The heterozygosity excess test (Cornuet and Luikart, 1996) was more sensitive to fluctuating population size than the  $M$  ratio test (Garza and Williamson, 2001), indicating significant deviation from mutation–drift equilibrium. Whilst it is possible that insufficient time has passed to observe the effects of population decline on measures of genetic diversity, it is more likely that our observations are explained by the delayed sexual maturity and longevity characteristic of crocodylians. Following cycles of popula-

**Table 1**

Estimates of contemporary effective population size and the period of time to which the estimate applies. 95% confidence intervals (C.I.) are indicated in brackets.

Method	$\tilde{N}_e$ estimate	$\tilde{N}_e/\tilde{N}$	Time period of estimate
Inbreeding effective size	477 (160–5534)	–	Former parental $N_e$ ( $t - 1$ to pre-exploitation)
Linkage disequilibrium (Hill, 1981)	89.2 (52–235)	0.04	Current parental generation $N_e$ ( $t - 1$ to $\sim t - 5$ )
<i>Variance effective size</i>			
Moment-based (Waples, 1989)	233.6	0.09	Harmonic mean $N_e$
Bayesian approach (Berthier et al., 2002)	249.8	0.10	$Gen^n$ (0) to ( $t - 5$ ) 2005 to $\sim 1945$



**Fig. 2.** Simulated loss of genetic diversity over time for different starting  $N_e$  values. (a) Observed allelic diversity ( $A_o$ ), and (b) observed heterozygosity ( $H_o$ ). In each simulation starting  $N_e$  was kept constant over time. Simulations suggest that in order to retain 90% of extant allelic diversity over the next 100–200 years, the minimum effective population size needs to be in the range of  $N_e = 150$ –250.

tion collapse, these life history traits act to stem the loss of genetic diversity, even though the effective size of the population may progressively decline. In such a scenario, longevity together with delayed sexual maturity would shorten the effective bottleneck period, and thereby buffer the expected rate at which genetic variability is lost. Furthermore, as with many hunted species, only adults were explicitly targeted during periods of intense exploitation, such that younger cohorts that remained were likely to maintain the presence of rare alleles in the population. Under this scenario we expect population decline to impact allelic distributions to a greater degree than the average number of alleles per locus (Kuo and Janzen, 2004); this is important as it may obscure the predicted genetic signatures of bottleneck events.

#### 4.2. Effective population size estimates and $N_e/N$ ratios

Overexploitation has significantly reduced the effective size of the Okavango Nile crocodile population. Long-term estimates of  $\hat{N}_e$  suggest a very much larger breeding population of crocodiles once occupied the Okavango River. This is not necessarily surprising given the numbers of adults that were systematically removed by hide hunters from the mid to late 20th century (Pooley, 1982; Craig et al., 1992). We estimated contemporary effective population size using a number of approaches to better approximate the effect of population decline on current  $\hat{N}_e$  and our data suggest that contemporary  $\hat{N}_e$  is at least an order of magnitude lower than long-term estimates. All estimates indicate that contemporary  $\hat{N}_e$  is also considerably lower than the current census adult population size. Estimates of the variance effective size, representing the har-

monic mean  $N_e$  for the period  $Gen^t$  (0) to  $(t - \sim 5) \sim 2005$ –1945, were very similar  $\hat{N}_e$  range  $\sim 208$ –222) and were intermediate to estimates of the inbreeding effective size i.e.  $N_e$  of the parental generations that gave rise to the adult and offspring cohorts under study (Waples, 1989, 2005); our results suggest that the Okavango population has experienced a five-fold decrease in  $N_e$  over this time period ( $N_e$  range  $\sim 480$  to  $\sim 90$ ). Approximations of  $N_e$  can differ if population size has not remained constant during the time period under study; over time greater levels of disequilibrium are predicted to accumulate in reduced populations, thereby limiting the frequency of novel gene combinations and resulting in divergent estimates of  $N_e$  (Hill, 1981). Given the recorded exploitation of crocodiles in the Okavango Delta it is not surprising that the two estimates of  $N_e$  differ and it's most likely that this difference reflects the population's recent demographic history. Before the onset of intensive hunting  $\sim 50$ –80 years ago the effective size was likely to have been substantially higher than today. Together, characteristic features of this population that include an historically large inbreeding effective size, reduced variance effective size, and low adult census size all suggest the rapid decline of a historically larger population of crocodiles (Templeton and Read, 1994).

