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

Jacqueline M. Bishop [a,*], Alison J. Leslie [a], Sven L. Bourquin [a], Colleen O'Ryan [b]

[a] *Department of Conservation Ecology and Entomology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[b] *Department of Molecular and Cell Biology, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa*

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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.

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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[*] Corresponding author. Present address: Department of Zoology, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa. Tel.: +27 21 6503631; fax: +27 21 6503301.

E-mail addresses: jacqueline.bishop@uct.ac.za (J.M. Bishop), aleslie@sun.ac.za (A.J. Leslie), bourquin@sun.ac.za (S.L. Bourquin), colleen.oryan@uct.ac.za (C. O'Ryan).

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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 crocodilian 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 Handling 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 $\leqslant$17 cm snout-vent length (SVL), yearlings 18–38 cm juveniles, 39–65 cm, sub-adults 66–115 cm, adults $\geqslant$116 cm. Blood was collected from the caudal vein in the tail from each crocodile and stored in lysis buffer at $-20°$ 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 $\tilde{N}_{adult}$ and total population $\tilde{N}$ 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 $\tilde{N}$ 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