Because of the increased significance of  $N_e$  in governing genetic processes in reduced populations, we were also interested in the  $N_e/N$  ratio for this population. While Frankham (1995) reports a mean ratio of 0.11 across a range of unmanaged populations,  $N_e/N$  is expected to vary widely depending on the life history of an organism (Nunney and Campbell, 1993; Nunney, 1995). The detrimental effects of population decline are much more immediate in populations with a reduced  $N_e$  relative to  $N$ , whilst the greatest reductions in  $N_e/N$  occur when populations, as in this study, experience repeated fluctuations in size (Frankham, 1995; Vucetich and Waite, 1999). The estimated  $\hat{N}_e/N$  ratio for Okavango crocodiles ranged from 0.04 (current parental generation) to  $\sim 0.1$  (temporal period harmonic mean), where adults account for  $\sim 25\%$  of the population. The  $\hat{N}_e/N_{adult}$  approximates 0.14, which is still considerably lower than previous studies indicating that long-lived species with overlapping generations generally support an effective size close to 50% of the breeding adult number (Nunney, 1993; Nunney and Elam, 1994). Together with annual nest survey data that suggest nesting effort is  $\sim 22\%$  (Bourquin, 2008), our findings may also reflect hitherto unrecognised levels of reproductive skew in Nile crocodiles. Many crocodylians are characterized by a polygynous mating system, but quantifying the degree of skew in natural populations is difficult; mating occurs in the water and can often involve groups of males and females, making clear observations of individual animals rarely possible (Lang, 1989). This finding has important implications for the long-term persistence of the Okavango population. If individuals do not breed for behavioural and environmental reasons then simply increasing the adult population size e.g. via a supplementation program, may not be sufficient to increase the population's effective size. Furthermore, the  $N_e/N$  ratio in mating systems characterized by reproductive skew is very sensitive to factors that reduce breeding opportunities for females (Frankham, 1995; Stiver et al., 2008). It is well established that increased variance in reproductive success of either sex will reduce  $N_e$  (Nunney, 1993; Hedrick, 2005); but in most mating systems variance in female reproductive success will have a greater influence on  $N_e$ ; this is because variance in female reproductive success is more likely to depend on the proportion of females that reproduce, together with the variance in offspring produced per successful female, than on the number of possible mates in the population (Nunney, 1993; Frankham et al., 2002). Increasing levels of habitat degradation currently limit nest site availability for breeding females while extensive human-induced nest disturbance occurs along heavily settled stretches of the Okavango River (Shacks, 2006). In order to mitigate these effects Shacks (2006) proposed

the establishment of 'no-go' breeding channels that could provide optimum nesting habitat for females, and may represent a valuable approach to increasing  $N_e$  in the absence of a supplementation program; nonetheless it first remains to be seen whether these channels will actually lead to increased nesting effort by adult females.

#### 4.3. Maintaining genetic diversity and implications for conservation

Evolutionary potential is conclusively linked to population size (Franklin and Frankham, 1998; Frankham et al., 2002). Nonetheless, the minimum effective size required to maintain genetic variation sufficient to prevent the negative short-term effects of inbreeding depression is difficult to determine and most likely varies considerably among species. Many authors champion the importance of allelic diversity; this is largely because of the role single alleles may play in changing environments (Allendorf, 1986; Fuerst and Muruyama, 1986; Spielman et al., 2004b). Our simulations suggest that the general goal of retaining 90% of current levels of allelic diversity over the next 200 years (~15 generations) can only be achieved if  $N_e$  were to increase and remain at  $\geq 250$ , in which case ~91% of the diversity currently present will be preserved; yet, an even more realistic shorter-term target of  $N_e \geq 150$  over a period of 100 years (~7 generations) would maintain allelic diversity at >90% of current levels ( $N_e \sim 150 = 92.6\%$ ). Based on estimates of the current  $N_e/N$  ratio, these values require a stable census population of ~4200 individuals and an adult population of ~1060 individuals over the next 100 years. Previous studies have identified adult survival as vital for long-term recovery of declining populations in long-lived taxa (Craig et al., 1992; Congdon et al., 1993), and achieving these  $N_e$  targets may require population supplementation in order to mitigate the effects of both ongoing removal of breeding adults and the inherently low population growth rate of crocodylians. Supplementing the current Okavango population is likely to be a major challenge. Under the current quota system, commercial ranching operations are required to return 5% of wild-collected clutches when young crocodiles reach approximately 1.2 m in length; at present this is not common practice and is unlikely to lead to a rapid increase in the adult breeding population. A further challenge to supplementing the current population using commercially reared animals is the incidence of disease in ranches and the risk this presents to wild populations. Most problems stem from poor sanitation, low water temperature and poor diet in commercial operations and include a high incidence of the pathogen *Salmonella* together with diseases associated with chlamydial infections (Dzoma et al., 2008; Lovely, 2007). There is also the added risk that increased harvesting pressure will follow on from the first signs of adult population increase resulting from supplementation or intrinsic recovery (Hutchings and Reynolds, 2004). Increasing the effective size of this population therefore requires a management approach shaped by: (i) the enforced return of healthy, disease free stock, (ii) reduced removal of adult female breeding stock, (iii) mitigation plans to improve nesting rates and ensure reduced nest disturbance, (iv) successful immigration of adults from the upstream Cubango and Quito rivers, and (v) an informed quota system based on the current population status.

## 5. Conclusions

This study reveals the problems implicit in using the retention of genetic variation alone as a proxy for population recovery and persistence in declining and endangered species. Whilst long-generation time may act to reduce the rate at which variation is lost in populations, the partial recovery of the Okavango Nile crocodile masks the reality that the effective size of this population has de-

clined. Additionally, the low  $N_e/N$  ratio of this population suggests that only a few individuals contribute successfully to the next generation, further enhancing its susceptibility to the effects of accelerated drift. These findings have complex implications for long-lived species; partial demographic recovery and an emphasis on the retention of neutral genetic variation in management planning of harvested species may be inadequate. Disregarding the effects of population decline on effective size may rapidly limit the evolutionary potential of once-abundant species and in due course obscure their inevitable decline and extinction.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2009.05.016.

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